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ON AGING, OLD AGE AND SENILITY

Conrado S. Dayrit
Emeritus Professor of Pharmacology,
College of Medicine, U.P.
Vice President, Medical Affairs Division,
United Laboratories, Inc.
Philippines

"The study of aging is in its infancy"
(Ray and Barrette, 1973)

Introduction

"Aging" is defined by Webster as the process of growing mature or old. This definition is biphasic. On the one hand, the process of maturation is a positive process that includes growth and progressive attainment of the individual's maximum potentials in structure and function. The second part of the definition of aging, is the process of growing old – a negatively directed process where catabolism, degeneration, wasting and atrophy exceed building and repair. The individual may show structural regression as well as a slowing down of various bodily functions, decreased compensatory reactions and a general deconditioning (Fig. 1).

It will be in the regression-degeneration sense that "aging" will be used in this paper.

Life Span

All living organisms have a determinable life-span (1.1, 1.3). Man's average life-span is listed as 90 years (Table 1) but it appears to have been undergoing significant changes during his existence on planet Earth. According to the Bible's Old Testament (2, 3), Adam, the first man, lived 930 years and his immediate descendants lived similar life-spans, the longest being Methusela's 969 years (Table 2). Noah was 600 years at the time of the Deluge and he lived another 350 years afterwards. Interestingly, the life-spans of post-Deluge people shortened significantly. Abraham who lived circa 2100 BC attained only 175 years, Moses 120 years, and David and Solomon (circa 1000 BC) could not have lived more than 60 or 70 years for they both reigned as king for only 40 years each.

The pharaohs of Egypt (1.5) who lived at about this time (3,000 to 1,000 years B.C.) reigned for approximately the same number of years as David and Solomon (Table 3) and very apparently did not have the life span of their con-

Table 2. Biblical life spans

	Years				*Years*
Adam	–	930	Paleg	–	239
Seth	–	912	Reu	–	239
Enosh	–	905	Serug	–	230
Kenan	–	910	Nahor	–	148
Mahalalel	–	895	Abraham	–	175
Jared	–	962	Sarah	–	127
Enoch	–	365	Ishmael	–	137
Methusela	–	969	Isaac	–	180
Lamech	–	777	Jacob	–	147
Noah	–	950 (600 at time of Deluge)	Joseph	–	110
			Moses	–	120
Shem	–	600	Joshua	–	110
Arpachshad	–	438	David	–	? + 40 as king
Shelah	–	433	Solomon	–	? + 40 as king
Eber	–	464			

From: The New American Bible (Illustrated). Translated from the Original languages with Critical Use of All the Ancient Sources by Members of the Catholic Biblical Association of America, 1970.

Table 3.

Early Dynastic Period (c. 2686) – 414 years

1st Dynasty – kings
2nd Dynasty – 6 kings — Average reign = 34.5 yrs

Old Kingdom (c. 2686 – c. 2160) – 526 years

3rd Dynasty – 5 kings
4th Dynasty – 3 " — Average reign = 24 yrs.
5th Dynasty – 7 "
6th Dynasty – 4 "
(Pepi II, child king, reigned 94 years)

1st Intermediate Period (c. 2160 – c. 2040) = 120 years

7th Dynasty – ?
8th Dynasty – ?
9th Dynasty – ?
10th Dynasty – ?
11th Dynasty – 3 kings

Old Age or the "Elderly"

Victor Hugo (1802-85) was quoted as having said "forty is the old age of youth and fifty is the youth of old age." The U.S. Senate Special Committee on Aging (1985-86) has classified the elderly into four groups (6): the "near elderly" (55-64 years), the "young old" or "elderly" (65-74 years), the "old old" (75-84 years) and the "very old" (85 years and over). Chronologically, therefore, old age is defined to start in the 5th to the 6th decade of life with about a 10 year difference between what was considered old in Victor Hugo's time and what is old now (Table 4).

Table 4. The "Elderly"

*Old age of Youth	40 – 50	
Youth of Old Age	50 – 60	**
	55 – 64	Near Elderly
	65 – 74	Young Old or Elderly
	75 – 84	Old Old
	85 +	Very Old

*"Forty is the old age of youth, fifty is the youth of of old age" – Victor Hugo

**Aging America: Trends & Projections. Prepared by the U.S. Senate Special Committee on Aging 1985-86 ed., Wash. D.C.

Retirement at 65

Retirement from one's job at age 65 is said to have been started by Bismarck (Prince Otto-Leopold von Bismarck Schonhausen (1815-98). Actually it appears that he really picked this up from Krupp's Armaments that chose 65 because almost nobody reached that age and yet the illusion of retirement could still be given (7). Another version was that Bismarck asked for an age that will ensure that not too many will draw retirement benefits for too long. Roosevelt's Special Security then helped entrench it in 1930s (7).

Retirement is acceptance of aging with its decreased physical and mental capacity and increased disease propensity. It is the employers protection against age-induced loss of efficiency and debility. On the other hand, it is also an opportunity for the elderly to enjoy a well-earned rest and for the still-young to embark on other projects or even a new life. The increasing life-span of the U.S. population has caused the retirement age to be raised to 70 years with no age limit in many industrial and academic institutions. Except for our Justices, we still retire our people at 65.

The Aging Population

The improvements in medicine and public health including maternal and child care has been extending the life-span upwards and decreasing infant deaths and birth accidents. Hence, the average life expectancy has been rising together with the number of the "elderly" (Table 5). The Philippine population is still a young one with about 40% children 0-14 years and 10% in the 50 years-and-over bracket. These figures are expected to change. By 1992, the 0-14 bracket is predicted to decrease to 37.8% while the 50-and-over segment will increase to 11.2%. It will take the Philippines perhaps 3 or more generations of good health care and sanitation and population control before we will approach the present population mix of the developed countries of Europe and the U.S. where the population growth is zero or near zero, where children are seldom seen around and the nursing homes are full of the aged and the infirm. Now they worry that there are too many aged and too few children.

Table 5. Philippine vital statistics

Population	*54,668,332*	*57,356,042*	*64,258,611*
0 – 14 yrs.	40.3%	39.7%	37.8%
15 – 49	49.2	49.6	57.0
50 & over	10.5	10.7	11.2
Male	50.2	50.2	50.2
Female	49.8	49.8	49.8
Ave. Life Expectancy (yrs)	63.1	63.7	65.2
Crude Death Rate/000	7.9	7.6	7.0
Infant Mortality Rate per 000 Live Birth	56.59	54.07	47.74

DOH's Health Plan for People's Health 1987-1992.

Stages of Life

All living organisms go through 3 stages during their life-span: growth, reproductive and senescent. The relative durations of each stage varies (1.1). In the semelparous, reproduction takes place near the end of the life span. The salmon, for instance, from their spawning grounds in Canadian and Alaskan rivers that empty into the Pacific Ocean, swim out to sea and after spending almost their whole lifetime roaming the oceans of the world they return guided by their ins-

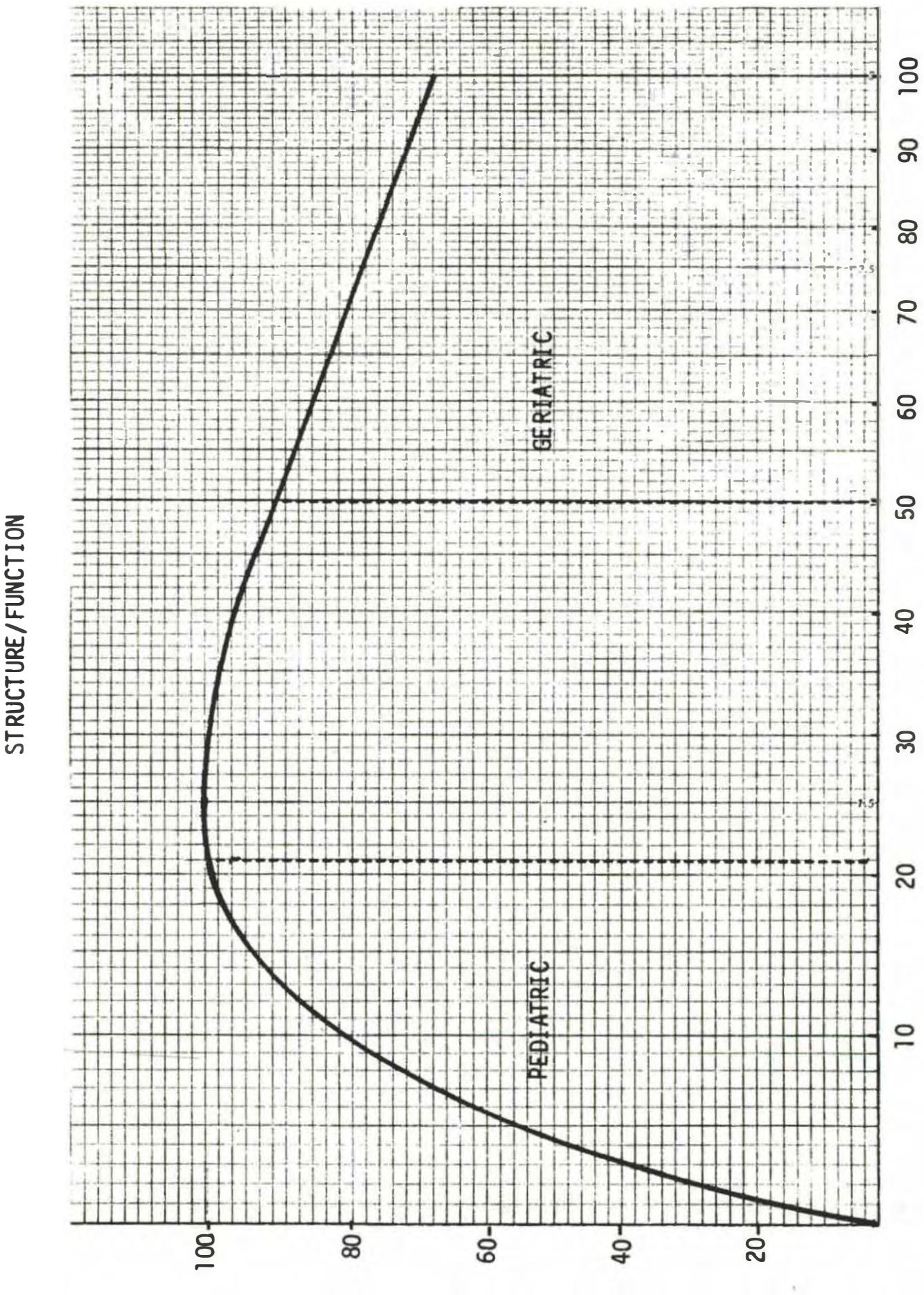

STRUCTURE/FUNCTION
100
80
60
40
20
GERIATRIC
PEDIATRIC
10
20
30
40
50
60
70
80
90
100
A G I N G (years)

Figure 1.

tincts, to the headwaters of their birth to deposit their eggs; after which they rapidly become senescent and quickly die. Man, on the other hand, is an iteroparous, and his reproductive period covers a major part of his total life-span. Senescence in the iteroparous is gradual in onset and progression, accompanied by a slow decline in reproductive performance, and accelerating with increasing age.

Aspects of Aging

Aging has been divided into: a) biological or physiological, b) psychological or behavioral and c) sociological or economic aspects. A 4th and the most commonly used as reference in the chronological aging which underlies the preceding three and, in the end, becomes the ultimate determinant if we believe that life-span on earth has been predetermined. The more poetic Greeks had their Fates: Clotho who spun the golden thread of life, Lachesis who measured it and Atropos who severed it.

All these facets of aging interact and influence each other in a positive or negative manner (Fig. 2). An optimistic outlook, for instance, can definitely benefit the sociological and physiological reactions of a person – just as mental depression can hasten physiological or biological decline. This is partly why there are the "young at 90," and the "old at 30," and those who "die of a broken heart." A sick patient has better chances of recovering when he has optimism. When he loses the will to live he starts to die.

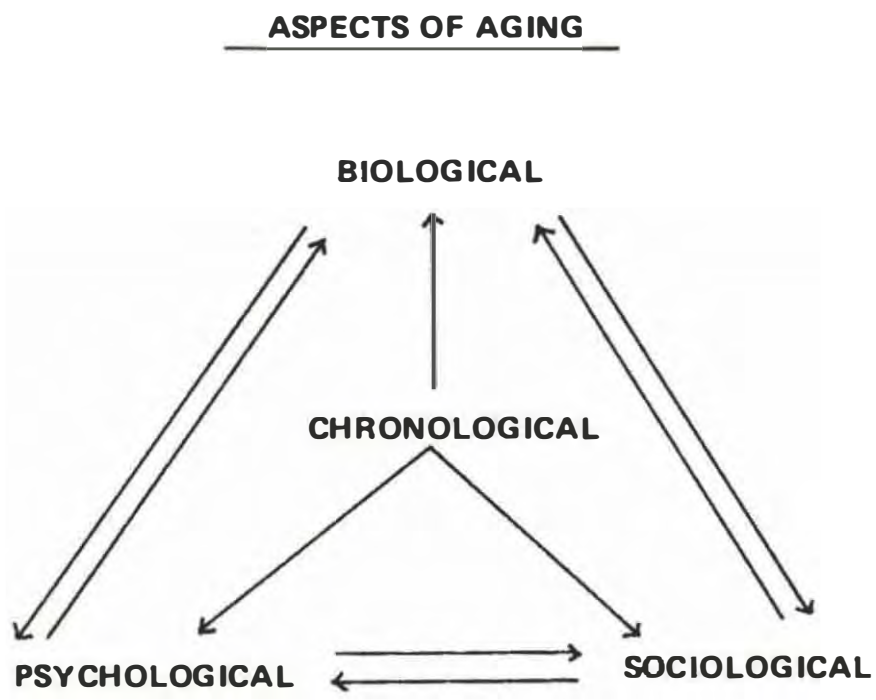


Figure 2.

Genes and Control of Growth and Development

All biological directions in living organisms are stored in the DNA and since all cells of an organism are the daughters, granddaughters and great granddaughters of a single fertilized ovum, they all contain the complete genetic material in their DNA. However, as cells differentiate and branch out towards more specialized cell types (e.g. neurones, muscle cells, white blood cells, connective tissue, etc.) and as they further mature into their specific target end-cell types (cortical pyramidal neurones, ventricular myocardial cells, T-killer lymphocytes, elastic or collagen fibers, etc.), they limit operation to selected genes. These genes are those that produce the characteristic proteins for the specific structures and functions of the cell at each stage of development. The amount of DNA information utilized by a fully mature cell is estimated to be only 0.4% of the total genetic information in the cell's DNA (8). The rest of the vast material in the DNA is repressed and may never be expressed for the life of the cell. Development of a cancerous state in a cell may derepress some genes and cause a cell to retrogress to a more primitive or immature state. Its specialized function is then lost but its rate of mitosis increased many many fold. In this state a cell line may survive in culture indefinitely without apparently being affected by aging, such as the HeLa cells which were obtained from the uterine cervical cancer of a patient named Helen Lane in 1952; these cells have been maintained in tissue culture up to the present as in their original state.

Normal cells, however, appear to be limited as to the number of divisions they can undergo. Hayflick's experiments (8) with fibroblast cells in tissue culture under carefully controlled conditions showed that human embryonic fibroblasts undergo a fixed 50 ± 10 population doublings within 7 to 9 months, at a doubling rate of about once per week, which slows down to 10 days as senescence develops. The cells finally fail to divide enough to reach confluence, show signs of degeneration, and die. The life-span of 50 doublings appears to be intrinsic to the embryonic fibroblast cells and they manifest this by a remarkable demonstration of "memory." For after interruption of cell division by freezing in liquid nitrogen for any length of time (in the case of one cell culture, 13 years) the cells when thawed out resumed their doubling to a lifetime total of 50 and no more. Fibroblasts obtained at autopsy from lungs of human adults 20 to 87 years, however, doubled only 14 to 29 times (8). Other cells, like those of the skin, liver, brain and smooth muscles have also been subjected to similar tissue culture studies by other workers and have been found to possess a similar limited capacity to divide. This is the genetic life span under experimental conditions. What it is in vivo, we do not know.

Tissue culture studies being essentially in vitro do not of course and cannot take into account all the in vivo factors, particularly bioamines and hormones, that may influence and delay the aging process. What these studies say is that there seems to be a built-in mechanism in every cell's DNA that counts the number of times it can divide and shuts off the whole machinery at the end of its pre-

determined critical limit. How far is men's present life-span from his genetic life span is the $64,000 question.

Some cell lines continue to divide for the duration of a man's life span; while others divide little if at all after reaching maturity (8, 9). Cells of the bone marrow, skin, connective tissues and the mucosal lining of the gut continue dividing and replacing the cells lost. Red blood cells, for instance, have a life span of 120 days and are replaced by new rbc from the bone marrow. Platelets live for only 10 days and are continuously replaced. Thus, the bone marrow is in a constant state of mitotic activity. Any depression of this activity leads to decreased number or count in the circulating blood: anemia, granulocytopenia, thrombocytopenia, agranulocytosis. This is not so with nerve cells, muscle cells, endocrine cells, and cells of the special senses. These cells stop dividing soon after birth, although they continue to grow in size up to maturity. The brain, cannot replace its neurones damaged by a stroke; nor can the heart replace its infarcted myocardium. Repair of these damaged areas is by scar tissue and the function lost may be compensated to greater or lesser degree only by hypertrophy of the remaining functional elements. And an eye lost is lost forever. A scarred cornea has to be replaced by a corneal transplantation. Malchus would have had to live the rest of his life without the ear Peter chopped off had Jesus not put it back.

It has been said that with the continuous division and replacement of cells, an individual is not the same individual he was a few years ago. This generalization appears to be untrue as far as cell identity is concerned – for the cells of the brain and nervous system, and those of the heart and endocrine glands are the self same ones that the individual had when he was born and the same ones that will age and die with him. From the molecular point of view, however, it has been argued that with the continuous replacement of the protein structures of the cell, it no longer is the same molecular identity. Perhaps so, but exception must have to be made for the cell's chromosomes and DNA strand and, more important, the genetic information and directions the DNA carries. Perhaps in the regulatory signals of the DNA, its operons, repressors, derepressors, etc. (Fig. 3) lie the secret of growth, aging and senescence.

Effects of Aging

The first evidences of aging are functional in nature, particularly those elicited under stress. They usually start slowly, imperceptibly about the age of 40, perhaps earlier in some, and are difficult to distinguish from the lows of normal function. The athlete tires a little more easily, performs less accurately and reacts more slowly a little more often than before. The executive has difficulty in reading the fine print and has to have reading glasses – and having acquired them he often forgets where he placed them. The audiophile complains that his fine HiFi stereo does not give out quite the same brilliance and presence as before. The males start complaining to their physicians about their sexual decline. The women, about half

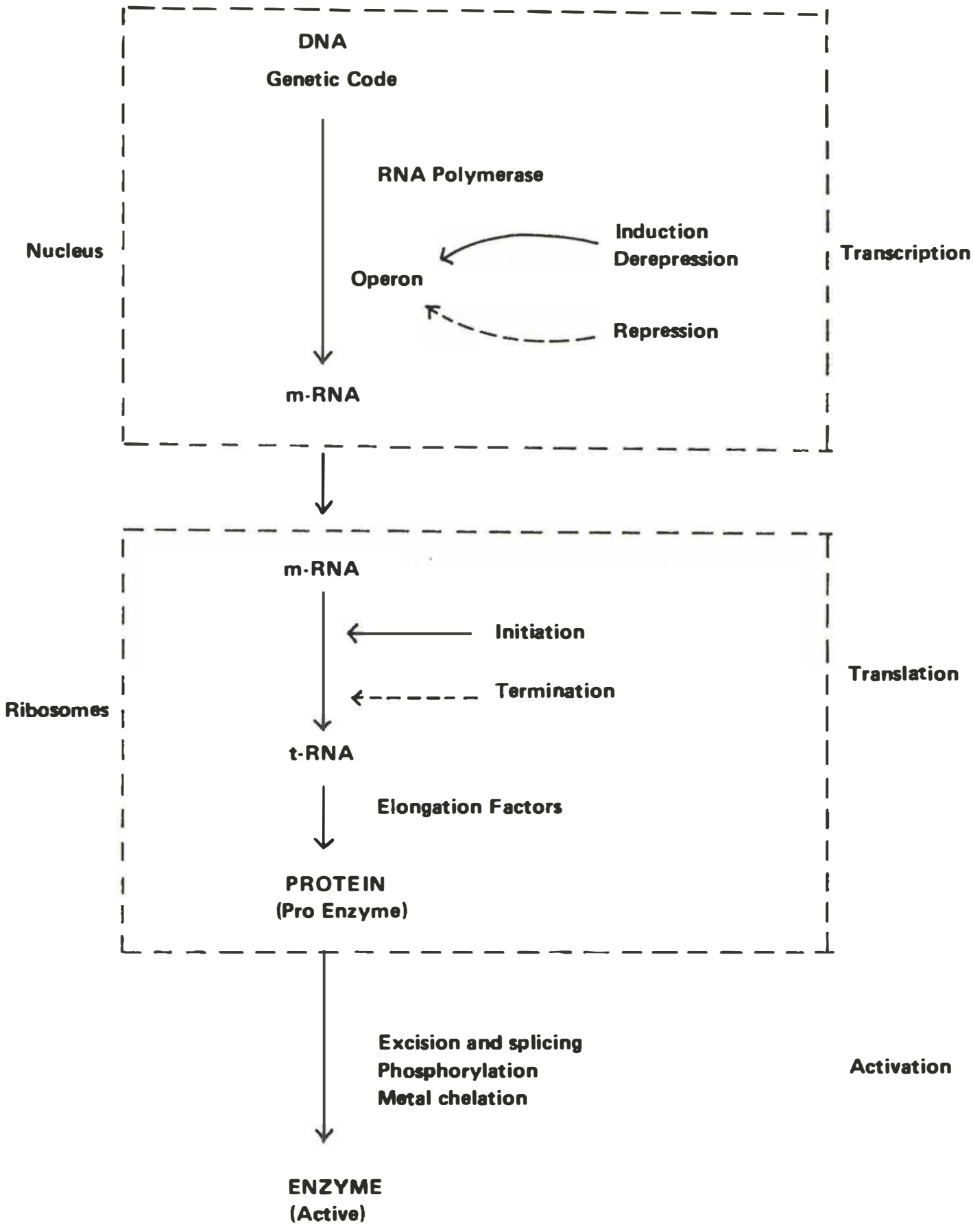

DNA
Genetic Code
RNA Polymerase
Nucleus
Induction
Derepression
Transcription
Operon
Repression
m-RNA
m-RNA
Initiation
Translation
Termination
Ribosomes
t-RNA
Elongation Factors
PROTEIN
(Pro Enzyme)
Excision and splicing
Phosphorylation
Metal chelation
Activation
ENZYME
(Active)

Figure 3.

of them, for their part, start to complain of hot flushes, increased nervousness or elevated blood pressure – signs of menopause.

Body Changes Found in Old Age

Table 6 (1.2, 9, 10, 11) summarizes the salient features of aging. Almost every organ and system of the body is involved in the process of degeneration. The degree of involvement, however, and the time they occur, differ from person to person. They may appear earlier in life, or later, or perhaps not at all. Most however do show the tell-tale signs of aging of skin and/or hair. A progressive increase in body fat with aging (12.2) (Fig. 4) has been reported by many but the data comes from developed countries. This finding is true also of the segments of our society whose caloric intake is well in excess of their physical activity – which happens when strenuous physical activities cease with the athletics of youth and the one exercise that easily is within every one's physical capacity is not done – that of pushing one's self from the table. Those who eat well-within their caloric requirements do not become obese – as many members of this venerable Academy can show as living proof.

But perhaps one of the most striking evidence that aging is really a retrogressive process is the gradual decrease in height of both sexes (Table 7) (12.1). In male whites this decrease starts about the age of 35 and in the females as early as 30. The obvious obesity of the population in this Table is seen in the slower fall in their weight. In time, the weight also follows as a result of decrease in muscle mass (13) from disuse atrophy and loss of bone mass (calcium, phosphates and protein matrix). Both changes are basically due to protein loss possibly due to alteration of enzyme activity. Decalcification and bone resorption begins around age 35 and results in thinning of the cortical layer of bone by 20% and 30%, respectively in men and women surviving to 90 years of age (14). This explains the predisposition of the hip bone to be fractured by minor accidental falls and of the vertebral bodies, the weight bearers of the body, to be compressed and fractured.

Effects on Collagen. Collagen is a structural protein that is the most abundant in the body, comprising 25 to 30% of total body protein. It is the principal component of tendons, ligaments, basement membrane and all connective tissues; it is also present in bone and cartilage, blood vessels, intervertebral disks and the cornea and lens of the eye (Table 8). Aging appears to alter the levels and activities of various enzymes (hydroxylases, glycosyl transferases, pro-collagen peptidases, lysyl oxidases) that act on collagen and thereby cause the polypeptide chains of collagen to become increasingly cross-linked, inflexible, and less soluble. Collagen being an extracellular protein, is not turned-over like other proteins and cannot be renewed; so that when it is cross-linked, the organ affected suffers degeneration of function and structure (9). In arteries, for instance, collagen change is one of the mechanisms involved in the atherosclerotic process. Presbyopia and cataract of the lens of the eye are directly linked to collagen degeneration. And weakening of the interver-

tebral disk and its nucleus pulposus explains why the aged spine becomes rigid and deformed. The same can be said of the synovial membrane of joints and development of arthritis in the aging person.

Table 6. Body changes and disease conditions found in old age

Organ/System	*Pathological/Biochemical changes*	*Function changes and symptoms*
GENERAL		
Growth	Decreased Wt and Ht from about age 40	
Fat	Increasing fat deposition (in heavy eaters)	Obesity, increased abdominal size
INTEGUMENTAL		
Skin	Decreased collagen and elastic fiber. Increased cross-linking of collagen (Types I,III) fibers. Decreased interstitial fluid	Wrinkling, thinning of skin
Hair	Hair follicle degeneration and atrophy	Graying, thinning, falling off, balding
BONES/JOINTS	Osteoporosis – loss of bone matrix and mineral components	Tendency to fractures
	Spondylosis	Back pains, kyphois
	Arthritis	Joint pains, deformity Difficulty in bending, squatting, standing up
SKELETA MUSCLE	Atrophy from disuse (decreased physical activity)	Decreased muscular strength Decreased lean body mass
NEURO-MOTOR SYSTEM	Decreased number of neurones Decreased number and sensitivity of receptors	Weakness, slow reaction time, tremors Decreased proprioception difficulty in balancing Decreased muscular control for fine movements
CARDIOVASCULAR SYSTEM		
Arteries	Atheroma formation; loss of elastin and collagen narrowing calcification thrombosis, occlusion	Reduced blood flow to organs supplied; ischemia, infarction of heart, brain, kidneys, extremities High blood pressure, esp. systol

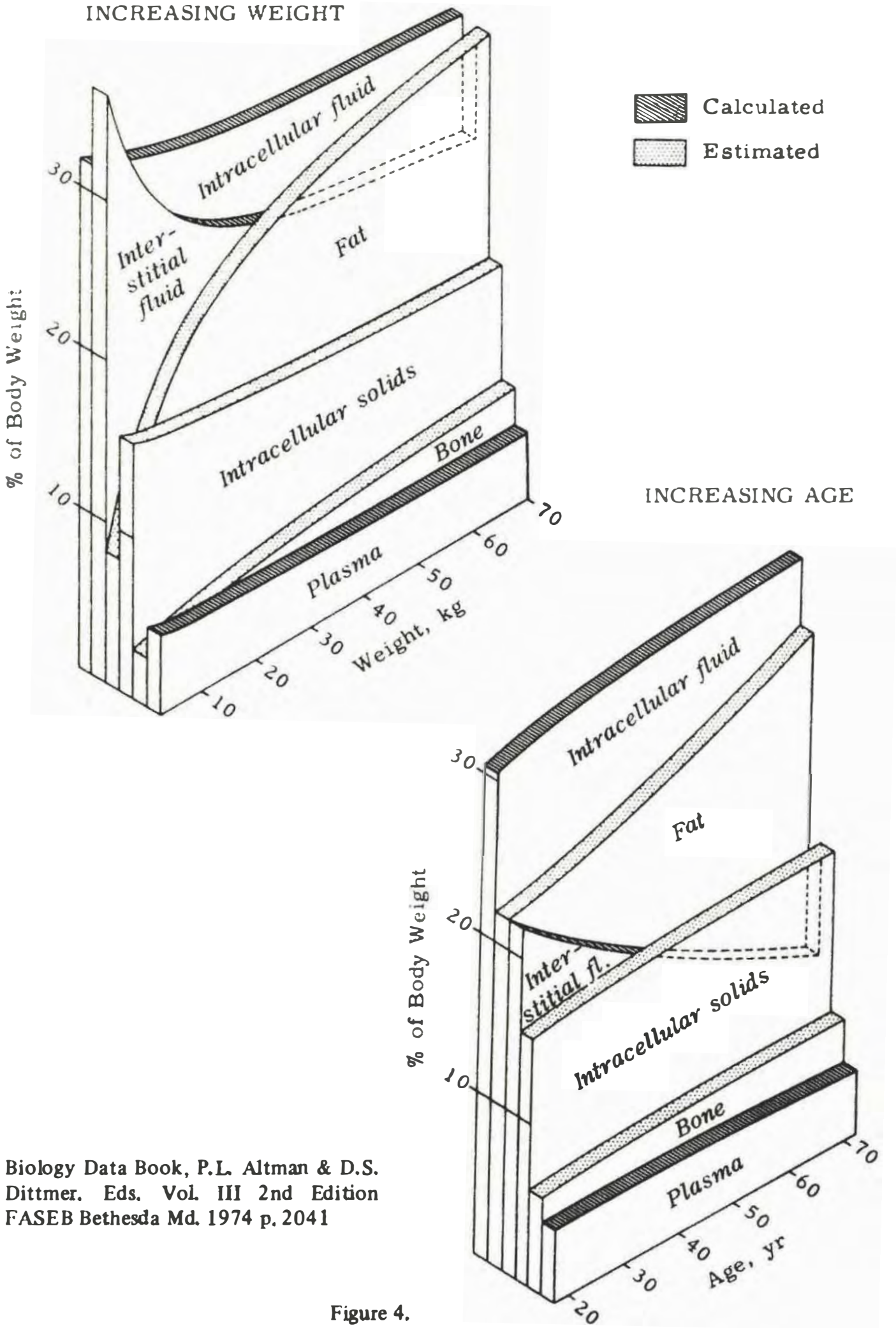
Heart	Myocardial ischemia, infarction	Angina pectoris
	Aortic and mitral valve thickening and calcification	Decreased stroke volume and cardiac output.
	Amyloid deposition	Arrhythmia, palpitations
	"Brown atrophy"	Weakness, shortness of breath congestive heart failure
Veins	Leg vein varicosities	Leg pain, edema
LUNGS/BRONCHI	Chronic bronchitis, bronchiectasis	Wheezing, dyspnea, cough
		Weakness
GUT	Decreased digestive enzymes	Appetite loss, indigestion
	Decreased bowel motility	Constipation
	Hemorrhoids	Anal pain, bleeding
LIVER	Decreased drug metabolizing enzymes	Increased drug toxicity
KIDNEYS	Decreased renal blood flow and glomerular filtration rate	Increased drug toxicity
PROSTATE	Benign prostatic hypertrophy	Urination problem
SPECIAL SENSES		
Eyes	Lens less elastic	Presbyopia
	Cataract	Blindness
Ears	Otosclerosis	Deafness, for high notes to total
	Vestibular hypersensitivity	Intolerance to tilting, tendency to faint
Olfactory Taste	Reduced number and sensitivity of receptors	Change in sense of smell and taste
ENDOCRINES	Cessation of ovarioan function; reduced estrogen secretion	Menopause; osteoporosis
	Decreased testosterone secretion	Decreased sexual potency
	Decreased insulin secretion	Late onset diabetes mellitus
	Decreased T_3 secretion	Overall decrease in metabolic activity
IMMUNOLOGIC SYSTEM	Thymus involution, Decreased Helper T-lymphocytes	Increased susceptibility to infections. Increased predisposition to cancer
	Decreased ability to distinguish "self" from "non-self"	Auto-immune diseases

BLOOD	Decreased erythropoiesis Increased total cholesterol and triglycerides Decreased fibrinolysis	Senile anemia Increased tendency to thrombosis
BRAIN	Decreased number of neurones Decreased number and sensitivity of receptors Cerebral ischemia and degenetion; stroke	Decreased reaction time Weakness Impaired memory Decreased learning solving ability Paralysis, paresis Parkinsonism Senile depression, psychosis Amyotropic lateral sclerosis Alzheimer's dementia

Table 7. Growth: height and weight by age

	Age	*WEIGHT*		*HEIGTH*	
		Male (kg)	*Female (kg)*	*Male (cm)*	*Female cm)*
U.S. (1960-62)	18-24	72.6	58.5	174.5	162.1
	25-34	77.6	61.7	175.5	161.8
	35-44	78.0	65.3	174.0	161.3
	45-54	78.0	66.7	173.2	159.8
	55-64	75.3	68.9	171.2	158.5
	65-74	72.6	66.2	169.9	156.2
	75-79	68.0	62.6	167.4	155.2
Caucasian	20-24	71.7	56.7	174.5	162.6
	25-29	73.9	57.6	174.5	162.8
	30-34	74.8	59.0	174.0	161.5
	35-39	75.3	61.7	173.7	161.0
	40-49	75.8	64.4	172.7	160.5
	50-59	74.8	67.1	170.9	159.5
	60-69	73.5	66.2	169.7	158.0
	70-79	71.2	65.3	168.9	157.0
	80-89	68.5		167.9	

From: PL Altam & DS Dittmer Eds. Fed. Am. Soc. Exptal Biol. 1972 Vol. 1 p. 201



Biology Data Book, P.L. Altman & D.S. Dittmer. Eds. Vol. III 2nd Edition FASEB Bethesda Md. 1974 p. 2041

Figure 4.

Table 8.

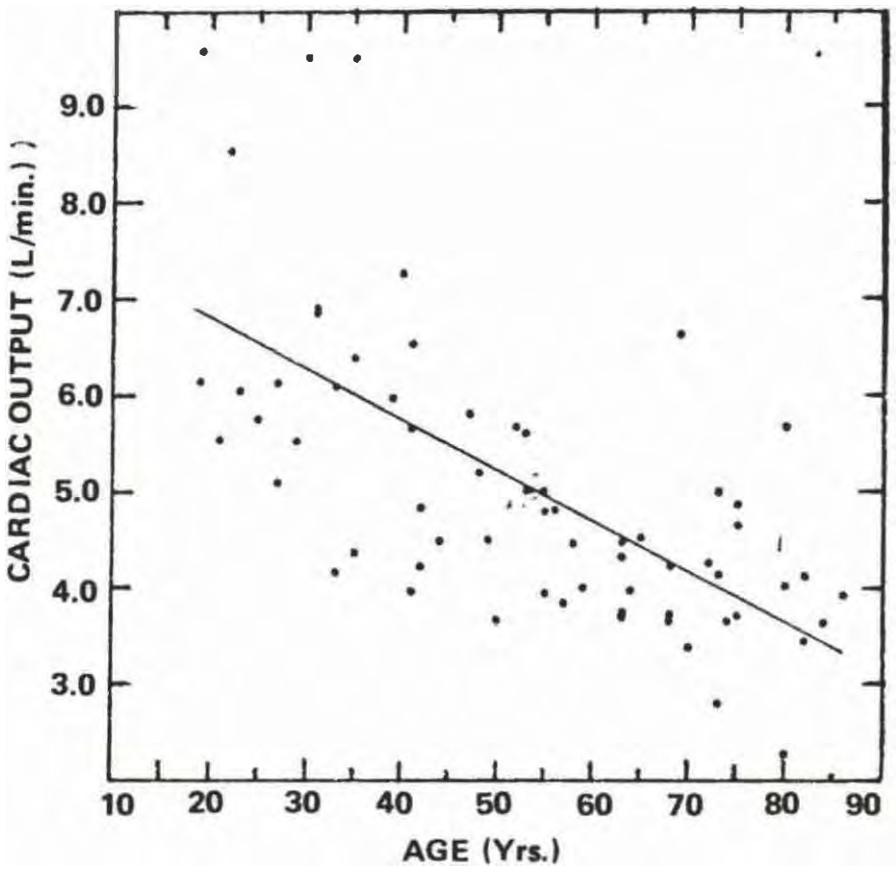
Collagen type	*Chain structure*	*Distribution*
I	$[\alpha 1(I)]_2 \alpha_2$	Bone, tendon, skin, connective tissue, intervertebral disk, blood vessels
II	$[\alpha 1(II)]_3$	Hyaline cartilage, eye lens, cornea, nucleus pulposus of the intervertebral disk
III	$[\alpha 1(III)]_3$	Skin, blood vessels, smooth muscle, synovial membrane of joints
IV	$[\alpha 1(IV)]$	Basement membrane

Modified from: MS Kanungo: Biochemistry of Aging. Acad. Press 1980 p. 137

Effects on the Cardiovascular System: The principal effects of aging are probably exerted mostly on the arteries, while the effects on the heart are secondary in nature. Microscopy may indeed show increase in collagen and elastic fibers in the old heart, and atrophy or hypertrophy of myocardial cells in different areas (11); but although these could be part of a senile cardiomyopathic process, the more probable is that they are due to the coronary sclerosis that invariably is present in these old hearts. The aphorism that "one is as old as one's arteries" still stands. Arteries carry life's sustenance to every organ in the body and it is inevitable that any interference with this conduit will cause damage and dysfunction of that organ.

The aging of the cardiovascular system starts very insiduously as early as the prime of life. Brandfonbrener *et al.*, (15) report a steady decline in the basal cardiac output of 67 males without cardiovascular disease starting about the age of 30, with the regression line of the cardiac output decreasing by 1.0% per year of age (Fig. 5). The regression line of the cardiac index (L/min/m body surface) decreases 0.79% per year of age. The arterial pressure, on the other hand, shows a rather steep increase of both average systolic and average diastolic pressures from infancy till age 20-24; whereupon, while the average systolic BP continues to increase more slowly till age 70-74 to hypertensive levels, the average diastolic BP curve remains fairly flat and normal at around 80 mm Hg (Table 9 and Fig. 6). The result is a systolic type of hypertension characteristic of the elderly and attributed to increasing rigidity of the large arteries of the body as a result of atherosclerosis. This is the simple picture when average systolic and diastolic pressures are examined. But when the upper ranges of BP readings per age group are studied (Table 9) it is evident that, considering the recently established BP standards of 135/85 as the

upper limit of normal and 140/90 as the lower limit of hypertension, the subjects in the studies of 1940s-50s cited in Table 9 and Fig. 6 unfortunately include systolic and diastolic hypertensives both male and female even from the age of 20-24 years. Does this vitiate the studies? The problem is a little like the chicken and the egg dilemma. Should subjects showing up readings of 140/90 or higher be excluded from the survey because they have hypertensive disease and will confuse the effects of aging? or should they be included for the reason that hypertension is a part of aging? Are hypertension and atherosclerosis a part of aging? The question is still moot.



The relationship between cardiac output and age in 67 males without circulatory disorder and during "basal" state. The line indicates the simple linear regression for the data. (From Brandfonbrener, M., Landowne, M., and Shock, N.W.: Changes in cardiac output with age, Circulation 12:557, 1955).

Figure 5.

Atherosclerosis causes hardening, narrowing and blocking of arteries resulting in myocardial infarction, stroke and death. These diseases are most often found in old age and thus have been considered as almost intrinsic to the aging process. Since the 1950s, however, concerted efforts of the world's scientists focused on this problem as it was reaching epidemic proportions. The strong predisposing causative roles of saturated fats, cholesterol and blood lipids, lack of exercise, smoking, hypertension, diabetes, etc. (Table 10) were one by one established. In the U.S. more than

Table 9. Arterial blood pressure

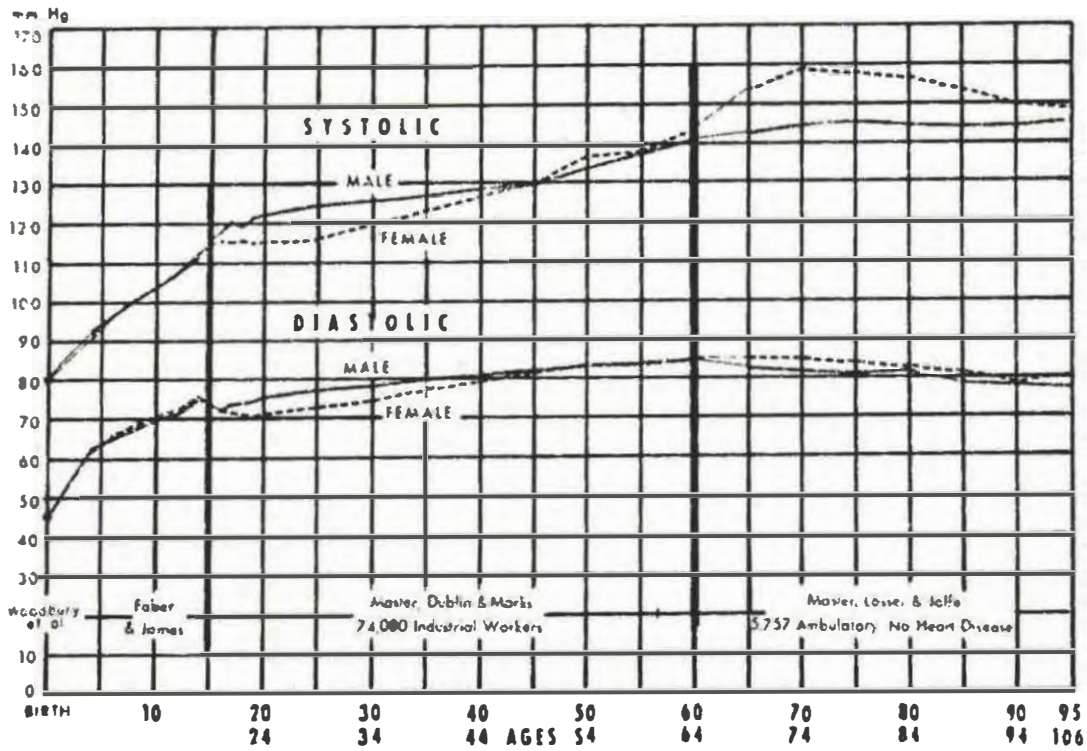
	Male		*Female*	
Age	*Systolic*	*Diastolic*	*Systolic*	*Diastolic*
20-24	123 (96-150)	76 (57- 96)	116 (93-139)	72 (53- 91)
25-29	125 (100-150)	78 (60- 95)	117 (94-139)	74 (56- 92)
30-34	126 (99-153)	79 (60- 98)	120 (92-147)	75 (54- 96)
35-39	127 (99-155)	80 (60-101)	124 (97-151)	78 (58- 98)
40-44	129 (100-159)	81 (63-100)	127 (94-161)	80 (59-100)
45-49	130 (97-163)	82 (51-103)	131 (92-169)	82 (59-104)
50-54	135 (97-172)	83 (61-106)	137 (96-179)	84 (59-108)
55-59	138 (101-175)	84 (62-106)	139 (97-180)	84 (61-106)
60-64	142 (100-183)	85 (60-109)	144 (100-188)	85 (60-110)
65-69	143 (92-194)	83 (64-102)	154 (97-211)	85 (58-112)
70-74	145 (93-197)	82 (52-112)	159 (108-210)	85 (55-115)
75-79	146 (104-188)	81 (56-106)	158 (106-210)	84 (58-110)
80-84	145 (95-195)	82 (63-101)	157 (102-212)	83 (57-109)
85-89	145 (98-192)	79 (50-108)	154 (99-209)	82 (48-116)
90-94	145 (99-191)	78 (54-102)	150 (104-196)	79 (55-103)

Ref.: AM Master, HA Lindsay, WS Hartroft (in Biology Data Book 2nd ED 1974 Vol. III p. 1714)

Table 10. Atherosclerosis: predisposing factors

1. Time and Aging – "wear and tear"
2. Genetic (hereditary)
3. Lack of exercise – sedentary
4. High caloric, high fat diet
5. High serum total cholesterol and LDL, low HDL
6. Hypertension
7. Diabetes
8. Smoking
9. Psychic, emotional stresses, Type A personality

half (51.4%) of all deaths in the 1950s were due to heart disease, principally arteriosclerotic-coronary (slide). Now, as a result of the drastic changes in the national diet (from about 3,300 daily calories with 40-45% animal fats to a much leaner diet) and the promotion of exercise, as jogging and walking (16), and the serious campaign against smoking (17), the cardiovascular mortality rate has come down to only 30% of total deaths and the average life-span has been increased to 74.5 years. Much still remains to be done but the important lessons to be derived is the differentiation possible between the aging itself and those diseases found in aging whose control can enable us to modify the aging process.



Mean blood pressure levels in apparently healthy people from birth to old age. (From Master, A.M., and Lasser, R.P.: Blood pressure elevation in the elderly. *In:* Hypertension: Recent Advances, Brest, A. N., and Moyer, J. H., editors. Philadelphia, 1961, Lea & Febiger, pp. 24-34.)

Figure 6.

Unfortunately, the process of atherosclerosis (Fig. 7) is much more complicated than just lipid deposition as proposed in the Insudation Theory of Rudolf Virchow and supported by the cholesterol-feeding studies in rabbits of N. N. Anitschkow. Firstly, even without formation of an atherosclerotic plaque, there is a wear-and-tear type of degeneration of the arterial wall seen with aging. This consists of (Fig. 8): a gradual thickening of the intima, a thinning of the muscular layer, disruption of the internal elastic membrane, increase in fibrous and collagen fiber deposition and an overall hyaline degeneration of structures.

In 1976, Benditt (18) found that even preceding any sign of lipid deposition, special smooth-muscle-like cells from the muscular layer migrate to the intima and initiate the plaque formation (Monoclonal Migration Theory). What induces those cells to migrate is not known but one plausible explanation is that endothelial injury may attract platelets to adhere and aggregate and to release, among other known substance, a migration or growth factor. The deposition of cholesterol and other lipids in the plaque appears to be in a later development. The lipids in lipoprotein form enter the wall of blood vessels from the blood stream and are deposited in the intima if they can not pass out thru the lymphatic channels of the vasavasorum (19). The formation of the plaque at this stage is complicated by stimulation of the clotting process – formation of layers of fibrin clots or thrombus over the plaque, together with calcification of the plaque, which further narrows and finally occludes the artery, choking off the circulation to the heart, brain,

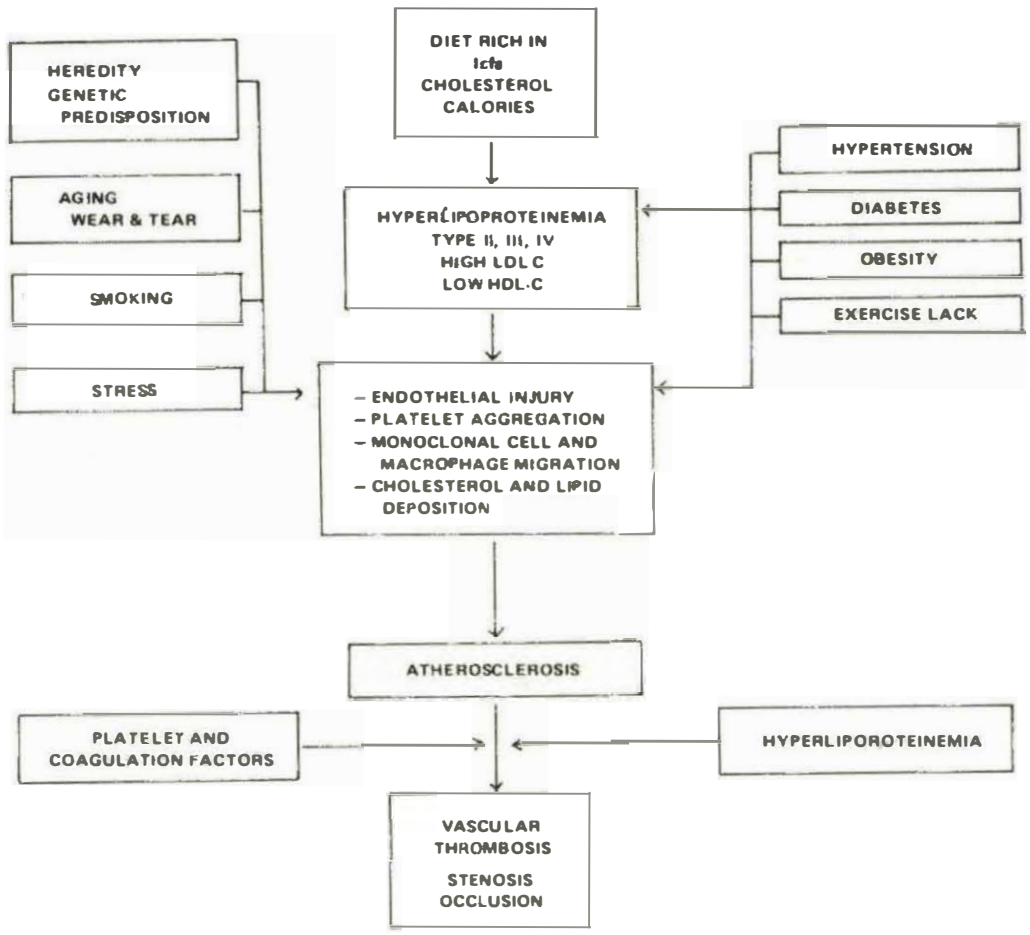


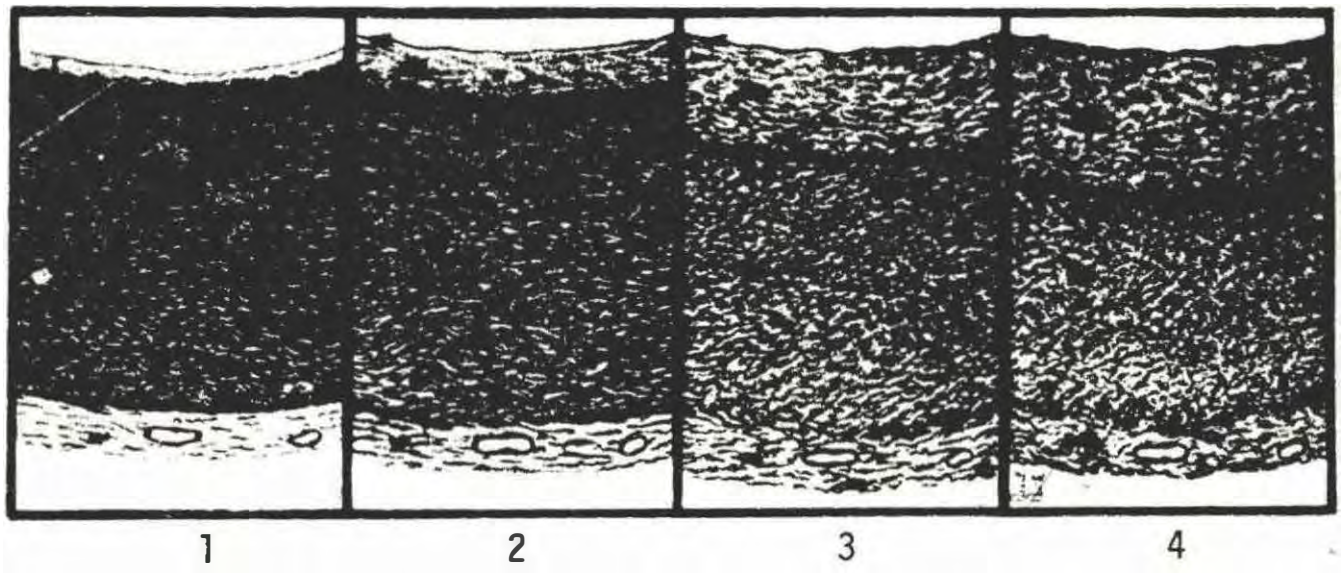
Figure 7.

kidneys or leg (Fig. 7). This last process, the clotting, also appears to be controllable to some extent now with antiplatelet aggregation agents, such as aspirin. Hence, the only process still beyond our control in what we know about atherosclerosis is the initiation of the plaque by these migrating monoclonal cells and the understanding of how the genes do it – why some persons develop atherosclerosis very early in life and some do not and are able therefore to break the age barrier.

Effects on the Communications System

On the Adrenergic System: The adrenergic system is the "fight or flight" division of the autonomic nervous system. Together with the adrenaline it causes to be released from the adrenal medulla, it prepares the body for action. Thus it makes the heart beat stronger and faster, the bronchi to dilate, the blood vessels to constrict, the blood pressure and the blood glucose to rise. Aging depresses the adrenergic control over these various organ systems (20-25). The heart rate at rest becomes slower and the ability of the heart rate to increase in response to exogenous adrenaline or isoproterenol is reduced. So also is the ability of the blood pressure to respond to baroreceptor reflexes, resulting in the well-known tendency of old persons to orthosthatic hypotension. The effects of aging on the heart and its nerves have been demonstrated in rats by electron micrography (Table 11). The older the rat is (and rats are old at 12 months and ancient at 24 months) the more degenerative findings are seen involving not only the structures of the myo-

Degenerative changes due to aging: Four states in the aging process of the aorta



1. Adolescent (thirteen to nineteen years of age). The intima A and subintima B are relatively fine and thin. The internal elastic lamina C is fine in texture. The medica D consists of good fleshy muscle. The adventitia E is loose and delicate in texture.
2. Young adult (twenty to thrity-nine years of age). The subintima has increase in size and become denser. The internal elastic lamina has become thicker. The media shows evidence that fibrous connective tissue has supplanted some of the smooth-muscle fibers, and there is progressive disorganization of elastic tissue. The adventitia is denser.
3. Older adult (forty to fifty-nine years of age). The subintima is the seat of diffuse and focal deposition and general fibrous thickening. The internal elastic lamina has split. In the media, the disorganization of elastic tissue has become more marked. A great deal of muscle tissue has been replaced by connective tissue and, on gross examination, seems to have lost much of its color. The adventitia has become cloudy, thick and tendinous.
4. Senile (sixty to eighty years of age). In senescence, the submintima is very thick and fibrous and now has a glassy opacity due to hyalinization. The internal elastic lamina has become frayed and fragmented, with tag ends in the adjacent media. The media has had most of its muscle fibers replaced by connective tissue and has the appearance of gristle. The adventitia is very coarse and tendinous.

From: Physician's Bulletin 23: 1:9 (Eli Lilly & Co.)

Figure 8.

Table 11. Electron micrographs of atria of fischer 344 rats

	3 months old	*12 months old*	*24 months old*
Cell membrane	Intact	Discontinuous in nerve & muscle	Lack of cell boundary definition
Sarcoplasmic Reticulum	Intact		Swollen
Mitochondria	Intact	Disrupted cristae	Empty
Myocardium	Intact		Degeneration
Axoplasms	Clear	Dark inclusions – cellular debris	
Noradrenergic Terminal	Dark Vesicle		Some still contain many & clearly identifiable NE vesicles. Many showed extensive vacuolization, advanced type lamellar degeneration.
Interstitium	Fibrous material		Collagen – packed

cardial fibers, but also the adrenergic nerves; the latter shows extensive vacuolization and lamellar degeneration (21). The decrease in adrenergic activity during aging may be explained by changes involving practically the whole of the system from the brain to the periphery (see Figs. 9 and 10). In the brain, there is loss of Alpha and Beta receptors from different centers as well as dopaminergic receptors from the corpus striatum; the loss is attributed to impaired synthesis (24, 25). Degeneration of peripheral adrenergic nerves has been reported (21). There is diminished norepinephrine content of the heart and various organs and impaired NE synthesis in the adrenergic neurone endings (22). These can best be explained by lack or decreased enzymatic activity of the hydroxylases or decarboxylases that convert tyrosine to DOPA, dopamine and NE. There is no change seen in NE release mechanisms and there is no decrease in the number of Beta receptors in the heart nor of alpha 2 receptors in the arterioles. But the responsiveness of these receptors to their agonist, NE, is decreased. Adenyl cyclase activity is also decreased. The explanation appears to lie in a change in interaction between the Beta receptors and the guanine nucleotid regulatory protein (Fig. 10), causing an impaired ability to form a high affinity state necessary for adenyl cyclase activation (22, 23). Furthermore, and contributing to loss of adrenergic control by aging is a decreased translocation in the membrane-bound phosphokinase (23). Basically, therefore, a change in the protein configuration of these structures must be occurring in aging.

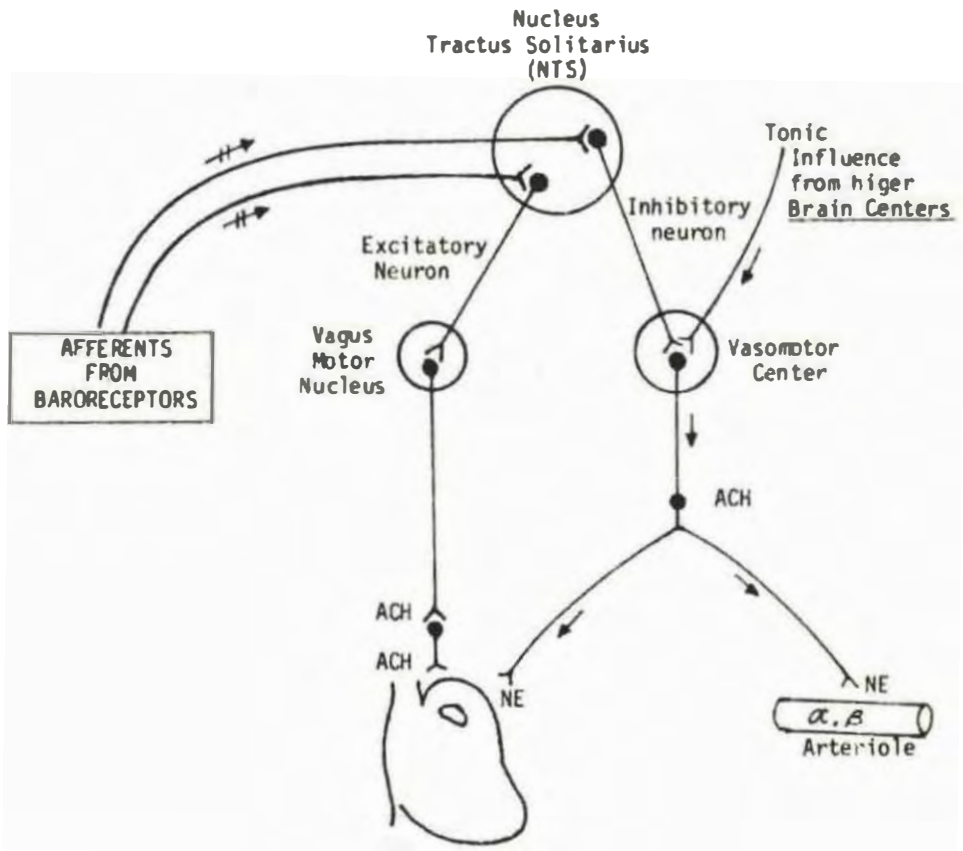


Figure 9.

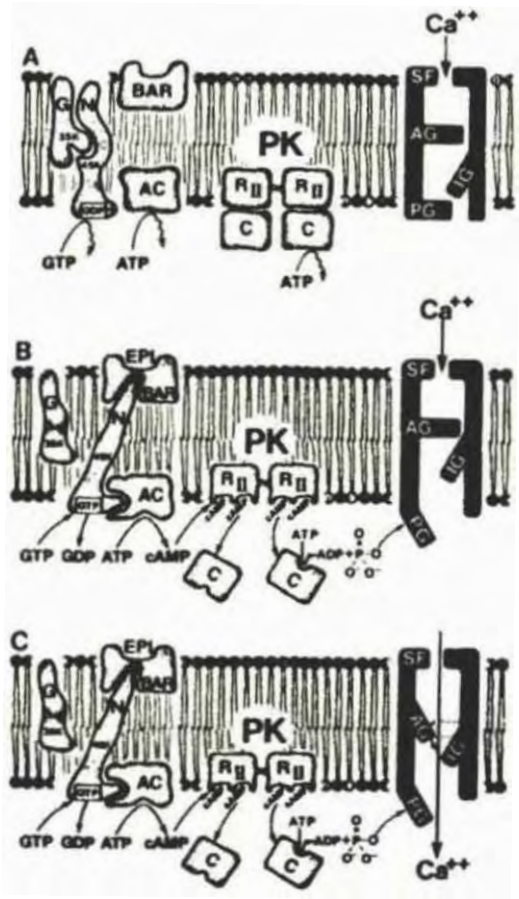


Figure 10.

On the Endocrines. A sharp fall in estrogen secretion occurs with menopause in females; in males the fall in androgens is gradual, extending well into old age (Table 12). These are the most important changes seen in the endocrines in aging (9). Being anabolic, the loss of sex hormones contribute significantly to the osteoporosis and loss of lean muscle mass that accelerates after age 40. Of lesser significance are the decreases in parathormone leading to calcium loss, of triodothyronine (T3) leading to a general metabolic decline, and alteration in insulin activity or secretion leading to decreased sugar tolerance.

Table 12. Changes in hormones blood levels in old age (human)

Testosterone	↓
Estrogen	↓
Al dosterone	↓
Glucocorticoids	↔
Growth Hormone	↔
Gonado tropin	↑
TSH	↔
Parathormone	↓
Insulin	↔
Glucagon	↔
T_4	↔
T_3	↓

On the Humoral System of the Body. The magnitude and scope of the body's system for intercommunication and control is still being unravelled. It is not only inter-organ and inter-tissue, as with the well-studied endocrine-hormones and secretogogues; it is even and inter- and intra-cellular. It utilizes its own neurotransmitters and chemical messengers which vary from the simple cAMP and cGMP to highly complicated peptides and proteins. It includes the blood clotting factors, chemotactic factors, prostaglandins, leukotrienes, kinins, renin-angiotensin-aldosterone, lymphokines, ANH (Atrial Natriuretic Factor), TNF (Tumor Necrosis Factor), MAF Macrophage Activating Factor) TIMP (Tissue Inhibitor or Metalloproteinases), and hosts of other active substances not to mention the antibodies for every specific antigen. These substances are produced by different cells and tissues, usually in situ where they are needed. The endothelium, that single layer of flat cells lining the intima of blood vessels which can barely be seen by the light microscope is not just a mechanical lining, but is now known to perform multiple functions of resisting or promoting thrombosis, vascular repair and active protection; in the process it manufactures and secretes at least 9 different substances such as proteoglycans, thrombomodulin, Protein C, prostacyclin, endothelial growth factor, Factor S.

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The effects of aging on this big family of hormones and humoral agents is not fully understood but will surely be elucidated as they are better understood: e.g. the role of the platelet factors, particularly that which has to do with atherosclerotic plaque formation; the role of the lymphokines, such as the interleukins 1 and 2 which may play a role in resistance to infection and cancer; the leukotrienes and asthma, chronic bronchitis and pulmonary aging.

Effects on Enzymes

Enzymes are the catalysts that make the whole body machinery function. They are synthesized by every cell, and apparently as needed or called for. Lactase is needed for the lactose of milk; and tends to disappear in non-drinkers of milk, hence their diarrhea with milk. The lever is the organ that detoxifies and metabolizes many substances including drugs. The production by the liver of drug metabolizing enzymes (DME) may be induced or inhibited by specific drugs resulting in faster or slower metabolism of those drugs that need the enzyme. Table 13 compares the biologic half-lives (T 1/2) and clearance rates of representative drugs in young and old persons (27). All drugs in the list are metabolized more slowly by the elderly and may therefore cause toxicity which explains the well known observation that old persons are more susceptible to drugs and should be given smaller doses. The reason that oral propranolol does not differ in its T 1/2 in the young and the old is because it is metabolized so very fast that barely 10% of the ingested dose can survive this first pass to reach the systemic circulation. Old age therefore can be seen to cause significant decrease in the liver's DMEs such as the oxidases, decarboxylases, deaminases, etc. The enzymes of the aorta, brain, and striated (skeletal) muscles also show some decreases on aging but the studies are still insufficient Table (14). Oxido-reductases of the aorta are definitely reduced as shown by 9 out of 10 reported studies. Hydrolases and lyases appear not to be decreased by aging (9).

Effects on the Brain

The early manifestations of aging on the brain, namely difficulties with memory and learning of new things, have been mentioned and listed (Table 6). Also already mentioned are the effects of disturbed circulatory flow leading to cerebral ischemia and strokes (cerebral thrombosis, embolism and hemorrhage). Degeneration of various parts of the brain may be explained by localized circulatory disturbances such as degeneration of the basal ganglia leading to Parkinsonism. Studies of the cerebral blood flow by Doppler Ultrasonic Technique has shown a discontinuous pattern in the elderly suggestive of increased cerebral vascular resistance (28). These are all effects of aging on the brain, part of senility. Senile, however, is a word that more specifically refers to, as Hayakawa puts it,

Table 13. Age-related pharmacokinetic data

	Age (yrs)	*T 1/2 (hrs)*	*% Change*	*Clearance Rate ml/min/kg*	*% Change*
ANTIBIOTICS					
Penicillin G (i.v.)	25	0.55			
	77	1.0	+82		
Procaine Penicillin G	25	10			
(i.m.)	77	18	+80		
Tetracycline	25	3.5			
	75	4.5	+29		
Dihydrostreptomycin	27	5.2			
	75	8.4	+62		
Amoxycillin	Young	1-1.5			
	89	2.67	+116		
Cefazolin (i.v.)	24-33	1.57			
	70-88	3.15	+101		
TRANQUILIZERS					
Diazepam	30	32			
	65	70			+118
Phenobarbital	20-40	71			
	70	107			+51
CARDIAC DRUGS					
Propranolol (oral)	29	3.58			
	80	3.61	0		
Propranolol (i.v.)	29	2.53		13.2	
	80	4.23	+67	7.8	−69
Metoprolol	23	3.5			
	67	5.0	+43		
Digoxin	27	51		1.11	
	72	73	+43	0.88	−26
	34-61	36.8		1.7	
	72-91	69.6	+89	0.8	−113
Quinidine	23-34	7.25		4.04	
	60-69	9.7	+34	2.64	−53
Lidocaine	24	1.34		7.6	
	65	2.33	+74	8.1	+7=0
ANALGESICS					
Morphine (i.v.)	26-32	2.9		14.7	
	61-80	4.5	+55	12.4	−19
Aspirin	21	2.38			
	77	3.71	+56		
Indomethacin	20-50	1.53			
	71-83	1.73	+13		

Paracetamol	24	1.82	
	81	3.03	+66
Phenylbutazone	26	81	
	78	105	+30

Ref.: DP Richey: Pharmacokinetics & Drug Disposition in Handbook on Pharmacology of Aging by PB Goldberg & J. Roberts p. 7, CRC Press 1983

the enfeebling effects of age on the mind; the second childhood (28). There is not only a serious loss of memory but also of cognition and reasoning. It may be accompanied by depression, psychosis, or dementia. However, ordinary senile psychosis which appears after age 65 years must be differentiated from Alzheimer's dementia which is familial and may start even as early as age 40. The neuropathological findings are similar but those of Alzheimer's Diseases are more severe and more extensive (30). In Alzheimer's the primary cellular degeneration consists of neurofibrillatory tangles, granulovacuolar changes, senile plaques between cells composed of enlarged nerve fibers and synaptic endings with degenerated mitochondria and with an amyloid core (31). The regional cerebral blood flow and metabolic rate of glucose as measured by Positron Emission Tomography (PET) are reduced throughout the brain in relation to the severity of the dementia and neuropsychometric deficits (32). The neurotransmitters in the brain are markedly reduced, starting with acetylcholine of the hippocampus, later followed by the monoaminergic, GABA-ergic and peptidergic systems (30). Unfortunately, simple replacement of the acetylcholine by choline containing compounds like lecithin is of no therapeutic value in preventing or delaying the progress of the disease. The enzyme choline acetyl transferase that synthesizes the acetycholine is somehow not produced in adequate amounts. Alzheimer's Disease is familial and recently (Newsweek 3/2/87) reported that a genetic marker for Alzheimer's has been located in chromosome 21 and that a gene also located in chromosome 21 has been identified as directing the manufacture of the amyloid protein deposited in Alzheimer brain plaques.

Lipofuscin in Aging

Lipofuscin is a pigment found deposited in the cytoplasm of aging hearts (11) and in neurones (33). The dark brown type is seen in the substantia nigra of the brain by age 3 years and does not increase by age. The light yellow type is chemically related to lipids, first detected in the cells of Clarke column in the brain by age 20 years and increases linearly with chronologic age. Lipofuscin appears to be nothing more than a waste product of mitochondrial degeneration and its removal by phagocytes can be enhanced by antioxidants and pigment-mobilizing drugs like meclofenoxate (33).

Genes, Enzymes and Proteins

It is evident that the aging process exerts its effects primarily on the proteins and peptides of the body and most importantly the enzymes. Almost nothing in the body can function, be synthesized or metabolized, without the intervention of one or several enzymes. Hormones depend on enzymes for their synthesis. The thyroid hormones T3 and T4, and the medullary adrenal hormones, epinephrine and norepinephrine, are synthesized from tyrosine by enzymes. The adrenal corticosteroids and the sex hormones are synthesized from cholesterol by enzymes, while cholesterol itself is built up from acetate by enzymes. Energy cannot be stored in ATP without enzymatic help, and ATP releases its tremendous store of energy only by enzymatic action. There are enzymes that bond or break up, enzymes that add or subtract, that transfer or transform, that regulate transport or act as receptors. In short, there are enzymes for every imaginable chemical and physiological process. Even the genes in the double-stranded DNA cannot be expressed without an RNA polymerase enzyme that separates the strands and transcribes the message into the messenger RNA. The mRNA then migrates to the ribosomes for translation of its base sequence according to the genetic code to determine the amino acid sequence of the protein. When the genes, through wear and tear, or the destructive effects of ultraviolet rays, free radicals, toxic materials or oxygen lack become damaged, again there are enzymes to repair and restore the DNA's integrity (34,35). There is an enzyme to reverse thymine dimerization photochemically; enzymes that incise the DNA strand and excise the damaged segment (specific endonocleases); enzymes that synthesize a new complementary strand (35) and insert it into the deleted region (DNA polymerases): and an enzyme that seals the DNA incisions (ligase). Because of such repair maintenance, bacterial genes can be duplicated as many as 100 million times before there will be a 50% chance that even one gene will be altered (34). The DNA polymerase has a special repair activity – that of a 3' – 5' proof-reading exonuclease activity which enables it to backtrack and edit or proofread any incorrect base-pair that may have been inserted and remove it. The average error frequency for base-pair substitution has been estimated to be in the order of 10^{-8} to 10^{-10} errors per base pair replicated in T4 bacteriophage and *E. coli*, respectively (37).

What are enzymes? Enzymes are proteins or polypeptides and, like all amino acid-composed substances, the information for their synthesis resides in the genes of the DNA. One enzyme may have its messages encoded in one or several genes that may not even be adjacent to each other.

Since the DNA cannot be transcribed without enzymes and enzymes are dependent on DNA for their synthesis, a chicken-and-egg question may be posed at this point: which came first – the DNA or enzymes?

The whole of the DNA of a person, as discussed before, is present in every cell of every organ of that person. Every cell's DNA contains all the information for the manufacture of every protein and peptide needed in that person's whole

life. Furthermore, the DNA is extravagantly redundant. Its genetic material is repeated, some as many as a million times (36). These repeated segments (which are identical or near copies of the gene) appear to be scattered throughout the DNA and could be transcribed into mRNA. As mentioned before, however, only about 0.4% of a cell's DNA may expressed or operant; the rest is unexpressed or suppressed; and that which is operant is not continuously expressed but is switched on and off depending on need. Hence, the control mechanisms of the DNA play a most important and as yet poorly understood role. What dictates the level of each enzyme in the cell and how are the constantly changing needs signaled "upstairs"? With the more than 2,000 enzymes in the human system, how does each signal its lack or excess? The post-translational controls, such as the phosphorylase reaction to activate a pro-enzyme to active form is most helpful in enabling the stocking up of raw material pro enzymes near where they will be needed. But what about the phosphorylase supply – since the half lives of enzymes is short? There is much still that is not known. Could the effect of aging be on the control or regulatory mechanisms? Unfortunately, while very much is now known about the DNA's "hardware" very little is known about its "software" – i.e. the programs that run it. Localizing the site of the aging program on the DNA regulatory mechanism is an attractive and logical hypothesis.

Table 14. Enzyme changes on aging (human)

Enzymes	*Organ*	*No. of studies showing*		
		Dec	*No change*	*Inc.*
Oxido reductases	Aorta	9	1	
Transferases	Aorta	1	1	1
	Brain	–	1	–
	Striated muscle	1	–	–
Hydrolases	Aorta	2	–	4
	Brain	–	1	1
Lyases	Aorta	1	2	2
	Skeletal muscle	1	–	–
Ligases	Aorta	1	–	–

Modified from M.S, Kanungo, Biochemistry of Aging, 1980

There are two principal effects of aging on proteins (a) decreased synthesis, whereby muscle and bone mass is lost and adrenergic receptors are decreased; and (b) structural alteration, leading to such changes as increased collagen cross-linking and alterations of enzyme activity. Enzyme lose their potency resulting in diminished biochemical reactions and metabolic processes, consequently, decreased physiologic functions (Table 6). Other proteins such as receptors are similarly

altered rendering them insensitive i.e. incapable of reacting with or accepting their agonists. Such alterations occur when critical changes in the tertiary conformations of the protein occur. The tertiary structure of a protein is that which is most thermodynamically stable for the primary structure and interactions of its side chains. Under proper conditions, proteins have been shown to spontaneously assume their tertiary foldings, which structure is stabilized by formation of disulfide bridges. Reduction of the disulfide bridges in a protein denaturates the protein and results in its losing both its native conformation as well as its biological activity (38). Many enzymes are composed of two or more subunits and take on a quarternary structure with a specific binding site for its substrate. Alteration in this quartenary structure will change the affinity of the binding site for substrate.

These alterations in proteins, enzymes and receptors with aging were at first attributed to errors in DNA transcripton or RNA translation (Orgel's Error Theory of Aging). More recent studies (on unaltered and altered enolase) have shown that alteration in enzyme activity cannot be due to errors in translation. Furthermore, cell-free studies indicate that the fidelity of translation does not decline with age (39). The alteration of enzyme activity in aging is probably a post translational event and may be the result of a subtle denaturation from what Rothstein calls an increased "dwell-time" of proteins in an aged cell that has a diminished protein turn-over (40).

Promotion of Health and Longevity

The present life span of human beings is markedly disease-limited, – limited particularly by infections, malnutrition, cardiovascular diseases, cancer, senility (Fig. 11). If there were no diseases to shorten life, life span will surely be much longer, although there still must be limits to man's longevity, since genetic determination is probably what controls the life spans of all creatures of this earth – whether flora or fauna, vertebrates or invertebrates, fish or fowl, one-celled or multicellular organisms. The important question we should like to answer, however, is not, how long can man's life span be lengthened but rather how can a healthy life be extended? By so doing, life span inevitably should also be increased and this is well and good – provided that the longer life span of people can be fruitfully integrated into the social life and economic practices of human society. Our ideal goal in other words, should be the attainment of a state of health for the majority of society where even the aged would be in full possession of his faculties and not a burden to his family or society but capable still of contributing his share in terms of physical or mental output – till the end of his life whether such will be 100 or 150 years, or perhaps even longer. (Although I would not personally like to live that long unless this world gets a whole lot better!). This scenario with an abrupt terminus is just like what Oliver Wendell Homes' logically related in "The Deacon's Masterpiece (or the Wonderful One-Hoss Shay"):

That was built in such a wonderful way
For the wheels were just as strong as the thills
And the floor was just as strong as the sills
And the panels just as strong as the floor
And the back-cross bar as strong as the fore

Seventeen hundred and fifty five
It ran a hundred years to a day
Eighteen hundred and fifty five
Just the hour of the Earthquake shock
What do you think the parson found
The poor old chaise in a heap or mound
As if it had been to the mill and ground.
How it went to pieces all at once
All at once and nothing first
Just as bubbles do when they burst.

Present man is not built that most logical way. There is always some *locus minoris resistentiae,* some weak spot, or organ – the brain, the heart, the kidney, the killer T-lymphocyte – that fails ahead of the others. And the cause of such failure is recognized as a disease of the organ. Seldom can we refer to a person as "dying of old age" – dying because he is too old and not because he was sick. But in some places that were visited by Alexander Leaf (41) in Ecuador, Pakistan and the Caucasus, there were more old old and very old people than most other places in the world; and these old people were still doing hard physical work although eating much less food than we do. Their food intake we would in fact rate as mal – or under-nourishment (Table 15). Unfortunately, Leaf's account was not a real epidemiological survey. We do not know if these old people were really all that healthy although so many were indeed very old – in Vilcamba, Ecuador, they comprised 16.4% of the population, and 1% of the population were centenarians!

There is no doubt that disease hasten the aging process and there are disease-producing factors that can deleteriously affect the DNA and its regulatory mechanisms for aging, as will be discussed below. Contrawise, health and the absence of disease undoubtedly can prolong life, perhaps even to the biologic genetically-determined limit. Whether the latter can be extended further is something for the future to decide as to feasibility and desirability.

How then can we live healthier longer lives? Before we can talk about the health problems of the industrialized society, we still have to mention that the Philippines is still in the Age of Infection. Our leading causes of morbidity and mortality are the infectious diseases – TB, bronchopneumonia, diarrhea, El Tor, intestinal worms – fortunately not yet AIDS! Our TB incidence is, according to our own Secretary of Health "a national calamity" and it is, since our ASEAN neighbors have all controlled theirs and we still have not! El Tor is endemic, diar-

Table 15.

	Ecuador Vilcamba Village	*West Pakistan Hunza Valley*	*USSR Caucasus*
Age			
Over 100 yrs.	9 of 819		4,500 – 5,000 1,844 (39/100,000) live in Georgia 2,500 (63/100,000) live in Azerbaijan 15,000
Over 80 yrs.			
Over 60 yrs.	16.4%		
Oldest	121 yrs.	110 & 105 yrs.	
Work	Hard – Agriculture	Intense – agriculture & mountain climbing	70% of 15,000 who are over 80 work in farms
Diet			
Total Calories*	1,200	1,923	
Proteins	35-38 g	50 g	
Fats*	12-19 g	36 g	40-60 g
Carbohydrates	200-250 g	354 g	
Animal type Protein & Fats	Very low	1%	
Alcohol	Yes-Moderate		Yes
Tobacco	Yes		

From: A. Leaf. Scientific Amer. Sept. 1973 pp. 45-52
*U.S. Diet (1960): Total Calories – 3,300/day
Fats (mostly animal) –157 g - 42% of daily caloric intake

rhea periodically epidemic and intestinal worms infecting more than 90% of our public school children. The control measures for these diseases are known. They are all taught in Hygiene and Sanitation classes. Yet water is not potable, sewage and garbage strewn about and flies, cockroaches and rats abound. Of 132 water samples tested from Manila, Legaspi City, Quezon, La Union, the Igorot Provinces, only 18(13.6%) were found potable. La Union and Kalinga-Apayao had no potable water among the samples tested (43,44).

Malnutrition, of the undernutrition type, is the lot of many of our poor people and their children. Many children will suffer irreparable brain cell loss and doomed to an IQ of a moron. One does not talk of a ripe old old age and longevity when there are obstacles like these. Control and remedy of these diseases and the

conditions that bring them about must be our highest health priorities. Continuing campaign to instill healthy habits and cleaning of surroundings and environments should reap untold benefits. Chlorination of wells and drinking water should be studied and applied all over the country. Garbage and sewage disposal, etc, etc. How can we talk of aging when our people never even have a chance to reach old age or reaching it are already in a stage of senility!

Control of Chemical Pollution: The world is in the midst of a chemical revolution and chemical pollutants abound: in the air, lakes, rivers, ground; in the food we eat and the water we drink and the air we breath, out in the open as well as in our homes, offices, not to mention conference rooms. Nicotine and cigarette tar have been proven to cause not only cancer of the lungs and coronary attacks but also cancer of the mouth and pharynx, urinary bladder, pancreas (44.1) not only in the smoker himself but also the people who inhale his smoke – side stream or passive smokers. Asbestos boards making up our ceilings or lining our air conditioning ducts should be now have all been removed and replaced after demonstration of the carcinogenicity of asbestos fibers on the lungs. Lead and mercury poison the central nervous system and cause tremors. Cadmium, nitric acid, nitrous acid, formaldehyde, hydrogen sulfide cause irritation of the eyes, nose, respiratory passages and mucous membranes. Carbon monoxide from car exhaust, gas ranges, wood stoves, bind hemoglobin and make it unavailable for oxygen transport. Ozone from automobile emissions and copying machines is irritant to lungs and eyes and nose although beneficial once it reaches the stratosphere where it shields the world from the carcinogenic ultraviolet rays (45). The recent reports of a hole in the ozone layers over Antartica caused by chlorofluorohydrocarbons should cause universal alarm because UV is not only carcinogenic especially to the skin but UV can produce immediate formation of pyrimidine dimers on DNA strands which if not repaired may result in failure or mis-transcriptions of genetic messages (44.2). The use of persistent bioaccumulative pesticides like DDT may be good for increasing the harvest but ensure that all our fishes in our rivers and the cattle who roam our fields will not get enough DDT to eventually poison us who eat their meat and the vegetables (44.3). And speaking of air pollution, Manila should rank among the top cities for this, what with our leaded gasoline, decrepit cars, buses and jeepneys emitting thick black smoke oblivious of the police who periodically announce to the public that smoke belchers will be apprehended but never do. The city dwellers' lungs at autopsy are significantly blackened with carbon. The provincial dwellers' lungs may probably look better because the dust and carabao and horse dung may not show up. But the high rate of respiratory infections is a sure sign that our lungs are insulted enough to keep our average life span where it now is – 65 years.

Diet and Exercise: "Man should eat to live, not live to eat". Malnutrition of the over-eating variety has long been shown to lead to hyperlipidemia, atheroschlerosis, hypertension, heart disease and strokes. This is particularly true if the high caloric intake is contributed to by a high saturated animal-type fat content and accompanied by a sedentary habitus. A low caloric diet below 2,000 /Cal. per day

with a low fat content is now known to produce the reverse effect – lowered blood lipids, cholesterol and blood pressure and decreased tendency to atherosclerotic complications and death. In laboratory animals, food restriction or undernutrition but not malnutrition, is said to be the most effective and reproducible method for increasing longevity, and for decreasing and delaying the occurrence of myocardial degeneration and fibrosis, periarteritis, spontaneous tumors, age-induced diminution of immunologic response as well as auto-immune diseases (39). In male Fischer F344 rats, the age related decrease in protein synthesis was suprisingly prevented by diet restriction – as if dieting acts as a physiological stress to stimulate gene expression (Lindell) (39). Leaf's report on the diets in the 3 regions he visited would appear to support these findings on experimental animals (Table 15). The Ecuadoreans ate a bare 1,200 calories with only 10% as fat, mostly vegetable. The Pakistanis ate more, 1,900 calories, but the fat was also mostly vegetable and formed only 17% of the daily diet. The Caucasian diet was not reported but the fat content was similar to that of the Pakistanis. And all 3 peoples did hard physical work. Perhaps those who say that exercise does not prolong life do not exercise enough. But it does look like that many of us are eating too much for our own good and smoking too much. That is why cardiovascular disease incidence among Filipinos is rising fast; it is now No. 2 as a cause of death (46).

How to Delay Aging

As can be gleaned from the previous discussion, the factors of aging are many. Looming over all would be one's inherited genes – how good are they and how efficient are their regulatory and repair mechanisms. Good genes can often resist or overcome all the insults and damages inflicted by pathogenes, toxins, toxic metabolites to which a person may be exposed and good genes can carry a person to a ripe old age in surprising good state of health. This means that the enzyme systems for repair of the cells of the important organ-systems must be good. Some enzymes are synthesized from relatively simple compounds like the vitamins; hence vitamins should be given in adequate amounts to ensure that the enzyme levels of all cells in the body are adequate. Other enzymes are peptides or polypeptides and require DNA-RNA activity. Control of this activity is beyond our present capabilities although gene splicing (genetic engineering) is developing very fast and soon science may be knocking on the door of genetic control of the aging process and perhaps uncover issues with legal or moral complications (47). Professors Niehans of Switzerland and Wiedeman of Germany practice injection of sheep embryo cells with the idea of "rejuvenating" the DNA of cells. Embryonic cells are used because they are said to be still non-antigenic and will not be rejected. Many famous people, some aging presidents and artists are said to have undergone this therapy and "were rejuvenated". The treatment is expensive and requires a long preparation and stay at the clinic isolated in beautiful scenic little towns; and the therapy has to be

repeated after every so many years. However, the rejuvenation appears to be more psychological than physical – but then psychology is a potent rejuvenating force.

The antioxidant and free-radical scavenging properties of Vitamin E appear most suited as protector from the damaging effects of free radicals on the phospholipids of cell membranes. This vitamin as well as selenium which can detoxify lipid peroxides, are becoming attractive in geriatric therapeutics.

Mind over Matter

The mind is the seat of intelligence, reason, cognition, memory and will. It is also the abode of wisdom. It must be in the brain but its location can not be found. It could be diseased and age prematurely as in Alzheimer's Disease. In many people it remains healthy till very late in life and may continue to develop even when the other body systems have started to retrogress. Such people grow in intellect and wisdom and create their greatest works and masterpieces at ages of 60, 70, 80 or 90. The power of the mind over the body has long been recognized and practiced by the peoples of the east. The fakirs of India walk on live coal of 2000°C temperature but do not suffer any burns; or stick long needles through various parts of their anatomy with no bleeding or apparent pain. An article in the *American Heart Journal* in the 1950s reported a "holy man" buried alive for 3 days as pre-planned, with continuous ECG monitoring of the cardiac activity. The ECG complexes gradually became smaller and disappeared for 3 days only to reappear and return to normal configuration when the subject was exhumed alive. Such control of the mind over the body is manifested in many other ways a little less spectacular. The karate artist who splits a concrete block with a blow of his hand delivered with the concentrated force of all the energies of his mind – and without suffering any injury. In a negative way, the power of the mind can also be seen in the sufferings of the hypochondriac who experiences pain and dysfunction of every organ in her body on which her fearful mind focuses. I treated a shell-shocked patient with complete hemiplegia during the entire duration of the Japanese Occupation 1941-45, only to see him get up and walk normally after the Liberation of Manila. There is no doubt that the mind can control the body reactions. The mind can control even the effectiveness and reactions of drugs. A patient who believes in his physician gets well or at least feels better with his medicines; while a patient distrustful of his doctor does not get any better and may develop adverse reactions.

"One is as old as he thinks he is". Can the mind control aging? The old young man is a defeatist, a pessimist and a perennial worrier. His stresses increase his corticosteroid levels, the breakdown of proteins by enzymes, formation of peptic ulcers, hypertension, anxieties and later depression. He is old at 40. The young old man on the other hand thinks positively and is not easily fazed by difficulties; an optimist, he seldom worriers and is seldom stressed. Mental, cardiovascular,

gastrointestinal, immunologic diseases, consequently have little opportunity to take a foothold on him. His active mind continues to seek for challenges. George Zukor at the age of 103, was asked to write the history of the movie industry of which he was a participant and innovator since the industry's infancy. He retorted – don't bother me, I'm very busy. As long as the mind keeps young and meets challenges positively, aging's effects are held back. When a man retires for a well-earned rest, or worse, if forcibly retired for any reason, then aging proceeds or may even be accelerated. One of these days, studies may show that indeed the DNA's regulatory mechanisms can be turned on or off by the mind, which already controls so many functions of the body.

Prescription for Youth in Old Age

Old age is chronologically dependent and time marches on and no one can hold back its hands (The time capsule going to negative time is another story).

Youth is prevention of aging by preservation of health, avoidance of pollution, prevention of disease, toning-up of the body and exercise of the mind and spirit. These having been achieved, our genes can take care of the rest.

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ON THE LEXICOGRAPHIC OF THE n-PERMUTATIONS

Severino V. Gervacio
MSU-Iligan Institute of Technology
Tibanga, Iligan City

ABSTRACT

We call $\pi = x_1 x_2 \ldots x_n$ an *n–permutation* of the elements 1, 2, . . . , n if each x_1 is a positive integer not exceeding n and $x_i \neq x_j$ for all $i \neq j$. We refer to x_1 as the *ith element* of the permutation π. If $\pi_1 = x_1 x_2 \ldots x_n$ and $\pi_2 = y_1 y_2 \ldots y_n$ are two n–permutations, we say that π_1 *precedes* π_2 if there exists a positive interger k not exceeding n such that $x_i = y_i$ for all $i < k$ and $x_k < y_k$. If no such integer k exists, then $x_i = y_i$ for all i and we say that the permutations are *equal*. We shall write $\pi_1 < \pi_2$ if π_1 precedes π_2, and $\pi_1 = \pi_2$ if they are equal. We know that there are exactly n! permutations on 1, 2, . . ., n. It is easy to see that for any two n-permutations π_1 and π_2, exactly one of the following holds: $\pi_1 = \pi_2$, $\pi_1 < \pi_2$, $\pi_2 < \pi_1$. Furthermore, if $\pi_1 < \pi_2$ and $\pi_2 < \pi_3$ then $\pi_1 < \pi_3$. Thus, $<$ is a linear ordering on the n-permutations. This is called the *lexicographic ordering.*

If exactly r distinct permutations precede π, we say that π has *rank* r + 1. Thus, 123 . . . n has rank n and n . . . 321 has rank n! We shall denote by rank (π) the rank of π.

Three main problems are dealt with in this paper. The first is the computation of the rank of a given permutation, the second is the determination of the permutation with a given rank, and the third is the lexicographic enumeration of n-permutations.

The Rank of an n-Permutation.

For convenience, let us define the *index* of the permutation $\pi = x_1 x_2 \ldots x_n$ to be $\text{ind}(\pi) = \tilde{x}_1 \tilde{x}_2 \ldots \tilde{x}_n$, where $\tilde{x}_i$ is the number of subscripts $j > i$ such that $x_j < x_i$. Clearly, $0 \leq \tilde{x}_1 \leq n-i$ for each i. In particular, $\tilde{x}_n = 0$. As an example, if $\pi =$ 316425, then $\text{ind}(\pi) = 203100$. It should be remarked that if $n > 9$, then we should use the notation $\pi = (x_1, x_2, \ldots, x_n)$ and $\text{ind}(\pi) = (\tilde{x}_1, \tilde{x}_2, \ldots, \tilde{x}_n)$ to avoid any confusion.

Theorem 2.1. *If $\pi = x_1 x_2 \ldots x_2$ is an n-permutation, then*

$$\text{rank}(\pi) = 1 + \sum_{k=1}^{n-1} \tilde{x}_k (n-k)!$$

Proof: Let $\pi' = y_1 y_2 \ldots y_n$ be any permutation which precedes π. Let us count the total number of such permutations π' as follows. Let k be a positive integer less than n. Set $y_i = x_i$ for all $i < k$. Choose any $j > i$ such that $x_j < x_k$ and set $y_k = x_j$. Note that we have a total of $\tilde{x}_k$ choices here. For the last n–k elements of π', we choose any of the (n–k)–permutations of $[1, 2, \ldots, n] / [y_1, y_2, \ldots, y_k]$. Hence, the number of such permutations π' we can construct corresponding to k is $\tilde{x}_k(n-k)!$. Clearly, two distinct values of k will give us non-overlapping sets of permutations that precede π. The theorem then follows.

Theorem 1 solves our first problem. As an illustration, if k = 316425, then ind(π) = 203100 and rank(π) = 1 + 2(5!) + 0(4!) + 3(3!) + 1(2!) + 0(1!) = 261. Thus, the permutation 316425 is the 261*st* permutation in the lexicographic list of all 6-permutations.

Finding the permutation with a given rank.

Lemma 3.1. *Distinct n-permutations have distinct indices.*

Proof: Let $\pi_1 = x_1 x_2 \ldots x_n$ and $\pi_2 = y_1 y_2 \ldots y_n$ be distinct n–permutations. Without loss of generality, we may assume $\pi_1 < \pi_2$. Then there exists a positive integer $k < n$ such that $x_k < y_k$ and $x_i = y_i$ for all $i < k$. Let $j > k$ and $x_j < x_k$. Then $x_j = y_t$ for some $t > k$ and $y_t < y_k$. It follows that $\tilde{y}_k \geq \tilde{x}_k$. But in addition, $x_k = y_s$ for some $s > k$ and $y_s < y_k$. Therefore $\tilde{y}_k > \tilde{x}_k$ and hence ind(π_1) $\neq$ ind(π_2) since they differ in their k*th element.*

Corollary. *Let $a_1 a_2 \ldots a_n$ be an ordered n-tuple of integers such that $0 \leq a_i \leq n-i$ for each i. Then there exists a unique n–permutation π with ind(π) = $a_1 a_2 \ldots a_n$.*

Proof: Consider the set P_n of all ordered n-tuples of integers $c_1 c_2 \ldots c_n$ where $0 \leq a_i \leq n-i$ for each i. Note that P_n has exactly n! elements. By the theorem, the mapping ind from the set of all permutations of 1, 2, . . . , n to P_n is one-to-one. It follows that ind is also an onto map since there are exactly n! n–permutations. Hence, any element $a_1 a_2 \ldots a_n$ of P_n is the index of a unique permutation π.

Although the above argument proves the existence of a unique permutation with a given index, it does not show us a way of constructing the permutation. We shall now develop an algorithm for constructing the permutation with a given index.

Let $a_1 a_2 \ldots a_n$ be a given index. Let $C_i = c_1^1 c_2^1 \ldots c_n^1 = 123 \ldots n$. Since $0 \leq a_i \leq n-i$, then $i \leq a_i + i \leq n$ and hence $1 \leq a_i + 1 \leq n-i+1$ for each i. Define x_1 to be the $(a_i + 1)$ *th* element of C_i and let $C_2 = c_2^2 c_2^2 \ldots c_{n-1}^2$ be the (n–1)–tuple obtained from C_1 by striking out the element x_1, the (a_1+1) *th* element. Recursively, we define $C_i = c_1^1 c_2^1 \ldots c_{n-1+2}^1$ to be the ordered (n–i+1)–tuple obtained from C_{i-1} by striking x_{i-1} out. Since the elements of C_i form an increasing

sequence, it is clear that the elements of C_i also form an increasing sequence, for each i. Let x_1 be the (a_i+1) *th* element of C_i and form the permutation $\pi = x_1\ x_2\ \ldots\ x_n$. We claim that $\text{ind}(\pi) = a_1 a_2 \ldots a_n$. Now, since x_i is the (a_i+1) *th* element of C_i and the element of C_i form a non-decreasing sequence, it follows from the construction of π that the number of elements x_j of π such that $j > i$ and $x_j > x_i$ is equal to the number of elements of C_1 to the left of the (a_1+1) *th* element. Hence, $\tilde{x}_i = a_i$ and $\text{ind}(\pi) = a_1 a_2 \ldots a_n$.

Lemma 3.2. *Every positive integer r not exceeding n! can be expressed uniquely in the form* $r = 1 + \sum_{i=1}^{n-1} a_i(n-i)!$, *where* $0 \leqslant a_i \leqslant n-i$ *for each i.*

Proof: Let $1 \leqslant r \leqslant n!$. Then r is the rank of a unique n–permutation $\pi = x_1 x_2 \ldots x_n$. Let $\text{ind}(\pi) = a_1 a_2 \ldots a_n$. Then $0 \leqslant a_i \leqslant n-i$ for each i and by Theorem 2.1, $r = 1 + \sum_{i=1}^{n-1} a_i(n-i)!$. To prove the uniquess of this representation, let us suppose $r = 1 + \sum_{i=1}^{n-1} b_i(n-i)!$, where $0 \leqslant b_i \leqslant n-i$ for each i. Let $b_n = 0$. By the corollary to Lemma 3.1, there exists a unique n–permutation π' with $\text{ind}(\pi') = b_1 b_2 \ldots b_n$. Bur rank $(\pi') = r = \text{rank}(\pi)$. It follows that $\pi = \pi'$ since there is a unique permutation with a given rank. From Lemma 3.1, it follows that $b_i = a_i$ for each i.

Theorem 3.1. *Let r be an integer such that* $1 \leqslant r \leqslant n!$. *Let* $r_o = r-1$ *and for each* $i < n$, *let* q_i *and* r_i *be the quotient and remainder, respectively, obtained by dividing* $(n-i)!$ *by* r_{i-1}. *Then* $r = 1 + \sum_{i=1}^{n-1} q_i(n-i)!$ *and* $0 \leqslant q_i \leqslant n-i$ *for each i.*

Proof: Consider the equation $r_{i-2} = q_i(n-i)! + r_i$. Suppose that $q_i > n-i$. Then $q_i \leqslant n-i+1)$. This implies that $r_{i-2} \leqslant (n-1)!\,(n-i+1) + r_1 = (n-i+1)! + r_i \geqslant (n-i+1)!$ This is a contradiction since $r_{i-1} < (n-1+1)!$.

Now, if we add all the equations $r_{i-1} = q_i(n-i)! + r_i$, where $i = 1, 2, \ldots, n-1$, then we get

$$r_o + \sum_{i=1}^{n-2} r_i = \sum_{i=1}^{n-1} q_i(n-i)! + \sum_{i=1}^{n-1} r_i,$$

which implies that

$$r_o = \sum_{i=1}^{n-1} q_i(n-1)! + r_{n-1}$$

But $r_{n-1} = 0$ since it is the remainder obtained by dividing r_{n-2} by 1!. The theorem then follows since $r_o = r - 1$.

Corollary. *Let* $1 \leqslant r \leqslant n!$ *and let* $q_1, q_2 \ldots, q_{n-1}$ *be the integers defined in the theorem. Then* $q_1 q_2 \ldots q_n$, *where* $q_n = 0$, *is the index of the permutation* π *whose rank is r.*

Example. Find the 6–permutation with rank r = 15. First we determine the quotients q_i.

$$14 = 0(5!) + 14 \Longrightarrow q_1 = 0,$$
$$14 = 0(4!) + 14 \Longrightarrow q_2 = 0,$$
$$14 = 2(3!) + 0 \Longrightarrow q_3 = 2,$$
$$2 = 1(2!) + 0 \Longrightarrow q_4 = 1,$$
$$0 = 0(1!) + 00 \Longrightarrow q_5 = 0,$$
$$\text{and set} \quad q_6 = 0.$$

Therefore ind(π) = 002100. Let C_1 123456. Using our algorithm, we have

$0 + 1 = 1 \Longrightarrow x_1 =$ 1*st* element of $C_1 = 1 \Longrightarrow C_2 = 23456$
$0 + 1 = 1 \Longrightarrow x_2 =$ 1*st* element of $C_2 = 2 \Longrightarrow C_3 = 3457$
$0 + 1 = 3 \Longrightarrow x_3 =$ 3*rd* element of $C_3 = 5 \Longrightarrow C_5 = 346$
$1 + 1 = 2 \Longrightarrow x_4 =$ 2*nd* element of $C_4 = 4 \Longrightarrow C_3 = 36$
$0 + 1 = 1 \Longrightarrow x_5 =$ 1*st* element of $C_5 = 3 \Longrightarrow C_6 = 6$
$0 + 1 = 1 \Longrightarrow x_6 =$ 1*st* element of $C_6 = 6$

Hence π = 125436 is the 6–permutation with rank 15.

It should be observed that the computation of the quotients q_i is not easy if n is large. A much easier method of computation is possible. Suppose $1 \leqslant r \leqslant n!$. Let $r_o = r - 1$ and let the results of the series of divisions be the following.

$$r_o = q_1\ (n-1)! + r_1 \qquad 0 \leqslant r_1\ \text{H}$$
$$r_o = q_2\ (n-1)! + r_1 \qquad 0 \leqslant r_1 < (n-1)!$$
$$r_1 = q_2\ (n-2)! + r_2 \qquad 0 \leqslant r_2 < (n-2)!$$
$$r_2 = q_3\ (n-3)! + r_3 \qquad 0 \leqslant r_3 < (n-3)!$$

$$r_{n-2} = q_{n-1}\ (1!) + r_{n-1} \qquad 0 \leqslant r_{n-1} < 1!$$

We shall use the fact that for any real number x and any positive integer n, [x/n] = [[x] /n], where [x] denotes the greatest integer not exceeding x (This is not so difficult to prove.).

Consider the following sequence of divisions.

$$r_o = q_1'(1) + r_1 \qquad 0 \leqslant r_1' < 1$$
$$q_1' = q_2'(2) + r_2' \qquad 0 \leqslant r_2' < 2$$

$$q_2' = q_3'(3) + r_3' \qquad 0 \leqslant r_3' < 3$$

$$\vdots$$

$$q_{n-1}' = q_n'(n) + r_n' \qquad 0 < r_3' < n$$

Observe that $q_1' = [r_o/1]$, $q_2' = [q_1'/2] = [r_o/2!]$, $q_3' = [q_2'/3] = [r_o/3!]$, . . . , $q_{n-1}' = [r_o/(n-1)!]$, $q_n' = [r_o/n!] = 0$. Multiply the second equation by 1!, the third by 2!, and in general multiply equation i + 1 by i!. If all the resulting equations are added, we get $q_1' = q_n'(n1] + \sum_{i=1}^{n-1} r_{n-1+1}'(n-i)!$. But $q_1' = r_o$ and $q_n' = 0$. Furthermore, $0 \leqslant r_{n-1+2}' < n-i+1$. By Lemma 3.2 (uniqueness), it follows that $r_{n-i+1}' = q_i$ for each i = 1, 2, . . . , n−1.

Example. Represent 15 in the form $1 + \sum_{1=1}^{n-1} q_i(n-1)!$, where $0 \leqslant q_1 \leqslant n-i$ for all i.

We have $r_o = 14$. Then we perform the following divisions.

14/2 = 7, remainder 0, $q_5 = 0$
7/3 = 2, remainder 1, $q_4 = 1$
2/4 = 0, remainder 2, $q_3 = 2$
0/5 = 0, remainder 0, $q_2 = 0$
0/6 = 0, remainder 0, $q_1 = 0$

So we have 15 = 1 + 0(5!) + 0(4!) + 2(3!) + 1(2!) + 0(1!).

4. Lexicographic Enumeration of n–Permutations.

There are five methods of enumerating n–permutations discused in [1], namely, the Tompkins-Paige method, the derangement method of M. Hall, the transposition method of M.B. Wells, the adjacent mark method of S. M. Johnson, and the lexicographic method of D. N. Lehmer. Of these methods, only Lehmer's method lists the n–permutations in lexicographic order. Essentially the method consists of listing the n–permutations starting from the one with rank 1, i.e., the permutation 123. . . n, and then incrementing the rank by one until the last permutation n . . . 321 is reached. For each given rank, the permutation is determined by means of a neat algorithm but the whole process takes time to complete because of the need to compute for the index of the permutation. Here we shall find a way to accomplishing the same task without the need for computing indices.

Let π_1 and π_2 be n–permutations. We say that π_2 is the *successor* of π_1 if $\pi_1 < \pi_2$ and there exists no n–permutation π such that $\pi_1 < \pi < \pi_2$. Let $\pi_2 = y_1 y_2 \ldots y_n$ be the successor of $\pi_1 = x_1 x_2 \ldots x_n$. Then by definition, there exists

a positive integer $k < n$ such that $x_i = y_i$ for all $i < k$ and $x_k < y_k$. Let $\pi = z_1 z_2 \ldots z_n$ be any n–permutation such that $\pi_1 < \pi$. Then there exists a positive integer $k' < n$ such that $x_i = z_i$ for all $i < k'$ and $x_{k'} < z_{k'}$. Clearly, $k' \leq k$. Therefore, to find the successor of an n–permutation π_1, we look for the n–permutation π_2 such that corresponding elements of π_1 and π_2 in all positions before the k*th*, for the maximum possible value of k.

Lemma 4.1. *Let $\pi_2 = y_1 y_2 \ldots y_n$ be the successor of $\pi_1 = x_1 x_2 \ldots x_n$ and let k be the positive integer satisfying $x_i = y_i$ for all $i < k$ and $x_k < y_k$. Then $x_k < x_{k+1}$, $y_1 < y_{i+1}$ for all $i > k$ and y_k is equal to the minimum x_j satisfying $j > k$ and $x_j > x_k$.*

Proof: Suppose $x_k > x_{k+1}$. Since $x_i = y_1$ for all $i < k$ and $x_k < y_k$, it follows that $y_k = x_j$ for some $j > k+1$. Hence, $x_{k+1}\ x_{k+2} \ldots x_n$ is not monotonic decreasing. Consequently, there exists a maximum $t >$ such that $x_t < x_{t+1}$. Let $\pi' = z_1 z_2 \ldots z_n$ be the permutation defined by $z_t = x_{t+1}$, $z_{t+1} = x_t$, and $z_i = x_i$ for all i different from t and t+1. Clearly, $\pi_1 < \pi$. But this is a contradiction since $t > k$. That $y_1 \leq y_{i+1}$ for all $i > k$ is proven using a similar argument. By definition of successor, y_k must be equal to the minimum x_j such that $x_j > x_k$ and $j > k$.

Lemma 4.1 gives us a way of constructing the successor of any given permutation, and hence, an algorithm to enumerate all permutations in lexicographic order. We illustrate this by means of an example. Let $\pi_1 = x_1 x_2 \ldots x_n = 261354$. The largest k for which $x_k < x_{k+2}$ is k = 4 corresponding to the element 3. The minimum element to the right of 3 which is greater than 3 is 4. Therefore, the successor of π_1 is $k_2 = 261435$.

A computer program in PASCAL that lists in lexicographic order all n–permutations is given below.

```
Program Permutation;
label       done, show;
type        values = 0..50;
var         i, j, k, order: values;
            perm: array [0..50] of values;

Procedure Search; { determine first element of permutation not equal to corresponding element in the successor}
begin       i: = order;
            perm [0] : = 0;
            repeat
                  i: = i – 1;
            until (perm [i] < perm [i + 1]); [ith element will be changed if it is not
            equal to 0]
end;
```

```
Procedure SortTail {arrange in increasing order the elements to the right of the
ith}
label       okey;
ebgin       if i = order-1 then go to okay;
            for j: i + 1 to order- 1 do
                  for k: = j + 1 to order do
                        if perm[k] < perm[j] then
                        begin     perm[0] : = perm[k] ;
                                  perm[k] : = perm[j] ;
                                  perm[j]  : = perm [0] ;
                        end;
okay:
end;
Procedure Adjust; [interchange the ith element and the least element to its right and
greater than the ith]
begin       j: = i;
            repeat
                  j: = j + 1;
            until (perm[j] > perm[1]);
            perm[0] : = perm[j] ;
            perm [j] : = perm[i] ;
            perm [i] : = perm [0] ;
end;

Procedure Display;{write on the screen the permutation }
begin       for i: = 1 to order do
            write (perm[1],'');
            writeln;
end;



end;
begin       write (Enter the order (1-50): ' );
            readln (Order);
            for i: = 0 to order do {define the initial permutation 123. . . n }
                  perm(i): = i;
show:       display;
            search;
            if i = 0 then go to done;{end program if there is no more successor}
            sorttail;
            adjust;
            go to show;
done:       write ('End of the lexicographic list!');
end.
```

Reference

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Trans. Nat. Acad. Sci. & Tech. (Phils.)
1987.9:51-63

DISTRIBUTED CONTROL USING A HIERARCHY OF COORDINATORS

Arturo I. Concepcion
College of Computer Studies
De La Salle University
Taft Avenue, Manila. Philippines

ABSTRACT

One of the central issues in the efficient and reliable operation of distributed computer systems (DCS) is distributed control. This issue deals with the decentralization of the control of the entities that provide the many functions of DCS. The absence of a central coordinator forces the entities to rely on timely information provided by other entities to decide on the best choice of action. But this scheme cannot be solved in real-time because of the overhead in computation and communication. Furthermore, the presence of unreliable communication and delays add more complexity to the issue. This paper proposes the use of a hierarchy of coordinators to solve the distributed control issue of DCS. The function of the hierarchy of coordinators is to ensure the timely arrival of information and to provide the global state of the DCS to the entities. The construct of coordinators also guarantees the computation of the decision process in finite time. This paper presents the distributed control algorithm based on the construct. To study its behavior, a test bed was implemented on a multiprocessor architecture, the Heterogeneous Element Processor (HEP). Simulation runs made on the HEP showed that the construct is viable solution.

Introduction

A current issue that generates much interest in research is the distributed control in distributed computer system (DCS). DCS offers several advantages over Von Neumann type of computers. Among the advantages are computation speed-up, high reliability, resource sharing, better utilization and extensibility [STAN84]. To achieve high reliability in DCS, functions such as scheduling algorithms, deadlock detection, routing and congestion control algorithms and concurrency control algorithms are distributed over several decision makers on nodes in the DCS. But having no central coordinator, control over these functions becomes a difficult task. Having all of these algorithms coordinate to achieve good overall performance is even more complex. Ho in 1980 [HO80] proposed to solve this problem through team decision scheme. But this algorithm involves a large overhead in terms of computation and communication. To reduce the overhead, estimation of the global

state requires some relaxation to the team decision scheme such as step delay approaches [HO80] and periodic coordination [LARS82].

This paper proposes a structure, the hierarchy of coordinators, to achieve distributed control in a DCS. This structure will guarantee that the computation of the global state can be done in finite time and satisfy time constraints, in the case of real-time systems. An overview of some of the proposed algorithms to maintain the correct and efficient operation of the DCS is presented in section 2. Section 3 of this paper discusses the mechanism of the hierarchy of coordinators in maintaining distributed control over a set of processes. Then section 4 presents the implementation of a test bed for the hierarchy of coordinators on the Heterogenous Element Processor (HEP). This machine is an MIMD computer. Simulation runs and analysis are described in section 5. Finally, Section 6 offers some future directions on our approach.

Deadlock, Global State and Roll-Back Issues

In a distributed environment, there are issues that must be addressed such as deadlock detection, global state determination and roll-back mechanism. These issues constitute a substantial overhead on the correct and efficient operation of the DCS. Research efforts have been directed to answer these issues in the past, [JEFF85, CHAN83, CHAN85, OBER82, MENA79].

For deadlock detection in a DCS, algorithms were proposed in [CHAN83]. The algorithms consist of an idle process initiating a deadlock query message to its dependent set. The dependent set consists of a linear order of processes where P_i is idle and is waiting for a resource held by Pi+1. Process P_j is dependent on process P_k if there exist a dependent set between processes P_j and P_k. P_j is deadlocked if it is dependent on itself. For every deadlock detection computation, the maximum number of messages sent for N processes is N*(N-1). Other distributed deadlock algorithms involve the construction of the hierarchical wait-for graph [MENA79, OBER82]. The global wait-for graph is constructed from lower level coordinators which form the wait-for graph of their respective constituents. This information is gathered by higher level coordinators until a single coordinator (the root) computes the wait-for-graph for the whole system.

In [CHAN85] an algorithm was proposed to determine the following state in a DCS: a computation has terminated, or the system has deadlocked, or a token has disappeared in the ring. The algorithm to detect any of the above conditions consists of a process (after recording its state) sends a marker along each channel incident to and directed away from the process. Each process receiving the marker on the channel will record its own state and the marker is sent along its incident channel. The algorithm terminates when all the channels have been traversed. To ensure that the computation of the global state is terminated in finite time, no marker will remain forever in a channel and its process will record its state in finite time.

Finally, [JEFF85] presented a roll-back scheme for the system of concurrent processes which does not synchronize. Each message has a timestamp and each process has a local virtual time. The process executes these messages according to a timestamp ordering. Since the process does not synchronize its execution with other processes, a straggler message (timestamp is less than local time of process) might arrive at some time later. When this happens, the scheme will initiate a roll-back to undo the previous computation back to a consistent state. This state corresponds to the state of the process just before the time indicated on the timestamp of the straggler message.

The determination of the global state of the system of processes is a hard task to perform since the processes are at a different local clocks and the processes communicate at will. The algorithm presented by [CHAN85] determines only specific types of global states (termination of computation, deadlock and loss of token) the DCS might be in. Deadlock can occur if there are no constraints in the manner in which the processes can wait for another process holding a resource. Allowing also a process to go ahead of its computation regardless of whether the process is in synchrony with other processes or not, results in the necessity of a roll-back scheme for re-synchronization. The algorithms presented in [CHAN83, CHAN85, JEFF85] contain overhead which could lead to unacceptable performance depending on the frequency of its invocation and the extent of rolling back.

Hierarchy of Coordinators

This paper proposes a distributed control algorithm for a DCS. The DCS is modelled as a system consisting of a set of processes communicating by message passing. There are no shared variables and each process is assumed to reside completely in a processor. Thus the terms process and the processor will be used interchangeably in this paper. The communication link between processes is assumed to be reliable and the probability of being down is remote.

Let $P = \{ P_i \}$ be the set of processes,
$i = 1, 2, \ldots, n$ where $| P | = n$.
Let $C = \{ C_{ij} \}$ be the set of coordinators,
$i = 1, \ldots, \gamma = 1, 2, \ldots, \eta$
where γ is the maximum level of hierarchy
and η is the number of partitions,
Let $[P = \{ [P_i \}$ be the set of partitions,
$i = 1, 2, \ldots, \eta$ and
$[P_i \epsilon P$ if level $= 0$
$[P_i \epsilon C$ if level > 0.

This assignment of coordinators to partitions of processes and coordinators produces a hierarchy of coordinators, see Fig. 1. The coordinator assigned to the root is $C_{\gamma\eta}$ where γ is the maximum level of hierarchy and η is the number of partitions.

It was mentioned earlier that a process completely resides in a processor. The coordinators may or may not be assigned to a separate processor. Thus several coordinators might reside in one processor. The hierarchy of coordinators, from level 1 to γ, is a logical interconnection among coordinators. To simplify our discussion, however, the coordinators are assumed to reside in separate processors.

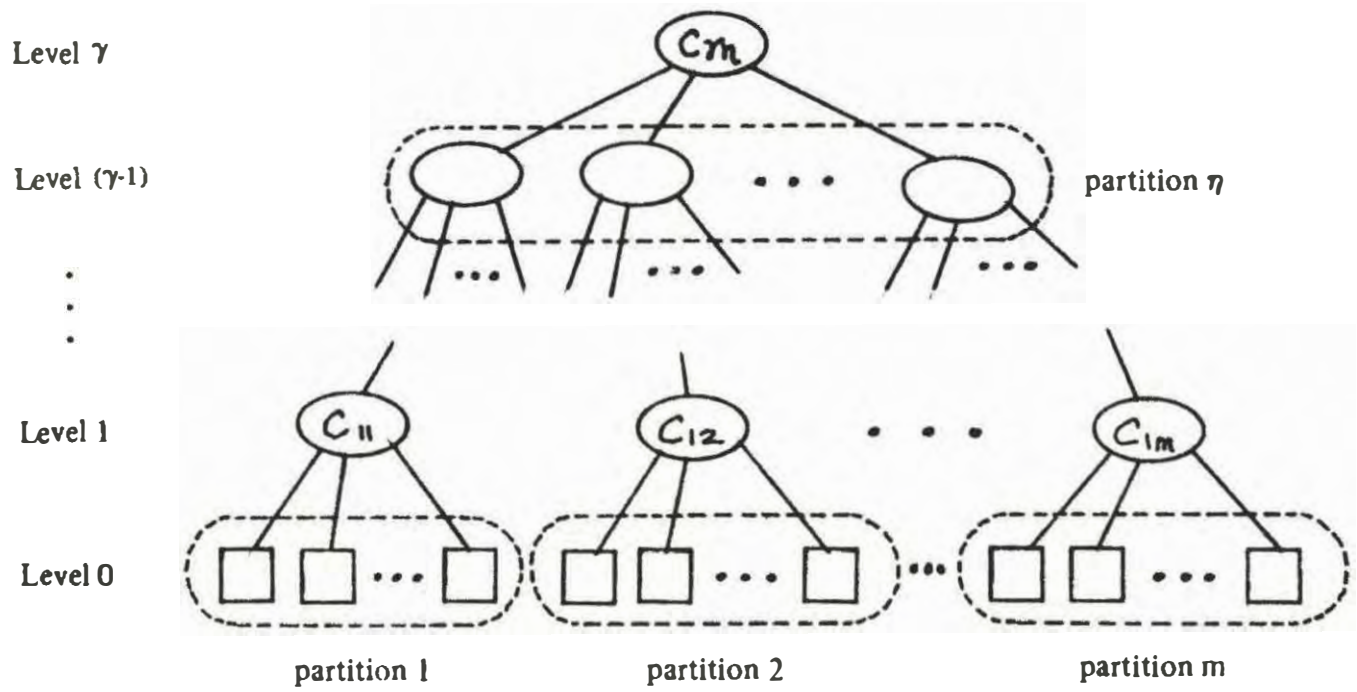

Fig. 1

3.1 Algorithms for a Coordinator and a Process

Each process has the following state variables:

t_L = timestamp of the last message received or time of last signal received.

t_N = estimate of the next time a process will send a message.

s = current state of a process.

A message contains 4 tuples, (spid, dpid, τ, msg), the source and destination process(es), the timestamp of the message and the actual message itself. Note that the timestamp on the message is the current global time. Whenever a process receives a message, the process' t_L is updated to τ and an estimate of the next time the process will send a message, t_N, will be computed. The process with the minimum t_N is allowed to communicate its results to another process(es). The process with the minimum t_N is called the imminent process.

The algorithm for coordinators ensures synchronization among processes by sending appropriate messages to its subordinates and to its upper level coordinator, if any. The coordinators maintain the following state variables:

T_L = timestamp of the latest message received or timestamp of the latest signal received (from an upper level coordinator).

T_N = the minimum t_N of its subordinates (which is either processes or coordinators).

S = collected states of subordinates.

Each coordinator has the following responsibilities: to route and scrutinize messages for consistency; to collect the states of its subordinates and build a partial global picture of the DCS; to synchronize its subordinates; and to guarantee that the global state computation terminates. The algorithms are shown in Figs. 2 and 3. Each algorithm consists of the actions taken when receiving an external message, emsg; a signal from a coordinator; or an output message from a process, ymsg, from subordinates. Note that the algorithms discard messages which are out of synchrony with the system.

The following gives a summary of the actions taken by either a process or a coordinator when it receives a message.

When a process receives a signal, it first checks whether the message time-stamp, τ, is within the range of allowable values. The process computes its output ymsg to its coordinator. Simultaneously, the process computes its t_N

Algorithm for a Coordinator

```
begin
   recv(spid, dpid, τ, msg)                    [ message is received ]

   select
   (T_L < = τ < = T_N and type (msg) = emsg)
   begin
      send (spid, dpid, τ, emsg)               [ send to all processes ]
      for all processes ϵ dpid                 [ in the set dpid and ]
         recv (spid, dpid, t_N, s)             [ wait until all the ]
         store T_N and s                       [ processes send their ]
      end for                                     [ t_N and s ]
   end

   (or τ = T_N and type (msg) = signal )
   begin
      send (spid, dpid, τ, signal)             [ where dpid is the ]
      parbegin                                 [ imminent process ]
         begin
```

```
            recv (spid, dpid, t_N, s)          [ received states from ]
            store t_N and s                    [ processes ]
         end
         begin
            recv (spid, dpid, τ, ymsg)         [ received out - ]
            if dpid ϵ subordinates then        [ put msg ]
            begin
               send (spid, dpid, τ, emsg)      [ send ymsg as ]
               for all processes ϵ dpid        [ emsg to the ]
                  recv (spid, dpid, t_N, s)    [ destination ]
                  store t_N and s              [ processes and ]
               end for                            [ wait for ]
            end                                   [ results ]
            else send (spid, dpid, τ, msg)     [ else, send ]
         end                                   [ msg to next ]
      parend                                   [ level coordi- ]
   end                                         [ nator ]

      (otherwise)                    [ if not within range, disregard ]
      begin                          [ message and continue ]
         discard msg
         return
      end
   end select

   T_L = τ                                    [ compute the state ]
   T_N = min (t_N of subordinates)            [ variables of the ]
   S   = compute state (s of subordinates)    [ coordinator and ]
   send (spid, dpid, T_N,S)                   [ send results ]
end.
```

Fig. 2

Algorithm for a Process

```
begin
   recv (spid, dpid, τ, msg)                    [ message was received ]
   if (t_L <= τ <= t_N) then t_L = τ
      else
         begin
            discard msg                         [ if not within range ]
            return
         end                                    [ ignore the message ]

   select
      (τ <= t_N and type (msg) = emsg)                  [ compute ]
         s = δ_ext (s, emsg)                            [ state ]

      (τ = t_N and type (msg) = signal)
      parbegin
         begin
            y = λ (s)                           [ compute output and ]
            send (spid, dpid, τ, ymsg)          [ send result to ]
         end                                    [ processes ]
         s = δ_int (s)                          [ compute state ]
      parend

   end select

   t_N = t_L + α(s)                             [ compute t_N and ]
   send (spid, dpid, t_N,s)                     [ send result, including s, ]
end.                                            [ to coordinator ]
```

Fig. 3

and its new state, s, which are sent to the coordinator.

When a coordinator receives a signal it checks first the value of τ for synchronization consistency. The signal is sent to the imminent subordinate (either a process or coordinate). After the signal is sent, the coordinator waits for the imminent subordinate to send its new state variables. Then the coordinator determines the new imminent subordinate.

When a process receives a emsg, it first checks τ for synchronization consistency. The coordinator then sends emsg to all destination subordinates. The coordinator then waits for all destination subordinates to send their new state variables. After which the coordinator determines its new state variables and the new imminent component.

When a coordinator receives a ymsg, it determines whether this message is destined for a subordinate(s) or not. If the destination is a subordinate(s), then the coordinator sends ymsg as a emsg, otherwise, the coordinator sends the ymsg to its upper level coordinator.

Implementation of a Test Bed on the HEP Computer

The architecture of the Heterogeneous Element Processor (HEP) has been described in [GAJS85, HWAN84]. As shown in Fig. 4, the main components are the Data Memory Module, the Packet Switch Network and the Process Execution Module. The HEP architecture is classified as a MIMD machine which can execute multiple instructions on multiple streams of data. A program consists of one or more tasks while each task consists of one or more processes. Each process is composed of a sequence of instructions. Both the tasks and processes are executed in parallel in HEP while the instructions of each process are executed in a sequential pipeline fashion. Each PEM has a program memory where active tasks and process instruction streams are selected for execution. Up to 50 instruction streams can be active at any given time. Notice that each PEM has a number of functional units which allow pipeline execution of multiple instruction streams for multiple data streams. For software support, HEP has the DENELCOR's Extended FORTRAN 77 [DENE 84]. This provides the parallel programming environment for the HEP computer.

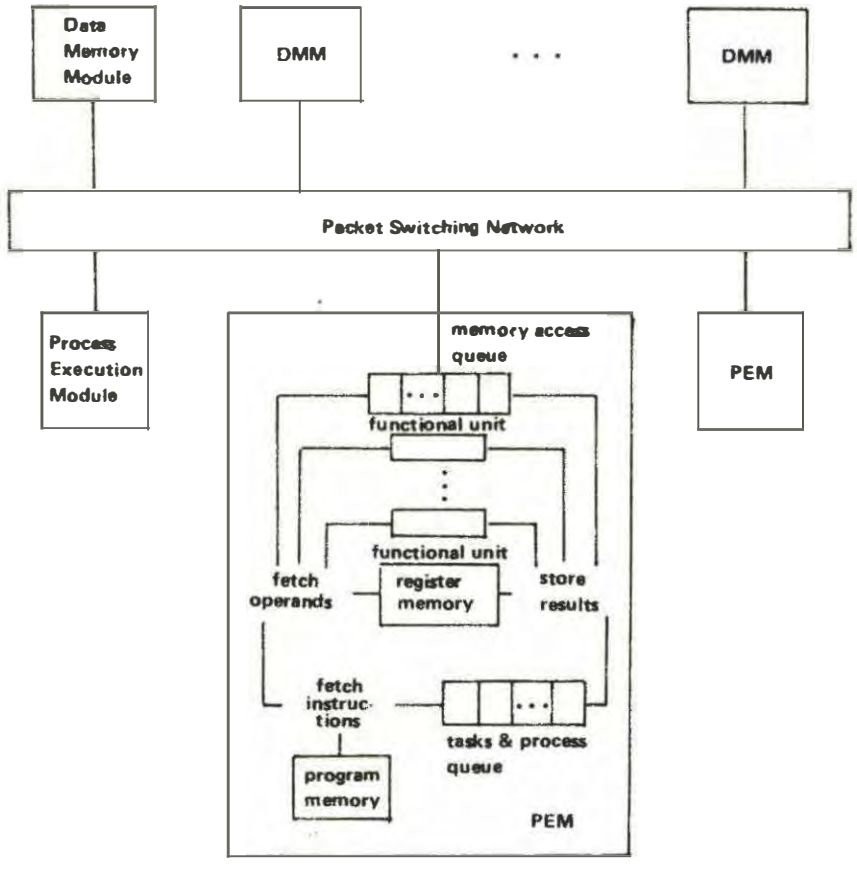

Fig. 4

The synchronization among parallel processes is done via the F/E (full/empty) bit that is tagged on special shared variables called asynchronous variables. These variables are prefixed with a "$" character. An instruction which uses the contents of an asynchronous variable must wait until the F/E bit of that variable is set to full. When the bit is set to full, the content of the variable is made available and its F/E bit is then reset to empty. This assures that no two processes can access an asynchronous variable at the same time.

The implementation on the HEP computer consists of translating the algorithms for a coordinator and for a process (see Figs. 2 and 3) into DENELCOR's Extended FORTRAN 77. Several hierarchical structures were implemented on the HEP with varying levels. Each structure was created in the main program by first requesting the number of levels in the hierarchy from the user. Then for each level, the user is asked to enter the number of children (if any). A node without any children is assumed to be a process and the rest of the nodes are coordinators.

Simulation Runs and Analysis

Several hierarchical structures were implemented on the HEP computer to measure the computation times of the algorithms. Each structure were simulated using the following run-time parameters:

a) Send emsg and ymsg messages to all the processes. In this case when a emsg or a ymsg are generated, all the processes will be in the set of destination processes. This parameter is equivalent to a DCS whereby message passing is heavy.

b) Send emsg and ymsg to a random number of processes. Here, the destination processes are selected by a random number generator. This choice of parameter simulates random message passing for a DCS.

c) Send emsg and ymsg to only one process. This parameter simulates the case where a process communicates with at most one process.

Another purpose is to find the effect of reducing the number of coordinators which subsequently reduces the number of levels in the computation of the global state. A structure with 8 processes were simulated with varying degrees of levels. The percentages of the number of signal to the number of emsg messages sent are varied to observe the effects of heavy load on the DCS.

Simulation runs

Three different tree structures with 8 processes and varying number of levels and coordinators, were implemented. The structures are shown in Figs. 5, 6 and 7. The first structure has a single coordinator controlling all 8 processes. The second structure is a binary tree with 7 coordinators and 8 processes. The third structure is one in which the level of the tree is reduced to eliminate 2 coordinators from the

previous binary tree construct. The results of the simulation runs are shown in Figs. 8-10. Comparing the different constructs, the single coordinator takes the longest time to compute for the global state. The slope of the curve varies directly as the number of signal messages goes up. Note that the receipt of a signal by a process results in the production of ymsg message. Comparing the binary tree construct and the reduced level tree, the computation times are not significantly different, although the reduced level structure takes a slightly longer computation time .

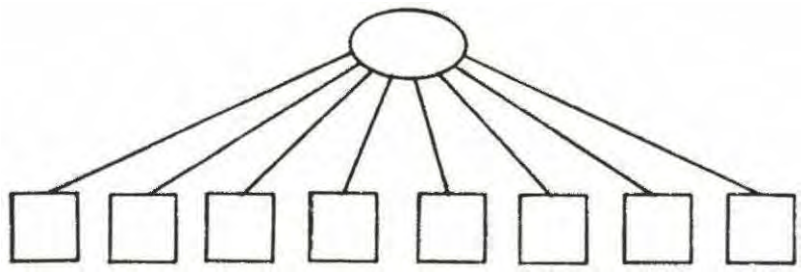

Fig. 5

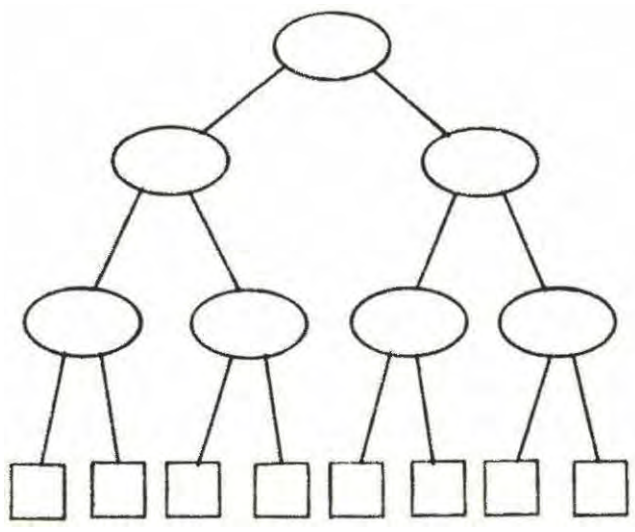

Fig. 6

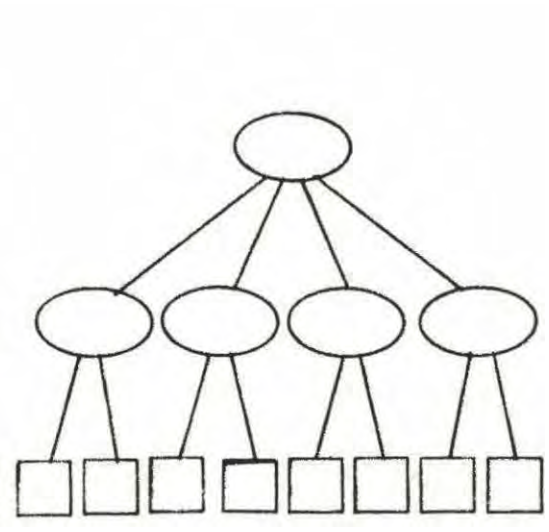

Fig. 7

Analysis of simulation runs

Shown in Figs. 8, 9 and 10 are the plot of simulation runs made for a DCS having 8 processes. Note that the simulation runs were made for 10%, 30%, 50%, 80% and 90% signal messages generated as compared to the generation of emsg messages. Five runs were made on each plot and the following are the parameters on each run:

a) Run 1 – this run has the messages ymsg and emsg sent to a random number of process(es). This run is represented in the line plot by squares.

b) Run 2 – this run has the messages ymsg and emsg sent to almost all the processes most of the time. This run is represented by '+' in the line plot.

c) Run 3 – this run has the messages ymsg and emsg sent to at most one process most of the time. This run is represented by diamonds in the line plot.

d) Run 4 – this run has the ymsg sent to the farthest process from the sending process most of the time while the emsg is sent to a random number of process(es). This run is represented by triangles.

e) Run 5 – this run has the ymsg sent to the nearest process from the sending process most of the time while the emsg is sent to a random number of process(es). This run is represented by 'x'.

Experimental Run in HEP

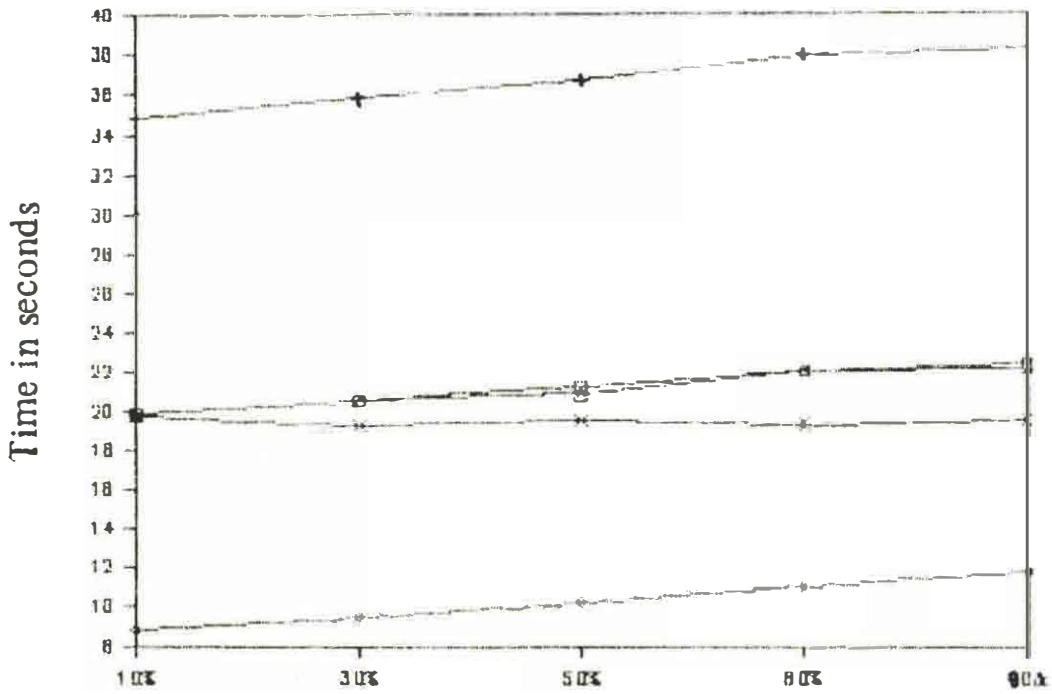

Fig. 8

Experimental Run in HEP

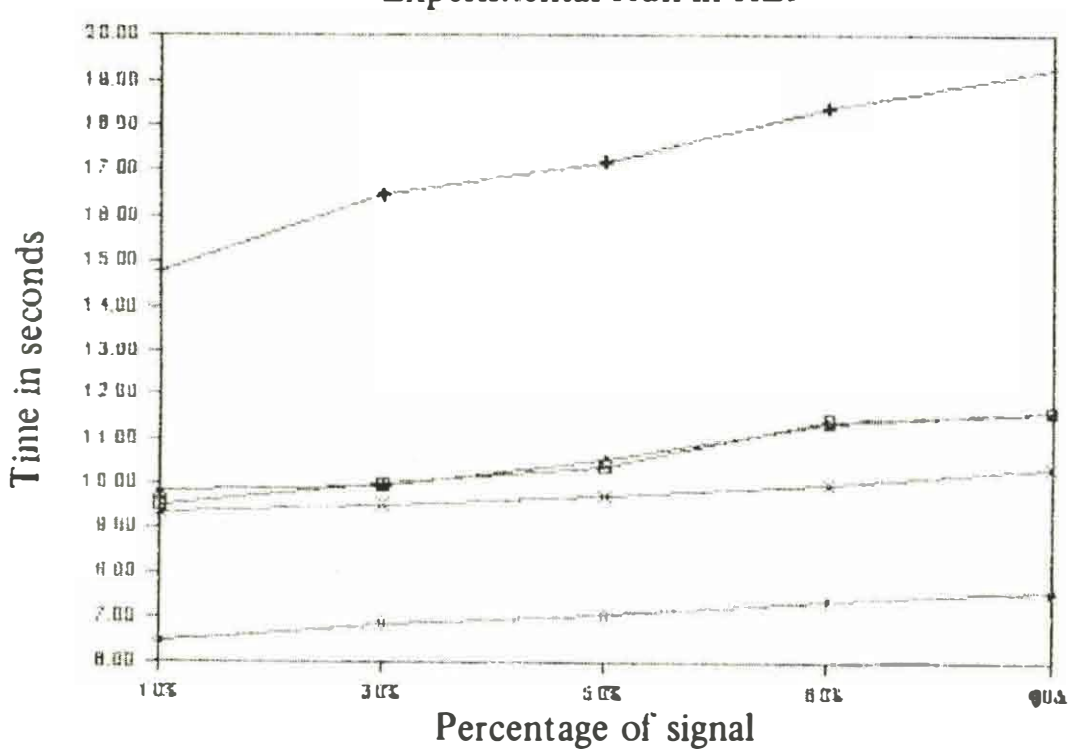

Fig. 9

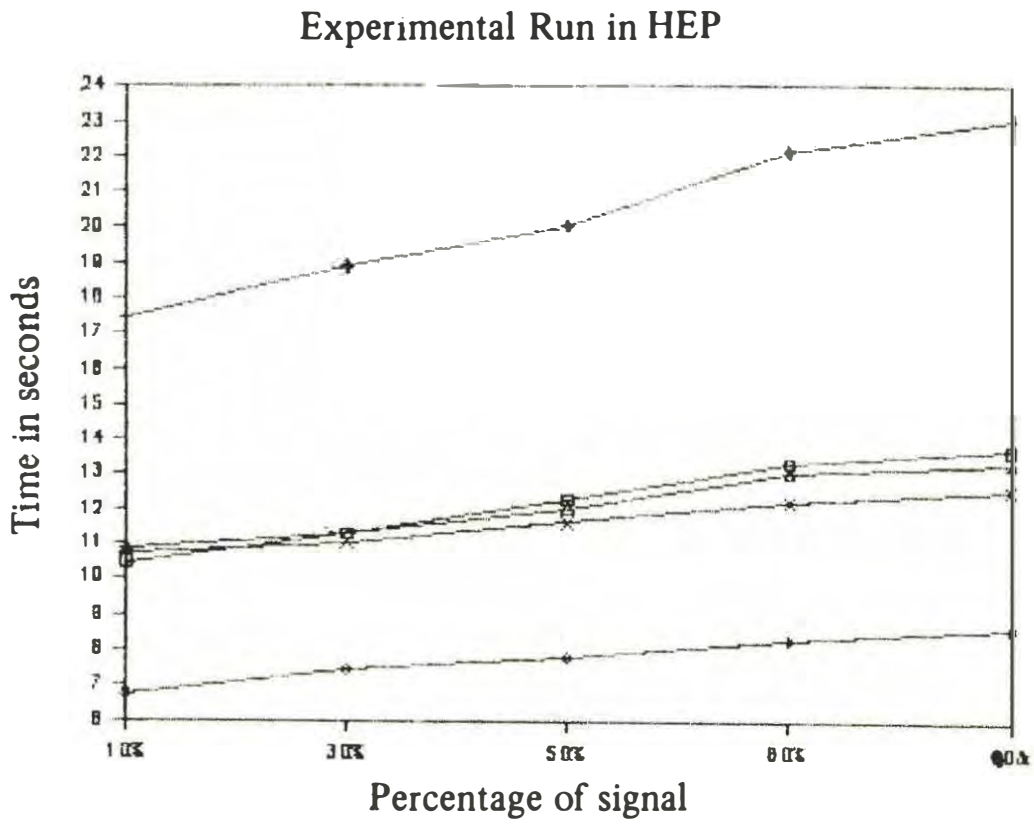

Fig. 10

Fig. 8 shows the simulation run for the construct with a single coordinator, Fig. 9 for the construct with 7 coordinators and Fig. 10 for the reduced level of hierarchy with 5 coordinators. All plots showed an increased in computation time as the percentage of signal messages generated increases. This result is expected since the receipt of signal results in the generation of ymsg. All plots also showed that Run 3 has the lowest computation time while Run 2 has the highest computation time. The reason is that Run 2 sends messages to almost all the processes while Run 3 sends a single message to just one process. Runs 1, 4 and 5 showed an average computation times as compared to the two extremes of Runs 2 and 3. The single coordinator showed the highest computation time because of bottlenecks at the single coordinator. Reducing the level of the hierarchy by one (from 7 to 5 coordinators) induces a slightly higher computation time than the construct with 7 coordinators. This indicates that having less number of levels might be equivalent in performance to the complete number of coordinators. This is a subject of further investigation.

Conclusion

This paper has proposed a hierarchy of coordinators for distributed control of a DCS. The coordinator's function is to collect information from its subordinates to compute the global state. Through the global state, optimal performance can be achieved because the complete information is available for accurate decisions. The price to pay for the computation of the global state is the synchronization and intercommunication schemes for the processes. The processes' communication are controlled by the hierarchy of coordinators. As the algorithm shows, only one

process (the imminent process) is allowed to communicate at any one time. The simulation runs have shown that the computation time varies directly as the number of levels increases (or the number of coordinators are increased).

The hierarchy of coordinators is a compromise between a centralized and a distributed approach. The centralized approach utilizes a single coordinator the failure of which results in a catastrophe for a DCS. The fully distributed approach sometimes lead to unacceptable performance because of the complexity of the computation and the overhead incurred due to roll-back procedures or re-computation of its state variables. The hierarchy of coordinators provides a structure whereby both the advantages of a central coordinator and distributed control are present. Failure of any coordinator can be handled using an electron algorithm and crash-recovery techniques.

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A FLEXIBLE PEEPHOLE OPTIMIZATION METHOD FOR INTERMEDIATE CODES[1]

Eliezer A. Albacea
Computer Science Laboratory
Institute of Mathematical Sciences and Physics
University of the Philippines at Los Baños

ABSTRACT

Object codes generated by compilers are usually not optimal. Several methods of improving object codes have been devised to further reduce the execution times of programs. This paper describes a new method under the class of peephole optimization technique. The method was implemented using a one-pass Pascal compiler and the intermediate language SLIM. The latter is described briefly in this paper. To evaluate the new method, a comparison of several methods under the peephole optimization technique is presented. In addition, the codes generated with and without the proposed method are compared.

Introduction

Tanenbaum *et al.* [9] stated that in a compiler consisting of a front end that translates to a common intermediate language and a back end that translates to a machine's assembly language, improvement of object code can be performed in three conceptual places.

The first conceptual place is to do the improvement in the front end. The decision to do it in the front end would consequently require that the translator be "highly specialized". What we mean by a "highly specialized" translator is a translator that attempts to generate the best code that it can possibly generate for a particular source code fragment. Usually this type of translator is too complicated to construct and thus would require a high development effort. In addition, such translators will increase the compilation time of source programs because the compiler will have to carry out numerous tests to get better object code for a certain source code fragment. But no matter how specialized the translator is, it will still miss some possible improvements in the source code fragments translated separately by the translator. For example, the Pascal statements a:= b + c and d:= a + d will be translated by a highly specialized translator (assuming that it translates each

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statement separately) to Lb + c Sa followed by La +d SD. Obviously, the translator will fail to detect the possible improvement of the instructions Sa La to simply Sa. To catch these possible improvements, however, the compiler should do further improvement on the intermediate code. This brings us to the second conceptual place, i.e., doing the improvement on the intermediate code.

Since doing the improvement in the front end may still require another pass through the intermediate code to catch every possible improvement, it is usually advisable to do all the code improvement on the intermediate code and merely construct a simple front end translator. In this way, the development of the translator will not involve too much effort. Moreover, since the intermediate language does not change, the optimization procedures will be the same for all front ends or back ends.

The last conceptual place is to do the improvement in the back end. This possibility seems to be the most profitable. The reason is that if the objective is to catch all possible improvements in the code, then improvement should be done in the code that is finally executed. But, this would mean that for every new back end, a new code improver must be written. Note that a possible improvement in one machine may not be possible in the other. For example, the sequence of instructions MOVE.L 4(A1), D0 and MOVE.L D0, 10(A0) are Motorola MC68000 instructions which can be improved to MOVE.L 4(A1),10(A0). But a similar set of instructions in another machine that does not allow memory to memory copy can not be improved at all.

Improvement is usually done on the intermediate code to avoid the greater development effort in doing the improvement in the front end or the back end. Although doing the improvement on the intermediate code will not catch all possible improvements, the difference compared to doing it in the back end is usually slight. This is because each intermediate code is usually mapped to the most efficient actual machine code.

There are several classes of methods devised to improve intermediate codes. But, we shall be concentrating only on one class, the peephole optimization technique. After considering the technique in general, a discussion of the implementations of a peephole optimizer including the new method will be given. To investigate the advantage of the new method, a time comparison of several programs compiled with and without the code improver will be presented.

The intermediate code Stack Language for Intermediate Machines (SLIM) and the SLIM code generated by a one-pass Pascal compiler will be used as basis for the discussion of the new optimization method. Hence, in the following sections the SLIM machine and its instructions will first be introduced before the discussion of the new method.

The SLIM Machine

SLIM is a simple one-accumulator, stack-oriented hypothetical machine. The

machine was first proposed by Fox[4], but was later enhanced by Peck[7]. The machine was designed with the following principal aims:

1. To reflect current machine architecture, if possible;
2. To obtain a reasonably simple machine such that it can be used for teaching the elements of computing;
3. To obtain a machine that is suitable as target machine for high-level languages such as BCPL;
4. To obtain a tool for achieving portability of systems programs; and
5. To obtain a machine on which it is possible to have an operating system.

SLIM is a machine very similar to a conventional computer in that it consists of a memory and a processor.

The memory of SLIM is a sequence of cells. Each cell contains 'n' bits with the value of 'n' implementation dependent. It may be 16 bits, 32 bits, or more, but the choice depends entirely on the number of bits required for an address on the target machine and the memory available. The cells are addressed consecutively starting from 0 to 'm'. The value of 'm' as with the number of bits per cell, is machine dependent. However, the size of 32K cells was found to be a comfortable size for many SLIM implementations, e.g., on Perkin Elmer 3230 Motorola MC68000, IBM 370 (Amdahl 470), etc.

SLIM has a total of seven (7) registers. These registers and their functions are given in Table 1.

Table 1. SLIM Register

Symbol	Register	Function
A	Accumulator	This is where all arithmetic and logical operations take place
E	Environment	Holds a pointer to the environment of a procedure
H	High Point	Points to the last useful cell on the stack
C	Program Counter	Holds a pointer to the instruction to be executed
G	Global	Holds a pointer to the first cell of a sequence of cells reserved for global variables
N	Interrupt	Holds information that is used to recover from an interrupt
S	Stack Limit	Holds a pointer to the last cell in the stack

Typical SLIM instructions may contain at most three fields – the operator, the operand modifier, and the raw operand. The operator field is always present in the instruction while the raw operand and the operand modifier may or may not be, depending on the type of instruction.

An operation may be L for load, i.e., move data from memory to accumulator (A), S for store, i.e., move data from accumulator (A) to memory, + for add, J for jump, and so on.

A raw operand is either an unsigned or signed number, a character, the H register, or a label (@n where 'n' is an integer).

The remaining field is the operand modifier. It is used, if it is part of the instruction, to qualify the meaning of the raw operand. An operand modifier is either E (modified by environment), G (modified by global), I (modified by indirection), IE (combination of environment and indirection), or IG (combination of global and indirection).

Table 2 shows a list of all the SLIM operators. The table gives a brief description of the operator, and the microcode for each operator to help visualize its actions. The microcode is written in BCPL. The table does not show all the possible operands and operand modifiers; instead we represent the operand, modified or unmodified, by the letter W.

Table 2. SLIM Operators

Instruction	Mnemonic	Microcode
Load cell	LW	A := W
Store cell	SW	!W := A
Load cell subscripted	L!W	A := A!W
Store cell subscripted	S!W	LET V = !H; H −:= 1; A!W := V
Load byte	L%W	A := A%W
Store byte	S%W	LET V = !H; H −:= 1; A%W := V
Load field	L:W	A := W of A
Store field	S:W	LET V = !H; H −:= 1; W OF A := V
Load device	L$r	A := A!r
Store device	S$r	LET V = !H; H −:= 1; A!r := V
Push and load cell	PLW	H +:= 1; !H := A; A := W
Jump	JW	C := W
True jump	TW	IF A = TRUE THEN C := W
False jump	FW	IF A = FALSE THEN C := W
Modify high point	MW	H +:= W
<dop>	<dop>W	A := A <op> W
<mop>	<mop>	A := <mop> A
Procedure call	CW	
Procedure return	R	C := E!O; H := E!(−2); E := E!(−1)
Push	P	H +:= 1; !H := A
Exchange	X	W := H!(−1); H!(−1) := A; A := W
Originate	O	
Void	V	no operation
Quit	Q	exit
Switchon sequential	?S	
Switchon indexed	?I	
Non-local access	U	

<dop> – dyadic SLIM operators
<mop> – monadic SLIM operators

Peephole Optimization

Peephole optimization has been used to improve intermediate and actual machine codes. The method works by looking at a small range of instructions, at least two instructions, and replacing them by more efficient instructions. This small range of instructions is referred to as the peephole. The code in the peephole may be continguous, e.g., the peephole SE2 LIE2 is replaced by SE2 or scattered, e.g., the peephole LE2 P . . . SH is replaced by . . . SE2. The nature of the technique is that the replacement code for a sequence of instructions can be used for further improvement. An example is given in Table 3.

Table 3. An illustration of peephole optimization

Sequence of Instruction	Peephole	Replacement
LIE2 P LIE3 +H <inst>	P LIE3	PLIE3
LIE2 PLIE3 +H <inst>	PLIE3 +H	+IE3
LIE2 +IE3 <inst>	+IE3 <inst>	

<inst> – remaining instructions in the sequence

One of the aims of code improvement is to improve the code in a manner that the run-time improvement is greater than the overhead introduced by the improvement procedures at compile time. The next section will discuss how this objective is approached by showing several methods (including the new method) adopted to implement a peephole optimizer.

Implementation of a Peephole Optimizer

Davidson and Fraser [3] described a method for improving assembler codes using two instructions in the peephole. The method works by examining a pair of instructions in the peephole and replacing them, if possible, with one instruction which has the same action. In case the pair of instructions can not be reduced to one instruction, the first of the two instructions gets emitted. The new instructions in the peephole then are the second instruction of the previous peephole and the instruction immediately following the previous pair of instructions. For example, consider the sequence of PDP-11 instructions MOV @R3, R2 and ADD #2 R3 which can be replaced by an equivalent one instruction MOV (R3)+, R2.

Tanenbaum *et al.* [9] developed a method which allows the number of instruction in the peephole to vary from one to any number greater than one. The method was used to improve the intermediate code EM and it employs the use of a pattern/replacement table. The table consists of a collection of lines, each line having a pattern part (peephole) and a replacement part. In contrast to the approach by Davidson and Fraser [3], which uses a constant number of instructions in the peephole, the pattern part (peephole) vary in number of instructions. Their method works by simply constructing the patterns and replacements in advance and

these are looked up in the table during compilation. To avoid missing new patterns created by the replacements, the method repeats the matching process until no more match is found. Examples of pattern and replacement lines are given in Table 4.

Table 4. Patterns and replacements in EM

Pattern	Replacement	Comment
LOC A LOC B ADD	LOC(A + B)	Add constants A and B
LOC 2 MUL	LOC 1 SHL	Change multiplication to shift

Note that the length of the pattern (number of instructions) varies and the replacement is not necessarily shorter in length than the pattern. It may have the same length but the replacement is known to be executed faster than the pattern, e.g., the change from multiplication to shifting.

The New Method

This new method can be used to improve the intermediate code SLIM.In fact, it is theoretically possible to use the method to improve other intermediate codes like PCODE, JANUS , or any intermediate code generated by a one-pass translator.

The method is an extension of the method employed by Davidson and Fraser [3] . The difference is that the number of instructions in the peephole is allowed to increase depending on the kind of source code the translator is translating. The method, therefore, combines the advantages of the methods by Davidson and Fraser [3] and Tanenbaum *et al.* [9].

The extension allowing more than two instructions in some code fragments is essential because the translator generates code which is impossible to improve with only two instructions in the peephole. For example, given the code fragment, a – b, where 'a' and 'b' are the first two local variables of the procedure, then the translation of the given code fragment is LIE2 P LIE3 PLH –H. Using only two instructions in the peephole, this can be improved to LIE2 PLIE3 PLH –H. The subsequent translation, however, can be improved to LIE2 –IE3 if three instructions are used in the peephole.

The problem of determining the number of instructions in the peephole for a particular source code fragment can actually be decided by the manner the translator translates the code fragment. Take for example the same code fragment, i.e., a – b, and suppose that the translator translates the right operand first, this would mean that only two instructions in the peephole are enough to improve the code to its best possible form. To illustrate this point, consider the translation when the right operand is translated first. The translation will be LIE3 P LIE2 –H which can be improved to LIE3 PLIE2 –H and finally to LIE+ –IE2.

Note that the foregoing is always true only for a "highly specialized" translator but not for a one-pass translator. A one-pass translator will usually translate code fragments from left to right; thus requiring longer patterns in the peephole.

The code improver will initially assume a size of two instructions in the peephole. Whenever a code fragment requiring more than two (2) instructions in the peephole is translated, the size of the instructions in the peephole should correspondingly increase. What arrangements then are necessary in order to allow the code improver to change the size of the peephole?

The approach taken in the implementation of this new method was to require the translator to send a signal to the code improver. The signal will inform the code improver that the translator is about to translate another source code fragment. Further, the signal can be in the form of a number. This number will give the code improver the necessary information on the number of instructions required in the peephole in order to improve the code fragment being translated to its best possible form. Another signal must be sent to the code improver once the translation of the current source fragment is through. The size of the peephole then must be sent back to the previous size, i.e., size of the peephole before the latest signal was received by the code improver. Note that the previous size is not necessarily equal to two.

Several advantages can be identified in having the foregoing arrangement. One is that the search for the patterns and their corresponding replacements will only be limited on the patterns whose size is equal to the current size of the peephole. This would consequently mean a significant reduction in search time compared to searching the pattern from all the available patterns that can possibly be improved.

In the case of a one-pass Pascal translator and SLIM as intermediate code, it was found that most translation of Pascal code fragments can actually be improved using only two instructions in the peephole. But for Pascal expressions, two instructions in the peephole are not enough. Three instructions in the peephole were necessary to improve expressions to their most efficient form.

One might argue that since the set of expressions is the only type of code fragment that needs more than two instructions in the peephole, why not use two instructions all throughout? This question makes sense, but if one can reduce the translation of an expression from five (5) to two (2) using three (3) instructions in the peephole, instead of from five (5) to four (4) using two (2) instructions in the peephole, then this will be a great improvement to the code. The reason is that an expression is actually a sub-fragment of almost all Pascal code fragments (not only Pascal but also C, BCPL, Algol 60, Algol 68, PL/I, and other high-level languages).

Adding a code improver of this nature to a one-pass translator will preserve its one-pass property and therefore overhead due to the introduction of the code improver during compilation will be tolerable. The translator will translate source code as before, but everytime an object code is generated it is first pass through the code improver which decides whether the code can already be emitted or not. In short, the code improver is just acting as a filter. The code improver maintains a peephole wherein everytime the translator produces a code it includes this code in the peephole. If the new peephole can be improved, it is replaced by its more efficient equivalent. Otherwise, the oldest instruction in the peephole will be emitted. The code improver will then wait for the translator to generate another object

code. The process is repeated until the translator generates the end of program code.

Patterns and Replacements

There are so many patterns in SLIM that can be improved but only those patterns that can possibly be generated by a one-pass Pascal translator will be shown. Usually, it is the compiler writer who has full knowledge of the patterns of instructions that the compiler generates. It is therefore his responsibility to identify all these patterns and replacements that can be included in the code improver. This is in addition to the requirement that he should know the number of instructions in the peephole necessary to improve a certain source code fragment.

Table 5 shows a summary of all the patterns and their corresponding replacements incorporated in the code improver employing the new method. The type of improvement can be classified into four general classes, namely: folding, rearrangement, strength reduction, and null sequences.

The first group of patterns and replacements constitute those instructions with operands whose values are known at compile time. When the values of all operands in an expression are known at compile time, that expression can be folded, i.e., replaced by a single value. For example, the instructions M5 M4 can be replaced by M9. The values of the operands 4 and 5 were replaced by their sum.

The group on rearrangement has the usual purpose of reducing the amount of temporary storage required during the evaluation of an expression. For example, the code L100 PL50 +H is improved to L100 +50. The unimproved pattern will first load the value 100 into the accumulator; push the value in the accumulator onto the stack; load the value 50 into the accumulator; and finally add whatever is stored in the accumulator with whatever is on top of stack. Compare this to the improved code where the value 100 is loaded into the accumulator and then it is added to the operand of the + operator, i.e., to 50. The use of temporary storage, i.e., the use of the stack, was eliminated.

The strength reduction group of patterns and replacements has the objective of replacing an expensive operation by a cheaper one. The replacement may not necessarily be shorter than the pattern of instructions to be replaced. One example is the replacement of multiplication by the shift operation, e.g., LIE2 *2 to LIE2 >>1.

Finally, the last group is the null sequences group. These instructions can well be deleted from the translation of the source program without affecting the correctness of the translation. In short, this is composed of instructions whose total effect is null. One example is the pattern LIE2 SIE2. The first instruction loads the value of the first local variable into the accumulator and the second instruction stores it back to where it came from.

It will be shown in the next section that the code improver described introduces a negligible overhead to the compilation of source programs but improves the execution time by a reasonable amount.

Table 5. Patterns and replacements in SLIM

Pattern	*Replacement*
Folding	
Lc–	L–c
Mb Mc	M(b+c)
Lφ ∿	L–1
L–1 ∿	LO
Rearrangement	
PLm <di>H	<di>m
PLmPLH <di>H	<di>m
Strength Reduction	
Lm *2	Lm >>1
Lm /2	Lm <<1
Lm *4	Lm >>2
Lm /4	Lm <<2
Lm *8	Lm >>3
Lm /8	Lm <<3
*–1	–
#*–1.0	–
/–1	–
#/–1.0	–
Null Sequences	
J@1 @1:	@1:
J@k J@1	J@k
P Lm	PLm
Mc R	R
R R	R
SEc LIEc	SEc
LIEc SEc	
+0	
# + 0.0	
–0	
# – 0.0	
*1	
# * 1.0	
/1	
# / 1.0	

b & c – integer constants
k & 1 – labels

m – SLIM modifier
<di> – dyadic SLIM operator

Comparison of Improved and Unimproved SLIM Code

The Pascal translator was used to compile several programs to investigate the effect of the code improver on the compilation and execution times of source programs. Of course, attempting to improve the code will almost certainly degrade the compilation of source programs. But, if the improvement will decrease the execution time by at least the same amount as the increase in compilation time then the improvement carried out on the code is certainly worthwhile.

To see whether the method used by the code improver results in an improvement, i.e., the decrease in execution time is greater than the increase in compilation time, the translator was used to translate the following programs:

1. Implementation of the date of Easter algorithm by Amman [2].
2. Sorting of 1000 data items using the quicksort algorithm.
3. Implementation of the eight queens problem by Wirth [10].
4. Multiplying a 20 by 20 matrix.

The programs were translated (with and without the code improver) and executed. The summary of compilation and execution times are given in Table 6 and Table 7, respectively.

Table 6. Compilation times in (seconds)

Program	*With the Code Improver*	*Without the Code Improver*
Date of Easter	0.46	0.41
Quicksort	0.71	0.67
Eight Queens	0.61	0.61
Matrix Multiplication	0.33	0.33

Table 7. Execution times (in seconds)

Program	*Improved SLIM Code*	*Unimproved SLIM Code*
Date of Easter	0.38	0.69
Quicksort	1.36	1.78
Eight Queens	3.16	4.07
Matrix Multiplication	0.96	1.11

It is clear from Table 6 and Table 7 that the degradation in compilation due to the introduction of the code improver is less than 0.1 second. But, the improvement in execution time is much more than 0.1 second. This shows that computing cost can be reduced with the introduction of the code improver.

The significant decrease in execution time can be attributed to the significant decrease in number of instructions in the improved SLIM equivalent. This is illustrated in the date of Easter program where the number of SLIM instructions is reduced from 235 instructions to 131 instructions. A significant difference of 104 instructions. This difference is 44.26% of the original number of instructions.

Conclusion

Although the new method was tested using a one-pass Pascal translator and SLIM as intermediate code, it is worth noting that the method can also be used for other high-level language translators generating intermediate codes. In addition, it can also be added to compilers generating assemblers instead of intermediate codes. But, of course, the method should only be employed in compilers generating assembler codes when it was previously determined that it will be impossible to improve the assembler equivalent of some source code fragments using only two instructions in the peephole.

Introducing the method to existing compilers will require the modification of the compiler itself and the writing of the code improver. The modification on the compiler is necessary because the method requires the translator (compiler) to send a signal about the type of source code it is about to translate.

