TRANSACTIONS of the NATIONAL ACADEMY of SCIENCE and TECHNOLOGY

Vol. 11, 1989

TRANSACTIONS of the NATIONAL ACADEMY of SCIENCE and TECHNOLOGY

VOL. II, 1980

National Academy of Science and Technology

The National Academy of Science and Technology or NAST was created by virtue of Presidential Decree 1003-A dated December 17, 1976. It was not until July 1978 that it was organized formally.

GOALS. The Academy aims to implement the government's policy of promoting scientific research and invention in the country; provide meaningful incentive to those engaged in scientific and technological work; recommend to the President for recognition worthy achievements in the scientific field and help professionalize government scientific and technological services.

MEMBERSHIP. PD 1003-A defined scientist "as an individual who has earned a doctoral degree in any field of the sciences in our accredited university and has demonstrated and earned distinction in independent research or significant innovative achievement in the basic and applied sciences. This includes agricultural, engineering and medical sciences, mathematics and social sciences as manifested by his published works in recognized scientific and technical journals. However, in highly meritorious and extremely exceptional cases the foregoing doctoral degree requirement maybe waived.

To this day, there are twenty-seven members, although the decree provides that the total membership of the Academy shall not exceed fifty at any one time.

Unless otherwise terminated for a cause or by voluntary resignation, membership in the Academy is by nomination and approval by majority members of the Academy with the NAST en banc election.

PRIVILEGES. Members of the Academy shall carry the title of "Academician." They are provided other benefits and privileges:

* Publication of scientific and technological works at the expense of the Academy.

* Travel support for attendance and participation in international conferences and such other incentives, financial or otherwise, designed to promote scientific and technological effort and achievements.

NATIONAL SCIENTISTS. The Academy recommends from among its members "annually for Presidential award not more than ten scientists for distinguished individual or collaborative achievement in science and for technology who shall be accorded by the President the rank and title of "National Scientist."

The named "National Scientists" shall each be given a gratuity in such amount to be fixed by the Academy and shall be entitled to other privileges as are enjoyed by the "National Artists." The NAST has the option to recommend or not at all the award for any given year.

Republic of the Philippines
National Academy of Science and Technology

FOREWORD

All publications including reference books and scientific and technological journals have all their useful places. The Second Annual Presentation of Papers of the National Academy of Science and Technology held July 1980 is part of what we accomplished. We believe that in order to reach out further relationships, we will be fulfilling a pretty important part, if we publish the proceedings in this form we call "Transactions".

In these pages are the latest knowledge and up-dated information prepared by renowned author-scientists, whose outlook are purely scientific and cultural.

Through the Transactions, therefore, the Academy intends to share the work of our scientists with the world. This intent and purpose pervade the "Transactions" and makes it a specially meritorious scientific publication.

We hope that familiarization with these works will add meaning to our scientific tasks, in the same way that it will inspire our friends in the scientific community. It should also broaden and deepen the dedication which is the hallmark of any true scientist.

PAULO C. CAMPOS
President

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POPULATION GROWTH AND HUMAN PROGRESS

By Paulo C. Campos, M.D., Academician

It is obvious that the population in the world is in a period of explosive growth, geometric in pattern (Figure 1). Whereas, it took a millenium before the earth could have the first billion population; the second billion took only a hundred years; the third bil-

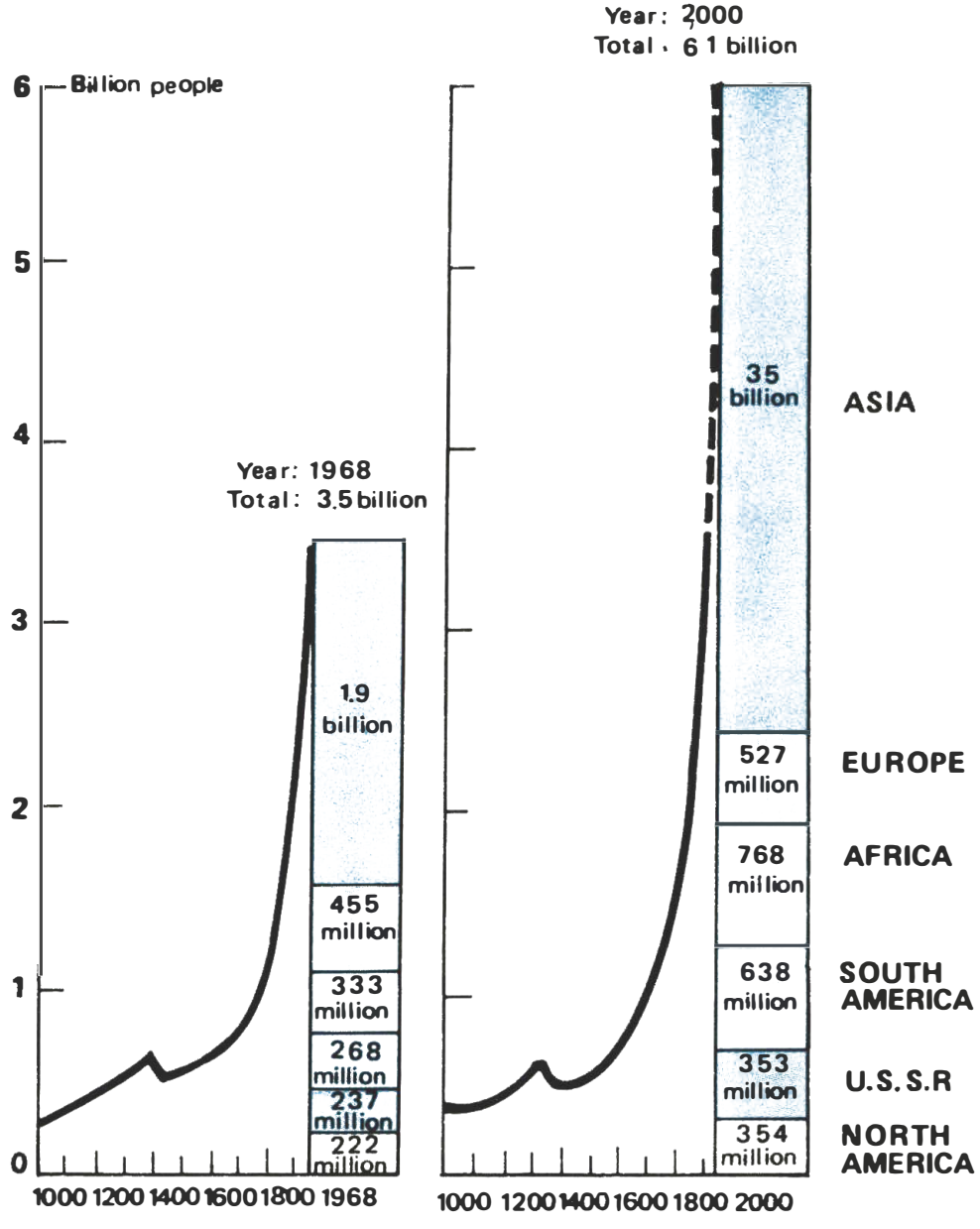

Figure 1. Population by world areas A.D. 1000-2000.

*Excerpt of address delivered by Dr. Paulo C. Campos, President of the National Academy of Science & Technology during the 2nd annual presentation of papers held on July 15, 1980 at the Philippine International Convention Center.

lion, 50 years; the 4th billion, 15 years; the 5th billion, only 9 years; the 6th million will only take 5 years, etc. (Figures 2 and 3). So one begins to wonder and worry about the future as the available food and resources on earth become scarce. At this juncture, we have to fall back on our observations on living systems we encounter in nature.

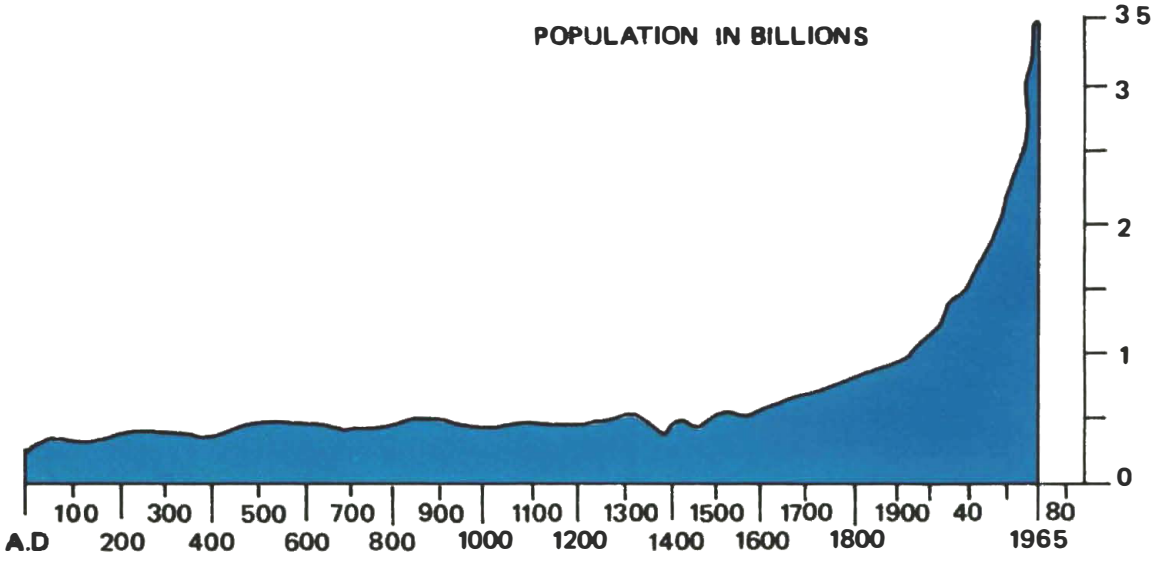

Figure 2. World population estimated A.D. 0-1965.

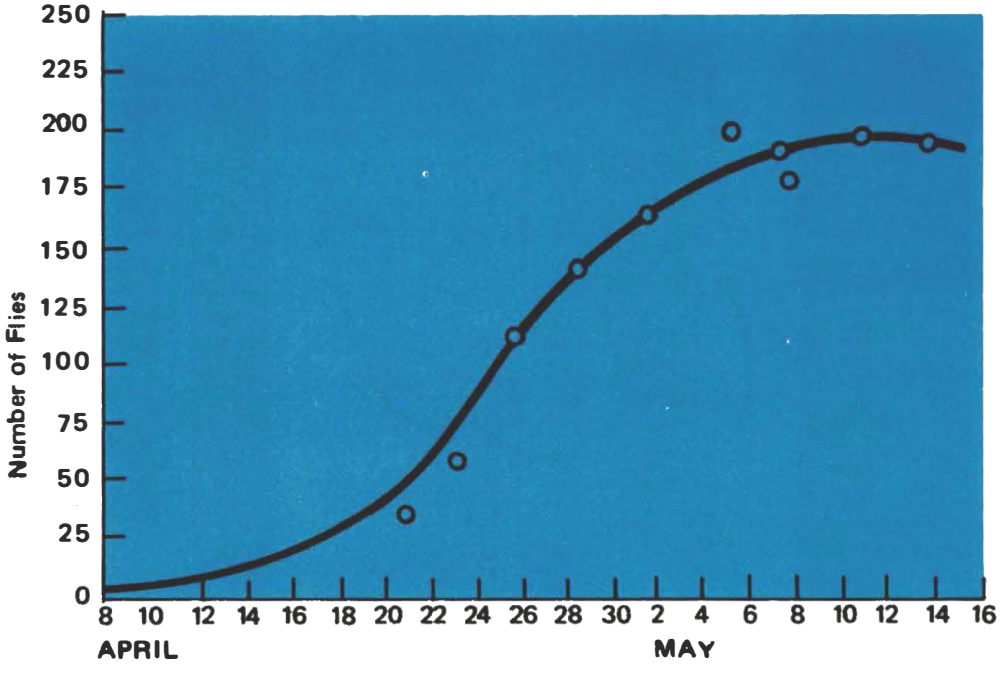

Figure 3. Growth of fruit-fly population.

A valid model is the growth curve of the fruitfly (Figure 4). It starts with a slow growth rate, then slowly accelerates into a more explosive growth rate. However, at some point, the growth rate starts to decelerate until it levels off in a plateau. In other words, it is an S-shape or sigmoid curve.

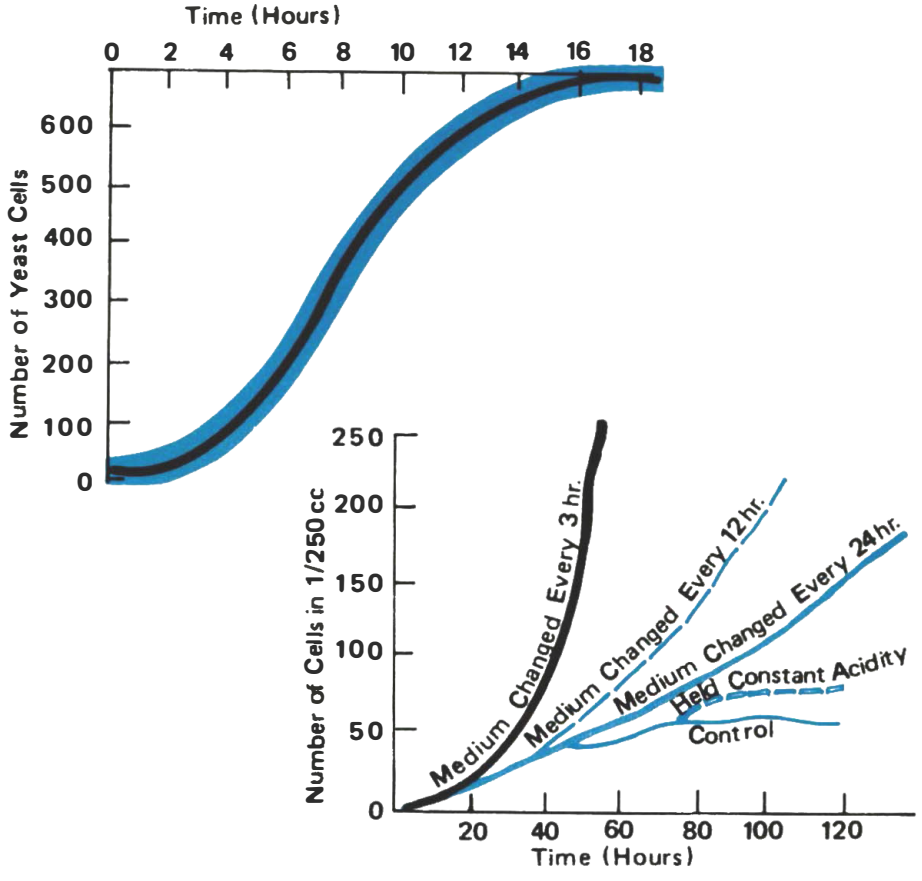

Figure 4. The upper graph depicts the growth curve of yeast cell grown in a laboratory culture and refers to the table immediately above. The lower graph shows the growth curves of yeast cells grown under varying environmental conditions.

This is the same observation in other living systems like the yeast (Figure 5). It has further been demonstrated that the growth of yeast cells in culture can be altered by changing the ph and the frequency with which the medium is changed.

Antibody responses follow a sigmoid curve but can be modified with the introduction of reinforcing substances (Figures 6 and 7).

The growth curve of the sheep population in Australia followed an initial sigmoid pattern followed by an equilitratory state (Figure 8).

If we are to project a possible trajectory for the human population growth curve, the sigmoid or "S" curve is one strong possibility.

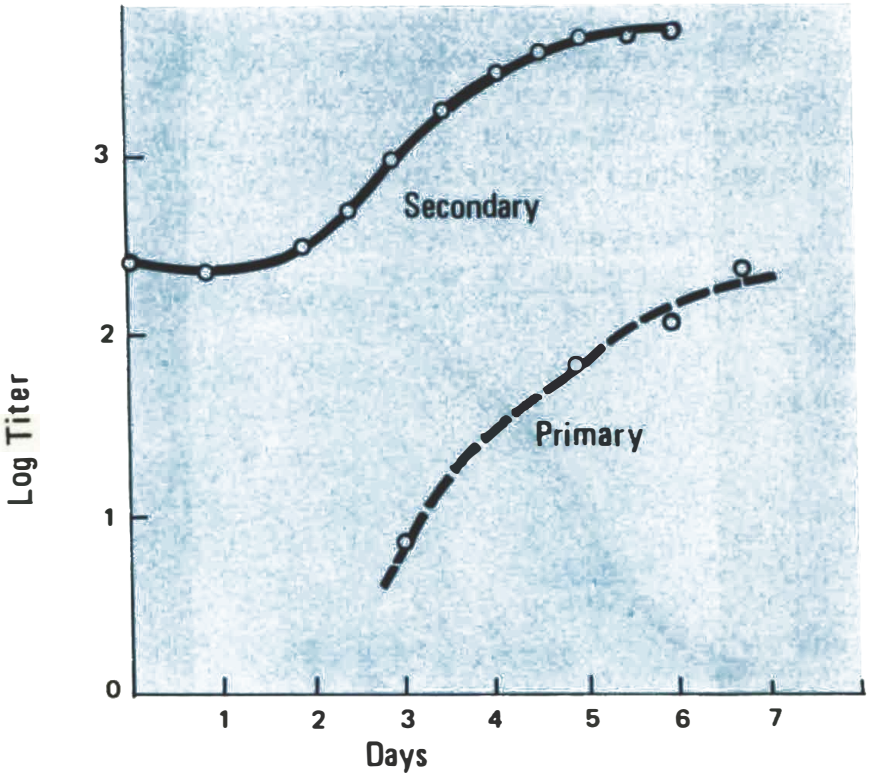

Figure 5. The primary and secondary antibody responses of two rabbits to intravenous injections of a "vaccine".

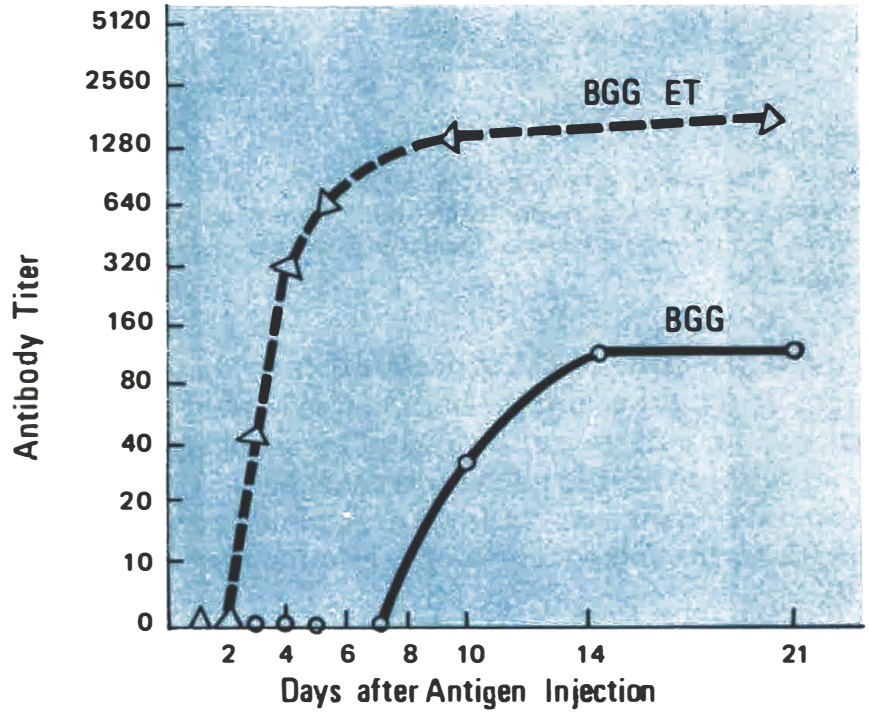

Figure 6. The primary and secondary antibody response in mice injected with bovine gemma globulin (BGG) with and without the reinforcing effect of another substance, endotoxin (ET).

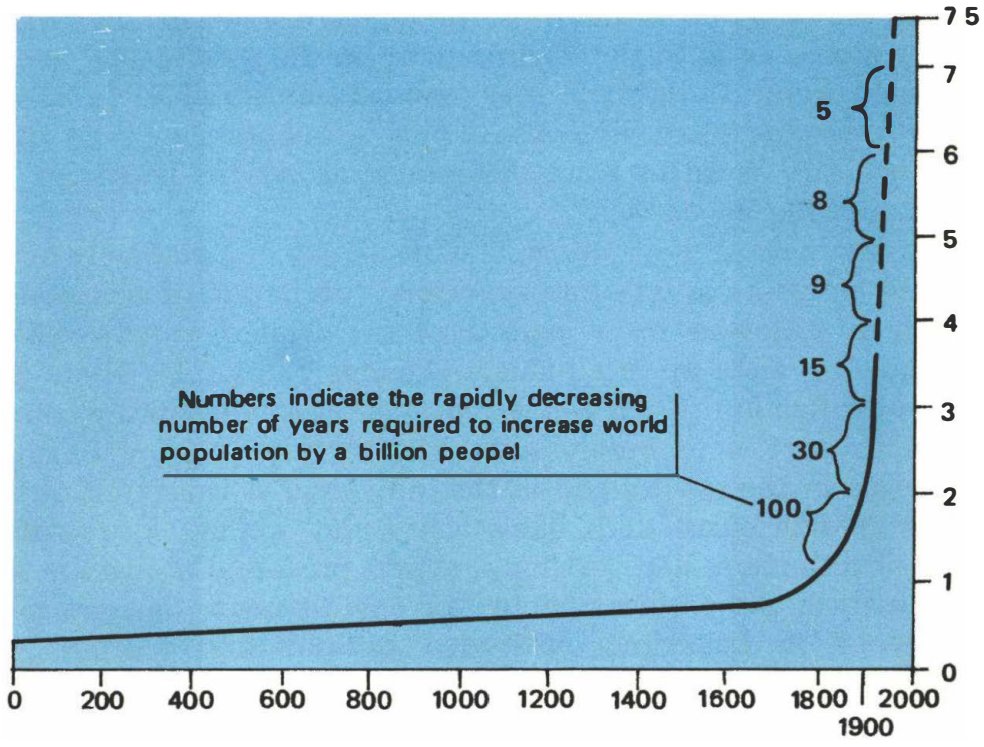

Figure 7. Adapted from "The Population Bomb".

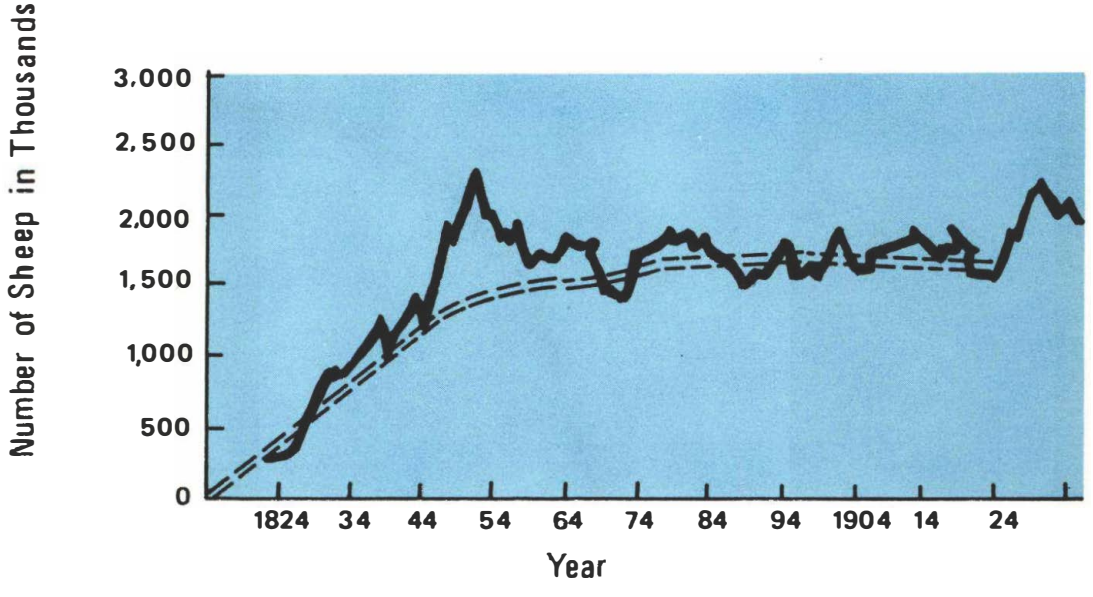

Figure 8. Growth curve of sheep following their introduction to an area. Note initial sigmoid pattern followed by approximate equilibrium.

The implication is that there are regulatory mechanisms in all living systems, both in the environment, in the species, and in the individual itself. These regulatory mechanisms are possibly coded in the germplasm but it is also highly probable that they have some intuitive sense to react differently at various points of its evolution along the curve.

If we look at the S curve, it starts slowly and gradually picks up then enters a period of acceleration. This period of accelerated growth is followed by a period of decelerated growth which subsequently ends up in a plateau (Figures 9, 10, 11). There is a point of inflection which we can imagine as a break in the course of evolutionary time. Surely and most certainly profound changes take place in the species and in the individual at this point of the human growth curve. It is likely that today we are close to that point of inflection; and we should expect profound changes in the individual, in the species and in our environment. Changes may initially be in behaviour, attitudes, outlook and reactions to stimuli, influences, and events.

What makes man a little more complex and difficult to understand, is that man is attributed with the capacity of ***learned behavior*** and ***individual will*** which is absent in lower animals. The point of greatest concern is how we (man) would and should exercise these attributes. In the past, the struggle is between man and his environment. Now, the battle is between man and his species. We are moving into the era when the struggle will have to be in the individual himself.

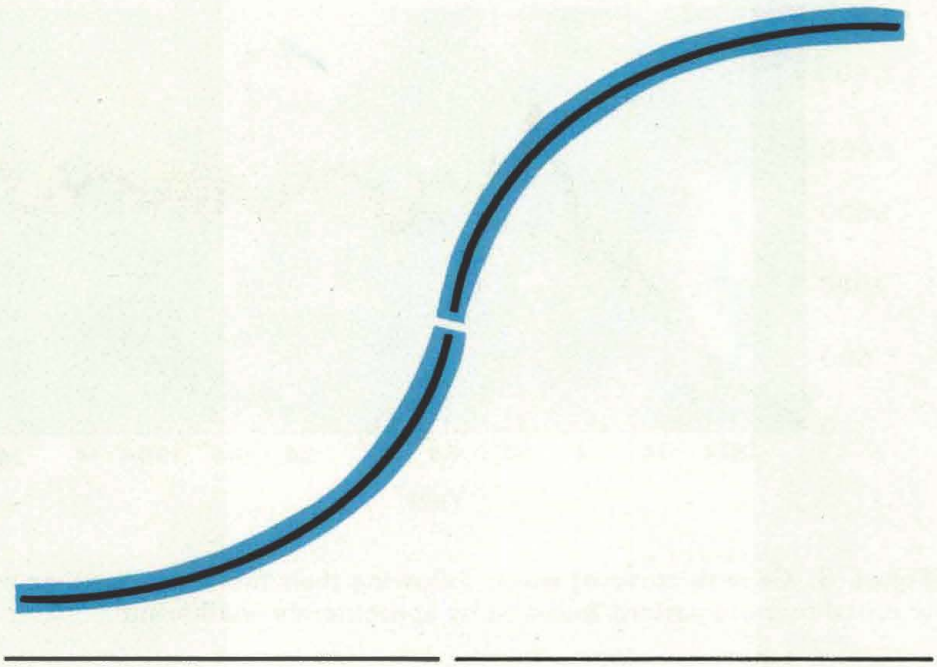

Figure 9.

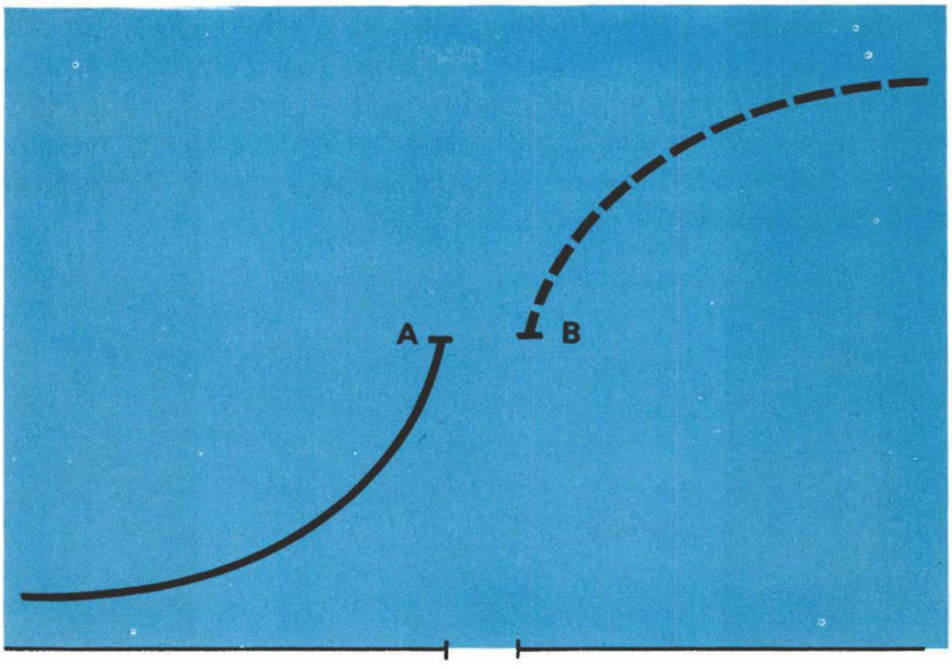

Figure 10.

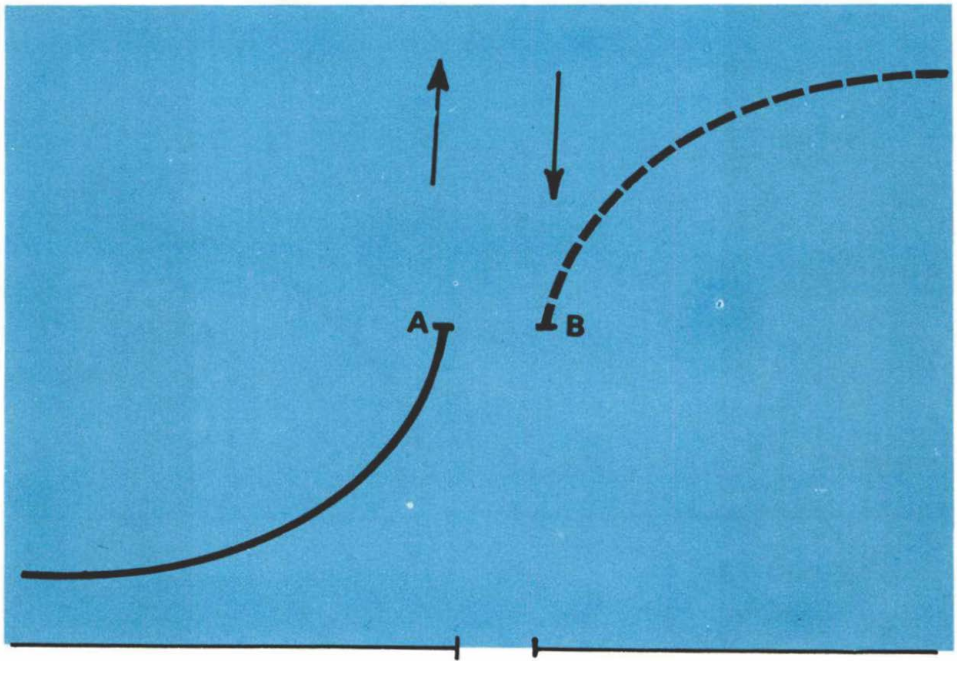

Figure 11.

Many of us are lost or confused, because each individual seem to be imbued with two patterns of behavioral development (Figure 12). It follows a dualistic pattern characterized by features and attributes similar to those in Figure A and another set of attributes similar to those found in Figure B (Figure 13). They tend to come into conflict in man, in his species, and in the individual itself. Man, however, by exercising judgement and his capacity to select, need not follow the patterns of unrestrained growth which would lead to his extinction.

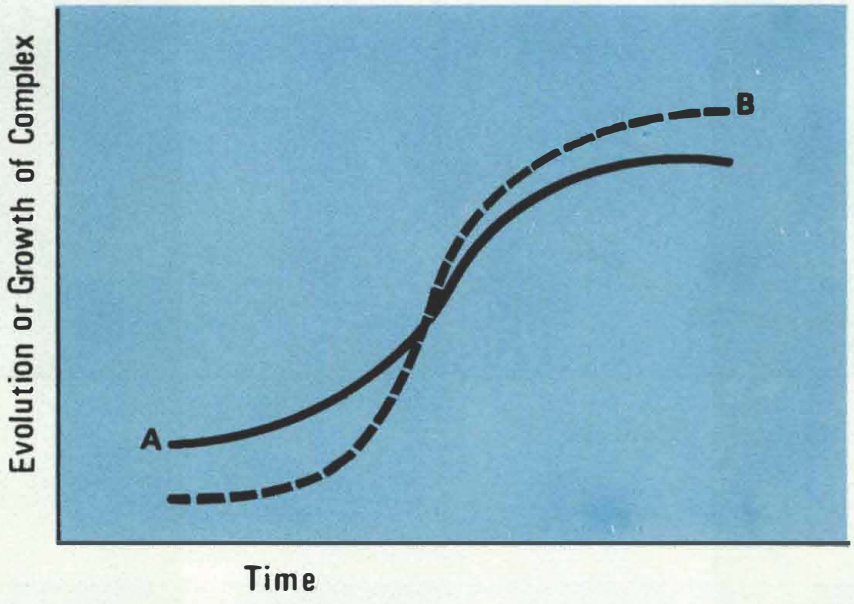

Figure 12.

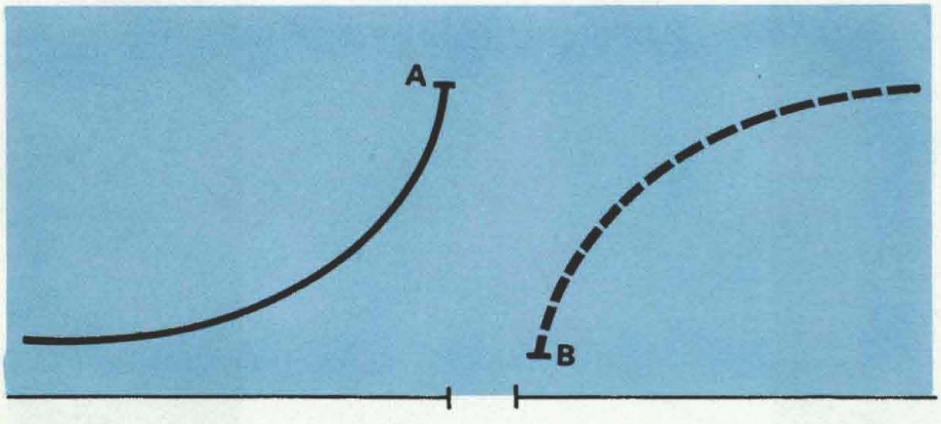

Figure 13.

We need not follow the pattern of the lemmings either (Figure 14) as was originally advocated by Malthus — war, famine, and pestilence — will keep population with the limits of earth's resources. We need not follow the sigmoid curve of living systems, as presented by the fruitfly model but we can follow a growth

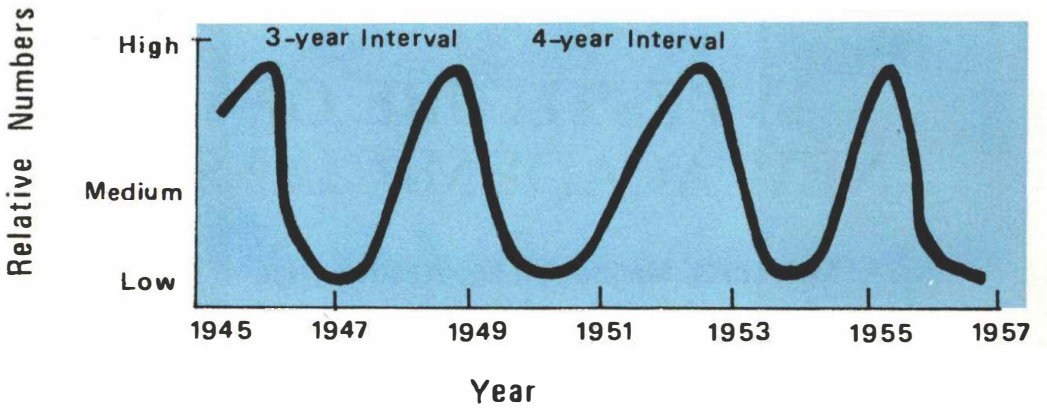

Figure 14. Generalized curve of the three-to-four year cycle of the brown lemming population.

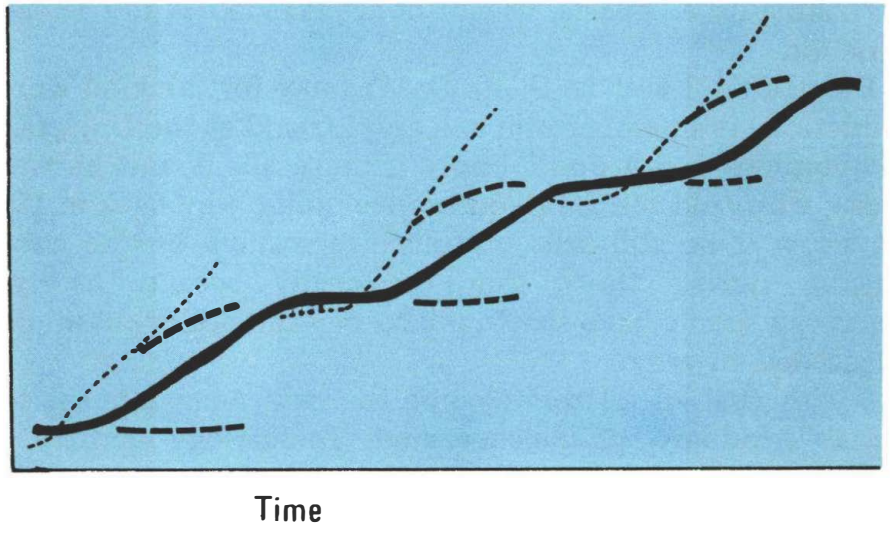

Figure 15.

curve where man can harness the environment, exercise control over his species and tame his dualistic nature. In resume, man has more than an alternative for his choice. We can observe three or four patterns of curves for human progress vis-a-vis population growth:

The first is one of unrestrained growth which, of course could only lead to extinction;

The second is the catastrophic growth curve as exemplified by the lemmings (Figure 14);

The third is the S or sigmoid curve; and,

The fourth is the curve where adjustments and adaptations play significant roles specially in species like *Homo sapiens* where judgement and the power to select is an attribute. Such a curve is Figure 15.

INVESTITURE OF NEW ACADEMICIANS

Melecio S. Magno, Ph.D., Academician
Minister of Science

Dr. Campos, President of the National Academy of Science and Technology; Dr. Lagmay, Vice-President; Dr. Mijares, Secretary; members of the Council, Dr. Velasquez, the National Scientists, Dr. Juan Salcedo, Jr., one of my predecessors in the National Science Development Board, Dr. Alfredo Santos; fellow Academicians, distinguished guests, friends of science, ladies and gentlemen and friends.

First of all, I wish to thank Dr. Lagmay for the kind words of introduction. I remember him as a good friend at the University of the Philippines and I don't know if he recalls it, but he was the first one who sold me a Life insurance policy way back in 1946. I don't know if he still sells insurance nowadays, but he was very persuasive during those times especially because as a young instructor, I didn't have the funds to pay the policy premium but he succeeded anyway.

I wish first of all to welcome the new Academicians to the National Academy of Science and Technology and to greet everyone of you present here tonight, a pleasant good evening.

Many of the Academicians had been my colleagues at the University of the Philippines and, of course, this is no surprise because most of the qualified manpower in science and technology are in the University of the Philippines. In fact, during my four years in the National Science Development Board, I have always depended on the University of the Philippines for expertise. We have some colleagues of course in the Board who have the same ability as our friends in the University of the Philippines but they did not have the opportunity and the chance for graduate training. I shall try to refrain from speaking too long and too seriously, since I know very well that you have already spent a long, serious but no doubt interesting day attending the presentation of scientific papers, and by now everyone must be looking forward to have an enjoyable meal.

Tonight, I guess, is as good a time as any to ponder on the Academy's reasons for being and how it has fared since 1977 or 1978, the year of its creation. The Academy was established by the President in response to the need to provide meaningful incentives to those engaged in scientific and technological research as well as to give due recognition to outstanding achievements of

science and technology. The creation of the Academy was envisioned then to professionalize the government's scientific and technological services in order to effect the promotion of scientific research and invention and the advancement of science and technology for national development. I've used the very same words in PD 1003-A, so that our terms of reference will be clear.

As a member of this august body, I do indeed feel honored and consoled that the government appreciates the scientist and his work. The status of the scientist, in effect, has been elevated to that of the artist and for this we are grateful to President Marcos and the First Lady. Their words and their deeds indicate their recognition that while their arts serve to uplift the human spirit, science and technology are instrumental in bringing about material progress. This new found responsibility of the scientist, therefore, carries with it a great responsibility . Far from putting the scientist on a pedestal, he or she is expected to help in solving the Filipino's basic needs of food, clothing and shelter. As the Minister of the NSDB, I cannot help but be acutely conscious of this. The national structure in the country's reservoir of scientific manpower must respond to national priorities. Membership in the Academy should not place the scientist above the rest of the community. If anything, because of this greater or rather higher educational attainment, greater expertise and greater capability is looked up to for solutions and explanations to almost every problem or phenomenon under the sun.

Research, therefore, should not stop at the publication of a scientific paper for only fellow scientists may understand this. It is incumbent upon the scientist to explain to the layman and to the rest of society, the importance of his work and how this maybe relevant directly or indirectly to everyday human existence as well as social and economic activity. The mystification of science must be initiated by the scientist himself if only to prove wrong the general impression that he lives in an ivory tower.

I wish to bring to your attention an important article in Science Magazine of the February 22, 1980 issue. This article is entitled "Science: Our Common Heritage", authored by Kenneth E. Boulding who is a distinguished professor of Economics of the University of Colorado in Boulder. He is, I think, the President of the American Association for the Advancement of Science. One of the things that he talks about in this important article is that even scientists, nay, even Academicians, have some misconceptions or illusions about the so-called science. I would suggest that each one of us, study this very seriously because it affects the concept of our own discipline.

I wish to thank Dr. Lagmay for saying that I am an objective person, objectivity, of course, is one of the qualities expected of a

scientist. But, this is not a monopoly of a scientist alone. There is one important point that was raised by Dr. Boulding which is a little different from the regard that the layman has with respect to the difference between Science and the Humanities. You've heard of the two cultures of Charles P. Snow, he says that the scientific culture and the humanities culture cannot mix together. The persons in the humanities do not appreciate Mathematics or for instance the second law of thermodynamics in Chemistry or Physics, but at the same time the scientist does not appreciate the humanities. So there is a big gap between the two. Dr. Boulding suggests that it is not correct to make such a distinction between the sciences and the humanities or between the physical sciences and the social sciences for that matter. Of course, we may disagree, some of us in the so-called "hard sciences" may feel that our sciences are superior to those in the other sciences. But I suggest, and Dr. Boulding suggests, that this might not be the right perspective for looking at the different sciences as such. What he is suggesting is a unity of knowledge, unity of the sciences themselves.

There is one important point that I would like to bring to your attention especially of the social scientist. And may I quote here: "Human knowledge becomes particularly insecure, when we move into unfamiliar regions of a field or a system. Experimental science tends to deal with the familiar for the laboratory, after all, is a clear descendant from the kitchen. This may sound a little insulting to the practitioners in the cathedrals of high energy physics which move into unfamiliar regions of science, in this case, very small. But even this region of experimental science, can deal only comfortably with events that are common within the field of the experiment. In extreme positions even of relatively familiar fields, strange things happen such as, pregozene, dissipated systems, (sorry I'm not familiar with this author). His work is relatively new, far from equilibrium.

The evolutionary process itself indeed is one in which rare events in unfamiliar parts of the field, are of extreme importance in explaining the overall pattern in time. While the sciences of the familiar are not very much of help, in probable events in a small field, they cannot be studied in a laboratory. This is perhaps, while experimental social psychology seems to be running into a severe crisis in fields where extreme positions are highly significant, the experimental method maybe of limited value, indeed, it is quite inappropriate. One of the unfortunate effects indeed of correlational statistics has been to divert attention from extreme cases which are simply rejected as deviations, whereas they may contain important knowledge about the extreme positions of the field. The uncritical transfer of statistical techniques which are entirely

appropriate in some epistemological fields in which they are quite inappropriate, has been the source of a great deal of western scientific efforts, especially in the social sciences. Statistical significance is by no means the same thing as epistemological significance and one of the underexplored frontiers in science is the tailoring of statistical methodology to particular epistemological fields.

Privately I have of course agreed with this, although I have not tried to argue with my social science friends, Dr. Lagmay or Dr. Encarnacion. But I have always expected that in the social sciences, it is not easy to quantify, because we are dealing with people, we are dealing with complex quantities. Maybe that's the reason why statistical methods are used rather than the methods of physics. But as Dr. Boulding finds out, there are pitfalls in this method. What he is suggesting is that for science to survive, we have to re-examine epistemologies of our different fields of science. And epistemology that works or that may work in a field like physics may not work in another field. So, well, that I cannot say much about this because I'm not an expert in the methodology of science, but I would suggest that the members of the Academy study this article and perhaps devote a session or two, to discuss it and perhaps to react to it if they find that it's not quite correct.

This is one activity which I would like to propose to the Academy. There are of course other activities in which the Academicians maybe involved that I would like to mention them now. Because of the rigid requirements for membership in the Academy it has been an association of the best scientific minds in the country. This is indeed quite a distinguished company. But as I have indicated earlier, membership in the Academy should be a source of not only pride but also humility for us, for the contributions to the people's well-being demanded of us are correspondingly greater. I nurture the hope that someday the equivalent of "barefoot doctors," will permeate all fields of science, so that the benefits of scientific information and technology may seep to the grassroots. Just as in the health sector, we have the rural health units of the barangay level manned by paramedics and auxiliary health personnel. So too should we "barefoot scientists" in all other areas of science. Perhaps this concept should be incorporated into the extension network. Such personnel would complement the professional practitioners and the scientist-researchers by way of insuring diffusion of scientific and technological knowledge to the end-users and providing feedback to them regarding the acceptability or applicability of technological solutions in the field.

I realize of course, that it's not the scientist's main job to disseminate research results and do extension work. Other segments of the society are responsible for these tasks. But the scientist has a great stake in the establishment of a mechanism for effective technology transfer and diffusion. Until such a circuit for the flow of technology is completed and made in good operating condition, then I guess the scientist will always be vulnerable to the charge of electism.

At this point, I am reminded of a story which the late President Eisenhower related in a convocation at the University of the Philippines. This was, I think, in the early 50's. He told a story of a man who went to the country and engaged the services of a hunting dog. The owner of that dog had the inclination to call his dog by the name of an instructor or an assistant instructor or an assistant professor and so on. I suppose he was a friend of the faculty of a certain university. And so when the hunter was asking how much the hire should be for one day's work of the hunting-dog, he was told, "Well, now, that he is an instructor, his fee, my fee for him is $3." So, he paid $3. The next year, the hunter came back and asked for the same dog, saying, "I liked his services last year. Could I hire him again? ". And the owner of the dog said, "Sir, well, you know he is now an assistant professor, so you cannot have him for $3, it's now $4." So, this dog was hired for $4. The next year, the dog was now called associate professor, so he was now hired for $5. And hence, the next year, he was now professor, $6. Alright, the man returned the next year and said, "Could I hire that dog again? It has been very serviceable and useful to me." But the owner of the dog said, "Well, sorry since that dog became college president, it does nothing but wag his tail and bark." Let it not be said of us Academicians, in order that we cannot do anything, but wag the whole tail and bark.

What I'm trying to say is that, we should keep abreast of the developments in our own fields. We are supposed to be the experts. You know, there are very few of us in the country, especially in a given field. There are very very few indeed and there are very few Academicians and in sum of duty, I think, to keep abreast of developments so we can inspire the young people. Of course there is a limit to this, I realize, but as long as we can do it, it is our duty, I think to be active in our own fields.

There is one other area of involvement to which I wish to invite the participation of the members of the Academy; that of the analysis of public policy. Our government, administration, the Cabinet, the Batasang Pambansa, or any of the ministries, make these policies. Sometimes they are controversial, sometimes they involve science and technology. Now, science and public policy and analysis are not quite the same thing and do not have the same

objectives. Many traditional scientists try to avoid policy analysis. While science has has its objective — the discovery of physical truth, good policy analysis aims to evaluate order and structure the current state of knowledge, although such knowledge maybe poor or incomplete, so as to allow decisions to be made with as complete understanding of what is known, its limitations and its implications.

Unlike science, in the exerscience, of which opinions, preferences and values play limited role, good policy analysis must deal with such opinions, preferences and values. But it does so in ways that are open and explicit and that allow different people with different opinions and values to use the same analysis in making their own decisions. Like science, however, good policy analysis does not draw hard conclusions unless they are warranted by an ambiguous data or well-founded theoretical insight. Just to cite a few examples, for instance, policy issues.

Take the case of the nuclear reactor. Our knowledge of the effects of radioactivity, radioactive wastes, nuclear fuel and all that, is quite incomplete. And ordinarily, scientists like us will not venture to advance an opinion. We can always say, "Well as a scientist, I'd rather suspend my judgment because I've known enough," which is of course, through the dispirit of science itself, because science awaits full understanding. It does not engage in speculation, except as, such speculation contributes to the design of future experimental and theoretical research. But, when the scientist is confronted with inadequate data, then he says, "that is not my line, I'd rather not venture into giving an opinion about the matter, because it would be unscientific". But, whether understanding is complete or not, about a certain subject, about a certain issue, the government or society has to make a decision, and it has to make that decision now, even if the understanding on the matter is quite incomplete. We can not forever postpone a decision on the Nuclear Power Plant. Otherwise, if we wait 10 years, 20 years, the cost of putting up a Nuclear Power Plant will have increased 5 times, 10 times, who knows. In fact, the delay in the putting up the Nuclear Power Plant has cost the government so much already. You might ask, "Well, it's easy to talk, when you don't live in Bataan, where the Nuclear Power Plant will be located". Alright, it is easy to desire that you will have a Nuclear Power Plant. But, how about the people of Bataan who will be closer? The matter, therefore, of deciding whether to have a Nuclear Power Plant or not, is a policy issue. It is not a scientific problem, it is a policy problem, and the government or the decision-makers have to make their decisions regarding these policy matters with incomplete knowledge. And they have to use, they have to depend on, as mentioned already, opinions,

preferences, values and two different people using the same set of knowledge. The same, or perhaps the same preferences, but different, say, values and attitudes will have different conclusions.

I nevertheless would like the members of the Academy, to involve themselves on issues like these, because they are looked up to by the leaders of the country. If you leave the decision-makers to non-scientists alone, then I'm afraid you'll always say, "maybe we made a mistake". (Baka nagkamali yung Minister, hindi n'ya alam lahat.) That will always remain a nagging problem. Of course, it's no guarantee that even with the use of the best or the most adequate science and technology, we will get a definitive answer to a problem. There will always be trade-offs, for instance, in the case of the Nuclear Power Plant. Which has more value to us: to have more energy or to have more protection against radioactive radiation or rays? And usually, the problem is not easy. But, as I said, society, the decision-makers have to make a decision. And I believe that the scientists and technologists should have them as much as possible by ordering, evaluating and assessing the available paper. Taking into account, even preferences, opinions and values.

I know that the Academy has been doing this in some cases, I have attended some meetings, for instance on the Nuclear Power Plant. The Academy did have some sessions on the matter. And, I don't know, but I like to think that the government's decision now to continue with the Nuclear Power Plant, has been influenced by the opinion of the top scientists and technologists in our country, as represented by the Academy and by other scientific organizations in our country. As I said already a number of policy involves science and technology. It is in the study of such problems where the members of the Academy, with their capability for thorough understanding of the technical issues involved and the ability to sort out good science from bad, are invited to participate. I've expressed these thoughts aloud not because I intended to spoil your appetites, but to give voice to a hope, which is as much a challenge and which I know the entire Academy shares the hope that in our country, science and technology may be harnessed effectively to contribute to national development.

DOCTOR T.H. PARDO DE TAVERA AND PHILIPPINE HISTORIOGRAPHY

By Encarnacion Alzona, Ph.D., Academician

With humility I present to the National Academy of Science and Technology my brief notes on Doctor T.H. Pardo de Tavera (1857-1925), eminent Filipino scholar and scientist, educator and statesman, and on some of his contributions to Philippine historiography.

He began his researches in Paris when he was a medical student at the Sorbonne. Undoubtedly he was inspired by the queries about his native country of European Orientalists he met in that center of learning. At that time in the civilized world very meager was the existing knowledge about these distant islands.

Philippine linguistics was his first obsession. To prepare himself for the scientific study of language he enrolled at the Ecole Speciale des Langues Orientales Vivantes at Paris from where be obtained a diploma.

His first work, *Contribución para el estudio de los antiguos alfabetos filipinos*, appeared in 1884 and was dedicated to Professor Ferdinand Blumentritt, noted Austrian Orientalist. It included the most important findings of E. Jacquet, a Belgian scholar, in his treatise *Considéracions sur les Alphabets des Philippines* (Paris 1831), the first scientific work on the subject, adding to them the results of his own research. Count Meyners, famous Orientalist, honored it with a French translation which was published in the review *Annales de l'Extrême Orient*, Paris 1886.

This monograph, his first contribution to Philippine historiography, was his debut in the universal society of scholars.

Again the attention of European scholars was focused on him when his article entitled *La médicine a l'Ile de Luzon, Archipel des Philippines* (The Practice of Medicine on the Island of Luzon, Philippine Archipelago) was published in the *Journal de Médicine de Paris* (1884, vol. VI, No. 2231). It was an account of the sorcery, witchcraft, etc. related to medicine as practiced by the natives of Luzon. A Spanish version of it, *La medicina en la Isla de Luzon*, made by Pedro de Govantes de Azcárraga, was published in the review *Dos Mundos*, Madrid 1884. Professor Blumentritt translated it into German with the title *Die Medicinischen Kenntnisse der eingeborenen der Insel Luzon* and published it in the review *Globus* (1885, XLVII) for the benefit of German scholars.

Also highly admired was his monograph entitled *El Sanscrito en la lengua Tagalog*, which he published in Paris in 1887. It consists of a list of words of Sanskrit origin in the Tagalog language with explanatory comments on their introduction into the Tagalog language. He dedicated it to Segismundo Moret y Prendergast,[1] enlightened minister of colonies of Spain. Orientalists lavished praise on this work of Pardo de Tavera. Rizal, after examining the complimentary copy that the author had sent him, exclaimed in admiration, "How I envy Pardo Tavera's knowledge of Sanskriti." And Rizal was already a master of several foreign languages. Such was the importance of Pardo de Tavera's monograph.

His knowledge of comparative linguistics was again demonstrated in his next work, *Considéraciones sobre el origen del nombre de los números en Tagalog*, which first appeared in 1889 in *La España Oriental*, published in Manila. Then reprints consisting of twenty pages were made. W.E. Retana considers it "a curious and instructive work."

In his search for documents relating to the early history of the Philippines, he discovered in the Franciscan convent at Manila a manuscript entitled *Las costumbres de los indios Tagalos de Filipinas* by the Franciscan missionary Father Juan de Plasencia. This manuscript, the only one in existence, is dated Nagcarlang, 24 October 1589, and is kept in the Franciscan convent in Manila. It was written upon the request of Spanish Governor Santiago de Vera (1584-1590) who was desirous of being informed about the customs and laws of the Tagalogs for his guidance in his administration. He wished to adopt as much as possible Spanish laws to those of the natives.

He published this rare manuscript in *Revista Contemporanea*, Madrid, 15 June 1892, with the title *Las costumbres de los Tagalos de Filipinas, segun el Padre Plasencia.* He enriched it with illuminating notes, as well as a biography of "this venerable Francis-

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[1]Segismundo Moret y Prendergast, minister of colonies from 1 April 1870 to 28 December 1870, promulgated the Moret Decrees which secularized education in the Philippines. They established an *Instituto Filipino* which would provide secondary education and created chairs of Tagalog and Bisayan languages in it; and they secularized the University of Santo Tomás, renaming it *Universidad de Filipinas*. The Domincian friars opposed these reforms and through their procurator at Madrid, Fr. Francisco Rivas, succeeded to convince Moret's successor Adelardo López de Ayala, to revoke the Moret Decrees, which he did in 1871. Father Rivas offered to introduce the medical course, which was not given then at the University of Santo Tomás, though, he said, it should be a shorter course than the one in Spain "on account of the limited intelligence of those natives."

For a brief reference to this matter see "Moret y el Padre Rivas" by Mariano Ponce in Jaime C. de Veyra y Mariano Ponce, *Efemérides Filipina*, Manila 1914, vol. I, pp. 46-49.

can missionary in the early years of the conquest who occupies a most important place in the history of the Philippines." Professor Blumentritt translated it into German which appeared in *Zeitschrift für Ethnologie* (January 1893) with the title *Die Sitten und Brauche der alten Tagalen. Manuscript des P. Juan de Plasencia, 1589. Herausgegeben und eingeleitet von Dr. T.H. Pardo de Tavera.* Dr. H. Kern, noted professor of Sanskrit at the University of Leiden, published a Dutch translation, *De ge woonten der Tagalogs op de Filippijnen volgens Pater Plasencia in Bijragen tot de taal-land en volkenkunde van Nederlandsch-Indie*, The Hague, 1892, vol. VIII.

Pardo de Tavera is also the author of *Plantas Medicinales*, a book of 341 pages in 8°, published in Madrid in 1892. Its first part consists of a list of medicinal plants with their scientific names, their popular names in the principal Philippine dialects spoken in the Islands, their uses, and their description. Then follows an index according to the qualities of the plants; the next part is a statement of their therapeutic value with an alphabetical list of the most common ailments for which the plants have therapeutic value. At the end is a general index to facilitate the use of the book.

His next notable research dealt with printing and engraving in the Philippines. No one else before him had undertaken such a study. Entitled *Noticias sobre la imprenta y grabado en Filipinas*, it first appeared in *Revista contemporánea* (Madrid, 1892, Nos. 426, 427, and 428. It was praised by Juan T. Medina of Chile in his book *La Imprenta en Manila desde sus orĩgenes hasta 1810*, Santiago de Chile, 1896.

A very valuable contribution to Philippine historiography was his study of Father Murillo Velarde's map of the Philippines, published in 1734, which was the first detailed map of the Archipelago, notable for its accuracy and neatness. Its engraver was a Filipino, the noted Nicolás de la Cruz Bagay. Pardo de Tavera stated that the first edition of the map was unknown in Manila and hence he believed it opportune to publish a commentary on it. His work consists of 19 pages in 8° with two engravings, and printed in Manila in 1894.

The closing years of the 19th century were tragic to Filipino aspirations. We had declared our independence from Spain and established a republic based on a written constitution. Then the United States of America, a great power, intervened, determined to extend her rule over our Archipelago, thereby securing a foothold in the Far East like the other great powers: Great Britain, France, Germany, and Russia.

Under the new regime Pardo de Tavera continued his scholarly pursuits; and in 1900 appeared his study of a rare historical

document: a report of Governor Simón de Anda y Salazar (1770-1776) to King of Spain entitled *Abusos o desordenes que se han criado en las Islas Filipinas.*[2] Pardo de Tavera's study consists of 102 pages in 8°. Anda's report fills 41 pages and the rest contain Pardo de Tavera's commentaries, or as he modestly calls them "Notes". As Anda related his opinion of the deleterious rôle of the friars in the Philippines, the commentator supplied facts and occurrences, without expressing his own opinion. While Pardo de Tavera's study illuminated the history of the Philippines of that period, it certainly did not endear him to the friars.

Though he was already a member of the Philippine Commission and occupied with numerous official duties, he still found time to engage in scientific research. In 1901 he published an interesting study entitled *Etimología de los nombres de razas de Filipinas* which he dedicated to Dean C. Worcester, a member of the Commission. Worcester was a former professor of zoology at the University of Michigan and had been in the Philippines on expeditions twice. He was the author of a book entitled *The Philippines Islands and their People*, New York, 1898.

The Americans recognized Pardo de Tavera as a distinguished gentleman and brilliant scholar; and when they decided that a brief history of the Philippines should be included in the Philippine census of 1903, they requested him to write it. This is his *Reseña histórica de Filipinas desde su descubrimiento hasta 1903.* (A Brief Review of the History of the Philippines since its Discovery until 1903). When he examined the Spanish edition of the Philippine Census, he discovered that it was not his original work that was used but a Spanish translation of the English translation. He found many errors in the Spanish translation and so he brought the matter to the attention of the Philippine Commission. In March 1906, the Commission approved a resolution ordering the publication of his original work. Though only 75 pages in 8°, by far it is the most illuminating brief survey of Philippine history that this speaker has read. However, it has been criticized adversely by a certain religious order for not extolling more profusely the rôle of the friars in the Philippines. The author had been fair, rendering praise and censure whenever they were due.

In the desire of Washington to gather information about its new possession the Library of Congress took steps to acquire books relating to the Philippines' past. Pardo de Tavera placed at the disposal of the Library of Congress for publication his own collection of valuable and rare works. Entitled *Biblioteca Filipina* it was published in 1903 jointly by the Library of Congress and

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[2]See José Montero y Vidal, *Historia General de Filipinas,* II, 236-281. Also, Manuel Artigas y Cuerva, *Historia de Filipinas* (1916), Manila, 252-263. In the footnote is a brief biography of Dr. Anda.

the Bureau of Insular Affairs. The chief merit of Pardo de Tavera's *Biblioteca* lies in the critical comments on each item which enhance its usefulness to the historical researcher.

In 1909, his health failing, he resigned from the Philippine Commission and went abroad, hoping to recuperate. Before his departure, his admirers, among them, Manuel L. Quezon, Juan Sumulong, and Jaime C. de Veyra, organized a farewell banquet on the night of the 17th of April 1909. His address at that banquet was printed in the original Spanish and the English translation.

The banquet was attended by distinguished Filipinos belonging to different political parties, some of whom had denounced the platform of the defunct Federal Party of which he was the president. On this occasion he clarified his stand, declaring that he then sincerely believed that statehood was preferable to the humiliating status of colony.

He also remarked that some politicians were injudiciously dividing our people into Filipinos *de cara y corazón* and Filipinos *de corazon* only, alluding to Filipinos with Spanish blood. There should not be such division among Filipinos. All should be Filipinos *de corazón*, regardless of color, shape of the nose, or whether or not they wore beards. (Pardo de Tavera wore a beard.) Nations are not composed of people with the same physical characteristics, but of people who have the same sentiments, common aspirations and ideals. If common physical characteristics are the basis of the union of primitive peoples, modern nations rest on more solid bases which are common aspirations and ideals. He cited the people of the United States of America, Canada, and the United Kingdom, by way of examples.

Pardo de Tavera was of mixed blood: Spanish and Tagalog. He was a native of the Philippines and had always regarded himself as a Filipino. In appearance he was comely and stately; in manners, he was courtly, continental.

A highly valued work of Pardo de Tavera was *El carácter de Rizal*, first published in *The Philippine Review*, a monthly publication edited by Gregorio Nieva. It was a masterly study of the character of our national hero who was his personal friend. The demand for it was so great that it became necessary to publish it in pamphlet form. Translated into English, Filipino educators believe it should be required reading in Philippine schools.

Future historians of the Philippines will find the lectures, addresses, and articles of this brilliant and patriotic scholar illuminating in the interpretation of the period to which they pertain.

He devoted serious thought to the economic development of his country, being aware of her vast economic resources and believing it to be of fundamental importance to her political future.

In an article entitled *La agricultura y la inmigración*, published in the *Philippines Free Press* of 5 October 1912, he elucidated his stand on the necessity of importing foreign agricultural laborers in order to hasten the cultivation of the vast fertile lands of the Islands, since Filipino laborers at that time were indifferent and unskilled, content to subsist on what the extraordinary fertility of the soil and the benign climate could provide them with little exertion. As a result landowners were not getting encouraging returns from their holdings.

He was president of the *Asociación Económica de Filipinas* dedicated to the promotion of agriculture. Among the letters of adhesion that he received was one from Manuel L. Quezon, then resident commissioner of the Philippines at Washington.

Another signifficant contribution to Philippine historiography is his lecture before the prestigious Philippine Columbian Association in Manila entitled *Resultados del desarrollo económico de Filipinas* (Results of the Economic Development of the Philippines) covering the pre-Hispanic, Hispanic, and contemporary periods. It was published in two issues of *The Philippines Free Press:* 7 December and 24 December 1912. It was a masterpiece, and its author was hailed as a great educator, a prodigious talent, etc. By public consensus he was the foremost scholar of his time. For a long time his lecture was the favorite topic of conversation of Filipino intellectuals, dealing as it did with the most vital problem of their country.

Another lecture of his that stirred prolonged discussion among the intelligensia was *El legado del ignorantismo* (The Legacy of Obscurantism),[3] delivered before the summer assembly of public school teachers held in Baguio on 30 April 1920. In this critical survey of public education during the Spanish era the lecturer revealed an extraordinary familiarity with the *novenas*, *corridos*, tales of miracles, and the like that then constituted the popular reading matter. It goes without saying that this lecture did not endear him to the religious orders which directed public education during the Hispanic epoch in our country.

Many of his admirers were the intellectuals belonging to what was then popularly called "the rising generation" — young people who were the products of the public schools established by the Americans. Two of them, Eliseo Quirino and Vicente M. Hilario who were members of the faculty of the University of the Philippines, included in their book *Thinking for Ourselves* (1924) the English translation of *El legado del ignorantismo*, *El alma filipina*,

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[3]The Legacy of Obscurantism", translation by Encarnacion Alzona and published in a booklet entitled *The Character of Rizal and The Legacy of Obscurantism*, with a biographical sketch of Pardo de Tavera by Encarnacion Alzona. Publication of the University of the Philippines, Diliman, 1960.

the concluding part of *Resultados del desarrollo ecoñomico de Filipinas*, and the preface to his book *Plantas medicinales de Filipinas*, and "The Conservation of the National Type", written in English, which was his commencement address (4 April 1921) to the graduates of the University of the Philippines. They believed that the ideas of Pardo de Tavera and other Filipino thinkers deserved to be disseminated among the Filipino youth who were justifiably being criticized for their greater familiarity with the ideas of American thinkers and writers rather than of their own countrymen.

Pardo de Tavera was cordial and sympathetic toward the younger intellectuals. This speaker remembers her elation upon receiving in the United States a handwritten letter of Pardo de Tavera in his elegant script.[4] In that letter he wrote that he had intended to ask me to write a history of the City of Manila and he regretted that I had left for abroad; but he added that he was also glad that I was pursuing advance studies. Upon my return to the Philippines, I presented him with a copy of my unpretentious doctoral dissertation, my debut in the society of scholars, and he acknowledged it with a commendatory letter in French,[4] a language of which he had a enviable command, like all highly educated men and women of his time.

He invited to his home on Buenavista Street in Santa Mesa, at that time a romantic residential district, the younger intellectuals, showing them his collection of rare books and manuscripts among which he had scattered black pepper as protection from termites. Some of his pamphlets were bound with Philippine woven cloth, done by himself. He said he was preparing to write a history of Philippine civilization, which undoubtedly would be authoritative considering his vast learning and scientific mind. Death, however, prevented him from carrying out his grand design to the nation's great loss. Nonetheless his scholarly contributions to Philippine historiography are more than sufficient to immortalize him.

Furthermore, he has other achievements that entitle him to the eternal remembrance of his countrymen. When he was a member (1901-1909) of the Philippine Commission, the legislative body in the first years of the civil government during the American regime, he contributed greatly toward the Filipinization of the government established by the Americans. At that time the Americans were abysmally ignorant of the Philippines and her people; and they were wise enough to seek the cooperation of educated Filipinos. Thus, through his recommendation many competent Filipinos were appointed to government posts.

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[4]A copy of the original of each of these letters is reproduced in the following pages.

DR. T. H. PARDO DE TAVERA
Manila

714 Pennsylvania Ave.
20 Agosto 1919.

Señorita Encarnación Alzona.
Chicago.

Distinguida señorita y amiga:
Después de mi vuelta del Japón, solamente esta mañana me entero por un diario de aquí que V. está en Chicago. Me alegro mucho por V., pues lo siento por mí porque precisamente estaba yo pensando recomendarle a V. que escribiera una Historia de Manila. Esta sería una obra muy importante y que encierra lo más notable de la historia nuestra de Filipinas.
He querido escribir a V. estas líneas para darle mi felicitación por haber sido pensionada para los EE.UU. en donde V. aprenderá mucho. Espero que, gozando de buena salud, [illegible] podré saludarla a su [illegible].
Suyo [illegible] atentísimo

T. H. Pardo de Tavera

P.S. Dé V. a la Srta. Carmen [illegible] [illegible] de mis recuerdos.

Manille le 29 Sep. 1933

A Madame Encarnacion Alzona.

Chère Madame:

J'ai, en fin, reçu votre thèse "Some French Contemporary Opinions of the Russian Revolution of 1905" que j'ai lue avec le plus grand plaisir et intérêt.

Je vous suis très reconnaissant d'avoir pensé à moi, et vous prie d'accepter mes sincères remerciements pour votre charmant envoi.

Vous avez fait là une étude très consciencieuse avec une analyse parfaite et juste: aussi je suis tout à fait enchanté d'avoir eu la bonne fortune de lire votre travail dans lequel j'ai beaucoup appris.

Recevez Chère Madame, l'expression de mes sentiments les plus respectueux

T. Pardo de Tavera

He was instrumental in the establishment of the first state medical school in this country. With other Filipino physicians he organized an association known as *Colegio Médico-Farmacéutico de Filipinas* of which he was the first president (1899-1900). This association brought to the attention of the Commission the country's pressing need for trained physicians, as the majority of the people was left to the mercy of herb doctors. With the support of Commissioner Dean C. Worcester he succeeded to convince the Commission to approve the establishment of the Philippine Medical School in 1905.

He cherished another project to promote the general welfare of his countrymen; the creation of a state university free from clerical control. His efforts were crowned with success when the Commission approved Act No. 1870, passed by the Philippine Legislature in 1908 providing for the establishment of the University of the Philippines. This was opened in 1909 and the Philippine Medical School became its College of Medicine in 1910.

He was appointed regent of the University for a term of five years. The presidency of the University was offered to him when it became vacant due to the resignation of its American president, but he declined the offer. Years later in a conversation relating to this matter, he remarked that he declined to accept it because it was difficult for any one to succeed in that position on account of political interference.

However, in 1923 he accepted the post of director of the Philippine Library and Museum, being the first Filipino to fill it. A bibliophile, the position fitted him to a T. The employes liked him and they elected him president of the Philippine Librarians Association whose formation was inspired by him.

The University of the Philippines conferred on this illustrious Filipino the degree of LL.D. *honoris causa* after serving the University as regent. Aside from this honor, he received no other from our traditionally noble and grateful nation, while minor intellectuals, mere compilers of other scholars' works or commentators have been conspicuously honored, some even with important highways named after them and others with their effigies adorning postage stamps.

In concluding may I add a postscript. The modern Filipino woman owes Pardo de Tavera a debt of gratitude. He was one of the very rare Filipinos of his time who supported the woman suffrage movement in our country. Although it was an unpopular issue, he gave generously his moral support to the few courageous women who led the movement. He openly advocated the enfranchisement of Filipino women and attended a legislative hearing on the question.

Serafin D. Quiazon, Ph.D., Discussant

Thank you Mr. Chairman, distinguished guests, Academicians.

It is only fitting that Dr. Encarnacion Alzona's lecture on Doctor Trinidad Pardo H. de Tavera and Philippine Historiography is conducted by the National Academy of Science and Technology and is directed towards an academic audience, for Doctor Tavera was a part of this atmosphere. In her paper, she presents information drawn from her thorough knowledge of and personal acquaintance with Doctor Pardo de Tavera, on the basis of which a psycho-historical dimension of his scholarly achievements and successful career is reconstructed and analyzed. She has taken time to pause in order to obtain a sufficient measure of Doctor Pardo de Tavera's inner spirit and his relations to his contemporaries and admirers and to find a method for neatly analyzing his writings to illuminate his full life and his times with depth, clarity, accuracy and economy.

No one can quarrel with Dr. Alzona that Doctor Pardo de Tavera contributed much to the intellectual content of Philippine culture, whether in linguistics, bibliography, history, literature and *materia medica*. Nor can we doubt the anti-friar attitude of Doctor Pardo de Tavera. Using his works, writing, speeches and other source materials, Dr. Alzona with careful eyes to reliability has put together an absorbing bio-bibliographical essay, sympathetic yet fair with, Doctor Pardo de Tavera, an "hijo del pais" and a native of Manila who became an influential voice and one of the central figures in the early American colonial administration. A scholar with such intimate knowledge of the Philippines was, indeed, a valuable asset to the American colonial government. In her efforts to categorize Doctor Pardo de Tavera, she has captured the quintessence of the man who in life was an amalgam of fine traits and whose wide range of active interests emerged in bolder relief.

I concur with Dr. Alzona's observation that while intellectual dwarfs had been lavished with honors, and some even with long avenues named after them and still others remembered with commemorative stamps, we have not done anything to project Doctor Pardo de Tavera's rightful place in our society from the limbo of anonymity into which he had fallen. Perhaps, the National Academy of Science and Technology can do something to rescue Doctor Pardo de Tavera from limbo and to restore him to the

high and honored place he deserves. In Latin America, the National Academy of Sciences devote their energies either in erecting monuments for their great scholars or in placing them in a more proper perspective in the field of intellectual history. Dr. Alzona's article and work on Doctor Tavera will be consulted as valuable source for contemporary and future scholars seeking to understand the man.

May I add in closing that I, too, like Dr. Alzona and many of us here today, are silent admirers of Doctor Tavera. When I assumed the headship of the National Library some fourteen years ago, I discovered his portrait in oil, together with that of Dr. Jose Rizal's by Fabian de la Rosa and our National Flag in water color by Juan Luna, deteriorating in a dingy, cockroach-ridden storeroom of the building. The first thing I did was to salvage his portrait and to hang it inside my office. Since then, this portrait of a socially, dignified, gentleman-scholar with a renaissance mind has been keeping me in good company in my spartan-looking office.

Mita Pardo de Tavera, M.D., Discussant

Thank you Dr. Lagmay and of course, Dr. Alzona and those who are here, and express my deep gratitude in my name and in that of my family for your splendid talk on what to me is a splendid man. Perhaps the most important single influence in my life. And Dr. Quiazon, thank you too for living these 12 years with the image of my grandfather. I'm so overwhelmed by so many Ph.D's, eminently but I would say this. I think that it's time for MD's to share ocassions like these. I think that MD's stand to have a very myopic view. And I think that this is what we need, to be more involved in all the influences that contribute to man. And I would like to say that I am one of those who think of man, matters or of medicine of my specialty not from the physical sense of view but to me, man or health. In other words, it is an expression of physical, social and mental well being of man, so I would say that occassion like this is very welcome to decisions like me who may have in our view.

Now I am certainly not a historian, by training or by vocation or by inclination, but I am a historian by birth. I was fed with history. I live under the shadow of that great tree. I heard history from the time that I could understand things. So, I would say, "think", but that makes me a historian in some sorts of way. Yes, it is in my blood so that because it is my blood, I cannot just stand as a by-stander and just watch even in a strict place. I feel that strong sense of history, and I feel the need to participate and in changes that are taking place in the evolution of our Philippine Society and the restructuring of our society. I cannot claim to have the vast knowledge to Dr. Alzona, but I can speak on certain intimate glimpses about the man that I revered. His role in history would be of one who saw the need to write down, if only to serve as a guide for those coming behind them as to what world of roots of the Filipino people. It appeared then that after 400 yrs. old of Spanish occupation that we as people had lost somehow an identity and this is why he felt a strong sense of recording facts that would point out the origin of the Filipino people. And I would think that what he wrote then, is certainly applicable today because we are even a more confused race and we were after 400 years of Spanish occupation we have had.

Some 70 years, 80 years of American occupation so we are indeed a very sour country that had lost its identity in many ways,

and this is what is being established and we are trying to remember or remind Filipinos as we are fellow Filipinos. Of the origin, he wrote about the Sanskrit, the influence of the Sanskrit in our language, the origin of the number, printing and engraving the customs. He wrote as much as he could about the character of Filipino and the roots of the Filipino. So, from that point of view, I would say that many of his writings are relevant today. Certain views that he may have expressed then, will no longer be applicable in other matters. But then, what he wrote about 55 years ago, may no longer hold true now as views change as structures change. Situation change as Filipinos represent part of the general structure as a nation among nations. I remember him very much as being a very serious sort of person.

You would think that a man who had seen so much examples with him in silent. At the age of 13, he and his family had to leave the Philippines and live in Paris. But before they found the homeland in Paris, they went from country to country looking for a nest. They were in exile because of his uncle's participation in the Cavite Revolt. He wasn't his father, because his father died when he was a young boy and so Joaquin Pardo de Tavera was the most important influence in his life. And as a clan, the whole clan moved on to Paris — Dr. Tavera, his brother Felix, and his sister Paz who later was to marry, Juan Luna. Stimulated by his love of country from the age of 13 he was able to return to the Philippines. When he returned to the Philippines, he was already a Doctor of Medicine. It was in his mother's home that the leaders of this rebellion then would gather in Paris to plan from afar the revolution that necessarily would have to take place.

In fact, I have pictures of that period taken in the home of his mother in Paris which was considered the meeting place of the Filipino "Liberals" as they were called at that time and of course, in France was a place where they found this haven because France was a liberal country and it gave a haven to people with liberal ideas. I think to this day Khomeini fought a revolution from Paris. Well, as I said he was a peerless person he should have seen the example of what happens when you engage in trying to go against the so-called "establishment" but he came back with only one desire and that was to serve his country in whatever way he could. He fought for justice, women's suffrage was a foremost side of justice, he wrote against injustice, pointing out the defects of the Spaniards, of the abuses of the friars of the Spanish regime. So that establishes in many ways his role but a safe position. There is a particular role that strikes me not only as curious but as very relevant. He spoke of the practice or the Medical or the folk medicine in the island of Luzon. And there are things, these are of great interest to me now and in the latter years of my life as a

physician, I started as a clinician, but now I find myself as a physician of people. I am interested in folk medicine, how it can be utilized to serve the interest or the needs of the great majority of the Filipinos. He wrote on medicinal plants and what makes it quite interesting is that in the purpose of his book, he pointed out 80 years ago that we should not dismiss this as just a source of witchcraft or something of primitive medicine, he pointed out that for certain common things, common treatment had a role and this is also my interest, strangely enough my present interest. So yes, he left a great deal, I was just rapidly looking through the list of all the many things he wrote about, referred to them as notes. He wrote monographs on a lot of subjects, I just stop counting at a hundred and twenty last night going through quickly through the list of what he has written about, and he has, I know, countless thoughts that he left unpublished numerous articles that I'm overwhelmed by his output. He was a sensitive writer. He was like Picasso. Picasso left a lot of canvases. Everything he saw with his eyes, he painted. I think in the case of my grandfather everything that he saw, he put them in words and in such beautiful words. So, I, too, keep those books with pepper.

I remember, Dr. Alzona say this, and I couldn't help but a sort of smile inside, because I still find myself the same thing. It's also nice to know that the university that he found help on it's also the university that many of our leaders in our present society have graduated from, and that I can say, I am proud to have also graduated from.

So, I want to thank the members of the National Academy of Science and Technology and Dr. Alzona for having thought of me of representing my grandfather. And perhaps one little secret, it's my maiden name, that if I have carry that maiden name through all these years, it was because in my own small way, I was given honor to this man who had served our country so well. Thank you.

Bonifacio Salamanca, Ph.D., Discussant

I would like first of all to add my congratulations to Dr. Encarnacion Alzona for her brief — but highly informative — paper on "Doctor T.H. Pardo de Tavera and Philippine Historiography", and to commend the National Academy of Science and Technology for providing the venue for its deli ery and presentation.

The timing could not have been better. From the standpoint of the Philippine Historical Association — of which Dr. Alzona was a founding member, first editor of its journal and now a revered member of its Board of Advisers — it is a challenge on this the 25th Anniversary of its birth, for it to embark on a project to examine the contributions of Filipino scholars, like T.H. Pardo de Tavera, to Philippine historiography. As the current PHA president, I shall prod my colleagues to start doing so, using Dr. Alzona's paper as a model. Dr. Alzona's lecture also came at a time when there is a rekindling of interest in Philippine history and a spate of historical writing, inspired to a certain extent no doubt by no less than the President of the Republic's example.

I think there is a little parallelism in the historical and writing careers of T.H. Pardo de Tavera and President Ferdinand E. Marcos. The latter started writing serious Philippine history at a moment and under circumstances when affairs of state are of such magnitude that by simply reflecting on what transpired the day before could overwhelm much younger men. T.H. Pardo de Tavera himself wrote at least two of his major and enduring contributions for the study of Philippine history at the time when he was deeply immersed in the task of nation-building or "nation-saving", by helping establish civil government for the country to replace the Military Regime under the American Government — as one of the first triumvirate of *ilustrados* in the Taft/Philippine Commission — a task which must have demanded so much of his time and energy.

I shall presume that since Dr. Alzona did not mention it, Dr. Pardo de Tavera undertook his writings without the benefit of a battery of research assistants and writers — as is now apparently the practice. We can therefore assume without question that his writings were entirely his own handiwork.

Perhaps, an account of the circumstances under which they were composed, Dr. Tavera's 20th century writings suffer from more than the usual dose of inaccuracies and deficiencies, which

have been convincingly pointed out by a colleague of mine in her master's thesis twenty-one years ago.* This is not to dismiss those works as completely worthless to the scholar. Rather they should be viewed as attempts at historical reconstruction by a many-sided genius whose initial training did not exactly prepare him to make solid contributions to Philippine historical writing — Pardo de Tavera was a doctor of medicine, not a Ph.D. in history — and who was thrust into an exacting political career at the time he wrote *Bibleoteca* and *Reseña historica.*

Historiography is the imaginative but not fictitious reconstruction of the past. It requires a critical examination of a variety of sources, written and oral. It demands an impartial evaluation of sources, written and oral. It demands an impartial evaluation of the evidence. Finally, if he is to be true to his craft, the historian must deduce conclusions and infer generalizations that do not do violence to his facts. Just as important, he must not be swayed by his emotions or bias. Pardo de Tavera's *Reseña historica* evidently does not fully measure up to what present-day historiographers would regard as the attributes of a respectable historical study.

That is not, however, the only way one should view Pardo de Tavera's works. He was, after all, a human being and a Filipino, who lived part of the epoch he wrote about — the 19th century, a most important one in our evolution as a nation; he could not have maintained complete indifference to the behavior of certain sectors of the society, like the all-powerful friars of that epoch. Neither did he have the advantage of hindsight — any more than we would, were we now to assess the New Society in the Philippines before the bar of history. Even those trained in the art of Clio which Dr. Pardo de Tavera was not — would find it impossible not to make judgments which a century from now would appear fully unwarranted and ridiculous.

Viewed in this light, Dr. Tavera's works become all the more impressive, or at least significant. They are there not so much as models, but rather as a lode from which the painstaking researcher may extract valuable nuggets of information — if not also insight.

The philosopher/historian Carl Becker has said that every man is his own historian; another historian has said that every generation writes its own history. Dr. Pardo de Tavera was one member of his generation which viewed Philippine history in its own light. He deserves to be commended to write what he thought was the truth — his own truth.

We owe Dr. Alzona, therefore, a lasting debt of gratitude for giving our generation a *tour de'horizon* into the contributions of T.H. Pardo de Tavera to Philippine historiography. We are intellectually richer thereby.

Algebraically-Closed Groups: An Application of Model Theory to Algebra

By Bienvenido F. Nebres, S.J., Ph.D.

This paper is a short introduction to a method (derived from logic and model theory) of constructing algebraic structures (groups, semigroups, rings, etc.) with given properties. It was first introduced by Abraham Robinson, inspired by Cohen's method of forcing in set theory. We shall show some applications to the theory of algebraically closed groups.

Some Preliminaries on Algebraically Closed Groups

In a 1951 paper [7], W.R. Scott introduced the notion of an algebraically closed group: *A group G is said to be algebraically closed if every consistent finite system of equations, with parameters in G, is solvable in G.* A system of equations is said to be consistent over G, if it has a solution in a group extending G. Scott proved that every group can be extended to an algebraically closed group.

In 1952, B.H. Neumann [5] showed that aside from the trivial group, we get the same class of groups if we replace "finite system of equations" to "finite system of equations and inequations". He also showed that an algebraically closed group is simple.

In 1971, B.H. Neumann [6] considered the isomorphism problem for algebraically closed groups and showed that it would be very difficult to tell two algebraically closed groups apart in the following sense. *Let H be a finitely generated group.* We shall say that H is *recursively absolutely presented* if H is given by a recursively enumerable set of equations and inequations in the generators. Neumann proved: *Any finitely generated group, which is recursively absolutely presentable can be imbedded in any non-trivial algebraically closed group.* This result makes it practically impossible to tell two algebraically closed groups apart by looking at their finitely generated subgroups.

The new results on algebraically closed groups come from applications of A. Robinson's *method of forcing* in Model Theory. The method allows us to construct many non-isomorphic countable algebraically closed groups with specified properties. We shall illustrate the method by giving the proof of the converse to B.H. Neumann's theorem above (due to A. MacIntyre [4]). It will be noted from the proof that the method applies as well to other algebraic structures, such as semigroups, commutative rings, division rings, etc. In fact, there are some very interesting results on the

structures of such algebraic structures, satisfying an analogous notion of algebraic closure (Cherlin[1], Hirschfeld and Wheeler [2]).

Forcing in Model Theory and Omitting Types

Definition. A *condition p* is a finite consistent set of atomic sentences $\varphi(a_1, \ldots, a_n)$ and negated atomic sentences $\neg\varphi(a_1, \ldots, a_n)$ (in our case, equations and inequations in $a_1, \ldots, a_n$).

Definition. Given a condition p and a sentence φ, we define "p forces φ" (written $p \Vdash \varphi$) as follows:

(1) If φ is atomic, $p \Vdash \varphi$ iff $\varphi \in p$

(2) If φ is $\varphi_1 \vee \varphi_2$, $p \Vdash \varphi$ iff $p \Vdash \varphi_1$ or $p \Vdash \varphi_2$.

(3) If φ is $\varphi_1 \wedge \varphi_2$, $p \Vdash \varphi$ iff $p \Vdash \varphi_1$ and $p \Vdash \varphi_2$

(4) If φ is $\exists \chi \ \varphi(\chi)$, $p \Vdash \varphi$ iff for some a occurring in p, $p \Vdash \varphi(a)$.

(5) If φ is $\neg\varphi$, $p \Vdash \varphi$ iff there is no condition $q \geq p$ such that $p \Vdash \varphi$.

(6) If φ is $\forall\chi\varphi(\chi)$, then $p \Vdash \varphi$ iff $p \Vdash \neg\exists\chi\neg\varphi(\chi)$.

Definition. Let G be a group. G forces a sentence φ iff some condition p true in G forces φ.

G is a *generic group* iff for any sentence φ defined in G, φ is true in G iff G forces φ.

The interest of forcing a generic group is as follows: We can construct a sequence of conditions

$$p_0 \subset p_1 \subset p_2 \subset \ldots \subset p_n \subset \ldots$$

in such a way that the union $\bigcup_{n=0}^{\infty} p_n$ is a maximal consistent set of equations and inequations. It determines a unique generic group G. It can be easily shown that generic groups are algebraically closed. By a careful construction of the conditions p_n, we can make G satisfy certain predetermined properties.

To get further refinements on the structure of G, we introduce the notion of "G omitting a type".

Definition. Δ is a quantifier-free n-type if

(i) Δ is a set of basic formulas with free variables $v_0, \ldots, v_{n-1}$ (basic means "atomic or negated atomic")

(ii) Δ is consistent with the axioms for groups

(iii) For any atomic formula $\varphi(v_0, \ldots, v_{n-1})$ either $\varphi \in \Delta$ or $\neg\varphi \in \Delta$.

Definition. A group G realizes a quantifier free n-type if $\exists\, a_0, \ldots, a_{n-1} \in G$ such that $\varphi(a_0, \ldots, a_{n-1})$ is true in G for all $\varphi \in \Delta$.

We now illustrated the use of forcing and omitting types by giving MacIntyre's proof of the converse of B.H. Neumann's theorem.

Applications of Forcing and Omitting Types

Main Theorem. Let Δ be a quantifier-free n-type, which is non-recursive. Then there exists a generic group G which omits Δ.

Corollary. Let H be a finitely generated group, which is imbeddable in all non-trivial algebraically-closed groups. Then H can be recursively presented with solvable word-problem.

Proof of Corollary. Let $a_0, \ldots, a_{n-1}$ be generators for H and let $\Delta = (Q(v_0, \ldots, v_{n-1}): \varphi(a_0, \ldots, a_{n-1})$ is true in H. Then Δ is a quantifier-free n-type. We claim that Δ is recursive. For suppose otherwise.

Then by the main theorem, there is a generic group G which omits Δ. But then this means that H cannot be imbedded, in G, (if we had an imbedding $a_i \to a_i'$ where $a_i' \in G$, then $a_0', \ldots, a_{n-1}'$ in G would realize Δ). Since G is generic, it is algebraically closed. We would then have a contradiction to the hypothesis. Finally, Δ recursive clearly implies that H is recursively presented with solvable word problem.

Proof of the Main Theorem. The proof depends on the construction of a sequence of conditions $q_0 \leq q_1 < q_2 \leq \ldots \leq q_m \leq \ldots$ and atomic formulas $\varphi_0, \varphi_1, \ldots, \varphi_m, \ldots$ the following satisfying properties:

Let φ_m be an enumeration of all sentences (in the language of groups with constants $c_0, \ldots, c_n, \ldots$), $\delta_0, \delta_1, \delta_2, \ldots, \delta_m, \ldots$ an enumeration of all possible finite sequences of terms. Then

(1) For each atomic sentence φ, either $\varphi \in \bigcup_m q_m$ or $\neg\varphi \in \bigcup_m q_m$

(2) For each m, either $q_{m+1} \Vdash_r \varphi_m$ or $q_{m+1} \Vdash_r \neg\varphi_m$

(3) If $\delta_m = \langle t_0, \ldots, t_{n-1} \rangle$ then
either (a) $\varphi_m(v_0, \ldots, v_{n-1}) \in \Delta$ and $q_{m+1} \Vdash_r \neg\varphi_m(t_0, \ldots, t_{n-1})$
or (b) $\neg P_m(v_0, \ldots, v_{n-1}) \in \Delta$ and $q_{m+1} \Vdash_r \varphi_m(t_0, \ldots, t_{n-1})$

The difficult part is to satisfy condition (3) and it is here that we use the fact that Δ is not recursive. The rest is straightforward.

From $\bigcup_m q_m$, we then get a generic group G. The elements of G are named by terms t and thus for any elements $a_0, \ldots, a_{n-1}$ of G we have from condition (3) that there exists a formula $\varphi(v_0, \ldots, v_{n-1}) \in \Delta$ such that $\varphi(a_0, \ldots, a_{n-1})$ is false in G.

Thus G omits Δ.

Further Results

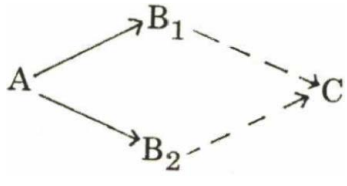
The method of forcing and omitting types can be used to construct generic groups omitting certain preassigned sets of types. Thus the method gives us a way of studying the structure of algebraically closed groups. Among the more interesting results are:

(1) Every countable algebraically closed group contains a proper copy of itself.

(2) Every countable algebraically closed group has $2^{\int v_0}$ automorphisms.

The method also applies to other algebraic structure, such as commutative rings, inverse semi-groups, division rings. It constructs generic (rings, semi-groups, division rings), which may be seen to be "algebraically closed" in an appropriate sense.

The results on division rings are particularly interesting. Analogous to the result above on algebraically closed groups, we have that: "Every countable algebraically closed division ring D contains a proper copy of itself." From a recursion-theoretic point of view, the theory of "algebraically- closed" division rings is very complex. More precisely, first-order arithmetic can be interpreted in the theory of "algebraically closed" division rings. The method of forcing we described above is called *finite forcing*, distinguishing it from another method called *infinite forcing*. The structures constructed by infinite forcing are called infinitely generic. If we take the theory of *infinitely generic division rings*, the second-order arithmetic can be interpreted in this theory. The results on division rings depend on a very important theorem of P.M. Cohn: *The theory of division rings has the amalgamation property*, i.e., in the category of division rings and embeddings, any diagram of the following type can always be completed:



References

1. Cherlin, G., *Model-Theoretic Algebra: Selected Topics*, Springer-Verlag Lecture Notes in Mathematics, #521, Berlin, 1976.

2. Hirschfeld, J. and Wheeler, W., *Forcing, Arithmetic, and Division Rings*, Springer-Verlag Lecture Notes in Mathematics, L454, Berlin, 1975.

3. MacIntyre, A., "On Algebraically Closed Groups", Annals of Mathematics (1973) pp. 53-97.

4. , "Omitting Quantifier-Free Types in Generic Stuctures", *Journal of Symbolic Logic*, Vol. 37 (1972) pp. 512-520.

5. Neumann, B.H., "A Note on Algebraically Closed Groups", *Journal of the London Mathematical Society*, vol. 27 (1952) pp. 247-249

6. , "The Isomorphism Problem for Algebraically Closed Groups", Proceedings of a Conference on Decision Problems in Group Theory, North-Holland, Amsterdam, 1971.

7. Scott, W.R., "Algebraically Closed Groups", Proceedings American Mathematical Society 2 (1951), pp. 118-121.

Heinrich Kuhn, Ph.D., Discussant

I would first like to say that this is clearly a very sophisticated area of mathematics and anybody who is a specialist in this area should serve our highest consideration. It's certainly correct to mention from the Australian Algebraes that this algebraic structures are so complicated that algebraes would not dare to. This would lead to my hope that in the future, there would be found more conventional truths of these results. By more conventional, I mean of course purely algebraic proof. But I think this is visual thinking and therefore one has really to say that the contribution made by the model theories especially, are really very impressive for algebraes and I personally find these things very hard to understand.

As an algebraic also, one would certainly be interested in knowing how this algebraically-closed groups are really constructed and then one would hope for getting an information on other types of isomorphisms of these groups and also this is probably almost helpless since one cannot only prove that many finitely generated groups can be imbedded in these groups but also certain types of group which are not finitely generated can be imbedded always in a non-trivial algebraically-closed group. So, all these questions are really of high complexity.

I would like to mention the fact that the result which was mentioned by Fr. Nebres, that the automorphism group has become very big and would lead to the question, how the automorphism group module slowly became part of the automorphism group. The so-called automorphism which look like, I would be very interested in knowing that, but I think nobody knows this. Also, the connection to verbally complete groups I would like to know, because these verbally complete groups are very closely connected to the algebraically-closed groups, but of course we cannot go to all these technical details.

I just wanted to mention certain things which I have been doing in this country, since coming to this country 3 years ago, I have tried to get some people here in the country interested in the things I have been doing, especially certain questions in the theory of finite groups which is in comparison to this algebraically-closed group, a lot easier and I am quite happy that I found a number of

people in the country who worked very well together with me and especially I would like to mention the on-going Ph.D. program in the country which seems to be successful.

I have been a visiting professor not only in the Philippines but also in other developing countries. I was in Brazil and I know their situation there relatively well. I have to say that the potential which is here in the country is bigger than in Brazil because I found that Filipinos are willing to accept tremendous effort in studying mathematics and I would really hope that these talented young people get all the support which is necessary for doing these things. I will probably stay a little bit longer in the country and I have more students who work together with me and I'm very glad at the outcome of these results, some of the results we got are impressive and I'm happy that I could be of help a little bit in this on-going effort and I would like to say finally, that I really hope that this program will go on and will get support from all the countries.

Jose Marasigan, Ph.D., Discussant

My comment would be of a more general nature. From the mathematical conferences I have attended. (I was at the International Congress of Mathematicians in Helsinki, and then in April this year, I was in Japan where I was fortunate enough to have been invited to attend the Spring meeting of the Japan Mathematical Society) I have been more and more convinced that algebra should be more developed in our country.

When you go to these conferences, you would notice, for example, that the language of algebra and geometry is presumed. If you want to be abreast of what's going on in these conferences, you need to know algebraic geometry. Also I would like to mention the fact that algebra is something that is not as useless as it has often been supposed to be. A very good example was the recent lecture of Dr. Eduardo Mendoza (University of Wuppertal) where he showed the applications of HADAMARD matrices in coding and information theory. Also when I was in Darmstadt a year ago, I found out that mathematical ideas considered before as purely abstract, are now being applied in areas like data structure, and data management.

In closing, I think there is a need in our country to develop algebra and I hope that the authority will give all the support we need in the development of algebra. We need the assistance of experts from other countries in our initial endeavor. From Japan, for example, we can invite many mathematicians. I have talked to some of them and they are willing to come and give lectures in algebraic geometry and other areas of mathematics.

ESSENTIAL OILS

By Luz O. Belardo, Ph.D., Academician

PART I
Introduction

It is a pleasure to present a paper on a fascinating area of natural products, the essential oils. The coverage of this presentation is limited to brief discussions on the nature, production, chemistry, and uses of essential oils; hence, it is by no means complete. Occasionally, some of the results of my chemical study on some new Philippine essential oils will be touched.

Essential oils are so-called, not because they are in a way necessary to the well-being of the users,. but because they are essences. The word essential in this case was derived from the Paracelsian concept (1493-1541) of *Quinta essentia* or *quintessence*, meaning the fifth element after the alchemist's mercury, sodium chloride, sulfur and lead. It was considered the last possible and most sublime extract of the most condensed form of the plant or drug.

Since antiquity, man has been mystified by essential oils and has made great efforts to obtain them. The whims and demands of royal families for perfumed baths, luxurious boudoirs and sumptuous banquets compelled the hot search for aromatic plants. It will be recalled that the quest for spices was concomitant with the ambition of early naval expeditions to discover and conquer new lands far across the seas.

Essential oil is also called volatile oil, because it is a greasy liquid, and volatile because the spot it leaves on brown wrapping paper loses its translucency and finally disappears.

Production

Essential oils are obtained from different parts of plants, rarely from animals. The traditional methods of extracting the oil are distillation, expression, extraction with low-boiling solvents, and enfleurage, each method being chosen according to the suitability to the plant material. Distillation is the most commonly used method. Expression is limited to the obtention of the oil by piercing or rasping the oil sacs in the rinds of fruits as in the case of oil of orange. Low-boiling solvents extract components of oil in practically intact condition that can produce perfumes of very fine quality reproducing to a remarkable degree the odor of the living flowers. Ilang-ilang oil that is used in the creation of the masterpieces of French perfumery is obtained, not by distillation, but by

extraction with low-boiling solvents. Enfleurage utilizes fats to capture the delicate bouquet of flowers where physiological activity of developing and giving off perfumes continue even after picking, as in jasmine. Each of the aforementioned methods has its merits and demerits.

A number of ways of extraction have been developed for special cases. Extraction with liquid carbon dioxide, vacuum-tight distillation, and warm fermentation prior to distillation are in use (1). A process that was introduced in the '70s and was found very useful in accounting for the greatest number of volatiles from plant materials is the headspace technique (2). The volatiles from a sample are trapped by a porous material. It is brought into equilibrium with a gas phase over it in a closed vessel after which a certain volume of the gas phase is injected into a GC for qualitative and quantitative purposes.

A method which is claimed to yield the genuine essential oil consists in piercing the oil sac of the plant and drawing the oil by means of a syringe-like glass capillary, and analyzing directly with GLC.