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SAMPLE SIZE DETERMINATION IN POST HARVEST EXPERIMENTS

Mariano B. de Ramos* and Aleli B. Olea**
Statistical Laboratory, Institute of Mathematical Sciences and Physics
***Post Harvest Training and Research Center, U.P. at Los Baños, College, Laguna, Philippines*

ABSTRACT

Nine sets of data representing nine characters or variables were analyzed with the objective of determining the optimum sample size for post harvest experiments on mango. The estimated values of the variance components σ^2_E and σ^2_S were used to relate the precision of a treatment mean with the number of replications r and number of subsamples s. For any desired degree of precision one can refer to the graph obtained to determine the sample size n which is the product of r and s. On the other hand, if the cost of an experiment is known or can be estimated, then the optimum numbers r, s and n can be determined from the tabular values.

Introduction

One very important consideration that a research worker has to consider in the planning of his experiment is sample size. In the case of comparative experiments, sample size may refer to the number of experimental units used per treatment which is also called replications as in a completely randomized design or it may refer to the combination of the number of replications and number of subsamples per replication as in a completely randomized design with subsampling. Researchers can be guided in this particular problem by examining and using the results of the statistical analysis of past experiments in which the variance components due to identified sources can be estimated. By using the estimated values of those variance components, the researcher can determine the precision of a treatment mean from which the sample size of future experiments can be determined. Thus, in this study, the focus of the analysis were some data from past experiments of the Post Harvest and Training Research Center (PHTRC) at the University of the Philippines at Los Baños with the aim of determining the appropriate sample size for future experiments.

In many of the experiments that have been conducted at PHTRC, the statistical design used is usually the completely randomized design with subsampling. In

such design, the experimental error variation of the data comes from two sources, namely, from the differences between the experimental units treated alike and from the differences between the sampling units within experimental units. Thus, if the experimental error variance of a character x is denoted by σ_x^2, then

$$\sigma_x^2 = \sigma_E^2 + \sigma_S^2$$

where σ_E^2 is the variance component due to the experimental units or replications and σ_S^2 is the variance component due to the sampling units. Without any knowledge of the magnitudes of these two variance components, the researcher would not know the appropriate sample size to use in order to obtain reliable and precise experimental results, hence, he may just utilize whatever materials are available. If the sample size used happened to be too small, then the experimental results may not be able to detect real treatment differences, while if the sample size used happened to be too large, the results of the experiment might be more precise than what would be required statistically.

In the light of the problems stated above, this study was conducted with the main objective of determining the appropriate and optimum sample size for mango post harvest experiments. The specific objectives of the study were: (i) to obtain estimates of the variance components due to experimental units and due to sampling units for various mango post harvest characters, (ii) to obtain the appropriate sample size for mango post harvest experiment that will yield results with certain degree of precision, and (iii) to obtain the optimum sample size for mango post harvest experiments that will give optimal results for a given fixed cost per treatment.

Review of Literature

Anderson (1947) used the analysis of variance to test the significance of variance components that affects the prices of hog meat in two markets. Marcuse (1949) obtained an estimate of the reciprocals of n_1, n_1n_2, and $n_1n_2n_3$. Anderson and Bancroft (1952) utilized a general estimation procedure for the variance components, such as the method of maximum likelihood.

Kempthorne (1952) derived the optimum number of secondary sampling units and optimum number of primary sampling unit for sampling in field experiments. Goldsmith and Gaylor (1970) used the three stage nested design for the estimation of variance components, however unbalanced the arrangements may be. Sahai (1976) studied various estimators of the variance components for the balanced three stage nested design.

In sampling for laboratory brix, Solivas (1978) found that in raw and adjusted sugar rendement, the variance components among the rows and the experi-

mental error variance component were significantly greater than zero. In the study of the avocado fruit characters, Ledesma (1983) found various variance components that gave higher contribution to the total variation. In sampling for coconut characters, Alforja (1983) found that the sample size n considered optimum varies depending with the uniformity of the cultivars. He also found that 24 palms is sufficiently enough to obtain reliable information for nuts per tree estimation.

Materials and Methods

The data

The data used in this study were obtained from the past post harvest experiments on mango that were conducted at PHTRC. Table 1 shows the statistical description of the experiments. There were four experiments and the number of treatments range from four to eight, the number of replications from three to four, and the number of subsamples per replication from three to nine. Nine post harvest characters or variables were measured, two from experiment I, two from experiment II, four from experiment III and one from experiment IV. These characters were color index at day 0 and 5, total soluble solids, titratable acidity, percent cumulative weight loss, firmness, disease incidence, PH and visual quality rating.

The statistical design and model

All the four experiments were conducted in a completely randomized design with subsampling. A typical example of such design is where the treatments are heating temperatures, the experimental units or replications are boxes of fruits, and the sampling units are the individual fruits in the boxes. An experiment may then involve, say, t treatments, r boxes of fruits per treatment, and s fruits per box. Thus, if a character x is measured on sampling unit, the statistical model is of the form

$$x_{ijk} = \mu + \tau_i + e_{ij} + d_{ijk} \qquad (2)$$

$$i = 1, 2, \ldots, t$$

$$j = 1, 2, \ldots, r$$

$$k = 1, 2, \ldots, s$$

Table 1. Description of the four post harvest experiments on mango from which the data of the study were obtained

Experiment No.	*No. of Treatments (t)*	*No. of Replications (r)*	*No. of Sub-Samples (s)*	*Characters Measured*
I	4	4	8	Color index at day 0 and 5
II	6	3	9	Total soluble solids and titratable acidity
III	8	4	3	Percent cumulative weight loss, firmness, disease incidence and pH
IV	6	4	4	Visual quality rating

Source: Post Harvest Training and Research Center, U.P. at Los Banos.

Table 2. Format of the analysis of variance for the nine post harvest characters of mango

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*Expected Mean Square*
Treatment	$t-1$	$rs \sum_i (\bar{x}i.. - x..)^2$	MS(Tr)	$\sigma^2_S + s\sigma^2_E + \sum_i \tau^2_i/(t-1)$
Experimental error	$t(r-1)$	$s \sum_i \sum_j (\bar{x}_{ij}. - \bar{x}_{i}..)^2$	MSE	$\sigma^2_S + s\sigma^2_E$
Sampling error	$tr(s-1)$	$\sum_i \sum_j \sum_k (x_{ijk} - \bar{x}_{ij}.)^2$	MS(SE)	σ^2_S
Total	$trs-1$	$\sum_i \sum_j \sum_k (x_{ijk} - \bar{x}...)^2$		

where x_{ijk} is the observed value in the kth sampling unit of the jth replication and ith treatment, μ is the general mean effect common to all observations, τ_i is the effect of the ith treatment, e_{ij} is the random error effects due to the ith experimental unit of the ith treatment, and d_{ijk} is the sampling error effect due to the kth sampling unit of the jth replicate. For each of the nine characters used in the model, it was assumed that the treatment effects τ_i are fixed and $\Sigma_i \tau_i = 0$. Also, the experimental error e_{ij} were assumed to be normally and independently distributed with mean 0 and variance σ^2_E or $e_{ij} \sim$ NID (0, σ^2_E), and the sampling error d_{ijk} were assumed to be normally and independently distributed with mean 0 and variance σ^2_S or $d_{ijk} \sim$ NID (0, σ^2_S).

Estimation of variance components

The variance components estimated for each character were those due to the differences between experimental units or replications which was denoted by σ^2_E and due to the differences between the sampling units within the experimental units denoted by σ^2_S.

The method used in estimated these variance components was by analysis of variance. Essentially, the steps involved in the estimation of σ^2_S and σ^2_E were:

(1) construction of the analysis of variance table (Table 2), and

(2) equating the actual mean square and the expected mean square for the sampling error and experimental error. Hence, if

(i) MS(SE) = σ^2_S

(ii) MSE = $\sigma^2_S + s\,\sigma^2_E$

then

$$\hat{\sigma}^2_S = \text{MS (SE)} \tag{3}$$

$$\hat{\sigma}^2_E = (\text{MSE} - \text{MS (SE)}) / s \tag{4}$$

where MSE is the mean square due to experimental error defined as

$$\text{MSE} = s \sum_i^t \sum_j^r (\bar{x}_{ij:} - \bar{x}_{i..})^2 / t(r-1), \tag{5}$$

and MS (SE) is the mean square due to the sampling units defined as

$$\text{MS (SE)} = \sum_i \sum_j \sum_k (x_{ijk} - \bar{x}_{ij.})^2 \; / \; tr(s-1) \tag{6}$$

In these formulas, the quantities

$\bar{x}... = \sum_i^t \sum_j^r \sum_k^s x_{ijk} / trs$, the grand mean

$\bar{x}_{i..} = \sum_j \sum_k x_{ijk} / rs$, the treatment mean, and

$\bar{x}_{ij.} = \sum_k x_{ijk} / s$, the replication mean.

Determining the sample size for a given degree of precision

The basis of determining the sample size which in this study is the combination of the number of replications r and the number of subsample s or $n = rs$ was by the use of the precision of a treatment mean. In a completely randomized design with subsampling, the variance of treatment mean $\bar{x}_{i..}$ is given by the formula

$$\text{var}(\bar{x}_{i..}) = \text{MSE}/rs \tag{7}$$

In terms of the estimated variance components $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$, the variance of a treatment mean is

$$\text{var}(\bar{x}_{i..}) = \frac{\hat{\sigma}_E^2}{r} + \frac{\hat{\sigma}_S^2}{rs} \tag{8}$$

Therefore, the standard error of a treatment mean is

$$\text{s.e.}(\bar{x}_{i..}) = \sqrt{\frac{\hat{\sigma}_E^2}{r} + \frac{\hat{\sigma}_S^2}{rs}} \tag{9}$$

In terms of the coefficient of variation, the precision is

$$\text{CV}(\bar{x}_{i..}) = \text{s.e.}(\bar{x}_{i..}) / \bar{x}_{...}$$

Thus, the final form of the precision formula used in this study is

$$CV(\bar{x}_{i\,\cdot\,\cdot}) = \sqrt{\frac{\hat{\sigma}_E^2}{r} + \frac{\hat{\sigma}_S^2}{rs}} \Big/ \bar{x}_{\cdot\,\cdot\,\cdot} \tag{10}$$

The values of the $CV(\bar{x}_{i\,\cdot\,\cdot})$ were then computed and their graphs were drawn against r and s for values of $r = 2, 3, 4, 5$ and $s = 1, 2, \ldots, 10$.

Determining the optimum sample size at a given experiment cost per treatment

Kempthorne (1952) defined the information on each treatment as

$$I = \frac{rs}{\hat{C}_S + s\,\hat{\sigma}_E^2} \tag{11}$$

By assuming a cost function of the form

$$C_0 = r\,(C_E + s\,C_S) \tag{12}$$

where C_0 is the cost of the experiment per treatment, C_E is the cost per experimental unit, and C_s is the cost per sampling unit. Solving for r in (12) and substituting the result in

(11) gave the formula for information as

$$I = \frac{(s)\,(C_0)}{(C_E + sC_S)\,(\hat{\sigma}_S^2 + s\,\hat{\sigma}_E^2)} \tag{13}$$

Minimizing (12) with respect to s gave

$$s\sqrt{\left(\frac{C_E}{C_S}\right)\left(\frac{\hat{\sigma}_S^2}{\hat{\sigma}_E^2}\right)} \tag{14}$$

The optimum value of r was then found to be

$$r = \frac{C_0}{\sqrt{C_E + C_E\,C_S\,\hat{\sigma}_S^2 / \hat{\sigma}_E^2}} \tag{15}$$

Since estimates of C_E and C_S were not available, various ratios of C_E to C_S were assumed and then the values of s and r were computed by formulas (14) and (15) using the known values of $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$ for each character.

Results and Discussion

The analysis of variance

The analysis of variance for the nine post harvest characters of mango showing only the degrees of freedom (DF) and mean square (MS) for treatment, experimental error and sampling error are given in Table 3. In these results the estimates for the mean square error, MSE and the sampling error mean square, MS (SE) may be considered as stable since these were based on sufficient degrees of freedom.

The mean squares for the treatment, MSTr are marked to indicate that they are either, significant *** or not significant (NS) as compared with the mean square error. Out of the nine characters, seven are identified for which the treatment effects were significant. Only two characters, color index at day 0 and 5 did not show the significant effects of treatments.

With respect to the magnitude of the mean error and mean square sampling error, it was noted that in all but one character the values of the former are larger than the latter. This would indicate that the error variance component in such characters are all positive. The only character which showed a negative estimate for the error variance component was pH. However, the test of significance for the experimental error variance component σ_E^2 resulted into only two significant mean square error and those were for color index at day 5 and total soluble solids. In case of the non-significant error mean squares, pooling of the mean square error and mean square sampling error may be in order.

Estimates of variance components

The two variance components, σ_S^2 and σ_E^2 were estimated by formulas (3) and (4) using the values of MSE and MS (SE) given in Table 3. These estimates of variance components, $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$ are given in Table 4, and they are expressed in absolute form or as percentage of their total. For instance, the values of $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$ for color index at 0 are .20 and .0055, respectively, or they are 97% and 3%, respectively, of the total variance component 0.2055. The results of these estimated variance components for the nine characters indicate that the variance component due to sampling units, $\hat{\sigma}_S^2$ were very much larger than the variance component due to experimental unit, $\hat{\sigma}_E^2$ by as much as 5 to 36 times. This would only show that most of the variability in post harvest characters of mango comes from the differences between the sampling units and very little comes from the differences

Table 3. Results of the analysis of variance for the nine postharvest characters of mango

Source Of	*CI(0)*		*CI (5)*		*TSS*		*TA*		*WL*		*F*		*DI*		*PH*		*VQR*	
Variation	*DF*	*MS*	*DF*	*MS*	*DF*	*MS*	*DF*	*MS*	*DF*	*MS*	*DF*	*MS*	*DF*	*MS*	*DF*	*MS*	*DF*	*MS*
Treatment	3	.19^{ns}	3	2.59^{ns}	5	4.5**	5	10.03**	7	.7**	7	.08*	7	2.39**	7	.43**	5	6.23
Experimental error	12	.25^{ns}	12	1.15*	12	.56**	12	.18^{ns}	24	.24^{ns}	24	.03^{ns}	24	.52^{ns}	24	.04^{ns}	18	1.49^{ns}
Sampling error	128	.20	128	.57	162	.18	162	.11	96	.07	96	.02	96	.44	96	.06	96	1.29

CI (0) – Color index at day 0
CI (5) – Color index at day 5
TSS – Total soluble solids
TA – titratable acidity
WL – Weight loss
F – firmness
DI – Disease incidence
VQR – Visual quality rating
DF – degrees of freedom
MS – Mean square error

Table 4. Estimate of experiment mean ($\bar{x}$), coefficient of variation (CV), and variance components for the nine postharvest characters of mango

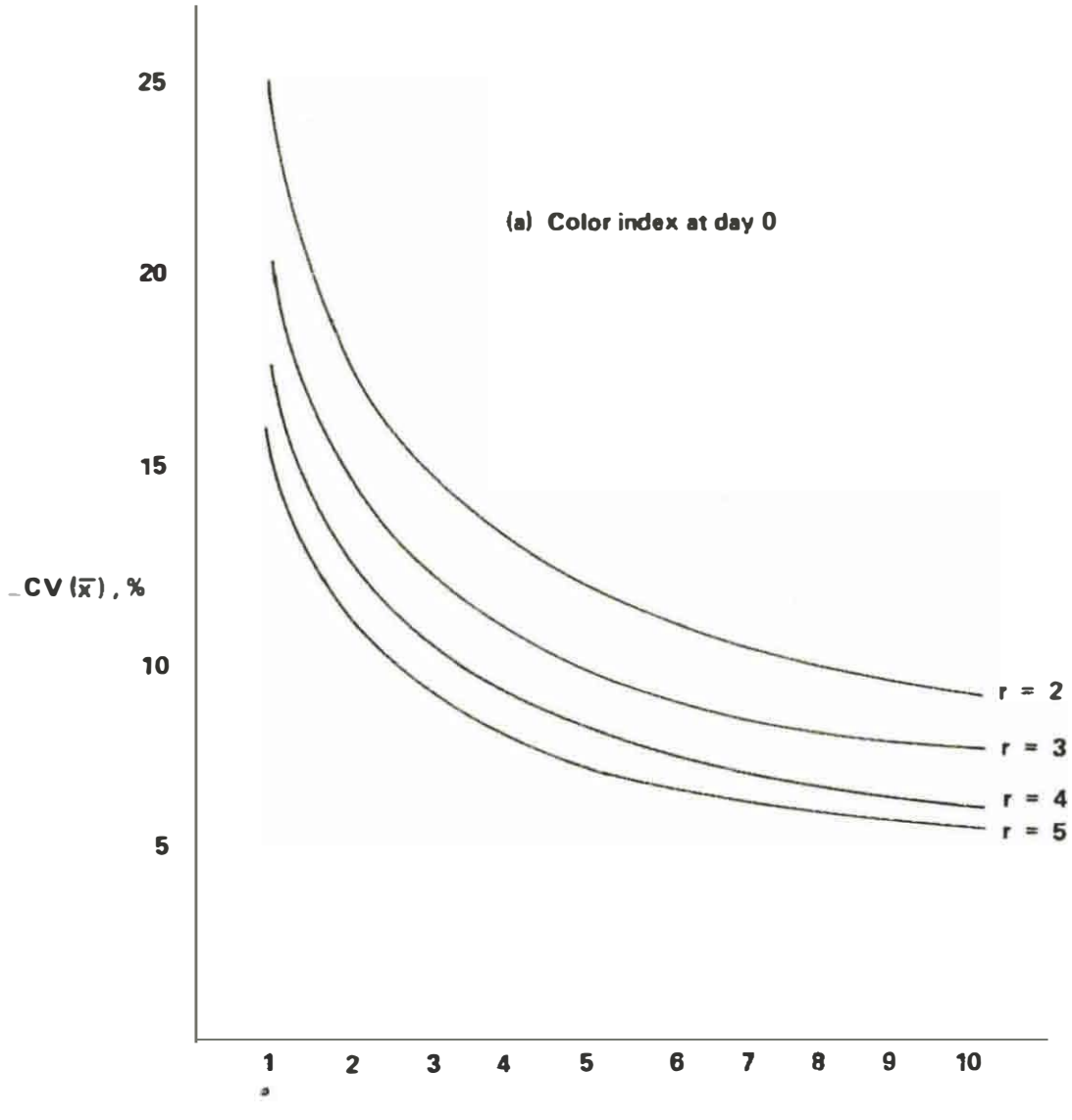
Character	*MEAN* ($\bar{x}$)	*CV (x)* (%)	*Variance components*					
			$\hat{\sigma}^2_S$	%	$\hat{\sigma}^2_E$	%	$\hat{\sigma}^2_x = \hat{\sigma}^2_S + \hat{\sigma}^2_E$	%
Color index at day 0	1.29	38.7	0.20	97	0.0055	3	0.2055	100
Color index at day 5	4.44	24.2	0.57	90	0.064	10	0.634	100
Total soluble solids	6.74	11.1	0.18	83	0.038	17	0.218	100
Titratable acidity	3.33	12.7	0.110	94	0.007	6	0.117	100
% Weight cumulative loss	.99	35.0	0.07	85	0.0125	15	0.825	100
Firmness	.99	17.5	0.02	89	0.0025	11	0.0225	100
Disease incidence	.42	171.1	0.44	96	0.02	4	0.46	100
PH	4.54	4.44	0.66	100	0	0	0.06	100
Visual quality rating	6.25	19.50	1.29	97	0.04	3	1.33	100

between the experimental units. In such cases, the implication is that in those experiments that were conducted, very little control have been given to keep the sampling units more uniform, such as using more uniform fruits with respect to weights or size, etc. The use of other experimental design, such as randomized complete block design with sub-sampling may even bring about a more efficient results.

The precision of a treatment mean as a function of sample size

The precision of a treatment mean may be expressed as a function of the number of replications r and number of sampling units s after having obtained the estimates of $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$. By using equation (11) and the values of $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$ given in Table 4, the values of coefficient of variation of a treatment mean, CV $(\bar{x}i \ldots)$ were computed for values of r ranging from 2 to 5 and s ranging from 1 to 10. The graphs of the CV $(\bar{x}i \ldots)$ values versus r and s were drawn and they are shown in Figs. 1 (a) to 1 (h). The graphs for each character is the function

$$\text{CV}\ (\bar{x}\ \text{i}\ldots) = \sqrt{\frac{\hat{\sigma}_E^2}{r} + \frac{\hat{\sigma}_S^2}{rs}}\ /\ \bar{x}\ \ldots)$$



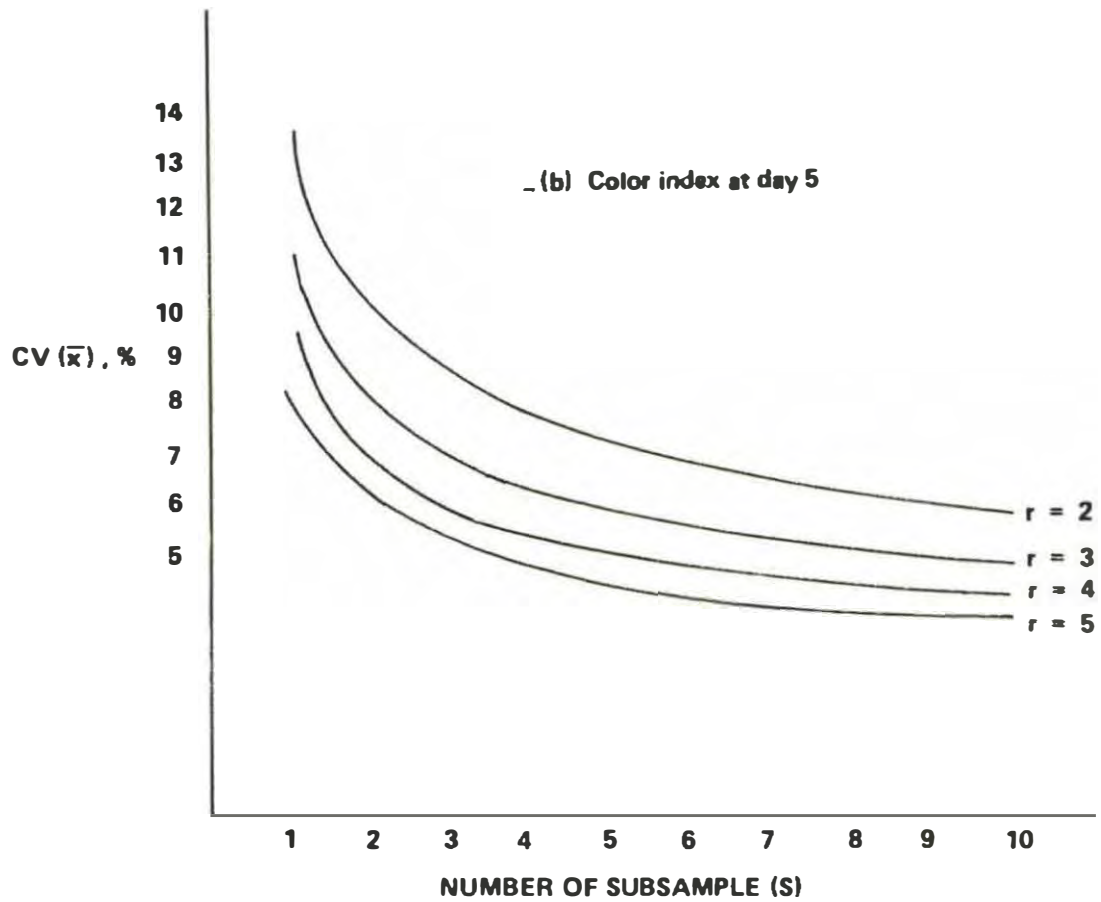
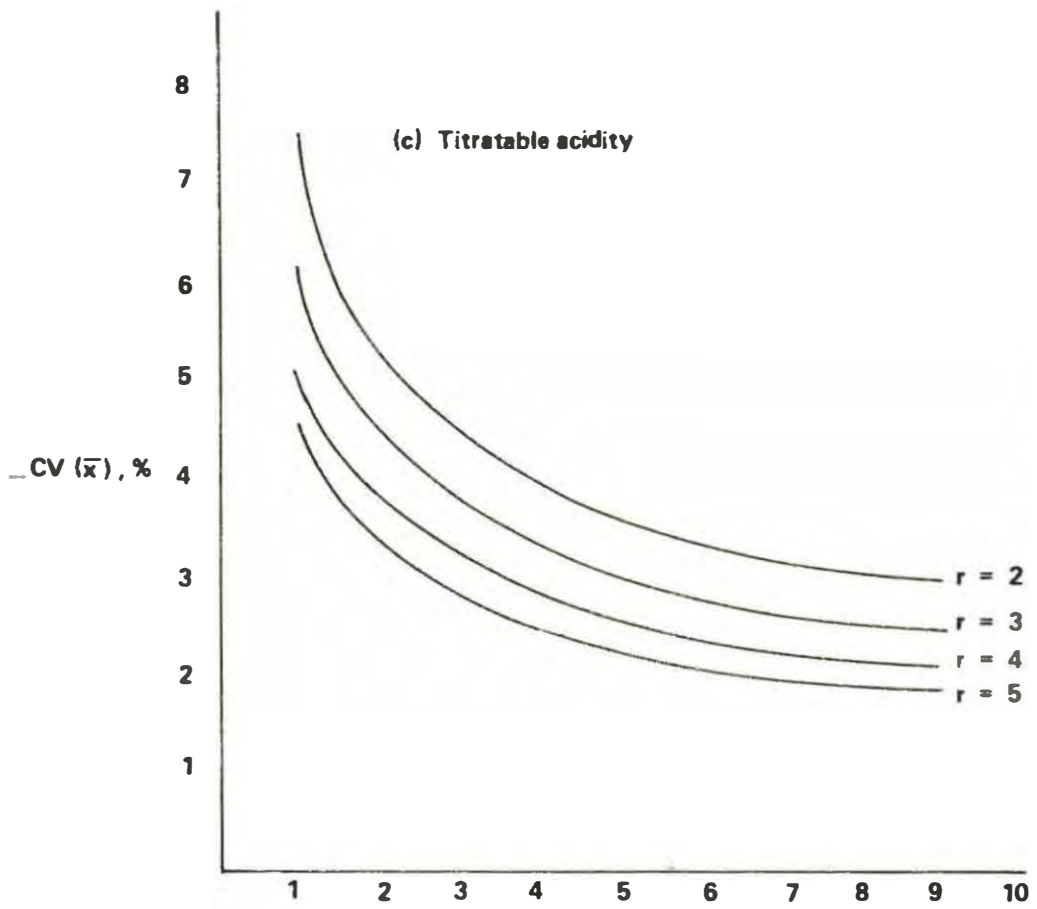

(b) Color index at day 5
CV (x̄), %
14
13
12
11
10
9
8
7
6
5
r = 2
r = 3
r = 4
r = 5
1 2 3 4 5 6 7 8 9 10
NUMBER OF SUBSAMPLE (S)

Figure 1


(c) Titratable acidity
CV (x̄), %
8
7
6
5
4
3
2
1
r = 2
r = 3
r = 4
r = 5
1 2 3 4 5 6 7 8 9 10

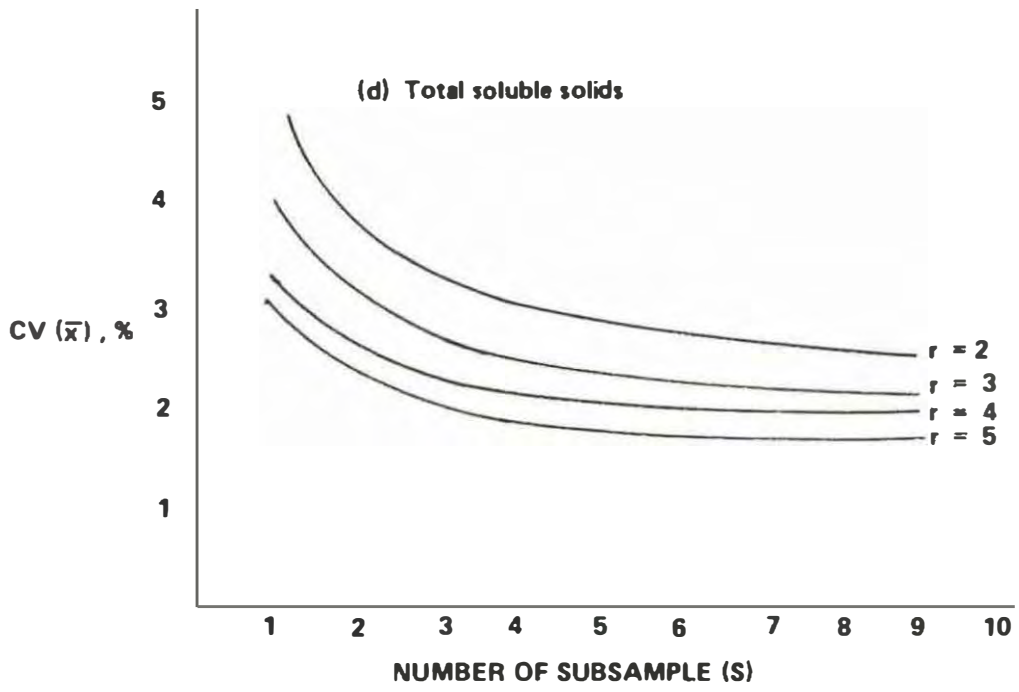
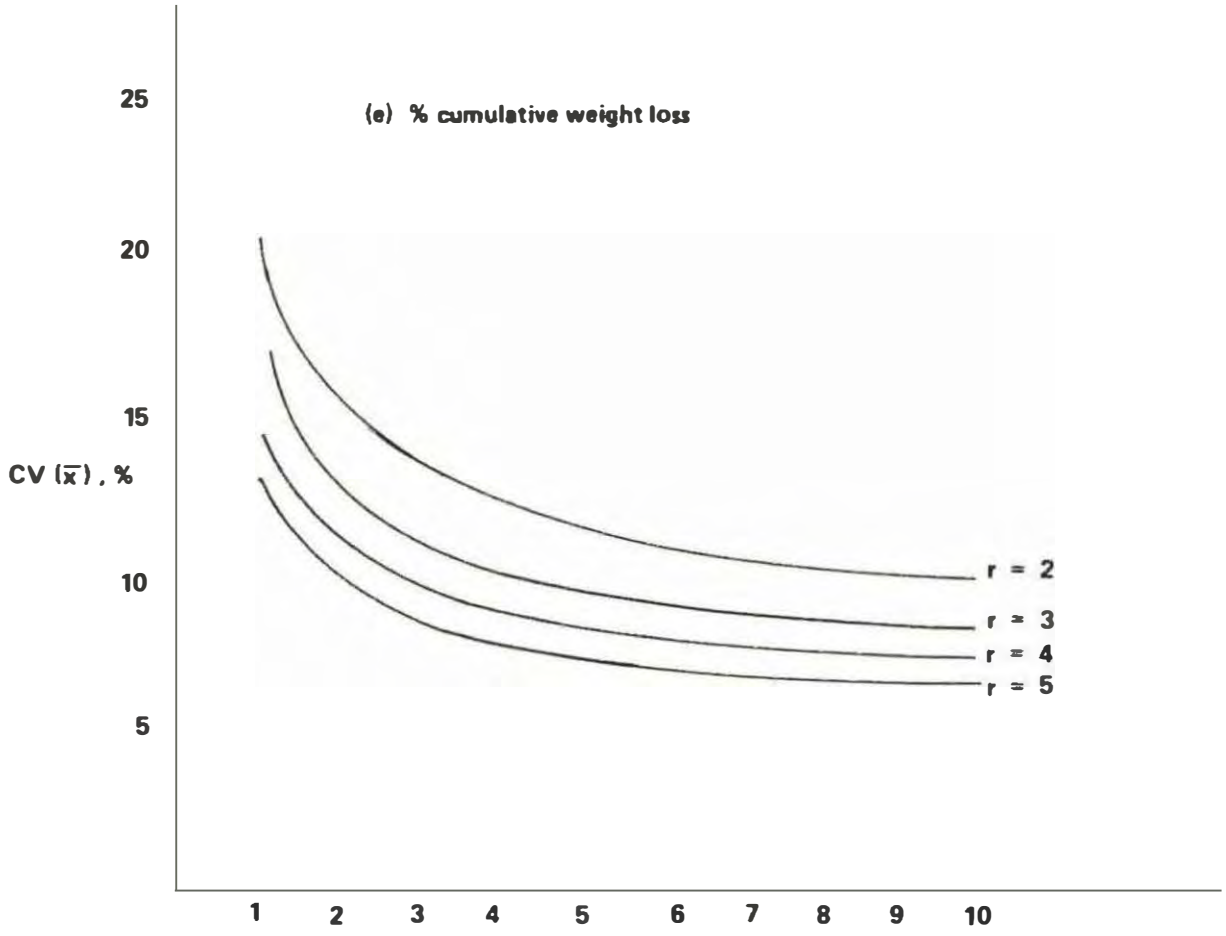

(d) Total soluble solids
CV ($\bar{x}$), %
5
4
3
2
1
r = 2
r = 3
r = 4
r = 5
1 2 3 4 5 6 7 8 9 10
NUMBER OF SUBSAMPLE (S)

Figure 1


(e) % cumulative weight loss
CV ($\bar{x}$), %
25
20
15
10
5
r = 2
r = 3
r = 4
r = 5
1 2 3 4 5 6 7 8 9 10

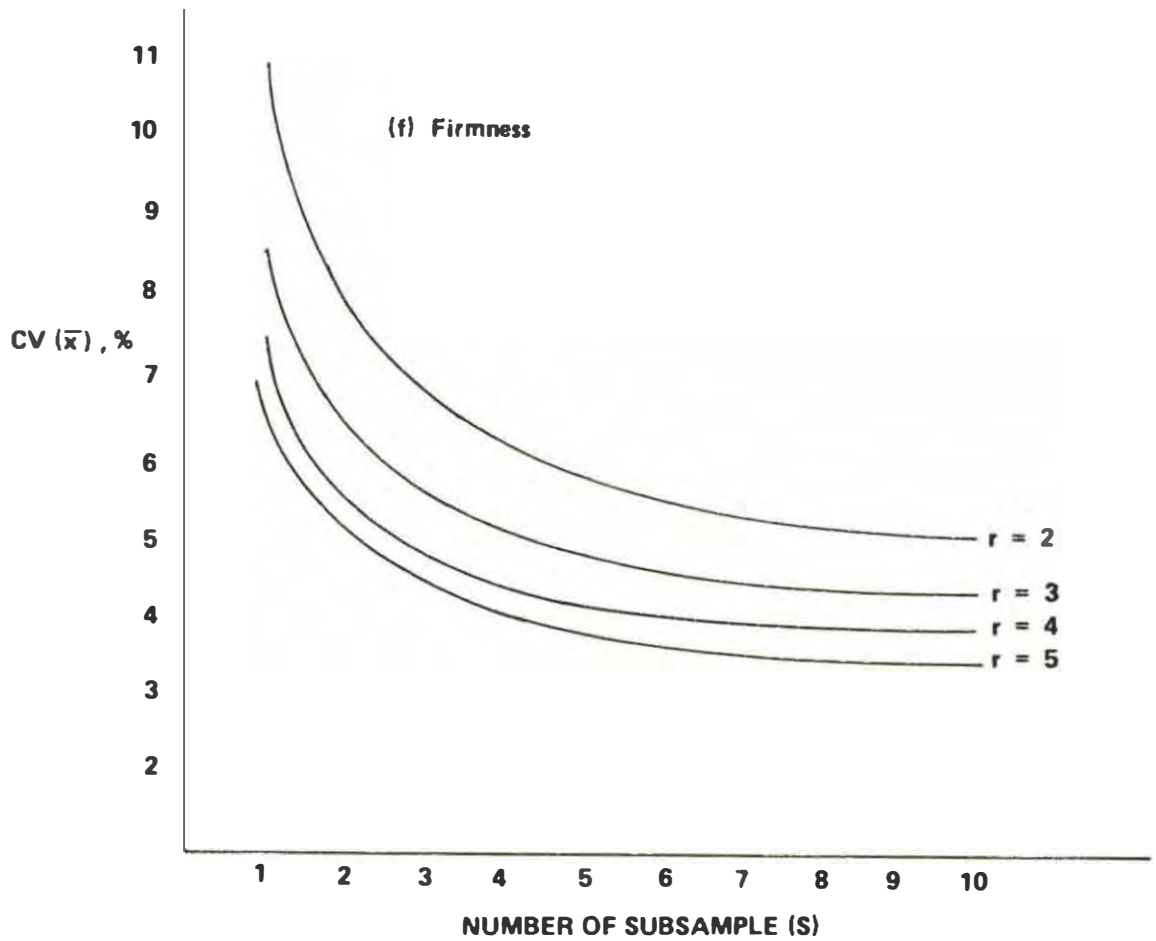
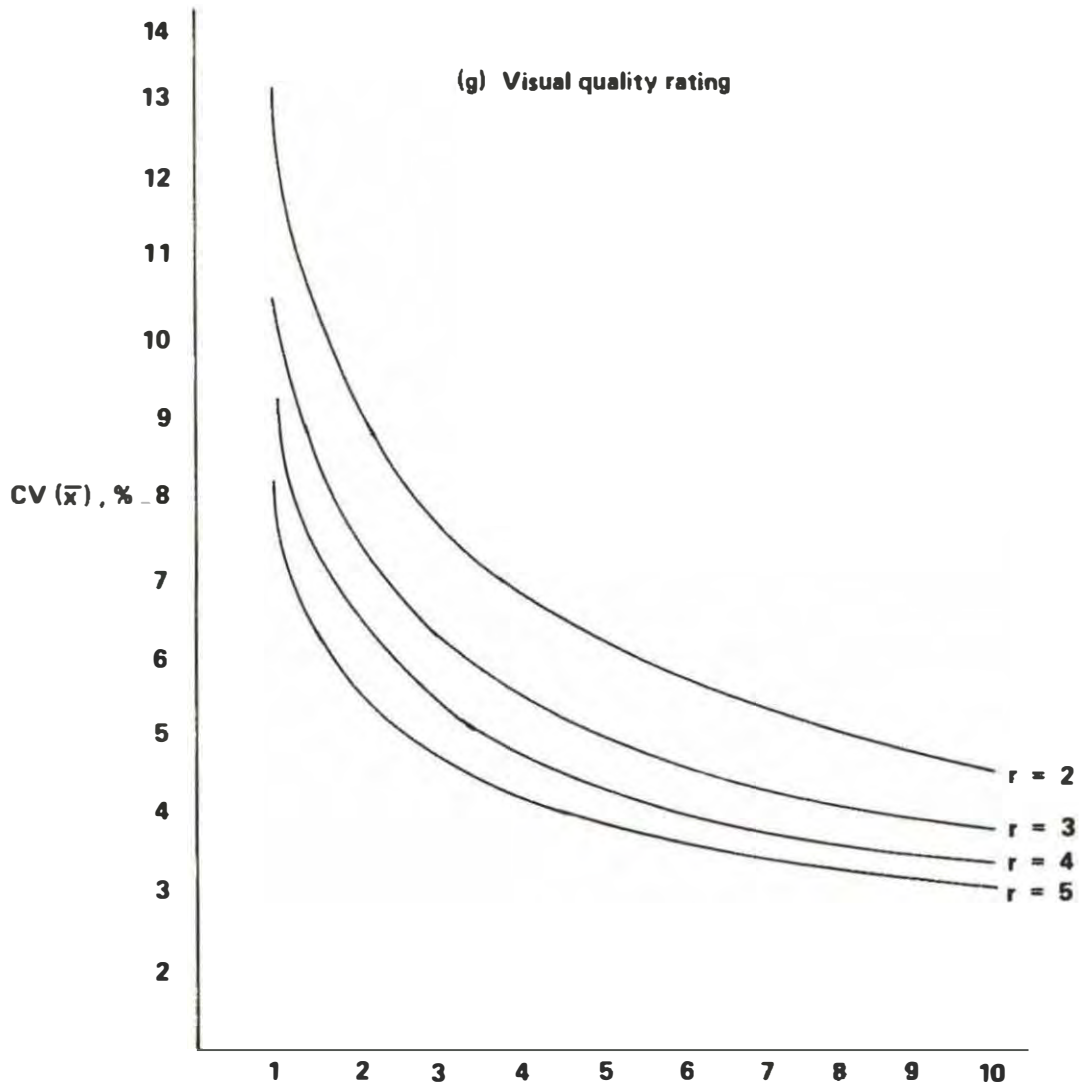

(f) Firmness
CV ($\bar{x}$), %
11
10
9
8
7
6
5
4
3
2
r = 2
r = 3
r = 4
r = 5
1 2 3 4 5 6 7 8 9 10
NUMBER OF SUBSAMPLE (S)

Figure 1


(g) Visual quality rating
CV ($\bar{x}$), %
14
13
12
11
10
9
8
7
6
5
4
3
2
r = 2
r = 3
r = 4
r = 5
1 2 3 4 5 6 7 8 9 10

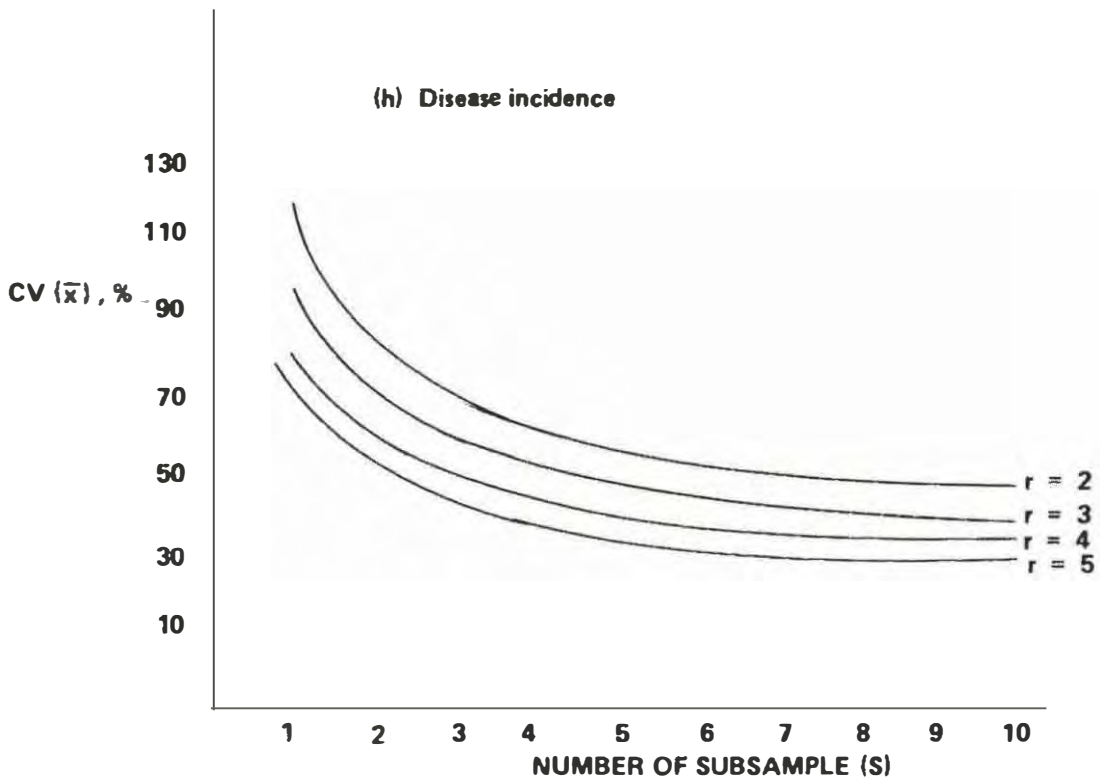


Figure 1

For total soluble solids, for instance, the graph shown in Fig. 1(d) is the function

$$CV\ (\bar{x}i\ldots.) = \sqrt{\frac{.038}{r} + \frac{0.18}{rs}} \qquad /\ (6.74)$$

where $r = 2, 3, 4, 5$ and $s = 1, 2, \ldots\ldots, 10$.

The graph of CV $(\bar{x}i\ \ldots\ldots)$ against the sample size s for a given number of replications r is like a negative exponential with maximum value at ∞ when $s = 0$, and a minimum value at $\sqrt{\frac{\hat{\delta}^2 E}{r}}$ at $s = \infty$. By proper choice of r and s, one can bring down the value of CV $(\bar{x}i\ \ldots)$ to any prescribed percentage, such as 10% or 5%. By using the graph for any character, the desired precision level can be set and then simply locate proper combination of r and s. For example, using Fig. 1 (a), one can make the precision of the treatment mean equal to 10% for choices of (r) (s) as (2) (7), 3(5), (4) (4) and (5) (3). This choices on the average led to a sample size $n = 15$.

The optimum sample size

The formulas for determining the optimum number of subsamples s and optimum number of replication r are given as

$$s = \sqrt{\left(\frac{C_E}{C_S}\right)\left(\frac{^2S}{^2E}\right)}$$

$$r = \frac{C_o}{C_E + \sqrt{C_E C_S \hat{\sigma}_S^2 / \hat{\sigma}_E^2}}$$

If one knows the cost of the experiment per treatment (C_o), the cost per experimental unit (C_E), and the cost per sampling unit (C_S), then the optimum value for s and r can be computed since $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$ are already known. To see the behavior of the estimates of s and r, certain ratio of the cost estimate as C_E C_S were given and then the values of s and r computed for each character. For example, if the ratio is 4:1, say, then

$$C_o = r' (4 + s' (1)$$

where r ' and s ' were the numbers of replications and samples in the actual experiment. Therefore, the value of

$$s = \sqrt{\left(\frac{4}{1}\right)\left(\frac{\hat{\sigma}_S^2}{\hat{\sigma}_E^2}\right)}$$

and $$r = \frac{r' (4 + s')}{4 + \sqrt{(4)(\hat{\sigma}_S^2 / \hat{\sigma}_S^2 / \hat{\sigma}_E^2}}$$

The computed values of s and r for the cost ratios 1:4, 1:2 1:1, 2:1 and 4:1 are given in Table 5. Thus for a cost ratio of 4:1, say, the optimum numbers r, s and n were computed as 3, 11, 33 for color index at day 0; 5, 6, 30 for color index at day 5; 5, 4, 20 for total soluble solids; 4, 7, 28 for titratable acidity; 3, 5, 15 for percent cumulative weight loss, 3, 8, 24 for firmness, 2, 10, 20 for disease incidence; and 2, 11, 22 for visual quality rating. As Kampthorne had pointed out, these numbers maximizes the information on each treatment mean for a given cost per treatment C_o.

Table 5. Optimum numbers of replications (r), subsamples (s) and sample size ($n = rs$) at a given cost ratio (C_E:C_S) for the eight post harvest characters of mango

Character	*SIZE*	*COST RATIO, C_E:C_S*				
		1:4	*1:2*	*1:1*	*2:1*	*4:1*
Color index of day 0	r	12	8	6	4	3
	s	3	4	6	8	11
	n	36	32	36	32	38
Color index at day 5	r	21	15	10	7	5
	s	2	2	3	4	6
	n	42	30	30	28	30
Total soluble solids	r	23	16	10	7	5
	s	1	2	2	3	4
	n	23	32	20	21	20
Titratable acidity	r	14	10	7	5	4
	s	2	3	4	7	7
	n	28	30	28	35	28
% Cumulative weight loss	r	9	6	5	4	3
	s	1	2	2	3	5
	n	9	12	14	12	15
Firmness	r	8	6	4	3	3
	s	1	2	3	4	8
	n	8	12	12	12	24
Disease incidence	r	5	3	3	2	2
	s	2	3	5	7	10
	n	10	9	15	14	20
Visual quality rating	r	6	4	3	2	2
	s	3	5	6	8	11
	n	18	20	18	16	22

Summary and Conclusions

Nine sets of data obtained from the experiments conducted at the Post Harvest Training and Research Center of U.P. Los Baños were analyzed using a completely randomized design with subsampling model for main purpose of obtaining estimates for two variance components that will be used for determining optimum sample size for post harvest experiments on mango. One set of data represent one post harvest character or variable and those characters were color index at day 0, color index at day 5, total soluble solids, titratable acidity, percent cumulative weight loss, firmness, disease incidence, pH and visual quality rating.

The analysis of variance of the characters showed that the mean square error (MSE) were larger than the mean square sampling error (MS (SE)) except for the characters pH. Those results meant that the estimates for the variance component due to the experimental unit or replication, $\hat{\sigma}_E^2$ were all positive. Further tests of significance, however, revealed that only two characters indicated significant estimates of $\hat{\sigma}_E^2$.

From the results of the analysis of variance, the value of MSE and MS (SE) were used to estimate the two variance components, $\hat{\sigma}_E^2$ and $\hat{\sigma}_S^2$ the variance component due to sampling units. The comparison of the two estimated variance components indicated that $\hat{\sigma}_S^2$ represents from about 85 to 97 percent of the experimental error variance among the nine post harvest characters. In terms of ratio, values of $\hat{\sigma}_S^2$ were larger than the values of $\hat{\sigma}_E^2$ by as much as 5 to 36 times.

By expressing the precision of a treatment mean, s.e. $(\bar{x}_i)$ in terms of the coefficient of variation of a treatment mean, $cv\ (\bar{x}_i)$, a function relating the $cv\ (\bar{x}_i)$ with the number of replications r and number of subsamples s was obtained for each character using the estimated values of $\hat{\sigma}_E^2$ and $\hat{\sigma}_S^2$. The graph of each function was then drawn for each character by varying the values of r from 2 to 4 and s from 1 to 10. The graphs showed that for a particular value of r, the values of the $cv(\bar{x}_i)$ decreases exponentially with increasing s and the points of inflection where somewhere between 4 and 6. Thus, if one wishes to obtain the right combination of r and s that will give the desired precision, he would simply refer to the graph of a particular character.

With respect to the determination of optimum sample size, the formulas derived by Kempthorne were used. Various ratios of the cost per experimental unit, C_E to the cost per sampling unit, C_S were used in the formula to get the optimum number of subsamples s and optimum number of replications r for each character. Thus, for any given cost ratio that is within the cost ratios used in this study, one simply refer to the tabular values of r and s to get the optimum sample size $n = rs$.

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A GENERALIZED ASYMPTOTIC THEORY OF MULTIVARIATE L–ESTIMATES*

Roberto N. Padua
Mathematics Department
De La Salle University

ABSTRACT

Statistics which can be expressed as a linear combinations of order statistics, called L–estimates, are considered in this paper. Much of the current theory on this subject deals with the case of univariate and identical parent populations. The present paper considers the general theory in which the parent populations are multivariate which may or may not be identical. The results of the previous authors are then shown as merely special cases of the present investigation in which the dimension is reduced to $p = 1$.

Introduction

The observation that the sample mean is unduly influenced by extreme observations has prompted present-day Statisticians to develop a class of statistics called robust statistics. This new field of Statistics includes the R, M and L estimates. The R estimates are estimates obtained by using the rank scores of the sample values. The M estimates are estimates obtained by minimizing some functions of $X_i - \theta$ where θ is the unknown parameter. On the other hand, L estimates are estimates of the form:

$$(1)\ldots \qquad \hat{\theta} = \sum_{i=1}^{n} C_i X_{(i)}$$

where $X_{(1)} \leq X_{(2)} \leq \ldots \leq X_{(n)}$ are the ordered sample values and the C_i's are weights.

Among the proposed competitors of the sample mean, the L estimates are the easiest to implement computationally. The R estimates may sometimes involved complicated mathematics and their efficiency, in general, is more difficult to assess. On the other hand, no closed forms of the M estimates can be given in general. The determination of the M estimates may, for example, involve the use of Newton-Raphson method.

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Because of the simplicity and mathematical tractability of the L estimates, much has been written on its asymptotic behavior in the univariate setting. Lloyd (1952) has derived an optimum L estimate for a fixed sample size. The asymptotic analysis has been linked with asymptotic normality through several approaches by Chernoff, Gastwirth, and Johns (1967), Stigler (1969, 1972), Shorack (1969, 1972), Boos (1977, 1979) and others. The asymptotic normality is derived under various restrictions on the underlying distribution from which the sample is drawn and the weights – generating function of the linear combination of the order statistics, giving the L–estimate.

The standard asymptotic theory of L–estimates deals with sample values which are *univariate* and has a common distribution. A few papers have been written on the case of variable distribution such as those by Shorack (1973) and Stigler (1974).

In the present paper, we develop a general asymptotic theory of L–estimates in the multivariate setting wherein the parent populations may or may not be identical. All the results of the previous authors will then be seen as special cases of the present investigation when the dimension is reduced to $p = 1$. Of particular interest in the case of the asymptotic distribution of the sample median which was derived by Mood (1941) and Lehmann (1984) and again by Padua (1986) under various setting.

Section 2 develops the asymptotic theory, Section 3 considers some applications and finally Section 4 gives some directions for future research.

Multivariate Distribution

Let $X_1, \ldots, X_n$ be n independent p–dimensional random variables with *cdf* $F_1, \ldots, F_n$, respectively. Let X_{ij} denote the jth component of X_i and $X_{(1j)} \leq \ldots \leq X_{(nj)}$ denote the ordered values of $X_{1j}, \ldots, X_{nj}$. Let $L = (L^*_{1n}, \ldots, L^*_{pn})'$, where

$$L^*_{jn} = \frac{1}{n} \sum_{1}^{n} C_i X_{(ij)}$$

$$C_i = n \int_{\frac{i-1}{n}}^{i/n} J(u)\, du,$$

J is a bounded integrable function on $[0, 1]$. For $y = (y_1, \ldots, y_p)$, let

$$H_{ij}(y) = \begin{cases} 0 \text{ for } y_1 < X_{1j} \\ \\ 1 \text{ for } y_1 \geq X_{ij} \end{cases}$$

First we consider the *i.i.d.* case when $F_1 = \ldots = F_n = F^*$, say. Let F_j^* denote the *cdf* of X_{ij}. We shall assume that

$$(2)\ldots \qquad \int_{-\infty}^{\infty} (F_i^*(x)\,(1-F_i^*(x))\,)\ dx < \infty, j = 1, \ldots, p.$$

Let $Z_i^* = (Z_{i1}^*, \ldots, Z_{ip}^*)'$, $\mu^* = (\mu_1^*, \ldots, \mu_p^*)$, and $\Sigma^* = (\sigma_{jk}^*)$, where

$$Z_{ij}^* = \int_{-\infty}^{\infty} (H_{ij}(X) - F_j^*(x))\ J(F_j^*(X))\ dx$$

$$\mu_j^* = \int_{-\infty}^{\infty} xJ(F_j^*(x))\ d\ F_j^*(x) \qquad \text{and}$$

$$^*jk = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} J(F_j^*(u))\ J(F_k^*(v))\ (\min(F_j^*(u), F_k^*(v)) - F_j^*(u)\,F_k^*(v)\ dudv.$$

The proof given in Padua (1986) for the derivation of the asymptotic distribution of L_n'' in the univariate case goes through for each component of L_n^*. Thus we have the asymptotic representation of $\sqrt{n}\ (L_n^* - \mu^*)$ as

$$(3)\ldots \qquad Z^* = \frac{-1}{\sqrt{n}} \sum_{1}^{n} Z_i^*.$$

From (3) and the multivariate central limit theorem we have

Theorem 1. Let J be bounded and continuous *a.e.* F^{*-1} on $[0, 1]$. If (2) is satisfied and Σ^* is positive definite then $\sqrt{n}\ (L_n^* - \mu^*) \xrightarrow{L} N(0, \Sigma^*)$ as $n \longrightarrow \infty$.

Theorem 2. Let J be bounded and continuous *a.e.* F^{*-1} on $[0, 1]$, $j = 1, \ldots, p$, such that $J(u) = 0$ for $0 < u < \alpha$ and $\beta < u < 1$. If the α and β quantiles of F_j^* are uniquely defined for each j, and Σ^* is positive definite then

$$\sqrt{n}\ (L_n^* - \mu^*) \xrightarrow{L} N(0, \Sigma^*) \text{ as } n \longrightarrow \infty.$$

Next we consider the non-*i.i.d.* case. Let F_{ij} denote the *cdf* of x_{ij} and let

$$\hat{F}_j^*(x) = \frac{1}{n} \sum_{i=1}^{n} F_{ij}(x),\ j = 1, \ldots, p.$$

We shall assume that $F_j^*(x)$ tends to a limiting distribution $F_j^*(x)$ for each x, as $n \longrightarrow \infty$.

*Proposition 1**. There exists a positive number N, such that

$$\sqrt{n} \int_{-\infty}^{\infty} |\hat{F}_j^*(x) - F_j^*(x)|\, dx \leq N,\ j = 1, \ldots, p.$$

for sufficiently large n.

*Proposition 11**. There exists a function $Q\,(0 < Q(x) \leq 1)$ and positive numbers a and b $(0 < b < 1)$, such that $Q^b(x)$ is integrable, and for sufficiently large n, $F_{nj}(x) \leq Q^2(x)$ for $x \leq -a$ and $1 - F_{nj}(x) \leq Q^2(x)$ for $x \geq a$, $j = 1, \ldots, p$.

*Proposition 111**. As $n \longrightarrow \infty$

$$\sqrt{n} \int_{-\infty}^{\infty} (F_j^*(x) - \hat{F}_j^*(x))\, J(F_j^*(x))\, dx \longrightarrow c_j\ ,$$

$$j = 1, \ldots, p.$$

where the c_j are constants, such that $-\infty < c_j < \infty$.

Let $\tilde{Z}_i = (\tilde{Z}_{i1}, \ldots, \tilde{Z}_{ip})'$ and

$\tilde{\Sigma}_1 = (\tilde{\sigma}_{ijk})$, given by

$$\tilde{Z}_{ij} = \int_{-\infty}^{\infty} (H_{ij}(x) - F_{ij}(x))\, J(F_j^*(x))\, dx$$

$$\sigma_{ijk}{}^2 = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} J(F_j^*(u))\, J(F_k^*(v))\, (\min(F_{ij}(u), F_{ik}(v) - F_{ij}(u) F_{ik}(v))\, dudv.$$

The proof given in Padua (1986) for the derivation of the asymptotic distribution of L_n'' in the case of variable distributions, goes through for each component of L_n''. Thus we have the asymptotic representation of $\sqrt{n}\,(L_n^*, \mu^*)$ as

(4) . . .
$$-\frac{1}{\sqrt{n}} \sum_{1}^{n} \tilde{Z}_1 + \tilde{c}$$

where $\tilde{c} = (\tilde{c}_1, \ldots, \tilde{c}_p)'$. We let

$$(5)\ldots \qquad \frac{1}{n}\sum_{1}^{n}(\tilde{\Sigma}_i) \longrightarrow \tilde{\Sigma}, \text{ as } n \longrightarrow \infty$$

where $\tilde{\Sigma}$ is a positive definite matrix. It is easy to see that Rao's condition (see Rao (1973), p. 147) for the application of the multivariate central limit theorem to the sum (4) is satisfied. Thus we have

Theorem 3. Let J be bounded and continuous *a.e.* F_j^{*-1} on $[0, 1]$ and $\hat{F}_j^*(x) \longrightarrow F_j^*(x)$ for each x, as $n \longrightarrow \infty$, $j = 1, \ldots, p$. If Propositions 1*, 11* and 111* are satisfied and (5) holds then $\sqrt{n}\,(L_n^* - \mu^*) \xrightarrow{L} N(\tilde{c}, \tilde{\Sigma})$, as $n \longrightarrow \infty$. The analogue of Theorem 2.5 in Padua (1986) for the multivariate distribution is given as follows. We omit the proof.

Theorem 4. Let J be bounded and continuous *a.e.* F_j^{*-1} on $[0, 1]$, $j = 1, \ldots, p$, such that $J(u) = 0$ for $0 < u < \alpha$ and $\beta < u < 1$. If $\hat{F}_j^*(x) \longrightarrow F_j^*(x)$ for each x, the α and β quantiles of F_j^* are uniquely defined, $j = 1, \ldots, p$, Propositions 1* and 111* are satisfied and (5) holds, then $\sqrt{n}\,(L_n^* - \mu^*) \xrightarrow{L} N(\tilde{c}, \tilde{\Sigma})$, as $n \longrightarrow \infty$.

Component-wise Sample Median: The sample median is a special case of a univariate L–estimate, and is treated separately from the general case (see, for example, Lehmann (1983), Theorem 5.3.2) in the literature, for simplicity. We consider similarly the component-wise sample median which is a special case of a multivariate L–estimate. For simplicity we assume that n is an odd integer. Let $\tilde{x} = (\tilde{x}_1, \ldots, \tilde{x}_p)'$, where $\tilde{x}_j$ denotes the median value of $x_{1j}, \ldots, x_{nj}$, $j = 1, \ldots, p$. Let F_{ij} and f_{ij} denote the *cdf* and *pdf*, respectively, of x_{ij} and for $b = (b_1, \ldots, b_p)'$ let $f_i(b) = (f_{i1}(b_i), \ldots, f_{ip}(b_p))'$, $F_1(b) = (F_{i1}(b_i), \ldots, F_{1p}(b_p))'$ and $F_{ijk}(b_j, b_k) = P\{x_{ij} \leq b_j, x_{ik} \leq b_k\}$.

Let $\Omega_i(b) = (v_{ijk})$, given by $V_{ijk} = F_{ij}(b_j)\,(1 - F_{ij}(b_j))$

$$V_{ijk} = F_{ijk}(b_j, b_k) - F_{ij}(b_j)\,F_{ik}(b_k).$$

Let $W_i = (W_{i1}, \ldots, W_{ip})'$ and $S_n = \sum_{1}^{n} W_i$, where

$$W_{ij} = \begin{cases} 1 & \text{if } x_{ij} > b_j / \sqrt{n} \\ 0 & \text{otherwise, } j = 1, \ldots, p. \end{cases}$$

We have $E(W_{ij}) = 1 - F_{ij}(b_j / \sqrt{n})$ and $\operatorname{cov}(W_i) = \Omega(b / \sqrt{n})$.

Clearly

$$(6)\ldots \qquad \sqrt{n}\ \overline{X} \leq b \Leftrightarrow S_n \leq \frac{n-1}{2}\ \underset{\sim}{e}$$

where $\underset{\sim}{e} = (1, \ldots, 1)'$ and $\leq$ means component-wise inequality,

$$E(n\underset{\sim}{e} - S_n) = \sum_1^n F_i(b/\sqrt{n})$$

$$= \sum_1^n F_i(\underset{\sim}{0}) + \frac{1}{\sqrt{n}}(b^* f_i(\underset{\sim}{0})) + 0(\sqrt{n})$$

where $a^*B = (a_1 b_1, \ldots, a_p b_p)'$, $\underset{\sim}{0}$ denotes a p–component null vector and

$$\operatorname{cov}(S_n) = \sum_1^n \Omega_i\ (b/\sqrt{n}$$

$$= \sum_1^n \Omega_i\ (\underset{\sim}{0}) + 0(n).$$

It is assumed that the x_{ij} have a continuous density at the origin. We make the following additional assumptions: As $n \longrightarrow \infty$

Assumption 1. $\frac{1}{n} \sum_1^n f_i(\underset{\sim}{0}) \longrightarrow f$

where f is a bound length vector with positive components.

Assumption 2. $\frac{1}{n} \sum_1^n \Omega_i\ (\underset{\sim}{0}) \longrightarrow \Omega$

where Ω is a non-null matrix, and

Assumption 3. $\frac{1}{n} (\sum_1^n) F_i\ (\underset{\sim}{0}) - \frac{n}{2}\ \underset{\sim}{e}) \longrightarrow (\underset{\sim}{0})$.

By the multivariate central limit Theorem (see Rao (1973), p. 147)

$$(7)\ldots \qquad \frac{S_n - E\ S_n}{\sqrt{n}} \xrightarrow{L} N(0, \Omega)$$

under Assumption 2. If Assumptions 1 and 3 are satisfied then from (2.25) we have for large n

$$(8)\ldots \qquad P(\sqrt{n}\ \bar{X} \leq b) = P\left(S_n \leq \frac{n-1}{2}\ e\right)$$

$$= P\left[\frac{S_n - E\,S_n}{\sqrt{n}} \leq -\frac{n+1}{2\sqrt{n}}\ \underset{\sim}{\theta} + \frac{1}{\sqrt{n}} \sum_{1}^{n} F_i(0) + \frac{1}{n} \sum_{1}^{n} b = f_i\ (\underset{\sim}{0})\right]$$

$$= P\left[\frac{S_n - E\,S_n}{\sqrt{n}} \leq b = f\right] .$$

Combining (7) and (8), we get

Theorem 5. If the x_{ij} have a continuous density at the origin and Assumptions 1, 2 and 3 are satisfied, then

$$\sqrt{n}\ \bar{X}^* = f \xrightarrow{L} N(0, \Omega), \text{ as } n \longrightarrow \infty .$$

We can rephrase Assumptions 1, 2 and 3 with reference to an arbitrary vector d in place of the null vector 0, and rephrase the given theorem accordingly, in an obvious manner.

L–estimate of regression coefficients. The study of robust estimation is particularly important for the general regression problem. In this regard Huber (1973) has noted that "just a single grossly outlying observation may spoil the least squares estimate, and moreover, outliers are much harder to spot in the regression than in the simple location case." Various types of robust estimates of the regression coefficients of a linear model have been considered in the literature. M–estimates of the regression coefficients have been considered by Anscombe (1967), Huber (1973) and Bickel (1975), among others. R–estimates of the regression coefficients have been considered by Adichie (1967), Jureckova (1971) and Maritz (1979). Some other types of robust estimates for the simple linear regression model have been proposed by Mood (1950), Theil (1950), Sen (1968) and Forsythe (1972). A type of M–estimate for the regression coefficients has been proposed by Koenker and Bassett (1978).

Consider the linear model

$$(9)\ldots \qquad Y = X\theta + \epsilon$$

where Y is an n–dimensional vector of response variables, X is an nxp matrix non-stochastic variables, θ is a p–dimensional vector of the regression coefficients and ϵ is an n–dimensional vector of errors. The components of ϵ are *i.i.d.* random

variables. We partition X into m submatrices, according to the rows of X. Let X_i denote the ith submatrix and let Y_i and ϵ_i denote the associated subvector of Y and ϵ, respectively. We assume that each X_i is of rank p. Let

$$\begin{aligned} \tilde{\theta}_i &= (X_i' X_i)^{-1} X_i Y_i \\ &= (X_i' X_i)^{-1} X_j' \epsilon_i + \theta \end{aligned}$$

denote the least squares estimate of θ, as obtained from the ith partition of (Y, X). Let $\tilde{\theta}_{ij}$ denote the jth component of $\tilde{\theta}_i$ and let $\tilde{\theta}_{(1j)} \leq \ldots \leq \tilde{\theta}_{(mj)}$ denote the ordered values of $\tilde{\theta}_{ij}, \ldots, \tilde{\theta}_{mj}$. For a robust estimate of θ, consider a multivariate L–estimate $\hat{\theta}$ whose jth component is given by

$$(10)\ldots \qquad \hat{\theta}_j = c_1 \tilde{\theta}_{(1j)} + \ldots + c_k \tilde{\theta}_{(mj)}$$

where c_1 are suitable constants. Simple estimates such as those for which $\hat{\theta}_j$ is a trimmed mean or a median value of $\tilde{\theta}_{ij}, \ldots, \tilde{\theta}_{mj}$ are particularly interesting. We considered the latter estimate for which

$$(11)\ldots \qquad \hat{\theta}_j = \text{median}\,(\tilde{\theta}_{1j}, \ldots, \tilde{\theta}_{mj}).$$

In many practical situations it is reasonable to assume that the components of ϵ in (9) are symmetrically distributed about the origin. We shall make this assumption here. Therefore, the $\tilde{\theta}_{ij}$ are symmetrically distributed about the origin, $j = 1, \ldots, p$ and $i = 1, \ldots, m$. Denoting by F_{ij} and f_{ij} the *cdf* and density function of $\tilde{\theta}_{ij}$, we get $F_{ij}(0) = \frac{1}{2}$. It can be generally assumed that the matrix X and the error distribution are such that the assumptions of Theorem 4 are satisfied. It follows that

$$\sqrt{m}\ (\hat{\theta} - \theta)^* f \xrightarrow{L} N(0, \Omega) \text{ as } m \longrightarrow \infty \text{ , where}$$

Ω is a positive definite matrix whose diagonal elements are each equal to $\frac{1}{4}$.

We need to find the values of f and Ω. Suppose that the rows of X are independently distributed according to a given distribution. Then given the common distribution of the components of ϵ, we can empirically determine the values of f and Ω by the Monte Carlo method, for example. To compare $\hat{\theta}$ with the least squares estimates we compare the covariances of the asymptotic distribution of

$$\hat{\theta} \text{ and } \bar{\theta} = \frac{1}{m} \sum_{1}^{n} \tilde{\theta}_i ,$$

where $\tilde{\theta}_i$ is the least squares estimate of θ, associated with the *ith* partition of X. In this regard we note that $\tilde{\theta}_j$ is distributed with mean θ and covariance

$$\sigma^2 (X_j' X_j)^{-1},$$

where σ^2 denotes the common variance of the component of ϵ. If the rows of X are generated from the normal distribution $N(\underset{\sim}{0}, V)$, then $(X_i' X_j)^{-1}$ is distributed according to the inverted Wishart distribution. Therefore, for large m

$$\frac{1}{m} \sum_{1}^{m} (X_{ii}' X_i)^{-1} \xrightarrow{p} \frac{1}{m} \sum_{1}^{m} E(X_i' X_i)^{-1}$$

$$= \frac{1}{m} \left(\sum_{1}^{m} \frac{1}{k_i - p - 1} \right) V^{-1}$$

where k_i denotes the number of rows in the ith partition of X. It is assumed that $k_i \geq p + 2$ for each i. If all the k_i are nearly equal to k, say, then by the multivariate central limit theorem

$$\sqrt{m}\,(\tilde{\theta} - \theta \xrightarrow{L} N\left(\underset{\sim}{0}, \frac{\sigma^2}{k-p-1} V^{-1}\right)$$

as $m \longrightarrow \infty$.

Future Research

Although the theory presented here is comprehensive, there are still some avenues for future research in L–estimation. Some of the more important ones are as follows:

1. Establish bounds for the error in normal approximation. Such bounds may be of the Berry-Esseen type which gives the maximum error that one may incur using the normal approximation.
2. Develop software packages which will incorporate L estimates of location parameters and regression coefficients.
3. In the application to robust regression, a theoretical research on the optimal block sizes is needed. Moreover, a theoretical research is also needed to see the effect of multicollinearity on the proposed regression estimates.

These are but a few of the research directions which may interest an applied statistician or a mathematical statistician.

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STATIONARY PROPERTY OF AN ARBITRARY FERMIONIC FUNCTIONAL AND CHIRAL U(1) ANOMALY IN STOCHASTIC FIELD QUANTIZATION

Danilo M. Yanga
National Institute of Physics
College of Science
University of the Philippines
Diliman, Quezon City 3004

ABSTRACT

Using the analogs of Novikov's Theorem for Grassmann variables, the stationary property of an arbitrary functional of fermionic fields is verified. The unregularized chiral U(1) anomaly is then derived as a subsequent application.

In a previous paper, the chiral U(1) anomaly[1] is derived by invoking the stationary property of the pseudoscalar density $\tau_5 \equiv \bar{\psi}\gamma_5\psi$ within the framework of stochastic field quantization. We now want to verify this stationary property not only of $\tau_5(x)$ but in general, any functional of fermionic fields based on a decomposition in terms of an orthonormal set of functions and an infinite number of Grassmann variables. We start by reviewing the derivation of the generalized Langevin equation for fermionic system.

Within the framework of Itô's differential calculus[2], the Langevin equations for the independent fermionic fields $\psi^\alpha(x, \tau)$ and $\bar{\psi}^\alpha(x, \tau)$ which describe a fermionic system are respectively,[3]

$$d\psi^\alpha(x\tau) = \frac{\delta S}{d\bar{\psi}^\alpha(x\tau)}\, d\tau + d\theta^\alpha(x\tau) \qquad (1a)$$

$$d\bar{\psi}^\alpha(x\tau) = \frac{\delta S}{d\psi^\alpha(x\tau)}\, d\tau + d\bar{\theta}^\alpha(x\tau) \qquad (1b)$$

In the above equations, the fermionic action functional is bilinear in the fields, $\delta/\delta\psi^\alpha$ and $\delta/\delta\bar{\psi}^\alpha$ are left derivatives while the Grassmann Wiener processes satisfy the correlation relations:

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Within the framework of Itô's differential calculus[2], the Langevin equations for the independent fermionic fields $\psi^\alpha(x, \tau)$ and $\bar{\psi}^\alpha(x, \tau)$ which describe a fermionic system are respectively,[3]

$$d\psi^\alpha(x\tau) = \frac{\delta S}{d\bar{\psi}^\alpha(x\tau)}\, d\tau + d\theta^\alpha(x\tau) \qquad (1a)$$

$$d\bar{\psi}^\alpha(x\tau) = \frac{\delta S}{d\psi^\alpha(x\tau)}\, d\tau + d\bar{\theta}^\alpha(x\tau) \qquad (1b)$$

In the above equations, the fermionic action functional is bilinear in the fields, $\delta|\delta\psi^\alpha$ and $\delta|\delta\bar{\psi}^\alpha$ are left derivatives while the Grassmann Wiener processes satisfy the correlation relations:

$$\langle d\theta^{\alpha}(x\tau)\rangle = \langle d\bar{\theta}^{\alpha}(x\tau)\rangle = 0$$
$$\langle d\theta^{\alpha}(x\tau)\, d\bar{\theta}^{\beta}(y\tau)\rangle = \langle d\bar{\theta}^{\beta}(y\tau)\, d\theta^{\alpha}(x\tau)\rangle = 2\delta^{\alpha\beta}\,\delta(x\text{-}y)\,d\tau \tag{2}$$

By doing a series expansion of an arbitrary functional of fermionic fields, $F(\bar{\psi}, \psi)$ and neglecting higher-ordered terms involving $o(d\tau^{3/2})$ we get the generalized Langevin equation:

$$dF(\bar{\psi},\psi) = \left[\frac{-\delta S}{\delta\bar{\psi}^{\alpha}(y\tau)}\,\frac{\delta F}{\delta\psi^{\alpha}(y\tau)} + \frac{\delta S}{\delta\psi^{\alpha}(y\tau)}\,\frac{\delta F}{\delta\bar{\psi}^{\alpha}(y\tau)}\right]d\tau \tag{3}$$
$$+\left[d\theta^{\alpha}(y\tau)\,\frac{\delta F}{\delta\psi^{\alpha}(y\tau)} + d\bar{\theta}^{\alpha}(y\tau)\,\frac{\delta F}{\delta\bar{\psi}^{\alpha}(y\tau)}\right]$$
$$+\; d\theta^{\alpha}(y\tau)\, d\bar{\theta}^{\beta}(z\tau)\,\frac{\delta^2 F}{\delta\bar{\psi}^{\beta}(z\tau)\,\delta\psi^{\alpha}(y\tau)}$$
$$+\;\frac{1}{2}\,d\bar{\theta}^{\alpha}(y\tau)\, d\bar{\theta}^{\beta}(z\tau)\,\frac{\delta^2 F}{\delta\bar{\psi}^{\beta}(z\tau)\, d\bar{\psi}^{\alpha}(y\tau)}$$
$$+\;\frac{1}{2}\,d\theta^{\alpha}(y\tau)\, d\theta^{\beta}(z\tau)\,\frac{\delta^2 F}{\delta\psi^{\beta}(z\tau)\,\delta\psi^{\alpha}(y\tau)}$$

Note that the integration symbol over the spectator variables is suppressed for convenience. Because of the anticommuting nature of the fermionic fields, it is possible to express them in terms of an infinite number of Grassmann variables $\alpha_A(\tau)$ and a complete set of orthonormal

functions $\phi^{\alpha}_{A}(x)$.[4] We have

$$\psi_{\alpha}(x\tau) = \sum_{A=1}^{\infty}\phi_{A\alpha}(x)\alpha_A(\tau);\ \bar{\psi}_{\alpha}(x\tau) = \sum_{A=1}^{\infty}\phi^{*}_{\alpha A}(x)\,\bar{\alpha}_A(\tau) \tag{4}$$

with orthonormality properties:

$$\int d^4x\,\phi^{*}_{\alpha A}(x)\,\phi_{B\beta}(x) = \delta_{\alpha\beta}\,\delta_{AB} \tag{5}$$
$$\sum_{A}\phi^{*}_{\alpha A}(x)\,\phi_{A\beta}(x') = \delta_{\alpha\beta}\,\delta(x\text{-}x')$$

Using these decompositions, the generalized Langevin equation can now be expressed entirely in terms of the Grassmann variables $\alpha_A(\tau)$:

$$dF(\bar{\alpha}, \alpha) = \left[-\frac{\partial S}{\partial \bar{\alpha}_A} \frac{\partial F}{\partial \alpha_A} + \frac{\partial S}{\partial \alpha_A} \frac{\partial F}{\partial \bar{\alpha}_A} \right] d\tau + \left[\frac{\partial F}{\partial \alpha_A} d\theta_A + \frac{\partial F}{\partial \bar{\alpha}_A} d\bar{\theta}_A \right] + d\bar{\theta}_A d\bar{\theta}_B \frac{\partial^2 F}{\partial \bar{\alpha}_B \partial \alpha_A} \tag{6}$$

with

$$\langle d\theta_A(\tau) \rangle = \langle d\bar{\theta}_A(\tau) \rangle = 0 \tag{7}$$

$$\langle d\theta_A(\tau) d\bar{\theta}_B(\tau) \rangle = 2\delta_{AB} d\tau,$$

and

$$S = \sum_{AB} \bar{\alpha}_A K_{AB} \alpha_B \tag{8}$$

$$K_{AB} = \int d^4x \, \phi^*_{\alpha A}(x) K^{\alpha\beta} \phi_{B\beta}(x).$$

Because of the correlation relations (7), Eq. (6) simplifies into

$$\left\langle \frac{dF(\bar{\alpha}, \alpha)}{d\tau} \right\rangle = \left\langle -\frac{\partial S}{\partial \bar{\alpha}_A} \frac{\partial F}{\partial \alpha_A} + \frac{\partial S}{\partial \alpha_A} \frac{\partial F}{\partial \bar{\alpha}_A} \right\rangle + 2 \frac{\partial^2 F}{\partial \bar{\alpha}_A} \tag{9}$$

Furthermore substituting (8) into (9) yields

$$\left\langle \frac{dF(\bar{\alpha}, \alpha)}{d\tau} \right\rangle = \left\langle -\sum_{AB} K_{AB} \alpha_B \frac{\partial F}{\partial \alpha_A} - \sum_{AB} \bar{\alpha}_B K_{BA} \frac{\partial F}{\partial \bar{\alpha}_A} \right\rangle + 2 \frac{\partial^2 F}{\partial \bar{\alpha}_A \partial \alpha_A} \tag{10}$$

The verification of the stationary property of $F(\bar{\alpha}, \alpha)$ starts by observing what the first two terms of the right member of Eq. (11) can be evaluated by using the analogs of Novikov's theorem for Grassmann variables,

$$\sum_D K_{AD} \langle \alpha_D F \rangle = \left\langle \frac{\partial F}{\partial \bar{\alpha}_A} \right\rangle$$

$$\left\langle \sum_A \bar{\alpha}_A F \right\rangle K_{AD} = -\left\langle \frac{+\partial F}{\partial \alpha_D} \right\rangle \tag{11}$$

Direct application of these relations yields

$$\left\langle \sum_{AD} K_{AB}\alpha_B \frac{\partial F}{\partial \alpha_A} \right\rangle = \sum_A \left[\sum_B K_{AB} \left\langle \alpha_B \frac{\partial F}{\partial \alpha_A} \right\rangle \right]$$

$$= \sum_A \left\langle \frac{\partial}{\partial \bar{\alpha}_A} \left(\frac{\partial F}{\partial \alpha_A} \right) \right\rangle = \sum_A \frac{\partial^2 F}{\partial \bar{\alpha}_A \partial \alpha_A} \tag{12}$$

$$-\left\langle \sum_{AB} \bar{\alpha}_B K_{BA} \frac{\partial F}{\partial \bar{\alpha}_A} \right\rangle = -\sum_A \left[\sum_B \left\langle \bar{\alpha}_B \frac{\partial F}{\partial \bar{\alpha}_A} \right\rangle K_{BA} \right]$$

$$= + \sum_A \left\langle \frac{\partial^2 F}{\partial \alpha_A \partial \bar{\alpha}_A} \right\rangle = -\sum_A \frac{\partial^2 F}{\partial \bar{\alpha}_A \partial \alpha_A} \tag{13}$$

Substituting (12) and (13) into (10) we get

$$\left\langle \frac{dF(\bar{\alpha},\alpha)}{d\tau} \right\rangle = 0. \tag{14}$$

This is the stationary property of $F(\bar{\alpha},\alpha)$ with respect to the fictitious time τ. Actually this property is to be expected since Novikov's Theorem applies to expectation values where

$$\langle \cdot\cdot \rangle = \int \prod_A d\bar{\alpha}_A d\alpha_A \, (\cdot\cdot) \exp\left[-\sum_{BC} \bar{\alpha}_B K_{BC} \alpha_C \right] \tag{15}$$

and $\exp\left[-\sum_{BC} \bar{\alpha}_B K_{BC} \alpha_C \right]$ is the equilibrium value of the action functional. However, whether at equilibrium or not, this proof holds because based on the decompositions (4) the stochastic property of the fermionic fields have been taken over by the Grassmann variables $\alpha_A(\tau)$.

As an application of what we have just developed suppose

$$F(\bar{\alpha},\alpha) = \sum_{CD\alpha\beta} S^{\alpha\beta}_{CD}(x) \bar{\alpha}_C \gamma_5^{\alpha\beta} \alpha_D, \tag{16}$$

where $S^{\alpha\beta}_{CD}(x)$ is some functions of $\phi(x)$. (9) takes the form

$$\left\langle \frac{dF(\bar{\alpha},\alpha)}{d\tau} \right\rangle = \left\langle \sum_{ABC\alpha\beta} \left[K_{AB}\alpha_B S^{\alpha\beta}_{CA} \alpha_C \gamma_5 - \bar{\alpha}_B K_{BA} S^{\alpha\beta}_{AC} \gamma_5^{\alpha\beta} \alpha_C \right] \right\rangle$$

$$- 2 \sum_{A\alpha\beta} S^{\alpha\beta}_{AA} \gamma_5^{\alpha\beta}. \tag{17}$$

Again applying Novikov's Theorem, we find

$$\left\langle \sum_{ABC\alpha\beta} K_{AB}\,\alpha_B\, S^{\alpha\beta}_{CA}\,\bar{\alpha}_C\,\gamma_5^{\alpha\beta} \right\rangle = -\left\langle \sum_{ABC\alpha\beta} \bar{\alpha}_B\, K_{BA}\, S^{\alpha\beta}_{AC}\,\gamma_5^{\alpha\beta}\,\alpha_C \right\rangle$$

$$= \sum_{\alpha\beta A} S^{\alpha\beta}_{AA}\,\gamma_5^{\alpha\beta} \tag{18}$$

Substituting (18) into (17) yields $\left\langle \frac{dF(\bar{\alpha},\alpha)}{d\tau} \right\rangle - 0$, or in effect

$$\left\langle \sum_{ABC\alpha\beta} \left[K_{AB}\,\alpha_B\, S^{\alpha\beta}_{CA}\,\bar{\alpha}_C\,\gamma_5^{\alpha\beta} - \bar{\alpha}_B\, K_{BA}\, S^{\alpha\beta}_{AC}\,\gamma_5^{\alpha\beta}\,\alpha_C \right] \right\rangle$$

$$= 2 \sum_{A\alpha\beta} S^{\alpha\beta}_{AA}\,\gamma_5^{\alpha\beta} \tag{19}$$

Now with

$$\begin{aligned} K_{AB} &= \int d^4x\, \phi^*_{\alpha A}(x)\, K^{\alpha\beta}\, \phi_{B\beta}(x) \\ K_{\alpha\beta} &= (-i\not{D} + m)_{\alpha\beta}\,;\; D_\mu = \partial_\mu + A_\mu\,, \end{aligned} \tag{20}$$

and the choice

$$S^{\alpha\beta}_{AB}(x) \equiv \phi^{*\alpha}_A(x)\;\phi^\beta_B(x) \tag{21}$$

(D_μ is the covariant derivative while A_μ the electromagnetic potential) we identify the RHS of (19) to be proportional to the unregularized chiral U(1) anomaly,[5]

$$\text{Anomaly Term} \quad \alpha\; 2 \sum_{A\alpha\beta} \phi^*_{\alpha A}(x)\,\gamma_5^{\alpha\beta}\,\phi_{A\beta}(x) \tag{22}$$

This agrees very well with previous results. It is a direct consequence of the stationary property of the generalized fermionic functional.

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OXYTETRACYCLINE PRODUCTION IN COCONUT WATER

Patrocinio Sevilla-Santos, A. M. Lozano,
L. Santiago, F. Mendoza, and D. Rosario
National Institute of Science and Technology
Pedro Gil, Manila, Philippines

ABSTRACT

Oxytetracycline (OTC) was produced on a very simple novel medium of coconut water. Buffering and corn steep liquor addition increased the antibiotic content to 145 μg/ml OTC after 5 days of fermentation. The producer organism is a local streptomyces isolate NIST S-70-24B. The fermentation brew did not exhibit vitamin B_{12} activity (*E. coli* factor). The antibiotic was extracted from the brew and identified as oxytetracycline by comparing its physical and chemical properties with the three tetracyclines.

A parallel study on chlortetracycline (CTC) production by *Streptomyces aureofaciens* NRRL 2209 gave a CTC yield of 0.09 μg/ml after 72 hours of fermentation and a vitamin B_{12} activity of 0.14 μg/ml.

Introduction

Oxytetracycline or commonly called Terramycin* is an antibiotic derived from *Streptomyces rimosus*. It belongs to the family of tetracycline antibiotics (compound consisting of 4 connecting rings with various group substitutions).

Terramycin is primarily bacteriostatic although high concentrations can be bactericidal. It is active against a wide range of bacterial infections caused by Gram positive and Gram negative organisms, certain viral and protozoan organisms in both human and animals. It has also found application in the fortification of animal feeds, in crop protection and food preservation.

The tetracyclines are generally produced by fermentation processes but details of media composition for industrial production are usually well-guarded secrets. However from a review one can conclude that the productive organisms assimilate lactose and glucose as carbon sources, soybean oil meal, distillers soluble, modified milk and other animal proteins as well as corn steep liquor, as sources of nitrogen, growth factors and mineral salts. Most of these culture media ingredients

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[1]Consultant, Research Center for the Natural Sciences, University of Santo Tomas, España, Manila.

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are not locally available, and coconut water a natural substance might be a good substitute because of its well balanced chemical components including the presence of vitamins, amino acids, mineral salts and growth factors. By using a local strain of Streptomyces S-70-24B, a proven Terramycin producer, instead of *S. rimosus,* (a foreign isolate), as the productive strain, this study was done to find whether coconut water can be used for oxytetracycline production. The present paper describes the results obtained in shake flasks fermentation experiments using coconut water for the production of oxytetracycline.

Materials and Methods

Microorganisms

A. Antibiotic producers. (1) NIST Isolate S-70-24B a streptomyces species obtained from a soil sample from Pampanga after dilution and tetracycline addition. This streptomycete is closely related or similar to *S. rimosus* (Lat *et al*; *in press*). It is comparable to oxytetracycline producing streptomycetes namely *S. armillatus, S. platensis, S. sayamaensis* and *S. rimosus* in its cultural, physiological and biochemical properties and morphological characteristics. (2) *Streptomyces aureofaciens* NRRL-2209 a lyophilized culture obtained from the Northern Regional Research Laboratories in Peoria, Illinois, U.S.A.

B. Test organisms used for antibiotic activity and assay: *Micrococcus pyogenes* var. *aureus* (ATCC 6538-P), *Bacillus subtilis* (ATCC 6633), *B. cereus* var. *mycoides* (ATCC 9634), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Alcaligenes faecalis,* Salmonella gallinarum,* Sarcina lutea* (ATCC 9341), *M. flavus,* Candida albicans,* Saccharomyces cereviseae** and *Fusarium moniliforme.**

C. Test organisms for determining vitamin B_{12} (*E. coli*) activity. *E. coli* M-500 an *E. coli* mutant.

Strain selection

The organisms, S-70-24B and *S. aureofaciens* 2209 were plated out on agar medium (Emerson agar) and incubated at room temperature. When colonies were fully developed, certain areas of the plates were cautiously flooded with a 24 hour broth culture of *E. coli.* The colony producing the biggest inhibition zone was selected as a high antibiotic producer.

Culture media

Coconut water was obtained from mature nuts. Before it was used, it was subjected to the following physico-chemical treatments: 1. base precipitation, 2.

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*Percentage of total amino acids.
*Local strain.

oxidative treatment with perchloric acid, 3. potassium permanganate addition, 4. charcoal adsorption, 5. ion exchange and 6. buffering with 0.2 M monobasic and 0.2 M dibasic sodium phosphate solution (Table 1). For the preparation of media for fermentation, other ingredients were dissolved in the treated coconut water or distilled water. Dilute NaOH or HCl solutions were used to adjust the pH if needed. Other constituents added consisted of corn steep liquor, copra meal, bijon (rice noodles) effluent, spent brewers grain, at a concentration of 3 percent and urea 1%. (Sevilla-Santos, 1984)

Table 1. Preparation of phosphate buffered coconut water (Colowick and Kaplan 1955)

Solution A (ml)	*Solution B (ml)*	*Resulting pH values*	
		Initial	*Before inoculation**
92.0	8.0	5.5	5.3
87.7	12.3	5.7	5.4
81.5	18.5	5.9	5.6
73.5	26.5	6.15	5.9
62.5	37.5	6.4	6.2
51.0	49.0	6.6	6.2
37.0	61.0		
28.0	72.0		

Soln A – 0.2M sol'n of monobasic sodium phosphate (27.8 g in 1000 ml coconut H_2O)
Soln B – 0.2M sol'n of dibasic sodium phosphate (53.65 g of $Na_2HPO_4.7H_2O$ in 1000 ml coconut water)
Add A and B and dilute to a total of 200 ml

Inoculum preparation

Two types of inoculum were tried. Inoculum A consists of an aqueous spores and mycelial suspension prepared by suspending a 7-day old agar slant culture of the producer organism in about 5 ml of sterile distilled water. Inoculum B is a preformed inoculum which takes a longer time to prepare. The 7-day old culture was first inoculated unto the seed medium (Emerson broth) and incubated by shaking for 24 to 48 hours at 30°C. A 5% to 10% of the preformed inoculum was added aseptically to the fermentation media.

Shake flask fermentation

For antibiotic production a 5% inoculum B (preformed) was added to 500 ml flask containing 100 ml of the specified culture media. Incubation was carried out at room temperature (± 30°C) in reciprocating shaker with a 1½ inch displacement at 120 strokes per minute or in Lab-line Orbit Environ shaker at 120 revolution per minute.

When inoculum A was used, the entire suspended contents of one agar slant was poured aseptically into a shake flask.

Determination of antimicrobial activity

The antimicrobial spectrum of sampled brew for S-70-24B and *S. aureofaciens* shaken cultures was determined by the agar dilution method using both agar cups and stainless steel cylinders. Standard curves were prepared from dilutions of pure oxytetracyclines and chlortetracycline powders (Grove and Randall, 1955).

Determination of vitamin B_{12} (E. coli) activity

This was determined in the sampled brew using the modified method of Harrison *et al.* 1951 and *E. coli* mutant as the test organism.

Isolation of terramycin from S-70-24B fermentation broth

There are two methods of recovering the antibiotic from the fermentation brew. One is by adsorption, the other by extraction. The procedure of Stoudt (1963) which is the second method was followed (Fig. 2) in isolating terramycin from the fermentation brew using phosphate buffered coconut water as the fermentation medium. That of Sobin *et al.* (1950) (Fig. 3) was used in isolating terramycin from the fermentation brew produced on soybean meal 2.5%, cornsteep liquor 9%, glycerin 1% and sodium chloride 0.25% with 1% calcium carbonate.

Results and Observations

Selection of S-70-24B as the producer organism

Seven isolates namely S-70-24B, OTC (1), OTC 1 (4), OTC 1 (7), OTC 1 (10), OTC 1 (13) and OTC 111 (1) from the NIST Antibiotic Section were revived. The choice was made from among the isolates which gave inhibition zones of 30.0 ± 2mm *B. cereus* var. *mycoides* plates (1982 assay). Among the isolates subcultured in Emerson agar two failed to grow and two were fast-growing, one of the latter was S-70-24B. The remaining three grew only after five days of incubation at room temperature.

Fermentation studies by shake flask in Emerson broth shows that S-70-24B was the best isolate for antibiotic production as shown in Table 2.

Thus, S-70-24B was chosen as the organism for oxytetracycline production.

Strain selection

To obtain a high-yielding strain for oxytetracycline fermentation, the two colonies which produced big zones of inhibition on the agar plate were picked out and inoculated to liquid media and incubated on a shaker. Isolate 1 and Isolate 3

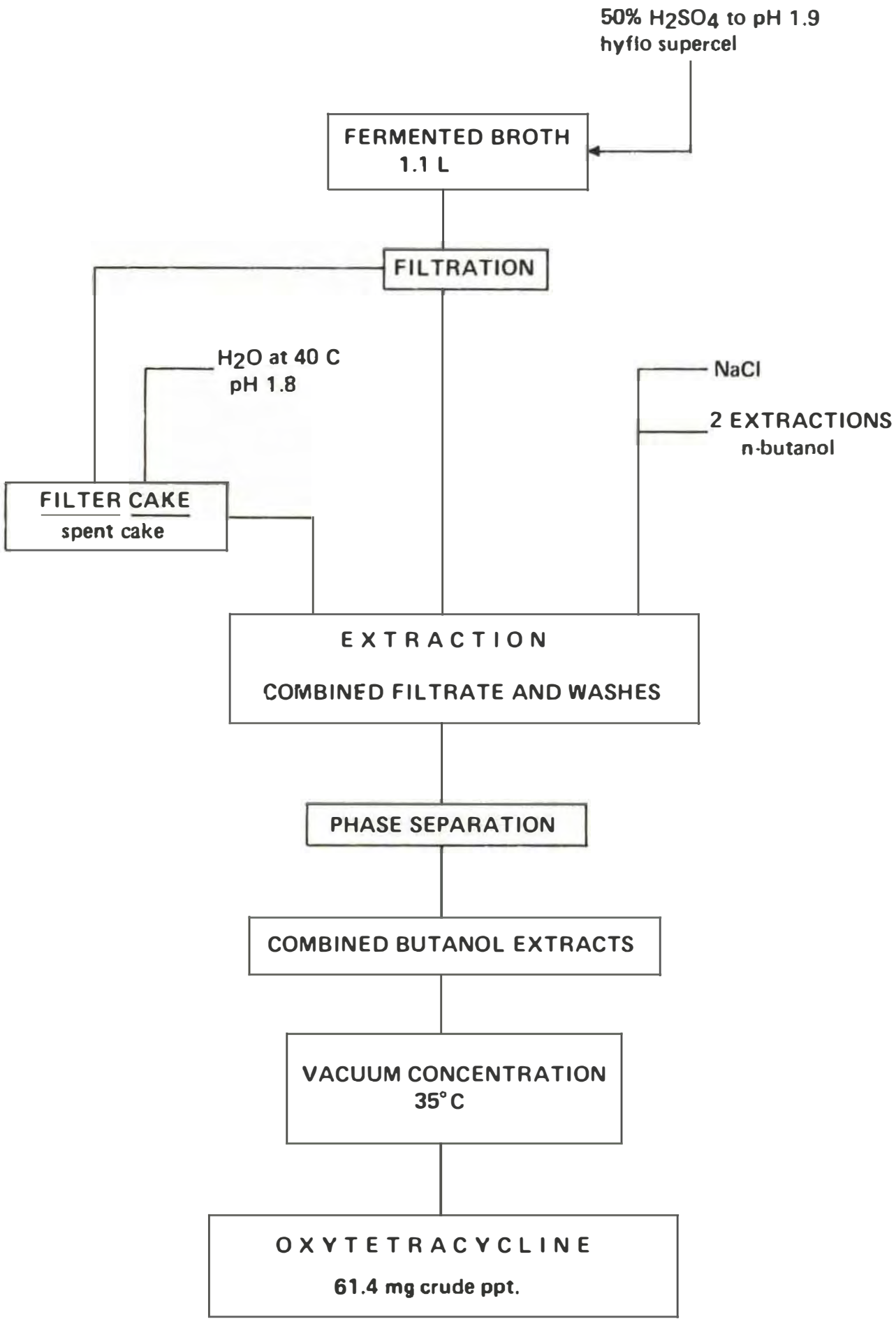

Fig. 2. Recovery of oxytetracycline, Stoud *et al.*, 1963.

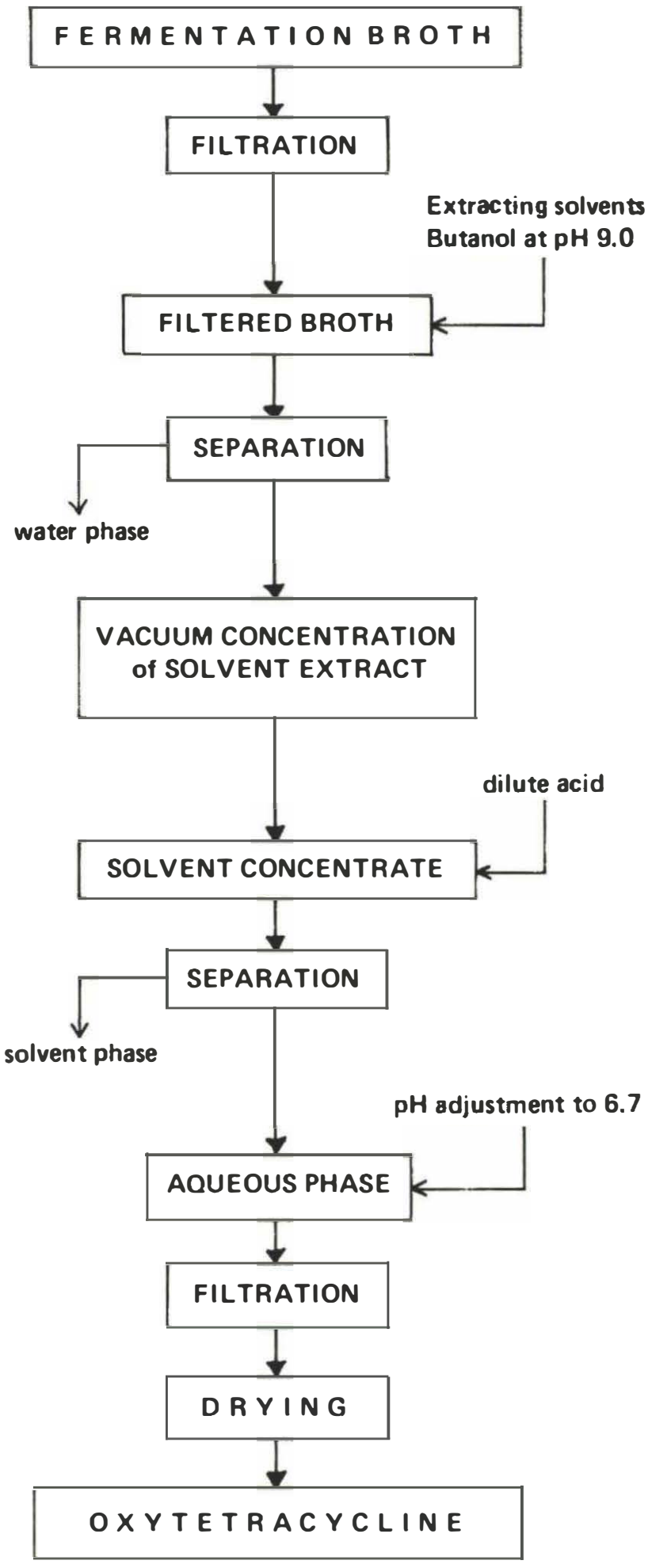

Fig 3. Oxytetracycline extraction process, Sobin *et al.*, 1950.

Table 2. Antibiotic activity of oxytetracycline-producing *Streptomyces* culture in Emerson broth

Streptomyces isolate	*Zones of inhibition (mm)*	
	Trial 1	*Trial 2*
S-70-24B	30.2	23.0
OTC 1 (1)	25.2	21.3
OTC 1 (4)	23.2	17.2
OTC 1 (13)	23.4	21.9
OTC 1 (10)	12.3	10.8

produced 29.0 and 28.0 μg/ml of oxytetracycline while the parent stock culture gave only 23.4 μg/ml. Isolate 1 was selected as the better antibiotic producer.

Shake-flask fermentation of S-70-24B in coconut water

As the fermentation media for shake-flask cultures (untreated) coconut water was used. A week-old brew was tested against six test microorganisms . It was shown that the antibiotic substance produced had a broad spectrum of activity as shown in Table 3.

Table 3. Antibiotic spectra of S-70-24B grown by fermentation in untreated coconut water

Test organisms	*Zone of inhibition* (mm)*
Bacillus cereus var. *mycoides*	28.1
Bacillus subtilis	28.4
Sarcina lutea	24.1
Escherichia coli	19.0
Candida albicans	19.4
Fusarium moniliforme	nil

*Average of three flasks

Shake-flask fermentation in base-treated coconut water

Coconut water after partial and complete base precipitation was compared with untreated coconut water as a culture medium for oxytetracycline production. The results from partial base precipitation does not differ so much from the untreated coconut water in terms of inhibition zones. After complete base precipitation, the medium was found unsuitable for antibiotic production. Perhaps the constituents of coconut water which enhance the production of the antibiotic

substances may have been removed by complete precipitation. The antibiotic activity of the partial and complete base precipitation is shown in Table 4.

Table 4. Antibiotic activity of S-70-24B growth on treated and untreated coconut water

Coconut water treatment	*Zone of inhibition* (mm)* *B. cereus* var. *mycoides* *Trial 1*	*Trial 2*
Untreated	15.6	28.1
Base precipitation (partial)	13.5	27.6
Base precipitation (complete)	8.0	10.5

*Average of 3 flasks

Shake-flask fermentation of S-70-24B in buffered coconut water

Coconut water which was buffered by addition of monobasic and dibasic sodium phosphate to pH values ranging from 5.3 to 6.4 was tried as fermentation medium. The inoculum used was one agar slant culture per 50 ml medium in 250 ml flask. Incubation was on a shaker. Table 5 shows the results of sampling on the 1st, 2nd and 3rd day of incubation.

Table 5. Antibiotic activity of S-70-24B grown on buffered coconut water

pH values *Before inoculating*	*before autoclaving*	*Zone of inhibition* (mm)* *B. cereus* var. *mycoides* *Day 1 (1:3)*	*2 (1:9)*	*3 (1:9)*
5.3	5.5	21:0 (9.6)	19.4 (18)	23.3 (54)
5.4	5.6-5.7	20.4 (8.1)	19.0 (17)	23.9 (63.0)
5.6	5.8-5.9	21.9 (12.0)	19.5 (18)	24.22 (68.4)
5.9	6.0-6.1	20.3 (7.8)	18.0 (12.6)	21.92 (36.0)
6.2	6.2-6.3	8.7 (0.4)	17.4 (10.8)	20.77 (26.1)
6.4	6.4-6.6		16.3 (8.1)	20.4 (24.3)

*Average of 3 trials
Figures in parenthesis represents antibiotic content in μg/ml.

Shake-flask fermentation of S-70-24B antibiotic

For antibiotic production, S-70-24B prefers a wide range of initial pH from 5.5 to 5.9. The highest antibiotic activity of 70 μg/ml was obtained on the third day of fermentation. Fig. 4 presents the data from shake flask fermentation, show-

ing antibiotic production, pH and fermentation time using buffered coconut water with various pH values. An initial pH ranging from 5.5 to 5.9 gave the same trend of the antibiotic production of 70 μg/ml in 3 days time.

Shake-flask fermentation in nitrogen supplemented coconut water

Since coconut water has only about 0.5% protein, an inorganic nitrogen in the form of $(NH_4)_2HPO_4$ was added to the coconut water medium. The result of the second trial which gave the same trend as in the first experiment is given in Table 6. Submerged fermentation in shake flasks containing 0.1% $(NH_4)_2HPO_4$ in coconut water gave the best inhibition of 24.8 mm equivalent to 20 μg/ml of oxytetracycline after 120 hours of fermentation. This is higher than that obtained in using plain coconut water as the fermentation medium.

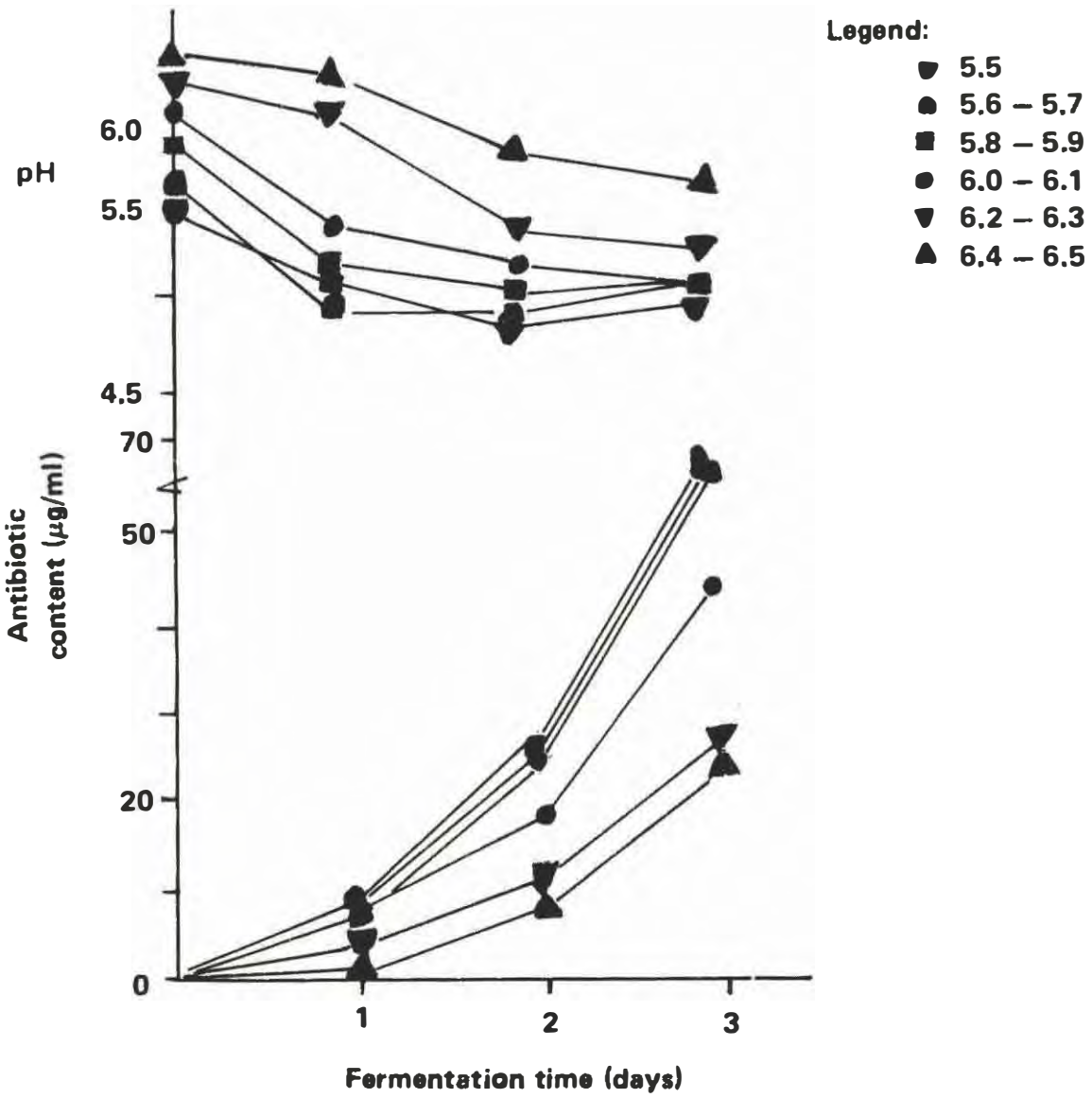

Fig. 4. Antibiotic content and pH of S-70-24B fermentation brew using different buffered coconut water.

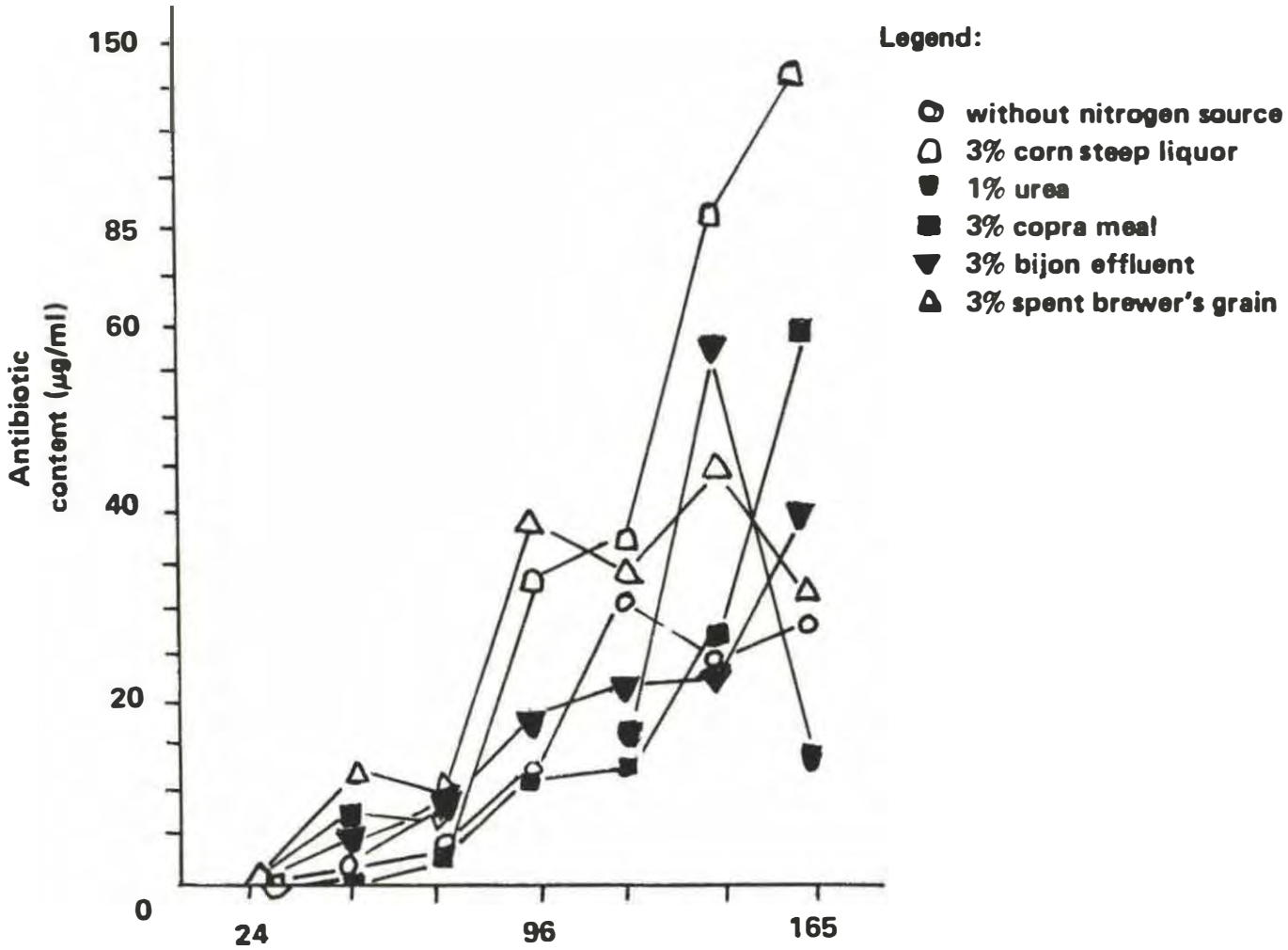

Fig. 5. Antibiotic content of S-70-24B brew using nitrogen supplemented buffered coconut water.

Table 6. Submerged fermentation of oxytetracycline production by S-70-24B utilizing nitrogen (inorganic) supplemented coconut water

Length of fermentation (hours)	*Inhibition zones* (mm)* Medium A	Medium B	Medium C	Medium D	Medium E
48	23.3 (20)	22.1 (16)	22.0 (16)	14.2 (3.5)	18.2 (75)
72	23.5 (22)	22.4 (18)	22.5 (19)	16.3 (5.3)	17.3 (64)
100	22.3 (17)	24.3 (27)	23.2 (20)	13.3 (2.6)	12.7 (23)
120	23.3 (20)	24.1 (24)	24.4 (26)	12.5 (23)	9.6 (1.8)

*Average of 3 trials

(Figures in parenthesis represents µg/ml)

Composition of fermentation media: Media A – coconut water (pH 5.1), Media B – coconut water (pH 7.2), Media C – coconut water + 0.1% $(NH_4)_2HPO_4$ (pH 5.7) Media D – coconut water + 0.1% $(NH_4)_2HPO_4$ (pH 7.2) and Media E – M-13 pH adj. to 7.2.

Composition of M-13 g/100 ml base-treated coconut water: corn steep liquor 4, sucrose 3, $CaCO_3$ 0.65, $(NH_4)_2SO_4$ 0.2, $MnCl_2$ 0.00033, $CuSO_4$ 0.00033 and $ZnSO_4$ 0.005.

Shake-flask fermentation of S-70-24B in buffered coconut water supplemented with organic nitrogen source

By supplementing the phosphate buffered coconut water with different nitrogenous substances, namely 3% corn steep liquor (45% solids), 1% urea, 3% copra meal, 2% spent brewers grain, 3% bijon effluent, it was observed (Fig. 5) that corn steep liquor at a concentration of 3% gave the highest antibiotic content of 145 μg/ml in 120 hours. At the start, the antibiotic production was nil. It was only on the 48th hour that the production began to rise and by 72nd, 96th and 120th hours, it increased rapidly. Antibiotic production was monitored by microbiological assay. Results are given in Table 7.

Table 7. Oxytetracycline production by S-70-24B in nitrogen (organic) supplemented buffered coconut water

Length of fermentation (Hours)	*Oxytetracycline content (μg/ml) Buffered Coconut Water Medium*					
	A	*B*	*C*	*D*	*E*	*F*
24	2.04	trace	5.85	trace	4.20	11.50
36	9.30	3.15	7.50	3.30	7.80	11.50
48	12.60	31.80	10.80	13.50	18.00	37.80
72	20.70	36.30	12.60	22.50	21.60	32.40
96	24.50	86.40	56.70	25.40	23.50	44.60
120	29.70	145.80	18.90	35.64	39.70	40.50

Medium
A – Buffered coconut water (BCW)
B – BCW with 3% cornsteep liquor (45% solids)
C – BCW with 1% urea
D – BCW with 3% copra meal
E – BCW with 3% bijon effluent
F – BCW with 2% Spent Brewer's grain

Isolation and identification of product

From 1,100 ml of pooled active brew, which was subjected to an isolation procedure for oxytetracycline see Fig. 4, 61.4 mg of antibiotic was isolated. The yield was small.

Chemical characterization

The isolated substance is a brownish yellow amorphous substance with a melting point of 213°C (microscope method). The compound is most soluble in water, ethanol, methanol and butanol; moderately soluble in acetone, ethyl acetate,

carbon tetrachloride and toluene; almost insoluble in chloroform, benzene, ether and petroleum ether.

By Rast method, the molecular weight was found to be 461.

Quantitative elementary analysis* gave the following composition: C–57.31%; H–5.28%; N–6.08% and O–30.85% and a molecular formula of $C_{22}H_{24}N_2O_9$ was obtained.

The physicochemical properties are summarized in Table 8.

Table 8. Physicochemical properties of S-70-24B antibiotic

M.W.	461
Molecular formula	$C_{22}H_{24}N_2O_9$
M. pt.	213°C
Solubility	
very soluble	water, methanol, ethanol, butanol
moderately soluble	acetone, toluene, ethyl acetate, carbon tetrachloride
poorly soluble	benzene, chloroform, ether, petroleum, ether
Specific rotation	−126° (MeOH)
Elementary analysis	C-57.31%, H-5.28%, N-6.08%, O-30.85%
Physical appearance	brownish yellow amorphous substance

Identification of antibiotic produced by S-70-24B as oxytetracycline was based on the comparison of its physical and chemical properties and its thin layer chromatographic results with the authentic samples of oxy-, chloro- and tetracycline.

The results of the test on the crude precipitate of S-70-24B and the three tetracyclines is given in Table 9 and shows that color reactions of S-70-24B are very similar to those of oxytetracycline. (Sevilla-Santos 1985) .

A comparison of the physical and chemical properties of the crude antibiotic with authentic samples of chlortetracycline, oxytetracycline and tetracycline is given in Table 10.

Table 9. Tests for identification of antibiotic produced by S-70-24B

Reagent	*Reaction*	*Antibiotic Powder Sample*			
		Chlortetra-cycline	*Tetra-cycline*	*Oxytetra-cycline*	*S-70-24B crude*
H_2SO_4 (conc.)	color	navy blue	dark violet	cherry red	old rose
2N NaOH	color	light yellow green	dark yellow green	dark yellow green	light yellow
not heated	UV	dark blue	none	green	light blue
heated 30 min.	color	pink orange	light yellow	yellow	light yellow
	UV	bright blue	light blue	light blue	bright blue
HCl (conc.)					
not heated	UV	none	none	yellow	yellow
heated	UV	none	none	yellow (greenish)	yellow

*UV fluorescence

As seen from the comparative data above, the antibiotic of S-70-24B is very similar, almost identical with oxytetracycline.

Thin layer chromatography

Table 11. shows the results of thin layer chromatography of the isolated compound S-70-24B and the authentic tetracyclines on silica gel G plates with a solvent system of organic solvent layer of Butanol-tartaric acid-water (100:6:100).

Antimicrobial spectrum

The antimicrobial spectrum of S-70-24B when tested against Gram positive and Gram negative bacteria, yeast and fungi by the agar dilution method is presented in Table 12. S-70-24B antibiotic exhibits good activity against the Gram positive bacteria with minimal inhibitory concentration of 0.1 μg/ml, and MIC against the Gram negative bacteria ranging from 1 to 10. In general, no activity was observed against *P. aeruginosa*, yeast and fungi.

Production of chlortetracycline by NRRL 2209

For the production of chlortetracycline by a known chlortetracycline producer, NRRL 2209, several fermentation experiments were conducted using as culture media or as diluent coconut water, or treated coconut water or buffered

Table 10. Physical and chemical properties of antibiotic S-70-24B and three tetracylines (Lat *et al., in press.*)

	Chlortetracycline	*Oxytetracycline*	*Tetracycline*	*Antibiotic S-70-24B*
a. Form	Golden yellow crystals	Yellow platelets	Yellow powder	Brownish yellow powder
b. Melting point	240°C (decomposed)	213-216°C	224-226°C	213°C
c. Specific rotation	−275.0 (MeOH)	−126.5 (MeOH)	−239 (MeOH)	−126 (MeOH)
d. Solubility	Soluble in water, slightly soluble in methanol, ethanol, butanol, acetone, ethyl acetate & benzene, insoluble in ether & petroleum ether.	Soluble in water, ethanol & methanol, slightly soluble in 2-propanol & toluene.	Soluble in water, slightly soluble methanol & ethanol, insoluble in ether.	Soluble in water, butanol, ethanol & methanol, slightly soluble in acetone, carbon tetracychloride, insoluble in benzene, chloroform, ether & petroleum ether.
e. Elementary analysis	C – 55.17% H – 4.84% N – 5.85% O – 26.73%	C – 57.39% H – 5.25% N – 6.08% O – 31.27%	C – 59.45% H – 5.44% N – 6.30% O – 28.8 %	C – 57.31% H – 5.28% N – 6.08% O – 30.85%
f. Molecular weight	478.88	460.44	444.43	461

Table 11. Mobility of tetracyclines and crude antibiotic S-70-24B

Antibiotic compound	*Spot*	*hRf values*
Chlortetracycline (CTC)	1	44
Oxytetracycline (OTC)	1	50
Tetracycline (TC)	1	32
Mixture of CTC, OTC, TC	3	44
		52
		31
Crude antibiotic S-70-24B		
Batch 1	2	49, 37
Batch 2	1	40
Batch 3	1	53
Batch 4	1	53
Batch 5	1	53

TLC plates: Silica gel G
Solvent system: Butanol-tartaric acid-H_2O (100:6:100)

Table 12. Antimicrobial spectrum of antibiotic S-70-24B

Test organisms	*Minimal inhibitory concentration (μg/ml)*
Micrococcus pyogenes var. *aureus*	0.1
Bacillus subtilis	0.1
B. cereus var. *mycoides*	0.1
Sarcina lutea	0.1
M. flavus	0.1
Alkaligenes faccalis	1.0
Escherichia coli	1.0
Salmonella gallinarum	10.0
Pseudomonas aeruginosa	> 100.0
Saccharomyces cerevisiae	> 100.0
Candida albicans	> 100.0
Fusarium moniliforme	> 100.0

coconut water. In some trials, protein sources locally available such as corn steep liquor, urea, fermented mother liquor, were added as protein supplement.

The use of coconut water as the sole fermentation media gave negative antibiotic activities. Buffered coconut water gave an antibiotic content of 0.09 μg/ml and 0.14 μg/ml of vitamin B_{12} activity for NRRL 2209 Isolate no. 1; and 0.02 μg/ml antibiotic with 0.08 μg/ml vitamin B_{12} activity for Isolate no. 2. The above results are very small and not practical.

With distilled water as diluent of a starch-corn steep liquor (CSL) medium, the antibiotic activity obtained was 0.56 μg/ml with a vitamin B_{12} activity of 0.14 μg/ml. Using buffered coconut water instead of distilled water as the diluent, no activity was observed.

Details of the study will be published later.

Discussion

It has been shown that S-70-27C, a local streptomyces isolate (both the parent and the mutant strain) could use 10% coconut water in the fermentation medium for tetracycline production (Joson *et al.* 1983). In the present study addition of 10% and 50% coconut water to the medium seem to inhibit the biosynthesis of the antibiotic. Certain substances present in the coconut water might have exerted some inhibitory action. To remove or inactivate the inhibitory factor, precipitation by addition of a base, mild and drastic oxidation, adsorption and ion-exchange treatment were tried. Only the base precipitation method gave favorable result.

To stabilize the pH during fermentation $CaCO_3$ was added. Phosphate buffer was also used to attain and maintain the desired pH values. For chlortetracycline production by NRRL 2209, coconut water as the sole culture medium gave negative results while for oxytetracycline production by S-70-24B, there was antibiotic production but with low activity. Buffering with phosphate (mono and di-basic) gave good results both for chlortetracycline and oxytetracycline production; the antibiotic produced by NRRL 2209 was very small in quantity while S-70-24B gave better results.

The results obtained in the supplementation of buffered coconut water with corn steep liquor confirmed previous findings (Evans, 1983). For S-70-24B, the antibiotic activity of the brew went up to about 145 μg/ml, a good and promising result.

For vitamin B_{12} production however, distilled water diluent seems to be better than buffered coconut water.

In the use of the seed or inoculum, both the spore suspension inoculum and the preformed inoculum could be used with the same end result. The only difference is that in the preformed inoculum using buffered coconut water the same amount of antibiotic was produced 24 hours earlier than the spore-suspension inoculum.

Summary and Conclusions

A local Streptomyces NIST S-70-24B (closely similar to *S. rimosus*) produced oxytetracycline 70 μg/ml in buffered coconut water with an initial pH of 5.5 to 5.9 on the third day of shake-flasks fermentation.

Addition of ammonium phosphate, or sodium phosphate and/or corn steep liquor increased the oxytetracycline titer to 145 μg/ml in a 5-day fermentation period.

Oxytetracycline was recovered from the fermentation brew and the yield was 61.4 mg of crude precipitate for every liter of the brew. It was identified as oxytetracycline by comparison of its properties with those of the three tetracyclines.

The antimicrobial spectrum of the product shows more activity against the Gram positive test bacteria than against the Gram negative ones.

Coconut water has been found to be a promising substrate for future development in a larger scale of oxytetracycline production but is not a very appropriate culture medium for chlortetracycline production.

Acknowledgment

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SOLID FUELS FROM THE COCONUT

Julian A. Banzon
Emeritus Professor
University of the Philippines Los Baños
College, Laguna, Philippines

ABSTRACT

Coconut shell and husk contribute 5.9 percent of Philippine energy needs, which is greater than that from locally mined petroleum oil (2.9%) or local coal (4.7%). Investments on nuclear and fossil fuels are huge in comparison with that on coconut. Coco shell, husk and leaf petiole from existing 400 million palms has the potential to supply up to 20 percent of the energy needs. It is suggested that these solid fuels be used for rural electrification.

Introduction

More or less accepted but not yet adopted because of cost, is the use of coconut oil as motor fuel. Large scale utilization in this manner would relieve the pressure of competition in the international market, by providing an alternate outlet. But costs must be reduced to be competitive with petroleum fuels; this may be attained by utilization of the hitherto largely unused products from the coconut palm.

The present study indicates that coconut shell, husk and leaf petiole, which are already being used in a limited scale commercially as solid fuels, have a potential of supplying a significant percentage of Philippine energy needs, if full advantage is taken of their presence.

The shortage of energy and effect on industry. While the shortage of energy is everybody's problem, it appears particularly acute in the Philippines. Contemporary newspaper reports attest to this.

Philippine energy consumption (1985): sources of energy and percentage contribution. This is shown in the following tabulation (1):

Imported oil		50.7%	47.2 M bbl
Conventional sources	Local Petro oil	2.9%	2.7 M bbl
	Local coal	4.7	4.38 M bbl
	Hydro	10.2	9.5 M bbl
	Geothermal	9.1	8.5 M bbl
		26.9%	25.1 M bbl

Non-conventional	Bagasse	4.94%	4.6 M bbl
	c-shell/husk	5.92	5.52 M bbl
	Rice hull	1.00	0.93 M bbl
	Wood/wood waste	5.17	4.82 M bbl
	Dendro	1.17	1.09 M bbl
		18.2%	16.96 M bbl

The above table shows that coconut shell/husk contributes 5.92% of national energy needs, which is a larger contribution than locally mined petro oil or coal. With the huge investments on petroleum exploration and development and the total loss of the Bataan nuclear plant, coconut shell/husk appears to be a better business deal.

Position of coconut shell/husk in the list of nonconventional energy sources. The contribution of coconut shell/husk is larger than that of bagasse or even that of wood/waste of the lumber industry. The coconut has characteristics that make it a more dependable energy supplier than sugarcane or timber trees or even ipil-ipil (dendrothermal).

Local petroleum oil production compared to present utilization of coconut shell/husk. Philippine petroleum oil fields are reported to have the following production (1985):

NIDO	0.381 M bbl (barrels)
MATINLOC	1.378 M bbl
CADLAO	1.23 M bbl

NIDO is said to be almost dry now. Inevitably, the other fields would also reach the same fate. The coconut is a better longtime supplier of energy. The sum of production of these 3 wells is 2.99 M bbl. Present usage of coconut shell/husk is 5.9 M bbl.

Energy potential of Philippine coconut fuels. It would be highly informative to know the energy potential of Philippine coconut solid fuels. There are at present, over 400 million coconut palms. The annual harvest is 10 to 16 billion nuts inspite of typhoons, droughts, pests and neglect. Assuming a yearly crop of 12 billion nuts and 16 x 400 million pieces of leaf petiole (palapa), we can calculate the energy potential. The following information are also needed (2):

	W/pc	MJ/kg
shell	0.193 kg	23.0
husk	0.242 kg	16.7
palapa	0.50 kg	16.7

yearly leaf production averages 16 (6)

bbl oil equivalent = 8,370 MJ/bbl

KWH equivalent = 3.6 MJ/KWH

Calculations for coconut shell:

Weight = 0.193 x 12 B = 2.316 B Kg

$$MJ = 2.316\ B\ Kg \times 23\ MJ/kg = 53.27\ B\ MJ$$

$$bbl = \frac{53.27\ B\ MJ}{8.370\ MJ\ bbl} = 6.36\ M\ bbl$$

$$KWH = \frac{53.27\ B\ MJ}{3.6\ MJ/KHW} = 14.80\ B\ KWH$$

where B = billion = 10^9

M = million = 10^6

The calculations for the total Philippine coconut production are summarized in the following table:

	Kg (B)	MJ (B)	bbl (M)	KWH (B)
shell	2.316	53.27	6.36	14.80
husk	2.904	48.61	5.81	13.50
palapa	3.200	53.57	6.40	14.88
TOTAL	8.42	155.45	18.57	43.18

Calculated worth of coconut fuels. In places where they are traded for cash, palapa sells for at least ₱0.10 per piece to as high as ₱0.25. Shell and husk are approximately ₱0.05 and ₱0.025, respectively. At these prices, the value of these fuels would be as follows:

shell	12 B	pieces x ₱0.05	= ₱0.6 B
husk	12 B	pieces x ₱0.025	= ₱0.3 B
palapa	400 M	pieces x 16 x ₱0.10	= ₱0.64 B
	Total		₱1.54 B

Calculated worth per barrel equivalent:

shell	₱0.6 B/6.36 M	= ₱94.33/bbl	= $4.72/bbl
husk	₱0.3 B/5.81 M	= ₱51.63/bbl	= $2.58/bbl
palapa	₱0.64 B/6.4 M	= ₱100/bbl	= $5.00/bbl

Energy calculations for one hectare of coconut palm. At present the average hectare of coconut contains 135 palms and yield about 40 nuts/palm-year. The annual harvest would then be 135 x 40 = 5,400 nuts and 135 x 16 = 2,160 pieces of palapa. Using the same values of weight per palapa, shell, etc. calculations may be made of the energy that can be provided by a hectare of coconuts. A summary is given in the following table:

	Kg	MJ	bbl	KWH
shell	1,042	23,970	2.86	6,658
husk	1,307	21,875	2.61	6,076
palapa	1,080	18,079	2.16	5,022
TOTAL	3,429	63,924	7.63	17,756

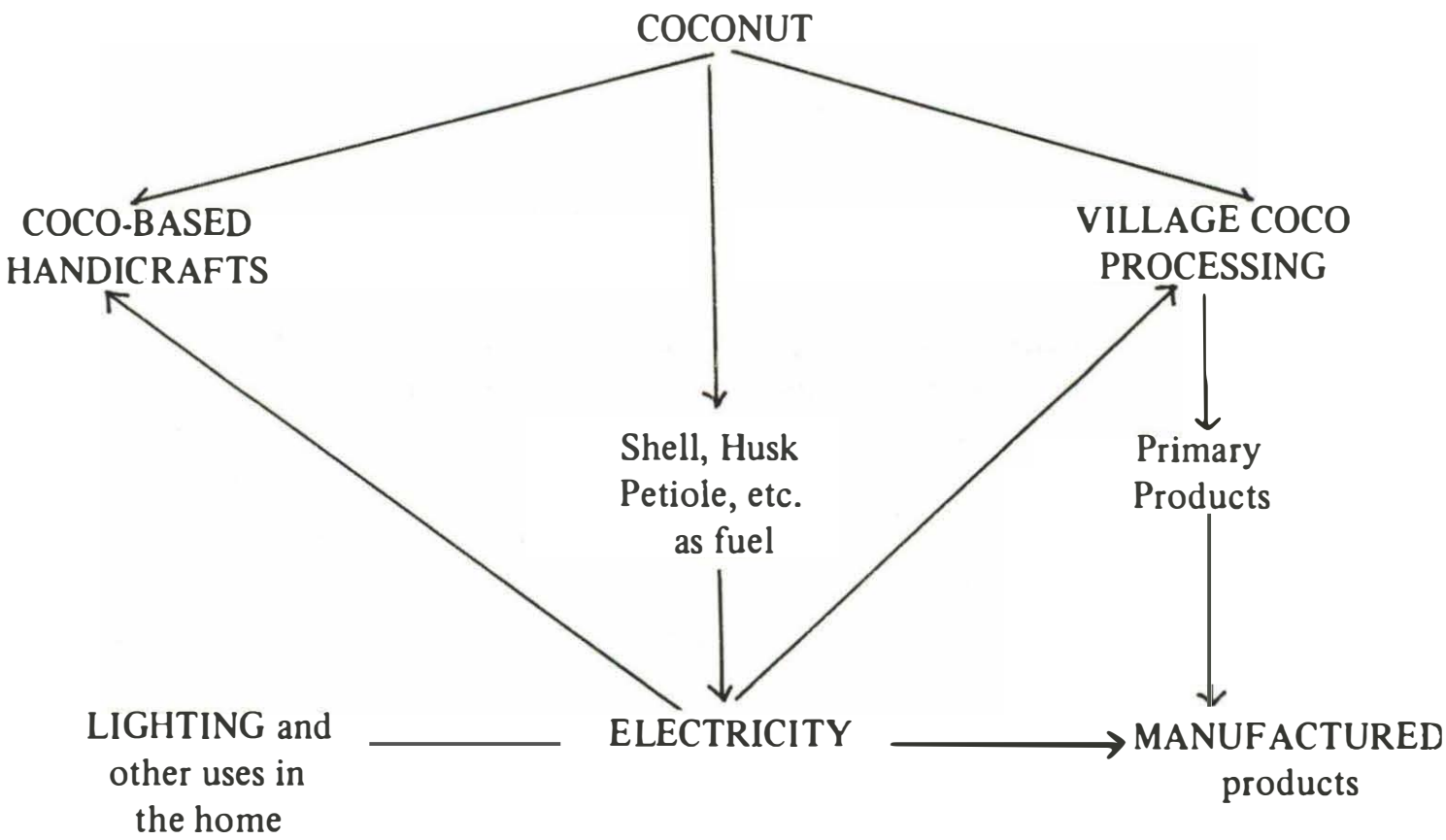
At 5% energy conversion to electricity, one hectare of coconut can yield 887.8 KWH per year or 74 KWH per month. A hectare of coconut can therefore provide the electrical needs of one village household.

Utilization of coconut-derived fuels. While there is an enormous quantity of coconut shell, husk and palapa which can be used as fuel, there is the problem of utilization. The most practical is probably electricity generation especially for villages that are outside the commercial lines. By placing the generators within the coconut groves, the problems of fuel transport may be minimized. Local labor can be used for gathering. Transmission lines among dwellings may use the coconut trees as posts.

Summary

Coconut shell, husk and petiole (palapa) which at present already contribute 5.9 percent of Philippine energy needs, may further be exploited. The energy potential is equal to 18.57 million barrels of petroleum oil equivalent or about 20 percent of national needs, at an average price of about $4/bbl. There are advantages in employing these coconut-derived fuels for local electricity generation.

The availability of electricity is probably most crucial in attempts of rural development. The present project on village coconut processing is hampered by lack of dependable supply of electricity. Coconut processing alone is not enough to bring relief to the farmers, it must be expanded to manufacturing and handicrafts using materials from the coconut palm. Summarized, these activities may be outlined in the following form:



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INHIBITORY EFFECTS ON SOMATIC AND GERM CELL GENOTOXICITY OF NITROSAMINES

Clara Y. Lim-Sylianco
Department of Chemistry, College of Science
University of the Philippines

ABSTRACT

Dimethylnitrosamine, diethylnitrosamine, dipropylnitrosamine, dibutylnitrosamine and N-nitrosopyrrolidine were shown to be genotoxic not only to somatic cells but also to germ cells. Genotoxicity to somatic cells was revealed by the micronucleus test. Genotoxicity to germ cells was indicated by the results of the dominant lethal test.

These nitrosamines induced the formation of micronucleated polychromatic erythrocytes in bone marrow cells. They also induced a reduction in fertility index, gestation index and implantation index. The % dead implants and females with resorptions were increased.

Vitamin A, vitamin C, vitamin E, niacin, riboflavin, thiamine, pyridoxine, biotin, folic acid and vitamin B_{12} reduced the genotoxicity to somatic and germ cells. The same observation was recorded of calcium, magnesium, manganese, copper, iron and zinc.

The genotoxicity of dimethylnitrosamine was reduced by expressions of fruits and vegetables.

Introduction

Nitrosamines have been found in tobacco smoke (1) alcoholic beverages (1), mushrooms (2). and foods such as grains, pasteurized milk and cheese (3) (4) (5) and nitrite treated cheese, fish, smoked fish and meat (2) (6). The occurrence of fairly high levels of nitrosamines in certain types of meat curing mixtures containing spices and nitrite indicates that some of the nitrosamines in cured meat products may originate from these curing mixtures (7). Nitrosamines in these premixes are apparently formed under dry conditions because of the interaction of amines in spices and nitrites both of which are major components of these formulations (8). There is evidence that man is exposed to nitrosamines either preformed in foods prepared with nitrite such as bacon and sausages or formed in the gastrointestinal tract (9). It has also been reported that nitrosamines may be formed from nitrite and secondary amines under the acidic conditions of the stomach.

Nitrosamines are highly carcinogenic in experimental exposures to animals and may represent an important cancer hazard to man (10). These are metabolized mainly by microsomal mixed function oxidases into reactive intermediates which

react with DNA. Nitrosamines have to be metabolized to a reactive intermediate by microsomal mixed function oxidases in order to have mutagenic and carcinogenic effect (11). There is a high specificity among various agents, tissues and cells in their capacity to metabolize nitrosamines.

Dimethylnitrosamine labelled with ^{14}C interacted with rat liver nucleic acids especially DNA in vivo to form 7-methylguanylic acid residues that could be released as 7-methyl guanine by hydrolysis (12).

Diethylnitrosamine has induced tumors of the respiratory tract, upper alimentary tract, and liver in mice, rats, hamsters, fish, birds, rabbits, dogs, pigs, guinea pigs and monkeys (13).

N-nitrosopyrrolidine, a carcinogen, is formed by cooking foods containing non-carcinogenic nitrosoproline (14). N-nitrosopyrrolidine occurs at levels of 1-80 ppm in fried but not uncooked bacon (6).

Experimental Methods

Somatic cell genotoxicity was studied using the micronucleus test (15). Germ cell genotoxicity was investigated using the dominant lethal test (16).

The experimental mice used were of Swiss Webster strain.

The vitamins were obtained from Sigma Chemical Co. Copper chloride, zinc chloride, manganese chloride, magnesium chloride, calcium chloride and ferrous sulfate, analytical grade, were obtained from Mallincdrott.

The nitrosamines and the vitamins were introduced simultaneously by oral gavage, in the micronucleus test. Simultaneous administration was also used for the mineral ions.

In the dominant lethal test simultaneous administration was done with nitrosamines, vitamins and mineral ions.

Assessment of somatic cell genotoxicity was based on the formation of micronucleated polychromatic erythrocytes in bone marrow cells. Inhibitory effects were based on the reduction of the formation of micronucleated polychromatic erythrocytes. Assessment of germ cell genotoxicity was based on the reduction of fertility index, gestation index, implantation index and increase in percentage dead implants and females with resorptions.

Results and Discussion

Table 1 shows that dimethylnitrosamine, diethylnitrosamine, dipropylnitrosamine, dibutylnitrosamine and N-nitrosopyrrolidinc induced the formation of micronucleated polychromatic erythrocytes in bone marrow cells of mice. Diethylnitrosamine induced the formation of more micronucleated polychromatic erythrocytes than dimethylnitrosamine because the carbocation released from diethylnitrosamine is more stabilized than the released from dimethylnitrosamine. This enhances the alkylating ability of diethylnitrosamine for DNA.

Table 1. Somatic cell genotoxicity of nitrosamines

	No. of micronucleated polychromatic erythrocytes
Negative control (distilled water)	1.22 ± 0.05
Dimethylnitrosamine, 10 mg/kg	18.75 ± 1.21
Diethylnitrosamine, 10 mg/kg	25.22 ± 1.37
Dipropylnitrosamine, 10 mg/kg	9.64 ± 1 06
Dibutylnitrosamine, 10 mg/kg	9.72 ± 0.91
Nitrosopyrrolidine, 10 mg/kg	9.67 ± 0.89

Table 2. Dose of vitamins and mineral ions administered to experimental mice

	Dose per kilogram weight
Vitamin A	150 mg
Vitamin C	150 mg
Vitamin E	150 mg
Niacin	150 mg
Riboflavin	150 mg
Thiamine	150 mg
Pyridoxine	150 mg
Biotin	150 ug
Folic Acid	150 ug
Vitamin B 12	150 ug
Calcium	150 mg
Magnesium	150 mg
Copper	15 mg
Iron	15 mg
Manganese	15 mg
Zinc	15 mg

Table 3 depicts data on germ cell toxicity of the five nitrosamines. All of them reduced the fertility index, gestation index, implantation index and increased percentage of dead implants and females with resorptions. They also induced the reduction in fetal weight. These effects can be a consequence of the metabolism of the nitrosamines to agents that alkylate DNA of the germ cells.

From the data in Table 4, it can be seen that the vitamins and mineral ions reduced the formation of micronucleated polychromatic erythrocytes induced by dimethylnitrosamine. This means that the fragmentation of the chromatin

Table 3. Germ cell genotoxicity of nitrosamines

	FI	*GI*	*II*	*DI*	*FR*	*FW*
Control	94.2	96.1	10.2	1.4	1.6	1.3 gm
Dimethylnitrosamine	22.1	58.3	7.3	27.4	100	0.8
Diethylnitrosamine	17.6	44.5	7.1	38.6	100	0.8
Dipropylnitrosamine	33.7	65.2	7.3	21.8	100	0.8
Dibutylnitrosamine	32.9	63.4	7.4	22.8	100	0.8
N-nitrosopyrrolidine	33.9	68.2	7.0	19.6	100	0.8

FI = fertility index = No. of females pregnant/No. of females mated X 100
GI = gestation index = No. of live implants/Total no. of implantations X100
II = implantation index = Total implantations/no. of females pregnant
DI = percentage dead implants
FR = percentage females with resorptions

Table 4. Effect of vitamins and mineral ions on somatic cell genotoxicity of nitrosamines

	Number of micronucleated polychromatic erythrocytes per thousand
Negative Control, distilled water	1.31 ± 0.12
Dimethylnitrosamine alone	18.75 ± 1.22
plus vitamin A	0.89 ± 0.08
plus vitamin C	0.32 ± 0.04
plus vitamin E	0.12 ± 0.05
plus niacin	1.12 ± 0.78
plus riboflavin	0.00
plus thiamine	1.22 ± 0.08
plus biotin	1.12 ± 0.03
plus pyridoxine	1.18 ± 0.09
plus folic acid	0.98 ± 0.04
plus vitamin B 12	0.98 ± 0.07
plus calcium	1.33 ± 0.08
plus magnesium	1.78 ± 0.06
plus copper	1.56 ± 0.13
plus iron	1.44 ± 0.07
plus manganese	2.22 ± 0.67
plus zinc	1.78 ± 0.06

material was reduced in the presence of vitamins and mineral ions. The alkylating tendency of dimethylnitrosamine for DNA was inhibited by vitamins and mineral ions. The same observation is recorded for diethylnitrosamine (Table 5) dipropylnitrosamine (Table 6), dibutylnitrosamine (Table 7), and N-nitrosopyrrolidine (Table 8).

Table 5. Effect of vitamins and mineral ions on somatic cell genotoxicity of diethylnitrosamine

	No. of micronucleated polychromatic erythrocytes per thousand
Negative Control, Distilled water	1.12 ± 0.08
Diethylnitrosamine alone	25.22 ± 1.37
plus vitamin A	1.53 ± 0.09
plus vitamin C	1.06 ± 0.11
plus vitamin E	1.40 ± 0.23
plus niacin	1.43 ± 0.05
plus riboflavin	1.22 ± 0.04
plus thiamine	1.60 ± 0.06
plus biotin	1.17 ± 0.09
plus folic acid	1.52 ± 0.07
plus vitamin B 12	1.33 ± 0.08
plus calcium	2.47 ± 0.30
plus magnesium	2.28 ± 0.46
plus copper	1.82 ± 0.09
plus iron	1.27 ± 0.04
plus manganese	1.40 ± 0.04
plus zinc	1.40 ± 0.23

Tabie 6. Effect of vitaminsand mineral ions on somatic cell genotoxicity of dipropylnitrosamine

	No. of micronucleated polychromatic erythrocytes per thousand
Negative control, distilled water	1.00 ± 0.12
Dipropylnitrosamine alone	9.64 ± 0.98
plus vitamin A	0.87 ± 0.03
plus vitamin C	0.98 ± 0.08
plus vitamin E	0.78 ± 0.11
plus niacin	1.22 ± 0.09
plus riboflavin	0.52 ± 0.05
plus thiamine	1.11 ± 0.07
plus biotin	1.22 ± 0.09
plus folic acid	1.32 ± 0.08
plus vitamin B 12	1.28 ± 0.06
plus pyridoxine	1.22 ± 0.07
plus calcium	0.86 ± 0.04
plus magnesium	0.79 ± 0.12
plus copper	1.11 ± 0.08
plus iron	1.02 ± 0.07
plus manganese	1.21 ± 0.09
plus zinc	0.82 ± 0.05

Table 7. Effect of vitamins and mineral ions on somatic cell genotoxicity of dibutylnitrosamine

	No. of micronucleated polychromatic erythrocytes per thousand
Negative control, distilled water	1.18 ± 0.05
Dibutylnitrosamine alone	9.72 ± 0.91
plus vitamin A	0.97 ± 0.03
plus vitamin C	1.21 ± 0.09
plus vitamin E	0.87 ± 0.08
plus niacin	1.11 ± 0.13
plus riboflavin	1.35 ± 0.08
plus thiamine	1.27 ± 0.06
plus biotin	1.09 ± 0.06
plus folic acid	1.42 ± 0.09
plus vitamin B 12	1.47 ± 0.23
plus pantothenic acid	1.22 ± 0.12
plus calcium	1.02 ± 0.04
plus magnesium	1.02 ± 0.12
plus copper	1.21 ± 0.09
plus iron	1.26 ± 0.07
plus manganese	2.11 ± 0.09
plus zinc	0.98 ± 0.08

Table 8. Effect of vitamins and mineral ions on the somatic cell genotoxicity of N-nitrosopyrrolidine

	No. of micronucleated polychromatic erythrocytes per thousand
Negative control, distilled water	1.22 ± 0.06
N-nitrosopyrrolidine alone	9.67 ± 0.78
plus vitamin A	1.23 ± 0.06
plus vitamin C	1.73 ± 0.05
plus vitamin E	1.87 ± 0.06
plus niacin	0.93 ± 0.05
plus riboflavin	0.89 ± 0.05
plus thiamine	1.33 ± 0.24
plus pyridoxine	1.01 ± 0.06
plus biotin	1.82 ± 0.09
plus folic acid	1.12 ± 0.08
plus vitamin B 12	1.11 ± 0.08
plus calcium	1.67 ± 0.07
plus magnesium	1.55 ± 0.33
plus copper	1.22 ± 0.07
plus iron	0.93 ± 0.09
plus manganese	1.10 ± 0.05
plus zinc	1.08 ± 0.08

Germ cell genotoxicity of dimethylnitrosamine was reduced by vitamins and mineral ions. This was shown in the increase of fertility index and gestation index and in the reduction of % dead implants and females with resorption (Table 9). The same observation is recorded of diethylnitrosamine (Table 10), dipropylnitrosamine (Table 11), dibutylnitrosamine (Table 12) and N-nitrosopyrrolidine (Table 13).

Expressions from fruits and vegetables reduced the somatic cell genotoxicity of dimethylnitrosamine (Table 14 and Table 15). Fruits and vegetables are natural sources of vitamins and mineral ions.

Summary

Dimethylnitrosamine, diethylnitrosamine, dipropylnitrosamine, dibutylnitrosamine and N-nitrosopyrrolidine are genotoxic to both somatic and germ cells. This genotoxicity can be reduced by vitamin A, vitamin C, vitamin E, niacin, riboflavin, thiamine, folic acid, biotin, vitamin B 12, pyridoxine, and pantothenic acid. Genotoxicity can also be reduced by calcium, magnesium, copper, iron and manganese. Expressions from fruits and vegetables can also reduce the genotoxicity of nitrosamines.

Table 9. Effect of vitamins and mineral ions on germ cell genotoxicity of dimethylnitrosamine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
Dimethylnitrosamine alone	22.1	58.3	27.4	100
plus vitamin A	88.2	90.6	2.0	2.1
plus vitamin C	91.4	88.4	1.6	2.0
plus vitamin E	87.6	87.9	1.4	1.6
plus niacin	93.1	92.3	1.8	2.0
plus riboflavin	92.6	93.4	1.3	1.6
plus thiamine	89.6	91.5	1.8	1.8
plus pantothenic acid	87.9	88.7	1.8	1.8
plus calcium	88.8	89.3	1.5	1.6
plus magnesium	91.2	88.4	2.0	2.0
plus iron	86.5	91.1	2.0	1.9
plus zinc	87.9	92.0	1.4	1.3

Table 10. Effect of vitamins and mineral ions on germ cell genotoxicity of diethylnitrosamine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
Diethylnitrosamine alone	17.6	44.5	38.6	100
plus vitamin A	92.2	89.9	2.1	2.1
plus vitamin C	88.9	92.3	1.6	1.4
plus vitamin E	87.5	88.6	2.1	1 9
plus biotin	91.6	87.6	1.7	1.8
plus thiamine	93.2	90.8	1.5	1.4
plus pyridoxine	94.2	91.8	1.7	1.5
plus calcium	96.2	93.1	1.2	1.5
plus magnesium	92.3	94.2	1.5	1.6
plus manganese	85.7	88.5	1.9	1.8
plus iron	83.9	98.1	1.4	1.3
plus zinc	89.7	91.8	1.5	1.6

Table 11. Effect of vitamins and mineral ions on the germ cell genotoxicity of dipropylnitrosamine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
Dipropylnitrosamine alone	33.7	65.2	21.8	100
plus vitamin A	86.7	88.6	1.4	2.1
plus vitamin C	88.5	89.7	2.1	2.2
plus vitamin E	91.1	91.5	1.2	1.5
plus niacin	92.3	88.9	1.9	1.6
plus riboflavin	90.1	89.7	1.9	1.6
plus folic acid	86.4	92.0	1.8	1.9
plus vitamin B 12	85.9	88.5	1.4	1.3
plus calcium	83.9	88.1	2.1	2.1
plus magnesium	86.7	91.2	1.9	2.1
plus copper	91.1	87.2	1.6	1.9
plus iron	88.4	85.8	1.9	2.1

Table 12. Effect of vitamins and mineral ions on germ cell genotoxicity of dibutylnitrosamine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
Dibutylnitrosamine	32.9	63.4	22.8	100
plus vitamin A	88.6	86.7	1.2	2.2
plus vitamin C	89.3	88.6	2.3	2.4
plus vitamin E	87.6	89.6	2.1	2.1
plus riboflavin	90.1	86.8	2.1	2.2
plus pyridoxine	91.8	90.3	1.8	1.8
plus pantothenic acid	87.6	88.9	1.9	2.1
plus biotin	86.7	86.9	2.1	2.2
plus calcium	89.6	94.1	1.1	1.8
plus copper	89.7	94.3	2.1	2.1
plus manganese	87.6	89.7	1.9	2.1
plus zinc	91.1	95.6	1.3	1.4

Table 13. Effect of vitamins and mineral ions on germ cell genotoxicity of N-nitrosopyrrolidine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
N-nitrosopyrrolidine	33.4	68.1	19.3	100
plus vitamin A	93.1	92.5	2.1	2.1
plus vitamin C	92.3	91.1	2.3	2.2
plus vitamin E	93.2	90.4	1.8	2.0
plus niacin	91.1	89.7	2.3	2.1
plus pantothenic acid	86.7	87.8	2.3	2.1
plus biotin	89.4	88.6	2.2	2.2
plus folic acid	88.7	90.7	1.9	1.8
plus calcium	90.1	89.8	2.1	2.1
plus magnesium	92.3	91.1	1.5	1.6
plus copper	93.1	89.7	2.1	2.2
plus iron	91.1	88.3	2.2	2.1
plus zinc	90.4	90.1	1.7	1.9

Table 12. Effect of vitamins and mineral ions on germ cell genotoxicity of dibutylnitrosamine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
Dibutylnitrosamine	32.9	63.4	22.8	100
plus vitamin A	88.6	86.7	1.2	2.2
plus vitamin C	89.3	88.6	2.3	2.4
plus vitamin E	87.6	89.6	2.1	2.1
plus riboflavin	90.1	86.8	2.1	2.2
plus pyridoxine	91.8	90.3	1.8	1.8
plus pantothenic acid	87.6	88.9	1.9	2.1
plus biotin	86.7	86.9	2.1	2.2
plus calcium	89.6	94.1	1.1	1.8
plus copper	89.7	94.3	2.1	2.1
plus manganese	87.6	89.7	1.9	2.1
plus zinc	91.1	95.6	1.3	1.4

Table 13. Effect of vitamins and mineral ions on germ cell genotoxicity of N-nitrosopyrrolidine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
N-nitrosopyrrolidine	33.4	68.1	19.3	100
plus vitamin A	93.1	92.5	2.1	2.1
plus vitamin C	92.3	91.1	2.3	2.2
plus vitamin E	93.2	90.4	1.8	2.0
plus niacin	91.1	89.7	2.3	2.1
plus pantothenic acid	86.7	87.8	2.3	2.1
plus biotin	89.4	88.6	2.2	2.2
plus folic acid	88.7	90.7	1.9	1.8
plus calcium	90.1	89.8	2.1	2.1
plus magnesium	92.3	91.1	1.5	1.6
plus copper	93.1	89.7	2.1	2.2
plus iron	91.1	88.3	2.2	2.1
plus zinc	90.4	90.1	1.7	1.9

Table 14. Effects of expressions from fruits on the somatic cell genotoxicity of dimethylnitrosamine

	No. of micronucleated polychromatic erythrocytes per thousand
Dimethylnitrosamine, 10 mg/kg	15.67 ± 1.56
plus Atis	1.66 ± 0.66
plus Avocado	2.10 ± 0.69
plus Duhat	1.86 ± 0.38
plus Kamatsile	2.49 ± 0.77
plus Lansones	3.08 ± 0.98
plus Melon	3.22 ± 0.18
plus Papaya	2.11 ± 0.21
plus Sineguelas	3.55 ± 1.01
plus Suha	2.33 ± 0.33
Control	1.76 ± 0.07

Table 15. Effects of expressions from vegetables on the somatic cell genotoxicity of dimethylnitrosamine

	No. of micronucleated polychromatic erythrocytes per thousand
Dimethylnitrosamine, 10 mg/kg	15.67 ± 1.56
plus Ampalaya fruit	3.58 ± 0.31
plus Ampalaya leaves	3.66 ± 0.33
plus Bell pepper, green	3.08 ± 0.16
plus Bell pepper, red	2.77 ± 0.38
plus Garlic	2.01 ± 0.09
plus Kamias	3.77 ± 0.68
plus Green mongo	2.57 ± 0.84
plus White onions	2.44 ± 0.55
plus Raddish	3.22 ± 0.18
plus Squash fruit	3.21 ± 0.76
plus Squash leaves	3.55 ± 0.69
plus Tomatoes	2.88 ± 0.76
Control	1.76 ± 0.07

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OBSERVATIONS ON THE SEA VEGETABLE ALGAE OF PANAY ISLAND, CENTRAL PHILIPPINES[1]

Paciente A. Cordero, Jr.
National Museum
Executive House
Rizal Park, Manila
Philippines

ABSTRACT

The eco-morphology and seasonality of the edible marine algae or Sea Vegetable Algae of Panay Island, Western Visayas is presented. A total of sixty-one (61) sea vegetable algae species belonging to three (3) taxa were identified: Green algae or Class Chlorophyceae is represented with twenty (20) species, brown algae or Class Phaeophyceae with nine (9), and red algae or Class Rhodophyceae with thirthy-two (32). Green *Caulerpa peltata* var. *macro-disca* is the most popular sea vegetable algae in the island.

Ecological parameters determined such as surface water temperature, hydrogen ion concentration and salinity were found not to vary significantly in the four (4) collecting stations/provinces of Aklan, Antique, Capiz and Iloilo. Likewise, they have insignificant effects on the morphologies and/or growth and development of the algae. However, topography and type of substrates appear to influence the growth and the general morphology of the test algae species selected for this study.

Introduction

The Philippines, considering its tropical location and topography coupled with attendant favorable ecological factors, finds its marine waters rich in living organisms. One such organisms are the seaweeds.

The central part of the Philippines, occupied by several islands forming the Visayan provinces is divided into Central, Eastern and Western regions. The physiography presents a picture of a phycologically interesting area. Outstanding of these marine plant organisms/resources are the edible seaweed species, more appropriately called as *sea vegetable algae.*

The present project, therefore, is an attempt to assess the potentials of the sea vegetable algae in Panay Island including notes on their biology.

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Review of Literature

To date there is no comprehensive documentation of the sea vegetable species of the country. Previous attempts were sporadic listings of few species such as those by Quisumbing (1951), Sulit *et al.*, (1952), Montilla and Blanco (1953), Domantay (1961), Galutira and Velasquez (1963), Cordero (1974), Modelo (1979) and Agngarayngay (1980), among others.

In 1981, Cordero described seventy six (76) species of useful seaweeds collected from select areas of the country. However, from the island of Panay, there is not a single literature on the sea vegetable algal species present. The few available literature containing incidental descriptions of algal materials from the island include the works by Carreon (1974) from Aklan, Aligaen (1977) from Guimaras Island, and Cordero (1978, 1980) from Aklan and Iloilo, respectively. Calmorin (1981) in his master thesis accounted for eighty nine (89) algal species gathered from northern Iloilo, of which seventeen (17) species are reportedly edible.

Materials and Methods

The choice and designation of collecting stations for the four provinces of Panay Island, viz., Aklan, Antique, Capiz and Iloilo, criteria ranging from physiography and topography to the type of habitat and substrates, presence of algal standing crop and some relevant ecological parameters were used in the determination of the study areas. Standard pre- and post- activities that treat on marine algae were adopted. The determination of ecological parameters like surface water temperature, hydrogen ion concentration and salinity were recorded using ordinary laboratory paraphernalia. Seasonal occurrence of the sea vegetable algae were monitored periodically during the dry and wet months of the year. Consequently, was the *in situ* observations on marked representative algal species in predesignated areas by taking into considerations of the aforementioned ecological factors. The extent of growth and morphological development of the test algal species were noted. Field interviews among fishermen and coastal inhabitants were conducted randomly by taking into account the species of edible algae present in the area, their seasonal occurrence and mode of utilization, common names, etc.