Nature of Essential Oils

Essential oil is characterized by an enormous chemical complexity. It is a commodity the chemistry of which, one might say, embodies a definition of organic chemistry, that is, chemistry of hydrocarbons and their derivatives. Almost every class of organic compounds is likely to be present (hydrocarbons, oxygenated, sulfuretted, nitrogenated and even halogenated compounds) in multiple combinations and in varying proportions. Each essential oil has its main component(s) and many minor ones. The predominant component of oil of peppermint is menthol; oil of turpentine, pinene; oil of anise, anethole.

Among the physical properties, odor and taste are preferentially looked into: odor by the perfumer, and taste by the flavorer.

The properties of each oil are imparted by the combined attributes of all its constituents. The chemical properties depend upon the functional group or groups present in each component.

Fractionation and Analysis

In the distant past, essential oil chemists depended solely on fractional distillation for the separation, and on solid derivatives formation for the identification of components of essential oils.

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(1) Jenkins, G. & W. Hartung, *The Chemistry of Organic Medicinal Compounds,* John Wiley & Sons, N.Y., 1949, page 283.

(2) Sirikulvadhana, S., W.G. Jennings & G. Vogel, *International Flavors & Food Additives, 6,* 126 (1975).

With the advent of chromatography came what may be called revolution in essential oil chemistry. Some findings that were obtained by methods of long long ago are, today, in need of review and re-evaluation because some of them are found wanting in the light of modern methods of analysis. Fifteen years ago, the bilberry fruit was reported to contain no more than ten volatile constituents. Lately, its gas chromatogram revealed a total of 109 components. From 1891 to 1976, jasmine flower was known to have only fifty volatiles. In 1977, one hundred and thirty compounds were reported (3). The many attempts at reconstituting the flavor of our breakfast beverages resulted in the discovery thru GC that coffee has six hundred volatiles; cocoa, three hundred twenty; and tea, three hundred sixty (4). Such is the wonder of the gas chromatograph.

Today, column chromatography, thin layer chromatography and gas liquid chromatography, and often a simultaneous combination of them are used for the fractionation of essential oils.

Chemical means of separating groups of components of essential oils have not been done away with. In some instances when the presence of phenols, lactones, and acids are indicated, the oil and/or the absolute is treated with sodium carbonate or sodium hydroxide before applying column chromatography.

A method which this author used with success in the analysis of the oil of *Peperomia pellucida* L. (HBK) (5), is a large automated steady state counter current distribution which has 100 tubes of 25 ml. capacity each. The beauty of the set-up lies in its capacity to hold much more quantity of oil than what preparative GLC can accommodate. Moreover, it does not need much attention as it can run by itself thru the night after which the fractions are picked up from the collectors in the morning.

In industry, however, fractionation in large scale is still used, employing giant fractionating columns under pre-tested conditions that would insure stability of specific fractions desired.

Other separation techniques include gel permeation chromatography which is a form of liquid chromatography that sorts polymer molecules in a gel-packed column according to their size in solution. Also, the droplet counter current chromatography which is an all liquid separation technique that is based on the partitioning of solute between a steady stream of droplets of moving phase and column of surrounding stationary phase.

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(3) *Proceedings of the VIIth International Congress of Essential Oils* (Kyoto, Japan, October, 1977) published March, 1979, page 473.

(4) Vitzthum, O.G. & P. Werkhoff, *Aroma Analysis of Coffee, Tea and Cocoa by Headspace Technique*, Hag, Hagstrasse, Bremen, W. Germany, Page 115. Thru *Int. Flav. & Food Additives, 10,* 79 (1979).

(5) Oliveros-Belardo, L., *Perf & Ess. Oil Record, 58,* 359 (1967).

In the search for and in the identification of new compounds of which essential oils seem to be inexhaustible sources (as the kind and number of organic compounds obtained therefrom are still on the increase) GLC is undoubtedly very useful. However, there are still a number of conservative and seasoned perfumers who are not yet completely sold to the efficacy of the gas chromatography. They say (6): —

> "It is true that the gas chromatograph has proved useful separating components of essential oils, but in dealing with trace elements that are the key factors in giving an essential oil its character, the best chromatograph is the nose with a detector far more sensitive than any detector."

It is a fact that all essential oils have numerous trace components and nuances, all of which contribute to the total aroma of the oil. Remove any of them and the odor of the oil changes. A gas chromatograph will show how many trace components there may be, but may not identify all of them, whereas a trained nose can tell if something is missing in or something is different in the usual oils that it sniffs from day to day. To this, I might say that the gas chromatograph and the nose can complement each other. The GLC is objective, the nose could be subjective. Besides there is still a lack of trained noses. It is for this reason that the art and science of olfaction are being developed and fully utilized now by big essential oil and perfumery houses because it is recognized that it is the nose that helps the perfumer envision and determine the scent that perchance would entice and satisfy the ever changing preferences of milady for varied creations in perfumery.

It has now become common practice to couple the GC with some auxillary identification apparatuses by leading part of the effluent material to other types of equipment. Thus, there is the GC-MS, GC-IR, and even GC-TLC. However, the coupling would give valid results only when an eluted peak represents just *one* compound. In the case of terpenic components where isomerism is a characteristic property, an eluted peak may consist of more than one substance. This problem has motivated the search for an innovative set-up and led to the development of what is called *Tandem Gas Chromatograph* (7). The system is actually made of two independent GC machines. The first one has the main column for survey of peaks. It has also a smelling port. The second machine has two columns, one of which is polar, and the other non-polar, The effluent from the first oven is led to the two columms of the

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(6) From Editorial, Flavor *Industry*, *1*, 284 (1970).

(7) *Proceedings of the VIIth International Congress of Essential Oils* (Kyoto, Japan, October, 1977) published March, 1979, page 323.

second oven where any complex peak is resolved into separated peaks and finally led to an attached IR spectrophotometer. This innovation satisfies the need, not only of the flavorer and perfumer for a "sniff test" thru the smelling vent, but also of the analytical chemist for his identification interest.

Spectroscopy has triggered an immense advancement in the identification of natural products. On account of the rapidity with which it can "thumb print" an organic compound, the spectroscopes have become indispensable tools in the study of essential oils, considering the fact that many of the liquid components of essential oils are unstable and need immediate analysis as soon as they are isolated.

Conclusions that are based on the short-cut methods of GC retention time, UV, IR, NMR, MS as compared with authentic standards, have been considered valid and are acceptable. It is obvious, however, that such comparison method will work with already known compounds but not with new unknown ones. It would still be necessary to coordinate spectroscopic results with identification of solid derivatives. Accomplishing its synthesis is a sure way of establishing the absolute configuration.

Uses of Essential Oils

Essential oils are used in medicine, pharmacy, industry and agriculture. Their extensive uses, however, are as odorant and flavorant.

They provide the elements for making perfumes — those favorite magical concoctions that evoke people, events and emotions. Perfumes are likened to melodies. Soft music in its perfect tonal harmony, is soothing to the nerves and inspiring to one's spirit. Similar elating effects are derived from perfume which is akin to a harmony, a pleasant blending of fragrant materials extricated from plants.

The possibilities for countless creations by the perfumer is tremendous. While the musician has only eight basic notes out of which thousands of melodies have already been composed, the perfumer has today no less than 4,000 fragrance ingredients at his disposal with which he can create millions of possible combinations (8).

Flavor oils, like oils of garlic, onions, cloves, cinnamon and pepper, are the donors of the appetizing odor of roasting barbecue

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(8) Lindsay, A. F., *Household and Personal Products Industry, 11,* 40 (1974).

and chicken *adobo* (9). The diner's appetite is further satisfied with a cup of ice cream the flavor of which is accented with essences from jackfruit and mango. Rounding up the elegant dinner is the brewed coffee whose enticing aroma emanates from its volatile oil.

PART II

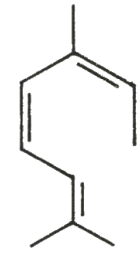
A. Types of constitutents found in essential oils, represented by selected terpenic and sesquiterpenic components.
B. Characteristic reactions illustrating the chemical properties of essential oils.
C. Gas chromatograms of some Philippine essential oils studied by L. Oliveros-Belardo.

TYPES OF CONSTITUENTS FOUND IN ESSENTIAL OILS

A. HYDROCARBONS, NON-TERPENIC AND TERPENIC.
   1. PARAFFINS. EXAMPLE, NORMAL HEPTANE found in *PITTOSPORUM RESIMFERUM* HENSL.
   2. OLEFINS.
      a. MONENES. (ONE DOUBLE BOND, F), EXAMPLE, OCTYLENE.
      b. DIENES (TWO F's), EXAMPLE, ISOPRENE.
      c. TRIENES (THREE F's), EXAMPLE, ACYCLIC TEPENES:

MYRCENE

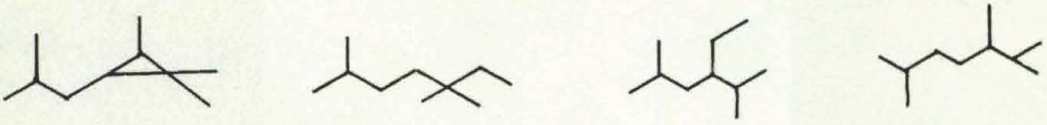
OCIMENE



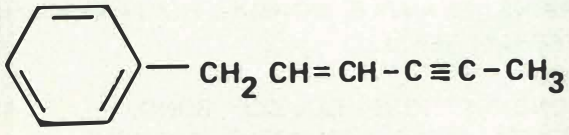
The skeleton formulas above obey Wallach's (1887) isoprene rule for terpenes.

(9) A Filipino native dish of chicken or pork cooked with salt, vinegar and spices, garlic predominating.

Some monoterpenes, like those found in *Compositae* plants, do not follow the isoprene rule. They have the following carbon skeletons:



3. CYCLIC
   a. AROMATIC
      EXAMPLES:
      p–CYMENE IN NUMEROUS VOL. OILS (turpentine, lemon, coriander, cinnamon, sage, origanum, etc.)
      STYRENE IN STYRAX AND HONDURAS BALSAM NAPHTHALENE IN OILS OF CLOVES, ORRIS ROOT AND STYRAX
      AN INTERESTING BENZENE DERIVATIVE IS AGROPYRENE, THE FIRST HYDROCARBON WITH AN ETHYLENIC AND AN ACETYLENIC LINKAGE OBSERVED IN NATURE.
      95% IN THE OIL OF *AGROPYRUM REPENS*

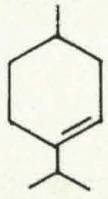


AGROPYRENE

95% in the oil of *Agropyrum Repens*

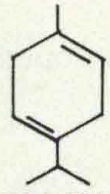
SELECTED EXAMPLES OF CYCLIC TERPENES FOUND IN ESSENTIAL OILS.

(1) MONO CYCLIC
(a) MONENES, ONE F

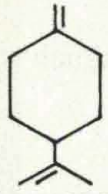


Δ^3 – MENTHENE (THYME OIL)

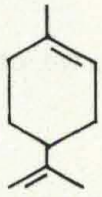
(b) DIENES, F'S BOTH INSIDE, OR OUTSIDE OR ONESIDE AND THE OTHER OUTSIDE OF THE CYCLE:



TERPINENE (OCIMUM OILS)

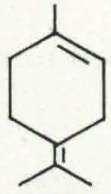


ℓ LIMONENE

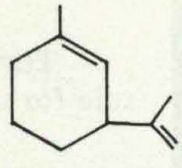


LIMONENE

(CITRUS OIL)



TERPINOLENE (MANILA ELEMI)



SYLVESTRENE (PINE NEEDLE)

(c) POSSIBLE ISOMERISM

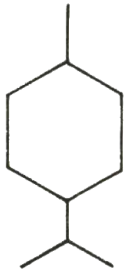
POSITION ISOMERS, DEPEND ON POSITION OF F'S.
OPTICAL (DEXTRO AND LEVO).
RACEMIC (OPTICALLY INACTIVE).
GEOMETRIC (CIS-AND TRANS-).
CONFORMATION ISOMERISM (CHAIR AND BOAT FORMS).

CHAIR CONFORMATION

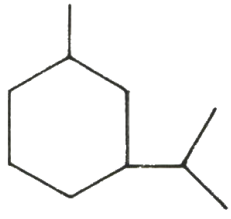
BOAT CONFORMATION

b. HYDROAROMATIC

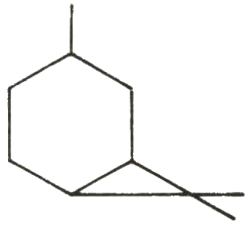
Some ring systems from which the structures of cyclic terpenes, $C_{10}H_{16}$, and their oxygenated compounds are derived:



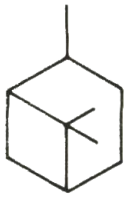
P-MENTHANE



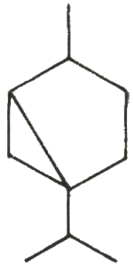
M-MENTHANE



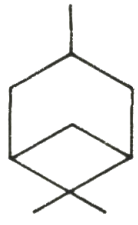
CARANE



PINANE

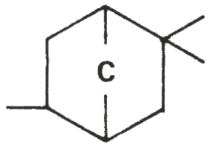


THUJANE

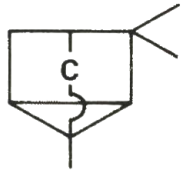


ORTHODANE

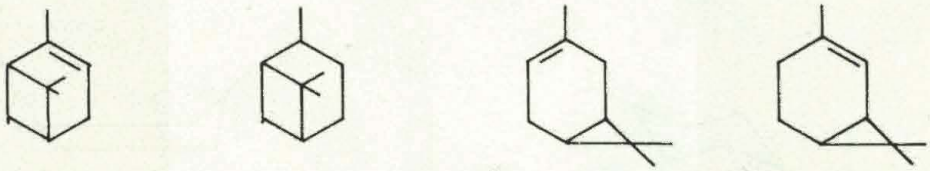
CAMPHANE



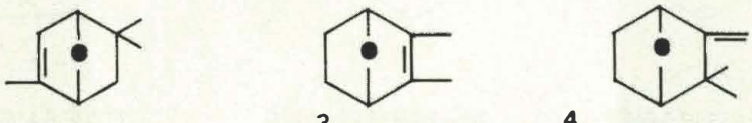
FERNCHANE



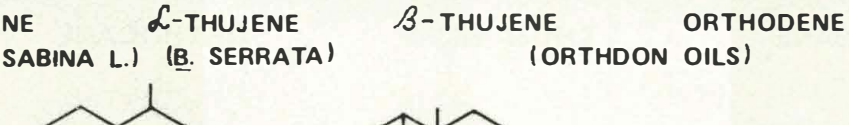
CYCLOFENCHANE



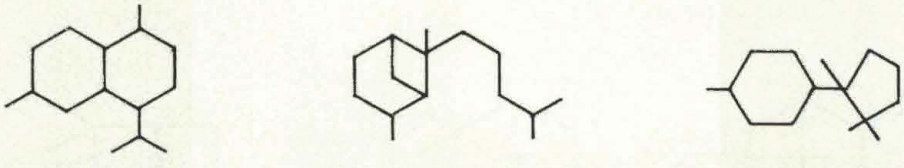
(2) BICYCLIC TERPENE HYDROCARBONS, TWO CYCLES AND ONE DOUBLE BOND.



α-PINENE β-PINENE (TURPENTINE OIL) Δ^3-CARENE Δ^4-CARENE (TURPENTINE OIL)

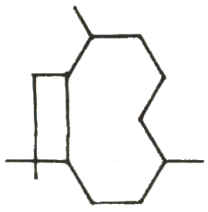


SABINENE (JUNIPERUS SABINA L.) α-THUJENE (B. SERRATA) β-THUJENE ORTHODENE (ORTHDON OILS)

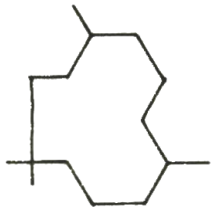


FENCHENE SANTENE (SANDALWOOD OIL) CAMPHENE (EUCALYPTUS OIL)

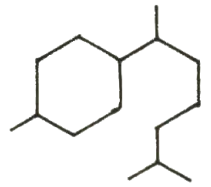
Each of these ring systems is the carbon skeleton for the Sesquiterpene Hydrocarbon or Hydrocarbons written below it.



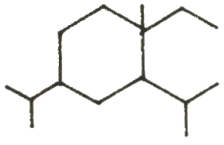
THE CARYOPHYLLENES



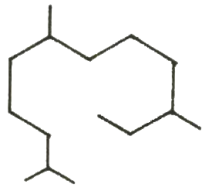
THE HUMULENES



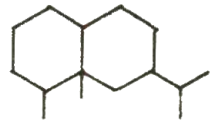
THE BISABOLENES
ZINGEBERENE
SESQUIPHELLANE
DRENES
THE CURCUMENES



THE ELEMENES



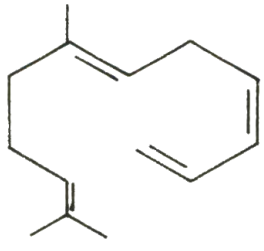
THE FARNESENES



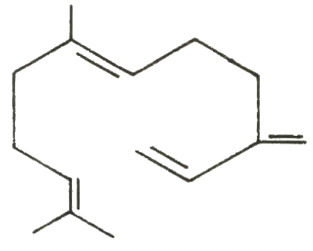
VALENCENE

SOME EXAMPLES OF SESQUITERPENE HYDROCARBONS, $C_{15}H_{24}$

1. ACYCLIC

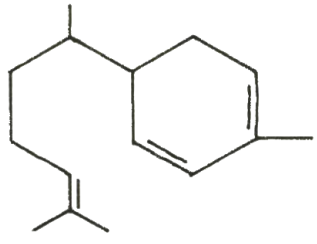


SESQUICITRONELLENE
(CITRONELLA OIL)

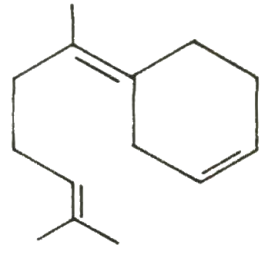


BETA FARNESENE
(YLANG YLANG)

2. MONOCYCLIC

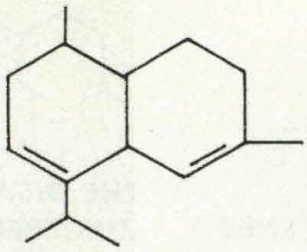


ZINGIBERENE
(OIL OF GINGER)

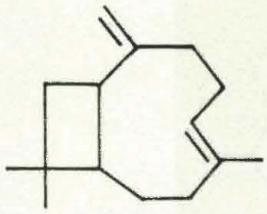


GAMMA BISABOLENE
(OIL OF MYRRH)

3. BICYCLIC

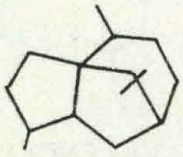


ALPHA CADINENE

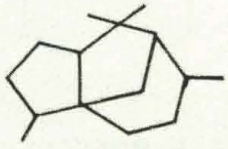


CARYOPHYLLENE

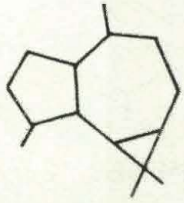
Each of the following ring systems is the skeletal structure for the Sesquiterpene Hydrocarbon or Hydrocarbons written be low it.



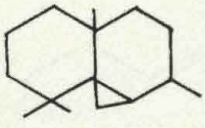
THE PATCHOULENES
CYPERENE



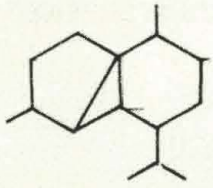
THE CEDRENES



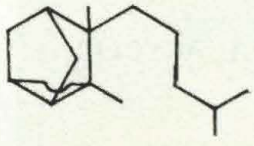
THE GURJUNENES
ALLO-AROMADENDRENE



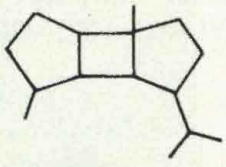
THUJOPSENE



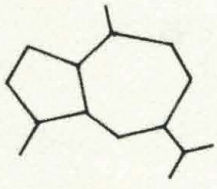
THE CUBEBENES



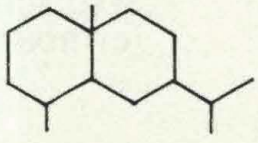
THE SANTALENES



THE BOURBONENES



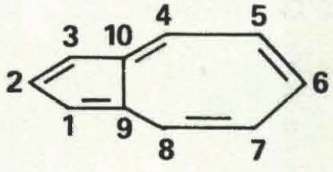
THE GUAIENES



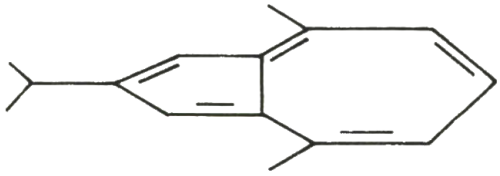
THE SELINENES

A group of Hydrocarbons, closely related to the Sesquiterpenes, are the *Azulenes.* The interesting feature of the basic compound is its pure blue color from which time the name azulene was derived. The colors of the derivative range from blue-violet, violet, red-violet or even green.

CARBON SKELETON :

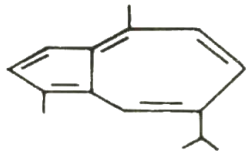


EXAMPLES:

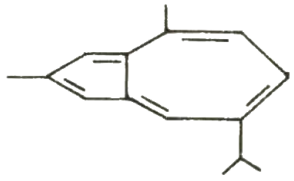


VETIVAZULENE

GUAIAC OIL (SESQUITERPENE FRACTION) —S, H_2→ BLUE GUAIAZULENE,

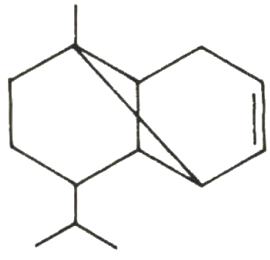


GUAIAC OIL (SESQUITERPENE FRACTION) —Se, H_2→ VIOLET GUAIAZULENE,

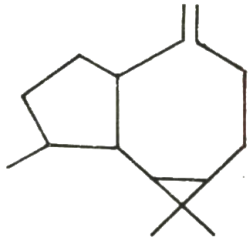


Occurence: Oils of chamomile (15%), Guaiac, Cubeb, Valerian, Galbanum, Wormwood, Eucalyptus, etc.

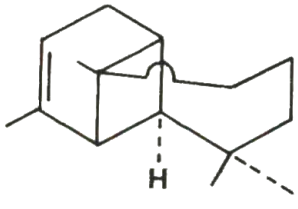
4. Examples of Tricyclic Sesquiterpene Hydrocarbons.



COPAENE
(70% IN THE OIL OF *SINDORA INERSIS* FROM DAVAO, PHILIPPINES).



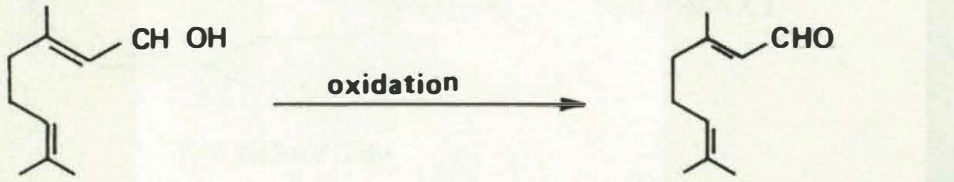
ALLO-AROMADENDRENE
(MAJOR COMPONENT OF OIL FROM WOOD OF PHILIPPINE *SANDORICUM KOETJAPE* (BURM. f.) M. ISOLATED IN 1969), by L.O. BELARDO



ALPHA-LONGIPINENE
(ISOLATED AS A NEW SUBSTANCE FROM SWEDISH PINE OIL IN 1965. ISOLATED AS ONE OF THE MAIN COMPONENTS FROM OIL OF THE WOOD OF *S. KOETJAPE* (BURM.f.) MERR. IN 1969), by L.O. BELARDO

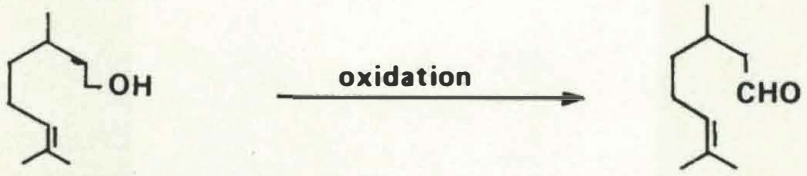
ALCOHOLS ALDEHYDES

OF CONSIDERABLE IMPORTANCE IN PERFUMERY:

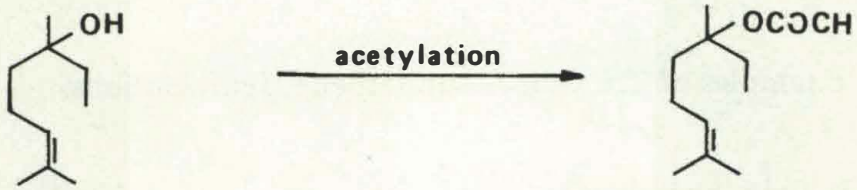


GERANIOL GERANIAL (CITRAL A)

NEROL NERAL (CITRAL B)

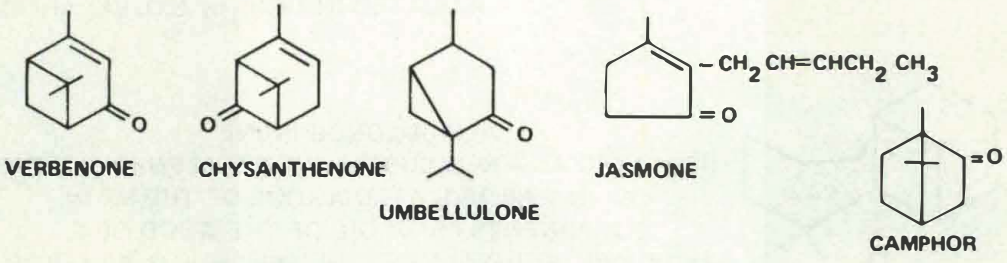


CITRONELLOL CITRONELLAL

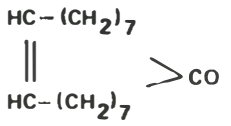


LINALOOL LINALYL ACCETATE

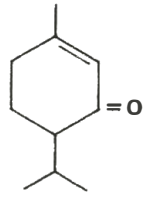
KETONES



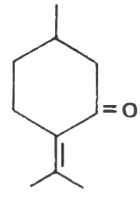
VERBENONE CHYSANTHENONE UMBELLULONE JASMONE CAMPHOR



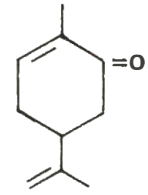
CIVETONE



PIPERITONE

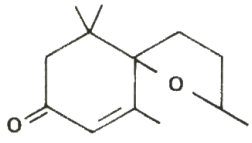


PULEGONE

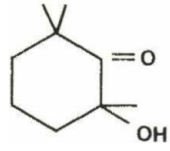


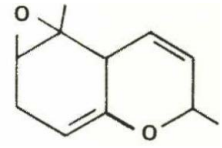
CARVONE

SOME KETONIC COMPONENTS OF BLACK TEA AROMA:



TEASPIRONE
(ALREADY SYNTHESIZED)





ESTERS

Esters are usually responsible for the fragrant odors of essential oils even if they are present in small quantities.

Some examples are:

Benzyl Acetate — in the flower oils of gardenia, jasmine and sampaguita.

Benzyl Benzoate — in ilang-ilang floral oil.

Cinnamyl Cinnamate — in cinnamon spice.

Geranyl Acetate — in oils of citionella, lemongrass, geranium, lavender, coriander, eucalyptus, etc.

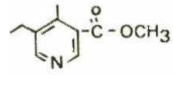
Linalyl Acetate — 70% in lavender floral oil.

Menthyl Acetate — in peppermint oil.

Methyl Anthranilate — in jasmine, tuberose, gardenia, ilang-ilang, champaca

Methyl Salicylate — in gaultheria and birch trees.

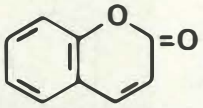
Methyl 4 — Methyl-5-ethylnicotinate, was found to be the Major basic component (29%) of *Jasminum sambac* from China.



LACTONES

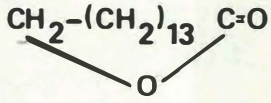
SOME EXAMPLES

1. COUMARIN



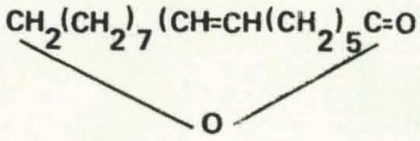
widely distributed in plants.
widely used in perfumes.

2. EXALTOLIDE



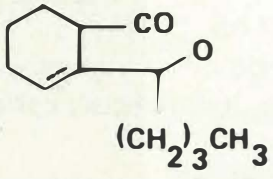
from angelica root
used in high grade perfumes.

3. AMBRETTOLIDE



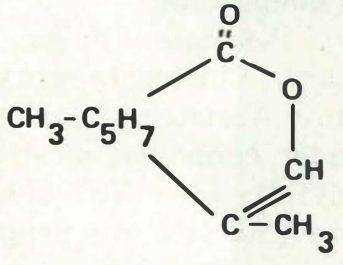
from ambrette seed.
Has the odor of musk.

4. SEDANOLITE



An odoriferous compound in
oil of celery seed.

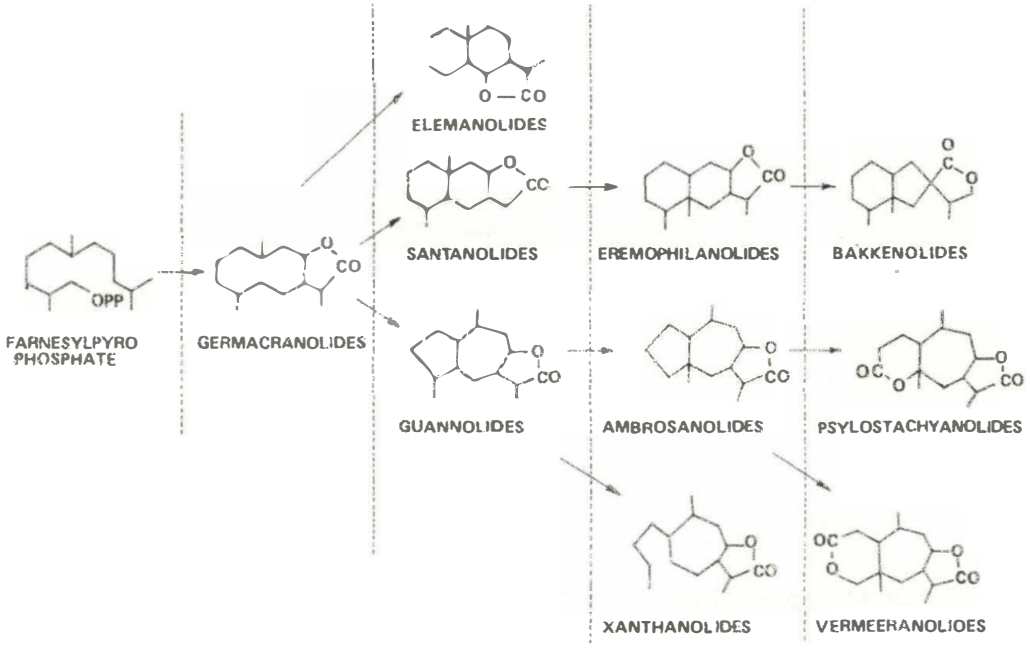
5. NEPETALACTONE



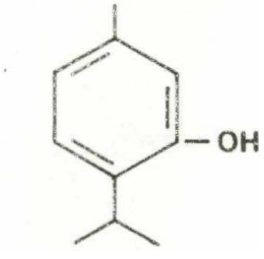
From oil of catnip plant
Attracts some species of cats
and makes them playfully excited.

SESQUITERPENIC LACTONES

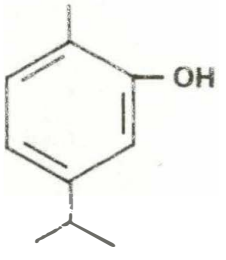
Scheme of the supposed biosynthesis of sesquiterpene lactones, each of which is derived from any of the Carbon skeletons below. Family *compositae* alone has 500 of these compounds of various types.



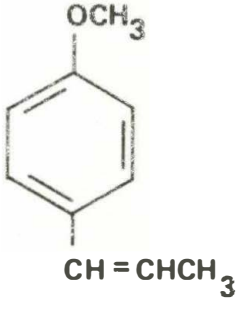
PHENOLS AND PHENOL ETHERS



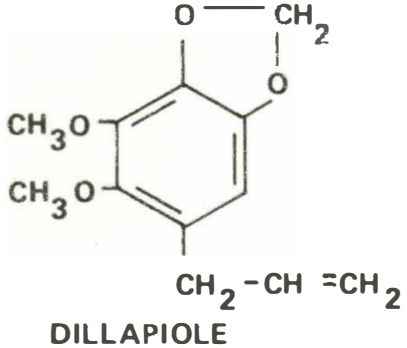
THYMOL



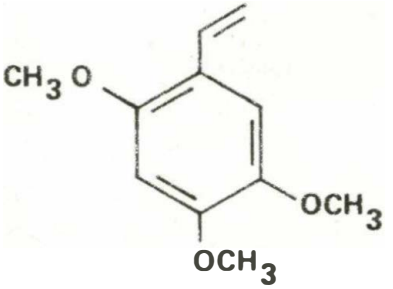
CARVACROL



ANETHOLE

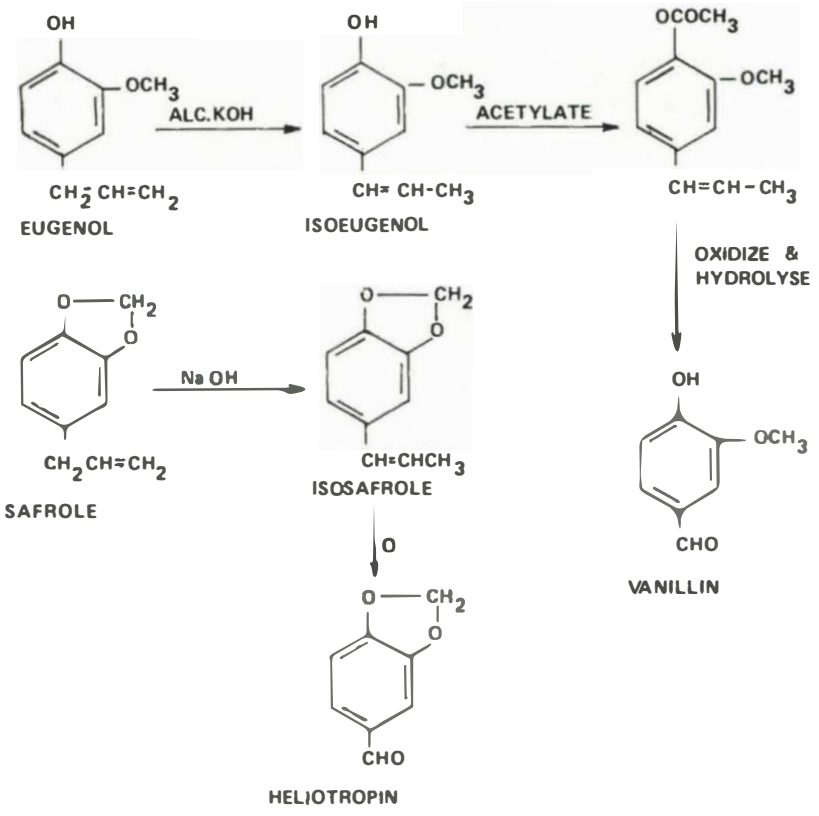


DILLAPIOLE



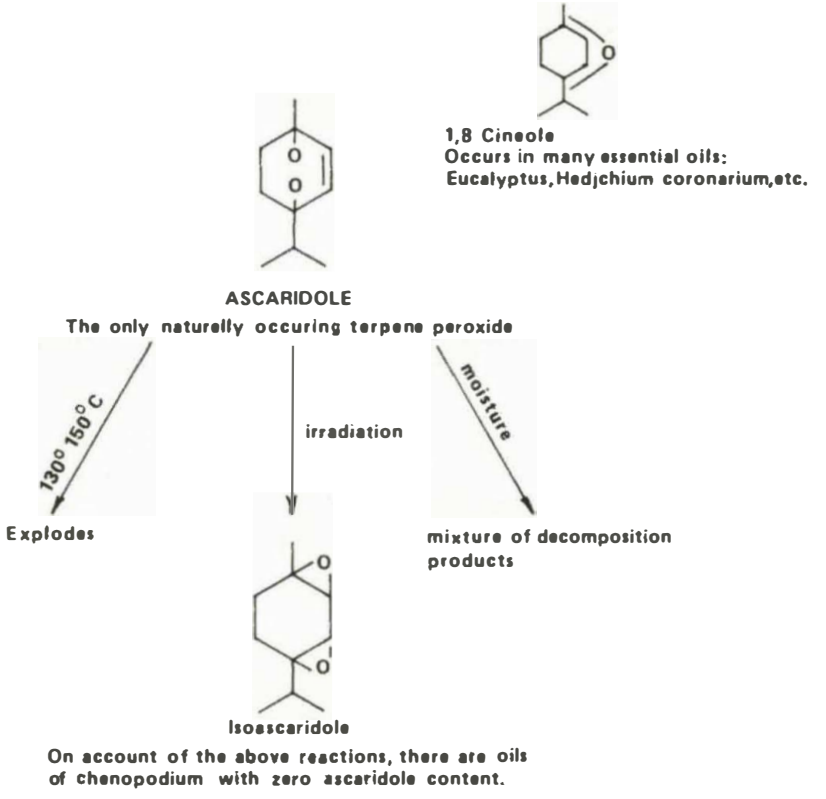
TRIMETHOXYSTYRENE

PHENOLS AND PHENOL ETHERS Continued



OXIDES IN ESSENTIAL OILS:

Ascaridole is used as anthelmintic. (It occurs in the oil of chenopodium.



SULFURETTED PUNGENT COMPOUNDS ISOTHIOCYANATES and DISULFIDES OBTAINED BY ENZYMIC ACTION ON GLYCOSIDES

1. ISOTHIOCYANATES

$R\text{-}C(\text{-}S\text{-}C_6H_{11}O_5) = N - O - SO_3^- K^+$ *enzyme*,

glucosinolate (thioglucoside)

$R - CN$ + [S] + glucose + SO_4

$R - NCS$

$R - OH + CNS^-$

R = allyl (sinigrin), in black mustard, cabbage, horse-radish
= p- hydroxybenzyl (sinalbin), in white mustard
= bensyl (glucotropaeolin), in garden cress
= phenethyl (gluconasturliin), in watercress, horse radish
= 4- methylthio-trans-3-butenyl, in radish

Thus:

$$CH_2 = CH\text{-}CH_2 - N = C(-S-C_6H_{11}O_5)-O.SO_2OK$$

sinigrin
enzyme
HoH

2. DISULFIDES

FROM ALLIIN, an amino acid in garlic *(Allium sativum)*

$$2\,CH_2 = CHCH_2\,SOCH_2\,CH(NH_2)\,COOH \quad \text{(Alliin)}$$

allinase

$$CH_2 = CH\text{-}CH_2\,SO - S - CH_2\,CH = CH_2 + 2NH_3$$

allicin (a thiosulfinate)

$$+ 2CH_3\,COCOOH$$

pyruvic acid.

(responsible for the typical, but not repulsive, odor of garlic)

disproportionation

$$(H_2 = CH - CH_2\,(O\leftarrow)\,S\,(\rightarrow O) - S - CH_2 = CH_2 +$$

$$CH_2 = CH - CH_2 - S - S - CH_2 - CH = CH_2$$

diallyl disulfide
(source of unpleasant smell of garlic)

+ other products

SOME SULFUR COMPOUNDS
IN ONION (*Allium cepa)*

1. FLAVORS:

   a. FRESHLY CUT, due mainly to propylpropanethiosulfonate.
   b. BOILED, due to propyl and 1-propenyl di-and trisulfides.
   c. FRIED, due to dimethylthiophenes.

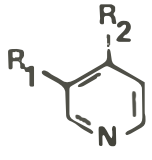
2. The LACHRYMATOR (Tear producer) is either

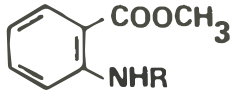
   Propenylsulfenic acid, $CH_3\ CH = CH - S \rightarrow O$
   H
   (Swian, 1963)

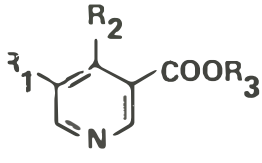
or

   Thiopropanal S — oxide, $CH_3\ CH_2\ CH = S (\rightarrow o)$
   (Nursten 1975)

SOME NITROGENATED COMPOUNDS IN ESSENTIAL OILS METHYL ANTHRANILATE (in jasmines, gardenia, ilang-ilang, etc.); INDOLE (jasmine, champaca, night bloomers); SKATOLE (civet cat). From 1965-73, only 6 basic components were known in the jasmines. In 1977-79, fourteen new bases and 3 known anthranilates were reported. The following bases were found in *Jasminum grandiflorum & J. sambac*: —



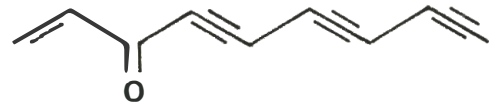




COMPOUND NO.	R	R_1	R_2	R_3	
1		C_2H_5	H		
2		$CH = CH_2$	H		
3		$C_2 H_5$	CH_3		
4		$CN = CH_2$	CH_3		
5	H				72-86% in J. grandiflorum
6	CH_3				
7	$COCH_3$				
8		H	H	H	10% in J. sambac
9		H	H	C_2H_5	
10		H	CH_3	CH_3	
11		C_2H_5	H	CH_3	
12		C_2H_5	H	C_2H_5	
13		$CH = CH_2$	H	CH_3	
14		$CH = CH_2$	H	C_2H_5	
15		$C_2 H_5$	CH_3	CH_3	29% in J. sambac
16		$CH = CH_2$	CH_3	CH_3	
17		C_2H_5	CH_3	C_2H_5	

CURIOUS COMPOUNDS, UNUSUAL STRUCTURES, AND NOVEL RING SYSTEMS, (REPORTED IN LITERATURE FROM 1969).

1.



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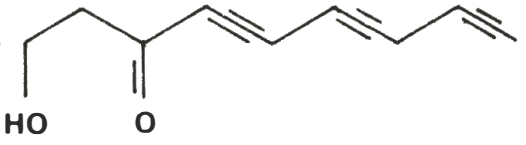
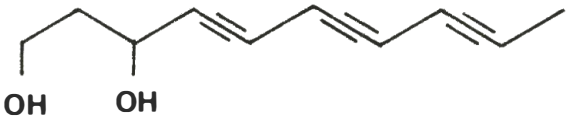
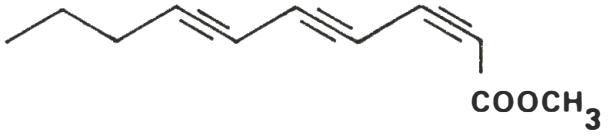


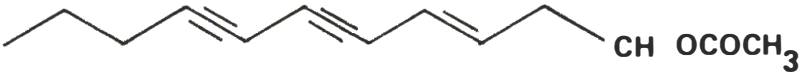
3.



4.



5.

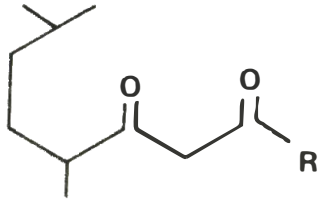


Aliphatic acetylenic compounds have, few years ago, been reported in nature. Compounds 1, 2, and 3 were isolated from the oil of garden lettuce. Compounds 4 and 5, from the oil of *Asterae* plants.

6.

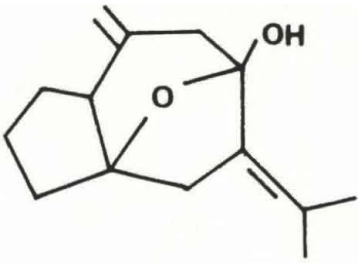
ODOROUS CONSTITUENT FROM GENUS DICTYOPTERIS.

7.



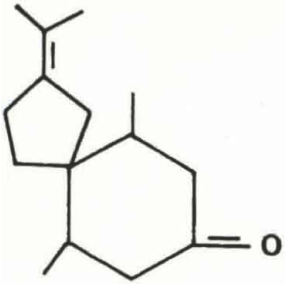
A BETA DIKETONE FROM THE OIL OF HELICHRYSUM ITALICUM.

8.



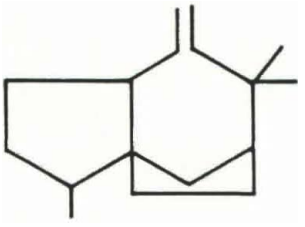
CURCUMENOL FROM
OIL OF
CURCUMA ZEDOARIA.

9.



VETIVONE

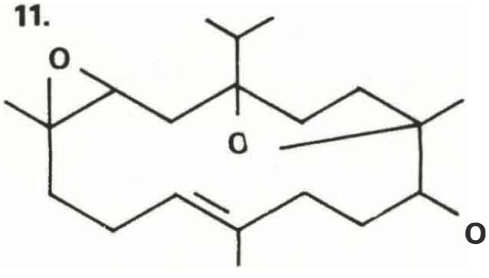
10.



COOH
(A NEW ACID)

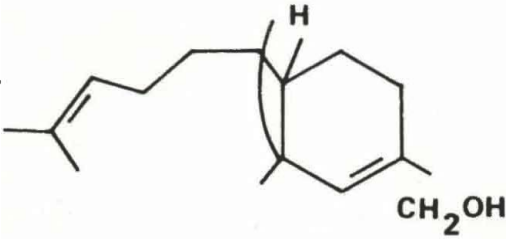
BOTH FROM VETIVER OIL

11.



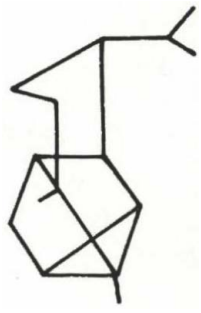
INCENSOLE OXIDE
FROM
FRANKINCENSE

12.



SIRENIN

13.

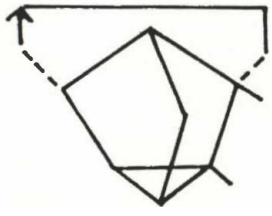


CYCLOSATIVENE
(OIL OF ABIES MAGNIFICA)

14.

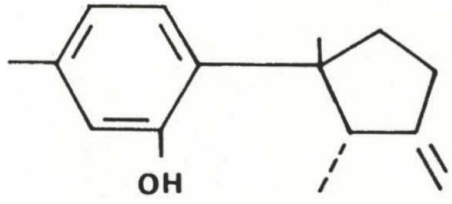
ALPHA CIS BERGAMOTENE

15.



LONGICYCLENE
THE FIRST TETRACYCLIC SESQUITERPENE ISOLATED FROM ESSENTIAL OIL.
(PINUS LONGIFOLIA)

16.

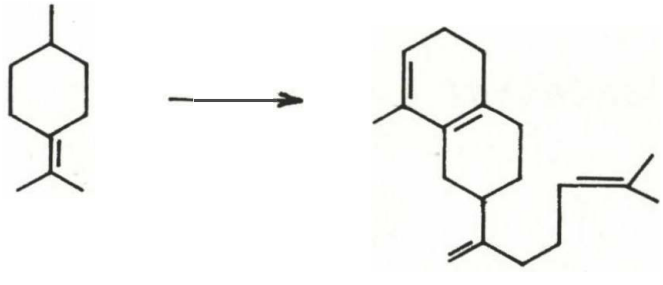


A BROMO SESQUITERPENOID.
(OIL OF LAURENCIA NIPPONICA)

CHEMICAL PROPERTIES OF ESSENTIAL OILS

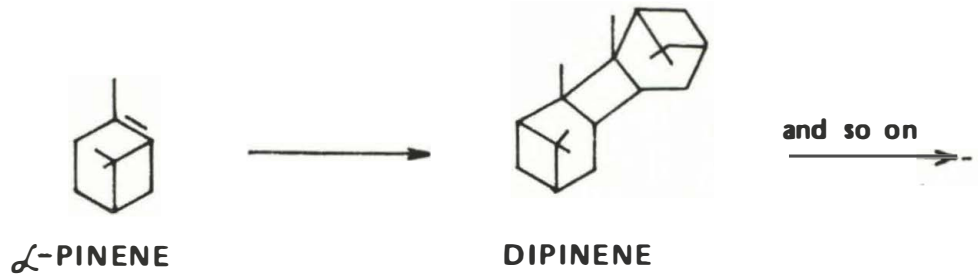
THE CHEMICAL PROPERTIES ARE SPECIFIC FOR CERTAIN OILS AND DEPEND UPON THE TYPE OF COMPONENT(S) OF THE OIL.
EXAMPLES GIVEN HERE ARE SIMPLE CASES.

1. *POLYMERIZATION:* This is shown by oils that are rich in terpene hydrocarbons where the hydrocarbon reacts by itself any number of times under certain conditions. Examples:



MYRCENE

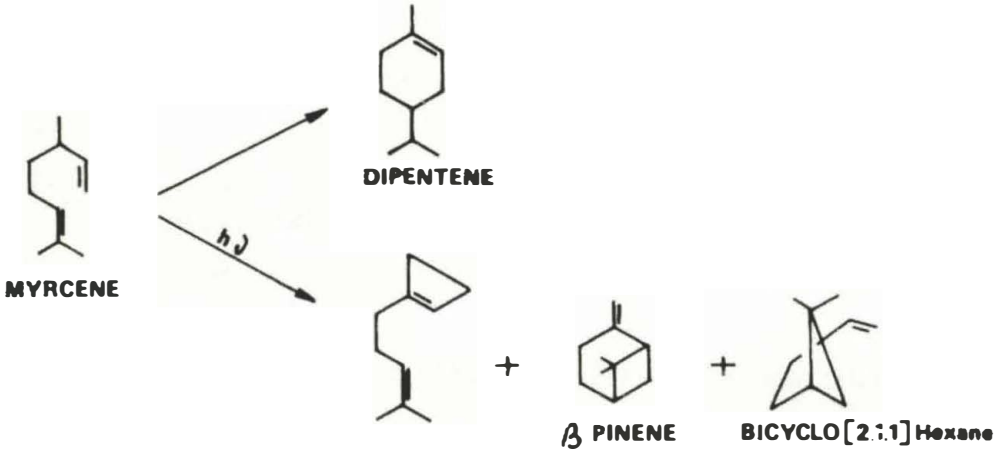
α-CAMPHORENE

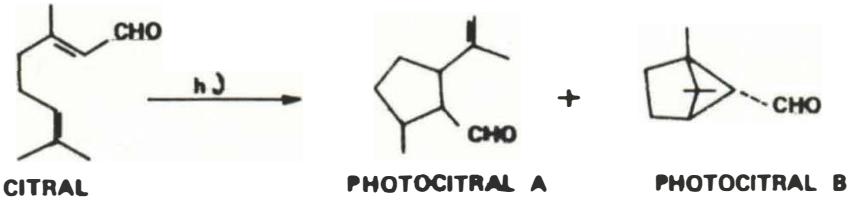


α-PINENE DIPINENE

As a whole, polymerization products in essential oils are undesirable because they come down as alcohol-insoluble residues with terebenthinate, unpleasant odor. Thus, terpeneless essential oils demand higher price than the whole oil.

2. *CYCLIZATION*





CITRAL PHOTOCITRAL A PHOTOCITRAL B

3. *REARRANGEMENT*

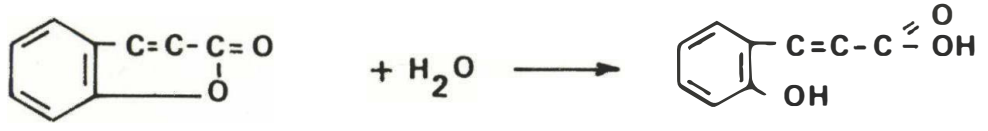
α-PINENE CAMPHENE

4. *CONDENSATION*

a. Ester formation from acid & alcohol; cinnamyl cinnamale in cinnamon oil.
b. Acetal formation
c. Ketal formation
d. Cannizaro's reaction

5. *HYDROLYSIS*

a. Indication of hydrolysis of methyl salicylate in oil of Wintergreen is its increase in acid value.
b. Indication of hydrolysis of linalyl acetate in Oil of Lavender is its lessened fragrance.
c. Lactones

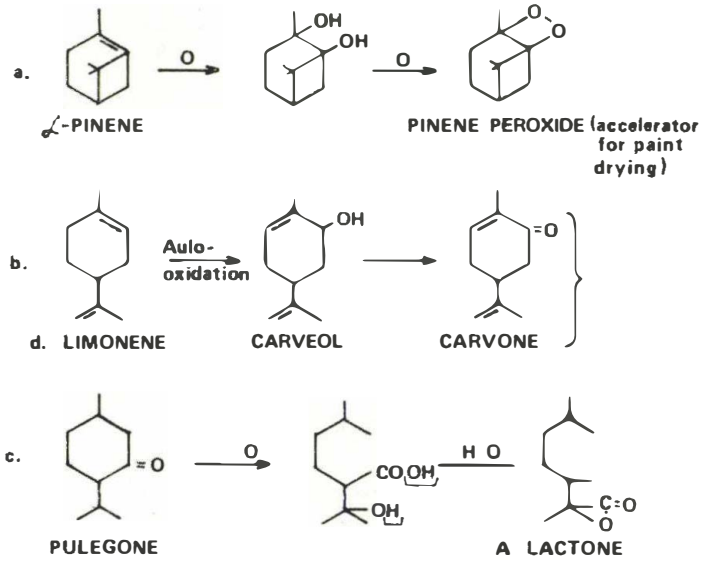


COUMARIN **O-Hydroxy Cinnamic Acid (Feebly odored)**

Coumarin odor is highly noticeable during cool evenings or early morning, but as T rises with the sunshine, the odor becomes faint or imperceptible due to hydrolysis. As twilight sets in and evening comes once more, the odor of coumarin returns.

6. *OXIDATION*

After storage at ordinary room conditions, none of the volatile oils is completely volatile anymore due to oxidation and/or polymerization products that form as residues.



This explains why citrus oils that are rich in limonene develop a spearmint-like odor due to the carvone formed.

d. The white residue that collects at the bottom of a bottle of Oil of Bitter Almond is benzoic acid formed by the oxidation of benzaldehyde, the latter from hydrolysis of amygdalin.

e. Geraniol $\rightleftarrows$ citral A

THE NEXT PICTURES WILL SHOW GAS CHROMATOGRAMS OF SOME PHILIPPINE ESSENTIAL OILS STUDIED BY L. OLIVEROS-BELARDO:

ESSENTIAL OIL OF *PEPEROMIA PELLUCIDA* (L.) HBK.

ESSENTIAL OIL OF WOOD OF *SANDORICUM KOETJAPE* (Burm. f.) MERR.

ESSENTIAL OIL OF FRUIT PEELING OF *ANONA SQUAMOSA* L.

ESSENTIAL OIL OF LEAVES OF *PSIDIUM GUAJAVA* L.

ESSENTIAL OIL OF LEAVES OF *CYMBOPOGON CITRATUS* (DC.) STAPF

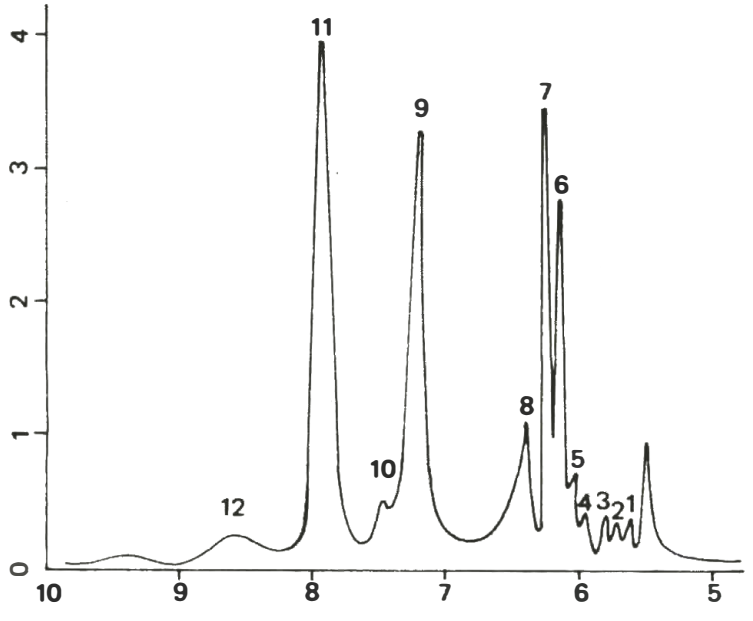


Fig. 2. Gas-liquid chromatogram of the volatile oil of Peperomia pellucida *(L.) HBK (6 ft. column of 15% polypropylene glycol, 140°—190° C., (at 5° per minute) Peaks studied:*

(6) a sesquiterpene hydrocarbon, $C_{15}H_{24}$

7, caryophyllene

9, sesquiterpene alcohol, $C_{15}H_{25}OH$

10, a solid trimethoxystyrene

11, allyldioxymethylenedimethoxybenzene

The field of studies on essential oils is so far-ranging and the volume of research work that has been accomplished is so extensive that for such a limited time allotted, one has the choice to either skim the surface in a very general manner or concentrate on one small segment of the whole. It is not surprising therefore, to note that one treatise on essential oils consists of six volumes.

This brief discussion, therefore, will focus on the further expansion of certain aspects of essential oils which, hopefully, will prove interesting to all.

Essential oils (volatile oils, ethereal oils) are regarded as odorous, more or less lipophilic, complex mixtures of substances which evaporate when exposed to air at ordinary temperatures and are stored by plants in secretory cells, cavities or canals, or excreted through glandular hairs. They are found distributed in the plant kingdom in some brown and red algae and fungi and in the following main groups of green land plants (1):

— mosses and moss allies (Bryophyta)

— vascular cryptogams (Pteridophyta, ferns)

— seed plants (Spermatophyta). Essential oils are unusually abundant in several unrelated plant families such as the Labiatae, Rutaceae, Geraniaceae, Umbelliferae, Compositae, Lauraceae, Myrtaceae, Graminae, and Leguminosae.

Occurence in the plant itself can vary greatly, some plants may contain volatile oils in all of the tissues (Conifers) in others, they are found in appreciable quantities only in specific plant parts such as: the flowers (jasmin, rose) flowers and leaves (lavender, peppermint), leaves and stems (patchouli, geranium), barks (cinnamon, sweet birch), woods (cedar, sandalwood), roots (vetiver, spikenard), rhizomes (calamus, ginger) fruit rinds (bergamot, lemon) seeds (fennel, anise) and oleoresinous exudations (myrrh, storax).

Composition and Qualitative Variation

With very few exceptions, the essential oils are generally mixtures of hydrocarbons and oxygenated compounds which differ greatly in chemical composition. The odor and flavor of these oils is mainly determined by the oxygenated constituents which are usually appreciably soluble in water.

In recent years, the perfume world's interest in the organic acids which are very minor constituents of essential oils has specifically and markedly increased. Even at very low concentrations, these acids were found to determine or shade, perfume notes. Separation and identification after the transformation into the methyl esters or methyl ethers in the case of phenols, follows the procedures already discussed in Dr. Belardo's paper.

The qualitative variation in essential oils may be considered on the level of individual plants. In most cases, the composition of the oil depends on the age of the plant and of the organ that stores the oil. The example is caraway where the oil obtained from the ripe fruits used as spice, varies pronouncedly in composition from that obtained from young unripe fruits. Another important factor is that, in many species, the various parts of the plant such as roots, stalks, leaves, flowers and fruits sythesize and store their own characteristic oils. In the cinnamon tree, the main constituents vary according to the source of the oil, thus; from the bark of the branch - 65 to 76 per cent cinnamaldehyde, from the leaves — 70 to 90 per cent eugenol, and from the root bark — mainly camphor. In the citrus family, the oil from the flowers is very different from that in the fruit rind or other plant parts, e.g. orange oil (fruit rind) should not be confused with oil of neroli (orange blossom oil) nor with petitgrain (from leaves and twigs).

Environmental factors may also have a marked qualitative effect on the essential oils. Grahle and Holtzel (2) showed that for *Mentha piperita*, menthol and methyl acetate were major constituents of the oil under long day conditions only. Under short-day conditions, menthofuran was preferentially formed and stored.

Other variation levels to be considered are local populations, species and taxa of higher or different rank (3).

Essential Oils in Medicine and in Industry

Many crude drugs are used in medicine because of their essential oil content but in many cases, the oils are used as drugs in themselves. Among the therapeutic properties or uses on record are (4, 5, 6, 7, 8).

Carminative — almost all the essential oils

Anthelmintic — chenopodium oil, santonin oil, rectified turpentine oil

Antimalarial — oil of wormwood

Antirheumatic — oil of dwarf pine needles, gaultheria oil, savin oil

Antipruritic — oil of bitter almond, menthol, camphor

Antispasmodic — thyme oil

Antiseptic and germicidal — oils of oregano, cinnamon, clove, bay, juniper, sassafras, tea tree, thyme

Diaphoretic — oils of sambong, eucalyptus, cajeput
Diuretic — cubeh oil, jumper oil
Counterirritant — oil of perppermint, thyme, turpentene, wintergreen
Expectorant — oils from anise, dwarf pine needles, eucalyptus, fennel, niaouli, thyme, lagundi
Emetic — oil of chamomile
Fungicidal — clove oil, mustard oil, thyme oil
Galactagogue —fennel oil
Local anesthetic — clove oil (for teeth) peppermint oil (for pharyngitis)
Insectifuge — citronella oil
Rubefacient — oils of garlic, camphor, cajeput, mustard, rosemary
Sedative — oil of valerian
Stimulant — oils of *Hyptis suaveolens*, buchu, cajeput, clove, dwarf pine needles
Stomachic — oils of anise, cardamom, copaiba, coriander, galanga, ginger,
Sudorific — oil of yarrow, oil of *Premna odorata*, artemisia, lantana
Parasiticide — rectified pine tar and birch tar oils, cajeput oil
Vermifuge — oils of niaouli, savin

A recent report from the PROC (9) is the clinical treatment of ringworm using a 15 per cent tincture of cloves in 70 per cent alcohol. Patients with at least 2-year old ringworm of the body and feet, unsuccessfully treated with other drugs, responded after 2 - 3 days treatment, i.e., the symptoms began to subside. In general, healing was accomplished in 3 - 5 days. The active principle was considered to be the volatile oil. The essential oil of celery, especially that from the seed was reported to have sedative properties in experimental animals.

The essential oil of *Vitex negundo* L. and two other varieties have been extensively studied in the Institute of Chinese Traditional Drugs, and found very effective for coughs. Marketed in soft elastic capsules, this volatile oil is extracted by steam distillation in communes, collected by the pharmaceutical "factory" and manufactured into dosage forms (10).

The importance of essential oils on the industrial front cannot be overlooked. One of the first plastics, celluloid, was compounded of camphor. Many products with disagreeable odors became acceptable after treatment with essential oils. Some products in which they are incorporated are — synthetic rubbers, glues and other adhesives, animal feeds, automobile finishing supplies, insecticides and repellents, furniture polishes, janitor supplies, paints, paper and printing inks, petroleum and chemical products, textile

processing materials and many other accompaniments of modern living (6).

Various industrial oils, for example, are obtained from citrus residues after juice extraction. "Citrus stripper oil" a by-product from processing orange and grapefruit peel and "Citrus peel oil" recovered from cannery refuse are used as sources of D-limonene a raw material for organic chemicals, and L-carvone which is used in paints, varnishes. plastics and soap perfumes. Cedarwood oil finds extensive use as insect repellent, clearing agent in microscopy, deodorant, and component of polishes while tea tree oil from Australia, is used in medicated soaps, dentrifices and certain medicinals. Fennel oil is useful in toothpastes and mouthwashes in pharmaceuticals and cough lozenges, liqueurs and confectionery and ginger oil in mouthwashes, ginger beverages and liqueurs.

One of the latest developments in essential oil utilization is the discovery of a perfume constituent which has deodorizing action. Thus, with the use of these perfumes, effective deodorants can be prepared without employing any of the customary active materials. This constitutent is reported to possess the following properties:

1. Natural occurence in a wide range of essential oils
2. A chemical structure which is in no way related to that of the usual deodorant agents
3. Experimentally demonstrated bacteriostatic effectiveness
4. A high degree of skin compatibility
5. A pleasant, rather neutral odor

Essential oils may be used as derived from nature as a fragrance ingredient or as raw material for the production of a wide range of aroma chemicals. A burgeoning industry in China, (11) the main products are natural methol crystals, USP and a fine quality dementholized peppermint oil (cornmint oil) from a particular variety of *Mentha arvensis*, *Litsea cubea* oil as source of citral and a whole range of ionones, and geranium oil. These products are readily accepted by American users in the toothpaste, mouthwash, cosmetics and toiletries industry.

Bibliography

1. Hegnauer, R. The Importance of Essential Oils in Plant Classification. Dragoco Report v 25: 204, 1978.

2. Grahle, A. and Ch. Holtzel. Photoperiodische Abhangigkeit der Bildung des Atherischen Oles bei *Mentha piperita* Naturewissenschaften 50: 552 1963.

3. Warnecke, H. M. Constituents of the Essential Oil of *Cistus ladaniferus* (Acids and Phenols) Dragoco Report v 25: 192 1978.

4. Kraus, A. and F.J. Hammerschmidt. An Investigation of Fennel Oils Dragaco Report v. 27: 31 1980.

5. Tyler, V. E. Brady, L. R. and J. E. Robbers. Pharmacognosy. Lea and Febiger. 1976

6. Schery, R. W. Plants for Man. Prentice-Hall, Inc. 1972.

7. Valenzuela, P. and J. A. Concha. Philippine Medical Plants Yielding Volatile Oil. Journal of the Philippine Pharmaceutical Association. v. 35: 385 1948.

8. Stecher, P. G., Ed. The Merck Index. Merck and Co., Inc. 1968.

9. Leung, A. L. Chinese Medicine — Potential Source for New Drugs. Drug and Cosmetic Industry, v. 125: 49 1979.

10. Personal Observation, 1979, during a study misssion on herbal medicine to the People's Republic of China.

11. Chaukin, W. China Looms as a Source of Essential Oils. Drugs and Cosmetic Industry v 126: 50 1980.

Alice A. **Buenconsejo, M.S., Discussant**

Dr. Belardo is to be commended on her work on essential oils, considering that prior to the advent of gas liquid chromatography, techniques on fractionation and characterization by physical and chemical means were resorted to by her. The use of large automated steady state counter current distribution with 100 tubes of 25 ml capacity each cited in her report, in fact made it possible for her to collect fractions which she was able to characterize and identify. With the advent of GLC, progress on essential oil chemistry has increased to a great degree. This has been of benefit to the Food and Fragrance Industry aside from the field of Pharmacy and Medicine.

On the application of essential oils, local industry today is in great need of perfumes for detergent and toilet preparations as well as flavors for a variety of foods. The work of Dr. Belardo on essential oils has revealed that the Philippines has a variety of sources for each oils, which can be used to advantage for concocting/compounding perfumes and flavors. It is just a matter of being able to produce these essential oils locally on commercial scale, such that they can be compounded into perfurmes and flavors.

In the field of perfumes for industrial products, the industrialist or Marketing man merely describes the characteristics of the scent he desires in layman's terms. It is the task of the perfumer to put together the various components, based on his knowledge of the chemistry of each of these components of essential oils, to arrive at what the industrialist desires. The industrialist or Marketing man then uses his nose to verify/judge if the perfumer's choice of components suits his needs/meets his requirements. Thus scientific techniques such as GLC to identify and obtain essential oil fractions, followed by the meticulous compounding by perfumers of components of essential oils obtained, go hand in hand with the olfactory assessment in order to arrive at the most suitable perfume for a particular use.

Persistence and zeal in the identification of the essential oils present in various local plants that have not yet been studied are

still required, as there are still a lot of plants that are potential sources of essential oils. Once identified however, there is the even greater need for the commercialization of the extraction and isolation of these essential oils and their components, for only then can the knowledge gained by the zealous experimentation of researchers like Dr. Belardo be of use to the local manufacturers and in turn to the ultimate consumer. The present day cost of perfumes and fragrance imported from foreign countries is very high, due to a 100% duty levied on fragrances. As a result, the ultimate cost of a perfumed product to the consumer is correspondingly high and this can certainly be reduced if fragrances resulting from the use of essential oils derived locally can be used as alternative to the presently procured foreign perfumes.

In the field of foods, delicate flavors like mango, calamansi, guava, passion fruit and those of other local fruits are in demand and to-date not a single flavour house has succeeded in duplicating the flavour of the local mango nor the calamansi. As mentioned by Dr. Belardo, the guava fruit peel oil is presently being characterized and may shed light on the components required to arrive at a desired guava flavour. Similarly, work on the characterization of oil from the peel or pulp of local fruits will still have to be pursued, if flavors from local fruits are to be a commercial possibility in the future.

Essential Oils are indeed a "must" in day to day living, being utilized in medicines, as expounded on by Dr. de Castro, in Detergents and Toilet Preparations which would be unappealing without the variety of scents available today and which are made possible by the various essential oil components and finally in the field of Food, where the variety of foods are made interesting and distinguishable from one another by the addition of spices, flavors, aromatic-giving substances that are all either derived or in some way linked to essential oils.

Alicia O. Lustre, Ph.D., Discussant

I consider it an honor to be allowed to participate in this program as a discussant. May I take this opportunity to bring out the significant aspects of the research that was just presented to us this morning.

First of all, I would like to congratulate the researcher for her work. At the outset, it shows that it is possible for dedicated and capable scientists to conduct essential oil research in this country and to achieve recognition at par with those of other investigators abroad, inspite of the fact that locally the gas-chromatograph and the mass-spectrometer are not yet ordinary laboratory tools.

The work contributes very significantly to our store of basic information on the fascinating constituents of our local fruits, flavors and natural plants. This contribution can go a long way in making the teaching of science and chemistry in our schools more interesting to the students and more rewarding to the teachers.

I know that, well at least, in food chemistry it can be very difficult to have to demonstrate food technology principles, using apples and pears as examples. With investigations of this nature, the day will come when we can have our own local books demonstrating scientific principles using examples more familiar to us, as guavas and our own sampaguita.

As far as the economic aspects are concerned, it is reported that there are at present 1,400 flavors being used in the food industry, both natural and artificial. As food additives, these compounds have had their share of questioning from the public. There are, however, two types of food products where the use of flavors will continue to be a consequence. These are the stimulated meat products and the fruit drinks. Stimulated meat products are what we now get as the meatless bacon. the meatless hamburgers, and fruit drinks. The paper read indicates that we have the resources to contribute in part of the world's need for natural flavors particularly fruit flavors. In this regard, one significant aspect worth investigating is our utilization of fruit processing wastes particularly peels as sources of local flavorants. Thank you.

Q — Torralballa: Calvin of California went to Brazil and found plants there from which he aptly isolated petro-

leum-like products, not volatile oils but petroleum-like products. Have you heard about this?

A — Belardo: Yes, Dr. Torralballa. In fact it was announced here but just for lack of time, I omitted it. It says here, in 7 months a plantation at the south coast field station in California has produced more than 10 barrels of oil per acre and production is still on an output gross acre.

Torralballa: That's right, he actually showed samples in which the oil is like crude oil.

Belardo: Yes.

Torralballa: And apparently, the main components then of that product are the straight hydrocarbons, instead of the turpenes.

Belardo: Yes. This is precisely the purpose of that project announced wherein . . . .

Torralballa: Yes. That's what I brought perhaps. Do you know the particular species then?

Belardo: There are several . . . There are several species and these are all found in the Philippines also.

Torralballa: Also?

Belardo: Yes.

Torralballa: Oh! it's wonderful.

Belardo: And it is very easy to grow.

Torralballa: And I knew that he went to Brazil and that was where he first found those plants.

Belardo: Yes, because Brazil and the Philippines are tropical countries.

Torralballa: of the same climate. . .
Yes, thank you very much.

Belardo: You are welcome.

PHYTOCHEMICAL RESEARCH AND DRUG DEVELOPMENT: RANDOM THOUGHTS

By Alfredo C. Santos, Dr. phil., Academician

A Basic Research and Tie-up with Developmental/Industrial Research

A close tie-up between basic research and developmental research either preceding industrial application, or in close cooperation with industry is necessary for the development of plant/natural products into medicaments. Basic research is carried out mainly in the universities, the main function of which is to advance the frontiers of knowledge [1]. Thus, when a university researcher/professor has elucidated the chemical structure of a natural product, or has determined its biological/pharmacological action and the work culminated in a publication in a journal of recognized/international standing, he is inclined to leave the problem there and move on to another basic problem. But at this stage, the compound is not ready for introduction in therapeutics/general use in medicine. A lot of developmental work is needed, and this is not a problem for the university, but it is rather the function of the research organization of pharmaceutical industries. This kind of research is very expensive, and to be able to maintain it, there is need to grant them exclusive rights in the form of patents.

In the Federal Republic of Germany, the AFG [2], a Federation of National Research Council carries out a considerable amount of pure and applied basic research in selected areas of interest. Their facilities, financial and personal requirements exceed that of the normal scope of the university. As soon as commercial application becomes feasible the projects are turned over to industry. In Australia, the CSIRO (Council of Scientific and Industrial Research Organization) has similar functions.

In this connection and in the inadequacy of such organizations in the Philippines,* the report of Dr. Paul Byerly, Jr. — the writing of which was stimulated by a request from the NSDB to the Director of Mission, FOA, for a research advisor concerning industrial research, stated in part: . . .

"A society's problems are largely capable of solution only by the results of competent research . . . .

*The Philippine Institute of Pure and Applied Chemistry (PIPAC) at the Ateneo University Campus, Loyola Heights, Quezon City offers Chemical Analytical, Technical Training-and Research Services in the field of chemistry.

"The Survey Group recommends that in the public service the primary responsibility for basic research and that part of applied research in agriculture (industry) requiring well-trained scientists be placed with the University of the Philippines, and that testing, demonstration, control and regulation, be left to the responsibility of the appropriate bureaus of the Ministry of Agriculture and Ministry of Natural Resources . . . ."

It may be mentioned here, that when Dr. Juan Salcedo, Jr., Academician and National Scientist, was the Chairman of both the NRCP and the NSDB, a delineation was marked, whereby the National Research Council of the Philippines is to take care of basic and fundamental researches and the NSDB of the applied and industrial researches.

Drugs from plants (?)

Improved technology in chemistry and pharmacology during the last few decades permits now a faster and more systematic research on medicinal plants. What before took years of painstaking experimental research work in the laboratory using classical chemical methods to isolate and determine the chemical structure of a natural product, can now be done, using physical tools, in a very much shorter time. In this connection, we would like to recall our own experience in the case of the alkaloids of *Phaeanthus ebracteolatus* (Presl) Merr [3].

In spite of these advances, drug development – although of great importance remains expensive. It is estimated that the chances for a new compound to become a drug are at least 3,000 to 1. A drug company is known to have spent more than $20 million without obtaining a marketable product. Among the criteria to be assessed are: intensity and specificity of action, side effects, acute and chronic toxicity, and disposition within the body. [4].

The need for *continuous research* may be seen further in the case of tetrandrine, the optical antipode of phaeanthine.

Although isolated long ago (in 1928) and its structure subsequently determined, it is only recently that its potentialities are being discovered as: (a) an effective antitubercular agent,[5] active vs. 16 strains of *Myobacterium tuberculosis in vitro*, at 8-33 ug/ml; and as (b) an anti inflammatory agent[6] it is at least as effective as cortisone.

It would be very interesting to test phaeanthrine if it would exhibit biological activities similar to those of tetrandine. In the case of phaeantharine, the findings of Leticia Angeles, are summarized in a paper presented in part in a Poster paper presented at the 6th FDA Science Symposium on Aquaculture

held in New Orleans, La. on February 12 to 14, 1980. Major advantages are:

(1) larvicidal, hence a more discrete and direct method of pest control

(2) less wasteful

(3) will not contribute to environmental pollution

(4) risks to non-target species are minimal

(5) development of resistance is practically nil

(6) will not accumulate in body fat

(7) it is not only non-carcinogenic but even exhibits some anti-tumor activity in experimental animals

(8) the chemical nature of the active principle suggests susceptibility to biodegradation and

(9) negligible cost.

Field trials on the crude preparations are suggested. Significant implications in agriculture (pest control) and medicine (disease vector control) cannot be over emphasized.

Synthetics or Medicinal Plant Preparations (?)
S.E.E.S. (Side effects eliminating substances)

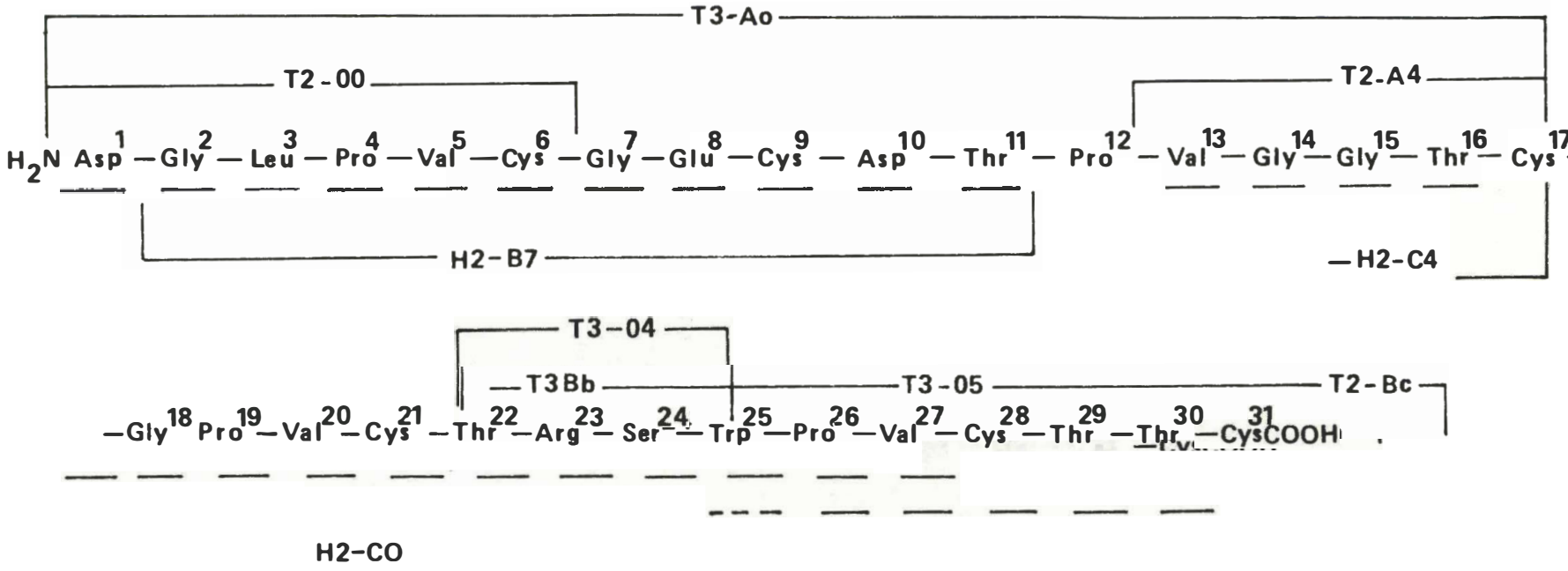
The experience in Zaire. Missionaries have found out that the natives have been using successfully a medicinal plant, which was found to be *Oldenlandia affinis*[7] (Fam. Rubiaceae) — to hasten parturition. Phytochemical investigations in Oslo, Norway, showed that the oxytocic effect is due to a number of polypeptides, among which one (cf. formula) was extremely active.[8] Unfortunately it produced very irregular heart beats, so the tests had to be abandoned. It would be very difficult to find the right/correct combination that would produce the desired effect of hastening parturition, without the side effects. Certainly for the people of Zaire, it would be much easier to use an aqueous extract of the plant.

A System of Phytochemical Research

The international journal "Phytochemistry" classifies research works published in the following areas: Biochemistry, Biosynthesis, Chemotaxonomy and Phytochemistry (proper).

For the utilization in therapeutics of medicinal plants products/preparations-phytochemical and biological, microbiological, pharmacological and clinical are necessary.

A selection at random, and mass biological screening of large number of plants is expensive and not advisable. In this connection, Norman R. Farnsworth,[9] of the University of Illinois, utilizing a computerized natural-product-data-bank was able to select 17 plants from a list of 575 plants suspected to possess sedative activity, with 9 out of the 17 yielding extracts with equal



Formula Kalata-peptide

or greater sedative activity than the selected model of pentobarbital sodium.

Search for New Drugs. A System of Phytochemical Research

In the search for new drugs there is a need for a combined chemical and biological/pharmacological cooperation. One usually begins by making an extract of the plant and subjecting this to a preliminary biological/pharmacological screening. For the purpose, ethanol would be a suitable solvent. A method for the preparation of plant extracts is described in "Phytochemical, Microbiological and Pharmacological Screening of Medicinal Plants.[10] Methanol has been used also, but in this case, the plant extract should be completely deprived of methanol, which itself is poisonous.

A method of Dr. Y. C. Kong, is based on the separation of constituents into non-polar, semi-polar and polar by sequential extraction with hexane or petroleum ether for non-polar constituents, such as oils, terpenes, followed by subsequent extraction with alcohol, and partition between chloroform and water, whereby the semi-polar constituents, like glycosides, go into chloroform, while the polar constituents, like glycosides, go into the aqueous phase. All crude extracts — ethanolic, or hexane, chloroform and aqueous extracts are subjected to biological screening. The individual extracts are subjected to biological-screening. The individual extracts possessing biological activities are then subjected to chromatographic separations, similar fractions, i.e., with the same Rf values are collected together and subjected again to biological testing. In the isolation process, the mother liquors should always be tested for biological activity, for the biologically active constituent may not be the nice crystals, but in the mother liquors. Fractions without biological activity may be discarded.

UNESCO, IFS Sponsored Programs in the Philippines

International agencies/foundations have been helping developing countries in the utilization of medicinal plants.

Unesco sponsored programs in the Philippines:
Phytochemical screening programs

(1) On phytochemical, biological, microbiological and pharmacological screening of Philippine medicinal plants. In cooperation with the UST Graduate School and Research Center, held in March 1978;

(2) On isolation and chemical studies and structure elucidation of natural products. In cooperation with the UP College of Pharmacy and UPLB, held in October 1979.

(3) A sub-regional workshop — a sequel to (1) above, was held recently in April 1980 in the Visayas, at the University of San Agustin, in Iloilo, and like the two above, with participants from Indonesia, Thailand, Hongkong, Singapore and Malaysia.

Problems confronting the utilization of Philippine medicinal plants and their products.

Why are UNESCO, IFS, WHO sponsoring/encouraging research on herbal medicine?

— Could it be because many synthetic drugs are petroleum based?

or — Could it be that rising prices make these drugs inaccessible to many in developing countries who need them, but can ill-afford their high prices?

What is the cause of rising prices?

In this connection, the views of Renato Constantino, Michael L. Tan and the report of the WHO that 50,000 brands of medicine here could be listed under 2,000 generic names only (Times Journal, May 9, 1980) offer interesting food for thought.

Diazepan, B. P., (generic name) costs only ₱0.21/5mg tablet whereas under the brand name Valium its price rises to ₱0.915/4 mg. tablet; Ampicillin B. P., costs only ₱2.331/500mg whereas when sold under the brand names of Pentrexyl, Ambicin, Penbritine — its price rises to almost six times for the same 500 mg tablets.[11]

In a developing country like ours, the main effort of health delivery should be concentrated on prevention rather than cure.

From the foregoing consideration, it would be desirable to consider the possibility of using dosage forms (tinctures, extracts, infusion, decoction) from Philippine medicinal plants for simpler ailment.

On the integration on the use of medicinal plants into the health care system, the opinion of the participants in the seminar held in Tokyo [12] in September 1977, were summarized in the WHO Report as follows:

> "In certain countries, integration of the use of medicinal plants into health care system has been successfully accomplished with both economic and therapeutic advantages.
>
> "In other countries, no such efforts have been made and the importance of choosing suitable approaches in attempting integration was stressed by many participants.
>
> "The participants noted that an alternative approach to an integrated approach, is the use of medicinal plants in

health care complementary to Western medicines. In certain cases due to consumer approval and/or economic necessity, medicinal plants are more acceptable than certain synthetic drugs and therefore a parallel and complementary system would appear to be a possibility that does not present great difficulty in implementation.

"In general, no opposing views on the potential usefulness of potential medicinal plants in health care were expressed. However, medicinal plants in any country's health care system would remain a national policy decision. Furthermore, WHO should stimulate greater awareness and understanding among national authorities, health personnel and people of the potential value of the use of medicinal plants."

In a paper presented by Dr. Quintin Kintanar before the Economic and Social Commission for Asia and the Pacific (ESCAP) Meeting last May 13, 1980 on the development and transfer of technology for the utilization of medicinal and aromatic plants in the Philippines, he enumerated the following requisites:

1) scientific studies to put its use on a rational basis
2) educational and promotion activities to legitimize the proper use of medicinal plants *vis-a-vis* modern drugs
3) public health programs utilizing medicinal plants in primary health care
4) agricultural and industrial production of selected plants at the community and national or regional level to meet the needs of the country for medicinal plants
5) technical assistance and cooperation among developing and developed countries in the further advancement and transfer of technology in this field.

A thorough study on the utilization of medicinal plants will involve a multidisciplinary approach.

1) the plant must be properly identified by a taxonomist, and a herbarium specimen be deposited in the institution. The pharmacognosist can determine the localization of the active constituents in the plant tissues/organs that will serve as a guide to the phytochemist in the selection of the proper plant part to be collected.
2) in the phytochemical studies where knowledge of chemical methods and physical tools is used in the isolation and structure elucidation, a thorough acquaintance in such specialized fields as the alkaloid, steroid, etc. is necessary.

3) on the biological studies, the cooperation with the microbiologist, biochemist, pharmacologist and clinician is indispensable.
4) the cultivation and propagation of medicinal plants will involve the agriculturist.
5) the pharmacist will take care of the preparation of the proper dosage forms.

The need for fundamental research to insure continuing scientific and technological process can not be overemphasised. Let us not utilize the well-trained researchers to do routinary/administrative work least we kill the goose that lays the golden egg.

Realizing the importance of basic research, the RTC-NAST has proposed the creation of the positions of career scientists, Senior and Junior Research Fellows of the Academy, for fulltime researchers.

The NAST-RTC in Chemistry

A Ph.D. program (studies leading to the Ph.D. degree) was presented before the RTC in Mathematics, Physics and Chemistry for endorsement/recommendation by the NAST to the NSDB for funding. In the field of chemistry, the proposed areas for research (thesis/dissertation) are: natural products' chemistry, and analytical chemistry.

One of the pressing need/problem is the lack of qualified thesis advisers who could assign an original problem: for only faculty researchers who are actually engaged in research and have been publishing contributions regularly in the journals of recognized standing (not newsletters, bulletins which are not abstracted in the Chemical Abstracts, Biological Abstracts, etc.) and are thus acquainted with the current literature, are in a position to act as thesis advisers. A tie-up with researchers/professors abroad who have the necessary physical tools may be necessary.

The next and even more important problem is — how to keep the now well-trained professor/researcher:

He should not be promoted to an administrative position, and be a loss to science.

He should not be lured to industry where, although his salary may be multiplied many times — his research contributions will only be available to serve the interests of the company he is working for/employer.

A researcher is usually wedded to his/her work and provided he/she is given a reasonable salary to maintain a decent — not necessarily luxurious living, and given some fringe benefits that would provide education for his children, housing and transportation facilities/allowances, he will stick to his research.

One of the main difficulties encountered in the experimental researches on the chemical and biological studies of Philippine medicinal plants is the difficulty of surveying the literature.

In a conference with the Technology Resource Center Staff, Dr. Paulo Campos, President of the National Academy of Science and Technology (NAST) indicated the need of a Computerized Data Bank to help researcher in the survey of the literature and keep them abreast of the more recent developments. In this connection, we have surveyed the world literature on the chemistry and biological/pharmacological studies of Philippine medicinal plants, up to 1980 under an NRCP Research Project I.D.-22 entitled "Philippine Medicinal Plants and Their Contained Natural Products: (Phase III) Biological and Pharmacological Survey."

We have been repeatedly asked by several researchers as to when will our survey be published. Even the Technology Resource Center (TRC) wanted to get our material to be fed on their computerized bank. Of course the data bank will only give the original title, author and journal reference and one has still to go to the various scattered libraries to read the article. In our survey, the main results are tabulated, and provided the material, usually needed in the abstract of the original article.

Career scientists, Research Professors, Fellows of the Academy:

The NAST, under the sponsorship of its President, Academician, Dr. Paulo C. Campos, has presented a program where selected scientists/researchers would be given a reasonable emolument, to enable them to concentrate mainly in research work. It is said that a university is only as good as its faculty. It is hoped that time will come when scholars from other countries would come to undertake research work in our universities/ institutions.

Literature Cited

1. Santos, Alfredo C. "Phytochemical Research and the University," *Acta Manilana*, series A No. 18 (28), 124 (1979).
2. Arbeits gemeinschaft dar Grossforschungseinrichtungen. "Scientific and Technological Cooperation with Developing Nations." Ahrstr. 45 D-5300 Bonn (02221) 30 22 49 and 30 22 50. Federal Republic of Germany.
3. Santos, Alfredo C. "Developments in the Investigations on the Chemistry of Natural Products." *Acta Manilana*, series A, no. 12 (19), 48 (1974).
4. Fastier, F.N. and Laws, G. F. "Drugs from New Zealand Plants (?) Search" v. 6, no. 4, 117 (1975).

5. Vichkanova, S. A. Makarova, L. U. and Solov'eva, L.F. Chemotherapeutic Properties of the Alkaloid Tetrandine in Experimental Tuberculosis Farmakol." Toksikol. (Moscow) 36 (1), 74-8 (Russian). Thru C.A. *78* (17) 106079 h (1973).
6. See No. 3.
7. *Methods in pharmacognosy.* A Baerheim Svendsen. "In Proceedings 4th Symposium/Pharmacognosy and Chemistry of Natural Products on Development Cooperation in the discovery and use of natural resources for drugs in the Third World." October 13-14, (1977). The State University of Leyden. Gorlarus Laboratories, Wassenaarseweg 76, Leiden. The Netherlands.
8. *Pharmacognosy of Today and Tomorrow.* Finn Sandberg. In Comprehensive Report on Phytochemical, Microbiological and Pharmacological Screening of Medicinal Plants/Workshop held at the UST Graduate School on March 27 —April 3, 1979. (47 pages).
9. N. R. Farnsworth. J. Pharm. Sci. *55*, 225 (1966). Through reference7.
10. Prepared by the UST Research Center Staff and published as a supplement of the *Acta Manilana*, March 1978 (iv;70p).
11. *Primer on the Philippine Drug Industry.* Ibon Research. P.O. Box 1283, Manila, Philippines. May 1979.
12. Final Report Seminar on the "Use of Medicinal Plants on Health Care", Tokyo, Japan. September 13-17, 1977 by WHO Regional Office for the Western Pacific, Manila, Philippines p. 4, 178-180.

Modesto T. Chua, Ph.D., Discussant

In this paper, Dr. Santos touches on many of the issues tied up with phytochemical research and with development of drugs from plants in the Philippine context.

Phytochemical Research in the Philippines Today

There is a lot of interest in natural products research on the part of chemists and pharmacists in the Philippines. This is in keeping with the present world-wide intense research activity on medicinal plants as a source of new drugs to replace or supplement synthetic drugs.

Fundamental research on medicinal plants, as Dr. Santos states, is best done in the universities. There are many examples of university-based productive research groups in many countries: Tchesche in Germany, Bacton in England, Kochethov in Moscow, Woodward in the United States, to name some highly visible ones.

Each of these research groups represents a dynamic scientific hierarchy. In Bonn, Germany, Tschesche is at the apex of his hierarchy. Below him are 3 or 4 assistants, who are themselves senior scientists working toward their professorships. Working with each assistant is a group of postdoctoral, doctoral and magisterial students, each working full-time on related researches. At the bottom of the hierarchy are the undergraduate students, who at some point in their training are asked to work on projects connected to the on-going graduate and postgraduate researches.

The research group "system" in the universities represents a very efficient way to conduct research, as can be gauged from the output in both scientific papers, as well as scientific workers. Research on natural products remains at it has been in the past, an excellent, demanding training ground for chemists, pharmacists and other scientists.

In the research group, scientific attitudes, techniques, feel for molecules, and information gathered over the years are efficiently transmitted. This constitutes the "living tradition" of the group.

If we are to aim for more effective progress in natural products research, where ability to carry out the isolation, testing and structural elucidation of *new* active principles is enhanced, then we need substantially increased investment in the advanced training of chemists and biologists in our universities.

The proposed Ph.D consortium among the University of the Philippines, La Salle University and Ateneo de Manila University is a step in the right direction. Through a pooling of scarce scientific manpower, the three institutions hope to reach a "critical mass", whereby self-sustaining growth may lead eventually to stable research groups in natural products.

Multi-Disciplinary Research on Medicinal Plants

For efficiency, this is the approach to take, a type of task-force method involving the phytochemist, biologist, biochemist, pharmacologist, clinician, the agriculturists.

There are many models for this: the one which springs quickly to mind is the Materia Medica Institute of the Chinese Academy of Sciences, Peking. This Institute has six departments:

Phytochemistry
Pharmacognosy/Botanical
Pharmacology
Synthetic Organic Chemistry
Experimental Plantations

These departments work together on plants of interest. The extensive data covered are fed to computers for easy retrieval.

The establishment of problem-oriented research institutes, such as this, will work only if the necessary highly-trained manpower and support are available.

Support from UNESCO and other International Organizations

UNESCO has supported the establishment of a Southeast Asian Network for Chemistry of Natural Products. This network has a program of activities directed towards the promotion of research in natural products. Aside from the workshops mentioned in the paper, there were two earlier UNESCO-sponsored ones: (1) a training workshop on Isolation and Structure Elucidation of Natural Products — 18 April — 28 May 1977 — at the Ateneo — 20 participants from various institutions. (2) Seminar/Workshop on the Application of Spectroscopic Techniques to Structure Elucidation of Natural Products. (Subregional activity, 5 participants from other Asian countries) 29 May — 9 June 1978, held at the Ateneo.

Vital Role of Access to Scientific Information in Research

This point is clearly made in this paper. Scientific researchers constitute a community, where links among workers in various forms constitute a vital life line. In certain cases, the links may even be formalized into cooperative researches.

The examples given where work done by others provides stimulus and guide for a researcher's own work illustrate this. The examples may be extended. For instance, (+)-tetrandrine (from *Stephania tetrandra*) has been found to have antihypertensive activity. Similarly, work done elsewhere detect broad-spectrum antibacterial activity in 4 compounds isolated from *Andrographis paniculata*. Most active is neoandrographolide.

In his paper, Dr. Santos highlights the many bright and some dark aspects of work on medicinal plants in the Philippines.

Speaking from the vantage point of many years of active research in this field, he points out the scientific as well as the economic benefits accruing from research and development work directed towards producing drugs and drug raw materials from plants. At the same time, he sees some difficulties and problems besetting the research effort. For some problems there are no easy solutions.

In any endeavor, one of the key ingeredients is the availability of human resources to pursue it. As is common with other research areas, research on medicinal plants suffer from too few trained researchers. In addition, these researchers are, as a whole, looking separately into the pharmacological, phytochemical and biological aspects of the plants. Moreover, there is no indication, with the present system, that the desired increase in number of researchers will come about in the foreseeable future. Earlier this morning, Dr. Campos was speaking of the sigmoidal curves of growth, where a low-level activity reaches a dynamic stage where growth suddenly expands and accelerates. The vital ingredient for this expansive growth of research has to be found in the system which motivates and trains new researchers.

Historically, universities have been the focus of research in natural products. Research is an integral and necessary part of univeristy activity, serving both to express and develop the creativity and skill of the teaching faculty as well as to train and educate young researchers. Skills, knowledge and insight are passed on whereby the new researchers become not clones of the teachers, but hopefully will surpass them.

In our universities today, with a few exceptions, the number of faculty members actively doing research has not increased, but has in fact diminished. Various pressures have led to this. Natural products research is demanding. It requires not only certain expertise in order to be productive, but also the ability to grow in skill and a continuing acquaintance with developments in the field.

This is best accomplished under situations where researchers do not work alone, but rather in tandem with others within the same field and also with related disciplines. This ensures that communication and enthusiasm do not diminish. Coordinated research

on the phytochemical, pharmacological and biological aspects of selected plants, for instance, would be most favorable.

If we wanted to modify our research structures in the universities, it would be useful to consider, as models, the set-ups in other countries, such as in Germany, the United States, Japan and England. The common feature in the productive universities in these countries is the way the "research group" is made up. The research group has a pyramidal structure, with a distinguished professor at the top. Working under him and with him are two or three lower level professors, usually also former students of him. Each of these professors has, in turn, a number of postdoctoral and doctoral students under their guidance. At the bottom of the pyramid are the graduate and undergraduate students.

In systems like this, you have the "infrastructure" where attitudes and skills, scientific traditions are efficiently passed down.

A recent move by the UP, Ateneo and De La Salle for a consortium towards a Ph.D. degree in Chemistry may be a start of a promise along this line. The NAST has lent its prestige towards supporting this move. This development may pull together the few professors from these universities towards a group, to do research in selected areas, including the chemistry of natural products.

We hope that this move gains ground as a concrete step promising accelerated research on medicinal plants in the future.

Quintin Kintanar, Ph. D., Discussant

As suggested by the phrase "Random Thoughts" in the title, this paper touches on many issues and subjects, some of which transcend the confines of Phytochemical Research and Drug Development — the main subject of the paper.

For instance, the issue of the role of basic research encompasses all of science and technology. On global scale and on a long time horizon, there is no question that advancement in any field will require basic research. The new knowledge and understanding of nature in the natural sciences and of man and his socio-economic institutions, in the social sciences, are the foundations of technological progress and national development. Any country in this modern age cannot afford to neglect basic research for long without paying the price of being left behind by the more enlightened societies who march with the increasing tempo of scientific and technological development. This is not to say that developmental and applied research is less important, for indeed they are necessary if we are to derive economics and social benefits from the fruits of basic research. Both types of research are necessary and must be supported. This is aptly demonstrated in the field of drug development discussed in this paper.

Some of the plans of NAST for a Ph.D. program in the breeder Sciences of Mathematics, Physics and Chemistry and an endowed career scientist or research positon mentioned by Dr. Santos, are indeed very laudable. This will help insure an adequate supply of highly educated and trained scientists who can devote their full energies and talents to scientific work without having to worry much about where the next meal is coming for him and his family. Most of the time it is the economic factor which induces a scientist to take on an administrative or management position or to go into private industry. Let us hope that these plans will find enough support so that it will not result in a still-birth.

The other aspects of the paper devoted to a discussion of research and development in the field of pharmaceutical and medicinal plants are too brief at times, but covers a lot of grounds.

The full development of a drug from plants as pointed out in the paper, is of course an expensive and time-consuming process which requires the expertise of agriculturists, botanists, pharmacognoscists, phytochemists, pharmacologists, clinicians, engineers, industrialists and managers. If all of these personnel can be put effectively under one roof, or at least under one integrated program, the time requirement per unit of output will of course materially diminish, resulting in greater over-all productivity.

The efforts of NSDB and NRCP in the field of medicinal plants are noteworthy. The NSDB-supported Integrated Medicinal Plants Research and Extension program brings together a multi-disciplinary group of medicinal plants researchers from the College of Pharmacy, College of Medicine and College of Agriculture of the University of the Philippines and from the National Research Council of the Philippines and the National Institute of Science and Technology, who work in cooperation with health planners of the Ministry of Health to promote the scientific application of medicinal plants in health care. The NRCP on the other hand is interested in establishing an Institute of Materia Medica which would serve as a Center under one roof, for the study and development of medicinal products from medicinal plants. Similar Centers exist in the large cities of the Peoples' Republic of China, where medicinal plants are widely used.