Results and Discussion

Study area

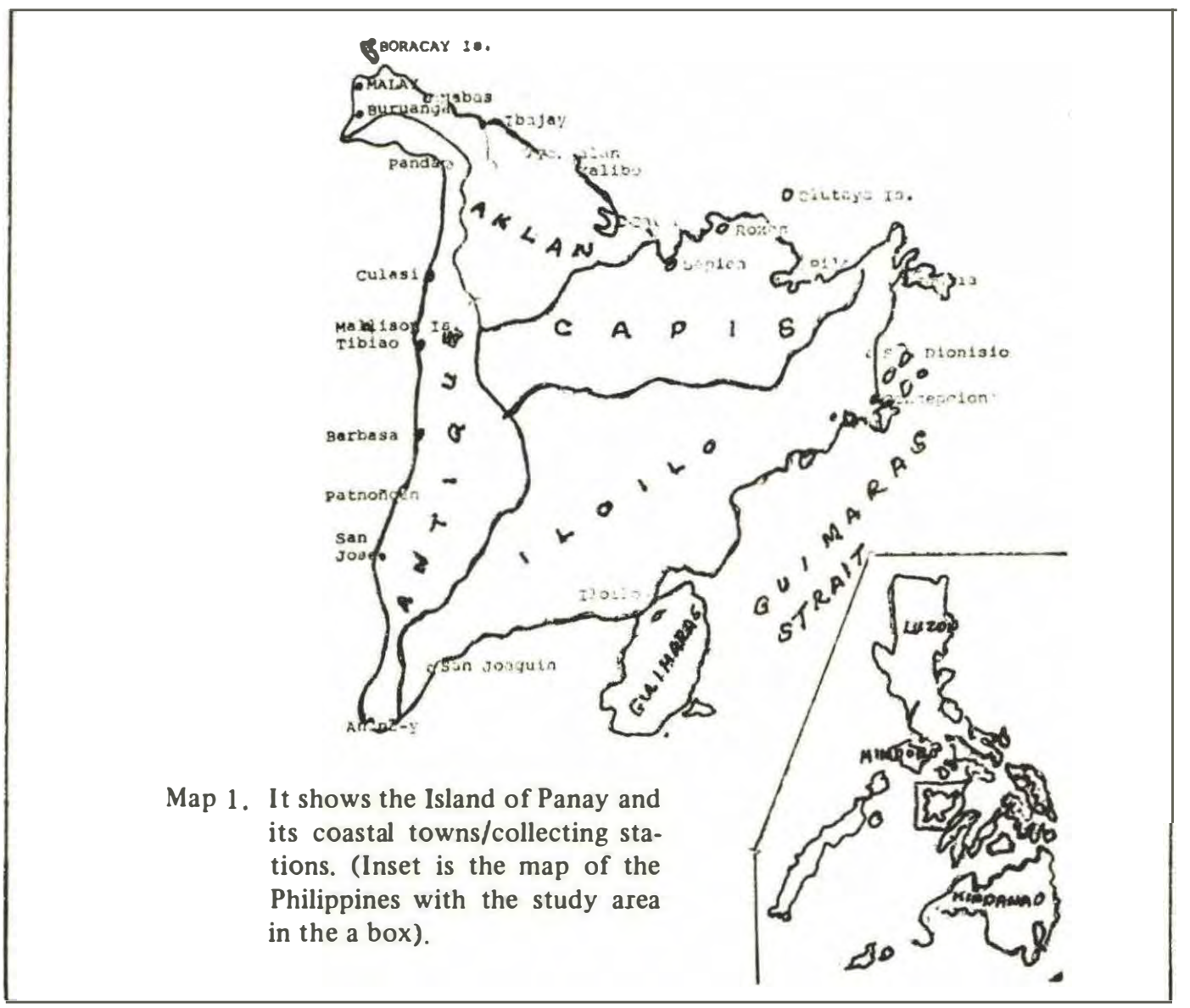
Panay Island, located south of Manila, is the sixth largest island in the Philippines. It is composed of four (4) provinces, namely: Aklan, Antique, Capiz and Iloilo (Map I).

For the present investigation the four provinces had the following number of collecting stations/towns and sub-collecting stations (Table 2). Thus, each collecting

station was artificially divided into north and south using towns as natural boundaries (Table 1).

Table 1. Subdivision of four provinces

A.	AKLAN	
	North	- Tañgalan to Buruanga
	South	- Makato to Batan
B.	ANTIQUE	
	North	- Tibiao to Pandan
	South	- Barbasa to Anini-y
C.	CAPIZ	
	North	- Sapian to Roxas
	South	- Panay to Pilar
D.	ILOILO	
	North	- Barotac Nuevo to Carles
	South	- Dumagas to San Joaquin



Map 1. It shows the Island of Panay and its coastal towns/collecting stations. (Inset is the map of the Philippines with the study area in the a box).

Table 2. *Summary of collecting/subcollecting stations in Panay Island*

I *AKLAN NORTH*

A. Buruanga
1. Bay/Poblacion
2. Santander
3. Bel-es
4. Tigum

B. Malay
1. Bay/Poblacion
2. Argao
3. Caticlan
4. Boracay Island
4.1 Balabag
4.2 Manok-manok

C. Nabas
1. Rizal
2. Union

D. Ibajay
1. Naisud
2. Bugtong-bato

E. Tañgalan
1. Jawili
2. Dumatad
3. Afga Point

II. *ANTIQUE NORTH*

A. Pandan
1. Mag-aba

B. Culasi
1. Malalison Island
2. Batbatan Island
3. Lipata Point

C. Libertad
1. Bay/Poblacion
2. San Roque

D. Tibiao
1. Bay/Poblacion

ANTIQUE SOUTH

A. Patñongon
1. Ipayo
2. Igbanwa

B. Anini-y
1. Bay/Poblacion
2. Negas

C. San Jose
1. Bay/Poblacion
2. Tiringting

D. Barbasa
1. Bay/Poblacion

III. *CAPIZ NORTH*

A. Roxas
1. Olutaya Island

B. Sapian
1. Lonoy
2. Culasi

CAPIZ SOUTH

A. Pilar
1. Punta Bang-ugay

IV. *ILOILO NORTH*

A. Estancia
1. Bituon-Point
2. Nabaye-tiknop

B. Ajuy
1. Bay/Poblacion

C. San Dionisio
1. Bay/Poblacion

D. San Joaquin
1. Bay/Poblacion

Taxonomic treatment

A total of sixty one (61) sea vegetable algae were identified. These belong to the three (3) major taxa: Class Chlorophyceae with twenty (20) species, Class Phaeophyceae with nine (9) and Class Rhodophyceae with thirty two (32).

Class : Chlorophyceae
Order : Ulvales
Family : Ulvaceae
Genus : *Enteromorpha* Link

Enteromorpha compressa (Forssk.) Greville
Enteromorpha prolifera(Muell.) J. Agardh

Genus : *Ulva* Linnaeus

Ulva lactuca Linnaeus
Ulva pertusa Kjellman
Ulva reticulata Forsskal

Order : Cladophorales
Family : Cladophoraceae
Genus : *Chaetomorpha* Kuetzing

Chaetomorpha crassa (Ag.) Kuetzing
Chaetomorpha spiralis (Ag.) Kuetzing

Family : Valoniaceae
Genus : *Dictyosphaeria* Decasine

Dictyosphaeria cavernosa (Forssk.) Boergesen

Family : Valoniaceae
Genus : *Valonia* Ginnani

Valonia aegagrophila (Roth) Agardh
Valonia ventricosa J. Agardh

Order : Caulerpales
Family : Caulerpaceae
Genus : *Caulerpa* Lamouroux

Caulerpa lentillefera J. Agardh
Caulerpa peltata var. *macro-disca* Decaisne
Caulerpa racemosa var. *lamourouxii* (Turn.) W. van Bosse
Caulerpa racemosa var. *occidentalis* (W. v. Bosse) Gilbert
Caulerpa racemosa var. *racemosa* Papenfuss et Egerod
Caulerpa serrulata (Forssk.) J. Agardh

Order : Siphonales
Family : Codiaceae
Genus : *Codium* Stackhouse

Codium adhaerens (Cabr.) J. Agardh
Codium fragile (Sur.) Hariot

Codium intricatum Okamura
Codium tenue Kuetzing

Class : Phaeophyceae
Order : Punctariales
Family : Scytosiphonaceae
Genus : *Colpomenia* Derbes et Solier

Colpomenia sinuosa (Roth) Derbes et Solier

Genus : *Hydroclathrus* Bory

Hydroclathrus clathratus (Bory) Howe

Order : Chordariales
Family : Leathesiaceae
Genus : *Leathesia* S. F. Gray

Leathesia difformis (L.) Areschoug

Order : Fucales
Family : Sargassaceae
Genus : *Sargassum* C. Agardh

Sargassum confusum C. Agardh
Sargassum duplicatum J. Agardh
Sargassum fulvellum C. Agardh
Sargassum gigantiefolium Yamada
Sargassum hemiphyllum (Turn.) J. Agardh
Sargassum piluleferum J. Agardh

Class : Rhodophyceae
Order : Nemaliales
Family : Helminthocladiaceae
Genus : *Helminthocladia* J. Agardh

(?) Helminthocladia australis Harvey

Genus : *Liagora* Lamouroux

Liagora boergesenii Yamada
Liagora ceranoides Lamouroux
Liagora farinosa Lamouroux
Liagora japonica Yamada

Family : Chaetangiaceae
Genus : *Scinaia* Bivona

Scinaia moniliformis J. Agardh

Order : Gelidiales
Family : Gelidiaceae
Genus : *Gelidiella* Feldmann et Hamel

Gelidiella acerosa (Forssk.) Feldmann et Hamel

Family : Gracilariaceae
Genus : *Gracilaria* Greville
Gracilaria arcuata Zanardini
Gracilaria blodgetti Harvey
Gracilaria coronipifolia J. Agardh
Gracilaria crassa Harvey
Gracilaria edulis (Gmel.) Silva
Gracilaria eucheumioides Harvey
Gracilaria incurvata Okamura
Gracilaria salicornia (C. Agardh) Dawson
Gracilaria verrucosa (Huds.) Papenfuss

Order : Cryptonemiales
Family : Cryptonemiaceae
Genus : *Carpopeltis* Schmitz
Carpopeltis crispata Okamura
Carpopeltis divaricata Okamura

Genus : *Halymenia* C. Agardh
Halymenia dilatata Zanardini
Halymenia durvillaei Bory
Halymenia harveyana J. Agardh

Order : Gigartinales
Family : Hypneaceae
Genus : *Hypnea* Lamouroux
Hypnea cervicornis J. Agardh
Hypnea charoides Lamouroux
Hypnea nidulans Setchell
Hypnea saidana Holmes

Family : Solieriaceae
Genus : *Eucheuma* J. Agardh
Eucheuma gelatinae Weber van Bosse
Eucheuma muricatum (Gmel.) Weber van Bosse
Eucheuma spinosum Weber van Bosse
Eucheuma striatum Schmitz

Order : Ceramiales
Family : Rhodomelaceae
Genus : *Acanthophora* Lamouroux
Acanthopora spicifera (Vahl) Boergesen

Genus - *Laurencia* Lamouroux
Laurencia okamurai Yamada
Laurencia papillosa (Forssk.) Greville

Ecological parameters

Basic ecological parameters such as surface water temperature, salinity and hydrogen ion concentration were recorded during the dry and wet seasons in the predesignated localities of Aklan, Antique, Capiz and Iloilo (Table 3).

Table 3. *Average ecological data measured in four representative localities* (1982-1983; 1983-1984)

LOCALITIES	DRY SEASON			WET SEASON		
	Water Temp.	pH	Salinity (PPT)	Water temp.	pH	Salinity (PPT)
A. Estancia, Iloilo	30°C	7.5	31	28°C	7.5	30
B. Tañgalan, Aklan	29°C	7.5	30.5	28°C	7.5	30
C. Pandan, Antique	29°C	7.5	31	28°C	7.5	30
D. Olutaya, Is., Capiz	29°C	7.5	31	28°C	7.5	30

An analysis of Table 3 tends to show that the four localities present almost identical average ecological parameters even during the dry and wet seasons. Earlier studies conducted by Cordero (1981) for Iloilo and Antique appear to duplicate the aforementioned data. It might be well to theorize that the significantly identical ecological data could be attributed to the physiographiic location of Panay Island. The island is classified under the inland water category which is neither directly affected by the activities of the South China Sea nor the Pacific Ocean. The interplay of ecological factors obtaining in these areas support the generally even distribution of algal species in the four provinces of Panay Island. The case of *C. peltata* var. *macro-disca* having been recorded, initially in Aklan, Iloilo and recently in Capiz and its 'absence' in Antique appears as an exception. An in depth study on the biology of this edible green alga is a challenge to future students of phycology. Correspondingly, are the exceptionally large *Ulva lactuca* specimens gathered from Boracay Island, Malay, Aklan.

Seasonal occurrence

Based on the data monitored during the wet months of 1983 and dry months of 1984, a study on the seasonal occurrence of select algal species in two provinces

is in order. As trace species, therefore, it was necessary to include both edible and non-edible forms naturally growing in Aklan and Antique (Table 4-a; 4-b).

Table 4-a. Seasonality of select marine algae in Aklan

	SPECIES	DRY SEASON/MONTHS	WET SEASON/MONTHS
A.	Class Chlorophyceae		
1.	*Ulva lactuca*	mature, fertile	young to juvenile
2.	*U. reticulata*	mature, fertile	young sterile
B.	Class Phaeophyceae		
1.	*Hydroclathratus clathratus*	young to juvenile	mature, fertile
C.	Class Rhodophyceae		
1.	*Amphiroa fragilissima*	young to juvenile	mature
2.	*Hypnea cervicornis*	young to juvenile	mature, fertile

Table 4-b. Seasonality of select marine algae in Antique

	SPECIES	DRY SEASON/MONTHS	WET SEASON/MONTHS
A.	Class Chlorophyceae		
1..	*Ulva reticulata*	mature, fertile	young to juvenile
2.	*Halimeda opuntia*	mature	young to juvenile
B.	Class Phaeophyceae		
1.	*Hydroclathratus clathratus*	young to juvenile	mature, fertile
C.	Class Rhodophyceae		
1.	*Actinotrichia fragilis*	mature, fertile	young to juvenile
2.	*Mastophora rosea*	mature, fertile	young

From northern Aklan, Buruanga, Bel-es, the following species were considered namely: *Ulva lactuca* and *U. reticulata* (Chlorophyta), *Padina arborescens* and *P. crassa* (Phaeophyta) and *Amphiroa fragilissima* and *Hypnea cervicornis* (Rhodophyta). While for northern Antique, Pandan, Mag-aba, had *Ulva reticulata* and *Halimeda opuntia* (Chlorophyta); *Hydroclathratus clathratus* (Phaeophyta) and *Actinotrichia fragilis* and *Mastophora rosea* (Rhodophyta).

The general picture on the seasonal occurrence of the representative algal species in the two provinces is significantly similar. This is so with the green and brown algae, but for the red group which has varied morphological and fertility data observed. Incidentally, of the four red species, three are calciferous, viz., *A. fragilissima, A. fragilis* and *M. rosea;* while *H. cervicornis* is membranaceous.

Eco-morphological observations

A. Chlorophyceae Test Species.

(1) *Caulerpa peltata* var. *macro-disca*

Initial collections of *C. peltata* var. *macro-disca* came from Estancia, Iloilo (back of then Western Visayas College of Fisheries presently known as the Northern Iloilo Polytechnic School), during the wet months of 1983. This green sea vegatable alga was observed growing luxuriantly in colony and in predominantly clayish soft bottom. The plant showed morphologically well developed growth with profuse branching, robust thalli/branches and its peltate disc averaging 20 (-25) cm in diameter.

The kind of growth and development exhibited by the green sea vegetable alga are attributed to the favorable ecological factors prevailing in the area. The topography of the natural habitat is such that it draws protection from the direct effects of wind-driven water movement owing to several island/islets surrounding it. The sandy, siltish to corally type of substrate favors the plant's fragile root-system to gain easy anchorage. Other physico-chemical factors noted periodically during the designated wet and dry months are deemed contributory to the presence of a good standing crop of *C. peltata* var. *macro-disca* in Estancia, Iloilo. These factors were the average reading of the pH at 6.5 and 6.8, salinity at 32 and 33 ppt., and the water temperature at 27 and 28°C, respectively, for the wet and dry periods. The differences in the factors measured are negligible for the two seasons of the year. It is the interplay of ecological factors that contribute to the favorable growth and development of marine algae. Incidentally, despite the proximity of the natural habitat of this particular test species to the landing area of motorized boats (fishing and commercial), there is no sign of oil pollutant accumulating in the area. This could be due to the continuous and moderate flow of water pushed by currents flowing through channels in between islands/islets in the vicinity.

It is worth mentioning that *C. peltata* var. *macrodisca*, like other marine algae, shows "migration" activity. Following a strong water disturbance caused by a tropical storm that hit Iloilo, the area marked as natural habitat of this green sea vegetable in 1983 was 'bare' in 1984. Thorough underwater investigation showed that the plant had migrated southernly into an underwater canal and growing in identical substrate composition as that found at the back of the WVCP or NIPS. Plants in the new habitat showed no morphological difference when compared with those collected in 1983 from the old habitat. Neither were there variations in the ecological factors between the old and the new habitats of the green sea vegetable.

Moreover, the ability of *C. peltata* var.*macrodisca* to migrate to another habitat with identical ecological parameters was exhibited by the plant in the Province of Aklan. This green sea vegetable used to inhabit the brackish water of Tinagong Dagat at the back of the Municipality of New Washington, Aklan, until the population slowly disappeared in the 1970's. Fishermen blamed the increase in the number of commercial ships and other forms of motorized banca dropping anchor in the area as well as the conversion of the nearby mangrove land into fishponds and the subsequent use of commercial pesticides, herbicides and other chemicals applied into the fishponds. The same chemicals are drained down into the Tinagong Dagat waters especially during the rainy days. The adverse effects of these chemicals upon the aquatic life in the area appears possible as the culprit for the disappearance of *C. peltata* var. *macrodisca* in New Washington. For so many years there was no trace of the green sea vegetable in Panay Island, but for those found in Estancia, Iloilo.

In early 1984, however, the research staff of the present project found another population of *C. peltata* var. *macrodisca* in Sapian, Capiz. The area is very close to New Washington, Aklan than to Estancia, Iloilo, prompting the staff to speculate that the spores of this sea vegetable could have been carried by water current from New Washington, Aklan and settled in the coastal water of Sapian – the first town of Capiz next to Aklan!

The *C. peltata* var. *macrodisca* plants in Sapian, Capiz are found in a more exposed habitat facing the Sibuyan Sea. However, it has a substrate similar to that in New Washington, Aklan and in Estancia, Iloilo. Ecological factors such as pH, salinity and water temperature shows little or no variations at all compared with those observed in Iloilo. Thus, pH had average readings of 6.8 and 7.0, salinity at 32 and 33 ppt., and water temperature at 28 and 30°C for the wet and dry periods, respectively.

However, there was marked difference in the gross morphology, growth and development of the sea vegetable from the later province, viz., not growing luxuriantly, less profusely branched with slender thalli/branches and not so widely spread in their growth. Also, the peltate discs have an average diameter measurement of only 8 (-10) cm across.

The relatively less morphologically developed plants from Sapian, Capiz could be explained in part to the topography of the area being exposed and often hit directly by strong water movement, a situation which disturbs the growth and development of plant life in the aquatic habitat. Also, instead of growing colonially as observed in most prostrate marine algae, *C. peltata* var. *macrodisca* from Sapian, Capiz are often observed in scattered narrow patches–a mode of distribution common among marine algae growing in exposed situation.

(2) *Ulva lactuca*

There is distinct gross morphological variation in the *U. lactuca* observed *in situ* located in Boracay Island, Malay, Aklan and in Jawili, Tañgalan, Aklan. Plants from the former island showed luxuriant growth and in scattered patches, had

broad thalli of up to 250 (-300) cm. at its widest portion. Those from Jawili are relatively smaller plants measuring barely 80 (-100) cm broad and growing thinly in scattered patches.

Again, these morphological differences could be traced to some ecological factors that determine the kind of growth and development of the sea vegetable. The topography at Boracay Island is such that the *U. lactuca* habitat is a cove protected from the effect of strong wind/water activities. It is located at the back of the white sandy beach of this resort island. The substrates for both study areas are practically alike, e.g. rocky, sandy. Their average pH, salinity and water temperature readings during the wet and dry months vary very negligibly. In Boracay Island the average pH reading was 7.0 and 7.5 salinity at 31 and 33 ppt., and water temperature at 27 and 30°C for the wet and dry months, respectively. In Jawili, pH were recorded at 7.0 and 7.5, salinity at 31 and 33 ppt., and water temperature at 28 and 30°C for the same period.

It is safer, therefore, to consider the physical locations of the study areas for *U. lactuca* that cause the marked difference in the growth and development of the papery-frond.

B. Phaeophycean Test Species:

(1) *Hydroclathrus clathratus*

The data show the morphological variations of *H. clathratus* found in Aklan and Capiz, viz., growth and development, being attributable to the varied ecological factors present. Of these, topography and substrate are more likely to have tangible effect(s) on the plant more than the pH, salinity and water temperature measurement.

The physical location and kind of substrate found in Aklan seem to favor the *H. clathratus* plants growth and development exemplified by the broadness of the net-like thalli. In Capiz, the less exposed topography and the soft sandy siltish substrate of the habitat of *H. clathratus* produce thin and frail-looking thalli. The later type of substrate is explained by the proximity of the habitat to a mangrove area and fishponds.

H. clathratus, being a saxicolous alga, grows better on rocky solid substrate. It continues to grow even when detached from an initial substrate and stays afloat and/or trapped on other larger marine plants (seaweeds, sea-grasses) and mangrove trees.

(2) *Sargassum piluleferum*

A comparative analysis on the gross morphology of *S. piluleferum* observed in Aklan and Capiz reveals some distinct variations in terms of growth and development. In Aklan *S. piluleferum* are moderately distributed, colonial or found mixed with other *Sargassum* species. It reaches maximum height of not more than four feet tall, heavily branched and bearing numerous air-bladders at maturity. This is in contrast to the same brown sea vegetable alga observed in Capiz, e.g. being few, stunted, moderately branched, bearing few air-bladders and barely 1.5 (–2) feet tall upon reaching maturity.

As an explanations to the aforementioned morphological differences of *S. piluleferum* reference may be made to the data under ecology. Hydrogen ion concentration, salinity and water temperature for both Aklan and Capiz show little difference as to affect greatly the morphology of the plant. Again, between topography and substrate, the latter could provide a better explanation to the varied data on the growth and development of *S. piluleferum.* Definitely, the Philippine *Sargassum* species are all saxicolous, their discoid shape rhizoidal structure needs a solid/hard bottom to anchor the plant and withstand strong water disturbance. The plant shuns away from soft shifting substrate such as that found in Sapian, Capiz. More significantly is the plant's low tolerance to habitats rich in hydrogen sulfide common in mangrove areas. In Capiz, *S. piluleferum* were found in few stands dictated by the number of scattered submerged rocks.

C. Rhodophyceae Test Species:

(1) *Gelidiella acerosa*

Data on the morphology of *G. acerosa* observed in Aklan and Capiz account for the different ecological parameters found in the respective habitats of the plant. This red sea vegetable alga grows moderately and are thickly scattered in the intertidal zone of Jawili, Tañgalan, Aklan. The plants are erect not more than 150 cm tall, moderately branched, dark green and wiry when dry. While the same plant found in Sapian, Capiz are few, usually stunted and limited to the narrow portion of the intertidal zone having rocky sandy bottom.

Of the various ecological parameters determined, only the type of substrate and to a lesser extent topography, could provide an explanation to the variation in the morphological features of *G. acerosa.* A soft, loose siltish type of substrate is very selective on the species of marine plants it could hold, e.g., prostrate/creeping seaweeds like *Caulerpa* or the shallow-boring algae with fibrous type of root-system like the codiaceous species of *Udotea* and *Avrainvillea.* Where the root system is discoidal and digitate in structure exemplified by *Sargassum, Gelidiella,* etc., their tendency is to inhabit rocky/solid bottoms for better anchorage.

Strong wave action on the intertidal marine algal population affects their growth and development, viz., producing stunted plants, less developed branches, ruffled or torn leaf-like and reproductive parts, etc. Both *G. acerosa* plants from Aklan and Capiz grow in similar topographic conditions attributing to their negligible height difference.

(2) *Acanthopora spicifera*

The case of *A. spicifera* from Aklan and Capiz follows that of *G. acerosa* as far as the effects of ecological factors on their gross morphological features are concerned. However, pH, salinity and water temperature are factors hardly to be considered vital in the instant case. The type of substrates found in the two study areas provide a better clue to explain the growth and development of the plant. *A. spicifera* from Aklan grows best in the lower intertidal zone and are relatively taller at 150 (–200) cm. The plants are erect and profusely branched, robust, with

well-developed digitate hold-fast anchored firmly on rocky bottom. The colors are predominantly dark purple with shades of green specially becoming green in the shallower portion of the intertidal. Likewise, the texture is consistently membranous. The same plants found in Capiz were few in number/population, short and frail-looking. They barely reach 110 cm tall, bearing few slender branches, but rather softer in texture. Again, the presence of hydrogen sulfide in the siltish bottom attributes to the relatively poor growth and development of the *A. spicifera* in Capiz.

Field interview

Similar set of questions were randomly posted to inhabitants of the coastal towns/barrios of Aklan, Antique, Capiz and Iloilo which produced almost identical answer. Thus:

1. *Presence of Sea Vegetable Algae Species.* We received very limited information on this matter. The abundance of sea vegetable algae species in Panay Island has remained unknown as to their uses. Of the nationally known sea vegetable only the following with local names are known to the inhabitants interviewed, so far.

A. Class Chlorophyceae
   a. *Caulerpa peltata* var. *macro-disca*
      - "laba-laba"
   b. *C. lentillefera*
      - "laba-laba"
   c. *C. racemosa* (including varieties)
      - "laba-laba";
      - "lato"

B. Class Phaeophyceae
   (no knowledge as to presence of edible species)

C. Class Rhodophyceae
   a. *Gracilaria verrucosa* - "gulaman"
   b. *Gelidiella acerosa* - "gulaman"
   c. *Eucheuma spp.*
      (E. spinosum, E. striatum
      and *E. cottinii* - "gulaman"
      - "guso"

2. *Uses and Methods of Preparations.* The only known use of the sea vegetable algae species mentioned above is as food for man. The method of preparation is in the form of salad. Another and so far, the most popular is to eat it fresh with broiled sweet potato.

3. *Occurrence of Sea Vegetables.* The highly priced sea vegetable to the inhabitants of Panay Island is *Caulerpa peltata* var. *macro-disca.* Years ago this

green sea vegetable used to abound in the Tinagong Dagat (hidden sea) at the back of the municipality of New Washington. The habitat, more marine than brackish even in the presence of the Lagatik River which mixes with the marine water, started to lose its *C. peltata* var. *macrodisca* population with the constructions of several fishponds in the vicinity of the Tinagong Dagat. The townfolks attributed the disappearance of this sea vegetable to the continued use of herbicides and pesticides by fishpond owners. They believe that these synthetic compounds have detrimental effects to the growth of the sea vegetable. To date, the only supply of *C. peltata* var. *macro-disca* comes from Estancia, Iloilo and a small amount from Sapian, Capiz. Other species of *Caulerpa* come from Capiz province and from the nearby province of Palawan.

So far, there are no traces of *Caulerpa peltata* var. *macro-disca* in both the northern and southern portions of Antique. However, the apparent absence of this popular green sea vegetable alga in Antique is ably compensated by the presence of the green algal species of *Caulerpa, Ulva, Enteromorpha* and *Codium;* brown *Hydroclathrus, Colpomenia,* and *Sargassum* and Gracilaria, Eucheuma, Hypnea, Liagora Acanthopora and *Laurencia* to cite some. Similarly, is the case of the collecting areas of northern Aklan.

4. *As Regard Seasonality of Some Sea Vegetables.* The Antiqueños interviewed generally believe that season does not affect the occurrence of sea vegetable algae. Thus, it is during the dry months (summer) when sea vegetable algae like *Caulerpa* and *Gracilaria* are harvested and sold in the markets.

Comparatively, however, and owing to the natural growth of *Caulerpa peltata* var. *macro-disca* only in select parts of N. Iloilo and S. Aklan, sea vegetable algae as food is popular in the former provinces than in Capiz and Antique.

Summary

The three-year project provided the following results:

1. A total of sixty-one (61) species of sea vegetable algae were collected and identified from the four provinces in Panay Island. There are twenty (20) species of green, nine (9) species of brown and thirty-one (31) are red species.
2. There is hardly significant variations in ecological parameters, viz., surface water temperature, hydrogen ion concentration and salinity randomly taken in the four provinces. Conversely, are their insignificant effects on the morphologies and/or growth and development of the sea vegetable algae observed. However, topography and type of substrates appear to show certain degree of influence in the mode of growth and the general gross morphology of test algal species.
3. The most popular sea vegetable algae accepted as food in the island are the green *Caulerpa peltata* var. *macro-disca, C. lentillefera* and *C. racemosa* including varieties. There are no reports as to edible brown algae though these are present in the four provinces. The reds have the most representations, but

only *Eucheuma spinosum* and *E. striatum* are eaten by the inhabitants. *Gracilaria verrucosa* and *G. coronipifolia* are hardly known for their acceptability as food.

4. The occurrence of sea vegetable algae showed negligible variations as to the time/season of observation in Aklan and Antique. Except for *Hydroclathratus clathratus*, both the green and red test species revealed similar results as to time of maturity and development of reproductive parts viz., mature and fertile during the dry season and young to juvenile during the wet season.
5. Part of the ecological study was to determine the algal species growing in association with the sea vegetable species. There were a total of fifty-six (56) species distributed as follows: seventeen (17) green, thirteen (13) brown, and twenty-six (26) red algal species, which were reported separately.
6. The plan to conduct test culture of *Eucheuma* species programmed for year III of the project had to yield to logistic insufficiency.

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IN VITRO HEPATIC MICROSOMAL ACTIVATION OF CHLORINE-SUBSTITUTED NAPHTHOQUINONE PESTICIDE DICHLONE IN CHANNEL CATFISH *ICTALURUS PUNCTATUS* L.

Armando A. Andaya
Department of Biology, College of Science
De La Salle University, Taft Ave., Manila, Philippines

ABSTRACT

The possible involvement of oxygen free radicals in the mode of toxic action of the pesticide dichlone (2,3-dichloro-1,4-naphthoquinone) to yearling channel catfish, *Ictalurus punctatus* L. was investigated. The median lethal concentration (LC50) of dichlone was estimated at 42 ug/L at the end of 96 hr exposure of the test fish species to the toxicant. The detection of NADPH-cytochrome P450 reductase activity in the hepatic microsomes indicated the presence of a superoxide-generating system in catfish liver. This microsomal reductase activity was found to be stimulated dose-dependently by dichlone with concomitant increase in the rates of superoxide-mediated reduction of exogenous cytochrome c and augmentation of oxygen uptake. The biochemical parameters studied were taken as indirect measures of superoxide generation via enzymatic activation of dichlone in catfish liver. These observed biochemical reactions lend support to the claim that free radicals may, at least in part, be involved in initiating toxicity of dichlone to channel catfish.

Introduction

Much information gaps exist about the toxicology of the quinone pesticide dichlone (USEPA, 1980). Dichlone (2,3-dichloro-1,4-naphthoquinone) is known to possess fungicidal (Owens, 1953), herbicidal (Sikka *et al.*, 1972), and algicidal properties (Sweig *et al.*, 1972). Studies on the environmental impact of dichlone application on non-target aquatic species are practically nil. While dichlone had been found to be acutely toxic to a number of fish species (USEPA, 1981), and other aquatic invertebrates (Metelev *et al.*, 1983), its mode of toxic action to non-target species has not been given attention. The present study was conducted to provide information on existing gaps regarding the mechanism of toxicity of dichlone to aquatic animals.

Dichlone possesses a quinone structure which confers on the molecule the capacity to participate in oxidation-reduction (redox) reactions. According to Pryor (1982), environmental toxins capable of redox cycling stimulates the partial reduction of molecular oxygen with concomitant generation of reactive oxygen free radical species. Handa and Sato (1975) had earlier demonstrated the involve-

ment of the flavin enzyme NADPH-cytochrome P450 reductase in the microsomal superoxide generation mechanism. Oxygen free radicals, such as, superoxide anion (O_2) and hydroxyl anion (OH.), and the oxidant hydrogen peroxide (H_2O_2), are known to cause widespread cytotoxic effects including lesions of DNA, inactivation of enzymes, alteration of cell redox status, and disruption of cell membrane permeability (Freeman and Crapo, 1982).

It seemed logical to assume that dichlone could exert its toxicity to fish via the creation of oxidative stress. If it were possible to detect a superoxide-generating system in fish and to quantitatively describe superoxide production, then it could be hypothesized that free radical-mediated injury might have been involved, at least in part, in the mode of toxic action of dichlone to any sensitive fish species. This study tested the validity of the free radical hypothesis of dichlone toxicity.

Materials and Methods

Test compound

Analytical grade dichlone was obtained from Eastman Kodak Company (Rochester, NY). On the day of use, known amount of dichlone was dissolved in acetone and nominal concentrations prepared with dilution water.

Test animal

Yearling channel catfish obtained from commercial hatcheries in North Carolina (Buck Trails Fish Hatchery, Lake Waccamaw and Cape Fear Fish Farm, Raleigh) were transported to the laboratory and kept in 50 L glass aquaria containing carbon-filtered tapwater for 4-6 days prior to dosing. Fish were fed to satiation with Purina Floating Catfish Chow (cage formula) every other day at least 1 hr prior to tank water replacement. During acclimation and exposure, tanks were maintained on a 12 hr dark, 12 hr light photoperiod and aeration was continuously provided. All aquaria were soap-washed, disinfected with hypochlorite, and thoroughly rinsed prior to filling with dilution water.

Acute toxicity tests

Static 96-hr bioassays were conducted for dichlone according to the procedures of Stephan (1975). Six nominal concentrations and a control consisting of dilution water only were set. No noticeable changes in behavior and gross body appearance of catfish were noted in the control tanks that received acetone in equal amount added to the test tanks. During exposure, tank water was changed completely with fresh medium every 48 hr. Average values of water quality parameters were as follows: temperature = 20°C, pH = 7.38, dissolved oxygen = 8.06 mg/l, total hardness = 26mg/l, and total alkalinity = 37 mg/l. Seventy fish were randomly distributed among seven aquaria (10 fish per treatment). The number

of dead fish were noted after 24, 48, 72, and 96 hrs of initial exposure to the toxicant. Death was indicated by failure of the fish to respond to gentle prodding with a glass rod.

Estimates of the median lethal concentrations (LC50), slope function (S), and their respective confidence limits (CL) were calculated according to Litchfield and Wilcoxon (1949). Chi-square tests were performed to test the goodness of fit of the dose-percent effect curves.

Microsome preparation

Whole livers were excised from channel catfish (30 – 60 g body weight), blotted dry, and weighed. The livers were homogenized in 1:4 w/v ice-cold 0.05 M Tris/0.20 M sucrose buffer by passing 4 – 5 times a mechanically driven Teflon pestle into a glass homogenizer vessel. The homogenate was centrifuged at 4°C for 20 min at 10,000 x g. The supernatant obtained was subsequently centrifuged at O°C for 60 min at 105,000 x g to yield a microsomal pellet. The pellet was washed and resuspended in ice-cold 1.15% KCl equal to the original weight of the liver. The protein content of the microsomal suspension was measured by the method of Lowry *et al.* (1951). Microsomes were stored in a freezer at -70°C in 0.5 ml portions for later use.

Biochemical analyses

Superoxide generation by catfish liver microsomes in the presence of dichlone was measured indirectly in terms of the rate of superoxide dismutase-inhibitable reduction of exogenous cytochrome c and the rate of stimulation of cyanide-insensitive oxygen uptake.

1. *Cytochrome c reduction assay.* Reduction of exogenous cytochrome c was measured according to Omura and Sato (1964). Microsomes were incubated at 24°C in a final volume of 1 ml reaction mixture containing in final concentrations: 200 uM cytochrome c, 100 uM NADPH, 10 uM KCN, 0.20 mg microsomal protein, and specified amounts of dichlone in 0.3 M Na/K phosphate buffer, pH 7.0. Absorbance at 550 nM was noted to determine the extent of cytochrome c reduction. A molar extinction coefficient of 1.9×10^4 $mM^{-1} cm^{-1}$ was used to calculate the concentration of reduced cytochrome c. Values were reported in nmoles per min per mg protein.

The amount of superoxide generated from the interaction of dichlone with liver microsomes was estimated from the difference in rates of reduction of cytochrome c before and after the addition of known amounts of SOD.

2. *Oxygen consumption assay.* Polarographic measurements of oxygen consumption were performed using the YSI Oxygen Monitor provided with a Clark-type oxygen electrode (Yellow Spring Instrument Co., Yellow Spring, OH). Oxygen consumption of microsomal suspension was measured at 24°C in 3 ml reaction mixture consisted of 10 mM NADPH, 10 mM KCN, and 0.2 mg micro-

somal protein in 0.3 M Na/K phosphate buffer, pH 7.0. Values were reported in nmole min per mg protein.

Results and Discussion

Data on the acute toxicity of dichlone to yearling channel catfish are presented in Table 1. Initial mortality was noted within 5 hr post-dosing with 50 ug/L. Complete mortality was attained within 20 hr post-dosing with 200 ug/L. From the probit of kill, dichlone was estimated to have an LC50 of 42 ug/L at the end of 96 hr exposure of catfish to the toxicant. A gradual decay in magnitude of LC50's estimated at 72 and 96 hr exposures were significantly lower than those recorded at earlier time points (p0.05).

The estimated LC50 of dichlone with channel catfish approximated those reported by Johnson and Finley (1980) for rainbow trout, *Salmo gairdneri* (49 ug/L) for bluegill, *Lepomis macrochirus* (41 ug/L). However, dichlone toxicity was relatively lower for the fathead minnow, *Phoxinus phoxinus* (150 ug/L). Similarly, dichlone was less toxic to amphibians (USEPA, 1980) and several invertebrate species (Metelev *et al.*, 1983) compared to the fish species tested. Concentrations ranging from 500 to 800 ug/L dichlone found to effectively inhibit the growth of

Table 1. Acute toxicity of the quinone pesticide dichlone (2,3-dichloro-1,4-naphthoquinone) to yearling channel catfish *Ictalurus punctatus* L.

Exposure conc.	*Fish per tank*	*Dead fish/tank after* 24 hr	48hr	72 hr	96 hr	*Percent mortality after 96 hr*
200 ug/L	10	10	10	10	10	100
100	10	8	10	10	10	100
72.5	10	3	7	9	10	100
50.0	10	1	2	6	7	70
37.5	10	0	1	2	3	30
25.0	10	0	0	1	2	20
0.0	10	0	0	0	0	0
LC50 ug/L		82	59	45	42	
95% confidence limits						
lower limit		61	50	36	30	
upper limit		111	69	56	59	
Slope of probit line		1.64	1.29	1.23	1.16	
95% confidence limits						
lower limit		1.19	1.11	1.23	1.16	
upper limit		2.24	1.50	1.66	2.61	
Chi-square value*		0.16	3.05	0.91	1.49	

*p > 0.05

filamentous algae and blue-green algae in fish ponds (Pal and Gopalakrishnan, 1968) would be extremely lethal to the fish species mentioned above. In fact, these investigators noted total mortality of fry of carp, *Cyprinus carpio* within 1 hr of exposure to these concentrations. While variation in relative sensitivity to dichlone was noted among fish species, the mode of toxic action of this pesticide is not known.

At the outset, it was necessary to demonstrate the existence of a superoxide-generating system in channel catfish in order to be consistent with the free radical hypothesis of dichlone toxicity. It had been shown earlier that such superoxide-generating system is localized in the microsomes of mammalian liver and that an NADPH-cytochrome P450 reductase forms the center of its activity (Handa and Sato, 1979). Various fish species possess a comparable flavin enzyme system as a functional unit of the mono-oxygenase biotransformation mechanism involved in the metabolism of endogenous substrates and environmental toxins (Chambers and Yardbrough, 1976).

The actual presence of a superoxide-generating system in channel catfish was demonstrated in the present study. By describing the reductase activity of liver microsomes it was likely that superoxide would be generated in the system. Because the flavin enzyme utilizes NADPH as a required source of electron for the partial reduction of molecular oxygen to superoxide anion radical, it was decided to investigate the effect of NADPH deficiency or its absence on the specific activity of the enzyme. By adding $NADP^+$ to the reaction mixture, an allosteric interaction with the enzyme could prevent NADPH consumption leading to reduction in the rate of reductase activity of the enzyme. As shown in Fig. 1, the addition of 100 um $NADP^+$ to the reaction mixture was sufficient to reduce the activity of the flavo-enzyme by as much as 82.65% of the rate recorded when NADPH alone was present. The feedback end-product inhibitory effect of $NADP^+$ is a specific test for the presence of NADPH-dependent cytochrome P450 reductase enzyme system (Strobel *et al.*, 1978).

The specific activity of catfish liver microsomal flavin enzyme NADPH-cytochrome P450 reductase was estimated to range between 17.09 to 18.89 nmoles per min per mg protein. These values are close to those reported for rainbow trout, 14.7 – 22.1 nmoles per min per mg protein (Stegeman and Chevron, 1980) but are comparatively lower than those recorded for marine fish species, 49.3 to 94.9 nmoles per min per mg protein (James and Bend, 1980). However, the enzyme activity of catfish liver reductase was relatively higher than those of crustaceans, 4.3 – 5.2 nmoles per min per mg protein (James *et al.*, 1979).

The activity of the NADPH-cytochrome P450 reductase in liver microsomes of channel catfish was found to be inducible by environmental pollutants (Fingermann *et al.*, 1983). As shown in this study, the interaction of dichlone with catfish liver microsomes was accompanied by dose-related increase in enzyme activity as expressed in terms of rate of reduction of exogenous cytochrome c (Table 2). Close to a 10-fold increase in cytochrome c reduction was noted with increase in

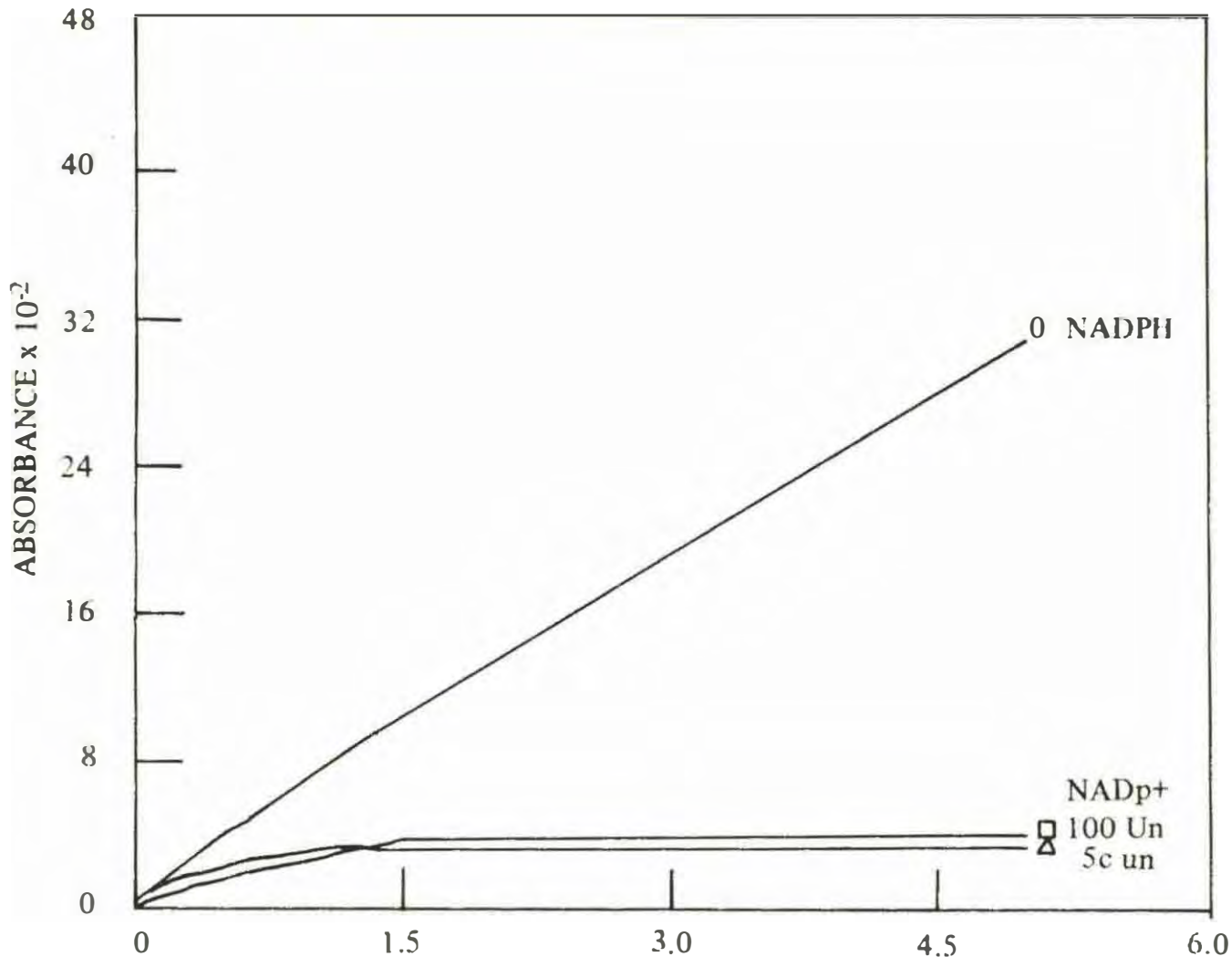

Fig. 1. Time-related inhibition of dichlone-stimulated reduction of cytochrome c in the presence of $NADP^+$ in the microsomal suspension of catfish liver.

Table 2. Dichlone-mediated reduction of exogenous cytochrome c and superoxide generation in the microsomal suspension of catfish liver. Values are means ± std. dev. of (n) number of determinations.

Additions	*cytochrome c reduction nmole/min/mg protein*	*superoxide generation nmole/min/mg protein*
o uM Dichlone	18.86 ± 1.77 (6)	
1	19.43 ± 0.88 (6)	
3	21.36 ± 1.90 (6)	
5	23.65 ± 1.04 (6)	
10	25.34 ± 2.64 (6)	
50	25.96 ± 2.06 (6)	
50 uM dichlone		
+ 15 ug/ml SOD	24.83 ± 0.66 (4)	1.13
30	23.80 ± 0.46 (4)	2.16
90	20.00 ± 1.10 (4)	5.96
150	17.57 ± 0.90 (4)	8.39
240	16.18 ± 3.16 (4)	9.78

nominal concentrations of dichlone in the reaction medium. This suggest that dichlone can induce the superoxide-generating system via the NADPH-cytochrome P450 reductase in the liver microsomes. To demonstrate that such assumption was likely, increasing amounts of SOD were added to the reaction mixture. Since SOD acts specifically on superoxide anion by dismutating it to $H_2O_2 + O_2$, then its presence in the reaction medium can inhibit the superoxide-mediated reduction of cytochrome c by scavenging the $O_2^{\cdot -}$ away from cytochrome c. Thus, the rate of reduction of cytochrome c could be inhibited in the presence of SOD. This reaction was demonstrated in the present study as shown in Table 2. Because the reduction of cytochrome from its ferric (Fe^{3+}) to the ferrous state (Fe^{2+}) via superoxide pathway proceeds at stoichiometric ratio of 1:1, the amount of superoxide anion generated in the presence of dichlone can be estimated from the rate of reduction of cytochrome c. Data presented in Table 2 revealed the increasing amount of $O_2^{\cdot -}$ generated with the addition of SOD into the reaction mixture.

The microsomal flavin enzyme NADPH-cytochrome P450 reductase also possesses an oxidase activity that is stimulated in the presence of quinone. It was shown that quinones can facilitate the flow of electrons from NADPH to molecular O_2 via the cyanide-insensitive respiratory pathway with concomitant generation of $O_2^{\cdot -}$ (Hassan and Fridovich, 1977). Thus, the rapid rate of oxygen consumption of fibroblast cells (Saxena *et al.*, 1974) and of intact mitochondria (Pritsos *et al.*, 1982) suggested the extent of diversion of electron flow through this respiratory pathway. Bachur *et al.* (1978) had demonstrated that the augmentation of oxygen consumption by quinone anti-cancer drugs was accompanied by generation of large quantities of $O_2^{\cdot -}$.

It is evident from the data presented in Table 3 that dichlone did stimulate the rate of consumption of oxygen in the microsomal suspension of catfish liver. This marked induction in O_2 uptake is consistent with the expected increase in $O_2^{\cdot -}$ generation in the microsomal system.

While both cytochrome c and molecular O_2 are suitable electron acceptors during the microsomal activation of dichlone in catfish liver, cytochrome c proved

Table 3. Dichlone-mediated consumption of oxygen in the microsomal suspension of catfish liver. Values are means ± std. dev. for (n) number of determinations

Dichlone addition	*Oxygen consumption nmole/min/mg protein*
0 uM	10.22 ± 0.47 (5)
20	29.86 ± 2.14 (6)
50	48.83 ± 7.16 (4)
100	65.36 ± 3.76 (4)
200	68.34 ± 7.99 (4)
500	67.44 ± 8.13 (4)
1000	68.11 ± 6.44 (4)

to be more effective in receiving electron via the flavin enzyme system than dismolecular O_2. The dismutation reaction utilizes half of the O_2 consumed whereas the reduction of cytochrome c via $O_2^{\cdot -}$ did not cause any consumption of O_2.

Summary and Conclusion

1. Dichlone is acutely toxic to yearling channel catfish under conditions of short-term laboratory exposure. Percent survival decreased dose-dependently with nominal concentration of dichlone in solution. The 96-hr LC50 was estimated at 42 ug/L.

2. Spectrophotometric analysis indicated the specific activity of the flavin enzyme NADPH-cytochrome P450 reductase in the microsomal fraction of catfish liver.

3. The reductase activity of the flavin enzyme was induced dose-dependently by dichlone addition as demonstrated by increased rates of reduction of exogenous cytochrome c. In addition, dose-dependent augmentation of oxygen uptake of liver microsomes was also noted.

4. The production of superoxide anion was demonstrated following *in vitro* interaction of dichlone with catfish liver microsomes.

5. The results obtained from this study are consistent with the hypothesis of free radical toxicity of the quinone pesticide dichlone to yearling channel catfish. However, it should be stated that other mechanism of toxicity may also be involved in the lethal effects of this pesticide to the test fish species.

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INTERSPECIFIC RELATIONSHIPS IN GENUS *ORYZA* OF THE SOUTHEAST ASIA-PACIFIC REGION

Joventino D. Soriano
Institute of Biology
University of the Philippines
Diliman, Quezon City, Philippines

ABSTRACT

The chromosomal structure, crossability, hybrid seed viability and morphological features of four species of genus *Oryza* believed to be indigenous to the region were studied. Except for tetraploidy in *O. minuta*, no other marked karyological difference was found among the various species. Interspecific crosses and hybrid seed viability were good only in certain combinations while the four species were more or less distinct morphologically. Based on the foregoing criteria, the *Oryza* species were classified into two regional groups.

Introduction

The genus *Oryza* is presently known to consist of some 21 or more species distributed in different parts of the tropical and sub-tropical regions. Two economically important crop plants belonging to this group are the so-called Asian rice *(O. sativa* L.) and African rice *(O. glaberrima* Steud.) The rest of the *Oryza* species are glass-like wild rices. The cytogenetic relationships among the cultivated and wild rices have been the subject of great interest among many plant investigators during the last 80 or so years in relation more or less to the origin of the staple crop species and understanding of a number of problems related to their improvement such as occurrence of intra- and interspecific sterility and the frantic search for new and useful germplasm needed for rice breeding work.

While previous studies on species interrelationships in the *Oryza* group have mostly dealt with plants occurring in distant geographical habitats, a regional approach might provide a more meaningful and undoubtedly less speculative analysis of the problem. The ancestors of present taxa then growing sympatrically at some given time invariably had a better opportunity for gene exchange then between those found in very distant lands. Many recent workers have recognized the important influence of environmental conditions in the success of interspecific crosses and their hybrids.

Materials and Methods

The four species of genus *Oryza* used in this study were the Australian wild rice (*O. australiensis* Domin.), *O. officinalis* Wall., *O. sativa* Linn., and the Philippine wild rice (*O. minuta* Presl.) Seeds of these species were originally obtained over a period of years from the International Rice Research Institute in Los Baños, Laguna through the kindness of Dr. Te-Tzu Chang and National Institute of Genetics in Misima, Japan. For *O. sativa* L., variety Peta, an *indica* rice was used. Seeds of a fifth species, *O. schlechterii* Pilger, reportedly an inhabitant of New Guinea, were not available for the study. Seeds of the four species were sprouted on moist tissue paper in petri dish and transplanted in pots at the Botany Experimental Garden, U.P. Diliman, Quezon City. Urea fertilizer was applied one week after tansplanting. Reciprocal crosses were made following the hot water emasculation method. The florets were bagged after artificial pollination. Data on crossability and hybrid seed viability were obtained. Karyological analysis was done on selected metaphases in root-tips of germinating seeds of the four species employing a modified squash technique using aceto-carmine stain. Microscopic measurements were done with a micrometer and photomicrographs were magnified about 20,000 times.

Results and Discussion

Karyological features. Data on chromosome number, total chromosome length, chromosome morphology and number of satellites are shown in Table 1. Three diploid species such as *O. australiensis, O. officinalis* and *O. sativa* were found to have the same chromosome numbers of 2N=24, while *O. minuta,* a tetraploid. has 4N=48. The results of the present chromosome counts confirm those of earlier reports (Rau, 1929; Sampath and Ramanathan, 1949; Hu, 1964) based on root-tip examinations. Although chromosome counts have also been made on meiocytes, particularly the pachytene stage where chromosomes are reportedly much longer and bigger than the mitotic figures (Morinaga, 1964; Shastry, 1964; Shastry and Rao, 1961: Li *et al.*, 1963) meiotic chromosomes do not stain very well and their centrometric attachments cannot be clearly located (Hu, 1964). A previous attempt by Yao, *et al.*, (1958) to analyze cryptic structural hybridity in intervarietal crosses using pachytene analysis did not succeed reportedly because of the difficulty of locating the centromeres. The mitotic genome, on the other hand, reflects the true somatic chromosome number directly, especially with the availability of suitable techniques some of which nowadays require very minimal pre-staining treatments (Marks, 1973).

Total chromosome lengths in the three diploid species ranged from approximately 36.2-41.3 micra. The differences in total chromosome lengths did not vary much from species to species although the chromosomes of *O. sativa* appeared to be

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Table 1. Some karyological features of four *Oryza* species

Species	*Somatic chromosome No.*	*Total chromosome length (micra)*	*No. of chromosomes*			*No. of satellites*
			Median	*Sub-median*	*Telo-centric*	
O. australiensis Domin.	24	41.8 ± 0.56	10	10	4	2
O. officinalis Wall.	24	39.5 ± 0.72	10	10	4	2
O. sativa Linn.	24	36.2 ± 0.83	10	10	4	2
O. minuta Presl.	48	–	–	–	–	3-4

slightly smaller than those of *O. australiensis* and *O. officinalis*. This observation appears to support the view that *O. sativa* is probably a more recent derivative than *O. australiensis* and *O. officinalis* since long chromosomes indicate a more ancient origin (Soriano, 1985). The domestication of *O. sativa* had probably to wait for the occurrence of some gene mutations in these or other wild rices for such characters as large grains, non-shattering panicles, uniform maturity and other features of value to man.

Measuring, much less identifying, the individual chromosomes of the four *Oryza* species was not presently possible inspite of the recent encouragements from Khan (1975) as well as Kurata and Omura (1978), due mainly to the very small chromatic figures and absence of suitable chromosome landmarks or markers. Exceptions were the longest chromosomes (Chromosome 1), shortest chromosomes (Chromosome 12), and a pair of satellited chromosomes (Chromosome 10) in the three diploid species which with unending patience could be handily identified from the many mitotic figures. Li *et al.*, (1963) reports having encountered a similar predicament with mitotic chromosomes of *Oryza* species. A similar difficulty of identifying individual chromosomes of *O. sativa* due to absence of suitable markers was previously encountered some 27 years ago (Soriano, 1959) in connection with the identification of chromosomal segments involved in interchanges in X-irradiated material. On the other hand, Shastry *et al.*, (1960) encouraged the adoption of pachytene analysis on the *Oryza* species.

It was not likewise possible to determine the total chromosome length of *O. minuta* which has twice the number of chromatic figures even in the slightly enlarged mitotic cells of the tetraploid, both in materials that were pre-treated and not pre-treated with colchicine.

Regarding chromosome morphology, no apparent difference was found in the form or shape of the chromosomes in the three diploid species. In well-spread out and stained metaphase cells, there were 10 V-shaped or metacentric chromosomes with median centromere insertions, 10 J-shaped or acrocentric chromosomes with sub-median centromeric positions, and four rod-shaped chromosomes with more or less terminally attached centromeres. The number and attachment of the

two satellites appeared somewhat similar in the three diploid species, each satellite being attached to or near the tip of a short chromosome (Chromosome 10) by a relatively long and distinct stalk. In a previous paper, Kurata and Omura (1982) reported similar chromosome lengths and centromere locations in *O. officinalis* and *O. sativa*. In the tetraploid species, *O. minuta*, from three to four satellites were found in root-tip cells. As a good majority of the metaphases had four satellites each, it was assumed that one of the satellites in the other cells may have been accidentally deleted.

Crossability between species

Table 2 shows the degree of crossability of the four species as percentage of successful crosses. Six reciprocal crosses were made which in most cases produced grains bearing features of one parent or a combination of both parents, except when *O. sativa* was the ovule parent when the hybrid seeds exhibited mainly the characteristics of cultivated rice. Deformed or empty caryopses were not considered good grains. Where the pistillate parent had the shattering character, as is the case with all the wild rices, care was observed to avoid any loss of grains by bagging the panicle.

Based on the data, the cross *O. officinalis* x *O. minuta* was the most compatible or crossable combination while *O. australiensis* x *O. officinalis* was the least successful, with only about 2.59% of the crosses producing good grains. Li *et al.*, (1963) successfully crossed *O. australiensis* with *O. officinalis* and other wild rice species but seed viability was similarly very low. In the cross between *O. sativa*

Table 2. Crossability and hybrid seed viability in four *Oryza* species

Reciprocal crosses	*Total crosses*	*Total good grains*	*% cross-ability*	*No. of viable seeds*	*% germination*
O. australiensis x *O. officinalis*	116	3	2.59	0	–
O. sativa x *O. australiensis*	145	16	11.03	3	18.75
O. australiensis x *O. minuta*	122	4	3.24	0	–
O. sativa x *O. officinalis*	133	2	1.50	0	–
O. officinalis x *O. minuta*	104	11	10.57	4	36.36
O. sativa x *O. minuta*	147	8	5.44	0	

and *O. minuta*, grains were formed only when cultivated rice was the pistillate parent. This is believed due to differences in floral structure where *O. minuta* has very small spikelet parts.

The capacity of two species to cross and produce hybrid seeds is invariably an indication of close genetic affinity between them. The integrity of species is a fundamental and universal concept and even among very closely related species, only a low degree of crossability is expected. It is probable that crossability is dependent on a number of morphological, physiological and genetic barriers between the two parental species. Closely related species in general would have fewer of these differences than distantly or completely unrelated forms.

In the four *Oryza* species, the percentage of successful crosses after artificial pollination ranged from approximately 2.59-11.03%. Natural hybridization would probably be radically less successful considering possible differences in pollen dehiscence and various barriers to fertilization and embryo formation. A crossability value of less than 10% would probably considered low under artificial hybridization as most of the unfavorable influence of environmental conditions have been minimized or entirely avoided.

Hybrid seed viability

The seed viability data (Table 2) may be used to indicate further the genetic affinity between two parental species. The capacity of a hybrid seed to germinate is undoubtedly related to the degree of cross compatibility between the two parents. None of the F_1 hybrid seeds resulting from the cross *O. australiensis* x *O. officinalis* was viable. Shastry *et al.*, (1961) found the F_1 hybrid plants from such cross to be completely sterile.

If an average of 14.6% germination of hybrid seeds is considered low (Morinaga, 1943), hybrid seed viability of about 18.75% from crosses of *O. australiensis* x *O. sativa* and 36.76% from *O. officinalis* x *O. minuta* are indeed good indications of genetic affinity between the parental species. With very low percentages of germinable hybrid seeds in most of the interspecific crosses, it is likely that much of the variability existing among these rices are probably due more to mutation than gene exchange. Mutations occur continuously in somatic and reproductive cells and may have accumulated throughout the long period of time the species has existed. Their perpetuation and expression of their phenotypic effects are undoubted subject to the various forces of natural selection.

Morphological features

The main vegetative features of the four *Oryza* species are shown in Table 3. The four species are phenotypically distinguishable by their growth habits. *O. australiensis* is a medium-sized plant with spreading purplish culms and long-peduncled panicle; *O. officinalis* is a relatively low, sparse plant with a few spreading light-green culms bearing slender panicles with brittle peduncles; *O. sativa* is a

tall green plant with many erect culms and leaves, compact panicles and large grains, and *O. minuta* is a very low-growing plant with radiating, almost procumbent, culms bearing dark-green leaves, fine panicles and very small spikelets. In a study of species interrelationships in Genus *Oryza*, Oka (1964) suggested that species resembling each other morphologically are highly correlated genetically.

Only *O. sativa* has compact and non-shattering panicles while all the wild rices have heavily shattering panicles. No sooner has a grain of wild rice attains a grayish or blackish color upon maturity than it falls to the ground with the slightest movement of the panicle. Thus, a wild rice panicle eventually becomes bereft of its grains as the plant approaches maturity. Indeed, the non-shaterring character of cultivated rice is probably one of the most important mutations that occurred in the origin of the staple crop. Among the three wild rice species, *O. australiensis* produced the biggest grains while *O. officinalis* and *O. minuta* gave the smallest grains.

In a paper on the origin of cultivated rice, Richharia (1960) points out that some primitive features in genus *Oryza* are a diploid genome, long stamen, large ligules, tall plant growth, perennial habit, large leaves, long spikelets, pigmented stigma, marked trichomes and awns and wide geographical distribution. Based on these criteria, *O. minuta* would come out as a relatively recent form. Morinaga (1943) classified *O. minuta* and other species as members of Section *Sativa* Roschev. of genus *Oryza* based mainly on morphological similarities such as presence of intersecting rows of small tubercles in their flowering glumes and the linear-lanceolate shape of their empty glumes. However, many other species groupings in genus *Oryza* were based solely on genome analyses determined from chromosome associations in the F_1 hybrids (Katayama, 1966; Oka, 1964; Shastry, 1964;

Table 3. Some vegetative features of four *Oryza* species

Plant features	*O. australiensis*	*O. officinalis*	*O. sativa*	*O. minuta*
Growth habit	Medium plant, many spreading culms	Low plant, sparse growth, spreading culms	Tall plant, erect culms and leaves	Very low plant with radiating culms
Culms				
Length range (cm..)	35-50	50-30	55-75	10-25
Diam. range (cm)	6-10	3-5	8-15	3-5
Angle	Open	Open	Erect	Spreading
Color	Purplish	Light-green	Green	Dark-green
Leaf blade				
Length range (cm.)	36-48	10-20	35-62	10-24
Width range (cm.)	10-18	4-8	14-20	5-8
Color	Purplish tips	Light-green	Green	Dark-green

Sampath and Rao, 1951). This aspect of the interspecific relationships of the four *Oryza* species is under study and will be presented in future reports.

Regional species groups

Although the genus *Oryza* is world-wide in distribution, much of the uncertainties in species interrelationships are probably due to features which have undoubtedly been brought about by selection factors typical of the geographic region of their origin. The data on somatic chromosomes, crossability, hybrid seed viability and morphological features presented above indicate that although species differences occur and are profound enough for them to retain their individuality and integrity as species, they possess certain vital similarities and affinities that will allow their being classified into regional groups. Their sympatric existence for long periods of time has no doubt played a major role in preserving common morphological features as they have been more or less subjected to similar forces of natural selection. They possess a certain degree of crossability and germinability of their hybrid seeds that have more or less allowed a certain amount of gene exchanges between the different species.

The four *Oryza* species are thus classified into two groups based on the foregoing criteria, as follows:

Regional Group I: *O. australiensis*
O. sativa

Regional Group II: *O. officinalis*
O. minuta

Table 4. Reproductive structures of four *Oryza* species

Reproductive structure	*O. australiensis*	*O. officinalis*	*O. sativa*	*O. minuta*
Panicles				
Type	Open	Open	Compact	Intermediate
Size	Long	Short	Medium	Short
Shattering	Shattering	Shattering	Non-shattering	Shattering
Spikelets				
Length range (min.)	4-5	3-4	10-12	2-3
Width range (mm.)	3-6	2-3	4-5	2-3
Grains				
Color	Black	Black	Brown	Black
Awn length	Long	Medium	Awnless	Long
100-grain wt. (gm.)	4-6	2-3	6-8	2-3

As no major karyological difference was found among the diploid species, the main criteria for the above relationships are crossability, germinability of hybrid seeds and similar morphological features. Thus, *O. australiensis* and *O. officinalis* belong to different groups due to their low percentage of crossability, inviability of their hybrid seeds and distinctly different morphological features. Using the same measures, *O. officinalis* and *O. minuta* gave a relatively high percentage of crossability of 10.57% and high viability of their hybrid seeds. Moreover, they are morphologically alike in panicle size and grain size.

The two regional groups given above were found to follow those proposed by previous workers (Morinaga, 1964; Sampath and Rao, 1951; Richharia, 1960; Katayama, 1966) based mainly on genomic analysis where *O. australiensis* and *O. sativa* were placed under the *Sativa* group of genus *Oryza* while *O. officinalis* and *O. minuta*, the *Officinalis* group.

Summary and Conclusions

1. The number and morphology of chromosomes of three diploid species, *O. australiensis*, O. *officinalis* and *O. sativa* were found to be essentially alike while *O. minuta* is a tetraploid with 4N=48.

2. A relatively high percentage of the crosses, *O. australiensis* x *O. sativa* and *O. officinalis* x *O. minuta*, produced good seeds while other cross combinations gave a low seedset.

3. Hybrid seed viability from the cross *O. australiensis* x *O. sativa* and *O. minuta* x *O. officinalis* was relatively high while seeds from other cross combinations were not viable.

4. In vegetative and reproductive features, plants of *O. australiensis* and *O. sativa* resembled each other more than those of *O. officinalis* and *O. minuta* which likewise appeared similar.

5. The use of chromosomal structure, crossability, seed viability and morphological features in determining possible affinity among the four species is briefly discussed.

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THE INSECTICIDAL ACTIONS OF SOME INDIGENOUS PLANTS WITH SPECIAL REFERENCE TO MAKABUHAI (*TINOSPORA RUMPHII* BOE RL.)

B. Morallo-Rejesus, H.A. Maini and C. M. Garcia
University of the Philippines Los Baños
College, Laguna, Philippines

ABSTRACT

The toxic and/or antifeedant actions of nine plants and the effectiveness of makabuhai in controlling rice insect pests in the field were investigated.

The volatile oils of lagundi (*Vitex negundo*), sambong (*Blumea balsamifera*), oregano (*Coleus amboinicus*) bulak-manok (*Aquatum conyzoids*), and manzanilla (*Chrysanthemum indicum*) were topically toxic to some of the test insects (red flour beetle, common cutworm, corn weevil, lesser grain borer, black army-worm and housefly). All these oils were potent against armyworm. Sambong and lagundi are the most toxic against common cutworm while the former was the most toxic against the red flour beetle. The water extracts of makabuhai was toxic to the green leafhoppers. The volatile oils of lagundi, oregano and sambong were antifeedant.

Laboratory and field studies indicate that the use of makabuhai aqueous extract for seedling root soaking and broadcasting or immersion of its vine are effective in controlling the major insect pests of rice. Makabuhai can substitute for two of the four insecticidal applications recommended for protecting rice against insect damage thereby reducing cost of production and ultimately increasing the net income of the farmer.

Introduction

An increase in agricultural production will continue to be of vital importance in the developing countries. In this context, great demand will be made on plant and post-harvest protection.

Pesticides are often considered to be the most potent control technology against insect pests. However, the large scale and continuous usage of synthetic pesticides created many problems. Among them are contamination of the biosphere, pest resistance and increasing costs. Consequently, farmers in many countries in the tropics like the Philippines cannot afford to buy pesticides. However, due to heavy pest outbreaks on various crops, control is needed to obtain reasonable yields. The presence of many plants in the country and the possibility to obtain extracts by simple and cheap means is a sound basis for conducting studies of the effects of natural products against important pests. Such studies of the senior author have been going on at UPLB since 1976 to isolate and investigate the insecticidal actions of

some indigenous plants and to what extent these could be used for controlling insect pests on agricultural crops.

Two principles from the roots of three marigold (*Tagetes spp.*) species were found to have different toxicities against housefly (*Musca domestica*), diamondback moth (*Plutella xylostella*) and rice green leafhopper (*Nephottix virescens*) (Morallo-Rejesus and Eroles, 1978). The mixture of the two principles produced either a potentiation or antagonistic action depending upon the source and the test insects. These principles were identified as 5-(3-buten-1-ynyl)-2,2' bithienyl and £ -terthienyl by infrared and ultraviolet spectral analysis (Morallo-Rejesus and Decena, 1982). A fraction from the leaves of *Tithonia diversifolia* characterized to be a lactone was found toxic against diamondback moth (Cariño and Morallo-Rejesus, 1982). The crude and semi-purified extracts of black pepper were topically more toxic than malathion against the diamondback moth larvae and adult houseflies and residually toxic on corn weevils, *Sitophilus zeamais* (Javier and Morallo-Rejesus 1982, 1986). The volatile oils from the leaves and flowers of *Lantana camara* and *Caesalpinia pulcherrima* and *T. diversifolia* were topically toxic on *M. domestica*, *Spodoptera exempta*, *Dysdercus cingulatus*, *P. xylostella*, *S. zeamais*, *Rhyzopertha dominica* and *Tribolium castaneum*. The oils were not toxic to *Ostrinia furnacalis* (Morallo-Rejesus and Tantengco, 1986).

This paper reports the toxic and/or antifeedant actions of nine plants and the effectiveness of makabuhai in controlling rice insect pests in the field.

Materials and Methods

Extraction

The leaves of caballero (*Caesalpinia pulcherrima*), lagundi (*Vitex negundo*), manzanilla (*Chrysanthemum indicum*), sambong (*Blumea balsamifera*) oregano (*Coleus amboinicus*), bulak-manok (*Ageratum conyzoides*), timbangan (*Aristolochia elegans*), malaubi (*Aristolochia tagala*) and marigold (*Tagetes erecta*) and the vine of makabuhai (*Tinospora rumphii*) were collected from the experimental garden at UPLB. Leaves and vines were dried to constant weight in the shade. The air-dried leaves or vines were ground to a fine powder (50 mesh) with an electric grinder. Extraction was made by standard procedure (using water or organic solvents/ e. g. petroleum ether, methanol, ethanol). The volatile oil was extracted by steam distillation.

Test insects

The larvae of diamondback moth (*Plutella xylostella*), common cutworm (*Spodoptera litura*), army worm (*Spodoptera exempta*), corn borer (*Ostrinia furnacalis*) and adults of cotton stainer (*Dysdercus cingulatus*), brown planthopper (*Nilaparvata lugens*), green leafhopper (*Nephottetix virescens*), lesser grain borer (*Rhizopertha dominica*) and red flour beetle (*Tribolium castaneum*) were used for the tests.

All insects were reared in the Department of Entomology, University of the Philippines at Los Baños in their natural hosts except corn borer and housefly which were reared in artificial diets.

Preparation of makabuhai aqueous extract

Fifty grams of dried ground makabuhai stems were placed in ball jars and added with different amount of water (125, 250 and 500 ml). The makabuhai water mixture was fermented for 24, 48 and 72 hours. This was sieved in nylon mesh and the solution obtained (filtrate) was then poured in petri dish with 14-day-old rice seedlings. The soaking time of seedling to the solution was from 6, 12, 24 and 48 hours prior to transplanting. Seedlings were transplanted in potted mud at 3-4 seedlings/hill at a depth of 1 cm right after each soaking time. Four days after transplanting, brown planthopper (BPH) were released at 20 insects/pot. Treated seedlings with insects were caged and mortalities were recorded for five consecutive days. A standard was provided using carbofuran solution following the recommended rate by Masagana 99 (740 cc Furadan 2F dissolved in 130 liters water/ha). Untreated check was also provided for comparison.

Laboratory bioassay

Contact toxicity. The toxicity of the volatile oils was evaluated by topical application using a calibrated microapplicator while the aqueous extract by leaf spraying. The oil extracts were diluted with acetone to the desired concentrations. One microliter of the diluted extract was topically applied on the thorax. For smaller insects such as leafhopper 0.2 ml was used. The treated insects were placed in petri dishes with the host plant. Treatment including control (treated with acetone only) were replicated three times. All mortality data were taken at 24 hours after treatment and corrected for natural mortality using Abbott's formula (Abbott 1975) and analyzed using probit analysis (Finney 1971) to determine the LD50.

Antifeedant action. Leaf squares measuring 50 x 50 mm were treated with the plant extracts at the desired concentrations (2.5 mg. 10 mg and 1 g/ml) according to the method of Morallo-Rejesus (1985). Feeding inhibition or repellancy were demonstrated by exposing *P. xylostella* and *S. litura* to treated cabbage leaf square and treated mulberry leaf squares, respectively.

The insects were introduced to either air-dried leaf (dry method) or just before and after spraying (wet method).

Toxicity of makabuhai

Spraying. Two-week old potted TN_1 rice plants were sprayed with the different aqueous extracts, (50:125, 50:250 and 50:500 gm/ml makabuhai-water

solution). The rice plant was sprayed either without (Method 1) or with insect on it (Method 2). For Method 1, the host was sprayed and allowed to dry for 24 hours prior to insect introduction, while in Method 2, the host was sprayed with the insect on it. Twenty adults were introduced per potted rice. Treated plants with insects were caged and mortalities were recorded for one week.

Root soaking. The 14-day old rice seedlings were soaked in the makabuhai filtrate derived from makabuhai fermented in water for 24, 48 and 72 hours. Four soaking time intervals were compared: 6, 12, 24 and 48 hours prior to transplanting. Immediately after soaking, the seedlings were transplanted in potted mud at 3-4 seedlings/hill at a dept of 1 cm. Four days after transplanting, the mylar cage was fitted to each pot and 20 adult brown planthoppers (BPH) were introduced.

Mortalities were recorded daily for five consecutive days. A standard using carbofuran slurry (740 cc Furadan 2F dissolved in 130 liters water/ha) and untreated check were also provided for comparison.

Soil application. The fresh makabuhai stems were chopped to about 50-60 cm in length. The chopped stems were applied at the base of a 2-week old potted rice plant at three rates: 60, 80 and 100 g/plot. After fitting the plant with mylar cages, 3-4-day old rice green leafhoppers were released.

The same procedures were followed in incorporating the chopped stems on potted bush sitao. Twenty one-day old adult aphids *(Aphis craccivora)* were released. Mortalities were taken daily for 7 days.

The toxicity of ground makabuhai stem by broadcasting on dapog seedbed was also evaluated. Ground makabuhai stems weighing 5, 10, 15 and 20 g were broadcasted separately in each dapog seedbed one day before sowing pre-germinated rice seeds (DBS). Another set of seedbed was provided wherein same amounts of ground makabuhai were broadcasted 10 days after sowing (DAS). Seedlings were transplanted in pots and labeled. Twenty adult BPH were introduced per caged pot. All treatments were replicated three times. Mortalities were recorded daily for 7 days.

Field evaluation of makabuhai

An area of 600 sq m rice field located at Central Experiment Station near Tabon Gate, UPLB was selected for field evaluation of makabuhai against rice pests. The area was divided into 3 blocks and each block was sub-divided into 9 equal plots measuring 4 x 5 m per plot. A one (1) meter canal was provided between blocks and planted with one strip of susceptible rice variety (TN 1) which served as inoculum plant. Treatments were replicated three (3) times and distributed randomly following RCBD. All recommended agronomic practices like weeding, herbicide application, water management and fertilizer applications were followed. A dry and wet season trials were conducted.

The following treatments were evaluated:

Treatments	*Seedling treatment*	*Timing of application*			
			25 DAT		*45 & 65 DAT*
T_1	MRS	+	SAFC	+	BS
T_2	MRS	+	BS	+	BS
T_3	BGM	+	SAFC	+	BS
T_4	BGM	+	BS	+	BS
T_5	BGM	+	ICFM	+	BS
T_6	CRS	+	SAFC	+	BS
T_7	CRS	+	SAG	+	BS
T_8	CRS	+	BS	+	BS
T_9	Untreated check				

MRS – makabuhai root soaking (overnight)
BGM – broadcasting of ground makabuhai on dapog seedbed 10 days after sowing pre-germinated seeds
CRS – carbofuran root soaking (24 hours)
SAFC – soil application of fresh and chopped makabuhai
SAG – soil application of ground makabuhai
ICFM – immersion of fresh and coiled makabuhai vines along water runways
BS – Brodan spraying

The toxicity of makabuhai was evaluated by counting the population build-up of BPH, GLH and deadheart caused by stem borer (SB) at 35 and 63 days after transplanting (DAT). Likewise, sampling was made at 84 and 91 DAT for BPH and percent whitehead, respectively. Yield were recorded and computed on per hectare basis. All data were analyzed using RCBD and differences between treatments were computed by DMRT.

The toxicity to golden snails were also evaluated using makabuhai and other plant extracts.

Results and Discussion

Toxicity

All the volatile oils tested (Table 1) were highly toxic to the lepidopterous larvae, *S. exempta* and *S. litura*, causing more than 90% mortality at 0.2 mg/ml except for sambong and manzanilla on *S. litura* which gave a 73% and 81.6% mortality at 24 hours. Lagundi, oregano and manzanilla were highly toxic also to *R. dominica*. Oregano was toxic to *Sitophilus* spp. but not to *T. castaneum* but the

Table 1. Topical toxicity of five plants containing volatile oils on different insects 24 and 48 hours after application

Test Insect	Concentration (mg/ml)	Percent mortality: Sambong 24 h	Sambong 48 h	Lagundi 24 h	Lagundi 48 h	Oregano 24 h	Oregano 48 h	Bulak-Manok 24 h	Bulak-Manok 48 h	Manzanilla 24 h	Manzanilla 48 h
T. castaneum[b]	0.2	91.7	91.7	56	56	33	33	38.3	38.3	13.3	13.3
	0.1	88.3	88.3	30	30	20	20	10	10	5	5
	0.01	23.3	23.3	3	3	0	0	0	0	0	0
S. litura[a]	0.2	73.3	73.3	100	100	100	100	93.3	93.3	81.6	81.6
	0.1	40.0	40.0	90	90	100	100	76.7	76.7	45	45
	0.01	0.0	0.0	18.3	18.3	18	18	11.7	11.7	3.3	3.3
Sitophilus spp.[b]	0.2	98.0	98.0	88.0	90.0	100	100	100.0	100.0	60	60
	0.1	95.0	95.0	66.0	78.0	98.3	98.3	65.0	65.0	26.7	26.7
	0.01	13.0	0.0	0.0	1.7	63.3	86.7	0.0	0.0	0	0
R. dominica[b]	0.2	93.0	-	96.6	96.6	93.3	93.3	0	0	98.3	98.3
	0.1	80.0	-	76.6	71.6	51.6	51.6	0	0	88.3	88.3
	0.01	1.6	-	8.3	8.3	20	20	0	0	1.6	1.6
S. exempta[a]	0.2	100	100	100	100	100	100	100	100	100	100
	0.1	97.7	96.7	90	90	100	100	100	100	100	100
	0.01	56.7	56.7	86.7	86.7	33.3	33.3	93.3	93.3	76.7	76.7

[a] 2nd instar larvae.

[b] adult.

reverse was observed with sambong. Oregano and sambong were highly toxic to cotton stainer (*D. cingulatus*, Hemiptera) and housefly (*M. domestica*) (Table 2). Lagundi was only highly toxic to *D. cingulatus* but not to *Musca*.

All test materials were toxic than malathion on *P. xylostella* and as toxic as malathion on *S. litura* (Table 3). Oregano was toxic as malathion on *R. dominica* but all materials were less toxic than malathion on *S. zeamais*.

The results show that the lepidopterous larvae (*S. litura*, *S. exempta* and *P. xylostella*) were the most sensitive to the volatile oils.followed by *D. cingulatus* (Hemiptera) and *M. domestica* (Diptera). The coleopterous species varied in the susceptibility to the volatile oils.

Table 2. Toxicity (mean percent mortality)[1] of volatile oil extracts at 24 and 48 hours to different test insects

Source	*Conc.*	*D. cingulatus* 24 h	*D. cingulatus* 48 h	*M. domestica* 24 h	*M. domestica* 48 h
Oregano	.05	93.33	96.66	60.00	100.00
	.10	95.00	98.33	96.66	100.00
	.20	100.00	100.00	–	–
Lagundi	.05	56.66	73.33	21.66	35.00
	.07	96.66	98.33	48.33	70.00
Sambong	.02	–	–	36.66	73.33
	.05	73.33	90.00	–	–
	.10	83.33	88.33	–	–
	1.00	–	–	100.00	100.00
Control		0.0	0.0	0.0	0.0

[1] 3 replicates

Table 3. Toxicity (LD_{50} mg/g body weight) of four volatile oil extracts 24 hours after topical application

Test Insects	*Plant* Bulak-manok	Oregano	Sambong	Lagundi	Malathion
P. xylostella	0.42	0.67	1.67	3.04	4.48
S. zeamais	0.62	0.34	1.06	0.90	0.005
R. dominica	1.04	0.59	2.18	–	0.4
S. litura	.13	.06	.68	48	0.32

Antifeedant action

All the crude extracts deterred feeding of the diamondback moth (DBM) (Table 4). Less feeding were observed when the crude extracts were sprayed on the cabbage leaf squares with the insect. Table 5 showed that in both methods *Tagetes* and sambong showed high mortality. The fact that higher mortality was observed on air-dried leaf squares treated with malaubi and timbangan indicate that these treated leaves did not only deterred feeding but toxic to the insects.

Lagundi extract was both toxic and feeding deterrent to DBM as shown in Table 6. It is more toxic than malathion. Oregano and sambong were both effective as antifeedant and contact poison to DBM (Table 7).

Table 4. The leaf consumption of the diamondback moth exposed for 24 hours on cabbage leaf squares treated with 1 gm/ml extract

Leaf Extract	*Spray Method*[1]	*Mean Area Consumed (cm^2)*[2]	*% Consumed*
Tagetes	A	1.00 a	4.00
Sambong	A	0.50 ab	2.00
Malaubi	A	0.50 abc	2.33
Timbangan	A	0.43 a	5.64
Caballero	A	0.33 ab	1.33
Tagetes	B	0.50 abc	2.00
Sambong	B	0.75 bcd	3.00
Malaubi	B	0.75 bcd	3.00
Timbangan	B	0.75 bcd	3.00
Caballero	B	0.92 cd	3.67
Control		1.83 e	7.33

[1]Wet method

A - Sprayed with the insects on the cabbage leaf squares.

B - Cabbage leaf squares sprayed and insects introduced after treatment.

[2]Average of 3 replicates; any two means having a common letter are not significantly different at 5% probability based on DMRT.

Toxicity of makabuhai

Spraying. The filtrate from makabuhai fermented in water for 24 hours has low toxicity to brown planthopper (BPH) and green leafhopper (GLH) when sprayed on rice seedlings with or without the insects. This indicates that the material has very low contact toxicity. Increasing the fermentation time to 48 hours did not improve the toxicity of the filtrate.

Table 5. The mean percent mortality of the diamondback moth after 24 and 48 hours exposure to the cabbage leaves sprayed with the extract

Leaf extract	*Spray method*[1]	*Percent mortality*[2] *24 h*	*48 h*
Tagetes	A	28.33 b	100.00
Sambong	A	55.00 a	88.33
Malaubi	A	21.67	33.33
Timbangan	A	18.33 cd	36.67
Caballero	A	13.33 de	15.00
Tagetes	B	23.33 c	100.00
Sambong	B	10.00 ef	85.00
Malaubi	B	13.33 cde	88.33
Timbangan	B	6.67 f	95.00
Caballero	B	10.00 de	11.67
Control		1.67 g	1.67

[1]Wet method

A – sprayed with insects on the cabbage leaf squares.

B – cabbage leaf squares sprayed and insects introduced after treatment.

[2]Average of three replicates; any two means having a common letter are not significantly different at 5% probability based on DMRT.

Table 6. The mean percent mortality and leaf consumption of the diamondback moth after 24 hours exposure to the lagundi volatile oil-treated cabbage leaves

Treatments[1] *(mg/ml)*	*Percent mortality*[2]	*Percent consumption*[2]
T_1 air-dried	23.33 b	5.67 ab
T_2 spray with insect	100.00 a	0 d
T_3 spray and introduced	80.00 a	1.67 c
T_4 malathion (0.05 gm/ml)	6.67 bc	7.00 a
T_5 control	0 c	5.33 b

[1]Dry and wet method

[2]Average of 3 replicates; any two means having a common letter are not significantly different at 5% probability based on DMRT.

Table 7. The mean percent mortality and leaf consumption of the diamondback moth after 24 hours exposure to the oregano and sambong volatile oils treated cabbage leaves

Treatment[1] *(1 gm/ml)*	*Percent mortality*[2]	*% Leaf consumption*[2]
Air-dried		
T_1 Oregano	100 a	0 a
T_2 Sambong	60 b	1.67 b
Spray with insect		
T_3 Oregano	100 a	0 a
T_4 Sambong	100 a	0 a
Spray and introduced		
T_5 Oregano	100 a	0 a
T_6 Sambong	100 a	0 a
T_7 Malathion (0.05 g/ml)	10 c	8.33 c
T_8 Control	3.33 c	4.33 c

[1]Dry and wet method.

[2]Average of 3 replicates; any two means having a common letter are not significantly different at 5% probability based on DMRT.

Root soaking. The systemic activity of makabuhai was evaluated by soaking the roots (RS) of rice seedlings before transplanting and exposure to BPH. More than 50% mortality was observed in five days in treated seedlings as compared to the 4.7% mortality observed in the untreated seedlings. Makabuhai RS was as toxic as Carbofuran RS (Table 8). The results indicate that the toxic principle is systemic. There was no significant differences in the toxicity of the seedlings soaked at 6, 12 and 24 hours interval.

Soil application. The application of chopped stem on the surface of the soil of the potted rice plant (enough water to cover the stems was maintained) indicated that makabuhai was toxic to GLH (Table 9). Higher mortality was observed with longer intervals between application and exposure of the insects. This again proved that the insecticidal principle is systemic.

The experiment was repeated using potted beans. The insecticidal principle seem to be absorbed and translocated in bush sitao when exposed to the plant one day after treatment.

Broadcasting of 20 g ground makabuhai on dapog seedbed measuring 4 x 4 x 2 was as effective as carbofuran broadcasting against BPH (Table 11). The lower dosage of 5-15 g were as toxic as 20 g but significantly less toxic than broadcasting

Table 8. The toxicity (% mortality[a/, b/, c/]) of makabuhai water extract fermented for 24 hours by root soaking or rice seedlings to brown planthopper

Treatment (T)	*Soaking time*	*Concentrations (wt/volume)*	*Days of observation* 1	2	3	4	5
T_1	06 hrs	50:125	29.53 bc	34.15 cd	39.12 bc	45.99 abc	57.29 ab
T_2	06 hrs	50:250	27.71 bc	31.07 d	36.18 c	40.17 c	50.85 ab
T_3	12 hrs	50:125	32.10 abc	37.99 bcd	40.00 abc	47.30 abc	56.15 ab
T_4	12 hrs	50:250	25.00 bc	30.78 d	34.99 c	42.09 bc	51.81 ab
T_5	24 hrs	50:125	35.25 ab	39.15 abcd	44.97 abc	53.87 ab	57.00 ab
T_6	24 hrs	50:250	25.00 bc	34.92 cd	37.87 bc	46.05 abc	55.01 ab
T_7	24 hrs	Carbofuran 2F	42.12 a	48.93 ab	53.93 a	57.86 a	64.01 a

[a]Cumulative average of three replicates.

[b]Computation based on percentage Arcsin transformation.

[c]Means followed by the same letters are not significantly different at 5% level of DMRT.

Table 9. Average percent mortality of rice green leafhopper[1] at various intervals after soil application of chopped makabuhai stem on rice plants[2] (Morallo-Rejesus and Silva, 1979)

	Days after soil application					
GMS/POT	*1*	*2*	*3*	*4*	*5*	*6*
60	1.03	7.09	39.06	65.88	86.58	100.00
80	4.00	12.08	34.06	75.29	91.46	100.00
100	15.46	39.06	67.03	87.06	98.78	100.00

[1]Twenty 3-4 day old adults/rep; 3 reps/treatment; two trials corrected for control mortality.

[2]Rice variety used - IR 1416; 44.5 cms (ht); 35-day old.

Table 10. Average percent mortality[1] of *A craccivora*[2] at various intervals after soil application of chopped makabuhai stem on bush sitao (Morallo-Rejesus and Silva, 1979)

	Days after soil application					
GMS/POT	*1*	*2*	*3*	*4*	*5*	*6*
60	7.00	4.25	29.58	76.19	86.90	91.20
80	39.00	92.55	100.00	–	–	–
100	57.00	77.66	80.46	96.43	100.00	–

[1]Corrected for control mortality based on two trials; observed 24 hours after exposure.

[2]Twenty one-day old adult/rep. with 3 reps/treatment.

with standard carbofuran. Although there was no significant differences in the toxicity observed between broadcasting one day before sowing pre-germinated seeds and ten days after sowing, the former method delayed the emergence of the seedlings for three days resulting in the non-uniformity in height of rice seedlings at transplanting.

Field Evaluation of Makabuhai Against Major Insect Pests of Rice

In the dry season trial, the application of makabuhai aqueous extract by root-soaking + soil application of fresh and chopped makabuhai (T1) or combinations of carbofuran root soaking + soil application of fresh and chopped ground makabuhai stems was not significantly different to standard treatment (T8-Carbofuran root-

Table 11. The toxicity (% mortality) of ground makabuhai stem broadcasted on dapog seedbed (4 x 4 in^2) to brown planthoppers

Treatments	*Dosage*	*Days of observation* 1	2	3	4	5	6	7
T DES[1]	5 g	5.00 ab	8.33 b	16.67 b	20.00 b	25.00 c	33.33 bc	38.33 b
T	10 g	6.67 ab	8.33 b	15.00 b	25.00 b	31.67 bc	35.00 bc	41.67 b
T	15 g	6.67 ab	8.33 b	16.67 b	25.00 b	26.67 bc	31.67 bc	45.00 b
T	20 g	6.67 ab	15.00 b	23.33 b	33.33 b	41.67 b	43.67 bc	56.67 ab
T DAS[2]	5 g	5.00 ab	8.33 b	13.33 b	21.67 b	25.00 c	28.33 c	36.67 b
T	10 g	5.00 ab	11.67 b	13.33 b	20.00 b	26.67 bc	33.33 bc	46.67 b
T	15 g	5.00 ab	13.33 b	18.33 b	25.00 b	31.67 bc	45.00 bc	51.67 b
T	20 g	3.33 b	16.67 ab	21.67 b	26.67 b	30.00 bc	50.00 b	63.33 ab
T Carbofuran 3G		18.33 a	35.00 a	53.33 a	61.67 a	71.67 a	76.67 a	85.00 a
T Untreated check		0 c	0 c	0 c	0 c	0 d	1.67 d	1.67 c

[1]Ground makabuhai stems broadcasted on dapog seedbed one day before sowing pre-germinated seeds.

[2]Ground makabuhai stem broadcasted on dapog seedbed 10 days after sowing.

soaking + Brodan spraying) in controlling the population of BPH, GLH and SB (stem borer) at 35, 63 and 91 DAT (Table 12-13). Comparable effects was also noted when fresh and coiled makabuhai vines were immersed in water runways. Broadcasting of makabuhai on dapog seedbed in combination with soil application of fresh and chopped stem or Brodan spraying at 25 DAT effectively control BPH and SB but not GLH at 35 DAT (Table 12). The treatments including the standard carbofuran treatment was not effective against BPH but effective against rice stem-borer.

Results of the second trial (Wet Season) showed no significant differences between makabuhai and the carbofuran treatments but significantly better than the control in suppressing the population of BPH, GLH and SB 35 DAT. This indicates that all the makabuhai treatments were as effective as the recommended insecticidal applications at seedling stage and at 25 DAT (Table 12). At 63 DAT, the treatments were not effective against GLH but were still effective against rice stemborers and brown planthoppers.

In terms of yield, the makabuhai treatments (T1-T5) were as high as compared to the insecticidal treatments (T8) and significantly better than the control. However, the pest population and yield in the second trial was lower than the first trial (Table 15). The successive typhoons during the wet season may have reduced the pest population but affected very much the growth of the rice plant that led to lowered yield.

The data showed that makabuhai seedling root soaking and soil application of freshly chopped makabuhai (T1) can substitute for the recommended carbofuran seedling root soaking and Brodan spraying at 25 DAT, respectively, thereby reducing the insecticidal application from four to two at 35 and 65 DAT, thus reducing the cost of production while increasing the net income. The broadcasting of ground powdered makabuhai on dapog seedbed and soil application of chopped makabuhai at 25 DAT (T3) or immersion of coiled vine can also substitute for the recommended insecticidal application (T8).

The golden snails that were introduced in the Philippines is becoming a serious problem in rice production by many farmers in the country. Makabuhai will control not only rice pests but the golden snail (Table 16). *Derris philippinensis* was also found to be very effective against the snails.

Conclusions and Recommendations

All the volatile oils evaluated were toxic to at least two of the test insects and were highly toxic to *S. litura* and *S. exempta*. The crude extract of *Tagetes*, Sambong, Malaubi, Timbangan and Caballero were feeding deterrent to *P. xylostella*.

The use of makabuhai aqueous extract for seedling root soaking and broadcasting of chopped or immersion of coiled vines are effective in controlling the brown planthopper, green leafhopper and rice stemborers of rice. It can substitute for two of the four insecticidal applications recommended for protecting rice from

Table 12. Average population count BPH, GLH and percentage deadheart at 35 DAT

Treatments	Dry season			Wet season		
	BPH	*GLH*	*% Deadheart*	*BPH*	*GLH*	*% Deadheart*
ST 25 DAT 45+65 DAT						
T_1 MRS + SAFC + BS	1.20 a	12.67 a	4.71 a	0.43 a	4.33 a	1.53 a
T_2 MRS + BS + BS	1.52 ab	19.00 cd	3.96 a	0.48 a	5.33 a	1.41 a
T_3 BGM_1 + SAFC + BS	1.45 ab	25.00 de	4.43 a	0.37 a	4.33 a	2.06 a
T_4 BGM_1 + BS + BS	1.43 ab	16.33 bc	3.63 a	0.37 a	4.33 a	1.77 a
T_5 BGM_1 + ICM + BS	1.70 b	12.00 ab	3.70 a	0.53 a	4.33 a	1.37 a
T_6 CRS + SAFC + BS	1.28 ab	12.00 ab	3.61 a	0.37 a	5.67 ab	1.97 a
T_7 CRS + BGM_2 + BS	1.43 ab	11.00 ab	3.40 a	0.65 ab	4.67 a	1.49 a
T_8 CRS + BS + BS (Std)	1.22 a	12.67 ab	4.21 a	0.57 ab	6.67 ab	1.94 a
T_9 Untreated check	1.65 c	33.67 e	10.77b	0.88 b	8.67 b	4.19 b

BS – Brodan spraying
BGM_1 – Broadcasting of ground makabuhai on dapog seedbed.
BGM_2 – Broadcasting of ground makabuhai on rice field
CRS – Carbofuran root soaking
MRS – Makabuhai root soaking
SAFC – Soil application of freshly chopped makabuhai
ICM – Immersion of coiled makabuhai along water-runway
BPH – Brown planthopper
GLH – Green leafhopper
ST – Seedling treatment
DAT – Days after transplanting

[1]Means followed by the same letters are not significantly different at 5% level of DMRT.

[2]Average of three replicates from 20 sample plants.

Table 13. Average population count of BPH, GLH and percentage deadheart at 63 DAT

Treatments	Dry season			Wet season		
	BPH	*GLH*	*% Deadheart*	*BPH*	*GLH*	*% Deadheart*
ST 25 DAT 45+65 DAT						
T_1 MRS + SAFC + BS	0.60 ab	2.19 bc	0.84 d	0.10 a	1.67 ab	2.77 a
T_2 MRS + BS + BS	0.66 bc	2.29 cd	0.82 bc	0.12 b	1.67 ab	2.94 a
T_3 BGM_1 + SAFC + BS	0.66 bc	2.67 d	0.80 a	0.12 b	2.00 ab	2.62 a
T_4 BGM_1 + BS + BS	0.57 a	2.40 cd	0.81 ab	0.12 b	1.67 ab	3.60 ab
T_5 BGM_1 + ICM + BS	0.59 ab	1.65 a	0.80 a	0.10 a	3.00 ab	2.83 a
T_6 CRS + SAFC + BS	0.70 c	1.83 ab	0.83 cd	0.12 b	2.33 ab	2.99 a
T_7 CRS + BGM_2 + BS	0.59 ab	2.41 cd	0.83 cd	0.18 d	0.67 a	2.78 a
T_8 CRS + BS + BS (Std)	0.69 c	2.18 bc	0.82 bc	0.13 c	2.67 ab	3.75 ab
T_9 Untreated check	0.93 d	3.18 e	0.93 e	0.23 e	3.33 b	5.25 b

BS – Brodan spraying
BGM_1 – Broadcasting of ground makabuhai on dapog seedbeed
BGM_2 – Broadcasting of ground makabuhai on rice field
CRS – Carbofuran root soaking
MRS – Makabuhai root soaking
SAFC – Soil application of freshly chopped makabuhai
ICM – Immersion of coiled makabuhai along water-runway
ST – Seedling treatment
DAT – Days after transplanting
BPH – Brown planthopper
GLH – Green leafhopper

[1]Means followed by the same letters in the same column are not significantly different at 5 % level of DMRT.

[2]Average of three replicates from 20 sample plants.

Table 14. Average population count of BPH (84 DAT) and percentage whitehead (91 DAT)

Treatments	*Dry season*		*Wet season*	
	BPH 84 DAT	*% Whitehead 91 DAT*	*BPH 84 DAT*	*% Whitehead 91 DAT*
ST+25 DAT+45+65 DAT				
T_1 MRS + SAFC + BS	0.74 abc	0.79 ab	0.03 ab	1.17 a
T_2 MRS + BS + BS	0.72 ab	0.80 bc	0.00 a	1.49 a
T_3 BGM_1 + SAFC + BS	0.74 abc	0.80 bc	0.05 ab	1.96 a
T_4 BGM_1 + BS + BS	0.75 bc	0.81 bc	0.03 ab	1.32 a
T_5 BGM_1 + ICM + BS	0.75 bc	0.85 d	0.07 ab	1.18 a
T_6 CRS + SAFC + BS	0.75 bc	0.83 cd	0.02 a	1.51a
T_7 CRS + BGM_2 + BS	0.74 abc	0.80 bc	0.07 ab	1.78 a
T_8 CRS + BS + BS (Std)	0.71 a	0.76 a	0.02 a	1.28 a
T_9 Untreated check	0.77 c	0.97 e	0.10 b	5.28 b

BS – Brodan spraying
BGM_1 – Broadcasting of ground makabuhai on dapog seedbed
BGM_2 – Broadcasting of ground makabuhai on rice field
CRS – Carbofuran root soaking
MRS – Makabuhai root soaking
SAFC – Soil application of freshly chopped makabuhai
ICM – Immersion of coiled makabuhai along water-runway

ST – Seedling treatment
DAT – Days after transplanting
BPH – Brown planthopper

[1]Means followed by the same letters in the same column are not significantly different at 5% level of DMRT.

[2]Average of three replicates from 20 sample plants.

Table 15. Average yield, gross income and net return of IR 64 resulting from the use of various combination of makabuhai and commercial insecticides (Dry and Wet Season - 1986)

Treatment	Dry season			Wet season		
	Mean yield/ha (Kg)	*Gross income (₱/Ha)*	*Net return[a]*	*Mean yield/ha (Kg)*	*Gross income (₱/Ha)*	*Net return*
ST+25 DAT+45 & 65 DAT						
MRS + SAFC + BS	5385.55 a	18849.42	1612.80	3268.75 a	11440.52	3783.78
MRS + BS + BS	5612.00 a	19642.00	1850.62	2756.77 a	9648.70	1437.11
BGM_1 + SAFC + BS	5296.94 a	18539.29	1302.66	3229.36 a	11302.76	3645.92
BGM_1 + BS + BS	5493.86 a	19228.51	1937.13	2825.69 a	9889.92	1678.33
BGM_1 +ICM + BS	5237.87 ab	18332.54	1095.92	3032.45 a	10613.58	2956.74
CRS + SAFC + BS	5631.69 a	19710.91	2417.37	2983.22 a	10441.27	2727.51
CRS + BGM_2 + BS	5631.69 a	19710.91	2417.37	3130.91 a	10958.18	3244.42
CRS + BS + BS (Standard Check)	5956.60 a	20848.10	2999.80	2973.38 a	10406.83	2138.32
Untreated Check	4607.75 b	16127.12	–	1870.67 b	6547.34	

BGM_1 – Broadcasting of ground makabuhai on dapog seedbed
MRS – Makabuhai root soaking
CRS – Carbofuran root soaking
BGM_2 – Broadcasting of ground makabuhai on rice field
SAFC – Soil application of freshly chopped makabuhai
ICM – Immersion of coiled makabuhai along water-runway
BS – Brodan spraying

ST – Seedling treatment
DAT – Days after transplanting

[a]Based on the untreated check

Table 16. Average percent mortality of golden snails found in the rice paddy treated with plant materials

Treatments	*Day of Observation*				
	1	*2*	*3*	*4*	*5*
T_1 Freshly Chopped Makabuhai (5 kg/20 m^2)	3.33 b	11.67 d	26.67c	43.33 d	73.33 bc
T_2 Ground Makabuhai (1.5 kg/20 m^2)	5.00b	65.00 b	76.67 b	81.67 b	85.00 b
T_3 Coiled Makabuhai Vine (6 m/20 m^2)	5.00 b	21.67 cd	48.33 c	60.00 c	73.33 bc
T_4 *Tagetes* Leaves (5 kg/20 m^2)	8.33 b	33.33	46.67 c	50.00 cd	60.00 c
T_5 *Derris* Roots (5 kg/20 m^2)	100 a	100 a	100 a	100 a	100 a
T_6 Standard (Carbofuran - 1 bag/ha)	11.67 b	23.33 cd	31.67 c	35.00 d	40.00 d
T_7 Untreated Check	0.00 b	0.00 e	8.33 d	10.00 e	11.67 e

[a]Average of three replicates.

[b]Means followed by the same letter in each column are not significantly different at 5% level of DMRT.

insect damage thereby reducing cost of production and ultimately increasing the net income of the farmer.

Many of the medicinal plants in the Philippines are selectively toxic to some insect pests. The research and development of botanical pesticide need to be accelerated at all levels for the direct use of the known pesticidal plant parts by the farmers for immediate use to the identification, synthesis and development of the chemical principle by the industry. The selectivity of naturally occurring pesticides makes them valuable in integrated pest management.

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SPERMATOGENESIS IN *TILAPIA NILOTICA*: AN ULTRASTRUCTURE STUDY

Annabelle A. Herrera
Institute of Biology
College of Science
University of the Philippines
Diliman, Quezon City, Philippines

ABSTRACT

Spermatogenesis in *T. nilotica* was studied using an electron microscope. At the time of hatching primordial germ cells (PGC) with their characteristic germinal dense bodies are identified in the gut, their extra-gonadal origin. By 8-9 days posthatching, the PGCs have reached to the bipotential gonad primordia via the splanchnic mesoderm.

At days 16-20, onset of testis differentiation occurs by the appearance of stromal cavities between PGC clusters. Seminiferous tubules with spermatogonia are first recognized at day 40, and by day 53, primary spermatocytes first appear. By day 88, abundant fully differentiated sperm cells appear in the testis ducts. The ultrastructure of spermatogenesis in *Tilapia nilotica* is basically similar to that of other male vertebrates.

Introduction

Tilapia has become a prominent aquaculture organism on a global scale. In Israel and other countries where reproductive activity stops during winter followed by regression and development, electron microscope study has been done on testis recrudescence (Grier and Abraham, 1983). In tropical areas where reproductive activity is year long, ultrastructure research on testis and ovary differentiation in relation to pituitary histogenesis has been done (Herrera, 1986) in addition to light microscope study of testis structure (Hyder, 1970).

This study presents the ultrastructure of the first spermatogenesis during ontogeny in *Tilapia nilotica*. It provides baseline information that aids aquaculturists in problems on sex reversal, induced maturity and artificial breeding.

Materials and Methods

Tilapia eggs and embryos were secured from the Institute of Fisheries Development and Research, University of the Philippines. These were cultured in plastic basins filled with dechlorinated tap water to about 2/3 their volume and aerated continuously.

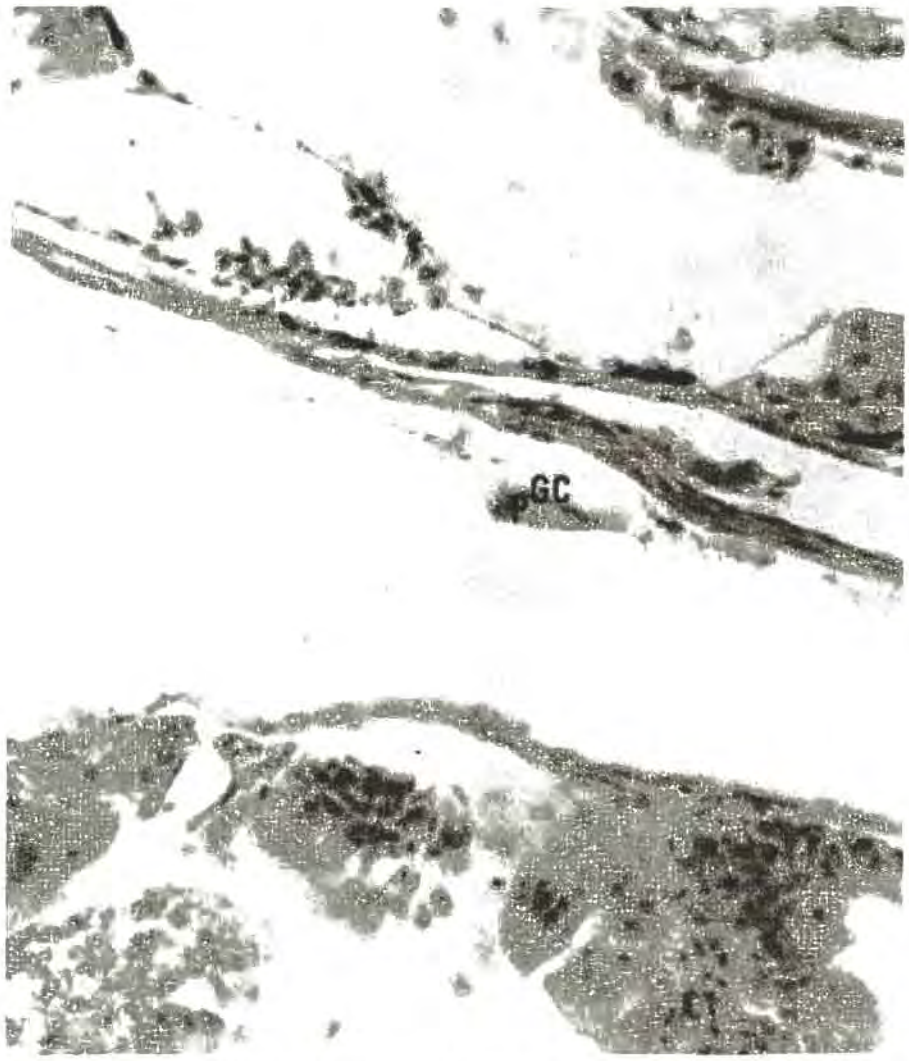


Fig. 1. The primordial germ cell in the dorsal mesentery on its way from the endoderm and splanchnic mesoderm. Hematoxylin-eosin. x 100.

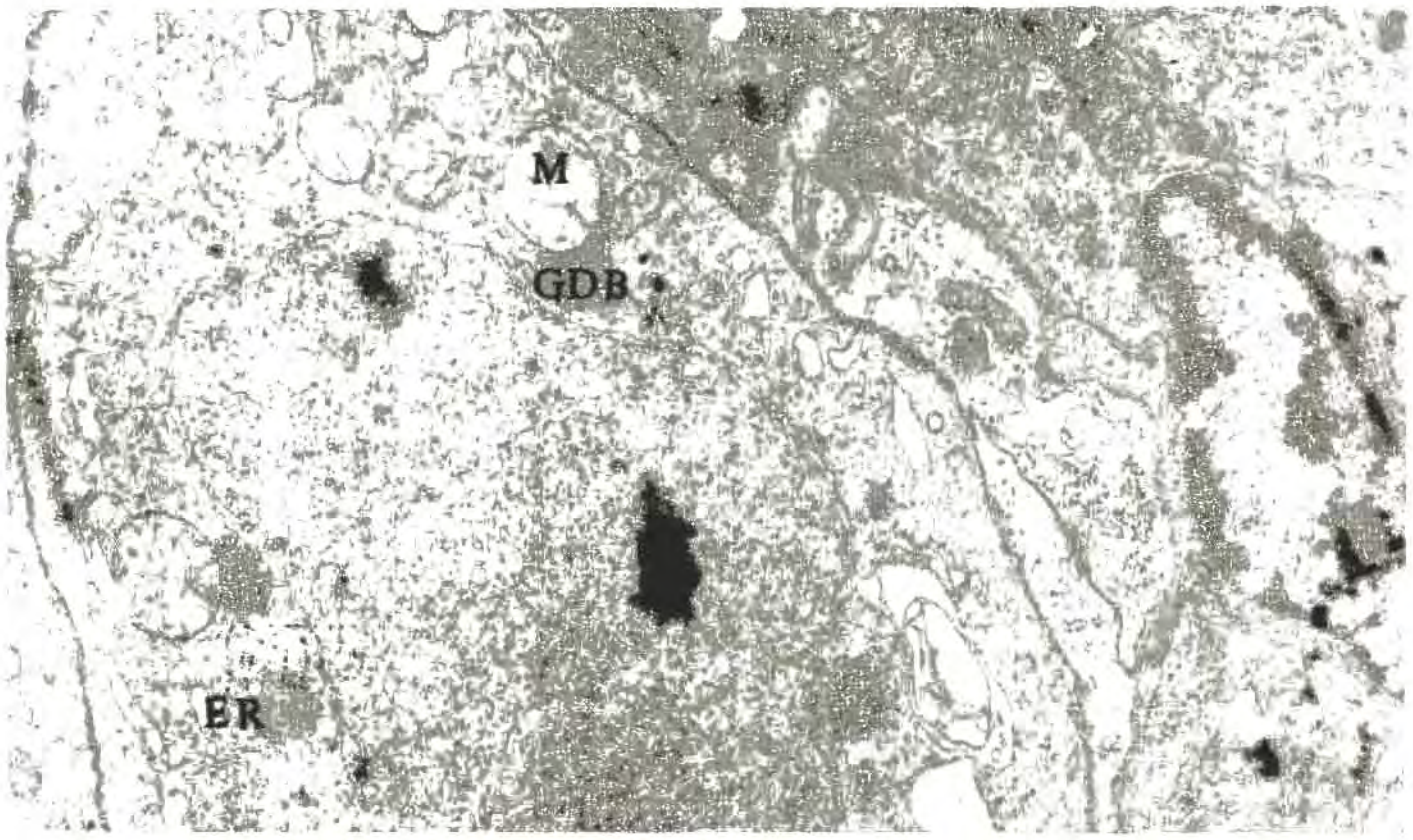
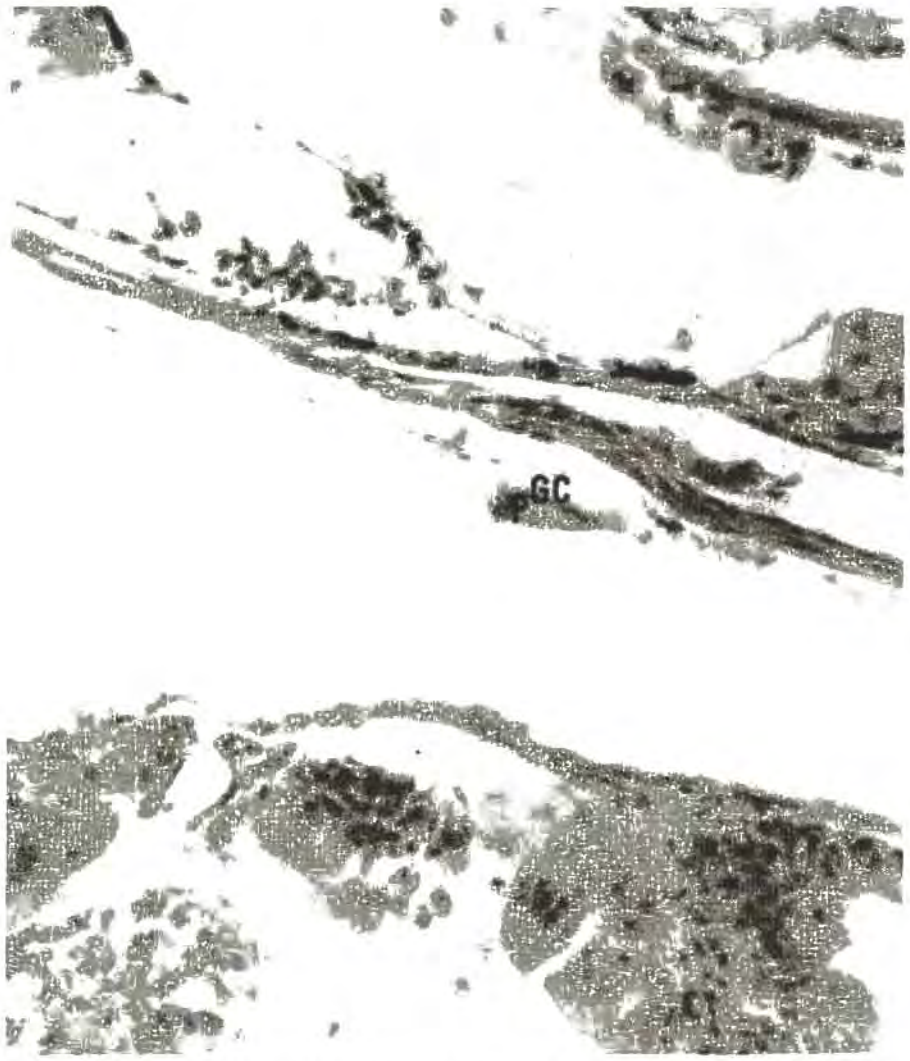


Fig. 2. Electron micrograph of a primordial germ cell (PGC). The cell and nuclear outlines are irregular. In the cytoplasm are large mitochondria (M), few dispersed ER lamellae (ER) and germinal dense bodies (GDB). x 30,000.



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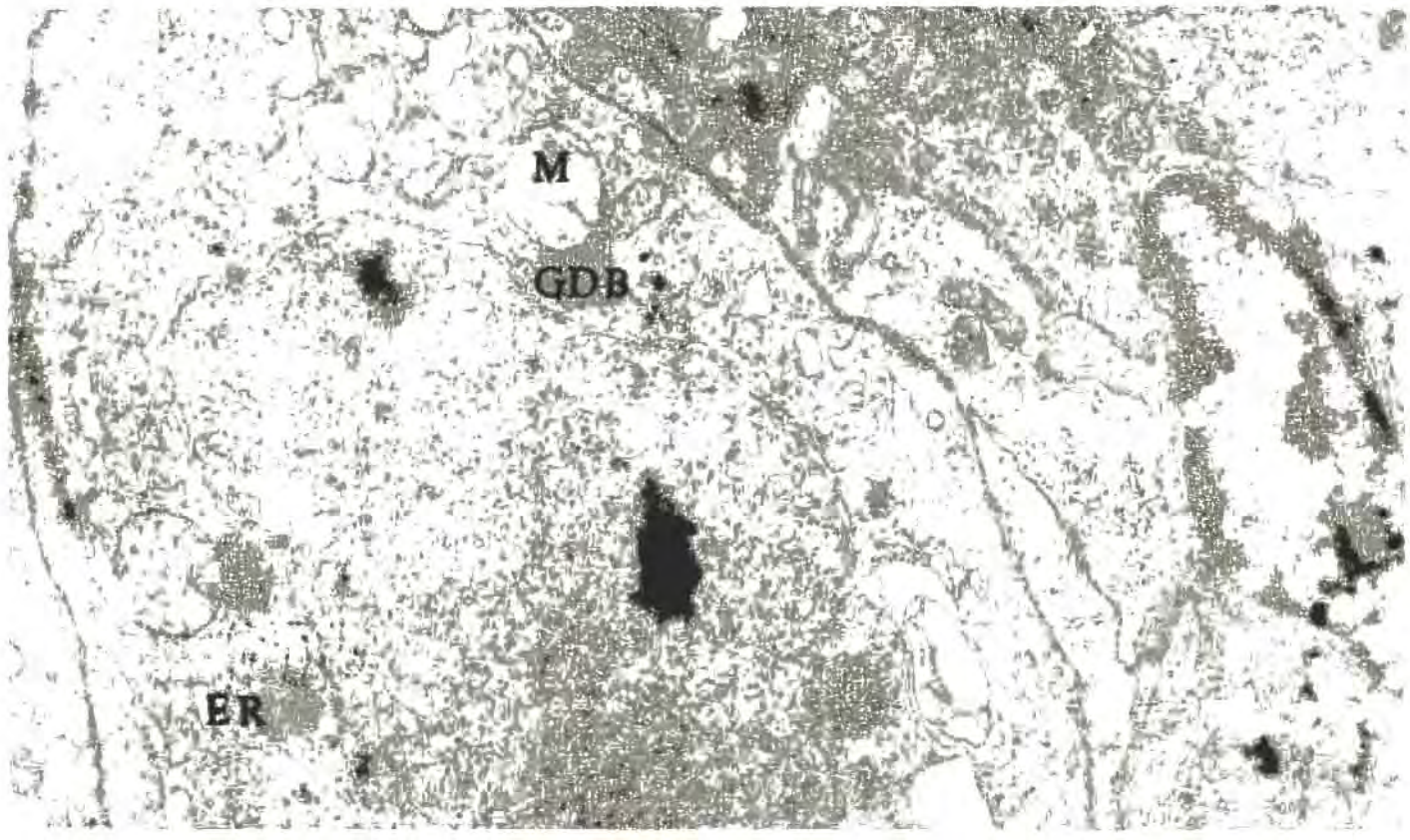

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Posthatch tilapia were kept in the same container until they reached the active feeding stage.

About 500 week-old fry were transferred to a "hapa" (nylon net enclosure) measuring 2 x 3 meters in one of the concrete fish ponds of the IFDR. Fish meal and "darak" (rice bran) were fed at about 4% body weight six times daily.

Histological techniques

From the time of hatching, 10 fry were fixed in Bouin's solution everyday for the first ten days and every two days for the succeeding weeks until the onset of gonadal maturity or the first appearance of fully formed sperm cells. For light microscopy, serial transverse sections of the region between the pronephros and the anus were stained with hematoxylin-eosin.

Based on the results of light microscopy, gonads of histologically significant developmental stages, were prepared for electron microscopy. The gonads were fixed in 2.5% glutaraldehyde, washed and soaked in phosphate buffer before post-fixation with 1% osmium tetroxide. Others were fixed in Karnovsky's glutaraldehyde-paraformaldehyde mixture in cacodylate buffer, washed and soaked in the same buffer before postfixation with osmium tetroxide. After dehydration in graded ethanol series, the specimens were embedded in Spurr's low viscosity epoxy resin. Ultrathin sections were mounted on uncoated grids and stained with uranyl acetate and lead citrate. These were examined with a Hitachi model H-300 electron microscope.

Results and Discussion

At the time of hatching

The primordial germ cells (PGC) at the time of hatching are in the gut endoderm, in the splanchnic mesoderm, or in the developing dorsal mesentery. The PGCs are clearly distinguishable from the somatic cells by their bigger size, larger nuclei, round to oval contour, and light cytoplasm (Fig. 1). In electron micrographs, the PGC has an irregular cellular and nuclear outline, a large heterochromatic to euchromatic nucleus, reticular nucleolus, few dispersed rough ER vesicles, and few to several large round mitochondria associated with germinal dense bodies (Fig. 2). The oval somatic cells flatten against the cell membrane of the PGC. The heterochromatic nucleus almost fills up the entire cell. The PGCs have a similar morphological appearance to the PGCs in *Oryzias latipes* described by Satoh (1974) characterized by the presence of germinal dense bodies interspersed with aggregations of mitochondria in the cytoplasm. The migratory pattern from endoderm to mesoderm to the genital ridges is similar to that of *Oryzias latipes* (Satoh and Egami, 1972) and *Cyprinus carpio* (Remojo, 1979).

Days 9-20 posthatching

At day 9, the PGCs establish themselves at the gonadal ridges (Fig. 3). Coloni-

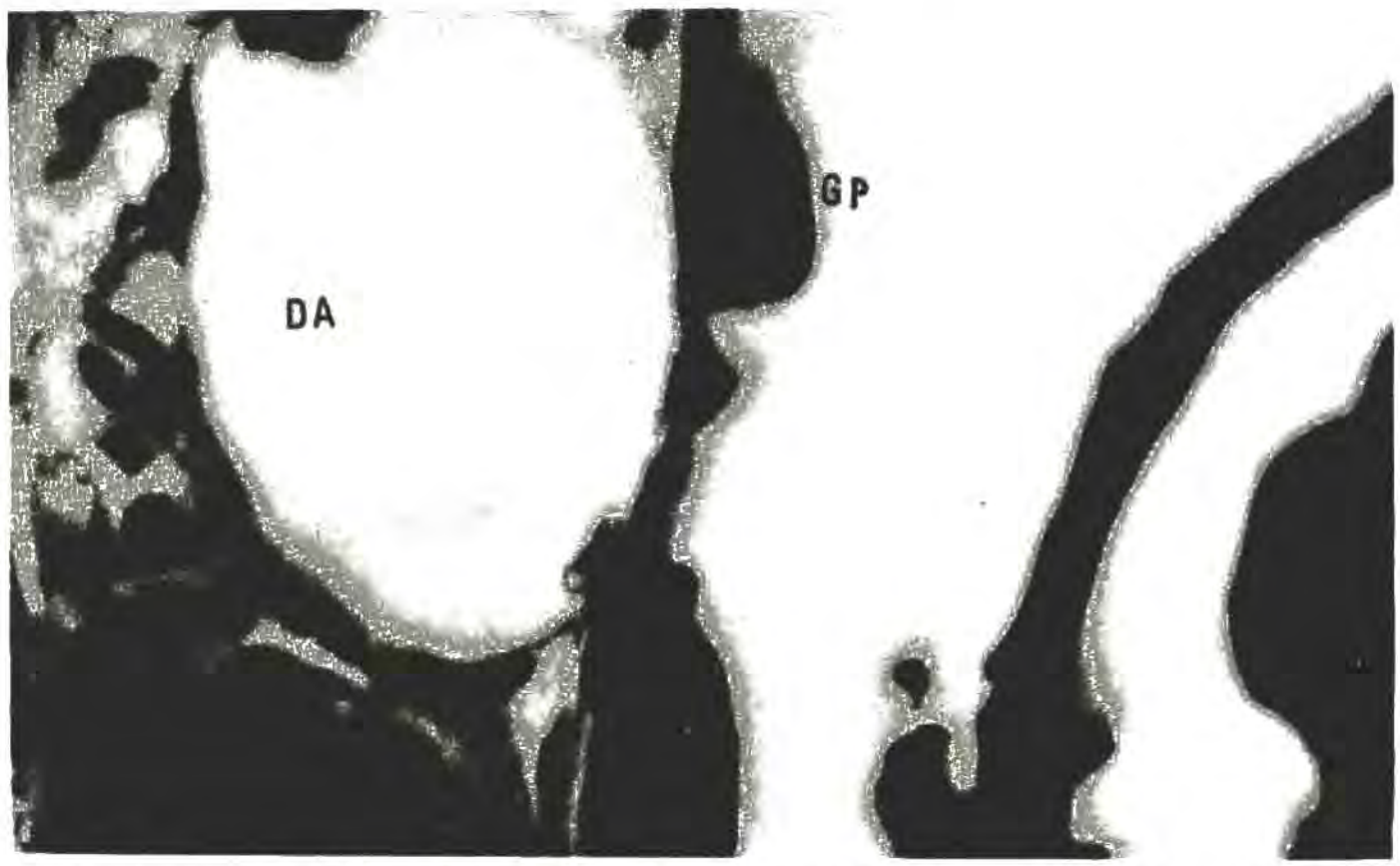

Fig. 3. The primodial germ cells (PGC) reach the gonadal ridges and form the gonad primordium (GP) which is composed of the PGCs and somatic cells (S). Hematoxylin-eosin x 400.

zation of the somatic tissues by PGCs with the formation of the paired gonadal primordia occurs at days 8-10 when the fry are about 11 mm. This coincides with the findings of Nakamura and Takahashi (1973) in *T. mossambica.* Eckstein and Spira (1965) observed it at day 8 in *T. aurea,* Boco (1977) at days 5-7 in *T. mossambica,* Yoshikawa and Oguri (1978) at day 6 in *T. zillii* and Brusle and Brusle (1978) at 8 months in *Mugil cephalus.* With the multiplication of the PGCs the gonadal anlagen enlarge. The gonad primordium consists of germ cells enveloped by somatic cells. The PGCs still look essentially the same as those of the newly-hatched fry. Fig. 4 shows a day 14 PGC. The cells have the same ultrastructural features as those of the PGCs of earlier developmental stages.

At about days 16-20, testicular differentiation occurs. In these gonads, germ cells are sparsely distributed, along the side facing the lateral peritoneal wall. Lumina (presumptive testocoel) are identified as splits in the stroma tissue (Fig. 5).

Electron micrographs show that there is already marked organization of the testis (Fig. 6). The somatic cells of the testis are elongated and irregular in shape, and show a notable aggregation near the mesogonium. The organization of the somatic cells bears a close resemblance to that of the day 40 testis. The nuclei are heterochromatic with irregular outlines. The nucleoli are granular. The cytoplasm contains mitochondria, scattered rough ER and vesicles.

Days 21-39

At this time, the testis still looks essentially the same as the 19-day old testis.

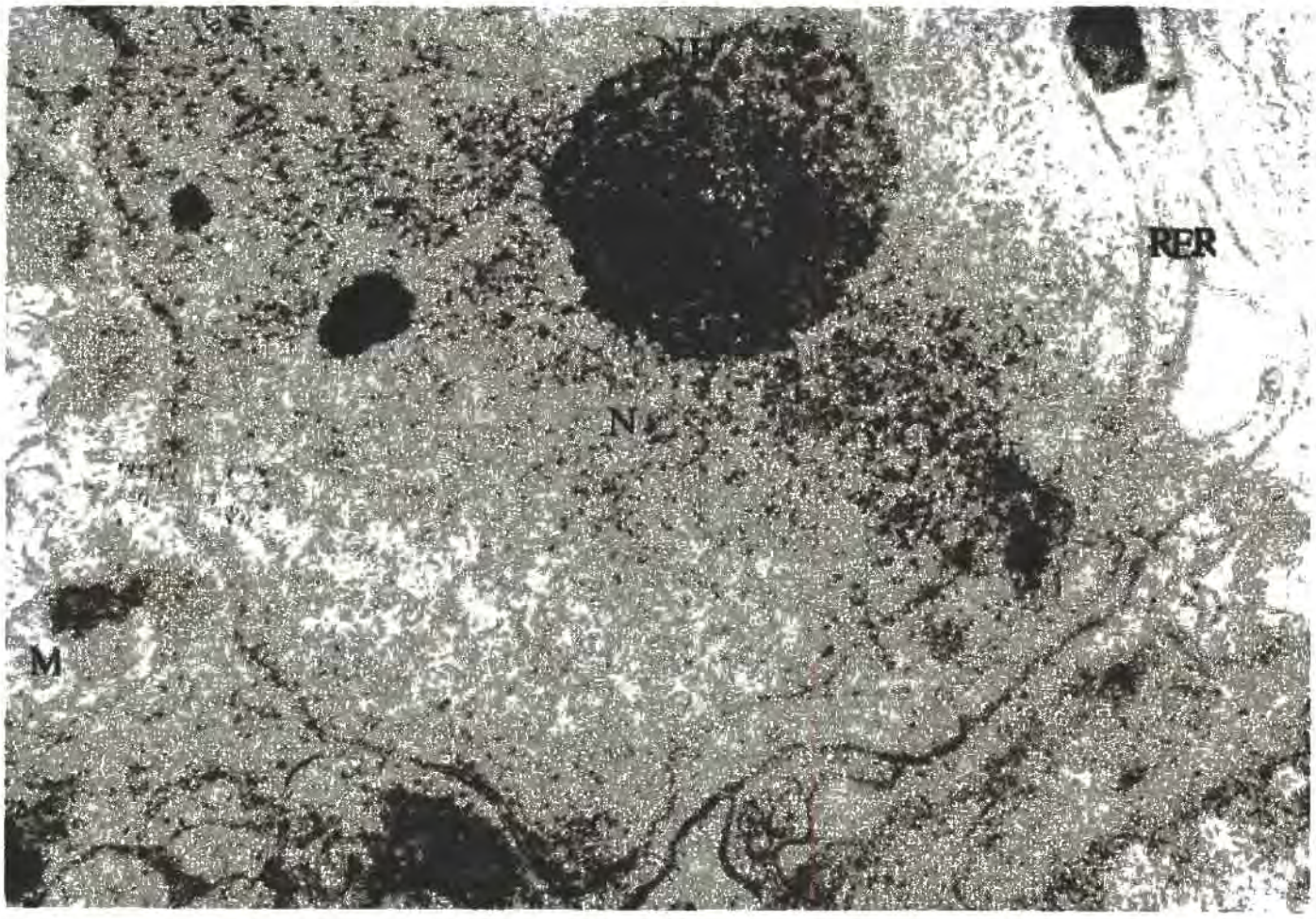

Fig. 4. Electron micrograph of day 14 PGC. N-nucleus, NU-nucleolus, RER-rough endoplasmic reticulum, M-mitochondria. x 14,000.

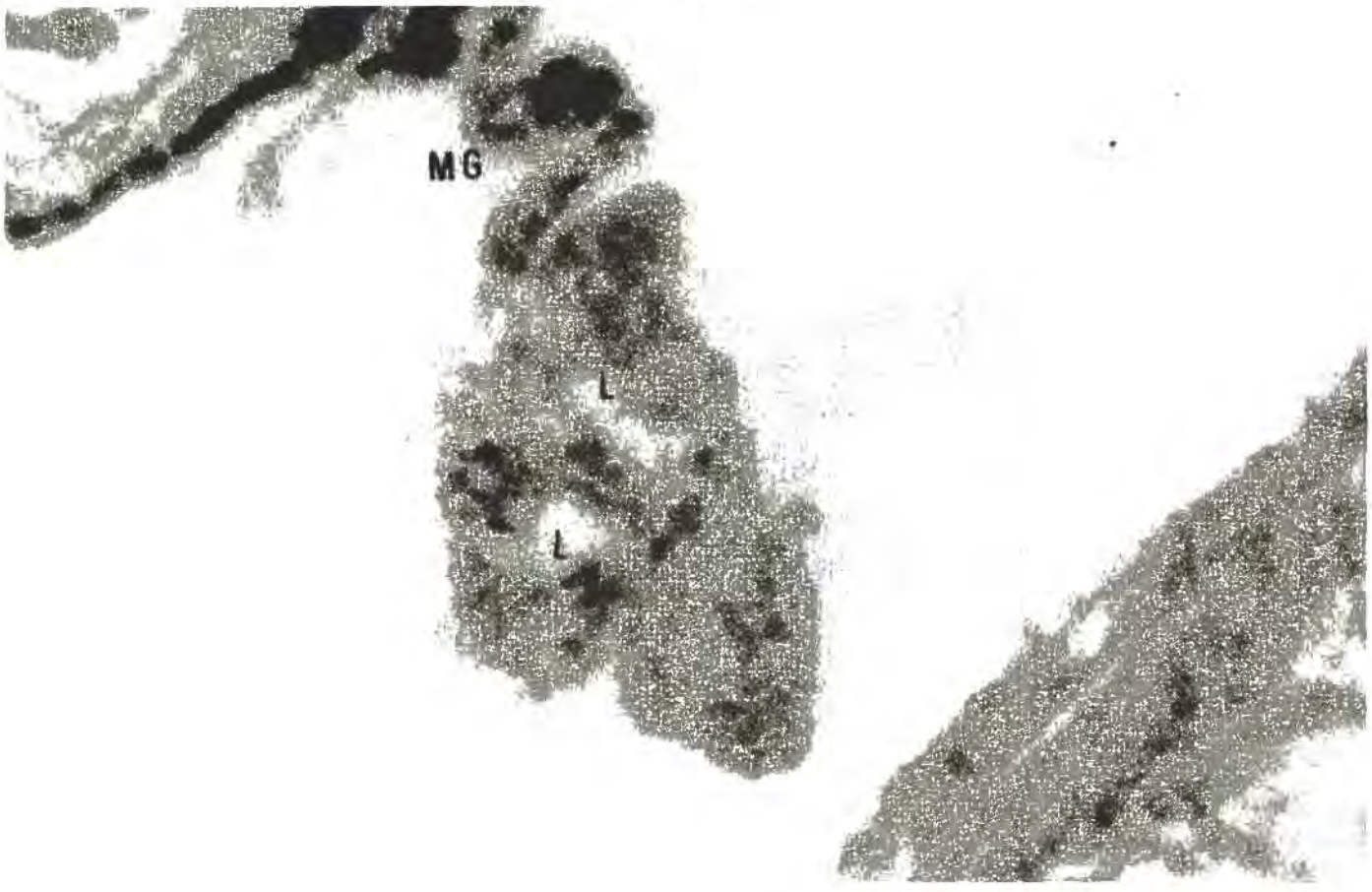

Fig. 5. The presumptive testis showing lumina (L) in the stroma tissue coincident with the efferent duct of the developing testis. MG-mesogonium, G-gut. Hematoxylin-eosin x 400.

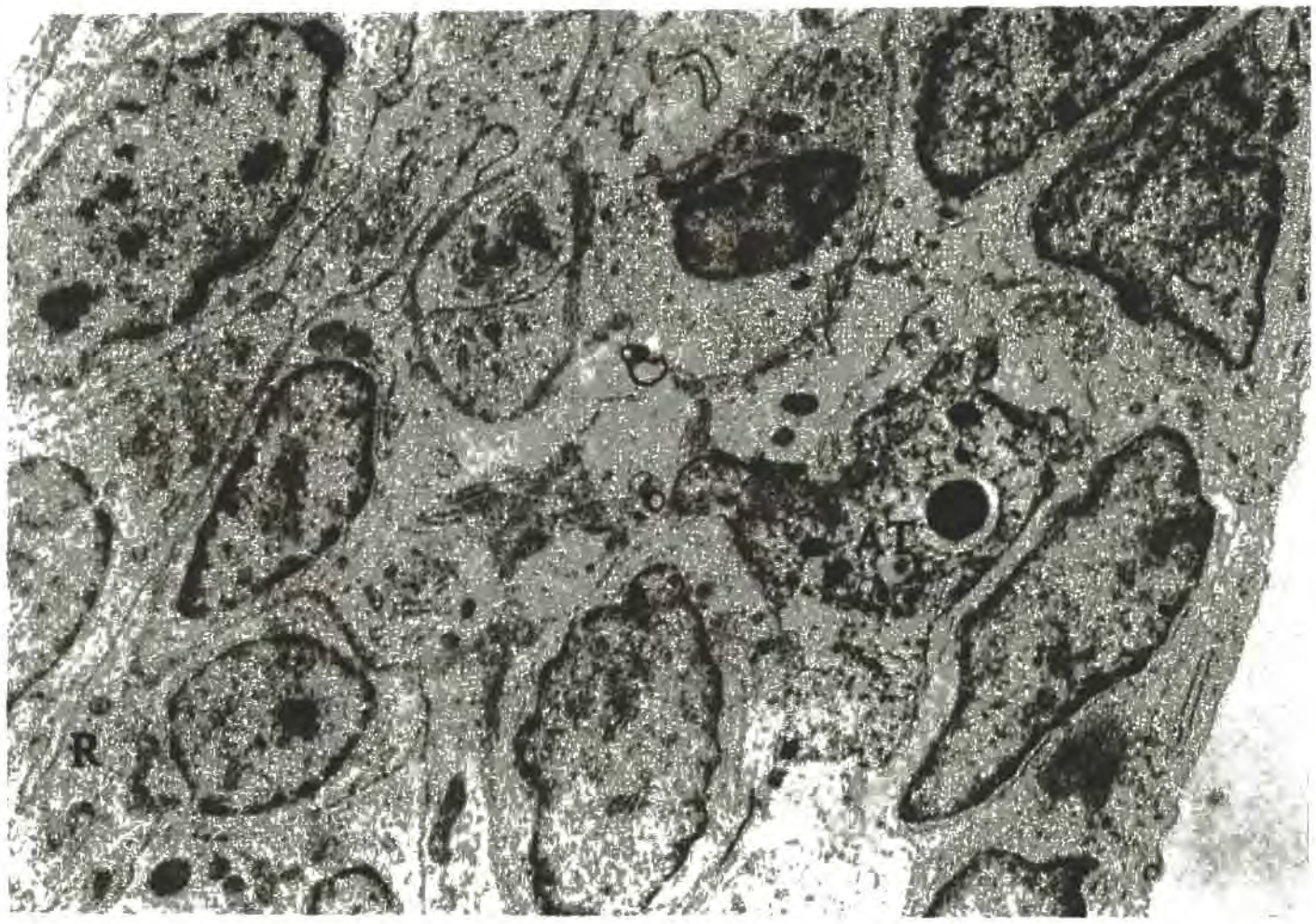

Fig. 6. Electron micrograph of a portion of the day 19 testis showing an aggregate of somatic cells with irregular cell and nuclear shapes, heterochromatic nucleus, dispersed ribosomes (R), vesicles and RER lamellae. AT-atretic cell. x 10,000.

Days 40-73

At about day 40, seminiferous tubules are more readily recognized in the testis (Fig. 7). Averaging 600 μm, the tubules are separated by myoid boundary cells. They are composed of large PGCs, spermatogenic and somatic cells (presumptive myoid boundary cells, Sertoli cells, interstitial cells). The PGCs have a very irregular cell and nuclear shape. In the cytoplasm are a few large mitochondria and some dispersed ER lamellae (Fig. 8).

The spermatogonia adjacent to the irregularly-shaped myoid boundary cells conform to the contour of the network. The spermatogonia farther away from the testis periphery where the myoid boundary cells abound have more or less regular cell outlines (Fig. 9). The heterochromatic nucleus is spherical to oval, and contains a prominent granular nucleolus. In the cytoplasm are several mitochondria, rough ER, Golgi bodies and ribosomes.

The somatic cells are much smaller than the germ cells. The myoid boundary cell body shows an irregular, heterochromatic nucleus. The interstitial cells are polygonal with heterochromatic, irregular nucleus. In the cytoplasm are several mitochondria, vesicles, rough ER and ribosomes (Fig. 10).

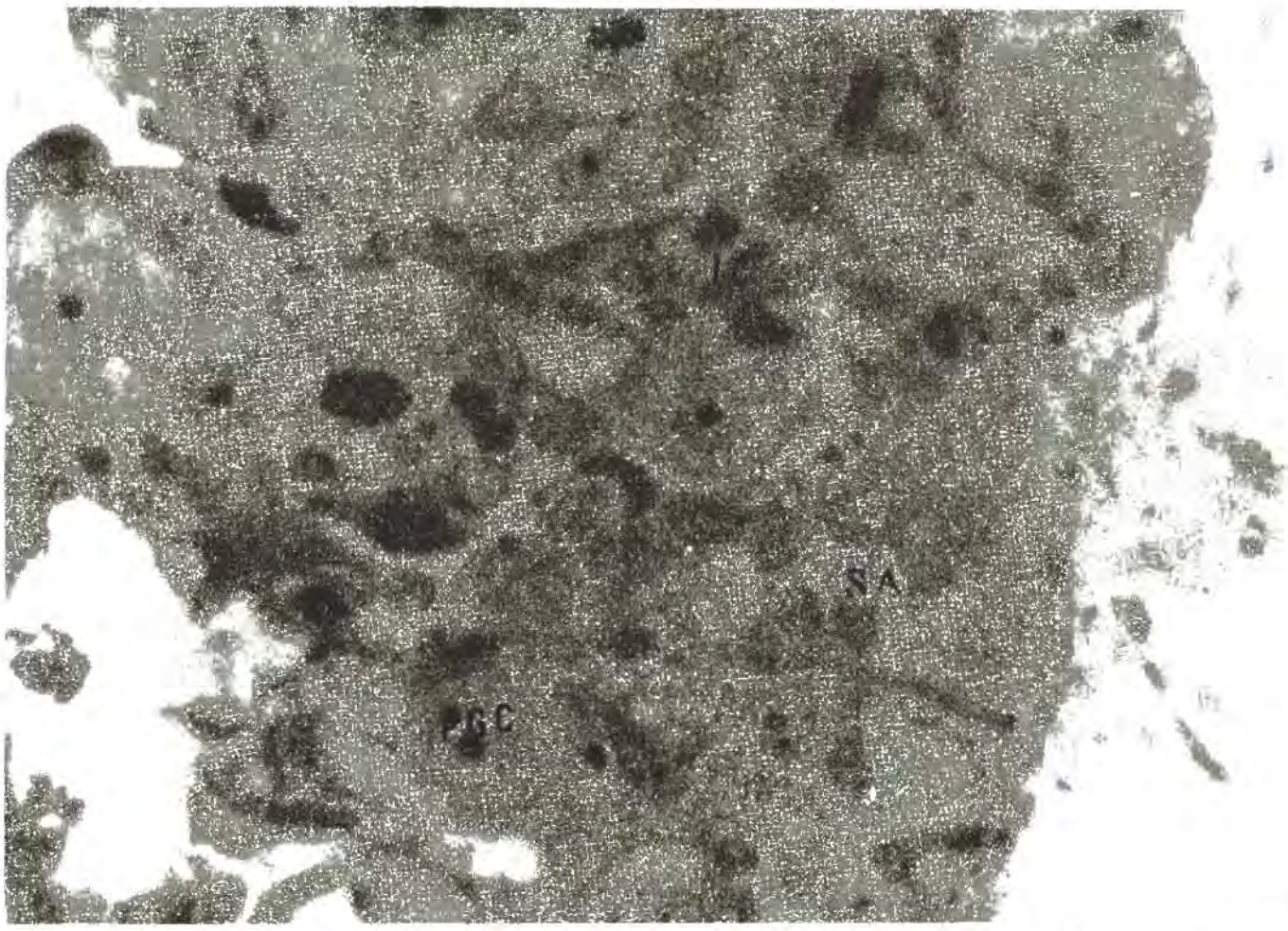

Fig. 7. The seminiferous tubules of the day 40 testis, IC-interstitial cell, SA-spermatogonia, PGC-primordial germ cell. x 1,000.

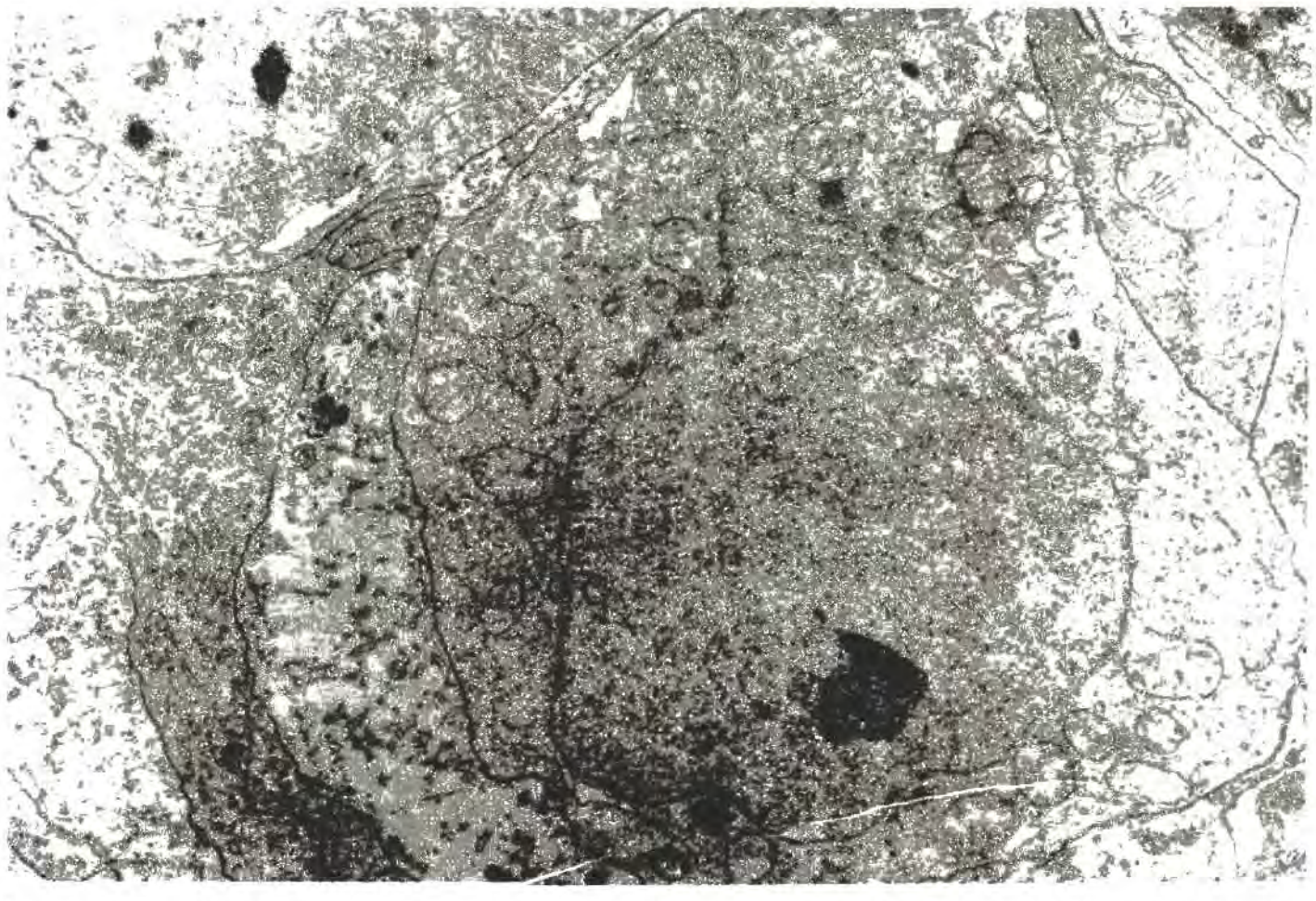

Fig. 8. Electron micrograph of a primordial germ cell at day 40. The cell and nuclear shapes are irregular. Germinal dense bodies (GDB) are present. x 13,600.

Fig. 9. Spermatogonia far from the myoid boundary cells have a more or less regular cell outline. VA-vacuole, M-mitochondria. x 13,300.

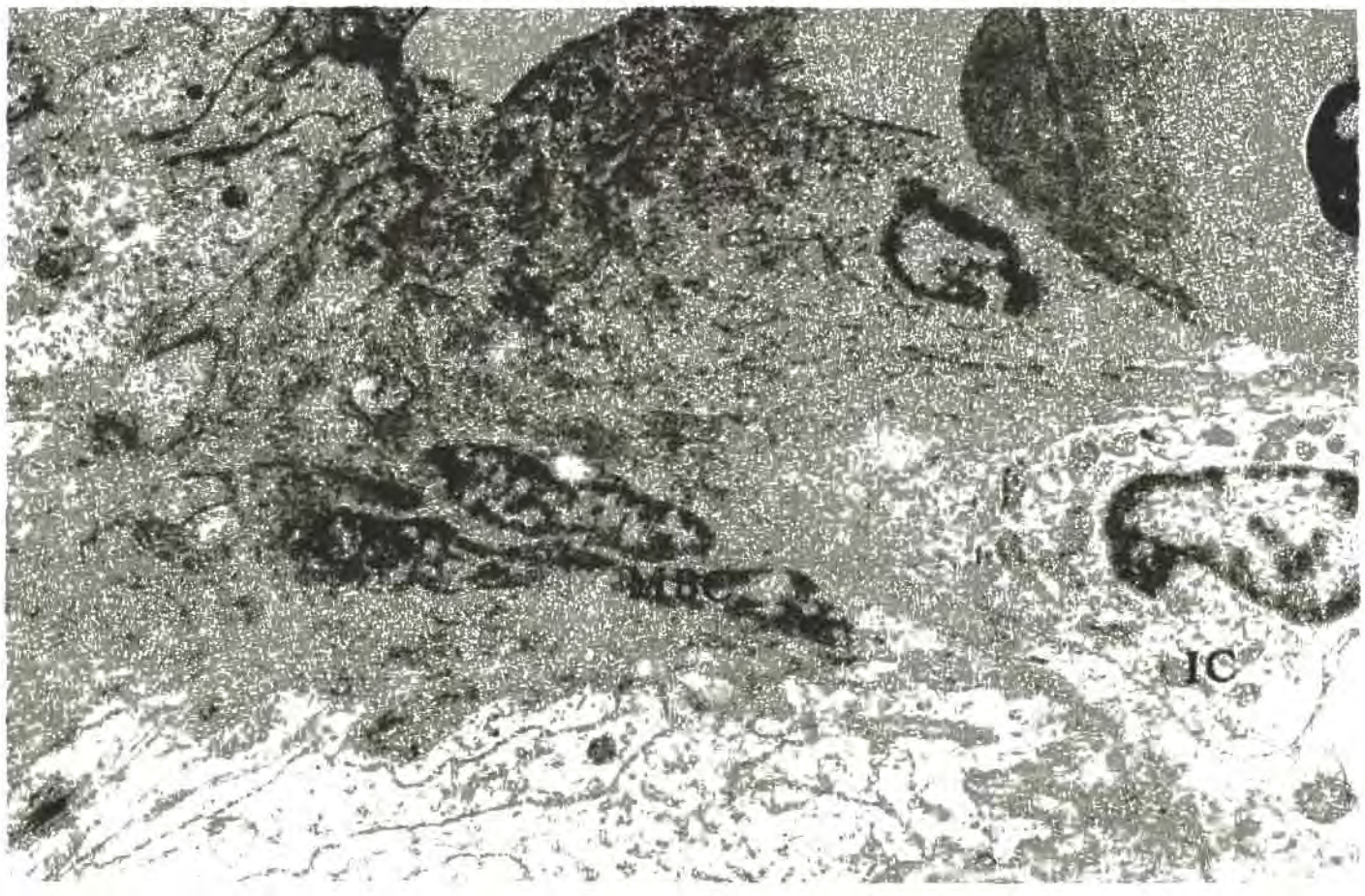

Fig. 10. Electron micrograph of the myoid boundary cells (MBC), and the interstitial cells (IC) of the day 40 testis. MBCs have cytoplasm extensions that serve as outer boundary of the seminiferous tubules. x 13,300.

By day 73 the testis is well into very active spermatogenesis. The cysts formed by the repeated division of gonia have become larger. With each division the cell sizes decrease and the cell group becomes larger. Chromatin of secondary gonia are highly condensed (Fig. 11). Mitochondria with parallel cristae are curved. Golgi bodies and rough ER are well-developed and ribosomes are scattered in the cytoplasm. The large round, heterochromatic nucleus almost fills up the entire cell. The primary spermatocytes, larger than the spermatogonia, are in various stages of meiotic prophase. Fig. 12 shows primary spermatocytes in pachytene stage. Chromosomes in synaptonemal complex can be easily recognized. In the cytoplasm are scattered vesicles, abundant free ribosomes, a few round to oval mitochondria and some rough ER lamellae. At no time after 66 days is a pure population of a single germ cell type present throughout the testis.

Days 88-100

The testis at this stage contains numerous large tubules filled with cells in varying stages of spermatogenesis (Figs. 13, 14, 15). Sperms fill the efferent ducts and main ducts ready for spermiation (Figs. 16, 17).

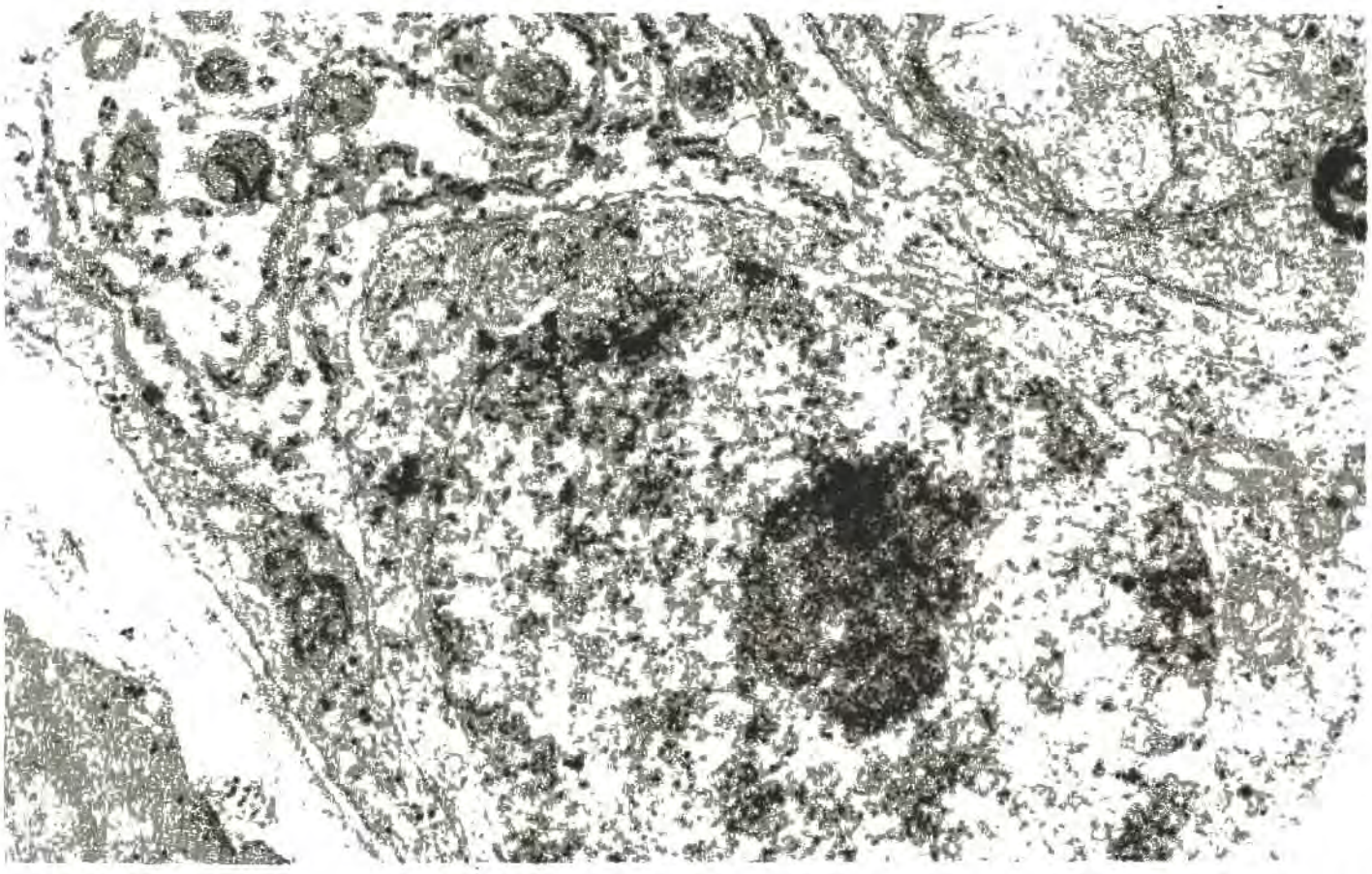

Fig. 11. Electron micrograph of spermatogonia at day 66. The cells have become smaller by the repeated division of a single spermatogonium. Mitochondria (M) with parallel cristae are observed. x 14,000.

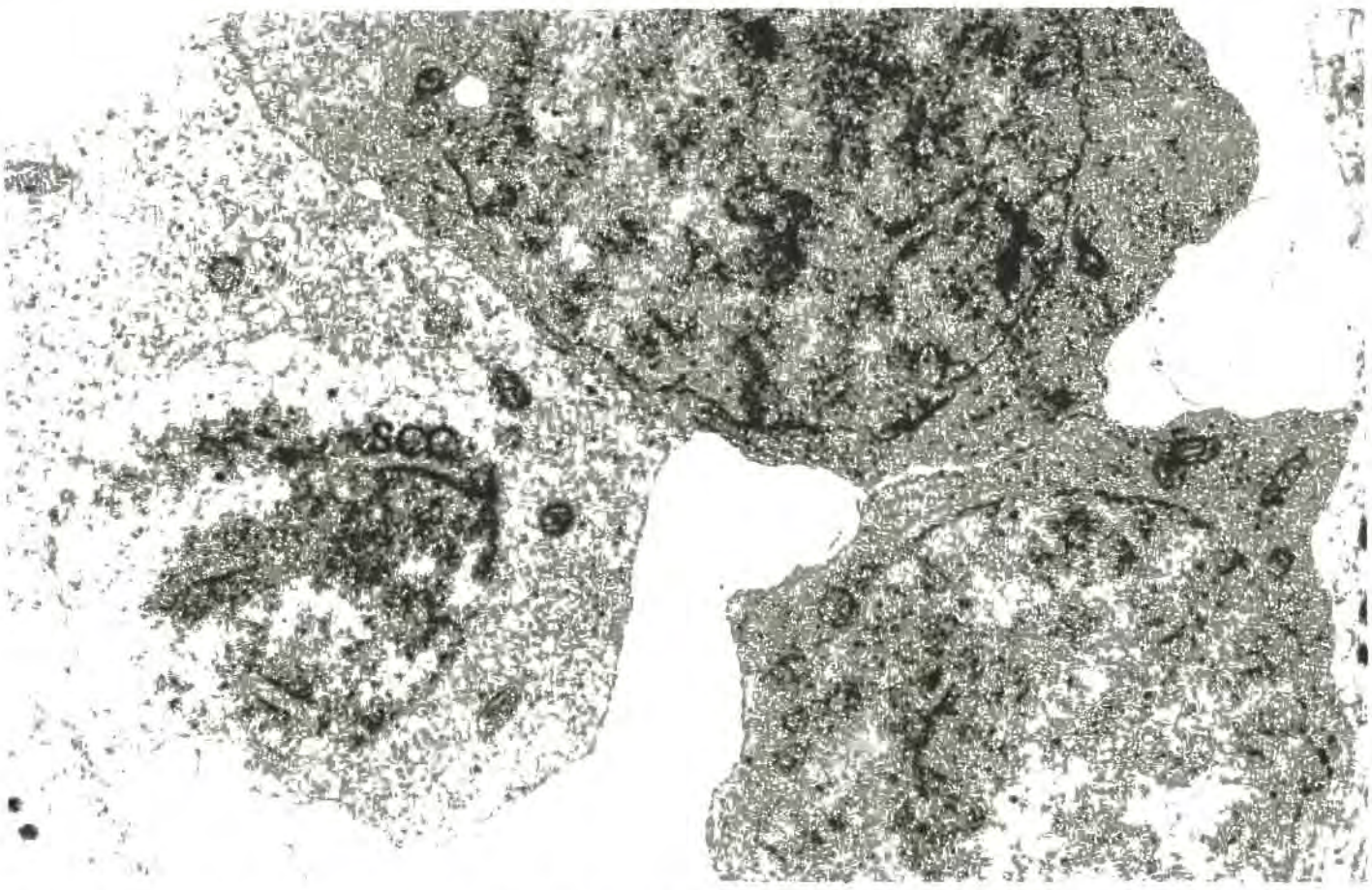

Fig. 12. Primary spermatocytes of the day 66 testis. The synaptonemal complex configuration (SCC) is clearly visible, the tripartite structure consisting in longitudinal section of two thick lateral elements and a fine central element running between them. The width of the complex is approximately 150 nm and the central element is about 10 nm in thickness. x 20,000.

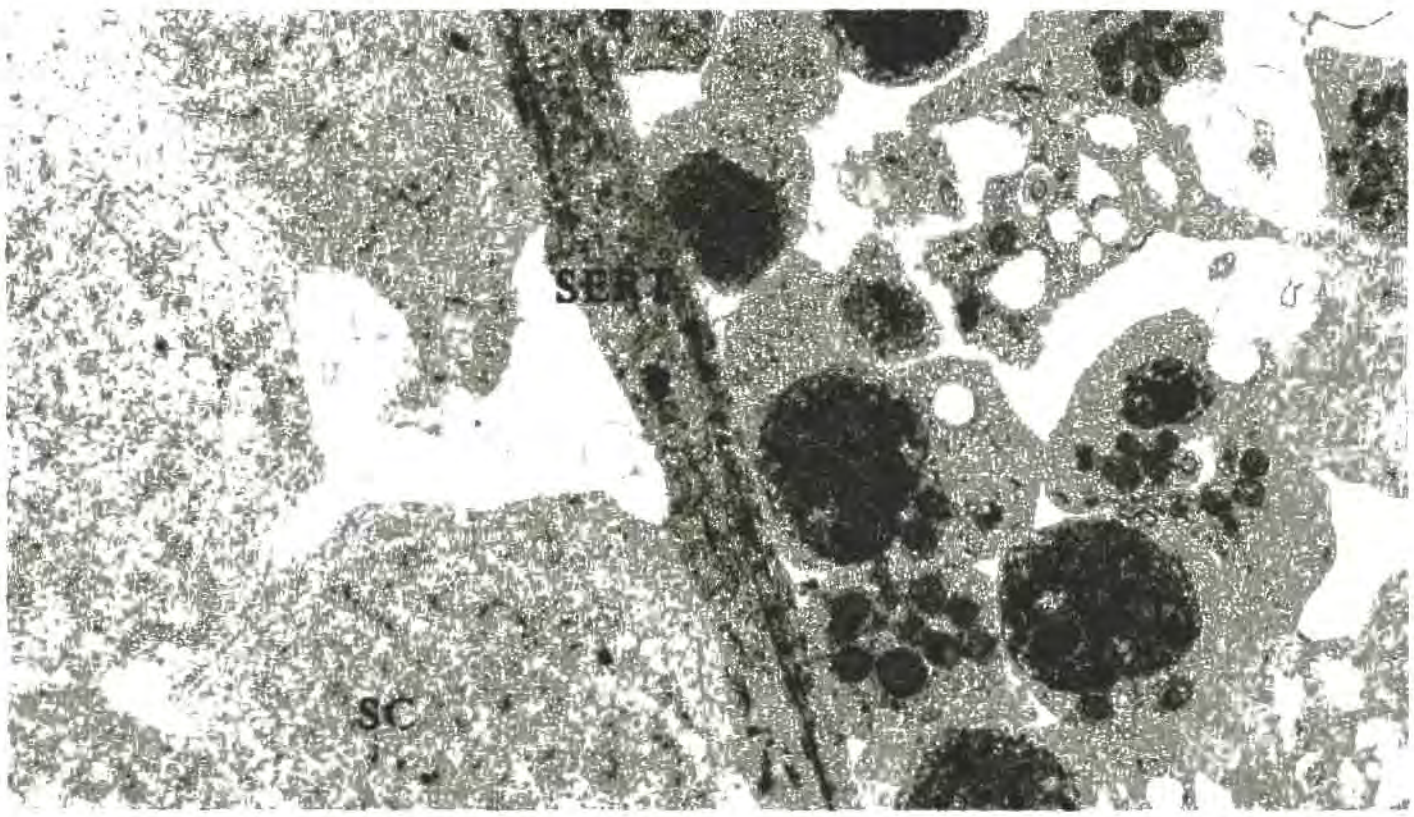

Fig. 13. Electron micrograph of primary spermatocytes (SC), spermatids (ST) and portion of a Sertoli cell (SERT). The primary spermatocytes are in zygotene. Mitochondria are proliferating to localize in the sperm midpiece during spermiogenesis. M-mitochondria, F-flagella. x 10,000.

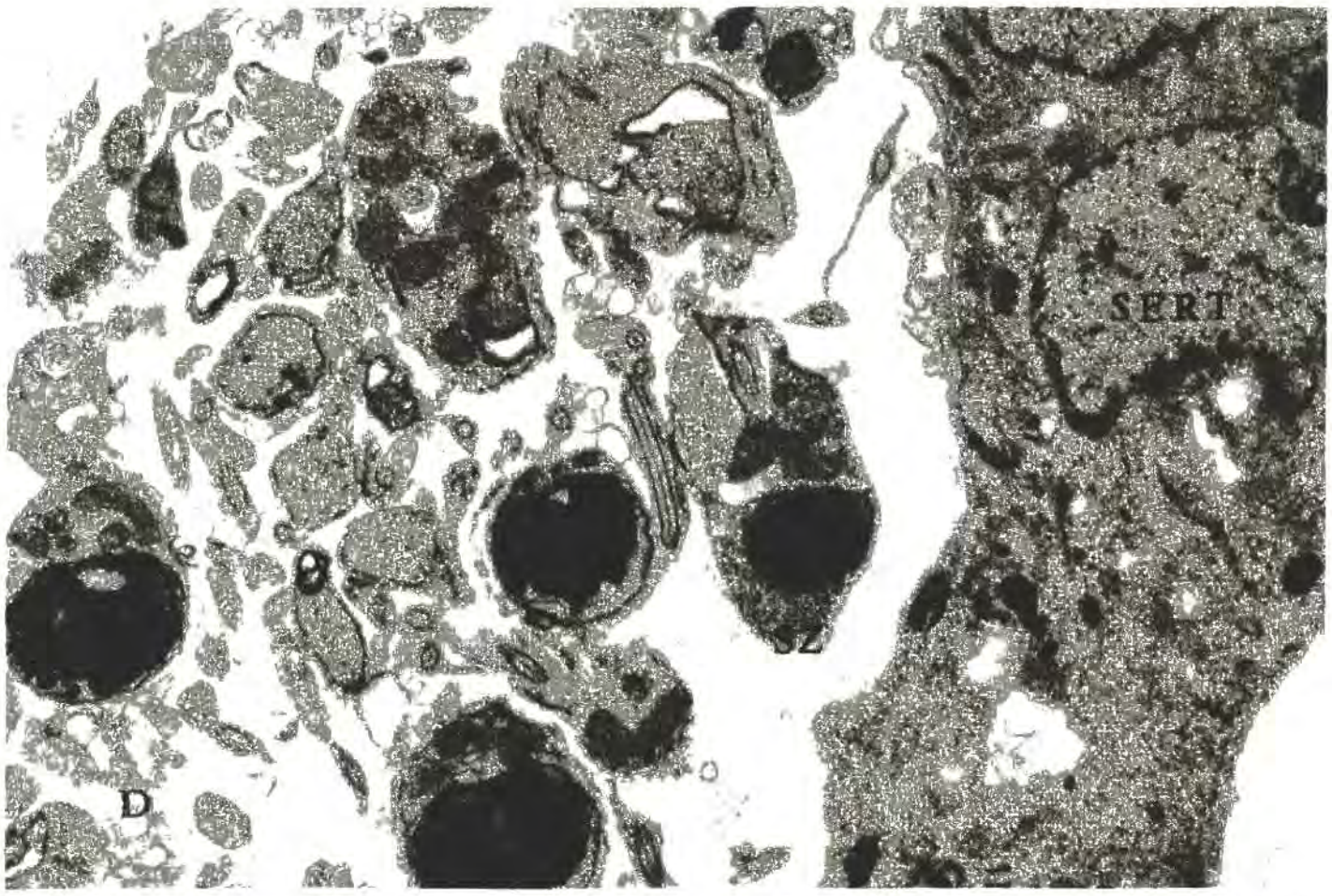

Fig. 14. Electron micrograph of a cluster of sperm cells (SZ) showing their condensed chromatin. Sertoli cells (SERT) line the efferent ducts. D-debris of cast-off cytoplasm of sperm. x 10,000.

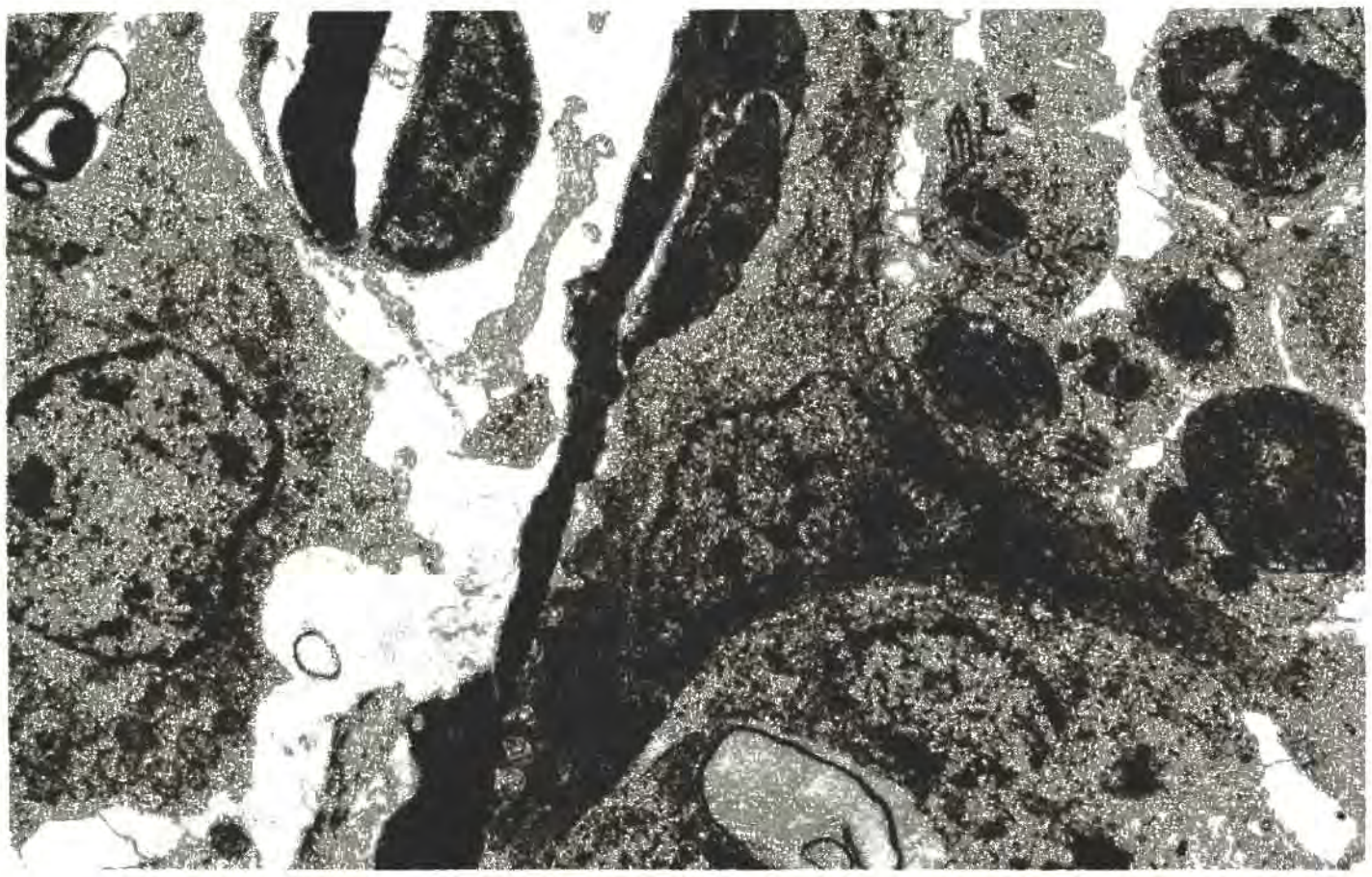

Fig. 15. Electron micrograph of a portion of a seminiferous tubule showing a Sertoli cell separating the cyst of spermatids in spermiogenesis from the cyst of spermatocytes. The myoid boundary cell lies outer to the Sertoli cell. AL-annulated lamellae. x 10.000.

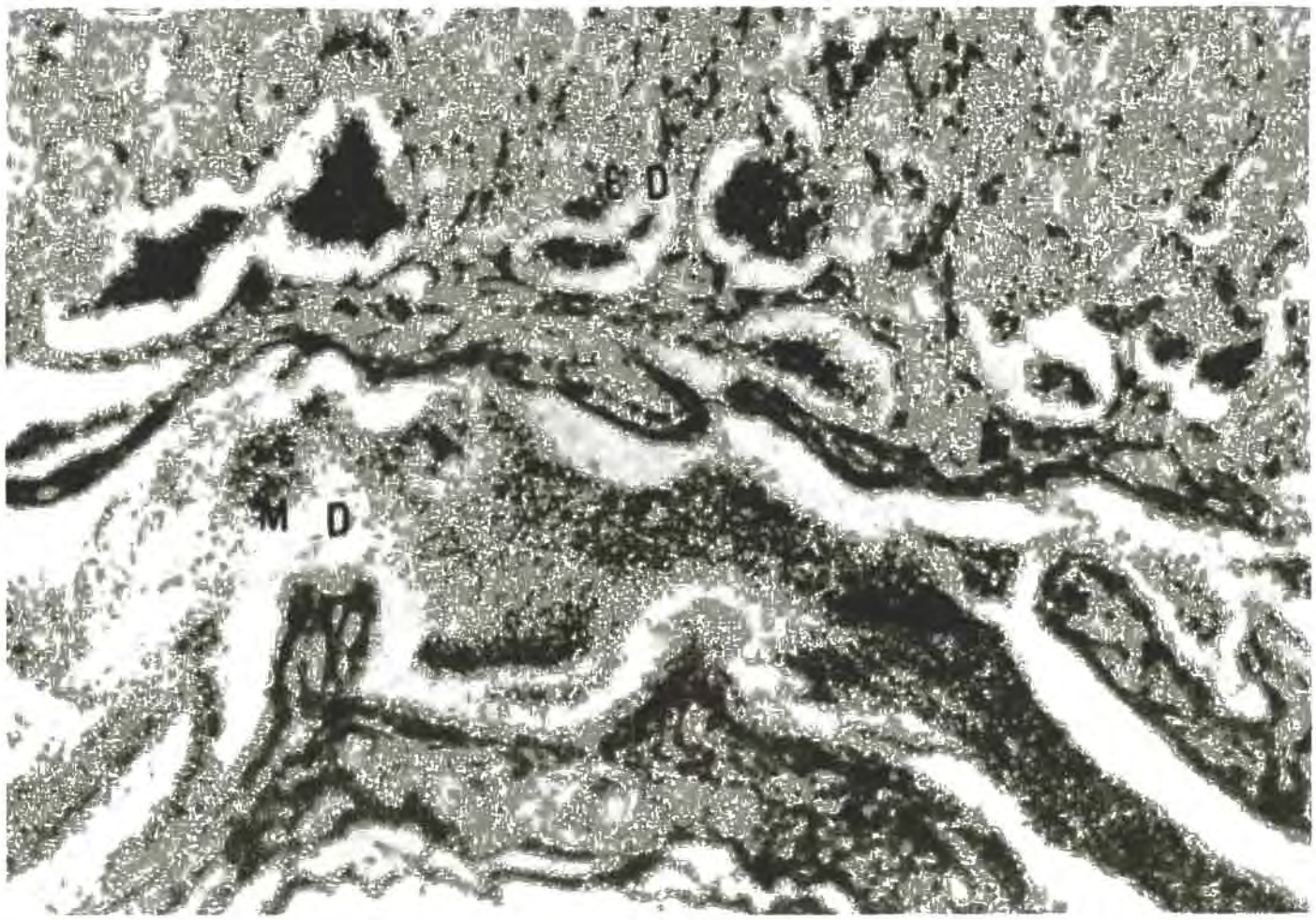

Fig. 16. The day 88 testis contains an abundance of spermatozoa (SZ) in the efferent ducts (ED) and main ducts (MD) of zone I. Hematoxylin-eosin. x 100.

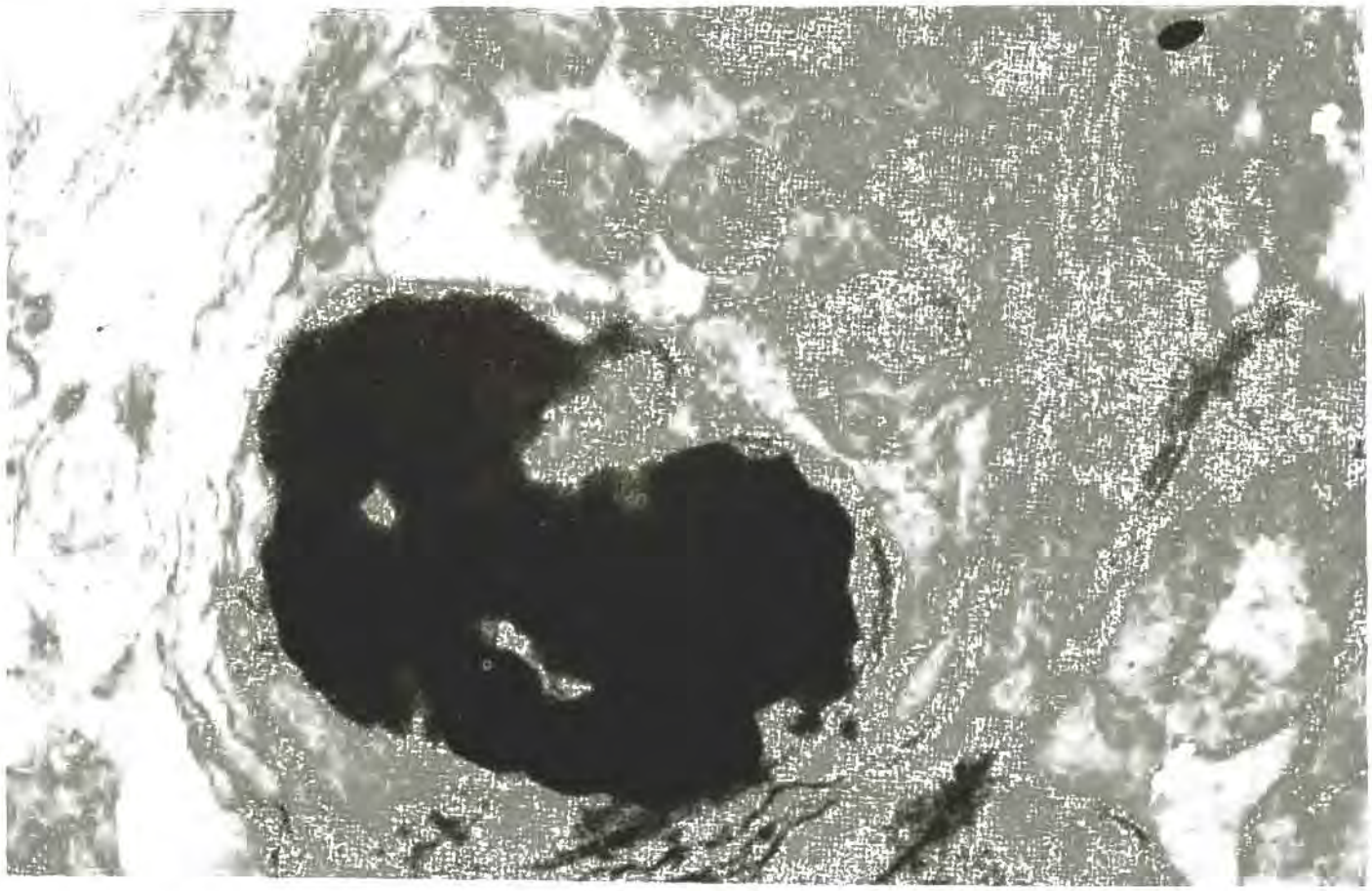

Fig. 17. A fully differentiated sperm. x 15,000.

Summary

The ultrastructure of spermatogenesis in *T. nilotica* is basically similar to that of other male vertebrates (Leeson and Leeson, 1985). The first batch of fully differentiated sperm formed during ontogeny are ready for spermiation by 88-100 days posthatching.

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UTILIZATION OF PROTOPLAST FUSION IN GENETIC ANALYSIS AND STRAIN IMPROVEMENT IN ANTIBIOTIC PRODUCING MICROORGANISMS

A.K. Raymundo, VG.A. Cadiz and N.T. Chua
Institute of Biological Sciences, University of the Philippines
Los Baños, College, Laguna, Philippines

ABSTRACT

Protoplasts of local streptomycin nitrosoguanidine treated strain of *Streptoverticillium mashuense* 2 CG_1 were prepared using lysozyme. Regeneration occurred at 2% on rich medium with stabilizers (Complete Regeneration Medium, CRM) only when polyvinylpyrrolidone (PVP), a plasma expander, was added. Regenerants were small and circular and emerged only on the 5th day of incubation as opposed to large fried egg morphology type of colonies of the non-protoplasts observed two days after incubation. Using a streptomycin resistant histidine requiring *Basillus polymyxa* mutant of an NRRL strain, protoplasts were also prepared with lysozyme and regenerated on HCP 1.5 also with PVP with a frequency of 1.82%. The protoplasts of this strain were fused with the protoplasts of a tyrosine requiring *B. polymyxa* strain. Of the 1000 regenerants analyzed, 26% were str^r his^+ tyr^-, 16.10% str^r his^-, 2.1% str^s his^- ,tyr^- and 0.6% str^s his^- tyr^+.

Introduction

The genetics of the streptomycetes and *Bacillus* species, the main antibiotic-producing organisms, has not been studied due to a lack of natural genetic transfer mechanisms. Conjugation, a plasmid-mediated transfer mechanism requiring cell to cell contact occurs only in some species. Transduction and transformation are other genetic transfer methods employed in other bacteria but the former requires the mediation of phages while the latter, a competence status, both of which are usually lacking in the above-mentioned bacteria.

Protoplast fusion appears to be the most feasible method for genetic studies of streptomycetes and *Bacillus.* This method consists of stripping cells of their wall to obtain protoplasts, fusion of the appropriate protoplasts using a fusing agent, and regeneration of the hybrid cells (fusants). During fusion, the cytoplasm and the whole chromosomes are brought together resulting in multiple gene exchanges and various hybrids that can be used for genetic analysis. In some cases, improved strains can be obtained.

At the Institute of Biological Sciences (IBS) and the National Institutes of Biotechnology and Applied Microbiology, a local streptomycin-producing isolate of

Streptoverticillium mashuense 2 CG_1 (Raymundo *et al.*, 1987a) and an NRRL strain of polymyxin-producing *Bacillus polymyxa* were studied. Except for a short report on regeneration of *Str. mashuense* SIP 5221 (Okanishi *et al.*, 1983) no other studies on protoplasts preparation, regeneration and fusion have been done on these two organisms.

A. *Streptoverticillium mashuense*

The strain, 2 $CG_{1\,4}$ nitrosoguanidine-treated and which was shown to be a potential high-yielding strain, (Raymundo *et al.*, 1987) was used in this study. The first problem was the establishment of a good protoplasting medium, one which would produce good growth with repressed sporulation, minimal pelleting and mycelial fragmentation. The Complete Precultivation Medium (CPM; Pigac *et al.*, 1982) proved to be the best among four media tested. In this medium, the transitional stage between the logarithmic and stationary growth phases reported to yield mycelia for optimal protoplast regeneration (Baltz, 1978) occurred after 48 hrs. Hence, mycelia at this stage was used for protoplasting.

Two types of enzymes, a) Sigma and b) Locally extracted by the Microbial Insecticide Lab (BIOTECH) were used for degrading the cell wall of bacteria in order to obtain protoplasts. Sigma lysozyme was required at 10 mg/ml to get 58% protoplast but only 3 mg/ml was needed of the local enzyme to get 99.9% protoplast. The difference is attributed to partial inactivation of the Sigma lysozyme.

Protoplasts are osmotically sensitive and thus burst when suspended in plain water. To check if the resulting cells after lysozyme treatment were indeed protoplasts, the suspension was serially diluted with plain water and plated. Only less than 1% grow in the medium indicating that most of the cells were indeed in protoplast form.

The sequence of events during protoplasting was noted. After one hr. of lysozyme treatment, initial mycelial fragmentation was observed under the microscope. The protoplasting medium became slightly cloudy due to release of protoplasmic contents. During the next hour, further mycelial fragmentation and the initiation of the protoplasting process starting from the ends of the mycelium occurred. After a total of three hrs, the mycelial fragments were almost completely converted to protoplasts.

The protoplasts obtained were spherical and transparent under ordinary light microscopy but were dark and surrounded by a halo under phase-contrast microscopy. They varied greatly in size, with the diameter ranging from 2.5-5.0 μm. These were easily differentiated from the uniform spores of *Strv. mashuense.* The protoplasts were also stable and were not lyzed by gentle centrifugation. This characteristic could be attributed to the stabilizing effect of the protoplasting Medium P which contained sucrose (osmotic stabilizer), $MgCl_2$ and $CaCl_2$ (cell membrane stabilizers) (Weibull, 1956; Tabor, 1962).

Regeneration of the protoplasts into cell-walled form is a critical step requiring the utilization of an appropriate regeneration medium. Preliminary experiments

were performed with the following regeneration media: R_2 (Okanishi *et al.*, 1974), RM (Ochi *et al.*, 1979), R_3 (Shirahama *et al.*, 1981), R_3M (Yamashita *et al.*, 1985a); R_2YE (Chater *et al.*, 1982), RM_2 (Ikeda *et al.*, 1982) and Ikeda's RM (Ikeda *et al.*, 1983). Protoplasts were plated using soft agar overlays containing 0.5% agar to prevent possible bursting of protoplasts which may occur if spread technique is used (Pigac *et al.*, 1982). After more than 2 weeks of incubation, colonies appeared on the regeneration medium but the number of colonies was more or less the same as those in the control plates. These colonies on the regeneration media were, therefore, inferred as non-protoplasts. No additional colonies grew on the above-mentioned media indicating non-regeneration of protoplasts.

To enhance regeneration, modifications were tried on the different media by changing sucrose concentration to 0.5 M $MgCl_2$ concentration to 3, 10, 25 and 50 mM $CaCl_2$ concentration to 3, 10, 25 and 50 mM; and by trying different combinations of these modifications. However, no regenerants were detected. Observation of the protoplasts plated on overlays of R_2 showed that they were stable and intact for up to one week. However, no morphological changes akin to regeneration were detected on the protoplasts. Further incubation resulted to progressive reduction in the number of protoplasts.

A further modification was the inclusion of polyvinylpyrrolidone (PVP), one of the known plasma expanders previously established as necessary for protoplast regeneration (Akamatsu and Sekiguchi, 1981) to a rich medium (Pigac *et al.*, 1982). Protoplasts emerged as feathery submerged colonies in the regeneration medium on the fifth day of incubation. With prolonged incubation, more putative regenerants appeared while early regenerants increased in colony size. After 10 days, the small and circular colonies of regenerants were easily differentiated from the much larger and fried-egg morphology of colonies of non-protoplasts which emerged two days after incubation. The difference in colony size is attributed to the growth advantage of non-protoplasts which did not need to synthesize cell wall prior to cell division. However, after 14 days of incubation, colonies from the regenerated protoplasts and non-protoplasted mycelia were no longer distinguishable. This indicated the necessity of monitoring the regeneration process during incubation. On a non-regeneration medium, colonies appeared after two days without the emergence of additional colonies showing that the putative regenerants were indeed regenerants. Fused colony morphology indicative of clustering of 5-6 colonies in a group was often observed among the regenerants. This was not observed in the control plates nor in the colonies of non-protoplasts. Percent regeneration was 2%.

Microscopic examination of the regenerating protoplasts showed that very short buds emerged after three days of incubation. These buds continued to elongate and as hyphal elongation progressed along with cell division, the protoplasts started to shrink until they were barely detectable. Staining of the protoplasts showed that even before the emergence of the hyphal bud, the spherical protoplasts have already synthesized cell walls since they were no longer affected by air-drying and heat-fixing which would have lyzed fresh protoplasts.

Fusion was not attempted with *Strv. mashuense* since markers have still to be established. However, no changes in streptomycin production and in morphology were observed with the regenerants. This indicates that genetic manipulation of protoplasts is feasible and changes in the fusants can then be attributed to gene exchanges during fusion.

B. *Bacillus polymyxa*

This study, being the first on protoplast preparation in *B. polymyxa*, necessitated an initial optimization of the conditions for protoplasting. A locally developed streptomycin resistant and histidine requiring (str^r his^-) (Ardales and Raymundo, 1986) marked mutant of the ATCC strain was used.

A range of temperature during lysozyme treatment was tried. The use of 37°C for a period of 2 hours resulted in 99.28% protoplasting efficiency. A temperature of 10°C also resulted in equally high percent of protoplasts (97.45%) indicative of non-inhibition of protoplast formation by low temperature. Increasing the temperature from 37°C to 42°C resulted in a decrease in percent protoplasts (94.20% and 81.44%, respectively). However, the decrease was not statistically significant indicating that a range of 10°C-42°C can be used for protoplast formation of *B. polymyxa*.

Conversion of cell to protoplast increased with increasing lysozyme (Sigma) concentration. At 1000 μg/ml concentration, 99.84% of cell were converted to protoplasts. Increasing lysozyme concentration to 200 μg/ml did not increase protoplasting efficiency. A concentration of 500 and 250 μg/ml likewise were effective with 96.89% and 84.36% protoplasting efficiency, respectively. A concentration of 100 μg/ml, however, resulted only in 70% protoplasts which was significantly lower than the above-mentioned results obtained with higher levels of lysozyme.

The use of optimum length of exposure to lysozyme is also important for efficient protoplasting of cells. At 30, 60 and 90 minutes of lysozyme treatment, 93.38%, 93.72% and 94.59%, 97.60% of the cells were already converted to protoplasts.

The age of cells suitable for lysozyme treatment was determined. Results after treatment of cells with 1000 μg/ml lysozyme for 120 minutes at 37°C indicated that cells from a culture grown for six and eight hours were the most susceptible to lysozyme treatment. A protoplasting efficiency of 95.34% and 95.09% were observed from cultures at 6th and 8th hours of growth, respectively. The cells at this stages of growth were approximately at the mid-logarithmic phase of growth.

The above results show that the highest protoplasting efficiency for *B. polymyxa* can be obtained by treatment of cells at mid-logarithmic phase (6th or 8th hours of growth) with 1.0 mg μg/ml lysozymes for 120 minutes at 37°C. However, a concentration of 0.25 mg/ml and 30 minutes incubation can also be used with minimal reduction in protoplasting efficiency.

The plating of protoplast on three different regeneration media showed that *B. polymyxa* str^r his^- growth can be observed after 24 hours of incubation on DM_3 and after 48 hours on HCP 1.5 medium. No growth was observed on the Hypertonic medium even after 7 days of incubation. The absence of regenerants on the Hypertonic medium could be attributed to the lack of complex compounds such as gelatin, horse serum or bovine serum albumin which have been established as necessary for protoplast regeneration (Gabor and Hotchkiss, 1979). DM_3 and HCP 1.5, on the other hand, are rich regeneration media and can provide the necessary carbon and nitrogen sources for cell wall synthesis and other metabolic requirements of the protoplasts during reversion. However, a regeneration frequency of 1.82×10^{-2} or 1.82% was observed on HCP 1.5 medium while only 3.25×10^{-3} or 0.32% was observed on DM_3 medium. Both media have the same composition except for polyvinylpyrrolidone (PVP) found on HCP 1.5 which could be responsible for the higher regeneration frequency of *B. polymyxa*. Akamatsu and Sekiguchi (1981) proposed that PVP stimulates division of protoplasts resulting in higher regeneration frequency.

Another locally developed strain which is tyrosine requiring (tyr^-), together with the str^r his strain, were used for the fusion experiment achieved by treatment of protoplasts with 40% PEG 3350 for two minutes. Based on the growth patterns of 1000 colonies on the different selective media the following fusion products were formed: 26% str^r his^+ tyr^-, 16.10 str^r his^- tyr^-, 2.10% str^s his^-tyr^- and 0.60% str^s his^- tyr^+ Of the parental types 31.60 were str^r his^- and 23.60% were tyr^-. No prototrophic colonies were observed.

The fusion products obtained after PEG treatment expressed partly the phenotypes of both parents indicating that recombination occurred.

The fusants were further analyzed to determine whether they are stable auxotrophic recombinants, complementing or non-complementing clones. Stable auxotrophic recombinants are haploids capable of maintaining their phenotypic characteristics even after several generations (Schaeffer *et al.*, 1976). Complementing clones possess the two parental genomes, phenotypically expressed, but would segregate after several generations resulting into colonies with parental phenotypes (Sanchez-Rivas, 1982). Non-complementing clones are those which carry both parental genomes but only one is expressed, the other being phenotypically silent. After several generations, segregation would occur and the unexpressed genome would become manifested (Hotchkiss and Gabor, 1980). Growth patterns of the progenies of these fusants showed that they were stable recombinant clones and hence are haploids.

Conditions for protoplasts preparation and regeneration have been established for both *Strv. mashuense* and *B. polymyxa*. In the latter, fusion was shown to be indeed possible with the production of hybrid colonies.

With this kind of technique, the genetic map could be started and essential genes can be identified. Strains possessing desirable characteristics could also be fused in the hope of obtaining high yielding strains and with perhaps better fermentation characteristics.

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ZOOGEOGRAPHICAL AFFINITIES OF PARAMPHISTOMIDS OF RUMINANTS

Salcedo L. Eduardo
Department of Veterinary Parasitology
College of Veterinary Medicine
University of the Philippines at Los Baños
College, Laguna

ABSTRACT

The distribution of the various species of the family Paramphistomidae is discussed.

Majority of species of the genus *Paramphistomum* occur in Asia and Europe and few are restricted to certain areas elsewhere. Previous records of some species were found to be cases of misidentifications. True records of *P. cervi* include only those in the north temperate regions. The species apparently is primarily a north temperate species and its distribution coincides with that of the intermediate host. Its introduction to south temperate areas is limited by the presence of suitable intermediate hosts as the species does not occur in South Africa and temperate parts of Australia but is now present in temperate Brazil.

The genus *Calicophoron* is predominantly African and of the 12 species, only four do not occur in Africa. All 12 species are restricted to bovidae. *C. calicophorum* is the most widespread species occurring in Asia, the USSR and Australia. *C. raja* has been recorded in 14 host genera all belonging to the bovidae, 9 of which are solely African. This species is one of the commonest in Africa but its intermediate hosts remain unknown. The species was recently identified in Cuba and was probably introduced in this country through importation of cattle and wild ruminants from Africa.

The genus *Gigantocotyle* is represented in Africa by three species and in Asia by only one. Two species occur in hippopotamus and two in ruminants. All three species of the genus *Explanatum* are Asian. Records of the presence of *E. explanatum* in Africa were cases of misidentification.

Members of the genus *Cotylophoron* occur in Africa, Asia and North and South America. Of the four African species, two were recorded outside the continent. Some earlier records of *C. cotylophorum* are also cases of misidentifications.

All 11 species of the genus *Orthocoelium* have been reported from Asia and only two species have been recorded also outside the continent. These species have probably been introduced through the zebu cattle and water buffalo from neighboring Asian countries to Australia, Kenya and Chad. All species of the genus *Leiperocotyle* and *Bilatorchis* are African and the host genera are solely African. Four paramphistomid genera characterized by the presence of pharyngeal diverticula are endemic in their occurrence as follows: *Balanorchis* in South America; *Stephanopharynx* and *Choerycotyloides* in Africa and *Olveria* in India.

Paramphistomids of ruminants probably have originated in tropical Asia. From here, they were dispersed by their hosts to several regions and in these regions evolved into genera and several species and flourished especially in

Africa where the climate is warm and a variety of ruminant hosts is present. It is believed that a land bridge existed during the late miocene between Asia and Africa and fossil records appear to suggest that families and tribes of animals invaded Africa from Asia. Despite however of extensive movements of their hosts, species of the family have not been widely dispersed by them because their establishment in new environments depends on the presence of suitable intermediate hosts.

Introduction

The superfamily Paramphistomoidae includes large assemblage of species occurring in almost all vertebrate host including mammals, birds, reptiles, amphibians and fishes. Those occurring in ruminants belong to two families, the Paramphistomidae (non-pouched amphistomes) and the Gastrothylacidae (pouched amphistomes). Generally, little is still known of the Paramphistomoidea but those occurring in ruminants are fairly known. With the recent revision of the family Paramphistomidae by the author after examination of enormous collections from various parts of the world, it is possible to discern the broad patterns of distribution and origin of the family Paramphistomidae. Gaps however still exist in certain areas. This paper deals only with those species of the family Paramphistomidae occurring in ruminants.

Materials and Methods

Specimens of Paramphistomids of ruminants were obtained from various sources. Majority of the specimens were from the collection of Dr. J. A. Dinnik; the late Dr. P.L. LeRoux, the London School of Hygiene and Tropical Medicine and the Commonwealth Institute of Helminthology (now CAB International Institute of Parasitology).

Materials were also examined from the reference collection of the British Museum (Natural History); London School of Hygiene and Tropical Medicine, University of London; Commonwealth Institute of Helminthology; Naturhistoriska Riksmuseet, Stockholm, Sweden; Musée Royal de l' Afrique Centrale, Tervuren Belgium; Instituto Oswaldo Cruz, Rió de Janeiro, Brazil; Institut für Parasitologie de Veterinärmedizinische Universität Wien, Austria and the Onderstepoort Veterinary Research Institue, Transvaal, Republic of South Africa.

Type specimens were re-examined on loan from the above institutions and Museum für Naturkunde (Bereich Zoologisches Museum) and der Alexander von Humboldt Universität zu Berlin, German Democratic Republic; Museum d' Histoire Naturelle Geneve, Switzerland; United States National Parasite Collection, USDA, Beltsville, Maryland and American Museum of Natural History, New York, U.S.A.

Additional materials were obtained from numerous individuals in various parts of the world who kindly provided specimens on request. These are given in

the taxonomic part published as series of papers (Eduardo 1982a, c; 1983; 1984; 1985a, b, c; 1986). Specimens from the Philippines are the author's own collection.

The host and origin of all materials examined were carefully noted. For specific identification, both hand thick and microscopic sections in the frontal, sagittal and transverse planes were prepared and representative specimens of some species were examined under the scanning electron microscope. The technique of hand thick section preparation and processing specimen for scanning electron microscopy are detailed in a separate paper (Eduardo, 1982b).

Results and Discussion

To present an accurate picture of the distribution of any particular group of parasites is difficult to provide especially when there is a dearth of information and when existing information contains inaccurate records of species due to misidentifications. Such is the case of the paramphistomids of ruminants where our knowledge of their geographical distribution is far from complete, gaps exist in certain areas and the life history of many species remains unknown. The situation is confused by many previous inaccurate records including reports of species by some authors who followed synonymies which were later proved to be valid species. Some misidentifications have been established by re-examination of the original materials or analysis of the original descriptions and accompanying illustrations but others could not be verified particularly when these records are incomplete or when the original materials are no longer available for re-examination.

All nine species of the genus *Paramphistomum* are parasitic only in ruminants, the majority of which occur in Asia and Europe and few are restricted to certain areas elsewhere. The exact distribution of *Paramphistomum cervi* is difficult to assess due to many previous dubious records. Maplestone (1923) confused the situation by placing eight species as synonyms of *P. cervi* and some subsequent authors based their identifications on this synonymy. Thus, the species has been recorded in various parts of the world giving a picture of a worldwide distribution. However, seven of the eight synonyms (some now belong to other genera) are in fact valid species. Therefore previous records of *P. cervi* which followed Maplestone's synonymy could also be any of the seven valid species. Subsequent re-examination of available original materials and investigation of recent collections have shown that the distribution of the species is not worldwide as was originally thought. Fischoeder (1903) was of the opinion that the species is purely of European distribution and Näsmark (1937) strongly endorsed this view. Dinnik and Dinnik (1954) have shown that what was previously recorded as *P. cervi* in East Africa by Dinnik (1951) was actually *P. microbothrium* (now moved to the genus *Calicophoron*). Record of the species by Joyeux and Baer (1928) in Dahomey and Stunkard (1929) in the Congo was regarded by Nasmärk (1937) as dubious identifications. He considered Stunkard's material as *P. clavula* (now moved to the genus *Calicophoron*) and concluded that *P. cervi* does not exist in Africa south of the Sahara.

Swart (1954) also claimed that previous records of *P. cervi* in the Republic of South Africa were infact *P. microbothrium.* Looss (1912) stated that what he described as *"Amphistomum conicum"* (=*P. cervi*) in Egypt in 1896 was actually *P. microbothrium.* Sey did not find *P. cervi* in his examination of amphistomes from Egyptian ruminants and stated that previous records of the species in the country could be *P. microbothrium.* Round (1968) concluded that none of the records of *P. cervi* in Africa is likely to be that of the species but of related ones.

Durie (1951) has shown that previous records of *P. cervi* in Australia were erroneous and these actually consisted of two species, *Ceylonocotyle streptocoelium* (now *Orthocoelium streptocoelium*) and *Calicophoron calicophorum.* Although *P. cervi* has been recorded in the Philippines (De Jesus, 1938; Tubangui, 1947), recent collections did not reveal the presence of this species there (Eduardo and Manuel, 1975; Eduardo, 1982c). Sey (1979) did not identify *P. cervi* in his examination of several collections of amphistomes of ruminants in India and specimens labeled *"P. cervi"* presented to him by various Indian authors were in fact either *P. epiclitum* or *P. gracile.* He came to the conclusion that previous records of *P. cervi* in the subcontinent could either be one of the two other species. Caballero y Caballero, Brenes and Jimenez-Quiros (1957) recorded *P. cervi* from *Bos taurus* in San Jose, Costa Rica but their description and illustration clearly indicate that their specimen was *Calicophoron microbothrioides.*

The result of the present study which consisted of examination of several collections from various parts of the world, both early and recent collections, also strongly indicates a limited distribution of *P. cervi.* The species was identified only in collections from some countries in Europe and from the only two samples from the yak (*Bos grunniens*) in Tibet. Recently, Velázquez-Maldonado (1976) recorded the species from cattle in Rio Grande du Sul, Brazil. The intermediate host of *P. cervi* in nature in Europe is *Planorbis planorbis* (Szidat, 1936). The distribution of this snail host includes Europe and western and northern Asia (Ellis, 1969; Frandsen personal communication). Other snails which were found experimentally to serve as intermediate hosts are *Anisus vortex, A. leucostomus, Bathyomphalus contortus, Hippeutis complanatus, Armiger crista* and *Segmentina nitida* (Kraneburg, 1977; Odening, Bockhardt and Gräfner, 1978). Since many previous records of *P. cervi* in tropical regions were found to be cases of misidentifications and true records include only those in the north temperate regions, the species apparently is primarily a north temperate species and its distribution coincides with that of the intermediate host. Its introduction to south temperate areas is limited by the presence of suitable intermediate hosts as the species does not occur in South Africa and temperate parts of Australia but is now present in temperate parts of Brazil.

Two species namely, *P. leydeni* and *P. hiberniae* which are closely related to *P. cervi* are also of European distribution. The former species has been recently recorded in Rio Grande du Sul, Brazil (Velázquez-Maldonado, 1976). The known snail hosts of both species serve also as intermediate hosts for *P. cervi, P. gracile,*

P. epiclitum, *P. ichikawai* and *P. gotoi* are primarily Asiatic species. The last two however extend to eastern Europe. *P. echikawai* also occurs in Australia, and *P. gotoi* has been recorded recently in Egypt from water buffalo (Sey, 1977). Both species and *P. gracile* have been recorded in Brazil (Velázquez-Maldonado, 1976) but the author's descriptions and illustrations clearly indicate that he was dealing with specimens of *P. leydeni* and *P. cervi*, respectively. The known intermediate host of *P. epiclitum* is *Indoplanorbis exustus* whose distribution includes India, Thailand, Malay Peninsula and Sumatra (Malek and Cheng, 1974). The record of the presence of *Cotylophoron indicum*, a species regarded here as a synonym of *P. epiclitum*, in Africa by Näsmark (1937) and Dinnik, Walker, Barnett and Brocklesby (1963) was a case of misidentification. The writer has re-examined Näsmark's material and his specimen was not of that species. Dinnik, Walker, Barnett and Brocklesby (1963) based their identification on Näsmark's description. The snail hosts of *P. ichikawai* are *Segnitilia* (now *Helicorbis*) *alphena* in Australia, *Helicorbis sujfunensis Gyraulus filiaris*, *Segmentina nitida*, *Polypylis largillieri* and *Hippeutis complanatus* in the U.S.S.R. and *Planorbis planorbis* in Hungary (Durie, 1953; Kiselev, 1967 and Sey and Vishnyakov, 1976).

Paramphistomum liorchis is so far known only in North and South America. It is mainly a parasite of the American deer belonging to the tribe Odocoeliein and its intermediate host is still not known. Only one species of the genus, *P. cephalophi* Eduardo, 1982 is so far known in Africa and it is a parasite of the black-fronted duiker (*Cephalophus nigrifrons*), whose present distribution is restricted only to Central Africa.

As judged from these records, the genus *Paramphistomum* is predominantly Euroasian and the genus has probably developed and radiated from this region. Despite extensive movements of the final hosts, species of the genus have not been widely dispersed by them because their establishment in new environment depends on the presence of suitable intermediate hosts.

The genus *Calicophoron* is predominantly African and of the 12 species of the genus only four, namely *C. calicophorum*, *C. papillosum*, *C. papilligerum* and *C. microbothrioides*, do not occur in Africa. All 12 species are restricted to the Bovidae. *C. calicophorum* is the most widespread species of the genus, occurring in Asia, the U.S.S.R. and Australia. Previous records of its occurrence in Africa are doubtful and probably are misidentifications. The description and illustration of Swart (1954) for the species based on specimens from the Republic of South Africa clearly indicate that his specimens were *Calicophoron raja*. Specimens labeled *"Paramphistomum calicophorum"* from the collection of the Onderstepoort Veterinary Research Institute were examined by the writer and these were found to be *C. raja*. Despite examination of extensive collections of amphistomes from various hosts and localities in Africa, *C. calicophorum* was not identified. The known intermediate hosts of *C. calicophorum* are planorbid snails, *Pygmanisus pelorius*, *Glyptanisus* (now *Gyraulus*) *gilberti* and *Segnitilia* (now *Helicorbis*)

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As judged from these records, the genus *Paramphistomum* is predominantly Euroasian and the genus has probably developed and radiated from this region. Despite extensive movements of the final hosts, species of the genus have not been widely dispersed by them because their establishment in new environment depends on the presence of suitable intermediate hosts.

The genus *Calicophoron* is predominantly African and of the 12 species of the genus only four, namely *C. calicophorum*, *C. papillosum*, *C. papilligerum* and *C. microbothrioides*, do not occur in Africa. All 12 species are restricted to the Bovidae. *C. calicophorum* is the most widespread species of the genus, occurring in Asia, the U.S.S.R. and Australia. Previous records of its occurrence in Africa are doubtful and probably are misidentifications. The description and illustration of Swart (1954) for the species based on specimens from the Republic of South Africa clearly indicate that his specimens were *Calicophoron raja*. Specimens labeled *"Paramphistomum calicophorum"* from the collection of the Onderstepoort Veterinary Research Institute were examined by the writer and these were found to be *C. raja*. Despite examination of extensive collections of amphistomes from various hosts and localities in Africa, *C. calicophorum* was not identified. The known intermediate hosts of *C. calicophorum* are planorbid snails, *Pygmanisus pelorius*, *Glyptanisus* (now *Gyraulus*) *gilberti* and *Segnitilia* (now *Helicorbis*)

alphena in Australia (Durie, 1956) and *Planorbis planorbis* and *Anisus* sp. in the U.S.S.R. (Katkov, 1973, Khaidarov, 1974). The species has probably been introduced to Australia through importation of water buffalo at various times from Indonesia and India. *Calicophoron papillosum* and *C. papilligerum* are so far known in India and the former also in Indonesia and their snail hosts are still unknown. The genus *Calicophoron* is represented by only one species, *C. microbothrioides* in North and Central America including the Carribean. The snail hosts of this species in the U.S.A. are lymnaeids, *Fossaria parva, F. modicella* and *Stagnicola cubensis. C. microbothrioides* also occurs in Eastern Europe. It has been recorded in the U.S.S.R. as "*Ceylonocotyle petrovi*" (Davydova, 1961) and has been introduced to Bulgaria through importation of cattle from the U.S.A. (Kamburov, Vasilev, Samnaliev and Kanev, 1977; Samnaliev, 1980). However, previous records of its occurrence in Albania (Erhardova, 1964) and Hungary (Kotlan 1958, 1960) were cases of misidentifications and these authors were dealing with specimens of *Calicophoron daubneyi* (Odening and Gräfner, 1979). *C. microbothrioides* has recently been recorded from cattle in the Philippines (Eduardo, Kaw and Javellana, 1987). It probably was introduced to the country through importation of cattle from the U.S.A.

The African species of *Calicophoron* are: *C. microbothrium, C. bothriophoron, C. raja, C. clavula, C. sukari, C. phillerouxi, C. daubneyi* and *C. sukumum.* Two of the above, namely *C. microbothrium* and *C. daubneyi* extend outside the continent. The former occurs in the Mediterrenean where it is the predominant species, in Portugal and in the Near East and the latter also in the Mediterrenean and Eastern Europe. The known intermediate hosts of *C. microbothrium* are bulinid snails. According to Dinnik (1965), *Bulinus truncatus* acts as the intermediate host for the species in North Africa, the Mediterrenean and the Near East and *Bulinus tropicus* and some species of the subgenus *Physopsis* in Africa south of the Sahara where these are widespread. On the other hand, the intermediate hosts of *C. daubneyi* are lymnaeid snails, *Lymnaea truncatula* in Kenya (Dinnik, 1962), *L. truncatula* and *L. peregra* in East Germany (Odening, Bockhardt and Gräfner, 1978) and *L. peregra* in Hungary (Sey, 1974). Dinnik (1962) has demonstrated experimentally that *C. daubneyi* failed to develop in bulinid snails which are intermediate hosts of *C. microbothrium*, likewise, the latter species failed to develop in *Lymnaea truncatula* which is the intermediate host of *C. daubneyi.* Sey (1974) failed to infect snails belonging to the same family as *Bulinus*, namely *Planorbis planorbis, P. spirorbis* and *Gyraulus crista* with miracidia of *C. daubneyi.* Both species appear to be strictly specific to their respective intermediate hosts. Although *C. microbothrium* has been previously recorded in Eastern Europe, i.e. Hungary (Kótlan, 1958; Sey, 1971), Bulgaria (Mereminskii and Vishnyakov, 1969; Vasilev and Samnaliev, 1974; Mikhailova, Gateva and Nedeva, 1972-73), the Balkans (Kótrla, Prokopič and Vishnyakov, 1974), recent investigations in these areas did not reveal the species but of another closely related one, *C. daubneyi* (Sey, 1974; Sey and Vishnyakov, 1976) and it is more likely that the above

authors were dealing with the latter species. This is supported by the fact that bulinid snails which serve as intermediate host for *C. microbothrium* do not exist in these areas, but lymnaeid snails which are intermediate hosts of *C. daubneyi* are present. In Europe, bulinid snails are distributed only in the south-western areas which include the Iberian Peninsula, southern France, Sardinia and Corsica (Haas, 1935; Mandahl-Barth, 1965). *Calicophoron raja* has been recorded in 14 host genera all belonging to the Bovidae, 9 of which are solely African. This species is one of the commonest in Africa but its intermediate host still remains unknown. Recently, the writer has identified the species in a collection of paramphistomes from cattle in Cuba. It probably has been introduced through importation of cattle or other wild ruminants from Africa and has established itself in the island due to the favourable climatic condition and presence of suitable snail host. *C. bothriophoron* has also been recorded in the neighbouring islands of Madagascar and Mauritius in domestic ruminants, it has been probably introduced from Africa through these hosts. The report of its occurrence in *Bos taurus brachycerus* in Bulgaria by Mikhailova, Gateva and Nedeva (1972-73) was a case of misidentification according to Odening and Gräfner (1979) who claimed that they were dealing with specimens of *Paramphistomum ichikawai*. *C. clavula* has been recorded in 8 host genera, all of the Bovidae, of which 5 are solely African wild ruminants. Its intermediate host in Somalia is *Bulinus abyssinicus* (Sobrero, 1962). Previous records of its occurrence in Hungary (Kótlan, 1958), Turkey (Güralp and Oguz, 1967) and Bulgaria (Mikhailova, Gateva and Nedeva, 1972-73) were misidentifications according to Odening and Gräfner (1979) and the species involved was in fact *C. daubneyi*. *C. phillerouxi* has been recorded in 8 host genera and with the exception of the genus *Bos*, all are solely African. Morphologically, the species is very closely related to *C. microbothrium* and could easily be mistaken for it. Dinnik (1961) has however demonstrated experimentally that the species does not develop in snail hosts which serve as intermediate hosts for *C. microbothrium* and *C. daubneyi*. Its known intermediate hosts are bulinid snails of the forskalii group (*Bulinus forskalii*, *B. senegalensis* and *B. cernicus*). *C. sukari* primarily occurs in domestic ruminants, but it has been recorded in *Syncerus caffer* and an unidentified antelope (Gretillat, 1964). Its known snail host is *Biomphalaria pfeifferi* and its subspecies (Dinnik, 1954; Dinnik, 1965; Dinnik and Dinnik, 1957).

From the above, the genus *Calicophoron* appears to have developed in Africa and from here radiated to other areas. It is also apparent that in species where the life cycle is known, many are highly specific to their snail hosts and their introduction to new environments is limited by the presence in nature of these intermediate hosts.

The genus *Gigantocotyle* is represented in Africa by three species, *G. gigantocotyle*, *G. duplicitestorum* and *G. symmeri* and in Asia by only one, *G. formosanum*. The first two species occur in the hippopotamus and the last two in ruminants. Round (1968) cited LeRoux (1933) to have recorded *G. formosanum* in cattle and *Kobus leche* in Zambia but this record has never been confirmed. As no additional

record of this species in Africa has appeared since then despite extensive surveys in recent years, it is more likely that LeRoux was dealing with a different species. Although existing hippopotamuses are restricted to Africa, their fossil remains were found in Eurasia from the late Pliocene and Pleistocene periods and in Madagascar from the Pleistocene.

All three species of the genus *Explanatum* are Asian, previous records of the presence of *E. explanatum* in Africa (Maplestone, 1923; Dubois, 1930; LeRoux, 1931) were misidentifications. It is clear from the illustrations of Maplestone (1923) and Dubois (1930) that they were dealing with a different species, most likely *Calicophoron raja*. Jansen, Pacenovsky and Krupicer (1974) recently reported the species from a *Damaliscus albifrons* that died in Rotterdam Zoo (although the origin of the host was not specified, it is an African host), but their illustration also clearly indicates that their specimen was *C. raja*. The known intermediate hosts of *E. explanatum* are: *Indoplanorbis exustus, Gyraulus convexiusculus* and *Lymnaea luteola f. australis* (Srivastava, 1944, Singh, 1958; Mukherjee, 1962, Agrawal, 1971). *Gyraulus convexiusculus* also serves as intermediate host for *E. bathycotyle* (Jain, 1969) in India.

Members of the genus *Cotylophoron* occur in Africa, Asia and North and South America. Of the four African species, two were also recorded outside the continent, *C. cotylophorum* in various areas in Asia and North America and *C. fuelleborni* in the U.S.A. as *C. noveboracensis*. Some earlier records of *C. cotylophorum* however were misidentifications. *C. cotylophorum* of LeRoux (1930) in the Republic of South Africa and of Krull (1934) and Bennett (1936, 1938) in the U.S.A. were found to be *Calicophoron microbothrium* and *C. microbothrioides* respectively (Dinnik, 1965; Price and McIntosh, 1944). The writer has also examined specimens labeled *"Cotylophoron cotylophorum"* from Puerto Rico and these were found to be *Calicophoron microbothrioides. Cotylophoron panamensis* is the common species of the genus in the new world. Its distribution includes the southern states of the U.S.A., Central America and the Caribbean and northern regions of South America. Asian species of the genus include *C. bareilliense* in India and the Philippines and *C. xiangjiangense* in China. The genus does not occur in Europe and Australia despite extensive movements of animal hosts. Previous records of *Cotylophoron* species in Australia, Europe including the U.S.S.R. were misidentifications. The known intermediate host of *C. cotylophorum* in India is *Indoplanorbis exustus* (Srivastava, 1937; Sinha, 1950).

All the 11 species of the genus *Orthocoelium* have been reported from Asia and only two species have been recorded also outside the continent, *O. streptocoelium* in Australia (Durie, 1951) and the Belgian Congo (now Zaire) (Van Strydonck, 1970) and *O. scoliocoelium* in Kenya (Dinnik, 1956) and Chad (present work). This species have probably been introduced through the zebu cattle and water buffallo *(Bubalus bubalis)* from neighbouring Asian countries. Erhardova (1964) recorded *O. scoliocoelium* in Czechoslovakia but Odening and Gräfner (1979) have shown that the material was in fact *Paramphistomum ichikawai*. The known intermediate hosts of *O. streptocoelium, O. dicranocoelium* and *O. scolio-*

coelium are *Glyptanisus* (=Gyraulus) *gilberti* in Australia (Durie, 1953), *Bulinus pulchellus* in India (Jain, 1969); *Anisus natalensis* (now *Ceratophalus natalensis*) in Kenya (Dinnik, 1951) and *Bulinus pulchellus* in India; *Gyraulus convexiuaculus* in the Philippines (Mukherjee and Chauhan, 1965; Jain, 1977, Jain and Srivastava, 1969; Eduardo and Kaw, 1986 respectively).

All three species of the genus *Leiperocotyle* are African and the host genera are solely African, *L. okapi* and *L. congolense* in the Okapi *(Okapia johnstoni)* and *L. gretillati* in *Syncerus caffer*. Porter (1947) (as cited by Round, 1968) has reported an unidentified *Paramphistomum* species from a giraffe *(Giraffa camelopardalis)* that died in the London Zoo and as far as the writer is aware, this is the only record of a paramphistomid in this animal. The giraffe is related to the okapi and both belong to the same family, the Giraffidae. Although their present distribution is restricted to Africa, fossil giraffids are known from Asia in the Meiocene and Pleistocene and several groups reached eastern Europe in the Lower Pliocene (Darlington, 1963).

The monotypic genus *Bilatorchis* has so far been recorded in only two African host genera, *Kobus* and *Limnotragus*.

Among the paramphistomid genera occurring in ruminants, four are characterized by the presence of pharyngeal diverticula and because of this, they may be regarded as primitive. These genera are endemic in their occurrence as follows: *Balanorchis* (monotypic) in South America; *Stephanopharynx* (with three species but two of which are regarded here as synonyms), *Choerocotyloides* (monotypic) in Africa and *Olveria* (with two species) in India.

Paramphistomids of ruminants probably have originated in tropical Asia. From here they were dispersed by their hosts to several regions and in these regions evolved into genera and several species and flourished especially in Africa where the climate is warm. It is believed that a land bridge existed during the late Miocene between Asia and Africa and fossil records appear to suggest that families and tribes of animals invaded Africa from Asia.

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BIOMASS PREDICTION EQUATIONS FOR GIANT IPIL-IPIL [*LEUCAENA LEUCOCEPHALA* (LAM.) DE WIT]

Leuvina Micosa Tandug
Forest Regulation and Utilization Division
Forest Research Institute, College, Laguna, Philippines

ABSTRACT

Aboveground biomass of 111 giant ipil-ipil trees with age 2-10 years from six provinces of the Philippines were determined to develop equations for estimating fresh and ovendry weight of the whole tree and its components.

Average ovendry weight of the total tree biomass ranged from 2.97 to 517.33 kg. The average tree contained 71.38 percent of the total dry weight in the merchantable bole, 3.45 in the foliage and 25.17 in the topwood, small and large branches and twigs.

Out of the seventeen regression models tested and evaluated for biomass estimation, an allometric model using two predictor variables provided the best estimates. Prediction equations based on this model and two others were derived in estimating fresh and ovendry weight of the whole tree and its components, viz., bole, topwood and large branches, stems and leaves.

Introduction

The current worldwide energy crisis has placed the less affluent nations to severe economic pressures. In an effort to remedy the situation, these countries have resorted to tapping what is available and preferably renewable energy sources, prominent of which is forest biomass. People turn to the forest to satisfy the demand for wood, causing great damage to the resource base and to the forest landscape in general. As a policy, maximum tree utilization was encouraged to alleviate the situation. What used to be wastes and residues, i.e., tops, branches, stumps and butt trimmings are now utilized for various productive purposes. For one, these are being used for generating power. They are likewise used as raw materials for pulp and paper, charcoal, and other products for industrial purposes. Lately, the leaves of some tree species, particularly ipil-ipil, are utilized as forage, leaf meal, and organic fertilizer to augment the shortage of animal feed and to cushion the high price of chemical fertilizers.

Over the great concern for the dwindling supply of wood, and the rate at which the forests are being exploited, establishment of industrial and energy forest plantations of fast growing species in the Philippines are being accelerated. Towards

this end, however, accurate strategies for estimating the biomass values of these plantations for effective management as well as for commercial business transactions, would become a problem.

Now, since we are aiming for a 100% use for every tree we cut, reliable estimation of the different components is of paramount concern. The study developed biomass prediction equations for estimating fresh and ovendry weight of the merchantable wood, tops, branches and leaves of giant ipil-ipil. Weight, as the universally adopted measurement for quantifying biomass of all components, was used.

Methodology

The test plant: giant ipil-ipil

Leucaena leucocephala (Lam.) de Wit, locally known as giant ipil-ipil, astonishes thousands of people with its fast growth, multiple uses and adaptability to various site conditions. Among the fast-growing and high-yielding varieties that have been disseminated and known to thrive well in the Philippines are the Salvador type from Hawaii. These Hawaiian giants particularly those which have been identified for wood production like k-28 and k-8 have been widely used for tree planting projects in the Philippines in the last decade (Revilla and Gregorio, 1983).

Giant ipil-ipil grows on almost any type of soil but thrives best on well drained soils. It is adversely affected by strongly acidic soils, i.e. at pH below 5.5 (Tilo, 1977). It is very sensitive to phosphorous and calcium deficiency in soils (National Academy of Sciences, 1977). It grows best where annual rainfall ranges from 600-1700 mm and lowland areas mainly below 460 m above sea level.

According to Mendoza (1975), giant ipil-ipil can attain a height of 9.5 m with a diameter of 6 cm in 1½ years time. Within 8 years, it can reach 13 m in height and 37 cm in diameter (Benge, 1975).

On a per hectare basis, yields vary at different locations under different management systems, especially at different stand densities. On an average plantation site, the average annual growth rate is about 15 cu m/ha (over five years). On very good sites, it is more than 50 cu m/ha (Revilla and Gregorio, 1983).

In a survey by Kanazawa *et al.*, (1982) of nine giant ipil-ipil (k-8 variety) plantations in Northern Mindanao, biomass of each part varied widely, at 11-155 cu m/ha for stem volume, 6-78 tons/ha for stem dry weight, and 8-96 tons/ha for total above ground weight. The leaves ranged from 0.7 to 3.6 tons/ha dry weight.

Field procedures

The study covered six locations in the Philippines (Fig. 1) representing climatic types I and III of the corona climatic classification system. A total of 111 sample trees were taken from established giant ipil-ipil plantations (Table 1). The

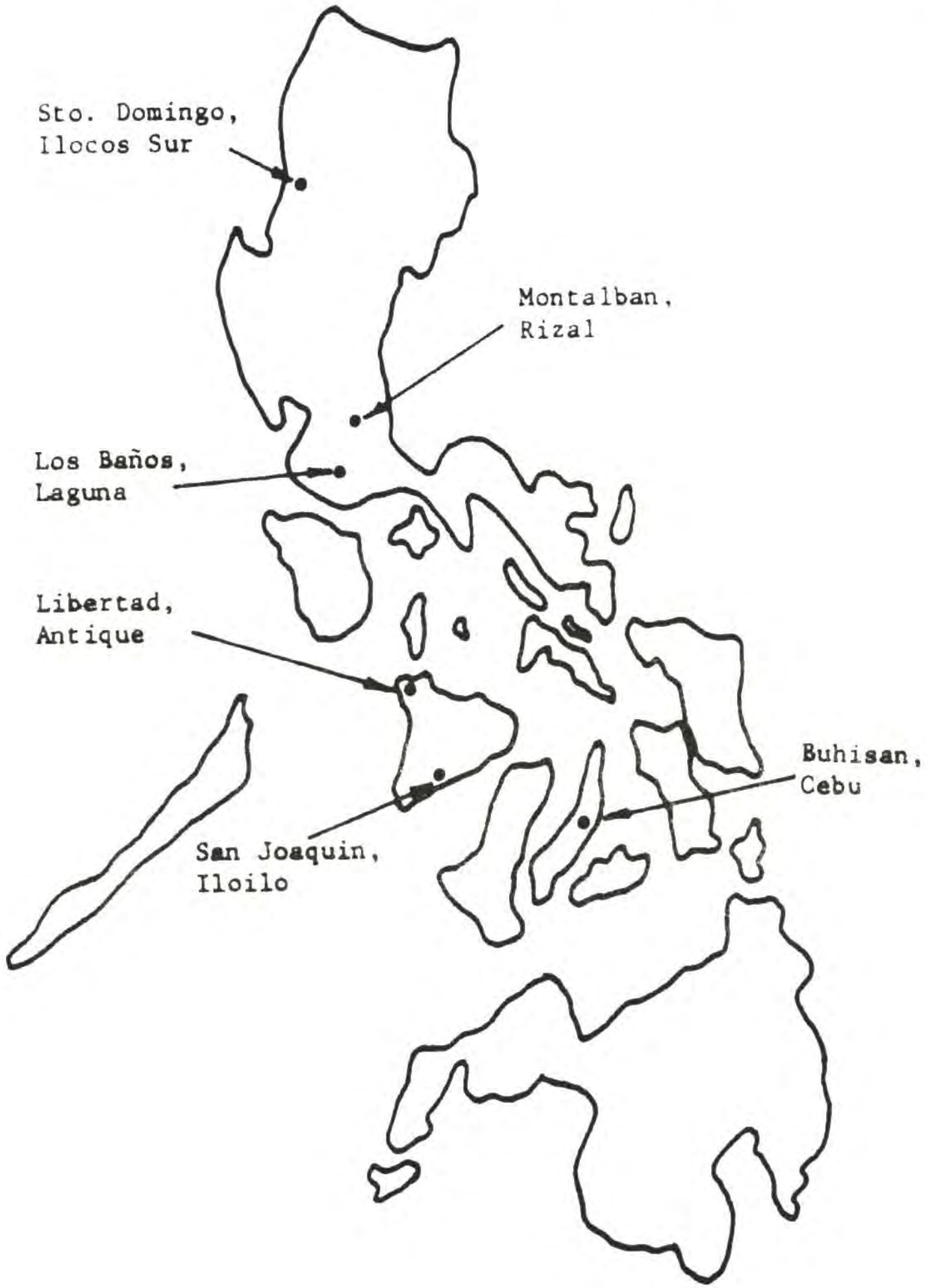

Fig. 1. Map of the Philippines showing location of the study sites.

Table 1. General description of the giant ipil-ipil stands in each location

CHARACTERISTICS		RIZAL	ANTIQUE	ILOCOS SUR	ILOILO	CEBU	LAGUNA
Age (Years)		2-4	4	7	5	10	9
Stand density (no. of trees/ hectares)	mean	8,926	10,742	8,140	648	1,500	459
	range	5,040-16,711	9,549-11,936	2,804-14,940	370-1,181	1.032-2,210	250-690
Mean Basal Area (m^2/ha)		16.60	22.59	45.62	22.53	90.25	9.89
Site index (BAGE 5 years)	mean	9.41	11.48	12.72	7.79	21.34	17.15
	range	3.33-12.18	9.42-11.70	11.36-14.18	5.84-9.00	10.60-25.60	13.73-20.49
Soil pH	mean	5.13	7.34	5.00	6.00	6.68	4.5
	range	4.90-5.80	6.60-7.65	4.90-5.10	5.50-6.60	6.40-7.10	4.2-4.8
Size of plantation (ha)		11.8	5.0	200.0	137.4	159.0	1.5

trees were felled about 15 cm above the ground, and measured for diameter at breast height (D), diameter at the base, diameter every 2 meters, merchantable height from the base to the minimum upper-stem diameter of 3 cm and total height. The trees were then cut into 2 sections from the base to the merchantable top. These were separated into components and weighed.

Disks of 3 to 5 cm thick were taken at the base of each section of the stem. These disks were labeled, sealed in plastic bags and taken to the Forest Research Institute (FORI) Laboratory for measurements. Samples of leaves (40-150 gm) and branches (10-200 gm) were likewise taken, labeled and sealed in plastic bags and brought to the laboratory.

Laboratory procedures

The fresh weight of the samples from the bole, branches and leaves were determined to the nearest 0.01 gm. All disk subsamples were debarked and weighed separately. The bark and wood from each disk as well as the sample branches were ovendried for 48 hours at 103° (±2°C) and then reweighed. On the other hand, the leaves were wrapped in aluminum foil, ovendried at 80°C to constant weight and then reweighed.

Disks collected from the boles were measured to calculate three ratios: (a) ovendry weight of disk to fresh weight of the disk (disk ODW/FW); (b) fresh weight of bark to fresh weight of the disk (bark FW/disk FW); and (c) ovendry weight of bark to fresh weight of disk (bark ODW/disk FW). Fresh weights of the sections were multiplied by ratios of appropriate disks to estimate ovendry weights. For each section, the average of the ratios calculated from disks collected at both ends of the section were computed and volume-weighted to obtain improved estimates. Estimated ovendry weight of sections were totalled to estimate bole wood weight and bole bark weight. For the uppermost segments, the ratios calculated from one disk were used to estimate ovendry weights. Ratio of fresh weight of bark to fresh weight of the disk was used to estimate fresh bark weights. The ovendry weights of branches were obtained by applying the corresponding ovendry/fresh weight ratios. The total ovendry weight of the leaves was obtained by multiplying the total fresh weight of the leaves by their sample ovendry/fresh weight ratio.

Statistical data on some of the measurements are shown in Table 2.

Analysis of data

Seventeen (17) regression models (Table 3) based on diameter breast height (D) and total height (H) were tested and evaluated for predicting biomass of each tree component.

The criteria used for selecting the "best" models and judging their suitability and/or fitness were as follows:

(a) highest coefficient of determination (R^2);
(b) smallest "index of fit" as proposed by Furnival (1961);
(c) how well the main assumptions underlying regression are satisfied;
(d) geometric reasonableness; and
(e) biological feasibility.

The coefficient of determination (R^2) was used initially to screen the 17 models. R^2 as the usual measure of goodness of fit is more suitable to compare equations that have the same dependent variable than when the dependent variables differ. In choosing between alternative models, Furnival (1961) however, recommended the use of likelihood comparisons rather than R^2 comparisons to evaluate the performance of several models including transformed or constrained models, e.g. logarithmic transformation or when the intercept (regression constant) is set to zero for estimating biomass. Accordingly, the logarithmic models not only assume multiplicative error term in the original power functions but its resulting R^2 in the standard way are in logarithmic scale. According to Furnival, these are not directly comparable with those obtained from the transformed models, hence his "index of goodness of fit" was used in the second screening.

For the "best" model which came out from the second screening, the main assumptions underlying regression, viz., homoscedasticity and normality were analyzed if they were really satisfied. In addition, the "best" model was examined

Table 2. Statistical data on diameter at breast height (D), total height (H), volume total green and ovendry mass of sample tree from the different study sites

CHARACTERISTICS	RIZAL	ANTIQUE	ILOCOS SUR	ILOILO	CEBU	LAGUNA
Sample Trees (No.)	27	13	18	14	21	18
D (cm)						
Mean	8.23	8.67	10.48	8.03	19.43	13.04
CV	0.464	0.345	0.436	0.310	0.333	0.370
Range	4.0-16.2	4.5-14.1	5.2-20.8	5.1-13.8	10.0-31.8	5.4-21.0
H (m)						
Mean	9.02	10.97	14.29	8.57	17.63	11.11
CV	0.330	0.167	0.248	0.192	0.204	0.275
Range	4.9-16.1	7.6-12.8	9.5-21.0	5.0-11.3	10.6-24.7	5.7-16.3
Volume (cu m)						
Mean	0.0379	0.0347	0.0705	0.0245	0.2272	0.0856
CV	0.998	0.680	1.096	0.774	0.633	0.736
Range	0.0032-0.1528	0.0075-0.0861	0.0115-0.2570	0.0047-0.0634	0.0359-0.5406	0.0076-0.2084
Total Green Biomass (kg)						
Mean	51.00	55.12	109.38	52.10	440.43	116.61
CV	1.096	0.708	1.168	0.818	0.680	0.752
Range	4.95-189.8	11.4-144.5	17.75-484.0	11.55-149.8	74.0-1,006.0	11-8-288.7
Total Ovendried Biomass (kg)						
Mean	29.45	24.28	67.23	26.78	243.51	60.12
CV	1.068	0.689	1.131	0.809	0.662	0.787
Range	2.97-98.01	6.99-78.89	10.10-270.30	5.73-73.02	33.88-517.33	5.84-145.32

if it ties into formulas for calculating volume (or weight) from lengths and diameters of cylinders, cones, paraboloids, etc. Lastly, the model was checked if it is biologically feasible. That is, if given a zero height or diameter, its predicted weight will not significantly differ from zero and if either D and H are increasing, its predicted weight will also be increasing.

Table 3. Regression models tested as possible candidates for tree and tree component weight equations

MODEL NO.	MODEL		REMARKS
1	$W = b_0(D^2H)^{b_1}$	or	$\ln(W) = \ln(b_0) + b_1\ln(D^2H)$
2	$W = b_1D^2H$		(No intercept)
3	$W = b_0 + b_1D^2H$		
4	$W = b_0 + b_1D^2H + b_2D^2H^2$		
5	$W = b_0D^{b_1}H^{b_2}$		$\ln(W) = \ln(b_0) + b_1\ln D + b_2\ln H$
6	$W = b_0 + b_1D$		(Simple linear model)
7	$\log(W) = b_0 + b_1D$		
8	$W = b_0 + b_1D^2$		(Basal area equation)
9	$W = b_0D^{b_1}$	or	$\ln(W) = \ln(b_0) + b_1\ln(D)$
10	$W = b_0 + b_1D + b_2D^2$		(Parabolic or quadratic model)
11	$W = b_0 + b_1D + b_2D^2 + b_3D^3$		(Cubic equation)
12	$\ln(W) = b_0 + b_1D + b_2H$		
13	$W = b_0 + b_1D^2 + b_2H + b_3D^2H$		
14	$W = b_0 + b_1D + b_2H + b_3D^2H + b_4D^2 + b_5D^3$		(Polynomial model)
15	$W = b_0e^{b_1D}$	or	$\ln(W) = \ln(b_0) + b_1D$ (Exponential Model)
16	$W = D/(b_1 + b_0D)$	or	$1/W = b_0 + b_1(1/D)$ (Hyperbolic equation)
17	$W/D^2H = b_0(1/D^2H) + b_1$		(Weighted model)

W = fresh or ovendry weight of tree or components (kg)
D = diameter at breast height (cm)
H = total tree height (m)
ln = natural logarithm
log = common logarithm
$b_0, b_1, b_2, b_3, b_4, b_5$ = regression coefficients

Results and Discussion

Out of the 17 regression models tested and evaluated, the allometric model

$$w = b_0 D^{b1} H^{b2}$$

where

w = fresh or ovendry weight of the whole tree or its components (kg)
D = diameter at breast height (cm)
H = total tree height (m)
b_0, b_1, b_2 = regression constants

provided the best estimates. Separate equations for fresh and ovendry weight of the whole tree and its components, regardless of location were derived from this "best" model (Table 4).

Among the tree components analyzed, weights of the bole were found to be generally predicted than the corresponding crown components. For giant ipil-ipil, the wood, bark, small branches and twigs, large branches and topwood and foliage comprised 67.14, 4.24, 5.61, 19.56 and 3.45 percent of total aboveground tree ovendry weight, respectively. Values for green weights are 60.45, 6.29, 5.98, 21.31 and 5.97 percent, respectively (Fig. 2).

The assumptions underlying regression of the above allometric model were checked by plotting the residuals (observed minus estimated values) around the fitted allometric model and by examining histograms of the residual errors. It was found that the model met the equality of variance and normality assumptions reasonably well. In addition to these checks, the estimated values over the observed values were plotted per study site to determine whether this model fitted the biomass data satisfactorily. It has been shown (Fig. 3) that the points closely gathered along the 45° line indicating that the model fitted the data quite well.

It is to be noted, however, that before the final equations were developed, the problem of underestimation of biomass estimates and non-additivity of component estimates accompanying the log-linear transformation of the allometric model, were corrected and solved, respectively.

The biases associated when transforming back the logarithmic estimates to their original units were removed by running the allometric model again, inserting b_1's and the b_2's from the first run, in the original model

$$w = b_0 D^{b1} H^{b2}$$

to obtain new and unbiased estimates of b_0 and consequently of the tree and component estimates.

The non-additivity of the component estimates (i.e., the predicted sum of the weights of component parts, based on individual equation for each part, being not exactly equal to the predicted total weight based on a single equation) was solved

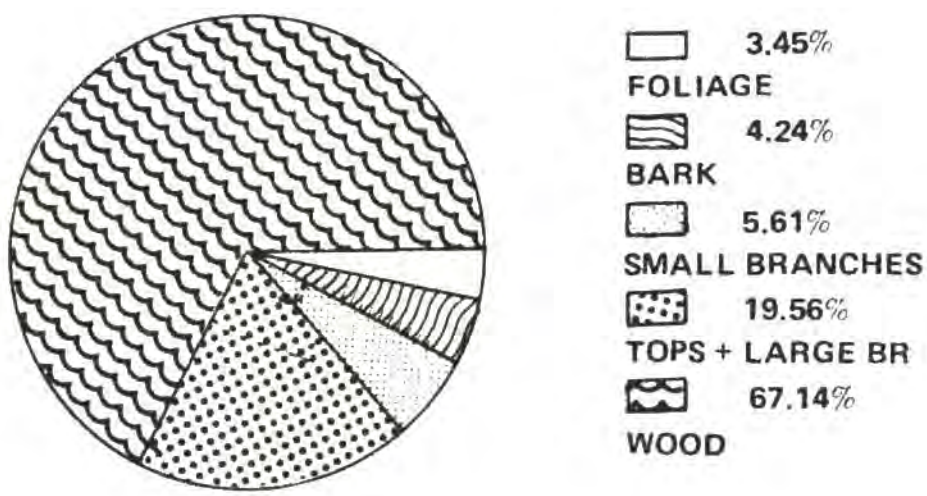

Fig. 2. Percent distribution of the different tree components.

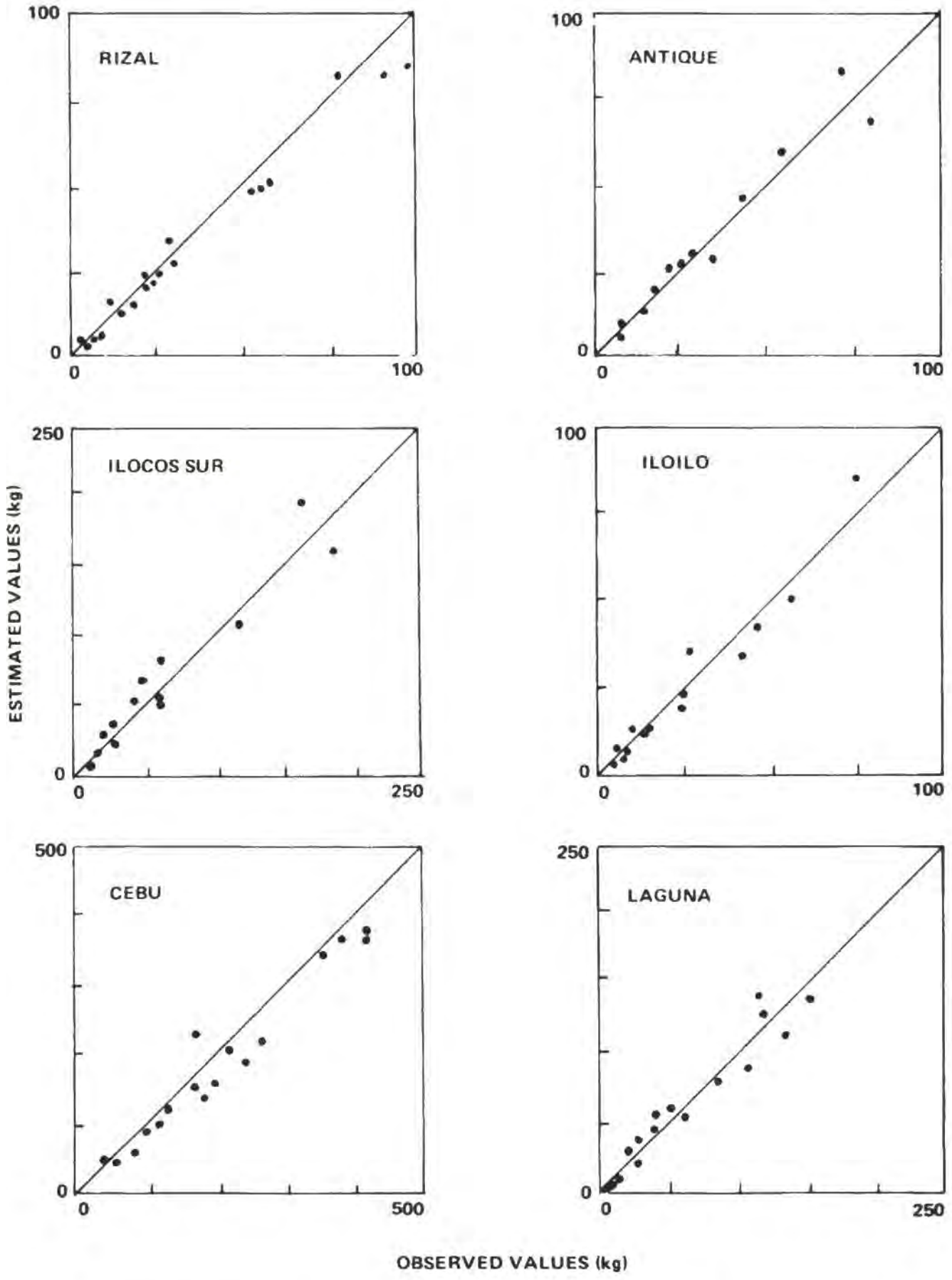

Fig. 3. Distribution of estimated values using Model 5 along 40° line in terms of total tree ovendry weight by location.

Table 4. Tree component weight regression coefficients for model $w = b_0 D^{b_1} H^{b_2}$

COMPONENT WEIGHTS (kg)	FIRST RUN				SECOND RUN			
	b_0	b_1	b_2	R^2	b_0	b_1	b_2	R^2
Fresh Weight								
Bole	−2.970	2.02	0.86	0.979	0.049730	2.02	0.86	0.937
Wood	−3.160	2.08	0.83	0.977	0.041644	2.08	0.83	0.936
Bark	−4.386	1.62	0.92	0.919	0.011548	1.62	0.92	0.798
Crown	−2.205	2.39	−0.10	0.907	0.140223	2.39	−0.10	0.920
Topwood and large branches	−5.328	3.09	0.14	0.864	0.005631	3.09	0.14	0.948
Small branches	−2.567	1.47	0.35	0.771	0.090488	1.47	0.35	0.862
Foliage	−2.242	1.95	−0.28	0.659	0.126849	1.95	−0.28	0.844
Whole Tree	−2.032	2.16	0.51	0.978	0.136561	2.16	0.51	0.977
Dry Weight								
Bole	−3.396	1.82	1.03	0.972	0.033760	1.82	1.03	0.955
Wood	−3.524	1.85	1.02	0.972	0.029525	1.85	1.03	0.953
Bark	−5.263	1.42	1.08	0.914	0.005745	1.42	1.08	0.943
Crown	−3.162	2.28	0.09	0.886	0.054459	2.28	0.09	0.913
Topwood and large branches	−5.884	2.94	0.25	0.858	0.003222	2.94	0.25	0.934
Small branches	−3.302	1.32	0.53	0.723	0.045140	1.32	0.53	0.839
Foliage	−3.426	1.85	−0.17	0.644	0.038815	1.85	−0.17	0.845
Whole Tree	−2.694	1.96	0.74	0.974	0.071563	1.96	0.74	0.973

by means of weighing using squares of the coefficient of variation imposed on the component equations. The component which is estimated with greater relative precision is given a lesser fraction of the discrepancy than with the other components. This allocation scheme was hold true to the bole as this was considered the largest and most important among the other tree components. Hence, since this part was given the least fraction of discrepancy, less distortion of its predictability was obtained.

Model $w = b_0 D^{b1}$, which is also an allometric model, performed equally well as the "best" model for the bole and total tree biomass but was slightly weaker for predicting the crown components.

The polynomial model

$$w = b_0 + b_1 D + b_2 H + b_3 D^2 H + b_4 D^2 + b_5 D^3$$

was found to be nearly as good as the allometric models in predicting biomass of the whole tree and component parts of giant ipil-ipil. As a regression model for biomass estimation, it offers the following advantages:

(a) It is linear and thus, provides various combinations of predicted component estimates by simply adding the existing coefficients in the prediction equations;
(b) It gives direct biomass estimates, thus obviating the need for adjustments and corrections as when allometric functions are used;
(c) It is simple to understand and easy to use, since it has no transformations.

However, the model should not be used for predicting biomass of trees with diameter at breast height and total height outside the range covered by the samples in the study. Unlike the allometric models, there is no trend in the prediction using this polynomial model. The predicted weight beyond the ranges of D and H in this study, could be erratic and it could hardly be explained by this polynomial model.

Regression coefficients of the prediction equations obtained from the three models by location are listed in Tables 5 to 7, respectively. These tables provide information for the ovendry biomass aboveground tree components of giant ipil-ipil.

Conclusions and Recommendations

1. Biomass measurement is a necessary step towards complete-tree utilization. For giant ipil-ipil, information on leaf biomass is useful for leaf meal and organic fertilizer production while biomass information on the stem, topwood and branches are necessary for charcoal, fuelwood, pulp and paper manufacture.
2. Aboveground biomass of giant ipil-ipil can be obtained by using simple measurements such as diameter at breast height (D) and total height (H) of

the tree. Results of the study from six provinces strongly prove the suitability of the allometric model $w = b_0 D^{b1} H^{b2}$ in estimating biomass of the whole tree and component parts of giant ipil-ipil with ages 2-10 years old. It is within these areas, and with this range of ages (plantation), therefore, that this model is highly recommended for use.

The biomass prediction equations developed from this model, however, could also be applied in areas approximating the geographical and ecological conditions of the study sites especially within the same climatic type. For other areas or sites, preliminary testing is advisable.

3. Model $w = b_0 D^{b_1}$ which is also an allometric model, performed equally well for the bole and total tree biomass, but was slightly weaker for predicting the crown components.

   This model could be considered for situation where D is the only available tree attribute measured and for obtaining first-approximation estimates of a giant ipil-ipil stand.

4. The polynomial model

$$w = b_0 + b_1 D + b_2 H + b_3 D^2 H + b_4 D^2 + b_5 D^3$$

was found to be nearly as good as the allometric models in predicting biomass of giant ipil-ipil. Although, it has five predictor variables and computationally tedious, it gives direct biomass estimates and a need for bias correction is totally eliminated as when allometric models are used.

As the polynomial model is linear, there is no problem of additivity of components estimates. With these considerations and ease of application, this model could be used as a practical compromise for estimating biomass of giant ipil-ipil. However, predictions should be limited for diameters at breast height and total heights within the ranges covered by the samples in this study.

5. The general biomass prediction equations using the best model, i.e., $w = b_0 D^{b1} H^{b2}$ developed are valid for application in the areas within climatic types I and III across the six provinces studied. However, a slightly better fit may be possible by using the individual province equations also developed in this study.
6. Biomass estimation of the whole tree and component parts using tree weight equations has been amply demonstrated for giant ipil-ipil. The procedures used in this study, therefore, could also be tried to other fast growing species.
7. For pulp, paper and fuelwood purposes, tree biomass estimation especially for branches and tops is a better method than the usual practice of using volume estimation. For this reason, biomass estimation is highly recommended to attain maximum and efficient tree utilization.
8. For refinement of the models, it is recommended that future studies along this line should consider collecting additional biomass data possibly by region

from other giant ipil-ipil plantations throughout the country. As other data shall have been available, follow-up studies could also be conducted to determine variation of biomass estimation among different site conditions considering different climatic and edaphic factors.

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Table 5. Regression coefficients for model $W = b_0 D^{b1} H^{b2}$ by location and tree component in terms of dry weight

LOCATION/ COMPONENT	FIRST RUN				SECOND RUN			
	b_0	b_1	b_2	R^2	b_0	b_1	b_2	R^2
Rizal								
Bole	−2.820	1.84	0.74	0.977	0.057903	1.04	0.84	0.968
Wood	−2.979	1.87	0.76	0.977	0.049218	1.87	0.76	0.981
Bark	−4.395	1.40	0.64	0.948	0.012243	1.40	0.64	0.970
Crown	−3.535	2.61	−0.05	0.956	0.031938	2.61	−0.06	0.948
Topwood and large branches	−6.045	3.50	−0.33	0.767	0.003457	3.50	−0.33	0.913
Small branches	−4.456	1.64	0.83	0.821	0.011023	1.64	0.83	0.878
Foliage	−3.685	2.61	−0.70	0.853	0.026548	2.61	−0.70	0.889
Whole Tree	−2.414	2.09	0.49	0.984	0.089167	2.00	0.49	0.905
Antique								
Bole	−3.430	1.56	1.32	0.972	0.032114	1.56	1.32	0.993
Wood	−3.585	1.58	1.33	0.970	0.027477	1.50	1.33	0.992
Bark	−5.154	1.28	1.22	0.979	0.005776	1.28	1.22	0.998
Crown	−0.006	2.73	−1.61	0.902	1.140902	2.73	−1.61	0.852
Topwood and large branches	−4.126	3.60	−1.15	0.844	0.020680	3.66	−1.15	0.740
Small branches	0.672	1.94	−1.64	0.828	2.077099	1.94	−1.64	0.936
Foliage	−0.453	2.22	−1.58	0.832	0.000236	2.22	−1.58	0.721
Whole Tree	−1.773	1.95	0.41	0.900	0.167912	1.95	0.41	0.983
Ilocos Sur								
Bole	−2.673	2.13	0.49	0.945	0.457711	2.13	0.49	0.932
Wood	−2.773	2.17	0.46	0.940	0.067366	2.17	0.46	0.976
Bark	−5.029	1.74	0.78	0.881	0.007355	1.74	0.79	0.966
Crown	−2.773	2.01	0.16	0.806	0.070321	2.01	0.16	0.918
Topwood and large branches	−4.651	2.45	0.22	0.835	0.010041	2.45	0.22	0.894
Small branches	−3.118	1.37	0.60	0.853	0.005583	1.37	0.60	0.927
Foliage	−3.118	2.67	−1.00	0.801	0.051593	2.67	−1.00	0.938
Whole Tree	−2.112	2.13	0.38	0.965	0.129951	2.13	0.38	0.977

Table 5 (Continued)

LOCATION/ COMPONENT	FIRST RUN				SECOND RUN			
	b_0	b_1	b_2	R^2	b_0	b_1	b_2	R^2
Iloilo								
Bole	−4.422	2.17	1.14	0.961	0.011823	2.17	1.14	0.934
Wood	−4.566	2.23	1.11	0.961	0.010208	2.23	1.11	0.984
Bark	−6.170	1.47	1.43	0.951	0.002082	1.47	1.43	0.990
Crown	−3.445	2.67	0.04	0.841	0.029732	2.67	0.04	0.926
Topwood and large branches	−7.856	2.42	1.91	0.835	0.000356	2.42	1.91	0.927
Small branches	−3.956	2.29	0.07	0.688	0.018651	2.28	0.07	0.842
Foliage	−3.012	3.25	−1.37	0.742	0.041154	3.25	−1.37	0.768
Whole Tree	−3.314	2.31	0.74	0.953	0.035039	2.31	0.74	0.976
Cebu								
Bole	−2.613	1.93	0.67	0.937	0.069663	1.93	0.67	0.951
Wood	−2.739	1.94	0.68	0.934	0.051231	1.94	0.68	0.959
Bark	−4.356	2.03	0.30	0.671	0.007867	2.03	0.30	0.963
Crown	−2.705	2.69	−0.31	0.912	0.070671	2.69	−0.31	0.965
Topwood and large branches	−3.055	2.75	−0.47	0.881	0.050071	2.75	−0.47	0.955
Small branches	−4.486	2.11	0.13	0.851	0.013436	2.11	0.13	0.922
Foliage	−4.853	2.22	0.02	0.872	0.007430	2.22	0.02	0.937
Whole Tree	−2.032	2.13	0.38	0.964	0.127002	2.13	0.38	0.987
Laguna								
Bole	−3.218	2.11	0.59	0.966	0.40776	2.11	0.59	0.964
Wood	−3.375	2.15	0.58	0.966	0.034863	2.15	0.58	0.961
Bark	−4.083	1.61	0.70	0.944	0.007639	1.61	0.70	0.966
Crown	−2.610	2.25	−0.33	0.914	0.075885	2.25	−0.33	0.951
Topwood and large branches	−3.492	2.68	−0.58	0.889	0.031757	2.68	−0.58	0.944
Small branches	−2.958	1.31	0.12	0.700	0.052293	1.31	0.12	0.937
Foliage	−5.624	1.15	1.10	0.803	0.001005	1.15	1.10	0.899
Whole Tree	−2.457	2.13	0.36	0.973	0.087710	2.13	0.36	0.975

Table 6. Regression coefficients for Model $w = b_0 D^{b_1}$ by location and component. n = 111

LOCATION/COMPONENT	FIRST RUN			SECOND RUN		
	b_0	b_1	R^2	b_0	b_1	R^2
RIZAL						
Bole	−2.191	2.32	0.968	0.107886	2.32	0.984
Wood	−2.341	2.36	0.968	0.092588	2.36	0.984
Bark	−3.854	1.81	0.937	0.020973	1.81	0.970
Crown	−3.583	2.57	0.956	0.030189	2.57	0.946
Topwood and large branches	−6.345	3.29	0.766	0.002626	3.29	0.900
Small branches	−3.757	2.18	0.810	0.022453	2.18	0.904
Foliage	−4.273	2.16	0.845	0.015219	2.16	0.894
Whole Tree	−2.003	2.41	0.980	0.133935	2.41	0.909
ANTIQUE						
Bole	−1.095	1.94	0.900	0.327528	1.94	0.969
Wood	−1.235	1.97	0.900	0.284273	1.97	0.967
Bark	−3.001	1.63	0.893	0.049539	1.63	0.900
Crown	−2.858	2.26	0.830	0.067132	2.26	0.785
Topwood and large branches	−6.164	3.32	0.827	0.002694	3.32	0.689
Small branches	−2.233	1.47	0.682	0.117511	1.47	0.878
Foliage	−3.256	1.76	0.730	0.040404	1.76	0.844
Whole Tree	−1.045	2.07	0.973	0.346400	2.07	0.984
ILOCOS SUR						
Bole	−1.904	2.36	0.941	0.164764	2.36	0.975
Wood	−2.047	2.39	0.944	0.142361	2.39	0.974
Bark	−3.815	2.10	0.869	0.025619	2.10	0.959
Crown	−2.524	2.09	0.886	0.090859	2.09	0.919
Topwood and large branches	−4.313	2.55	0.834	0.014262	2.55	0.898
Small branches	−2.562	1.65	0.841	0.090408	1.65	0.902
Foliage	−4.156	2.16	0.780	0.009105	2.16	0.941
Whole Tree	−1.517	2.31	0.962	0.239943	2.31	0.977

Table 6 (Continued)

LOCATION/COMPONENT	FIRST RUN			SECOND RUN		
	b_0	b_1	R^2	b_0	b_1	R^2
ILOILO						
Bole	−3.050	2.69	0.916	0.046196	2.69	0.970
Wood	−3.231	2.74	0.920	0.038416	2.74	0.969
Bark	−4.450	2.12	0.846	0.011704	2.12	0.974
Crown	−3.399	2.68	0.841	0.031071	2.68	0.925
Topwood and large branches	−5.556	3.29	0.764	0.003539	3.29	0.942
Small branches	−3.869	2.31	0.688	0.020313	2.31	0.840
Foliage	−4.665	2.63	0.691	0.008349	2.63	0.815
Whole Tree	−2.421	2.65	0.933	0.084780	2.65	0.966
CEBU						
Bole	−1.294	2.13	0.910	0.256513	2.13	0.952
Wood	−1.383	2.14	0.905	0.233804	2.14	0.950
Bark	−4.257	2.12	0.667	0.014024	2.12	0.953
Crown	−3.322	2.49	0.907	0.038787	2.49	0.964
Topwood and large branches	−3.989	2.61	0.872	0.020560	2.61	0.955
Small branches	−4.201	2.15	0.850	0.016115	2.15	0.920
Foliage	−4.819	2.23	0.872	0.007689	2.23	0.937
Whole Tree	−1.287	2.24	0.956	0.264360	2.24	0.984
LAGUNA						
Bole	−2.706	2.47	0.954	0.067558	2.47	0.975
Wood	−2.868	2.50	0.955	0.057508	2.50	0.975
Bark	−4.279	2.03	0.921	0.013913	2.03	0.970
Crown	−2.898	2.06	0.909	0.057853	2.06	0.963
Topwood and large branches	−3.993	2.34	0.877	0.019959	2.34	0.946
Small branches	−2.851	1.38	0.699	0.058058	1.38	0.935
Foliage	−4.669	1.81	0.744	0.010147	1.81	0.847
Whole Tree	−2.143	2.35	0.968	0.119273	2.35	0.980

Table 7. Tree component weight ipil-ipil in 6 areas in terms of dry weight

COMPONENT DRY WEIGHT (kg)	REGRESSION STATISTICS						
	b_0	b_1	b_2	b_3	b_4	b_5	R^2
RIZAL							
Bole	−6.9877	−0.1035	1.4175	0.0013	0.0803	0.0074	0.98
Wood	−6.6844	0.0348	1.2715	0.0019	0.0595	0.0073	0.98
Bark	−0.3033	−0.1380	0.1460	−0.0006	0.0208	5.99E-5	0.94
Crown	20.3965	−10.7923	1.4512	−0.0144	1.2118	−0.0222	0.94
Topwood and large branches	18.2442	−9.0383	1.0421	−0.0104	0.9551	−0.0179	0.91
Small branches	0.4000	−1.7030	0.7558	−0.0053	0.0246	−0.0021	0.87
Foliage	1.7523	−0.0509	−0.3467	0.0014	0.0522	−0.0021	0.82
Total Tree	13.7121	−10.7573	2.7227	−0.o125	1.2714	0.0148	0.99
ANTIQUE							
Bole	−26.4859	12,0090	−0.4681	0.0435	1.4449	0.0349	0.98
Wood	−26.0617	11.9827	−0.5329	0.0419	−1.4490	0.0365	0.98
Bark	−0.4242	0.0264	0.0648	0.0015	0.0041	−0.0006	0.99
Crown	0.6052	−32.2033	9.1519	−0.1504	4.4592	−0.0644	0.95
Topwood and large branches	0.9911	−20.0974	7.4360	−0.1130	3.8850	−0.0655	0.93
Small branches	−0.5325	−3.4271	1.1382	−0.0223	0.5800	−0.0072	0.93
Foliage	−8.8534	1.3212	0.5770	−0.0143	−0.0057	0.0084	0.90
Total Tree	−25.4565	−16.2206	8.6190	−0.1085	3.0103	−0.0289	0.98

Table 7 (Continued)

COMPONENT DRY WEIGHT (kg)	REGRESSION STATISTICS						
	b_0	b_1	b_2	b_3	b_4	b_5	R^2
ILOCOS SUR							
Bole	47.0346	−10.8079	−0.8795	0.0134	1.0371	−0.0171	0.96
Wood	50.1668	−12.0752	−0.8200	0.0115	1.1539	−0.0203	0.96
Bark	−3.1323	1.2673	−0.0599	0.0019	−0.1169	0.0032	0.95
Crown	−33.6035	4.7967	1.9703	−0.0240	−0.3438	0.0359	0.93
Topwood and large branches	−20.5880	2.1008	1.3787	−0.0165	−0.1183	0.0207	0.89
Small branches	−10.9390	2.0496	0.5659	−0.0064	−0.1746	0.0122	0.96
Foliage	−2.0845	0.6463	0.0250	−0.0011	−0.0509	0.0031	0.93
Total Tree	16.5634	−7.2785	1.1500	−0.0125	08102	0.0156	0.96
ILOILO							
Bole	28.9467	−11.5943	0.0039	0.0185	1.4472	−0.0507	0.97
Wood	27.9954	−11.0679	−0.0695	0.0178	1.3753	−0.0481	0.96
Bark	0.9513	−0.5265	0.0634	0.0008	0.0719	−0.0026	0.97
Crown	85.9706	−34.0997	0.0302	−0.0157	4.3983	−0.1500	0.93
Topwood and large branches	34.6425	−16.3166	0.9985	−0.0269	2.1395	−0.0590	0.96
Small branches	20.9529	−7.0584	−0.4655	0.0081	0.8905	−0.0374	0.76
Foliage	30.3752	−10.7240	−0.5028	0.0030	1.3684	−0.0522	0.86
Total Tree	113.9660	−45.1676	−0.0293	0.0021	5.7736	−0.1981	0.97
CEBU							
Bole	275.5171	−56.8482	0.1185	0.0055	3.8917	−0.0687	0.94
Wood	263,8162	−54.6009	0.1111	0.0050	3.7435	−0.0561	0.94
Bark	11.7809	−2.2473	0.0074	0.0805	0.1481	−0.0025	0.91
Crown	−61.9108	14.8430	−1.3609	0.0014	−0.7769	0.0190	0.93

Table 7 (continued)

COMPONENT DRY WEIGHT (kg)	REGRESSION STATISTICS						
	b_0	b_1	b_2	b_3	b_4	b_5	R^2
Topwood and large branches	-95.5391	21.1854	-0,4103	0.0015	-1.1746	0.0250	0.93
Small branches	42.6088	-7.3580	0.0281	0.0005	0.4011	-0.0062	0.83
Foliage	8.9804	1.0156	0.0213	-0.0086	-0.0034	0.0002	0.88
Total Tree	201.9055	-39.7579	-1.2498	0.0063	2.9666	-0.0471	0.98
LAGUNA							
Bole	93.0811	-33.2136	4.0933	-0.0148	2.8101	-0.0527	0.95
Wood	88.6501	-31.5626	3.8644	-0.0142	2.6642	-0.0497	0.95
Bark	4.5310	-1.6511	0.2289	-0.0006	0.1469	-0.0030	0.93
Crown	13.1506	-3.2900	-0.2041	4.14E-5	0.3380	-0.0066	0.87
Topwood and large branches	14.2221	-3.7638	-0.1503	-0.0010	0.3557	-0.0065	0.88
Small branches	-1.7863	0.6361	-0.0483	0.0003	-0.0327	0.0005	0.71
Foliage	0.7149	-0.1620	-0.0065	0.0007	0.0150	-0.0006	0.75
Total Tree	101.7008	-34.8522	3.6602	-0.0142	3.0021	-0.0563	0.96

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IDENTIFICATION OF A CLONED METHIONINE BIOSYNTHETIC GENE OF THE CYANOBACTERIUM *SYNECHOCOCCUS* PCC7942 BY HETEROLOGOUS DNA HYBRIDIZATION

Debbie O. Co
The Institute of Biology, College of Science
University of the Philippines
Diliman, Quezon City
Philippines

ABSTRACT

A cloned methionine biosynthetic gene, *met1*, of *Synechococcus* PCC7942 was identified by using defined heterologous DNA hybridization probes. Using the Southern hybridization technique, homologies were examined between restricted DNA of *Synechococcus* PCC7942 and the *met* genes of *E. coli* and *S. typhimurium* Regions of *Synechococcus* DNA were found to hybridize, albeit weakly, to probes containing the following enterobacterial genes: *metA*, *met'JBLF*, *metC* and *metE*. Cross hybridization was detected between the 6.0 kb *Eco*RI *met1* fragment of *Synechococcus* and the probe carrying the *met'JBLF* cluster of *E. coli*. Reciprocally, only the 5.0 kb DNA fragment carrying *met'JBLF* of *E. coli* hybridized *with a met1* probe. These results, along with previous physiological and biochemical data, reinforce the hypothesis that *met1* corresponds to a gene coding for an enzyme intervening in a terminal step of the methionine biosynthetic pathway, and more precisely, to a gene analogous to the *metF* gene of *E. coli*.

Introduction

Recent developments in recombinant DNA technology are making possible molecular genetic studies of cyanobacteria. These microorganisms, formerly referred to as blue-green algae, are the only prokaryotes that perform an oxygenic, "higher plant" type of photosynthesis, are important contributors to soil fertility and are potential resources for food and energy production.

With the goal of elucidating gene structure, function and possible new regulation mechanisms in cyanobacteria, a long-term project of isolating and cloning cyanobacterial genes was undertaken. Initial effort was concentrated on the cloning of a methionine biosynthetic gene of the unicellular cyanobacterium *Synechococcus* PCC7942 by a new strategy involving the use of transposon Tn*901* (Tandeau de Marsac *et al.*, 1982). A *met1::*Tn*901* mutant (SPcMET1) was isolated,

its chromosomal DNA fragments were cloned in the *Escherichia coli* plasmid vector pACYC184, and the resulting recombinant plasmid carrying the inactivated *met1*::Tn*901* gene was selected after transformation to *E. coli*. This cloned *met1*::Tn*901* DNA fragment was then used as a probe to select the corresponding *Synechococcus* wild type *met1* gene from a gene library prepared in *E. coli* in the shuttle cosmid vector pPUC29. When transformed into SPcMET1, the plasmid pTH225 (pPUC29:*met1*) allowed the mutant to grow prototrophically. A 6.0 kb *Eco*RI fragment from pTH225 was subcloned into the hybrid vector pUC303 to give rise to pME1 (Fig. 1) and functional complementation was also achieved following transformation into SPcMET1.

Subsequent biochemical and physiological studies of SPcMET1, involving enzyme assays and growth analyses in the presence of various intermediates of the biosynthetic pathway of methionine, established that Tn*901* must have inserted itself in a locus corresponding to *metE* and/or *metF* (Co, 1983; 1986). This indicates that the 6.0 kb *Eco*RI insert of pME1 carries either or both of these genes.

The aim of this study was to distinguish whether *met1* is equivalent *to metE or metF* or both by reciprocal heterologous hybridization with known *E. coli* and *Salmonella typhimurium met* genes. Since there is strong evidence that the methionine biosynthetic pathway in *Synechococcus* PCC7942 is similar to that in *E. coli* (Co, 1987), perhaps enough homology might be retained in the DNA of these genes to allow heterologous hybridization. The genes *metA*, *metC* and the cluster *met'JBLF* of *E. coli* and *metE* of *S. typhimurium* have been cloned in pBR322 (Table 1). Each of these clones have been either partially or completely characterized. The gene *metA* codes for homoserine-O-transsuccinylase, *metB*, for cystathionine-γ -synthase, *metC*, for β-cystathionase, *metE* is the structural gene for a vitamin B12-independent homocysteine transmethylase, and *metF* codes for methylenetetrahydrofolate oxydoreductase. The *metJ* gene codes for a regulatory factor and *metL* for aspartokinase II-homoserine dehydrogenase II, enzyme likewise involved in threonine and isoleucine production (Flavin, 1975).

Materials and Methods

Strains and plasmids

E. coli strains carrying plasmids with *met* genes were grown in LB medium or minimal medium 63 supplemented with glucose (2 $g.l^{-1}$) and vitamin B1 (5$\mu g.ml^{-1}$) (Miller, 1972). Solid media contained 15g agar per liter. Ampicillin (50 $\mu g.ml^{-1}$), tetracyclin (10 $\mu g.ml^{-1}$) or chloramphenicol (25 $\mu g.ml^{-1}$) were added depending on the type of plasmid harbored. The plasmids used are listed in Table 1.

Preparation of plasmid DNA

Large-scale plasmid isolation from *E. coli* was done as described by Maniatis

et al. (1982) with slight modifications. The clear lysate was concentrated by precipitation in the presence of 10% polyethylene glycol and 0.5 M NaCl before subjecting the sample to CsCl density gradient centrifugation. The ethidium bromide was extracted with propanol-2 saturated in Tris-HCl 10 mM/EDTA 1mM, pH 7.5. The sample was dialysed against a buffer containing Tris-HCl 10 mM/EDTA 0.1 mM, pH 7.7 and precipitated with 95% ethanol. Rapid isolation of plasmid DNA of *E. coli* was done according to the technique of Holmes and Quigley (1981) while that of *Synechococcus* PCC7942 followed the method described by Kuhlemeier *et al.* (1981).

Table 1. Plasmids

Plasmids	*Relevant Characteristics*	*Reference/Source*
pME1	pUC303::*Synechococcus* PCC7942 DNA (6 kb *Eco*RI fragment); 17 kb; Sm^r	Pasteur Institute
pIP26	pBR322::*metJBLF* of *E. coli* (EcoRI/*Sph*I fragment); 9 kb; Ap^r	Pasteur Institute
pMA6	pBR322::*metA* of *E. coli* (*Bam*HI/*Sal*I fragment); 8.2 kb; Ap^r	Michaeli and Ron, 1984
pIP29	pBR322::*metC* of E. *coli* (*Hind*III/*Pvu*II fragment); 4.4 kb; Ap^r	Belfaiza *et al.*, 1986
pGS69	pBR322::*metE* of *Salmonella typhirmurium* (*Pst*I fragment); 8.8 kb; Tc^r	Schulte *et al.*, 1984

Restriction digests and electrophoresis

Restriction endonuclease *Bam*HI, *Sph*I, *Eco*RI, *Hind*III, *Pst*I, *Pvu*II (New England Biolabs) were used according to the manufacturer's instructions. Restriction DNA fragments were electrophoresed on 0.4% to 1.5% agarose horizontal gels (16 hours, 3 V/cm) in 89 mM Tris-borate buffer pH 8.3, containing 2 mM EDTA.

Preparative purification of restricted DNA fragments

Preparative low melting point agarose gels were used to isolate restricted DNA fragments. After electrophoresis, DNA bands were visualized under ultraviolet light, cut out and transferred to siliconed Corex tubes containing up to 0.5 ml elution buffer (Tris-HCl 0.1 M/EDTA 1 mH, pH 8). By heating this at 65°C for 5 to 15 minutes, the DNA from the agarose band is solubilized then purified by 3 successive phenol extractions followed by ethanol precipitation.

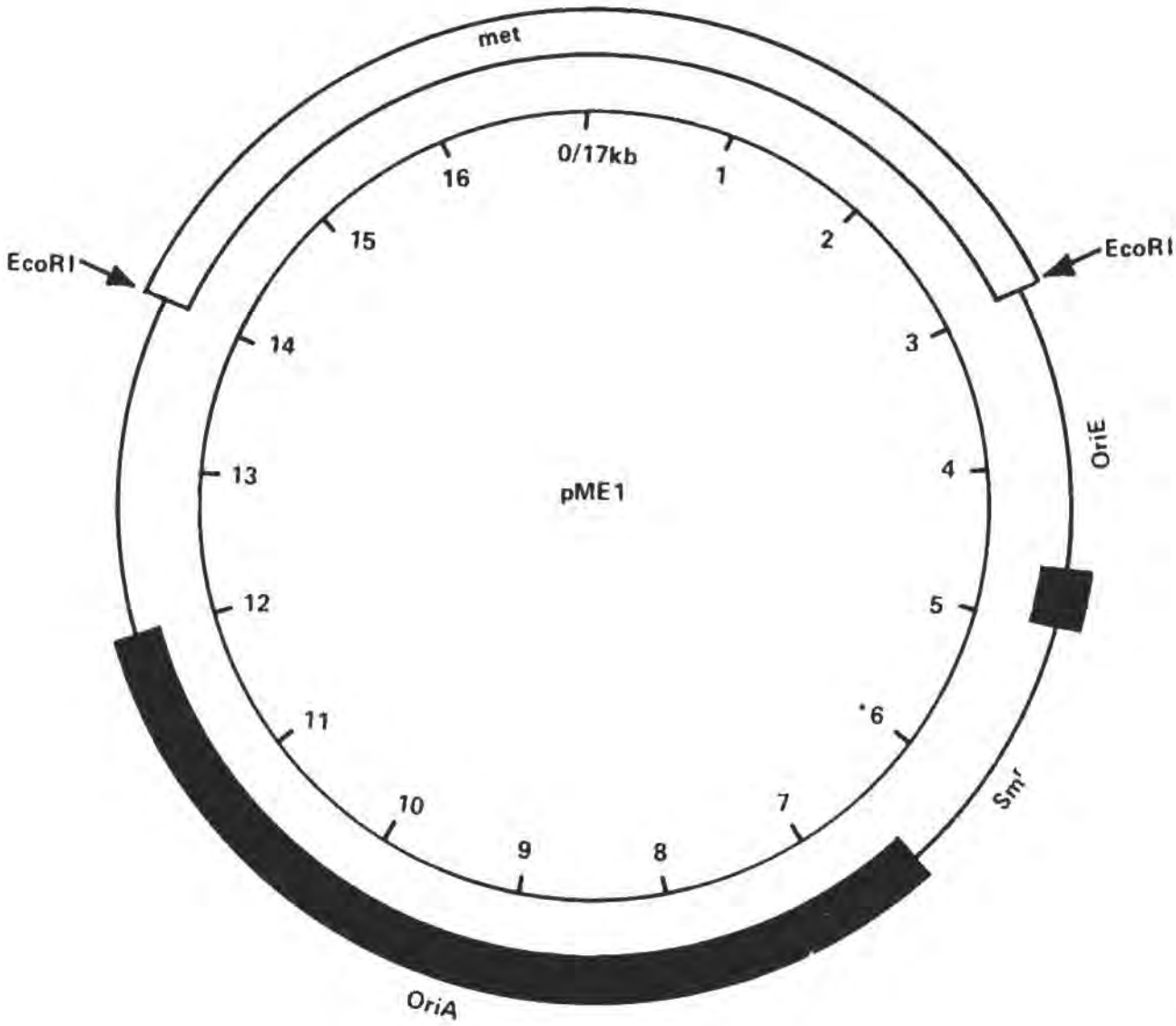

Fig. 1. Schematic representation of the hybrid plasmid pME1. OriA, origin of replication of plasmid pUH24 of *Synechococcus* PCC7942; OriE, origin of replication of plasmid pRI46 of *E. coli*; ▬▬, *Synechococcus* PCC7942 DNA; ——, *E. coli* DNA; ▭, 6.0 kb "*met*" insertion; Sm^r, resistance to streptomycin.

In vitro labeling of cloned met DNA

The DNA was incubated with DNA polymerase I (Boehringer Mannheim) in the presence of [α-^{32}P] dCTP (300 Ci/mmol, Amersham, 1 Ci = 3.7 x 10^{10} becquerels) as described by Maniatis (1982). Labeled DNA was separated from unincorporated nucleotides on a Sephadex G-50 column equilibrated with 0.01 M Tris-HCl, pH 8.0/ 0.001 M Na_2EDTA. The labeled DNA was denatured by heating at 95°C for 10 minutes in the presence of 15 $\mu g.ml^{-1}$ herring sperm DNA.

Southern transfers, DNA hybridization and autoradiography

After electrophoresis of DNA restriction fragments on agarose and DNA denaturation *in situ* by two successive immersions of 15 minutes each in a solution of NaOH 0.5 N and NaCl 0.6 M, the gel was neutralized by two successive

30-minute treatments in a buffer containing Tris-HCl 1M, NaCl 0.6 M, pH 7.4. The DNA was then transferred on a nitrocellulose filter (Schleicher and Schull, BA85) according to the technique of Southern as modified by Smith and Summers (1980). After 16 hours, the filters were washed in 2XSSC and dried at 80°C for two hours. The nitrocellulose filters were then soaked in 2XSSC (NaCitrate 15 mM, NaCl 0.15 M) and prehybridized at 65°C for one to four hours in a solution containing 6XSSC, Denhardt (0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.2% bovine serum albumin, 0.02% Ficoll), 0.5% SDS and 15 $\mu g.ml^{-1}$ denatured herring sperm DNA. In the actual hybridization step, the filters were transferred to a similar solution containing the denatured radioactively labeled probe. Hybridization conditions were adjusted to allow less stringent base pairing of heterologous DNA. For this, 10% of deionized formamide, 10% v/v dextran sulfate, 250 $\mu g.ml^{-1}$ denatured herring sperm DNA were added to the hybridization buffer composed of SSC, Denhardt and SDS as mentioned above. Incubation temperature was 42°C. The filters were washed at room temperature in 0.1XSSC containing 0.5% SDS (thrice) for 1 hour each, then in 1XSSC (twice) for 15 minutes each. The dried filter was placed in a cassette with Kodak X-Omat ARI film and an intensifying screen, at −80°C for at least one week and the film was then developed.

Results and Discussion

Plasmids carrying defined *met* genes of some enterobacteria were used as heterologous probes in identifying the specific functional nature of *met1*. The first step was to find if sufficient hybridization signals can be detected between the DNA of *Synechococcus* PCC7942 and the various purified DNA fragments carrying enterobacterial *met* genes.

Total chromosomal DNA of *Synechococcus* PCC7942 was digested with *Eco*RI, subjected to agarose gel electrophoresis, transferred to nitrocellulose paper, and hybridized with ^{32}P labeled DNA probes carrying either *metA*, the *metJBLF* cluster, *metC* or *metE*. In each case, low stringency hybridization conditions sufficient to allow detection of DNA hybrids homologous to as low as 30% had to be imposed. Although the actual homology between enterobacterial and cyanobacterial *met* genes is weak, DNA fragments which carry the different corresponding *met* genes were identified. Each of the DNA probes used hybridized with restriction fragments of different lengths. These results preclude the idea that all the *met* genes of *Synechococcus* PCC7942 are not clustered but the possibility that *metJBLF* exists as an operon cannot as yet be ascertained. Figure 2 shows the banding patterns obtained for each of these probes. The probe carrying the *metA* gene *of E. coli* was able to hybridize to two *Eco*RI fragments of *Synechococcus* PCC7942 (Fig. 2, canal 1). The *metJBLF* probe consistently displayed hybridization bands of 6.0 and 5.6 kb (Fig. 2, canal 2). The other bands are due to partial digestion

of chromosomal DNA in this particular sample. Similarly, bands of 1.9 kb and 4.9 kb were revealed after hybridization with *metC* and *metE* probes, respectively.

The low hybridization profiles indicate that the methionine biosynthetic genes have widely diverged during the course of evolution of those two prokaryotes. Among the different *met* genes of *Synechococcus* PCC7942, varying band intensities were observed depending on the probe used suggesting that the comparative degree of homology with heterologous DNA can vary among the *met* genes of one species. The hierarchy of hybridization, from highest to lowest, can be established as follows: *metA* > *metE* > *metC* > *met'JBLF*. The relatively dominant hybridization signal observed with the *metA* probe is in accord with our previous observation that homoserine-O-transsuccinylase (the product of the *metA* gene in *E. coli*) present in *Synechococcus* PCC7942 has a specific activity tenfold higher than that observed for the enzymes supposedly encoded by the other *met* genes (Co, 1987).

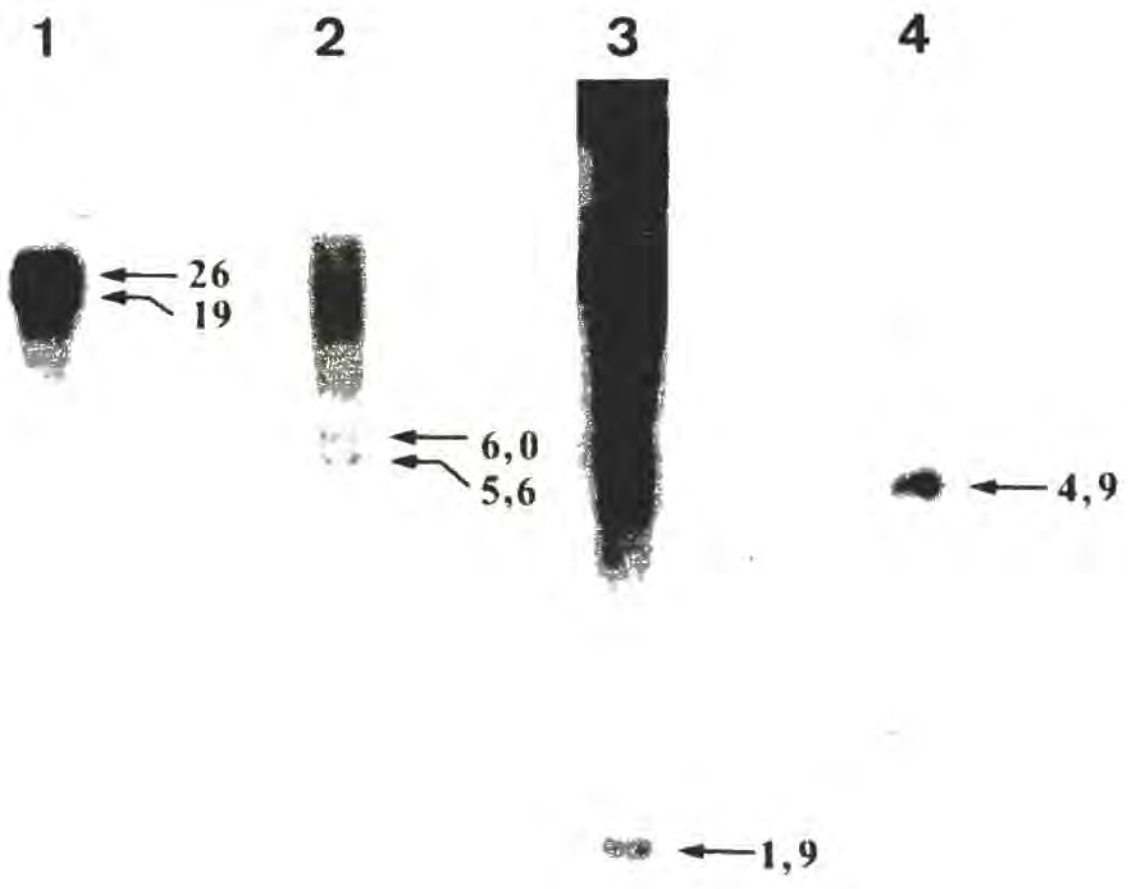

Fig. 2. Identification of *Eco*RI restriction fragments of total *Synechococcus* PCC7942 DNA carrying sequences homologous to *met* genes of enterobacteria. The DNA probes used were: the 2.6 kb *Bam*HI-*Sal*I fragment carrying the *metA* gene of *E. coli* (canal 1), the 5.0 kb *Eco*RI-*Sph*I fragment containing the *met'JBLF* cluster of *E. coli* (canal 2), the 2.0 kb *Hind*III-*Pvu*II fragment carrying the *metC* gene of *E. coli* (canal 3), and the 4.8 kb *Pst*I fragment carrying the *metE* gene of *S. typhimurium* (canal 4).

The *met'JBLF* probe was the only one which hybridized with a 6.0 kb *Eco*RI insert containing *metJ* in the plasmid pME1 (Fig. 1). This is one of the first indications that *metJ* is equivalent to *metF* which codes for a methylenetetrahydrofolate oxydoreductase in *E. coli*. Further evidence is manifested in Fig. 3 which shows the electrophoretic pattern of plasmid pME1 after hydrolysis by *Eco*RI and *Hind*III and of pTH225 (see Introduction) after hydrolysis by *Eco*RI, with the corresponding autoradiogram. Positive hybridization was detected with a 6.0 kb *Eco*RI fragment of pTH225 (Fig. 3, canal 2') and a 3.9 kb *Eco*RI-*Hind*III fragment of pME1 (Fig. 3, canal 1'). This latter result indicates that the gene or at least the homologous portion of the gene is localized in a 3.9 kb *Eco*RI-*Hind*III fragment. A second intense hybridization band measuring 5.6 kb was observed in one insert of pTH225 (Fig. 3, canal 2'). This band was also visible in the DNA hybridized with the same probe (Fig. 2, canal 2). It is therefore highly probable that this 5.6 kb *Eco*RI fragment carries a gene corresponding to other genetic components of the *met'JBLF* cluster present in the probe.

In the experiment presented in Fig. 3, difficulty in obtaining hybridization between DNA fragments sharing very little homology was encountered. Positive signals are also obtained with the vector components of the plasmid (V). However, despite the appearance of vector bands, the specificity of these results was demon-

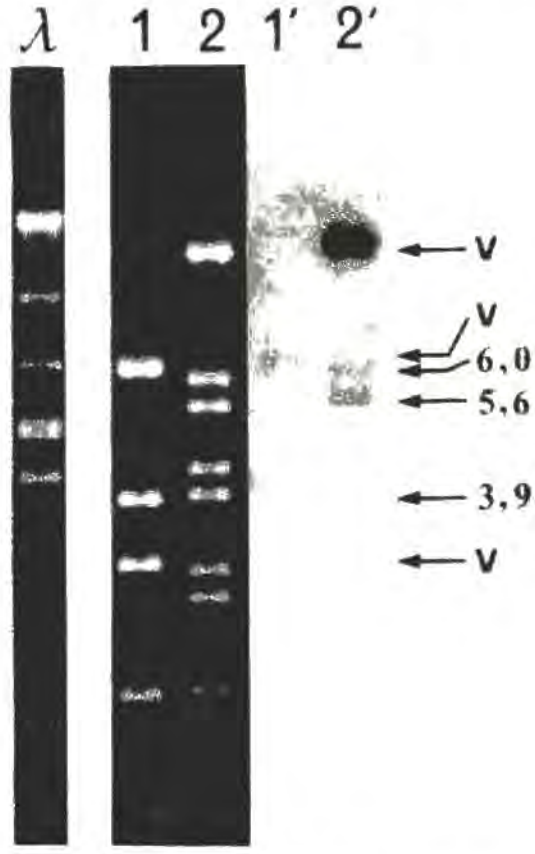

Fig. 3. Hybridization of plasmids pME1 and pTH225 with the 5.0 kb *Eco*RI-*Sph*I probe carrying the *met'JBLF* genes of *E. coli*. Agarose gel electrophoresis of pME1 hydrolyzed by *Eco*RI and *Hind*III (canal 1) and pTH225 hydrolyzed by *Eco*RI (canal 2), and corresponding autoradiogram (canals 1' and 2'). Lambda (λ) DNA was hydrolyzed by *Hind*III and *Hind*III-*Eco*RI and serves as the molecular weight marker. V=bands belonging to the vector pUC303 or pPUC29.

Fig. 4. Hybridization of the plasmid pIP26 carrying the *met'JBLF* genes of *E. coli* with the 6.0 kb *Eco*RI probe carrying *metI*. Agarose gel electrophoresis of plasmid pIP26 hydrolyzed by *Eco*RI and *Sph*I (canal 1) and corresponding autoradiogram (canal 1'). Lambda (λ) DNA, hydrolyzed with *Hind*III and *Hind*III-*Eco*RI, was used as molecular weight marker.

strated in an independent hybridization experiment wherein pBR322, the vector from whence the various *met* probes were derived, was used as a probe against restriction fragments of pME1 and pTH225. Only the vector interacted with pBR322 (data not shown). It is possible that pBR322 was present in trace amounts in the probe preparation. It has been shown here that pBR322 interacts with sequences on the pUC303 and pPUC29 vectors. Thus, the observed 6.0 kb and 5.6 kb bands of pTH225 and 3.9 kb band of pME1 in Fig. 3 resulted from specific binding to the *met'JBLF* probe.

Reciprocally, the 6.0 kb *Eco*RI insert in pME1 which carries *metI* was labeled with ^{32}P. Using the same hybridization conditions as the preceding, only the 5.0 kb *Eco*RI-*Sph*I insert of pIP26 (which carries the *E. coli met'JBLF* gene cluster) hybridized, albeit very weakly (Fig. 4). Similar reciprocal hybridizations using *metI* as probe were done against the other enterobacterial *met* genes but no hybridization was obtained. These results reinforce our hypothesis that the cloned *metI* gene corresponds to a gene coding for an enzyme that intervenes in one of the final steps in the methionine biosynthetic pathway, and more precisely, to a gene analogous to the metF gene of *E. coli*. *E. coli* and cyanobacteria occupy divergent evolutionary branches, yet their *metF* genes have been sufficiently constrained from diverging so as to allow detection of *Synechoccus met* genes with *E. coli* probes or vice versa.

This work has also demonstrated that heterologous DNA can be used in certain cases as a probe for genes in which evolutionary constraints have been exercised. This is true for *metA*. The percentage homology is relatively high for the gene encoding for homoserine-O-transsuccinylase of both *Synechococcus* PCC7942 and *E. coli* as evidenced by the strong hybridization signal observed. One can thus envisage the *metA* gene of *E. coli* as a feasible tool in the search for corresponding sequences in cyanobacterial gene libraries. On the other hand, given the relative difficulty in detecting hybridization signals with probes carrying *metC* and *metE*, it is suggested that these may not serve as useful probes in subsequent screening of gene libraries by colony hybridization.

Gene cloning is a prerequisite in the understanding of gene structure and expression mechanisms. According to sparse physiological data, it seems that the control by repression of amino acid biosynthetic genes, phenomenon commonly occurring in enterobacteria does not apply to cyanobacteria. So far, only tryptophan synthesis in the cyanobacterium *Agmenellum quadruplicatum* has indirectly been found to be subjected to repressive control at the genetic level (Ingram *et al.*, 1972). A complete understanding of amino acid biosynthesis requires therefore a detailed knowledge of the number of genes involved, the organization and control of these genes and the identification of the function of each gene. These will in turn permit an evaluation of evolutionary trends of biosynthetic genes.

In *E. coli* for instance, recent comparative nucleotide sequence data revealed that in the evolution of its methionine biosynthetic pathway, the *metB* and *metC* genes may have originated from a common ancestral gene (Belfaiza *et al.*, 1986). The original gene may have duplicated and subsequent mutations may have occurred on each copy leading to specialization of the encoded proteins. According to a proposal put forward by Horowitz (1945) about 40 years ago, the biosynthetic pathways as we know them today may have been progressively built backwards from the final metabolite of the pathway. More detailed intergenetic hybridization studies in *Synochococcus* PCC7942 and other cyanobacteria may bring more light into this interesting hypothesis about the possible evolutionary events that have occurred for biosynthetic pathways.

Acknowledgment

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PRODUCTION OF ANTISERUM AND INITIAL HYBRIDOMAS AGAINST PAPAYA RING SPOT VIRUS

T.M. Espino, S.B. Exconde, N. Minamiura, M. Izuka
and K. Ito
National Institutes of Biotechnology and Applied Microbiology (BIOTECH), University of the Philippines at Los Baños College, Laguna, Philippines

ABSTRACT

Papaya ring spot virus (PRSV) was isolated and purified from infected papaya leaves. The particles were shown to be threadlike and filamentous measuring about 750 nm in length and 12 nm in diameter. The average yield of PRSV per 100 g samples was 443 ug.

The antiserum against PRSV was used for rapid screening of infected papaya plants from the provinces of Cavite, Batangas and Laguna using the enzyme linked immunosorbent assay (ELISA) double antibody sandwich technique. Four hybridomas which gave consistent positive reaction when assayed by the ELISA technique and fast grower were obtained from the 2 fusion experiments. Positive PRSV hybridomas were stored in liquid nitrogen for the cloning experiments and eventually for mass production of monoclonal antibodies.

Introduction

Papaya (*Carica papaya* L.) is one of the most important crops in the Philippines because of its several uses as food, medicine and in industry. Papaya fruits are available throughout the year. It ranked 6th in the area planted and in quality and value of production among fruits grown in 1975 as reported by BAECON (Philippine Recommends for Papaya, 1977). Both fruits and the enzyme papain from papaya show great economic potential for local consumption and for export.

Papaya is not free from disease attacks. Some diseases include papaya mosaic, a minor disease which is transmitted mechanically and by a vector, *Myzus persicae.* The other disease, leaf curl is presumed to be caused by tobacco leaf curl virus and transmitted by whitefly (Philippine Recommends for Papaya, 1977). Both diseases do not cause serious damage to the papaya trees grown locally.

Recently, a new destructive disease identified as papaya ring spot virus (PRSV) disease was reported in epidemic proportion in Silang, Cavite affecting about 200 hectares of papaya farms. The disease is reported to have spread in the papaya plantations in the Southern Tagalog region especially in Cavite, Batangas

and Laguna. The disease causes severe mottling, leaf malformation, reduction of laminae, streaks and ringspot symptoms especially in fruits. Initial studies were initiated at UP Los Baños and DAF Regional Crop Protection Center in Region IV and confirmed the viral nature of the disease. The virus is known to be transmitted by mechanical inoculation with 60-70% efficiency, by granting and by four species of aphids.

Papaya ring spot virus is made up of filamentous particles about 800 nm in length and 12 nm in diameter (Herold and Weibel, 1962; Purcifull, 1972). It is stylet-borne by aphids, mechanically transmissible and belongs to the potyvirus group (de Bokx, 1965; Harrison *et al.*, 1971; Brandes and Berks, 1965). The virus is serologically related to watermelon mosaic virus (Webb and Scott, 1965; Milne and Grogan, 1969; Purcifull and Hiebart, 1979; Gonsalves and Ishii, 1980).

A number of techniques to prevent the spread of plant viral diseases are available such as the use of tolerant or resistant varieties, vector control, use of virus – free planting materials, sanitation and cultural control (Bar-Joseph and Garnsey, 1981). There are no resistant papaya cultivars and attempts to develop effective control measures for PRSV were unsuccessful. Although roguing offers a feasible solution to the prevention of the disease, the method is not a permanent solution for other areas that do not have a geographical isolation and where the disease has become endemic (Yeh and Gonsalves, 1984).

In the Philippines, the diagnosis of plant viral diseases is done via conventional methods which are less rapid and less sensitive. Studies on the production of specific antisera and monoclonal antibodies against plant diseases are very limited. Hybridoma technology involves the use of monoclonal antibodies to sensitively detect the proteins of plant viral genes. This technology has recently been applied to plant virus research in several laboratories (Briand *et al.*, 1982; Diaco *et al.*, 1983; Halk *et al.*, 1982; Hsu *et al.*, 1983; Tremaine and Ronald, 1983). The use of of hybridomas offers many important benefits such as production of a highly purified antibody and the certainty that each hybridoma line is a clone producing antibodies to a single antigenic site. Monoclonal antibodies have also several advantages over polyclonal antibodies which include the availability of an unlimited supply of antibody, uniform antibody preparation and production of antibodies of predetermined specificity.

The main objective of this work is to produce antiserum and monoclonal antibodies against PRSV which can be used in the effective and rapid diagnosis of PRSV-infected papaya plants all over the Philippines.

Materials and Methods

Isolation and purification of the antigen (PRSV)

Papaya ring spot virus was isolated from infected papaya leaves collected from the nurseries of the Dept. of Horticulture and Institute of Plant Breeding, UPLB and from surrounding areas in Laguna where papayas were planted. The

purification procedure described by Gonsalves and Ishii (1980) was used in the isolation of PRSV. Minor modifications were made in the purification of PRSV. They included the following: (1) In the pelleting of the virus particles after PEG precipitation, centrifugation was done at 14,500 g instead of 10,000 g resulting in increased recovery of the virus precipitated; (2) In the final purification of PRSV using density gradient centrifugation, a 10-40% sucrose gradient was substituted for 10-40% cesium sulfate gradient due to the high cost and unavailability of cesium sulfate. Continuous isolation and purification of PRSV are done to have enough supply of antigen for immunization and for ELISA of antiserum and initial hybridomas.

Isolation and purification of host proteins from healthy papaya leaves were also done. Healthy papaya leaves were subjected through the same purification scheme as the infected papaya leaves except the sucrose density gradient centrifugation to obtain host proteins for cross absorption with antibodies. The concentration of the PRSV preparation was determined by assuming $A_{260} = 2.40 = 1$ mg/ml. The average value was obtained from the different absorbance values at 260 nm for reference viruses of the potato virus Y group to which PRSV belongs (Table 1).

Table 1. Viruses of the potato virus Y group with $A_{260} = 1$ mg/ml as reference values for determining PRSV concentration

Virus	*A_{260nm}*	*Reference*
Carnation vein mottle	2.10	Hollings and Stone, 1971
Dasheen mosaic	2.38	Zetter *et al.*, 1978
Pea seed borne mosaic	2.50	Hampton and Mink, 1975
Peanut mottle	2.60	Bock and Kuhn, 1975
Average	2.40	

Electron microscopy

Purified fractions from healthy and infected papaya leaves were negatively stained with 2% phosphotungstic acid (PTA), pH 7.2 and examined under the transmission electron microscope.

Production of antiserum and initial hybridomas

Culture of myeloma cells for cell fusion. The non-secreting mouse myeloma cell line used was P3-x63 – Ag8-U1. The myeloma cells were cultured in RPMI 1640 + HT + 12% FCS (Gibco) in an atmosphere of 5% Co_2 at 37°C.

Immunization of test animals

Each mouse (BALB/c AnNCrj, male, 4-11 weeks old) was injected intraperitoneally with an emulsion consisting of 0.1 ml purified antigen (75-100 ug) and 0.1 ml Freund's complete adjuvant. After four and three weeks interval, a second and third injections respectively of 0.1 ml antigen was given to each mouse. The mice were killed 3 days after the last booster injection.

Cell fusion

The immunized mouse was killed by neck bone dislocation. The blood was pumped from the heart and kept at 4°C overnight as a source of antiserum. The immunized spleen was removed under a sterile hood by spraying the mouse with 70% ETOH. Other tissues connected with the immunized spleen were removed to prevent contamination. The spleen was placed in about 40 to 50 ml DMEM and macerated until almost all spleen cells were in suspension. The methods described by Kohler and Milstein (1975), Miura (1980) and Ito *et al,* (1985) were followed for the fusion experiments.

After two or three weeks, hybrids growing in 96-well plates were screened for antibody activity using the enzyme linked immunosorbent assay (ELISA) double antibody sandwich technique. The procedure was adapted from the method described in Hybridoma Techniques (EMBO, SKMB Course 1980, Basel). Positive hybridomas were transferred in 2-cm dishes, cultured in RPMI 1640 + HT + 12% FCS, incubated at 37°C in a 5% CO_2 incubator, allowed to multiply and reassayed by the ELISA technique. Positive hybridomas were then kept in the above culture medium containing 10% DMSO and frozen in liquid nitrogen for cloning experiments using the limiting dilution technique.

For the assay of hybridomas by the ELISA technique, the antiserum against PRSV was used as the positive control whereas PBS buffer, mouse normal serum and purified extracts of healthy papaya leaves were used as negative control.

Results and Discussion

Isolation and purification of PRSV from infected papaya leaves

Infected papaya leaves (Fig. 1) were used to isolate PRSV. Fig. 2 shows the scanning patterns of the purified preparation of infected papaya leaves after density gradient centrifugation in 10-40% sucrose. A ten to 40% sucrose gradient with 0.1 M phosphate buffer containing 0.01 M EDTA, pH 7.0 was used as blank.

The purified preparation of PRSV gave a single peak which was located near the meniscus indicating its slow sedimenting property. The homogeneous preparation of PRSV showed the presence of threadlike, filamentous particles measuring about 750 nm in length and 12 nm in diameter (Fig. 3) when examined under the transmission electron microscope. The result conformed with the PRSV prepa-

ration obtained by Herold and Weibel (1962) and Purciful (1972). No virus particles were seen under the transmission electron microscope when the purified preparation of healthy papaya leaves was examined.

The average yield of 443 ug was obtained per 100 g samples. The concentration of the virus preparation was 419 ug/ml by assuming A_{260} = 2.40 = 1 mg/ml as indicated in the materials and methods. The $A_{260/280}$ value of 1.19 for PRSV was obtained and was comparable with viruses of the potato virus Y group to which PRSV belongs (Table 2).

Table 2. Comparison of the $A_{260/280}$ values of the viruses belonging to potato virus Y group* and PRSV

Virus	*$A_{260/280}$*
1. Bearded iris mosaic	1.12
2. Carnation vein mottle	1.15
3. Carrot thin leaf	1.18
4. Clover yellow vein	1.29
5. Dasheen mosaic	1.09-1.19
6. Henbane mosaic	1.10
7. Hippeastrum mosaic	1.21
8. Iris mild mosaic	1.25
9. Pea seed-borne mosaic	1.14-1.18
10. Peanut mottle	1.24
11. Pepper veinal mottle	1.25
12. Papaya ringspot virus	1.19

*Values were obtained from the C.M.I./A.A.B. Descriptions of Plant Viruses.

Fig. 1. Symptoms of papaya ringspot virus on papaya leaves collected fron the field and used as source of inoculum.

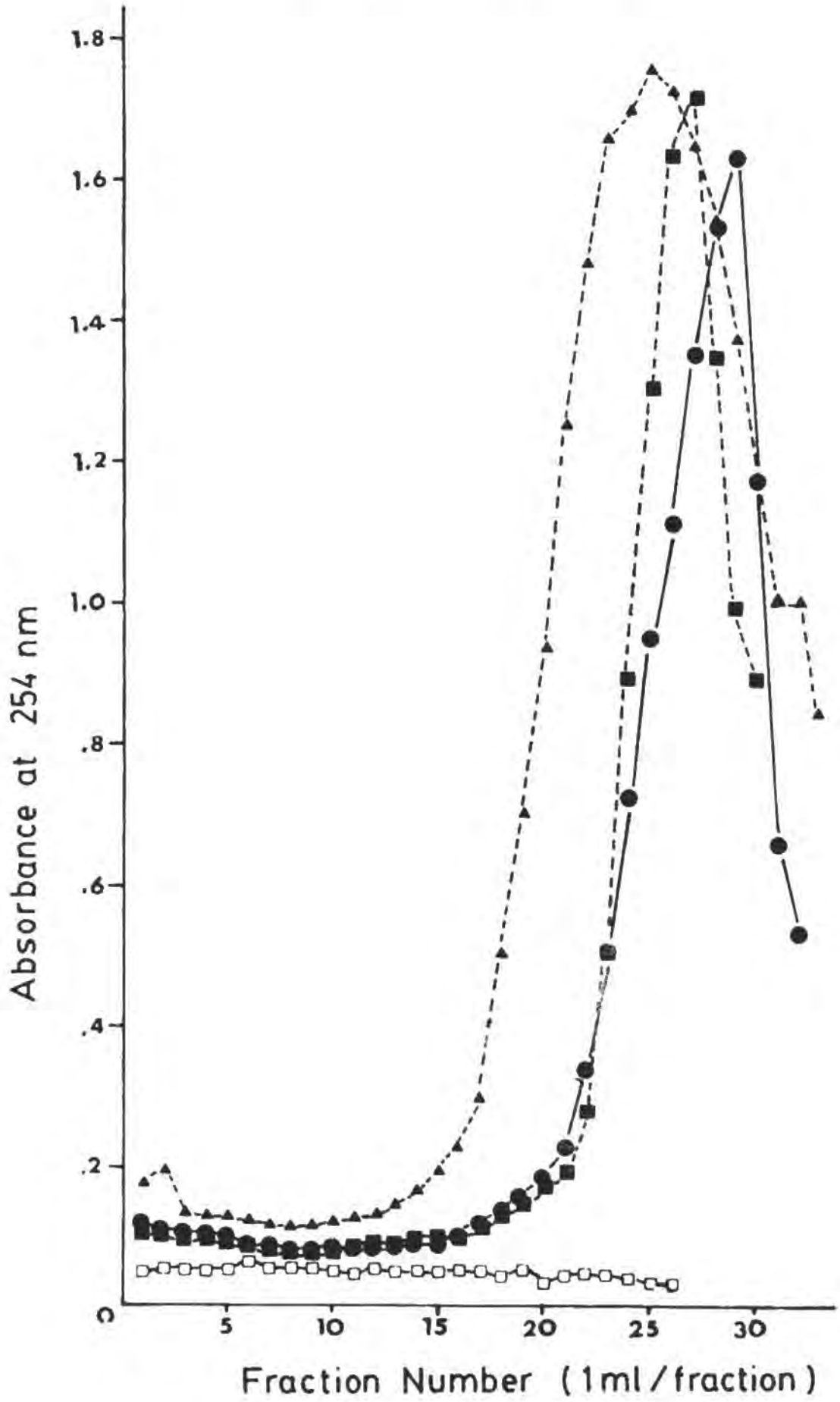

Fig. 2. Scanning patterns of a PRSV preparation after centrifugation in 10-40 percent sucrose gradient, △, □, ○, PRSV purified preparation; □, phosphate buffer (0.1 M containing 0.01 M EDTA, pH 7.0).

Fig. 3. Electron micrograph of papaya ringspot virus particles purified by sucrose density gradient centrifugation (72,000x).

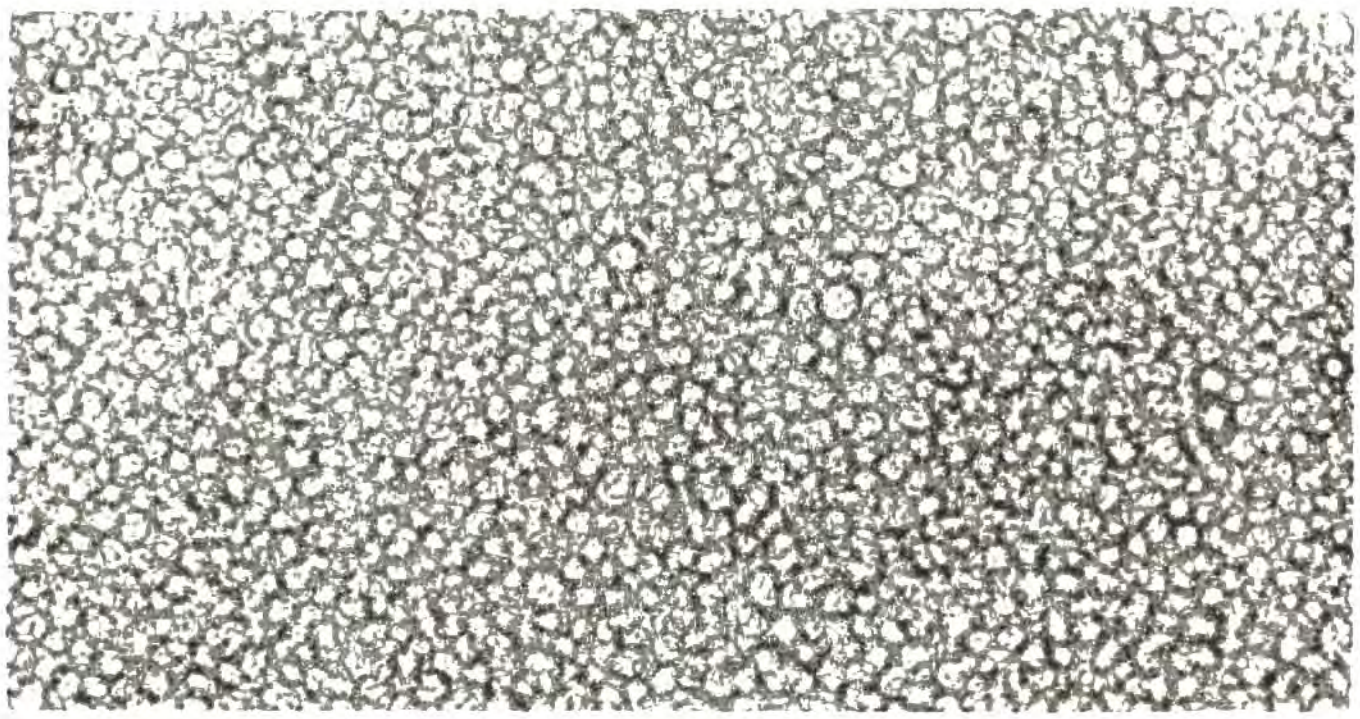

Fig. 4. Myeloma and spleen cells after fusion (200 x).

Production of antiserum and hybridomas against PRSV

Ninety six well plates containing fused cells were examined under the microscope (Fig. 4). The myeloma cells are bigger than the spleen cells as shown in Figs. 5 and 6. On the 3rd to 5th day, both myeloma and spleen cells were dying out while the hybridomas started to grow. A colony of PRSV hybridomas is presented in Fig. 7. After 12 days, the 3-96 well plates were assayed using the ELISA double antibody sandwich technique. The appearance of yellow color due to p-nitrophenol

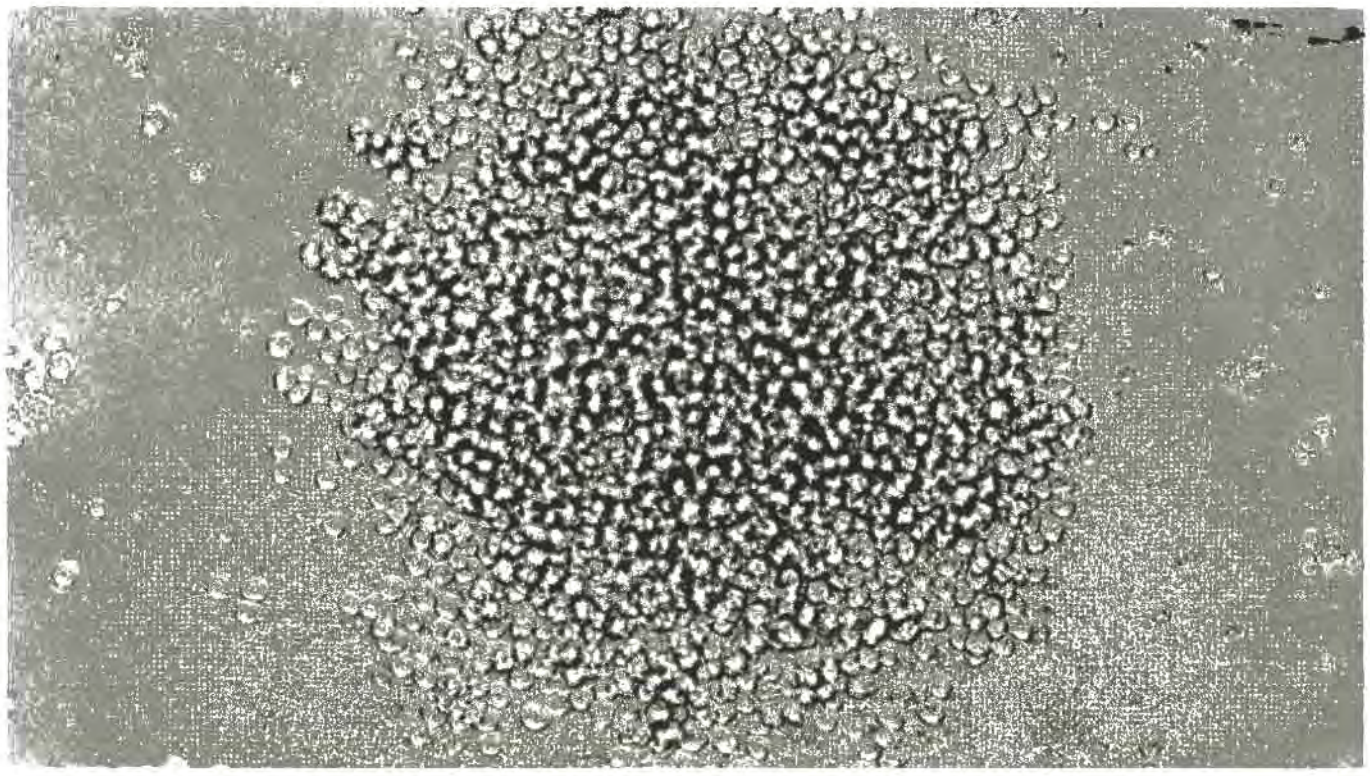

Fig. 5. Photomicrograph of myeloma cells cultured in RPMI 1640 + HT + 12% FCS + 8 – azaguanine (200 x).

Fig. 6. Spleen cells from immunized mice seen under the phase contrast microscope (200 x).

formation indicated positive reaction. Results of the assay are shown in Figs. 8 and 9. Thirteen positive hybridomas were obtained in the first screening with 2 hybridomas showing high antibody activities as indicated by the dark-yellow color. A second and third assays of the positive hybridomas reduced the number to 8 and 2, respectively. Only those hybridomas that gave consistent positive reaction and fast grower were multiplied and stored in liquid nitrogen for the cloning experiments. Table 3 summarizes the results obtained for the 2 fusion experiments. Four positive

hybridomas (Table 4) were obtained from the 2 fusion experiments with their corresponding absorbance readings at 410 nm. The negative controls were PBS buffer, healthy papaya sap and mouse normal serum while the positive control was PRSV antiserum.

Fig. 7. PRSV hybridomas in a 96-well plate seen under the phase contrast microscope (200 x).

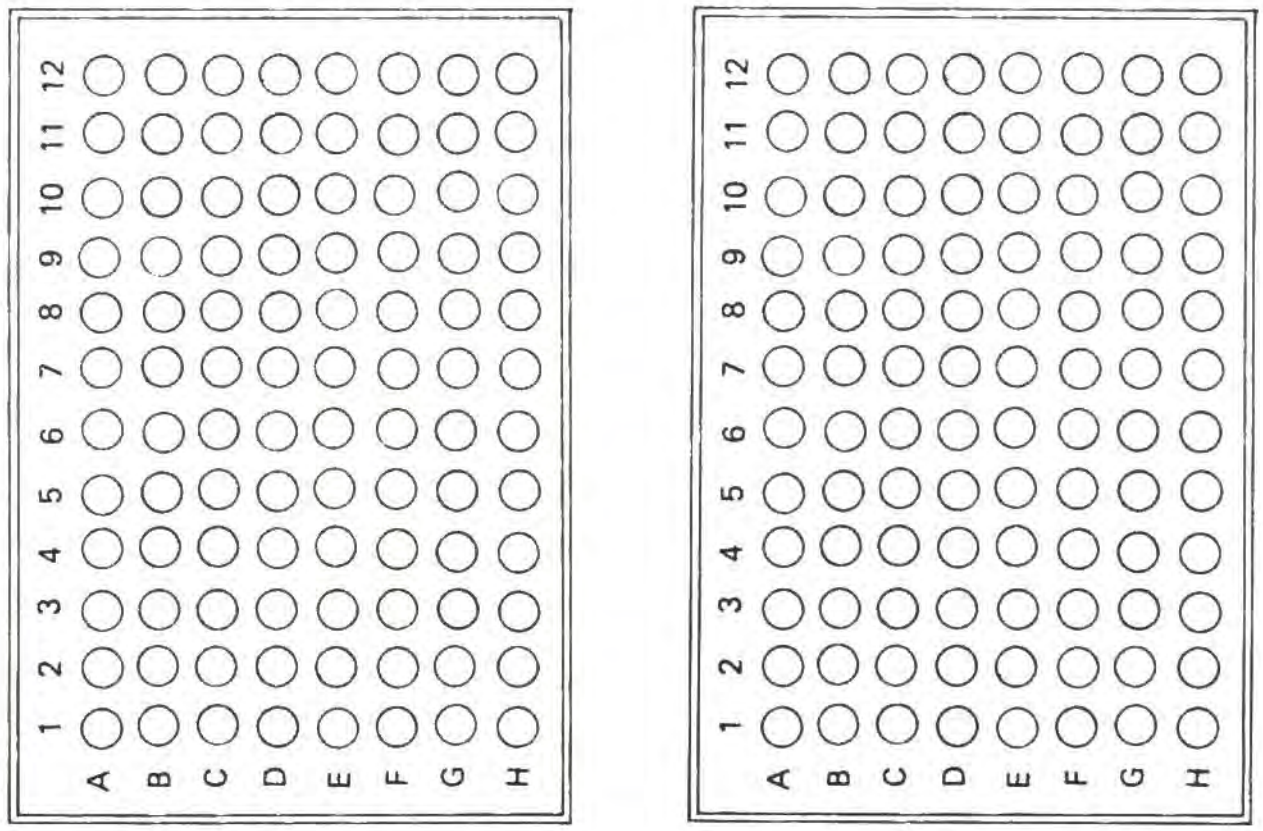

Fig. 8. Initial screening of nos. 1 and 2 PRSV – 96 well plates for positive hybridomas using the double antibody sandwich ELISA technique.

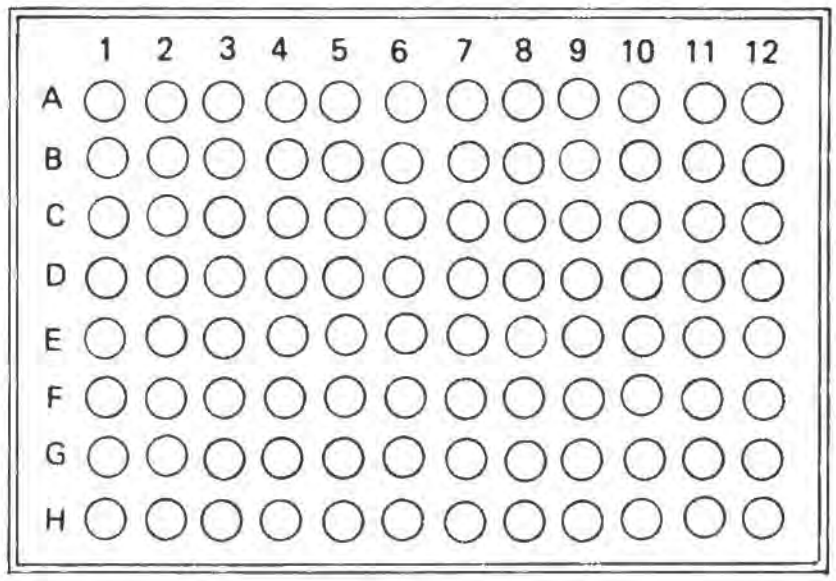

Fig. 9. Initial screening of no. 3 PRSV – 96 well plates for positive hybridomas using the double antibody sandwich ELISA technique.

Table 3. Production of stable PRSV hybridomas after 2 cell fusion experiments

Fusion No.	*Number of wells tested*	*Number of well containing antibody producing hybridomas*			*Total number of hybridomas obtained for cloning experiments*
		1st screening	*2nd screening*	*3rd screening*	
1	285	13	8	2	2
2	380	17	6	2	2

Table 4. Absorbance readings at A_{410} nm of PRSV positive hybridomas with PBS buffer, healthy papaya sap, mouse normal serum and antiserum as controls

	$A_{410\ nm}$
Negative control	
1. PBS buffer	0.057
2. Healthy papaya sap	0.049
3. Normal serum	0.065
Positive control	
1. Antiserum	0.991
Hybridoma samples	
1. $PRSV_J - 3$	0.249
2. $PRSV_J - 5$	0.606
3. $PRSV_B - B$	0.447
4. $PRSV_B - C$	0.291

Table 5. Rapid screening of the crude saps of infected papaya plants collected from different areas with PRSV antiserum using the ELISA double antibody sandwich technique

Sample source	A_{410nm}
Tanauan, Batangas	0.374
San Pablo City, Laguna	0.297
Tagaytay City, Cavite	0.335
Healthy papaya sap	0.100
PBS buffer	0.097

The antiserum against PRSV was then used for rapid screening of infected papaya plants in Cavite, Batangas and Laguna. Results in Table 5 indicate that the papaya plants from the 3 provinces were positive for PRSV as shown by their high absorbance readings which were 3x higher than the PBS buffer and healthy papaya sap (negative controls).

With the development of the rapid screening technique using PRSV antiserum and eventually monoclonal antibodies from PRSV hybridomas for detection of virus infection of papaya plants, we can now look forward to screening for tolerant papaya varieties under Philippine condition. Furthermore, the use of tolerant papaya varieties may lead to the control of the disease.