Towards the end of his paper, Dr. Santos quotes the statement of a WHO-sponsored seminar regarding the two strategies that can be used in the utilization of medicinal plants namely 1) complete integration into Modern Medicine or 2) mere complementation of modern medicine with medicinal plants for economic reasons or because of end-user's preference. The situation actually varies from one country to the next, calling for different strategies. In some countries there is a long tradition of Oriental herbal medicine which has not been eroded by the onslaught of scientific modern medicine. In these countries there has been a substantial uninterrupted and well-accepted use of medicinal plants, such as China and Sri Lanka.

On the other hand, in other countries like the Philippines, the western scientific influence has largely displaced the traditional modes of treatment at least in the urban areas. In these countries a major effort is needed to legitimize the use of medicinal plants particularly in the eyes of doctors educated in Western-type schools.

As I have pointed out recently, in order to maximize the benefits from medicinal plants in the Philippines, there is a need for:

1. Scientific studies to put its use on a rational basis.
2. Educational and promotional activities to legitimize the proper use of medicinal plants vis-a-vis modern drugs.
3. Public Health Program utilizing medicinal plants in primary health care.
4. Agricultural and industrial production at the community and national level to meet the needs of the country for medicinal plants.
5. Technical assistance and cooperation among developing and developed countries in the further development and transfer of technology in this field.

Magdalena C. Cantoria, Ph.D., Academician, Discussant

In connection with the paper of Dr. Santos, there are three "random" ideas which I would like to discuss very briefly:

1. The magnitude and complexity of the process of bridging the gap between phytochemical discovery and drug development,

2. The trend in recent systematic studies of testing biologically every fraction of the extract of each of the different plant parts before isolation of the constituents, and

3. The value of participation in national, regional, and international gatherings to phytochemical research and drug development.

The discovery and development of new drugs is a team process. It represents the cooperative efforts of biologists, pharmacists, phamacologists, clinicians, toxicologists, engineers, and, of course, businessmen. Pharmaceutical research is difficult, time-consuming, and it has become increasingly expensive. Drug development is very vital to modern life but its details are poorly appreciated by the lay public, including the media people, the politicians, and even by the scientific and academic community.

The research involved in bridging the gap between discovery of new compounds from natural products and delivery of useful therapeutic agents may be even of a greater magnitude than that involved in discovery. Academic laboratories play an important role in fundamental discoveries, while industrial laboratories have the task of taking a discovery in the field of medicine and pharmacy and developing it to the stage of therapeutic utility. Success results in a new product with new therapeutic value and represents commercial profit that will allow the continuation of the discovery and development cycle.

Every drug that is made available is the result of an extensive development program following the initial discovery of a natural product. A few examples drawn from the areas of pharmacognosy and physical pharmacy in which I have some experience will illustrate the extent of such a program.

a. For the production of the ergot alkaloids, the cultivation of fields of rye inoculated with the parasitic fungus is not always acceptable to farmers. A procedure now developed is to grow a selected strain of the fungus in artificial culture. Alkaloids are produced, which, although lacking the desired pharmacologic activity, may be converted by semisynthesis to the medicinally useful ergot alkaloids.

b. A steroid hormone that occurs in minute quantities in very tiny glands in cattle, sheep, and swine is very expensive to isolate from natural sources. The development of a practical method of production utilizing plant constituents as starting material has substantially reduced the cost.

c. A drug that has been shown to possess beneficial therapeutic activity is not practical if it is so irritating that it can not be tolerated by the body. By modifying its chemical structure, or by synthesizing a derivative or a related compound, or by manipulation of the pharmaceutic dosage form, the harmful side effects are eliminated.

d. A drug that is effective for only a few minutes in the body is not of much use. The proper designing of a formulation will extend the duration of the activity and a prolonged-action pharmaceutical results.

e. The evaluation of drugs in all test systems is another vital link in the process of drug development. In this area the progress is reflected in the emergence of such new terms as pharmacokinetics, bioavailability, blood-level curves, biophase, indepth stability, LADME (liberation, absorption, distribution, metabolism, excretion) system, and others. The researchers in this field must have a thorough background in mathematics to develop models for predicting drug systems, in physical chemistry to predict means by which drugs are absorbed, and in biology to be able to design suitable dosage forms which are well-absorbed or whose absorption is controlled in certain ways to produce very specific blood level patterns and which possess the expected efficacy and a predictable half-life.

It has been the conventional phytochemical practice to study those compounds which are most readily separated from a plant extract. These principles are usually those present in large quantities and which crystallize readily, or those which represent the researcher's field of interest, like alkaloids, terpenoids, glycosides, and others. After workshops, seminars, symposia, training courses, and the like. Such gatherings provide forums for stimulating discussions and exchange of ideas and excellent opportunities to interact personally with colleagues in the field, not to mention the mutual encouragement and inspiration derived. One gets a deeper insight into the details of researches going on in other laboratories which are not readily gathered by merely reading the scientific publications emanating from these laboratories. The knowledge thus gained helps in the individual's researches and the contacts made may lead to cooperative research, exchanges of materials, sharing of laboratory facilities, and even life-long friendships.

IN QUEST OF CERTAINTY: AN ODYSSEY INTO THE CADANG-CADANG PROBLEM

By Jose R. Velasco, Ph.D.

Introduction

Dr. R. B. Espino and I became introduced into the cadang-cadang problem in 1950 when we were made members of the Special Committee on Cadang-cadang of Coconut and Mosaic of Abaca in the Bureau of Plant Industry, DANR. I was made a member upon the suggestion of Dr. Espino, who perhaps thought that I should have something to contribute — I, having just returned from advanced training abroad. (I felt uneasy for being accorded a high estimate, when all I had was a little more book-learning.)

In our report on a first visit to the Bicol Region, we stated among others the following: "The weakness of the roots and the progressive reduction in the number and size of fruits seem to point to a defect in the water-balance of the plant, i.e., an unhealthy root system may cause a diminished water supply, while the few and small fruits may be the effect of a water deficit. (It is possible that the pathogenic organism hinders either the absorption of water or the upward movement of water, or both.) Faced with a gradually increasing water deficit, the plant undergoes a system of self-pruning. The older leaves turn yellow, dry up, and fall; so that, this yellowing of the leaves, which begot the name cadang-cadang is just a stage in "leaf-fall" (Espino and Velasco, 1950).

At that time, the consensus was that the disease was caused by a pathogenic organism; and since no bacteria or fungi could be isolated from the diseased parts, a virus was postulated as the causal organism. With this background, we nonchalantly assumed in our report that cadang-cadang was caused by a pathogenic organism. In retrospect, we may regard our first report as a rough approximation of the situation. The emphasis on the water-balance might be overdrawn but water-balance was certainly an important element in the picture. Of course, there other aspects of the disease which needed to be considered.

Diffidence at the Rubicon

For some time we did not probe into our hypothesis because we felt that a decision to work on the disease was almost as momentous as crossing the Rubicon. History tells us that Cesar

made the momentous decision of crossing the River Rubicon and, in so doing, started a world empire. (The parallel in our case stops at making a momentous decision — not at starting a world empire). We considered the decision to study cadang-cadang quite momentous because it would tie up a major part of our time and resources. Thus, if we have to have cultures which should be shielded from stray infection by a virus, we must be ready to grow coconut trees in huge screened cages or huge green-houses. If we should duplicate the field incidence of the disease we must be ready to work and wait for 50 years or more. These unattractive prospects stymied our desire to probe into the veracity of our hypothesis; hence, we found ourselves in an uneasy time of indecision.

In the meantime, we indulged our tendency to re-examine premises and hidden assumption — the less euphemistic term is "tendency to quibble". For example: Much importance was given to the claim that the disease was spreading. The facts on which the claim was based were that the initial report of a case was made in San Miguel Island. Then there were subsequent reports of cases in several other places in the Bicol Region. The tacit assumptions in the claim were that, (1) the reports were contemporaneous with the incidence of the disease and (2) the later cases were offshoots of the initial case in San Miguel Island. The first assumption can be shown to be weak if we take as an analogous situation the discovery of the islands, which became known as the Philippines. We cannot presume that the islands were non-existent before Magellan discovered them. The second assumption is (logic-wise) fallacious. It is like saying that all the Filipinos who became born after the coming of Magellan were offsprings of Magellan.

The more we thought about that trackless void in our knowledge of the disease and about the insurmountable impediments which were likely to be met in its study, the more forbidding the task appeared; and yet, the more tempting and challenging was the problem.

To indulge the "itchiness to stick our finger" into it, we thought we would like to dig some roots to see if they were in fact unhealthy. Then, we thought we would do just one more thing, and no more: We would just spray some trees with minor elements to find if they would recover from cadang-cadang.

But our resolve "not to do anything more with cadang-cadang" had to be broken — only to be replaced by another resolve. After a few more resolves, we came to realize, much to our helpless consternation, that we were deeply involved in the study of cadang-cadang. By indulging "our itchy finger" we drifted through the *Rubicon* without making the momentous decision.

Of green spectacles and green hay

In our examination of hidden assumptions we became reminded of the tale about a farmer who induced his cows to eat more hay by fitting them with green spectacles. The poor animals thought all the while that they were eating green grass.

Of course, specialists are far from having the mentality of cows; however, they are sometimes accused of being carried away by the spectacles of their specialization. On the other hand, if they speak outside their line, they are branded as interlopers. In terms of the common cliche, they are in "between the two horns of dilemma". A way out is for specialists to determine if the subject is within the area of their competence; and if not, they should be candid enough to say so. Another way out is for decision-makers to weigh the merits of the pronouncements of each specialist; then pick out the aspects which are most common-sensical.

Looking back over the years, one may note that the country's effort in solving the cadang-cadang problem was not free from the bias of specialists. For instance: When the owners of San Miguel Estate asked in 1931 the U.P. College of Agriculture for assistance, the college authorities decided that since cadang-cadang was a disease, a plant pathologist would be in the best position to study it. (The tacit assumption was that the disease was caused by a pathogenic organism; it was rare that anybody would associate a disease with a physiogenic cause). The plant pathologist who was sent to study the disease was Dr. G. O. Ocfemia, a noted virologist who had just worked out the virus natures of abaca mosaic and of the bunchy top of abaca. After some years of cogitation, he came out with a short paper (Ocfemia, 1937), stating that there was a strong likelihood that cadang-cadang was viral in nature. Apparently, the main basis of his judgement was the similarity between the water-soaked specks of cadang-cadang and the pin-prick yellow spots produced by grass mosaic viruses, such as corn mosaic. With the stature of Dr. Ocfemia, other local plant pathologists, and for that matter other scientists, deemed it hard to ignore (if at all) his considered opinion.

Another instance illustrating the influence of specialization on the nature of one's judgement is the following: Randles and his colleagues (1975; 1979) observed some unusual nucleic acid particles in the cells of cadang-cadang affected coconuts. This led them to postulate that cadang-cadang was caused by a "viroid". The methods used for isolating the viroid were ion-exchange chromatography, gel filtration and gel electrophoresis — standard methods in biochemistry for isolating nucleic acids. The questions which may be raised are: (1) Are all nucleic acid fragments viroid in nature? (2) Assuming that they are, can we further assume that

the viroid is the cause of the disease? — or that the viroid and the disease arise from a common cause? They are attempting to transmit the viroid inoculum using the water-soaked specks as criterion of success.

A moot subject in the elucidation of cadang-cadang concerns its diagnostic symptoms. By narrowing down on the water-soaked specks,[1] the plant pathologists probably missed some other (and perhaps more relevant) manifestations of the disease. In the early stages of the study, the workers would have done well to focus on the field aspects of the disease. For instance, by looking for, and finding water-soaked specks in the trees outside the Bicol Region, or in trees which are not visibly affected by cadang-cadang (as Velasco and Fertig, 1956, have in fact reported water-soaking in their culture) one may gain the impression that the specks are not unique to plants affected by cadang-cadang. By noting that in some patches, the trees die of the disease while relatively young (about 2 meters tall) in contrast with those in other patches where they die at a ripe old age (some 20 meters tall), a researcher may think of charting the course of dying-off in a few illustrative patches. He may find that in the plot of age on deaths, the intercept and the slope of the curves do differ from one patch to the other. He may even decide that the two parameters are a more relevant criterion of the severity of the disease than the occurrence of water-soaked specks.

These views, which are in the nature of hindsights, are being mentioned in order to underscore the need to be wary about wearing one's "colored spectacles" early in the exploration of an unfamiliar problem.

To heed a siren's fickle call.

When a mariner starts to hear an enticing, lilting song — now in front of him, and now behind him — this is a sure indication that he is lost at sea. For a long, long time, we had seemed to be in a similar state in our effort to track down the nature of cadang-cadang. Of course, in the same way that the mariner is convinced that he is at sea (and not up in the air), we were convinced that we needed to study the soil. The following are a few indications that

[1]Kent (1953) summarized the symptoms used by plant pathologists as follows:

"The most characteristic symptoms of the disease can only be seen by removing one of the leaves about 1/3 to 1/2 the way back from the center of the crown. The pinnae from such fronds when viewed in reflected light first show small water-soaked lesions on the underside only. These lesions range from minute dots to a size of 2mm wide x 3mm long. At first the lesions are limited by the veins, but more dot-like, water-soaked areas appear which coalesce to produce larger, irregular, elongate, and blotchy water-soaked areas. While the water-soaked lesions tend to occur on the blade of the pinnae, they are also found on the midrib and petiole."

the soil could have an important contribution to the manifestation of the cadang-cadang syndrome: First, the disease varies in severity from one place to another. Rothkirch (cited by Sill, 1964) had this to say:

> "We made another interesting observation on this trip. In the northern part of Camarines Norte, around Paracale and Jose Panganiban, cadang-cadang manifestation seems to have come to a standstill. When I first saw this area in 1946, about 1 to 2 percent of the trees were infested; in 1960, infestation had increased to approximately 3 percent, and today it is still about the same. What is surprising is the fact that only about 15 kilometers from this area — around Vinzons and Talisay — large areas of coconuts have been practically wiped out by cadang-cadang during the past 10-12 years".

Secondly, we fertilized some affected trees with "heroic doses" of ammonium nitrate and they deteriorated very fast. On the other hand, those given ammonium phosphate became vigorous and productive (Velasco et al, 1965). This could mean that a detrimental soil constituent was more soluble as a nitrate and much less soluble as a phosphate.

Thirdly, many species of plants, other than coconut, were abnormal and died-off prematurely (Canoy and Velasco, 1964). Citrus trees (fig. 1) and a shade trees (*Erythrina fusca*) were con-

Figure 1. Citrus trees in various stages of deterioration. Like the coconut, they die off.

Figure 2. Neighboring coconut trees. One is in the terminal stage of cadang-cadang while the other is healthy or perhaps, in the incipient stage.

spicuous when they died-off because they were planted in solid stand. (It is seldom, if at all, that a pathogenic organism could be so wideranging in host relationship).

Once convinced that we should search the soil for the cause of cadang-cadang, our first impulse was to analyze the soil. But it was easier said that done, because we did not know what to analyze for.

To institute some semblance of a system in our approach, we postulated that the abnormality could be due to: (1) a deficient essential element, (2) an excess of an element, like aluminum, which is non-toxic in moderate amounts, and/or (3) an element which is toxic even in trace amounts.

We toyed for some time with the first alternative and obtained data on the comparative statuses of nitrogen, phosphorus and potassium (table 1). There seemed to be no striking differences in NPK contents between the diseased and the non-affected sample.

Hand in hand with our study of the major elements, we studied the essential minor elements (i.e., boron, copper, manganese, molybdenum and zinc.) At first we thought that copper was deficient in the affected soil (Velasco et al, 1957). Our bio-assay for copper gave clear indications that this was so. But then, we found that the procedure was not specific for copper, and that high levels of aluminum can produce results on the test organism similar to copper deficiency (Velasco et al, 1960).

Table 1. The Nitrogen, Phosphorous and Potassium Content of Soil Samples from Los Baños and Guinobatan

Soil Sample	*Per cent Moisture*	*Constituents in per cent dry basis*			
		NH_4—N	P_2O_5	K_2O	MgO
Los Baños	14.12	0.13	0.089	0.91	—
Guinobatan (healthy tree)	16.92	0.17	0.13	0.17	2.85
Guinobatan (cadang-cadang)	23.39	0.42	0.15	0.80	2.22

As an offshoot of our effort to bio-assay for copper, we became curious about aluminum, and analyzed for it using aluminum as our reagent. We seemed to get higher levels in Bicol soil (Velasco *et al*, 1959) but the red-to-pink tinge was quite different.

Even before we could get definite indications for, or against the essential elements, we caught ourselves spilling over into the non-essential elements. We knew that we should be systematic in our approach, yet we could not restrain ourselves from proceeding unsystematically. (We were like a boxer who knew the rules; but, just the same, boxed below the belt — once in a while). Lest we leave the impression that we regard system and logic as the exclusive paths towards extracting information from Nature, we should emphasize that there are other paths. Our only excuse for guiding by them is that they are a means of keeping our sanity in the venture into the confusing labyrinths of the unknown.

By way of making up for the weakness in our data we retraced our steps and analyzed more soil samples for as many elements as our facilities would allow. The total and available amounts of the elements are presented in table 2. Aluminum tended to be high, but not consistently so. Organic matter, or loss on ignition was consistently higher in the Bicol Region.

To avail of more modern facilities, soil samples were sent to foreign laboratories for analysis with the emission spectrograph. Table 3 was kindly reported by Dr. R. Mitchell of the Macaulay Institute of Soil Research, U.K. It will be noted that none among the elements determined showed a tendency to be higher in the cadang-cadang samples (i.e., Guinobatan, Pili and Tigaon).

Another attempt was made to analyze for various elements, this time in the leaf ash. The data of Wallihan *et al*, (1965) show that silver tends to be higher in the cadang-cadang sample (Table 4). However, little importance may be attached to this observation

Table 2. Average of Chemical Constituents in the Different Soil Analysis

Province[2]	*N*	*AL*	*Ca*	*Fe*	SiO_2	*OM*[3]
	A. Total amount, in per cent					
Sorsogon	0.24	19.91	20.17	3.00	53.66	9.95
Albay	0.25	23.09	8.63	7.01	49.35	9.60
Camarines Sur	0.24	13.80	11.75	6.42	55.93	8.88
Quezon	0.17	14.74	6.78	5.78	54.97	6.53
Laguna	0.16	16.66	8.21	7.45	50.04	5.56
	B. Available amount, in mg/100 gram/soil					
	P	*AL*	*K*	*Fe*	*Mn*	*Cu*
Sorsogon	4.6	5.2	15.3	0.9	0.8	0.04
Albay	6.3	4.9	17.0	0.7	0.5	0.13
Camarines Sur	10.8	2.5	27.3	0.5	0.5	0.08
Quezon	4.3	2.8	22.6	0.8	2.4	0.11
Laguna	13.3	2.4	31.6	0.4	1.6	0.20

[1]Data derived from Velasco *et al*, 1959.
[2]The average for Sorsogon consisted of samples from Prieto Diaz (2 samples), Gubat (2), Castillo and Pilar; for Albay, samples from Albay town (2), Guinobatan, Ligao and Polangui; for Camarines Sur, samples from Bato, Iriga and Baao; for Quezon, samples from Atimonan, Pagbilao, Lucena, Sariaya and Tiaong; and for Laguna, samples from San Pablo, Calauan and Los Baños.
[3]Organic matter was obtained as loss on ignition.

not only because the order of magnitude is very low, but also because silver has not been known to cause problems in field culture of plants. Among the essential elements, boron was given some attention because the thick, brittle spear leaves of cadang-cadang diseased plants were suggestive of boron deficiency. It will be noted from Table 4 that, if at all, boron was higher in the diseased coconuts.

Since 1965, when the paper of Wallihan *et al* was published, our group had been groping for some likely soil constituent on which to pin our hope. At various times we determined organic matter, then germanium, the platinum metals, selenium, antimony, beryllium, the earth acids (titanium, niobium and tantalum), and group IIIb (gallium, indium and thallium). Sometime before we focused on the earth acids, we had some indications that there could be a difference between the diseased and the non-affected samples in the amount of rare earths. However, when we analyzed for the group according to the prescribed procedure we obtained inconsistent results.

In all our wandering across the periodic table, one question which kept coming back to us like a bad dream was, When do we drop work on one element? If we obtain one negative result from

Table 3. Elements Found in Soil from Various Coconut Groves, (in ppm. dry weight)[1]

Element	*Non-affected*		*Cadang-cadang*		
	College Laguna	*Tiaong Quezon*	*Guinobatan Albay*	*Pili, Camarines Sur*	*Tigaon, Sur*
Silver	1	1	1	1	1
Barium	600	1500	400	400	400
Beryllium	3	3	3	3	3
Cobalt	40	30	25	20	20
Chromium	30	30	8	10	15
Copper	200	150	100	100	80
Gallium	30	20	30	25	30
Germanium	10	10	10	10	10
Lanthanum	100	150	100	200	150
Lithium	25	20	15	15	20
Manganese	2000	2000	2000	1500	1500
Molybdenum	2	6	1	1	2
Nickel	15	25	10	10	15
Lead	30	30	30	30	30
Rubidium	300	300	300	300	300
Scandium	6	10	10	4	8
Tin	3	3	3	3	3
Strontium	300	2000	1000	600	1000
Titanium	9000	6000	6000	4000	5000
Vanadium	250	200	200	200	150
Yttrium	30	30	30	30	30
Zinc	1000	1000	1000	1000	1000
Zirconium	400	300	200	200	250

[1]Kindly determined by Dr. R. Mitchell (1963), Macauley Institute of Soil Research, U.K.

a test, is it enough indication that the element is not present in the system? The latter question is relevant because as you and I know, a negative result can indicate one of several situations: (a) the method was not pertinent, (b) a mistake was committed in the manipulation (c) the reaction was masked by an interfering substance or (d) the element was absent. Most of the time, we had to rely on our hunches — what Polanyi calls "tacit knowing" . . . And on several instances, we found that we did not know what we thought we tacitly knew.[2] And this was the reason why we returned to some elements a second or a third time. That was what we did as regards the rare earths.

One consolation we had in this repetitive orbiting on an element was that each time we picked up the task we learned what

[2]This is just to indicate that our tacit knowledge is far from infallible — not a repudiation of Polanyi's.

Table 4. Chemical Analysis of Coconut Palm Leaflets, In Per Cent Dry Weight (After Walliham *et al*, 1965)

Element	*Condition of trees*		
	Non-affected[1]	*Cadang-cadang*[2]	*Unhealthy*[3]
Calcium	0.26	0.29	0.30
Magnesium	0.18	0.21	0.23
Potassium	0.91	1.58	0.82
Sodium	0.07	0.02	0.12
Phoshorus	0.14	0.14	0.13
Iron (ppm)	33.6	26.9	32.3
Manganese (ppm)	34.9	16.8	75.5
Zinc (ppm)	6.7	7.5	3.1
Copper (ppm)	2.5	3.7	2.7
Molybdenum (ppm)	0.85	0.45	0.20
Boron (ppm)	9.3	12.6	11.2
Non-essential elements in leaf ash determined by arc spectrograph (ppm)			
Silver	0.01	0.03	
Aluminum	12.00	10.00	
Barium	1.20	0.95	
Cobalt	0.30	0.10	
Chromium	0.20	0.06	
Nickel	0.50	0.20	
Lead	0.80	0.76	
Tin	0.40	0.25	
Strontium	5.00	1.90	
Titanium	3.0	1.9	
Vanadium	1.0	0.6	

[1]Healthy trees in San Pablo and College, Laguna.
[2]Trees in medium stage of cadang-cadang in Ligao, Albay.
[3]Unthrifty trees in Sariaya, Lucena, Pagbilao and Atimonan, Quezon.

mistake to avoid and what aspect needed further exploration. It was as if each little effort — each little push — made the pendulum travel a bigger and bigger arc of a circle. We amused and consoled ourselves by recalling that it was the concept which guided Lawrence in designing his cyclotron.

And we felt rewarded when we learned that iron had to be removed from the system if consistent results should be obtained in precipitating the rare earths as their oxalate (Velasco *et al*, 1977). Table 5 shows that the rare earths in the affected samples were in the order of six times as much as those in the non-affected samples.

Not much later, we came across some information in the literature which led us to pick up again the study of thallium. By removing the bulk of insoluble hydroxides (iron, aluminum, etc.), we were able to neatly precipitate the sulphide of thallium (Velas-

Table 5. The Amount of Rare Earths in Various Soil Samples

Locality	*Miligram per 200 gram soil*
Cadang-cadang affected:	
Bacon	443.8
Guinobatan	629.9
Pili	771.3
Non-affected:	
Los Baños	109.9
Pagbilao	96.7
Silang	84.5

Table 5a. Elements in the Crude Isolate (Per Cent)[2]

Sample	*La*	*Ce*	*Pr*	*Nd*	*Sm*	*Gd*	*Total*
Cadang-cadang affected:	0.02	0.07	0.02	0.06	0.01	0.01	0.19
Non-affected:	0.01	0.02	nd.	0.01	nd.	nd.	0.04

[2]Kindly determined by Dr. D. J. Bland, Institute of Geological Sciences, U.K.

co *et al*, 1978). Our results showed that while thallium was consistently present in the cadang-cadang samples, it was not detectable in the non-affected samples (Table 6).

Instead of getting one element to relate to cadang-cadang, we now have a group plus one. One element was hard enough to relate to the disease; by having the rare earths and thallium, we multiplied our task. We are still in a quandary.

However, we seem to see a beginning of "crystallization", as it were, in the chaotic, supersaturated solution. This seems to occur in group III of the periodic table. We may recall that the malformed, thick, brittle spear leaf is characteristic of the tree in the terminal stage of the cadang-cadang. This is reminiscent of boron deficiency. Boron is the first element in group III; the rare earths are a special offshoot of group IIIa and thallium is in the main group IIIa. It is likely that boron could have been displaced from active sites in the plant through competitive inhibition by the rare earths and/or thallium; hence, the boron deficiency symptoms. Besides this involvement with boron, the rare earths have other chemical and physiological properties. Their capacity to change valence can involve them in the oxido-reduction system of the plant. For its part, thallium is highly toxic to animals and plants. It will not be surprising if thallium would be demonstrated as the main cause of toxicity in areas where the disease is severe.

Table 6. Crude Isolate as Thallium Chromate
(in grams per 20 grams of soil)

Sample	*Grams/20 grams*	*Per Cent*
Cadang-cadang affected:		
San Miguel	1.7226	6.7
Guinobatan	1.3928	5.4
Pili	0.8180	3.1
Non-affected:		
Los Baños	n.d.	
Silang	n.d.	
Pabilao	n.d.	

We hope that the task would appear interesting enough for other researchers to venture into.

To out-do Doubting Thomas

In the course of our laboratory analysis, we often had to check ourselves and make sure that we did not get carried away by our enthusiasm for a given element. For example, at the time we were trying to probe the presence of beryllium, every precipitate we got with ammonium hydroxide appeared to be the substance we were after. Our isolate seemed to give the expected color reaction with curcumin; also, with quinalizarine. Hence, we had to remind ourselves constantly of the precept that "We see what we believe we ought to see." We deemed it necessary to confirm our observation by following an entirely different (if parallel) procedure. Adverting to the account about St. Thomas in the Bible, it was not enough that he saw the resurrected Christ; he should as well be able to touch His wound.

We should check and double check our data in chemical analysis because we deal with properties which are not exclusive to the element. As Lundell and Hoffman said, (1938) ". . . probably no method of chemical determination, whether it be gravimetric or volumetric, is based on a reaction that is peculiar to a single compound." The need to maintain a certain reserve of skepticism in assessing one's own data is even more imperative in instrumental analysis because in most cases the properties measured are simply electrical impulses.

Within grasp of the "will-o'-the-wisp."

In this odyssey, we experienced various moods. There were times when we felt uncontained elation over promising leads; there were periods of drabness, doing routine "busy-work"; then we

experienced periods of resignation, and we asked ourselves, "Why bother? " This last mood got heightened when our failure to probe into our hypothesis was coupled with frustration over unavailability of funds, facilities and materials.

One will-o'-the-wisp which we pursued for sometime was the profuse frothing of the affected soil when treated by an oxidizing acid (say, nitric acid). We searched the literature for the elements which could exhibit this property. By turns, we analyzed for organic matter, selenium, tungsten, molybdenum. It was many years and many mistakes later that it dawned upon us that the frothing could be due to a high content of sulfide in the affected soil.

It appeared that the oxidizing acid converted the sulfide into elemental sulfur. The colloidal sulfur caused the frothing; furthermore, it made the lather stay longer when the solution was stirred vigorously by the stirring rod. Quite vexingly, the colloidal sulfur did not get brought up to a higher oxidation state (say, sulfate) by treatment with oxidizing acid — or at any rate with great difficulty. Hence, the persistence of the frothing.

Unhappily, our subsequent observations told us that the sulfide could not entirely account for the frothing. Samples fused with Na_2CO_3 and picked up in water frothed when treated with HCl.

In a sense the "wasted" effort was not wasted in vain, because it opened to us another window. It strengthened our resolve to look more closely at the thallium status of the affected soil. This is because the literature states that commercial thallium is obtained from sulfuric acid plants. Thallium is said to be associated with sulfur in its deposits; when sulfur is burned to make sulfuric acid, thallium is vaporized. On cooling it condenses and gets mixed with the clinkers.

This incident, wherein one activity leads into another, emphasizes to us the inter-connections and open-endedness of research.

To publish and/or perish

On the subject of publishing one's results no matter how tentative, opinions vary widely. Most of us in the Philippines tend to be perfectionists. We do not want to publish results unless we have proven beyond doubt that we have solved a problem. This would mean that our group should not publish on our effort to elucidate the cadang-cadang problem because we have no definite information for, or against our hypothesis. It was cautioned that if we made many false starts (like crying wolf a little too often), our credibility might greatly suffer. On the other hand, in the United States and other advanced countries, scientists go by the dictum — "publish or perish". In view of the contradiction, we felt that the

dice is loaded against us: if we don't publish, we perish; if we publish what could be the mistakes, we perish just the same. For better or for worse, we opted for the latter alternative.

By way of rationalization, we told ourselves that a research undertaking has at least two aspects: the advancement in information and the advances towards gaining information. This is another way of saying that the two legs of science are contents and process. When we publish tentative results, we hope to attain the latter. We take the reader on a "guided tour" into the intricacies of that aspect of Nature that we are exploring; and incidentally, into the highways and by-ways of our thought process. The thought process could be defective, inadequate and debatable. If so, we expect other people to point this out. To be able to attract the critical view of the scientific community — to be able to start a bid in the market place of ideas — this is concession enough. If colleagues will just do better than turn a cold shoulder.

To tell the people.

Publishing is one important first step in communicating our results. To be of some value, the information which is communicated needs to be accepted, appreciated and applied. A large part of these latter processes rest with our public — that is, fellow scientists may or may not give credence to our finding. The intelligent lay public will have to attach some value of the findings; and the pertinent people will have to utilize the findings. Otherwise, our effort may come to naught.

History is replete with accounts of society's failure to attach value to scientific information. Mendel's discoveries on the inheritance of plant characters lay buried in an unknown publication in a remote library until the phenomenon (and his paper) were rediscovered. A more unfortunate fate was the lot of Galileo, who persisted in presenting proofs, that the earth revolves around the sun, contrary to accepted tenets.

Our experience in presenting our results of cadang-cadang has not been as unfortunate as that of Galileo; but we did meet with disappointments. To disagree with a renown plant pathologist in his observation and interpretation of cadang-cadang is not an easy task. The task is made even harder by the unhappy coincidence that all the experts funded by AID and FAO have chosen to prove that cadang-cadang is caused by a virus or a viroid. The following list of experts is virtually a "Hall of Fame" in plant pathology and virology. Otto Reinking (1950), Donald de Leon (1951-1953), George Kent (1953); R. S. Vasudeva (1955), C. S. Reddy (1956), W. C. Price (1956-1957; 1968-1973), Frank McWhorter (1958-1959), Karl Maramorosch (1960), Francis O. Holmes (1961-1962), W. H. Sill Jr. (1963), A. N. Nagaraj (1963-1967), F.

E. Nitzany (1967-1968), N. S. Wilson (1972), B. Zelasney (1976 to date) and J. W. Randles (1973 to date). The country owes them a debt of gratitude for their time and effort.

On the Filipino side, the following researchers made their unstinted contributions towards the total effort, either independently or in collaboration with Dr. Ocfemia and the foreign experts: A. E. Bigornia, A. Bustrillos, C. A. Calica, B. S. Castillo, M. S. Celino, T. G. Fajardo, A. B. Magnaye, J. L. Naron, D. B. Protacio, E. A. Rasa, E. P. Rillo, and M. S. del Rosario.

The usual reaction of the public is that with all this expertise and the amount of money and material being poured into the undertaking, it is hard to think that the experts could be anything but right.

Literature Cited

1. Canoy, C. S. and J. R. Velasco 1964. Survey of Plant Species in the Cadang-cadang and Non-cadang-cadang affected coconut groves. Philippine Agric. 47(9): 506-533.
2. Celino, M. S. 1947. A Preliminary Report on a Blight Disease (Cadang-cadang) of Coconut in San Miguel Estate, Albay Province. Philippine Jour. Agric. 13:31-35.
3. De Leon, D. 1951. Report on Studies of a Disease (yellow mottled or Infectious kadang-kadang) of the Coconut Tree in the Philippines. Mimeographed.
4. Espino, R. B. and J. R. Velasco 1950. The Cadang-cadang Disease of Coconut in Albay. Report to the Special Committee on Cadang-cadang of Coconut and Mosaic of Abaca. Bureau of Plant Industry, DANR, Unpublished.
5. Kent, C. C. 1953. Cadang-cadang of Coconut. Philippine Agric. 37(5-6);1-13.
6. Lundell, G.E.F. and J. I. Hoffman 1938. Outline of methods of chemical analysis. 4th printing 1945. New York: John Wiley and Sons, Inc.
7. Ocfemia, G. O. 1937. The probable nature of cadang-cadang disease of coconuts. Philippine Agric. 26:338-340.
8. Randles, J. W. 1975. Association of Two Ribonucleic Acid Species with Cadang-cadang disease of Coconut Palm. Phytopathology 65:163-167.
9. Randles, J. W. G. Boccardo, J. S. Imperial, P. Palukaitis and T. Hatta, 1979. Cadang-cadang: Evidence for a Viroid Etiology. Fifth Session, FAO Technical Working Party on Coconut Production, Protection and Processing 3-8 Dec. 1979. Manila.
10. Sill, W. H., 1964. Report to the government of the Philippines on cadang-cadang disease of coconut. No. 1861. FAO. Rome.
11. Velasco, J. R. and S. M. Fertig 1956. Progress report: Sand culture experiment with coconut, Philippine Agric. 40(2): 57-68.
12. Velasco, J. R., E. M. Palmquist and E. S. Mamawal 1957. Copper content of some soil samples from coconut groves of Laguna and Quezon Province. Philippine Agric. 41(4): 241-243.

13. Velasco, J. R. 1957. Copper deficiency in Bicol soil as a possible cause of cadang-cadang of coconut. Philippine Agric. 41(3): 157-170.
14. Velasco, J. R., Alice Holasco, R. S. dela Peña, E. Pantastico and V. F. Guevara 1959. Aluminum and its possible relationship to cadang-cadang of coconuts. Philippine Agric. 43: 177-199.
15. Velasco, J. R., R. K. dela Fuente, E. B. Pantastico, R. S. dela Pena, and Araceli Alfonso Alejar 1959. Chemical composition and physical properties of soil samples from coconut groves. Philippine Agric. 43: 433-455.
16. Velasco, J. R., R. K. dela Fuente and R. S. dela Peña 1960. The interference of aluminum in the Mulder bioassay method for copper. Philippine Agric. 43: 587-588.
17. Velasco, J. R. 1961. A re-examination of the coconut cadang-cadang question. Philippine Agric. 45(4): 145-164.
18. Velasco, J. R., E. B. Pantastico, C. S. Canoy, C. L. Barile and D. B. Guevarra 1965. Fertilization of coconuts in the Bicol Region. Philippine Agric. 49: 573-588.
19. Velasco, J. R., Leonora E. Domingo, Zenaida N. Sierra, and Fe F. Coronado, 1977. Rare earths in coconut groves affected by the cadang-cadang disease. The Philippine Jour. Coconut Studies 2(2): 1-6.
20. Velasco, J. R., Leonora E. Domingo, and Fe F. Coronado, 1978. The Thallium content of soil from coconut groves affected by the cadang-cadang disease. Philippine Jour. Coconut Studies 3(1): 11-15.
21. Velasco, J. R., Leonora E. Domingo, Albine Lansangan and Zenaida N. Sierra, 1979. Cultural Studies on Coconut Cadang-cadang: Reaction of Plants to the Rare Earths, Thallium and Certain Soil Samples. Philippine Jour. Coconut Studies 4(1): 1-13.
22. Wallihan, E. F., J. R. Velasco, R. C. Shorples, and G. R. Bradford. 1955. Mineral composition of leaflets of the coconut palm in Southern Luzon, Philippines. Philippine Agric. 49: 174-179.
23. The following colleagues participated at various times in this study, although their reports were not cited in this paper: Oscar Baldovino, Teofila Cabrera, Elias Canapi III, Marlito Cardenas, Emma Crudo, Cecilia Enriquez, Luz de Guzman, Gilberto Layese, Feliciano Manuel, Bonifacio Mercado, Restituta Pamintuan, Natividad Platon and Marcos Vega. To them goes my grateful appreciation.

Avelino Bigornia, Ph.D., Discussant

The paper of Dr. J. R. Velasco, entitled "In quest of certainty: An odyssey into the cadang-cadang problem", is on the whole an admirable, most candid account of the frustrations, disappointments and meanderings of most scientists that worked with cadang-cadang research. While sharing with Dr. Velasco all the disappointments and frustrations inherent to working in this basic research, this commentator does not agree on a number of important points raised in the paper, among other things, the following:

1. The spread of the disease is put in doubt since "the claim that disease is spreading" is based only on the fact that "subsequent reports were actually subsequent cases" and the later cases were offshoots of the initial case in San Miguel Island". On the contrary, the main basis why the disease is said to be spreading are the results from the studies on the epidemiology of the disease, consisting of annual trip surveys of the Bicol region and epidemiology plot studies (7 plots of 5 ha each) for nine years, 1951-1959. Published papers on the epidemiological studies of the disease (Bigornia, et al. 1960 and Price, Bigornia, 1969) are unfortunately not included or ignored by this paper.

2. On "A moot subject in the elucidation of cadang-cadang concerned its diagnostic symptom", doubt is also implied since "more relevant" manifestations of the disease should have been observed to better understand the malady. In this regard, it should be pointed out that the current diagnostic visual symptoms currently in use today had been found most reliable by most cadang-cadang research workers, in the absence of a more precise method or technique. The small, numerous, irregularly-shaped yellow spots without necrotic centers, that are translucent by transmitted light but appears olivaceous or water-soaked by reflected light on the leaves, had been found most practical and reliable in field diagnosis, *as long as the increase in number and size of these spots follow the phyllotaxy of the palm.* The visual symptomatology of cadang-cadang may be found in a published paper of C. A. Calica and A. E. Bigornia, 1960. The biochemical essay for ccRNA is now currently in use at the ARC for more precise diagnosis of cadang-cadang, since this technique reveals cadang-cadang infection 17 months before leaf symptom expression.

3. Doubts are also raised as to the involvement of nucleic acid fragments being viroid in nature and/or causative of cadang-cadang. Evidence so far gathered at the ARC, PCA, have shown the following:

a. Close association of the ccRNA with disease.

b. Transmission tests using ccRNA as inoculum had produced visual cadang-cadang symptoms in some of the inoculated young palms.

c. The ccRNA may be recovered from inoculated test palms showing positive cadang-cadang foliar symptoms.

All these, quite follow KOCH's postulates.

Lastly, it is regretable that Dr. Velasco with his initiative and drive should retire from the "field to lick his wounds", the exploration in the involvement of elements in the periodic table must remain incomplete.

Julian Banzon, Ph.D., Discussant

I condensed my five page comments to one paragraph. What I gathered from the paper of the speaker is that it's very apparent that it's the effort and not the result that makes his paper very interesting. Whether cadang-cadang is due to the rare earths or to the biological factor or even to extra-terrestial beings, the cause of cadang-cadang remains an incomplete odyssey. May I say however that the oral presentation did very poor justice to the written paper. Please read the paper. It is an unusually excellent scientific assay, as rare as the rare earths.

ANTIMUTAGENIC EFFECTS ON AFLATOXIN B-1, AFLATOXIN G-1, DIMETHYLNITROSAMINE, METRONIDAZOLE, AND MITOMYCIN C

By Clara Y. Lim-Sylianco, Ph.D., Academician

Introduction

Worldwide, so much concern has been expressed about environmental mutagens which are substances that alter the structure of DNA. When DNA of somatic cells is altered and the defect is not repaired, neoplasm may result after a period of twenty years or so. Damage to DNA of germ cells can result in sterility and genetic defects which may appear in future generations. Damage to DNA of cells during organogenesis may result in birth defects. Thus, mutagens can lead to genetic disorders; mutagens can be carcinogens and mutagens can be teratogens.

Environmental mutagens have been identified in moldy peanuts, moldy cereals, broiled meat and fish, cigarette smoke, drugs, pesticides, polluted air, drinking water, hair dyes, and birth control pills. Because of their widespread occurrence, it is not possible to stay away deliberately from all mutagens. It is not possible to have an environment that is completely free from mutagens. This realization led to the search for antimutagens, substances that can either lessen or remove the effects of mutagens.

In these studies antimutagenic effects of vitamin C, vitamin E, vitamin A, riboflavin and thiamine are studied. The test mutagens used are aflatoxin B-1, aflatoxin G-1, dimethylnitrosamine, metronidazole and mitomycin C. Aflatoxin B-1, aflatoxin G-1, dimethylnitrosamine and metronidazole are well known carcinogens.

Aflatoxins are mycotoxins that may appear in the human diet produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus oryzae*. Some aflatoxins are mutagenic in *Drosophila melanogaster* (1), in *Bacillus subtilis* (2), and in *Neurospora crassa* (3). Aflatoxin B-1 and aflatoxin G-1 are carcinogenic in four animal species inducing tumors of the liver and some other organs following administration by several routes (4). The proposed active metabolite is the 2,3 — epoxide which is formed as a consequence of the metabolic oxidation of the vinyl ether double bond (5). Its carcinogenic properties depend on binding to DNA of the active metabolite (6), (7). Teratogenic effects in hamsters were observed with aflatoxin B-1 (8)

Dimethylnitrosamine which has been found in nitrite — treated meat (9) and in cigarette smoke (10) has been reported to cause tumors in various animal species (11). It was shown to be mutagenic in *Salmonella typhimurium* strains TA 1630 and G 46 in the presence of liver enzyme (12). Activated dimethylnitrosamine was also shown to be mutagenic in E. coli WP2 (13). The mutagenic effect upon metabolic activation stems from its ability to alkylate DNA (14). Protein free diet which depresses dimethylnitrosamine metabolism decreases its mutagenicity (15).

Metronidazole is the active principle of a well known antitrichomonal drug. It is used to treat amoebic dysentery (16), (17). In fluctuation tests, it increased the mutation frequency of bacteria several times above the spontaneous mutation rate (18). It has been shown to induce lung tumors in mice (19). Its metabolism in mice is similar to that in humans (20).

Mitomycin C, an antibiotic, induced micronuclei in root cells of *Vicia faba* (21). It also induced dominant lethals in Hobrobracon (22). It is a potent alkylating agent of DNA (23) that has been used as an antitumor agent.

Mutagenic and antimutagenic effects were assessed using the Micronucleus test of W. Schmid (24). In this method mutagens cause the formation of micronucleated polychromatic erythrocytes in bone marrow cells of the experimental mice. Mitotic bone marrow cells with chromatid breaks or chromatid exchanges suffer from disturbances in anaphase distribution of their chromatin. Chromosome pieces lag in the anaphase. After telophase, a sizeable portion of the displaced chromatin is not included in the nuclei of the daughter cells. Instead, they form single or multiple micronuclei in the cytoplasm of these cells. In preparations from animals treated with mutagens, micronuclei are found in different cell types. The majority, however, is observed in polychromatic erythrocytes. Young erythrocytes, less than 24 hours old, stain differently from older ones. These polychromatic erythrocytes stain bluish instead of red.

Methods and Materials

Aflatoxin B-1, aflatoxin G-1, fetal calf serum (lyophilized) and the vitamins were obtained from CALBIOCHEM. Dimethylnitrosamine was an Aldrich product. Metronidazole was obtained from Metro Drug. Mitomycin C was a gift from Dr. Walderico M. Generoso, Oak Ridge National Laboratory. The mice were obtained from Alabang Stock Farm.

The micronucleus test of W. Schmid (24) was used in these studies. Briefly, the highest tolerated dose of mutagens (in dimethylsulfoxide) was given intraperitoneally, while the vitamins (water suspension) were given orally. The mutagens were adminis-

Table 1. Antimutagenic Effects of Vitamin C*

Mutagen	*Dose of Mutagen*	*Number of micronucleated polychromatic erythrocytes per thousand***			
		No vitamin C	*With vitamin C*		
			Same time	*1 Hr. before*	*1 Hr. after*
Aflatoxin B-1	7mg/kg	22.66	1.62	2.10	11.96
Aflatoxin G-1	10mg/kg	14.61	2.90	3.42	10.32
Dimethylnitrosamine	70mg/kg	24.00	0.32	0.78	13.33
Metronidazole	10mg/kg	13.33	2.53	2.06	1.54
Mitomycin C	3mg/kg	25.00	2.06	3.24	4.06

* Vitamin C was given orally at a dosage of 3mg/kg

** Values are corrected for controls

Table 2. Antimutagenic Effects of Vitamin E*

Mutagen	*Dose of Mutagen*	*Number of micronucleated polychromatic erythrocytes per thousand***			
		No vitamin E	*With vitamin E*		
			Same time	*1 Hr. before*	*1 Hr. after*
Aflatoxin B-1	7mg/kg	22.66	0.94	6.23	17.10
Aflatoxin G-1	10mg/kg	14.61	1.97	2.11	9.85
Dimethylnitrosamine	70mg/kg	21.67	0.12	0.28	3.27
Metronidazole	10mg/kg	13.33	1.53	1.04	2.72
Mitomycin C	3mg/kg	25.00	2.23	2.82	8.23

*Vitamin E (dl alpha tocopherol) was given orally at a dosage of 150 mg/kg

**Values are corrected for controls.

tered twice, 30 hours and 6 hours prior to the preparation of the bone marrow. Bone marrow of the femur was flushed into a test tube containing fetal calf serum and centrifuged. The air-dried smears were stained and examined for micronuclei in erythrocytes.

Results and Discussion

Aflatoxin B-1, aflatoxin G-1, dimethylnitrosamine, metronidazole, and mitomycin C in maximum tolerated dosages, cause the formation of micronuclei of erythrocytes of experimental mice (Tables 1,2,3,4,5). This indicates that these substances affected the DNA of the chromatin material of dividing red blood cells.

Table 3. Antimutagenic Effects of Vitamin A*

Mutagen	*Dose of Mutagen*	*Number of micronucleated polychromatic erythrocytes per thousand***			
		No vitamin A	*With vitamin A*		
			Same time	*1 Hr. before*	*1 Hr. after*
Aflatoxin B-1	7mg/kg	22.66	1.04	1.23	3.24
Aflatoxin G-1	10mg/kg	14.61	3.14	2.09	5.78
Dimethylnitrosamine	70mg/kg	21.67	0.89	0.76	3.27
Metronidazole	10mg/kg	13.33	1.22	2.74	1.82
Mitomycin C	3mg/kg	25.00	0.23	0.65	3.86

*Vitamin A was given at a dosage of 150mg/kg.
**Values are corrected for controls.

Table 4. Antimutagenic Effects of Riboflavin*

Mutagen	*Dose of Mutagen*	*Number of micronucleated polychromatic erythrocytes per thousand***			
		No Riboflavin	*With Riboflavin*		
			Same time	*1 Hr. before*	*1 Hr. after*
Aflatoxin B-1	7mg/kg	22.66	0.45	0.56	4.67
Aflatoxin G-1	10mg/kg	14.61	0.00	0.89	3.45
Diemthylnitrosamine	70mg/kg	21.67	0.00	0.00	4.32
Metronidazole	10mg/kg	13.33	2.13	2.86	1.26
Mitomycin C	3mg/kg	25.00	1.22	2.34	3.89

*Riboflavin was given at a dosage of 150mg/kg
**Values are corrected for controls.

This effect led to the displacement of some chromatin fragments which formed micronuclei in the cytoplasm of these cells. Thus, not only are these substances mutagenic but also clastogenic.

Vitamin C, vitamin E, vitamin A, riboflavin, and thiamine reduced micronuclei formation of aflatoxin B-1, aflatoxin G-1, dimethylnitrosamine, metronidazole and mitomycin C (Tables 1,2,3,4,5).

With aflatoxin B-1, aflatoxin G-1, dimethylnitrosamine and mitomycin C, vitamin C, vitamin E, vitamin A and riboflavin were most effective if given at the same time or one hour before the mutagen. The timing of the administration of the vitamins did not matter at all with metronidazole. The vitamins were effective

Table 5. Antimutagenic Effects of Thiamine*

Mutagen	*Dose of Mutagen*	*Number of micronucleated polychromatic erythrocytes per thousand***			
		No thiamine	*With thiamine*		
			Same time	*1 Hr. before*	*1 Hr. after*
Aflatoxin B-1	7mg/kg	22.66	1.87	1.67	3.89
Aflatoxin G-1	10mg/kg	14.61	1.45	1.98	3.22
Dimethylnitrosamine	70mg/kg	21.67	2.20	2.45	2.34
Metronidazole	10mg/kg	13.33	3.39	2.93	2.99
Mitomycin C	3mg/kg	25.00	1.76	1.65	2.03

*Thiamine was given at a dosage of 150mg/kg
**Values are corrected for controls.

whether given at the same time, an hour before or an hour after the mutagen.

Thiamine was as effective when given at the same time, an hour before or an hour after.