Acknowledgment

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ECONOMIC AND DEMOGRAPHIC ADJUSTMENTS TO ECONOMIC STRESS: THE CASE OF THE URBAN POOR

Alejandro N. Herrin
School of Economics, University of the Philippines, Diliman, Quezon City

ABSTRACT

Household survey data from selected urban poor communities suggest several interesting hypotheses regarding the mechanisms poor households adopted to cope with increased unemployment and reduced incomes following the economic crisis of 1983. These mechanisms include increased labor force participation of spouses and other adult members, reduced schooling participation of children, reduced consumption expenditures notably on food, and limitation of additional children. The desire to limit fertility, however, has not been totally matched by contraceptive use. It is hypothesized that this discrepancy is largely due to the lack of access to family planning services.

Introduction

Much of what has been written about the impact of the 1983 economic crisis refer to changes in macroeconomic indicators such as output, employment, income and prices. Very little effort has been made to examine the impact of the crisis at the household level, particularly in terms of the various adjustments households adopted to cope with economic stress and the implications of these adjustments for the welfare of the household members affected.

In 1985, a household survey in selected urban poor communities in Davao and Cebu was conducted as part of the monitoring evaluation activity of a pilot project to strengthen the provision of maternal and child health care and family planning (MCH/FP) being implemented by the Population Center Foundation for the Commission on Population since 1983. The survey was principally designed to inquire about MCH/FP concerns and the extent to which specific project services were reaching the target population. However, it was felt by the project evaluators that contemporaneous events, namely aspects related to the economic crisis, are bound to confound the influence of project activities on potential improvements in MCH/FP. More practically, there was a need for information to help the project staff fine-tune project activities in response to possible changes in the magnitude and urgency of MCH/FP needs arising from the effects of the economic crisis. As a result, a limited set of questions were added to the survey to provide such information. More particularly, the survey additionally inquired into (a) the employment of adult household members and household income; (b) perceived changes in household incomes and changes in consumption patterns during the past two years;

(c) school participation of children of various ages; and (d) fertility plans, current contraceptive use, and perceived access to family planning services.

Preliminary analysis of these additional data suggests several interesting hypotheses regarding the impact of the economic crisis on the poor which could provide bases for detailed and more focused investigations using more representative samples in the future. The hypotheses may be briefly described as follows: Worker terminations due to shutdowns and retrenchment of industrial establishments as well as to the general slowdown in business activity following the 1983 economic crisis have adversely affected the urban poor in general more than the rest of the urban work force and, among the urban poor, the poorest of the poor. Adjustments to the loss of employment of household heads and to the consequent reduction in incomes took several forms. These included increased labor force participation of spouses and other adult household members; reduced schooling participation of children; reduced consumption expenditures, notably on food; and fertility limitation. Current fertility plans, however, could not be effectively realized due to the lack of access to family planning services.

The next section briefly describes the socio-economic characteristics of the urban poor in selected areas in Davao and Cebu. Although the poor have many things in common, they are also a heterogenous group. To capture this heterogeneity, we examine the characteristics of different types of households as identified by the occupation of household head. The third section presents data and inferences regarding various adjustments households adopted to cope with difficult times. The major hypotheses are summarized in the final section.

Socio-Economic Characteristics of Urban Poor Households

Identifying social groups

Social groups are identified by occupation of the household head (Table 1) and subsequent data are analyzed with attention to these different groups of households. The urban poor households in Cebu generally belong to the following major categories: sales, services, transport and craftsmen. In Davao the major categories are sales, transport, craftsmen and fishermen/farm workers.

Income profile

Table 2 shows the income profile of households by occupation of the household head. Household income was computed by summing up (a) the wage income of the household head, the wife and all other adult members of the household (i.e. 15 years old and over) during the month prior to the survey; and (b) all other income from various non-wage sources over the past year divided by 12. The total household income from all sources is, therefore, reckoned in terms of monthly income, with last month's wage income taken as an average for the year.

The mean monthly household income in both areas is ₱1,308: ₱906 in Cebu and ₱1,607 in Davao. Taking into account household size, the mean per capita

Table 1. Distribution of households by major occupation of the household head, selected urban poor communities in Cebu and Davao cities, 1985 (In Percent)

	Occupation of Household Head	*All Areas*	*Cebu*	*Davao*
1.	Professional, technical & administrative workers	3.9	3.6	4.2
2.	Clerical workers	3.4	4.2	2.7
3.	Sales & related workers	21.0	23.3	19.2
4.	Service & related workers	9.2	10.3	8.3
5.	Transport & communication workers	15.4	18.5	13.1
6.	Craftsmen & production workers	27.9	27.9	27.9
7.	Fishermen & farm workers	16.8	8.6	22.8
8.	No jobs	2.6	3.5	1.9
	Total	100.0	100.0	100.0
	(Total Number of Households)	(1,551)	(660)	(891)

household income in both areas is ₱202: ₱133 in Cebu and ₱256 in Davao. Household incomes vary not only between areas but also between different categories of households. In both areas, the highest mean household, and mean per capita household, incomes are found among the "professional" and "clerical" categories, while the lowest are among the "fishermen" and "occupation not reported" categories. In Cebu, the highest mean household income is 3.2 times the lowest figure, while in Davao, the corresponding factor is 2.3. Thus between areas and between households within each area, there are wide variations in income among poor households. (Using a poverty line of ₱600 per capita monthly income derived by transforming Abrera's (1974) annual poverty threshold estimate for a 6-member household in "Other Urban Areas," practically all of the households in both areas fall below the poverty line.)

Employment profile

Table 3 shows the employment profile (past week prior to the survey) of adult household members by category of households. In both areas the overall employment ratio, i.e. the proportion of all household members age 15 years and over who were reportedly employed during the week prior to the survey, is 47 percent. In contrast, the employment ratio for the national urban population in 1985 based on the Integrated Survey of Households (3rd quarter) is 58 percent.* The employment ratio in the sample urban poor areas is thus lower than the national urban average by 11 percentage points. Part of the difference may be due

*This is obtained by multiplying the labor force participation rate of 59.4 percent and the employment rate of the labor force of 98.3 percent.

Table 2. Income by occupation of household head, selected urban poor communities of Davao and Cebu, 1985

Indicator	*Type of Household By Occupation of Household Head*								*All households*
	Prof/Tech/ Adm.	*Clerical*	*Sales*	*Services*	*Transport*	*Craftsmen/ production*	*Fishermen/ farmers*	*No occupation reported*	
Mean Monthly Household Income									
All Areas	2,255	1,767	1,452	1,202	1,145	1,365	1,006	810	1,308
Cebu	1,875	1,587	814	881	599	1,111	563	582	906
Davao	2,502	1,977	2,026	1,497	1,714	1,554	1,130	1,117	1,607
Mean Monthly Per Capita Household Income									
All Areas	324	278	215	203	173	217	154	114	202
Cebu	241	214	114	140	89	171	81	85	133
Davao	391	386	314	269	263	254	176	151	256
*Poverty Incidence (Percent)**									
All Areas	95.1	96.2	97.5	97.9	97.9	96.5	98.1	100.0	97.4
Cebu	91.7	100.0	99.4	98.5	100.0	97.3	98.2	100.0	99.7
Davao	97.3	91.7	95.9	97.3	95.7	96.0	98.0	100.0	96.5

*₱600 per capita poverty threshold.

Table 3. Employment profile of adult household members, selected urban poor communities of Davao and Cebu, 1985

Indicator	*Type of Household By Occupation of Household Head*								*All households*
	Prof/Tech/ Adm.	*Clerical*	*Sales*	*Services*	*Transport*	*Craftsmen/ production*	*Fishermen/ farmers*	*No occupation reported*	
A. *Percent of Household Members 15 Years Old and Over Who Worked During Past Week*									
1. *All Areas*									
Household head	82.0	96.2	83.1	92.3	87.4	78.7	67.7	0.0	79.0
Spouse	19.7	30.8	36.9	31.7	30.1	31.2	30.8	60.0	32.5
Other adults	28.4	21.1	33.3	20.3	29.4	36.9	34.0	41.1	32.0
Total	40.3	46.3	49.4	47.7	49.2	49.0	44.8	34.6	47.4
2. *Cebu*									
Household head	75.0	96.4	82.5	92.6	88.5	83.2	77.2	0.0	81.8
Spouse	16.7	28.6	35.7	27.9	29.5	31.0	38.6	65.2	32.7
Other adults	32.2	15.4	32.4	16.7	24.2	36.3	43.3	32.1	30.5
Total	38.3	39.8	48.0	44.4	49.3	49.9	52.9	32.4	47.3
3. *Davao*									
Household head	86.5	95.8	83.6	91.9	86.3	75.4	65.0	0.0	77.0
Spouse	21.6	33.3	38.0	35.1	30.8	31.5	28.6	52.9	32.3
Other adults	24.0	36.8	34.1	24.3	33.1	37.4	30.3	50.0	33.4
Total	41.9	56.7	50.7	50.9	37.4	48.3	42.2	37.1	47.5
B. *Number of Working Members per Household (past survey week)*									
All Areas	1.52	1.56	1.64	1.45	1.46	1.46	1.27	1.17	1.46
Cebu	1.71	1.54	1.64	1.40	1.37	1.52	1.61	1.04	1.51
Davao	1.41	1.58	1.63	1.50	1.56	1.42	1.17	1.35	1.43

to the difference in the reference period used by each survey. The urban poor survey used the "past week" while the national survey used the "past quarter". The use of the latter reference period tends to produce higher employment rates than the former. However, it is possible that the lower employment ratio among the urban poor could be due either to lower labor force participation rate of the working age population or to lower employment rate of the labor force. Since we expect a higher labor force participation rate among the poor, i.e. more household members will be seeking work to supplement their low household incomes, it is reasonable to infer that the urban poor did in fact experience lower employment rates than the rest of the national urban work force. If this inference proves to be correct, this means that the worker terminations due to shutdowns and retrenchment of industrial establishments as well as to the general slowdown in business activity following the 1983 economic crisis adversely affected the urban poor more than the rest of the urban work force.

Between household heads in different occupational categories, lower employment ratios (lower than the overall average for the sample of 79 percent) are exhibited by the professional/technical administrative workers (mainly managers and working proprietors) in Cebu, craftsmen and production workers in Davao, and fishermen/farm tenants or workers in both areas. Those whose occupations were not reported were all without jobs during the survey period. In contrast, higher employment ratios are exhibited by clerical and service workers in both areas.

Other household characteristics

Table 4 shows data on selected household characteristics which have implication for current welfare. Lower income households generally tend to have a larger dependency burden, i.e. percent of household members less than 15 years of age. Moreover, lower income households generally exhibit lower educational levels of both household heads and their spouses than higher income households. One implication of this is that the former's capacity to find more secure employment at higher wages are lesser than the latter.

Economic Stress and Household Adjustment

Table 5 shows data on reported income change during the past two years (1983-1985). Of the total households in both areas, 47 percent reported that their incomes have declined while another 36 percent reported no improvement in incomes. The data also reveal a higher percentage of lower income households reporting income declines than higher income households. This average pattern might simply mean that those with lower current incomes are precisely in such a situation because of declines in incomes. On the other hand, it could also mean that households with lower current incomes have characteristics, i.e. low level of

Table 4. Household characteristics, selected urban poor communities in Davao and Cebu. 1985

Indicator	*Type of Household by Occupation of Household Head*								*All households*
	Prof/Tech/ Adm.	*Clerical*	*Sales*	*Services*	*Transport*	*Craftsmen/ production*	*Fishermen/ farmers*	*No occupation reported*	
Mean Household Size									
All Areas	6.95	6.37	6.76	5.92	6.6	6.28	6.53	7.08	6.49
Cebu	7.79	7.43	7.12	6.29	6.70	6.51	6.75	6.83	6.80
Davao	6.41	5.12	6.44	5.57	6.51	6.12	6.41	7.41	6.26
Percent of Household Members Age 0-14 Years									
All Areas	45.5	47.1	51.0	58.6	55.1	52.6	57.3	51.9	52.5
Cebu	42.8	48.1	51.9	50.0	58.6	53.2	56.1	52.9	53.1
Davao	47.7	45.5	50.1	50.7	51.4	52.1	56.9	50.8	52.0
Mean Age of Household Head									
All Areas	36.6	35.5	34.1	33.7	35.1	33.8	33.7	38.4	34.4
Cebu	34.5	36.8	34.4	34.0	34.2	33.2	33.7	38.4	34.2
Davao	37.9	34.0	33.8	33.5	36.1	34.3	33.7	38.4	34.5
Mean Age of Spouse									
All Areas	32.8	31.7	31.3	30.2	31.9	31.0	30.6	33.5	31.3
Cebu	32.7	32.9	32.0	30.8	31.7	30.9	31.5	33.7	31.6
Davao	32.9	30.4	30.7	29.6	32.2	31.1	30.3	33.3	31.0

Table 4 (Continued)

Indicator	*Type of Household by Occupation of Household Head*								*All households*
	Prof/Tech/ Adm.	*Clerical*	*Sales*	*Services*	*Transport*	*Craftsmen/ production*	*Fishermen/ farmers*	*No occupation reported*	
Mean Years of Education Completed of Household Head									
All Areas	10.9	11.0	7.6	8.8	7.2	7.9	6.0	7.3	7.7
Cebu	9.4	10.4	6.9	7.9	6.1	7.2	4.7	6.7	7.0
Davao	11.9	11.7	8.3	9.7	8.4	8.5	6.4	8.0	8.3
Mean Years of Education Completed of Spouse									
All Areas	9.5	9.2	7.4	8.3	7.0	7.6	6.6	6.7	7.5
Cebu	8.5	7.6	6.5	7.6	5.7	6.4	6.4	6.4	6.5
Davao	10.2	11.1	8.2	8.9	8.3	8.5	6.7	7.1	8.2

Table 5. Indicators of economic stress and household adjustments, selected urban poor communities of Davao and Cebu, 1985

Indicator	*Type of Households by Occupation of Household Head*								*All households*
	Prof/Tech/ Adm.	*Clerical*	*Sales*	*Services*	*Transport*	*Craftsmen/ production*	*Fishermen/ farmers*	*No occupation reported*	
A. *Current Income Situation Compared to Past Two Years*									
1. *Higher*									
All Areas	29.5	28.8	11.7	23.9	20.5	18.3	10.4	10.0	17.0
Cebu	16.7	17.9	9.1	14.7	18.0	14.7	7.0	8.7	13.3
Davao	37.8	41.7	14.0	32.4	23.1	21.0	11.3	11.8	19.8
2. *Same*									
All Areas	27.9	40.4	33.8	38.0	38.1	35.9	37.7	25.0	35.8
Cebu	33.3	46.4	31.8	41.2	38.5	39.1	36.8	26.1	37.0
Davao	24.3	33.3	35.7	35.1	37.6	33.5	37.9	23.5	35.0
3. *Lower*									
All Areas	42.6	30.8	54.5	38.0	41.4	45.8	51.9	65.0	47.1
Cebu	50.0	35.7	59.1	44.1	43.4	46.2	56.1	65.2	49.7
Davao	37.8	25.0	50.3	32.4	39.3	45.6	50.7	64.7	45.2
B. *Spending Patterns of Households Whose Income Declined Over Past Two Years*									
1. *Maintain food, reduce others*									
All Areas	34.6	50.0	40.7	31.5	27.3	36.9	31.1	23.1	34.7
Cebu	33.3	50.0	24.2	20.0	15.1	24.7	6.3	6.7	21.0
Davao	35.7	50.0	58.1	45.8	41.3	46.0	38.8	45.5	45.9

Table 5 (Continued)

Indicator	*Type of Household by Occupation of Household Head*								*All households*
	Prof/Tech/ Adm.	*Clerical*	*Sales*	*Services*	*Transport*	*Craftsmen/ production*	*Fishermen/ farmers*	*No occupation reported*	
2. *Reduce food, maintain others*									
All Areas	11.5	0.0	11.3	11.1	15.2	16.2	4.4	3.8	11.4
Cebu	25.0	0.0	19.8	16.7	28.3	32.9	15.6	6.7	22.9
Davao	0.0	0.0	2.3	4.2	0.0	3.5	0.4	0.0	2.0
3. *Reduce food and others*									
All Areas	53.8	50.0	48.0	57.4	57.6	47.0	64.4	73.1	53.9
Cebu	41.7	50.0	56.0	63.3	56.6	42.4	78.1	86.7	56.1
Davao	64.3	50.0	39.5	50.0	58.7	50.4	60.2	54.5	52.1
C. *Percent of Children Out of School*									
1. *Age 6-12 years*									
All Areas	14.3	16.7	17.7	20.1	20.5	19.8	29.9	26.8	22.1
Cebu	19.0	17.1	18.5	27.2	29.4	27.8	48.1	29.6	29.0
Davao	11.4	15.8	16.0	9.7	10.3	13.7	24.9	1.4	16.6
2. *Age 13-16 years*									
All Areas	16.7	11.8	31.6	41.5	43.5	37.6	58.3	50.0	41.9
Cebu	27.3	11.8	37.5	46.2	67.4	46.8	80.0	46.2	50.0
Davao	7.7	–	0.0	33.3	22.4	28.2	51.3	57.1	34.2
3. *Age 17-21 years*									
All Areas	66.7	77.8	76.9	50.0	72.4	65.7	78.9	83.3	70.3
Cebu	85.7	87.5	75.0	72.7	95.7	64.7	90.9	100.0	80.7
Davao	40.0	0.0	80.0	22.2	57.1	66.7	74.1	60.0	60.8

education of household members, that make them more vulnerable to adverse changes in the urban economic environment.

The declines in income appear to be related to the greater unemployment rates of household heads. This is particularly true among the households in the fishing/farming, no occupation, and sales categories in both areas, craftsmen/production workers category in Davao, and professional/technical/administrative workers category in Cebu. These households had the lowest employment ratios of household heads (from 0 to 84 percent) and the highest percentages reporting income declines (from 50 to 65 percent). Of further interest is that these same households, with the exception of the professional/technical/administrative workers category in Cebu, exhibit generally higher employment ratios for spouses and other adult household members than the other households. This employment pattern suggests that an adjustment mechanism is being adopted, namely, when household heads become unemployed, their spouses and other adult members attempt to compensate for the loss of income of the unemployed household head by increasing their own labor force participation and taking on whatever jobs that are available. However, because the jobs that are available are most likely to be low paying jobs, their contributions to total household income are not high enough to fully compensate for the loss of income due to the unemployment of the household head, hence total household income declines.

As a result of declining incomes, further household adjustments are made. One such adjustment is to modify expenditure patterns as suggested in Table 5 which shows data on household spending patterns among those who reported income declines during the past two years. In general, reduced income meant reduced total consumption expenditures. Given initially low levels of income and a higher proportion of income spent for food, a reduction in income is bound to affect food consumption. In fact, the data show that 65 percent of all households who reported income declines also reported they had to reduce expenditures for food. The percentage is higher in Cebu than in Davao: 79 vs. 54 percent. Overall, a higher percentage of the relatively lower income households reported reduction in food expenditures than the higher income groups. This is much more evident in Cebu than in Davao.

Another adjustment to economic stress that can be inferred from the data shown in Table 5 relates to the schooling of children. The data reveal that the percentage of children of specific schooling ages who are out of school is much higher in Cebu than in Davao. For children ages 6-12 years, the percentages of children out-of-school are 29 in Cebu and 17 in Davao; for children ages 13-16 years, the percentages are 50 and 34, respectively. The data also reveal higher percentages of children of specific ages who are out of school among lower income groups than among higher income groups in each area.

For the entire sample, of the out-of-school children ages 6-12, 25 percent are reported to be out of school either because of financial constraint or because the children were needed in work. The corresponding percentages of children ages

13-16 and 17-21 years who were out of school due to the above reasons are 59 and 73, respectively. In view of the close correlation between income levels, income declines, and percentages of out-of-school children, one could infer that one adjustment households adopt to cope with economic stress is to take children out of school both to reduce schooling expenditures and to encourage older children to work to supplement household incomes. The latter response is consistent with the data which showed higher employment rates of other household members age 15 years and over among lower income households than among higher income households, or among households with higher proportion reporting income declines.

Table 6 shows data on fertility plans. Overall, 75 percent of all household respondents reported they plan to stop childbearing completely. Another 14 percent reported they plan to postpone childbearing. Thus altogether about 90 percent of all households plan either to postpone or to stop childbearing completely.

Variations in fertility plans between areas are noteworthy. The percentage of households reporting they plan to stop childbearing completely is higher in Cebu than in Davao: 81 and 72, respectively. Correspondingly, only 10 percent of households in Cebu and 18 percent in Davao plan to postpone childbearing. This pattern in fertility plans between Cebu and Davao households is interesting when one takes into account the fact that, in general, Cebu households compared to those in Davao have lower current incomes and greater proportion indicating income declines during the past two years. Thus it would appear that, at least at the area level, current economic stress are associated with greater desire for more drastic fertility limitation. Between households, these relationships are more readily apparent in Davao than in Cebu.

Are fertility plans matched by contraceptive use? Table 7 reveals that only 53 percent of couples are practicing any method of contraception, and only 30 percent are using modern program methods (i.e. pill, IUD, and sterilization) while, as we have mentioned earlier, 90 percent of households plan either to postpone or to stop childbearing completely. In Cebu where the percentage of households indicating they plan to postpone or to stop childbearing completly is 91, the percentage of households where couples practice any method of contraception is only 47. In Davao, the percentages are 88 and 58, repectively. The use of modern contraceptive methods, however, is higher in Cebu than in Davao: 34 vs. 28 percent.

Is the discrepancy between fertility plans and current contraceptive practice due to response bias (i.e. untruthful reporting among households regarding either fertility plans or contraceptive use) or to factors related to declining fecundity, or is the discrepancy an indication of unmet needs? There appears to be no readily apparent reason why respondent households would provide untruthful answers to questions regarding fertility plans or contraceptive practice. If they had a reason and did provide untruthful responses as a result of such motivation, their answers to both questions while biased would nevertheless tend to be highly consistent. But this in fact is not the case. It is also possible that a certain number of women

Table 6. Fertility plans, selected urban poor households of Davao and Cebu, 1985

Indicator	*Type of Households by Occupation of Household Head*								*All households*
	Prof/Tech/ Adm.	*Clerical*	*Sales*	*Services*	*Transport*	*Craftsmen/ production*	*Fishermen/ farmers*	*No occupation reported*	
1. *Stop completely*									
All Areas	73.8	73.1	75.1	72.5	80.3	72.7	76.5	85.0	75.4
Cebu	83.3	85.7	86.4	76.5	82.8	77.7	71.9	87.0	80.9
Davao	67.6	58.3	64.9	68.9	77.8	69.0	77.8	82.4	71.6
2. *Postpone*									
All Areas	18.0	17.3	14.5	16.9	9.6	16.0	15.5	10.0	14.4
Cebu	12.5	0.0	6.5	11.8	9.0	13.6	12.2	8.7	10.0
Davao	21.6	37.5	21.6	21.6	10.3	17.7	14.8	11.8	17.7
3. *Bear less than planned*									
All Areas	4.9	7.7	4.6	6.3	5.9	5.6	3.8	5.0	5.2
Cebu	4.2	10.7	3.2	7.4	5.7	5.4	8.8	4.3	5.6
Davao	5.4	4.2	5.8	5.4	6.0	5.6	2.0	5.9	4.8
4. *Not plan/Others*									
All Areas	3.3	1.9	5.8	4.2	4.2	5.6	5.0	7.5	5.0
Cebu	0.0	3.6	3.9	4.4	2.5	3.3	7.0	0.0	3.5
Davao	5.4	0.0	7.6	4.1	6.0	7.7	3.9	0.0	5.8

Table 7. Family planning practice and access, selected urban poor communities in Davao and Cebu, 1985

Indicator	*Type of Household by Occupation of Household Head*								*All households*
	Prof/Tech/ Adm.	*Clerical*	*Sales*	*Services*	*Transport*	*Craftsmen/ production*	*Fishermen/ farmers*	*No occupation reported*	
Percent of Currently Married Women Age 20-44 Currently Using Any Family Planning Method									
All Areas	72.1	59.6	51.4	50.0	55.6	54.6	50.4	35.0	53.0
Cebu	54.2	60.7	52.6	44.1	51.6	46.2	31.6	26.1	47.4
Davao	83.8	58.3	50.3	55.4	59.8	60.9	55.7	47.1	57.7
Percent of Currently Married Women Age 20-44 Years Using Family Planning Methods									
1. *Modern Program Methods*									
All Areas	32.8	36.5	33.2	25.4	34.7	28.7	27.7	25.0	30.4
Cebu	29.2	42.9	42.9	22.1	40.2	32.6	22.8	21.7	34.4
Davao	35.1	29.2	24.6	28.4	29.1	25.8	29.1	29.4	27.5
2. *Other Program Methods*									
All Areas	27.9	21.2	15.1	19.0	15.5	17.6	15.8	7.5	16.8
Cebu	20.8	17.9	9.1	20.6	9.8	11.4	8.8	4.3	11.7
Davao	32.4	25.0	20.5	17.6	21.4	22.2	17.7	11.8	20.7
3. *Non-Program Methods*									
All Areas	11.5	1.9	3.1	5.6	5.4	8.3	6.9	2.5	6.1
Cebu	4.2	0.0	0.6	1.5	1.6	2.2	0.0	0.0	1.4
Davao	16.2	4.2	5.3	9.5	9.4	12.9	8.9	5.9	9.5

Table 7 (Continued)

Indicator	*Type of Household by Occupation of Household Head*								*All households*
	Prof/Tech/ Adm.	*Clerical*	*Sales*	*Services*	*Transport*	*Craftsmen/ production*	*Fishermen/ farmers*	*No occupation reported*	
Percent of Currently Married Women Age 20-44 Years By Access to Family Planning Services									
1. *Easy access, can afford*									
All Areas	57.4	50.0	34.5	32.4	25.1	32.4	36.5	12.5	33.5
Cebu	37.3	39.3	26.0	23.5	18.9	26.1	28.1	8.7	25.0
Davao	70.3	62.5	42.1	40.5	31.6	37.1	38.9	17.6	39.7
2. *Can't afford, have access*									
All Areas	34.4	44.2	51.4	54.9	57.3	49.8	48.8	52.5	50.9
Cebu	50.0	50.0	55.2	54.4	56.6	46.2	49.1	56.5	52.0
Davao	24.3	37.5	48.0	55.4	58.1	52.4	48.8	47.1	50.1
3. *Can't afford, have no access*									
All Areas	8.2	5.8	14.2	12.7	17.6	17.8	14.6	35.0	15.7
Cebu	12.5	10.7	18.8	22.1	24.6	27.7	22.8	34.8	23.0
Davao	5.4	0.0	9.9	4.1	10.3	10.4	12.3	35.3	10.2

perceive themselves to be no longer fecund and, hence, would feel that they no longer need to practice contraceptive. If this is the case, their numbers are bound to be very small. The women in the sample are within the ages 20-44 years, their average age is only 31 years, and their past fertility have been quite high. The number of children ever born per woman in Cebu is 4.7 while in Davao is 4.1. During the past two years, Cebu women bore 0.568 child per woman, while Davao women bore 0.502 child per woman. Thus perceived declining fecundity associated with age or past fertility is less likely to be a factor responsible for the less than expected contraceptive use among couples on the basis of their fertility plans. Thus if neither of the two factors just mentioned appear important in explaining the apparent discrepancy between fertility plans and current contraceptive use, then the discrepancy might indeed be due to unmet needs and related factors.

Data on household's access to family planning services shown in Table 7 reveal that only 34 percent of households in both areas reported that they can easily afford, and have easy access to, family planning services. The percentage is higher in Davao than in Cebu: 40 vs. 25. Affordability and easy access to family planning services are clearly related with income whether one looks at the relationship between areas (Cebu vs. Davao), or between households in all areas, or between households within each area.

Among those who reported they can not afford the cost of family planning services, 24 percent claimed "no access" as well, i.e. no ready access to free or low cost services from government programs. In Cebu this percentage is higher than in Davao: 31 vs. 17. Altogether, 16 percent of all households claimed "can't afford and no access"; the percentages in Cebu and Davao are 23 and 10, respectively. Differences between households within each area can also be noted. Thus it would appear at this point that the apparent discrepancy between fertility plans and contraceptive use could in fact be partly due to the lack of effective access to family planning services at least as perceived by the households themselves.

Summary

The information just described provides interesting insights into the various adjustments households adopt to cope with adverse economic situation. The major insights are taken here as hypotheses to be tested using larger and more representative households. These may be summarized as follows:

First, households in the urban poor communities are indeed poor: practically all of them have incomes falling below the poverty line. However, even among the poor there are wide disparities in income by occupation of the household head. Worker terminations due to shutdowns and retrenchment of industrial establishments as well as to the general slowdown in business activity following the 1983 economic crisis have adversely affected the urban poor more than the rest of the urban work force.

Adjustments to declines in income took several forms: increased labor force participation and employment of spouses and other adult members; reduction in

consumption expenditures, notably on food; lower schooling participation of children; and fertility limitation. Current fertility plans, i.e. to postpone or to stop childbearing completely, however, are not matched by contraceptive use. The discrepancy is related to lack of access to family planning information or to services that are in accord with couples' preferences.

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THE FILIPINA LOOKS AT HERSELF: A REVIEW OF WOMEN'S STUDIES IN THE PHILIPPINES*

Amaryllis T. Torres
College of Social Welfare and Community Development
University of the Philippines
Diliman, Quezon City, Philippines

ABSTRACT

This paper examines the content of studies and publications on women which have been undertaken in the Philippines since the early twentieth century. These studies may be classified as belonging to the following periods: (1) the period of the first feminist struggle (1905-1937); (2) the post-war years (1940-1970); (3) the Development Decade – the '70s; and (4) the new decade of feminism - the '80s. An analysis of the patterns and trends in these materials concerning the status, views and actions of the Filipina indicates the following: first, the concerns of women *as women* are emphasized by writers of the first and second feminist decades, while those in the other periods deal with gender issues in relation to either more general social science concerns, or in terms of social development goals; second, education is considered to be a potent factor in enlarging the perspectives of women about themselves; third, by-and-large, the average Filipina considers home-making to be her primary function; and fourth, not all development projects were able to help women positively, even during the International Decade for Women. In conclusion, the author stresses the point that scholarship about women should not be dissociated from the advocacy goals of the women's movement, so that its findings may be useful and relevant to the pressing needs of the Filipina.

Introduction

This paper is based on a study which aimed to develop an anthology of studies on the Filipino woman. The project was undertaken under the auspices of UNESCO and involved the collection of studies on women from various local sources to depict her conditions, status and roles in Philippine society. Interpretative essays were then written to analyze the trends, issues and conceptual frameworks used by the different authors to understand the Filipina.

I coordinated this project and supervised the collection and annotation of the materials. Four other colleagues joined the editorial committee and contributed critical essays. They included: Dr. Ma. Luisa Camagay, Prof. Judy Carol Sevilla, Prof. Rosario del Rosario and Dr. Cynthia Rose Bautista.

A total of about 360 books, articles and bibliographies on women were identified from various libraries of universities and from the resource materials of

women's groups. A sizable number were written by women scholars, and represent written literature from 1928 to 1985. Two distinct publications proved especially helpful. The first one is the book entitled "A Profile of Filipino Women" by Isabel Rojas-Aleta, Teresita Silva and Christine Eleazar. The second is a bibliographic collection of materials concerning "The Status of Women in the Philippines" by Ofelia Angangco, Laura Samson and Teresita Albino.

In today's presentation, the results of the review will be interpreted to answer the following questions:

First, what is the interplay between social forces (such as the women's movement, social development concerns, academic concerns) and the themes of women's studies in the Philippines?

Second, what portrait is painted of the Filipina through different significant periods of this century?

Prior to a discussion of these issues, however, allow me to provide a brief overview of the meaning and development of "women's studies" in contemporary terms.

Women's Studies and the Women's Liberation Movement

Interest in women as a separate sector, a distinct focus of research and teaching, emerged along with the North American movement for women's liberation. As such, the concerns of women's studies in the Western countries are those faced by the movement, and the "subject of research is defined in relation to concepts of women's oppression and their treatment as second-rate citizens underlying the organization of society. . ." (Vogel-Polsky, in supplement # 18, n.d., p. 4). The ultimate goal of the feminist movement and therefore of feminist research is to achieve gender equality within each society.

Given these concerns, women's studies are defined to be "an analysis of the subordinate position of women and the relationship between the division of labor between men and women and social evolution in a broader sense" (Supplement # 18, n.d., p. 6). In simpler terms, studies on women from the women's lib standpoint assume that there is unequal power in societies between men and women. Empirical data may then be treated in either of two ways: first, to portray the "social realities" of gender oppression (Supplement # 18, n.d., p. 6), or second, to examine knowledge and data from a frame of reference "in which women's different and differing ideas, experiences, needs and interests are valid in their own right. . ." (Bowles, G. and R. Klein, 1983, p. 3).

Advocacy as scholarship

Inasmuch as a concern for women's studies emerged from a social movement, it is to be expected that feminist scholars fail to depict the traditional "objective" and "impartial" researchers who are "disassociated" from their data. For one, it is usually the case that those who engage in feminist research are individuals com-

mitted to the goals of the movement (Papanek, H. 1984); therefore, they have clearly aligned intentions in pursuing women's studies. Secondly, studies on women should be useful to the movement's action objectives; thus the advocacy role of the researcher is also priorly defined. Feminist scholars, therefore, generally seek to build a social science which "does not set apart researcher and researched", and instead, strive to produce data with "an impact upon the world" (Bowles, G. and R. Klein, 1983. pp. 37-38).

The action orientation of women's studies places it on a parallel with other social development studies which seek to generate social information useful to the disadvantaged sectors under study. The desire to bridge the knowledge gap between the student/researcher and the researched group is a common concern of scholars seeking to implement participatory approaches to problems of social equity. These disciplinary trends encourage the testing-out of innovative methods for social research.

Methodology of women's studies

Since gender oppression can be expressed in a diverse number of ways, studies on women cannot be confined to any one of the social science disciplines. It is not the sole concern of economics or sociology or psychology, etcetera. Rather, women's studies, by definition, need to be multi-disciplinary and interdisciplinary (Bowles, G. and R. Klen, 1983; Supplement # 18, n.d.).

Another circumstance which contributes to the multi- (or/inter) disciplinary nature of women's studies is that gender differences and gender relationships stem from changes in social economic and political structures and processes (Papanek, H., 1984, p. 133). Papanek cites, for example, how modernity in India has increased the demand for the entry of educated women in the labor force, thus altering gender relationships among the educated middle classes. Simultaneous with this phenomenon, however, is the other fact that technological innovations have resulted in the loss of earning opportunities for the uneducated women of the lower classes who also fail to compete for new jobs for women requiring new skills. Instead, men (who fail to enter the more lucrative labor market) or machines have taken over the traditional jobs of these lower class women, thus enhancing differences in economic activity.

The differential influence of exogenous factors on affected sectors of men and women means that a thorough understanding of gender inequality requires familiarity with these complex events in the social rubric. Thus, a multidisciplinary perspective is important. Moreover, since women's studies is a relatively new discipline, it has still to fashion its own categories of phenomena and approaches to investigation. Meanwhile, manifestations of women's oppression are interpreted according to the perspectives of the older social disciplines.

Theoretical perspective

It was earlier stated that women's studies are premised on an assumption of gender inequality. Is it then the case that feminist research merely seeks to establish the differences between the sexes in relation to a host of other variables?

The answer of feminist scholar is "no." Merely to add knowledge about women to existing knowledge about men still perpetuates "Men's studies". Such an approach remains androcentric (men-as-the norm) and assumes that "the environments emits the same signals for men and women" (Bowles, G. and R. Klein, pp. 90-91).

To continue, Klein argues:

> "Such research . . . ignores the historical perspective, the fact that over millenia women and men have internalised feminine and 'masculine' needs . . . in which he is norm and she is 'the other' (Bowles, G. and R. Klein, 1933, pp. 90-91).

Papanek (1984) postulates that gender differences can be a major variable in examining social change and assessing its consequences. Social phenomena such as class differentiation, employment, education, and employment, and the impacts of technology are better understood in relation to gender. Nonetheless, the simple addition of gender as a variable to models of social change will not lead to new perspectives. Like Klein, she argues:

> "The addition of (gender as a) variable is insufficient to reverse the effect of the many unstated assumptions about gender differences and gender relations that are already embedded in the social sciences. Developing new paradigms that incorporate gender will require, as a first step, that these unstated assumptions be exhumed and examined". (Papanek, H. 1984, p. 135).

The theoretical stance of a feminist scholar, therefore, is dialectically linked with her commitments to women's liberation. Women's studies should properly be research *for* women (not research *on* women) and be framed within *her* own experiences, interests, and needs (Bowles, G. and R. Klein, 1983, p. 90). To do so without falling back on androcentric categories and comparisons requires tremendous creativity – both in terms of developing suitable paradigms for analyzing data and in terms of selecting (or evolving) methodologies that are truly feminist in orientation.

These, therefore, are the motives, methods, and philosophy of feminist scholarship. Against this backdrop, women's studies in the Philippines will be reviewed in terms of their contents and intentions.

Women's Struggle and Women's Studies in the Philippines

The nature of studies about the Filipino women is inextricably linked with historical factors in both the national and global settings. The keystones in Philip-

pine history which weld together studies of different periods may be described as follows:

1. the movement for women's suffrage in the first quarter of the century;
2. an orientation of "objective" scholarship among the researchers in the fifties and sixties;
3. the social development strategy of the seventies which attempted to link special programs and interventions to the people's felt needs, leading to;
4. a re-invigorated movement to organize women for the improvement of their situation in Philippine society.

A. *The first Feminist Movement struggle for enfranchisement of women (1905-1937)*

Among the earliest materials written in this century concerning Filipino women, two were published in 1928 and 1934.

The first monograph is entitled "The Development Progress of the Filipino Women" and was authored by Ma. Paz Mendoza-Guazon, Filipina, who enjoyed the distinction of many "first's" as a woman. She was the first Filipina to receive a high school diploma from the public school, the first woman to graduate as a doctor of medicine, the first to be appointed a lady professor at the University of the Philippines, and the first woman member of the Board of Regents of the same university (P.V. Kalaw, in the Introduction to the book, 1928). She was also the first president of the *Liga Nacional de Damas Filipina* and the founder of the Philippine Association of University Women, two organizations which led in the struggle for the recognition of the Filipina's right to vote. Thus, Ma. Paz Mendoza-Guazon was a doctor, a wife and mother, a scholar and a suffragist.

The other book on "The Filipino Women" was written by Encarnacion Alzona, an eminent historian. Like Dr. Guanzon, she was one of the first graduates of the University of the Philippines and eventually became a Professor of history in this institution. Dr. Alzona was the first woman delegate of the Philippines to UNESCO and was the first woman to serve as Chairman of its Subcommission on the Social Sciences, Philosophy and Human Studies. She was a member of the Philippine Historical Committee and wrote various books and prize-winning historical articles. When Dr. Alzona wrote her monograph, she was a Barbour Fellow (a *pensionada*) at the University of Michigan. Being an active advocate of women's suffrage, she wrote to prove that the Filipina of the twentieth century was "eminently qualified to hold her place in a modern and intricate society." (Author's Note, 1934). In 1985, Dr. Alzona was cited as a Distinguished National Scientist by the National Academy for Science and Technology. She, too, was an advocate and a scholar at the time of the first feminist movement in this country.

What did these early feminists say about our woman? In both monographs, the following themes dominate:

First, the egalitarian nature of gender relationships during Philippine pre-colonial history in social, economic and political activities;

Second, the emergence and institutionalization of gender differences during Hispanic rule; and

Third, the re-awakening of Filipinas to their civic, political and social rights as twentieth-century educated women.

1. *Changes in the role and status of women*

Women of these islands in the pre-colonial period are portrayed as enjoying enormous rights and privileges. Women became rulers over the barangays, acted as priestesses, and even as military leaders. Women participated fully in economic life and were artisans, craftswomen and livestock raisers. Marriages were generally monogamous and either partners could dissolve a problematic relationship. Wives retained their maiden names and were consulted by their husbands on contracts and agreements. In matters of inheritance, legitimate sons and daughters received equal shares while wives retained half of the conjugal property. Thus, women were regarded as equal to men and received protection from the laws of their society.

The intrusion of European androcentric values altered the position of women in society. Government was then perceived as the domain of men. Educational opportunities became uneven and "schooled" women were taught Christian doctrine, some reading and writing skills (enough to do prayers) and a lot of needlework. Women often aspired to be teachers, nuns or spinters. In economic life, women contributed to the export trade earnings of Spain through their needlework, while they serviced the needs of local residents through their retail businesses. Some women also helped in the administration of farms.

Marriages remained monogamous but divorce was prohibited. Spouses could legally separate but could not remarry. Spanish law deprived wives of "their right to dispose of their paraphernal properties, to engage in business without the husband's consent, and to hold any public office except the office of teacher" (Alzona, p. 39). Instead, Filipino women were encouraged to be devout, to do charitable work and to avoid politics.

The advent of the Revolution and the American colonial period modified the status and roles of Filipina women anew. The most dramatic change, however, occurred in their education. Whereas Spanish educational policy sought to confine women to home arts, the more progressive American educational philosophy opened the doors to tertiary education for young women. Thus, women could become profes-

sionals – doctors, lawyers, nurses, etc. – and were no longer restricted to the teaching profession. They became active in civic affairs, from rendering assistance to impoverished mothers, organizing puericulture centers, working with out-of-school youth and prisoners, to lobbying for Philippine independence and women's suffrage.

2. *Factors which influenced the Feminist Movement*

In retrospect, the advent and development of the Filipino women's struggle for enfranchisement may be traced to three factors: (1) opportunities which allowed the Filipina to be active outside of the sphere of her home; (2) the influence of feminist ideas from abroad; and (3) greater confidence in herself as a person and as a member of society.

From Alzona's account, the first advocate of women's suffrage in the Philippines was Apolinario Mabini who drafted a constitution which gave "female taxpayers who have attained the age of 21 years. . . the right to vote for public office" (Alzona, p. 67). However, his constitution was not adopted and the one approved by the Malolos Congress was silent on suffrage for women. Neither did the women in the revolutionary movement aspire for this right.

In 1905, an American anti-imperialist, Mr. Fiske Warren was reported to have suggested to a young Filipina (Concepcion Felix) that a political party be organized in order to work for the enfranchisement of women. The idea was rejected because, as Ms. Felix saw it, the Filipina was still unprepared to use the ballot. Instead, an association devoted to social welfare work, and which encouraged the appointment of women to school boards, was founded by Ms. Felix. This was the *Associacion Feminista Filipina*, which later changed its name to *La Gota de Leche*.

Later, on 1912, feminists – an American, Mrs. Carrie Catts and Dutchwoman, Dr. Aletta Jacobs – spoke before Filipino women to interest them in suffrage. Again, they met with a negative response, but another association of women was formed which also engaged in social work. This was the precursor of the Manila Women's Club.

Thus, although no Filipina by this time had as yet spoken for enfranchisement, many educated women had joined in associations which engaged in civic and charitable work. Inevitably, these activities drew the Filipina away from home, children and husband and swept her into situations wherein she was encouraged to take interest in public and political affairs and to use her talents and education for the country's welfare.

While women were engaged in these civic activities, many more politicians spoke for the benefits of female suffrage, including the then President of the Commonwealth, Manuel L. Quezon. In 1919, women

finally endorsed the moves in the Philippine Legislature to enfranchise women. At the same time, they conducted a massive and continuous educational campaign through newly-founded women's associations to convince other Filipinas of the merits of suffrage. Pro-suffrage groups at this time included the *Liga Nacional de Damas Filipinas,* the National Federation of Women's Clubs, the Women's Citizen League, and the Philippine Association of University Women.

Finally, in 1936, the Constitution provided that "the National Assembly shall extend the right of suffrage to women, if in a plebiscite . . . no less than 300,000 women. . . should vote affirmatively on the question (Alzona, p. 95). So, the women renewed and intensified their educational campaign for suffrage. When the plebiscite was held on April 30, 1937, 447,725 women voted "yes" to suffrage – more than a hundred thousand votes beyond the required margin. After 20 years of struggling for enfranchisement, the Filipina then won the right to the ballot.

3. *Feminist demands of working women*

Much of what have been written about the efforts of Filipino women in the first half of this century concern the suffrage movement. Less is known about the situation of working women at that time. Yet, evidences are slowly emerging which show that many women were in the labor force.

In a recently completed work (Camagay, L., 1986) it was historically proven that in the late 19th century, a sizable number of women had work outside of their homes. The livelihood of women usually included work as *criadas* (domestic helpers), *maestras, matronas* (midwives), *cigarreras, buyeras, bordaderas* and *sinamayeras.* Historical records also show that gender discrimination existed even then. For instance, maestras received lower wages than maestros. Women also suffered from sexual harassment – from their male *amos* and even from the *frailes.* Sometime in the latter years of the past century, the women in the tobacco industry also held strikes (or *alborotos*) to demand for better working conditions.

In 1918, the Philippine census counted about 700,000 women engaged in various industrial pursuits, including work done at home (e.g. weaving, dressmaking, embroidery, hatmaking, shoemaking, laundry). Alzona also reports that, by 1930, more than 8,000 women were employed in various industrial establishments, 3,000 of whom were members of labor organizations.

Evidences of the activity of organized labor are also in the literature. For one, an Act which required employers to provide seats for women workers and to install separate "closets and lavatories" for men

and women was passed in 1927 (Alzona, E. 1937). In 1930, a grass-roots women's organization was founded and called *Liga ng Kababaihang Filipina* (Del Rosario, R. 1986). It fought for suffrage and for the improvement of the rights of working women. Most likely, these women joined male workers in 1936, in a series of demonstrations which demanded for 'equal pay for equal work' among men and women, the prohibition of child labor and for the free education of the children of the poor (Tribune Manila, 1936).

Hence, *pensionadas* were not alone in the struggle for women's rights at this time. While educated women advocated political rights, working women worked at their side for suffrage and for the upliftment of their own economic conditions.

B. *The post-war years: studies on women* (1940-1970)

Literature on the Filipina woman in the generation following the Second World War may be characterized in three ways: *one*, anecdotal materials (usually appearing in magazines and journals) which either extoll the virtues of the Filipina or exhort her to do more for home and nation: *two*, sociopsychological conceptualizations, experiments and field observations of the roles, statuses, values, attitudes and aspirations of Filipino women (usually in contrast to those of Filipino men); and *three*, socio-anthropological observations of marital and family relationships, including decision-making processes, power and authority dynamics, and child rearing practices (see listing in Angangco, O., *et al.*, 1980). Few articles were written which concern feminist views, and most of these were autobiographical and retrospective accounts of the earlier suffrage movement (Kalaw, P., 1952; Castrence, P.S., 1957; Subido, T. 1955).

1. A dissection of the Filipina

Certain common themes emerge from the collection of women's studies in the thirty years following the Second World War. These include:

a. A confirmation by feature writers, feminists, and scholars alike that the Filipina's main concern is maintaining a well-knit and orderly family.

For instance, Nakpil (1963) asserted that the Filipina has the best of both worlds. She makes man believe that she is pliant and submissive, therefore keeping him happy, while un-obstrusively asserting her own desires, thereby fulfilling herself. Person (1957), while presenting the plaftform of the Civic Assembly of Women in the Philippines, stressed that dual role of the Filipina – as nationbuilder and as homemaker. Flores (1969) reported that working wives were beset by household problems, such as:

"Husbands get upset when their clothes are not darned properly. They feel. . . neglected.

When the house is not in order, the children not dressed neatly and the meals not prepared correctly, wife gets jittery and self-conscious because she is aware that her husband is not happy about the situation.

In-laws and. . . parents criticize women leaving homemaking to the servants. . ." (Flores, P., p. 120).

Orosa (1963) ventured to give practical advice to Filipino housewives on how the objectives of Rizal's *La Liga Filipina* could be implemented in their families. These objectives of fostering family unity, patriotism, education and the application of reforms, in her way of thinking, could be achieved if women acted as partners of their husbands in the home, if they exercised thrift and economy, and by patronizing local products and local markets.

Domingo (1961), Nurge (1965) and Nydegger (1966) did anthropological observations of child rearing practices in various Philippine communities. They confirmed that Filipino women spent a lot of time on work related to the household and that an important aspect of motherhood is child care. Filipino parents were observed to be over-protective of children and reinforced sex-related behaviours. Thus, girls were trained to assist their mothers in household chores and in babysitting while pre-adolescent boys were slowly integrated into farm-related activities.

In her analysis of the gamut of studies on the woman and the family, Sevilla infers that the "ideal wife" in Philippine literature is:

"a loving and loyal mate to her husband; she is responsible for keeping the marriage intact by her patience, hard work, submission and virtue. Aside from whatever outside employment she may hold, she is also expected to be a diligent housekeeper and . . . budgets the money . . . for family and household needs. The husband. . . has the larger voice in decisions involving the family. He is not expected to do household chores, except for occasional repairs. . . to allow time for more 'manly' activities like relaxing, drinking, and socializing with friends outside the home (Sevilla, J. 1986).

b. The second theme which emerges from most of the studies of this period dwells on the increasing assertiveness and expressiveness of the Filipina, as opposed to her caricature as a passive and inarticulate maiden in Hispanic times. This change in gender

character is often attributed to the 'positive' influences which American education and culture provided our women.

Benavides (1958) reiterates the views forwarded earlier by Guazon and Alzona that the Filipina has undergone changes in her status and roles through history and she emphasized how the American educational system helped open greater horizons for the modern Filipina through opportunities for higher education. Nakpil (1952) pursues a similar thesis when describing "The Filipina Woman" and attributes the complexity of her character to the influences of pre-colonial and colonial cultures: while Spanish culture produced a "shy, diffident and puritanical Filipina", American influence "gave her independence of character" (as annotated in Angangco, O. *et al*, 1980, p. 63). Similar observations are given by Isidro (1969). Castrence (1951), and Laureta, E. (1951).

c. The third outlook on women which may be derived from materials of the '50s and '60s, concerns man-woman distinctions and relationships. Most of the literature on this topic, moreover, are social science researches.

In general, the various studies described how early socialization fosters sex – roles stereotypes among Filipino children (Domingo, F., 1961; Flores, P., 1969). Not surprisingly, therefore, boys and girls manifested sex-related behaviours and even occupational preferences (Castillo, G., 1961; Flores, P., 1969; Flores, P. and Gonzales, M., 1969; CYRC, nd.).

Socialization in sex roles resulted in particular role expectations from men and women. Thus, women who ventured to go into careerwork were either lauded or castigated. Amor (1966) believed, for instance, that a working mother courted alienation from her children and neglected her "traditional role of fostering a happy and healthy family atmosphere" (in Angangco, O. *et al.*, 1980, p. 35). Castañeda (1953) averred that "the participation of women in industry has adverse effects on the welfare and progress of society" (p. 22), while Benito (1952) expressed concern over the negative effects on men's employment resulting from women's work. Vice-versa, Arceo-Ortega (1963) and Nakpil (1963) commended the Filipina careerwoman, who is able to fulfill herself through her work while helping augment family income, and remained "a tolerant wife and a good mother" (in Angangco, O. *et al.*, 1980, p. 75).

Men's views on the changing roles of the Filipina are also documented (Flores, P., 1969; Castillo, G. and Guerrero, S., 1965). Husbands of women in the professions tended to be supportive of their working wives, especially if their earners were economically rewarding. They perceived each other as 'partners' and shared in most decisions concerning family affairs. Nonetheless, husbands continued to be perceived as the ones who should be concerned with public and national affairs, while wives (after work) should look after their homes and children. Critical decisions in the family were also made by the husbands.

Thus, women's power in the home was exercised to the extent that she was in charge of the children's activities, household budget, and routinary affairs related to household tasks. Decisions related to the children's education, family savings and recreational activities were shared with the husbands. In cases where the wives worked, they expressed readiness to give up their occupations if their husbands and children's welfare needed more of their time (Sevilla, J., 1986).

1. Scientific objectivity in women's studies

The bulk of research literature spawned in the 30 years described by this section used methods and analytic perspectives popular at the time. Thus, surveys, anthropological and psychological studies were employed to obtain information concerning the Filipinos, her husbands and children. In interpreting the amassed information, researchers chose to remain "close to their data". Hence, descriptive studies were generalized on this level and were sometimes compared to other materials with objectives akin to its own.

No conscious effort was made, therefore, to transcend data in order to make statements concerning the impacts of observed gender roles on women's rights and potentials. Proposed ways to improve woman's position in society were generally found in articles divorced from data and maintained the view that the Filipino woman should seek a balance between her role as homemaker and her fledgling aspirations for professional fulfillment.

3. Continuing action for women's rights

Materials pertaining to the women's movement were scant, and provide few insights about the continuing feminist struggle during the '50s and '80s.

In a recent publication of the NCRFW, it was reported that women in the immediate postwar period felt the need for a duly-organized women's group to ensure the coordination and consolidation of women's efforts for the continuity of their action programs for more effective results (NCRFW, 1985). Hence, in 1947, the existing organizations banded together into the

"Women's Civic Assembly of the Philippines", later renamed the Civic Assembly of Women in the Philippines (CAWP). The CAWP acted as an umbrella organization for different groups, such as the Girls Scouts of the Philippines, the National Federation of Women's Clubs, Catholic Women's Club and the Rural Improvement Clubs.

Through the years, the CAWP has been engaged in educational activities (family life, health, livelihood) and in other social welfare and public affairs affecting women.

Tarrosa Subido (1955) also provides information on the continuing activities of the feminists following the grant of suffrage in 1937. From her book, it appears that feminism sought expression through women's participation in politics.

In the elections, following the passage of the Women Suffrage Law, several women won seats as mayors and councilwomen. In 1947, President Roxas invited the CAWP to participate in the Independence Day ceremonies, and more women consequently found themselves in responsible positions within government. Women's groups likewise aligned themselves with political parties, such as the Women's Auxiliary of the Liberal Party and the Women's Magsaysay-for-President Movement.

Subido also credits the efforts of the older feminist groups and newer women's associations for the passage of legislations favorable to women. These include among others: The Charity Sweepstakes Bill (to subsidize welfare agencies); Paraphernal Property Law (empowering a married woman to dispose freely of her paraphernal property); Women and Child Labor Laws; and, most importantly, the passage of the New Civil Code in 1950, which removed or modified an antiquated provision adopted from the Civil Code of Spain which restricted the affairs of married women. At the time she wrote her book, women's groups were lobbying for the creation of juvenile and domestic courts, a women's and child's Bureau and further improvements in the Election Code. Since then, these recommendations have been implemented.

C. *The development decade: the seventies*

The decade of the seventies spawned new views of society and social responsibilities brought about by the increasing polarization of developed and underdeveloped economies. Many so-called "Third World" countries emerged as newly-liberated states (freed from colonialism) but found themselves in dire need of social, economic and political reforms.

The consultative process for development became a mandate among the developing and underdeveloped nations since past experiences showed that a 'felt needs' strategy' and 'popular participation' were critical for the success of developmental programs. Aware of the explosive possibilities of these new outlooks for development, the countries of the First World geared

to retain their influence over former colonies by offering "development aid". Thus, foreign assistance poured into Third World countries for infrastructure improvement, for social innovations in technologies and institutions, and for social development research.

The disadvantaged position of women in many traditional societies was recognized early in this decade, leading to the UN's Declaration of 1975 as the International Women's Year, and the next 10 years as the International Decade for Women. The goals of the Declaration were threefold:

(1) to promote equality between men and women;
(2) to support the integration of women in the total economic, social and cultural development effort; and
(3) to recognize the contribution of women to the promotion of friendly relations and cooperation among nations and to the strengthening of world peace.

1. *The focus of women-in-development studies*

In the Philippines, government assumed the position that over-population, poverty and unemployment are restraining factors to its development as a modern industrializing nation. Hence, it was important that systematic steps be taken to reduce family size, to generate income and to create employment. It was in this context that many new studies on the Filipina were undertaken.

Taken together, studies which aimed to examine the conditions of women in relation to their development are called Women-in-Development Studies.

2. *Conditions related to women's participation in development*

The plethora of social science techniques for social research helped considerably in generating a substantial body of literature on women during the seventies. The Filipina was studied from all angles, and her portrait differed drastically from the old caricature of simpering Maria Clara.

What new image of the Filipina emerged?

a. The new wave of studies showed clearly *that the Filipino woman was not a unitary being.* Rather, her characteristics and situation in life were affected by a plurality of variables (Bautista 1986). Castillo re-evaluated the average statistical observation concerning women by presenting diversities brought about by geographical origins, marital status, labor force participation and other social factors (1976). A similar approach was used by Aleta, Silva and Eleazar (1977) when they reconstructed the Profile of Filipino Women on the basis of sketches drawn by different researchers.

Among the many observations derived from these studies are the following:

(1) Women tend to have fewer children if they live in rural and agricultural communities, marry early, work only at home and live in nuclear households. However, children were valued by most women and they usually had more children than they have originally planned to have.

(2) Men and women in the Philippines are at par in terms of literacy and educational attainment. However, there are sex differences in career aspirations.

(3) In 1976, women made up a third of the labor force, with a greater proportion coming from rural areas. However, while the absolute size of the female labor force increased over the years, the labor force participation rate (LFPR) of women declined over a 20 year period, especially for rural women. Educational attainment was also found to be related to LFPR of women, and certain occupations were more feminized than others. Thus, women were frequently found in services (as domestic helpers), in professional, technical and sales occupations.

(4) Of the women in the labor force, almost half are married. Nevertheless, about a third of single working women stopped to work after marriage, and married working women would stop if their husbands earned enough for family needs or if their incomes were considerably less than those of the men.

While a considerable number of researches were conducted on the abovementioned factors, there was an almost equally large volume devoted to other concerns. Unfortunately, a more thorough discussion of the field of women's studies during the Development Decade is not possible in this paper. Suffice it to say that other studies analyzed the conditions of women's lives in terms of the following factors: LFPR and fertility; fertility and family decision-making; migration and employment; the status, roles and problems of specific sectors (e.g. rural women, tribal women; working women; women in professions); profile of women's participation in development programs; legal status of women; and women in public/political affairs.

b. Another important finding from the WID studies is that, in most cases, *Filipinas were content with their lot and accepted the traditionally ascribed roles of home makers* (Castillo, G., 1976; Montiel, C. and Hollnsteiner, M., 1976; Licuanan, P. and Gonzales, A., 1976; Aleta, I. *et al.*, 1977; Manalang, P., 1983).

Over the years, from one generation to the next till the seventies, Filipinos were socialized into the firm belief that womanhood was equated with home, husband and children. Even work was secondary to this concern. The normative force of this view is best seen when even the law prescribes that "the husband is responsible for the support of the wife" while "the wife manages the affairs of the household" (Romero, F., 1977).

More recently, in a study commissioned by the NCRFW, it was discovered that women from the various Philippine regions still clung to "pre-modern" values. (UPS-CE-NCRFW, 1984). Manalang attributes the findings to the Filipina's orientation for home and family.

> Instead of many life worlds, they have one principal life world, their definition of reality are focused on the family and its survival, they take their identity from being mothers and wives. . . Nor do they distinguished between a public and a private life (Manálang, 1984, p. 13).

Eviota (1978) reacted to this gender role with alarm and argued that house-keeping isolated women from public affairs, thereby diminishing the scope of their social power to effect meaningful changes for themselves. This role, moreover, obscured the possibility of organizing them for feminist goals. She stated:

> Identification with one's own sex and alliances based on shared interests, similar personal needs, and the same grievances against men are often perceived by women as outside the framework of household responsibilities and as conflicting with the traditional female role. (This) is aggravated by the belief that Providence ordains that their place is beside their husbands. Thus, women have an apparent moral justification for. . . refusal to acknowledge female solidarity. . . (Eviota, B., 1978, p. 154).

These findings, therefore, emphasize that women require alternative roles which will dissipate their efforts away from household chores in order to take direct interest in their development (Makil, P. 1981, Aleta, I. *et al.*, 1977; Eviota, E., 1978).

c. *A real contribution of the WID studies is found in the development of innovative measures of women's contributions to society.* (Castillo, G., 1976; Illo, J.F., 1985; Miralao, V., 1980)

Miralao (1980) demonstrated how an analysis of the use of time by men and women can shed new information on their contributions to household and economic activities. Measures of effort or time-inputs, for instance, showed that in many

comparisons, women's total production time is higher than that of men.

Illo arrived at the same conclusion using a different measure (1985). In her analysis, the value of woman's production is seen to be higher than that of a man's if one were to consider the production of use values as the criterion rather than the generation of exchange values. In this conceptualization, women's activities in the home (cooking, child care, etc.) are given values in the same way that man's farm labor inputs are evaluated.

The impact of WID studies

a. Earlier, it was stated that the rationale for WID Studies was to generate information so that these may provide the benchmarks for developmental policies and social instrumentations. As a result, many *agencies of government engaged in programs or projects geared especially to the needs of women.* For example, livelihood projects were spearheaded by the NCRFW, the Rural Improvement Clubs and other women's organizations in order to provide additional sources of income to women and thereby also draw them out of the confinement of household work. On the legal front, legislative and codal reforms were drafted, proposed and enacted – such as an improved Child and Youth Welfare Code, and specific provisions protecting women workers in the Labor Code (NCRFW, 1980; UP-IIR Workshop on Women, 1986). Skills training and literacy programs were initiated by women's groups, while an intensive population control program was launched in order to provide married women a broader lattitude in defining their family aspirations (Aleta, I., 1977; NCRFW, 1984).

b. *Despite these moves,* both the Official Country Report on the Achievements of the Decade for Women and the NGOs Alternative Country Report point to the *continuing problems of Filipino women in various sectors.* Likewise, feminists find the WID framework inadequate because it focused on "efficient development which implies simply the infusion and increased productivity of 'neglected sources' such as women" (Saltmas and Liamson, 1985). Hence, questions of gender relationships in the home and work place have not been addressed. Alternative employment strategies have also failed because women have not been relieved of their household chores. Instead, the economic crisis has led to the further degradation of women, who have lately been used as cheap sources of labor in garments and

electronics manufacturing (Del Rosario, 1985) and as cheap entertainers for foreign tourists on sex tours (Dela Cruz, 1985).

c. In addition to these impacts, *the WID efforts have opened the vistas for further efforts in women's studies* in 2 ways.

First, to a great extent, the women scholars who have sought to describe and understand the situation of the Filipina have themselves become feminized. A greater appreciation of women's conditions (as women) has emerged, as evidenced by innovative approaches to the study of the woman question (Illo, J., 1985; Miralao, V., 1980); and explicit recommendations that women must seek public exposure and organize into associations with common goals in order to advance their positions in society (Eviota, E., 1978; Castillo, G., 1985).

Second, the five details on the situation of women which the research literature provided in the 'seventies has also been useful to feminist groups, who are now able to re-interpret these information within their own frameworks for action (PWRC, 1985). Moreover, women's groups have started to use research tools themselves as an instrument for educating and organizing various women's sectors.

D. *The new decade of feminism: the 'eighties'*

The easing of restrictions over organizing efforts as a result of the "lifting" of Martial Law in 1981 spawned the formation of various women's organizations (Maranan, A., 1981; Del Rosario, R., 1986). These associations have since then been involved in various issues concerning women and the nation, and are presently engaged in a unified battle to enlarge women's rights through the Constitution. Part of their strategy has been the use of research to be able to reinforce their feminist demands.

1. *The impact of development on women*

Del Rosario (1986) calls the women's studies in the new decade as *Impact of Development on Women* (IDW) studies. These efforts have often been expressed in 3 ways: (1) as situationers on the conditions of women in specific sectors (e.g. migrant female workers, women in industry, women in agriculture); (2) as case studies of women in various areas and work situations; and (3) as comparative studies of women within different geographical regions of the country and of Asia.

Generally, IDW studies note the following conditions affecting women in the Philippines: (Del Rosario, 1986; PWRC, 1985)

(1) Women workers' situation has been aggravated by the demands of global capitalism. For instance, rural women have been further impoverished by the Green Revolution technology and agri-business penetration into the countryside. Women in cottage industries apply their traditional skills (in sewing, embroidering, weaving, etc.) for the export market yet remain in the informal labor sector without protection from labor laws. Women in garments, electronics and other export-oriented industries are given low wages and exposed to repetitive, hazardous and regimented work condition.

(2) Women's problems are aggravated by "her double burden which is rooted in traditional gender discrimination of society" (Del Rosario, 1986, p. 45). The inequality between the sexes has substantially continued, principally because males fail to share house work and child care and because institutional support for economic activities of women remain scarce. The outlook on women as housewives and mothers has been reinforced by media, thus entrenching traditional views among men and women. Few governmental attempts have also been undertaken to provide daycare centers, health care and economic support for women.

(3) Specific and concrete steps must be taken to alleviate the oppressed conditions of women. These steps must involve both government and women's groups and focus on women's problems in their various spheres of undertaking. The organization of women into sectoral groups is also important so that "women's forces can strengthen themselves and develop within or alongside material and class forces" (PWRC, 1985).

Studies within the framework of IDW continue in the mid-'80s. Recently, Pagaduan, M. and others completed a participatory research on "The Awakening of Peasant Women." It attempts to depict the potentials of and hindrances to, consciousness-raising among women. Two ongoing studies of women in agriculture use statistical procedures, oral histories, case studies and observations to study the status of women in farming (Bautista, C., 1986). Researches are also being conducted on Filipina entertainers in Europe (Arcinas), on mail-order brides (Cooke), and on working women in governments and textile industries (Samson).

Apart from these efforts of university women scholars, women's groups themselves continue to study and document the conditions of their sectors (Del Rosario, R., 1986).

2. *Sources of women's oppression*

The oppression of the Filipina is perceived to be rooted in three factors: gender inequality, class domination and national subservience to foreign interests (PWRC, 1985). Thus, the feminist movement in the present decades chooses to struggle for the improvement of women's rights along these three dimensions. The Philippine Women's Research Collective states:

> "A women's movement which ignores national and class questions will remain limited, ineffectual and isolated from . . . the motive forces which are the sources of structural change. On the other hand, a women's movement which permits the relegation of women's issues to the background is in fact delaying or negating the full liberation and empowerment of women – an end. . . attained (by) the final uprooting of ideas and institutions which perpetuate inequality between the sexes . . .

Initial efforts to concretize this feminist framework are found in the recently completed series of monographs by the PWRC. Essentially, these monographs find that:

(a) Export-oriented industrialization has pushed rural women into marginalized lives, while women in industry have received extremely low wages; (Ofreneo, 1985; Del Rosario, 1985).

(b) Widespread poverty and unemployment resulting from the economic recession in recent years have pushed women into degrading positions – as prostitutes, as domestic helpers and entertainers abroad, and as mail-order brides (Dela Cruz, P., 1985; Orozco, W., 1985).

(c) Despite declarations that women should be relieved of household work, it remains the main obstacle to her active participation in development. Ironically, mass media has helped perpetuate the image of Filipina women as homemakers, and consumers, rather than as active producers and leaders in their own right (David, R. and Dela Cruz, P., 1985).

These observations find confirmation in other researches. The marginalization of landless rural women as an offshoot of new farming systems has been confirmed by Illo (1985) and Castillo (1985). Official labor statistics describe the outflow of Filipinas as domestic workers, nursing aides and entertainers and provide data on the continuing decrease in the LFPR of women (BVM, 1985). Samson (1977) had earlier also spoken of the escapism in terms of the mass media and the degradation of the image of the Filipina.

3. *Unity of theory and practice*

Developments in recent years have drawn women scholars closer to the women's movement, and vice versa. It may, therefore, be safe to say that studies on the Filipina in subsequent years will remain attuned to the objectives of the feminist movement.

Women's studies and the women's movement have gone through a full circle in 60 years. Early studies on the Filipina were written to show her capabilities in order to win a political right. In this decade, studies were conducted to demonstrate the structural roots of the Filipina's continuing oppression in Philippine society (Bautista, C., 1986) so that our women can learn more about themselves and join in the women's rights movement. This trend is expected to continue.

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APPROPRIATE FEEDING STRATEGY INVOLVING UNTAPPED FEED RESOURCES: THE KEY TO ACCELERATE MEAT AND MILK PRODUCTION IN THE TROPICS

Le Trong Trung
Dairy Training and Research Institute, College of Agriculture
University of the Philippines at Los Baños, College, Laguna

ABSTRACT

The role of forage pasture in the development of smallhold-based dairy-beef industry is re-examined. Likewise, the relevance of feeding standards to ruminant feeding in the tropics is critically analyzed, taking into consideration quality and availability of feedstuffs and other nutritional factors.

Untapped feed resources for ruminants in the Philippines are identified vis-a-vis their utilization as feeds in selected Asian countries. Nutritional constraints of these feedstuffs and their remedies are mentioned. Current knowledge in ruminant nutrition relevant to tropical conditions is discussed focusing on the creation of a favorable rumen environment for efficient fiber digestion, estimating feeding value, and the provision of dietary bypass-protein for post-ruminal digestion and absorption.

A practical feeding concept involving maximum utilization of fibrous agricultural residues with limited supplementation for optimal productivity is presented.

Finally, a strategy and socio-economic considerations to extend this technology to farmers are suggested.

Introduction

Cattle, buffalo, goat and sheep in the developing countries account for 66%, 99%, 96% and 56% of the world's livestock populations, respectively (Madamba, 1987). In spite of the high concentrations of ruminants in these countries, total production has always been low. Likewise, human population in this part of the world is also high, representing 75%. As a result, there is always a general dependency on meat and/or milk importation by the developing countries from developed countries.

In the past decade, the Philippine population increased from less than 43M to 55M while ruminant population decreased and the number of pigs and chicken increased very slightly (Table 1). Assuming that per capita food consumption was constant, simple arithmetic clearly reveals increased dependency on meat/milk importation through the years. In the early '80's, the Philippines imported annually over US$100 million worth of dairy foods (99% import dependent) and US $12-

20M worth of meat (FAO Yearbooks, 1980-82). With respect to dairy production, the question of whether to develop the industry has been answered as early as 1962 with Republic Act No. 4041, the first dairy law, amended by RA 4718 in 1966. After over a decade without much progress, a second dairy law (BP #21) was again enacted. Struggling for the last 7 years, the dairy situation has not made much headway and now is back to square one.

Table 1. Human and livestock populations (x 10^6) of the Philippines

Population	*1970*	*1975*	*1980*	*1985*
Human	37.54	42.57	48.32	54.50
Livestock				
Buffaloes	4.43	5.05	2.87	4.33
Cattle	1.68	2.25	1.88	1.90
Goats	0.77	1.35	1.45	1.93
Pigs	6.45	9.70	7.93	8.01
Chickens	57.00	46.50	52.76	57.00

FAO Production Yearbooks (1970-1985).

History clearly demonstrates that it takes a firm and lasting political will to develop the dairy industry. This is recently shown by some neighboring countries such as India and Indonesia. A useful index is import requirement in relation to local milk production and human population (Table 2). The local milk production of Indonesia and India steadily increased in the last 5 years against constant figure for the Philippines (FAO Production Yearbooks, 1980-85). As a result, these two countries, in spite of being much more populous than the Philippines spent much less dollars for dairy importation. We must follow their footsteps considering the current debt burdens and the many benefits that would result from development of

Table 2. Local milk production (10^6 metric tons) dairy importation (10^6 US dollars) and current population (10^6) of selected Asian countries

Country	*Milk production*		*Daily import*		*1985*
	1980	*1985*	*1980*	*1985*	*Population*
Philippines	.031	.031	111	72	55
Indonesia	.079	.162	74.8	61.5	166
India	31.5	41.0	73.4	53.8	759

FAO Production Yearbook (1980-1985).

an indigenous dairy industry, e.g. foreign exchange conservation, employment generation and improved living standard in the rural areas, remedied malnutrition problems, etc.

This paper deals specifically with issues on feeds and feeding strategy to support local production of meat and milk.

Relevance of Pasture to Small-Hold Ruminant Production

Ruminants have special importance in the conversion of natural resources that are inedible to humans, into precious protein foods. Thus, ruminant production has always been viewed as an effective means of utilizing vast areas of natural grassland in poor/acidic soil regions with seasonal rainfall or in areas where crop production is impractical. It has become, therefore, a matter of tradition to think about pasture establishment whenever ruminant production is to take place. In the case of local dairy/beef projects (DTRI, PDC, BAI, MSH-KKK, etc.), the recommendation is for participants to plant not less than 1,000 m^2 of land to forages for every cattle received. Unfortunately, not many farmers can satisfy such a requirement because they are mostly landless; if ever they possess small pieces of land, cash crops are planted, not grass.

Since about 80% of the ruminants in the developing countries, including the Philippines, are kept by smallhold crop farmers (Madamba, 1987), development programs should center on the smallholders if maximum impact is to be attained. And since land is limited for majority of livestock farmers, forage pasture is not the suitable feed resource. Rather, crop residues and by-products or whatever left in the field after the crops are harvested and green forages available in marginal non-cropped areas must constitute the bulk of feed requirements for ruminants.

A Critical Look at the Feeding Standards

A great deal of research funds has been spent through the years in many tropical countries to establish "feeding standards" for their animals. Recently, there have been efforts to put together pieces of research findings from different countries in the tropics (Kearl, 1982). Farmers, particularly smallholders do not use those data while researchers and college professors feel more comfortable with publications of the US-NRC (1983) or UK-ARC (1980). They must have very good reasons to trust foreign standards. Subsequent discussion will focus on the inappropriateness and irrelevance of feeding standards to the tropical countries. This is not the first paper along this line. More detailed arguments against feeding standards have been published (Jackson, 1980; 1981; Trung, 1986a).

The environment and voluntary feed intake

A cool environment is more conducive to voluntary feed intake (VFI) than a hot one. Heat increment (HI) on one hand is useful for animals in the temperate

zone to keep their body warm; on the other hand, extra energy (otherwise useful for the animal's productivity) is required for its dissipation in order to maintain feed intake under tropical conditions. Another factor is relative humidity, high relative humidity renders evaporative heat loss less effective. Hence, it further limits VFI in the humid tropics.

Thus, given the same kind of feed, animals in the tropics would theoretically eat less, hence there is less nutrient intake. But animals in the tropics actually need more energy to fight heat, e.g. dissipating HI. These are rather basic considerations in feeding ruminants in the tropics.

Feed availability and quality

In most tropical countries, feed resources are limited, consisting variety of by-products of cereal and export crops. As said earlier, little land is available for the growing of forages or feeds intended only for livestock. The by-products available in largest amounts in most tropical countries are characterized by a low content of total nitrogen and the energy components which are largely cell-wall carbohydrates (Trung, 1986). There is no argument, therefore, that compared to temperate forages, tropical feedstuffs are of inferior quality. This further complicates the problem of lower VFI. In effect much lower nutrients, especially energy, are available for ruminants from roughages in the tropics.

In temperate and developed countries, cereal grains form the basis of live stock feeding systems. These are rarely available for tropical ruminants because of scarcity and high price, quite apart from the fact that these grains are usually reserved for monogastrics as well as human consumption. Concentrate ingredients in most tropical countries are industrial by-products which are again made available largely to feedmills catering to swine and poultry growers.

So the irony is that while animals in the tropics theoretically require more energy, among other nutrients, for their productive functions, the hot and humid conditions, coupled with low quality forages are major constraints for them to obtain adequate intake of nutrients. In this situation, the formulation of conventional balanced livestock diet is difficult because of the limitations, quantitatively and qualitatively, of the feeds available. It appears, therefore, that the only solution for tropical ruminants to meet their nutrient requirements is through the use of superior quality feedstuffs having low HI values. This would mean the massive importations of quality feedstuffs, which obviously is very undesirable if not impossible under the prevailing economic conditions of most developing countries.

Other nutritional considerations

Energy value

The limitations of total digestible nutrient (TDN) as an energy unit are well established. Net energy (NE) on the other hand while being the most accurate way

of expressing energy value, is far from being appreciated in the tropics. Even in the temperate countries, the application of NE is constrained by the sophistication involved in its determination in feeds. The use of "estimated NE" values is perhaps as inaccurate as the adoption of book values. Neither of these methods would provide useful data for practical feeding of ruminants in the tropics. The same argument holds true with metabolizable energy values.

Protein evaluation

With the advent of protein nutrition of ruminants in recent years, it is now accepted that evaluation of protein feeds does not simply involve knowing their protein contents (%N x 6.25). A more sensible method of protein evaluation is understanding its role in providing fermentable nitrogen for functions of rumen organisms and estimating how much protein would escape rumen fermentation and undergo digestion and absorption in the lower gastro-intestinal tract.

Simply multiplying the protein content of a feed with its amount to be consumed by ruminants to come up with the required amount of protein is usually misleading. It is well established that protein is utilized by ruminants with different degrees of efficiency: rumen fermentation is only half as efficient compared to bypass protein.

Associative effects of dietary feed ingredients

The usual practice of summing up nutrients contributed by individual ingredients to arrive at the required amounts for the animals does not mean much in many cases. The association effect among the ingredients interacting/complementing with one another in a ration is not taken into consideration in ration formulation using tables of feed composition and nutrient requirements. The associative effect means a lot in so far as digestibility and intake are concerned. Hence nutrients available to the animal are also influenced. Recent research on utilization of fibrous residues with minimal concentrate supplementation obtained dramatic responses in production, comparable to those of liberal concentrate feeding. This is a very good example of what conventional feeding standards can not explain.

Untapped Feed Resources and their Role in Development

In terms of land use in the Philippines (Table 3), the trend for the last decade was that arable land increased while pasture land decreased (FAO Production Yearbooks, 1975; 1985. This trend is unlikely to be reversed with ever increasing population and the urgency of providing staple foods. Furthermore, with land reform program to be fully implemented, it is envisioned that more and more small farmers will till their land for cash crops. Thus, realistic livestock development program must turn to smallhold farmers planting rice, corn, sugarcane and cash crops, who should be able to effectively exploit their limited land resources for both crop and livestock. That is to fully and efficiently utilize their crop residues to feed their animals.

Table 3. Land use and production of major crops and their residues in the Philippines

Land use/crop	*Hectarage* 10^6 *Ha*	*Main product*	*Residue*
		10^6 *Tons*	
Total land	30	–	–
Pasture land	1.14	–	–
Arable land	11.3	–	–
Rice	3.2	8.2	8.2
Corn	3.3	3.5	3.5
Coconut	3.3	3.0	*
Sugarcane	0.4	3.2	0.6

*Assuming a carrying capacity of 0.25 animal unit/ha, 0.8 M animal units can be raised under coconut.

FAO Production Yearbook (1985); Phil. Stat. Yearbook. NEDA (1986).

The presence of ruminants in crop-based farming systems would close the cycle of nutrient utilization hence realizing the symbiotic relationship among animals, crops and man. Ruminants complement crop production by providing manure, utilizing by-products and residues that otherwise would have little value. From the system, meat, milk, draft power and plant products go to mankind. The current practices of burning rice straw, allowing corn stover to rot in the field, and burning sugarcane field before harvesting must be put to a stop.

Availability of unexploited feedstuffs and economic implication

There are potential feed resources available locally. From the major crops of rice, corn, and sugarcane alone, about 12 million tons of crop residues can be expected annually (Table 3). If fully utilized,this would mean that 4 million (M) more cattle/buffaloes can be raised. In other words, the present ruminant population could be doubled through full utilization of the 3 crop residues as feeds. Additionally, another 0.8 M cattle can be supported by coconut plantations. This does not take into account other crops planted on the remaining 1M hectares of the arable land. Consequently, one can easily figure out how much dollars can be saved from importation of meat and milk, how many jobs can be created and how much improvement in living standard of rural people can be effected.

Some selected Asian experiences

To support the contention that it is not forage pastures but rather crop residues that build the meat and milk industry in many tropical countries, some examples of neighboring countries are herein given.

India (Singh and Rangnekar, 1986; Kunju, 1986). In India, where 181 million (M) cattle and 61.4 M buffaloes produce 40 M tons (T) of milk in a year, the avail-

able feeds are 26 MT of concentrate, 351 MT of green fodder and 350 MT of crop residues (The ratio in dry matter basis is 1:2.7:21.2 for concentrate:forage:residues). With all crop residues (which have been burnt or ignored in the past) now fully utilized, the Indians still experience a perennial feed shortage to support a progressive dairy industry.

Bangladesh (Saadullah and Barton, 1986). With a land area of 14.4M ha (less than a half of the Philippines), Bangladesh has to support almost twice the Philippine population. Competition of land among crops is a serious problem and pasture does not have a place in this country. Notwithstanding this limitation, the country maintains 22M cattle, 0.5M buffaloes, 10.5M goats and 0.5M sheep (5 times the Philippines') which produce 0.33M tons of milk and 39,000 tons of milk products. Rice straw currently contributes over 80% of the total available feeds for livestock. There is approximately 2 kg dry straw and 88 g of concentrate available per head of cattle per day.

Sri Lanka and the Southeast Asia. Following the examples set by India and Bangladesh, research and development activities in other Asian countries on utilization of fibrous agricultural residues have been greatly intensified in the last 6 years. This may be attributed to the increased awareness of the political leadership and partly to the intensified activities of members of the "Australian-Asian Fibrous Agricultural Residues Research or AAFARR Network" which covers Sri Lanka, Malaysia, Indonesia, Thailand and the Philippines. Leading the group is Sri Lanka where a "Straw Utilization Development Project" has been going on for 6 years with financial assistance from the Government of the Netherlands. In this country, rice straw which is the traditional feed for draught cattle, has been extended to beef and recently, to dairy cattle. Urea treatment with limited supplementation is the centerpiece of the technology extended mostly to dairy farmers. Various degrees of acceptance from end-users at different geographic locations in relation to climatic conditions and availability of feedstuffs have been reported (Ibrahim and Schiere, 1986). Considering many similarities between Sri Lanka and the Philippines, valuable lessons may be drawn from this project.

Indonesia (Nari, 1985) may come next and this can be attributed to the aggressive development of the dairy industry in recent years. Java, accounts for only 7% land area but hosts over 65% of the country's cattle and buffalo population, can speak for Indonesia in terms of dairy development and crop residues utilization. It is estimated that Java produces about 50% of the Indonesian rice. This means that 35M tons of rice straw is generated yearly. At present approximately 23% of the said amount is being fed to ruminants. Across Java, 75% of the available corn stover and sugarcane tops (8M tons) are being utilized as feeds by farmers. With the fast growing dairy industry, it is envisioned that Indonesia will soon utilize whatever crop residues available to feed their ruminants.

The Philippines is perhaps one of the remaining few countries in Southeast Asia where rice straw is freely burnt in the field. It is rather ironic to observe in the Philippines that many of over a thousand recently imported Holstein-Sahiwal

cows are dying of starvation in the field. It is starvation amidst an abundance of crop residues and by-products, which are handy, if at all utilized to feed the animals. Little can be done about this situation so long as there is such a belief that it is cheaper to import milk so why bother developing the dairy industry?

Dietary Manipulation to Maximize the Utilization of Fibrous Feeds

Nutritional constraints of crop residues

Crop residues are generally lignocellulosic in nature, hence are poor quality feeds. Lignin in crop residues acts as a physical barrier to microbial breakdown, rendering their polysaccharides less digestible. Minerals such as silica, phosphorus, calcium, potassium and sulphur of rice straw are insoluble hence not available to the animals and thus further impeding the digestion of cell wall materials. Crop residues contain crude protein (CP) levels lower than 7% which is recommended for acceptable voluntary intake. Detailed discussion on nutritional constraints of crop residues and overcoming these limitations have been published; the options to remedy these contraints include pretreatments (physical, chemical, biological) and/or supplementation (Doyle *et al.*, 1986; Trung, 1986b; Trung and Devendra, 1987).

Creation of a favorable ruminal environment

As mentioned earlier, crop residues contain less than 7% CP. A dietary protein of 11% CP is required for the generation of adequate ammonia (7 mg/100 ml) for rumen microbial synthesis and activity. The rumen microorganisms, fortunately, require only cheap and readily available feeds such as urea, poultry manure, legume forages, copra meal, etc. for the purpose.

Rumen microorganisms also need fermentable carbohydrate for their survival and functions. Fibrous residues provide mainly structural polysaccharides which are not readily available to the microbes. Hence provision of molasses, rice bran and other industrial by-products is essential for cell growth. This is particularly important when non-protein nitrogen (NPN) is fed to the animals. Digestion of cell wall components however decreases with high dietary levels of fermentable carbohydrate (in excess of 0.5% liveweight).

Mineral supplementation benefits both the host animal and rumen microbes since crop residues are deficient in most mineral elements. Sulphur supplement is important when NPN is included in the diet.

Estimating animal response: Intake and digestibility

An efficient feeding system is the one which maximizes the rate and efficiency of growth of rumen micro-organisms. It must provide reasonable amount of fermentable carbohydrate and adequate nitrogen and minerals. A minimum

degree of fibrousness is needed to provide stimulus necessary for rumen contractions, the strength and frequency of which are important determinants of rumen turn-over rate. All the above factors are important for high voluntary feed intake which in turn is governed by digestibility, the rates of digestion and passage, and the potential rumen volume.

Digestibility is the most important factor affecting animal performance in situations where the supply of feedstuffs is rather scarce. On the other hand, when roughages are available abundantly, voluntary feed intake is the key. Under any given set of conditions, therefore, digestible dry matter intake (DDMI) is a reliable indicator for predicting animal's response to a diet. The DDMI would give us the idea of how much is the potentially available energy for the animal's utilization hence performance. This is much more meaningful compared to TDN (total digestible nutrient) as a measure of energy and hence deserves more attention among scientists working on ruminants in the tropics.

The role of bypass protein

Theoretically, ruminants need only NPN for the synthesis of microbial proteins which in turn are utilized by the host animal in the lower digestive tract. In reality, however, part of the dietary protein is true protein which may escape rumen fermentation and hence digested and absorbed directly by the animal. As mentioned earlier, this is a more efficient way of utilizing preformed proteins, particularly those with high biological values. Naturally occurring supplements that are capable of escaping rumen fermentation are fishmeal, meat meal, dried legume forages and some oil cakes.

Appropriate Feeding Strategy and its Evidence of Success

Understanding the discussion above, it must be clear by now that the overall objective of ruminant feeding is not to formulate "balanced rations" with desired nutrient contents, but rather to know how to use the basic feed resources available in the country.

The sensible feeding strategy, therefore, is to manipulate the limited amounts of high-quality feed supplements in a way that will maximize the utilization of the more abundant feedstuffs and at the same time allow bypass nutrients to be utilized directly by the host animal.

High quality feed supplements need not be expensive ones. Oil meals, bran, and molasses are found all over the tropics. They are valuable energy and protein supplements; and yet, a number of developing countries are not exporters of these by-products (Madamba, 1987). Another abundant yet unexploited protein and mineral supplement is legume trees/shrubs. Perhaps, Nepal is one of very few developing countries that have fully exploited the usefulness of fodder trees (Trung, 1985). Potentials and feeding values of tropical fodder trees have been published (NAS, 1979).

Satisfactory performance of growing-fattening cattle using the above-mentioned feeding strategy has been reported throughout Asia. It should be pointed out that the rice straw-based experimental diets in the said studies contained energy and/or protein levels way below the US-NRC feeding standard (1978). Obviously, when designing those experiments, the researchers did not worry about feeding standards but rather about proper supplementation for maximized utilization of fibrous feeds and nutrient intakes hence, optimal productivity.

As shown in Table 4, in spite of the low protein and energy contents of most experimental diets, intake has been exceptionally high. Over 3% liveweight of dry matter intake from rice straw-based diets is a very interesting observation of most studies. As earlier mentioned, it is generalized that for a diet to be readily consumed by the animal, protein content must be 11% CP to generate adequate ammonia nitrogen concentration for microbial functions. This generalization may not be necessarily true as proven by research findings from South and South East Asian regions (Table 4).

Table 4. Dry matter intake (DMI) as percent liveweight (% LW) and average daily gain (ADG) of cattle fed straw and limited supplementation with crude protein (CP) and total digestible nutrients (TDN) computed from published data

No.	*Reference*	*CP*	*TDN*	*DMI*	*ADG*
		% DMI		*(% LW)*	*(Kg)*
	US NRC Standard (1978)	12	60	–	0.9/ 0.7
1.	Trung et al (1987)	7.5	52	2.2	0.5
2.	Khan & Davis (1982)	4.6	50	3.1	0.3
3.	Saadullah *et al.*, (1982)	4.6	46	3.4	0.3
4.	Chauhan (1982)	7.5	[illegible]	3.4	0.34
5.	Jaiswal *et al.*, (1983)	12.5		3.4	0.33
6.	Cheva-Isarakul & Kanjanapruthipong (1986)	9.4	[illegible]	2.6	0.7

1. Philippines – Urea treated straw (UTS) + 1 kg grass + 0.5 – 1.0 kg conc.
2. Bangladesh – UTS + 1 kg grass + 0.4 kg concentrate
3. Bangladesh – UTS + 1 kg grass + 0.3 kg sesame oilcake
4. India – UTS + 0.2 kg fish meal (FM)
5. India – UTS + FM/cotton seed meal/leucaena (30% protein requirement)
6. Thailand – UTS (or untreated straw + urea-molasses) + 1 kg conc.

Technology Transfer and Socio-Economic Consideration

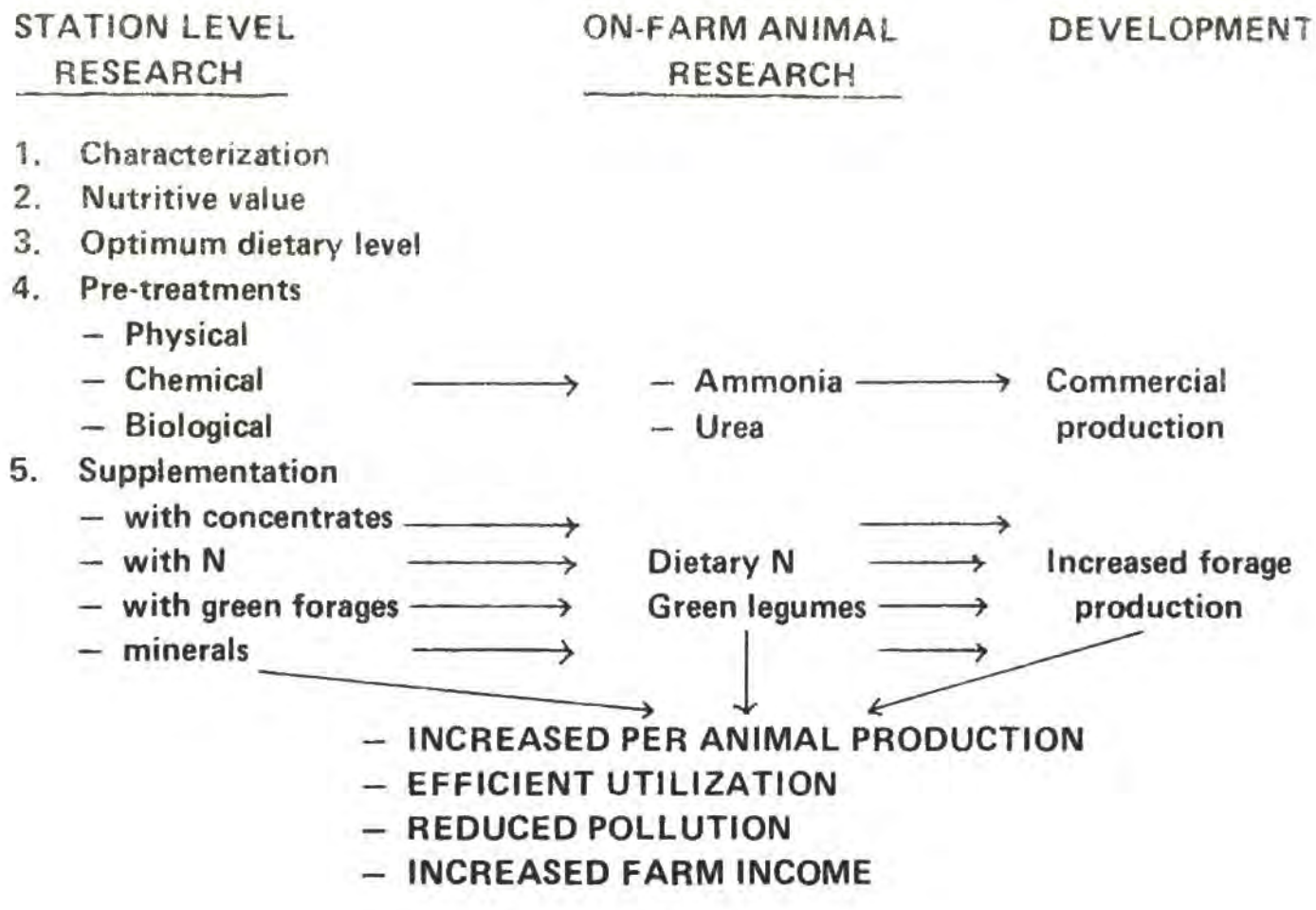
At this point in time, research data generated in Asia are sufficient to bring about confidence in extension workers who would introduce various technologies to the end-users. In relation to the utilization of crop residues, it must be stressed that no single technology is universal to all farm conditions. One may be very suc-

cessful in a certain area but may not be so in the adjacent one. Hence many factors, e.g. agroclimatic, social, economic, class of animal, etc. must be properly considered before extending a technology to farmers. But certain principles are now evident. Fibrous crop residues, properly treated and/or supplemented have important roles in ruminant nutrition for small holder production systems. Traditional approaches relying on forage pastures and Western-style feeding standards must be changed.

On-farm trials

The farmer is perhaps in the best position to tell his neighbors whether or not to follow his footsteps. Devendra (1987) outlined steps that would eventually lead to large scale utilization of straw. At the moment, information generated by research at the "station level" is considered adequate for "on-farm" animal research. The technologies to be tried on-farm include ammonia-urea treatment/spraying and/or supplementation with locally available protein-rich feedstuffs. The success of on-farm trials would lead to more development activities that would eventually result to increased production and farm income (Fig. 1).

Fig. 1. A strategy for station level research, on-farm animal research and development exemplified by the utilization of rice straw in Asia (Devendra 1987).



On-farm trials are probably the only accurate assessment of whether new technology packages are acceptable both economically and socially to the farmers as they take into account all of the interacting components of unique small farm systems. They are a means of identifying and addressing the constraints to adoption of new feeding systems. In many instances in Asia, the importance of such trials far out-weighs the need for further documentation of the effects of supplementation or pretreatments (Doyle *et al*., 1986).

Class of animals and economic consideration

Experiences in India, Bangladesh and to a smaller extent, Sri Lanka and Indonesia indicate that dairy farmers are the most receptive to new technologies. This is particularly true in areas with pronounced dry seasons and/or limited forage production.

Benefits derived from a new technology can be readily appreciated with high production potential animals, such as lactating cows or those required to provide draft power. In milking cows, both delayed age at first calving and prolonged calving intervals increase the proportion of non-productive life and feeds for such period, hence reduced profits.

A technology is readily welcomed by farmers only if it is economic. Obviously this means added benefit must more than offset additional cost brought about by the new technology. Oftentimes, some cost and benefit factors are difficult to quantify and vary greatly from one to another place. Excellent examples are how to quantify benefits derived from "shortened unproductive life" as earlier mentioned; or how to account for additional cost involved in handling crop residues when the farmer is unemployed or underemployed.

Conclusion

There is an obvious need to safisfy protein requirements of the ever increasing Philippine population. Import dependency of meat and milk is definitely not the answer. The ruminants in the Philippines, just like in many other Asian countries, are found in the hands of small holders who are basically landless crop farmers. Appropriate development strategy must focus on this clientele using whatever available after harvest seasons as the bulk of feeds for their animals. Supplements, cheap and locally available, must be given in small quantities to optimize the utilization of the principal diet which is crop residues. Finally, there is a need to determine technology packages which are simple, economic, agroclimatically specific to different areas and appropriate to the smallholder livestock farmers.

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CORN PATHOLOGY RESEARCH OF PIONEER IN THE PHILIPPINES

S. C. Dalmacio, G. Lozano, F. Cordero and A. Yadao
Pioneer Overseas Corporation, San Isidro, Cabuyao, Laguna
Philippines

ABSTRACT

Basic studies relating to *Diplodia macrospora*, downy mildew, bacterial rot and banded leaf disease have been conducted at Pioneer.

Inoculation experiments proved that *D. macrospora* was the cause of leaf blight, ear rot and stalk rot of corn. The leaf blight phase appears to be initiated first, followed by either the ear rot or stalk rot phase, depending upon the location of the leaf blight phase. Symptoms of the three phases of infection are described. This study constitutes the first report on the occurrence of the ear and stalk rot phases of *D. macrospora* and the destructive potential of the disease in the Philippines.

Studies on metalaxyl fungicide showed that the chemical was capable of eradicating established downy mildew infection on corn. The implications of this result in DM resistance breeding and other research areas are given. Studies also showed that metalaxyl fungicide gave complete control of downy mildew at rates as low as 0.2 g a. i./kg seed. On the other hand, the recommended rate of 2 g a. i./kg showed some detrimental effect on seed germination.

Using a stalk inoculator for bacterial rot screening, we have identified good sources of resistance to the disease among existing Pioneer corn inbreds. On the other hand, presently grown commercial corn hybrids showed inadequate resistance to bacterial rot with infection ranging from 53 to 85%. Yield losses from these infection levels ranged from 36 to 82%. There was good correlation ($r = 0.83$) between percent infection and yield loss. Results of a study on the comparative virulence of three isolates of *Erwinia carotovora* pv *chrysanthemi* showed isolate IPB 24 as the most virulent and isolate R8, (an isolate from rice) as the least virulent.

Among the eight methods tried for inoculating corn with *Rhizoctonia solani*, the whorl inoculation using sclerotial bodies showed the highest percent infection (98%), followed by leafsheath inoculation using infested sorghum grains (92%) and whorl inoculation using infested sorghum grains (67%). Of these three, leafsheath inoculation method was adopted as the standard method in screening for resistance to banded leaf disease because it simulates natural infection pattern by the fungus. Results of the most recent trial showed great variation in all parameters measured. Thus, lesion length ranged from 35 to 71 cm, ear rotting from 28 to 100%, visual rating from 1 to 8 and ear height (measured from the point of inoculation to the base of the ear) ranged from 5 to 65 cm. Correlation analyses involving these parameters showed that the extent of ear rotting, which

reflects resistance to banded leaf disease, was more closely correlated with ear height than lesion length. Close correlation between visual rating at 20 days after inoculation and ear rotting also suggests that visual rating can be used in evaluating resistance to banded leaf disease, thus, eliminating the tedious job of counting infected ears at harvest or measuring lesion length.

Introduction

Diseases constitute a great hazard to corn production particularly in tropical areas where staggered planting over one or more cropping seasons is practiced. Pioneer realized this between 1974 and 1976 when it introduced to the Philippines corn hybrids developed in Jamaica. Though these hybrids showed high yield, they all proved highly susceptible to downy mildew, long considered the most destructive disease of corn in the Philippines. Breeding for high-yielding corn hybrids with acceptable resistance to corn diseases, primarily downy mildew, therefore, became the main thrust of Pioneer research when it establish a research station in General Santos City, South Cotabato in 1976. Within three years, the first downy mildew resistant hybrid, X076, was released to the farmers. This hybrid was replaced by Pioneer 6181 the following year (1980) and became so popular among many farmers because of its yield stability that despite competition from other Pioneer and competitor hybrids, it is still in the market. The development of Pioneer 6181 coupled with the discovery of metalaxyl fungicide highly effective against downy mildew led to the adoption of corn hybrid technology by many corn farmers. However, this also gave rise to other disease problems like stalk and ear rots, several foliar diseases and viral diseases which may have long been overshadowed by downy mildew. Realizing the need for hybrids with multiple disease resistance trait to attain high-yielding potential, Pioneer intensified its corn pathology research starting 1985. The ultimate goal is to minimize losses due to diseases, primarily through disease resistance breeding and secondarily, through other disease control measures. This paper aims to present research accomplishments of Pioneer on corn pathology in the Philippines.

Materials and Methods

Diplodia macrospora

In 1985, *D. macrospora* was found associated with severe incidence of ear rot and leaf blight. Since then, these two phases of the pathogen have been regularly encountered in most farmers' fields in South Cotabato and at our research stations in General Santos City and Cabuyao, Laguna. To understand some aspects of the fungus' biology, pathogenicity tests were conducted. Three types of inoculum were used, namely: infected leaf tissues, artificially infested sorghum grains and spore suspension from naturally produced pycnidia. Infected leaf tissues and infested sorghum grains were introduced to the whorl of 40- to 50-day old plants while spore

suspension was injected into the second internode of stalk of 70- to 80-day old corn plants. Inoculated plants were regularly observed for the appearance of initial and the development of the typical symptoms. Moreover, field observations on the natural incidence of the disease were also made to relate results of artificial inoculation.

Downy mildew

Severe incidences of downy mildew were encountered in some isolated areas in Isabela and Northern Mindanao in 1985 on supposedly DM-resistant materials. Considering that this could be a case of 'breakdown' of DM resistance, studies on metalaxyl fungicide, long considered as the most effective chemical against DM, were conducted. To study the eradicative effect of metalaxyl on DM infection, seeds of a DM-susceptible corn were planted in seedboxes. One week after planting, seedlings were artificially inoculated with DM fungus, *Peronosclerospora philippinensis*, using spore suspension. Seedboxes were then divided into groups. In one experiment, seedlings were sprayed with Ridomil MZ at various times after inoculation, viz. one, three, five or seven days after inoculation. In another experiment, the seed dressing (Apron 35 SD = 35% metalaxyl) and the sprayable (Ridomil MZ = 10% metalaxyl) formulations were sprayed on seedlings one week after inoculation. In both experiments, inoculated seedlings but not sprayed with metalaxyl were provided to serve as check. Seedboxes were placed on benches outside the greenhouse and seedlings were observed for DM infection. Final DM count was taken 25 days after inoculation.

To determine if metalaxyl can cure plants showing more advanced stages of infection, surviving plants left after final count was taken were sprayed with Apron 35SD. Plants were then observed for disappearance of DM symptoms until flowering.

To study the effect of varying rates of metalaxyl on seed germination and DM control, seeds of Pioneer hybrids were treated with Apron 35SD ranging from 0.2 g a. i. to 6.0 g a. i./kg seed. Treated seeds were tested for seed germination at different intervals using the ragdoll method. Efficacy of Apron 35SD against DM was determined by planting the treated seeds in the DM nursery using three to five replications. Untreated seeds were also planted as check.

Bacterial rot

Bacterial rot occurs at all stages of crop growth, causing top rot, stalk rot and ear rot, depending upon the tissue or organ affected. Field observations made since 1985 suggest that it is causing substantial damage on corn, hence, given considerable attention. Main emphasis of Pioneer research concerning this disease include evaluation of resistance and pathogen variation. To evaluate for resistance to the disease, test materials were planted in two-row plots, 5 m long. Plants were inoculated at different growth stages with the bacterial suspension applied through the whorl or through the stalk using an inoculator. Number of dead plants was taken 10 to 15 days after inoculation and percent mortality computed. Yield loss due to the

disease was also determined on hybrids by comparing the yield of inoculated and uninoculated rows. Correlation coefficient between percent infection and loss was also determined.

Banded leaf disease

This disease, which is caused by *Rhizoctonia solani*, is prevalent in almost all corn growing areas during periods of high precipitation. The fungus is soil-borne and initiates infection from the base spreading upwards. Substantial losses occurs when ears become infected. At Pioneer, various inoculation methods were compared to determine the best method to use in screening for resistance to the disease. Eight methods of inoculation were initially compared, as described below:

T1 = soil inoculation using 20 artificially infested sorghum grains per hill at planting time

T2 = leafsheath inoculation by inserting 20 infested sorghum grains between leafsheath and stem, two to three nodes above the ground

T3 = whorl inoculation using 20 infested sorghum grains

T4 = whorl inoculation using mycelium suspension

T5 = whorl inoculation using chopped infected leaf materials

T6 = band application of chopped infected leaf materials

T7 = whorl inoculation using five naturally infected corn kernels

T8 = whorl inoculation using sclerotial bodies

T9 = no inoculation

Except for T1, all inoculations were made 35 DAP using a Pioneer corn inbred known to be susceptible to the disease. Efficiency of methods was determined based on percentage infected plants.

Using the most appropriate inoculation method, germplasm materials of Pioneer were evaluated for resistance to BLSD. Plants were grown in two-row plots, five meters long in two replications. Inoculation was done about 40 days after planting by placing 5-10 infected sorghum grains at the axil of the third leaf from the ground. Lesion length, measured from the point of inoculation upwards, ear height and visual rating were taken at 20 days after inoculation while number of rotten ears was taken at harvest time. Correlation coefficients were determined among ear height, lesion length, visual rating and percent ear rot.

Results and Discussion

Diplodia macrospora

All pathogenicity tests showed positive results, clearly indicating that *D. macrospora* is the cause of leaf blight, ear rot and stalk rot. The prevalence and the nature of *D. macrospora* infection also indicate the destructive potential of the fungus on corn production in the country.

Based on field observations and inoculation experiments, the description of symptoms for the different phases of infection are given as follows. On the leaf, infection starts as a small, circular to oval chlorotic spot which later enlarge and become necrotic with distinct gray center and brown margin. Lesions continue to enlarge lengthwise reaching a length of 10-15 cm. Mature lesions resemble those of Stewart's wilt (caused by *Erwinia stewarti*) and Northern leaf blight (caused by *Helminthosporium turcicum*). However, leaf blight caused by *D. macrospora* is distinguishable by the presence of small, black, globose structures (pycnidia) scattered over the central portions of the lesion. On the ear, small, circular lesions start usually at the base of the outer husks, which later spread outward and into the inner husks. Husks of infected ears dry up prematurely and become pasted together by the fungus mycelium. On severely infected ear, fungus mycelium ramifies over the kernels and on the cob between the kernel rows. Kernels of affected ear assume pale and shrivelled appearance and become soft. Pycnidia of the fungus appear on the husks, kernel surface and on the cob. Infection on the stem usually starts at the nodal portion where the leaves are attached. Stem become discolored, starting from the nodal region and later spread both upward and downward. At a later advanced stage, leaves at and above the affected node wilt and dry up.

In many instances under natural conditions, several lesions were observed to occur at a common origin, which suggests that infection is initiated in the whorl when there is free moisture to allow spore germination and infection. This was confirmed by artificial inoculation through the whorl. In almost all cases, leaf infection precedes both ear and stalk infections. It appears that spores of the fungus produced on the leaves are washed down to the ear and/or leaf axils subsequently causing ear and stalk infection. This implies that controlling the leaf blight phase would substantially reduce ear and stalk infection. Screening for resistance to the leaf blight would therefore be more appropriate in *D. macrospora* resistance breeding. Nevertheless, correlation of resistance among the three phases of infection needs to be established.

Although *D. macrospora* is known to cause the three phases of infection in other countries, this is the first documented report on the occurrence of the ear and stalk rot phases in the Philippines. This also constitutes the first report on the destructive potential of the disease in the country. The paper by Stevens and Celino in 1931 was the first and only report that we are aware of on *D. macrospora* as causing leaf blight in the Philippines.

Downy mildew

On the eradicative effect of metalaxyl in the first experiment, all plants sprayed with metalaxyl (Ridomil MZ) within five days after inoculation did not show systemic infection while 16.5% of plants sprayed with metalaxyl seven days after inoculation showed systemic infection. Such systemic infection, however, disappeared within one week; infected plants resumed normal growth thereafter. Plants that were not sprayed with metalaxyl showed 59.5% infection. In the second experiment, all plants except those used as check were sprayed with either Apron 35SD or Ridomil MZ at six days after inoculation. Plants sprayed with Apron showed 0.7% infection while those sprayed with Ridomil showed 9.1% infection. These infected plants, however, completely recovered from DM infection within one week. Unsprayed plants showed 97.6% infection. At the time of assessment which was 15 days after inoculation, infected plants in two seedboxes of control treatment were each sprayed with Apron 35SD and Ridomil MZ; infected plants in the remaining seedbox were left unsprayed. Observations made 15 days later showed all sprayed plants to have completely recovered from DM infection. At this time, only four out of 28 infected plants in the remaining control treatment were surviving but showing complete chlorosis and spindly growth. When these were sprayed with Ridomil, all plants recovered their normal green color and were able to produce tassel and ear shoot when the experiment was terminated.

Results presented above clearly demonstrate the eradicative effect of metalaxyl on DM infection. While these results tend to disagree with the findings of Exconde (1982), eradicative action of metalaxyl is expected because of the systemic nature of the fungicide. Results also indicate high biological activity of metalaxyl considering that symplastic movement in foliar treatment is usually not more than 1-2% of the applied metalaxyl (Zaki *et al.*, 1981). This high biological activity is confirmed by our recent study where complete control of DM was obtained with seed treatment at the rate of 0.2 g a. i./kg, which is only 10% of the recommended rate. Likewise, Odvody and Frederiksen (1984) obtained 100% control of *P. sorghi* in corn and sorghum at seed treatment rates as low as 0.05 g a. i./kg seed. Results also indicate that both the foliar spray (Ridomil) and the seed treatment (Apron) formulations are equally effective when sprayed to infected plants. However, Apron would be more economical to use because of its more concentrated form and the absence of another active ingredient present in the spray formulation. The appearance of systemic infection on some plants sprayed with either Apron or Ridomil at six to seven days after inoculation may be explained by the delayed action of fungicide on the fungus that has already become established in the shoot apex. The normal incubation period for systemic symptom expression on plants inoculated one week after planting is about one week (Dalmacio and Exconde, 1969). In terms of DM control, the seed treatment approach is undoubtedly preferred over foliar treatment, however, spraying has important implications in DM resistance breeding. Under too much DM pressure, important breeding lines may be saved from complete elimination by DM through foliar spraying. We are also presently utilizing

foliar application to determine the stability of DM resistance under DM and DM-free conditions for parent seed production. Foliar spraying may also be utilized in studying the inheritance of DM resistance by allowing crosses between truly susceptible (infected plant of a susceptible inbred) and resistant (uninfected plant of a resistant inbred) plants.

Results of study on the effect of metalaxyl on seed germination are presented in Tables 1 and 2. Results showed that seed germination was affected by both metalaxyl and length of storage. The effect of storage on seed germination may be attributed to seed moisture resulting from the water used in the slurry treatment. Perhaps, the initial moisture of the seed used was already high that the addition of water even at the rate of 0.5% activated physiological processes of the seed leading to decreased seed vigor. Comparison of the seed germination of treated and untreated seeds within a given storage period, however, clearly indicate the detri-

Table 1. Effect of Apron 35SD rate and length of storage period on corn seed germination of two Pioneer corn hybrids

Length of storage (days)	*Apron 25 SD Rate (g a.i./kg)* 0	2	4	*Mean*
0	95.45[a/]	91.80	82.8	90.02
7	86.65	66.15	31.66	61.49
14	71.30	38.30	25.00	44.87
Mean	84.47	65.41	46.49	65.46

[a/] Average of 300 seeds tested

Table 2. Effect of Apron 35SD rate and length of storage on corn seed germination of two Pioneer hybrids (2nd experiment)

Length of storage (days)	*Apron 35 SD Rate (g a.i./kg)* 0	0.5	1.0	1.5	2.0	*Mean*
0	95.5[a/]	92.5	90.0	86.5	77.0	88.3
7	93.0	86.5	82.7	78.0	70.5	82.1
14	90.0	84.2	76.2	71.2	62.0	76.7
30	89.2	85.5	80.2	67.0	61.5	76.7
60	78.5	74.7	71.7	66.5	60.2	70.3
90	62.2	51.7	45.2	33.5	20.5	42.6
Mean	84.7	79.2	74.3	67.1	58.6	72.8

[a/] Average of 400 seeds of two hybrids subjected to ordinary and cold storage conditions.

mental effect of metalaxyl on seed germination. The lowest rate of metalaxyl used (0.5 g a.i./kg) showed the least effect on seed germination. According to Hairston (1986, personal communication with Alex Paez of Pioneer), the effect on seed germination is associated with seed vigor, rate of application, storage conditions and use of other seed treatment, e.g. herbicide safeners. On sorghum, he cited reports of reduction in germination on seed stored for 5-6 months treated at 0.6 g a. i/kg seed, storage temperature and duration. However, water used in the slurry in excess of 10 ml/kg seed affected germination of both treated and untreated seed. In view of the conflicting data reported in the Philippines, further study using freshly harvested seeds and constant monitoring of seed moisture before and after seed treatment and during storage need to be conducted.

Tables 3 and 4 show the results of the study on the effect of different rates of metalaxyl on DM infection. In the first trial, DM infection was quite low, averaging only 26% infection in the untreated materials. Nevertheless, no infection was observed in the most DM-susceptible hybrid (untreated seed gave 71% infection) even at the rate of 0.5 g a. i./kg. In the second experiment where there was tremendous DM pressure (90% infection on a fairly resistant hybrid), no infection was observed even at the rate of 0.2 g a. i./kg, which is only 10% of the recommended rate of 2.0 g a. i./kg. Results of these experiments clearly demonstrate the effectiveness of metalaxyl against DM as has been reported by a number of investigators. Results also show that lower rates can be safely used thus, minimizing detrimental effect of metalaxyl on seed germination as well as reducing the cost of corn production. At the present price of ₱1,450.00 per kg of Apron 35SD, cost of the chemical good for 20 kg seed per ha amounts to only ₱16.60 compared to ₱166.00 per ha using the recommended rate of 2.0 g a.i./kg seed.

Table 3. Effect of varying rates of metalaxyl on downy mildew infection (1st experiment)

Rate (g a. i./kg)	*Hybrid*			
	3228	*6181*	*X306B*	*Mean*
0	3.4	4.0	71.0	26.0
0.5	0	0	0	0
1.0	0	0	0	0
1.5	0	0	0	0
2.0	0	0	0	0

[a] Percent downy mildew infection; mean of 5 replications of about 42 plants per replication.

Bacterial rot

With the breakthrough in DM control, bacterial rot now appears to be the most important disease of corn in the Philippines. As much as 40% incidence of the

Table 4. Effect of varying rates of metalaxyl on downy mildew infection (2nd experiment).

Rate (g a.i./kg)	*Replication* 1	2	3	*Mean*
0	96.0	91.0	83.0	90.0
0.2	0	0	0	0
0.4	0	0	0	0
0.6	0	0	0	0

[a/] Percent downy mildew infection based on 100 seeds planted per replication.

disease was observed in South Cotabato in a 3-hectare field in 1981 (IPB, 1981). In 1985, about 20% incidence was again observed in the same area. We have also observed as much as 80% infection on some breeding materials at our research stations in General Santos City and Cabuyao, Laguna.

Inbreds developed by Pioneer stations in the Philippines and other Asian countries are being screened for bacterial rot resistance since 1985. Results of a recent trial are presented in Table 5. Infection ranged from 0-100% which indicates good disease pressure. Out of 107 inbreds evaluated, 25 showed good resistance (less than 10% infection). Some of these inbreds have consistently shown resistance in previous trials and may now be considered for utilization in bacterial rot resistance breeding.

Table 5. Reactions of Asian inbreds to bacterial rot at Cabuyao station during the 1986-87 season

Inbred No.	*Replication* I	II	*Total Plt.*	*%BSR*
001	5.6	0	38	2.8
002	0	60.0	37	30.0
003	30.4	22.7	45	26.5
004	0	0	15	0
005	0	4.5	38	2.3
006	25.0	30.8	29	27.9
007	0	18.2	24	9.1
008	45.5	57.1	43	51.3
009	79.2	57.2	45	68.2
010	38.1	61.9	42	50.0
011	11.1	18.2	40	14.7
012	13.6	45.5	44	29.6
013	78.9	100.0	42	89.5
014	27.3	0	42	13.7
015	82.6	95.5	45	89.1

Table 5. (Continuation)

Inbred No.	*Replication* I	II	*Total Plt.*	*%BSR*
016	14.3	0	34	7.2
017	68.8	85.7	37	77.3
018	36.8	30.4	42	33.6
019	65.0	72.2	38	68.6
020	58.8	50.0	31	54.4
021	40.0	50.0	40	45.0
022	100.0	85.7	25	92.9
023	66.7	89.5	37	78.1
024	56.0	72.7	47	64.4
025	33.3	75.0	11	54.2
026	0	33.3	13	16.7
027	17.6	11.8	13	14.7
028	100.0	33.3	10	66.7
029	80.0	93.3	25	86.7
030	82.4	76.2	38	79.3
031	9.5	10.5	40	10
032	0	23.8	43	11.9
033	5.6	0	42	2.8
034	75.0	26.3	31	50.7
035	27.3	9.1	33	18.1
036	51.7	47.8	52	49.8
037	23.8	89.5	40	56.7
038	38.1	90.9	43	64.5
039	77.8	73.9	41	75.9
040	0	0	6	0
041	21.4	8.3	26	14.9
042	5.3	36.4	30	20.9
043	81.8	89.5	41	85.7
044	0	41.2	34	20.6
045	0	0	13	0
046	40.9	53.3	34	38.1
047	84.6	60.0	28	72.3
048	63.6	84.2	30	73.9
049	86.7	89.5	34	88.1
050	100.0	100.0	29	100.0
051	100.0	46.2	17	73.1
052	38.9	58.8	35	48.9
053	42.9	66.7	19	54.8
054	85.7	47.6	28	66.7
055	72.7	50.0	42	38.9
056	100.0	94.1	23	97.1
057	100.0	100.0	10	100.0
058	60.0	41.7	22	50.9

	Replication				Total	
	I	*II*	*III*	*IV*	*Plt.*	*%BSR*
059	0	0	6.3	0	65	1.6
060	16.7	63.0	9.5	22.7	80	28.0
061	75.0	83.3	84.2	88.2	52	82.7
062	26.1	13.6	31.8	37.5	91	27.3
063	33.3	14.3	16.7	18.2	40	20.6
064	25.0	0	0	7.7	38	8.2
065	84.2	64.7	68.8	90.5	73	77.1
066	34.8	52.4	88.9	24.0	87	50.0
067	5.3	26.1	23.8	31.8	85	21.8
068	70.0	50.0	84.2	77.8	77	70.5
069	42.1	71.4	82.4	65.0	70	65.2
070	5.9	0	5.3	15.8	65	6.8
071	0	4.3	5.3	10.0	83	4.9
072	50.0	100.0	42.9	78.3	83	67.8
073	0	15.4	6.3	4.8	70	6.6
074	100.0	0	25.0	50.0	20	43.8
075	94.7	100.0	95.2	100.0	80	97.5
076	91.7	90.0	61.1	36.4	84	72.3
077	27.3	71.4	33.3	0	25	33.0
078	100.0	80.9	79.2	87.5	89	89.2
079	59.1	22.7	37.5	22.7	90	35.5
080	44.4	8.9	22.2	31.8	81	26.8
081	75.0	81.3	7.7	50.0	47	36.0
082	9.1	4.3	9.5	36.8	85	14.9
083	0	0	0	0	54	0
084	0	0	0	0	81	0
085	50.0	33.3	85.7	58.3	51	56.8
086	43.8	70.6	38.1	52.4	75	51.2
087	0	0	4.8	0	76	1.2
088	89.5	84.4	89.5	86.4	79	87.5
089	0	4.3	9.5	0	73	3.5
090	0	0	28.6	0	30	7.2
091	14.3	15.8	5.0	0	86	8.8
092	0	10.0	13.3	0	67	5.8
093	70.0	50.0	57.1	91.7	69	67.2
094	20.0	25.0	6.3	0	56	12.8
095	45.0	33.3	–	70.0	55	49.4
096	100.0	78.3	90.5	88.9	88	89.4
097	86.7	90.0	91.7	90.5	68	89.7
098	52.6	65.0	66.7	60	85	61.1
099	0	0	31.6	20.8	84	13.1
100	100.0	92.3	100.0	81.2	78	93.4
101	10.0	1.7	0	4.5	67	4.1
102	90.0	90.2	72.2	86.4	81	84.8
103	95.0	78.9	77.8	68.2	79	80.0
104	90.9	90.9	72	73.9	92	81.9
105	94.7	100.0	90.0	94.1	80	94.5
106	92.3	86.7	100.0	92.9	55	90.0
107	0	0	–	0	44	0

Results of inoculation on precommercial and commercial hybrids of Pioneer and two commercial hybrids of a competitor company are shown in Table 6. Infection ranged from 53-85% with yield loss ranging from 36-82%. Correlation analysis between infection and yield loss gave an r value of 0.83, which indicate good correlation. However, this is expected from the experiment since plants were inoculated after flowering and yield of infected plants could not be compensated by neighboring healthy plants.

Table 6. Mean percent bacterial rot infection, yields of inoculated and uninoculated plots and percent yield loss due to bacterial rot on nine precommercial and commercial hybrids

Hybrid	*Percent rot a*	*Yield (kg/plot) b*		*Percent Loss*
		Inoculated	*Uninoculated*	
3208	71.2	0.8	2.0	66.7
3224	84.8	0.4	2.2	81.8
3228	53.0	1.1	2.3	52.2
3274	78.0	0.8	1.8	55.6
XCG51	58.6	1.6	2.5	36.0
YCG55	58.7	1.2	2.3	47.8
YCH55	78.8	0.8	2.5	68.0
Competitor 1	75.8	0.8	2.2	63.6
Competitor 2	84.0	0.6	2.2	72.7

[a] Average of 4 replications.

[b] Average of 3 replications of paired rows of inoculated and uninoculated plots.

Results indicate that corn hybrids presently grown in the Philippines do not possess adequate resistance to bacterial rot and are highly vulnerable to the disease under conditions favorable for disease development like those during periods of heavy precipitation. Apparent absence of resistance to bacterial rot among presently grown hybrids and varieties may be attributed to inadequate effort in bacterial rot resistance breeding.

Various screening techniques have been tried and these include whorl application with bacterial suspension, injection of bacterial suspension into the stalk and pricking of stalk with sharp nail previously dipped in bacterial suspension. For screening of germplasm materials, Karganilla and Exconde (1973) recommended pricking method. On the other hand, researchers at the Institute of Plant Breeding (IPB) are using whorl inoculation. At Pioneer, we have tried both methods and found these to be slow. We also found pricking method as unreliable because of contamination of inoculum during dipping of inoculating instrument after every

pricking of stalk. We found the use of an inoculator (designed and fabricated in the U.S. for inoculating corn stalks with fungal pathogens) as the most convenient with equally reliable results. As many as 600 plants can be inoculated per hour per person. What remains to be determined, however, is the inoculum concentration most appropriate in inoculating plants in the breeding nursery. Too high concentration may completely wipe out a population while too low concentration may result to more escapes that would make selection for resistance ineffective. Other factors to be considered should include the type of isolate and age of the plant.

Banded leaf disease

Results presented in Table 7 showed infection ranging from 2 to 98%. Among the eight methods tried, whorl inoculation with sclerotial bodies and leafsheath inoculation with infested sorghum grains gave 98% and 92% infection, respectively. While whorl inoculation using either sclerotial bodies or infested sorghum grains is more efficient, it does not simulate natural infection in the field. *Rhizoctonia solani* is soil-borne which initiates infection from the base and spreads upwards to the ear where it causes ear rot. Hence, whorl inoculation would not be the appropriate screening method. The best alternative appears to be the leafsheath inoculation using either sclerotial bodies or infected sorghum grains. It is quite slow but each plant is assured of being inoculated, thus, minimizing, if not eliminating, escapes.

Using the leafsheath inoculation method, we have screened Pioneer materials for banded leaf disease (BLSD) for the past three seasons. Results of our most recent trial are presented in Table 8. Lesion length varied from 35 to 71 cm, visual rating from 1-8%, ear rot from 28-100% and ear height (measured from point of inoculation) from 5-65 cm. Based on the BLSD resistance criteria used, there exist

Table 7. Effect of various inoculation methods on percent infection by *Rhizoctonia solani* on corn

Treatment	*Replication* 1	2	3	4	*Mean*
T1	31	22	43	28	31 a
T2	93	95	92	90	92 b
T3	56	68	62	82	67 c
T4	10	19	8	16	13 d
T5	37	17	32	52	34 a
T6	8	10	32	18	17 d
T7	43	5	33	49	32 a
T8	100	90	100	98	98 b
T9	2	0	0	7	2 c

Note: Means followed by the same letter are not significantly different of 0.05 level using LSD. Treatment descriptions are given in the text.

variation in BLSD resistance among the inbreds. However, no inbred appears highly resistant based on lesion length. This is to be expected for diseases caused by a pathogen with very broad host range.

To understand what factors contribute to the degree of ear rotting, correlation analyses were made among lesion length, ear height, visual rating and % ear rot. Results showed that both ear height and visual rating were highly correlated with percent ear rot with r values of −0.68 and −0.59, respectively. Likewise, there was high correlation between ear height and visual rating (r = 0.76). On the other hand, no correlation was found between lesion length and percent ear rot (r = −0.05) and between lesion length and visual rating (r = 0.10). Results imply that resistance to BLSD is more of the function of ear height rather than lesion length, despite observed differences in lesion length among inbreds. Resistance to BLSD in corn has been reported in India (Singh and Sharma, 1976, Ahuja and Payak, 1978), however, in such studies nothing was mentioned about the effect of ear height on resistance. But assuming that inbreds with different ear height are infected with the fungus at the same rate, it is quite obvious that the inbred with lower ear placement would be affected more than the inbred with higher ear placement. And since variation in ear height (σn = 12.32) is greater than variation in lesion length (σ n = 6.71), it could be expected that ear height would influence the incidence of ear rot more than lesion length.

Table 8. Incidence of *Rhizoctonia* ear rot and visual disease rating of corn inbreds as influenced by ear height and lesion length (PA, 1986)

Inbred No.	*Ear height (cm[a])*	*Lesion length (cm)*	*Visual rating*	*% Ear rot*
1	30	55	1	86
2	23	53	1	100
3	31	57	1	95
4	39	60	2	92
5	42	53	3	49
6	36	53	3	94
7	33	56	3	33
8	33	52	3	78
9	32	57	3	100
10	26	45	1	100
11	12	53	1	100
12	34	55	1	38
13	38	58	2	47
14	19	53	1	100
15	31	53	1	83
16	26	42	1	76
17	30	51	1	69
18	14	77	1	100

Table 8. (Continuation)

Inbred No.	*Ear height (cm[a])*	*Lesion length (cm)*	*Visual rating*	*& % Ear rot*
19	25	58	1	79
20	51	55	6	29
21	43	56	5	74
22	45	71	2	85
23	37	59	4	72
24	49	46	6	33
25	30	51	4	63
26	26	58	2	94
27	46	61	4	44
28	20	57	1	100
29	47	50	5	37
30	36	66	3	37
31	41	55	3	40
32	35	56	3	87
33	31	54	1	86
34	41	50	5	74
35	47	49	2	65
36	32	53	1	95
37	48	53	5	28
38	48	51	7	28
39	24	51	1	90
40	65	56	5	31
41	23	55	1	76
42	7	41	1	100
43	18	54	1	87
44	57	56	8	61
45	48	67	3	66
46	35	56	1	93
47	28	53	2	97
48	28	59	1	100
49	30	56	2	88
50	5	47	1	100
51	11	48	1	95
52	27	47	1	91
53	15	46	1	97
54	36	57	2	81
55	45	35	8	87
56	18	44	1	59
57	36	57	2	86
58	20	54	1	93

[a]Ear height measured from point of inoculation.

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GENOTYPE-ENVIRONMENT INTERACTION IN FLUE-CURED TOBACCO (*NICOTIANA TABACUM* L.)

Philbert S. Bonilla
Philippine Tobacco Research
and Training Center
Mariano Marcos State University
Batac, Ilocos Norte, Philippines

ABSTRACT

Eight randomly chosen tobacco cultivars were grown in three locations for two seasons. Significant differences were found among cultivars for all characters. Genotype x location and genotype x year interactions were small and nonsignificant for most traits. Genotype x location x year interactions were highly significant for three traits and usually greater than the first order genotype x environment interaction. However, the magnitude of genotype x environment interactions were only a small fraction of varietal differences.

Comparison of theoretical variances of treatment means with varying plot allocations revealed that increasing the number of years is more effective than increasing locations or replications in reducing the standard error. But adding more years costs more in time than adding locations. The acceptable optimum plot allocation for tobacco testing was found to be 3 replications, 6 locations in 2 years.

Another six selected cultivars were grown in six locations for two seasons. Significant genotype x environment interaction were found for the five characters. All the varieties except one were found adapted to wide range of environments based on b-value. Cultivars differed in stability based on b-value and s^2_d. NCBY was found to have high yield potential, adaptable and stable, hence an ideal check genotype for varietal testing.

Introduction

Genotype-environment (GE) interactions are of major importance to be considered in testing and evaluating varieties. Comstock and Moll (1963) have shown statistically the effect of large genotype-environment interactions in reducing progress from selection. Because of GE interactions, evaluation requires repeated testing in both time and space. A major emphasis has been to maximize the effects of such interactions and still adequately measure the genetic worth of the cultivar.

In the process of evaluating varieties, the breeder must ascertain performance of a variety in comparison with other varieties in a) yield level, e.g., the overall average yield compared to the overall yields of the others, b) adaptation, e.g., whether the variety is better adapted to one type of environment than to another,

and c) stability, e.g., the consistency of the performance relative to the yield performance of the other cultivars.

Different methods have been proposed to solve the problems created by genotype-environment interactions. An analysis of variance that combines years and locations was amply demonstrated by several workers (Sprague and Federer, 1951; Comstock and Robinson, 1952; Hanson *et al.*, 1956; and Comstock and 1963). This technique, however, could provide information only on the existence and magnitude of GE interaction but unable to give any measure of the contribution of individual genotypes to components of interaction.

Interest has been centered on regression techniques as an alternative method of analyzing GE interaction as proposed by Yates and Cochran (1938), developed by Finlay and Wilkinson (1963) and refined by Eberhart and Rusell (1966). Bilbro and Ray (1976) used *b* values as a measure of adaptation and proposed coefficient of determination (R^2) as a more logical parameter for stability. In this study, the method of Eberhart and Russell (1966) was adopted in determining stable genotypes because it considers two derived quantities, b and s_d^2, as measures of stability.

Trials to obtain the necessary information for proper evaluation of tobacco varieties and advanced breeding lines are both costly and time consuming. The question as to optimum allocation of replications, locations and years of testing necessary to obtain an estimate of a variety's potential in tobacco in the Philippines has received only limited attention.

The present study has the following objectives: 1) Ascertain the magnitude of GE interaction and its components and the relevance of each in testing procedures, 2) Determine optimum allocation of resources in conducting yield tests for flue-cured tobacco, 3) Estimate adaptation (b value) and stability (b and s_d^2) parameters for each of the different breeding lines used.

Materials and Methods

Cultivars and test locations

There were two sets of cultivars used in this study. The first set (Set I) was used to ascertain the magnitude of GE interaction through the variance component analysis, and in determining the optimum allocation of resources. It was composed of eight randomly selected cultivars representing high (Balikbayan, NCBY and Coker 254), medium (Buyer's Choice, WR-5 and Yellow Special), and low (Bissetes Special and Coker 298) yielding groups. It was grown in Batac, Ilocos Norte; Sta. Maria, Ilocos Sur; and Balaoan, La Union for two consecutive years (crop season 1982-1983 and 1983-1984).

The second set (Set II) was composed of selected cultivars entered in the advanced test for untopped flue-cured tobacco trials. It was composed of Balik-

bayan, NCBY, Coker 86, Coker 254, Coker 258 and Reams 266. The adaptability and stability of each variety were estimated. It was grown in Batac and Marcos, Ilocos Norte; San Juan and Sta. Maria, Ilocos Sur; Pidigan, Abra; and Balaoan, La Union for the same years as in set I.

Field experiment and data collection

All experiments were laid out in a randomized complete block design with four replications. A plot was composed of five rows 0.8 m apart. Plants were 0.5 m apart within rows. The following data were collected: cured yield, grade index, leaf width and length, number of days to flower, plant height and number of harvestable leaves.

Statistical analysis

A combined analysis of variance over location-year combination was computed for each set. For parameters with heterogenous variances based on Barlett's tests, log transformation was used. In some cases, locations were deleted to achieve homogeneity of variances.

Set I: Random Model

The statistical model is as follows:

$$x_{ijkr} = \mu + g_i + \ell_j + y_k + (\ell y)_{jk} + b_{rjk} + (g\ell)_{ij} (gy)_{ik} + (g\ell y)_{ijk} + e_{ijkr} \quad \text{(Eq. 1)}$$

where:

x_{ijkr}, is the observed value of the i^{th} genotype in the r^{th} replicate in the j^{th} location in the k^{th} year;

μ, the over all mean;

g_i, the i^{th} genotypic effect;

ℓ_j, the j^{th} location effect;

y_k, the k^{th} year effect;

$(\ell y)_{jk}$, the interaction effect of the j^{th} location with the k^{th} year;

b_{rjk}, the block effect of the r^{th} replication in the j^{th} location in the k^{th} year;

$(g\ell)_{ij}$, the interaction effect of the i^{th} genotype with the k^{th} year;

$(gy)_{ik}$, the interaction effect of the i^{th} genotype with the k^{th} year;

$(g\ell y)_{ijk}$, the interaction effect of the i^{th} genotype with the j^{th} location in the k^{th} year;

e_{ijkr}, the experimental error.

Variance components. The variation components for the seven parameters were estimated based from the expected mean squares, derived considering Eq. 1.

Optimum allocation of resources. The theoretical variance of a variety mean was computed for characters with significance $\hat{\sigma}^2_{gy}$ or $\hat{\sigma}^2_{g\ell}$ or $\hat{\sigma}^2_{gy\ell}$ or their combination. The theoretical variance of the genotype mean (Jones, 1960) was computed using the following:

$$V_{\bar{x}} = \hat{\sigma}^2_{gy/y} + \hat{\sigma}^2_{g\ell/} + \hat{\sigma}^2_{gy\ell/y\ell} + \hat{\sigma}^2_{e/r\ell y} \qquad \text{(Eq. 2)}$$

where:

$V_{\bar{x}}$, the theoretical variance of genotype mean;

y, the number of years;

ℓ, the number of location;

r, the number of replications.

Estimates of the variance components obtained from set II materials were substituted into the formula with varying number of years, locations and replications, hence providing a basis for the comparison of the allocations with respect to the sizes of the resulting variances.

Three dimensional drawings were used to present the effects of various plot allocations. The joint effect of changing the number of years, location, and replication was visualized by the over-all slope of the surface. In all cases, the height arising from the base was the $V_{\bar{x}}$ as computed from the formula.

Actual variance of a variety mean of the Philippine Tobacco Research and Training Center's plot allocation was compared to the proposed plot allocations. Increase in percentage error and the number of plots reduced was simultaneously considered in recommending the optimum resource allocation.

Set II. Fixed Model

The statistical model is as follows:

$$x_{ijr} = \mu + \ell_i + g_j + b_{ri} + \ell g_{ij} + e_{ijr} \qquad \text{(Eq. 3)}$$

where:

x_{ijr}, is the observed value for the j^{th} variety at the r^{th} replication of the i^{th} environment;

μ, the overall mean;

ℓ_i, the i^{th} environment;

Variance components. The variation components for the seven parameters were estimated based from the expected mean squares, derived considering Eq. 1.

Optimum allocation of resources. The theoretical variance of a variety mean was computed for characters with significance $\hat{\sigma}^2_{gy}$ or $\hat{\sigma}^2_{g\ell}$ or $\hat{\sigma}^2_{gy\ell}$ or their combination. The theoretical variance of the genotype mean (Jones, 1960) was computed using the following:

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$$x_{ijr} = \mu + \ell_i + g_j + b_{ri} + \ell g_{ij} + e_{ijr} \qquad \text{(Eq. 3)}$$

where:

x_{ijr}, is the observed value for the j^{th} variety at the r^{th} replication of the i^{th} environment;

μ, the overall mean;

ℓ_i, the i^{th} environment;

where:

s_d^2, the deviation from linear regression of the i^{th} variety;

α_{ij}, the variance due to deviation from regression of the i^{th} variety at the j^{th} growing conditions;

s_e^2, mean square for pooled error;

e, the number of growing conditions.

A variety is stable when b = 1 and $s_d^2 = 0$.

Results and Discussion

Set I: Random Model

Variance components

The estimates of variance component for set I is presented in Table 1. The $\hat{\sigma}_y^2$ and $\hat{\sigma}_\varrho^2$ were not significant for all tobacco traits studied except for $\hat{\sigma}_y^2$ of cured yield. However, highly significant $\hat{\sigma}_{y\varrho}^2$ was observed for all characters. This implies that the ranking of the different locations based on the mean performance of the genotypes used, differed from year to year.

Large varietal differences ($\hat{\sigma}_g^2$) were present for all the traits. The $\hat{\sigma}_{gy}^2$ was significant for grade index and leaf width while $\hat{\sigma}_{g\varrho}^2$ was significant only for grade index. Significant estimates of $\hat{\sigma}_{gy\varrho}^2$ were obtained for days to flower, plant height and number of harvestable leaves. Similar results were obtained by Jones *et al.* (1960) for flue-cured tobacco at North Carolina.

Significant $\hat{\sigma}_{g\varrho}^2$ and $\hat{\sigma}_{gy}^2$ and nonsignificant $\hat{\sigma}_{gy\varrho}^2$ were found for grade index. This indicates that the eight varieties performed differently from year to year when averaged over locations and likewise from location to location when averaged over years. Presence of significant $\hat{\sigma}_{gy\varrho}^2$ and absence of significant $\hat{\sigma}_{gy}^2$ and $\hat{\sigma}_{g\varrho}^2$, as in days to flower, plant height and number of leaves, indicates that the interaction of varieties with environment arose from the distinct and exclusive conditions existing in a particular experiment (e.g. year-location combination). Results also suggest that years need not be consecutive and that locations in different years may not be in the same immediate area.

Estimates of $\hat{\sigma}_g^2$ were much greater than those of $\hat{\sigma}_{gy}^2$, $\hat{\sigma}_{g\varrho}^2$ and $\hat{\sigma}_{gy\varrho}^2$ for all characters except for grade index where $\hat{\sigma}_{g\varrho}^2$ was slightly greater than σ_g^2. Furthermore, $\hat{\sigma}_g^2$ was significant for yield but not for components of genotype x environment interactions. In tobacco, there are three phases of yield testing namely preliminary, general and advanced trials. The implication of small $\hat{\sigma}_{ge}^2$ components relative to $\hat{\sigma}_g^2$ is that, general trial can be deleted.

Optimum allocation of resources

Figure 1A illustrates the change in variance when year was fixed at 2 for grade index. Increasing the number of locations was evidently more effective than increasing the number of replications. Although actual variances cannot be determined from the surface figure, the illustration clearly shows the small increment in the decrease of variance when replication increases. This can be supported by the almost horizontal line depicted from replications 1-5 with location fixed at a particular number.

When number of years was increased as replication was fixed at 4 (Fig. 1B), a considerable decrease in variance was observed. Such decrease in variance was evident from 1-3 years though showing a decrease in increment with every additional year. It is more effective increasing the number of years than increasing the location or replication. However, more plots would be necessary through additional location or replication to attain the desired efficiency. Increasing the number of years would also mean delay in releasing new varieties. Results for leaf width, days to flower, plant height and number of harvestable leaves (Figs. 2, 3, 4 and 5, respectively) were similar with those for grade index. Another feature of the 5 graphs is that an optimum is reached as the surface starts to level off, this means that from that point an increase in the number of test will provide only a small gain in precision, and eventually, wasteful.

The Philippine Tobacco Research and Training Center uses 2 years, 7 locations, and 4 replications for their advance test. After the test, an outstanding variety is recommended to the Philippine Seed Board. Table 2 presents the actual standard error for 4 replications, 7 locations and 2 years (PTRTC procedure) as compared with the proposed number of replicates, locations and years for each of the tobacco trait with significant GE interactions. Relative efficiency was computed as the ratio of the theoretical variance of a variety mean over actual variance. A maximum increase in cv of 10% and the number of plots reduced was considered simultaneously in looking at the different plot allocation.

For grade index, the actual cv using the center's procedure was 5.75. The least number of resource allocation acceptable was 3 replications, 6 locations and 2 years with an increase of 6.16 (cv) or 7.13% with 20 plots reduction. The actual cv for leaf width was 0.0393 and using 3 replications, 5 locations and 3 years it was reduced by 2.04% with 11 plots reduced. Result for leaf width was also similar for plant height and indicates that increasing the number of years is more effective than increasing location. But the time constraint is much more important, hence 2 years of test is adequate and consideration was given to varying number of locations and replications relative to a testing period of two years.

Based on the above argument, the optimum plot allocation for leaf width, days to flower, plant height and number of harvestable leaves are (sequence of numbers represent replication, location, year), 4, 5, 2; 3, 6, 2; 4, 6, 2; and 4, 6, 2, respectively).

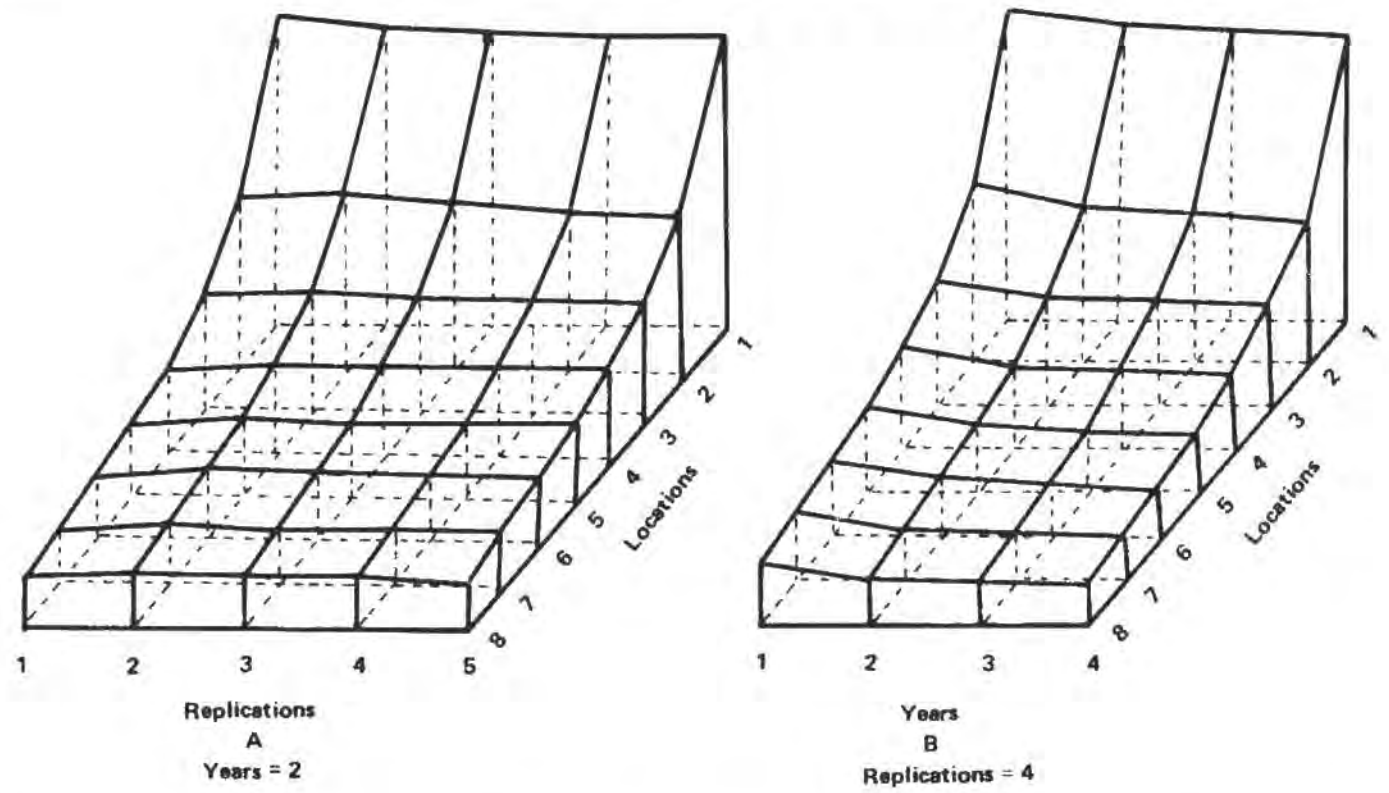

Fig. 1. Surface representing the theoretical variance of a variety mean resulting from various plot allocations for grade index. Variance is the height from the base.

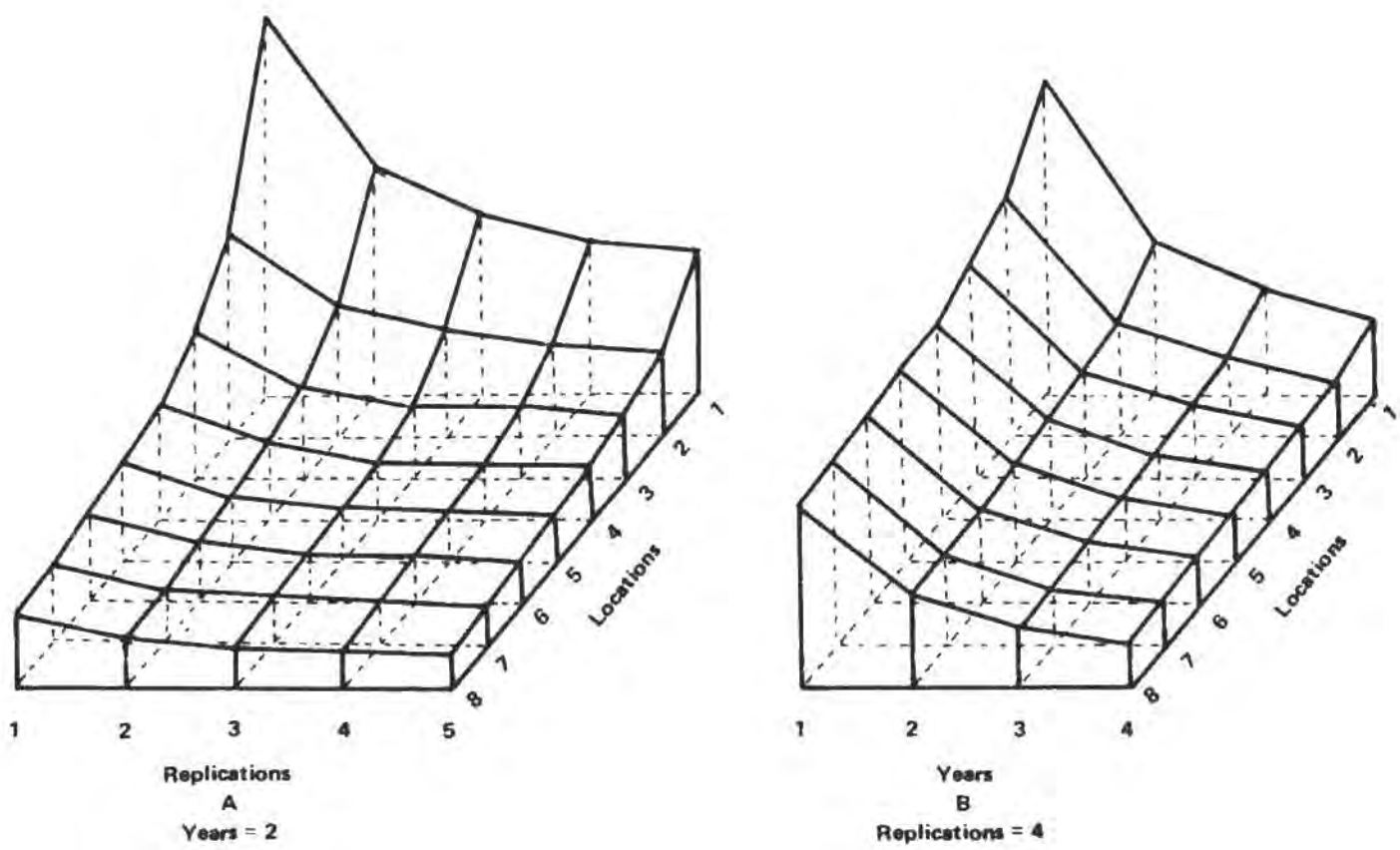

Fig. 2. Surface representing the theoretical variance of a variety mean resulting from various plot allocations for log leaf width. Variance is the height from the base.

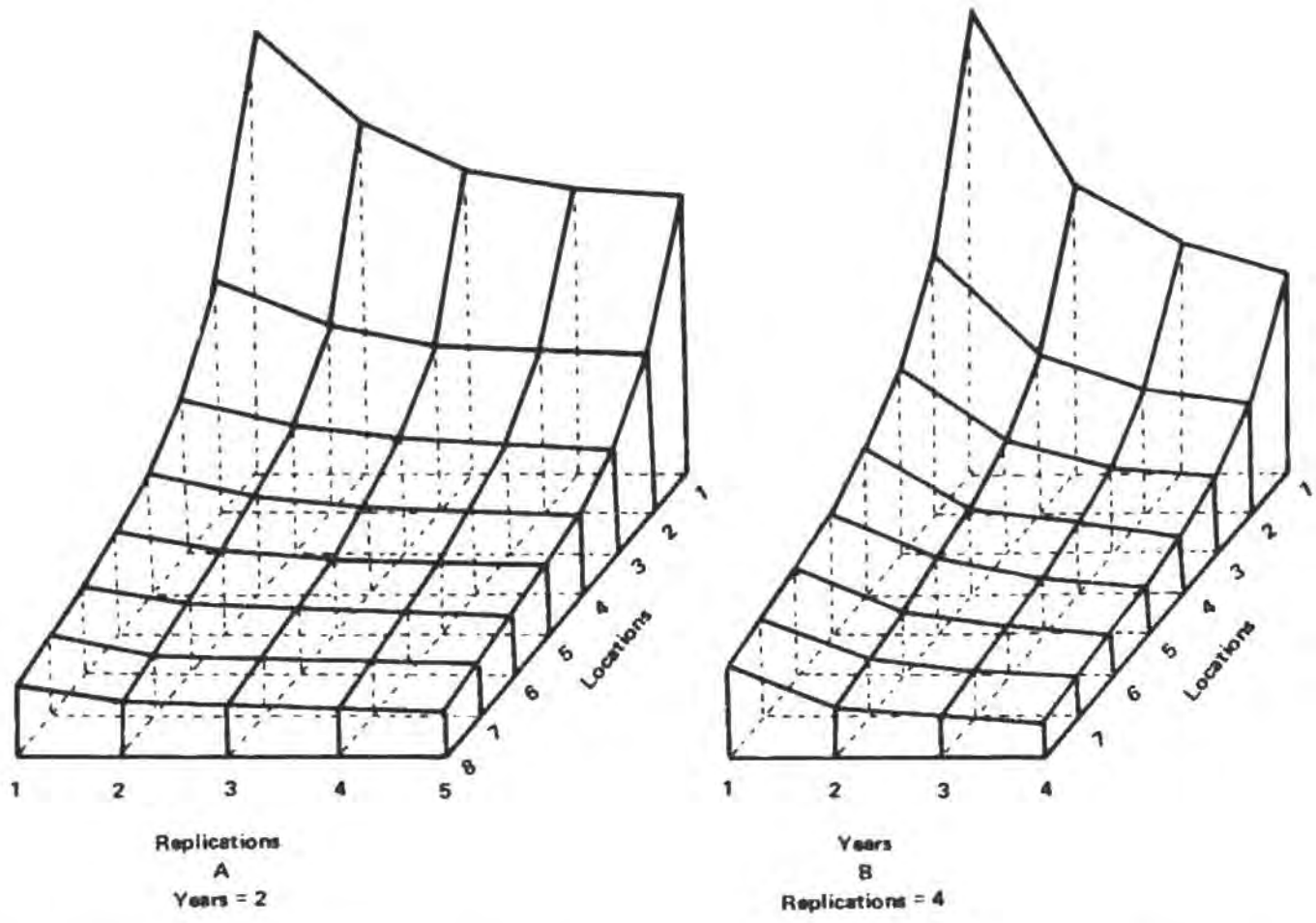

Fig. 3. Surface representing the theoretical variance of a variety mean resulting from various plot allocations for days to flower. Variance is the height from base.

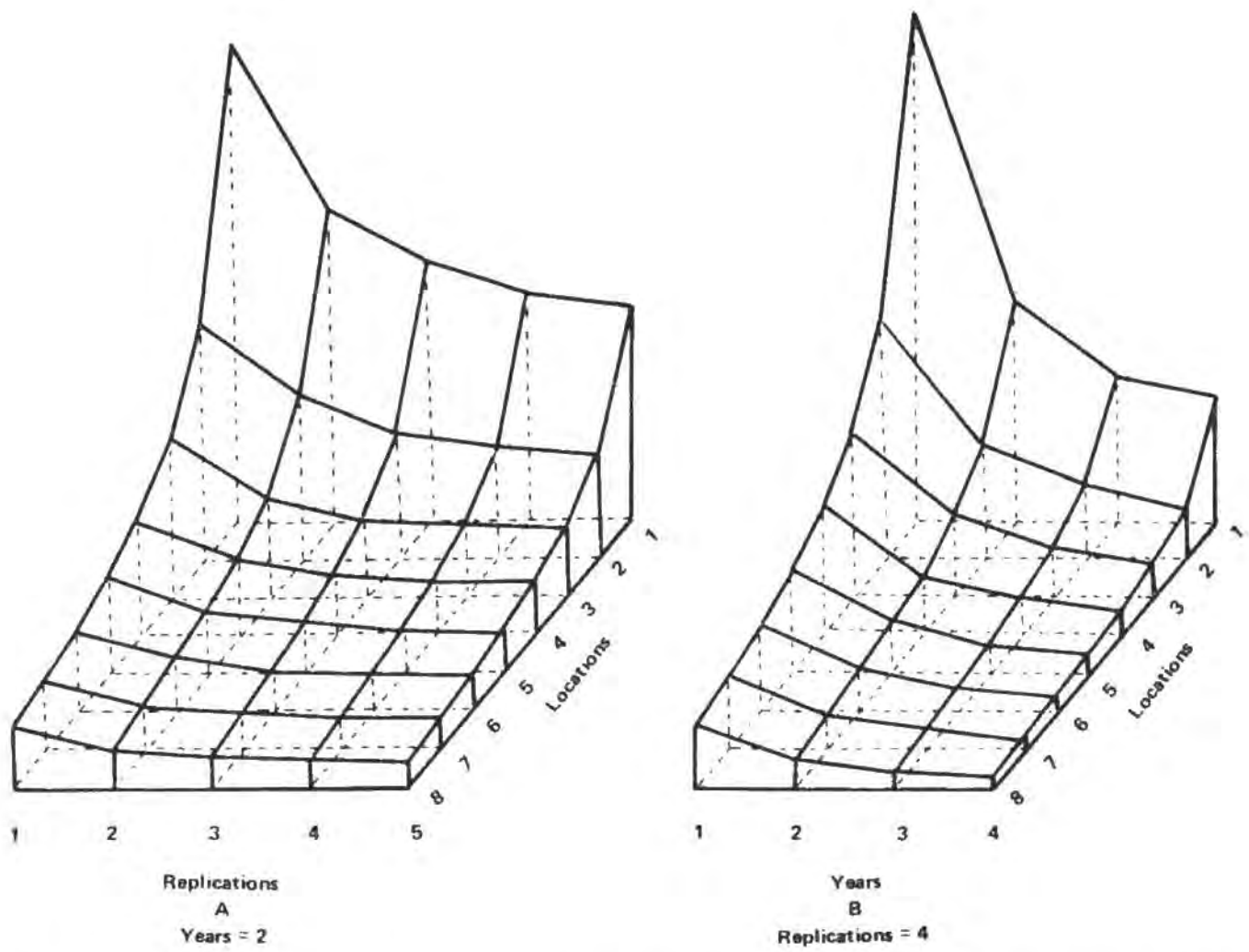

Fig. 4. Surface representing the theoretical variance of a variety mean resulting from various plot allocations for plant height. Variance is the height from the base.

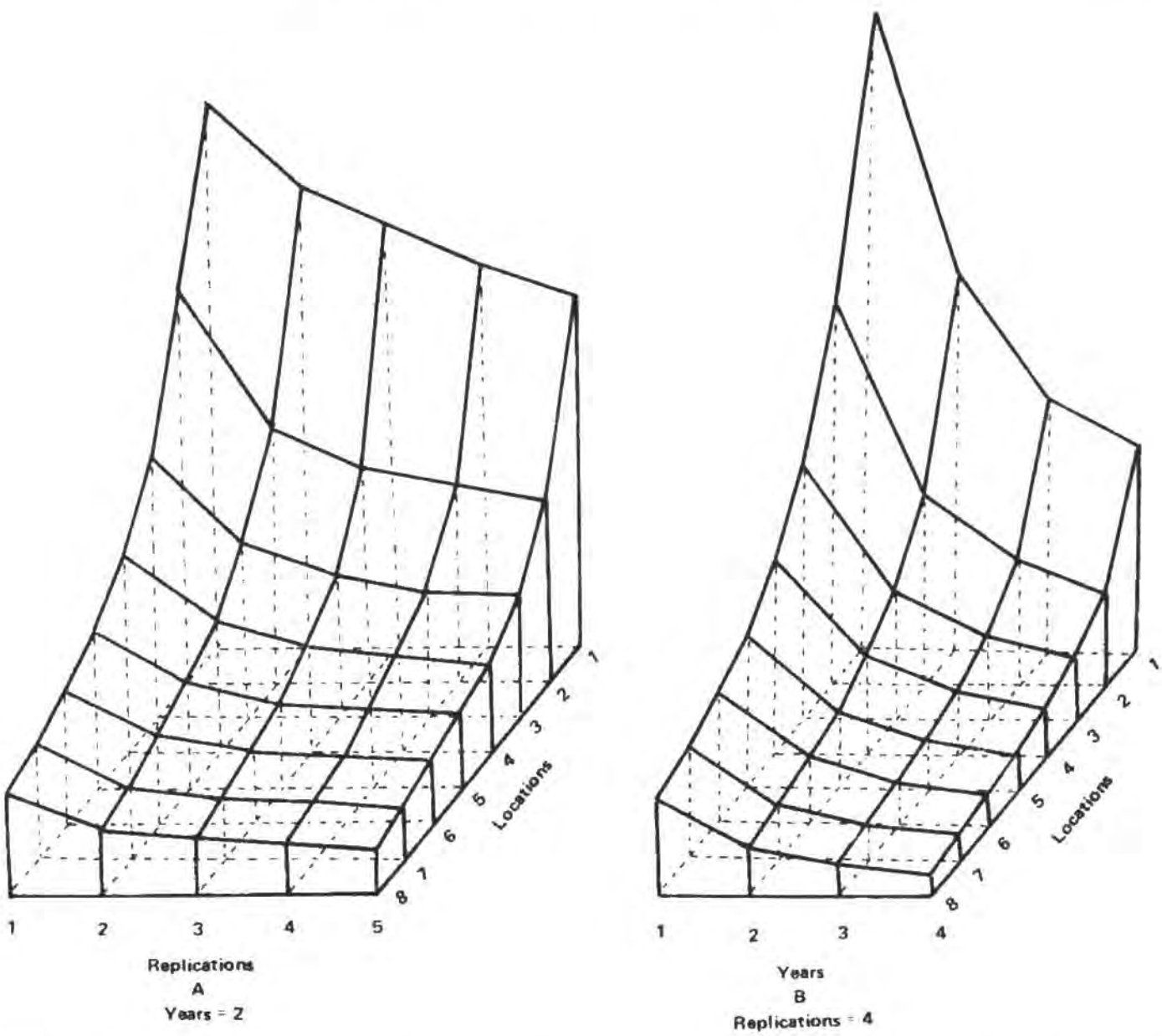

Fig. 5. Surface representing the theoretical variance of a variety mean resulting from various plot allocations for number of harvestable leaves. Variance is the height from the base.

Table 1. Estimates of variance components for 7 parameters of tobacco varieties (Set I)[1]

Variance Components	*Parameters*						
	Cured yield	*Grade index*	*Leaf width*	*Leaf length*	*Days to flower*	*Plant height*	*Number of harvestable leaves*
$\hat{\sigma}^2_y$	.24701**	.02208	−.00031	3.97857	21.3738	−168.68565	−.39168
$\hat{\sigma}^2_\ell$	.00287	−.01621	−.00060	9.06526	75.08249	−159.93801	1.60536
$\hat{\sigma}^2_{y\ell}$	.06981*	.4119**	.0428**	14.37088**	67.54576**	1,177.35835**	1.40106**
$\hat{\sigma}^2_g$	.06373**	.17102**	.0023**	21.6330**	49.71614**	192.16590**	8.95154**
$\hat{\sigma}^2_{gy}$	−.00032	.02199*	.00014*	.69762	.63095	0.31483	−.22372
$\hat{\sigma}^2_{g\ell}$	−.00755	.24232**	−.00009	.00784	2.09859	−3.19896	−.35742
$\hat{\sigma}^2_{gy\ell}$	.01442	−.00789	.00017	−.06568	4.79539**	33.97257**	1.0707**
$\hat{\sigma}^2_e$	.07779	.04008	.00110	9.63331	6.91294	68.14784	2.38105

[1]* and ** significantly different at 5% and 1% level, respectively.

Table 2. Standard errors for variety means under the PTRTC testing procedure (4 replications, 7 locations and 2 years) and with the several pertinent combinations of replications, locations and years

*Combination**			*No. of plots reduced*	*Grade index*		*Leaf width*		*Days to flower*		*Plant height*		*No. of harvestable leaves*	
R	*L*	*Y*		*CV*	*% Increase*	*CV*	*% Increase*	*CV*	*% Increase*	*CV*	*% Increase*	*CVCV*	*% Increase*
PTRTC Procedure													
4	7	2	–	5.75	–	.0393	–	1.366	–	1.081	–	1.493	–
–													–
3	7	2	14	5.80	.87	.0416	5.85	1.398	2.34	1.145	5.92	1.579	5.76
4	6	2	8	6.12	6.43	.0403	2.54	1.451	6.22	1.164	7.68	1.614	8.10
3	6	2	20	6.16	7.13	.0418	6.36	1.479	8.27	1.233	14.06	1.705	14.20
4	5	2	16	6.58	14.43	.0417	6.11	1.554	13.76	1.270	17.48	1.7663	18.30
3	5	3	11	6.38	10.96	.0001	(2.04)	1.386	1.46	1.065	(1.48)	1.526	2.21

*R, L & Y denotes replications, locations and years, respectively.

Giving equal importance to the five traits, the percent increase in cv was averaged to compare the different plot allocations. Reducing the number of replications from 4 to 3 and maintaining 7 locations and 2 years gave an average of 4.15% increase in cv for all traits and a reduction from 56 to 42 plots. Given 4 replications and 2 years and reducing 7 locations to 6, there was an average increase of 6.19% and this supports the relatively large effect of reducing locations rather than replications. For the 5 parameters, the increase in the standard error of 3 replicates, 6 locations, 2 years had an average of 10.0% with plots reduced from 56 to 36 or 20 plots cheaper. The same result was found by Bonilla (1983) in a blank test where the optimum number of replications was 3 using Smith's index of soil heterogeneity (b) with 10% degree of precision.

The last combination is 3 replicates, 5 locations, and 3 years with an average increase of 2.22%, the smallest increase from the actual allocation and a reduction from 56 to 45 plots. Increasing the number of years at 3 replicates and reduction to 5 locations would mean a decrease in variance of variety mean for leaf width (2.04%) and plant height (1.48%).

The relative efficiencies discussed did not directly consider the cost because the total number of plots reflects the relative cost of gathering the necessary data. The 3 replications, 6 locations and 2 years gave a slight increase in the coefficient of variation or an average of 10% for the five characters and its recommendation can be justified because of a reduction of one-third in the total number of plots.

Set II: Fixed Model

Environment and genotype main effects were highly significant for all characters. Significant GE interaction were found for all traits except for leaf width and length. Partitioning of the genotype x environment sum of squares based on Eberhart and Russell (1966) analysis to sum of squares due to regression and due to deviation from linearity of response from mean sum of squares for traits with significant GE interaction is presented in Table 4.

Adaptability analysis

Table 5 presents the mean value and b-value of the 6 varieties for cured yield. However, no b-value was significantly different from 1.0 (see Fig. 6) hence, all varieties were adapted to all environments with respect to their yield potential.

The mean grade index of the 6 tobacco varieties and their b-values are summarized in Table 6. Within this range of b-values, it can be detected that Coker 258 was adapted only to favorable environments (Fig. 7) and the rest of the varieties had adaptability to any kind of environment.

The varieties flowered at different times of the season. Balikbayan was a late flowering variety while Coker 86 was an early flowering one (Table 7). The regression lines of the 6 varieties is shown in Fig. 8.

Table 4. Analysis of variance and deviations from their regression of the 6 tobacco varieties based on mean sum of squares for the five traits with significant GE interaction (Set II)

Source of Variation	*D.F.*	*Mean Squares*[1]		
		Log cured weight	*Log grade index*	*Log days to flower*
Varieties	5	3.03×10^{-2}**	.1203**	3.40×10^{-3}**
Env. + (Varieties x Env.)	66(30)	2.78×10^{-2}**	1.3×10^{-2}**	2.12×10^{-3}**
Env. (Linear)	1	1.3528**	.5404**	.0578**
Varieties x Env. (Linear)	5	9.5×10^{-3}**	.0130**	4.0×10^{-5}**
Pooled deviation	60(24)	7.3×10^{-3}**	4.26×10^{-3}**	2.0×10^{-4}**
Balikbayan	10(4)	3.93×10^{-3}	4.14×10^{-3}*	6.25×10^{-4}**
NCBY	10(4)	2.22×10^{-3}	2.29×10^{-3}	1.5×10^{-4}
Reams 266	10(4)	5.25×10^{-3}	3.75×10^{-3}*	7.5×10^{-5}
Coker 86	10(4)	1.91×10^{-2}**	2.54×10^{-3}**	3.25×10^{-4}*
Coker 254	10(4)	9.15×10^{-3}**	5.03×10^{-3}**	7.5×10^{-5}
Coker 298	10(4)	3.83×10^{-3}	7.84×10^{-3}	2.25×10^{-4}
Pooled Error	216(108)	3.15×10^{-3}	1.82×10^{-3}	1.25×10^{-4}

[1]* and ** significant at 5% and 1% level, respectively.
( ) – degrees of freedom for days to flower and number of harvestable leaves.

Table 5. Summary of variety means, adaptation and stability parameters for log cured weight of 6 tobacco varieties grown at 12 environments (Set II)[1]

Variety	*Mean performance (tons/ha)*	*Adaptability (b-values)*^{ns}	*Standard deviation from regression* (s^2_d)	*Classification*
Balikbayan	2.51a	0.8744	7.8×10^{-4}	stable
NCBY	2.37ab	0.7956	9.3×10^{-4}	stable
Reams 266	2.25b	0.9126	2.1×10^{-3}	stable
Coker 86	2.29b	1.3232	1.6×10^{-2}**	unstable
Coker 254	2.23b	0.9125	6.0×10^{-3}**	unstable
Coker 258	2.19c	1.1812	6.8×10^{-4}	stable

[1]Any two means having a common letter are not significantly different at the 5% level of significance.
**Significantly different from zero (0) at 1% level of significance.
ns – not significant

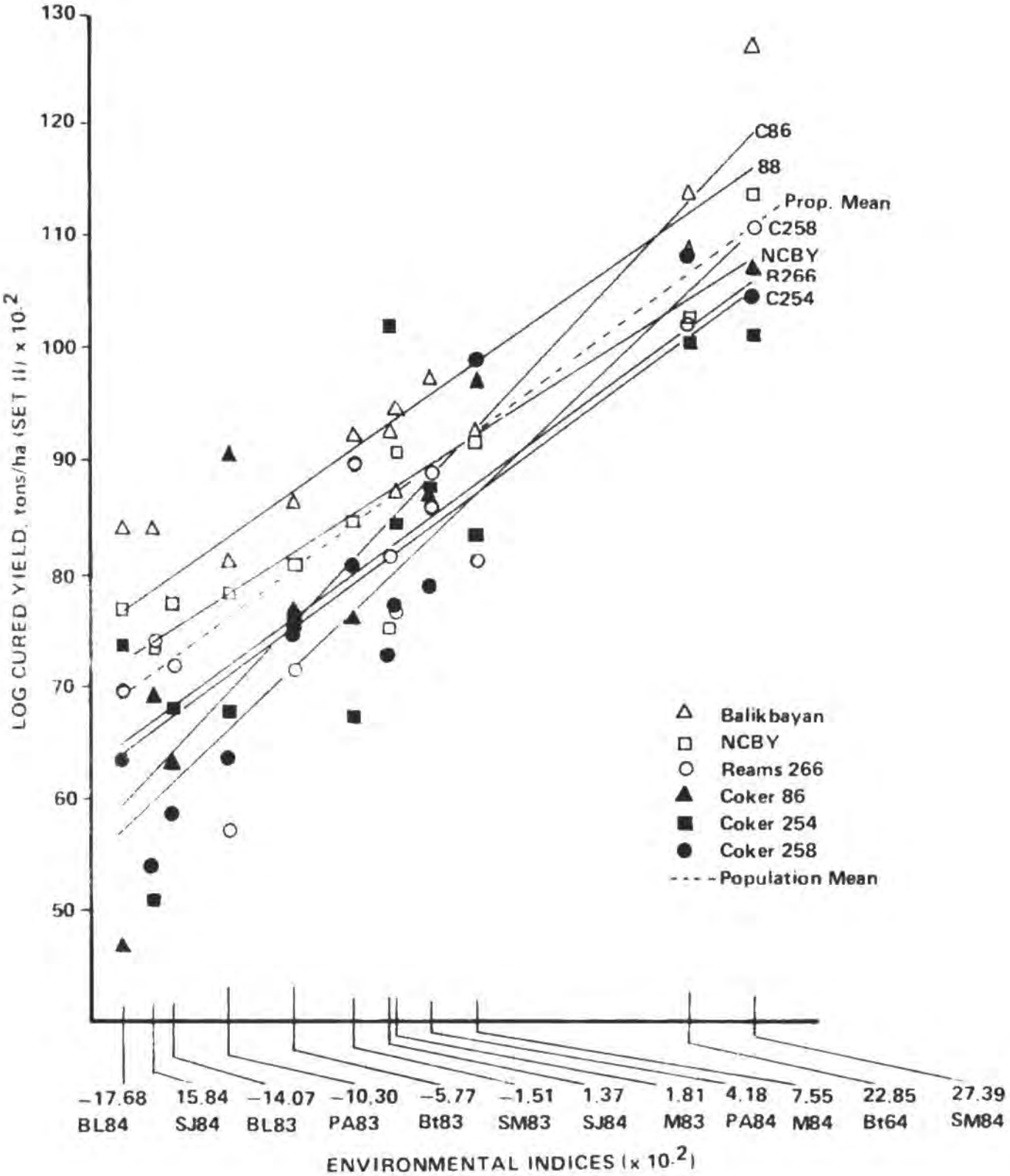

Fig. 6. Regression lines (SET II) showing the relationship of log cured yield of 6 tobacco varieties and population mean grown at different location and years – Bt., Batac, Ilocos Norte; M., Marcos, Ilocos Norte; S.J., San Juan, Ilocos Sur; Sm., Sta. Maria, Ilocos Sur; P.A., Pidigan, Abra; BL., Balaoan, La Union; 83 and 84 represent crop year 1982-83 and 1983-84, respectively.

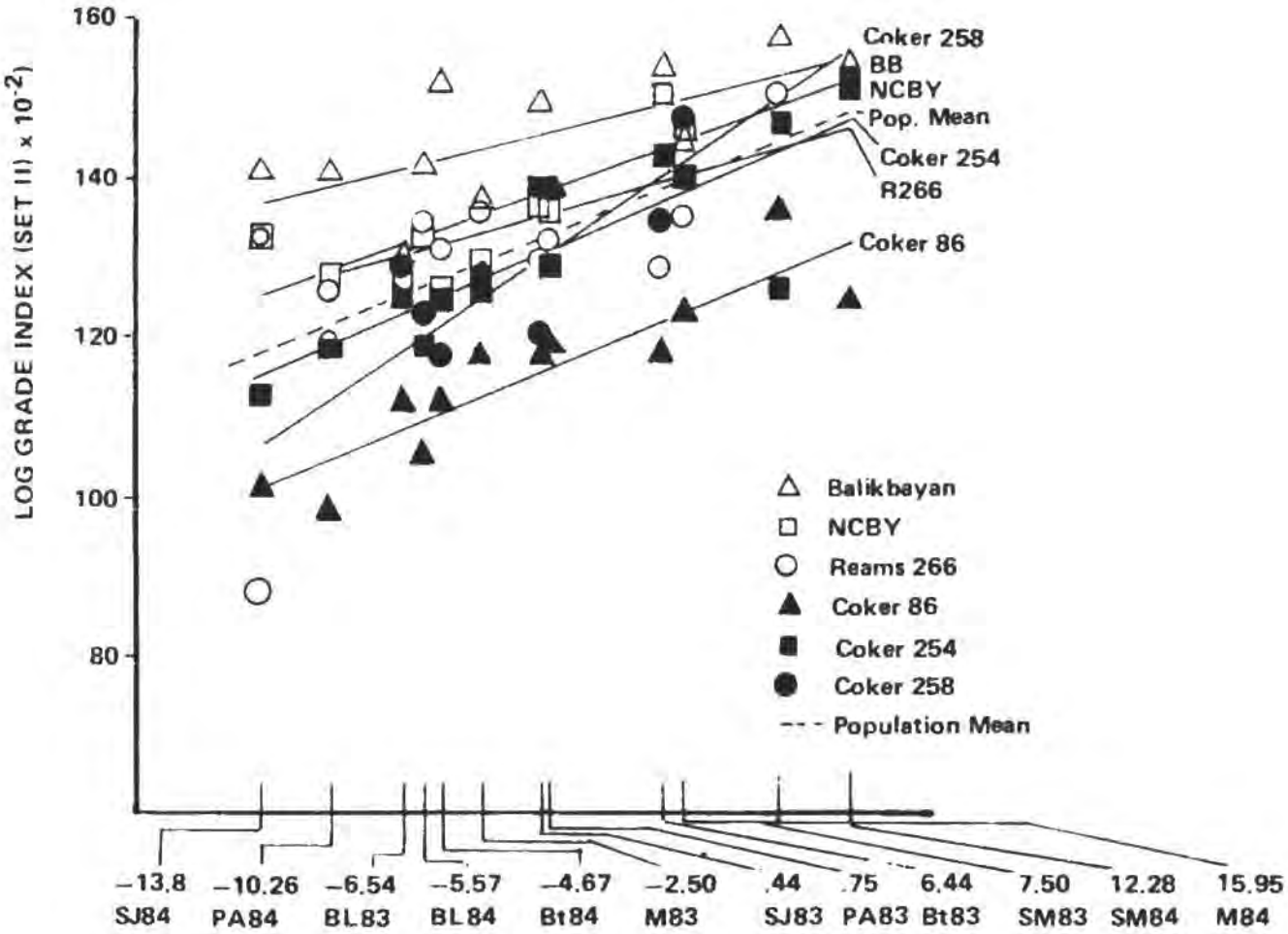

Fig. 7. Regression lines (SET II) showing the relationship of log grade index of 6 tobacco varieties and population mean grown at different location and years – Bt., Batac, Ilocos Norte; M., Marcos, Ilocos Norte; SJ., San Juan, Ilocos Sur; SM., Sta. Maria, Ilocos Sur; PA., Pidigan, Abra; BL., Balaoan, La Union; 83 and 84 represent crop year 1982-83 and 1983-84, respectively.

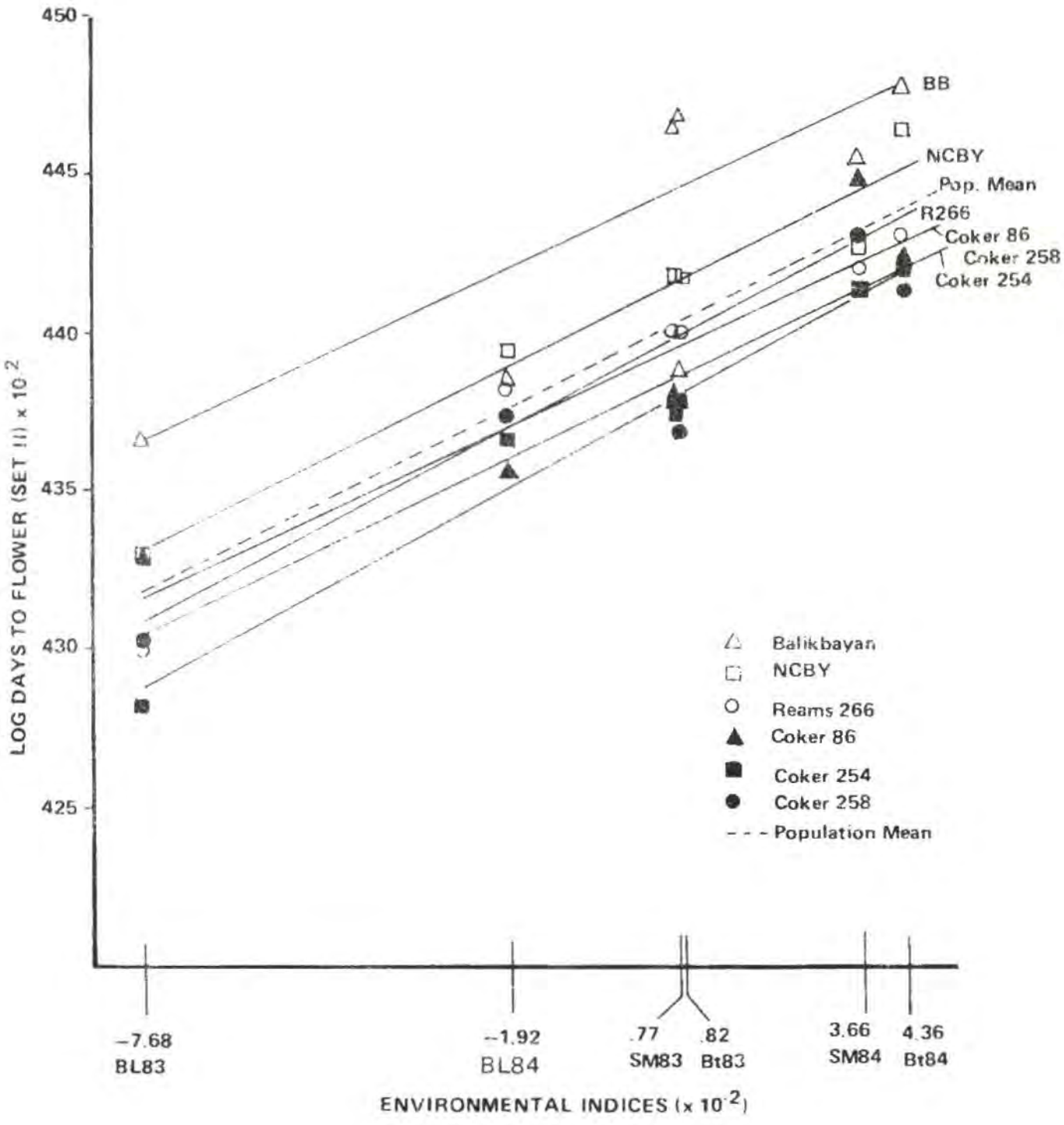

Fig. 8. Regression lines (SET II) showing the relationship of log days to flower of 6 tobacco varieties and population mean grown at different location and years – Bt., Batac, Ilocos Norte; SM., Sta. Maria, Ilocos Sur; BL., Balaoan, La Union; 83 and 84 represent crop year 1982-83 and 1983-84, respectively.

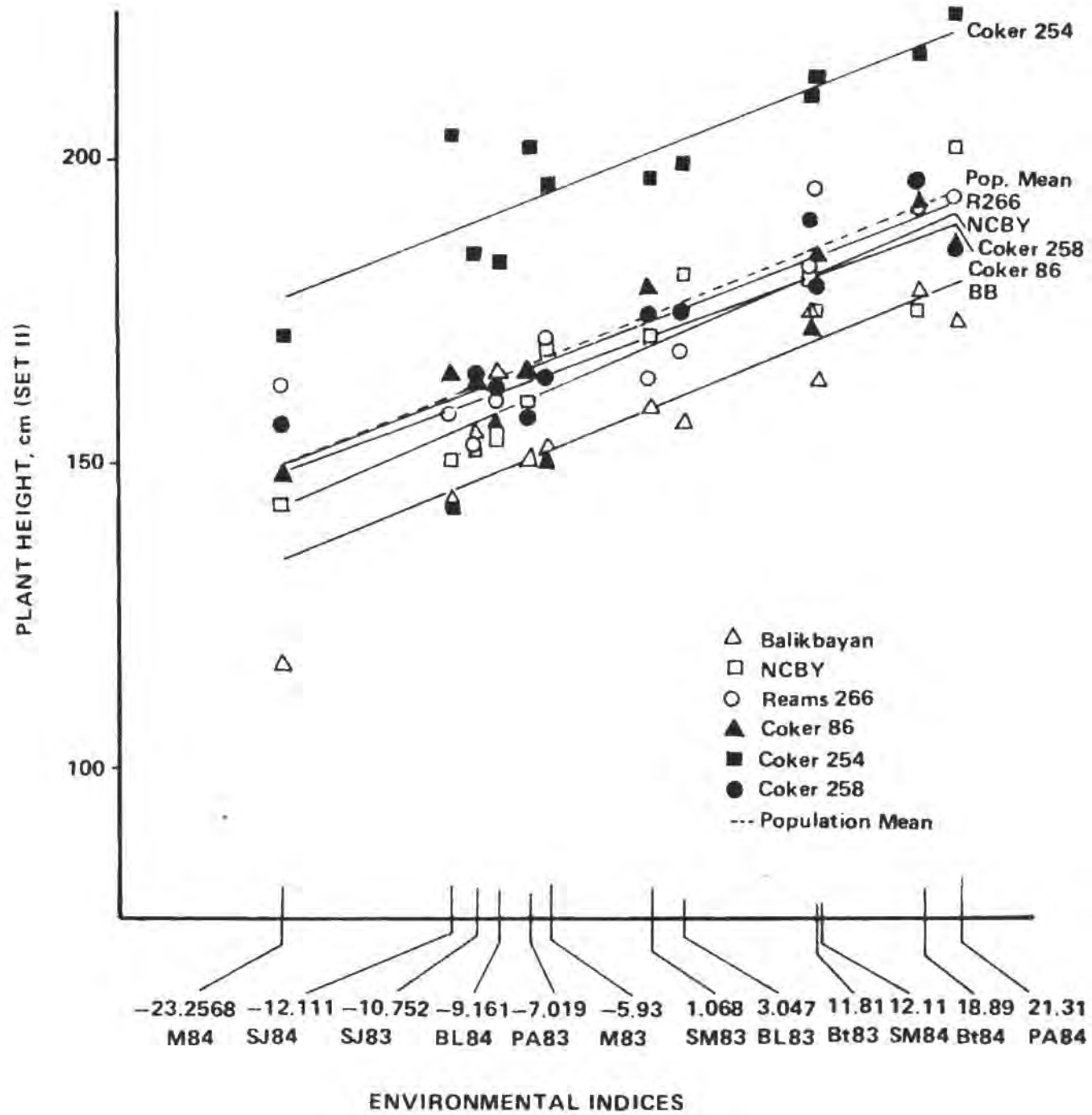

Fig. 9. Regression lines (SET II) showing the relationship of plant height of 6 tobacco varieties and population mean grown at different locations and years – Bt., Batac, Ilocos Norte; M., Marcos, Ilocos Norte; S.J., San Juan, Ilocos Sur; SM, Sta. Maria, Ilocos Sur; PA., Pidigan, Abra, BL., Balaoan, La Union; 83 and 84 represent crop year 1982-83 and 1983-84, respectively.

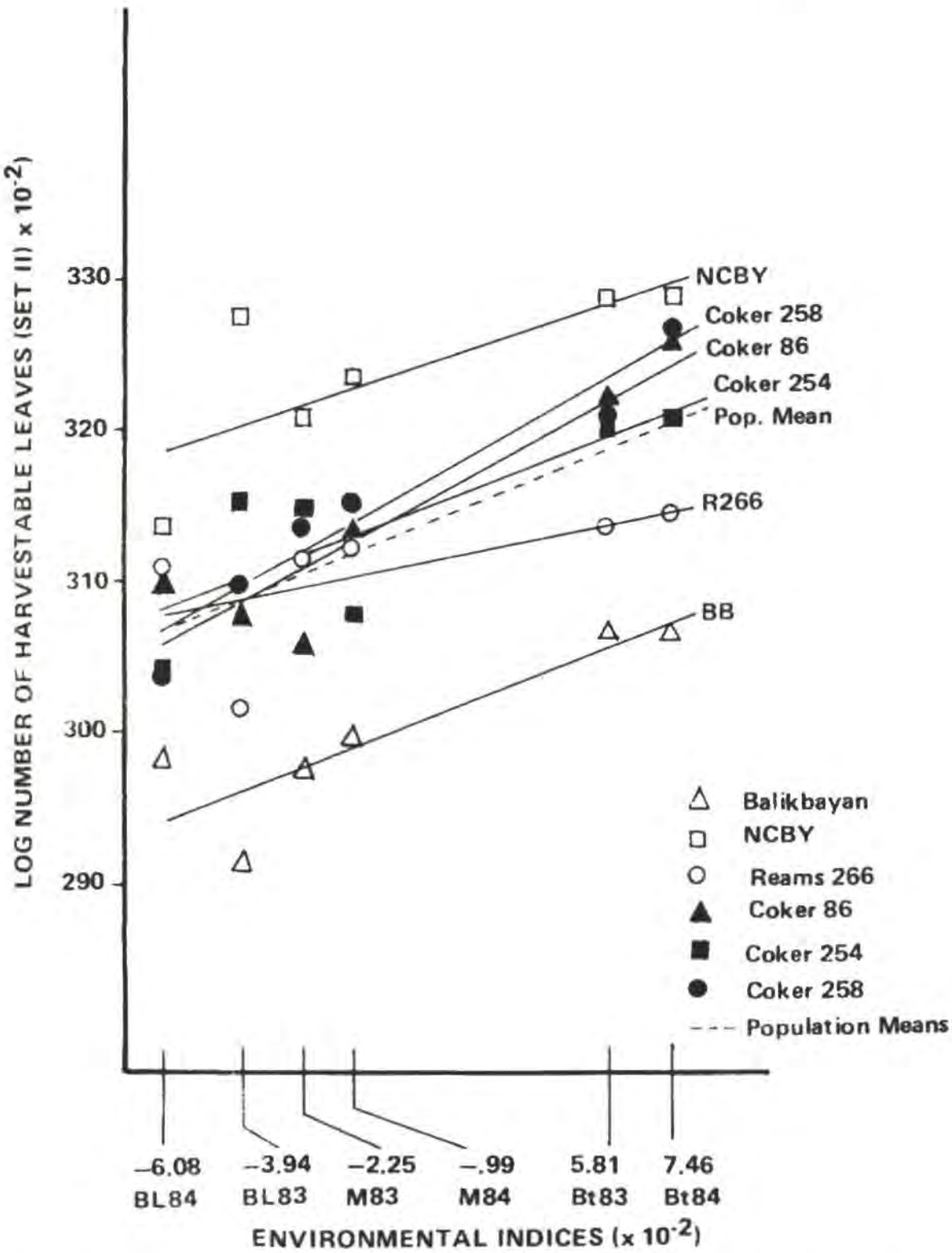

Fig.10. Regression lines (SET II) showing the relationship of log number of harvestable leaves of 6 tobacco varieties and population mean grown at different location and years – Bt., Batac, Ilocos Norte; M., Marcos, Ilocos Norte; S.J., San Juan, Ilocos Sur; PA., Pidigan, Abra; BL., Balaoan, La Union; 83 and 84 represent crop year 1982-83 and 1983-84, respectively.

Table 7. Summary of variety means, adaptation and stability parameters for log days to flower of 6 tobacco varieties grown at 6 environments (Set II)[1]

Variety	*Mean performance (tons/ha)*	*Adaptability (b-values)*^{ns}	*Standard deviation from regression* (s^2_d)	*Classification*
Balikbayan	84.41	.9646	5.0×10^{-4}**	unstable
NCBY	82.20b	.9990	2.0×10^{-5}	stable
Reams 266	80.66bc	1.0833	-4.0×10^{-5}	stable
Coker 86	80.50bc	0.9175	0.2×10^{-4}*	unstable
Coker 254	79.28c	1.0891	-4.0×10^{-5}	stable
Coker 258	79.70c	0.9444	1.0×10^{-4}	stable

[1]Any two means having a common letter are not significantly different at 5% level of significance.
* and ** – significantly different from zero (0) at 5% and 1% level, respectively.
ns – not significant

Table 8. Summary of variety means, adaptation and stability parameters for plant height of 6 tobacco varieties grown at 12 environments (Set II)[1]

Variety	*Mean performance (tons/ha)*	*Adaptability (b-values)*^{ns}	*Standard deviation from regression* (s^2_d)	*Classification*
Balikbayan	157.86c	1.0290	42.6916*	unstable
NCBY	162.99b	1.0901	19.0737	stable
Reams 266	172.12b	.9662	21.4069	stable
Coker 86	170.02b	.9207	11.8852	stable
Coker 254	200.51a	1.0008	17.4160	stable
Coker 258	170.76b	.9933	21.6453	stable

[1]Any two means having a common letter are not significantly different at the 5% level of significance.
*Significantly different from zero (0) at 5% level of significance.
ns – not signficant

Ideal genotype

An ideal genotype, may be defined as one with maximum yield potential, adaptable to a wide range of environments and stable. Stable genotype shows minimum variation in a wide range of environments.

Among the six varieties, Balikbayan and NCBY ranked first in cured yield, and both varieties were adaptable to any environment and stable. Balikbayan had the best cured leaf quality but unstable based on s^2_d. Also in grade index, NCBY and Reams 266 ranked second but the latter was unstable. Hence considering

cured yield and cured leaf quality, NCBY meets the definition of an ideal genotype. In addition, NCBY had the most number of harvestable leaves which indicated its good vegetative potential.

Table 9. Summary of variety means, adaptability and stability parameters for number of harvestable leaves of 6 tobacco varieties grown at 6 environments (Set II) [1]

Variety	*Mean performance (tons/ha)*	*Adaptability (b-values)*	*Standard deviation from regression* $(s^2_d)^{ns}$	*Classification*
Balikbayan	29.09c	.94779	2.0×10^{-5}	stable
NCBY	25.47a	.80234	1.02×10^{-3}	stable
Reams 266	22.39b	.50669	6.8×10^{-4}	stable
Coker 86	23.16b	1.36445	2.5×10^{-4}	stable
Coker 254	23.10b	.97218	1.02×10^{-3}	stable
Coker 258	23.39b	1.40691@	-6.0×10^{-4}	unstable

[1] Any two means having a common letter are not significantly different at the 5% level of significance.
@ – significantly different from 1.0 at 5% level of significance.
ns – not significant.

Summary and Conclusion

$\hat{\sigma}^2_y$ and $\hat{\sigma}^2_\ell$ were not significant for all the tobacco traits except for $\hat{\sigma}^2_y$ of cured yield. Highly significant $\hat{\sigma}^2_{y\ell}$ was observed which indicates that the ranking of the different locations based on the mean performance of the genotypes used differed from year to year. Genotypic differences were present. The small $\hat{\sigma}^2_{gy}$ and $\hat{\sigma}^2_{g\ell}$ and for most of the traits were not significant. $\hat{\sigma}^2_{gy}$ was significant for days to flower, plant height and number of harvestable leaves but it was a small fraction of the genotype variance.

The results indicate that there was some differential response to environments, but it was not accounted for by the location or year grouping. The large $\hat{\sigma}^2_{y\ell}$ and significant second order interaction ($\hat{\sigma}^2_{gy\ell}$), compared to first order interaction ($\hat{\sigma}^2_{gy}$ and $\hat{\sigma}^2_{g\ell}$) suggest that the variation of the environment falls under the unpredictable category of interaction.

Estimates of $\hat{\sigma}^2_g$ were much greater than those of $\hat{\sigma}^2_{gy}$, $\hat{\sigma}^2_{g\ell}$ and $\hat{\sigma}^2_{gy\ell}$ for all characters except for grade index where $\hat{\sigma}^2_{g\ell}$ was slightly greater than $\hat{\sigma}^2_g$. The implication of small $\hat{\sigma}^2_{ge}$ components is that promising lines identified in preliminary trials (single season-location test) can be entered into advanced trials.

It was observed that when number of replications and years were kept at a fixed point, and the number of location was increased, the variance of a variety

mean decreases. The increment in the decrease of variance decreased when it reached the optimum plot allocation. Substantial reduction in variance from addition of a single year for a given number of replication and location reduces the expected variance more effectively than increasing the number of replication.

Several plot allocations were compared to the PTRTC testing procedure (4 replications, 7 locations and 2 years). Giving equal importance to the five parameters, the acceptable optimum plot allocation for tobacco varietal testing would be 3 replications, 6 locations for 2 years but a reduction of plots from 56 to 36 (20 plots reduced) and with an average of 10% increase in cv from the PTRTC procedure.

In the fixed model (set II), the adaptation and stability for all traits with significant GE interaction was estimated. All of the tobacco varieties were highly adaptable to a wide range of environment except for Coker 258 which is adaptable only to favorable environments considering grade index and number of harvestable leaves.

An ideal genotype is one with maximum yield potential, adaptable to a wide range of environment and stable. NCBY met this criteria considering cured yield, grade index and number of harvestable leaves and was therefore recommended as the check variety for PTRTC varietal improvement trials.

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THE DEVELOPMENT OF A MONITORING SYSTEM FOR CORN BORER *OSTRINIA FURNACALIS* (GUENEE)

B. F. Cayabyab and E. A. Benigno
National Crop Protection Center
University of the Philippines at Los Baños
College, Laguna, Philippines

ABSTRACT

A monitoring study for corn borer, *Ostrinia furnacalis* (Guenee) was conducted at the Central Experiment Station, U.P. at Los Baños during 1985 wet season and 1986 dry season.

Kerosene light trap, crude pheromone extract and no bait were tested for 1985 wet season. Statistically, the light trap was significantly higher than the rest.

Virgin female, light trap, crude pheromone extract and no bait were studied for 1986 dry season. Statistically, the most efficient was the virgin female, followed by kerosene light trap and the crude pheromone extract. Except for the 50 female tips extracted from acetone, all the extracts were not significantly different from the control.

To relate the monitored data with practical insect pest management, a model that can predict a single field population of the corn borer by age class was utilized. Simulation were done on 7, 9, and 14 days catches by 25 female tips extracted from heptane and light trap during the wet season. Another run of the model using pest management sub-routine and the same immigration data were processed. The same immigration days were used for the virgin female data during dry season.

The observed adult peak timing was correctly predicted during wet season. The simulated peak egg deposition was nearly predicted in both season when compared to the observed peaks.

Introduction

Except for cultural practices and varietal resistance which are still wanting when it comes to corn borer control, all the other methods share a common feature which is the presence of infestation prior to the initiation of control. It seems that monitoring system in the said methods is wanting. Also lacking are the most necessary environmental data that coincide with the coming of adults and the resulting development trend.

The use of pheromone to monitor population of lepidoptera is very attractive. The simplicity of construction and maintenance of the traps and their species-specificity give them many advantages over other methods (Campion and Nesbit 1983). Of the various monitoring methods for corn borer adults, the use of phero-

mone traps seems to be effective and inexpensive (Benigno, 1983). Sweeping is limited at a certain stage of the corn plants. It may also cause unnecessary damage to the plant. Suction devices are cumbersome to operate and laborious. Light traps are unreliable due to fluctuating luminiscene. In addition, fuel is costly and electricity is not always available. Passive traps are also non-workable (Jackman *et al.*, 1983).

The virgin female corn borer moth is known to produce a sex pheromone which Klun *et al.* (1980) identified and synthesized as (Z) and (E)-12-tetradecen-1-01 acetate in 1:1 geometric proportion. Thus, the pheromone component can be used for monitoring. The monitored data from corn borer bait can be utilized for population simulation.

Modelling of the corn borer mainly based on study by Camarao (1976, a & b) Jackman *et al.* (1984) with the incorporation of results of recent biological studies Saito and Oku (1976), Saito (1979), Saito (1980), Magalit (1983) and Lizarondo (1983), proved that more realistic modelling and simulation could be achieved. Lately, Lynch *et al.* (1984) found that heptane extract of ovipositors from lesser corn stalk females contain 10 compounds. Test with combination of these compounds showed that a mixture of these 10 compounds was as effective as virgin females in luring males into traps. This might also be true in corn borer.

This paper presents the comparative performance of three monitoring methods for corn borer. It also describes the utilization of trap catones data from the said methods for computer simulation of corn borer population.

Materials and Methods

Experimental area and experimental plots

A 4500 square meters plot was utilized for the rainy season experiment, while a 3500 square meters area was utilized for dry season. IPB Var 1 was used in both trial. Planting, weeding, cultivation, fertilization and yield determination were based on the current recommended practices (UPLB-NFAC) Countryside Program. Control plots were also maintained.

Insect traps

Empty one gallon ice cream plastic cans were used throughout this study. A gallon is 20.3 cm in diameter and 12.7 cm deep. The gallon was placed in a 50 cm x 50 cm wood frame with an identical frame for cover and protection of bait supported on four corners by posts. The trap can be easily adjusted to the height of corn plants at a given stage by nails on the post. The posts used were ipil-ipil (*Leucaena leucocephala* Lam de Wit) and kakauate (*Gliricidia sepium* (Jack). The basins were filled with water at 3 cm and 1 teaspoon of commercial detergent was added.

Baits

Only crude pheromone extracts from male and female corn borers were used during the wet season. The females and males were reared individually in vials from larvae and pupae that were either mass-reared in the laboratory of field-collected.

Extracts were prepared by cutting at least 1-3 cm of the tip of abdomen of both the male and female corn borer with the use of ordinary nail cutter.

Extraction was done on rubber septa (Jackman *et al*, 1983, 1984) using three different solvents replicated three times per rate density of tip of abdomen. The solvents consisted of acetone, acetone after heptane and heptane alone. Extraction time was for 60 seconds instead of the 10 seconds method employed by Jackman *et al.* (1983, 1984).

The adult corn borers were 1-3 days old at the time of excision. The dry season experiment utilized three types of bait. These included virgin females, no bait and crude pheromone extracts. Virgin females were maintained individually in vials under ambient laboratory conditions. Healthy moths were selected as baits when deployed in the field. These were at least 1-2 days old. The moths were replaced when they perished.

The usual procedure for crude pheromone extraction was done. This time extraction was only on females at a rate of 25 and 50 tips of abdomen. The same solvent extraction time and storage were maintained. In both dry and wet season experiment, unbaited traps were deployed for the control. Likewise, a kerosene light trap was maintained for further comparison in the duration of the experiment.

Trap location

Traps were placed at the periphery or around the edges of corn fields from the date of emergence up to seven days before harvest. A total of 30 traps were deployed during the rainy season while 24 traps were used during the dry season. The traps were approximately 6 meters apart. Each rate or density of tip of abdomen and the virgin females were randomly deployed and replicated three times.

Trap maintenance

Pheromone crude extracts from both male were housed separately in a 3 cm by 10 cm wire screen cage suspended at 4-8 cm above the center of the water surface during the rainy season.

The virgin female was utilized in addition to the septum during the dry season. A 10% honey in water solution on a cotton swab served as food of the virgin female bait. The virgin females were changed whenever they were found dead. Water levels, bait and trap construction materials were maintained during the trapping duration.

Insect data collection

The collection of trapped corn borers was on a daily basis. Egg masses, larvae and pupae were counted 2-3 times a week.

Data analysis

The HP9845 B desktop computer statistical package was used for data analysis. The trap catches were transformed using the equation $\sqrt{x + 0.5}$ (Steel and Torrie, 1960). A one way ANOVA and two way ANOVA were employed for comparing overall trap performance.

A model that can predict a single field population of the corn borer *Ostrinia furnacalis* (Guenee) by age class through time was adopted from Jackman and Benigno (1983). The model can compute a time series of population density by age class form the input of temperature and immigration data.

The corn borer model

The model summarizes the ecological research on corn borers. The equations for development, oviposition and survival rates were derived from the studies of Camarao (1976a and b) and Saito and Nakayama (1981).

Results and Discussion

Wet season monitoring

Table 1 describes the comparison of efficiency between the individual traps.

Table 1. Multiple comparison of different individual monitoring methods. Wet Season, CES (June 16 – August 19, 1985)

Trap Method	*Mean**
5A**	.0367 a
5H	.0367 a
5AA	.0733 a
25A***	1.5144 a
25H	1.4078 a
25AA	1.2211 a
25MA****	.2567 a
25MH	.0367 a
25MAA	.0733 a
Kerosene light trap	12.8889 b
Control	.1100 a

*Means with the same letter are not significantly different at a = .05
**5 female tips extracted in A = acetone, H = heptane and AA = acetone after heptane
***25 female tips extracted in A = acetone, H = heptane and AA = acetone after heptane
****25 male tips extracted in A = acetone, H = heptane and AA = acetone after heptane

The tip rates of crude pheromone extracts are not significantly different from each other. On the other hand, the kerosene light trap is statistically significant with the rest of the traps. The kerosene light trap is the most efficient trap that monitored the adult corn borer.

Nevertheless, a closer look into the traps monitored data of the 25 female tips using the solvents, acetone, heptane and acetone after heptane showed that these have the highest catch among the extracts. These extracts likewise approximated the catch of the kerosene light trap in terms of consistency/nightly trapping including the quantity of catch. The extracts even attracted more male corn borer during the early entry/immigration of corn borers into the experimental corn field. The appearance of females particularly in the septa with male extracts suggests that there are also extractable compounds in the male tips that can readily attract females and this was confirmed by Atkinson (1981) in the case of the African sugarcane borer, *Eldana saccharia*. The presence of occasional catches in the control was due to simple blundering (Roeloffs and Carde, 1977).

Dry season monitoring

The relative efficiency of the individual traps is depicted in Table 2.

Table 2. Comparison of individual monitoring methods. Dry Season, CES (January 30 April 16, 1986)

Trap Method	*Mean Catch**
25A**	.0744 ab
25H	.0522 ab
25AA	.1044 ab
50A***	.3799 b
50H	.0522 ab
50AA	.1488 ab
Virgin Female	4.6095 d
Kerosene Light Trap	1.2947 c
Control	0 a

*Means with the same letter are not significantly different at a = .05
**25 female tips extracted in A = acetone, H = heptane and AA = acetone after heptane
***50 female tips extracted in A = acetone, H = heptane and AA = acetone after heptane.

All 25 female tips extracts are not significantly different with the 50 female tips extracts and the control. However, the 50 female tips extracted from acetone is statistically significant when compared with the control. The kerosene light trap is statistically different with all the extracts and the control while the virgin female is statistically significant with the rest.

It is interesting to note that in the absence of the virgin female baits, the extracts and light traps caught more in the wet season.

Wet season simulation

Table 3 shows the simulated and actual peak timing of egg and adult corn borer without pest management from the immigration data of a septum with 25 virgin female tips extracted in heptane.

The peak egg deposition for simulation using 7 days immigration input was 55 days after emergence. This true also for the 9 and 14 immigration days. The actual data for peak egg deposition was at 51 days. Thus, the predicted simulated value was 4 days late.

The simulated adult peak timing of emergence using 7 days immigragion input was at 51 days after emergence. Again the remaining two sets of immigration inputs showed the same peak. Nevertheless, the 14 days immigration entry yielded two adult peaks at 51 and 61 days after emergence. The observed peak adult emergence was 51 DAE. Hence the three runs duplicated the actual peak.

The simulated peak of egg deposition and adult appearance with pest management subroutine once more showed the usual peak egg deposition at 55 DAE and peak adult density at 51 DAE.

The only perceptible difference between the two runs of the model is that there was a reduction of egg density in the model with pest management subroutine. From an original 3111.94 egg density, this decreased to 2964.47. Other than this, the egg and adult simulated densities using 7, 9 and 14 days immigration inputs were the same. In short, the model can utilize either inputs and still generate the same peaks. However, a higher number of immigration input is better since it can simulate two or probably more adult peak instead of one as in 7 and 9 days immigration input. These peaks in 14 days immigration input can be used when compared with the observed peaks, that is there are more peaks that can be validated against the actual data.

Table 3 also shows the simulated peak timing of 14 days immigration input from kerosene light trap's male corn borer catches.

The run model with no pest management generated two simulated egg peaks at 52 and 54 DAE. These peaks are 1 day and 3 days late from the observed peak. It is clear that the kerosene light trap input provided the nearest predicted egg peak timing value as compared to the other runs from the 25 virgin female tips extracted from heptane. The run model with pest management predicted the adult peak emergence at 51 DAE like the other simulated run using 25 virgin female tips extracted from heptane. It was likewise noted that the observed peak of emergence from the 25 female tips and kerosene light trap coincide at 51 DAE with 17 and 5 male catches respectively for the said traps. Moreover, like the 14 days immigration input from the 25 female tips extracted from heptane, there was also corresponding increase of adult peaks in kerosene light trap.

The model shows that with increased immigration days input in both monitoring methods, there is a parallel increase in peak emergence of adult corn borers when compared with the actual data. In like manner, Nakasuji and Fijita (1980)

Table 3. Comparison of simulated and observed peak of corn borer population based from 25 female tips extracted in heptane and kerosene light trap catches. (Up to 65 days after emergence). Wet Season, CES (June 26 – August 19, 1985)*

Pest management practice	*Peak stage*	*25 Heptane*												*Kerosene Light Trap*					
		Simulated									*Observed*			*Simulated*			*Observed*		
		7 days immigration			*9 days immigration*			*14 days immigration*						*14 days immigration*					
		Date	*DAE*	*Number*	*Date*	*DAE*	*Number*	*Date*	*DAE*	*Number*	*Date*	*DAE*	*Number*	*Date*	*DAE*	*Number*	*Date*	*DAE*	*Number*
None	Egg	Jul. 18	(55)	3111.94	Jul.18	(55)	3162.98	Jul.18	(55)	3162.98	Jul 14	(51)	17	Jul. 15	(52)	1681.05	Jul. 14	(51)	17
								Jul. 14	(51)	19.94	Jul. 14	(51)	17	Jul. 17	(54)	1701.08	Jul. 14	(51)	5
	Adult	Jul. 14	(51)	19.94	Jul.14	(51)	19.94	Jul. 25	(61)	3.30	Jul. 16	(53)	24						
Four Spray** + One Detasseling	Egg	Jul. 18	(55)	2964.47	Jul 18	(55)	3162.98	Jul. 18	(55)	3162.98	Jul. 14	(51)	17	Jul. 12	(49)	8.77	Jul. 14	(51)	17
	Adult	Jul. 14	(51)	19.94	Jul. 14	(51)	19.94	Jul. 14	(51)	19.94	Jul. 14	(51)	17	Jul. 14	(51)	11.33	Jul. 14	(51)	5
								Jul. 25	(61)	3.30	Jul. 16	(53)	24	Jul. 21	(58)	5.81			

*Observed egg density based from 200 random samples while observed adult density was based from the average of 3 crude pheromone extract bait and light trap.
**Sprays at 20, 39, 53 and 51 DAE, detasseling at 44 DAE.

Table 4. Comparison of simulated and observed peaks of corn borer population based from virgin female trap catches* (Up to 65 days after emergence) Dry Season, CES (January 30-April 16, 1986)

Pest management practice		*Simulated*									*Observed*		
	Peak stage	*7 days immigration*			*9 days immigration*			*14 days immigration*					
		Date	*DAE*	*Number*	*Date*	*DAE*	*Number*	*Date*	*DAE*	*Number*	*Date*	*DAE*	*Number*
None	Egg	Mar. 20	(60)	52790.65	Mar. 23	(63)	89234.93	Mar. 21	(61)	168832.02	Mar. 12	(53)	15
	Adult	Mar. 18	(58)	306.83	Mar. 12	(52)	95.90	Mar. 18	(58)	1067.43	Mar. 5	(45)	25
					Mar. 15	(55)	269.13	Mar. 20	(60)	946.04	Mar. 22	(62)	25
					Mar. 21	(61)	520.81	Mar. 24	(64)	689.08			
One Spray at Whorl**	Egg	Mar. 20	(60)	50568.02	Mar. 23	(63)	88294.76	Mar. 21	(61)	161054.27	Mar. 12	(53)	15
	Adult	Mar. 19	(59)	285.48	Mar. 12	(52)	24.03	Mar. 18	(58)	1018.34	Mar. 5	(45)	25
					Mar. 15	(55)	224.99	Mar. 20	(60)	910.57	Mar. 22	(62)	25
					Mar. 21	(61)	504.16	Mar. 24	(64)	670.56			
Detasseling***	Egg	Mar. 20	(60)	50568.62	Mar. 23	(63)	88294.76	Mar. 21	(61)	161054.27	Mar. 12	(53)	15
	Adult	Mar. 19	(59)	285.48	Mar. 12	(52)	24.03	Mar. 18	(58)	1018.34	Mar. 5	(45)	25
					Mar. 15	(55)	224.99	Mar. 20	(60)	910.57	Mar. 22	(62)	25
					Mar. 21	(61)	504.16	Mar. 23	(63)	658.73			

*Observed egg density based from 200 random samples; adult based from average of 3 virgin female baits.
**Spray at 16 DAE against cutworm and 43'DAE for economic threshold against corn borer.
***Spray at 16 DAE; detasseling at 53 DAE.

found in their simulation test that the capturing rate of males or mating rates of females during a short period is not advisable to use as input.

Dry season simulation

Table 4 shows the simulated and actual observed peaks of eggs and adult with and without pest management practice.

The simulated peak egg deposition for 7, 9 and 14 days immigration input were 60, 63 and 61 days after emergence, respectively. The simulated adult peaks emergence are 58 days after emergence for 7 days immigration 52, 55, and 61 days after emergence for 9 days immigration and 58, 60 and 64 days after emergence for 14 days immigration input. In contrast, the actual egg peak deposition was 53 days after emergence while the adult peak emergence were at 45 and 62 days after emergence. The obvious disparity in peak egg deposition between the predicted and observed date can be attributed to the three day sampling interval of egg count. It was possible that the observed peak was missed in the process.

The actual peak egg deposition was very near the 7 days immigration input, while the adult peak density especially the second was approximated by the 9 and 14 days immigration input. Hence, as previously mentioned it is noteworthy to run the model based on several immigration days to find the most appropriate number of immigration. This is important when comparing the actual peak (observed) from the simulated peak.

This computer simulation model is essential in timing insect post management controls, assessment of control efficacy and a deeper analysis of the effects of natural enemies and physical factors such as temperature, wind velocity and others. These factors can be easily incorporated into the model and enhance the predictive value of the generated peak timing.

It is likewise noteworthy to explore the possibility of using virgin females as actual control measures for mass trapping due to their unusual ability to attract a large number of male corn borers. Moreover, it is important to test new synthetic extracts derived from corn borer with the use of different solvents in view of the findings of Jackman *et al.* (1984) where different response to different solvents was observed. Furthermore, it is essential to increase the number of tips to be extracted in crude pheromone extracts in order to see if increasing tip extraction will really increase catch. Finally, the newly explored topic of mating disruption as a promising control measure must also be given due attention.

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RAISING THE YIELD POTENTIAL OF RICE

Benito S. Vergara
International Rice Research Institute, Los Baños, Laguna, Philippines

ABSTRACT

With the introduction of the modern high yielding rice varieties and appropriate cultural practices, yields have increased in the last two decades. Subsequent efforts to improve yielding ability by increasing photosynthetic rate, increasing biomas production and increasing harvest index have not resulted in significant increase in yields. An approach being pursued to achieve this end is the manipulation of the weight of single grains. Results of studies have shown the following.

Increasing the number of high density (HD) grains can increase yield potential. HD grains result in better milling recovery and higher head rice recovery. Varietal differences in the number of HD grains per panicle exist.

Within a panicle, certain spikelets invariably develop into HD grains. Most spikelets on the primary branches are HD grains; spikelets on the secondary branches have low grain weights. Leaves near the panicle are more important in grain filling. Removal of the 4th leaf from the top increased grain weight and number of HD grains.

Lower temperature or higher photosynthetically active radiation after anthesis result in higher number of HD grains. Applied nitrogen fertilizer had no effect on the number of HD grains.

Limitations in grain filling may be the result of several factors. Although sufficient carbohydrate is available, not all spikelets develop into HD grains. Factors limiting grain filling include structure of the pedicel, the spikelets, and growth regulators.

In view of the above findings, a new plant type is proposed to break the yield ceiling. Further studies are being conducted to identify the limitations of the current varieties in order to develop the new plant type being proposed.

Introduction

Rice yields have greatly increased in the last two decades mainly through crop improvement and the accompanying cultural practices. With the development of IR8 and subsequent cultivars of similar type, rice yields increased in the tropics; but now yields have apparently reached a plateau (Flinn *et al.*, 1982). Subsequent efforts to improve yielding ability have not resulted in visible gains.

The present efforts to raise the yield potential focuses on increase in photosynthetic rates, in biomass production, and in harvest index (HI) (IRRI, 1982).

Increase in Photosynthetic Rate

Research on high photosynthetic rates in the last several years has not really benefited or increased grain yields in most crop plants. Identification of varietal differences in chlorophyll content (Kariya and Tsunoda. 1980; Sasahara *et al.*, 1983; Yamakawa and Oshima, 1977) and photosynthetic rates (Murata, 1957; Murata and Iyama, 1963) has not led to improvement in rice grain yields. Varietal improvement in rice through the years showed no improvement in photosynthetic rates (Evans *et al.*, 1984). There is no clear-cut evidence that a cultivar with high leaf photosynthetic rate has improved yield potential (Yoshida, 1972). Accompanying changes such as better translocation and partitioning of photosynthates might be necessary for an improvement in photosynthesis to be effective. Many have tried to isolate cultivars with high photosynthetic rates, but the advantages of such varieties have yet to be demonstrated or used by plant breeders. For all the research conducted on photosynthesis, it is yet to be proven that increase in photosynthetic rates of a cultivar will increase grain yield.

Increase in Biomass Production

Varietal differences in biomass production, more specifically in crop growth rates, have been studied but no improvement has been reported (Evans *et al.*, 1984). The theoretical limit for biomass production has not been reached, but available data suggest that the present high production can be effectively increased only if the growth duration is increased and proper partitioning is obtained. Without proper partitioning, increase in biomass only leads to higher proportion of non-photosynthesizing plant parts or increase in plant height. Without a strong and thick culm, such increases in biomass would only result in lodging and mutual shading and eventual decrease in grain yield instead of the desired increase. This is the case in traditional varieties whose high biomass production in the early stages results in mutual shading so that the mean photosynthetic rate per unit leaf area and crop growth rate decrease.

Increase in Harvest Index

The HI has increased from less than 0.10 to 0.55 in the modern varieties (IRRI, 1978; Evans *et al.*, 1984). This is one of the main features responsible for the yield increase. The increase in HI resulted in less straw or less non-photosythesizing plant parts and a decrease in plant height, which increased lodging resistance (Tanaka *et al.*, 1966). Further increase from 0.55 to 0.60 generally did not improve grain yields. Plants with 0.60 HI are generally very short with telescoping leaves, low tiller number, and low spikelet number. Because an increase in biomass production tends to lower HI, further increase in HI does not look promising.

Increase in HI through increase in sink size has been tried (IRRI, 1978; Rahman, 1984; Takeda, 1984), either by increasing the number of spikelets per panicle or increasing the spikelet size. This approach has so far not met any success.

Many research institutions have not stopped exerting efforts to increase the yield potential of rice. Since IR8, however, yield potential has not increased. The suggested pathways for increasing yield potentials do not look promising, but we still need to look into them until we find other possible pathways.

Yield Components

Another way of looking at the possibility of increasing the yielding ability is to examine the yield components.

Grain yield is the product of the number of panicles per unit area x number of spikelets per panicle x percent fertility of the spikelets x weight of a single grain.

Normally and under tropical conditions, an increase in panicle number per unit area reduces the number of spikelets per panicle and vice versa (IRRI, 1968). Although agronomic practices can improve the number of spikelets per unit area, the maximum possible has already been achieved and further increase is very difficult (Takeda, 1984).

Increasing the number of spikelets per panicle often results in a large number of empty spikelets (Matsushima, 1957; Kumura and Takeda, 1962; Wada, 1969; Venkateswarlu *et al.*, 1981). This is apparently due to the reduced supply of carbohydrates in relation to the total demand of the spikelets. The optimum number has been reached for the present plant type.

Increasing spikelet size to increase yield potential has also been tried (IRRI, 1978; Rahman, 1984; Takita, 1986), but without success so far. Generally, an increase in spikelet size resulted in a lower number of spikelets per panicle or square meter (IRRI, 1978). There is also a tendency for large spikelets to have only partially filled grains (Takita, 1986, Xiong *et al.*, 1986).

A high percentage of spikelet fertility has already been achieved in the modern cultivars (Yoshida *et al.*, 1972), most of which have around 85% fertility. According to Matsushima (1966), a fertility percentage of around 85 is the correct balance. A percentage lower than 85 indicates a possible source limitation and one higher than 85, a sink limitation. One could aim for 95% fertility that would increase yield by at most 10%. This increase would have to come from better pollination and better development of the spikelets. The former is greatly modified by environmental conditions such as wind, rain, and high and low temperatures. One has very little control of these environmental conditions.

Another alternative in increasing grain yield is to increase weight per grain within a variety. Very little work has been done along this line because workers have accepted the fact that grain weight is the most stable character of a variety (Matsushima 1970), and hence, variability within a variety is very small (Yoshida, 1981). A medium grain variety will always produce medium grains regardless of

environment and cultural practices. Studies by Venkateswarlu and others (1986b) have shown, however, that weight per grain within a variety is highly variable (Fig. 1). One could therefore increase grain yield by increasing the number of heavy or high density (HD) grains.

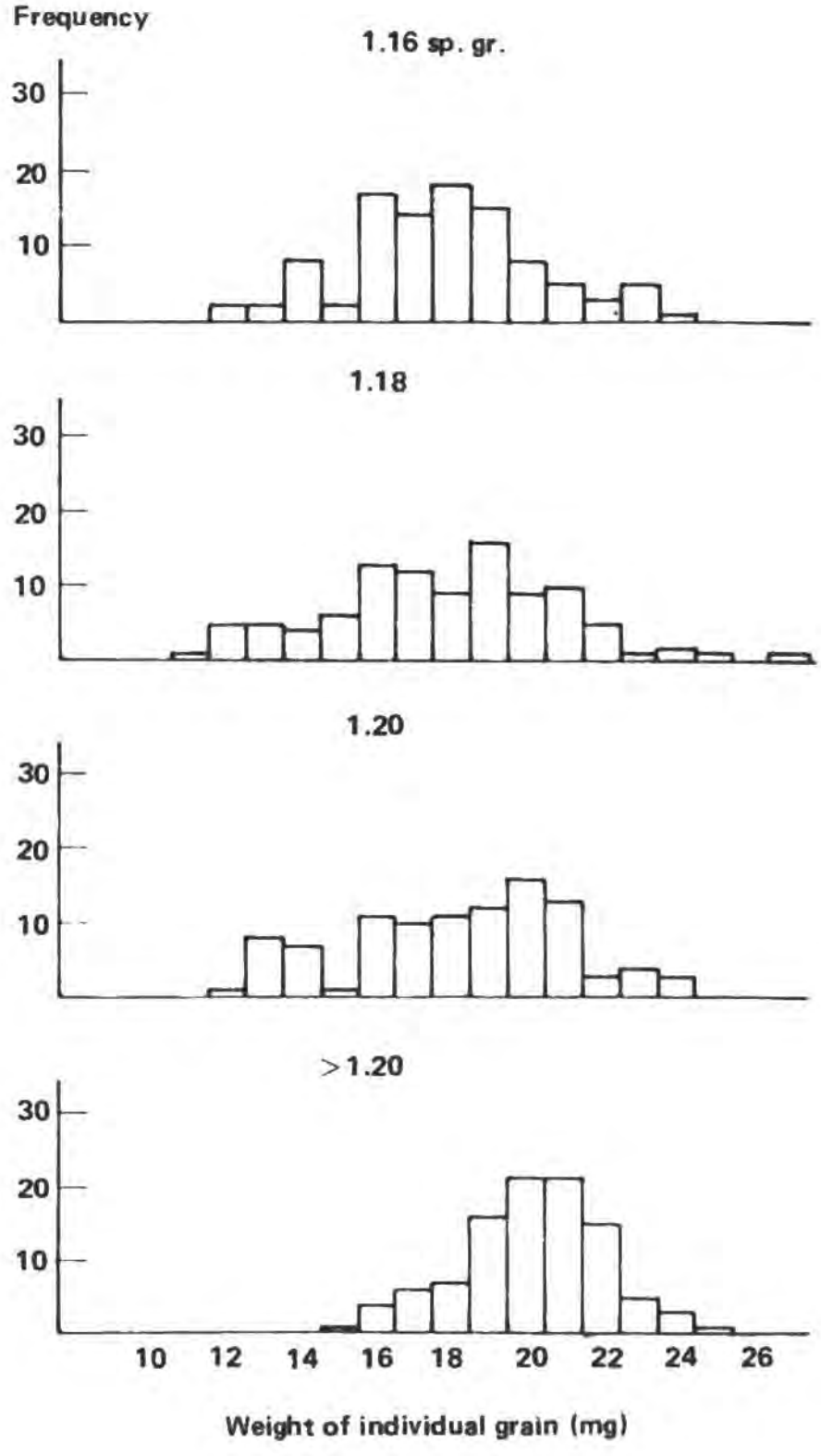

Fig. 1. Frequency distribution of weight of individual grains of IR36, with 1.16 to 1.20 sp. gr. when exposed to 780 μmol $m^{-2}s^{-1}$.

Higher Percentage of High Density Grains

Within a panicle, some grains are heavier and also have higher density (Fig. 2). Usually the 5th and 6th spikelets in a panicle branch have HD grains (Nagato and Chaudhry, 1969; Ahn, 1986). Thus, if we improve the density of the other filled spikelets, one can increase grain yields by as much as 30% in IR8 (Venkateswarlu *et al.*, 1986b). HD grains have not only higher volume and weight (Venkateswarlu *et al.*,, 1986b) but also higher milling and head rice recovery (Venkateswarlu *et al.*, 1985a), which is the final market yield of rice.

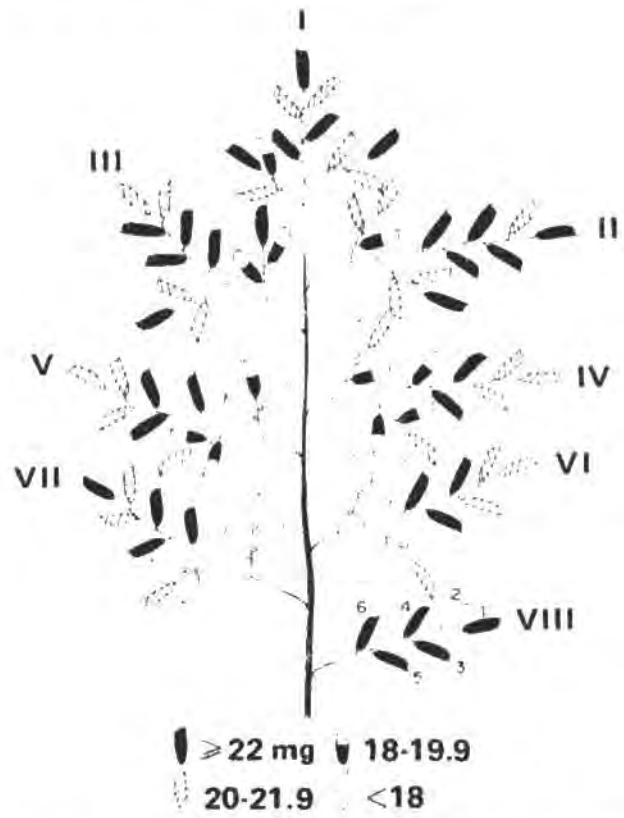

Fig. 2. Location of grains of different weights in a panicle of IR58 (Ahn, 1986). Roman numerals indicate branch numbers, Arabic numerals indicate spikelet numbers.

Table 1. Varietal differences in high density grains. IRRI, 1985 dry season

Designation	*High density grain index (%)*
IR29725	63
IR42	57
IR28222	55
IR28178	50
IR29744	48
Peta	44
IR58	40
IR8	39
Binato	22

HD grains, however, have lower protein and crude fat content (Juliano and Ibabao, personal comm.). Increase in grain weight is due to an increase in starch content. Varieties differ in the deposition of starch. In the indicas, the central part of the endosperm is compact and hard; in the japonicas, the compact starch is on the peripheral region (Nagato and Chaudhry, 1969). This property may be responsible for the lower milling loss of japonicas.

The possibility of increasing the number of HD grains is confirmed by recent research results especially those from the Plant Physiology Department at the International Rice Research Institute.

Varieties differ in the number of HD grains per panicle; therefore, selections for varieties with HD grains can be made (Table 1). The HD grain character is heritable and showed increases in some F_1 hybrids (Fig. 3). Late maturing varieties

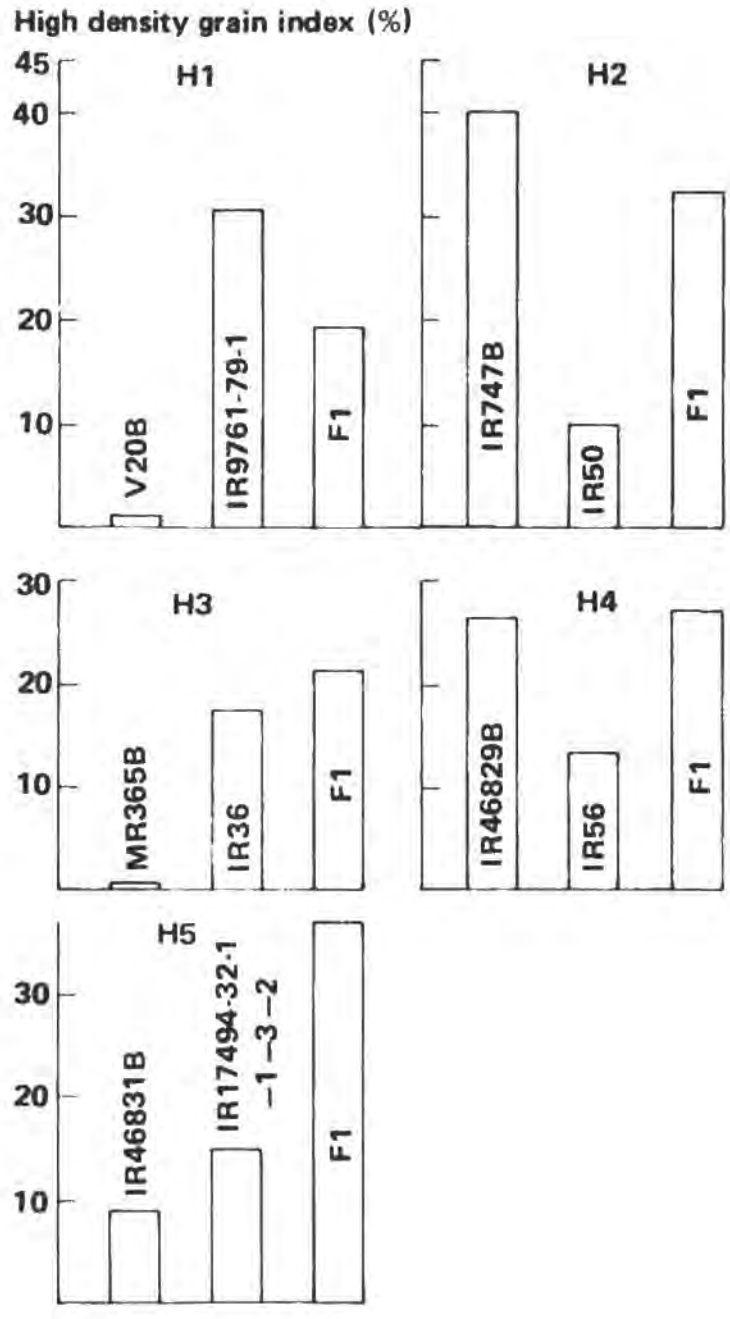

Fig. 3. High density grain index in parents and hybrids of rice (Venkateswarlu, 1986b).

have more uniformity in grain filling than early maturing varieties, and this uniformity resulted in a higher percentage of head rice (Jongkaewattana and Geng, 1986). The occurrence of HD grains had no correlation with 1,000-grain weight in the range of 20.0 to 28.0 g (Venkateswarlu *et al.*, unpublished paper). This would mean that rice grains of varying sizes can be developed while maintaining a high percentage of HD grains.

Contrary to expectations, increasing N application from 0 to 250 kg/ha did not decrease the number of HD grains (Fig. 4). In IR28178, the number and percentage of HD grains actually increased with increase in nitrogen applied.

Wada (1969) reported that increased N fertilization increased spikelet number because of the increase in spikelets on the secondary branches. However, this increase resulted in a higher number of low density grains. However, varietal responses to N fertilization in terms of HD grains produced differ (Venkateswarlu *et al.*, unpublished paper). The non-decrease or increase in HD grains with N fertilization may be the result of a varietal increase in spikelets on the secondary branches accompanied by a higher degree of grain filling. This varietal trait needs further study as it is important in future selection of breeding lines.

Studies on environmental factors such as temperature showed that low temperature or a longer ripening period resulted in a higher number of HD grains (Fig. 5). This indicates that production of HD grains is partly dependent on duration of the ripening period. In the tropics where temperatures are higher, production of HD grains would be hampered because the ripening period is shorter.

Higher photosynthetically active radiation (PAR) from anthesis to harvest greatly increased the number of HD grains (Fig. 6). Low PAR can be a limiting factor in increasing HD grains during the rainy season. HD grains were not realized in all the filled spikelets irrespective of PAR level.

Kato (1986) reported that low PAR resulted in lower weight of all grains in a large-grain variety. In a small grain variety, the grains on the secondary branches and lower branches decreased in weight while the rest remained constant.

Within a panicle, certain spikelets invariably had HD grain (Fig. 2). Spikelets on the secondary branches had low grain weights and removal of other spikelets did not increase the individual weights of spikelets on the secondary branches (Fig. 7). HD filling of spikelets on the secondary branches is not completely related to the amount of available photosynthates.

The HD grains or vigorous spikelets generally flower earlier and fill up earlier (Choi, 1986).

Limitations on Grain Filling

The factors that affect or limit grain filling in obtaining HD grains need further studies.

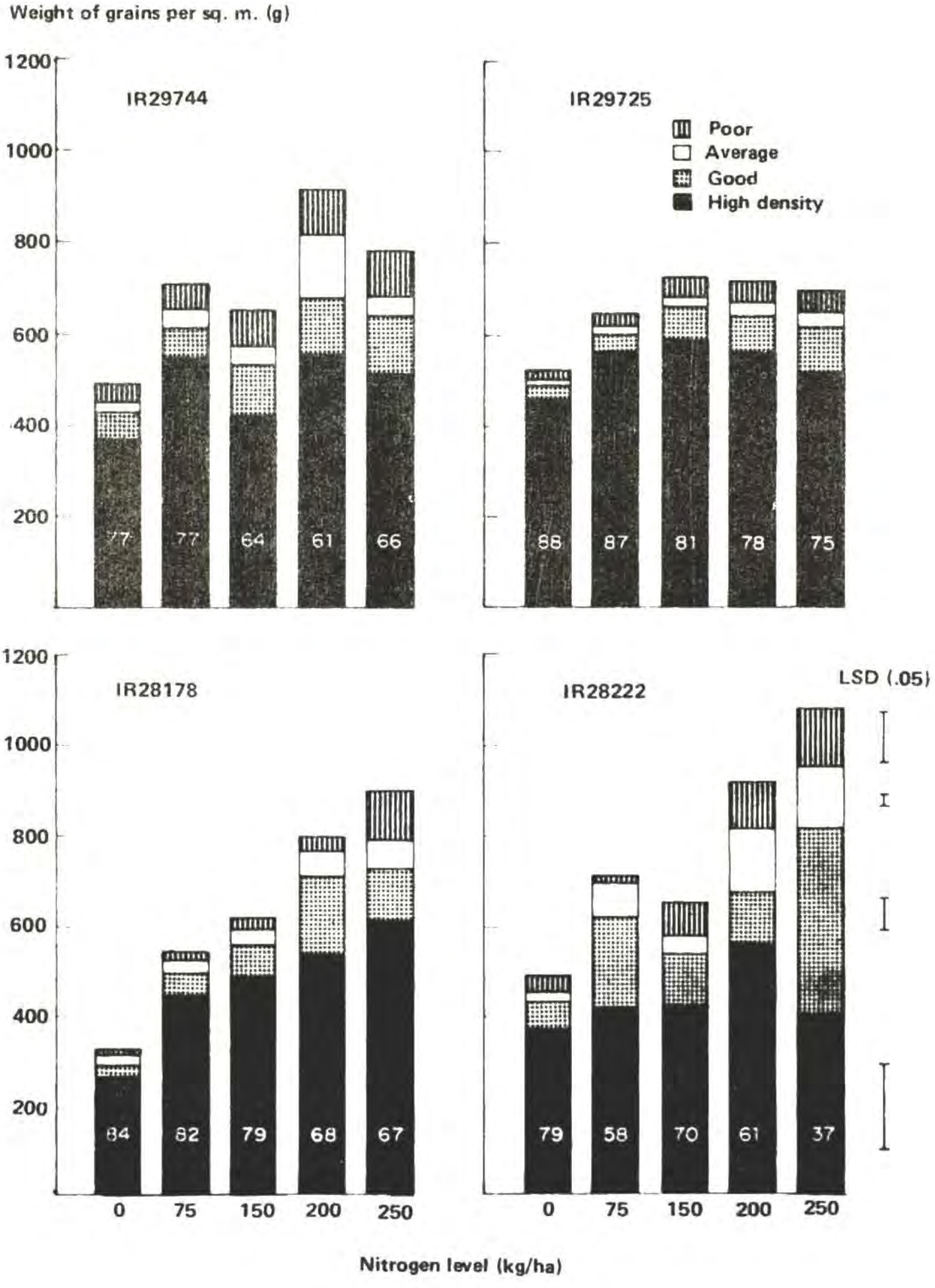

Fig. 4. Number of grains of different grades at varied nitrogen levels (Venkateswarlu, 1986b).

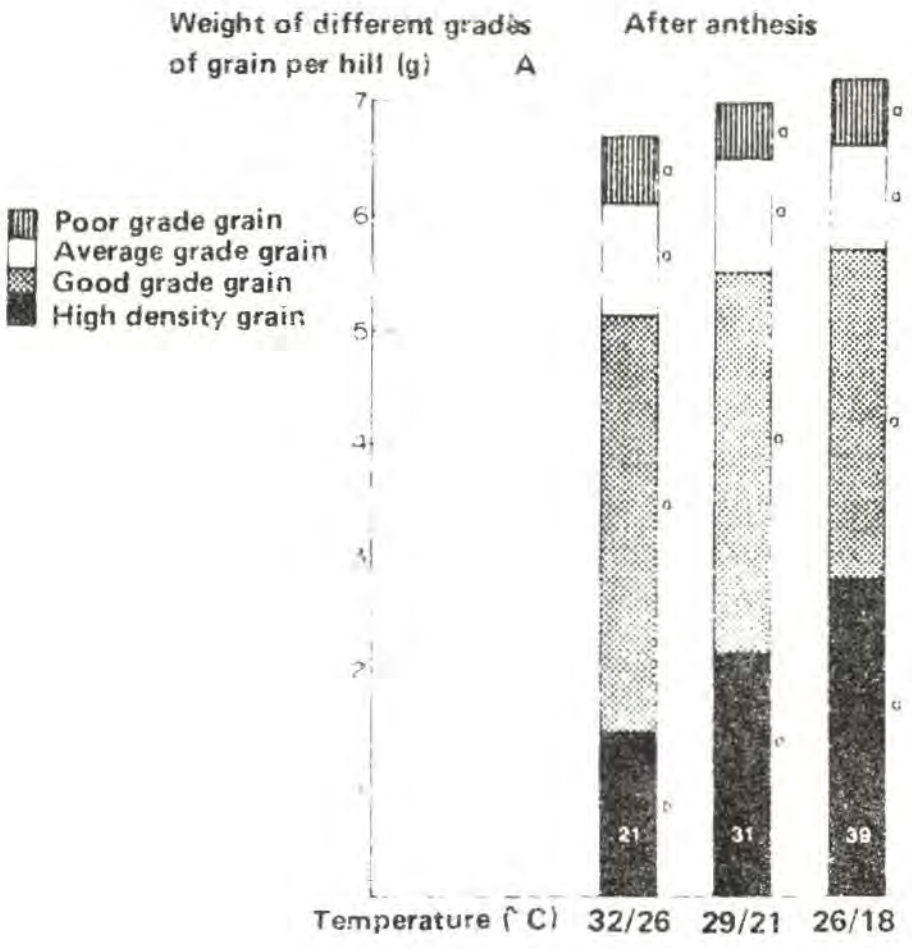

Fig. 5. Influence of temperature regimes on the weight of different grades of grain in IR36 (Venkateswarlu *et al.*, unpublished). The figures in dark shade are percent values. Bars of the same shade followed by the same letter are not significantly different at the 5% level.

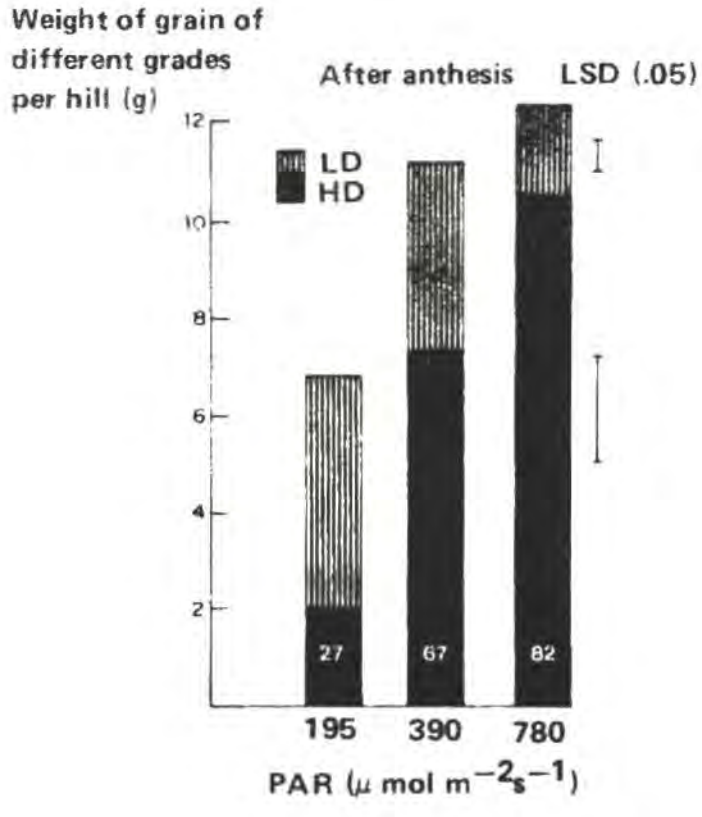

Fig. 6. Weight of low density (LD) and high density (HD) grains of IR36 when exposed to different PARs after anthesis (Venkateswarlu *et al.*, unpublished data).

Carbohydrate supply

The leaves are important in grain filling, depending upon their position on the tiller (Fig. 8). The flag leaf and penultimate leaf supply most of the assimilates to the grains. Removal of the 4th leaf from the top increased grain weight and number of well-filled grains (Ahn, 1986). In the present plant type, carbohydrate is not a limiting factor in obtaining HD grains (Fig. 8). Reduction of sink size by removing various spikelets did not increase the weight of the grains that are normally lightweight (Fig. 7). This was also reported earlier with different varieties of various 1000-grain weights (IRRI, 1978). Kato (1986), however, reported varietal differences: the large grain varieties showed a significant increase in their final grain weight while the small grain varieties did not have any increase.

The supply of sugar precursors did not limit starch accumulation in the grain (Singh and Juliano, 1977). Something else is limiting in the small and medium grain varieties, in some cases, it is as simple as having smaller or poorly developed spikelets to start with.

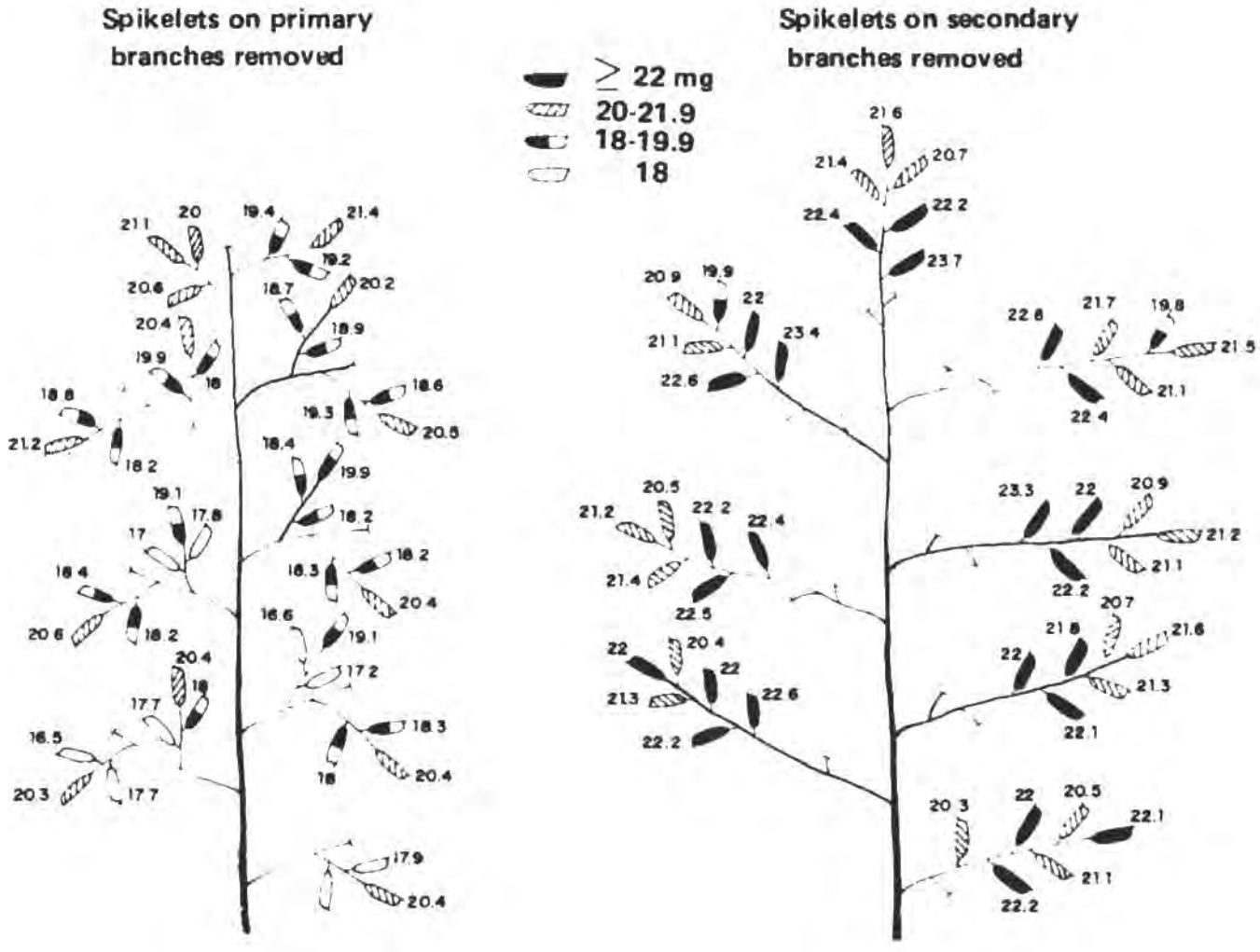

Fig. 7. Effect of sink size and position on the location of IR58 grains of different grades (Ahn, 1986).

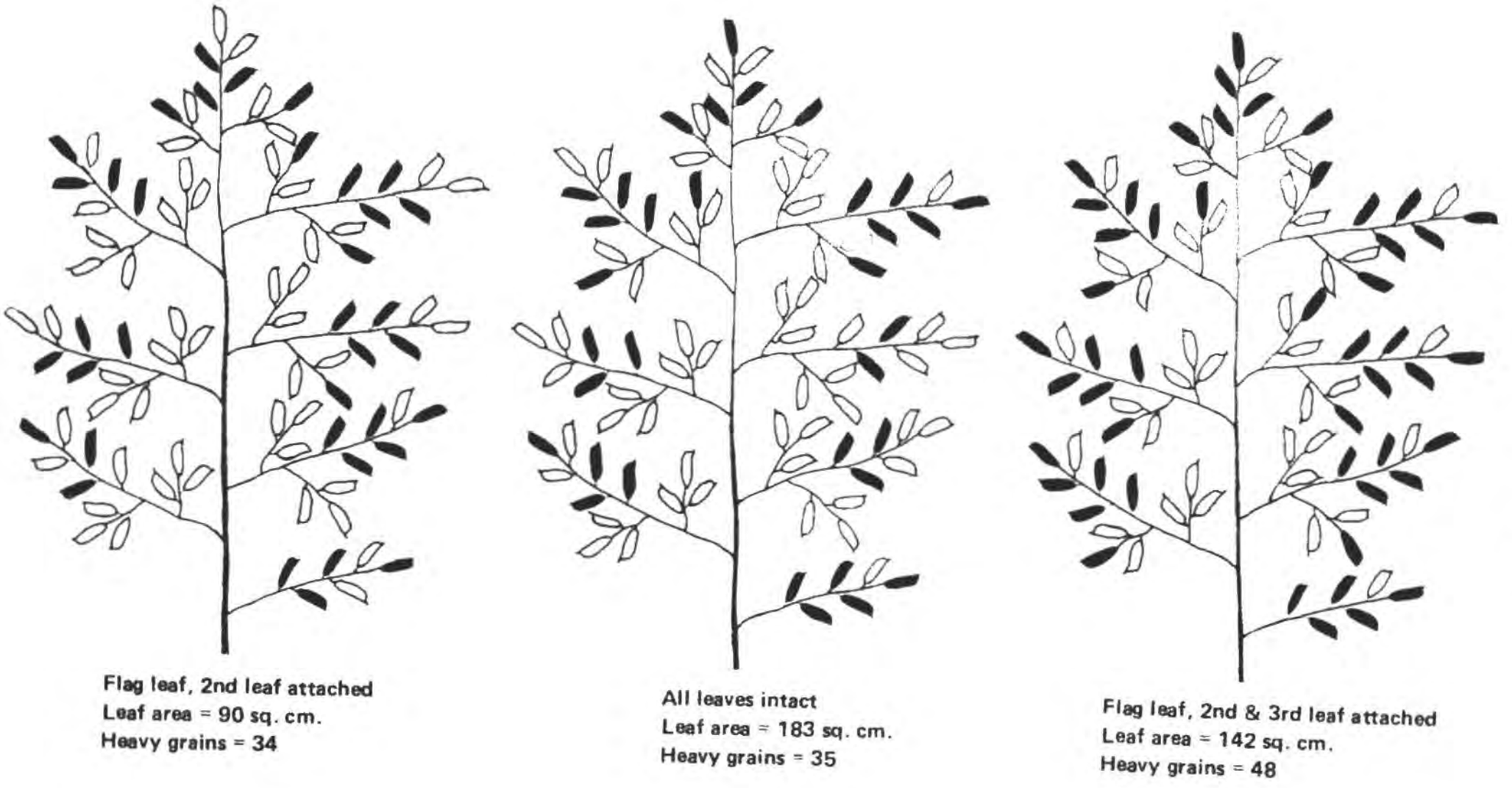

Fig. 8. Effect of leaf removal at flowering on the number of high density grains produced in IR58 (Ahn, 1986).

Rate of filling

Spikelets are filled to capacity within 11 to 21 days (Singh and Juliano, 1977; IRRI, 1978). The large grains (40 g) mature in 16 to 21 days, small grains (<18 g) in 11 to 12 days, and medium size grains (20-30 g per 1000 grains) in 11 to 21 days (IRRI, 1978). Indica varieties mature earlier than japonicas (Nagato and Chaudhry, 1969; Choi, 1986).

Grain filling rate and duration are positively correlated with grain size (Jones *et al.*, 1979; Fujita *et al.*, 1984). Grain filling duration was shorter (12 to 18 days) in the primary branch than in the secondary branches (12 to 29 days). Spikelets on the secondary branches had lower grain filling rate and lower final weight (Ahn, 1986). This would indicate that rate of grain filling affects grain density.

Low "sink-pulling" force

Although sufficient carbohydrate is available, many of the spikelets do not fill up to HD capacity. Whether or not growth regulators are involved, as suggested by Thorne (1974) on wheat and barley, needs further studies. Preliminary data show that spikelets resulting in HD grains have high IAA content and the peak occurs early in the development of the grains (Robles *et al.*, unpublished data). Respiration measurements showed high rates for HD grains (Shanghai Teachers College, 1978).

Spikelets on the primary branch had greater sink strength than those on the secondary branch. The lower spikelets (5th and 6th) on the primary branch were heaviest. On the secondary branch, the topmost spikelet was always heavier (Ahn 1986). Even with all leaves removed at flowering, the same spikelets filled up first.

Structural limitations

In rice, the transport of assimilates from the vascular bundle to the endosperm is mediated by the pigment strand. At 12 days after anthesis, no structural evidence in the pigment strand was found to restrict the flow of assimilates to the endosperm (Oparka and Gates, 1981 and 1984). Whether or not the pigment strand becomes sealed off during grain filling would have importance in assimilate translocation.

The spikelets with HD grains have bigger pedicellar vascular bundles, specifically, larger phloem (Nishiyama, 1983) and more and better developed vascular bundles (Chaudhry and Nagato, 1970). Phloem size decreased by acropetal succession in the primary branch except the top spikelet. On secondary branches, the topmost spikelets had the thickest. Spikelets on the primary branches had thicker phloem than those on the secondary branches. This would partly explain the greater density of grains in the primary branches than in the secondary.

Chaudhry and Nagato (1970) reported that although the vascular bundles in all primary branches were similar, the 1st secondary branches developed better than the 2nd secondary branches on the same primary branch. This would also explain the lower density of grains on the secondary branches and the reason for

the suggestion that cultivars with no secondary branches on the panicle should be selected.

The number of large vascular bundles in the peduncle is correlated with the number of primary branches (Dana *et al.*, 1969; Matsushima, 1970; Hayashi, 1976; Joarder and Eunus, 1980). Panicles with large numbers of vascular bundles should be selected to increase primary branches and compensate for the decrease in spikelet number with the removal of the secondary branches.

Indica rices have more vascular bundles than japonica (Hayashi, 1976). Indica/japonica crosses were found to have more and larger vascular bundles than japonica varieties (Lee *et al.*, 1985).

Thick culms have more vascular bundles. There is a high correlation between the diameter of the first node at the top of the culm and the length of the primary rachis branch and also the number of grains per panicle (Hayashi, 1980). The secondary tillers have one less vascular bundle than the primary tillers. The tertiary tillers have two less vascular bundles (Hayashi, 1976). This suggests a low tillering plant type if the aim is to have high number of vascular bundles.

PadmajaRao (in press) reported that HD grain index was generally higher among primary tillers than in secondary/tertiary tillers, especially in the early maturing varieties.

Increased nitrogen fertilizer application resulted in an increase in the number and size of the vascular bundle, number of primary and secondary branches of the panicle, and number of spikelets per panicle (Lee *et al.*, 1985).

Suggested Plant Type

In line with the new concept of increasing the number of HD grains, the following plant type is suggested:

1. Low tillering type. Only primary tillers should develop. This would ensure a higher number of vascular bundles (Hayashi, 1976), higher number of HD grains (PadmajaRao, in press; Choi and Kwon, 1985) and facilitate the production of heavy weight tillers. Vigorous or large tillers result in more HD grains; higher sink/source ratio; and higher spikelet number, percent filled spikelets, leaf area/tiller, and sink capacity (Choi and Kwon, 1985).

Low tillering by denser planting will not be practical since this method, using modern high tillering varieties, results in light weight tillers with thin culms. The resulting panicle is relatively small.

2. Panicle weight type. Large panicles will be needed to compensate for low tillering. Data from 86 varieties tested showed no significant negative relationship between spikelet number per panicle and HD grains (Samantasinhar and Sahu, 1986). It is possible to have a high HD grain index with a large panicle for stable and sustained grain yield.

3. Thick culm for more vascular bundles, less lodging, support of bigger panicle, and carbohydrate accumulation.

4. Panicles with primary branches only. Primary branches have mostly HD grains and fewer empty and half-filled spikelets. The percentage of ripened grains is governed mainly by the degree of ripening of the spikelets on the secondary branches. Matsushima (1976) suggested that, to raise the percentage of ripened grains, the number of secondary branches should be reduced.

5. Large pedicellar vascular bundle for better transport of assimilates. There are no scientific data on rice to support this aspect. But, if the transport system is limiting, larger vascular bundles might enhance movement of the assimilates.

6. Medium size grains (IR8 size) with less white belly (Takita, 1985), which is essentially low-density grain. White belly is positively correlated with grain width in indica cultivars (Takita, 1986). Large grains have low density and usually are not completely filled (Takita, 1986).

7. Erect and thick leaves (Yoshida, 1972) for better light distribution and higher photosynthetic rate per unit leaf area.

8. High photosynthesis under low PAR so that carbohydrate supply will not be limiting during the monsoon season.

9. Low maintenance respiration. Converting the rice plant from the C_3 to the C_4 system would be difficult. To increase net assimilation rate, maintenance respiration can be decreased. Higher shoot/root ratio may also result in a decrease in the maintenance respiration of roots.

10. Medium growth duration is needed so that carbohydrate accumulates before heading (Takeda and Murata, 1956, Vergara *et al.*, 1964; Yoshida, 1972). This accumulated carbohydrate would be useful in the production of larger panicles and heavier grains.

11. Intermediate plant height with HI of 0.55. This will not only make the plant lodging resistant, decrease maintenance respiration but more important the optimum partitioning of the carbohydrate to the grains.

Major Development Needs

1. Select donor parents with a high number of HD grains. A simple procedure using a seed blower for screening cultivars with HD grains has been devised (Venkateswarlu *et al.*, 1986a).

2. Select plants with a high number of vascular bundles or of primary branches in the panicle and testing for HD grains. Choi (1985) suggested that sink size/tiller is an effective indicator of high yield potential. This aspect should also be considered in plant selection.

3. Identify plants with low tillering ability. If such plants are not available, breeding for that character should be started. Use of tissue culture and other methods to produce a low-tillering plant type should be explored. Unless such a plant type is developed, its usefulness and potential cannot be tested.

4. A low-tillering type will need different cultural management practices that should also be studied. The use of a row seeder should be evaluated.

5. Study the role of cytokinin, gibberellin, and auxin on carbohydrate accumulation in the spikelets.

The movement of water and of assimilates in the dorsal region of the grain seem to be linked. Oparka and Gates (1984) suggest that studies be made to determine whether the rate at which water is removed from the grain influences the movement of assimilates out of the phloem. Silica deposition on the lemma and palea might play an important role in transpiration and translocation.

6. Study the role of slow senescence and low maintenance respiration on grain filling. Indications are that leaf area at 30 days after heading correlates positively with grain weight (Shin and Kwon, 1985).

7. Study the limiting rate of translocation to the endosperm and compare varietal differences in translocation efficiency.

8. Conduct genetic studies on inheritance of HD grains, tillering, branching of the panicle and number of vascular bundles to improve these plant traits.

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A STUDY ON THE *GLIRICIDIA SEPIUM* (JACQ.) WALP. GERMPLASM COLLECTION IN VISCA

E.C. Bumatay, R.G. Escalada and C.R. Buante
Visayas State College of Agriculture
Baybay, Leyte, Philippines 6521-A

ABSTRACT

Gliricidia sepium (Jacq.) Walp germplasm collection was undertaken to determine the phenological characteristics and growth habit of the different accessions and to evaluate the growth rate and survival of this species propagated through seeds (sexual) and cuttings (asexual/vegetative).

Different accessions were collected all throughout the Philippines and from abroad. Local accessions collected were usually bushy, shrubby and short-statured having short, broad leaves. Foreign accessions were arboreal with lesser side branching and the leaves were small to large in size and elliptically-shaped. Number of leaflets per rachis varies from branches to branches and from tree to tree.

In terms of plant growth among those propagated through seeds, Accession No. VGs 15 grew to 4.55 m, the tallest among the accessions and also developed the biggest diameter increment (3.08 cm) at one year of growth. However, at the second and third year of growth, Accession No. VGs 6 was noted as the tallest and the biggest diameter increment obtained, 6.73 m high and 4.75 cm dbh and 7.58 m high and 5.63 cm dbh, respectively.

On the other hand, those propagated through cuttings, Accession No. VGc was found to be the tallest (4.26 m) and biggest diameter (2.83 cm) at one year of growth. However, during the second and third year of growth, Accession No. VGc 2 prominently grew faster, 6.11 m high and 4.50 cm dbh and 6.63 m high and 4.89 cm dbh, respectively.

This development showing promising results prompted the researchers to undergo studies on flowering and fruiting behavior, hybridization and rapid propagation techniques to develop new promising hybrids of *G. sepium*.

Introduction

Gliricidia sepium (Jacq.) Walp is a fast-growing leguminous tree of the Family Fabaceae and found as a good material for reforestation programs. It can survive in impoverished soil and can easily be propagated by cuttings. The normal development of the root system, however, is slow and the mortality rate is rather high during transplanting. Despite these drawbacks, asexually propagated plants are superior than seedlings because they develop shoots, leaves and branches in a very much shorter time the moment they get established.

Wherever *Gliricidia sepium* grows, its hard, heavy wood is used for fuel. Although not tall, the tree produces much branch wood and coppices easily. Its calorific value is 4,900 kcal per kg (NAS, 1980).

Sumberg (1983) cited that in any trials of tree plantations, local species should always be given top priority since they are already adjusted to the existing environmental conditions. Those trials might provide the germplasm materials to start local tree plantation for pulp and paper manufacture, firewood and other research purposes.

A germplasm collection has been established and this paper reports the study on the phenological characteristics and growth habit of the different accessions and the growth rate and survival of plants propagated through seeds and cuttings from different localities in the Philippines and abroad.

Materials and Methods

Collection of planting materials

The *G. sepium* seeds and cuttings were raised in the nursery for 1½ months and then hardened for a month in hardening beds for preconditioning of the seedlings. The seedlings were watered from time to time. Nursery data were recorded. In some cases, direct planting of cuttings in the plot was done.

Field site preparation and planting

Before planting, the area was thoroughly prepared. Existing vegetation and weeds were removed to prevent competition for light, soil nutrients and soil moisture between the seedlings and weeds. Planting was done at the onset of rainy season. Weeding of the area was performed periodically or whenever necessary.

In outplanting, 20 seedlings and/or cuttings in each accession were selected randomly for field trials. The spacing used was 1 m x 1 m and the sample seedlings in each accession were then planted in the plots.

Soil sampling and analysis

The following soil properties were determined initially and at the third year of experiment.

1. soil pH
2. organic matter
3. total nitrogen
4. available phosphorus
5. extractable potassium
6. soil texture

Data collection

The data collected were the following:

1. percentage survival of seedlings/cuttings
2. plant height (m)
3. stem diameter (cm)

The initial plant height and diameter were taken immediately after outplanting and every 3 months thereafter. Succeeding measurements were done at 6 months interval when the seedlings had attained 1 year or more in growth.

Sample accessions were selected among the collection. Three accessions were selected to represent local collection propagated through seeds and three accessions for those propagated through cuttings (local). Three accessions were used also to represent the collection from abroad.

The selection of sample plants was based on the growth dominance, age and superior tree characteristics attributed to trees for hybridization and improvement of varieties according to specific use.

Hybridization

The breeding work considers the characteristic traits of the accessions–both foreign and local. These traits include branching habit, growth form–either erect or bushy; disease and drought resistance; leaf characteristics among others.

Results and Discussion

Nursery and field trials

Planting stocks of *G. sepium* were raised on the nursery for evaluation of germinative capacity of seeds and cuttings of different accessions. Conditioning of the seedlings was done before conducting outplanting in the field. Selection of vigorous seedlings for field trial planting was performed to ensure survival rates in actual field conditions. Most of the accessions propagated through seeds were found to be vigorously growing at 80-100% survival rate. On the other hand, those propagated through cuttings showed high mortality during the seedling stage. This might be due to long drought which occurred sometime in 1983 during the initial stage of the study. Inspite of this drawback, those accessions that survived and got established were already growing vigorously.

In the early part of 1985, some local accessions started to flower but failed to produce pods. This was attributed to the fact that these accessions were still too young hence, the probability of the pollination process not to proceed normally, resulting in the failure of the plants to bear pods. It was observed also that pollens were shed off or anthesis took place while the flowers were still unopened. This means that either the species was cleistogamous or there was a strong self-incompatibility mechanism present. However, during the first to second quarter of the

third year (1986), several accessions both local and foreign, had borne flowers and developed pods but the seeds produced were aborted. Further verification of this phenomenon has been undertaken to induce the production of viable seeds.

Plant growth and development

Plant height and diameter at breast height of representative *G. sepium* accessions were noted (Table 1). At one year old, Accession No. VGs 15 was observed to be the tallest (4.55 m) and the biggest in terms of diameter increment (5.08 cm, dbh) among the selected accessions. Accession No. VGc 2 (Fig. 2) on the other hand, was the shortest (3.33 m) with Accession No. VGs 13 having the smallest diameter increment (2.20 cm, dbh) compared to other accessions selected. At two years of growth, Accession No. VGs 6 (Fig. 3) was noted to grow very fast and developed bigger diameter, 6.73 m and 4.73 cm, dbh, respectively, while Accession No. VGs 13 and VGs 2 were the shortest 5.33 m and 3.49 cm. dbh and 5.42 m and 3.48 cm, dbh, respectively.

Table 1. Mean annual plant height and diameter growth of representative *G. sepium* accessions in the germplasm collection[1]

	One Year of Growth		*Two Years of Growth*	
Accession number[2]	*Plant height (m)*	*Diameter at breast height dbh (cm)*	*Plant height (m)*	*Diameter at breast height dbh (cm)*
VGs 2[3]	3.27	2.22	5.42	3.48
VGs 4	3.46	2.33	5.44	3.61
VGs 6	4.03	2.73	6.73	4.75
VGs 13	3.43	2.20	5.33	3.49
VGs 15	4.55	3.08	6.46	4.54
VGs 16	4.39	2.36	5.75	3.51
VGs 1	3.43	2.56	5.78	3.99
VGs 2	3.33	2.51	6.11	4.50
VGs 4	4.26	2.83	6.05	4.36

[1]Based on mean of 20 sample trees per accession
[2]VGs – *G. sepium* propagated through seeds.
VGc – *G. sepium* propagated through cuttings.
[3]VGs 2 – Caniaw, Bantay, Ilocos Sur (northern part of the Philippines)
VGs 4 – Buhisan, Cebu City (Central part of the Philippines)
VGs 6 – Nicaragua
VGs 13– Catbalogan, Samar (Eastern part of the Philippines)
VGs 15– Central America N118, OFI #31/83
VGs 16– Chaing Mai, Thailand N 79
VGc 1 – Mabalodbalod, Tigaon, Camarines Sur
VGc 2 – Kagiang, Buhisan, Cebu City
VGc 4 – Camp 7, Osmena Reforestation Project, Minglanilla, Cebu

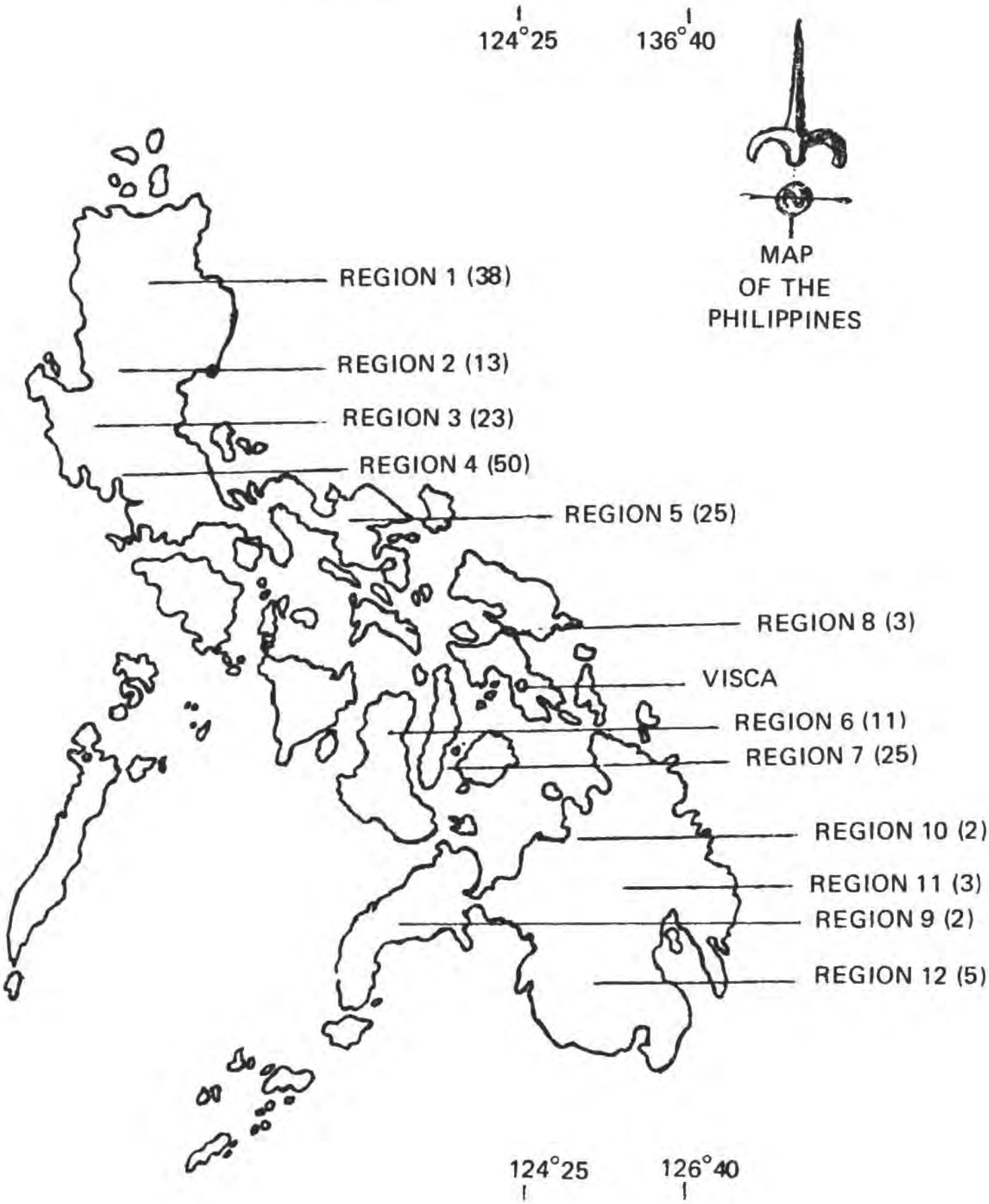

Fig. 1. Relative locations where *G. sepium* were collected in the country. Numbers in parenthesis are number of accessions in each place.

Fig. 2. Philippine accessions of *G. sepium* more than 2 years old showing branchy characteristic in the germplasm collection trial (Gc 2).

Fig. 3. Foreign accession of *G. sepium* more than 2 years old in the germplasm collection trial (Gs 6).

In terms of mean annual growth/increment, Accession No. VGc 2 showed significant increase in plant height (2.78 m) while Accession No. VGs 6 had significant increase in stem diameter (2.02 cm, dbh) compared to others. If this trend in growth of *G. sepium* appears to be normal then the trees can be harvested in 8-10 years for pulpwood or fuelwood production which could alleviate the dwindling supply of the latter particularly fuelwood.

Accession selection for varietal improvement

The germplasm collection of *G. sepium* is large and perhaps the largest in the country which is feasible for scientific exploration–species trial for fuelwood and pulpwood production, herbage production for fertilizer material (2.19% N; 0.18% P; 2.03% K) as compared to *L. leucocephala* (2.04% N; 0.30% P; 1.04% K) and varietal improvement to produce superior varieties and effectivity as alley cropping with due basis on its plant characteristics such as growth form, leaf size and shape, flowering and fruiting habit and response to rapid propagation techniques.

Soil sampling and analysis

Table 2 shows the initial chemical and textural soil analysis of the experimental area. The soil is a mixture of 13.08% sand, 37.70 % silt and 48.50% clay. The initial chemical analysis showed that N, P, K and Zn are high in the top soil than in the subsoil.

After 3 years of growth of *G. sepium* in the germplasm, soil analysis revealed an increase in nitrogen and potassium content of the soil but decreasing in the amount of phosphorus. The soil has a pH of 5.30 which means it is an ideal for crop production.

Summary and Conclusions

Gliricidia sepium grows well even in very impoverished soil condition. However, planting them directly using either seeds or cuttings in the field, resulted in poor survival rate. To prepare seedlings for outplanting, nursery operations have to be followed. Seedlings raised in the nursery exhibited favorable growing performance particularly those propagated through seeds while those propagated through cuttings somehow had a high mortality rate especially during the long drought that occurred at the early stage of the project.

Several accessions of *G. sepium* had already flowered. However, in the first flowering season, no pods were developed. In the succeeding flowering season, though many pods and seeds had developed, many of the latter were aborted. Pollens were shed off while the flowers were still unopened showing that either the species were cleistogamous or there was a strong self-incompatibility mechanism present.

In terms of plant growth, the test accessions showed promising results. Accession No. VGs 15 (Central America N118, CFI #31/83) grew to the tallest (4.55 m)

Table 2. Soil analysis of the *G. sepium* germplasm collection

Soil samples		*Chemical*				*Textural*			
		%N	*P (ppm)*	*K (me/100 g soil)*	*Zn (ppm)*	*% sand*	*% silt*	*% clay*	*Grade*
A.	Initial								
	Top soil	0.33	3.16	0.38	6.10	C o m p o s i t e			
	Subsoil	0.20	1.76	0.25	5.80	13.80	37.70	48.50	Clay
B.	After 2 years of growth								
	Subsoil	0.34	1.18	1.69	5.30				

and developed the biggest in diameter increment (3.08 cm, dbh) at one year of growth. During the second year of growth, Accession No. VGs 6 (Nicaragua) was noted to be the tallest and had the biggest diameter increment (6.73 m and 4.75 cm, dbh, respectively). Higher increase in mean annual growth based on plant height was noted in Accession No. VGc 2 (Caniaw, Bantay, Ilocos Sur) with 2.78 m and in Accession No. VGs 6 based on diameter (dbh) increment at 2.02 cm.

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RESIDUAL EFFECTS OF CORN (*ZEA MAYS* L.) RESIDUES ON SUCCEEDING CROPS UNDER DIFFERENT TILLAGE LEVELS

Geronima B. Medina
Agriculture Division, Ateneo de Davao University
Jacinto Street, Davao City, Philippines

ABSTRACT

The growth and yield of corn, soybean and mungbean crops grown to a field previously planted to corn, and subjected to zero, minimum and conventional tillages were evaluated. Pot experiments using soil samples collected at 10, 17, 24, 31 and 52 days after corn residue application were also made. Favored growth and higher yield of the test crops were obtained when grown to a field with corn residues. Except for corn, tillage levels did not significantly influence the performance of the test crops. Stimulatory and inhibitory effects were observed with the pot experiments. The possible causal factors of corn residue inhibition or stimulation on growth of the test crops are discussed.

Introduction

Allowing crop residues to decompose in the field after harvest and the immediate planting of the subsequent crops in the same field has been a common practice by several farmers. The return of crop residues to the soil is beneficial to crop growth and development since this improves the soil organic matter. However, such practice sometimes results in deleterious effects of succeeding crops.

Well-documented evidences have shown that crop residues left on the field after harvest extremely reduce growth and development to succeeding crops. Decomposing crop residue releases organic substances inhibitory to the growth of the plants usually resulting to swollen seeds, abnormal radicle which lack root hairs and necrosis of the root tips (Patrick and Koch, 1958; Patrick, 1971; Chou and Patrick, 1976). Garcia (1983) has also reported that soils previously planted to corn and have corn residues left after harvest have strong allelopathic effects on the growth of succeeding corn seedlings. The effects are manifested in terms of shorter plants with chlorotic leaves and lower root, shoot and biomass weights.

Recognizing allelopathy as one of the constraints in crop production, there is a need to study the effects of corn residues left in the field after harvest on growth and yield performance of subsequent crops.

The objectives of the study were: 1) to determine the stimulatory or inhibitory effect of corn residues on the growth and yield of corn, mungbean and soybean; 2) to evaluate the effects of tillage on inhibitory or stimulatory effect of corn residues to subsequent crops; 3) to estimate the best time of planting subsequent crops such that inhibitory effect due to corn residues is no longer active.

Materials and Methods

Field experiment

The experimental area of 3,036 square meters which was plowed and harrowed once was divided into two main plots with an area of 1,516 square meters per plot. One of the plots was fallowed for one season while the other was planted to corn for the establishment of crop residues. At harvest, the stalks were chopped and spread on the soil surface. Thereafter, each mainplot was divided into four blocks and each block was subdivided into three subplots. Each subplot had an area of 26.25 square meters with seven furrows maintain at 0.75 meter between rows and 5 meters long. Zero, minimum and conventional tillages were established at random in each block. Corn residues were incorporated into the soil at various depths and proportion depending upon the tillage used. The experiment was arranged in a split-plot randomized complete block design with four replication. Corn residues application and tillages were assigned to the mainplot and subplot, respectively. Zero tillage means that planting was done directly into the field (drill method); minimum, only furrows were established before planting while conventional, one plowing, one harrowing before furrowing were made before planting. Agronomic characters and yield were recorded from corn, mungbean and soybean as test crops. The Duncan's Multiple Range Test at 5% level of probability was used to determine significant differences among treatment means.

Pot experiment

Soil samples were collected at 10, 17, 24, 31 and 52 days after corn residue application in the field. Soils including corn residues were randomly collected from four replicate plots at approximately 10 cm. depth from the soil surface for all tillages. Soils from each tillage represents a treatment. Soil samples were mixed with sieved river sand in a ratio of 50% soil samples and 50% sieved sand (v/v), placed in a clay pots (size 8) and bioassayed using corn, mungbean and soybean as test crops. Planting was done every after each sampling. Experimental units were arranged in a split-plot complete randomized design with ten replication having one pot one plant per replicate. The conventional, minimum, and zero tillages as the source of soil samples with corn residues and the control (fallow soil) were the main plots while days after corn residue application as the subplots. Weekly plant height was recorded. Four weeks after planting, plants were harvested. Dry root, shoot and biomass weights of each test plants were recorded and expressed as percent of control in order to compare the periodic response of test plants to different stages of corn residue decomposition.

Table 1. Corn, soybean, mungbean plant height planted to a field with or without corn residues

	Weeks After Planting																
		1			2			3			4			5		6	7
Treatments	C	S	M	C	S	M	C	S	M	C	S	M	C	S	M	C	C
With corn residue	25[a]	12[a]	12[a]	45[a]	21[a]	20[a]	74[a]	32[a]	32[a]	109[a]	46[a]	48[a]	1,500[a]	60[a]	65[a]	201[a]	238[aa]
Without corn residue (control)	22[a]	10[b]	10[b]	14[a]	21[a]	19[a]	68[a]	31[a]	29[a]	96[a]	45[a]	46[a]	139[a]	64[a]	65[a]	188[a]	228[a]

Means in a column with the same letter are not significantly different at alpha 0.05 using DMRT. This will be followed in succeeding tables unless otherwise specified.

C = corn S = soybean M = mungbean

Results and Discussion

Field experiment

There was no significant difference in plant height from the control five weeks after planting except for the first and fourth week for mungbean, soybean and corn, respectively (Table 1). The possible causal factors affecting the difference in soybean and mungbean plants might be due to the residual fertility of the soil previously cropped to corn crop that stimulated growth. The significant difference observed during the fourth week of corn growth could not be ascertained if it was due to the presence of corn residues or to other undetermined growth factor.

Table 2 shows that corn grain yield and its earlength were significantly higher when planted to a field with corn residues. This difference might be accounted for the presence of corn residues in the field since percent unfilled ear, percent barren plants and the average number of ears showed non-significant.

The number of plants harvested per plot was determined to possibly explain lower yields in plots with thin stand. As shown in Table 3, mungbean planted to a field with corn residues at thinner stand (560 plants) gave a significantly higher number of pods per plant as compared to mungbean planted to a field without corn residue at thicker stand (644 plants). This might be the reason why mungbean grain yield (760 kg/ha) at thinner stand is comparable to the yield (771 kg/ha) of mungbean without corn residue having thicker stand.

As to the effect of tillage, zero till influences significantly bigger mungbean seed formation and longer corn earlength as compared to conventional tillage. Minimum tillage effect, however, is non-significant with zero till (Table 4). Plant height and yield of test crops were not significantly affected by tillage used.

Pot experiment

Soil samples from the field previously planted to corn and with corn residues left after harvest with three tillages (conventional, minimum, zero) inhibited corn and mungbean growth as shown in shorter plants with conventional tillage which is comparable to those planted in a fallow soil having no corn or other plant residue at all. Stimulation, however, was observed in soybean plants from the three tillages as shown in taller plants (Table 5). Furthermore, the same soil with corn, soybean and mungbean crops currently growing sampled 10 days after corn residue application stimulated growth of the same crops whereas those sampled 17, 24, 31 and 52 days after residue application inhibited growth as evidenced by lower dry root, shoot and biomass weights.

It should be noted that during the 31 and 52 days sampling period, crops in the field were already one and two-month old, respectively. The inhibitory effect of 17, 24, and 31-day soil samples might suggests that corn residues in the field were already undergoing decomposition process and its products directly affecting root growth (Borner, 1960; Guenzi nd McCalla, 1966; Wang *et al.*, 1967; Chandramohan *et al.*, 1973; Chou and Patrick, 1976; Cochran *et al.*, 1977; Bhowmik and Doll,

Table 2. Corn earlength, percent barren plants, average number of ears and grain yield planted to a field with or without corn residue

Treatments	*Earlength (cm)*	*Percent unfilled Ear*	*Percent barren Plants*	*Average number of ears*	*Grain yield (kg/ha)*
With corn residues	16.74[a]	12[a]	13[a]	0.96[a]	3,046[a]
Without corn residues (control)	15.99[b]	15[a]	15[a]	1.01[a]	2,064[b]

Table 3. Number of plants per pot, pods per plant, 100-seed weight and grain yield of mungbean planted to a field with or without corn residue

Treatments	*Number of plants/ plot*	*Number of pods per plant*	*100-seed weight (gm)*	*Grain yield (kg/ha)*
With corn residue	560[b]	21[a]	3.8[a]	760[a]
Without corn residues (control)	644[a]	17[b]	3.9[a]	771[a]

Table 4. Corn earlength and 100 seed weight of mungbean grown in a field with corn residue subjected to different tillages

Tillages	*Corn earlength (cm)*	*Mungbean weight per 100-seeds (gm)*
Conventional tillage	15.8[b]	3.80[b]
Minimum tillage	16.3[ab]	3.85[ab]
Zero tillage	16.9[a]	3.93[a]

1982) resulting to inhibited plant growth and development. Inhibition might also due to the indirect effect of nitrogen immobilization by soil microorganisms (Henderson *et al.*, 1955; Norman, 1959; Kimber, 1973a; Turner and Rice, 1975).

The inhibitory effect of the soil sampled 52 days after residue application might be due to the allelopathic substances released by the currently growing corn, mungbean and soybean crops through root exudation or rain-leached substances from the above-ground plant parts (Guenzi and McCalla, 1962; Kimber, 1973b; Ballester *et al.*, 1982; Garcia, 1983), accumulated in the soil and were included in the bioassay.

Table 5. Corn, soybean and mungbean plant height (cm) from first, second, third and fourth week after planting to soils with corn residue subjected to different tillages

Treatments	*Plant height (cm)*											
	Week 1			*Week 2*			*Week 3*			*Week 4*		
	C	*S*	*M*	*C*	*S*	*M*	*C*	*S*	*M*	*C*	*S*	*M*
With corn residue												
Conventional till	10^{a}	6.4^{a}	8.3^{ab}	31^{bc}	15.2^{a}	14.5^{ab}	53^{b}	26.9^{a}	23.5^{bc}	73^{b}	38.9^{a}	35.2^{bc}
Minimum till	10^{a}	6.9^{a}	8.4^{ab}	34^{ab}	16.2^{a}	15.1^{ab}	59^{a}	27.1^{a}	24.4^{ab}	81^{a}	37.7^{a}	36.7^{ab}
Zero till	11^{a}	6.9^{a}	8.8^{a}	35^{a}	16.1^{a}	15.7^{a}	60^{a}	27.3^{a}	25.5^{a}	80^{a}	39.3^{a}	38.6^{a}
Without corn residue	10^{a}	4.9^{b}	7.7^{b}	30^{c}	12.5^{b}	13.7^{b}	53^{b}	22.4^{b}	22.2^{c}	75^{b}	33.1^{b}	33.4^{c}

The irregular growth pattern observed in dry root, shoot and biomass weights may suggest a periodic production of phytotoxic products of decomposition (Kimber, 1973b; Cochran *et al.*, 1977; Garcia, 1983) as influenced by the manner of tillage used (McCalla and Haskins, 1964; Doran, 1980).

The increasing growth expressed in terms of dry root, shoot and biomass weights when corn and mungbean were planted to soil sampled 52 days after residue application might be due to the mineralization effect (Tack *et al.*, 1972; Turner and Rice, 1975).

Conclusion

A field previously cropped to corn and had corn residues left after harvest favors growth in terms of plant height in corn, mungbean and soybean crops planted ten (10) days after residue application. This condition also increased yield and caused longer earlength in corn employing zero or minimum tillages. The favored growth and yield might be due to the stimulatory effect of corn residues.

Timing of planting, tillages to be used and kinds of crop to be planted are very important. To escape the inhibitory effect of corn residues in a field previously cropped to corn and had crops currently growing, the following may be observed:

a) Soybean should be planted 10 days after corn residue application in the field using zero, minimum or conventional tillages.
b) Mungbean can be planted 10 or 24 days after corn residue application using any of the tillages mentioned, 17 days after corn residue application using zero tillage.
c) Corn can be planted 10 or 52 days after corn residue application using zero or minimum tillages.

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CRITICAL TIME FOR THE OCCURRENCE AND DEVELOPMENT OF TUNGRO INFECTION IN THE FIELD

E. R. Tiongco, R. C. Cabunagan, Z. M. Flores, and H. Hibino
Department of Plant Pathology, The International Rice Research Institute, Los Baños, Laguna, Philippines

ABSTRACT

Field trials were conducted at IRRI farm to determine the time tungro infection likely occurs and how it spreads in the field.

Tungro infection, at very low rates, occurred in plants from uncovered seedbeds. However, no difference in infection to any of the tungro viruses occurred between the plants from covered and uncovered seedbeds after transplanting in the field. Although no symptoms were discernible, RTSV infection was detected in the plants by latex test at 14 DAT. Tungro symptoms were manifested by the infected plants between 22 and 35 DAT coinciding with the detection of both RTBV and RTSV. Tungro infection on IR62 and IR64 also occurred in the same period of time.

At 37 DAT, no difference in infection in the three distance classes of surrounding (direct neighbor, diagonal, distant) hills was obtained in TN1, IR36, and IR54 plants. With time, more direct neighbor hills of TNI plants were infected while the infection rates in the three distance classes in IR36 and IR54 plants did not differ. Under controlled conditions in field cages, viruliferous leafhoppers spread tungro to rice plants nearer the virus source. Hence, the spread of tungro infection is more likely to occur in plants in proximity to the infected plants of a susceptible variety.

The possible role of RTSV and the seedbeds in tungro epidemiology is discussed.

Introduction

One of the major constraints to rice production in South and Southeast Asia is tungro. It is transmitted by several species of leafhoppers in a semipersistent or transitory manner (Ling and Tiongco, 1979). The most efficient vector species is the rice green leafhopper, *Nephotettix virescens* (Distant) (Ling, 1972).

Based on the new understanding that tungro is a composite disease caused by rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (Hibino *et al.*, 1978; Omura *et al.*, 1983), significant findings on tungro transmission (Hibino *et al.*, 1979; Hibino, 1983) and symptomatology (Hibino *et al.*, 1978; Hibino, 1983) improved our knowledge about the disease and in turn gave a clearer picture of tungro epidemiology.

The results of field experiments which indicate the time tungro infection likely occurs and how it spreads in the field is reported.

Materials and Methods

Field experiments

A preliminary trial was conducted from October 1986 to January 1987 to determine the time of occurrence of tungro infection in the field. Seeds of Taichung Native 1 (TN1), a variety susceptible to tungro and the green leafhopper (GLH) were sown on uncovered and covered seedbeds. After 26 days, half of the seedlings from each seedbed were transplanted in a screenhouse while the other half transplanted in the field. The seedlings were spaced at 20 x 20 cms. Tungro infection in the entire plant population was assessed based on symptoms 22, 37, and 65 days after transplanting (DAT). A similar but improved trial was conducted in January to April 1987. The seedlings were sown and apportioned in the same manner as above and transplanted in 5 x 5 m plots in the field laid out in a randomized complete block (RCB) design with four replications. Seedlings transplanted in a screenhouse were planted in 4 x 4 m plots arranged in RCB with two replications. At weekly intervals starting 14 DAT, percentage tungro infection was assessed based on symptoms, and leaf samples were collected and indexed by latex test to determine the tungro-associated viruses in the plants from the field and from the screenhouse. Number of GLH on the plants in the field was recorded weekly starting 21 DAT. Data were taken in 5 sample areas per plot at 16 rice hills per sample area.

Tungro incidence on GLH-resistant varieties was determined using IR62 and IR64. One month after sowing, the seedlings were transplanted with 20 x 20 cm spacing in 10 x 10 m plots laid out in RCB design with four replications. Visual readings of tungro infection in 10 sample areas with 25 rice hills per plot were done at 14, 28, and 37 DAT.

The spatial spread of tungro infection in the field from the initial infected hills to three distance classes of surrounding hills was determined using three varieties with different levels of resistance to GLH. The three distance classes were designated as: a) direct neighbor – rice hill at 20 cm distance parallel or vertical from the initial infected hill, b) diagonal – rice hill at oblique direction approximately 28 cm from the initial infected hill, and c) distant – rice hill other than the first two. TN1 (susceptible), IR36 (moderately resistant), and IR54 (resistant) were transplanted with 20 x 20 cm spacing in 2 x 2 m plots laid out in RCB design with four replications. Visual assessment of the initial infection in the three varieties was recorded at 30 DAT and its spread at weekly intervals thereafter.

The spread of tungro disease was also studied under natural conditions and in field cages measuring 4 x 4 x 1.5 m. One month after sowing on a covered seedbed, TN1 seedlings were transplanted in 4 x 4 m plots and spaced at 20 x 20 cm. The plots were arranged in RCB design with four replications. Four plots were

covered with fiberglass-screen field cages immediately after transplanting. Each plot accommodated 361 plants including one TNI plant infected with both RTBV and RTSV planted at the center of each plot to serve as virus source. Sixteen days after transplanting, each infected plant was covered with a mylar cage and 20 male virus-free *N. virescens* were introduced. After 4 days acquisition access time, the mylar cage was slowly removed to release the insects. After one week, insecticide was applied. Two weeks after insect-release, all plants were scored for symptoms, indexed for infection by the latex test, and hill position of infected plants plotted.

Latex test

Latex particles (Difco Bacto-latex 0.81) were sensitized with partially purified immunoglobulin (IgG) to RTBV or RTSV following the procedure of Omura *et al.* (1984). About 10 cm of the second youngest leaf of each test plant was cut and homogenized separately in 1 ml of 0.05 M Tris-HCl buffer, pH 7.2, using a combined leaf and bud press (Erich Pollahne, Wennigsen, The Federal Republic of Germany). Equal amounts (50 μl) of plant sap and sensitized latex suspension were placed in a small test tube and shaken at 160 oscillations/minute for 30 min. The presence of viruses were indicated by clumping of latex particles observed at 100X magnification using a light microscope.

Results

Seedbed infection

Preliminary trials showed that no tungro infection was observed 22 DAT in all plants from the covered seedbed while infection rates of 0.2% were recorded in plants from the uncovered seedbed planted in the screenhouse and 0.1% in the field. At 37 DAT, the plants in the field from the covered seedbed had 13% disease incidence and those from the uncovered seedbed had 12% which increased to 77 and 79% at 65 DAT. No increase in tungro incidence was observed in plants transplanted in the screenhouse from either seedbeds at 37 DAT (Table 1).

When latex test was used in the improved trial to determine tungro infection, only RTSV infection of 0.02% was obtained 14 DAT in plants from uncovered seedbed transplanted in the screenhouse and none in plants from the covered seedbed. However, plants from both seedbeds transplanted in the field registered 0.94% RTSV infection at 14 DAT and increase to 61% at 35 DAT. Thereafter, a corresponding increase in double infection with RTBV and RTSV was observed as RTSV infection decrease (Fig. 1). Infection with RTBV was low.

An average of 1.1 GLH per plant hill was recorded at different observation time on plants from covered and uncovered seedbeds planted in the field. No difference in the number of GLH per hill was obtained between treatments and over time (Table 2).

Table 1. Percentage tungro infection in Taichung Native 1 rice plants from uncovered and covered seedbeds at different days after transplanting in a screenhouse and in the field

Treatment	*Seedlings transplanted to:*	*% Tungro incidence days after transplanting*		
		22	*37*	*65*
Covered seedbed	screenhouse	0	0	–[a]
	field	0	13	77
Uncovered seedbed	screenhouse	0.2	0.2	–
	field	0.1	12	79

[a]No scoring conducted.

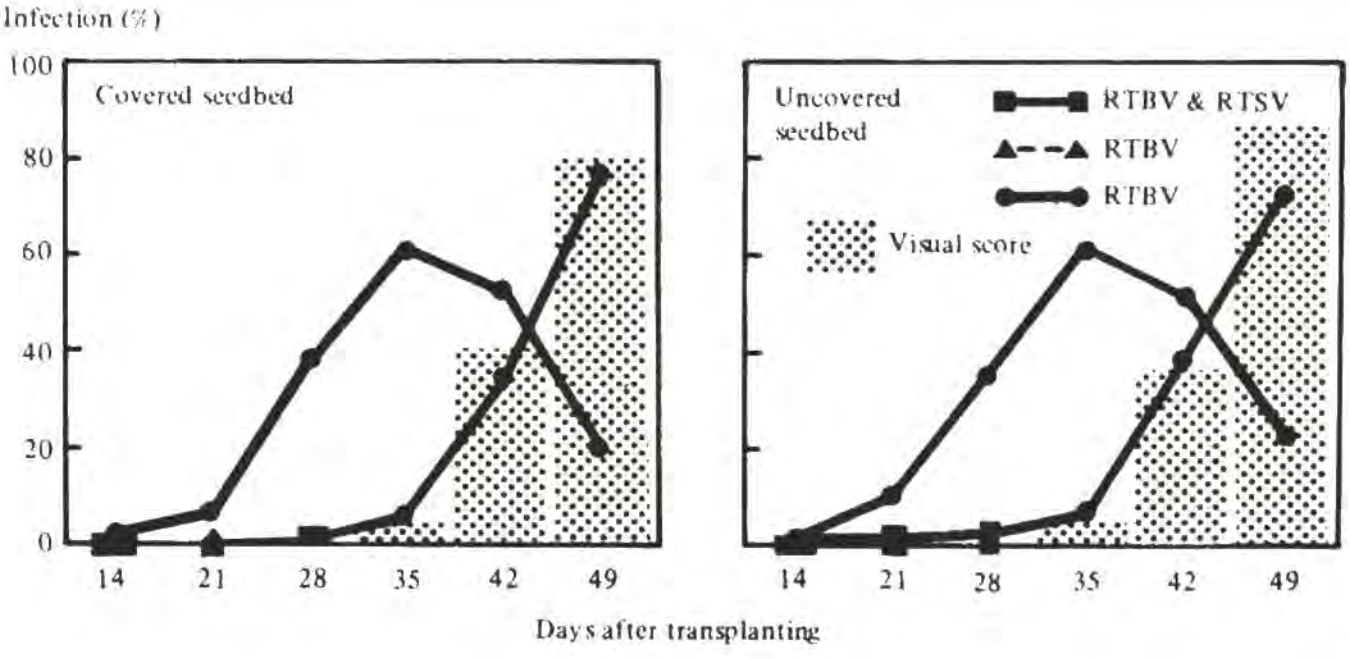

Fig. 1. Percentage infection with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) as detected by the latex test and visual scores of tungro-infected Taichung Native 1 rice plants from covered and uncovered seedbeds transplanted in the field and scored at different time after transplanting.

Tungro infection on IR62 and IR64

No tungro infection was observed on IR62 and IR64 at 14 DAT. Infection rates of 2.19 and 2.83% were recorded at 28 DAT and increased to 3.50 and 6.85% at 47 DAT. In IR64, difference in infection levels occurred in all observation dates (Table 3).

Table 2. Average number of rice green leafhopper per hill in plots planted to Taichung Native 1 from covered and uncovered seedbeds at different time after transplanting.

Treatment	*Days after transplanting*			
	28	*35*	*42*	*49*
Covered seedbed	1.45 a[1]	0.86 a	0.89 a	0.98 a
Uncovered seedbed	1.37 a	1.02 a	1.04 a	1.09 a

[1]In a column, means having a common letter are not significantly different by DMRT at the 5% level.

Table 3. Average tungro infection in IR62 and IR64 rice varieties at different days after transplanting.

Variety	*Days after transplanting*		
	14	*28*	*47*
IR62	0 a[1] (a)[2]	2.19 a(ab)	3.50 a(b)
IR64	0 a (a)	2.83 a(b)	6.85 b(c)

[1]In a column, means having a common letter are not significantly different by DMRT at the 5% level.

[2]In a row, means having a common letter are not significantly different by DMRT at the 5% level.

Spread of tungro infection

The spatial spread of tungro infection on TN1, IR36, and IR54 plants was studied in the field. No difference in the occurrence of infection in the three distance classes was obtained in all varieties at 37 DAT, although higher levels of infection was recorded on TNI plants (Table 4). At 44 DAT, more direct neighbor hills of TN1 plants were infected than those of diagonal or distant hills while infection rates of the three distance classes in IR36 and IR54 plants did not differ. However, infection rates between direct neighbor and distant hills differed among the varieties. More diagonal hills of TN1 plants were infected than IR36 and IR54 plants. At 44 DAT, the same results were obtained.

In a week's time, viruliferous *N. virescene* under controlled conditions in a field cage infect rice plants close to the virus source (Fig. 2). The 20 leafhoppers were able to infect an average of 11 rice plants with both RTBV and RTSV and 1.25 plants with either RTBV or RTSV. The farthest distance an infected plant was

Table 4. Percentage tungro infection in three distance classes from infected hill of three varieties with different levels of resistance to the rice green leafhopper at different days after transplanting

DAT[1]	*Distance classes*	*TNI*	*Variety*[2] *IR36*	*IR54*
37	Direct neighbor	1.78 a[3](a)[4]	0.52 a(b)	0 a(b)
	Diagonal	1.31 a (a)	0.20 a(b)	0 a(b)
	Distant	1.46 a (a)	0.46 a(b)	0 a(b)
44	Direct neighbor	2.95 a (a)	0.82 a(b)	0 a(c)
	Diagonal	1.56 b (a)	0.39 a(b)	0 a(b)
	Distant	1.91 b (a)	0.81 a(b)	0 a(c)
51	Direct neighbor	4.04 a (a)	1.48 a(b)	0 a(c)
	Diagonal	1.78 b (a)	0.72 a(b)	0 a(b)
	Distant	2.06 b (a)	1.25 a(b)	0 a(c)

[1]Days after transplanting.

[2]Reaction to green leafhopper of TNI-susceptible, IR36 – moderately resistant, and IR54-resistant (Heinrichs et al., 1985).

[3]In a column, means having a common letter are not significantly different by DMRT at the 5% level.

[4]In a row, means having a common letter are not significantly different by DMRT at the 5% level.

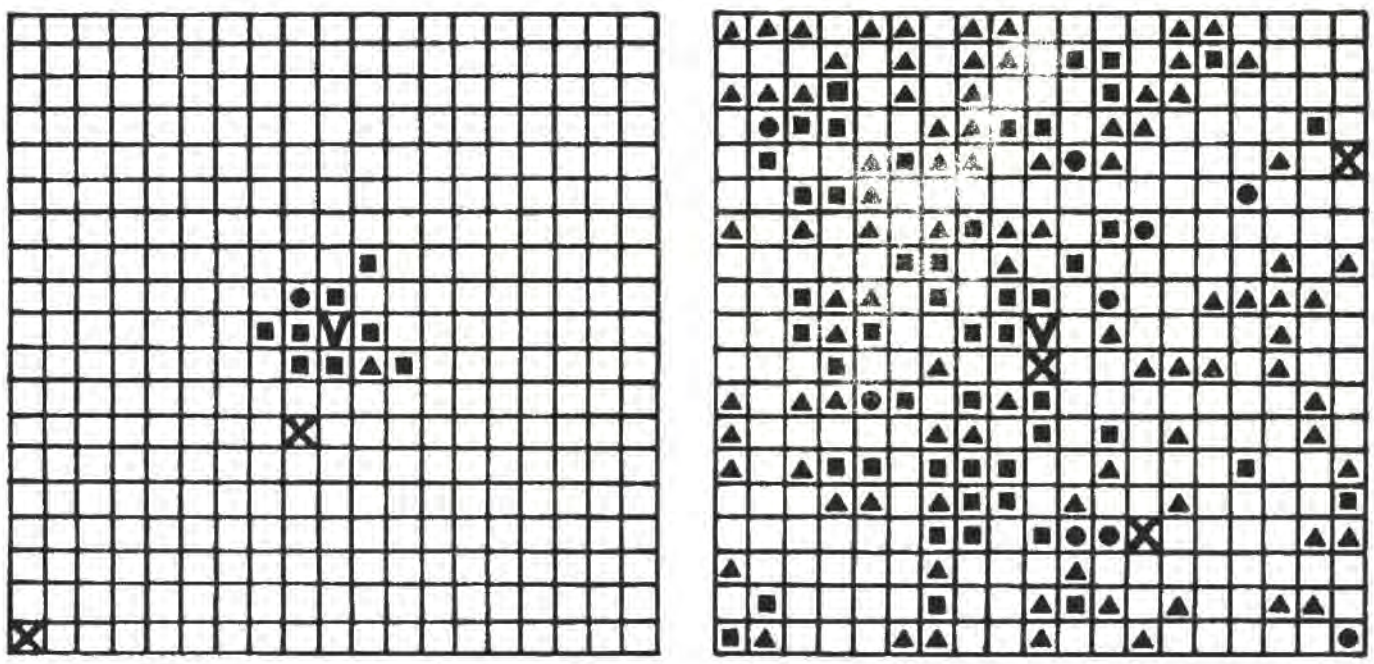

Fig. 2. Representative plots showing the spread of tungro infection by 20 RTBV and RTSV-viruliferous *N. virescens* in 7 days under controlled conditions in field cage (left) and under natural conditions at 30 DAT (right). Symbols: ■ – plants showing symptoms infected with both RTBV, and RTSV, and ● = with RTBV; ▲ = plants without symptoms but infected with RTSV; X = dead plant; and V = virus source.

recorded from the virus source was 128 cm. However under natural conditions, no distinct pattern of spread occurred.

Discussion

Since RTBV and RTSV were incriminated with the tungro disease, little information on the role of these viruses on tungro epidemiology is available.

Results of experiments in the covered and uncovered seedbeds showed that tungro infection, although very low, occurred in plots planted for the uncovered seedlings. Similar results were obtained in another trial wherein infection with RTSV at low rate was observed in plots planted for uncovered seedlings. These are indirect evidences that demonstrate tungro infection occurred, to some extent, in the seedbed. However, after transplanting in the field, no difference in infection rates with any of the viruses were observed between plots planted for the covered and uncovered seedlings. RTSV infection reached its peak within a month after transplanting and gradually decreased thereafter. Meanwhile, plants infected with both RTBV and RTSV increased remarkably within two weeks. The same trend in the development of RTBV and RTSV was obtained in a trial in wet season 1985 (Tiongco, *et al.*, 1986). However, the question on how RTBV gets into the disease cycle remains to be answered.

Although no symptoms were discernible, RTSV infection was detected in the plants by the latex test at 14 DAT. The characteristic symptoms of tungro, like yellowing and stunting, were manifested by the infected plants between 22 and 35 DAT coinciding with the detection of infection with both RTBV and RTSV. Tungro infection in IR62 and IR64 also occurred in the same period of time. These observations concurred with the findings of Hibino *et al.* (1978) that plants infected with both RTBV and RTSV, or RTBV alone showed varying degrees of yellowing and stunting whereas those infected with RTSV were generally symptomless.

RTSV is an important entity in tungro epidemiology. It acts as a "helper" for the transmission of RTBV by leafhopper vectors (Hibino *et al.*, 1978), it occurs widely in farmer's fields (IRRI, 1985; Bajet *et al.*, 1986), it can be transmitted at high rates by leafhoppers that fed on plants infected with RTSV alone (Hibino *et al.*, 1979; Hibino, 1983) and it infects, at high rates, most IR and other rice varieties with resistance to GLH (Daquioag *et al.*, 1984; 1985; Hibino *et al.*, 1987). This study showed RTSV occurred in the seedbed and in newly transplanted fields as a latent virus which limits diagnosis.

Results showed viruliferous leafhoppers in field cages spread tungro to rice plants close to the virus source. The distance of 128 cm obtained in this study showed the capability of viruliferous *N. virescens* to infect plants from the virus source under controlled conditions in field cages in a week's time.

Reports on movements of rice leafhoppers under different conditions have been made (Ling and Carbonell, 1975; Miyashita *et al.*, 1984). Under greenhouse conditions, Ling (1975) observed that seedlings in the proximity of the virus

source had higher infection rates. Similarly, seedling to seedling movements of *N. virescens* in cages were higher between adjacent seedlings (Ling and Carbonell, 1975). Hence, proximity to the virus source is a factor in the spread of tungro infection. Under natural conditions in the field, other factors can contribute to the increase in the amount and extent of spread which may result to an unexpected outbreak of the disease often with little warnings.

The results of these experiments pointed to RTSV as an important element in the initial stages of tungro infection. The absence of discernible symptoms in plants infected with RTSV limits and delays the diagnosis of infection to at least three weeks after transplanting. Early detection of infection is important for a successful control and the use of serodiagnosis is well suited for this purpose.

Some plants from the uncovered seedbeds in this study became infected. This placed the unprotected seedbeds suspect as the staging point in the spread of the disease. Ling *et al.* (1982) pointed that leafhoppers may move to the seedbeds where they lay eggs and transmit tungro. However, it remains to be seen up to what extent the initial infection in the seedbed influenced the disease spread in the field.

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EFFECTS OF ADVENTITIOUS ROOT REMOVAL ON THE GROWTH OF FLOODED TROPICAL PASTURE LEGUMES

Reynaldo R. Javier
Department of Agronomy and Soil Science
Visayas State College of Agriculture
Baybay, Leyte, Philippines

ABSTRACT

Macroptilium lathyroides and *Vigna luteola* with either intact or removed adventitious roots from the immersed stem, were flooded continuously for 15 and 30 days from the start of flowering.

The removal of adventitious roots from the immersed stem of the flooded plants hastened leaf chlorosis and abscission. Dry matter yield (shoots and roots) and nodule dry weight were reduced to a considerable extent in *V. luteola* but only to a minor extent in *M. lathyroides*.

All flooded plants survived with increase in flooding duration. The rapid formation of adventitious roots noted in these species soon after immersion provided the adaptive mechanism for plant survival and growth under flooding.

Introduction

The presence of special adaptations in plants to withstand waterlogging injury has not been given much attention. Nevertheless, plants have to adjust physiologically and morphologically by showing changes not only in the root system but also in the stems if they have to persist under anaerobic conditions.

The formation of adventitious roots is an important excess-water-tolerance strategy (Kramer, 1951). Gill (1970) stated that flood tolerant species adapt to waterlogging by production of functional adventitious roots. Because root aeration is critical in flooded soil, any plant species or cultivar with high proportion of adventitious roots would be at an advantage. In clover, the Yarloop cultivar has higher proportion of near-surface roots than the Mr. Barker cultivar which makes the former more flood tolerant than the latter (Francis and Devitt, 1969).

Since adventitious root formation on the stem tends to be characteristic of woody species native to periodically flooded habitats, it is therefore often seen as an adaptation conferring flooding tolerance. However, experimental evidence for this is lacking. This study was thus conducted to determine whether the adventitious root system formed during flooding can sustain and extend the growth of the plant at times of flooding.

Materials and Methods

Macroptilium lathyroides and *Vigna luteola* are both short-lived perennials (Sherman, 1977) and are normally mesophytic pasture legumes that have the ability to persist and produce well on seasonally wet soil (Whiteman, 1977). They could show rapid initiation and growth of adventitious roots from the immersed stem a few millimeters below the water level, and further growth beyond the flowering period (Chudasama, 1981) hence, they were the species chosen for this study.

The experiment was conducted in a glasshouse using a split-split plot arranged in a randomized complete block design with four replications. The two pasture legume species (*M. lathyroides* and *V. luteola*) served as the main plots, three flooding durations (0, 15 and 30 days) as the subplots, and three adventitious root removal treatments (C = control or unflooded, F_1 = flooded plants with adventitious roots intact, and F_2 = flooded plants with adventitious roots removed from the immersed stems as soon as they were visible) as the sub-subplots.

Before flooding, the 36 pots planted to each species were divided into groups according to plant height and allocated such that plant size in each treatment was uniform. Flooding treatments were imposed at the onset of flowering so that comparison could be made at the same physiological stage. Each of the pots was placed in 20-liter drums filled with water. A water depth of 5 cm above the soil surface was maintained.

The plants subjected to 0 flooding duration were harvested on the day when flooding was begun while those in the other two durations were harvested after 15 and 30 days. At each harvest, plants in unflooded pots for each flooding duration were simultaneously harvested for comparison.

Visual symptoms during flooding periods were observed. At each harvest, the oven dry weight of shoots, roots and nodules were also recorded. Every 4 days, the diffusive resistance on the abaxial surface of young fully expanded leaves of each plant was measured between 11:00 a.m. and 12:00 noon using a leaf porometer.

Results

The glasshouse day and night temperatures ranged from 21 to 32°C and 16 to 26°C, respectively. Relative humidity varied from 51 to 96%. Daily water temperature at 9:00 a.m ranged from 17 to 20°C.

Visual observation

Fifteen days after flooding of *M. lathyroides*, the leaf color of the flooded plants (F_1 and F_2) was similar to that of the control.

In *V. luteola* plants, leaf chlorosis was evident particularly in those where adventitious roots were removed (F_2).

After 30 days, adventitious roots in the flooded plants (F_1) of both species

became well-nodulated but main roots were evidently rotten particularly in flooded *V. luteola* plants.

In flooded *V. luteola* plants (F_1 and F_2), leaf chlorosis spread with time so that after 30 days, a large proportion of the leaves senesced and abscissed. Flooded *M. lathyroides* plants which showed less leaf chlorosis initially recovered such that after 30 days flooding, the plant color was similar to that of the control.

Dry weight (g/plant)

The total dry weight of F_1 plants after 15 days flooding was higher than that of the control by 11% in *M. lathyroides* and 17% in *V. luteola*. However, F_2 plants of *M. lathyroides* and *V. luteola* had total dry weights which were lower by 5 and 8% respectively than that of the control. Extending the flooding duration to 30 days significantly increased the total dry weight of *M. lathyroides* F_1 plants by 16 and 12% over those of the F_2 and control plants, respectively. In *V. luteola*, the total dry weights of F_1 and F_2 plants were lower than that of the control by 1% and 26%, respectively.

The shoot dry weight followed the same trend as that of the total dry weight. With increasing duration of flooding, the shoot dry weights of *M. lathyroides* were higher than the control while those of F_2 plants were lower. The shoot dry weight in the former treatment was higher by 14% than in the latter. In *V. luteola*, the shoot dry weight increased in the flooded treatment by 19% (F_1) and 0.5% (F_2) over the control after 15 days flooding. With the extension of flooding to 30 days, shoot growth in flooded plants (F_1) did not differ significantly from that of the control but was markedly higher than that in F_2 plants.

The root dry weights of the two species were more affected by the treatments than the shoots. *M. lathyroides* maintained a significantly greater root growth than the control in the flooded treatments (F_1 and F_2) up to 30 days flooding. On the average, the root dry weight significantly increased by 26% in the F_1 plants and by 10% in the F_2 plants over the control. In *V. luteola*, the F_1 plants increased their root dry weight by 13 and 16% over the control at 15 and 30 days flooding, respectively. In contrast, the root dry weights of F_2 plants were significantly reduced by 33 and 57% compared with the control at 15 and 30 days flooding, respectively. This could be attributed to the decay of some of the original roots.

The two species differed in their pattern of nodulation with increasing flooding duration. In *M. lathyroides*, nodulation in the flooded treatment (F_1 and F_2) was higher than in the control with increasing flooding duration. After 30 days of flooding, it became significantly higher in F_1 plants than in the control by 26% due to further nodulation in the adventitious roots. In F_2 plants, the increase over that of the control was only 18% (Table 1). Nodulation was markedly reduced in *V. luteola* with increasing duration of flooding. At 15 days flooding, nodulation in the flooded treatments (F_1 and F_2) was lowered by 15% relative to that of the control. Extending the flooding duration to 30 days significantly lowered the nodule dry weight by as much as 28% in F_1 and 61% in F_2. As flood-

ing duration increased, nodulation was reduced due to the decay of the nodules that appeared at the time of flooding.

Leaf diffusive resistance (s/cm)

The leaf diffusive resistance values at each sampling period over 30 days flooding treatment are presented in Table 2. Over the entire period, the leaf diffusive resistance values ranged from 1.4 to 3.6 s/cm in *M. lathyroides* and from 2.4 to 3.7 s/cm in *V. luteola.*

The leaf diffusive resistance of flooded *M. lathyroides* plants was lower than that of the control and this was maintained particularly in those whose adventitious roots were still intact. The leaf diffusive resistance of the flooded *V. luteola* plants (F_1 and F_2) was statistically similar to that of the control.

Discussion

Physiological response to the flooding treatments varied between the two species and may be related to their flooding tolerance. Generally, there was a good relationship between the ability of the plant to produce adventitious roots and its survival under flooded condition. The rapid production of adventitious roots noted in both *M. lathyroides* and *V. luteola* plants soon after immersion provided an adaptive mechanism for their continued growth under flooded conditions.

Removal of all adventitious roots as they emerged from the immersed stems delayed the growth of the two species. However, growth reduction was more severe in *V. luteola* than in *M. lathyroides* as the flooding duration was extended. The dry weights of shoots and roots were significantly reduced by flooding. This confirms the results obtained by Jackson (1955) that the primary role of adventitious roots in both flooded tomato and sunflower plants is to act as absorbing organs.

Root growth was reduced to a greater extent in flooded *V. luteola* and to a lesser extent in flooded *M. lathyroides.* Root weight in *V. luteola* F_2 plants was 64% less than that in F_1 plants after 30 days flooding. This is probably due to oxygen deficiency in the root environment (Conway, 1940) due to removal of adventitious roots. Harris and van Bavel (1957) reported that of all plant activities, root respiration is the most sensitive to soil aeration. With impaired root respiration, nutrient and water uptake as well as energy for root growth are limited.

M. lathyroides maintained an almost similar growth rate in the flooded as well as in the control treatments. Flooded plants with excised adventitious roots were almost similar in appearance to those of the other treatments. This suggests that *M. lathyroides* has other mechanisms aside from prolific production of adventitious roots which enable the species to survive under submerged condition and tolerate waterlogging. Probably, the unaerated soil condition brought about by flooding triggered the production of highly differentiated stems and roots which are anatomically and physiologically different from those in well-aerated soil (Kramer, 1951). Bryant (1934) found that barley roots produced in unaerated cultures had

Table 1. Dry weights of flooded *Macroptilium lathyroides* and *Vigna luteola* plants as affected by adventitious root removal

Species	Treatment: Flooding duration (days)	Treatment: Adventitious root removal[1]	Dry weight (g/plant): Total	Shoot	Root[2]	Nodule
M. lathyroides	0	C	5.36	3.65	1.49	0.22
		F_1	5.68	4.10	1.37	0.21
		F_2	5.73	4.03	1.48	0.22
	15	C	12.08	10.02	1.73	0.33
		F_1	13.56	10.64	2.50	0.42
		F_2	11.45	8.98	2.08	0.39
	30	C	20.76	17.62	2.77	0.37
		F_1	23.54	19.27	3.77	0.50
		F_2	19.77	16.25	3.07	0.45
V. luteola	0	C	7.46	5.46	1.59	0.41
		F_1	8.38	6.03	1.83	0.52
		F_2	8.36	5.93	1.98	0.45
	15	C	14.61	10.82	3.20	0.59
		F_1	17.53	13.35	3.68	0.50
		F_2	13.50	10.87	2.13	0.50
	30	C	26.84	20.50	5.57	0.77
		F_1	26.63	19.45	6.63	0.55
		F_2	19.74	17.05	2.39	0.30
Species x Flooding x Adv. Root-Removal Treatment	LSD	.05	2.82	ns	0.72	0.13
		.01	3.79	ns	0.96	0.18

[1]C = control (unflooded), F_1 = flooded plants with adventitious roots, F_2 = flooded plants without adventitious roots.

[2]Adventitious roots in F_1 were excluded to justify comparison.

more and larger air spaces in the cortex, thinner cell walls and a greater tendency to be differentiated along the sides and tip than those in aerated cultures.

The yellowing and abscission of the leaves of flooded *V. luteola* plants may have been due to dessication or to poisoning by toxic substances moving up from the dying roots (Kramer, 1951). These toxic substances may have escaped from the dying cells in the roots or they may have been produced by microorganisms in the

Table 2. Diffusive resistance of flooded *Macroptilium lathyroides* and *Vigna luteola* plants as influenced by adventitious root removal

Species/ Treatment	*Leaf diffusive resistance (s/cm)*										
	Days after imposition of flooding										
	-1	*3*	*5*	*8*	*11*	*14*	*17*	*20*	*23*	*27*	*30*
M. lathyroides											
C	3.5	3.6	2.2	2.4	2.6	2.9	2.5	2.6	2.3	2.9	2.5
F_1	3.5	2.9	2.2	1.4	2.2	2.1	2.4	2.4	1.8	2.9	1.8
F_2	3.6	2.6	2.9	3.0	2.3	2.9	3.1	3.2	2.3	3.0	2.7
LSD .05	ns	0.6	0.7	0.8	ns	0.8	ns	0.8	ns	ns	0.6
V. luteola											
C	2.7	2.9	2.8	2.8	3.3	3.3	3.5	3.0	2.7	2.7	3.2
F_1	2.7	3.3	2.4	3.4	3.1	2.8	2.9	2.9	2.4	2.6	3.1
F_2	2.8	3.3	3.3	3.7	3.1	3.4	3.3	3.1	2.8	2.9	3.1
LSD .05	ns	ns	ns	0.8	ns	ns	ns	ns	ns	ns	ns

C = control (unflooded)
F_1 = flooded plants with adventitious roots
F_2 = flooded plants without adventitious roots

roots or in the soil. Rowe and Beardsell (1973) reported that under anaerobic condition, there is much greater production of compounds such as sulfides and nitrites which are toxic to the roots and which when carried upwards in sufficient amounts might poison the leaves. It is likely that these factors responsible for the changes in flooded *V. luteola* plants became operative even before the appearance of the adventitious roots and remained operative for some time after the adventitious roots have developed (Jackson, 1955).

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PLANTING DATE-RELATED FACTORS ON THE GROWTH OF COTTON, BATAC, ILOCOS NORTE

Leonila M. Tolentino
Department of Crop Physiology
Cotton Research & Development Institute
Batac, Ilocos Norte, Philippines

ABSTRACT

Cottonseeds of UPL-C2 were sown during crop years 1982-85 every 1st and 16th day of the month: (1) to relate the growth and development of plants to different planting dates and to the prevailing climatic conditions and (2) to relate the occurrence of insects to different planting dates. Plant growth, number of days to squaring, flossing and seedcotton yield were significantly affected by planting dates and planting date-related factors. Maximum yields were obtained when the crop was grown from April 16th to January 1st. The occurrence of insect pests was likewise significantly influenced by the different planting dates.

Introduction

Cotton plants require adequate soil moisture during its stages of vegetative growth (PCC, 1978). Planting should be done between September and October to take advantage of the remaining soil moisture and allow enough dry weather for the bolls to mature and to be harvested by March-April undamaged by rain. Moreover, Cabangbang (1984) reported that in the Ilocos provinces, planting is normally done in August or September so that harvesting falls within January to February. High quality fibers are produced from this crop because harvesting falls within the driest part of the year. However, in Batac, Ilocos Norte we could not fit cotton into this planting date because of the prevailing cropping pattern, hence the importance of this study.

This study was conducted to relate the growth and development of the plants to the different planting dates and to the prevailing climatic conditions and to relate the occurrence of pests to the different planting dates.

Materials and Methods

Seeds of UPL-C2 were sown in a 4.5 m^2, 12.0 m^2 and 18.0 m^2 plots during the crop years 1982-83, 1983-84 and 1984-85, respectively every 1st and 16th day of the month from September 1982 to August 1985.

Variations in climatic factors of weekly mean relative humidity, maximum and minimum temperature, daylength or photoperiod, total bright sunshine, wind-speed and weekly total rainfall were achieved by the different planting dates.

A 75 - 25 - 25 kg N, P_2O_5 and K_2O per hectare was used in the experiment. Twenty five kg N, P_2O_5 and K_2O per hectare was applied at planting and the remaining N was sidedressed 35 days after emergence. Weeding, irrigation and spraying were done when the need arose.

A scatter diagram of the data was made to determine the best regression model fitted to each observation. Regression (simple linear, second degree and stepwise regression models) and correlation analysis (simple linear) were used in analyzing the data.

Results and Discussion

Vegetative growth

Plant height, length and number of sympodial branches which were used as measures for vegetative growth were greatly influenced by the different planting dates (Table 1). Cotton plants planted from June to December were taller than those planted from January to May, perhaps due to higher soil moisture content and relatively lower temperature during those months. These conditions probably favored cell growth especially elongation of individual cells thus consequently producing more robust plants. Longer sympodial branches were observed from May to October planting indicating that lateral growth was more extensive during these months.

Table 1. Relationship between the agronomic characters of cotton and dates of planting

Agronomic characters	R^2 value
Length of sympodial branches	0.6335**
Number of sympodial branches	0.6890**
Plant height	0.8587**
Average boll per plant	0.7479**
Average weight per boll	0.5898**
Seedcotton yield	0.5059**
Days to squaring	0.4071**
Days to flossing	0.3391**

**Significant at 1% level

Vegetative growth was likewise influenced by the different climatic variables during the growing season (Table 2). The different climatic variables i.e. total bright

sunshine, rainfall, relative humidity, photoperiod, windspeed, maximum and minimum air temperature played a major role in the growth and development of the cotton plants. Water for example is the major constituent of physiologically active plant tissue and is an essential element for the maintenance of plant turgidity, necessary for cell enlargement and growth. Maung Mar (1979) showed the significance of lower temperature in the development of vegetative branches.

Table 2. Relationship between the different agronomic characters of cotton and the different weather variables.