Since aflatoxin B-1, aflatoxin G-1, dimethylnitrosamine, and mitomycin C are well known alkylating agents of DNA, the results in these studies suggest that their alkylating power is reduced in the presence of vitamins. It is possible that the reduction in alkylating power is a consequence of interaction of mutagens or their metabolites with the vitamins or the vitamins stabilize DNA against mutagenic influence. The lesser effectivity of some vitamins when given an hour after suggests that the mutagens or their metabolites alkylate DNA very rapidly or the vitamins were not effective in inducing repair of genetic damage.

The results with metronidazole show that the vitamins were effective even when administered an hour after. It is possible that either metronidazole is a slow acting mutagen or the vitamins induced repair of genetic damage effected by metronidazole.

It is very explicit from these results that the vitamins tested have antimutagenic and anticlastogenic properties. That these vitamins have protective ability is clearly suggested. Protective activities of some vitamins have been reported. Vitamin C was found (25) to prevent nitrosamine formation from amines and nitrites. Vitamin C also protected treated rats against teratogenic (26) as well as transplacental, (27) carcinogenic effects of nitrosoethylurea. Riboflavin was found to inhibit liver carcinogenesis in rats treated with 4-dimethylamino-azo benzene (28). Reduction of chromosomal breaks induced by 7,12-dimethylbenzanthracene in the presence of ascorbic acid and d1-alpha tocopherol has been reported (29).

Conclusion

Aflatoxin B-1, aflatoxin G-1, dimethylnitrosamine, metronidazole and mitomycin C are mutagenic and clastogenic since they induced the formation of micronucleated polychromatic erythrocytes in mice.

Vitamin C, vitamin E, vitamin A, riboflavin and thiamine induced the reduction of micronuclei formation indicating their antimutagenic and anticlastogenic effects.

References

1. Lamb, N.J. and Lilly, L.J., Mutation Res. 11: 430-433 (1971)
2. Maher, U.M. and Summers, W.L., Nature (London) 225: 68-70, (1970)
3. Garner, R.C. and Wright, C.M., Brit. J. Cancer 28: 544-551 (1973)
4. Garner, R.C., FEBS Lett. 36: 261-264 (1973)
5. Scheontal, R., Nature London 227: 401, (1970)
6. Patterson, D.S.P., Food. Cosmet. Toxicol. 11: 287-294 1973)
7. Sporn, M.P., Dingmar, C.W., Phelps H.L. and Wogan, G.M. Science 151: 1539-1542, 1971
8. Ellis, D. and Di Paolo, J.A., Archives Pathol. 83: 53-57, (1967)
9. Crosby, N., J.K. Foreman, J.F. Palframer and R. Sawyer, Nature (London) 238: 342-343 (1974)
10. Montessano, R. and H. Bartsch, Mutation Res. 32: 179-228 (1976)
11. Clapp, N.K., A.W. Craig, R.E. Torza Sr., J. Nat'l Cancer Inst. 41: 1213-1227 (1968)
12. Gabridge, M.G. and Legator, M.S., Proc. Soc. Expt'l. Biol. and Med. 130: 831-834 (1969)
13. Green, M.H.L. and Muriel, W.J., Chem. Biol. Interactions 11: 63-65 (1975)
14. Magee, P.M. and Hutton, T. Biochem. J. 83: 106 (1962)
15. Venkatesan, N., Arcos, J.C. and Argus, M.F., Cancer Res. 30: 2563-2567 (1970)
16. O'Holohan, D.R. and Hogoe-Mathews, J., Ann. of Tropical Medicine and Parasitology 66: 181-186 (1972)
17. Miller, M.J., Scott, F., and Forter, E.F., Am. J. Trop. Med. Hyg. 21: 400-403, (1973)
18. Voogd, C.E. J.J. van Der Stel and J.J.J. A. A.A. Jacobs, Mutation Res. 26: 483-490 (1974)
19. Rustia, M. and Shubick, P., J. Nat. Can. Inst. 48: 721-729 (1972)
20. Stambough, J.E., Feo, L.G. and Manthel, R.W., J. Pharmacol. Exp. Therap. 161: 373-381, (1968)
21. Arora, O.P., Shah, U.C., and Rao, S.R.U., Exptl. Cell Research 56: 443-448, (1969)
22. Smith, R.H. Mutation Res. 7: 231-234 (1969)
23. Kobayashi, J. Cytologia 25: 280-288 (1960)

24. Schmid, W., Agents and Actions 3: 77-85, 77-85 (1973)
25. Mirvish, S.S., et al. Science 177: 65 (1972)
26. Ivankovic, S., et al. Z. Krebsforsch. 79: 525 (1973)
27. Ivankovic, S., et al. Naturwiss 60: 525 (1973)
28. Kensler, C. J., et al. Science 93: 308 (1941)
29. Shamberger, R.J., Baughman, F.F., Kalchert, S.L., Willis, C.E. Hoffman, G.C. Proc. Nat. Acad. Sci. (Wash) 70: 1461-1463 (1973)

Jesusa A. Concha, Ph.D., Discussant

Due to time constraint, my comments on Dr. Sylianco's presentation will be very brief.

The subject that Dr. Sylianco has presented is indeed very timely, especially because people today are very much aware that the food we take in and the environment may cause cancer, genetic disease, and other disorders/deformities which may be manifested many years later. For example, those of us who love to have our steak fish, and pork broiled, will have to be very careful because studies have revealed that improper preparation can cause mutagenesis. The present study is corollary to the investigations on mutagens which Dr. Sylianco and co-workers have made, among others, on a number of plants with medicinal uses, including a number of plants found in the Philippine National Formulary.

This study presents to us a way of combatting the mutagens that we may be exposed to. The utilization of vitamins C, E, A, riboflavin, and thiamine, as antimutagenic agents is a good choice because these are easily available. We may get them from the food we eat or through medication. For example, we should subject our vegetables to too much boiling because the vitamins therein, especially vitamin C, can be destroyed by heat. A number of vegetables should preferably be taken raw to prevent destruction of their vitamin content. We may not like "darak" but we know that it is rich in vitamins. The Food and Nutrition Research Institute and the National Institute of Science and Technology have prepared cookies using "darak" as the main ingredient. Aside from these natural sources, there are a number of pharmaceutical preparation or dosage forms containing one or several vitamins. They are generally prescribed for pediatric and genetric patients, although a number of individuals take them whether prescribed or not.

Dr. Sylianco's study has shown that vitamin C is antimutagenic in very small dosages (3mg/kg body weight) while the other vitamins required about 150 mg/kg of body weight. Utilizing the results of her investigation, protection from some mutagens may be accomplished through the adequate use of the vitamins from food and/or pharmaceuticals.

We congratulate Dr. Sylianco on her studies on antimutagens. She has shown a way through which one may offset mutagenesis through vitamins.

Ruben Umaly, Ph.D., Discussant

I would like to congratulate Dr. Sylianco for the very nice paper. But, I feel I should not be here because I belong to Dr. Sylianco fans club. She is my favorite teacher and I am a very ardent admirer of Dr. Sylianco. So, most probably my comments would be mostly in favor of Dr. Sylianco's work.

But seriously, I had a look at some of the mutagens that she has been discussing and would like to correlate it with the voluminous presidential decrees, letter of instructions, and creation of several government agencies purposely to minimize pollution. Inspite all this seeming concern, we allow industries, for example the Kawasaki Smeltering Plant in Mindanao to be constructed here in the Philippines even if we know that established mutagens like sulfur dioxide and nitrous oxide are two of the most important pollutants from these industries.

We still import a lot of chemicals like pesticides, hair dyes, food additives that have been banned a long time by other countries. The construction of the nuclear plant was only temporarily suspended not because of their concern for our health and not because of the possible mutation that radioactive waste may cause, but most probably because of political or economic reasons that is because Carter ordered suspension of nuclear plant construction or we do not know where to get the ₱.4 billion additional money. So, we are still very much exposed to a number of sources of pollution in our country. Although it is very gratifying to know that we have our DNA repair mechanism, our immune system charged with the removal of the mutated somatic cells, and that we still have some antimutagens that we can rely on when everything fails and more strict governmental policies may be even better.

Going back to the work of Dr. Sylianco, I am sure it is only because of the short available time, that she limited her presentation to work on the micronuclei test. I think she and I agree that in the testing of mutagens, it should be a tierred system of analysis, meaning it should not be only to one group of organism but to a wider range, i.e. from bacteria to humans. But this would mean much involvement in manpower, as well as a large amount of

money and resources. Most probably, this is where a consortium of researchers and research institutions could work together. Perhaps the National Research Council of the Philippines and National Academy of Science and Technology can help researchers who are involved in this kind of work.

And lastly, as a student of genetics, I would be very much interested if Dr. Sylianco in her future lectures could explain in detail the mechanisms involved in the prevention of mutation by antimutagens. We have done some work on Vitamin C, but this is on radiation protection. We found out that we can prevent wing mutation and cephalothorax mutation on Drosophila if we incorporate vitamin C in the media that the larva would eat prior to irradiation.

The results seem to suggest that there is similarity between the mode of action of some of the chemical antimutagens and some of the chemical radioprotectors. Both should be present in optimal concentration at the time of exposure to radiation or to the mutagens. There are other factors in the cells that will most probably affect their efficiency. I am sure Dr. Sylianco could enlighten us on these points. I am looking forward to her next lectures on these topics.

Miraflor Gatchalian, M.S., Discussant

I think I should start with thanks to the National Academy of Science and Technology for having given me the honor, to be one of the discussants this afternoon. I join my other two discussants in congratulating Dr. Sylianco for such a wonderful paper and like Dr. Umaly I may also be giving a biased opinion. I too am one of the members of the fan club for the works of Dr. Sylianco on antimutagens.

Perhaps at this point many of us are beginning to fear how long, they may yet survive knowing that everything in the atmosphere could possibly result to the formation of mutagens. We all know that particularly for aflatoxin and nitrosamines we can be eating these all the time, without knowing it. We know that most cured meat products starting from hotdogs, corned beef, ham, and bacon, and other things you probably like most such as peanut butter, cabbage, cauliflower almost everything that we eat everyday are sources of mutagens and carcinogens, particularly aflotoxin and the nitrosamine group.

So in essense there can be much fear for those who live in ignorance because then you would not know which carcinogen will now attack you, and which carcinogen will eventually cause your death. Now, despite being an avid fan of Dr. Sylianco I would like to raise some questions which I think may also be bothering some of us here present. But at this point, may I emphasize that I feel the work of Dr. Sylianco is one of a heroic job, one which we need very badly at the present. Because for these only a little in-depth study has been done in the area of antimutagens. Now it is very good to know that at least we can make use of vitamins to fight this kind of disease in our country. In this regard, therefore, I have some questions on this work presented.

1. What are the statistics on mutagens in moldy peanuts, moldy cereals, broiled meat and fish? Are there means of destroying the mutagens in food before ingestion? If so, could you please explain? If these questions require another lecture series, then perhaps we should attend your next lecture for this information.

2. Will all broiled meat and fish contain mutagens? Or are the mutagens formed only under certain conditions? For instance, there are special types of heat, special types of charcoal etc., which could eventually accelerate the formulation of mutagens?

3. How much daily ingestion of mutagenic substances would eventually lead to cancer? Quantitatively how many kilos of infested food must be eaten to accumulate enough to cause the patient to be sick? Are the carcinogenic effects of mutagens cummulative in nature? How long will it be before one taking in mutagens suffer from these dreaded diseases?

4. What are the possibilities of mutagens in cured commodities (nitrate/nitrite treated products) especially in cured meats? What would be the level of nitrate/nitrite in the products ingested by man before we could say it may eventually lead to cancer?

II. Then referring to tables 1 to 5, I have also a few questions:

1. When each of the vitamin was added, was there an assumption that the diet was free of the other vitamins? Or is it possible that all 3 vitamins or all 5 vitamins could be working together and mutually "helping" each other?

2. How was each dosage given determined per vitamin? Did you have a basis for the choice or was this an optimal level experience by other people? Were effects of other levels also studied, in the past or in your work-related activities?

3. What was the basis for the dosage of mutagens? Would the vitamins be as effective if the mutagens dosage were less? What about if there was more?

4. Which of the vitamins would you consider the most effective antimutagens? Your conclusions indicate all of them are effective on all mutagens. Although after one hour of ingestion it seems like it had lost its effect as shown in your table.

5. And my last question, would you consider Vitamin A, Riboflavin and Thiamine as more effective antimutagens than Vitamin C, and E in general? Well, I feel sure now that to have all those questions answered, another lecture series will have to be organized. I am, however certain that if Dr. Sylianco will continue her work, most of us may hope to expect longer life.

BIOLOGICAL PATHWAYS

By Geminiano de Ocampo, M.D., Academician

The concept of biological pathways was formulated from clinical ophthalmological observations, experimental works and theoretical considerations. This presentation is a theoretical mental expedition. The concept maybe stated as follows: There are five intracellular pathways for biological processes. These are viability, development, differentiation, protection and proliferation. Metabolic processes along the metabolic pathways occur in all the biological pathways. The inward pathways start from the molecules in the outer cell wall while the outward pathways start from the genes and other intracellular organelles. These pathways convey biological inward, outward and intraorganelle messages upon which the biological processes depend. Many of the structural stations of these pathways have been identified. Their intermolecular connections however, are largely conjectural. The complete demonstration of the network of these pathways challenges biomedical research.

Clinical background. Corneal Viability — The questions are on corneal transplantation and deal with the desirability of the graft. How much of it is alive? How many of its cells are alive or dead? Are non-cellular membranes and stroma fibers alive or not?

Clinical Observations:

The sooner a graft is transplanted the more chances it has to take and remain alive. Direct transfer is best. At 4°C the graft remains suitable longer. It is most desirable when obtained within two hours after death. The more favorable the recipient eye is the more chances the graft will take and remain viable and clear. Better soil and good seed lead to more chances of survival and growth.

Self Renewal Concept of Biology. The essence of life and the living state that distinguishes the living from the non-living and the dead is self renewal. Self renewal is the common function of most life processes. The needs and the demands for self renewal determine the quantity and the quality of life processes.

The smallest living unit consists of organized molecules and the molecular complexes with the capability for self renewal. It may be called the biological unit or organelle. Examples are the gene, the mitochondriom, the cell membrane, ribosome, Golgi apparatus, mitotic apparatus, lysosome and the virus. Others are the extracellular membranes such as Descemet's membrane and Bowman's membrane.

The unit of renewal of the organelle maybe the atom, molecule, molecular complex or the whole organelle itself. On the control, speed, balance, nature and manner of self renewal depend the different biological phenomena and the various states of health.

Application of the concept. Self renewal serves to differentiate the living from the non-living. It is useful in relating observations about biological phenomena. It serves as a theoretical approach to a logical system of the cells and noncellular structures of a living organism. It is a broad unifying and basic principle in understanding diseases.

Using it as a common indicator and a unique dimension of biology it should be tested in investigation on the mechanism of life processes and the quantity, the form and the behaviour of living matter. Molecular tagging is the most useful tool of testing the concept.

Viability of corneal crafts. The focal point in corneal grafting as in all other biological transplantation is the graft's state of living and the crucial question is how can this living graft be kept alive in the recipient eye. We used in these experiments human cadavers, rabbits and monkeys.

Embryonation Experiments. I reversed the question how can one tell whether a cell is alive to how can one tell whether a cell is dead. A biological test is embryonation. If a corneal specimen is placed in the allantoin of a 9-day old chicken egg, after 48 hours all the dead cells in the epithelium, stroma and endothelium would disappear leaving only the living cells. By this procedure we could produce the acellular cornea. We made physiological, biochemical and physical tests on acellular cornea and obtained proofs that the acellular corneal fibers and membranes were alive but could not multiply. They are viable because they could exchange metabolites but like parasites they are dependent on the interspersed cells. They differ from artificial lifeless membranes. We made clinical and laboratory experiments using acellular cornea. The results support a different concept of viability of the cornea and other living tissues. We found incorporation studies which support the concept of the viability of the noncellular portion of the cornea. All these experiments and observations gave clinical, pathological, biochemical, physiological and biological evidence that the noncellular formed structures of the cornea are viable in the sense that viability means the capability to "survive with life" with or without the property to "propagate" life.

Death and the dead state. To understand further biological survival, viability and the living state, it is necessary to probe into the different aspects and tests of biological death and the dead state. We made experimental tests on cell death by the methylene

blue decolaration of conjunctival and endothelial cells and acridine orange test of the corneal endothelium.

Basic experiments on corneal necrosis. Many laboratory experiments gave the following conclusions: In corneal necrosis there is a massive and pathologic disintegration of the collagen framework in addition to a disorganization and disintegration of the ground substances as well as death and disintegration of the stroma cells. Corneal necrosis maybe produced by bacteria, chemicals, enzymes and physical agents like heat. Necrotizing substances are found in the toxins of p. aeruginosa and croton oil. Trypsin in dilute solutions affect only the ground substances. In higher concentration it further causes disintegration of the collagen fibers although this does not occur after freezing the cornea because of the alteration of the ground substances and probably also of the cement substances.

Collagen staining is lost in necrosis. The collagen fiber can exist in a necrotic state distinct from its native nennecrotic condition. Metachromasia is always absent in necrosis although it may disappear without necrosis. Changes in the ground substances and interfibrillar cement substance may cause softening or malacia and swelling without necrosis. Corneal opacities may be necrotic or not. Necrotic opacities may be delineated and demonstrated histologically and by histochemistry. Necrotic tissue may be debrided by enzymes (0.25% trypsin and fibrinolysin with DNAase) leaving the viable tissue intact.

A new concept of corneal viability. This may be stated as follows: All the formed and organized parts of the cornea, cells as well as the noncellular structures are viable. The only nonviable portion of the cornea is the amorphous and unorganized ground substances.

The collagen fibers and membranes of the cornea can exist in a native state as well as in a dead or necrotic condition. The viable native collagen fiber differ morphologically, histochemically, optically and biologically from necrotic collagen, reconstituted or extruded collagen strips, altered collagen catgut, gelatin or hydrolyzed collagen gelfilm.

In the preserved glycerinized cornea, the cells are dead but the collagen fiber and membranes are still viable. The acellular corneal graft used for keratoplasty can respire, dehydrate, incorporate S^{35} and remain transparent only by preserving its viability, unlike the unchanging and nonexchanging transparency of inert plastic or glass. Not only those structures that can grow or multiply or alive. The propagation of life is a different phenomenon from the maintenance of life or the ability to stay alive. The viable structures of the cornea show different degrees of vulnerability and what will preserve the life of the fibers may not

conserve that of the epithelium or the stromal cells or the endothelium. The endothelial cells are the most delicate and Descemet's membrane is the most hardy. It is therefore more conducive to meeting of minds and understanding if the cornea is considered not only as a single and whole viable structure but also to view it as composed of different viable parts with varying characteristics of viability.

Biological Unit. The quantum is for energy as the biological unit is for life. It deals with the how much of life and death at the subcellular levels and extracellular sites. The smallest unit of life is the biological unit. This is that which is capable of changing and exchanging its molecules and molecular complexes. While the cell may be the smallest unit that shows all the characteristics of life the biological unit is the tiniest unit that renew itself and possesses the most essential and basic property of living things, viability and self renewal. This is the organelle. Each organelle is composed of repeating organized subunits of organic with or without inorganic molecules of similar and/or different kinds. The biological subunit or molecular complexes compose the different formed structures of cells and noncellular structure. They make up the various particles and the organelles of the bacteria and the cell membranes. The free molecules of the tissue fluids and amorphous ground substances in between the formed structures, are not part of the biological subunits, but they serve as the reservoir which supply and receive the continuous exchange of molecules or molecular complexes.

Biological pathways and biological messengers. These are found in the cell. The inward pathways start from the outer surface of the cell wall and end at the genes while the outward pathways start at the genes and end at the inner surface of the cell wall. The terminals of the pathways are at the cell wall and the genes. The pathways form a network inside the nucleus across the nuclear membrane and in the messwork of the cytoplasm. They are collectively called smooth and rough endoplasmic tubules. They serve as the pathways to and from the cell wall and the nuclear wall. The secondary messengers that carry or transmit messages or molecules are those that carry biological messages from the cell wall to the nuclear wall. Some believe that prostaglandins could act as secondary messengers. There must be also some tertiary molecular messengers in the nucleus to carry biological messages from the nuclear messengers in the nucleus to carry biological messages from the nuclear membrane to the genes probably thru the supragenic molecules. I am not aware concrete observations and reports of this nature.

While the prostaglandins are considered inward secondary messengers the nature of their messages are still conjectural. If

they are connected with the inflammatory process as some think, they probably carry proinflammatory or prophlegestic messages. The corticoids are antiinflammatory or antiphlegistic. It is along this thinking that the existence of inflammatogenic genes is premised.

The concept of biological pathways attempts to suggest a framework for the existence of a structural basis for distinct biological activities in any cell. The discovery of the prostaglandins as the secondary biological messengers suggests the presence of primary biological messengers in the cell wall and membranes as well as tertiary messengers inside the cell nuclei.

Viability or survival pathway. This subserves the functions and needs for self renewal. It is the primary, initial, widest and constantly open biological pathway.

Survival is within and after one's life span. Viability refers to and concern only with life within the life span. In the biological organization of the organism those portions for survival are always active except perhaps in the state of suspended animation. Hence, there is always activity, however, minimal in the viability pathways. It can therefore be aptly called the primary biological pathway.

The other biological pathways subserve special functions other than mere survival. They are therefore designated secondary biological pathways within the cell and are open only when there are needs for self development, differentiation and specialized products, protection to self and other cells and proliferation. It is evident that more than one biological pathway could be open at anytime.

There are two kinds of metabolites — the primary metabolites which are essential for survival and the secondary metabolites needed for specialized functions. The latter travel through the secondary biological pathways. Some bacteria suspend differentiation products like toxins while they are proliferating or dividing.

The recent findings of biological messengers strengthen the assumption for the existence of intracellular biological pathways. These biological entities are postulated on the existence of molecular receptors many of which are now identified on the surface of the cell organelles. Transmitter molecules are produced at the receiver surface of the target structures. These intermediary transmitter molecules seem to have very short life span.

Biological development. This is a marvel of design and execution. Nature and Creator must be behind it. The factors for biological development are genetic and environmental. If there are (10^7) 10,000,000 genes in man these could theoretically be divided into 60% survival genes, 10% developmental genes, 10%

differentiative genes, 10% proliferative genes and 10% protective genes. The concept of biological development could be stated as follows. Full development of any biological unit from organelle to organism is the attainment of its nature structure, substance and function by the information from and control by developmental genes, the interaction with other biological pathways and the proper environmental factors. Completeness is premised on an adult design stored in the genome and built by the orderly, timely and sufficient supply of raw materials.

The new ideas are the postulation of the existence of adult design in the genome, developmental genes, development and proliferation of DNA themselves by nonmitotic replication and interaction with other genes and pathways.

This concept could be an approach to the search for the most effective vaccine and most helpful adjuvant as well as the most proper timing, combination and sequence of vaccination procedures.

Ocular developmental anomalies and biological pathways. These could be temporal, structural, functional, biochemical, biological and multidimensional. Glaucoma is shown by statistics to have polygenic inheritance. Defective genes responsible for developmental anomalies could be qualitative and/or quantitative in number of similar and dissimilar genes involved. The injury could be from ultraviolet light, gamma rays, some dyes like acridine or toxic molecules or drug and catabolic products, bacteria and virus.

Examples of developmental ocular anomalies are spatial (ectopia lentis, displaced or misdirected lashes, ectopia irides) temporal (persistent hyaloid artery), dacryostenosis, developmental and congenital glaucoma, ptosis, functional amblyopia, squint, ametropia, biochemical congenital night blindness, albinism, aniridia, iris coloboma, iridoremia, hypoplasia of the optic nerve; multidimensional (microphthalmos and anopthalmos). Corticoid glaucoma has a theoretical genetic basis. There are anomalies of development in myopia, congenital cataract, amblyopia and squinting, macular degeneration and underdevelopment of the fusion center.

The phenomenon of immune tolerance in undeveloped immune systems support the thesis of the development of the metabolic and biological pathways during the embryonic and fetal stages.

Embryonic antigens support the information theory of immunity, that is, there is a prestored information for the antibody in the genes of the antibody producing cells. This theory suggests that the genes have evolved in kind and number of the different species.

The development of the metabolic and biological pathways start from the inherited genes. The number of each kind of genes in the fetus, infant, adult or senile are not equal although their chromosomes may be similar. This could be due to differences in the number of copies of each genes. The immunological cells and the response to antigens by the fetus and neonates are different from that of the adult and the senile.

Biological differentiation. The kinds of differentiation are form, structure, substance and functions for survival or special functions. There must be different and proper qualitative and/or quantitative stimuli for the different biological functions along the different biological pathways. The prime purpose of differentiation from organelle to system is for specialization of function.

Examples of high specialization are the light receptors in the retina. These are specialized receptors for the different light waves in the rods and three kinds of genes in each cone as red genes, blue genes and green genes or three kinds of cones for red, blue and green.

Cultural media and differentiation. The problem we have met in the culture and sensitivity tests for bacteria had in one way contributed to the conceptualization of biological pathways. Some staphylococci do not develop pigment in a particular medium. The staphylococcic toxin may be absent in some media or may vary in amount and potency in other media. These observations suggested the existence in the genome of some mechanism with morphological basis. In other words there must be a genetic basis for differentiation and differentiation products.

Then we had some observations on the changing sensitivity of staphylococci to antibiotics. We observed over a period of five to ten years a definite decline in the sensitivity of conjunctival staphylococci to chloromycetin. After many more years this sensitivity returned. This is true with other antibiotics. This makes the choice of antibiotic a hit and miss affair without the aid of culture and sensitivity test. There are survival culture media and differentiative culture media for bacteria, virus and fungi or any plant and animal cell for that matter. All these must have a genetic basis in the differentiative and a proliferative biological pathways.

Differentiation like any biological phenomenon must have physical, chemical, morphological and molecular bases. The genes, paragenic molecules, gene complexes and genome segments that subserve differentiation must be physically proximate. Genomic mapping is advanced in lower forms of life but meager yet in man. This is a challenge to molecular biologists.

Other areas of investigation are the inhibition and promotion of differentiation, vaccine adjuvants, live and attenuated vaccines, relationship between pathogenicity and differentiative pathways,

virulence and differentiation, specificity or nonspecificity of the supragenic molecules, mutagenesis and resistance to antibiotics.

Protective pathway and subpathways. The concept of biological pathways assigns a portion or segment of the genome consisting of one or several chromosomes for the immunological system. The kinds of genes of the immunological genome must therefore be preformed or predetermined in a certain species in the fetal stage of development. However, it seems that the replicas or copies of each kind of the immune genes must be determined in the later stages of development of the organism. Hence while the immune genes in a single lymphocyte during the fetal state may be inherited and limited, the number of copies of the immune genes may be more or less in the child and the adult. Moreover the number and kind of lymphocytes may fluctuate depending on the need and the stimuli. Their proliferative activity also depends upon appropriate stimuli. On this concept the role of adjuvants for immunity vaccines could be based and sought for. For example, this could explain the role and usefulness of kock's adjuvants for cancer cell vaccine. All these could not only potentiate but even prolong the effectivity of our current bacterial vaccine for infectious diseases.

Theoretical consideration on immunity. It seems from studies that the almost total absence of trachoma among Filipinos may have some genetic element of natural immunity. The phenomenon of immunologically competent lymphocytes may be due to difference in the supragenic molecules and/or in the cell surface receptor molecules to antigens. The competency or incompetency of any lymphocytes to respond depends in the kind of antigens. The cell surface molecules, the recipients for antigen molecules may have variation. This may be the explanation for the effectivity of mixed vaccines. There must be a different lymphocyte population that respond to a different antigen. This may explain the differences in potencies and duration of vaccines according to their mode of preparation.

One basic question in immunology is where and how are the antigenic molecules produced. The concepts that may help clarify this are those of the mosaic molecular structure of the cell surface, the biological molecular messengers and supragenic repressor-derepressor molecular mechanism for inhibition and stimulation of genic activity. The concept of biological pathways will help clarify also the phenomenon of virulence and attenuated live vaccine.

Local immunity is closely related to that of inflammation. One way to the exploration of the problem of keloid is through the biological subpathways of immunity and inflammation.

Other problems that can be approached through the concept of biological pathways are: adjuvants to anticell vaccine like BCG

and levamisole, booster doses in immunotherapy of infectious diseases, suppression of immunity in corneal and organ transplantation, separation of inflammation from immunity, desensitization in immunotherapy, anticancer immunity and immunotherapy, fetal cell therapy and antirejection vaccine in tissue and organ transplantation.

A Theory of Inflammation. The inflammatory diseases are the most frequent of all human diseases. And yet inspite of countless observations, numerous experiments and voluminous literature on its manifestation, diagnosis and management there could be found such expression as "inflammation studies are largely descriptive." The problem is to find a unified hypothesis to account for much of the morphological data available without doing harm to physiologic evidence. In all inflammatory processes, agreement is on a few basic facts but there is disagreement on most of the critical issues. There is an overwhelming wealth of details but there is a conceptual confusion. In order to understand well inflammation it seems worthwhile to consider its theoretical aspects. We must pay attention to integration, a sort of intellectual synthesis with more emphasis on meaning and values than on mere facts. All these have induced this attempt at formulating a theory of inflammation. I took into account semantics, the definition, perspective, levels, phases, areas, cells, noncellular structures, basic observations, beneficial and deleterious effects and inflammatory cells. I also included biological postulates about primary and secondary metabolites, biological pathways, inflammatogenic agents, inflammatogenic injury and the existence of inflammatory genes for protection and repair. Then I formulated a theory of inflammation as follows: Inflammation is a network of reparative, protective and destructive physicochemical, physiological and biological reactions and interactions usually after inflammatogenic tissue injury. The inflammatory mechanism has genetic and environmental aspects.

The new ideas introduced are inflammatory genes, primary and secondary metabolites, biological pathways, inflammatogenic agents and injury and interaction. It seems that the inflammatogenes could elaborate inflammatory mediators without direct or indirect cell injury from changes or imbalance in the regulatory systemic or local neurohormonal mechanisms. These could be brought about by stresses which may be behind recurrent inflammation of obscure origin without evident inflammatogens.

The test of the theory is to demonstrate the existence of inflammatory genes responsible for early or late inflammatogens. These would necessitate demonstrating inflammatogenes similar to immunogenes, mutagenes, teratogenes and carcinogenes. Mapping of the chromosomal and extrachromosomal genes would also test

the theory. Another approach is the search for antiinflammatory agents against the various parts of the inflammatory mechanism.

Induced inflammation. Induction of inflammation is a form of therapy, for inflammatory and noninflammatory diseases in ophthalmology. Examples are inducing reactive inflammation for retinal detachment or retinal degeneration which may lead to detachment, thrombosis of retinal vein, ciliary ablation for intractable glaucoma, antiglaucoma filtering operation, tissue implant for torpid corneal ulcers, nonulcerative keratopathies and induction of fibrillogenesis.

A theory of carcinogenesis. If we must control cancer we have to understand it in all its aspects from a holistic perspective thru the biological pathways.

Fundamentally, carcinogenesis is a transformation of a cell in any stage of its life span. It consists essentially of transmissible imbalance of control of the proliferative and other biological pathways. It is brought about by endogenous and exogenous carcinogens acting directly or indirectly upon the proliferative control genes. These become the cancer genes which manifest abnormal primary and secondary metabolism.

The new ideas in this theory are: The concept of biological pathways, the concept of specialized segments of the genome for each biological pathway and the uninhibited proliferative control genes becoming the cancer genes.

During the early development of the blast cell the pathways for propagation are normally open but that for differentiation are still closed. At a later maturation stage the pathway for proliferation may be very active while that for differentiation may be minimally open. The diptheria bacilli do not elaborate diptheria toxin while it is proliferating. A mature brain cell and a rod or cone cell of the retina are highly differentiated but their proliferative pathways are closed.

In non-neoplastic growth, there is a temporary increase in positive derepressor supragenic molecules from injury and hormone, vitamin or mineral imbalance. These are found in inflammation, vitamin, mineral or trace metal deficiency or hormonal imbalance. Examples are Bitots spots in hypovitaminosis A, pterygium and inflammatory granuloma.

In neoplastic growth there is a permanent decrease or loss of negative repressor supragenic molecules brought about by carcinogens from internal environment or from external factors such as ingestants, inhalants, contactants, radiation or vibration and from genetic deficits.

Uses of the theory in the fight against cancer. The recognition of cancer cells by morphological means should be supplemented by physical, biochemical and biological methods.

Anticancer vaccines, vaccine adjuvants, cytacids and a cytostatics and restoration of balanced cell control should be continuously explored. Natural systematic vaccination could be attained through freezing and irradiation. Chemical and immunological detection of residual cancer cells after any form of therapy should be continuously investigated. The warhead principle of tagging the specific anticancer antibody should be pursued. The timing, the method and the extent of the surgery should be reevaluated in conjunction with the other modalities of therapy. The concept of biological pathways could serve as the framework for the all-out offensive against cancer.

A concept of biological degeneration. This is not a biological pathway but a deterioration occurring in any one or several biological pathways. It is a negative balance of self-renewal initially in the cell or noncellular structures. It may involve directly or indirectly any one or more of the metabolic and biological pathways. Metabolic disease must necessarily lead to degenerative diseases. Biological degeneration may also follow defective supply of raw material (nutritional) or inefficient degradation (uric acid) and elimination (neuropathy) or deficient detoxification (hepatopathy) of harmful intermediate and end metabolites.

Biological degeneration may be primary or genetic in origin and secondary or environmental in source. Defective elimination and degradation may be manifested by deposition of normal or abnormal intermediate or end metabolic products. This occurs in amyloid, lipsid proteinaceous, keratinoid, fibrinoid, hyaloid, osseous degenaration inside or outside the cells. Metaplasia like abnormal keratinization is degenerative. Genetic degeneration may be due to deficiency in number or kind of genes. Many diseases due to genetic defect in kind are well studies while those due to deficient number of genes are less known. Mascular degeneration of the juvenile, adult, or senile variety are most likely due to deficiency in number of genes. The secondary or environmental degeneration could be due to unhealthy internal environment or milieu interior from defective metabolism, elimination or transport. Abnormal external environment from radiation or ultrasonics, humidity, atmospheric pressure, oxygen deficiency or severe temperature could lead to secondary degeneration. Postinflammatory degeneration should be categorized under secondary degeration. The susceptibility of different cells and organelles to the external factors of degeneration is variable. Dystrophies are mainly nutritional degeneration. Vitamin deficiency and hormonal imbalance lead to secondary degeneration.

Molecular biology and biological pathways. Separation of the component molecules of the cells, nuclei, chromosomes, genes and

paragenic particles is the route of investigation on the different aspects of the biological pathways. I hope this would attract the interest of foreign and local molecular biologists. There is much literature on gene mapping in lower animals. There is, however, little work and much challenge for genetic molecular probing in man.

Application of the concept. Ophthalmology. I have communicated on biological pathways in ophthalmology.(1) (2) Among the areas where it could be useful are: Ocular hypovitaminosis A; vitreous hemorrhage, vitreous cells and vasculogenesis, diabetic retinopathy, glaucoma involving biological filters, fibroblastosis and cautery, preglaucoma, nonsurgical management of senile cataract, bullous keratopathy, corneal transplantation, uveitis, retinal detachment, maculapathy, retinitis, pigmentosa, squinting, optic atrophy, dacryosystitis, culture media and contact lenses.

Medicine. Among the areas where it may be helpful are organ transplantation, diabetes, hypertension, cardiopathy, infection, immune diseases, cancer, biological vaccines and degenerative diseases including senility.

Botany and Zoology. I hope the concept of biological pathways will stir some interest among plant and animal scientists.

References

1. de Ocampo, G., — Biological Pathways in Ophthalmology PJO 9:153-162, October-December, 1977
2. de Ocampo, G., Theoretical Bioophthalmology (Awaiting publication)

An extraordinary increase in information, knowledge and concepts concerning these pathways has occurred during the past two decades. Until the 1950's very little was known concerning either the cellular or molecular mechanisms involved in the antibody formation and cell-mediated immunity. Today, various scientific meetings, symposia and congresses dealing with the various aspects of these mechanisms and their applications to the broad area of biological science has tremendously increased the interest in these mechanisms.

Viability

The evidences presented by Dr. de Ocampo based on his clinical and experimental observations on acellular cornea has led him to the meaning of viability as "the capacity to 'survive with life with or without the property to' propagate life".

The graft versus host (GvH) reaction is influenced by the condition of the surrounding tissues.

What was the nature of the host tissue?

Which of the endocrine glands can be explored for biochemical and biological properties which may influence success of corneal grafts?

Biological pathways and biological messengers

While it is known that prostaglandins (PG) or sometimes known as macromolecules from mononuclear phagocytes of the series PGE_1 and PGE_2 are most often associated with inflammation, recent evidence has been presented that prostacyclin (PGI_2) may augment inflammatory reactions. (Robinson et al., 1970). However, prostaglandins may also inhibit immune-induced inflammation which is largely related to the stimulation of adenylate cyclase. They in some way function as those of corticosteroids, glucosteroids, etc.

Mononuclear cells in human peripheral blood produce a factor (MCF) that regularly stimulate the production of PGE and collagenase from resting ASC (adherent synovial cells). Robinson et al. (1979), in their in-vitro experiments indicate that rheumatoid inflammation involves interaction between the monocyte-macrophage, lymphocytes and synovial cells regulating the production of prostaglandins and other factors.

Did you observe such reactions in your experiments on corneal grafts?

Biological development

Can Dr. de Ocampo explain the theoretical divison of 10^7 (10,000,000) genes in man into 60% survival genes, 10% developmental genes, 10% differentiative genes, 10% proliferative genes and 10% protective genes?

Biological differentiation

Information on resistance to antibiotics support the concept that there is a genetic basis.

Are there mechanisms involved in the expression of a genetic trait?

Dr. de Ocampo has mentioned several developmental anomalies. He said that "corticoid glaucoma has a theoretical genetic basis." In his studies, what is the ratio of the expression of this ocular anomaly? What factors are involved?

Protective pathway and subpathways

Interferon was earlier known as an antiviral agent. It is also considered as an anti-cancer agent.

Can you explain the regulatory role of interferon regarding developmental and congenital glaucoma?

Dr. de Ocampo has enumerated the areas in opthalmology and medicine where the concept could be useful. He also hopes that the concept "will stir some interest among plant and animal scientists." These concepts have been explored by zoologists using many experimental animals (mice, rats, guinea pigs, dogs, cats, etc.), however, I cannot speak for the botanists.

Salvador Salceda, M.D., Discussant

Thank you Dr. Campos. My assignment scares me; I have no napoleonic complex intellectually.

As a student and an associate of Dr. de Ocampo in his numerous researches in ophthalmology, I have become aware of the many observational data cited so richly in his paper — serving as they do the foundation of the concept on Biological Pathways.

I share his anxiety and dissatisfaction over the lack of some unifying concept of the many morphological and descriptive observational data. As a researcher too, I welcome his efforts in formulating the Biological Pathways.

Discussing and commenting on the paper however, runs the risk of getting into details from which it is difficult to extricate.

I share the view that the smallest biological unit is not the cell nor is it the molecule — but something in between. An organelle is perhaps a prototype of what Dr. Ocampo believes as the smallest biological unit. Outside of the cell however, e.g., fibers and membranes, I am still at a loss what the biological unit is, considering that Dr. Ocampo believes that the formed non-cellular components of tissues are also alive and viable.

As to the five postulated biological pathways: *viability* being the most elemental and subserved by self-renewal, *developmental* for maturation, *differentiation* for specialization, *protective* for survival and *proliferation* for growth and multiplication, I have a few questions to ask.

1. Are there structures assigned to these pathways? If so what and where are they?
2. If these structures referred to by the author as "stations" how are the communication system brought about? How are messages sent to and from these stations?

SEROEPIDEMIOLOGIC STUDIES OF THREE VACCINE-PREVENTABLE VIRAL DISEASES IN PHILIPPINE URBAN AND RURAL COMMUNITIES

By Fe del Mundo, M.D., Academician

Introduction

A recognized achievement in Medicine and Public Health has been the development of safe, potent and effective vaccines against diseases that have caused high morbidity and mortality, particularly among children. The classical and historical smallpox vaccine has taken the lead and for many years it seemed like it would be the only effective viral immunization. However in recent years, in fact within the past two decades, new ones have been successfully developed, particularly against viral diseases, namely, poliomyelitis, measles, rubella and mumps.

Epidemiology is essential in the description and knowledge of each disease and with the advent of mass vaccination programs, such studies have become more important in order to formulate policies, identify priorities and prepare logistics. It is further suggested that epidemiologic surveys be undertaken periodically on an ongoing basis, even if the general patterns of the epidemiology are already known. Periodic surveys will determine new policies and directions as conditions change.

Epidemiologic studies may be either statistical or serological, the latter is the more recent method, the former requires less skilled staff but reporting and diagnosis can be problematic so that accuracy of statistical data leaves much to be desired. The seroepidemiologic method requires technical skills but specificity and accuracy of results compensate for laboratory skills required. Besides, serologic tests are more easily and more readily available now than in the past.

This paper presents seroepidemiologic studies before three viral vaccines were introduced in the Philippines. These were on Poliomyelitis (1963), Measles (1972) and Rubella or German Measles (1973). While these studies may appear retrospective, they were all undertaken by the same author in this institution. This paper presents prevalence of the three diseases prior to vaccination and also serves as baseline data for subsequent studies.

Poliomyelitis

Hammond in 1956 first reported on the polioantibody status of children: 156 in Manila, 73 close to Clark Airbase, and 173 in Negrito Villages of Fort Statsemberg. The tests were performed in

the U.S. Public Health Service. His findings showed the same patterns as those 7 years later, presented in this paper.

Our interest in the polioantibody status of Filipinos was stimulated upon introduction of polio vaccine in the country in 1962-1963. Sera of 1078 healthy individuals in Manila and neighboring provinces were tested for neutralizing antibodies against each of the three types of polio virus. The tests were performed in the Viral Laboratory of the Health Department. Individuals tested had not been vaccinated against polio. They ranged in age from 1 month to 25 years and over; the majority were children between 1 to 9 years old. The following is a summary of the findings in the 1963 studies:

— Poliomyelitis infection is practically endemic (91.75%) in localities in Manila, Quezon City and neighboring provinces where a total of 1078 sera were collected (1963).

— Infants from 1 to 3 months old gave a polio antibody prevalence of 100% which declined to 46.15% at 11 months of age. (Fig. 1).

— The greatest number of susceptibles was in the age group between 4 to 12 months at which ages the percentage of positive was lowest.

— Above 20 years old, 100% of those tested were all positive for the polio antibodies with type 3 less prevalent than the others. (Fig. 2).

— Observations in this survey may indicate that in 1963, mass polio immunization was not necessary in the locality where sera were collected. However, selected groups particularly between 4 to 12 months would be benefited by polio immunization.

— Undoubtedly it would be more informative if this survey could be extended to other areas of the country, and repeated in subsequent years.

Measles

In the Philippines, measles, as in many developing countries, continues to be a severe illness and a major hazard. Mortality and morbidity are high particularly in densely populated communities. It may not be impressive in statistical reports because the disease is usually taken for granted as common disease which every child will contract. Thus it is under reported while deaths are listed under the complications and not under measles.

In view of the lack of reliable information and accurate statistics on the epidemiology of measles in the Philippines, the author felt that an antibody profile in different areas of the country would be helpful, not only as baseline data of the disease but also as a guide to health authorities and to medical practitioners in future immunization programs.

With the availability of a safe and effective vaccine and its well documented beneficial effect against this previously called "world killer infection", it became imperative in 1972 to gather more reliable and scientific information on the disease and its occurence in the Philippines. The present study precisely has this for its main purpose.

Collection of specimens for this study was started in March 1972. Blood samples were obtained from well individuals who had not been measles vaccinated, ages 6 months to 40 years, in the following provinces and cities:

Bulacan	Marinduque
Pampanga	Cagayan de Oro City
Rizal	Quezon City
Mindoro Oriental	Manila
Quezon Province	

A total of 322 serum specimens were obtained. Measles antibody titer was determined by hemaglutination-inhibition (HI) test in these specimens.

The ages of these individuals ranged from 5 months to 42 years, with 198 residing in rural areas while 200 were urban residents.

The results show that approximately 1/4 of rural and 1/2 of urban infants 2 years and below contract the disease. This rate doubles at preschool age at which time when malnutrition is highest in the Philippines. At *school age*, 69% in rural areas and 76% or 3/4 of urban children have contracted the disease. At *adolescence* (13-19 years) 95% to 100% show immunity to the disease.

The study indicates that for immunization programs in the Philippines, the under-5 may be considered priority targets. It further gives data which may not only allow the evaluation of measles as a health and medical problem in the Philippines but also as guides in forthcoming immunization programs, to take advantage of an available safe, effective, and accepted vaccine against the disease.

Rubella (German Measles)

In the Philippines, rubella hardly appears in local or national morbidity or mortality statistics and this is so even in teaching hospitals and medical centers. To some extent this may be due to the mildness of the disease and also to the fact that it may easily be misdiagnosed as measles or as an allergic reaction. A more important reason is lack of awareness of the disease or an

impression that rubella does not occur in the Philippines, oftentimes from its synonym, German measles.

As in many other countries, a significant awareness and interest in rubella have been triggered by two serious rubella epidemics documented in medical history: that of 1941 in Australia when the terategenic potential of rubella became evident; and the coast to coast devastating epidemic in the USA in 1964-65. The awesome 1964 epidemic which left 20,000 babies with congenital defects and a tremendous loss to families and to society, stands as a nightmare to all. These two epidemics have made rubella a disease of scientific and public health interest.

Even in the Philippines efforts are now noted to include rubella in the differential diagnosis of rashes with fever. More commonly, retrospective diagnosis of rubella is made upon observing multiple congenital anomalies in the neonate. This however is considered an inaccurate way of obtaining incidence. In fact retrospective studies for rubella are not acceptable.

Improvement in serologic testing has made up for difficulties and inaccuracies in diagnosis of rubella. This diagnostic tool has also facilitated determination of susceptibility based on the frequency of antibody among individuals.

The nightmarish dread for rubella plus the fact that a safe and effective vaccine is now in use have prompted the author to determine the seroepidemiology of the disease in the Philippines, particularly as an opportunity became available in 1972, to do the HI antibody test.

Whole blood specimens (5 to 10 cc) were obtained by venipuncture with disposable needles and syringes in vacutainers, from individuals ranging in age from 5 months to 42 years in rural as well as urban communities, as follows:

Pampanga	Rizal
Cagayan de Oro	Manila
Mindoro Oriental	Quezon City
Bulacan	Caloocan City

The choice of these localities was not based on any particular criteria. They were sites where the authors had colleagues who could cooperate with friends and explain and convince them to submit to a venipuncture.

The subjects were males and females up to age 15 years. Beyond 15 years, the subjects were women.

A summary and the conclusions that were obtained in this study are as follows:

-- Lack of data on the incidence of rubella in the Philippines together with known facts that this is a poorly reported disease and subclinical infections occur significantly, prompted the

author to delineate the epidemiology of the disease by 3 serologic survey.

— The seroepidemiology of rubella in different areas of the Philippines was determined in this study (1972) by HI antibody test among 723 individuals, both rural (377) and urban (346).

— The 387 children in the 0-9 year groups constitute one of the most extensive rubella antibody surveys reported for this age period.

— In all the age groups rubella antibody was found positive, with an incidence of 6.2% in the 0-14 year group but double (12.6%) among urban children of this age. (Fig. 5)

— Thereafter, seropositive titers steadily increased to a peak of 78.9% in the 20-29 year group and almost persistently remained so, up to 40 years. (Fig. 6)

— In the present study, out of 241 women 15 to 40 years old, 179 or 74% gave positive titers so that 26% or about one fourth of women in the child-bearing age are devoid of rubella antibody in both rural and urban populations. In a study of the World Epidemiology of Rubella (1969), the finding was that more than one-fifth of women in the reproductive period do not possess rubella antibody and are therefore susceptible to the infection.

— It was found in this survey that overall rubella immunity is more prevalent in urban than in rural areas.

— It is evident in this study that rubella is a seroepidemiologic entity in the Philippines, although this does not appear to be so clinically and by statistical reports.

— The results of this study may serve to increase and stimulate awareness of the disease in the Philippines, particularly on potential teratogenic or embryopathic effects of the disease.

— The results obtained in this study provide information which may be useful in the event that future control program may be deemed necessary.

— Further, this serologic survey provides additional information to those that have been previously gathered, for international comparison and contrast.