Agronomic characters	R^2 value
Plant height	0.6606**
Number of sympodial branches	0.8333**
Length of sympodial branches	0.8258**
Average boll per plant	0.8792**
Average weight per boll	0.9095**
Seedcotton yield	0.7707**
Days to squaring	0.6130**
Days to flowering	0.4981*
Days to flossing	0.7537**

*Significant at 5% level
**Significant at 1% level

Fruiting

Number of days from emergence to squaring and flossing were significantly affected by planting dates (Table 1). Cotton planted from September to December 1st produced squares earlier (about 25-30 days after emergence) compared with those planted from December 16th to August (about 31-45 DAE), brought about probably by the differences in photoperiod observed throughout the growing season. Boll maturation as manifested by the number of days to flossing was shorter with those planted from January to July 1st (81-97 DAE) as compared with those planted from July 16th to December (101-117 DAE). The presence of lower soil moisture being made available to the growth of the plants could have contributed to this phenomenon. Chang (1968) reported that water deficiencies reduced yield, changed the pattern of growth, but enhanced boll maturity.

All the climatic variables considered except for rainfall had significant contribution to number of days to squaring and flowering (Table 3). These two phenomena were enhanced. Number of days to flossing, on the other hand, was significantly affected by relative humidity and temperature. Low relative humidity coupled with high temperature enhanced boll opening. Gipson *et al.* (1968) and Rijks (1967) found that relatively high temperature six weeks after sowing had

significantly increased the rate of development of the fruiting points. Other studies (Mauney and Philipps, 1963; Mauney, 1966; Manuel, 1982) showed evidences that the different climatic/environmental factors had significant effect on the production of squares or flowers buds.

Yield

Seedcotton yield was greatly affected by dates of planting (Table 1). Maximum yields (1.71-6.07 tons/ha) were obtained from April 16th to January 1st plantings while low yields (0.88-1.53 tons/ha) were obtained from January 16th to April 1st plantings. Lower yields during those months might be due to relatively smaller plants with fewer and shorter sympodial branches. Average boll per plant and average weight per boll were likewise significantly affected by planting dates.

Response of the cotton plant to the different climatic factors is shown in Table 3. Seedcotton yield was significantly affected by windspeed, rainfall, photoperiod and maximum temperature. Previous researchers have similar results (Dunlap, 1945; Eaton and Ergle, 1954) where they observed yield reduction in reduced light intensities. Likewise, Manuel (1982) noted the importance of photo-

Table 3. Regression equations on the different agronomic characters of cotton and the different climatic variables

Equation			R^2 value
Dsquaring	=	−1184.70 + 64.69 WS + 7.62 RH + 0.28 TBS − 29.81 PP + 12.47 Tmax + 15.22 Tmin	0.6130**
Dflowering	=	− 1607.81 + 86.78 WS + 11.97 RH + 0.27 TBS − 29.39 PP + 14.95 Tmax + 13.39 Tmin	0.4931*
Dflossing	=	− 414.10 + 7.11 RH + 1.29 Tmax − 5.51 Tmin	0.7537**
Ave. boll/ plant	=	29.34 − 20.05 WS + 0.19 TBS + 5.70 PP − 3.90 Tmax	0.8792**
Ave. wt/boll	=	56.57 + 2.28 WS − 0.03 TBS − 0.004 RR − 1.12 PP − 0.91 Tmax	0.9095**
Seedcotton yield	=	94985.4 − 5459.19 WS − 13.82 RR + 2595.8 PP − 3403.98 Tmax	0.7707**

*Significant at 5% level
**Significant at 1% level
WS – windspeed
PP – photoperiod
RH – Relative humidity
TBS – Total bright sunshine
Tmax – Maximum temperature
Tmin – Minimum temperature

period in obtaining maximum yields. Although Chang (1968) observed the significance of relative humidity in photosynthesis, seedcotton yield was not affected by relative humidity in the present study.

Results obtained indicate that the different climatic factors are vital in the attainment of maximum yield. Wind is necessary in facilitating the entrance of carbon dioxide, the raw material for photosynthesis, within the plant canopy. The rate of photosynthesis increases with the supply of CO_2, which in turn is favored by turbulence. Water, on the other hand, is fundamental to crop growth because of its role in photosynthesis. Plant water stress directly reduces the photosynthesis process because dehydrated protoplasm has lower photosynthetic capacity. Once the leaves lose their turgidity, the stomata close, thus preventing any further intake of CO_2 for photosynthesis. Air temperature is known to markedly influence growth and productivity of cotton. Thus, for a successful crop, cotton is best grown in areas where growing season has warm days and cold nights (Rijks, 1967). Maximum yield per plant had been obtained with day and night temperature of 29°C and 16°C, respectively.

Insect pests

The different insects observed in the experimental area had varied responses to the different planting dates (Table 4). Aphids, leafhoppers, flowerweevils, and pink-bollworm, were significantly influenced by the different planting dates. Their degree of occurrence, however, varied, i.e. aphid population was very high during the early part of the growing season and it decreased progressively as the season progressed. Leafhoppers, on the other hand, were abundant from March to June plantings. During the rest of the growing season, their degree of occurrence was negligible. High leafhopper population might be brought about by the dispersion of insects from the other plantings nearby. Bollworms were not observed from January to May 1st plantings. Probably, prevailing conditions during those months were not conducive for bollworm infestation.

Table 4. Relationship between insect population and planting dates

Insect population	R^2 value
Aphids	0.8659**
Leafhoppers	0.2548*
Flowerweevils	0.6758**
Bollworms	0.0144^{ns}
Phinkbollworms	0.9330**
Cottonstainers	0.2185**
Thrips	0.1186^{ns}
Spidermites	0.0841^{ns}

*Significant at 5% level
**Significant at 1% level
ns not significant

Table 5. Correlation analysis between insect population and the different variables

Insect population	Climatic Variables						
	Wind speed (km/hr)	Relative humidity (%)	Total bright shine	Rainfall (mm)	Photo-period (hrs.)	Air temperature maximum (°C)	Air temperature minimum (°C)
Aphids	0.264^{ns}	-0.282^{ns}	-0.655^{**}	-0.536^{**}	-0.636^{**}	-0.395^{ns}	-0.681^{**}
Leafhoppers	0.108^{ns}	-0.113^{ns}	0.142^{ns}	-0.131^{ns}	0.154^{ns}	-0.288^{ns}	-0.172^{ns}
Bollworms	-0.292^{ns}	0.349^{ns}	0.043^{ns}	0.049^{ns}	-0.354^{ns}	-0.433^{*}	-0.185^{ns}
Pinkbollworms	0.140^{ns}	-0.104^{ns}	-0.116^{ns}	0.035^{ns}	0.312^{ns}	0.105^{ns}	0.155^{ns}
Bollweevils	0.406^{*}	-0.307^{ns}	0.712^{**}	-0.650^{**}	-0.653^{**}	-0.513^{*}	-0.776^{**}
Cottonstainers	0.503^{*}	-0.361^{ns}	0.563^{**}	-0.604^{**}	-0.362^{ns}	-0.243^{ns}	-0.591^{**}
Spidermites	-0.083^{ns}	0.044^{ns}	0.194^{ns}	-0.012^{ns}	-0.293^{ns}	-0.251^{ns}	-0.199^{ns}
Thrips	-0.160^{ns}	0.063^{ns}	0.098^{ns}	-0.007^{ns}	-0.262^{ns}	-0.260^{ns}	-0.132^{ns}
Semilooper	-0.358^{ns}	0.359^{ns}	-0.201^{ns}	0.215^{ns}	-0.131^{ns}	-0.248^{ns}	0.062^{ns}

*Significant at 5% level
**Significant at 1% level
ns Not significant

Of the insects observed in the experimental area, only bollworm and bollweevil were found to be greatly affecting seedcotton yield. Variations in the yield could be explained by bollworm and bollweevil populations by as much as 81%. Seedcotton yield decreased with increasing bollworm and bollweevil population. The decrease resulted from damage of squares, flowers and bolls by these insects.

Insect population was likewise greatly affected by the different climatic variables except for spidermites, thrips and semilooper (Table 5). The different insects had varied responses to the different climatic factors. Bollweevil was positively correlated with windspeed, total bright sunshine and photoperiod while a negative correlation was observed with rainfall and air temperature. This finding indicates that bollworm would prefer cooler temperatures, a main reason no bollworm larva were observed on leaf surfaces towards noon time. They preferred to stay inside the squares and bolls of the cotton plants.

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HEALTH SCIENCES

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TRENDS IN THE HEALTH AND NUTRITION OF FILIPINO CHILDREN (0-19 YEARS) IN THE DECADE 1973 TO 1983

Fe del Mundo, Thaddeus Evangelista and Mario Arciaga
Children's Medical Center, Philippines
Banawe, Quezon City

ABSTRACT

A ten-year survey of the health and nutrition of Filipino children, from birth to 19 years old for the decade 1973 to 1983, is presented to determine the attainments and achievements of agencies and organizations in and out of government and look into current problems and possible solutions.

Of the total population in 1983, the sector below 20 years old (52.9%), showed a Life Expectancy of 62.5 years, an Infant Mortality Rate of 59.3 per 1000 live births and a Literacy Rate of 87.7. The nutritional status of preschoolers with weights higher than 75% of Filipino standard weight has improved to 8% in 1983. The morbidity and mortality rates have declined particularly for the immunizable diseases. Maternal Death Rate was 1.0 or a decrease of 28.6.

In general the basic health and development indicators have show improvement but these are still far from significant or satisfactory, even as compared to countries around the Philippines.

Introduction

All over the world there has been an encouraging and laudable increase in concern and attention given the health and well being of children. This is not only because the young continue to constitute the greater proportion of the population particularly in developing countries, but also because many adverse factors have occurred in recent years such as the economic recession, social unrest, population pressures and soaring prices.

Interest in child health was highlighted during the International Year of the Child (IYC) in 1979. This event served as an impetus for new or innovative or revitalized programs, projects and activities for children. The theme of the IYC celebration, MANKIND OWES TO THE CHILD THE BEST IT HAS TO GIVE, and a continuous reminder that children are tomorrow's future, have provided incentives that encouraged countries to persist in the care of children.

The historical global Alma Ata Conference (1978) or Primary Health Care influenced changes in the focus, directions, targets, strategies and policies in child health care. Impressive and significant priorities have been given to devel-

oping countries, particularly the underserved and unreached through the use of low-cost, simple and unsophisticated procedures and methods, the shift from hospitals to community-based activities, emphasis given to breakthroughs that are affordable, accessible, acceptable and their practical implementation. Training and recruitment of health manpower have undergone changes from highly technical professionals and experts to paraprofessionals and even lay persons, thus allowing delivery of services more readily than in the past, to remote and difficult areas.

Yearly, the United Nations Children's Fund (UNICEF) through its Executive Director, presents a vivid and comprehensive analysis and report of the STATE OF THE WORLD'S CHILDREN which includes what have been accomplished or achieved in different regions or in individual countries. There have served as inspiring and encouraging models to all. The International Pediatric Association as an organized body or through its regional members has also reported on the status of children in Asia, Europe and other sectors. Other international or national groups have commendably followed suit.

In the Philippines, health surveys have been sporadically prepared. The present survey has gathered information and data for the decade (1973-1983), from various sources as the Department of Health (DOH), the National Economic Development Authority (NEDA), the National Census and Statistics Center, as well as Annual reports of different health and nutrition organizations and centers and situational studies of government and private concerns particularly that of the Council for the Welfare of Children (1974 and 1985). Problems in gathering and coordinating the different findings are due to the fact that some are actual figures while others are estimates or projections.

Besides available statistics, the authors have looked into government policies related to health and nutrition and strategies for action to meet needs and problems that would operationalize such policies.

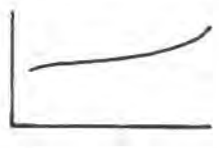
Objectives

It is the purpose of this survey to determine the health and nutrition status of children and their trends in a developing country, the Philippines, where many unfavorable and even unexpected events and factors have affected and played a part in the overall situation of the young.

Although improvements have been noted following the universal trend, these have not been significant nor impressive in the Philippines. In fact, notwithstanding concerted efforts on the part of the government and private concerns to give our children the best they deserve comparable to those of developed countries, situational health studies of the Philippines show there is much to be desired.

It is expected that this particular review of a decade may give a picture of the present health status of children (0-19 years) in the Philippines. This may

STATUS OF FILIPINO CHILDREN (0-19 YEARS)
1973 – 1983

	POPULATION OF CHILDREN (0-19 YEARS) INCREASED FROM 21,426,000 TO 27,017,135 26.1% INCREASE
	CRUDE BIRTH RATE INCREASED FROM 26.1 TO 29.0 11.1% INCREASE
	CRUDE DEATH RATE DECREASED FROM 7.0 TO 6.3 10.0% DECREASE
	INFANT MORTALITY RATE DECREASED FROM 76.7 to 59.3 22.5% DECREASE
	NEONATAL MORTALITY RATE DECLINED FROM 30.2 TO 17.4 42.4% DECREASE
	MATERNAL DEATH RATE DECREASED FROM 1.4 TO 1.0 28.6% DECREASE
	*COMMUNICABLE DISEASES DEATH RATE DECREASED FROM 297.7 TO 212.6 28.6% DECREASE
	TUBERCULOSIS MORTALITY RATE DECREASED FROM 64.7 TO 55.0 15.0% DECREASE
	CARDIOVASCULAR DISEASES DEATH RATE DECREASED FROM 8.03 TO 8.01 0.94% DECREASE
	MALIGNANT NEOPLASM DEATH RATE INCREASED FROM 1.04 TO 5.07 387.5% INCREASE
	LIFE EXPECTANCY INCREASED FROM 61 YEARS TO 625 YEARS 2.4% INCREASE

serve as a basis for the identification of past and current problems, to clarify future action and direction in the formulation of policies and recommendations and to assist in the development of programs and in the implementation of projects which may benefit our children.

Specifically, this study aims to: a) provide information and indicators to measure the progress and impact of the delivery of services for children; b) to identify the sources and statistical areas of concern and provide corresponding recommendations; c) to broaden awareness of various agencies engaged in child development on roles each agency can play; d) to assist funding and supporting agencies to plan and program proper allocation of resources for children.

General Considerations

Demographic trend among children (0-19 years old)

In the Philippines, there has been a 26.1% increase in the 0-19 age group from 1973, an increase which more or less parallels that of the total population (29.2%). This age group comprises more than half of the total population (Fig. 2), reflective of the pyramidal structure of the population in developing countries. In 1975, they comprised 55.74% of the total population but by 1980 this age group comprise about 53% of the total population (Table 1). The gradual decline in the percentage of this group may be due to a gradual decrease in the crude birth rates which was noted in 1980 up to 1983. The proportion of male to female births was 1 male for every 1.1 female as of 1983.

Table 1. Actual children population (0-19-years) Philippines

Age group	*1975*		*1980*		*1984*
All Ages	42,070,660	100.0	48,098,460	100.0	100.0
Under 1	1,213.577	02.88	1,742,912	03.62	3.56
1 – 4	5,267,189	12.51	5,923,285	12.31	12.31
5 – 9	6,330,637	15.09	6,605,446	13.73	13.74
10 – 14	4,950.580	11.76	5,255,641	10.93	10.93
Under 1 to 19	23.43 M	55.74	25.45 M	52.96	52.9

While the Philippine population program had reduced the growth rate from 3.0%, in the 1960's to 2.45% in 1980-83, the child sector continues to be sizeable. Since 1977 about 1.5 million are born every year.

The pyramidal shape of the population graph implies a substantial burden of dependency imposed on those who work. The 1978 census shows that the country's dependency ratio was 88 dependents per 100 persons of working age. This

trend exerts tremendous pressure on the productive sectors of the economy to provide the basic needs of a predominantly young population.

With a total land area of 300,000 square kilometers the population density of the country is 140 per one (1) sq. km. In 1975 around 58 out of 100 Filipinos belonged to the age category below 21. On the average therefore there are about 81 children for every 3 square kilometers in the country.

National budget

It will be noted that in the Philippine allocation for health has always been the lowest among four services and considering that the population increases by 1.2 to 1.5 million yearly, it is not surprising that health services are correspondingly impaired.

Table 2. National budget (in millions of pesos)

	1970	*1975*	*1978*	*1983*
Total	4,053	19,049	27,808	53,729
Economic Services	1,283	8,672	11,272	15,587
% of Total	(21.6%)	(45.5%)	(40.5%)	(29.0%)
Defense	615	3,932	4,542	6,521
% of Total	(15.1%)	(20.9%)	(06.3%)	(12.1%)
Education	1,133	2,212	3,582	6,381
% of Total	(27.9%)	(11.6%)	(12.8%)	(11.2%)
Health	226	785	962	2,525
% of Total	(5.5%)	(4.1%)	(3.4%)	(4.6%)

Health and nutrition manpower resources

The 1973 Health Manpower Resource of the MOH showed that there were 17,417 health professionals including sanitary health inspectors. Of this number 23.6% were nurses, 18.7% physicians, and 4.5% dentists. Also 2,980 were midwives who in the Ministry of Health are the mainstay of services in most communities. Regionwise, the survey registered:

Physicians	13,107
Nurses	8,283
Midwives	6,915

Of the 1,506,356 births registered in 1983, 853,011 (56.6%) had the benefit of medical attendance, an increase of 6.2% ever that of 1973. Majority (52.1%) were attended by midwives, 44.9% by physicians and 3% by nurses. A total of 653,345 birth (43.4%) were attended by either non-medical personnel or none at all. There were 1,130,157 births delivered in the homes and 375,199 delivered

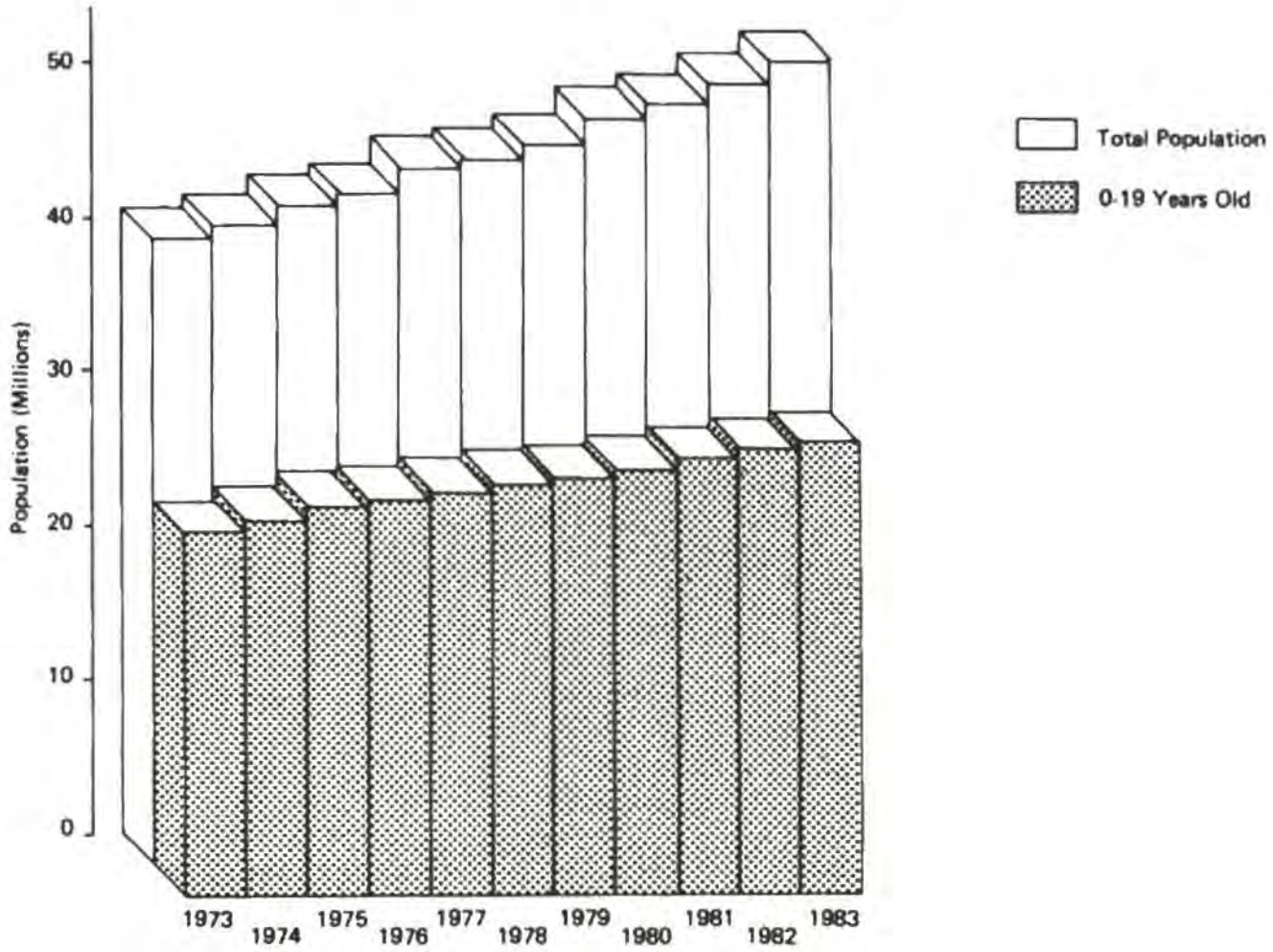


Fig. 2. Total population and the population
of 0-19 years old
Philipines, 1973-1983

in the hospital representing 75% and 25% of the total respectively as depicted in Figs. 3 and 4.

Table 3. Medical manpower, Philippines (1984)

Philippine Medical Association

Region		
Luzon	7,029 (70%)	1:3700
Visayas	1,739 (15%)	1:7000
Mindanao	1,763 (15%)	1:7200
Total MDs	11,331	1:5000
Total Population	53,673,000	

Philippine Pediatric Society (1984)

Metro Manila	554	(approx. 4 million children)
Provinces	294	(approx. 22 million children)
Total	848	pediatricians
Children 0-19 years	26,535,937	

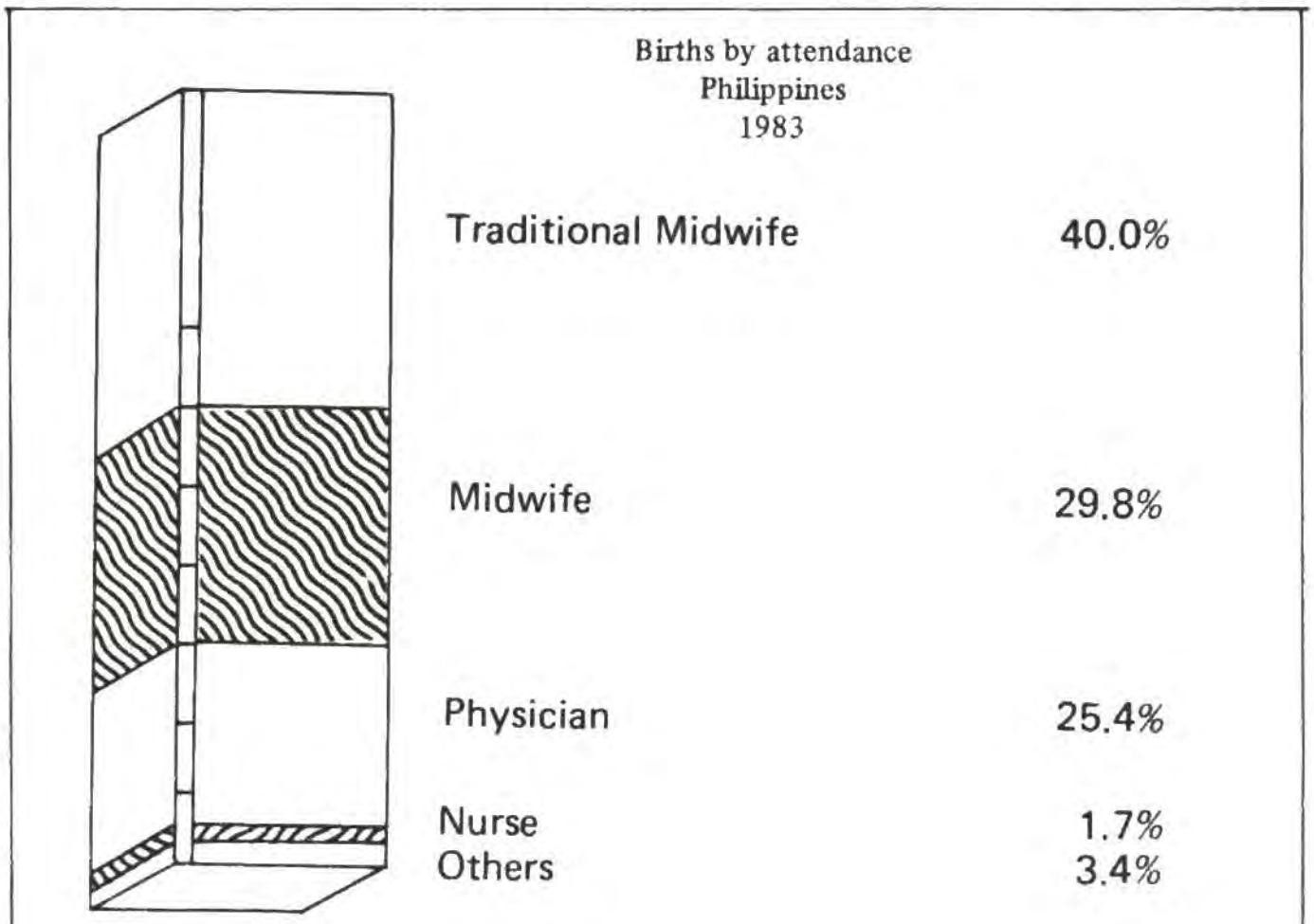


Figure 3.

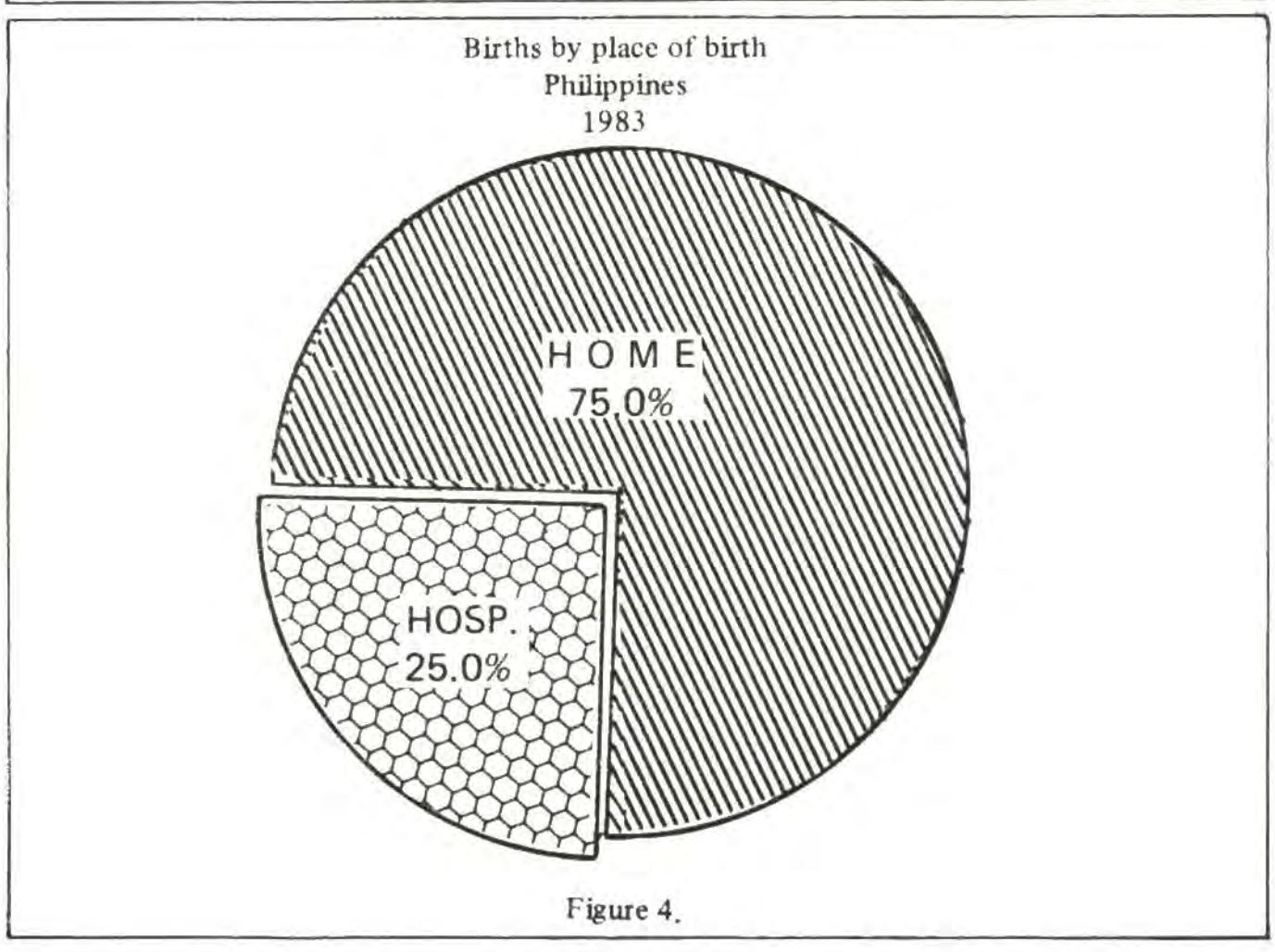


Figure 4.

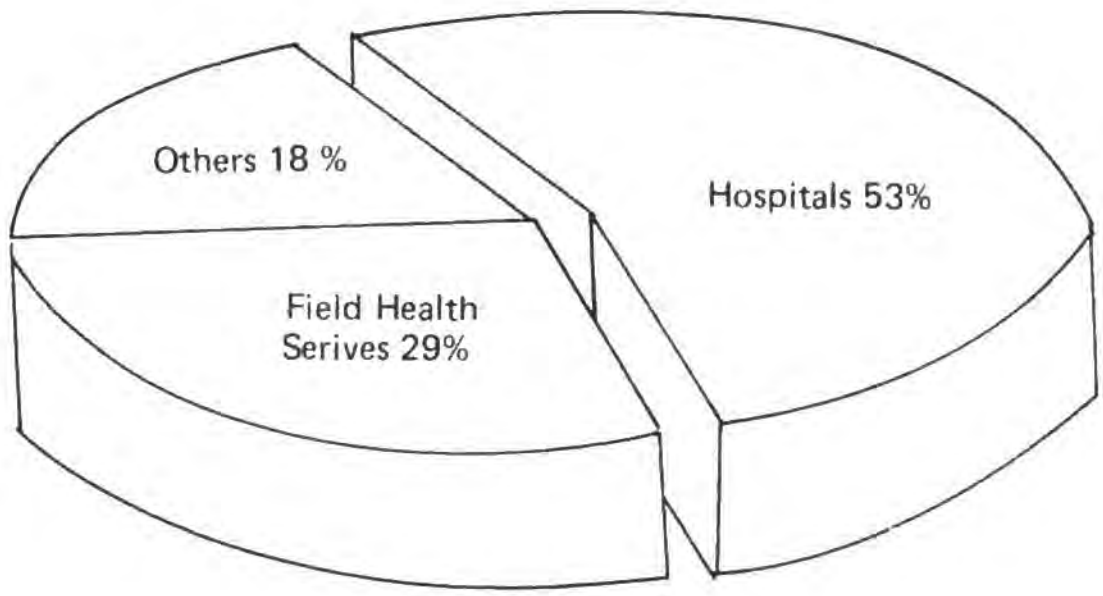


Fig. 5. Share of hospitals in health budgets

PHILIPPINES
(1982)

Source: World Bank and WHO

Table 4. Number of health facilities, Philippines 1980 and 1984

Facility	*1980*	*1984*
Hospitals*	345	367
Rural Health Units	1,991	1,991
Barangay Health Stations	7,353	7,991
Sanitaris	8	8
Chest Clinics	21	17
Skin Clinics		
Travelling	16	16
Stationary	6	6
Family Planning Clinics	1,743	1,542
Social Hygiene Clinics	32	30
Mental Hygiene Clinics	23	25
Dental Clinics	520	652
Malaria Units	33	32
Schistosomiasis Units	23	23
Filariasis Central Units	3	3
Nutriward Units	168	188

*Hospitals under the MOH only.

In the government subsystem in 1976 the health facilities are as enumerated above. Supportive are some 743 Puericulture Centers (private Maternal and Child Health facilities with Family Planning), 300 health centers, 51 maternity houses,

mobile hospitals, 8 Sanitaris and 5 static clinics. The RHUs are the basic field health units of the Ministry of Health located in municipalities with their satellite barangay (village) health stations. The latter are more accessible to the rural population, particularly in depressed areas.

There has been a continuing increase in the number of barangay health stations and rural health units through the years.

Of all the health facilities and services, hospitals get the biggest share from the national health budget (Fig. 5).

As of 1983, there were a total of 1,705 hospitals all over the country, seventy percent (70%) of which are privately owned. There was a 122.0% increase in the total number of hospitals built from 1973 to 1983.

Although 70% of the total number of hospitals are privately owned, the government hospitals held the bigger share (54%) of the total bed capacity.

Morbidity

Philippine Statistics on the notifiable disease presented by the Disease Intelligence Center, Department of Health were collected from the morbidity reports received weekly from all provincial and city health offices throughout the country.

A ten-year average (1973-1983) of the rates of the 10 leading causes of morbidity specific for the age group 0-19 years old (Fig. 6), in accordance with notifiable disease specified by the Department of Health (Act 3573).

Bronchitis had the highest morbidity rates (821.5 per 100,000 population of 0-19 years old) followed very closely by diarrheas (all forms) with a rate of 819.8. It will be noted that bronchitis was not included as a notifiable disease in 1974 and 1975. Diarrhea includes cholera, typhoid, and paratyphoid fevers and other salmonella infection, food poisoning, all forms of dysentery (bacillary and amoebic) and non-specific diarrheas. Influenza, with a rate of 550.9 is a far third. Pneumonias which ranked fourth, include both viral and bacterial etiologies. Measles ranked fifth with a rate of 117.4 per 100,000 population of 0-19 year olds. Whooping cough, malaria and tuberculosis included all clinical forms but it has been noted that approximately 96% of illnesses due to tuberculosis in general, are respiratory in nature. Schistosomiasis, although endemic in a few areas, ranked 9th with a rate of 7.8. Malignant neoplasms ranked 10th with a rate of 7.1 and include all growths on any site of the body. This did not include leukemias.

There were only minimal changes in the rates and ranking of the 10 leading causes of morbidity in 1973 and 1983. Malaria rose from 8th to the 6th rank although there was a decrease in the rate. Tuberculosis and schistosomiasis which ranked 6th and 9th in 1973, dropped to 8th and 10th respectively, in 1983. Their rates also decreased. Malignant neoplasms rose from the 10th in 1973 to 9th place in 1983, with a corresponding increase in rates. (Fig. 7).

It is noteworthy that except malignant neoplasm, all these leading causes of morbidity in the age group of 0-19 years, are communicable or infectious. Of

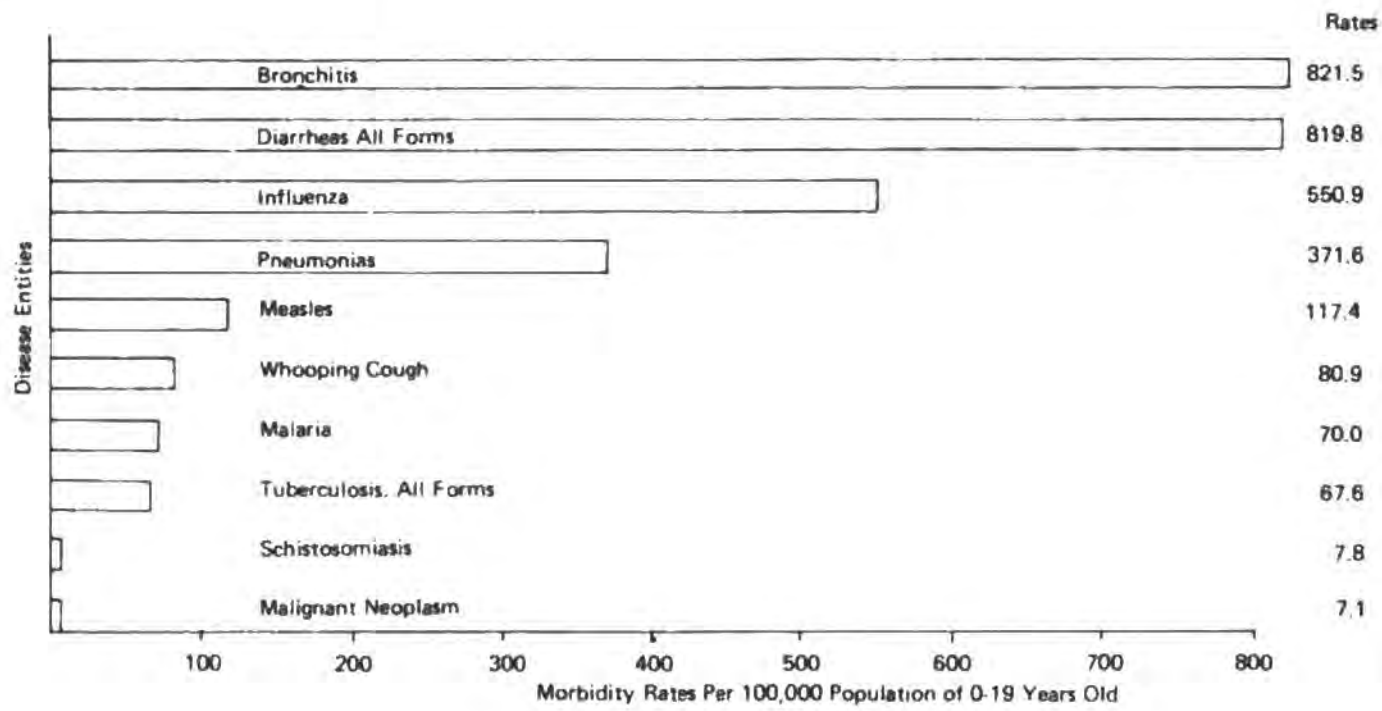


Fig. 6. Leading causes of morbidity among 0-19 years old: a 10-year average (1973-1983) Philippines.

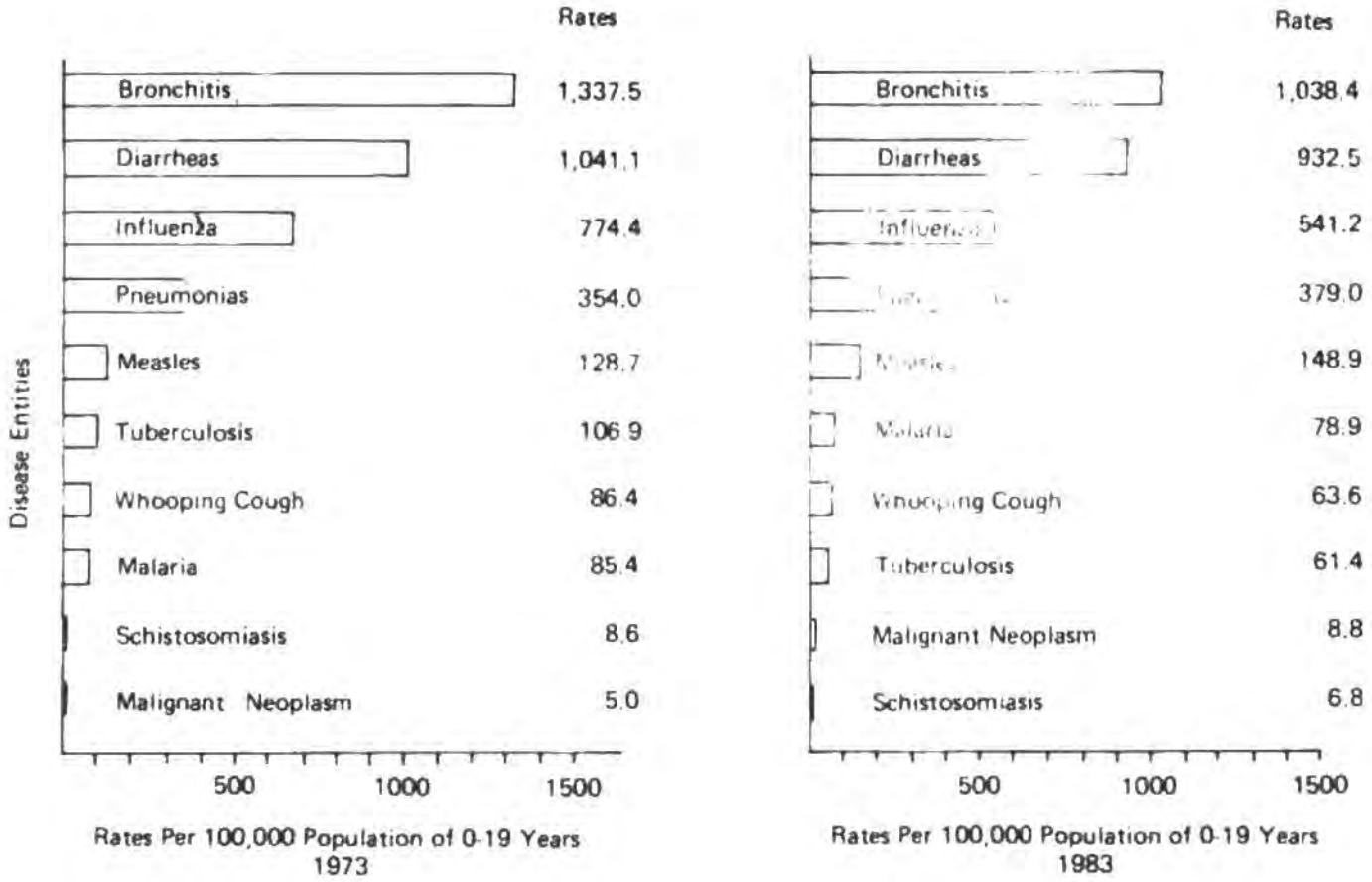


Fig. 7. Comparison of leading causes of morbidity among 0-19 years old in 1973 and 1983 Philippines.

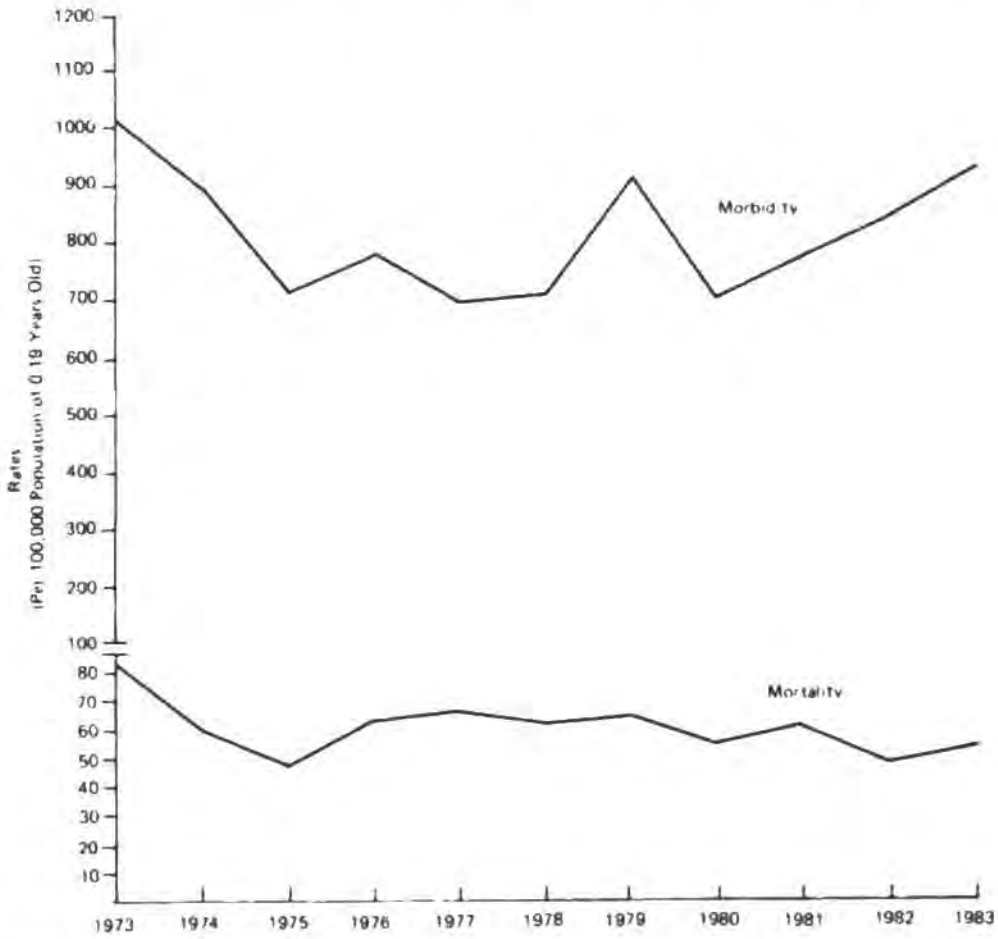


Fig. 8. Diarrhea, all forms: morbidity and mortality trends among 0-19 years old Philippines 1973-1983.

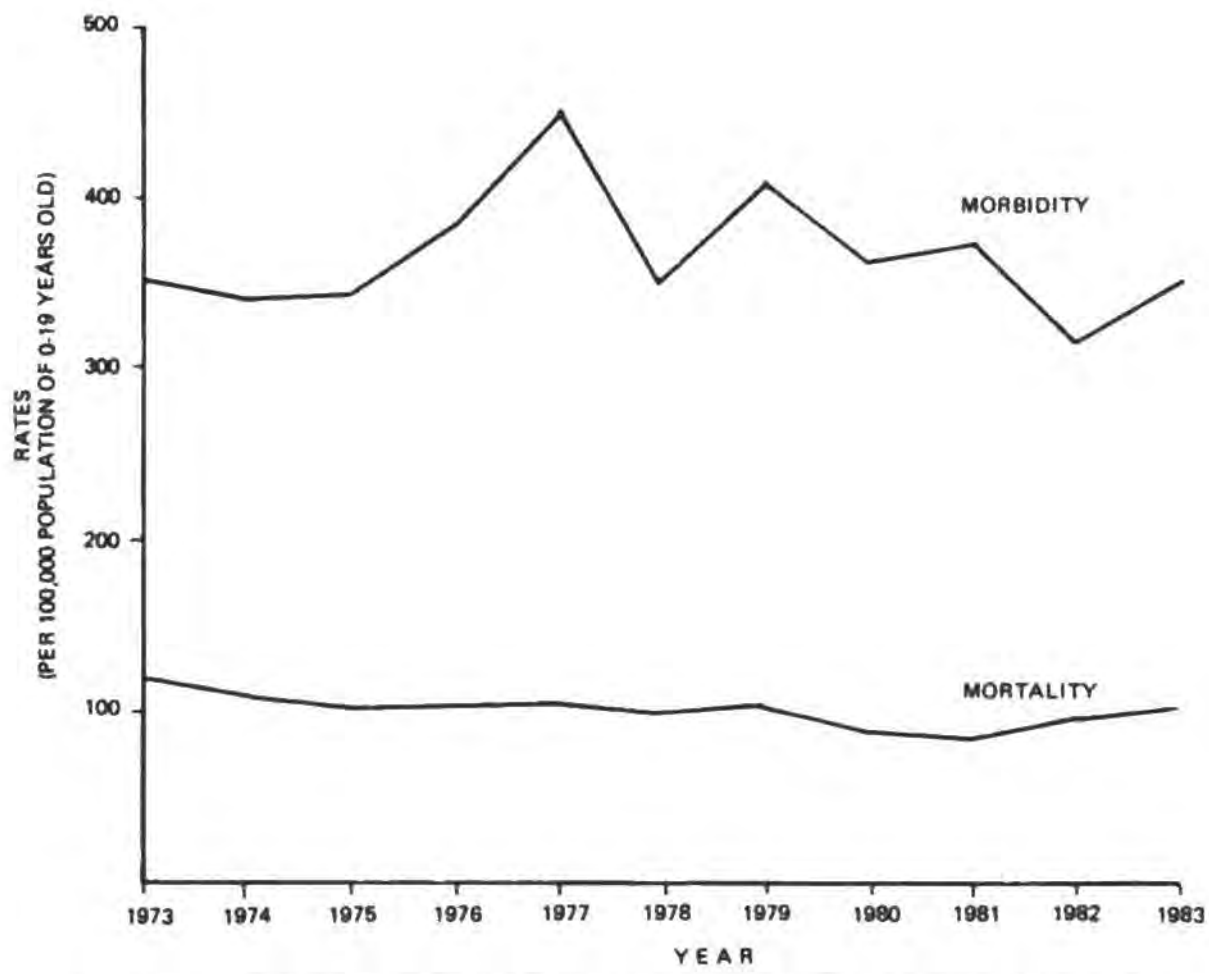


Fig. 9. Pneumonia: morbidity and mortality trends among 0-19 years old. Philippines 1973-1983.

the infectious diseases, 4 involve the respiratory system (bronchitis, pneumonias, whooping cough and tuberculosis), one involves the gastro-intestinal tract (diarrheas), 2 are viral (influenza and measles), and 2 are parasitic (malaria and schistosomiasis).

The incidence (and mortality) from most of these notifiable diseases showed a continued decline from 1973 to 1983. Although the incidence of diarrhea (Fig. 8), bronchitis and pneumonia (Fig. 9), in general were high in 1973, there was a declining trend, the lowest rate being in 1977 after which there was an increased rate up to 1983. All forms of dysentery and food poisoning, however, showed an increasing trend during the 10-year period. Leprosy showed a similar trend causing the increase in cases. The incidence of tetanus, whooping cough and polio decreased from 1973 to 1983. Measles and influenza have irregular trends due to outbreaks at almost yearly intervals. Malignant neoplasms have increased in incidence and mortality.

Mortality

The 10-year average of the leading cases of mortality specific for the age group 0-19 years is shown in Fig. 10.

Pneumonias top the list as a cause of mortality among the 0-19 age groups and in all age groups. There are, however, differences in the ranking of diseases as diarrheas, nutritional deficiencies and avitaminosis and measles. Accidents are more common among the 0-19 year olds, than among all groups, as a whole. On the other hand, heart and vascular disease, malignant neoplasm and tuberculosis are less common causes of death among children less than 19 years old than in the general population. Bronchitis and tetanus ranked 5th and 7th among the leading causes of mortality among children less than 19 years old but not among the 10 causes of mortality in the general population.

Among the 10 leading causes of mortality in children less than 19 years old, 6 were communicable. Of these, 3 are immunizable diseases through our Expanded Program of Immunization. The other 4 are non-communicable. Nutritional deficiencies include goiter without throitoxicosis, avitaminosis, kwashiorkor, nutritional marasmus and other protein-calorie malnutrition.

Most of the communicable diseases (pneumonia, bronchitis, tetanus and tuberculosis) had a decrease in mortality and morbidity rates from 1973 to 1983. Diarrheal rates were variable with slight improvement noted from 1979-1982 (Fig. 8). Measles, had a variable course, the lowest rates being noted in 1974 and the highest in 1983 with variable rates in between.

Mortality rates due to accidents increased from 1974-1978, then a decrease from 1979-1983. A similar trend was noted fro nephrosis, nephritis, nephrotic syndrome and infection of the kidney. Mortality rates from avitaminosis and other nutritional deficiencies showed a declining trend from 1973-1983. In general, the mortality rates of communicable diseases as a group, presented a generally

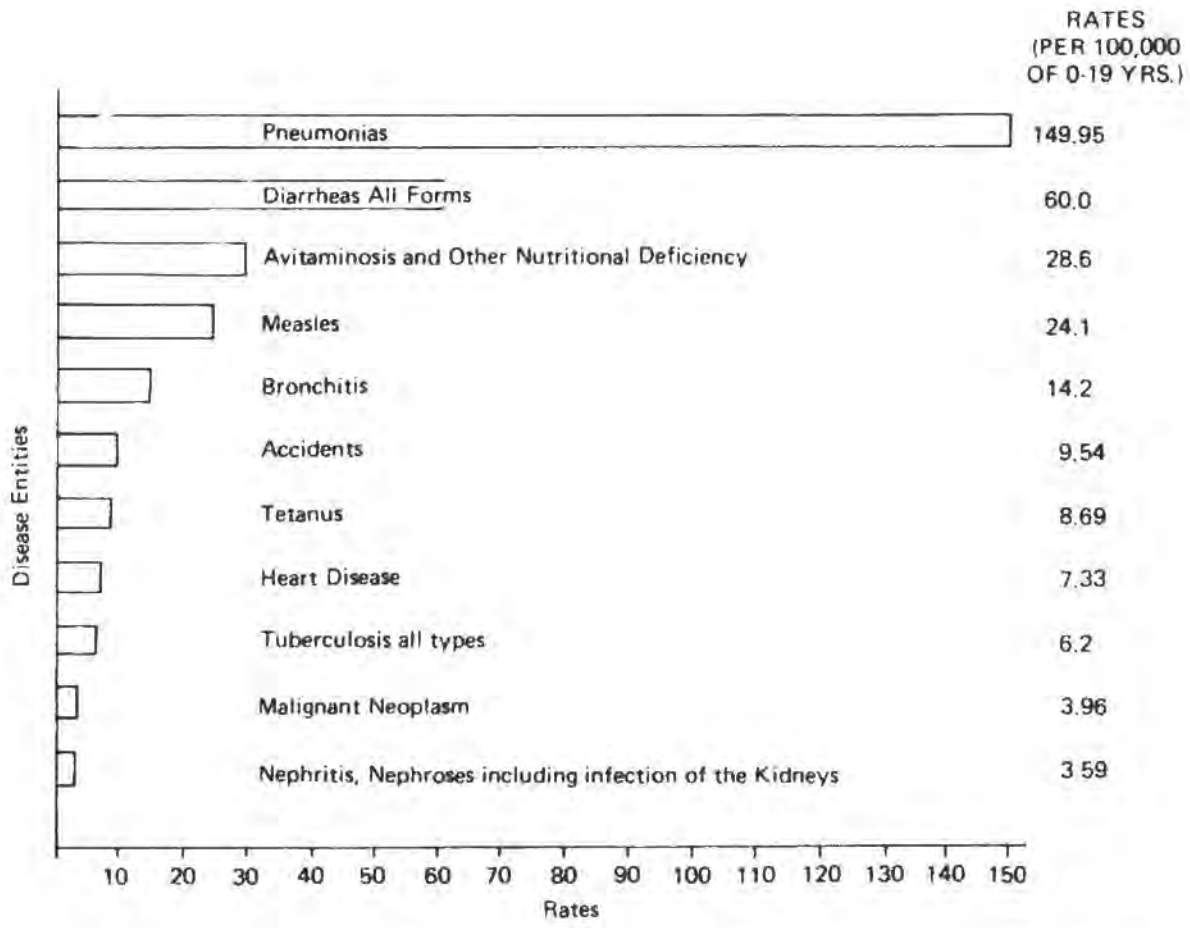


Fig. 10. Leading causes of mortality among Filipino children 0-19 years old: a 10-year survey Philippines 1973-1983.

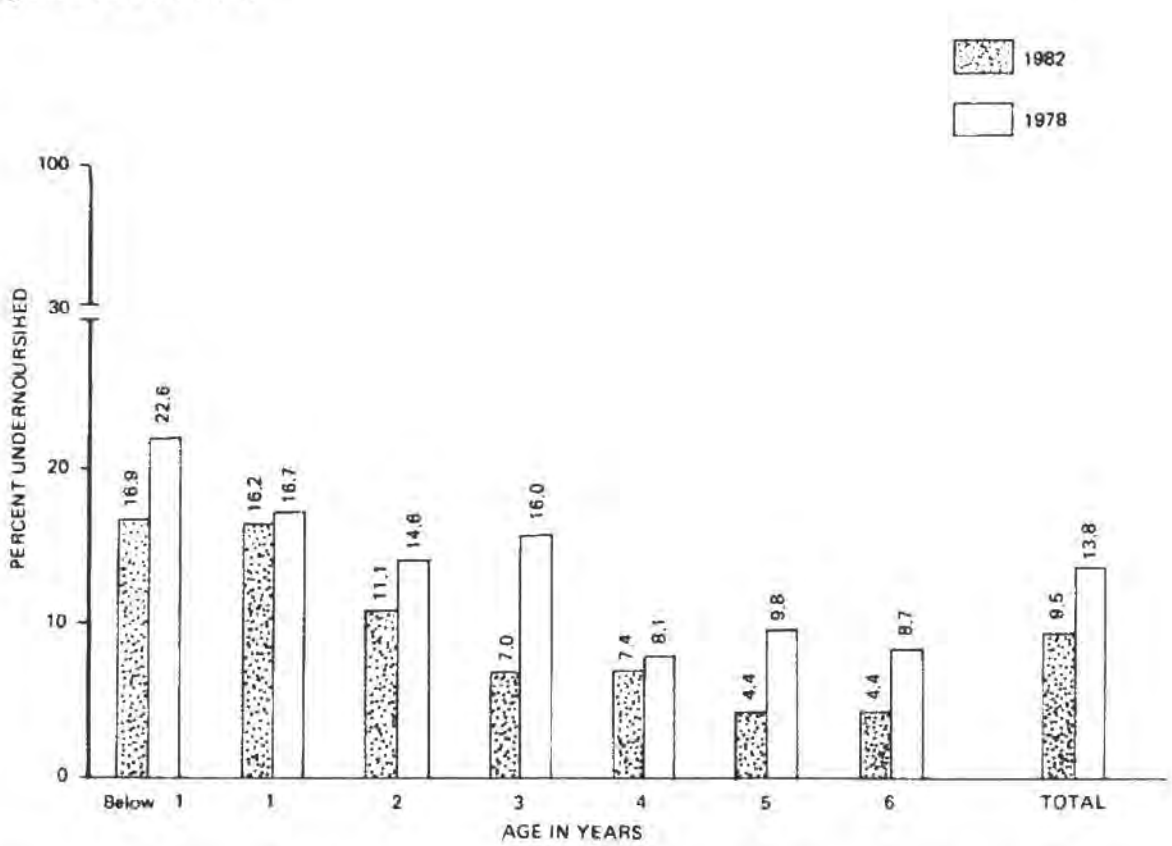


Fig. 11. Comparison of distribution of undernourished 0-16 years old children using weight for height standard by age.
Philippines, 1978-1982.

Source: Phil. Journal of Nutiriton, April-June, 1985

decreasing pattern while heart diseases and malignant neoplasms showed a gradually increasing trend.

Accidents include a wide range of spectrum namely: railway, motor vehicle and traffic accidents, water, air and space transport, poisoning, falls, fires and flames, submersion, suffocation and foreign bodies, accidents due to natural environment, adverse effects of drugs and medicaments, biological substances and late effects of accidental injury.

Cardiac diseases include acute rheumatic fever, chronic rheumatic heart diseases, ischemic heart diseases, hypertension with heart involvement, and other forms of heart diseases.

Nephritis, nephrosis, nephrotic syndromes and infection of the kidneys and malignant neoplasm show very similar rates and both occupy the 10th rank.

Nutritional status of children

From 1973 to 1983, there have been several number of surveys to assess the nutritional status of Filipinos, notably the Department of Health, Food and Nutrition Research Institute and the National Nutrition Center, with different findings which are in Table 5. To standardize data, we chose those of the Food and Nutrition Research Institue, in two (2) nationwide nutrition surveys of 1979 and 1982, which have been divided in 3 groups: food consumption, anthropometry, and clinical assessment.

a) Food consumption. In general, there has been an increase in the consumption of almost all food groups from 1978 to 1982. Highly significant increases in consumption were noted for sugars; meat and poultry (including eggs); dried beans, nuts and seeds; green leafy and yellow vegetables; miscellaneous foods. The overall increase in the consumption of these food groups have been attributed largely to the following: considerable focus by both government and private sectors on production of these commodities; massive campaign on the importance of green leafy and yellow vegetables in the diet through the Philippine Nutrition Program; and continued encouragement given to home production.

Reduced consumption were reflected in 2 food groups: cereals and cereal products and Vitamin-C rich foods.

Comparing nutrient intakes of the 2 survey periods, highly significant improvements were noted for riboflavin, protein and fat. Energy intake had no significant change. Ascorbic acid intake decreased from the 1978 intake level with the significant reduction in the consumption of Vitamic-C rich foods.

As regards nutrient sources of energy, the Filipino diet has remained characteristically carbohydrate in nature. In both surveys, carbohydrates constituted about 3/4 of the total one-day per capita.

b) Anthropometric measurements. The nutritional status of pre-schoolers (0-6 years old) was based on weight-for-height, weight-for-age and height-for-age indices. The pattern of malnutrition changes with each index for they measure different things.

Weight-for-height measurements provide a picture of current, acute malnutrition and is age independent until the age of 10-11 years. Using a cut-off point of less than 85% of the standard weight-for-height findings, there were 9.5% undernourished (moderate and severe) pre-schoolers out of the 3,615 subjects examined. These children may be suffering from acute malnutrition, needing priority action. The highest percentage of children found underweight-for-height were the 2-year olds and below. A downward trend in the prevalence of malnutrition among the undernourished children was found in the 1982 survey as compared with that of 1978 (Fig. 11). Highly significant decreases in the overall proportion of children with acute malnutrition was shown in 1982, representing a 31.2% nutritional status improvement over the 1978 survey. The overall proportion of under-weight-for-age children in the 1982 survey revealed a highly significant decrease of 21.5% than that of the 1978 survey.

Among pre-schoolers the rate was 20.6% as measured by height-for-age using a cut-off point of less than 90% of standard height for age. Height deficit tended to parallel the increase in weight deficit among the 1-3 years children. The height deficit found in infants may be the consequence of small size at birth rather than an indication of postnatal nutrition because it takes some time to develop skeletal deficits.

The nutritional status of school-age children (7-14 years old) was assessed using the percentage of standard weight-for-age. Using a cut-off of 70% of standard, 18.5% of these children were under weight for age. Height-for-age is a measure of past or chronic malnutrition and may be indicative of the history and effects of malnutrition in their early years of life. Among the 7-14 years old children, 24.6% were found below 90% of the standard height-for-age. About 14.7% of children 7-14 years old surveyed were both underweight and stunted. There were however, not enough data during the 1979 survey for comparison.

c) Clinical Assessment

1. Anemia assessment. The overall prevalence of anemia in the population is 26.6%. Among children, the prevalence of anemia was highest among the age group below 1 year (51.3%), as shown in Fig. 12. Their mean hemoglobin level of 10.68 g/dl was the lowest among all age groups. This may be due to an abrupt decrease in erythropoiesis and depletion of iron stores during the stage of rapid growth concomitant with intake of either breastmilk or unsupplemented cow's milk with low iron content. The iron requirement of infants, despite their much smaller body size is almost as high or higher than that of the adults.

2. Vitamin A deficiency. Clinical assessment of vitamin A deficiency in the 1982 survey included nightblindness and Bitot's spots among 2-6 years old Filipino children which were 1.6% and 1.4% respectively, indicating that vitamin A deficiency is a public health problem. The highest prevalence in nightblindness was among the 5-6 years old while that of Bitot's spots was on the 3-5 years old (Fig. 13). The highest prevalence of both nightblindness and Bitot's spots were in the 3-year old. The clinical findings of vitamin A deficiency may be closely

interrelated with the prevalence of undernutrition among pre-schoolers, using weight-for-height index. It could be presumed that those wasted and stunted pre-schoolers were deficient in protein, calories and fat which are very important in the conversion of B-carotene to arrive vitamin A and in its transport, absorption and storage. As vitamin A is important in maintaining the integrity of epithelial tissue, its deficiency may lead to signs and symptoms of early xerophthalmia among pre-schoolers.

3. Goiter prevalence. The highest goiter prevalence using the WHO criteria, was noted among the lactating women 13-20 years old (7.7) compared to the total goiter prevalence of 3.1 in the survey group. More females than males were noted to have goiter implying that the thyroid gland tends to increase in some until the end of the reproductive period because of the increased iodine requirements during child bearing and lactation. Thyroid enlargement noted among the non-pregnant, non-lactating women could be due to genetic predisposition and intake of goiterogenic substances.

4. Assessment of parasitological infection. One of the indirect ways to assess environmental sanitation status in the population is the parasitic prevalence rates. Intestinal parasitism was found in 69.3% of all subjects examined, with the 1-12 years old subjects having highly significant prevalence rates (Table 6). Ascaris was the most common parasite (51.6%) in the population.

A number of factors may play a role in these findings, to wit: poor hygienic practices of children and even adults, source of water supply and manner of garbage disposal.

Maternal health

Mother and child constitute one biologic unit. Directly or indirectly the health and nutrition status of the mother affects her offspring. Hence any discussions on child health from the period around birth to adolescence involves the mother.

A study of the Philippine maternal nutrition status presented the levels of biochemical parameters among pregnant woman with respect to iron, carotene, vitamin A, vitamin C and proteins. The study also presented the average per capita nutrient intakes computed to recommended dietary allowance (RDA). The results showed that 20 to 28 per cent of the subjects were "deficient to low" in vitamin A, 13 to 59 per cent in serum carotene, and 4 to 7 per cent in vitamin C.

As regards dietary intake, the mean levels of calories intake of subjects ranged from 83.5 to 101.6 per cent of RDA, while the intake of protein was 14.8 and 14.9 per cent, respectively, in excess of the recommended amounts for the subjects in the first and second trimesters of pregnancy. Iron intake not only 78.2, 82.4, and 61.8 per cent of RDA for the groups, respectively. The calcium intake for the third trimester subjects was quite low (47.3 per cent of RDA). The intake of vitamin A was 69 per cent of RDA for the second trimester subjects. Ascorbic acid intake was only 51 to 53 per cent of RDA.

Table 5. Prevalence of type of intestinal parasitism by age, sex and physiologic state of subjects: Philippines, 1982

Age, Sex and Physiological State	*Number Examined*	*Types of Intestinal Parasitism*									
		Ascaris lumbricoides	*Trichuris trichiura*	*Hook-worm*	*Enterobius vermicy-laris*	*Histo-lytica*	*Eschericia coli*	*Giardia lamblia*	*Schistosoma japonicum*	*Others*	*Any Parasite*
		Percent Positive									
Below 1 years	424	10.7	3.9	0.4	-	0	0.1	0	0	0	13.8
1 – 6 years	2,920	63.9	39.6	9.1	20.0	0.2	0.5	0.3	0.2	0.3	78.3
7 – 12 years	2,705	65.3	52.5	12.4	-	0.3	0.2	0.3	0.1	0.1	79.7
13 – 59 years											
(males	3,703	44.6	36.0	19.6	-	0.1	0.2	0.2	0.1	0	66.2
pregnant, non-lactating females)	3,420	46.1	37.6	12.3	-	0.2	0.3	0.1	0.1	0.1	65.3
60 years and over	886	35.0	35.7	20.0	-	0.3	0.1	0	0.4	0	61.1
Pregnant	246	59.1	36.9	14.3	-	0	0	0	0	0	69.1
Lactating	435	58.0	39.8	13.3	-	0.1	0.1	0.1	0	0	72.9
All Ages	14,739	51.6	39.3	13.7	-	0.2	0.3	0.1	0.1	0.1	69.3

[1]A subject may be positive for more than one type of parasite.
[2]Examination one only on 1 – 6 year old children.

The maternal mortality rate in 1975 was 1.4 per 1000 live births and 1.1 in 1979. The leading causes of maternal mortality in 1975 were hemorrhages (51.3%), hypertension (13.8%) and abortion (11.3%). By 1981 74.3% of birth occurred in the homes and 17.0% in hospitals, 2.5% in clinics and 6.2% in puericulture centers.

Basic health indicators

These are measures of progress commonly referred to as health indicators and include infant mortality, life expectancy and literacy. They represent aspirations of most nations and are efforts for wider progress as well as measures of specific achievements. Thus infant Mortality Rate reflects availability of safe water, mother's health and nutrition and quality of the home environment. Literacy rates represent the percentage of those over 10 who can read and write but for the poor to contribute to and benefit from the process of development. Life expectancy conveys quality of life and chances of death.

These indicators were incorporated in the targets of the International Development for the 1980s and were adopted by the UN General Assembly as goals for all nations to aim at by the year 2000, specifically IMR of 50 or less an average life expectancy of 60 or more and acquire lasting literacy requiring that every child should have at least 4 years of primary school education.

Table 6. Philippine development indicators on health.

Indicators	*1972*	*1982*	*1983*
Life Expectancy (in years)	57.0	62.6	62.5
Infant Mortality Rate (per 1,000 live births)	78.4	60.6	59.3
Crude Death Rate (per 1,000 population)	10.3	8.4	8.2
Hospital Bed-Population Ratio	1:815	1:612	1:615
No. of Rural Health Units (cumulative)	1,705*	2,019	–
No. of Barangay Health Stations (cumulative)	3,023*	7,250	–
MEDICARE			
Coverage (million persons, cumulative)	7.3	19.5	22.8
Beneficiaries (thousand persons)	55.4	1,384.2	1,443.3

*1975

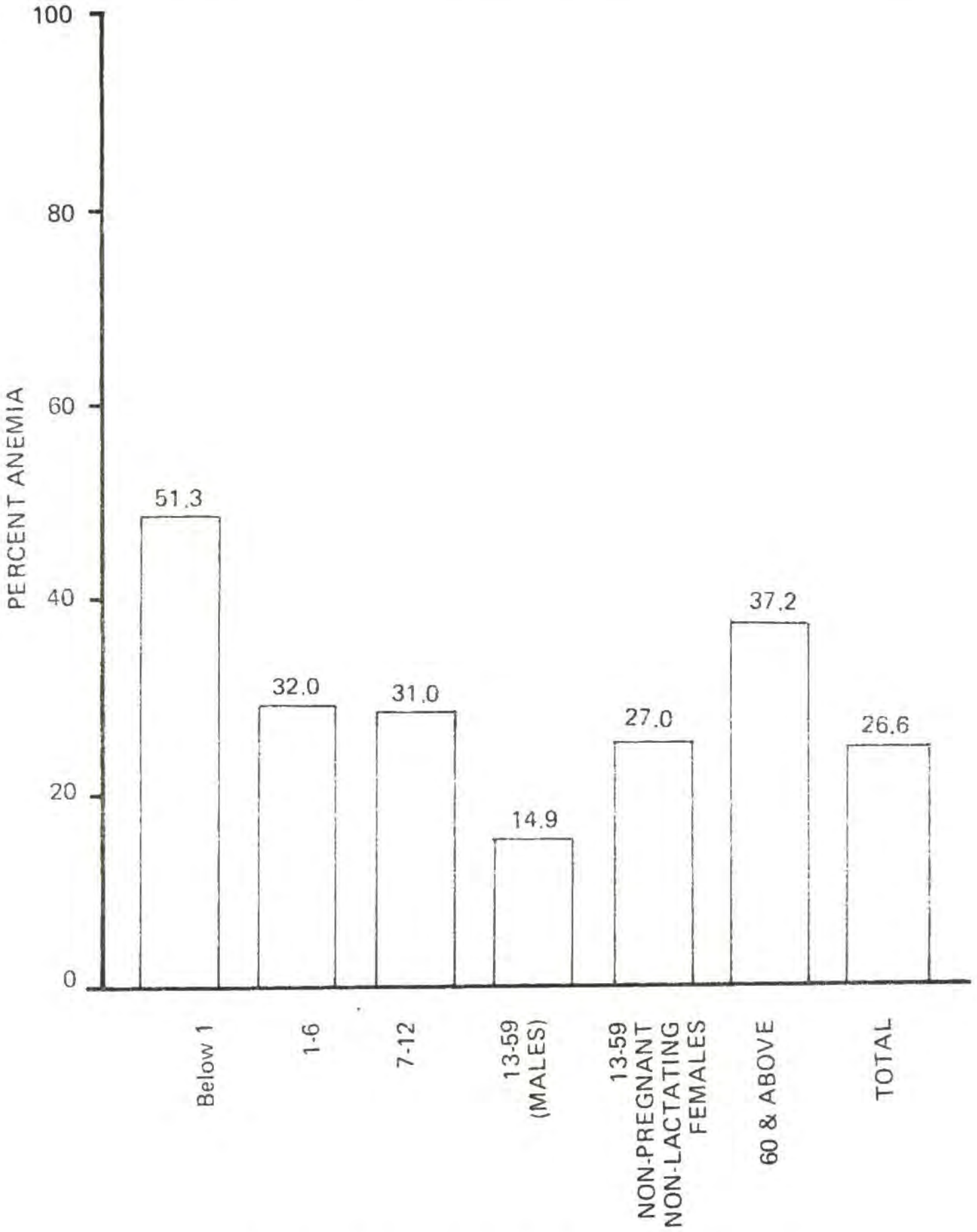


Fig. 12. Prevalence of anemia by age, sex and physiological state Philippines, 1982.

Source: Phil. Journal of Nutrition, April-June, 1985

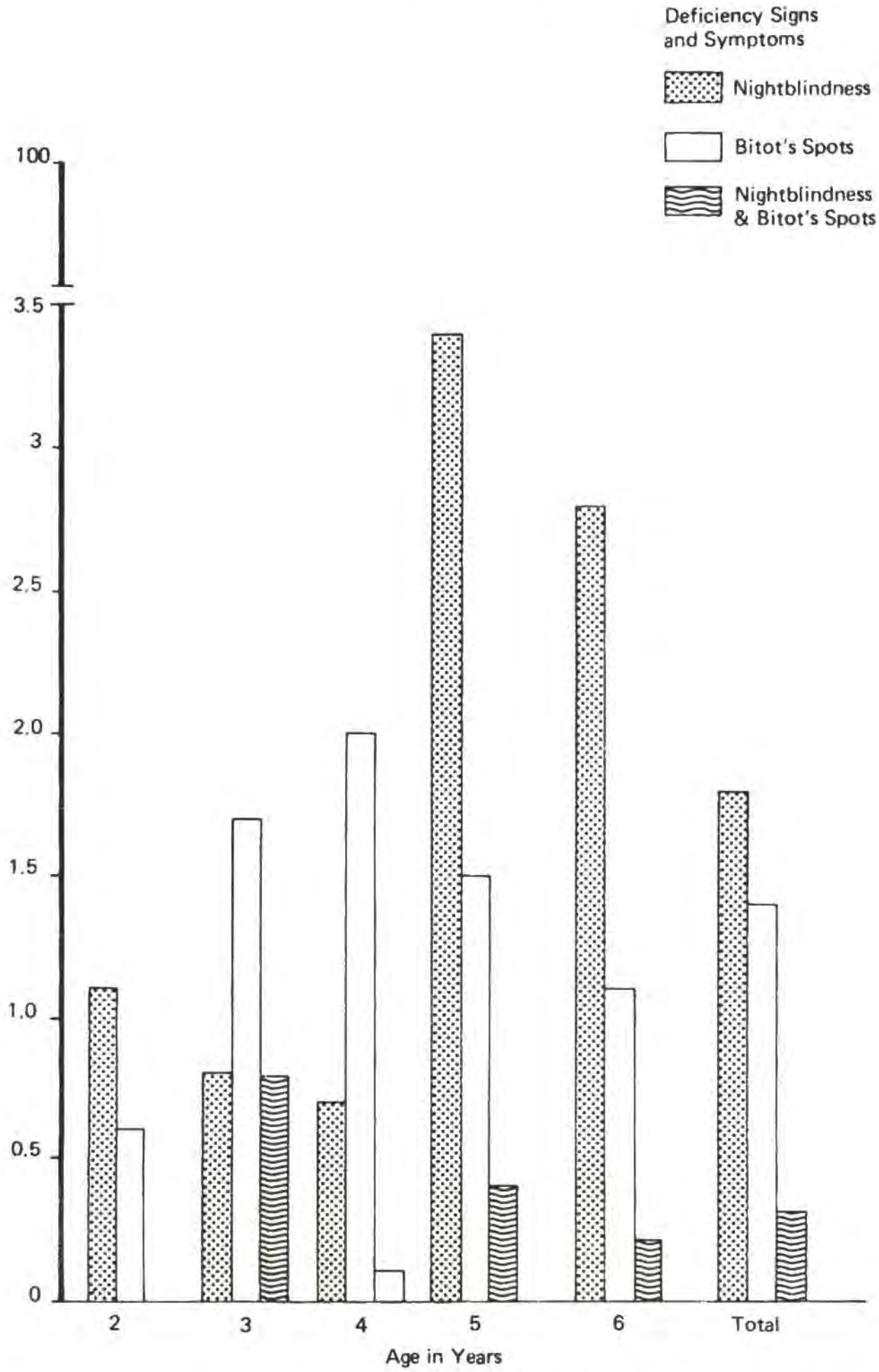


Fig. 13. Prevalence of nightblindess and bitot's spot among 2-6 years old Filipino children by age.
Philippines, 1982.

Infant mortality rates (IMR)

Infant mortality rate, or the number of deaths below one year per thousand live births, is considered one of the most sensitive indicators of progress in children. It has been fittingly stated that IMR is the most revealing measures of how well a society is meeting the needs of its people. It reflects not only per capita stocks of food, clean water, and medical care but also the actual availability of such amenities to all members of the population.

IMR is increasingly important not only because of the health aspects and effects on the quality of life but also because of its social and economic implication and effects on national development. A graphic presentation of IMR in selected Asian countries is shown in Fig. 14.

In the Philippines, the IMR has decreased over the 10-year period under review, from 76.5 in 1973 to 59.3 in 1983 (Fig. 1). The infant death rate curve has shown an irregular but marked declining trend. An all time low IMR for the country was observed in 1978 (53.1). Mortality during the first 6 days of life account for 32.8% of infant deaths.

The leading causes of infant mortality are shown in Fig. 15 in practically the same order and with very slight differences from year to year. Pneumonias and gastroenteritis take the lead each year; seven (7) out of the ten causes are due to infections.

Life expectancy

Estimates indicate that the expectation of life at birth, or the average number of years that a baby born during a specific period can expect to live, has accelerated to reach around 61 years in 1973; the increase has been slow but steady to 62.5 in 1983.

Table 7. Life expectancy in years (Projection only)

Year	*Male*	*Female*	*Difference*
1973	57.04	61.75	4.75
1974	57.90	61.75	3.83
1975	58.06	61.90	3.84
1976	58.53	62.39	3.86
1977	58.98	62.84	3.86
1978	59.39	63.25	3.86
1979	59.77	63.61	3.84
1980	59.80	63.4	3.6
1981	60.10	63.7	3.6
1982	60.4	64.0	3.6
1983	60.7	64.3	3.6

Literacy rate

The national literacy rate of Filipinos 10 years and over was 83.4% in 1975 and 82.7% in 1980. The urban literacy rates were higher than the rural rates with an average urban-rural difference of 12.9%. Despite this impressive record, studies show that college freshmen are poorly prepared for language skills, mathematics and the sciences.

The proportion of private household population 10 years old and over are able to read and write has slightly decreased from 83.36% to 82.72%. This however may be due to sampling errors. There was no difference in the literacy rate of males (82.82%) and females (82.63%) as of 1980. There is a higher rate of literacy in urban areas than the rural areas which is to be expected in view of the concentration of schools and accessibility in urban areas.

Table 8. *Private household population 10 years old and older who were able to read and write

	1970	*1980*
Total	83.36%	82.70%
Male		82.62%
Female		82.63%

*Private household population 7 years old and over by highest grade completed. Philippines 1975-1980.

	1980	
	Urban	*Rural*
Male	93.04%	76.70%
Female	92.0%	76.35%

Crude birth rate

The crude birth rate which indicates the general magnitude of the fertility level of the population, has increased from 26.1 per 1,000 population in 1973 to 29.0 per 1,000 population in 1983, registering an 11.1% increase in the 10-year span. The rates increased gradually with the highest (30.7%) rate being noted in 1979, followed by a gradual decline up to 1983 (Fig. 16). The lowest crude birth rate recorded was 24.8 per 1,000 population in 1972. Although the crude birth rate has shown a general declining trend since 5 decades ago, changes were rather slow and irregular.

Crude death rate

The crude death rate is a measure of the average risk of death of the population at large. It has decreased by 10% from 7/1000 population in 1973 to 6.3/1000 population 1983 as shown in Fig. 16 representing 327,260 deaths. This pattern of a general downward trend with slight thoughts as has been the pattern for the past 4 decades.

Programs and Strategies

Primary health care (PHC)

It will be recalled that in 1978 a global conference was held in Alma Ata, Russia, in which PHC was promulgated and adopted by 130 nations. A basic approach was developed towards the provision of health services that are accessible, affordable and sustainable by the community. This brings health care closer to the children and their families and reaches out as far as possible to where people live, work and survive. PHC has been adopted and implemented in the Philippines as a national program and a major health activity since October 19, 1979. DOH was directed to design and develop programs which will focus on health development at the community level particularly in rural areas and utilizing PHC system to control and eradicate the immediate and specific health problems in Philippine communities.

In general the objective of PHC is to mobilize communities and make them participate effectively in identifying their health needs and in providing their solutions through self-reliance and self-determination. Components of PHC are: a) health education; b) MCH and family planning; adequate food supply and proper nutrition; c) environmental sanitation; including adequate supply of safe water; prevention of communicable diseases; the use of essential drugs.

National program for the control of diarrheal diseases

The National Program of the Control of Diarrheal Diseases (CDD) of the Ministry of Health (MOH) was formerly launched in October, 1980 with the following objectives. 1) to reduce mortality from the diarrheal diseases among children less than 5 years old by 75% in 1987 through extensive use of Oresol and 2) to bring down diarrhea morbidity by 50% in 1987 through strengthening the components of Environmental Sanitation, Nutrition, Maternal and Child Health, Surveillance, Epidemic Control and Health Education.

Oresol production, was started in 1977 on a small scale and in 1980, its production has increased 12.6 times.

After 4 years of implementation, the impact of the CDD Program was evaluated jointly by representatives of the MOH, WHO, UNICEF and USAID from January 28 – February 11, 1985. A report on the comprehensive program review has shown that some impact on the mortality from diarrheal diseases has been

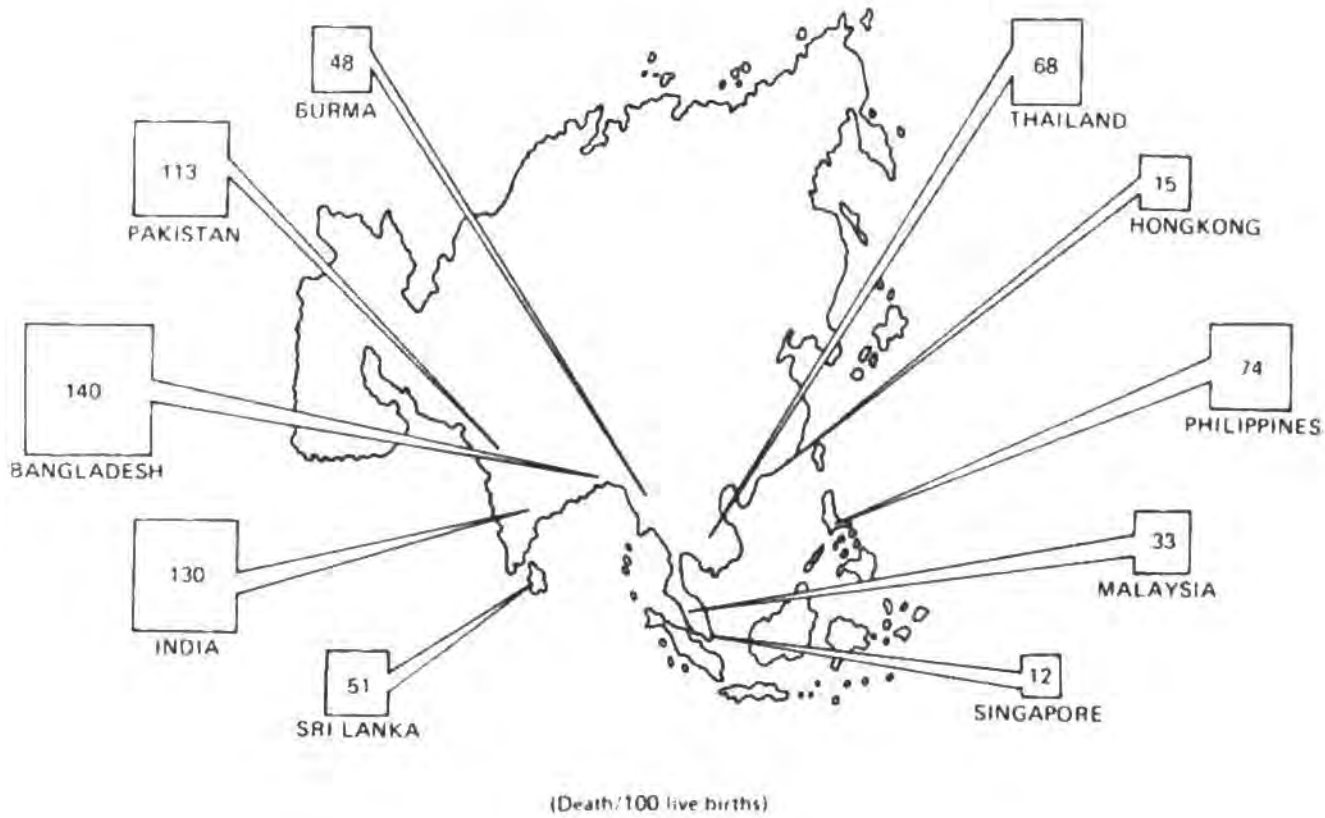


Fig. 14. Infant mortality in selected Asian countries 1975.

observed among children less than 5 years old based on data collected by the Disease Intelligence Center, MOH. Despite under-reporting, the reduction in the mortality rates by 52% from 1978 to 1982 reflects to some extent the impact of the program, considering the fact that was only a 17.8% reduction in the mortality rate from all causes in the same period. This reduction in mortality in diarrheal diseases is inversely proportional to the amount of Oresol produced (Fig. 17).

Important to note is that the data of this review came from 2 different sources; the National Census and Statistic Office and the Disease Intelligence Center.

Expanded program of immunization

The Ministry of Health with the assistance of UNICEF and WHO launched its Expanded Program on Immunization (EPI) on July 12, 1976 with the following objectives: 1) to reduce morbidity and mortality of the 6 EPI diseases (tuberculosis, diphtheria, pertussis, tetanus and measles, poliomyelitis) by increasing the proportion of fully immunized children in their first year of life; 2) to reduce the incidence of neonatal tetanus by providing pregnant women with tetanus toxoid immunization.

The first effort of the Philippine program was to give BCG to school entrants. In 1977, BCG and DPT were given to 3-14 months old children in priority areas and in 1979, and BCG and DTP program were expanded nationwide.

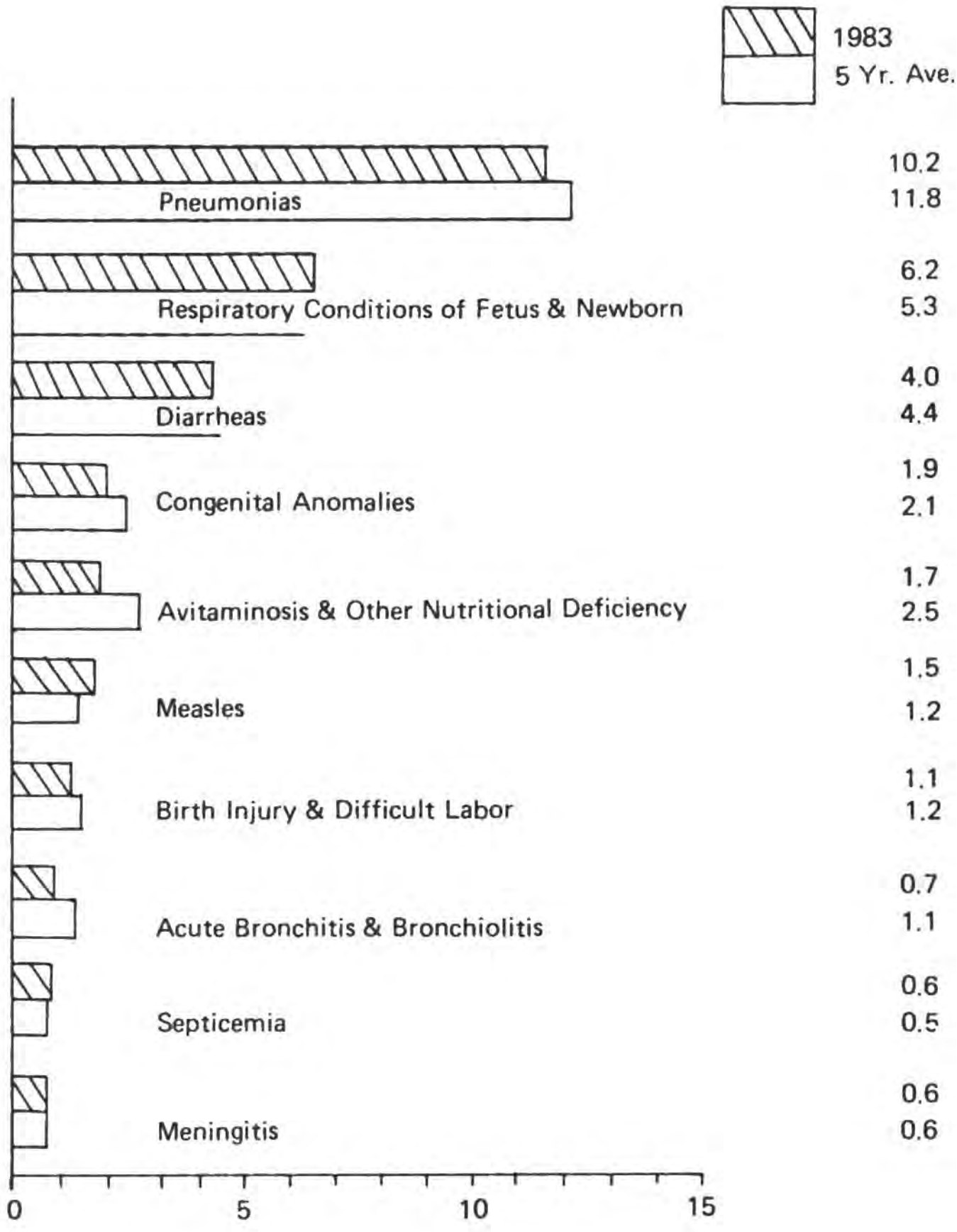


Fig. 15. Infant mortality: leading causes rate per 1,000 live births Philippines.
5 yr. ave. (1978-1982) and 1983.

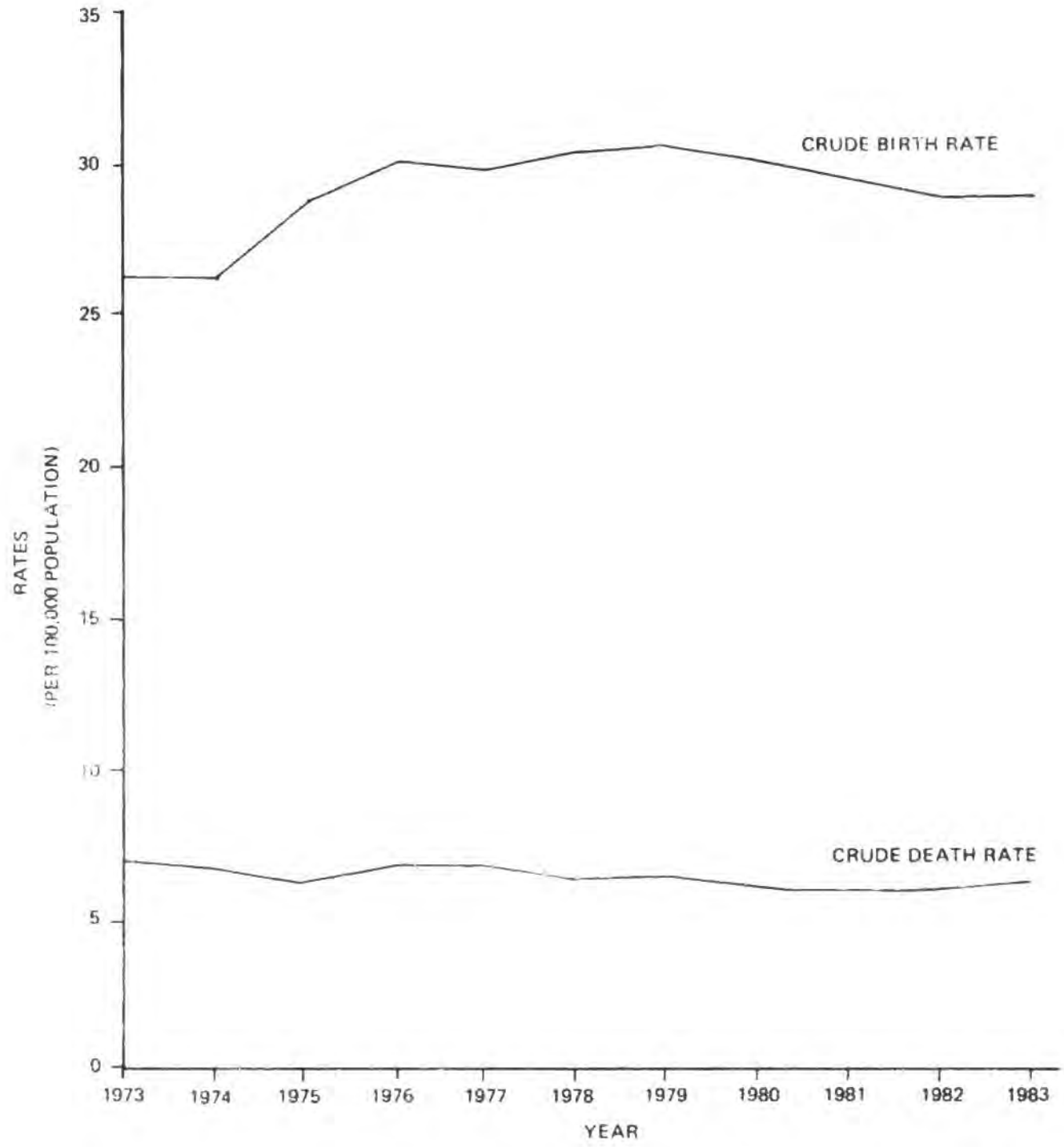


Fig. 16. Crude birth and death rates.

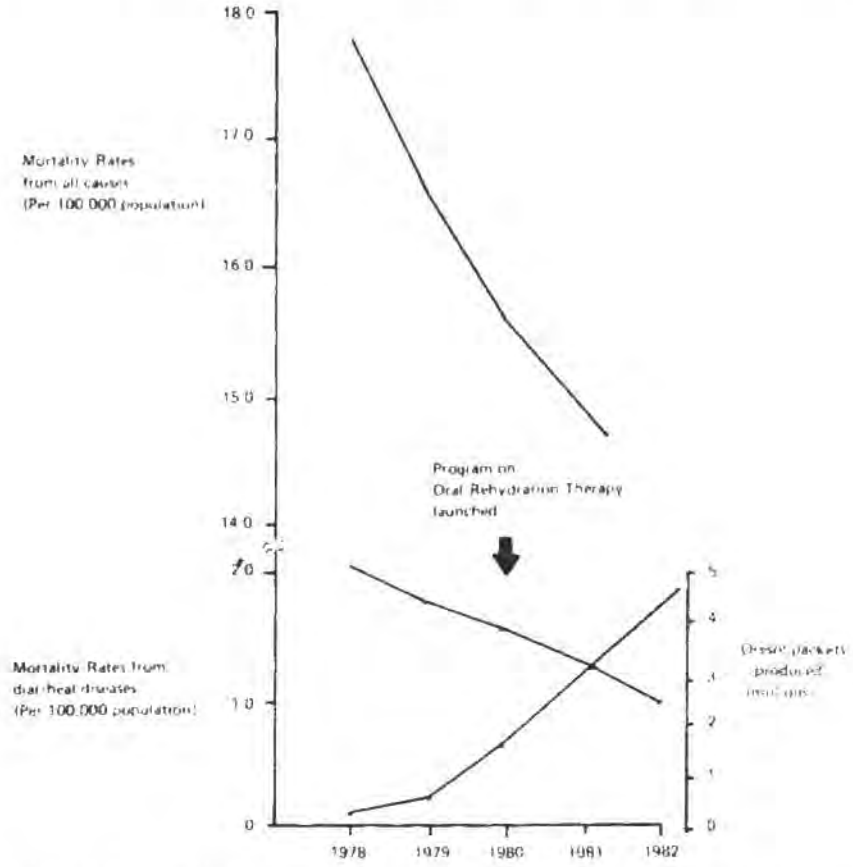


Fig. 17. Relationship of mortality rates from all causes and from diarrhea diseases with oresol* production, Philippines, 1978-1982.

Oral polio vaccine was started in 1980 among 3-14 months old children. In the same year, tetanus toxoid for pregnant women was given nationwide. Measles vaccination started recently in 1982. Figs. 18-23 are shown the morbidity and mortality rates of the 6 immunizable diseases among children 0-6 years old from 1973 to 1983. Superimposed on these are the percentage of coverage of the vaccines in their targeted population.

There is a significant and sustained drop in the morbidity and mortality rates of poliomyelitis, diphtheria, pertussis and tuberculosis noted after the start of immunization campaigns on the specific diseases in a nationwide scope. Anti-measles immunization started in 1982 so that it would be too early to assess its effect on the morbidity and mortality rates of the disease.

Promotion of breastfeeding

Aware of the universally recognized fact that the unfavorable trend from breast to bottlefeeding is detrimental to progress in nutrition and survival of infants, a campaign to promote breastfeeding has been undertaken in the Philippines, first through private individuals and groups but eventually under government leadership, through retraining of its personnel, using mass media to publicize superiority of breastfeeding over artificial formulas and instructions in hospitals and health centers. The present program in urban communities aims to change lay and medical attitudes, including hospital routines adverse to breastfeeding promotion.

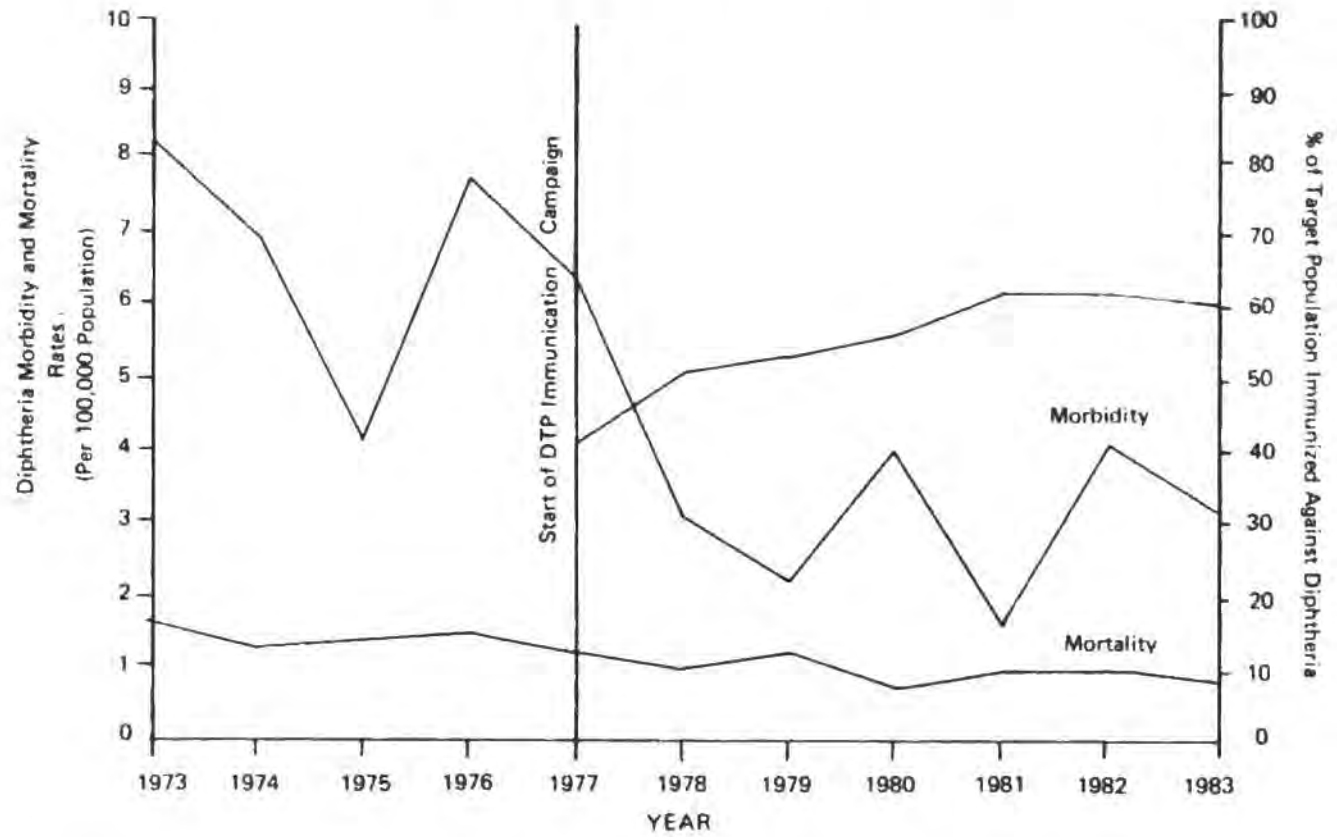


Fig. 18. Morbidity and mortality rates of diphtheria and percentage of target population* immunized against diphtheria.**

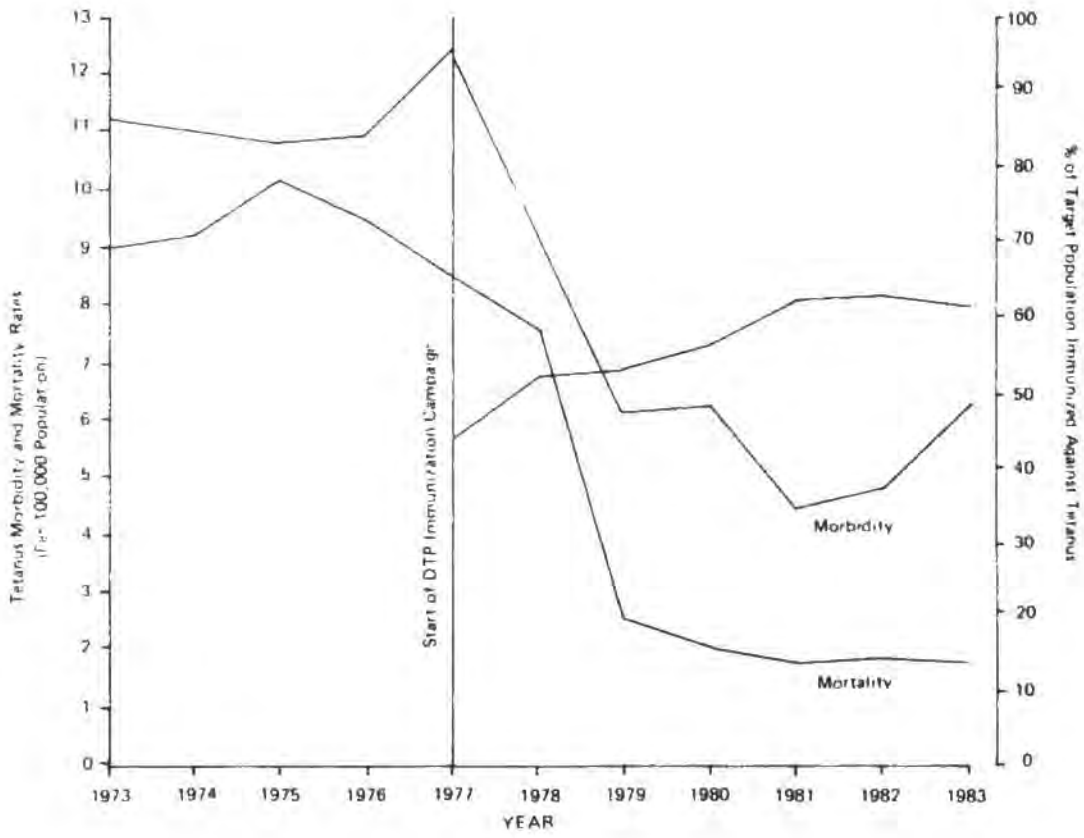


Fig. 19. Morbidity and mortality rates of tetanus and percentage of target population* immunized against tetanus.**
Philippines, 1973-1983.

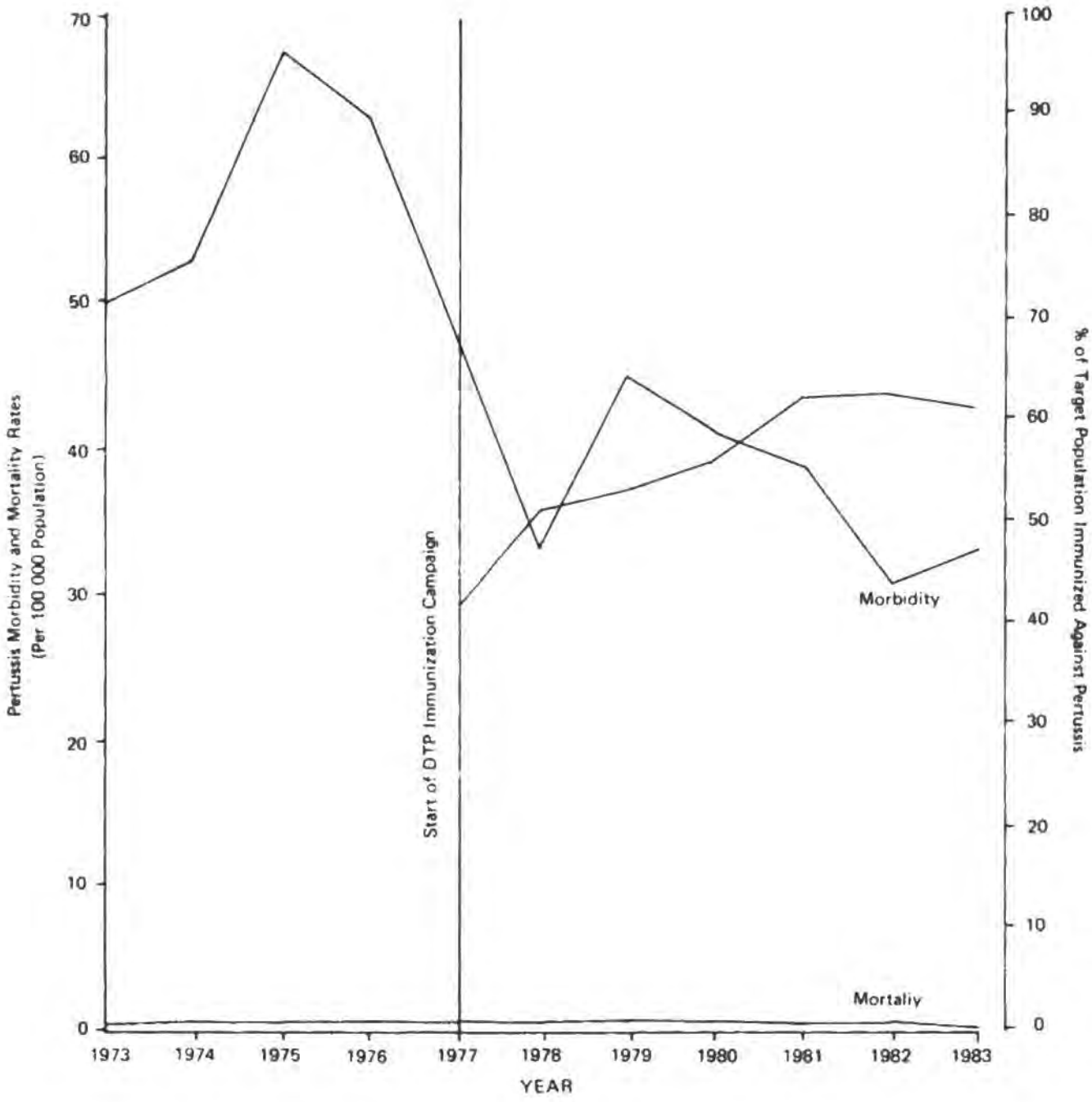


Fig. 20. Morbidity and mortality rates of pertussis and percentage of target population* immunized against pertusis.**

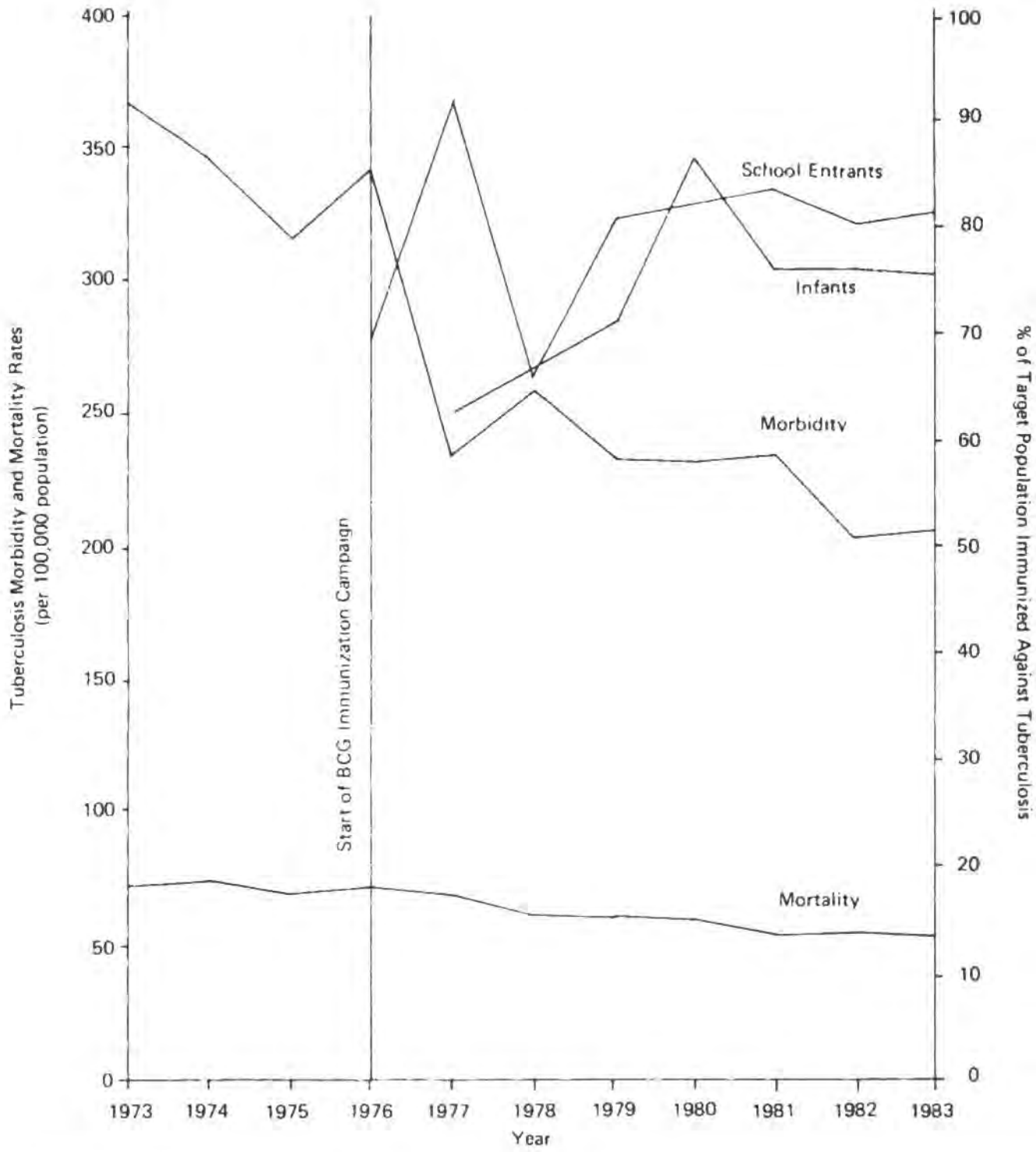


Fig. 21. Morbidity and mortality rates of tuberculosis and percentage of target population* immunized against tuberculosis.
Philippines, 1973-1983.

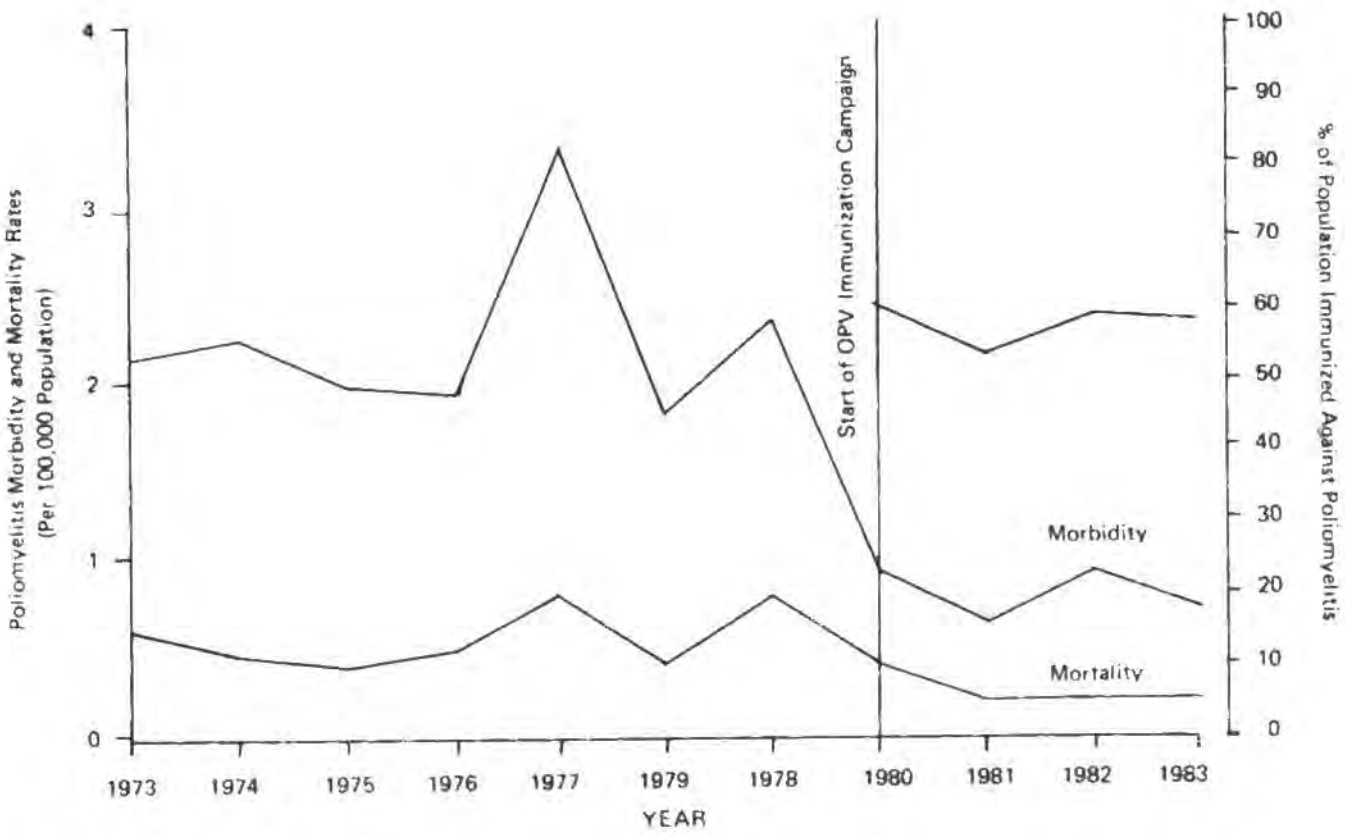


Fig. 22. Morbidity and mortality rates of poliomyelitis and percentage of target population immunized against poliomyelitis.
Philippines, 1973-1983.

Quoted in the 1984 UNICEF report is the observation that in the Philippines by encouraging breastfeeding instead of bottlefeeding among newborns at Baguio General Hospital, clinical infections were reduced by 88%, diarrheas by 93% and infant mortality by 95%.

A study of the incidence of breastfeeding in Luzon in 1974 indicated that this is practised by only 52% of mothers in Luzon, with 2½ times by percentage of breastfeeding mothers in rural than in urban areas. On the other hand, mixed and bottle feeding are practised more often by urban than by rural mothers. Notably there are low diarrheas cases in rural areas where by getting is still the practice.

The International Code on the Marketing of Breast Milk substitutes which was readily adopted by 35 nations in 1981 to change marketing procedures has increased to 130. Political will has decidely given momentum to the promotion of breastfeeding. Favorable effects have been observed and commended as an effective measure to reduce the "most unnecessary malnutrition of all". In the Philippines, the Code was finally signed by the President in January, 1987.

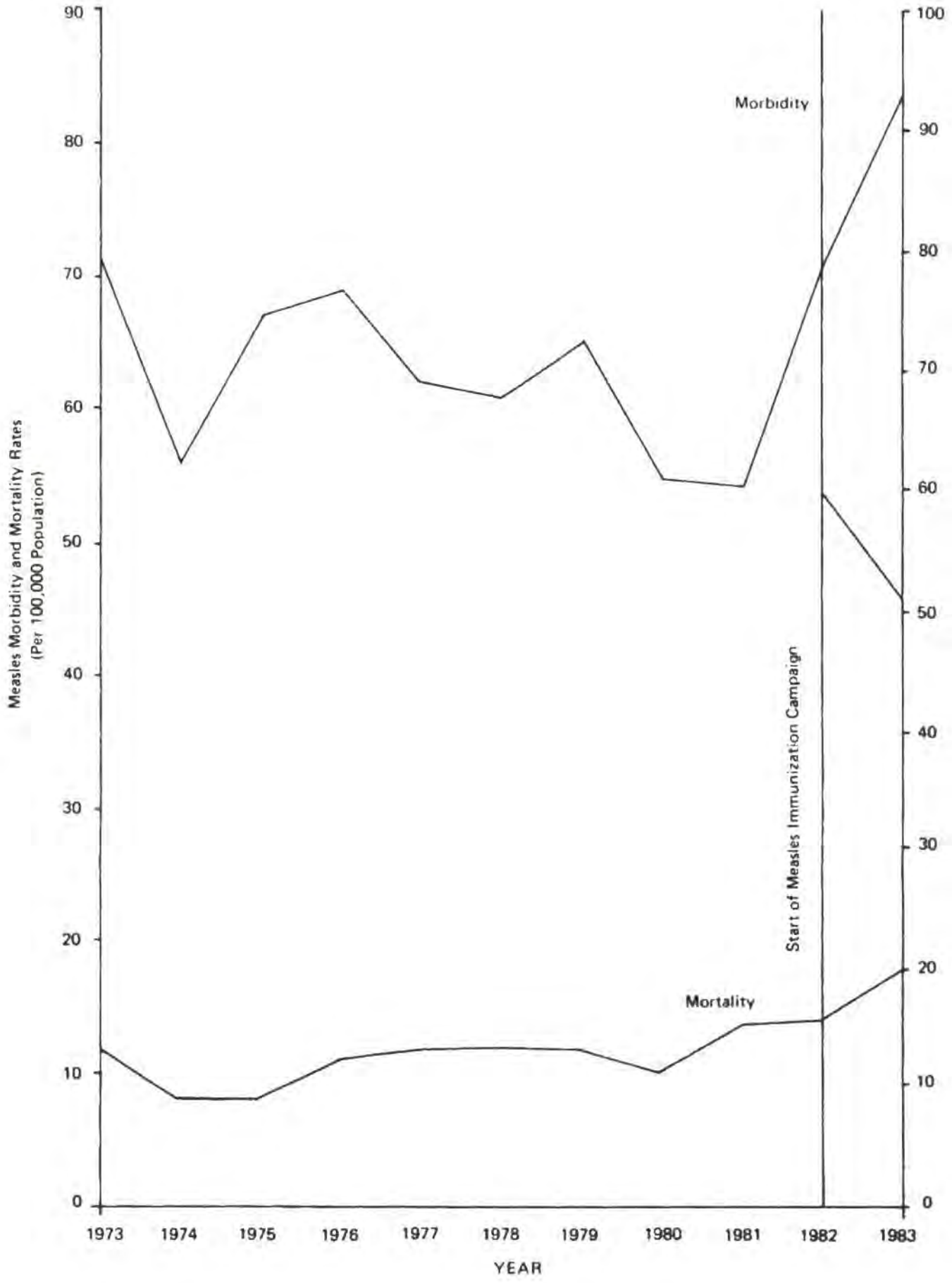


Fig. 23. Morbidity and mortality rates of measles and percentage of target population* immunized with antimeasles vaccine.
Philippines, 1973-1983.

Summary

A study of the trend in the health and nutrition of Filipino from birth to 19 years old was conducted for the decade 1973 to 1983. The main objective is to gather data and information on the attainment and achievement of a decade by agencies in government or in private concerns, whether as individuals or in groups and to determine problems and possible solutions to improve quality of life of the future citizens and resources of the country.

As in most developing countries, children in the Philippines constitute the large proportion of the population (52.9%) in 1983. This large young dependent sector necessarily have exerted pressures on the resources and facilities of the country.

Basic health and development indicators of the country show a life expectancy in 1983 of 62.5 years (an increase of 2.4% during the decade); an Infant Mortality Rate of 59.3 (a decrease of 22.5) and a Literacy Rate of 82.7.

Trends in these indicators show an improvement in the quality of life brought about by many factors including improved health and nutrition services and increased health education activities.

The morbidity and mortality rates have declined, particularly for the immunizable diseases (TB, diphtheria, tetanus, whooping cough, poliomyelitis and measles and also tetanus for mothers). The use of oral rehydration therapy has helped reduce mortality from diarrheas. In general, communicable diseases death rates decreased by 28.6. In the case of tuberculosis, mortality in this age group decreased by 15%. Pneumonias remain as a major health problem.

The nutritional status of pre-schoolers with weights higher than 75% of Filipino standard weight for age has improved by 8% in 1983.

Maternal death rate has decreased by 28.6% during the period under review. The leading causes of deaths were hemorrhages 51.3%, hypertension (13.8%) and abortion (11.3%). More mothers delivered in the hospitals (74.3%) which was 75 to 78% early in the decade. Maternal nutrition studies has shown that a small percentage had low levels of Vit. A and Vit. C while iron take was less than the recommended dietary allowance for the 3 trimesters, the lowest was in the last trimester.

An overall improvement in the health and nutritional status of children 0-19 years in the decade 1973 to 1983 is reported, although this may not be impressive nor highly significant.

Acknowledgment

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We are indebted to the authorities in charge of statistical data and annual reports of different ministries and children's organizations that accommodated us in these valuable information that are included in this ten-year survey.

Last but not least the authors are grateful to the National Academy of Science and Technology for encouraging us, in particular the senior author, for presenting a ten-year health survey of children in the Philippines. Without encouragement and support of NAST, this presentation would not have been realized.

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PROPOSED WEIGHT AND HEIGHT STANDARDS FOR 0-19 YEAR OLD FILIPINO CHILDREN

Rodolfo F. Florentino, Emilie G. Flores, Josefina A. Magbitang,
Ophelia M. Mendoza, Teresa S. Mendoza
and the
Philippine Pediatric Society

ABSTRACT

This is a collaborative study between the Food and Nutrition Research Institute and the Philippine Pediatric Society with the objective of developing anthropometric standards for nutritional and clinical evaluation of 0-19 year old Filipino children. A three-stage cluster sampling was utilized in defining the study population. The sample totalled 26,961 children from all regions of the country except Region IX and XII. Of these 23,660 children adjudged clinically and physically healthy with an ethnic background of less than 1/4 foreign blood were included in the construction of the proposed standards. Descriptive analysis was used to characterize the data. Using the cubic spline technique to smooth out the observed data, centile tables and curves from birth to 19 years for weight-for-age, height-for-age, and weight-for-height were derived. These tables and curves representing the distribution of the current crop of apparently healthy Filipino children would be useful in assessing the growth of a given child. On a community basis, these reference data may be used to define the extent and severity of over- or under-nutrition. The data, however, will need to be validated in the field to define cut-off points for defining malnutrition before they can be used for the latter purpose.

Introduction

Standards for growth provide a means by which to check whether a child's body size is increasing, entering a period of stability, or declining[1] In the clinic, the pediatrician utilizes growth standards to assess nutritional status or monitor growth of individual children. Deviations from normal standards, curves and channels of growth may indicate pathological conditions. Thus, failure to gain weight in children may indicate malnutrition or some other disease.

In the community, standards are used to define the extent and severity of under- or over-nutrition. Such information will, in turn, provide basis for nutrition program planning and screening for intervention programs. A good example is Operation Timbang where all children in the community are weighed and the proportion of children belonging to various categories of weights compared with standards are used for community diagnosis. Even more recently, the National Nutrition Council has adopted growth monitoring of individual children in the

community as a preventive measure against growth faltering of children and as an entry point for nutrition and health interventions.

Review of Literature

International standards

For many years, the so-called Boston and Iowa standards were used in many parts of the world including the Philippines to assess growth of children. The Boston or Stuart standards were taken from data collected in 1930-39 on a small sample of relatively well-nourished Caucasian children in the United States[2]. These data are often combined with the Iowa or Meredith reference population data compiled from a survey done in 1923 on a small population of Caucasian school age children.

In 1974, the National Center for Health Statistics (NCHS) came out with standards derived from a large sample of healthy children in the U.S. from all ethnic and socio-economic groups.[3] The data were made available in percentile and SD scores using the least squares cubic spline technique of DeBoor and Rice[4] to smooth the data. These values, together with additional values from birth to three years from the Fels Research Institute, were then recommended by the World Health Organization for international use for infants and young children irrespective of ethnic group.[5] This was based on the finding that the variability arising from ethnicity in the early years of life were much less significant than the variability arising from socio-economic and nutritional factors. Thus, it was averred that infants and young children from developing countries could reasonably use such standards as reference values especially in the absence of good local standards. For older age groups, however, the effect of genetic potential becomes increasingly apparent thereby making local standards necessary.

Local standards

The weight-for-age and height-for-age standards for Filipino children recommended by Bulatao-Jayme *et al.* in 1971[6] were based on nationwide nutrition survey data collected in 1958-1968 on more than 9,000 individuals of all ages. Since the data included undernourished children, the values recommended as reference standards for Filipino children were the 90th percentile of the data except for ages 0-6 months where sliding percentiles from P_{50} to P_{90} were used. The decision to use the 90th percentile was based on comparisons with limited data from high income children as well as with some weight-for-height indices of nutritional status. The standards were recommended as temporary pending collection of data on healthy children. Nevertheless, these standards have been used extensively since they came out in 1971. It should be borne in mind, however, that these standards conform to the growth pattern of high income children with which the data set was compared and not necessarily to the growth pattern of healthy Filipino

children irrespective of socio-economic status and geographic location. At best they represent the potential that Filipino children can reasonably achieve given the conditions of children of high socio-economic status.

More recent studies in FNRI confirmed the position of the 1971 standards particularly with respect to boys. Data from high income children collected by Magbitang *et al.*[7] on 4-18 year olds and Baltazar *et al.*[8, 9] on 0-18 month olds and 4-12 year olds showed that growth curves of young boys from high income families more or less coincided with the 1971 recommended Philippine standards. However, for girls especially at the older age groups, the curves were lower than the standards by as much as 6 cm for height and 3 kg for weight. Comparison of these data with NCHS curves showed that even high income children could only come up to the 25th percentile of the international standards.

Briones *et al.*[10] did a longitudinal study from 1969 to 1981 covering the age group birth to 12 years. There were 490 subjects initially, but this number went down to less than a hundred towards the end due to missing observations. Only healthy, well nourished children with no genetic or developmental aberrations were included in the study. The parameters collected were weight, height and head circumference.

Santos-Ocampo *et al.* have recommended the use of data they collected from Filipino infants and children belonging to middle socio-economic classes as a useful guide in the assessment of a child's development from birth to 14 years of age[11]. The data on infants were collected from well-babies followed up from birth at monthly intervals up to 2 years. The data on older children were taken at half year interval in a group of boys and girls from 2 to 14 years of age. About 100 per sex and age group were included in the derivation of standards.

Construction of local standards

In 1972 the International Union of Nutritional Sciences (IUNS) proposed several criteria that should be followed in the construction of local and international reference standards.[12] The task force recommended that the data set from which the standards are to be derived should be large with a minimum of 100-200 children per age group and representative of the healthy children population. The data should be cross-sectional. The sampling procedure should be defined and reproducible. Measurements should be carefully taken and recorded by trained observers, using equipment of well-tested design and calibrated frequent intervals. Furthermore, local reference standards should be representative of the ethnic and genetic mix of the population being studied. In addition, local standards should be monitored every few years to detect secular changes.

None of the present standards and data sets available in the Philippines today satisfy the above criteria. The 1971 FNRI standards were arbitrarily set at the 90th percentile of the general population distribution which included non-healthy children. As mentioned above, the 90th percentile at best represents the growth pattern of high income children and not of healthy children from all socio-economic strata.

As a matter of fact, studies on high income children showed that the standards seem to be too high especially among girls and older children. The data of Magbitang *et al.* and Baltazar *et al.* were again from high income children in the Metro Manila area. The data of Briones *et al.* and Santos, Ocampo *et al.* were based on longitudinal observations in the Metro Manila area on inadequate number of subjects.

Thus the present study, a collaborative effort between the Food and Nutrition Research Institute and the Philippine Pediatric Society, was conceived to develop new anthropometric standards of Filipino infants, children and adolescents based on a sufficiently large cross-sectional sample of healthy children from as wide a geographic distribution as possible and irrespective of socio-economic class. Specifically, the objective was to derive reference tables and curves for length/height-for-age, weight-for-age, weight-for-height, crown-rump length/sitting height-for-age, skinfold thickness and circumferences of mid-upper arm, chest and head, that might serve as standards for clinical and nutritional evaluation. The standards are intended to serve as reference with which to compare children with the distribution of the current crop of apparently healthy Filipino children. This paper describes briefly the methods that were followed in the derivation of the proposed standards and the results for the first three parameters. Those for the other parameters will be presented in a subsequent report.

Methods

Sampling design and selection

For the preschoolers, a stratified three-stage cluster sampling design was used, with the province as the primary sampling unit, the municipality and the city as the secondary sampling unit and the barangay as the tertiary unit. Using a complete list of preschoolers in the sample barangay, all households with preschoolers were notified to bring their 0-6 year old children at the barangay center or health unit. All children were examined and measured for the study. When necessary, several rounds of collection were made in order to include all children in the community.

In the case of the school age group (i.e., those aged 7-19 years), the same procedure was followed for the first three stages of selection, but some modifications were adopted after the tertiary level. Instead of gathering children from the households, it was deemed more convenient and practical to locate children in these age groups in schools. Since, in almost all cases, a barangay had either only one school in it or none at all, the rule followed in sample selection was to take the school located in the sample barangay, or the school nearest to it in the case of barangays without schools. The same rule was followed in the selection of high schools and colleges or universities where adolescents could be located. After the school had been selected, a random sample of classes were selected from each grade. A list of students from the selected classes were then requested from the teachers, from which a systematic random sample was drawn for inclusion in the study.

The target population for the survey were those aged 0-19 years in all provinces nationwide, except for those in Samar Island and the provinces in Regions IX and XII. The provinces in these areas were excluded due to peace and order problems which made it risky for data collection. The stratification variable for the survey was the region. The survey covered 11 strata corresponding to 11 out of 13 regions in the country including Metro-Manila.

Estimation of sample size

The sample included two sample provinces per region, two municipalities or cities per province, and two barangays as well as two schools per municipality or city. In Metro-Manila, two municipalities or cities were randomly selected. The decision to take a sample size 2 per sampling unit was a compromise between the need to contain the geographic areas covered by the survey in order to meet budgetary and time constraints, and the need to include more than one primary sampling unit per stratum to enable the estimation of standard errors for the corresponding estimates derived from the survey. In the case of Region VIII, however, only one province (Leyte) was included since Samar Island was earlier excluded due to peace and order problems.

Since the ultimate end of the survey was to derive standards for each age and sex group, these were considered as the domains of analysis and were regarded as such in estimating sample size requirements. This means that a separate sample size was computed for each age-sex category. All in all, the survey dealt with 62-sub-groups which included 31 age categories (0-11 months by single months and 1-19 years by single years) for each sex. For each of the 62 sub-groups, sample sizes were computed using the standard deviations of the estimates or weight-for-height by age and sex derived from the Nationwide Nutrition Survey conducted by FNRI in 1982[13]. For purposes of sample size commutations, the maximum permissible error was set at 5% of the corresponding estimates derived in the 1982 survey, with reliability coefficients also set at 5%. After the sample size requirements were determined using purely statistical considerations, further modifications were made to fit budgetary and logistic constraints. The actual sample size covered varied slightly as a natural outcome of cluster sampling as well as other factors.

Initial sample size estimates using 95% precision are shown in columns 2 and 3 of Table 1. These were rounded off to the nearest tens (col. 4 and 5) resulting in a grand total of 19,120. Percent of total for each sex-age group was computed (for less than one year, for example, 150/19120 x 100 = 0.785%). These percentages were then used to compute the targeted sample size with 28,000 as the total target (0.785% x 28,000 = 220). These sample sizes were later proportionally allocated to the different provinces/schools based on actual population in the area.*

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*The target sample size of 28,000 was arrived at because of the need to come up with a weight-for-height table where a minimum of 500 subjects by age and sex group was deemed necessary. In addition, allowance was made for the inclusion of "non-healthy" children.

Table 1. Sample size estimation

Age	Initital sample size 95% precision		Modified sample Size[1]		Targeted sample size[2]	
	Male	Female	Male	Female	Male	Female
Less than 1	142.42392	164.061559	150	170	2860[3]	3237[4]
1	74.608708	167.031852	100	175	146	256
2	120.29085	79.78631207	130	100	190	143
3	320.2316744	124.5542482	310	130	454	188
4	160.9410398	159.298171	165	165	242	244
5	173.621432	197.5327681	175	205	256	299
6	129.8601949	341.7764462	135	350	198	513
7	192.2286725	301.7764462	200	310	293	454
8	355.5327541	384 6040913	350	400	513	586
9	324.283951	486.8854225	350	500	513	732
10	372.2657325	704.7478499	400	710	586	1040
11	220.8583621	612.3766259	230	620	337	908
12	582.1888427	546.2780	600	560	879	820
13	474.05333	873.987167	500	900	732	1318
14	527.05333	524.35578	530	535	776	783
15	441.10396	546.48341	450	555	659	813
16	560.40412	593.48072	570	600	835	879
17	419.77244	838.85235	425	845	622	1237
18	406.45988	553.16843	410	560	600	820
19	509.73764	508.85006	515	515	754	754

[1]Initial sample size rounded-off
[2]Based on percent of total target (28,000) by age and sex
[3]Sample size of 220/month for 0-11 months.
[4]Sample size of 249/month for 0-11 months.

Field data collection

A. *Teams*

A total of eight survey teams spread out in all regions of the country except Regions IX and XII to examine the subjects and do measurements. Each team was composed of an FNRI physician, two researchers and another physician provided by the Philippine Pediatric Society (PPS). The latter physician who was either a PPS member or a pediatric resident joined the survey either on a part-time or full-time basis and did the physical examination of the subjects independently and declared the health status of the children separately from the FNRI physician in the team. Before the actual field data collection, the physicians and researchers were trained for two weeks including a field practicum on conducting physical examination and taking of anthropo-metric measurements.

Two teams were assigned in a province, and each team in turn covered one municipality or city as the case may be. In all, 131 physicians were provided by the PPS from all over the survey areas in the Philippines.

B. *Mechanics and activity sequence*

The weighing scales were first calibrated in the Test and Standards Laboratory of NSTA before field data collection and were again calibrated in the field before each weighing session using a 2 kg weight. The weighing scales were recalibrated by the Test and Standard Laboratories of NSTA in Cebu City.

The collection of data was generally done in fairly the same systematic way by each team in all the survey areas covered.

The equipment and instruments were first set up before the parent or subjects were interviewed to facilitate systematic flow of data collection. General information (name, birthday, sex, school or home address, ancestry, etc.) was first obtained for each subject. Using standard methods of Jelliffee[14] described in the manual of instructions, anthropometric measurements (weight, length/height, arm length, crown-rump length/sitting height, and mid-upper arm, chest and head circumferences) were carefully taken and recorded successively by the FNRI technicians. Weight was taken in light clothing without shoes with a platform weighing scale and read to the nearest 0.02 kg. Recumbent length was taken of children less than 2 years of age with a portable measuring board, while standing height was taken of children 2 years and over using a steel tape attached to a smooth wall. Readings of length and height were made without shoes to the nearest 0.1 cm. Afterwards, the FNRI physician took the skinfold measurements and proceeded to a physical examination of each child using a standard form. The physician evaluated each subject's general appearance and body build and if normal in these respects, proceeds to do a complete physical examination (including assessment of sexual maturation) to detect any pathological condition or abnormality. History of recent illness was also obtained, and finally the physician assessed whether the findings could likely affect the child's growth and development. The PPS counterpart did an independent examination of all subjects. Any disagreement between them with regards to the health status of the child would disqualify the child in the data analysis for construction of standards. However, as per experience of some teams, cross examination could not always be possible due to the limited time that the PPS doctors could stay with the team in the collection of data. However, for subjects who were examined by both PPS and FNRI physicians the percent agreement in terms of whether the child was healthy or not was 95%.

Clinical examination and description of subject population

The subjects who were included in the creation of the standards were only those who were apparently healthy as judged by the physicians. They were those considered mesomorphic* neither asthenic (+ or ++) nor endomorphic, neither

*Mesomorphic – individuals with an average or good muscular development and of the athletic type characterized by strong robust, active body with good agility. Asthenic (+) – individuals with slight or slender body build, fragile but active, Asthenic (++) – individuals who are markedly thin, with slight muscular development, weak, debilitated and wasted. Endomorphic – individuals with pyknic type of body build characterized by shortness of stature, broadness of girth and with powerful musculature.

pathologically short nor tall, and without debilitating illness like heart, lung and kidney diseases or physical deformity that can affect height and weight. The child in the previous two weeks should not have experienced any diarrheal episodes or other illness that may have an impact on his weight. Their foreign blood if any should be less than one-fourth.

In general, children suffering from conditions that may clinically affect the nutritional status of the child were excluded in the creation of standard. However, physicians proceeded with the physical examination even if the child was diagnosed in physical appearance as asthenic or endomorphic, and all anthropometric measurements were still taken.

Hence the subjects that were finally included in the creation of the standards were clinically and physically healthy at the time of examination, with an ethnic background of less that 1/4 foreign blood.

Mode of data analysis

Estimation of Parameters:

Age was computed by taking the difference between the date of collection and birthdate and expressing it in completed years and months.

For each type of anthropometric measure considered in the survey, the following computations were done:

A. *Computation of percentiles*

The specific percentiles computed for each anthropometric measure were P_3, P_{10}, P_{25}, P_{50}, P_{75}, P_{90}, P_{97}. The observed figures for each of these were then smoothed (see below) and compared with corresponding values in the existing standards (e.g., NCHS).

B. *Computation of the mean of each parameter*

In addition to the percentiles, the mean and standard deviation of each anthropometric measure considered were also computed. Since the sampling design used did not result in equal probabilities of selection for the different sampling units, it was necessary to compute and apply sampling weights in order to come up with unbiased estimates of the sample mean. Unfortunately, the computation of sampling weights was possible only for the estimates for the pre-school age groups but not for the school age groups because for the latter, not all the necessary data were available or were recorded.

C. *Computation of the standard errors of the mean*

In addition to the computation of the means of the different parameters, it was necessary to compute for the corresponding standard errors in order to assess the precision of the derived estimates. Moreover, such standard errors were needed in order to construct confidence interval estimates for the parameters or do tests of significance.

Since the survey used a complex sampling design, the standard errors were computed by applying Taylor's linearization procedure and the concept of "ultimate clusters" as defined by Hansen, Hurwitz and Madow (1953).

D. *Curve fitting*

The cubic spline technique was used to fit smooth curves to the data collected.[16] This procedure was used because it was observed that data on anthropometric measures vary in such a way that a single polynomial equation may not to able to describe it. The equation was applied on three successive portions of the curve, 0-3.25 yrs. 3.25-10.25 yrs, and 10.25-19.75 yrs. Since this resulted in discontinuities at the junctions of the curves, these junctions were smoothed out by visual inspection and arithmetic interpolation.

E. *Assessment of the precision of the estimates*

One way of evaluating the usefulness of the estimates derived from this survey is to determine their precision. The more precise they are, the more confident one will be in applying the results to similar or comparable groups.

To measure the precision of the estimates derived from this survey as an index of the statistical quality of the estimates, the coefficient of variation of the estimate of the mean of each parameter for each age and sex group was computed.

Results and Discussion

Of the 26,961 subjects examined, 23,660 were included in the analysis for the construction of standards, of which 44.9% were boys and 55.1% were girls. Excluded were the asthenic (+) children (9.33%); asthenic (++) (0.96%); endomorphic or obese (0.59%); those who were not healthy due to organic pathology (0.98%); those who had more than 1/4 foreign blood (0.04%).

The distribution of subjects by age and sex is shown in Table 2.

Observed values

Tables 3 and 4 give the observed values of weight and height by age at for both sexes. It can be noted that the mean of the observations were very close to the median, indicating general absence of skewness in the data. Tests of normality at each age also indicated normal distributions, again pointing to the homogeneity of the data. Except for the first month, the coefficients of variation were way below 30% (range, 1% to 17% for the various age groups indicated), indicating reasonably good precision of the estimates. The NCHS data show coefficients of variation of about 9% to 16%. Incidentally, the coefficients of variation at early infancy and during the pubertal period are higher than at other periods of childhood, indicating greater variability of data during the former periods.

Table 2. Distribution of subjects by age and sex

*AGE**	*Males*	*Females*	*AGE*	*Males*	*Females*
Months			7.0	267	348
0	56	42	7.5	204	207
1	27	31	8.0	350	432
2	31	58	8.5	234	280
3	55	39	9.0	307	430
4	67	35	9.5	266	320
5	52	39	10.0	364	548
6	36	51	10.5	277	377
7	62	44	11.0	303	529
8	37	45	11.5	245	387
9	43	40	12.0	404	531
10	59	35	12.5	383	414
11	61	35	13.0	433	625
Years			13.5	321	510
1.0	195	191	14.0	360	471
1.5	178	207	14.5	341	411
2.0	193	180	15.0	321	429
2.5	194	208	15.5	351	477
3.0	178	184	16.0	430	532
3.5	196	191	16.5	309	402
4.0	156	157	17.0	334	468
4.5	190	214	17.5	264	363
5.0	170	171	18.0	244	252
5.5	177	221	18.5	198	186
6.0	185	227	19.0	261	307
6.5	191	238	19.5		

*Completed month or year

Table 3. Observed values of weight-for-age, boys and girls

	BOYS				*GIRLS*			
*AGE**	*Median*	$\bar{X}$	*SD*	*CV*	*Median*	$\bar{X}$	*SD*	*CV*
Months								
0	3.38	3.41	0.55	30.69	3.10	3.23	0.65	24.50
1	4.90	4.88	0.84	4.11	4.45	4.40	0.52	2.45
2	5.62	5.68	0.85	1.28	5.00	5.15	0.79	5.73
3	6.00	6.05	0.84	7.40	5.40	5.61	0.67	14.19
4	6.60	6.63	0.89	10.20	6.05	6.13	0.77	6.15
5	7.05	7.18	0.84	5.17	6.60	6.61	0.96	8.78
6	7.10	7.12	0.99	7.00	6.90	6.82	0.86	6.13
7	7.60	7.56	0.80	9.74	7.02	6.91	0.66	3.84

Table 3 (continuation)

	BOYS				GIRLS			
*AGE**	*Median*	$\bar{X}$	*SD*	*CV*	*Median*	$\bar{X}$	*SD*	*CV*
8	7.75	7.83	0.83	4.91	7.10	7.20	0.86	6.79
9	8.10	8.10	1.07	6.22	7.40	7.47	0.98	6.60
10	7.90	8.04	1.16	7.67	8.00	7.80	1.07	10.90
11	8.20	8.14	1.08	5.41	7.60	7.82	1.46	5.33
Years								
1.0	8.90	8.33	1.16	13.10	8.10	8.24	1.07	12.97
1.5	9.70	9.88	1.28	12.95	9.00	9.13	1.20	13.14
2.0	10.60	10.70	1.31	12.28	10.15	10.28	1.32	12.82
2 5	11.50	11.59	1.61	13.86	11.00	11.14	1.48	13.31
3.0	12.50	12.55	1.48	11.79	11.70	11.90	1.65	13.87
3.5	13.25	13.30	1.59	11.96	12.60	12.92	1.78	13.77
4.0	13.70	13.89	1.84	13.24	13.65	13.72	1.69	12.30
4.5	14.55	14.84	2.05	13.78	14.10	14.24	1.81	12.70
5.0	15.10	15.33	1.92	12.53	14.80	14.97	1.88	12.52
5.5	16.10	16.12	1.71	10.64	15.70	15.85	1.87	11.78
6.0	16.78	16.94	1.84	10.88	16.50	16.60	1.90	11.45
6.5	17.80	17.96	2.10	11.72	17.50	17.66	2.23	12.63
7.0	18.60	18.63	1.88	10.06	17.90	18.08	2.32	12.82
7.5	19.40	19.41	1.89	9.72	18.60	18.73	2.39	12.63
8.0	20.30	20.40	2.31	11.32	20.00	20.13	2.37	11.84
8.5	20.62	21.06	2.52	11.95	20.80	21.06	2.49	11.73
9.0	22.30	22.53	2.89	12.82	22.20	22.50	2.64	11.88
9.5	23.00	23.32	2.76	11.85	22.50	22.99	3.25	13.94
10.0	24.00	24.31	2.70	11.10	24.40	24.72	3.62	14.69
10.5	25.88	25.33	2.90	11.45	25.50	26.22	4.42	16.76
11.0	26.80	27.31	3.73	13.67	27.60	28.24	4.70	16.69
11.5	27.30	27.36	3.16	11.56	29.20	30.05	5.22	17.32
12.0	29.20	29.92	4.64	15.50	31.70	32.27	5.68	17.65
12.5	30.25	31.78	5.28	16.60	34.05	34.20	5.47	15.94
13.0	34.05	34.96	6.10	17.43	36.30	36.61	5.44	14.95
13.5	35.90	36.56	6.45	17.65	38.18	38.24	5.83	15.20
14.0	39.50	39.50	6.27	15.87	40.00	40.33	5.52	13.76
14.5	41.10	41.49	6.23	15.01	41.10	41.51	5.49	13.22
15.0	43.90	44.43	6.18	13.90	42.70	42.78	5.16	12.10
15.5	46.00	46.16	6.08	13.17	43.22	43.52	4.89	11.23
16.0	47.78	48.14	5.98	12.42	44.00	44.26	5.10	11.51
16.5	49.12	49.23	6.11	12.41	44.90	45.15	5.34	11.86
17.0	50.00	50.02	5.77	11.53	45.40	45.54	5.06	11.13
17.5	50.40	50'88	6.11	12.01	45.20	45.73	5.23	11.62
18.0	52.00	52.33	5.99	11.45	45.90	45.11	5.67	12.31
18.5	51.15	51.67	6.06	11.74	45.55	46.23	5.19	11.24
19.0	53.35	53.29	6.13	11.51	46.30	46.24	5.46	11.87
19.5	52.92	53.41	7.44	13.93	45.20	45.25	4.86	10.75

*Completed month or year

Table 4. Observed values of height-for-age, boys and girls

	BOYS				GIRLS			
*AGE**	*Median*	*X*	*SD*	*CV*	*Median*	*X*	*SD*	*CV*
Months								
0	51.60	51.09	2.57	4.68	49.40	49.41	3.12	11.50
1	55.20	56.07	3.33	4.47	54.60	54.70	2.42	0.64
2	59.00	59.09	3.09	2.70	57.50	57.72	3.12	2.30
3	60.50	60.62	3.06	6.49	59.60	59.80	2.39	2.10
4	63.20	63.37	2.85	3.45	61.00	60.68	3.38	2.22
5	65.00	64.52	3.20	2.78	62.90	62.88	3.06	2.12
6	65.50	65.93	2.74	1.62	64.50	64.35	2.49	2.10
7	67.50	67.46	3.00	3.78	66.15	66.19	3.46	1.73
8	69.00	69.86	3.72	1.59	67.80	67.55	2.39	2.73
9	70.60	70.96	3.22	1.93	68.90	69.16	4.00	3.13
10	69.75	70.33	3.39	1.73	70.60	70.05	3.67	3.65
11	70.20	70.52	3.50	2.10	70.00	69.69	3.88	1.17
Years								
1.0	73.90	73.99	3.72	5.03	72.80	72.81	3.76	5.17
1.5	78.00	78.25	3.89	4.97	77.20	77.43	4.09	5.28
2.0	81.85	82.39	4.50	5.46	81.50	81.69	5.04	6.17
2.5	86.10	85.74	5.34	6.23	85.15	85.09	4.89	5.74
3.0	89.80	89.80	5.01	5.57	88.20	88.28	4.96	5.62
3.5	92.25	92.54	4.48	4.84	92.20	92.10	4.93	5.35
4.0	95.60	95.35	4.95	5.20	95.00	95.13	4.66	4.89
4.5	99.50	99.32	5.75	5.79	97.70	98.15	5.42	5.52
5.0	101.50	101.35	5.00	4.93	101.05	100.86	4.97	4.93
5.5	104.10	104.51	5.32	5.09	104.10	104.09	4.77	4.58
6.0	107.50	107.39	4.78	4.45	107.00	107.01	5.22	4.88
6.5	110.00	110.43	5.37	4.87	110.25	110.35	5.14	4.65
7.0	112.72	112.65	4.19	3.72	111.20	111.37	5.19	4.66
7.5	114.20	114.26	4.28	3 75	113.15	113.06	5.22	4.59
8.0	117.50	117.43	5.29	4.51	117.00	117.23	5.20	4.46
8.5	118.10	118.48	5.06	4.27	119.15	119.23	5.16	4.31
9.0	122.70	122.69	5.50	4.48	122.70	122.82	5.03	4.12
9.5	123.65	123.85	4.98	4.02	122.90	123.66	5.88	4.73
10.0	126.95	126.98	5.02	3.95	127.00	127.44	5.96	4.69
10.5	127.90	128.40	5.36	4.17	129.80	130.15	6.66	5.11
11.0	131.95	132.10	5.95	4.50	133.80	134.08	6.57	4.91
11.5	132.20	132.32	5.71	4.31	136.35	136.88	7.24	5.29
12.0	135.80	135.90	6.92	5 07	139.60	139.58	7.12	5.11
12.5	138.85	139.53	7.55	5.41	141.70	141.80	6.43	4.53
13.0	144.10	144.28	8.08	5.60	145.05	144.77	6.03	4.18
13.5	146.50	146.61	8.43	5.75	146.40	146.09	5.79	3.95
14.0	152.00	151.14	8.07	5.34	148.50	148.61	5.66	3.82
14.5	154.00	153.65	7.54	4.91	148.95	149.00	5.44	3.65
15.0	156.00	156.37	7.31	4.67	150.00	150.16	5.16	3.44
15.5	158.50	158.39	6.86	4.33	150.40	150.58	5.43	3.61
16.0	160.60	160.62	6.12	3.81	150.70	151.06	5.13	3.40
16.5	161.00	161.26	6.08	3.77	151.00	150.74	4.87	3.23
17.0	161.00	161.36	6.21	3.85	151.00	151.26	5.21	3.45
17.5	161.90	161.76	6.04	3.73	151.30	151.28	5.56	3.67
18.0	162.50	162.85	5.79	3.56	152.00	151.71	5.28	3.48
18.5	163.30	163.11	6.01	3.68	151.55	151.71	5.10	3.36
19.0	163.40	163.37	5.60	3.43	151.68	151.59	4.93	3.25
19.5	162.85	163.11	6.58	4.03	151.40	151.24	4.45	2.94

*Completed month or year

Smoothed values-weight- and height-for-age

Figs. 1 and 2 give the smoothed median values for weight and height by age for both sexes. These figures clearly show the rapid growth in infancy, followed by a rather linear growth until the pubertal spurt which occurs at abut 10 – 10 1/2 years in girls and about 12 years in boys. Growth then levels off at about 16 1/2 years for girls, while the boys are still increasing in weight by 19 years of age.

Boys are heavier than girls from birth to 9 years by about 1% to 10%, at which point girls get heavier. The girls, however, are overtaken by the boys at 14 1/2 years. Thus at birth the boys are heavier than girls by about 1/3 kg., and at 19 years, they are heavier by 6.3 kg.

Birth length of boys is greater by 0.7 cm than for girls. Boys double their length before the age of 5 years, while girls do so earlier at age 4 1/2 years.

As in the case of weight, boys are taller than girls from birth to about 10 years (but only by less than 1%), and then again from 14 years on. By 19 years of age, boys are taller than girls by 11 cm.

The smoothed percentiles of weight-for-age (Figs. 3 and 3a) show that the 3rd and 97th percentiles are, on the average, 21% and 27% below and above the median, respectively; the 10th and 90th percentiles are 15% and 17% below and above the median; and the 25th and 75th percentiles are both 8% below and above the median.

On the other hand, the smoothed percentiles of height-for-age (Figs. 4 and 4a) show that the 3rd and 97th percentiles are both, on the average, only 8.5% above and below the median, respectively; the 10th and 90th percentiles both 6% above and below the median; and the 25th and 75th percentiles are both 3% above and below the median.

It can also be noted that during puberty, there is an "expansion" in the curves (increase in difference between P_3 and P_{97}), a reflection of the increase in standard deviation during this period as mentioned earlier. This is because of the differences in the age at which puberty starts and in the rates of growth at this time among individual children.

Smoothed values-weight-for-length/height

Weight increases more or less linearly with length from 0 to 23 months (Fig. 5). Median weight-for-length for both sexes closely approximate each other with a maximum difference of only half a kilogram at 70 cm. On the other hand, the increase in weight with height from 2 to 10 years is somewhat curvilinear (Fig. 6) with a more rapid increase at higher heights than at lower values. Again the curves for both sexes approximate each other except at tall heights. The latter effect is probably due to the inclusion of early maturers among girls beyond 8 or 9 years of age.

For ages greater than 10 years, weight-for-height is no longer age independent because of large differences in pubertal spurt among children. Separate tables for these ages will be presented in a subsequent report.

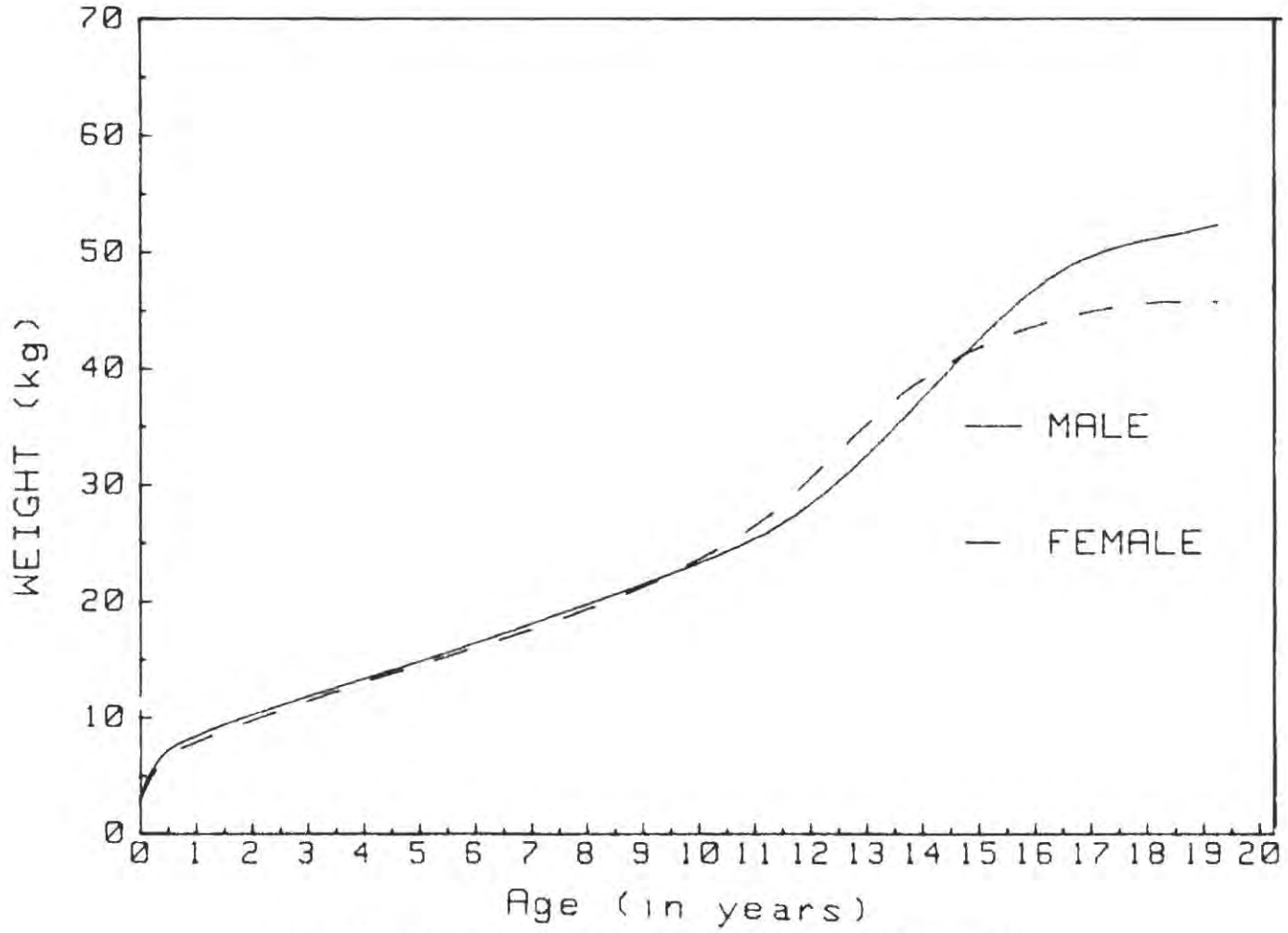

Fig. 1. Median weight - males vs females (0-19 yrs old).

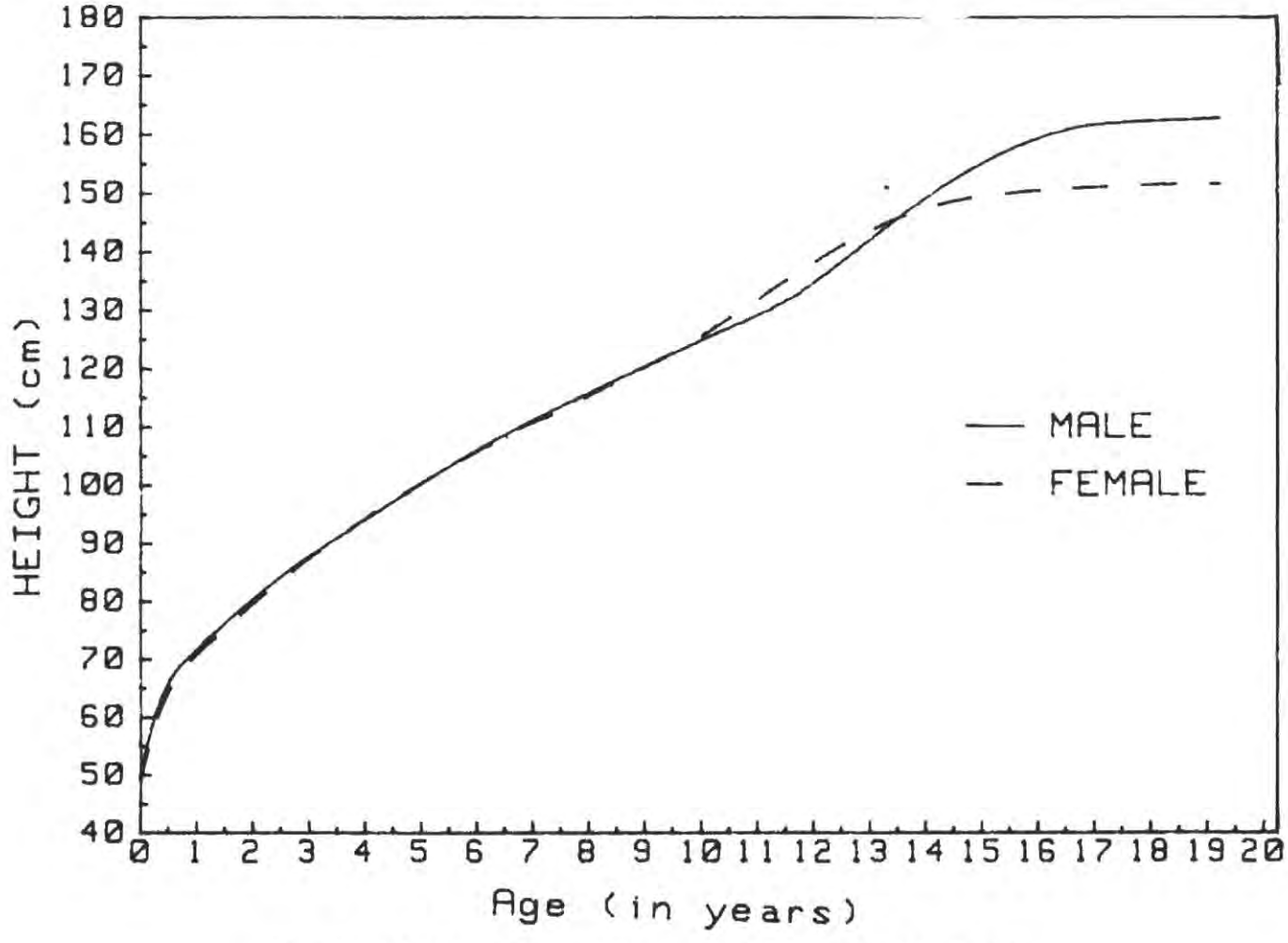

Fig. 2. Median height – males vs females (0-19 yrs old).

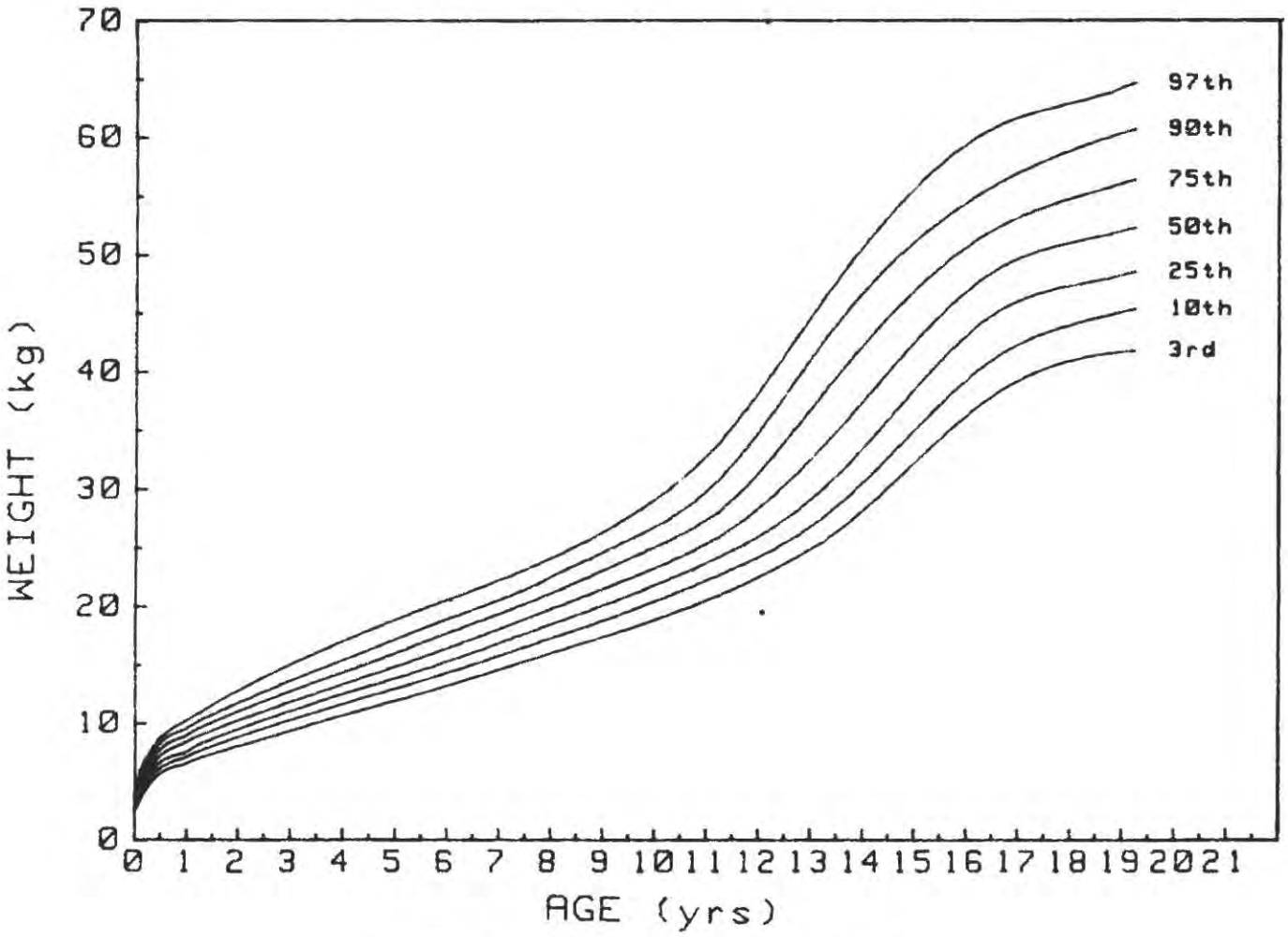

Fig. 3. Weight, males (0-19 yrs old).

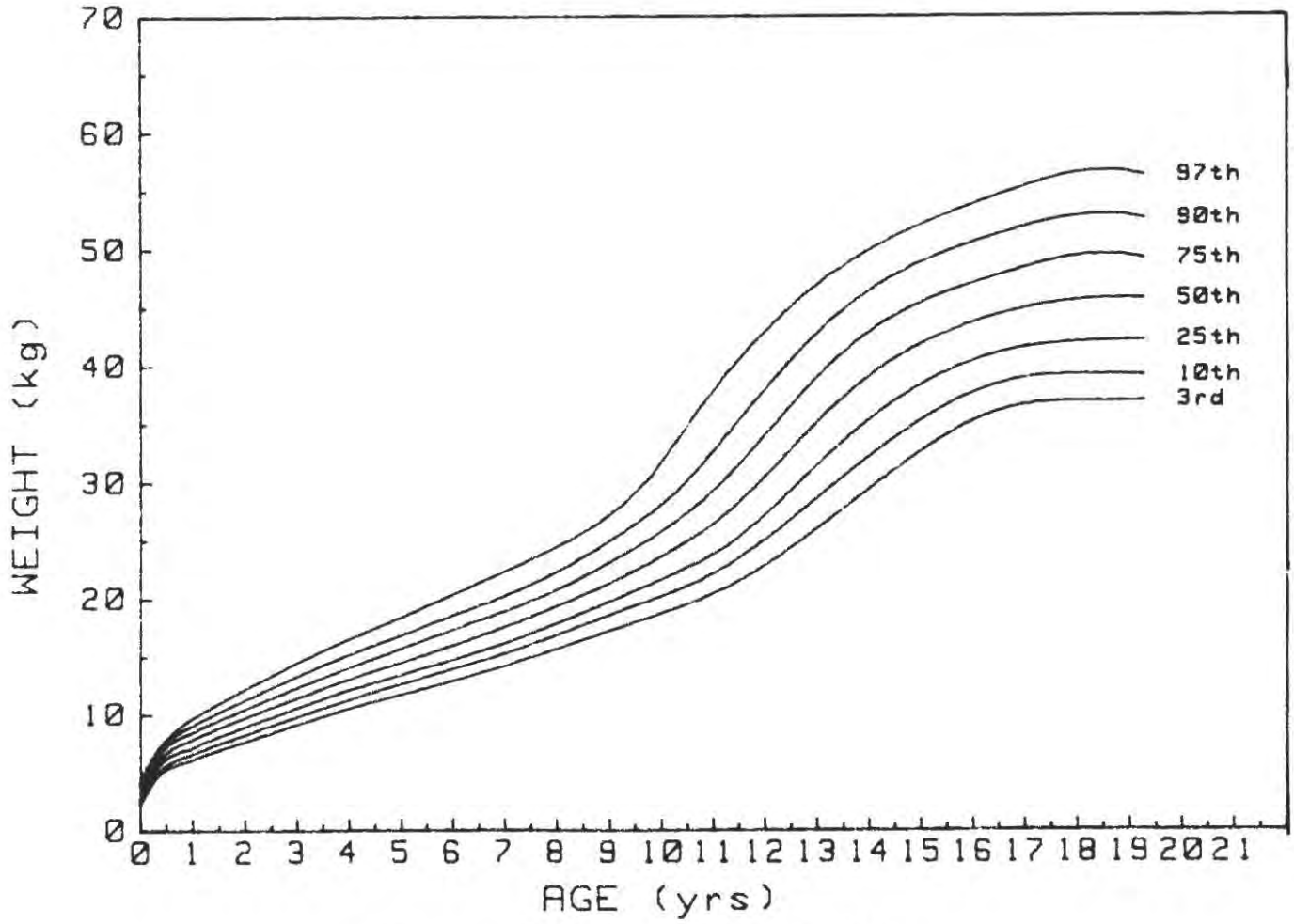

Fig. 3a. Weight, females (0-19 yrs old).

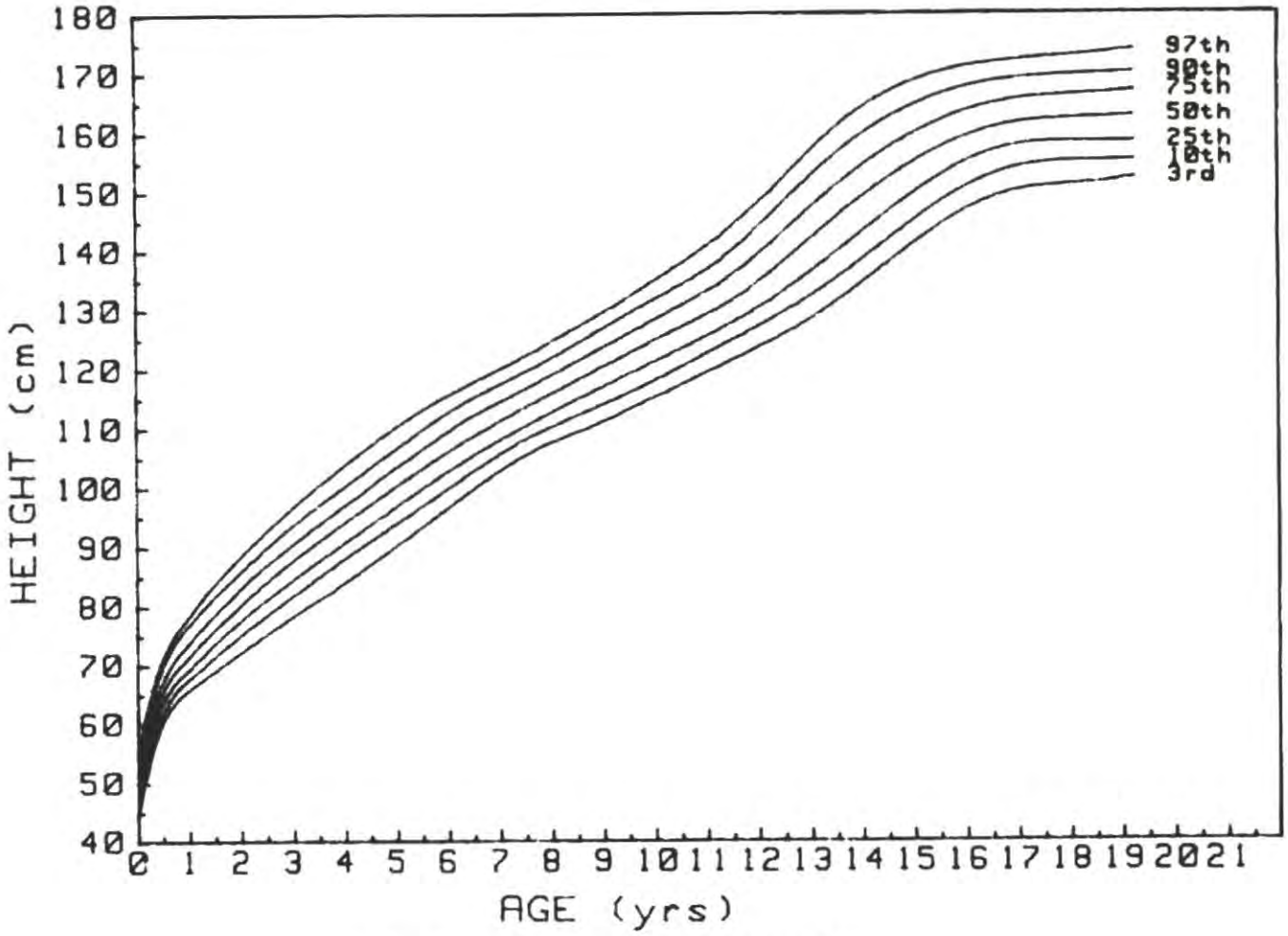

Fig. 4. Height, males (0-19 yrs old).

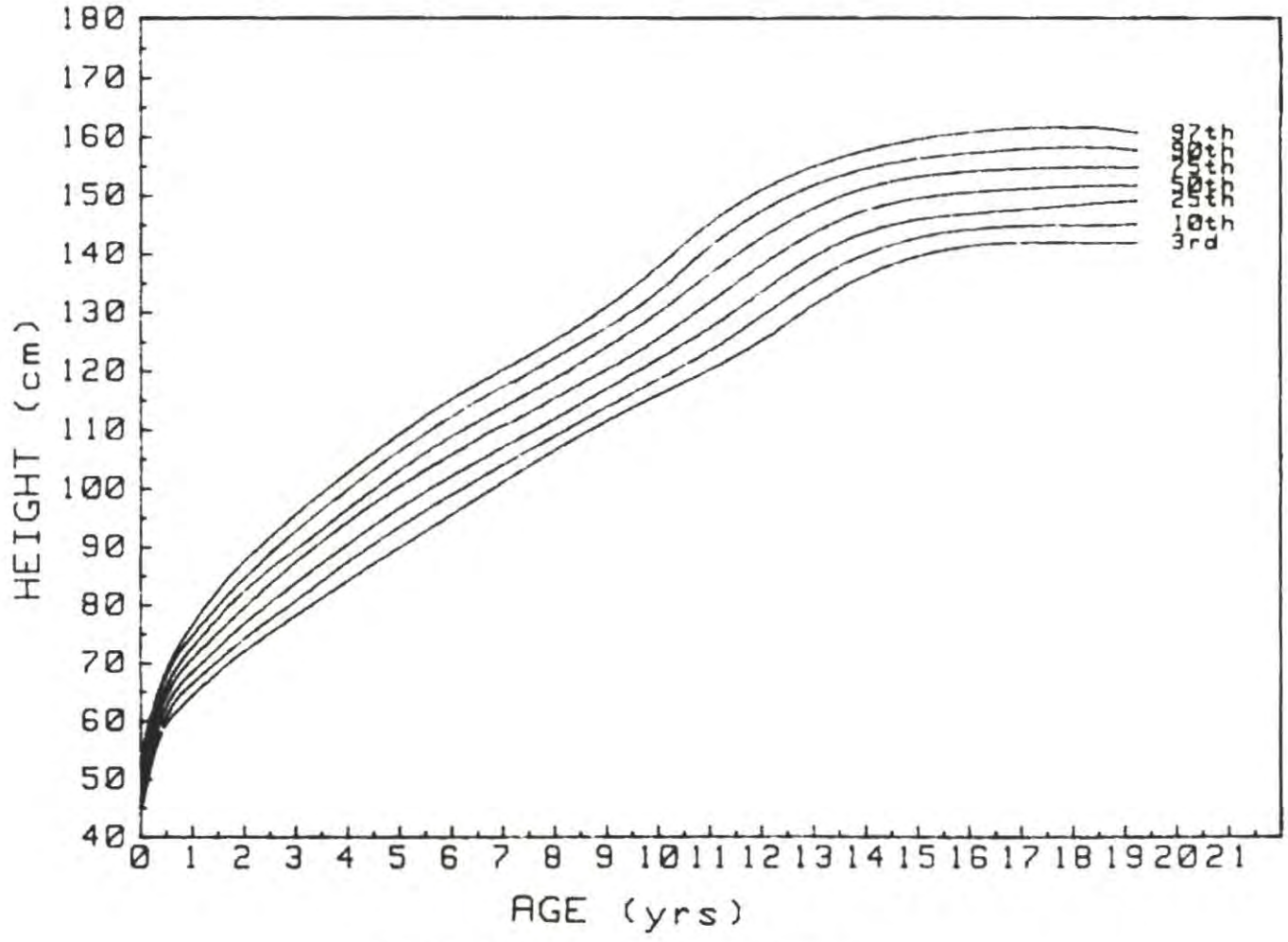

Fig. 4a Height, females (01-9 yrs old).

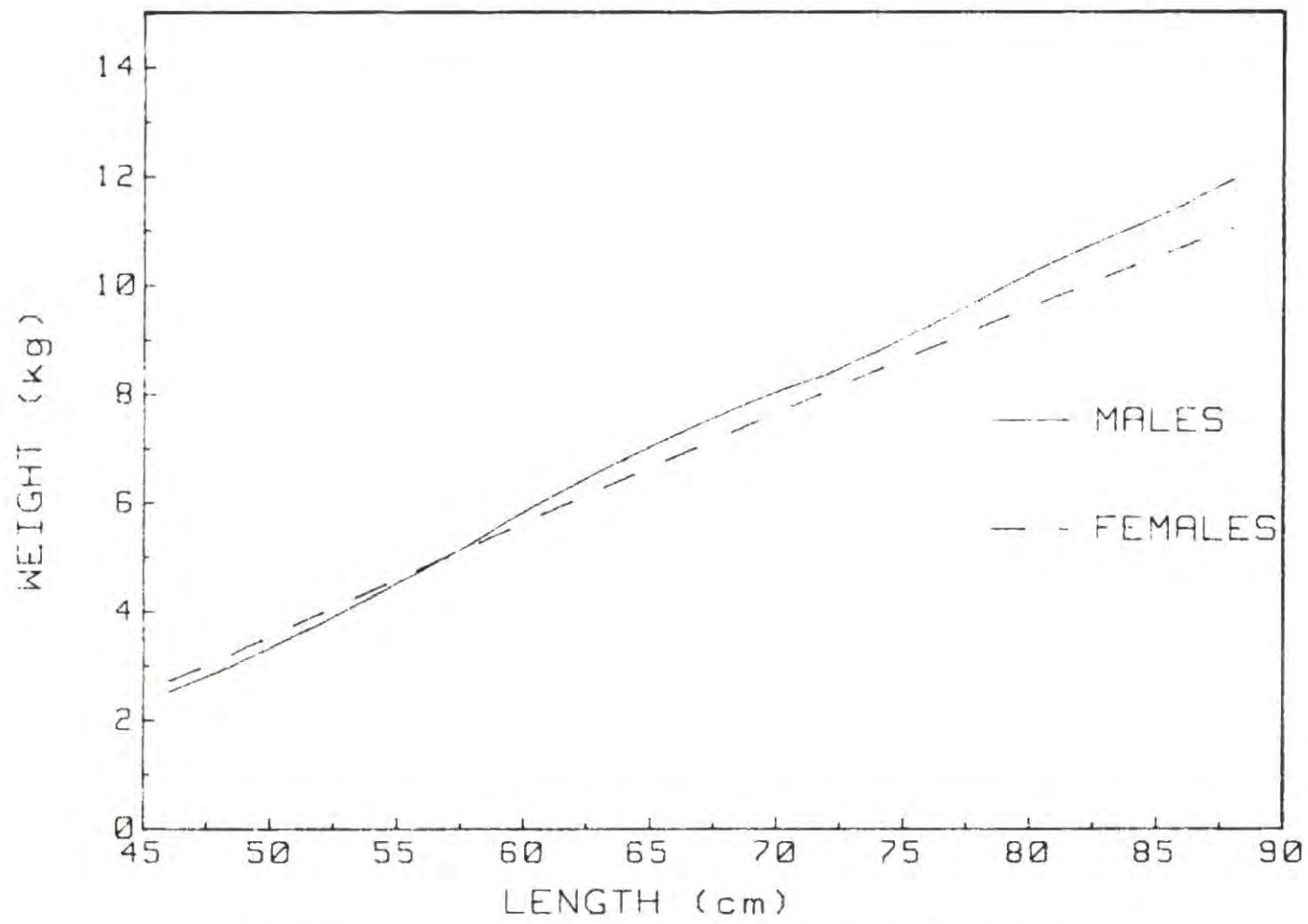

Fig. 5. Median weight for length – male vs female (0-23 months old).

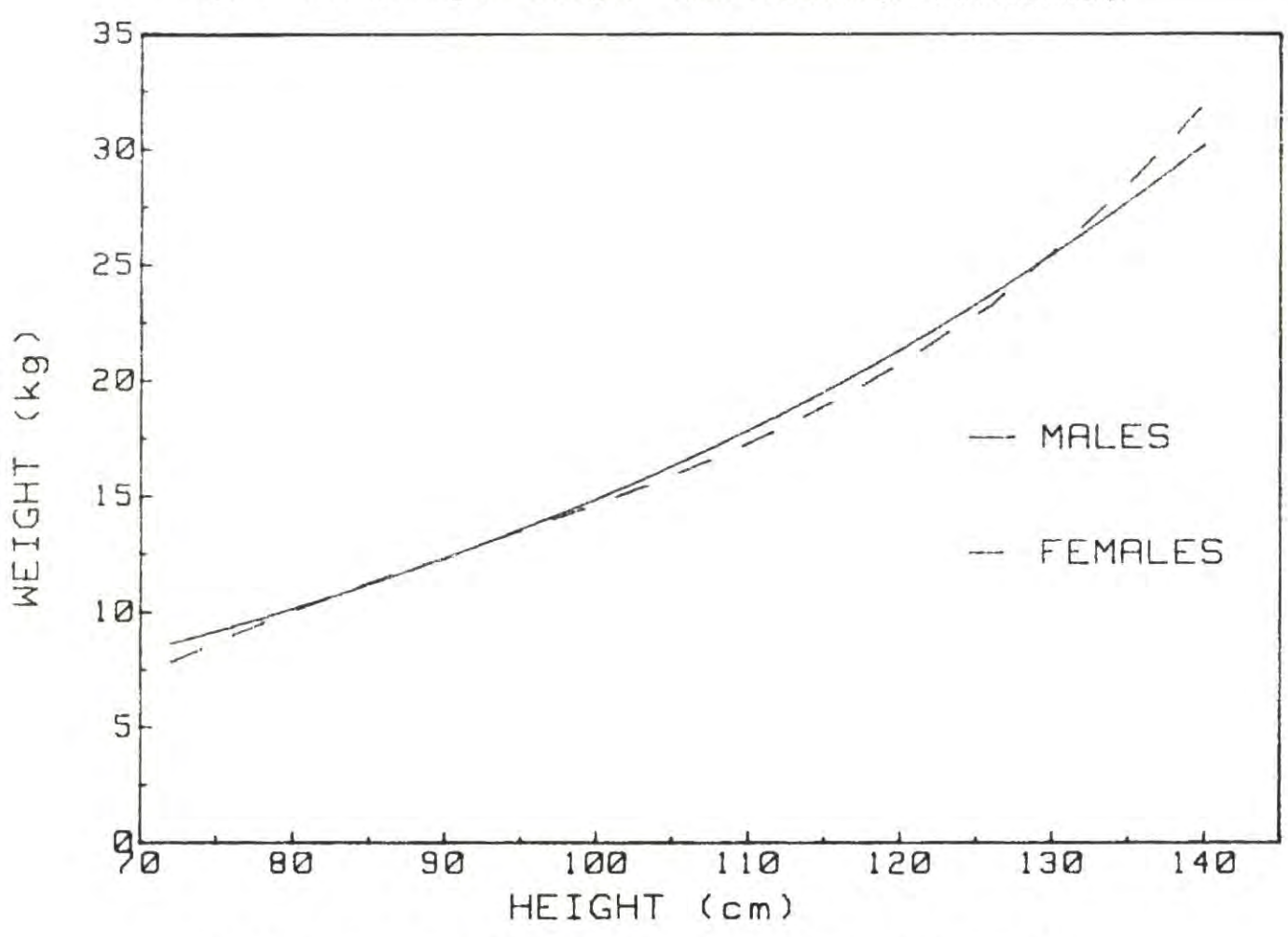

Fig. 6 Median weight for height – male vs female (2-10 yrs old).

Comparison with the 1971 FNRI standards

Comparison of the median weight-for-age values of the present study with the 1971 FNRI standards[6] showed that the two sets are comparable only from birth to 6 months (Figs. 7 and 7a). Beyond that, the present values are definitely lower by about 14%, on the average, for both sexes. At the middle of the seventh month (6.5 months of age), the difference is 0.06 kg in boys and 0.57 kg in girls. At the middle of the seventh year (6.5 years of age), the difference is 2.13 kg in boys and 2.47 kg in girls, and at 18.5 years, the difference is 6.82 kg in boys and 8.26 kg in girls.

The 1971 weight standards (which, except in infancy, was the 90th percentile of the population during the 60's), is seen to lie between the 75th and 90th percentiles of the present data. Similarly the median of the population from which the 1971 standards were derived is found to lie between the 25th and 50th percentiles of the present data. The differences may of course be due to the inclusion of only apparently healthy subjects in the present study, but it may also indicate a positive secular trend from the 60's to the present.

In terms of height, the deviations of P_{50} values of the present study from the 1971 standards are lesser for both sexes compared to those in the case of weight, but the values are still lower than the standards at all ages (Figs. 8 and 8a). The differences are about 6% for both sexes.

Again, the 1971 FNRI height standard is seen to lie between the 75th and 90th percentiles of the present data. Also, the median height of the population from which the 1971 standards were derived lies between the 25th and 50th percentiles of the present data. The same conclusion may be reached as in the case of weight differences between the 1971 standards and the present data.

Comparison with international standards

Except for the first half of infancy where the differences are slight, the present median values of weight are much lower than the NCHS standards (Figs. 9 and 9a). Percentage deviations from 1 year to 18 years ranged from 17% to 28% in both sexes (average, 22%). On the whole, the differences are slightly lower for girls than for boys. It should also be noted that the NCHS median curve lie between the 90th and 97th percentiles curves of the present data.

Differences of median height-for-age in the present study from international standards are lower than in the case of weights, although again the present values are lower than NCHS standards except in early infancy (Figs. 10 and 10a). After the first year, the present values are about 7% to 10% lower (average 8%). Again, the NCHS median curve lie between the 90th and 97th percentile curves of the present data.

As a whole the present values of weight-for-length and weight-for-height are much closer to the NCHS standards than the two parameters just discussed (Figs. 11 and 11a and 12 and 12a). In fact for shorter recumbent lengths, weight-for-length values in the present study are slightly higher than the NCHS standard for both

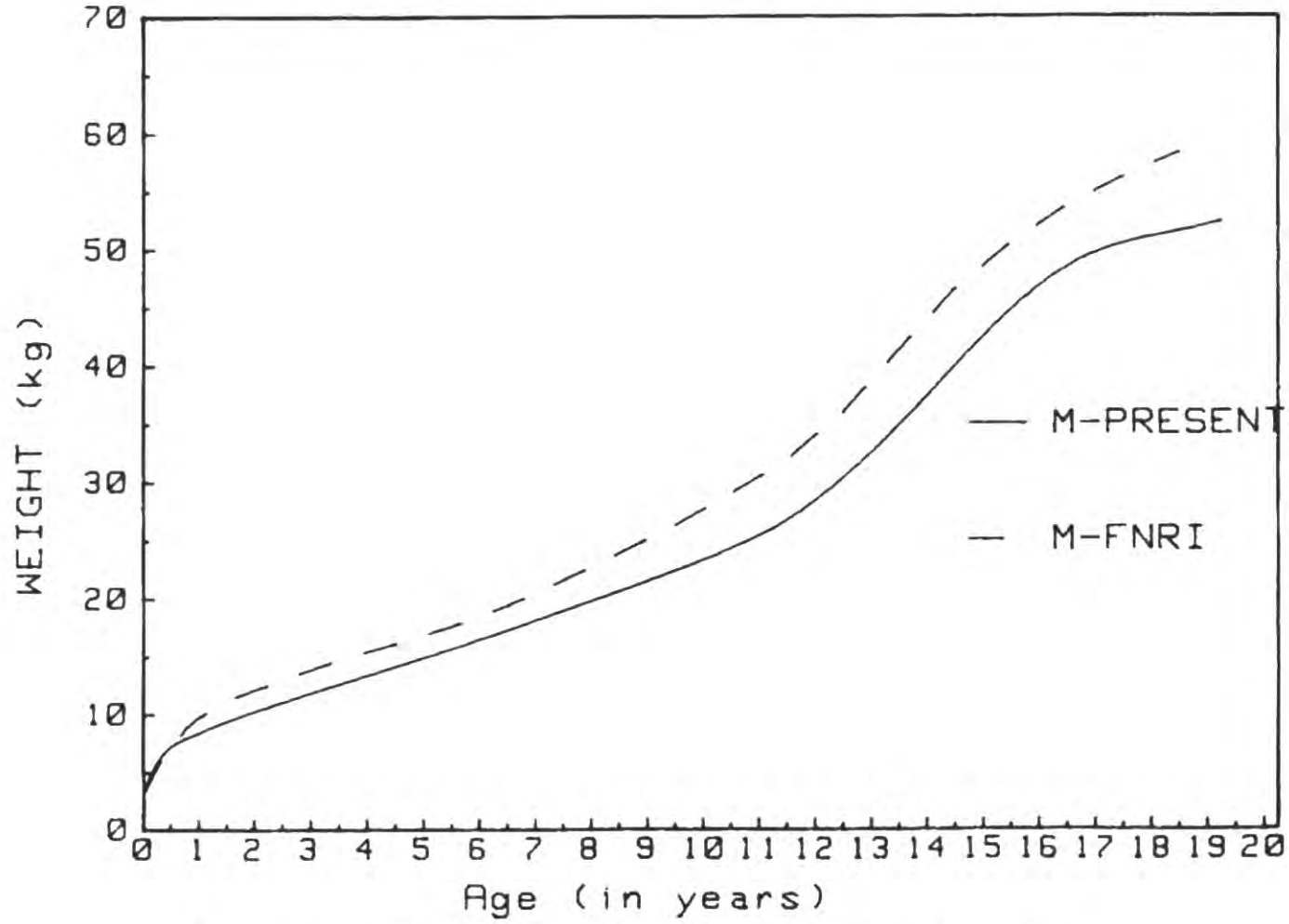

Fig. 7. Present median vs FNRI STD weight – males (0-19 yrs old).

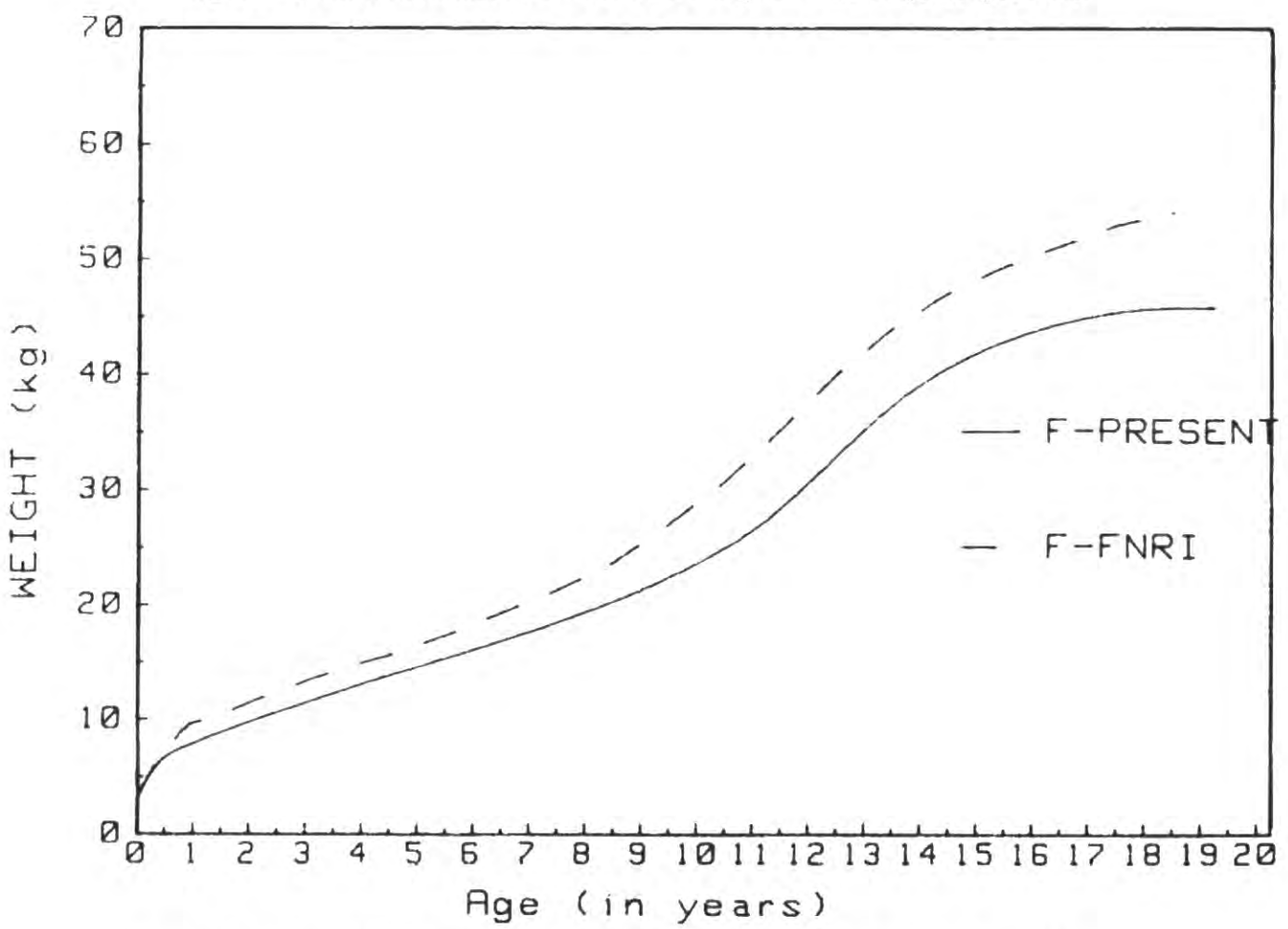

Fig. 7a. Present median vs FNRI STD weight – females (0-19 yrs old).

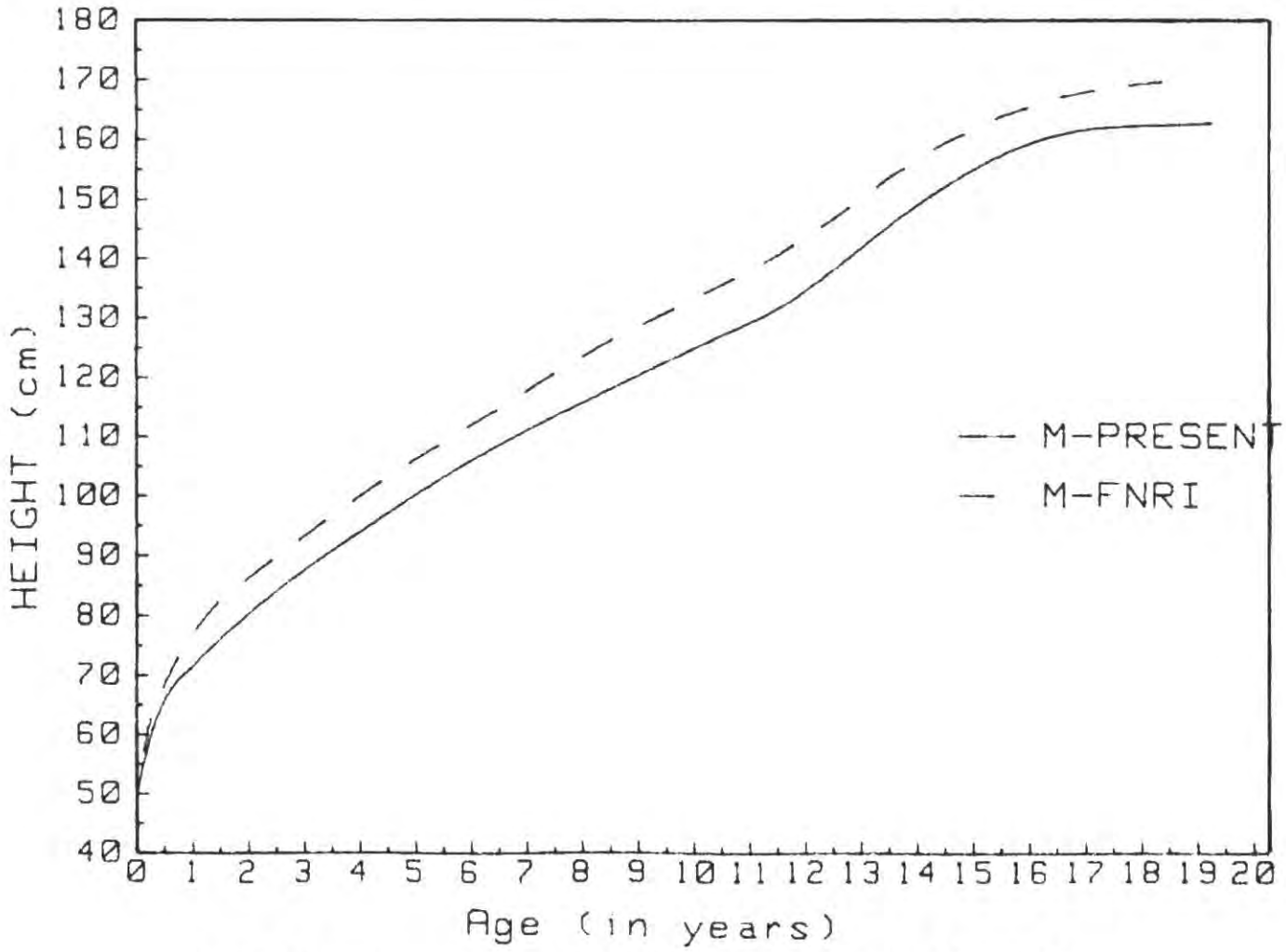

Fig. 8. Present median vs FNRI STD height – males (0-19 yrs old).

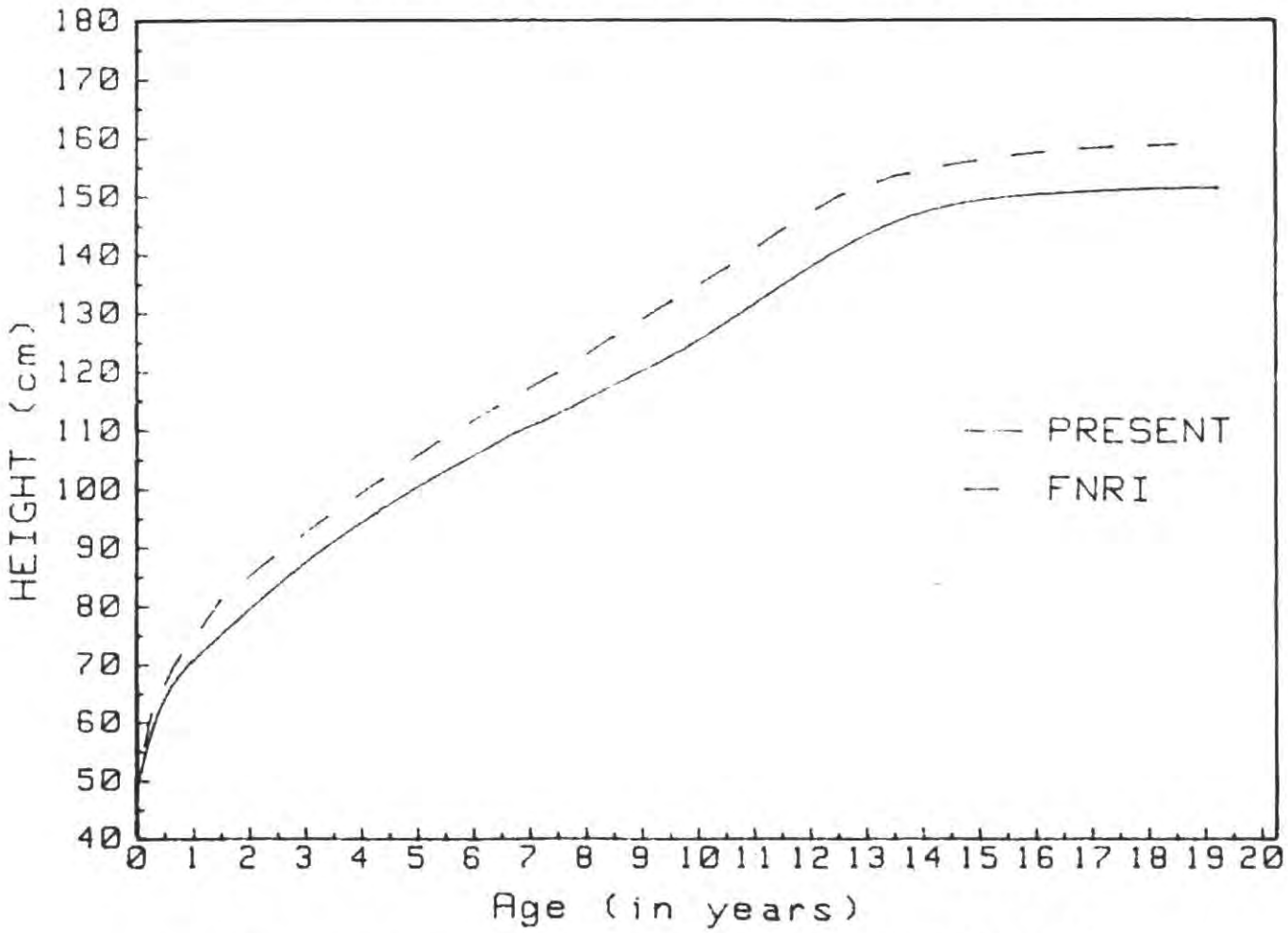

Fig. 8a. Present median vs. FNRI STD height – females (0-19 yrs old).

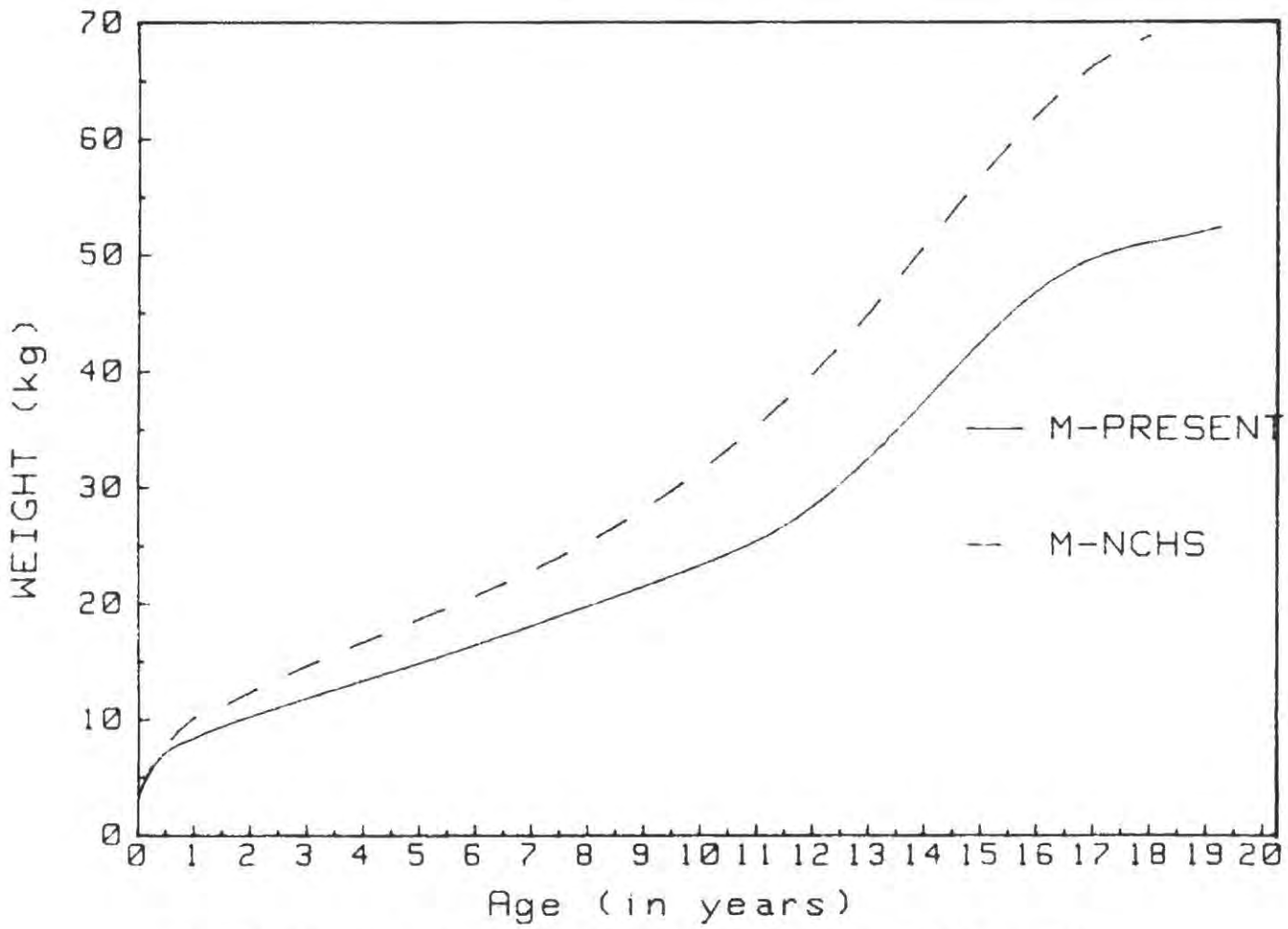

Fig. 9. Present median vs NCHS STD weight – males (0-19 yrs old).

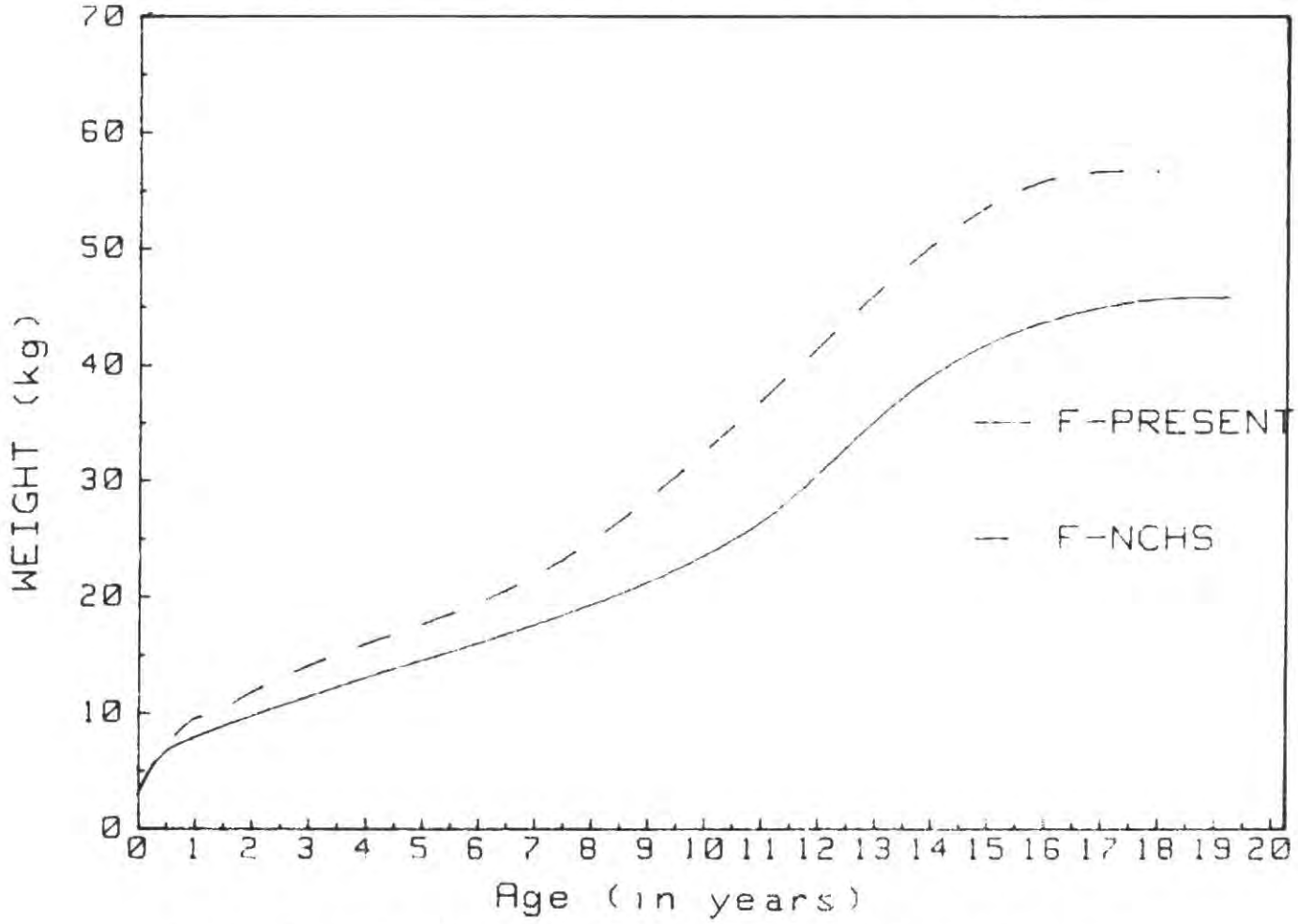

Fig. 9a. Present median vs NCHS STD weight – females (0-19 yrs old).

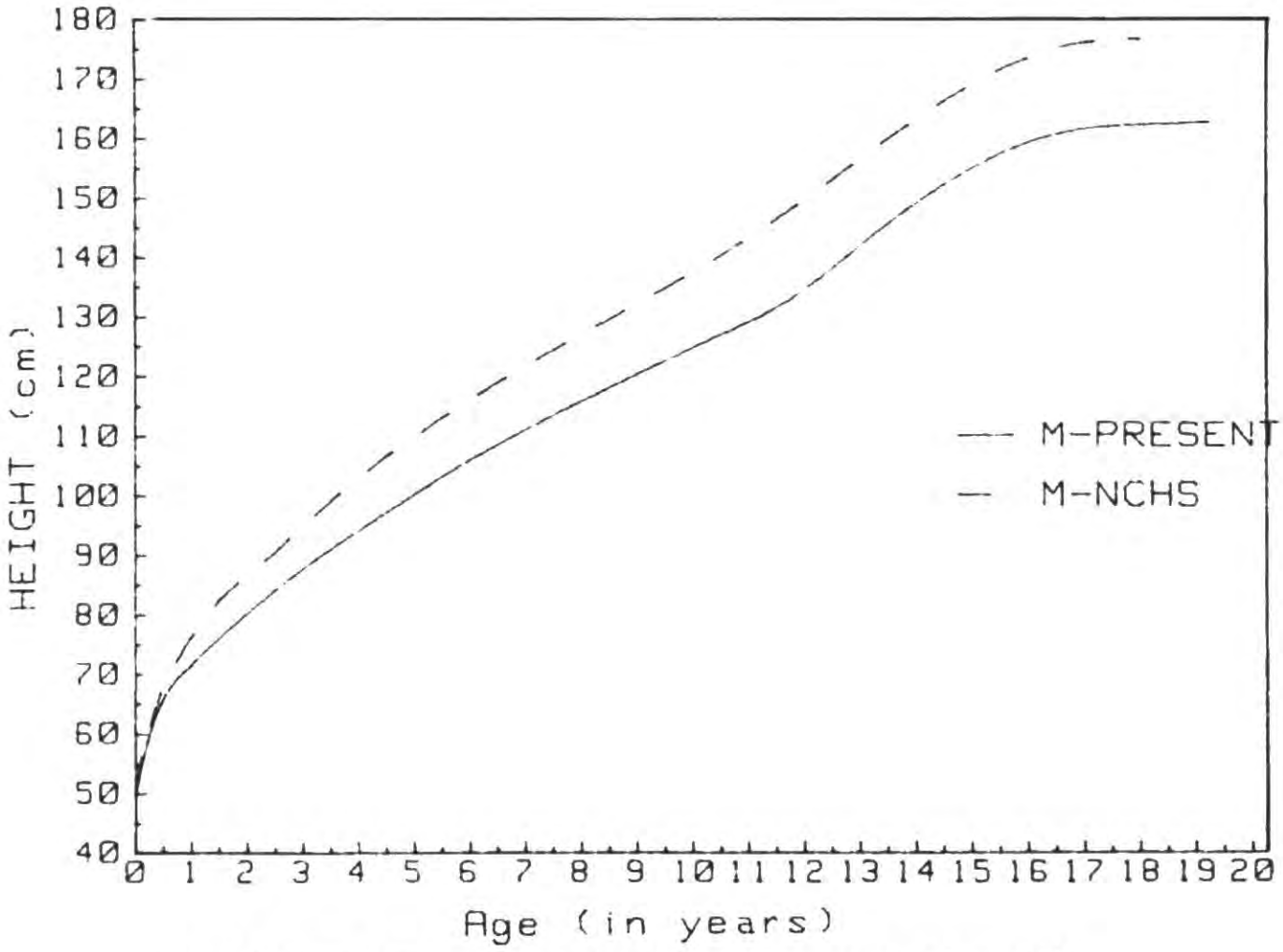

Fig. 10. Present median vs NCHS STD height – males (0-19 yrs old).

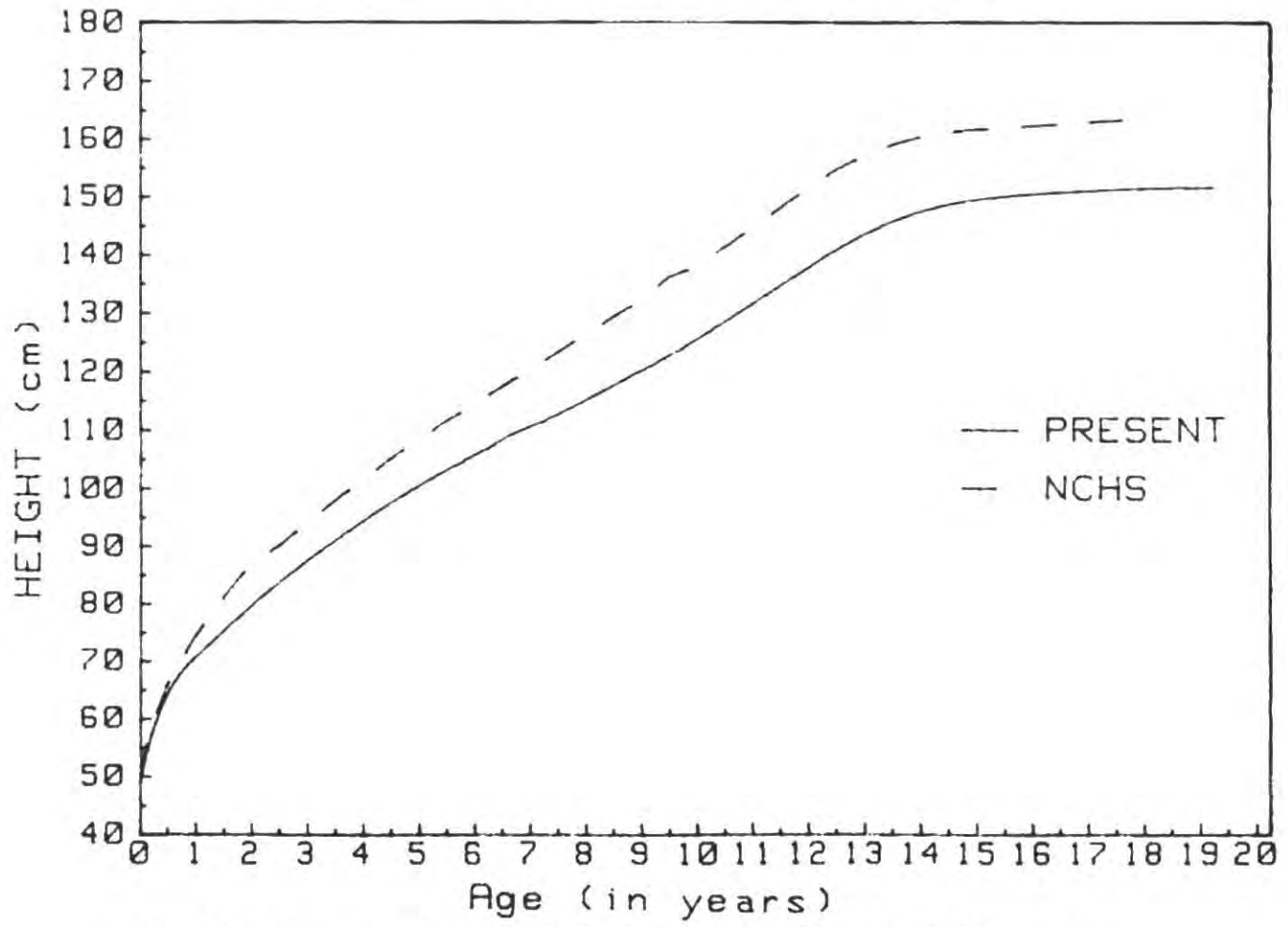

Fig. 10a. Present median vs NCHS STD height – females (0-19 yrs old).

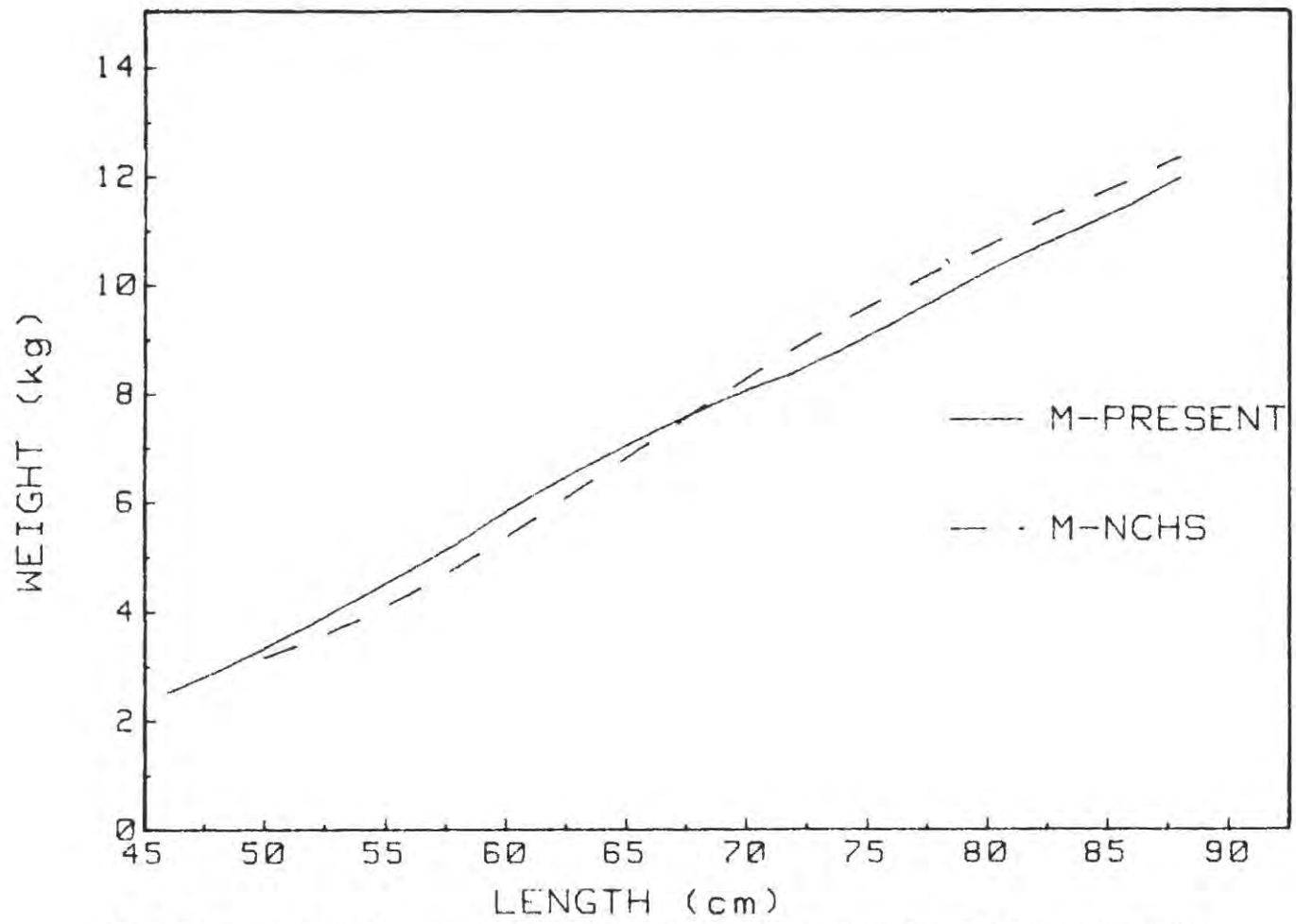

Fig. 11. Present median vs. NCHS STD weight for length males (0-23 months old).

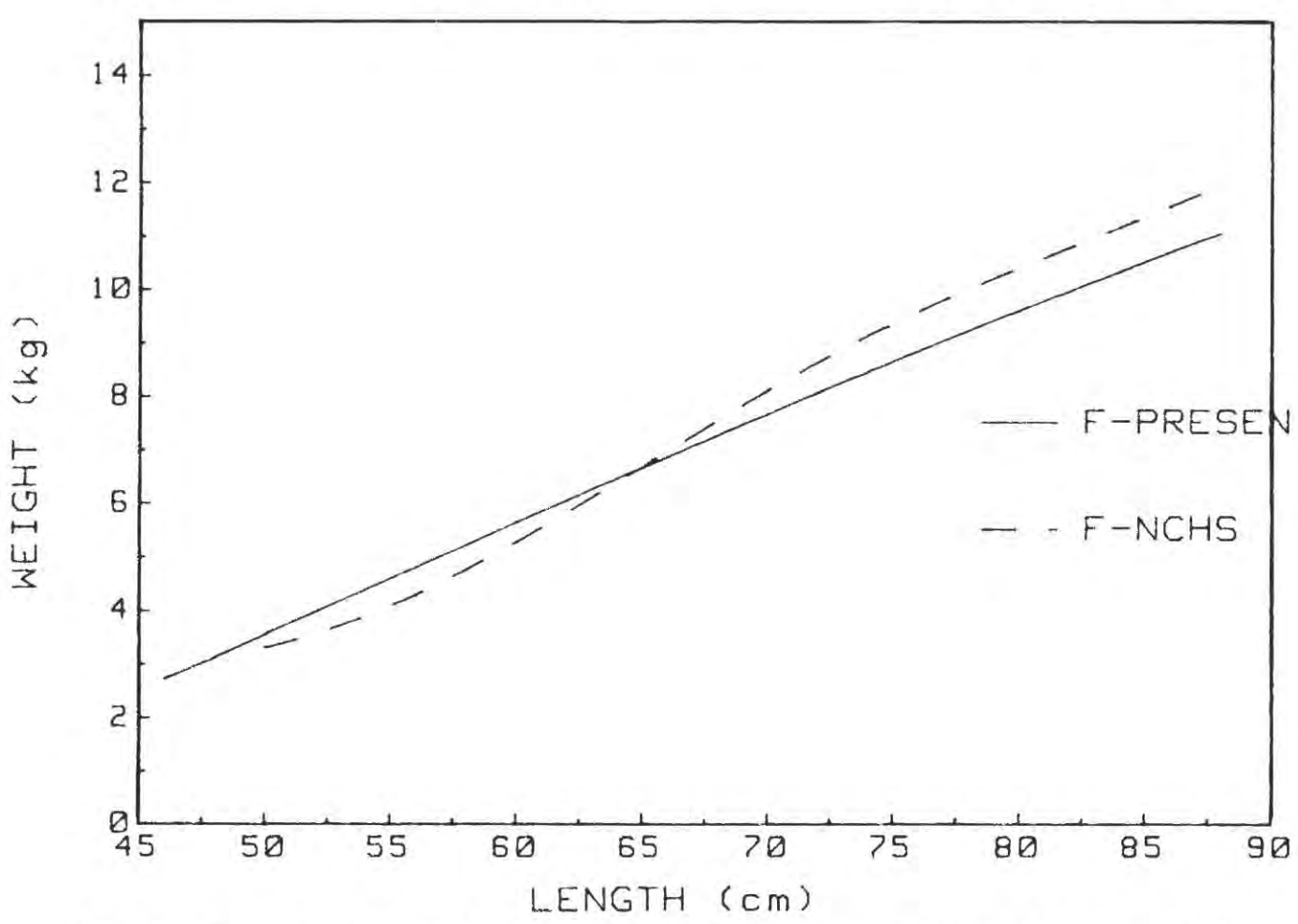

Fig. 11a. Present median vs. NCHS STD weight for length – females (0-23 months old).

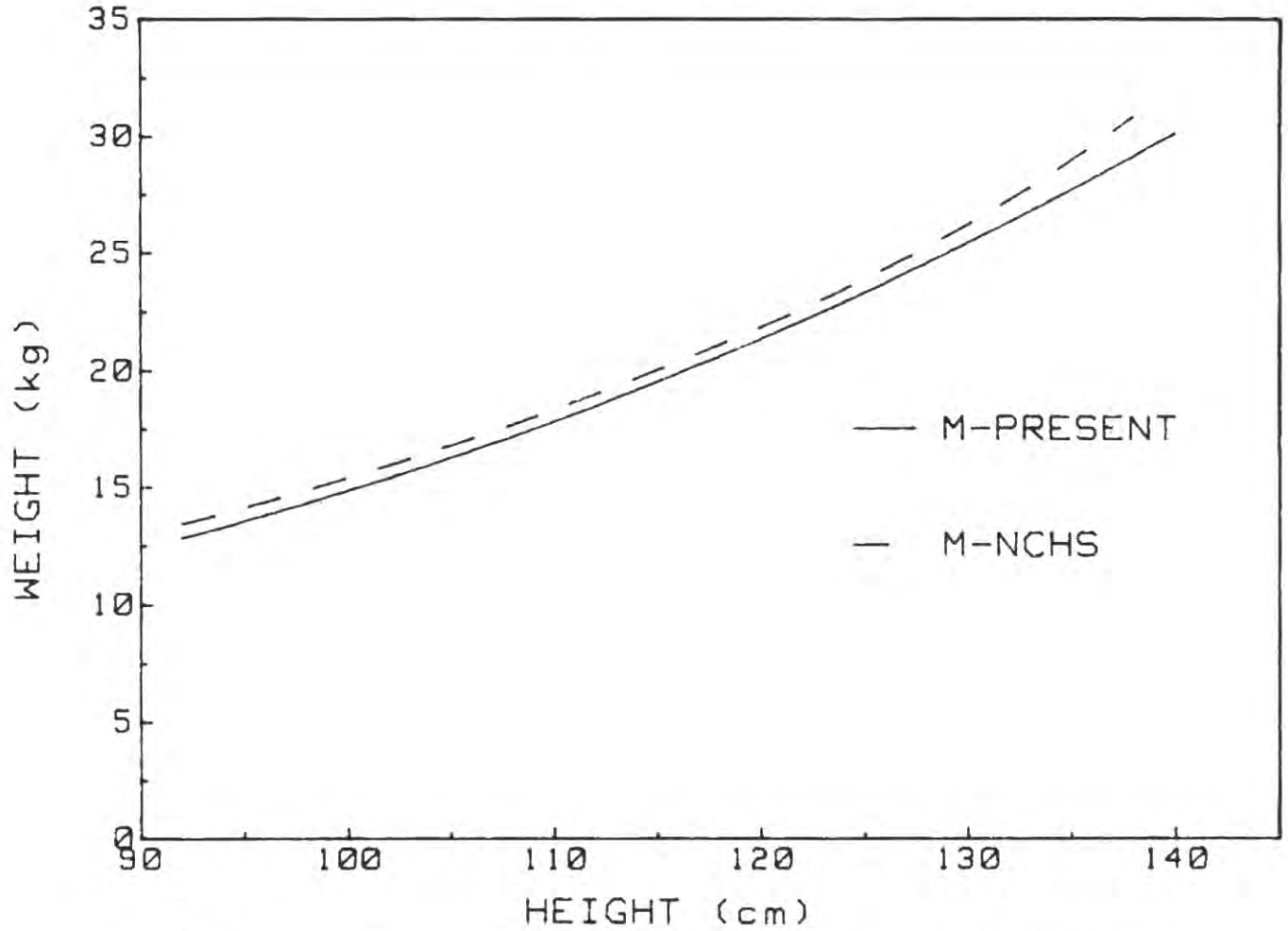

Fig. 12. Present median vs NCHS STD weight for height – males (2-10 yrs old).

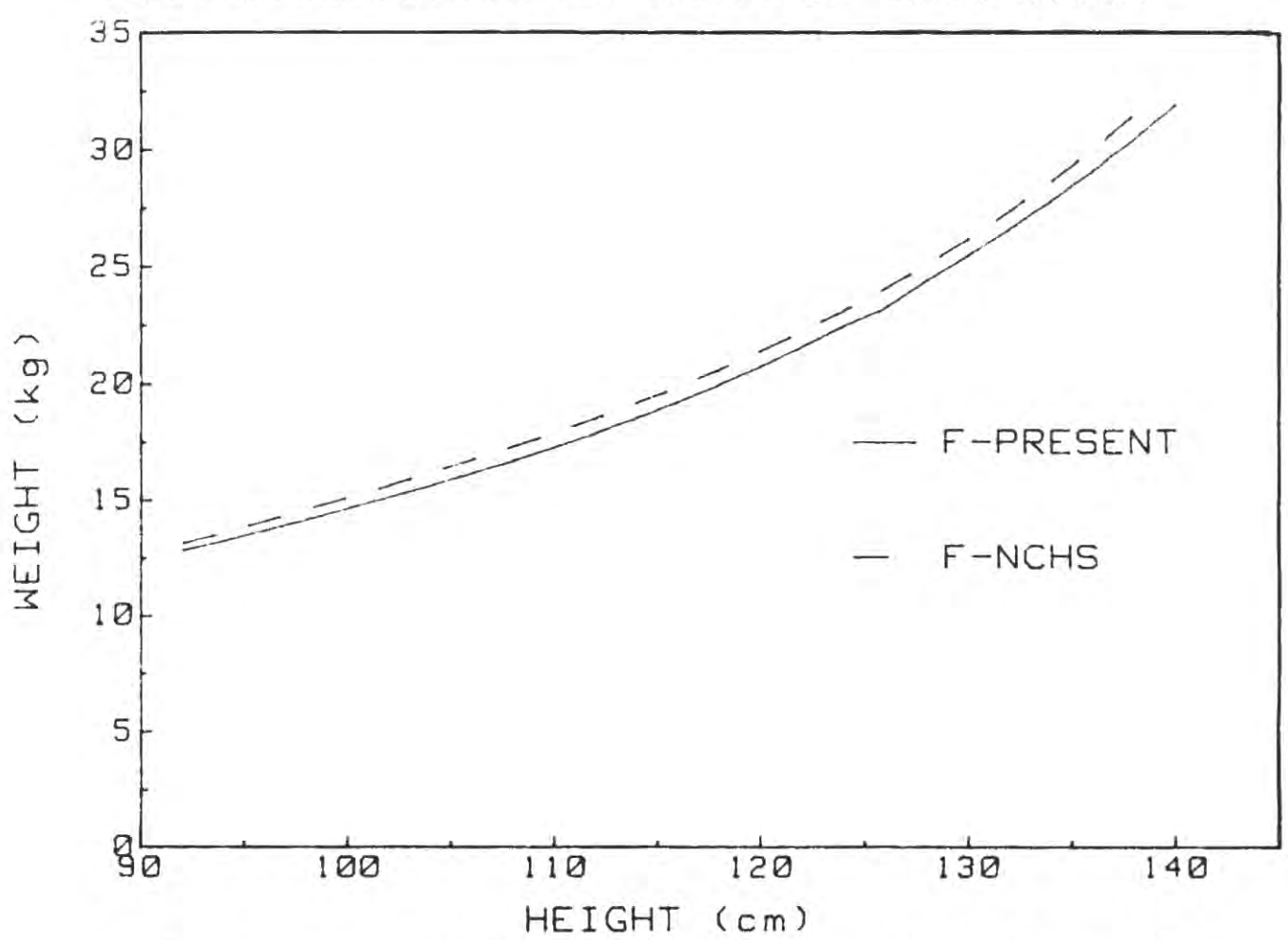

Fig. 12a. Present median vs NCHS STD weight for height – females (2-10 yrs old).

sexes. For taller lengths/heights, the NCHS standards are higher than the present values by only about 2 to 4%. It is apparent that as far as weight-for-height measurements for 2-10 year old children are concerned the values in the present study closely approximate the international standards.

Comparison with other local studies

In general, median values of weight and height in present study are higher than the average measurements of Filipino children of corresponding ages found in the 1982 national nutrition surveys (13). The median weight measurements of boys in the present study at age 6 months are higher by 9.5%, at 3.5 years by 2.5%, and at 17.5 years by 1.3%. In girls, the differences in median weight between the two sets of data are much smaller than in boys, with 0.75% difference at 2 years and 0.18% at 6.5 years.

The deviations between the two sets of data in terms of height are much lower than in the case of weight. Nevertheless, the mean heights for both sexes in the present study are also higher than the average heights at corresponding ages in the 1982 survey.

The median values were also compared with selected groups of children in the 1978 FNRI nutrition surveys[17] who belong to households with above average income and those above 90% energy adequacy intake level. Results showed that for age group 0-6 years, values in the present study are higher for both height and weight by as much as 6.65% for weight at 0.5 years, boys, and 6.3% for weight at 6.5 years, girls. Likewise, height values in the present study are higher for both sexes except at ages 6 1/2 years for boys and 4 1/2 years for girls.

Summary and Conclusion

In order to arrive at anthropometric standards for 0-19 year-old Filipino children useful for nutritional and clinical evaluation, 26,961 randomly selected children from nearly all regions of the country were clinically examined and measured for weight, height and other anthropometric parameters. A total of 23,660 healthy children of no more than 1/4 foreign blood were included for the derivation of standards. Percentile curves of weight-for-age, length/height-for-age and weight-for-length/height were constructed using the cubic spline technique to fit curves to the data. The coefficients of variation indicate very stable estimates for practically all of the age groups. Normal distribution was found for the different parameters at all ages.

As expected, the weight and height of boys are greater than those of girls for the same age up to the time the latter starts their pubertal growth spurt when the girls overtake the boys. The boys start to get taller and heavier again by 14 yrs of age.

The curves of weight-for-length/height for both sexes approximate each other except at tall heights.

Except in early infancy, the present values for weight-for-age are lower than the 1971 FNRI standards by as much as 15% for both sexes. Height-for-age values are lower than the same standards by about 6% for both sexes. On the other hand, the 1971 standards for weight and height as well as the median values of the population from which the standards were derived are seen to lie below the corresponding P_{90} and P_{50} values of the present data. While this may be due to the inclusion of only apparently healthy subjects in the present study, it may also indicate a positive secular trend from the 60's to the present.

Except in early infancy, present values were considerably lower than the NCHS standards (range of deviation, 18% to 28% for weight-for-age and 5% to 8% for height-for-age). Weight-for-length/height values, however, closely approximate the NCHS standards.

It is concluded that the present data constitute the best available data for use in the construction of new growth standards for Filipino children. It is however, recommended that these values are validated in the field among health and malnourished subjects to delineate the levels below which malnutrition is likely to be present.

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HEALTH HAZARDS IN THE PLASTIC INDUSTRY PROBLEMS IN RISK ASSESSMENT

Quintin L. Kintanar
National Academy of Science and Technology
Bicutan, Taguig, Metro Manila, Philippines

ABSTRACT

The work environment and the health of workers in the Plastic Industry in the Philippines were studied in the last two years using a multiple-step stratified sampling method.

The study indicated that only 5 out of a total of 312 companies and 52 respondents registered in the Association of Plastic Industry companies use the toxic plastic Monomers Vinyl Chloride (VCM) or Styrene (SM) as raw materials or intermediates in their production process. All the others either use polymers from these local companies or import intermediate raw materials in the form of plastic polymer powders and pellets, and fabricate them into various finished products.

The findings show that the work environment in the plastic industry in the Philippines is inadequate with respect to general physical conditions and occupational health and safety measures. These inadequacies notwithstanding, there was no evidence of the Vinyl Chloride Diseases Syndrome characterized by signs and symptoms of peripheral vascular disease particularly in the hands, or increased incidence of pulmonary fibrosis from chronic exposure in dust, in exposed workers.

The evaluation of the finding of an apparent increase in some companies of liver disorders, including hepatocarcinoma will be discussed with respect to the problems of multifactorial risk assessment in the Philippine setting.

Introduction

Since the 1960's as a sector, worldwide, the Plastic Industry has been growing faster than most other sectors. From a world production of 24 million tons in 1969, it grew to 58 million in 1980, and is forecast to reach 100 million tons in 1990 (Encyclopedia of Occupational Health and Safety, 1983).

This extremely rapid growth was brought about by the great usefulness of a variety plastic products in industry and at home, and the availability in great abundance of cheap base chemicals from oil and natural gas. Through the process of cracking and distillation the petrochemical industry has provided monomeric raw materials which when polymerized are transformed to the familiar thermoplastics like polyvinylchloride or polyethylene and the less familiar thermosetting resins used in paints and glues.

Starting in the 1960's small, medium and large plastic companies were established in the country which numbered more than 300 registered companies in 1981.

But along with the benefits obtained from the multitude of plastic products, come the cost and the hazards. Some time in the 1970's a clinical syndrome characterized by clubbing of the fingers and acro-osteolysis demonstrable by X-ray, was associated with chronic exposure to Vinyl Chloride Monomer (VCM). This Syndrome came to be known as the Vinyl Chloride Disease Syndrome. (N.Y. Acad. of Sci., 1975). More ominous than this was the association of chronic high level VCM exposure with the development of liver disorders including primary liver cancer particularly a specific type-Angiosarcoma of the Liver (Creech and Johnson, 1974).

With the continuous growth of the plastic industry in the Philippines, it became important to know the status of the work environment and the health of workers in the industry. Thus, this study funded by the Philippine Council for Health Research and Development, was undertaken starting in 1984.

Materials and Methods

A modified multi-step stratified sampling method was used in the selection of companies and subjects included in the sample studied.

Three hundred thirteen (313) registered Plastic companies were surveyed of which only 52 responded positively. From this 52 responders, 23 companies representing small, medium, and large companies located in Luzon, Visayas and Mindanao were selected. Of the 23, 10 companies engaged in the production or use of VCM, polyvinyl chloride (PVC) styrene or polystyrene, were included for in-depth studies of the work environment and the health of workers.

The studies/measurements/activities done under this project were the following:

a) Survey of the plastic industry with special attention to the work environment and the health of workers. n = 52

b) Plant visits to selected companies representing a cross section of the plastic industry. Majority of these companies are located in Metro Manila, others in Cebu, Davao and Iligan. n = 23

c) Review of medical records on Accidents, Absenteeism Morbidity and Mortality from 1980-1983. n = 10

d) Measurement of the concentration in the air of vinyl chloride and styrene in the production and control areas of plastic companies. n = 10

e) Physical medical examination of selected subjects in control and experimental groups. n_t = 241

Laboratory examination of control and experimental groups including:

n_t = 241

1. Blood Chemistry
   - SGPT
   - BUN
   - LDH

2. Blood Counts
   - RBC and Hct.
   - WBC and differential
   - Platelets

g) X-rays of Control and Experimental Groups. $n_t = 241$
   1. Chest
   2. Hands

h) Retrospective Review of the National Central Tumor Registry
i) Education and Information Campaign (Posters)

Results and Findings

Profile of plastic companies

The results of the survey show that most of the companies are engaged in processing and fabrication (18) and only (5) are in polymer or resin manufacturing either solely (1) or in combination with processing and fabrication (4). Table 1.

Table 1. Classification of plastic companies by types of production

Type of production	*Number*	*%*
A. Polymer/Resin Manufacturing	1	4.4
B. Processing and Fabrication	18	78.3
C. Combination of A & B	4	17.4
Total	23	100

The range of raw materials used in the 23 companies included in this study are listed in Table 2.

Three or 13% used VCM, 5 or 22% used PVC and the majority 13 or 57% used a combination of two or more raw materials.

Table 2. Distribution of plastic companies surveyed according to raw materials used

Raw materials	*No. of plastic companies*	*%*
Vinyl Chloride Monomer	3	13
Poly Vinyl Chloride	5	22
Styrene	2	8
Combinations:		
Styrene Polystyrene Polyethylene Etc.	13	57

Work environment and health facilities

Qualitative assessment of the physical condition of the work in the 23 companies visited by the project team showed that 83% has satisfactory lighting, 74% has satisfactory cleanliness and housekeeping.

Table 3. State of the work environment in plastic companies visited

Parameter	n	*Assessment*			*%*
		Satisfactory	*%*	*Unsatisfactory*	
A. Ventilation	23	9	39	14	61
B. Lighting	23	19	83	4	17
C. Cleanliness and Housekeeping	23	17	74	6	26

As far as facilities, protective measures and health services for the employees are concerned, 86% had satisfactory medical staff mostly on retainer arrangement on part-time basis, 59% maintained a medical clinic, and 45% kept some medical records. However, from all indications, the occupational health program and the quality of medical services offered still left much to be desired even in those companies where facilities were available.

Toilet and canteen facilities were also largely inadequate in most companies except for the largest ones.

Protective devices were provided in 55% of the companies visited but were not of high quality.

Record of accidents, morbidity and mortality

The accident rate in 1980 ranged from 25 to 42 per 1000 persons at risks. The morbidity records among the workers on the plastic industry showed the

normal pattern of illnesses in the general population (Table 4). According to Reverente (1982) only 3-5% of all sick leaves in local companies including industries, other than plastic are directly work-related. Ninety five percent (95%) of absences from work are due to non-occupational illnesses such as viral respiratory infection, etc.

Table 4. Morbidity cases of 23 plastic companies visited for the period 1981-1983

Health Problem	*1981*	*1982*	*1983*	*Total*
Respiratory	2,869	4,149	1,544	8,562
Gastrointestinal	1,111	1,341	304	2,756
Dermatological	376	173	198	717
Hypersensitivity	177	186	45	408
Ophthalmological	248	94	31	373
Cardiovascular	108	219	84	411
Gynecological	98	126	58	282
Skeletal	56	79	19	154
Central Nervous System	48	52	42	142
Hematological	11	26	8	45

Concentration of vinyl chloride and styrene monomer in air samples from various locations in the work environment

The qualitative measurement of Vinyl Chloride and Styrene monomer in production and control areas using the Kitagawa Detector tubes and Sampling Pump showed that Threshold Limit Value (1 ppm) were frequently exceeded in some companies engaged in the polymer manufacturing using vinyl chloride monomer as raw materials (Table 5). In one company engaged in both the synthesis of vinyl chloride monomer and production of polyvinyl chloride the short time exposure limit or ceiling concentration was also exceeded in the automated control room for the production process.

From a report of the U.S. Environmental Protection Agency the typical air concentration in various locations in a Vinyl Chloride/Chloride Polyvinyl Chloride plant are shown in Table 6.

Signs and symptoms of acute poisoning

The progressive signs and symptoms associated with various concentrations of Vinyl Chloride Monomer is shown in Table 7. The odor threshold is already at the fairly high concentration of 2,000 ppm. Behavioral effects and subjective sensations of elation, asthenia and heaviness of the legs are seen with concentration in the range of 5,000 to 8,000 ppm and possible death at 120,000 ppm. In our investigation, there was one reported case of a worker falling accidentally into the reactor vessel while cleaning it. Whether this was due to acute intoxication remains to be proven.

Table 5. Concentration of vinyl chloride and styrene monomers in various areas of ten (10) companies under study

Area	*Gas Sample Concentration*										
	Vinyl Chloride					*Styrene*				*Acrylo-nitrile*	
	PPM					*PPM*				*PPM*	
Extruder	5	.05	–	–	–*	–	–	3	5	5	neg.
Mixing	1	–	–	3	–	–	3	–	–	–	–
Molding	–	–	–	–	–	–	–	4	10	2	4
Reactor	–	–	neg.	–	–	–	–	–	–	–	–
Engineering	–	–	–	neg.	–	–	–	–	–	–	–
Polymerizer	–	–	–	1.5	–	–	–	–	–	–	–
Gas Holder	–	–	1.8	–	–	–	–	–	–	–	–
Kettle	–	–	4	–	–	20	–	–	–	–	–
Blend Tank	–	–	–	–	3	–	–	–	–	–	–

Table 6. Typical concentration of vinyl chloride**

	mg/m^3	*ppm*
Reactor prior to ventilation	7,800 mg/m^3	3000
Reactor during scraping	130-260	50-100
Near hands during scraping	1,560-2,600	600-1000

* Monitoring results were taken by the company dated April 18 and May 1, 1984.
**Reference: U.S. Environmental Protection Agency, 1974.

Table 7. Signs and symptoms of acute poisoning with vinyl chloride monomer*

Concentration	*S & S*
100 ppm	Not Perceptible
2,000 – 5,000 ppm	Odor Threshold = Sweetish Odor
5,000 ppm	Elation Asthenia Heaviness in Legs and Somnolence
8,000 – 10,000 ppm	Vertigo
16,000 ppm	Impaired Hearing and Vision
70,000 ppm	Narcosis
120,000 ppm	May be Fatal

*Reference: New York Academy of Science: 246; 1975, pp. 1-322.

Physical and medical examination

The physical and medical examination of both 97 control in non-production areas and 144 experimental subjects in production areas showed essentially normal and findings without any evidence of hepatosplenomegaly of peripheral vascular disease characteristic of vinyl chloride disease as described in the literature.

Laboratory examination

Blood chemistry namely SGPT, BUN, and LDH, and blood counts, namely RBC and hematocrit, WBC and Differential and Platelet counts, showed results in control and experimental groups which were mostly within normal range and not significantly different from each other (Table 8-SGPT, BUN, LDH and Table 9-RBC, hemacrit, WBC, Differential Platelets).

Table 8. Mean values with standard deviation of mean for blood chemistries of both exposed and unexposed groups

Blood Chemistry	*Number*	*Unexposed Group*	*Number*	*Exposed Group*
BUN	97	4.0582 ± .113	144	4.0801 ± .110
ALAKALINE PHOSPHATASE	97	31.106 ± 1.228	144	32.662 ± 1.031
LDH	27*	99.734 ± 7.303	41*	108.141 ± 5.816
SGPT	97	17.325 ± 2.1084	144	19.866 ± 1.844

Table 9. Mean values with standard deviation of mean for blood count for both exposed and unexposed groups

Blood Test	*Number*	*Unexposed Group*	*Number*	*Exposed Group*
Hemoglobin	97	12-586 ± .243	144	13.513 ± .1834
Hematocrit	97	43.248 ± .626	144	42.986 ± .511
WBC	97	8,001.389 ± 344.896	144	8,005.4622 ± 221.14
Lymphocytes	97	39.528 ± .9212	144	40.555 ± .8566
Segmenters	97	58.083 ± .925	144	56.244 ± .8096
Monocytes	97	.375 ± .0801	144	.4538 ± .068
Eosinophil	97	2.125 ± .2712	144	2.7981 ± .3399
Platelet	97	265.750 ± 11.701924	144	254.773.109 ± 9.224466

*Only 27/97 of unexposed group and 41/144 of exposed group were tested for LDH due to unavailability of reagents.

X-rays of the lungs and hands

The X-rays of the lungs and hands of subjects belonging to the control (not at risk) and experimental (at risk) groups showed no tell-tale findings suggestive at lung fibrosis or acroosteolysis which is characteristic of vinyl chloride disease syndrome.

Retrospective review of liver cancer

As a side study, a review of the National Tumor Registry of the Philippine Cancer Society from 1980-1983 was undertaken, which showed 313 cases of liver cancer including 24 cases of hepatocellular and one case of angiosarcoma of the liver (Table 10). Of the total, 97 cases of various types of hepatic cancer were investigated further by going back to their medical charts in 8 hospitals in Metro Manila. In no case was there a definite occupational history suggestive of plastic chemical induced cancer.

Table 10. Hepatic cancer cases taken from the National Central Tumor Registry of the Philippine Cancer Society for the period 1980-1983

Kinds of Liver Cancer	*Year*				
	1980	*1981*	*1982*	*1983*	*Total*
Hepatoma	31	38	52	5	126
Hepatocarcinoma	13	21	26	1	61
Carcinoma	13	10	12	2	37
Hepatocellular CA	3	6	14	1	24
Adenocarcinoma	2	6	8	2	18
Liver Cell Carcinoma	8	2	3	2	15
Malignant Cells	4	–	2		6
Sq. Cell Carcinoma	–	–	4	1	5
Fibrosarcoma	1	–	2		3
Liver Cancer		–	3	2	5
Hepatic Carcinoma	1	–	1	1	3
Carcinoma Sm. Cell	1	–	–	–	1
Metastatic Anaplastic CA	1	–	–	–	1
Metastatic Cell CA	–	1	–	–	1
Analplasticism Cell CA	–	1	–	–	1
Hepatic Cell Carcinoma	–	1	–	–	1
Islet Cell Tumor	–	1	–	–	1
Hepathocholangid CA	–	–	1	–	1
Epidermal Cancer	–	–	1	–	1
Angiosarcoma	–	–	–	1	1
Total	77	87	131	18	313

However, further investigation in one hospital in Cebu City showed that there were two cases of hepatoma and a single case of pathologically proven angiosarcoma of the liver, which was possibly work-related.

The first case of occupational liver angiosarcoma was discovered in the USA in 1961 in a plastic industry worker with 15 years exposure but the work-relatedness of angiosarcoma of the liver to plastic chemical exposure was not substantiated until 1974 by the work of Creech and Johnson.

Subsequently, cases from other parts of the world have been reported totalling now about 90 cases of occupational angiosarcoma of the liver.

Summary and Conclusion

a) Occupational health and safety is not given sufficient attention and priority in the plastic industry as evidenced by the less than adequate health services offered and the fair to poor conditions of the work environment in most of the establishments, as well as evidence that threshold limit value for vinyl chloride monomer in air has been exceeded in some instances.

b) Despite this situation however, the health of the workers at risk compared with a control group was not significantly worse based on the accident rate, morbidity and mortality records from 1981 to 1983, and the results of blood chemistry, blood counts, physical and medical examination, and x-ray of the lungs and hands. These findings suggest that there is still time to take preventive and ameliorative measures to avoid the known hazards of hazardous and toxic chemicals in the plastic industry, particularly vinyl chloride monomer.

c) There was no evidence of vinyl chloride disease such as sclerotic syndrome, acroosteolysis, thrombocytopenia, hepatosplenomegaly and abnormal hepatic function tests among workers in the production areas of plastic companies.

d) From a restrospective side study involving a review of the National Central Tumor Registry and a search made of the medical records of various hospitals in the country, a single case was found of possible occupational angiosarcoma of the liver.

e) In some of the companies especially those using VCM there was evidence of higher incidence of hepatitis and liver cancer than would be expected from the general population.

The problems of primary liver cancer risk assessment

One of the more interesting findings of this study has to do with the apparent increase in liver problems among workers in some plastic companies.

In Company X using VCM as raw material, the Company physician and nurse related to us that at one time in the past, there was a high incidence of liver disorders among workers as. evidenced by elevated blood SGOT enzyme levels. But when we asked for the records of these cases they could not produce them.

In another Company Y using styrene monomer as raw material, located nearby and down wind to Company X, the workers related to us that there was also a time when a large number of workers had complaints related to the liver, and they attributed this to air pollution coming from Company X.

In both cases, there was no record on which to base any conclusion and so these reports remain anecdotal. When we examined the blood enzyme levels of a

sample from the exposed and control groups of these two companies, the levels of SGOT and SGPT were within normal limits and not significantly different between experimental and control groups. Perhaps, had there been a good monitoring and recording system at the time there was the alleged high incidence of liver complaints among workers in these two companies, an epidemic of work-related liver disorders or infectious hepatitis would have been documented.

The problem of risk assessment becomes even more difficult when one deals with a complex condition such as primary liver cancer or hepatocellular carcinoma which is known to have several etiologic factors.

To illustrate this difficulty in risk assessment, take the case of a third large plastic Company Z using VCM as raw material. In this Company Z, during the 3-year period under study, there were 6 documented cases of hepatitis and 5 cases of primary liver cancer.

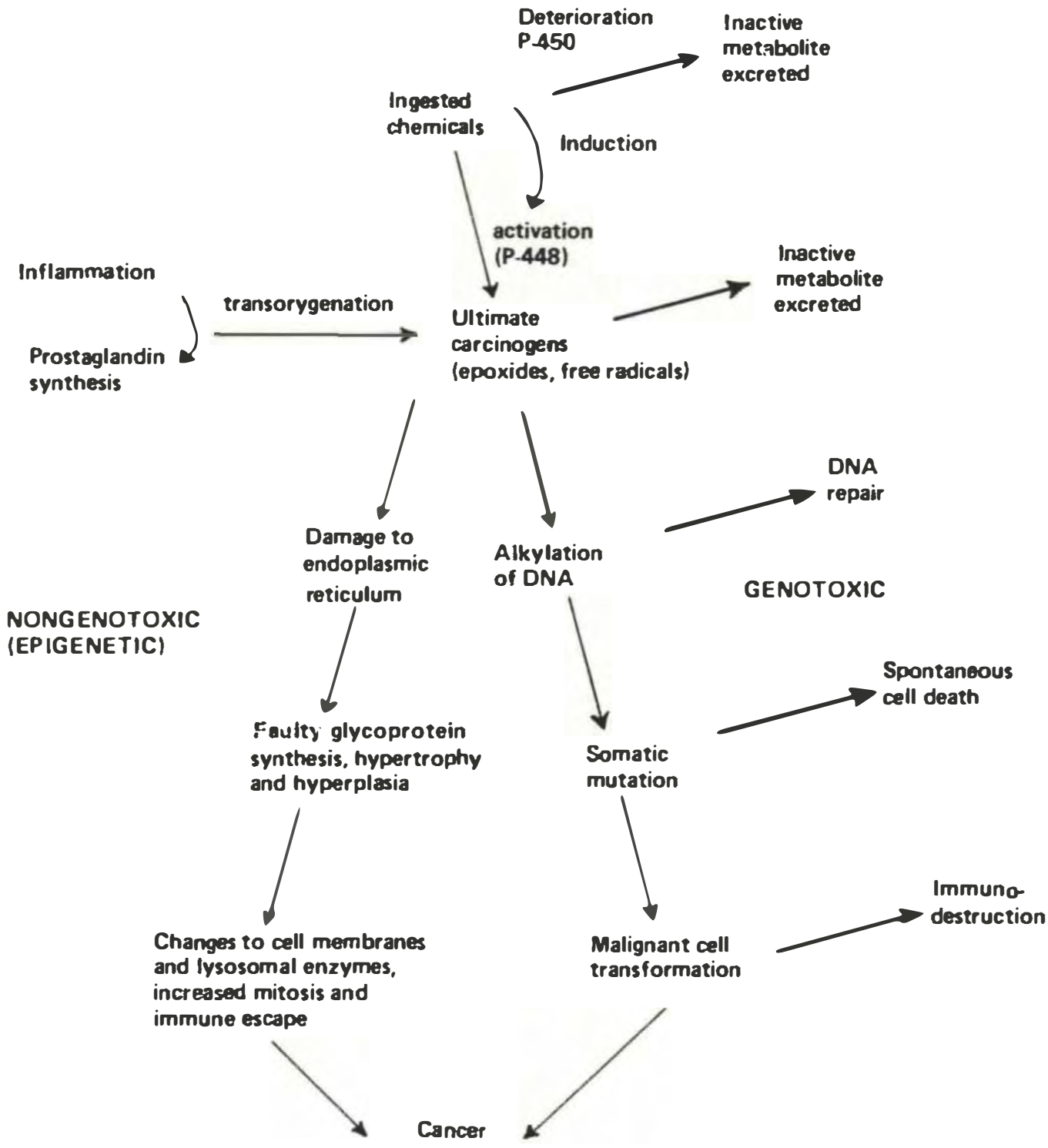
Going by the best estimates of the incidence of Hepatitis B and Hepatocellular Carcinoma coming from the Comprehensive Study of Primary Carcinoma of the Liver and other related Liver Diseases in the Philippines (Domingo, E. *et al.*, 1985) and other published estimates, the incidence of Hepatitis and Hepatocellular Carcinoma that we found in Company Z is way in excess of expectation as shown in Table 11.

At this point, in order to appreciate better the complexity of risk assessment in human cancer, I would like to present the current state of knowledge of the molecular mechanisms of chemical carcinogenesis as summarized by Dr. D.V. Parke in 1983. (Fig. 1 – Chemical carcinogenesis)

If we start at the point when the ultimate carcinogens impinges on the organism, we can see that *free radicals and active oxygen* as produced for instance by exposure to ionizing radiation are active initiating factors as are *chemical reactive intermediates,* such as *epoxides* of Benzo pyrene which can covalently bind to DNA and thus result in somatic mutations. According to recent theory, one hit at DNA is insufficient, and a major damage to DNA involving substantial transposition of genetic material and multiple invitations are required before malignant cell transformation can occur.

As shown at the top of the diagram, ingested potential chemical carcinogen may be *detoxified and inactivated* through the usual P-450 dependent microsomal mixed-function oxidase, *or activated* by Cytochrome P-448, if its stereochemistry allows sterically hindered oxygenation. For instance, pre-treatment of animals with phenobarbital leads to induction of cytochrome P-450 and the inactivation of the potential carcinogen benzopyrene to the ultimate carcinogen the *reactive epoxide form.* The sequence of events that lead to cancer may be through genetic damage (GENOTOXIC) or the so-called initiation phase, or through the promotion stage – which is often associated with non-genotoxic or epigenetic mechanisms, or with enzyme-inducing chemicals which promote the propagation of any malignant cell transformation through induction of hypertrophy and hyperplasia. The non-genotoxic mechanisms involve interaction and damage to subcellular structures such

Fig. 1. Molecular mechanism of chemical carcinogenesis*



*By D. V. Parke

Table 11. Incidence of hepatitis and hepatocellular carcinoma in Company Z compared with estimates of incidence rates published in the literature

Condition	*Incidence Rate*	*Investigators*	*Expected Number for 419 employees of Company Z*	*Adj. 3 yr.*		
				Actual	*Exp.*	*Act./Exp.*
Hepatitis B	70/100,000/yr.	Domingo *et al.*, 1985	0.29/yr.	6	.87	6.9x
Hepatocellular Carcinoma	40/100,000/yr.	Domingo *et al.*, 1985	0.17/yr.	5	.51	9.8x
Primary Liver Cancer Among Males	20/100,000/yr.	Bulatao-Jayme *et al.*. 1982	0.084/yr.	5	.25	20x

as endoplasmic reticulum mitochondria and cysosomes. Thus, malignant cell transformation results from an extensive generalized damage of the cell and not just from the alkylation of DNA.

Various chemicals and factors may enhance or diminish carcinogenesis by affecting any or a combination of the molecular mechanisms as shown by the processes to the right of the dark arrows on the figure.

Keeping this figure in mind, we can now examine some of the etiologic factors which may be operative in the primary liver cancer among workers in the plastic industry in Philippine setting.

1) *VCM exposure*

It is well accepted that chronic exposure to high levels of VCM, say at 5-10 ppm for 10-15 years, is associated with increased risk to liver disorders, including primary liver cancer. The occurrence of a specific type of malignancy, namely Angiosarcoma of the Liver, is considered almost pathognomonic of VCM exposure. The spot sampling of air for VCM showed that the Threshold Value Limit was exceeded in some places in this Company.

From this study, the only documented pathologically diagnosed care of Angiosarcoma of the Liver, slides of which I had presented earlier, was in a mason worker from Pardo, Cebu. Although he worked with plastic compounds as a mason, he was never an employee in a VCM-using plastic Company. The other case of Angiosarcoma of the Liver was included in the Central Tumor Registry of Metro Manila Hospitals, but we could not trace the medical records of the case to get further specific information on the case.

The molecular mechanisms of carcinogenesis by vinyl chloride is still unknown but it can be both at the genotoxic or non-genotoxic stages.

2) *Hepatitis B*

According to Dr. C.W. Chan of Hongkong, the majority of hepatocellular carcinomas in Southeast Asian countries are now considered causally related to the prevalence of Hepatitis B virus in the region. The comprehensive Liver Study estimates the prevalence of Hepatitis B in Filipinos at 8.7% of the population, one of the highest in the world. The 6 cases of Hepatitis over 3 years in Company Z is 7x more than the expected number in the general population.

The molecular mechanism of hepatocarcinogenesis by Hepatitis B Virus infection is still unknown but liver hepatoma is one of the cancers now considered to be etiologically associated with a virus infection.

3) *Aflatoxin*

A series of reports from Bulatao-Jayme, J. *et al.* (1978-1982) has established the probable contribution of dietary load of the mycotoxin-aflatoxin in the increased risk of developing primary liver cancer. Using the odds ratio as an estimate of relative risk, she has estimated that the very heavy aflatoxin dietary load of 7 mcg or higher per day increased the risk of developing Primary Liver Cancer 17 times as shown in Table 12. (Bulatao-Jayme, *et al.* 1982)

Table 12. Dose-response relationship in terms of relative risk of developing PLC by category of overall mean aflatoxin load

Category of Overall Mean Aflatoxin Load	*Number of subjects*		*Relative risk*[b] *(Odds Ratio)*
	Cases (90)[a]	*Controls (90)*[a]	
Light (0 – 3 mcg)	20	74	1.0
Mod. Heavy (4 – 6 mcg)	15	4	13.9*
Very Heavy (7 mcg & over)	55	12	17.0*

( ) a = number of subjects
* = significant
b = Relative Risk/Odds Ratio $= \frac{\text{Cases}_H \times \text{Controls}_L}{\text{Cases}_L \times \text{Controls}_H}$

Together with nitrosoamines, aflatoxin constitute the two major carcinogens that contaminate human foodstuff particularly in the tropical regions of the world. Improvement in agricultural and storage practices can greatly reduce the levels of contamination from levels measured in parts per million to lower levels measured in parts per billion with a commensurate reduction in cancer risk.

Our export of copra meal to Europe is now being threatened because our aflatoxin levels in copra may not meet the stricter standards being contemplated by the European community.

4) *Alcohol*

Bulatao-Jayme in the same paper cited above, found that the combination of aflatoxin and alcohol intake produced a strong synergistic carcinogenic effect to as high as 35x with Heavy Aflatoxin and Heavy Alcohol Intake compared to Light Aflatoxin and Light Alcohol Intake as shown in Table 13.

Table 13. Relative risk of developing PLC from the combined effects of aflatoxin load and alcohol intake

Category of aflatoxin load and alcohol intake	*Relative risk*
Light Aflatoxin, Light Alcohol	1.0
Light Aflatoxin, Heavy Alcohol	3.9*
Heavy Aflatoxin, Light Alcohol	17.5*
Heavy Aflatoxin, Heavy Alcohol	35.0*

* = significant

Alcoholic beverages contain nitrosamines mycotoxins and other known carcinogens in significant amounts. Additionally alcohol has been shown to inhibit prosta-glandin activity and mucus production in the GI Tract and may thus promote carcinogenesis.

Other factors like cigarette smoking and nutrition may also play significant roles in the development of liver cancer in the Philippine setting.

Nicotine and other components of tobacco smoke resulting in the depletion of intracellular glutathione resulting in the opening of tight junctions of epithelial cells, thus carcinogens may penetrate to the sensitive basal cell layer.

Malnutrition and protein deficiency results in inadequate synthesis of detoxifying enzyme systems and cellular defense mechanisms dependent on glutathione.

With all four etiologic factors apparently operative, how much can one attribute to any or a combination of any of these factors. Are the factors when present in a given case simply additive or do they interact in a synergistic way.

Given the complexity of human disease and the multiplicity of factors that determine the tendency or risk of getting it ranging from the genetic or constitutional factor to the myriad environmental influences, risk assessment of human disease conditions will always remain problematic.

Although risk analysis and risk benefit analysis using probabilistic Risk Assessment have been proven useful in many diverse fields of application such as nuclear power plant accidents, dam operation accidents (Elizabeth-Pate, 1983) or even in land use planning (Popper 1983) its use in multi-factorial risk assessment such as in Primary Liver Cancer appears a long way off, if at all it is possible.

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1.	Dr. Eulalia L. Venzon	—	Assistant Project Leader; Co-researcher
2.	Dr. Francisco R. Jose	—	Consultant
3.	Lucila C. Sumalinog	—	Research Assistant
4.	Yolanda C. Paras	—	Research Assistant
5.	Alexandra J. Fojas	—	Research Assistant
6.	Ingrid R. Llave	—	Research Assistant

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AN ATTEMPT TO ERADICATE ASCARIASIS AND HOOKWORM INFECTION IN AN ISLAND IN SORSOGON, PHILIPPINES
(A Progress Report)

B.D. Cabrera, M. Lorenzo, W. Abrantes, C. Ortiz
and T. Go
Institute of Public Health
University of the Philippines,
Pedro Gil, Ermita, Manila

ABSTRACT

Being an isolated island with only 900 population living in 147 houses we have attempted to eradicate rather than control ascariasis and hookworm infections. The baseline data for ascaris, trichuris and hookworm prevalence rates were 78, 81 and 42 percent respectively. The protozoan infection prevalence rates were rather low with striking absence of *E. histolytica* and very low prevalence of *G. lamblia*. *E. coli* was only 18%. Multiple infection however was quite frequent. Pyrantel pamoate and at times Oxantel-pyrantel were given every 4 months for 3 years at 5-10 mg/K body wt. single dose, followed by 2 months after treatment with stool examination. Blanket treatment was used so as to reduce both the intensity of infection and the number of eggs in the feces, resulting in the reduction of rate of transmission. From 231 children we collected 2,429 ascaris worms giving a worm load of 10.5 worms per subject and a sex ratio of 1.7 females for every one male worm. In addition to drug treatment, health education, personal hygiene and construction of water-scaled toilet in each house were applied in the barangay.

Introduction

Several attempts have been made to control some of the soil-transmitted helminthiases among inhabitants of villages that are adjacent to each other. (1-8) In these adjacent villages, the population movement cannot be controlled, hence both local inhabitants of one village and people coming from adjacent villages contribute in the indiscriminate pollution of the soil with human feces. It is for this reason that the term "control" appears to be safest and most appropriate word to use.

Sablayan island is located within Sorsogon bay and it takes an hour and 15 minutes by motorized banca to reach the place from the town of Castilla, Sorsogon Province (Fig. 1). The entire island is about seven kilometers long and two kilometers wide. The barrio or barangay of Sablayan consists of five sitios or hamlets, namely: Buri, Intombahan, Babatngon, Bucana and Batang. However, in

this report, sitio Batang has been excluded inasmuch as it is actually located outside of Sablayan island per se (Figs. 2 & 3). There are approximately 872 people living at present in this island with fishing as their principal livelihood. There is of course, limited amount of farming such as coconut, bananas and taro plantation.

Our census revealed that there are 147 houses in the entire island and of this number only 30 houses (20.4 percent) have toilet facilities. The source of water supply comes from eight drilled wells and from springs coming from the elevated portions of the island. There is one school house where majority of school age children go for schooling up to grade six.

Materials and Methods

Inasmuch as the aim is eradication rather than control, we have to apply a combination of methods such as: drug treatment, health education and environmental sanitation with potable water supply.

Preliminary meetings were held with the provincial health officer and his staff the rural health physician and her health educator, filariasis control unit and staff, Juban town Mayor, Sablayan barangay captain and his councilors. During these meetings, we discussed the purpose of the program, the benefits that will be derived from the project, and the role that local officials and each individual person have to play to arrive at the objective of the project. The target dates for the start of census, stool collections and treatment of the entire population were also discussed.

Sealable plastic bags, 4 1/2 x 3 1/2 inches in size with a wooden applicator inside, were distributed to each household. All members were instructed to collect stool sample about a "thumb size" with the use of the applicator. The specimen was then placed inside the bag together with the stick. The bags were then sealed and the I.D. number, age and sex of each subject were written on the outside of the plastic bag. Residents of the barrio proper dropped their specimens inside a basket hanging in some strategic places and later collected by our technicians. In the four sitios, the specimens were collected by the councilor of each respective sitio then submitted to our technicians stationed at our temporary laboratory.

A portion of the stool specimen was used to prepare smears for Kato technique and some for Kato-Katz. The rest of the specimen was emulsified thoroughly in a screw-cap vial containing ten percent formalin for concentration technique. All specimens were then packed and later shipped to the College of Public Health in Manila where all the necessary laboratory examinations were performed. The Kato technique was done to determine prevalence rates while the Kato-Katz technique was used to determine intensity of infection prior to the start of the treatment. The prevalence rate of helminthic and protozoan infections were determined by the formalin-ether concentration technique.

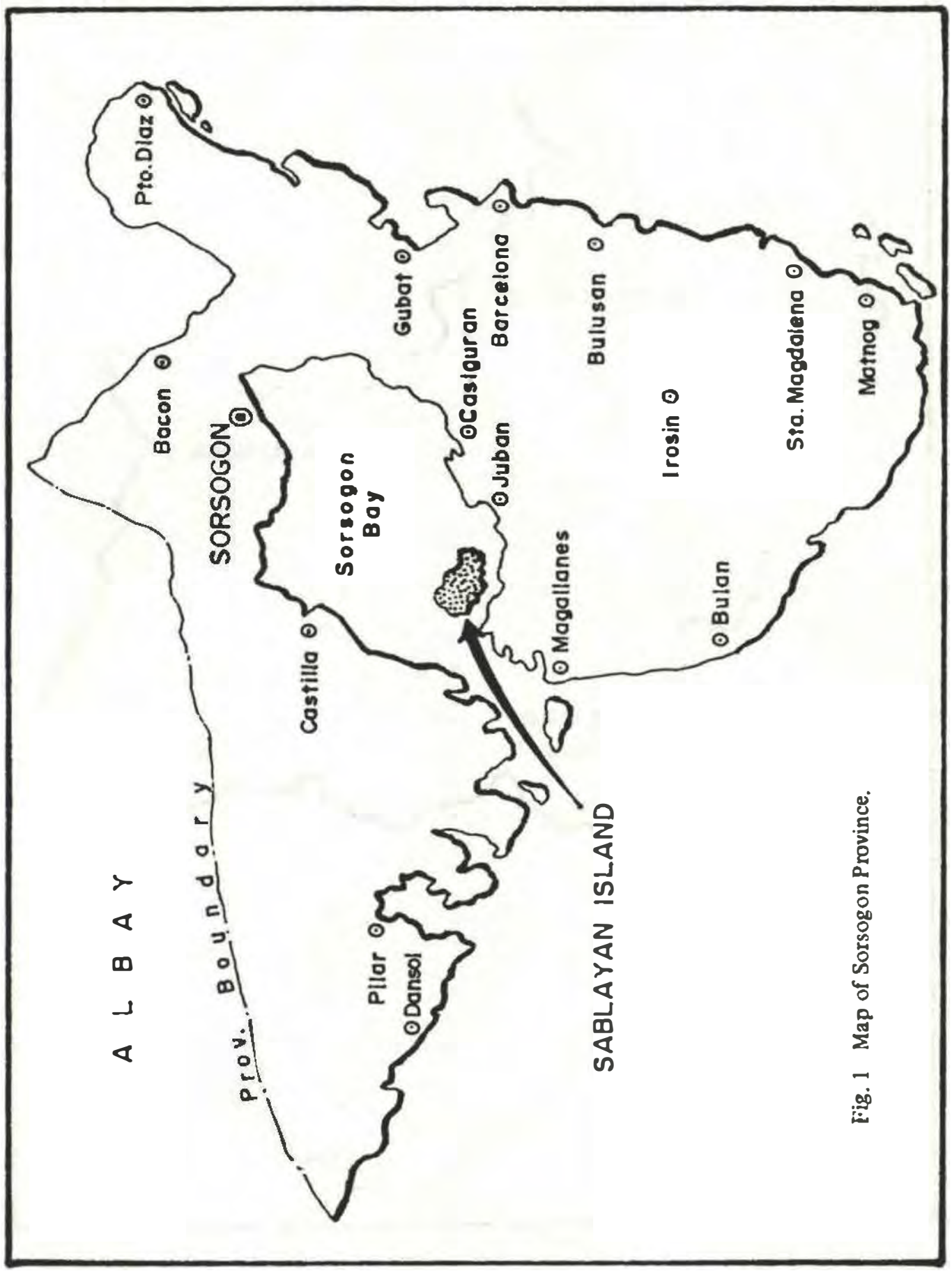

Fig. 1 Map of Sorsogon Province.

Fig. 2. Map of Sablayan Island.

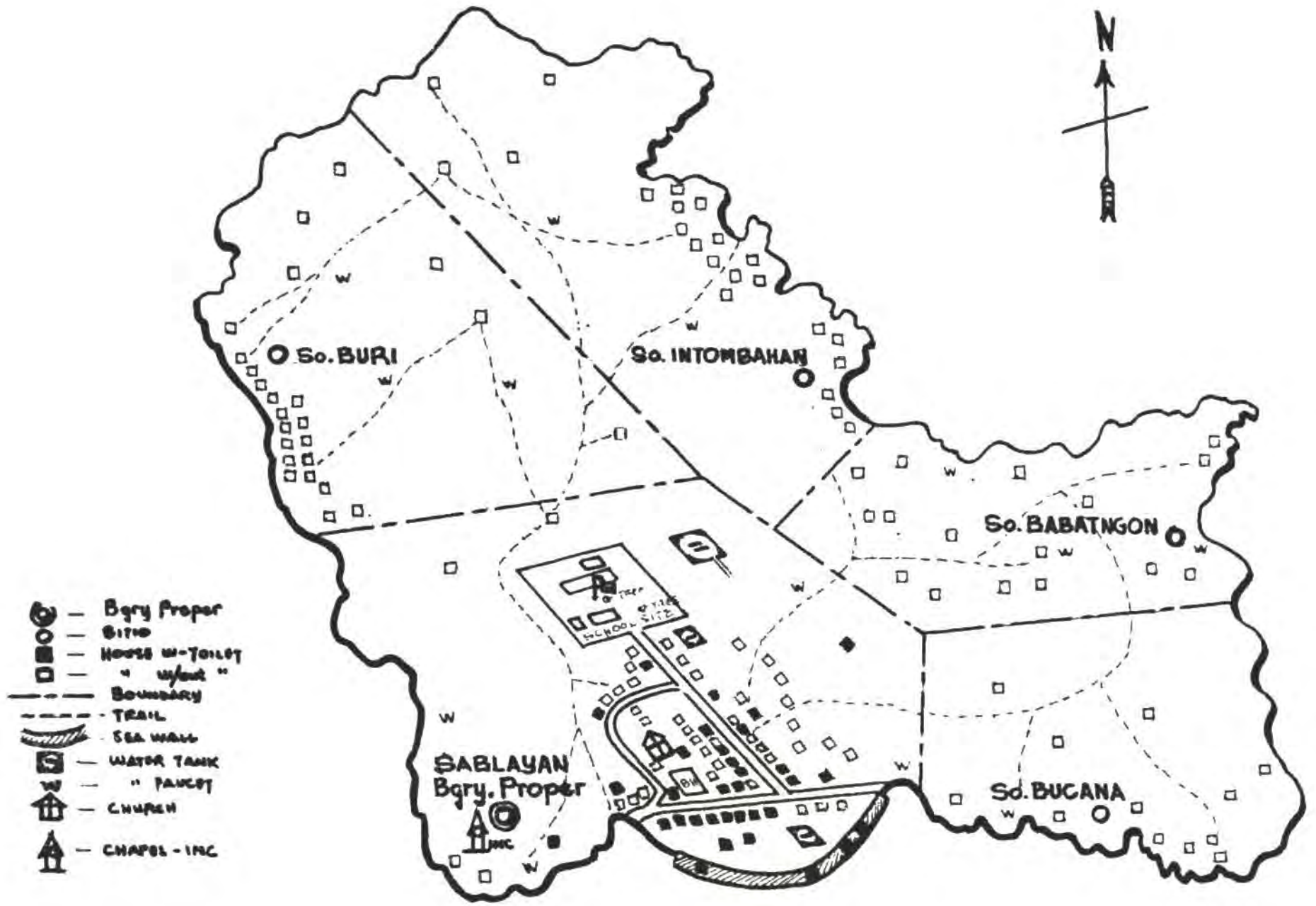

Fig. 3 Spot map of Barrio Sablayan.

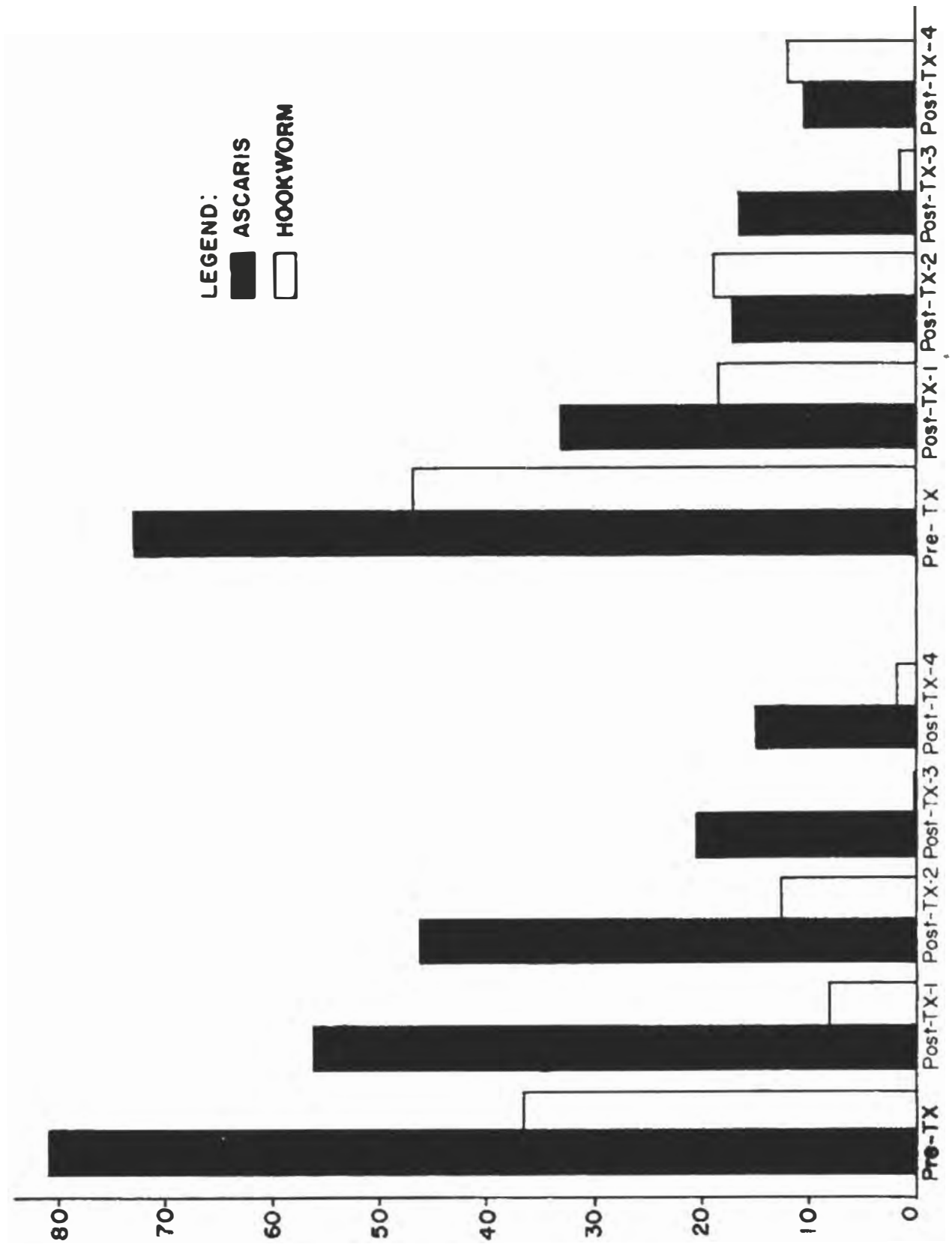

Fig. 4. Pre-treatment prevalence as compared to post-treatment prevalences of ascaris and hookworm infections, by age, Sablayan Island, Sorsogon, Philippines 1985-1987.

In addition to the stool collection, a number of children were weighed prior to the administration of the drug and will be weighed again at an appropriate time. Inasmuch as our aim is eradication rather than mere control of ascaris and hookworm infections, we have applied "blanket" treatment. Hence, all residents of Sablayan island were given anthelmintic treatment irrespective of the results of stool examination. Blanket treatment therefore, will reduce the worm burden or intensity of infection and at the same time reduce the number of eggs discharged with the feces which are deposited in soil indiscriminately. These two factors in turn will indirectly reduce the rate of transmission in the entire population by the elimination of the sources of eggs.

The drugs used are pyrantel pamoate (combantrin) and Oxantel-pyrantel pamoate (Quantrel), using the emulsion for the children and the tablets for the treatment of adults. The treatment schedule was given every four months or 3 times a year for 3 years while the post-treatment stool follow-up was done two months after each treatment. During the first treatment, the subjects were instructed to save the first two bowel movements following the taking of the drug. They were to defecate directly into large plastic bags distributed right after the drug administration. The plastic bags containing the specimen were collected. The ascaris worms were counted separating the males from the females, measured and weighed and the degree of maturity or development were determined.

In the meantime a series of meetings were conducted on health education, life cycles of the worms and method of infection and spread. On the environmental sanitation aspect, our sanitary engineer has taught the people how to build the water sealed toilets. The Department of Health thru my request has donated 150 toilet bowls enough to supply one toilet per household in the entire island.

Results

Out of the 872 individuals residing in Sablayan island, only 569 or 65 percent submitted stool samples for our baseline data. The pre-treatment prevalence of the soil-transmitted helminthiases among males is shown in Table 1, while among females, it is seen in Table 2. Table 3 shows the prevalence of soil-transmitted helminthiases of both sexes by age. The pretreatment mean egg count for ascaris among 90 children using Kato-Katz technique was 21,815.31 egg per gram (EPG), while that of trichuris was 2,039.25 EPG. Egg counts for hookworm was not done due to prolonged shipping time. The pre-treatment prevalence among children and adults by sex is shown in Table 4, while the distribution of patients according to the type of parasitic infection is shown in Table 5. The pre-treatment prevalence of protozoan infection is shown in Table 6.

The first post-treatment prevalence of ascaris and hookworm infections among males, among females and for both sexes are shown in Tables 7, 8 and 9 respectively, while the data on the expelled ascaris worms are shown in Table 10.

Table 1. Pre-treatment prevalence of soil-transmitted helminthiases among females, by age, Sablayan, 1985

Age group	*Total exam.*	*Ascariasis*		*Trichuriasis*		*Hookworm infection*	
		No. (+)	*% (+)*	*No. (+)*	*% (+)*	*No. (+)*	*% (+)*
0–4	48.0	37.0	77.1	37.0	77.1	7.0	14.6
5–9	78.0	62.0	79.5	68.0	87.2	40.0	51.3
10–14	46.0	41.0	89.1	34.0	73.9	29.0	63.0
15–19	15.0	10.0	66.7	7.0	46.7	5.0	33.3
20–29	17.0	12.0	70.6	13.0	76.5	7.0	41.2
30–39	25.0	17.0	68.0	19.0	76.0	10.0	40.0
40–49	31.0	25.0	80.6	25.0	80.6	19.0	61.3
50–59	20.0	15.0	75.0	16.0	80.0	13.0	65.0
60–69	6.0	2.0	33.3	5.0	83.3	4.0	66.7
70 +	2.0	1.0	50.0	1.0	50.0	2.0	100.0
TOTAL	288.0	222.0	77.1	225.0	78.1	136.0	47.2

Table 2. Pre-treatment prevalence of soil-transmitted helminthiases among females, by age, Sablayan, 1985

Age group	*Total exam.*	*Ascariasis*		*Trichuriasis*		*Hookworm infection*	
		No. (+)	*% (+)*	*No. (+)*	*% (+)*	*No. (+)*	*% (+)*
0–4	43.0	36.0	83.7	29.0	67.4	8.0	18.6
5–9	74.0	62.0	83.8	66.0	89.2	24.0	32.4
10–14	34.0	24.0	70.6	32.0	94.1	12.0	35.3
15–19	14.0	11.0	78.6	10.0	71.4	5.0	35.7
20–29	31.0	24.0	77.4	30.0	96.8	12.0	38.7
30–39	27.0	23.0	85.2	24.0	88.9	13.0	48.1
40–49	28.0	20.0	71.4	22.0	78.6	11.0	39.3
50–59	12.0	10.0	83.3	8.0	66.7	7.0	58.3
60–69	12.0	7.0	58.3	10.0	83.3	6.0	50.0
70+	6.0	4.0	66.7	3.0	50.0	3.0	50.0
TOTAL	281.0	221.0	78.6	234.0	83.3	101.0	35.9--

The second post-treatment prevalence of ascaris and hookworm infections among males, females and for both sexes are shown in Tables 11, 12 and 13.

The third post-treatment prevalence of these two nematode infections among males, females and for both sexes are shown in Tables 14, 15 and 16.

The fourth post-treatment prevalence of ascaris and hookworm infections among males, females and for both sexes are shown in Tables 17, 18 and 19.

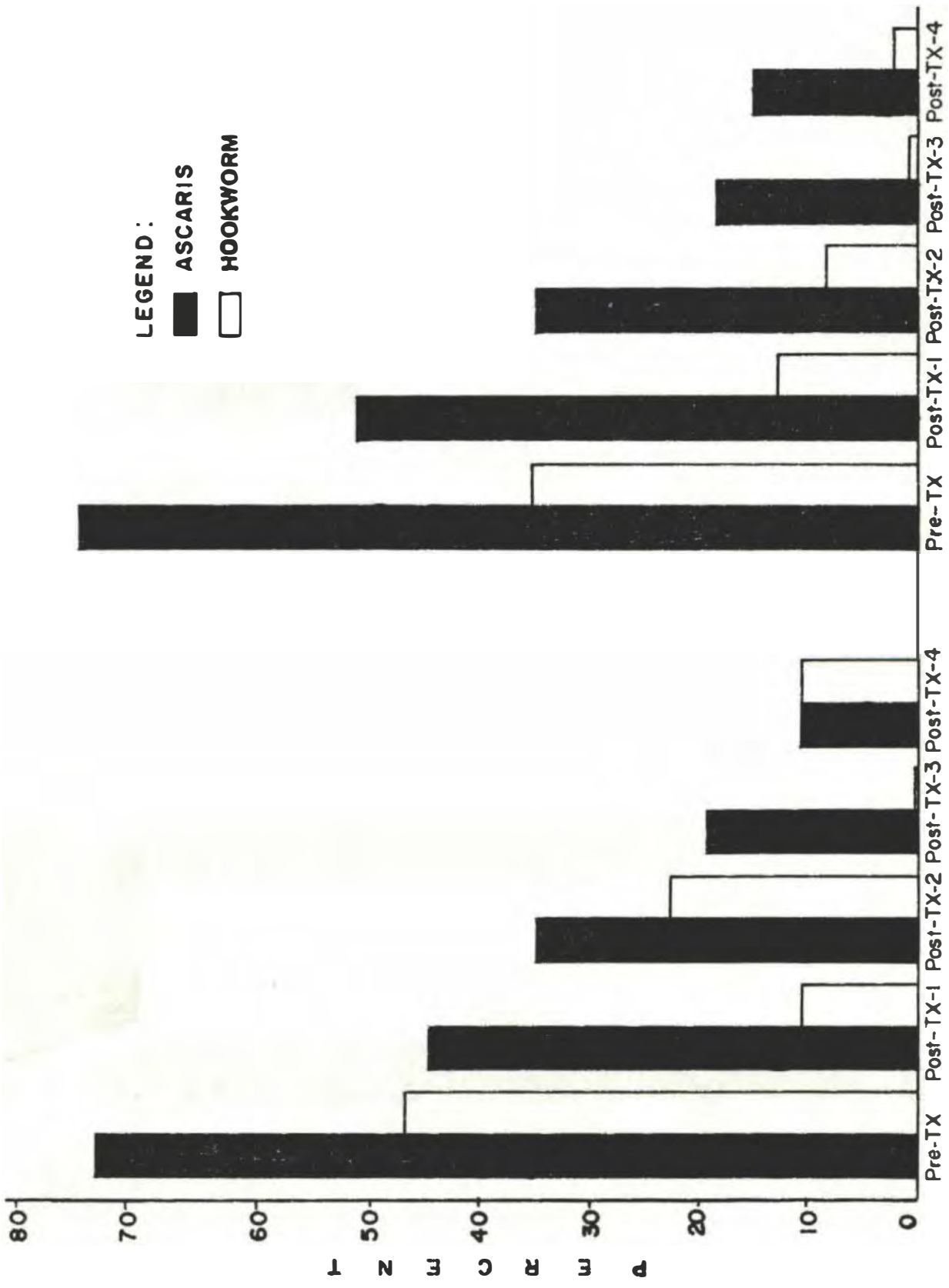

Fig. 5. Pre-treatment prevalence as compared to post-treatment prevalences of ascaris and hookworm infections by sex, Sablayan Island, Sorsogon, Philippines 1985-1987.

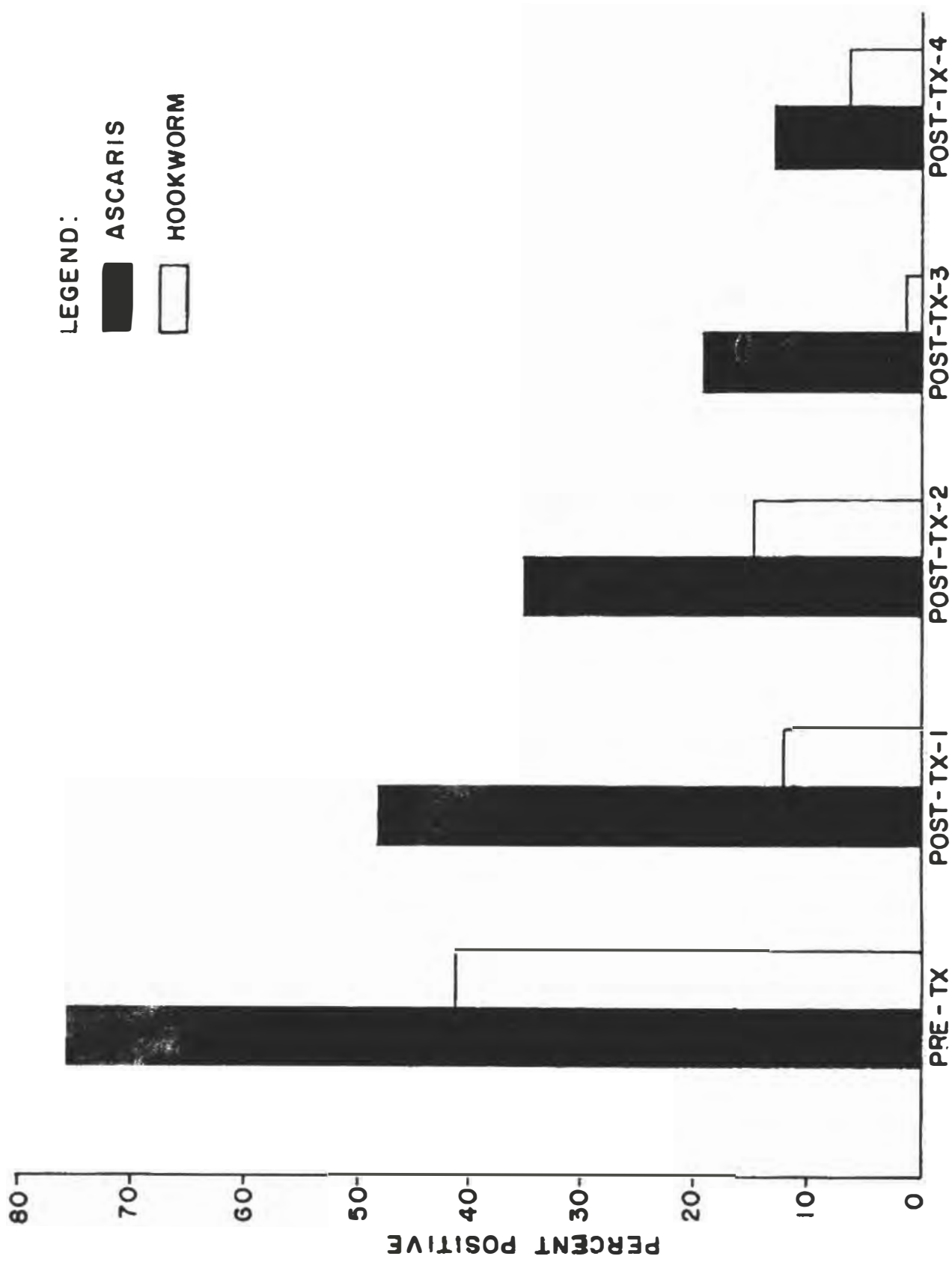

Fig. 6. Pre-treatment and post-treatment prevalences of ascaris and hookworm infections. Sablayan Island, Sorsogon, Philippines 1985-1987.

Table 3. Pre-treatment prevalence of soil-transmitted helminthiases of both sexes by age, Sablayan, 1985

Age group	*Total exam.*	*Ascariasis*		*Trichuriasis*		*Hookworm infection*	
		no. (+)	*% (+)*	*no. (+)*	*% (+)*	*no. (+)*	*% (+)*
0–4							
0–4	91	73.0	80.2	66.0	72.5	15.0	16.5
5–9	152.0	124.0	81.6	134.0	88.2	64.0	42.1
10–14	80.0	65.0	81.3	66.0	82.5	41.0	51.3
15–19	29.0	21.0	72.4	17.0	58.6	10.0	34.5
20–29	48.0	36.0	75.0	43.0	89.6	19.0	39.6
30–39	52.0	40.0	76.9	43.0	82.7	23.0	44.2
40–49	59.0	45.0	76.3	47.0	79.7	30.0	50.8
50–59	32.0	25.0	78.1	24.0	75.0	20.0	62.5
60–69	18.0	9.0	50.0	15.0	83.3	10.0	55.6
70 +	8.0	5.0	62.5	4.0	50.0	5.0	62.5
TOTAL	569.0	443.0	77.9	459.0	80.7	237.0	41.7

Table 4. Pre-treatment prevalence of soil-transmitted helminthiases among children and adults by sex, Sablayan, 1985

Age	*Sex*	*No. exam*	*Ascariasis*		*Trichuriasis*		*Hookworm Infection*	
			no. (+)	*% (+)*	*no. (+)*	*% (+)*	*no. (+)*	*% (+)*
	Male	172.0	140.0	81.4	139.0	80.8	76.0	44.2
0-14	Female	151.0	122.0	80.8	127 0	84.1	44.0	29.1
	Total	323.0	262.0	81.1	266.0	82.4	120 0	37.2
	Male	116.0	82.0	70.7	86.0	74.1	60.0	51.7
15 +	Female	130.0	99.0	76.2	107.0	82.3	57.0	43.8
	Total	246.0	181.0	73.6	193.0	78.5	117.0	47.6
All	Male	288.0	222.0	77.1	225.0	78.1	136.0	47.2
Ages	Female	281.0	221 0	78.6	234.0	83.3	101.0	35.9
	Total	569.0	443.0	77.9	459.0	80.7	237.0	41.7

A comparison between the pre-treatment prevalence and the post-treatment prevalences of ascaris and hookworm infections by age is shown in Table 20 and Fig. 4. A similar table by sex is shown in Table 21 and Fig. 5.

A comparison between the total pre-treatment prevalence and total post-treatment prevalences of ascaris and hookworm infections is shown in Table 20 and Fig. 6.

Table 5. Distribution of patients according to type of infection Sablayan Island, 1985

Type of infection	*No. of patients*	*Percent*
Ascaris alone	42.0	8.0
Trichuris alone	58.0	11.0
Hookworm alone	8.0	1.5
Ascaris and Trichuris	191.0	36.3
Ascaris and Hookworm	19.0	3.6
Trichuris and Hookworm	23.0	4.4
Ascaris, Trichuris and Hookworm	185.0	35.2
Total Positives	526.0	100.0
Total Negatives	43.0	7.5
TOTAL	569.0	.0

Table 6. Prevalence of protozoan infection by age and sex, Sablayan, 1985

	No. exam	*E. coli*		*E. nana*		*G. lamblia*	
Children 0-14		*(+)*	*%*	*+*	*%*	*+*	*%*
Males	172.0	22.0	12.8	5 0	2.9	2.0	1.2
Females	151.0	25.0	16.6	2.0	1.3	.0	.0
Both sexes	323.0	47.0	14.6	7.0	2.2	2.0	.6
Adults 15 +							
Males	116.0	25.0	21.6	7.0	6.0	.0	.0
Females	130.0	29.0	22.3	5.0	3.8	.0	.0
Both sexes	246.0	54.0	22.0	12.0	4.9	.0	.0
All ages							
Males	288.0	42.0	14.6	12.0	4.2	2.0	.7
Females	281.0	54.0	19.2	7.0	2.5	.0	.0
Both sexes	569.0	96.0	16.9	19.0	3.3	2.0	.4

Discussion

In Table 1, among male children, ascariasis prevalence was highest among the 10-14 years age group followed by the 5-9 age group. It was lowest among the 0-4 age group. In trichuriasis however, the prevalence was highest among the 5-9 years age group followed by the 0-4 years age and lowest among the 10-14 years age

Table 7. First post-treatment prevalence of ascaris and hookworm infections among males by age, Sablayan, 1986

Age group	*No. exam*	*Ascaris*		*Hookworm*	
		No. (+)	*% (+)*	*No. (+)*	*% (+)*
0-6	42.0	23.0	54.8	2.0	4.8
7-14	23.0	11.0	47.8	3.0	13.0
15 +	29.0	8.0	27.6	5.0	17.2
Total	94.0	42.0	44.7	10.0	10.6

Table 8. First post-treatment prevalence of ascaris and hookworm infections among females by age, Sablayan, 1986

Age group	*No. exam*	*Ascaris*		*Hookworm*	
		no. (+)	*% (+)*	*No. (+)*	*% (+)*
0-6	38.0	29.0	76.3	4.0	10.5
7-14	39.0	17.0	43.6	3.0	7.7
15 +	45.0	17.0	37.8	9.0	20.0
Total	122.0	63.0	51.6	16.0	13.1

Table 9. First post-treatment prevalence of ascaris and hookworm infections of both sexes by age, Sablayan, 1986

Age group	*No. exam*	*Ascaris*		*Hookworm*	
		no. (+)	*% (+)*	*No. (+)*	*% (+)*
0-6	80.0	52.0	65.0	6.0	7.5
7-14	62.0	28.0	45.2	6.0	9.7
15 +	74.0	25.0	33.8	14.0	18.9
Total	216.0	105.0	48.6	26.0	12.0

group. In the case of hookworm infection, as expected, the prevalence was highest among older children 10-14 years followed by the age group 5-9 years and the lowest being in the age group 0-4 years. Among the adult males, the highest prevalence was among the age group 40-49 years for both ascariasis and trichuriasis. This was followed by age group 50-59 years and lowest among age group 60-69 years for ascariasis. The next highest in trichuriasis was in the age group 50-59 while the lowest was in the age group 15-19 years. For hookworm infection, the

Table 10. Length and weight of 2429 ascaris worms collected from 231 treated children, Sablayan Island, 1985-86

Stage of development	*No. of worms*	*Mean length (cm)*	*Mean weight gm*
Mature			
Male (11-27.1 cm) [15-31cm]	873	21.5	2.6
Female (13-36.8 cm) [20-35 cm]	1434	24.04	3.8
Immature			
Male (< 10 cm) *f*			
Male (< 10 cm) [< 15 cm]	43	8.27	.39
Female (< 12 cm) [20 cm]	79	8.68	.43
TOTAL	2429	0	0

( ) = according to the present study
[ ] = according to Craig & Faust's Clinical Parasitology, 8th edition

highest prevalence was among male adults 50 years and over, followed by age group 40-49 years and lowest of the age group 15-19 years.

In Table 2 among female children, ascariasis was highest in both age groups 0-4 and 5-9 years and lowest in the age group 10-14 years. In trichuriasis, the age group 10-14 years had the highest prevalence rate followed by the age group 5-9 years. The lowest prevalence was found among the age group 0-4 years. In hookworm infection the highest prevalence came from the age group 10-14 years followed by the age group 5-9 years. The age group 0-4 years had the lowest prevalence rate.

It appears that ascariasis prevalence was higher among female children than among males in age groups 0-4 and 5-9 years. However, in the age group 10-14 years, male children had higher prevalence rate than females. Trichuriasis prevalence was higher among female children age groups 5-9 and 10-14 years than among males. However, male children in age group 0-4 years had higher prevalence than females. In hookworm infection, the prevalence rates among males, age groups 5-9 and 10-14 years were higher than among females. However, female children have slightly higher prevalence rate than males in the age group 0-4 years.

Among adult females the highest ascariasis prevalence rate came from the age group 30-39 years followed by the age group 50-59 years. The lowest prevalence was among age group 60-69 years. In trichuriasis, the age group 20-29 years had the highest prevalence rate followed by the age group 30-39 years. The age group 50-59 had the lowest prevalence rate. In hookworm infection, the highest prevalence was among the age group 50-59 years followed by age group 30-39 years. The lowest prevalence was among the age group 15-19 years.

It appears that ascariasis prevalence was consistently higher among adult females than among males. In general, the trichuriasis prevalence was higher also among females than males. In hookworm, the prevalence rate among males appear to have a tendency to be higher than among females.

In Table 3, among children of both sexes, ascariasis and trichuriasis were highest in the 5-9 years age group and lowest in 0-4 age group. In hookworm infection the age group 10-14 years had the highest prevalence rate and lowest among the 0-4 years age group. Among adults, ascariasis was highest among the 50-59 years age group and lowest at age group 60-69 years. In trichuriasis the prevalence rate was highest among the 20-29 years age group and lowest at 15-19 years. In hookworm infection, the highest prevalence rate was among the age group 50-59 years while the lowest was among the age group 15-19 years.

In Table 4, the pre-treatment prevalence rate among children showed that in ascariasis the rates among males and females are equal. Among adults, females had higher prevalence rate than males. Children had slightly higher prevalence than adults. In trichuriasis, females of both age groups had higher prevalence than males and children had slightly higher prevalence rate than adults. In hookworm infection, both male children and male adults had higher prevalence than females; and adults have higher prevalence than children.

In Table 5, the most common type of infections are a combination of ascaris and trichuris and combined ascaris, trichuris and hookworm. The least common was hookworm alone.

The prevalence of protozoan infection before treatment was started, was determined using the formalin-ether concentration technique (Table 6). The unexpected negative finding of *Entamoeba histolytica* cysts was rather striking. *Entamoeba coli* cysts were found in 17.8 percent while *Endolimax nana* cysts had 3.3 percent prevalence. *Giardia lamblia* cysts had only 0.4 percent prevalence. Females appear to have slightly higher prevalence rate for *E. coli* than males, and adults have higher prevalence for *E. coli* and *E. nana* than children. In *G. lamblia,* male children are the only group found harboring this protozoa.

Due to the unavailability in the market of anthelmintics with high efficacy against trichuriasis, we have decided to exclude this parasite temporarily in this particular project.

In the first post-treatment prevalence rate among males (Table 7) the prevalence rate for ascariasis among male children was 52.3 percent as compared to the 27.6 percent among adult males. In hookworm infection, the prevalence rate among

Table 11. Second post-treatment prevalence of ascaris and hookworm infections among males by age, Sablayan, 1986

Age group	*No. exam*	*Ascaris*		*Hookworm*	
		no. (+)	*% (+)*	*no. (+)*	*% (+)*
0-6	26.0	10.0	38.5	3.0	11.5
7-14	27.0	11.0	40.7	7.0	25.9
15 +	26.0	7.0	26.9	8.0	30.8
Total	79.0	28.0	35.4	18.0	22.8

male children was 7.7 percent as compared to 17.2 percent among adult males. The total prevalence among male subjects for ascaris and hookworm infections were 44.7 and 10.6 percent, respectively.

Among female children (Table 8) the ascariasis prevalence rate was 59.7 percent as compared to 37.8 percent among adult females. For hookworm infection, the prevalence rate for female children was 9.1 percent as compared to 20.0 percent for adults. The total prevalence among female subjects for ascaris and hookworm infections were 51.6 and 13.1 percent, respectively.

In the prevalence rates for both sexes (Table 9), ascariasis prevalence among children was 56.3 percent as compared to 33.8 percent among adults. In hookworm infection children had 8.4 percent prevalence rate as compared to 18.9 percent among adults. The total prevalence rates for ascaris and hookworm infections were 48.6 and 12.0 percent, respectively.

The usual classification of mature and immature ascaris worms is based mostly on the length and weight, however, we have used other criteria in addition to those suggested by Craig and Faust (Table 10). Our lower limit for mature and immature worms for both sexes are lower than those of Craig and Faust. Then, again, our criteria for mature and immature worms were not only based on the length and weight of the worms but also on the dissection for mature eggs and sperms of the worms. A total of 2429 ascaris worms were expelled from 231 children or an average worm load of 10.5 per child. There were 1513 females (62.3 percent) and 916 (37.7 percent) males or a sex ratio of 1.7 females for every one male. Among the female worms, 1434 (95 percent) were mature and 79 (5 percent) were immature; among the male worms 873 (95 percent) were mature and 43 (5 percent) were immature. This indicates that the rate of maturation or development of both sexes are the same. The mean length of mature and immature females were 24.04 cm. and 8.68 cm., respectively while the mean length of mature an immature males were 21.5 cm. and 8.27 cm., respectively. The mean weight of mature females were 3.8 gm. and 0.43 gm. respectively, while mature and immature males were 2.6 gm. and 0.39 gm., respectively.

In the second post-treatment prevalence rate among males (Table 11), the prevalence rate for ascariasis among male children was 39.6 percent as compared to 26.9 percent among adults males. In hookworm infection, the prevalence rate among male children was 18.9 percent as compared to 30.8 percent among male adults The total prevalence among male subjects for ascaris and hookworm infections were 35.4 and 22 8 percent, respectively.

Among female childr·n (Table 12). the ascariasis prevalence rate was 54.3 percent as compared to 11.1 percent among adult females. For hookworm infection, the prevalence rate for female children was 6.5 percent as compared to 11.1 percent for adult females. The total prevalence among females for ascaris and hookworm infections were 35.4 and 8.5 percent, respectively.

In the prevalence rate for both sexes (Table 13), ascariasis prevalence among children was 46.5 percent as compared to 17.7 percent among adults. In hookworm infection, children had a prevalence rate of 13.1 percent as compared to 19.4 percent among adults. The total prevalence rates for ascaris and hookworm infections were 35.4 and 15.5 percent, respectively.

Table 12. Second post-treatment prevalence of ascaris and hookworm infections among females by age, Sablayan, 1986

Age group	*No. exam*	*Ascaris*		*Hookworm*	
		no. (+)	*% (+)*	*no. (+)*	*% (+)*
0-6	23.0	17.0	73.9	.0	.0
7-14	23.0	8.0	34.8	3.0	13.0
15 +	36.0	4.0	11.1	4.0	11.1
Total	82.0	29.0	35.4	7.0	8.5

Table 13. Second post-treatment prevalence of ascaris and hookworm infections of both sexes by age, Sablayan, 1986

Age group	*No. exam*	*Ascaris*		*Hookworm*	
		no. (+)	*% (+)*	*no. (+)*	*% (+)*
0-6	49.0	27.0	55.1	3.0	6.1
7-14	50.0	19.0	38.0	10.0	20.0
15 +	62.0	11.0	17.7	12.0	19.4
Total	161.0	57.0	35.4	25.0	15.5

In the third post-treatment prevalence rate among males (Table 14), the prevalence rate for ascariasis among male children was 24.5 percent as compared to 12.4 percent among adult males. In hookworm infection, the prevalence rate among male children and adults were both negative. The total prevalence among male subjects for ascaris and hookworm infections were 19.7 and 0.0 percent, respectively.

Among female children (Table 15) the ascariasis prevalence rate was 17.1 percent as compared to 20.6 percent among adult females. For hookworm infection, the prevalence rate for female children was zero percent as compared to 2.8 percent for adult females. The total prevalence among females for ascaris and hookworm infections were 18.6 and 1.2 percent, respectively.

In the prevalence rates for both sexes (Table 19) ascaris prevalence among children was 15.5 percent as compared to 10.4 percent among adults. In hookworm infection, children had a prevalence rate of 0.0 percent as compared to 1.5 percent among adults. The total prevalence rates for ascaris and hookworm infections were 19.1 and 0.6 percent, respectively.

Table 14. Third post-treatment prevalence of ascaris and hookworm infections among males by age, Sablayan, 1986

Age group	*No. exam*	*Ascaris*		*Hookworm*	
		no. (+)	*% (+)*	*no. (+)*	*% (+)*
0-6	75.0	22.0	29.3	.0	.0
7-14	72.0	14.0	19.4	.0	.0
15 +	97.0	12.0	12.4	.0	.0
Total	244.0	48.0	19.7	.0	.0

Table 15. Third post-treatment prevalence of ascaris and hookworm infections among females by age, Sablayan, 1986

Age group	*No. exam*	*Ascaris*		*Hookworm*	
		no. (+)	*% (+)*	*no. (+)*	*% (+)*
0-6	56.0	11.0	19.6	.0	.0
7-14	84.0	13.0	15.5	.0	.0
15 +	107.0	22.0	20.6	3.0	2.8
Total	247.0	46.0	18.6	3.0	1.2

In the fourth post-treatment prevalence rate among males (Table 17), the prevalence rate for ascariasis among male children was 15.2 percent as compared to 4.1 percent among adult males. In hookworm infection, the prevalence rate among male children was 3.2 percent as compared to 24.7 percent among male adults. The total prevalence among male subjects for ascaris and hookworm infections were both 11.1 percent.

Among female children (Table 18) the ascariasis prevalence rate was 15.9 percent as compared to 15.4 percent among adult females. For hookworm infection, the prevalence rate for female children was 0.9 percent as compared to 4.4 percent for adult females. The total prevalence among females for ascaris and hookworm infections were 15.7 and 2.4 percent, respectively.

In the prevalence rates for both sexes (Table 19) ascaris prevalence among children was 15.5 percent as compared to 10.4 percent among adults. In hookworm infection, children had a prevalence rate 2.1 percent as compared to 13.4 percent among adults. The total prevalence rates for ascaris and hookworm infections were 13.4 and 6.7 percent, respectively.

The comparison between the pre-treatment prevalence and the post-treatment prevalences including percent decrease of the two parasitic infections by age is shown in Table 20 and Fig. 4. It appears that the percent decrease in post-treatment prevalence in ascariasis among adults was slightly better than among the children. The reverse is true when it comes to hookworm infection. The percent decrease in the post-treatment prevalences was slightly better among children than among adults. In the total prevalences, the percent decrease in hookworm infection was higher than that of ascariasis.

A similar table to Table 20, by sex, is seen in Table 21 and Fig. 5. It appears that in ascariasis the percent decrease in males is slightly better than in females. In hookworm infection the percent decrease among females is slightly better than among males. In the total prevalences, the percent decrease, in hookworm infection was better than that of ascariasis. The total prevalences of pre and post-treatment showed a gradual but consistent decrease in prevalence of both ascaris and hookworm infections, (Fig. 6).

Table 16. Third post-treatment prevalence of ascaris and hookworm infections of both sexes by age, Sablayan, 1986

Age group	*No. exam*	*Ascaris*		*Hookworm*	
		no. (+)	*% (+)*	*no. (+)*	*% (+)*
0-6	131.0	33.0	25.2	.0	.0
7-14	156.0	27.0	17.3	.0	.0
15 +	204.0	34.0	16.7	3.0	1.5
Total	491.0	94.0	19.1	3.0	.6

In the fourth post-treatment prevalence rate among males (Table 17), the prevalence rate for ascariasis among male children was 15.2 percent as compared to 4.1 percent among adult males. In hookworm infection, the prevalence rate among male children was 3.2 percent as compared to 24.7 percent among male adults. The total prevalence among male subjects for ascaris and hookworm infections were both 11.1 percent.

Among female children (Table 18) the ascariasis prevalence rate was 15.9 percent as compared to 15.4 percent among adult females. For hookworm infection, the prevalence rate for female children was 0.9 percent as compared to 4.4 percent for adult females. The total prevalence among females for ascaris and hookworm infections were 15.7 and 2.4 percent, respectively.

In the prevalence rates for both sexes (Table 19) ascaris prevalence among children was 15.5 percent as compared to 10.4 percent among adults. In hookworm infection, children had a prevalence rate 2.1 percent as compared to 13.4 percent among adults. The total prevalence rates for ascaris and hookworm infections were 13.4 and 6.7 percent, respectively.

The comparison between the pre-treatment prevalence and the post-treatment prevalences including percent decrease of the two parasitic infections by age is shown in Table 20 and Fig. 4. It appears that the percent decrease in post-treatment prevalence in ascariasis among adults was slightly better than among the children. The reverse is true when it comes to hookworm infection. The percent decrease in the post-treatment prevalences was slightly better among children than among adults. In the total prevalences, the percent decrease in hookworm infection was higher than that of ascariasis.

A similar table to Table 20, by sex, is seen in Table 21 and Fig. 5. It appears that in ascariasis the percent decrease in males is slightly better than in females. In hookworm infection the percent decrease among females is slightly better than among males. In the total prevalences, the percent decrease, in hookworm infection was better than that of ascariasis. The total prevalences of pre and post-treatment showed a gradual but consistent decrease in prevalence of both ascaris and hookworm infections, (Fig. 6).

Table 16. Third post-treatment prevalence of ascaris and hookworm infections of both sexes by age, Sablayan, 1986

Age group	*No. exam*	*Ascaris*		*Hookworm*	
		no. (+)	*% (+)*	*no. (+)*	*% (+)*
0-6	131.0	33.0	25.2	.0	.0
7-14	156.0	27.0	17.3	.0	.0
15 +	204.0	34.0	16.7	3.0	1.5
Total	491.0	94.0	19.1	3.0	.6

Table 17. Fourth post-treatment prevalence of ascaris and hookworm infections among males by age, Sablayan, 1987

Age group	*No. exam*	*Ascaris*		*Hookworm*	
		no. (+)	*% (+)*	*no. (+)*	*% (+)*
0-6	59.0	9.0	15.3	1.0	1.7
7-14	66.0	10.0	15.2	3.0	4.5
15 +	73.0	3.0	4.1	18.0	24.7
Total	198.0	22.0	11.1	22.0	11.1

Table 18. Fourth post-treatment prevalence of ascaris and hookworm infections among females by age, Sablayan, 1987

Age group	*No. exam*	*Ascaris*		*Hookworm*	
		no. (+)	*% (+)*	*no. (+)*	*% (+)*
0-6	49.0	12.0	24.5	.0	.0
7-14	64.0	6.0	9.4	1.0	1.6
15 +	91.0	14.0	15.4	4.0	4.4
Total	204.0	32.0	15.7	5.0	2.5

Table 19. Fourth post-treatment prevalence of ascaris and hookworm infections of both sexes by age Sablayan, 1987

Age group	*No. exam*	*Ascaris*		*Hookworm*	
		no. (+)	*% (+)*	*no. (+)*	*% (+)*
0-6	108.0	21.0	19.4	1.0	.9
7-14	130.0	16.0	12.3	4.0	3.1
15 +	164.0	17.0	10.4	22.0	13.4
Total	402.0	54.0	13.4	27.0	6.7

Table 20. Pre-treatment prevalence compared to post-treatment prevalence and (% decrease) of ascaris and hookworm infections, by age, Sablayan Island. Sorsogon, 1956-1987

Age group	*ASCARIS PREVALENCE*					*HOOKWORM PREVALENCE*				
	Pre-Tx	*1st Post-Tx*	*2nd Post*	*3rd Post-Tx*	*4th Post-Tx*	*Pre-Tx*	*1st Post-Tx*	*2nd Post*	*3rd Post-Tx*	*4th Post-Tx*
Children	81.1	56.3 (30.6)	46.5 (42.7)	20.9 (74.2)	15.5 (80.9)	37.2	8.5 (77.4)	13.1 (64.8)	0 (100.0)	2.1 (9.4)
Adults	73.6	33.8 (54.1)	17.7 (76.1)	16.7 (77.3)	10.4 (85.9)	47.6	18.9 (60.3)	19.4 (59.2)	1.5 (96.8)	13.4 (71.8)
Total	77.9	48.6 (37.6)	35.4 (54.6)	19.1 (75.5)	13.4 (82.8)	41.7	12 (71.2)	15.5 (62.8)	.61 (98.5)	6.7 (83.9

Table 21. Pre-treatment prevalence compared to post-treatment prevalences and (% decrease) of ascaris and hookworm infections, by sex, Sablayan Island, Sorsogon, 1956-1987

Age group	*ASCARIS PREVALENCE*					*HOOKWORM PREVALENCE*				
	Pre-Tx	*1st Post-Tx*	*2nd Post*	*3rd Post-Tx*	*4th Post-Tx*	*Pre-Tx*	*1st Post-Tx*	*2nd Post*	*3rd Post-Tx*	*4th Post-Tx*
Males	77.1	44.7 (42.0)	35.4 (54.1)	19.7 (74.4)	11.1 (85.6)	47.2	10.6 (77.5)	22.8 (51.7)	0 (100.0)	11.1 (76.5)
Females	78.6	(51.6 (34.4)	35.4 (55.0)	18.6 (76.3)	15.7 (80.0)	35.9	13.1 (63.5)	8.5 (76.3)	1.2 (96.7)	2.5 (93.0)
Total	77.9	48.6 (37.6)	35.4 (54.6)	19.1 (75.5)	13.4 (82.8)	41.7	12 (71.2)	15.5 (62.8)	.61 (98.5)	6.7 (83.9)

Summary

An attempt to eradicate ascaris and hookworm infections in an isolated island, in Sorsogon Province, with a population of less than nine hundred is hereby presented. There are 147 houses in the entire island but only 30 or 20.4 percent have toilet facilities. Initial stool survey of the population using Kato, Kato-Katz and FECT were done prior to blanket treatment. Pyrantel pamoate and Oxantel-pyrantel, given 3x a year for 3 years and stool collection and examination were done two months after each treatment schedule.

Pre-treatment prevalence of ascaris, trichuris and hookworm infections were 77.9, 80.7 and 41.7 percent, respectively. Multiple parasitism with these parasites was quite common. The prevalence rates of these soil-transmitted helminthiases among children were slightly higher than among adults except in hookworm infection.

Blanket treatment of the entire population was applied so as to reduce both the intensity of infection and the number of eggs in the feces. When such feces is deposited in soil, fewer helminths eggs develop, thus the rate of transmission is reduced. Partial report of soil examination for ascaris eggs revealed that six out of ten (60 percent) of soil samples were found positive.

A total of 2,429 ascaris worms were collected from 231 children or a worm burden of 10.5 worms per subject. The sex ratio was 1.7 females for every male worm.

Health education, personal hygiene and environmental sanitation were also introduced together with the technology of water-sealed toilet construction in each household.

At the end of each treatment schedule and stool follow-up, a gradual but consistent reduction in prevalence of both ascaris and hookworm infections resulted. The percent decrease in hookworm infection was better than that of ascariasis.

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