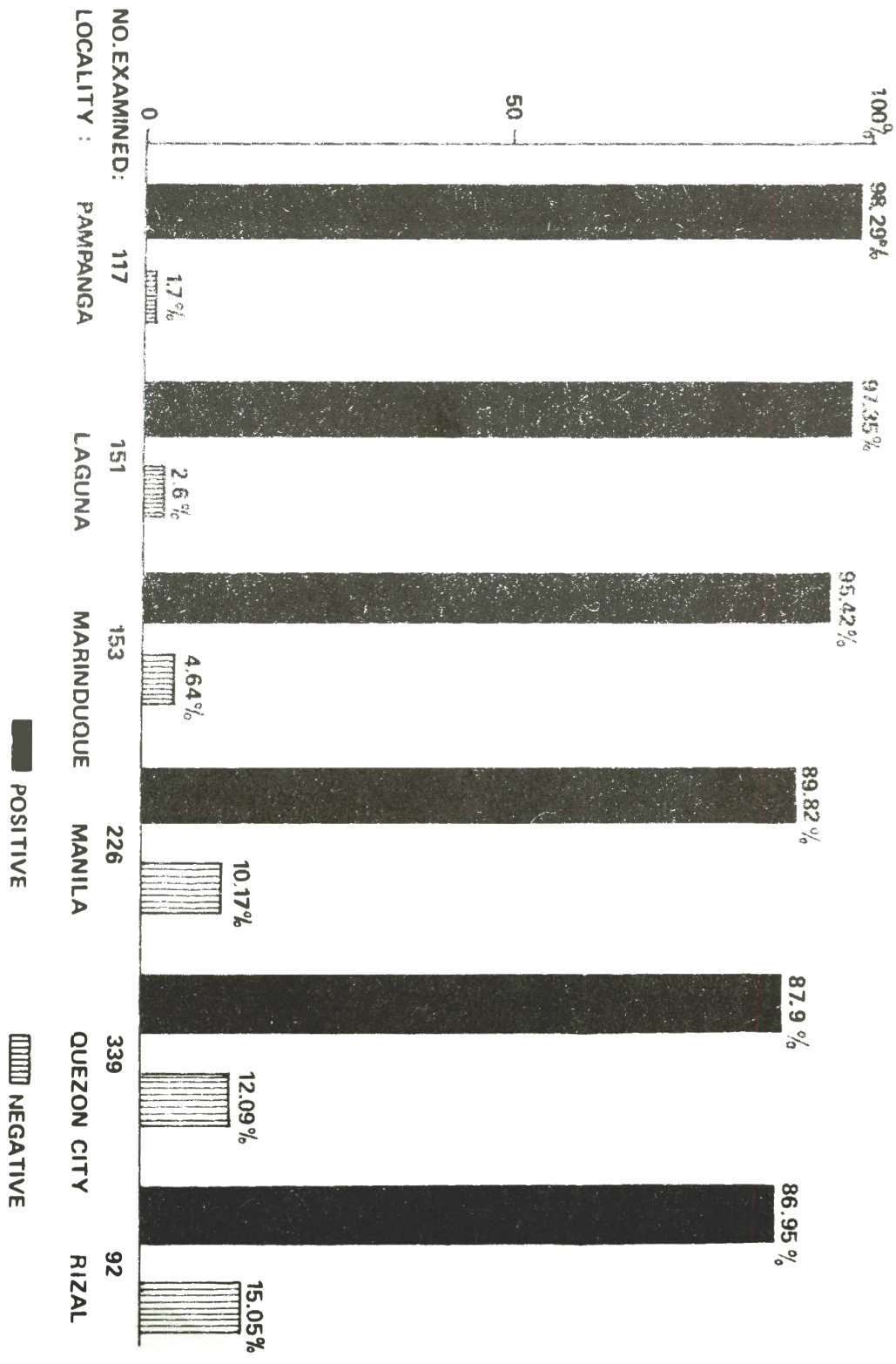


Figure 1. Frequency distribution of Poliomyelitis neutralizing antibodies by locality, Philippines 1963.

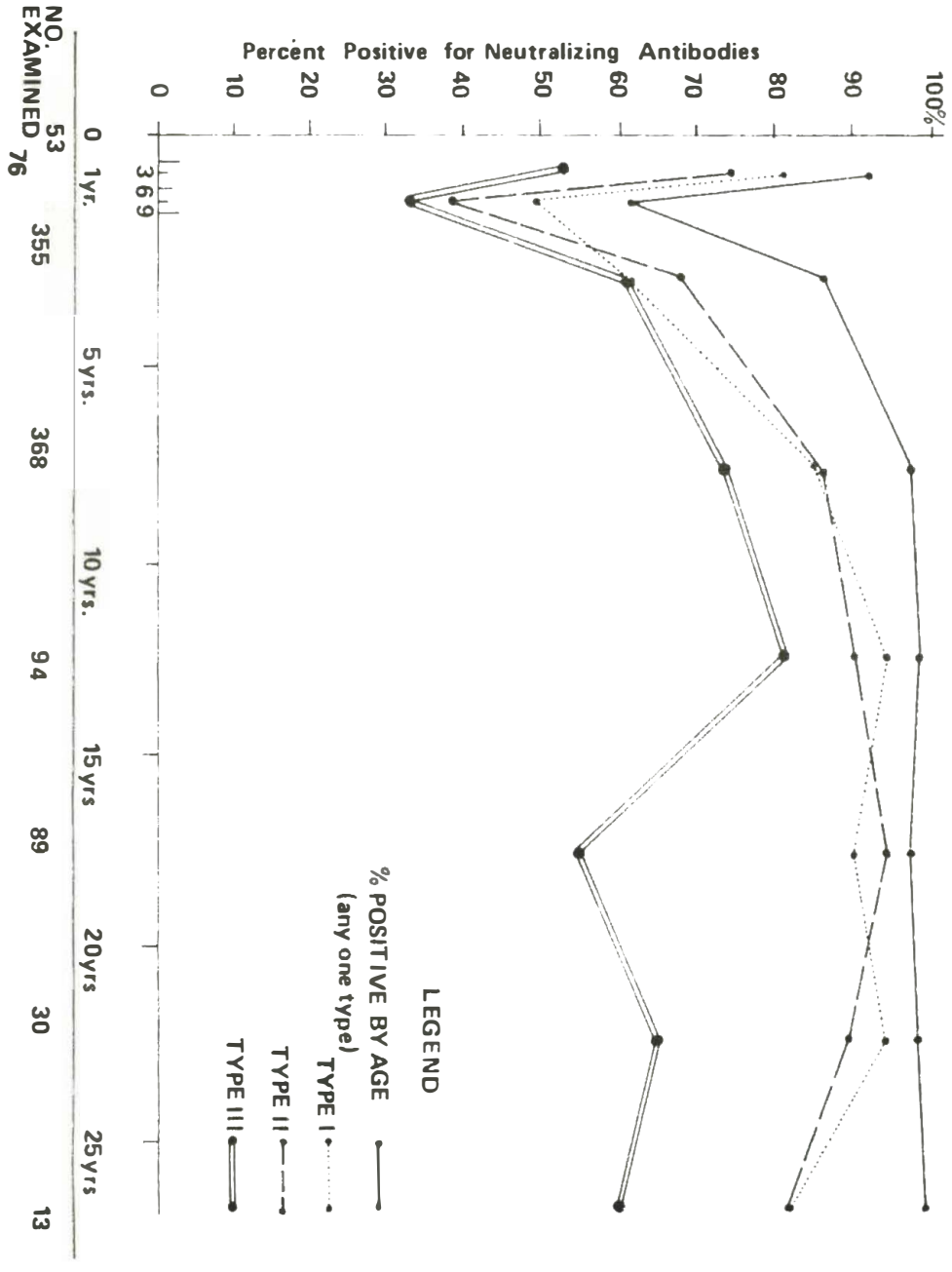


Figure 2. Frequency distribution of Poliomyelitis neutralizing antibodies by age group and serotype, Philippines 1963.

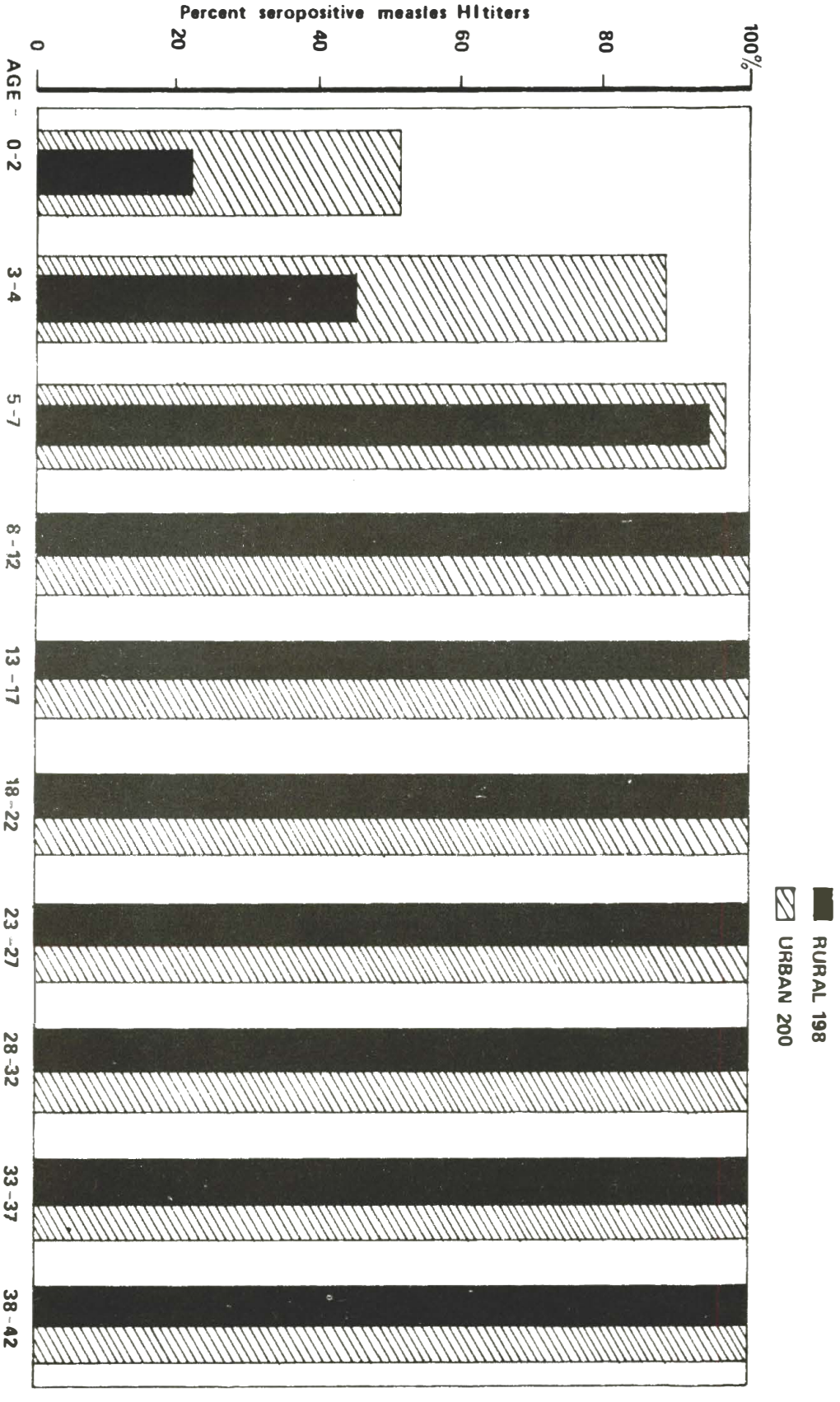


Figure 3. Percentage of Seropositive Measles Titers by Age group in Urban and Rural Populations, Philippines, 1972.

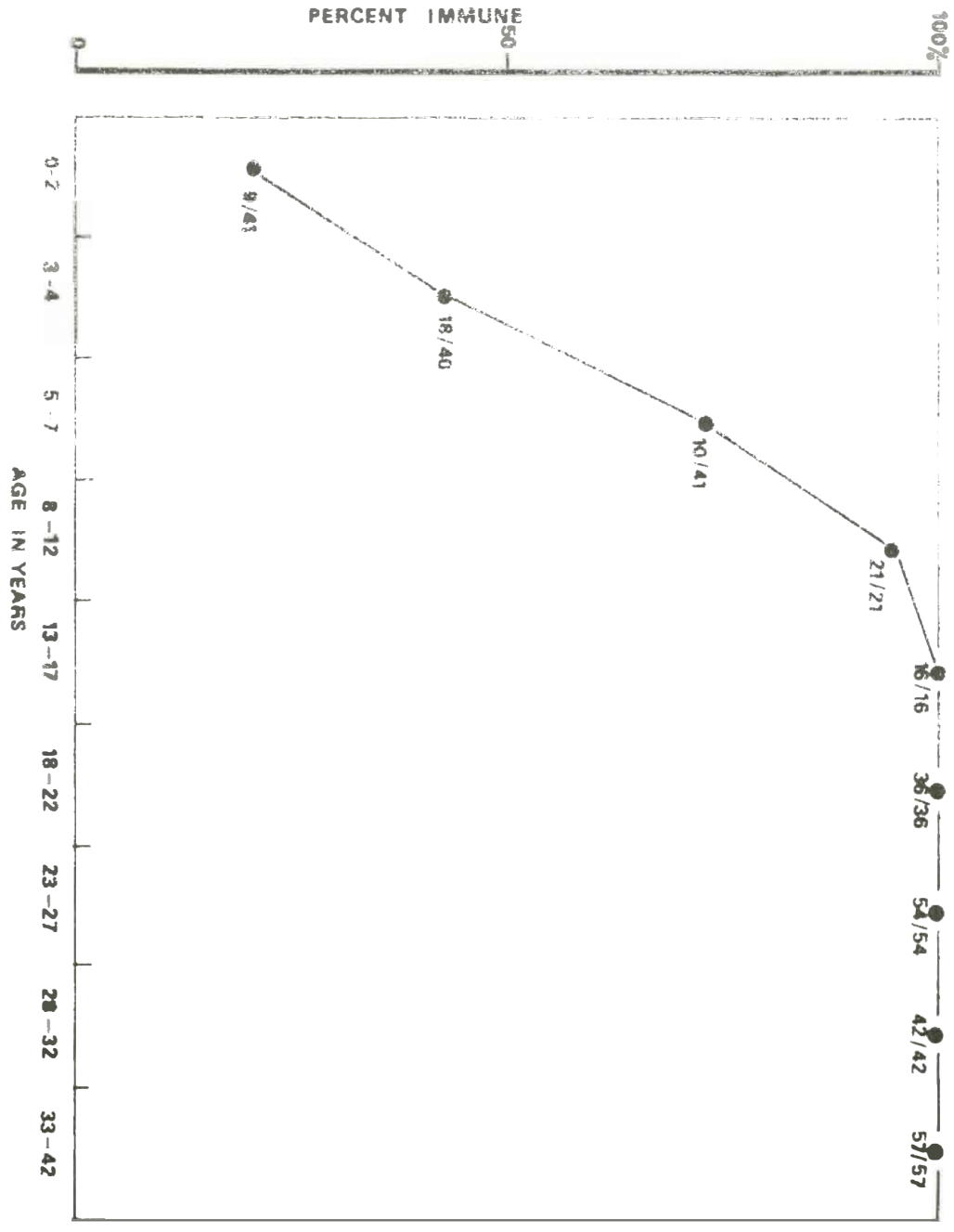


Figure 4. Acquisition of Immunity in the Philippines as Based on Measles HI Antibody Determination of 322 Sera from Urban and Rural Communities.

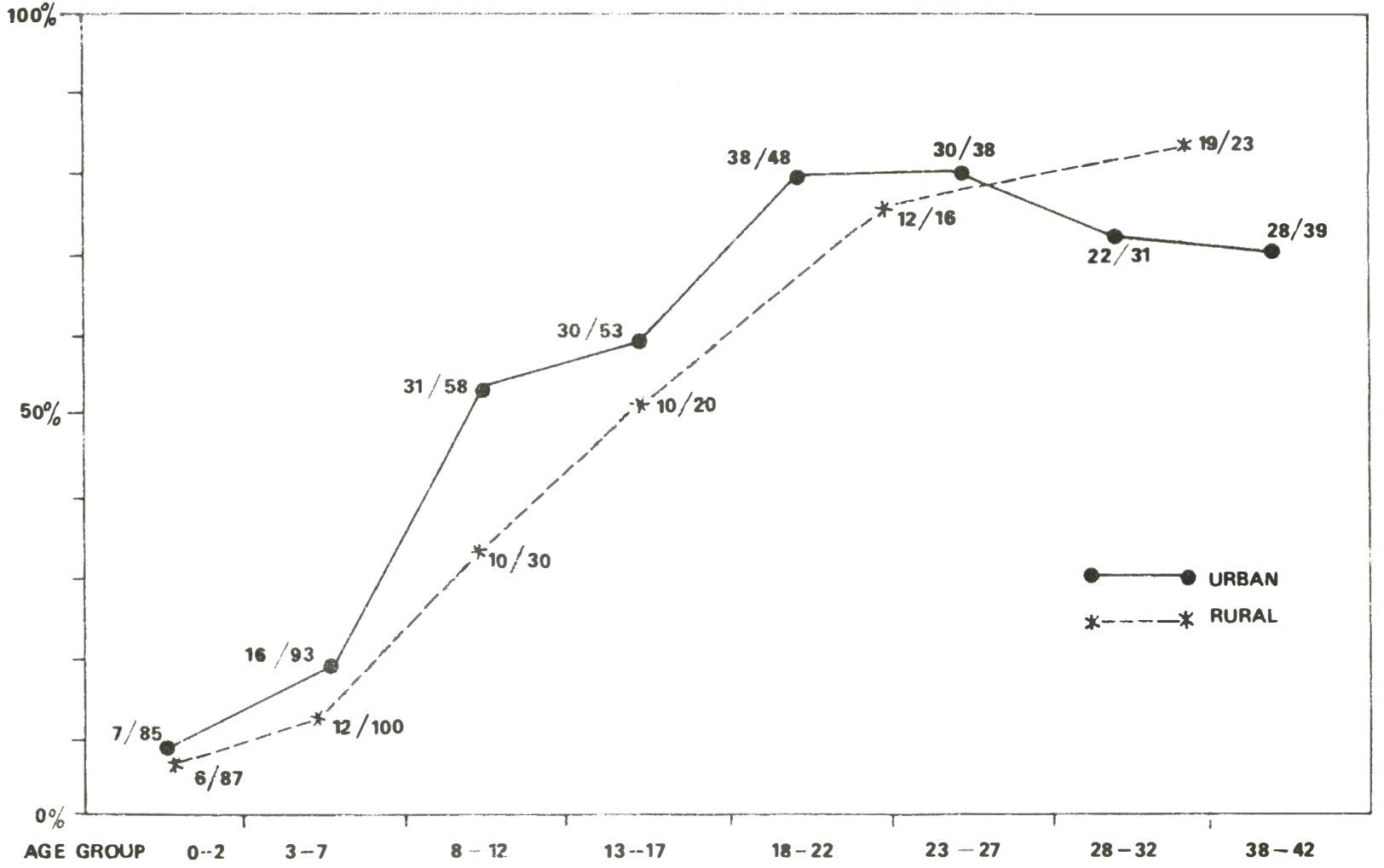


Figure 5. Acquisition of Rubella Immunity by HI antibody determination in Urban and Rural Populations in the Philippines, 723 sera.

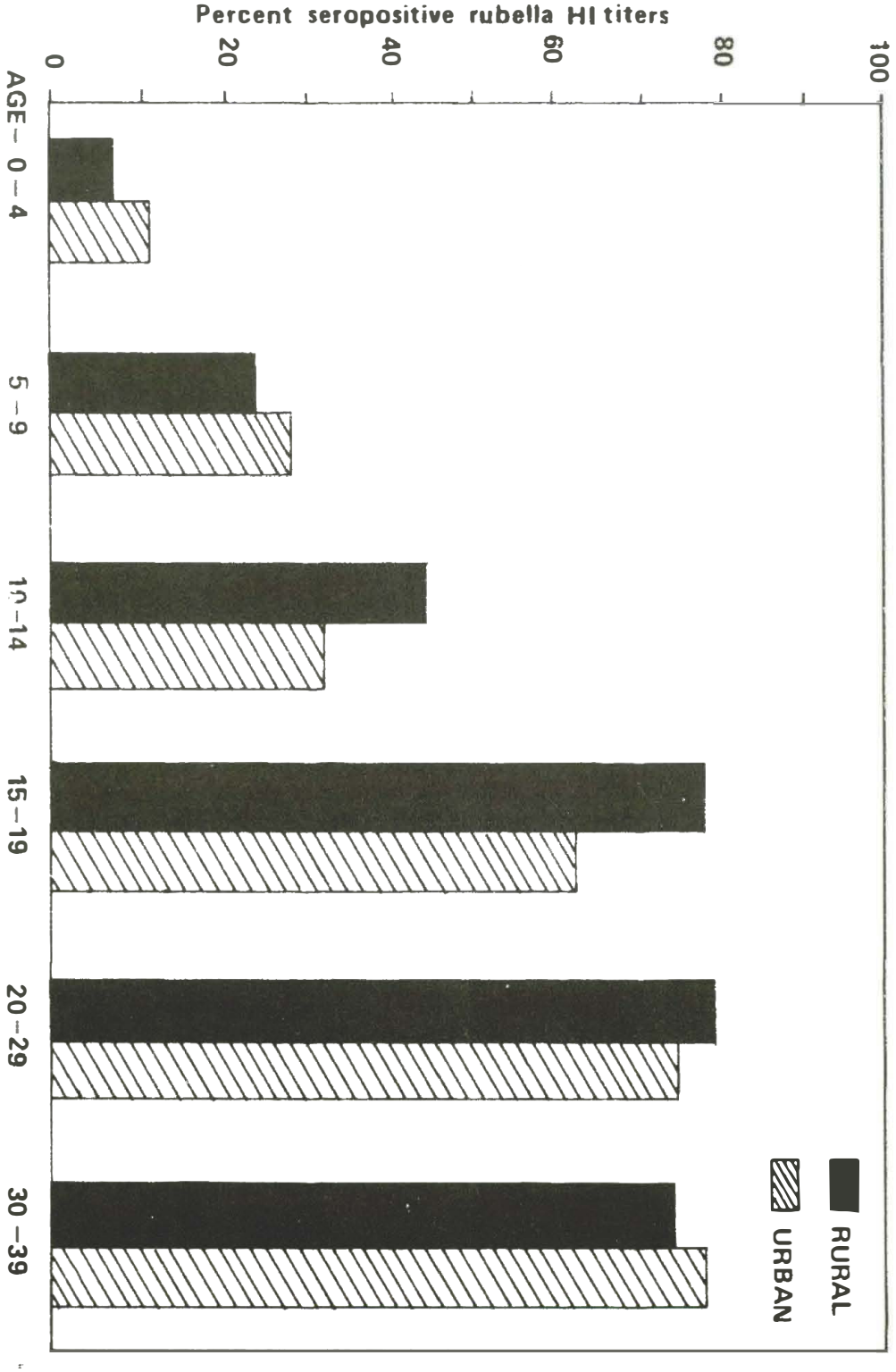


Figure 6. Percentage of seropositive rubella HI titer by Age Group in Urban and Rural Populations, Philippines, 1972.

BIBLIOGRAPHY

Poliomyelitis

Del Mundo F., Barrion LE: An institutional outbreak of acute poliomyelitis in Novaliches, Rizal J. Philipp Med/ss 39: 566, 1963

Del Mundo F., Ocampo T. Barrion LE, et al: An antibody survey as an index of immunity to poliomyelitis among Filipinos. J Philipp Med Ass 40: 1001, 1964

Paul JR.: Poliomyelitis, knowledge and trends in poliomyelitis a summary. Papers and Discussions at the Second International Poliomyelitis Conference, September 3, 1961. pp. 376-81

Poliomyelitis. WHO Pub. Monograph Series no. 26, 1955

Reddi YE: Acute anterior poliomyelitis, clinical aspects of the 1960-61 epidemics of Andhra Bradesh, J Indian Pediat Soc 1:43, 1962

Measles

Del Mundo F.: Cost analysis of measles in hospital practice and an estimate of economic loss from the disease in the Philippines. J Philipp Med Ass 49: 434, 1973

Del Mundo F., Buendia L: The epidemiology of measles in the Philippines based on the antibody profile in rural and urban areas. J Philipp Med Ass 49: 399, 1973

Langmuir AD: Medical importance of measles. Am J Dis Child 103: 224, 1962

World Health Annual, 1967. WHO, 1970, p 71

Philippine Health Statistic, 1975

Rubella

Del Mundo F.: The serodiagnosis of rubella in the Philippines based on hemagglutination-inhibition determinations in rural and urban population. Philipp J Pediat 22: 105, 1973

Desmyter J.: Serological diagnosis of rubella. Br Med J 4: 119, 1970

Lucas RT Jr: Rubella — measles, JAMA 215: 1156, 1971

Plotkin SA, Mellman WJ: Rubella in the distant past as a possible cause of congenital malformations. Am J Obstet Gynecol 108: 487, 1970

Siegel H. Fuerst HT, Guinee VF: Rubella epidemicity and embryopathy. Am J Dis Child 121: 469, 1971

Virginia Basaca-Sevilla, M.D., Discussant

I. POLIOMYELITIS

Dr. Fe del Mundo and the other scientists and researchers like Dr. W. McD. Hammon, Dr. Lourdes Espiritu-Campos, Dr. Pilarica Ejercito, Dr. Angel Ocampo, Dr. Veronica Chan, Dr. Eulalia Venzon and Dr. Higineo Nidea have to be congratulated for their interest and initiative in starting the studies on poliomyelitis, which to date still serve as the basic laboratory studies on this disease in the Philippines. Their studies contributed much in the formulation of the immunization policy.

Since 1976, the Ministry of Health has embarked on an Expanded Immunization Program involving children of ages 3 to 8 months from twelve health regions and Metro Manila. The immunizations were initially, for diptheria, pertussis, tetanus and tuberculosis prevention. In 1980, poliomyelitis immunization has been started countrywide and for practical and logistic purposes tied up with the DPT immunization. The schedule required three doses. The first dose is given to children 3 to 8 months, at the same time as the first dose of DPT and BCG are given; the second dose 2 to 3 months later, between immunization rounds; and the third dose to children 9 to 14 months, at the same time as the second dose of DPT.

The rationale for this may be traced to the findings of Dr. del Mundo and her group that infants 1 to 3 months old had a 100% prevalence for polio antibody. This prevalence started to decline at 4 to 6 months. Dr. Espiritu-Campos, in her 1963 study of 70 clinical and confirmed polio cases confined at the San Lazaro Hospital, found that two of the cases positive for viral isolation were 4 month-old infants showing that the loss of maternal antibody may occur as early as 4 months after birth. In separate studies Drs. del Mundo and Espiritu-Campos have found that further decline in antibody may occur from 6 to 12 months. Thereafter, it rises continuously by year with 95.65% being positive among those 4 years of age. These findings explain the polio immunization schedule that has been adapted.

The findings of Dr. Ejercito and her group of an enterovirus isolation rate of 18.2% from among 730 children below 1 to 6 years of age with diarrhea and 9.04% from the control group is quite significant. She found further that of the enteroviruses isolated among the patients 3.42% were polio virus, 3.91% coxsackie, and 10.82% ECHO viruses. In the control group it was 1.1% each for polio and coxsakie while 6.75% of the total isolates were ECHO viruses.

Likewise, Dr. Ocampo, in a study of 208 children of ages 1 to 14 years, had shown thru neutralizing antibody detection an 80% and 38% prevalence for ECHO and coxsackie respectively. In a study of 129 children, Ocampo demonstrated the effect of a single dose of Oral Sabin Vaccine. He found that only 77 or 48% showed a 4-fold conversion. The rest were negative or showed a 2-fold conversion. These studies on viral isolation among children with diarrhea and among those without diarrhea and rate of positive antibody conversion following immunization showed the role coxsackie and ECHO viruses can play in interfering with the multiplication of oral attenuated polio virus. Hence, the multi-dose administration of the vaccine. So that our immunization policy has been influenced by the laboratory studies actively done in the Philippines involving the detection of polio antibody in the population especially in children, enteroviral isolations and antibody detection following immunization.

In the light of the studies of Dr. del Mundo and others, one cannot overemphasize the need for a regular serological antibody survey of the children in all immmunization areas as well as a laboratory surveillance of clinical polio cases. Now that we have started a countrywide polio immunization, there is also a need to be aware of the experiences in the past that where polio immunization has been enforced for sometime in certain areas and is withdrawn, disease outbreak follows a few years after.

II. MEASLES

The interest and attention that the laymen and physicians give to measles and its prevention is not directly related to its morbidity rate in this country in contrast to poliomyelitis as proven by the country-wide immunization against poliomyelitis. The 5-year average morbidity rate for measles for 1970-1974 was 58.3 per 100,000 and for 1975 it was 66.3. As Dr. del Mundo pointed out, statistics for measles are quite inaccurate in the sense of under-reporting. The medical practitioners as well as the paramedics, the nurses, midwives, and sanitarians many times do not report all the cases as they see it especially in peripheral field practice and even in private clinics. The under-reporting for measles is similar to that of influenza.

Measles has always been regarded as part of childhood. If we consider the findings of Dr. del Mundo, that ¼ of the rural and 1/2 of the urban children of 2 years and below, contract the disease, with the rate doubling at pre-school age at which time when malnutrition is highest.

It is time that very serious consideration be given to immunization especially as a most effective preventive measure not only for the disease itself but for the consequent complications of malnutrition among the chidren especially of the rural area. While the disease may be lightly considered by some, the consequent malnutrition and the difficulties to rehabilitate the malnourished children compound the cases of malnutrition due to other causes. Some thoughts should be given to the inclusion of measles immunization in the EPI Program. This can be viewed with optimism when the price of the vaccines can be brought down ideally to the level of the cost of polio vaccine. The stability of the current vaccine assures us of potent vaccines for the use of our far-flung rural areas.

III. RUBELLA

Dr. del Mundo has stated well the widespread lack of awareness of the existence of rubella in the Philippines to the point that there are actually no morbidity and mortality statistics on it. She has well pointed out the need of one-fourth of our women in the childbearing age for protection against the disease. No one has actually gone deeply into the causes of multiple congenital anomalies in the neonate. We accept however, the probable embryopathic effects of the disease when contracted by the mother and that the infection may persist and the virus may be shed for many months after birth and therefore there may be damage done even during post-natal life.

In view of this, the need to prevent the disease is there and the protection of the foetus is the ultimate objective. The protection is afforded by the presence of circulating antibodies in the blood of the mother. This may be accomplished either by immunizing young girls aged 11-12 or adult women found to be susceptible. This needs investments in time and money.

The three diseases, poliomyelitis, measles and rubella can all be effectively prevented through immunization. It will require, however, a thorough analysis of their relative importance to the public, taking into full account the efficacy and cost effectiveness of the immunization procedure and our overall health priorities.

Geminiano de Ocampo, M.D., Academician, Discussant

I would like to ask the following:

1. Explain the site and mechanism of action of multiple vaccines containing measles.

2. What is the difference between attenuated and dead vaccines, measles vaccine being live but attenuated?

3. What is the basis for the difference in duration of effectivity of different vaccines?

4. As a preventive against embryopathies including rubella cataract, would it be effective of child bearing women to be vaccinated against rubella?

5. Why don't we have a real complete virus laboratory in the Philippines?

6. Blindness associated with measles is intimately connected with malnutrition. I would suggest as a preventive measure some antiseptic eye medication for children with measles.

IN VITRO CULTURE STUDIES ON COCONUT: PROGRESS AND PROBLEM

By Emerita V. de Guzman, Ph.D., Academician

Embryo Culture

Cutter and Wilson (1954) undertook the *in vitro* culture of coconut embryos to elucidate on the role of the endosperm during the post embryonic growth of the embryo. From their work which covered mainly the phase prior to visible germination they concluded that as the endosperm matures it develops substances that are inhibitory to the growth of the embryo. Subsequent attempts to grow excised coconut embryos in vitro succeeded in obtaining germination and seedling growth thus permitting observations on cultural requirements at later developmental stages.

The root was found to require more specialized conditions. In solid medium germinating embryos generally produced leaves but not roots (Abraham and Thomas, 1962; de Guzman and del Rosario, 1964). Root development in seedlings without or with rudimentarily developed roots could be induced by transfer to liquid culture (de Guzman, 1969). Concurrent and faster growth of both organs occurred when a reverse sequence of culture, that is from liquid to solid was employed (Balaga and de Guzman, 1971). With the liquid-solid sequence of culture the germination process could be separated into two phases: general enlargement which occurs in liquid culture and visible germination and seedling growth which take place in solid culture. Apparently histological and physiological changes that favor root growth occur during the liquid culture. In vitro seedlings without roots or where root growth has ceased responded to transfer in a liquid medium by initiation or reactivation of root growth.

During the initial liquid culture the physical state rather than composition of the medium is the primary consideration. The same general effect was obtained with two types of mineral formulation although improved results could be obtained by modifying the composition of the medium such as by addition of KCl and NaCl to White's liquid medium (Miniano and de Guzman, 1978). On the other hand, growth during the solid culture was greatly influenced by media composition. The concentration of dextrose during the solid culture exerted a great effect on the growth of the root and shoot. Root growth was greatly promoted and shoot growth inhibited by a high sugar supply (de Guzman, et

al., 1971). That the root has a higher sugar optima compared with the shoot was observed in embryo cultures of other species (Yates and Curtis, 1949; Reitsema et al., 1953; Ameniya et al., 1956). These results show that sugar plays a role not only in root formation (Gautheret, 1966) but also in the control of root growth. The promotive effect of sugar is dependent on its quantity and on the composition and concentration of the mineral salts in the culture medium. As reported by del Rosario and de Guzman (1978) high sugar greatly enhanced root growth when the mineral formulation used was that of Murashige and Skoog's (MS) but not with that of White's. There was no enhancement when MS was combined with low sugar. For maximum root development both high sugar and MS salts should be present; the two factors must be acting in a synergistic manner to produce the stimulation. The superiority of MS over White's medium in promoting growth and differentiation has been shown in other in vitro systems such as carrot root tissue culture for somatic embryogenesis (Reinert, 1968), Singapore orchid tissue culture (Payawal and de Guzman, 1972) and oil palm embryo culture (Jones and Dethan, 1973). This superiority of MS has been attributed to its high salt content especially nitrogen. MS has also high potassium and chloride contents and these elements are especially beneficial to the growth of coconuts. KCl fertilization of coconut palms resulted in shorter pre-flowering stages and greater yield (Magat, 1978).

Addition of growth regulators or of natural supplements is not necessary for the culture of mature coconut embryos into viable seedlings. However, results of preliminary experiments indicate that treatment with auxins increases percentage germination and faster seedling growth. In the germination of excised mature oil palm embryos better seedlings were obtained in media supplemented with auxins (Jones and Dethan, 1973). The beneficial effect of exogenous auxin applications does not necessarily imply that the mature and germinating embryos have not yet developed autotrophy with respect to growth regulators. In the case of auxins, the limiting endogenous level might be due to an increased degradation under *in vitro* conditions. As shown for the oil palm, addition of ferulic acid, a phenolic compound inhibitory to IAA oxidase, promoted root growth.

The in-vitro germinated coconut seedlings like those developed in *situ* exhibit leaf heterophylly producing the different types of leaves in the normal sequence. A marked difference in morphology is the non-development of the haustorium in the former. In coconut the in vitro conditions that enable development of the axial organs are still inadequate for the development of the cotyledon. This observation dramatizes the difference in the growth requirements of the different parts of the

embryo which in turn results from localized biosynthesis. A similar observation was made with *Zizania aquatica* L. (La Rue and Avery, 1938) with which regular growth of the cotyledon was not obtained even in cultures of the mature embryo although growth of the primary root was normal.

Slight swelling of the cotyledon in coconut has been observed in some cultures grown in media supplemented with cytokinins (tables 1 and 2). Cytokinin promotion of haustorial growth was also observed in oil palm cultures (Jones and Dethan, 1973). These observation indicate that among the conditions limiting growth of the cotyledon *in vitro* is cytokinin deficiency.

Table 1. Effect of cytokinins on the enlargement of haustorium of coconut embryos cultured in vitro. Observations were taken 16 weeks after initial inoculation.

Treatment[1]	Duration (weeks) liquid	solid	% enlargement
1.0 Ki	1	7	7.69
	2	6	14.64
2.0 Ki	1	7	0
4.0 Ki	1	7	8.39
2.5 BA	1	7	10.0
5.0 BA	1	7	23.07
10.0 BA	1	7	0
	1	7	23.64
2,4-D, GA, Ki	2	6	34.29
(Eeuweens, 1976)	2	6	33.16

[1]Growth regulator treatments were the same for liquid and solid cultures. Modified White's minerals were used for all liquid media. The solid media contains 1% Carbon and MS minerals except the last treatment which used Eeuween's. Embryos were precultured for 8 weeks in modified White's basal medium, then transferred to experimental medium.

Incidentally, the use of cotyledons as a bioassay material for cytokinins indicates the dependency of growth of this tissue on cytokinins of which it has a low endogenous content. During germination within the coconut seednut the cotyledon develops into a large bulbous structure. The cytokinin requirement for this extensive development could very well be directly provided by the endosperm. During germination the embryo proper emerges outside the shell whereas the cotyledon retains its intimate contact

Table 2. Effect of cytokinins given during the initial liquid culture on the enlargement of haustorium of coconut embryos. Observations were taken 16 weeks after initial inoculation.

Treatment	Duration in liquid culture (weeks) Wo	Expt'l.	% enlargement
1.0 Ki	8	0	23.64
	6	2	19.60
	4	4	25.49
	2	6	27.24
	4[1]	4	16.67
	2[1]	6	0
2.5 BA	8	0	33.33
	6	2	6.25
	4	4	0
	2	6	0
	4[1]	4	14.29
	2[1]	6	14.29

[1]Wo (without cytokinins) before experimental medium; for all others, experimental media (with cytokinins) were used before Wo.

Embryos were transferred to solid media using MS + 0.1 2,4-D + Ki or MS + 0.1 2,4-D + 2.5 BA, both with 1% C.

with the endosperm. Coconut endosperm especially the liquid portion is a rich source of cytokinins. Two of the naturally occurring cytokinins, zeatin and zeatin riboside, have been isolated in pure state from coconut water (Letham, 1968; van Staden and Drewes, 1975).

Callus Induction

Callus growth can be induced in cultures of plant parts consisting of tissues that may vary from the meristematic to the fully differentiated. In the case of dividing cells the pattern of meristematic activity and cellular development is so altered such that the original specialized structure of the explants is lost and instead a mass of unorganized tissue (callus) is formed. With mature or dormant tissues callus formation requires first the reversion (dedifferentiation) of the non-dividing cells to the meristematic condition. Yeoman (1970) divided callus development based mainly on storage parenchyma tissue cultures into 3 phases namely: induction, division and differentiation. The

initiation of cell division is preceded by profound metabolic and cytological changes in the cell. During the divison phase there is a great increase in cell number, a decrease in cell size and progressive return to the meristematic ground state. The callus may be kept in a continued proliferating state forming additional growth centers deeper in the tissue. However, considerable cyto-differentiation may occur at the end of the division phase producing vascular tissues and sometimes growth centers for the organization of apical meristems.

It has been recognized that different tissues vary in their growth regulator requirement for dedifferentiation (Gautheret, 1966). Except for a few cases callus growth in excised tissues or organs requires the exogenous application of growth regulators. For some tissues, the addition of auxins alone suffices to promote callus growth. However, there are those tissues where callus formation depends upon the addition of both an auxin and a cytokinin. A positive interaction between auxin and gibberellin was shown with excised citron fruit tissues cultured *in vitro* (Schroeder and Specter, 1975). Finally there are tissues in which the growth regulator requirement is satisfied by cytokinin application alone.

As a mechanism for dedifferentiation Oostron et al. (1975) proposed that the first event in the reversion to cell division is the coupling of the auxin with a cellular binding site or receptor. They found in pith explants cultured in medium with IAA a receptor protein which specifically binds IAA. Bogers et al. hypothesized that the hormone-receptor complex migrates to the nucleus where it binds with chromatin, thus making new DNA regions available for transcription. The first result of this binding was speculated to be the synthesis of new m-RNA species.

In coconut, attempts to produce callus cultures were carried out with embryos, inflorescence, endosperm tissues and sub-apical stem explants, using powerful auxins as the major growth substances. Apavatjrut and Blake (1977) used 2,4-D and kinetin as the growth regulators for the culture of sub-apical stem explants from mature palms. Cell divisions were observed throughout the explants producing a cambial-like tissue. At the periphery cells in a pro-embryo like arrangement can be identified. Eeuweens (1978) obtained a marked stimulation of growth of coconut inflorescence tissues with the use of NAA or 2,4-D alone. However, even at near optimal concentration of the auxin, growth could be further stimulated with the addition of a cytokinin. In our laboratory the standard media for callus induction contains 2,4-D and kinetin although the proliferative growth responses described below can be obtained with 2,4-D alone.

Growth and Morphogenesis using Embryo Explants

Addition of the appropriate concentration of auxins to the culture medium for embryos during either the liquid or solid culture disrupted normal germination growth. Indolebutyric acid (IBA) (Table 3) was less adverse to germination and seedling development but was also less potent in callus induction. The herbicidal auxins, 2,4-D, picloram (Table 4) and MCPB (Table 5) were much more effective in inducing cell and tissue proliferation.

Characteristic effect of 2,4-D treatment on embryos though variable was the rupture of the intact embryo at the base of the root and on the upper haustorial region. In the first site the exposed tissue showed surface callusing whereas in the latter, there occurred a proliferation of elongated bodies (root-like) with a callused surface. The root-like structures originated from the procambium at the upper haustorial regions especially at the shoot-root primordial-haustorial tissue junction. During seedling development the procambial cells differentiated into vascular tissues but in media supplemented with 2,4-D or NAA they instead became actively dividing giving rise to broad bands of meristematic tissues (Sajise and de Guzman, 1972). With lower concentrations of NAA the meristematic bands could be differentiated into roots resulting in the formation of a profusion of adventitious roots. With NAA at higher concentration and more potent auxins the meristematic bands could undergo partial differentiation forming root-like bodies. As described by Ubalde (1974) on the second week in solid culture the inner portion of the procambial meristematic bands contained mature cells whereas the outer cells retained their meristematic appearance. In rice the callus initiated from seedling nodes and cotyledonary nodes were observed to arise from the procambium whereas those initiated from the leaf sheaths and coleoptile originated from cells close to the immature vascular bundles (Wu and Li, 1971). The procambial tissues around the shoot-root junction and at the upper haustorium proper portion in the coconut embryo therefore make a good target for callus induction. Up to the present, however, all attempts to induce the root-like bodies in coconut embryos to proliferate into a continuously growing unorganized mass have failed. The use of higher auxin concentration reduced instead of increased the extent of proliferation of the root-like bodies (Table 6). Although suitable auxins could bring about cell division it seems that the support of other factors are required to sustain it and to bring about the varied changes associated with the formation of a vigorous callus. The formation of nests of meristematic cells serving as additional growth centers which is a feature of actively developing callus cultures has not yet been observed.

Table 3. The comparative effects of 2,4-D and IBA on the growth of intact embryos and different tissue explants in first transfer and subculture, respectively.

Cultured Explant	MS + 0.25 ppm 2,4-D + 1.00 ppm Ki				MS + 20 ppm IBA			
	Intact Embryo	Shoot	Cotyledonary Sections	Leaf	Intact Embryo	Shoot	Cotyledonary Sections	Leaf
Percent (%) Survival	40.4 ,	31.2	42.1	66.7	85.4	66.7	78.0	82.4
Fresh weight (g)	0.532	0.478	0.793	0.251	1.173	0.302	1.245	0.507
Percent (%) Germination	31.6	—	—	—	82.9	—	—	—
Percent Root Formation	5.26	—	—	—	41.5	—	—	—
Percent Root-like Callus Formation	5.26	—	—	—	2.4	—	—	—
Percent with Surface-callus	—	40.0	62.5	0	—	6.25	31.25	0

Table 4. The effect of different levels of picloram* on the callusing response of normal coconut embryos during the first (I) and second (II) transfer

ppm picloram	% Survival[1]		% Germination[2]		% Collusing[2]	
	I	II	I	II	I	II
0.1	100	100	0	0	31.8	100
0.25	100	100	0	0	30	100
0.5	86	90	0	0	42	100
1.0	77	88	0	0	41	100

	Degree of Root-like proliferation[3]					
	Slight		Moderate		Profuse	
	I	II	I	II	I	II
0.1	100	30	0	40	0	30
0.25	35	10	65	30	0	60
0.5	28	22	72	11	0	66
1.0	17	11	11	44	0	55

[1]Based on the number of uncontaminated cultures
[2]Based on the number of surviving cultures
[3]Based on the number of cultures showing proliferative growth
*Picloram: 4-amino — 3,5,6-trichloropicolinic acid

Another region of the embryo that was stimulated to proliferative growth was the cotyledonary sheath (CS) (de Guzman et al., 1978). In seedling development the CS develops as a thin non-fleshy structure. In media supplemented with 2,4-D it could undergo hyperdevelopment becoming thick and collar-like or producing masses of nodules. High NAA (20-40 ppm) favored the occurrence of CS proliferation over that of the root-like bodies (Table 6). In this respect it can be said that CS proliferation has a higher auxin threshold. Fragments of highly proliferating nodular tissues could be severed and sub-cultured a number of times to produce more nodular masses. The nodules appeared to be discrete or conjoined bodies consisting of cells that were small, prominently nucleated and compactly arranged. Some showed shoot-primodia-like forms. It is possible that these discrete bodies are units of organization which may be manipulated to produce organs or embryos. Thus in coconut tissues in addition to

Table 5. The effect of different levels of MCPB* on the callusing response of normal coconut embryos during the first (I) and second (II) solid transfer.

ppm MCPB	% Survival[1]		% Germination[2]		% Callusing[2]	
	I	II	I	II	I	II
0.1	93	86	0	0	20	58
0.25	92	86	0	0	54	92
0.5	80	92	0	0	77	92
1.0	81	86	0	0	82	100

	Degree of Root-like proliferation[3]					
	Slight		Moderate		Profuse	
	I	II	I	II	I	II
0.1	100	57	0	29	0	14
0.25	20	16.6	80	33.3	0	50
0.5	15	11	85	9	0	72
1.0	36	4	64	4	0	92

[1]Based on the number of uncontaminated cultures
[2]Based on the number of surviving cultures
[3]Based on the number of cultures showing proliferative growth
*MCPB: 4-(4-chloro-o-tolyl) oxy) butyric acid

organogenesis via callus formation direct organ regeneration also takes place.

On further subculture, however, the nodular mass may change over to a single smooth-surfaced lump of tissue. In this case cell division has been superseded by cell enlargement. The cells became large and highly vacuolated.

Growth and Morphogenesis Using Inflorescence Explants

The developing coconut inflorescence has been shown to be a promising source of explants either for organ or callus culture (Blake et al., 1975 and Eeuweens, 1976, 1978). In the following

Table 6. The effect of previous treatment with NAA on the percentage and degree of proliferation of coconut emryos[1]

Previous treatment ppm NAA	Cotyledonary Sheath Proliferation																Percent showing root-like Proliferation			
	Percentage (Total cultures)				Percentage of culture showing different degree of proliferation															
					Slight				Moderate				Profuse							
	I	II	III	Ave.	I	II	III	Ave.	I	II	III	Ave.	I	II	III	Ave.	I	II	III	Ave.
2.5	12.5	16.7	10	13.1	50	100	100	83.3	50	0	0	16.7	0	0	0	0	87.5	50	40	59.2
5.0	12.5	0	72	6.57	50	0	100	50.0	50	0	0	16.7	0	0	0	0	87.5	0	35.7	41.1
10.0	23.1	50	9.1	27.4	66.7	100	100	88.9	33.3	0	0	11.1	0	0	0	0	84.6	0	27.3	37.3
20.0	30.8	33.3	55.6	39.9	75	0	40	38.3	25	100	40	55.0	0	0	20	6.67	84.6	0	0	28.2
40.0	18.2	83.3	11.1	37.5	0	100	0	33.3	50	0	0	16.7	50	0	100	50.0	81.8	0	0	27.3

[1]Observations were taken after 8 weeks in 3rd transfer culture using MS + NAA + 2,4-D as culture medium.

discussion will be described our observations on the growth responses of explants from very immature inflorescence.

In media supplemented with 2,4-D and kinetin and other growth regulators substantial increase in size and fresh weights of floral explants were obtained (Table 7). A few cultures provide convincing evidence of reversion from reproductive to vegetative. Some of the resulting vegetative shoots have elongated, rolled scale leaves reminiscent of the leaves of in-vitro seedlings treated with gibberellic acid (Figure 1). In one culture the leaves have advanced from the scale to the true-leaf type (Figure 2). When this shoot was transferred to a medium without auxin a spike-like structure emerged instead of leaves. This rereversion from foliar to floral development was observed in several other cultures (Figure 3). Evidently in the cultures the direction of development of the shoot apex has not yet been firmly determined.

Table 7. Growth of coconut rachillae explants in different culture media. Observations (average fresh weight) were taken after 4 weeks in experimental media[1]. (Total age of culture — 8 weeks)..

Supplement to Experimental media (ppm)[2]	Fresh weight of all cultures (gm)	% alive	Fresh wt. of alive cultures (gm)
100 CH	0.6697(65)	35.38	1.1333(23)
500 CH	0.5655(78)	32.05	0.9821(25)
1000 CH	0.4165(78)	18.18	0.5851(8)
2000 CH	0.4008(35)	8.57	0.8731(3)
2.5 BA	0.4252(78)	25.64	0.9119(20)
5.0 BA	0.3078(66)	15.15	0.5376(10)
10.0 BA	0.6062(73)	53.42	1.0979(39)
1 NAA	0.3117(69)	15.94	0.7867(11)
2.5 NAA	0.3412(44)	15.90	0.8832(7)
5.0 NAA	0.2106(67)	0	—
1.0 NAA	0.3068(75)	8.0	0.7419(6)

[1] Explants were initially cultured in MS (4D) + 0.25 ppm 2,4-D + 0.02% C for 4 weeks and then transferred to experimental media. Average fresh weight of freshly excised explant was 0.050 gm.

CH = casein hydrolysate

[2] MS (4D) + 0.25 ppm 2,4—D + 0.02% C is common to all experimental media.

Likewise preliminary results indicate that the floral meristems have varied morphogenetic potentials. In some cultures

Figure 1. Coconut cultures showing development of floral meristems into vegetative shoots. Note the elongated rolled scale leaf in the left most culture.

Figure 2a. Shoot derived from a coconut floral meristem cultured in vitro. Note the presence of true leaves and the emergence of spike-like structure.

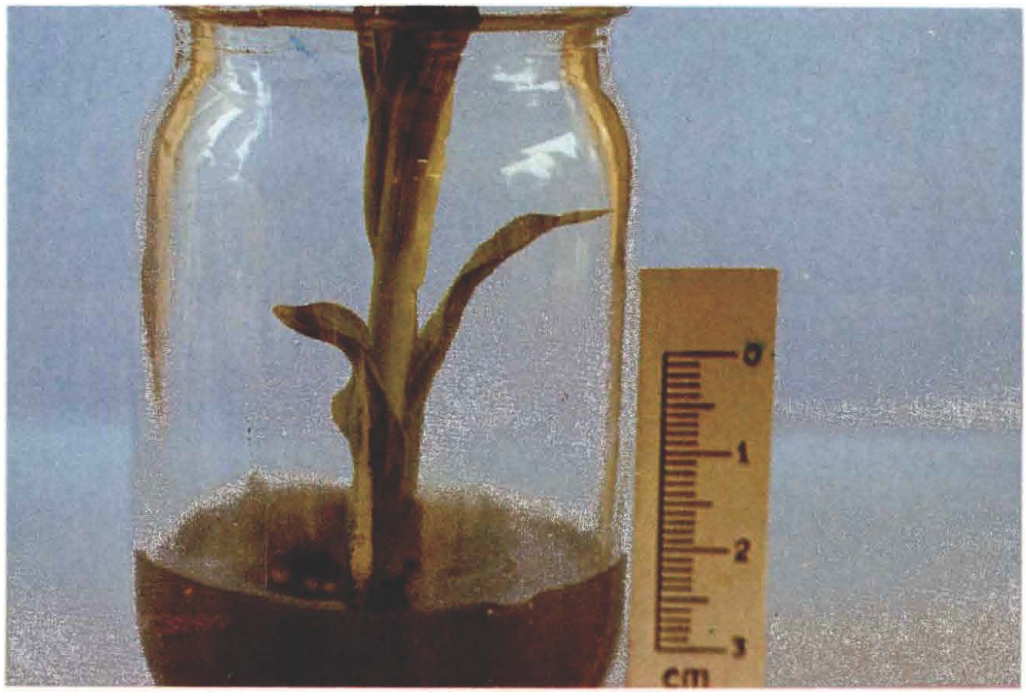


Figure 2b. Same shoot seen in Fig. 2a showing a better view of the true leaves.

Figure 3. Shoots developed from coconut floral meristems showing rereversion to reproductive state.

Figure 4a. A branched shoot developed from a single floral meristem.

Figure 4b. A culture derived from a single floral meristem. Note that the leaves are elongated and rolled; four shoots can be identified.

Figure 5. Branching in coconut seedling derived from an excised embryo cultured in vitro. Note that the branches are on the same node.

Figure 6. Culture derived from a single floral meristem with 2 shoots and a root. Root and shoot are not connected.

more than one vegetative shoots were produced from a single floral meristem (Figure 4). Since in coconut axillary buds are not produced except reproductive ones the additional shoot(s) may be presumed to have originated from terminal rather than lateral branching. Coconut appears to have a predisposition to terminal branching, a striking example of which is a palm with double dichotomy (Velasquez, 1975). Another case is the production of twin shoots from what appears to be a single embryo cultured in vitro (Figure 5). Terminal branching by subdivision of the vegetative shoot apex has been described in *Nypha* and is believed to occur also in other palms (Tomlinson, 1973). In the case of the vegetative shoots derived from floral meristems the induced growth in culture may have resulted in disturbance of apical integration leading to the organization of new apices.

Differentiation of roots from tissues of floral meristem origin has been reported by Eeuweens (1978) and observed in our laboratory in one culture (Figure 6). These demonstrate the ability of the floral meristem tissues to regenerate roots. Callus growth has been observed inconsistently in some cultures. Induction of cell division leading to cellular proliferations have been induced but not sustained to produce a vigorously growing callus.

General Discussion

With our present method of culture excised embryos can be grown into viable seedlings that can grow to maturity. It has enabled the propagation of the makapuno seednut which under natural conditions is non-germinating. It can be seen from the growth responses that an interplay of nutritional, hormonal and physical factors influence the extent and direction of growth of the embryo which in turn determine the morphology of the seedling. The present technique needs improvement to achieve more rapid and an in situ-like pattern of development. Further understanding of the relationship between the embryo and the endosperm and of the role of the special environment existing within the coconut seednut during germination will provide much needed directions in future embryo culture studies.

A major difficulty with efforts to induce callus and other forms of proliferative growth in both embryos and inflorescence tissues is the cessation of growth, erratic behavior, browning and eventual death of the tissues. In the subcultured proliferating CS tissues the older tissues turn brown and die. This restricts overall growth and further development by reducing the quantity of viable tissue and eliminating older tissues which are "ripe" for differentiation. In addition, the products of browning and tissue senescence may be self-inhibitory to the growth of the cultures.

The addition of activated charcoal to presumably absorb these inhibitory substances has greatly improved root development in seedling cultures and made possible continued growth in subculture of the CS proliferations for sometime. However, it has not completely eliminated the problem of browning and senescence and brought about the problem of reversion of type of growth from nodular to smooth. In a comparative culture of coconut, oil and date palm tissues Eeuweens and Blake obtained genuine callus formation only with the oil palm. This shows that coconut tissues pose greater resistance to growth manipulations *in vitro*. On an optimistic note, however, it can be said that the different morphogenetic responses that have already been elicited *in vitro* are positive signs that this resistance will eventually be overcome.

References

ABRAHAM, A. and K.J. THOMAS. 1962. A note on the in vitro culture of excised coconut embryos. The Indian Coconut Journal 15(2):84-67.

AMENIYA, A.H. 1956. Cultural conditions and growth of immature embryo in rice plant. Studies on the embryo culture in rice plant. Cited in "Plant Embryo Culture" by S. Narayanaswami. Botanical Review 30:587-628, 1964.

APAVATJRUT, P. and J. BLAKE. 1977. Tissue culture of stem explants of coconut (*Cocos nucifera* L.). Oleagineux 32(6):267-269.

BALAGA, H.Y. and E.V. DE GUZMAN. 1971. The growth and development of coconut "makapuno" embryo in vitro. II. Increased incidence and growth in response to sequential culture from liquid to solid and to media composition. Phil. Agric. 53:566-579.

BLAKE, J. et al. 1975. Progress in the vegetative propagation of coconut using tissue culture techniques. Proc. 4th Session FAO Technical Working Party on Coconut Production, Protection and Processing. Kingston, Jamaica.

BOGER, R.J. et al. The effect of indoleacetic acid on RNA synthesis in tobacco pith explants. Colloques Internationaux C.N.R.S. no. 261.

CUTTER, V.M., Jr. and K.S. WILSON. 1954. Effect of coconut endosperm and other growth stimulants upon the development in vitro of embryos of *Cocos nucifera*. Bot. Gazette. 115:234-240.

DE GUZMAN, E.V. and D.A. DEL ROSARIO. 1964. The growth and development of *Cocos nucifera* L. "makapuno" embryo in vitro. The Phil. Agr. 48:82-94.

———. 1969. The growth and development of coconut "makapuno" embryo in vitro. The induction of rooting. Phil. Agric. 53:65-78.

——. A.G. DEL ROSARIO and E.C. EUSEBIO. 1971. The growth and development of coconut "makapuno" embryo in vitro. III. Resumption of root growth in high sugar media. Phil. Agric. 53:566-579.

———, ——— and E.M. UBALDE. 1978. Proliferative growth and organogenesis in coconut embryo and tissue cultures. Phil. Jour. of Coconut Studies. 3:1-10.

DEL ROSARIO, A.G. and E.V. DE GUZMAN. 1978. The growth of coconut makapuno embryos in vitro as affected by mineral composition and sugar level of the medium during the liquid and solid cultures. Phil. Jour. of Science 105:215-222.

EEUWEENS, C.J. 1976. Mineral requirements of cultured coconut tissues. Physiol. Plant. 36:23-28.

———. 1978. Effects of organic nutrients and hormones on growth and development of tissue explants from coconut (*Cocos nucifera* L.) and date (*Phoenix dactylifera*) palms cultured in vitro. Physiol. Plant. 42:173-178.

GAUTHERET, R.J. 1966. Factors affecting differentiation of plant tissues grown in vitro. In "Cell Differentiation and Morphogenesis." Beerman et al., eds. North-Holland Publishing Company. Amsterdam.

JONES, L.H. and S.K. DETHAN. 1973. Establishment of oil palm plants from aseptically grown excised embryos. Paper for submission to Eucarpa.

LA RUE, C.D. and G.S. AVERY, Jr. 1938. The development of the embryo of *Zizania aquatica* in the seed and artificial culture. Bull. Torrey Bot. Club 65:11-21. Cited in "Nutrition, Growth and Morphogenesis of Plant Embryos" by V. Raghavan. Biol. Rev. 41:1-58. 1966.

LETHAM, D.S. 1974. Regulators of cell division in plant tissues. xx. The cytokinins of coconut milk. Physiol. Plant. 32:66-70.

MAGAT, S.S. 1978. A review of fertilizer studies on coconut. Phil. Jour. Coconut Studies. 3:51-72.

MINIANO, A.P. and E.V. DE GUZMAN. 1978. Responses of non-makapuno embryos in vitro to chloride supplementation. Phil. Jour. Coconut Studies. 3:27-44.

MURASHIGE, T. and F. SKOOG. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

OOSTROM, H. et al. 1975. Cited in "The effect of indoleacetic acid on RNA synthesis in tobacco pith explants" by R.J. Bogers. Colloques. Internationaux C.N.R.S. no. 261.

PAYAWAL, P.C. and E.V. DE GUZMAN. 1972. Growth and organ formation in orchid tissue culture as influenced by growth regulators and the mineral composition of the media. Kalikasan, Phil. Jour. of Biology. 1:155-166.

REINERT, J. and D. BACKS. 1968. Control of totipotency in plant cells growing in vitro. Nature 220:1340-1341.

REITSEMA, J., S. SATINA and A.F. BLAKESLEE. 1953. the effect of sucrose on the growth of *Datura stramonium* embryos in vitro. Am. Jour. Botany 40:538-545.

SAJISE, J.U. and E.V. DE GUZMAN. 1972. Formation of adventitious roots in coconut macapuno seedlings grown in medium supplemented with naphthalene acetic acid. Kalikasan, Phil. Jour. Biology 1:197-206.

TOMLINSON, P.B. 1973. The monocotyledons; their evolution and comparative biology. VIII. Branching in monocotyledons. The Quarterly Review of Biology 48:458-466.

UBALDE, E.M. 1974. Effects of naphthalene acetic acid and 2,4-dichlorophenoxyacetic acid on the development and anatomy of coconut 'Makapuno' embryos cultured in vitro. Undergraduate Thesis. College of Agriculture, U.P. at Los Baños.

VAN STADEN, J. and E.S. DREWES. 1975. Identification of zeatin and zeatin riboside in coconut milk. Physiol. Plant 34:106-109.

VELASQUEZ, G.T. 1975. Coconut palm displays double dichotonomous branching in Thailand. Turtox News 52:2 p. 6.

WU, L. and H.W. LI. 1971. Induction of callus tissues initiated from different somatic organs of rice plant by various concentrations of 2,4-dichlorophenoxyacetic acid. Cytologia 36:411-416.

YATES, R.C. and J.T. CURTIS. 1949. The effect of sucrose and other factors on the shoot-root ratio of orchid seedlings. Am. Jour. of Botany 36:390-396.

YEOMAN, M.M. 1970. Early development in callus culture. International Review of Cytology 29:383-406.

WHITE, P.R. 1963. The cultivation of plant and animal cells. The Ronald Press Co. New York. 288 pp.

Ramon C. Barba, Ph. D., Discussant

Most of the recent breakthroughs in research on agricultural production recognized here and in international forums are those on plant breeding — varieties that are resistant to diseases, better producers, earlier maturity, etc. However, the very few significant researches in the regulation of plant growth to improve production can stand on their own compared to similar efforts in plant breeding. Some of these include the induction of flowering in mango or pineapple to control time of production.

While these breeding efforts are important, and should in fact be intensified, I would like to see more researches on physiology to control plant growth to increase production like the efforts of Dr. de Guzman on the coconut.

One of the significant researches on the coconut is that on macapuno. Dr. de Guzman has single handedly worked with concentrated effort and successfully, to say the least. There are other areas wherein tissue culture can be a significant tool with respect to the coconut including objectives related to breeding or objectives being pursued in breeding researches. Let me elaborate. The breeders recognize the importance of improving coconut production through breeding. It has so many problems — variations in stand, low production, late maturity, etc. But there are problems in breeding the coconut. One is the lengthly juvenile phase so that the breeding cycle will easily outlast the life span of the researcher. It has a low multiplication rate and the area needed for breeding is extensive. It is often cross pollinated and heterozygocity is widespread. There is also a wide geographical spread of the coconut and the nuts are not easy to transport because of bulk and loss of viability.

There are superior existing varieties that don't come true to type from seeds, the only present means of coconut propagation. If only it can be propagated vegetatively like the camote or mango, then all that is needed is to cut and root or graft branches, respectively, to get plants with identical characteristics. Effective vegetative propagation of the coconut seems possible only by tissue culture although a lot of problems exist. For instance, it

seems that the ability to regenerate by tissue culture (cloning) is related to the ability of the plant to produce shoots and roots in their natural habitat. For example, the camote is theoretically easy to propagate by tissue culture because it is easy to regenerate camote plants by cutting stems and sticking these in the ground. Seldom does the coconut produce branches. If you cut a coconut trunk midway you do not produce new branches as in many trees but produce a dead trunk.

Perhaps it is possible to convince the coconut, as Dr. de Guzman said, to form adventitious shoots and reproduce by itself. But communicating with the coconut is in itself a problem. If I were a communications expert I might try that approach but being a plant physiologist I will put my money on tissue culture.

In spite of the apparent difficulty and cost of coconut tissue culture research, because of its importance, this specific area of research should be intensified by bigger grants and involving more experts whether in the same or separate laboratories. The investment of say, a million-peso worth of research, if successful, would easily reap dividends in a few years.

In other countries like The Netherlands, no less than a whole institute is created to solve specific problems. An "Institute for Potato Blight Research" is not far fetched, if the blight becomes severe enough to affect their potato production. While I do not advocate creating an Institute for Tissue Culture of Coconut, much more effort than presently exist should be channeled in this area of research.

Enrique P. Pacardo, Ph. D., Discussant

There are two interrelated points of the paper which I would like to focus my comments. One is related to the basic phenomenon of morphogenesis and the other pertains to the practical application of the knowledge of morphogenesis. Since the time a free somatic cell of carrot root was successfully cultured and grown into a full-grown plant in Steward's laboratory, several studies on different species have been reported. This should be a welcome development for indeed, tissue culture technique has enabled the experimenters to investigate both internal and external factors that regulate growth and differentiation of cells.

By observing painstakingly the various responses of coconut embryo under various combinations of these factors, Dr. de Guzman has uncovered a few secrets of coconut embryo. It has been shown that proper balance of salts and sugars in the medium and the physical state of the medium itself are necessary and may be indispensable for embryonic development to full-grown seedlings. The paper has provided evidence for the potential potency of coconut embryo, particularly macapuno seed nut which seemed to have lost its potency for further growth when being germinated *in situ.*

Under natural field condition, all physiological requirements of a germinating coconut embryo except water perhaps seemed to be supplied by the endosperm. However, in the present work there seemed to have been no attempt, at least no mention was made in the paper, to culture embryo in a medium containing coconut milk or a medium of coconut milk only. This approach might provide additional clues as to the growth requirements of the embryo since coconut milk could readily lend itself to chemical analysis.

One of the foremost questions in morphogenesis is that concerning the developmental potency of individual cells. Earlier experiments indicated that the potential towards organized growth is present in individual cells which are either somatic or reproductive. Their potentials are realized only under a proper balance of factors that promote growth and differentiation. In the present paper of Dr. de Guzman, no mention has been made

on whether or not experiments along this line, that is using individual free cell from embryo has ever been attempted. This may be due to some difficulties in isolating single cell but once this is done successfully, it would mean greater efficiency in the commercial propagation of macapuno, that is for one embryo, several potential free cultures could be made.

The role of growth regulators in a germinating embryo does not appear to be clear as yet. In one case, the application of 2, 4-D or NAA changed the mode of growth from differentiation to cell division.

The paper of Dr. de Guzman more or less summarizes the enormous work, time and effort, not to mention tons of coconut nuts used, spent during the last one and a half decades in her small tissue culture laboratory. And no doubt we are now in a better position than before to understand how a few basic processes in morphogenesis take place.

However, fundamental questions as to why such processes occur at a certain points of time remain as elusive as before. Why does an embryo undergoing differentiation suddenly revert back to meristimatic activity with high dosage of hormone? Why does root development take place only when a germinating embryo is transferred from solid to liquid medium?

In other words, why do these phenomena happen the way they do? These are fundamental questions in which right answers do not seem to be in sight yet. Perhaps new methodologies and approaches are needed. But with most preliminary works already done, Dr. de Guzman and her active school of tissue culture might provide us with some tentative answers to these questions during the next decade.

PHYSIOLOGY AND BIOCHEMISTRY OF THE VOLATILE OILS OF *MENTHA SPECIES* (FAM. LABIATAE) GROWN IN THE PHILIPPINES

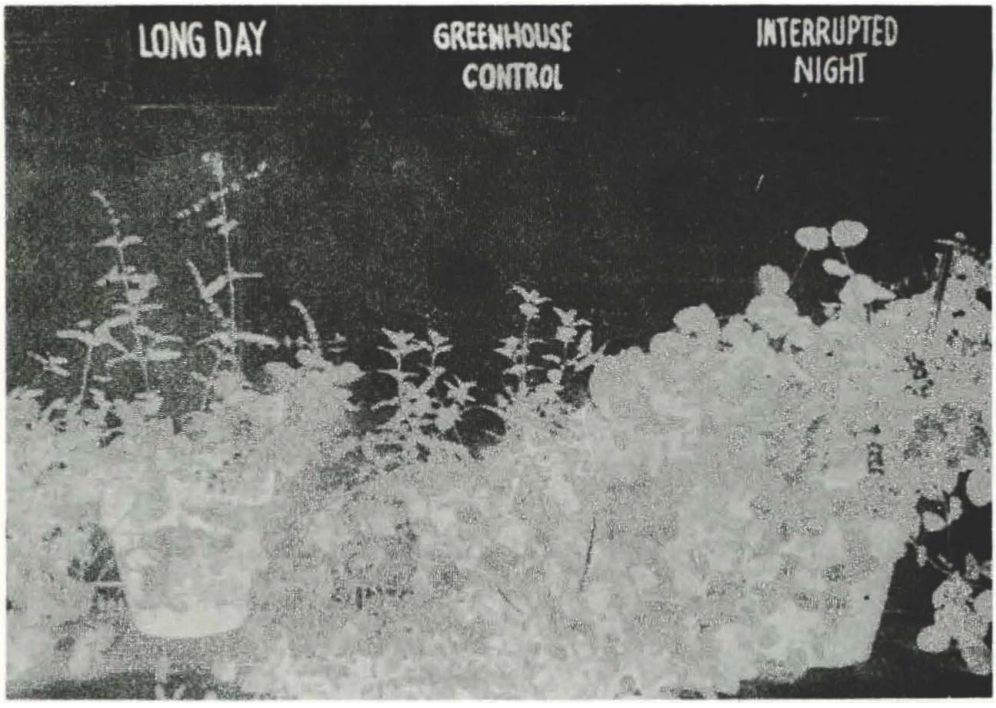
By Magdalena C. Cantoria, Ph. D., Academician

In his ENUMERATION OF FLOWERING PLANTS, Merril (1923) lists two species of Mentha, namely: *Mentha arvensis* L. *(Mentha crispa* Blanco) and *Mentha javanica* Blume. The first is known locally as *yerba buena* and the second, as *polihos* or *polio* (corruptions of the Spanish *poleo* meaning pennyroyal, *Mentha pulegium* L.). Burkill (1935) states that the *M. javanica* of Blume is M. *arvensis* L.

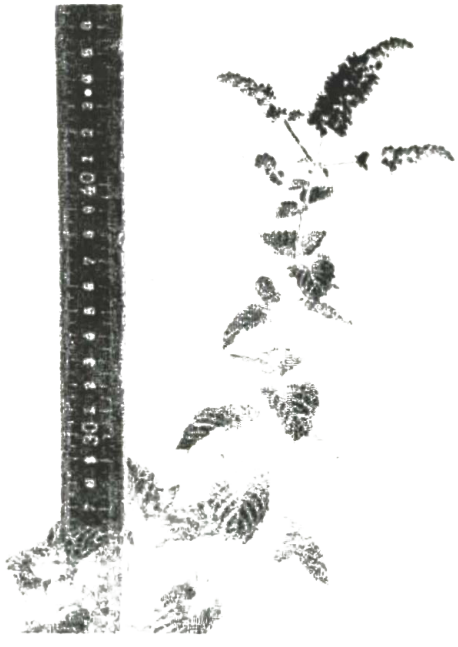
Yerba buena is widely scattered in cultivation in the Philippines. Polios, as the second plant is called in the Bicol area where it was collected, has also been noted in Bulacan but does not appear to be as widely known or cultivated as yerba buena. Both plants are grown for medicinal and ornamental purposes and for use as a flavoring.

Studies were originally undertaken to confirm the identity of yerba buena which does not flower under Philippine climatic conditions.

Slide 1 — Yerba buena grown outdoors remains vegetative under natural daylengths in the Philippines.



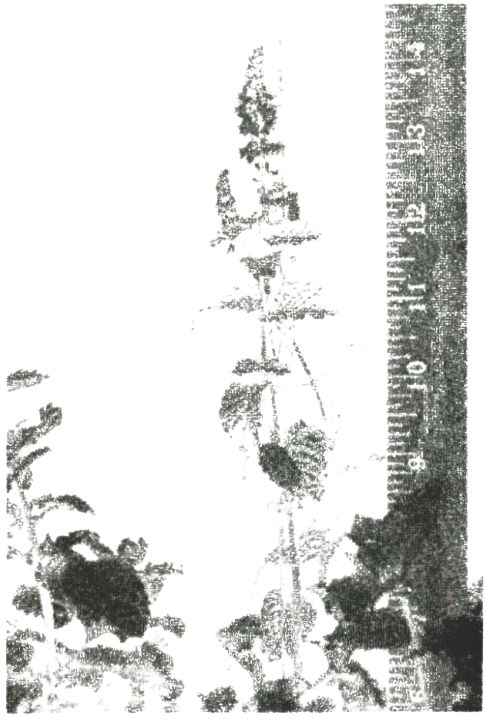
Slide 2 — Cuttings from one plant were grown under three photoperiodic treatments: 9-hour days, 18-hour days, and 9-hour days supplemented with 2 hours of light in the middle of the dark period. The plant flowered in response to the 18-hour days and under the 9-hour days supplemented with 2 hours of light in the middle of the dark period (Cantoria, 1970). This shows that the plant belongs to the group which plant physiologists refer to as long-day plants. They require a certain critical duration of dark period, which is a short one, before they flower.



Slide 3 — The inflorescence is a terminal slender spike which later branches. This type of inflorescence has not been reported in

Mentha arvensis L. which was the name previously given to yerba buena in the Philippines.

Slide 4 — Plants growing outdoors were subjected to an interrupted-night treatment which is the equivalent of a short dark period. Two floodlights were turned on for two hours in the middle of the night and the plants flowered.



Slide 5 — Close-up of yerba buena in slide 4.

Slide 6 — Stem apices were collected from yerba buena plants about to flower and sectioned. The differentiation of the apical cells into floral structures may be noted.

TABLE 1

Growth of Mentha arvensis *L. under two different daylengths*

		A 11.9-hr day	B 8-hr day	A/B × 100*
Top	Fresh weight (g	6.69 ± 0.55	2.86 ± 0.33	134
	Dry weight (mg	616.0 ± 70.71	260.70 ± 34.64	136
	Number of internodes	11.8 ± 0.4	10.3 ± 0.3	15
Internode	2nd			
	Dry weight (mg)	21.00 ± 0.78	14.05 ± 1.22	49
	Increase in length (cm)	3.58 ± 0.22	4.39 ± 0.35	
	Dry weight (mg)	22.83 ± 2.81	9.78 ± 0.98	133
	6th			
	Length (cm	3.29 ± 0.12	3.29 ± 0.04	—
Leaf	Dry weight (mg	9.50 ± 0.95	7.53 ± 0.80	—
	Length (cm)	3.25 ± 0.11	2.92 ± 0.15	—
	3rd Width (cm)	1.94 ± 0.11	1.93 ± 0.08	—
	Length: width	1.69 ± 0.06	1.53 ± 0.05	—
	Palisade number[a]	20.0 ± 0.2	17.2 ± 0.1	16
	Dry weight (mg)	25.65 ± 2.08	11.29 ± 1.37	127
	Length (cm)	5.34 ± 0.12	3.75 ± 0.15	42
	7th Width (cm)	3.34 ± 0.10	2.61 ± 0.12	28
	Length: width	1.62 ± 0.05	1.44 ± 0.02	13
	Palisade number[a]	16.7 ± 0.1	14.3 ± 0.1	17
Oil gland number[b] of 8th leaf	Lower surface	3.5 ± 0.1	3.7 ± 0.1	—
	Upper surface	2.0 ± 0.2	2.1 ± 0.2	—

Means of 8 plants with the standard errors of mean (±).

* Values are presented where differences between A and B are significant at 1% level.

[a] Per 0.011 mm².

[b] Per 0.48mm².

Slide 7 — In attempts to bring yerba buena to flower, plants were subjected to two different daylengths which was all that could be done with the facilities available at that time before the photoperiod chambers could be set up. Uniform cuttings were grown in quartz sand and watered regularly with Hoagland's solution in the greenhouse under natural daylengths (11.9 hours)

and under 8-hour days. The data shown in the table were collected (Cantoria and Mabasa, 1965).

Fresh and dry weights of the tops, leaves, and internodes were taken, ratios of leaf length:width were calculated, palisade numbers and oil gland numbers of the leaves were determined.

The effect of daylength was most remarkable in the weights of internode, leaf, and entire top. The longer daylength was significantly more favorable for growth than the 8-hour day. Plant physiologists attribute this kind of response to the amount of light energy received by the plant.

The palisade number has been used in microanalysis for the identification of leaf drugs. In this study, the palisade number is the average number of palisade cells within an area of 0.011 mm^2. In yerba buena, the palisade number was lower under the 8-hour day than under the 11.9-hour day. The oil gland number was not significantly affected by the daylength.

The plants did not develop flowers under these two daylengths.

TABLE 1.—*Growth of Mentha cordifolia Opiz under two different light intensities.*

			A Normal Intensity	B Reduced Intensity	$\frac{A-B}{B} \times 100$%
Total		Fresh weight (g)	18.90 ± 1.17	10.28 ± 0.06	84
		Dry weight (g)	2.10 ± 0.07	1.06 ± 0.07	98
Number of pairs of leaves			23.80 ± 0.92	22.80 ± 0.25	9
Percentage oil yield of fresh weight			0.147	0.117	--
Oil gland number [b]	5th	Upper epidermis	3.98 ± 0.08	4.59 ± 0.18	--
		Lower epidermis	9.08 ± 0.24	9.46 ± 0.23	--
	8th	Upper epidermis	4.08 0.13	5.29 0.18	--
		Lower epidermis	10.08 0.28	10.15 0.21	--
	11th	Upper epidermis	5.34 0.16	5.10 0.38	--
		Lower epidermis	11.20 0.18	10.37 0.19	8
	14th	Upper epidermis	4.08 0.13	5.25 0.19	27
		Lower epidermis	10.68 0.30	11.32 0.31	--
Oil gland diameter (mm) [c]	5th	Upper epidermis	0.132 0.0014	0.134 0.0013	--
		Lower epidermis	0.130 0.0009	0.133 0.0008	--
	8th	Upper epidermis	0.149 0.0008	0.145 0.0012	--
		Lower epidermis	0.147 0.0010	0.142 0.0014	4
	11th	Upper epidermis	0.147 0.0010	0.146 0.0016	--
		Lower epidermis	0.145 0.0013	0.144 0.0017	--
	14th	Upper epidermis	0.145 0.0014	0.141 0.0014	--
		Lower epidermis	0.143 0.0010	0.136 0.0006	4

Slide 8a — The growth of yerba buena under two different light intensities in the greenhouse was observed (Cantoria and Cuevas-Gacutan, 1974). On the basis of dry weight production, growth was better under normal greenhouse light intensity (25% of the outdoors) than under the reduced light intensity (2% of the outdoors). There was no distinct effect on the number of oil glands per unit area nor on the size of the oil glands.

Slide 8b — Prolonged exposure to reduced light intensity resulted in a change in leaf shape and in etiolation. The plants did not flower under the two light intensities used. The plant may be

considered a shade-tolerant species as it was observed to grow well inside the greenhouse where the normal light intensity is 25% of the outdoors.

TABLE 1. *Monthly variation in oil yield and air-dry weight of Mentha two-dioica Opiz grown in the field*

	Air dry weight of 100 tops (Two pairs of leaves)	Young tops		Mature tops	
		Air-dry weight	Moisture-free weight	% oil	Moisture-free oil
	gm	Per cent	Per cent	Per cent	Per cent
1968					
June	12.53	2 297	2 573		
July	13.07	1 588	1 971		
Aug.	10 08		2 095		
Sept.			1 687		
Oct.		1 100	1 045		
		1 905	1 943		
	11 01	1 480	1 109		
1969					
Jan.	14.53	1.738	1 784		
Feb	15 75	1.288	1.563		
	16 09	1 886	2.008		
	14 93	1 853	2 075		
May	11 76	2 110	2 405		
June	9 69	2 319	2 519		
July	8 72	1 936	2.326		
	7.28	1 694	1.817		
	7.50	1 826	1.674		
	12 24	1 624	1.805		
		1 479	1 586		
Dec.		1 696	2.079		
1968					
		1 903	2 101		
		1 810	1.958		
	11 39	1 711	1 809		
Apr.	11 94	2 046	2 161		
May	10 24	1 879	2.209		
June	11.48	1 964	2.471		
			2 073		

Slide 9 — Yerba buena plants were cultivated in the field and monthly variation in oil yield and air-dry weight of young tops and older tops was determined. The table shows the data collected (Cantoria, 1974).

Slide 10 — The results are plotted in the figure. Oil yield is highest during the period of maximum growth as measured by dry-weight production. This period of maximum oil yield starts

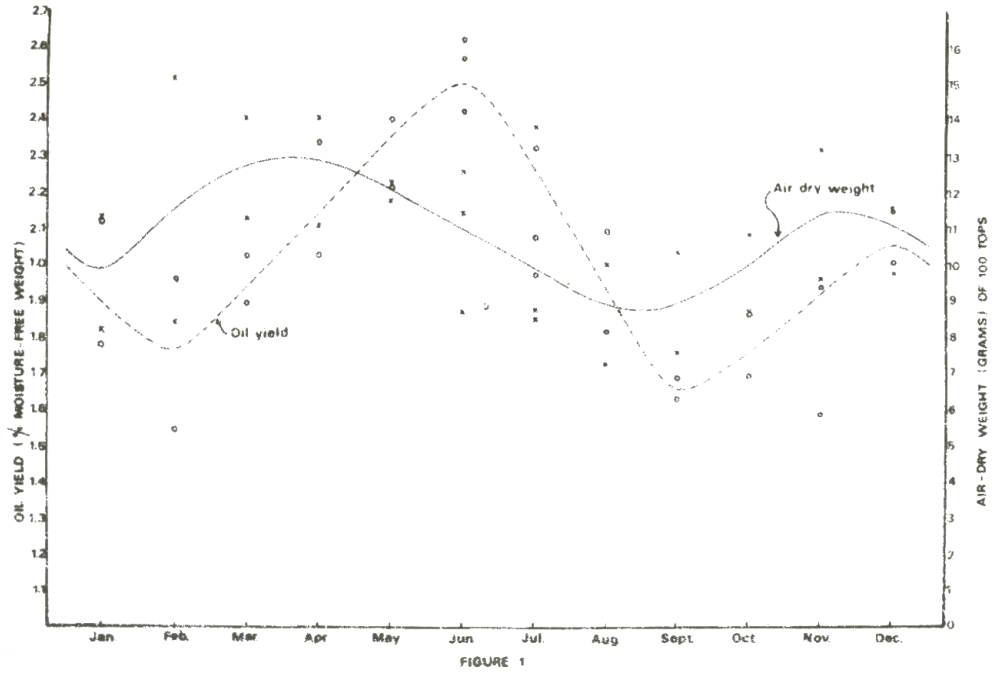
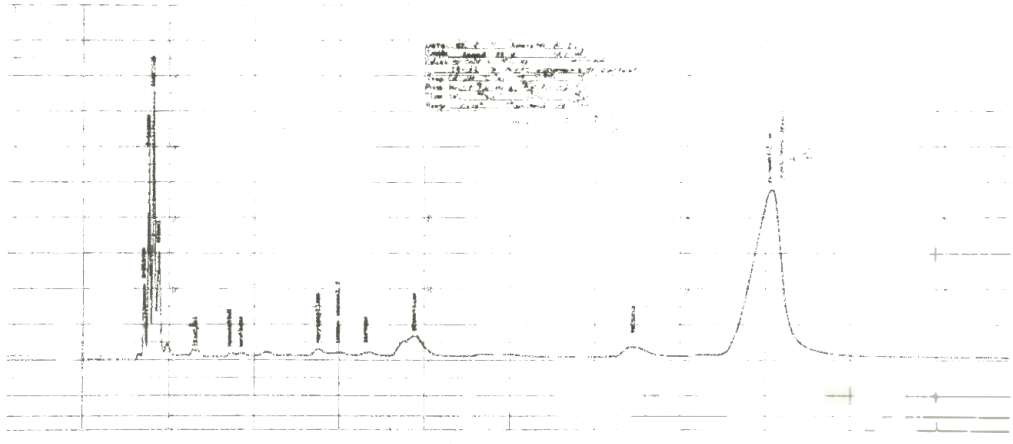


FIGURE 1

just after dry-weight production begins to increase. Oil yield reaches a peak during the months of March to June with a secondary peak in December. Dry-weight production reaches a peak during the months of February to June with a secondary peak in November. Heavy rains during July to September affect growth of the plants and oil adversely. Young tops yield more oil than older parts and it has previously been shown that flowering tops yield more oil than vegetative tops.



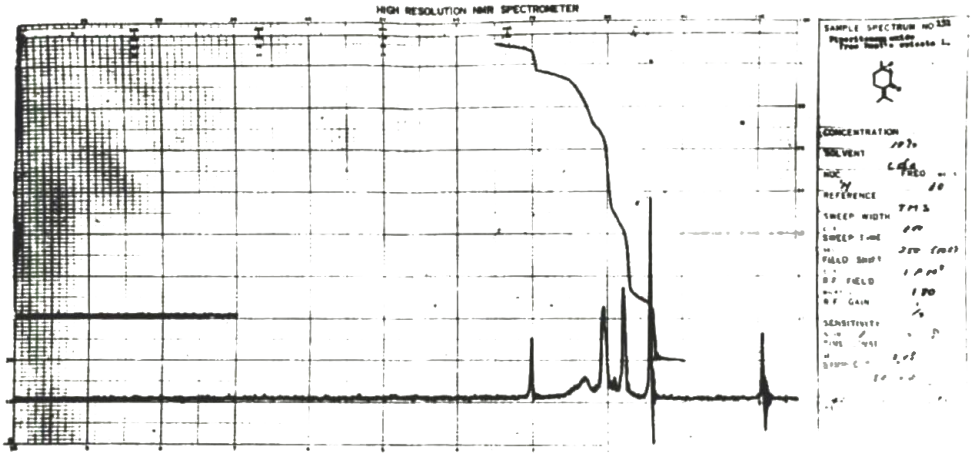
Mentha cordifolia Opiz (Philippine strain)
Oil from vegetative younger tops
Field grown
May 1970

Slide 11 — Preparative gas chromatography of the oil of yerba buena shows that piperitenone oxide is the principal

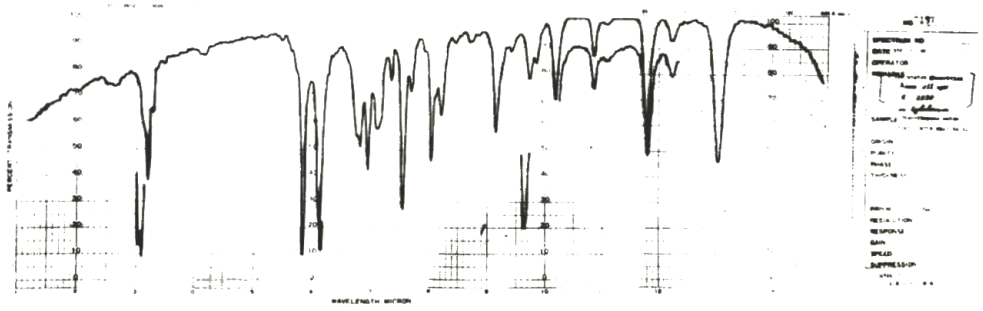
constituent. This compound was previously reported in *Mentha rotundifolia* by Reitsema (1956) and by Shimizu (1956) and in *Mentha spicata* L. by Shimizu and Ikeda (1961).

Mentha cordifolia Opiz (Philippine strain)
Oil from older vegetative parts
Field grown

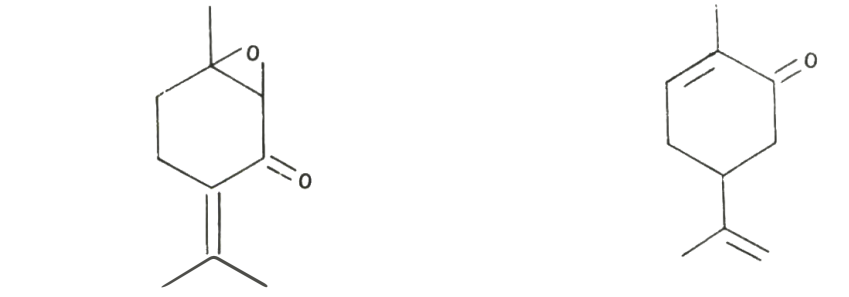
Slide 12 — Gas chromatogram of the oil from older vegetative tops.



Slide 13 — The identification of piperitenone oxide is confirmed by its NMR and IR spectra. Its structure is 1,2-epoxypulegone or 1-methyl-4-isopropylidene -1,2-epoxycyclohexanone-3.



Slide 14 — The IR spectrum of piperitenone oxide.



Piperitenone oxide

Mentha rotundifolia (L) Huds.

Carvone

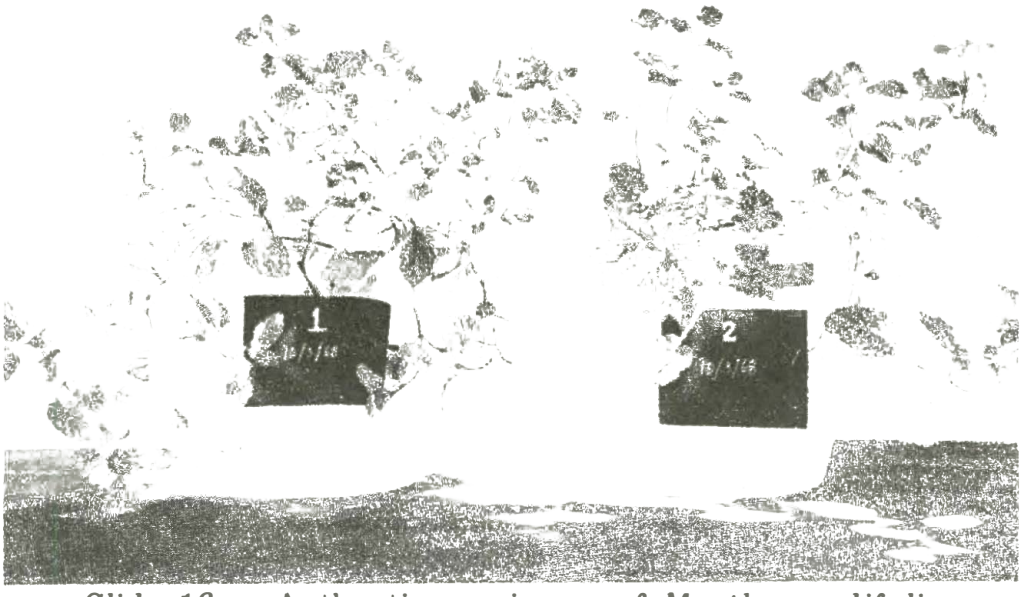
Mentha spicata L

Mentha cordifolia Opiz

Philippine plant: Piperitenone oxide

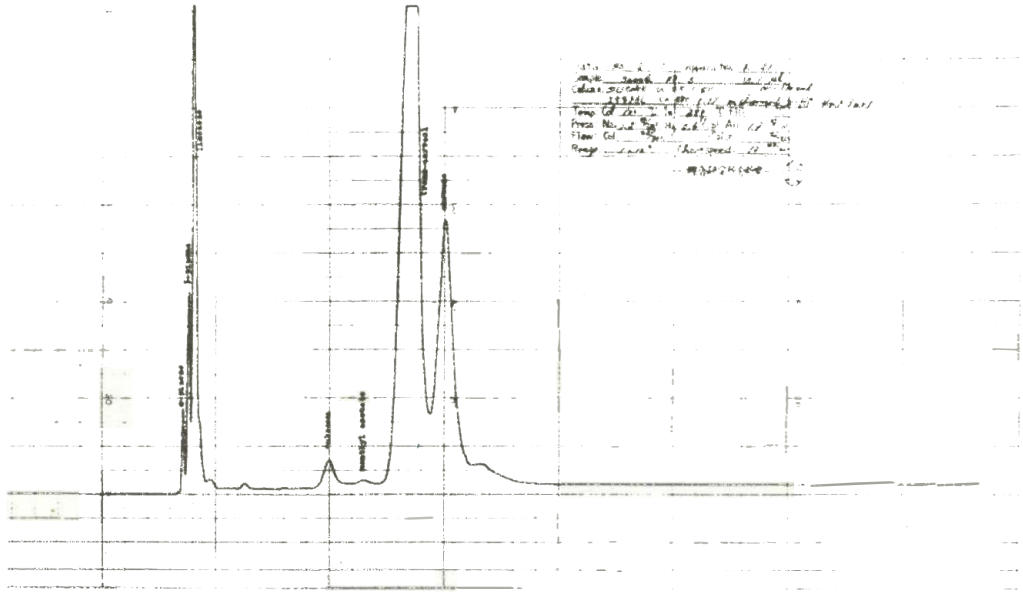
American plant: Carvone

Slide 15 — On the basis of its flowering characteristics and the composition of its volatile oil, yerba buena is identified as *Mentha cordifolia* Opiz. This plant is a cross between *M. rotundifolia* (L.) Huds, x *M spicata* L. (Clapham *et al.*, 1952). The inflorescence of both these species is a terminal spike. The principal component of the oil of *M. rotundifolia* is piperitenone oxide and that of *M. spicata* is carvone (Handa *et al.*, 1964).



Slide 16 — Authentic specimens of *Mentha cordifolia* were received from the Todd Company in Michigan and grown in the Philippines. The plant resembles the Philippine strain and, grown side by side, the two plants can not be distinguished.

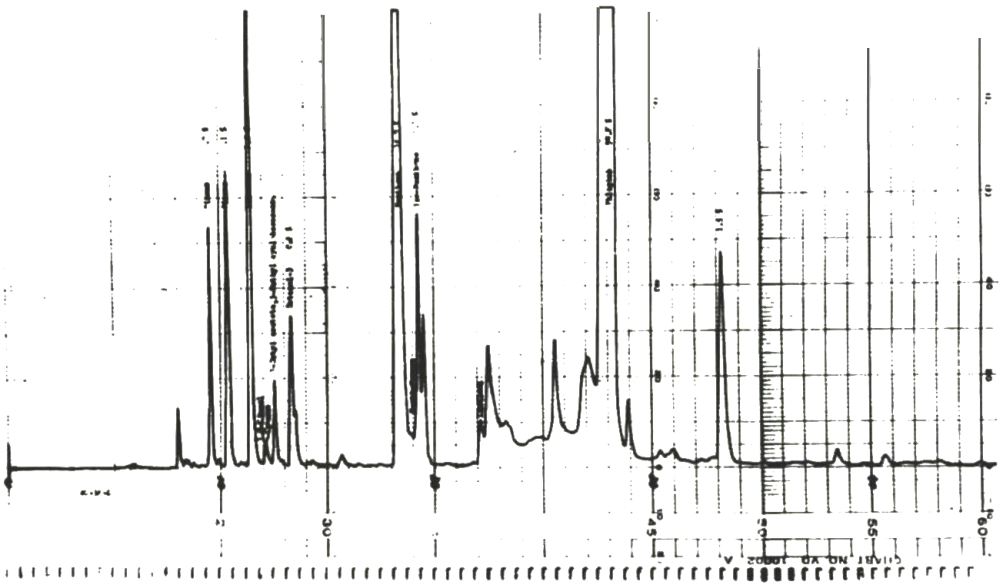
Slide 17 — Philippine and American strains of *M. cordifolia* in flower under interrupted-night or long-day conditions.



Plant No.1 grown from irradiated seeds of Mentha cordifolia Opiz
Oil from younger vegetative tops
Field grown
April 1970

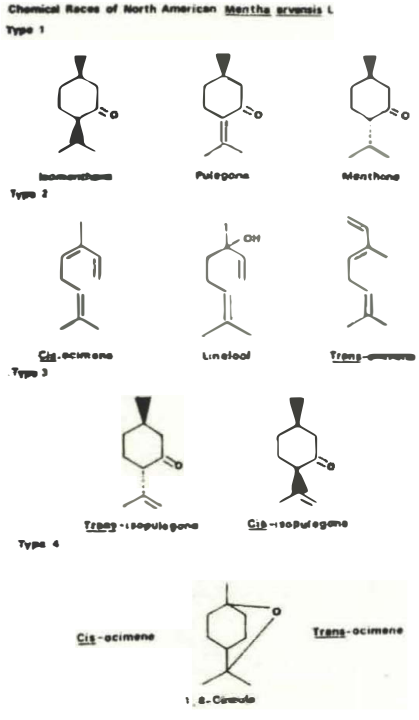
Slide 18 — The principal component of the volatile oil of the American strain is carvone which resembles that of the parent, *M. spicata*. On the other hand, the oil of the Philippine strain resembles that of the *M. rotundifolia* parent.

Slide 19 — Polios was obtained from the Bicol area a few years after the work on yerba buena was started. Under field conditions it develops flowers in axillary clusters characteristic of *Mentha arvensis* L.



Mentha arvensis L.
Polius
19 August 1975

Slide 20 — An analysis of the oil of polios shows that its principal components are pulegone and menthone. Its identification as *M. arvensis* L. is confirmed.

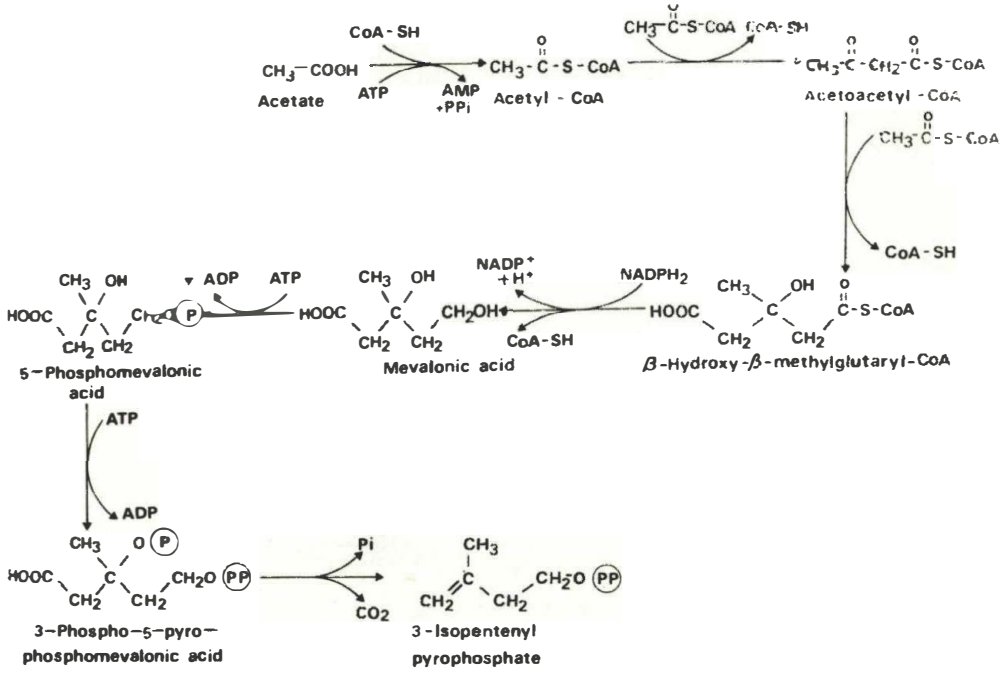


Slide 21 —*Mentha arvensis* L. is a very variable species, widely distributed in North America and temperate Eurasia (Stewart, 1944). In Europe and Asia, *M. arvensis* hybridizes freely with other species. Several of these hybrids are partially fertile and produce hybrid swarms in which morphological identification is impossible. In such cases, chromosomes number and chemical data

usually provide a clue to identity. Among the North American varieties, four chemotypes are recognized on the basis of volatile oil composition (Gill *et al.*, 1973).

Type 1 — high in pulegone, isomenthone, and menthone
Type 2 — high in linalool, *cis*-ocimene, and *trans*-ocimene
Type 3 — high in *cis*-isopulegone and *trans*-isopulegone
Type 4 — high in *cis*-and *trans*-ocimene and in 1,8-cineole

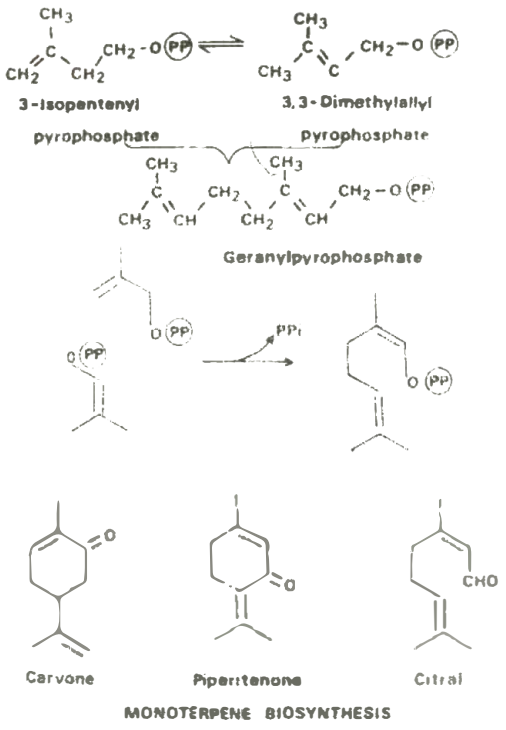
This classification excludes *var. piperascens* Malinvaud, Japanese or Brazilian peppermint, which is widely cultivated for its menthol content. Philippine polios belongs to Type 1. The oil of polios contains mainly pulegone and menthone, some isomenthone, and a very slight trace of menthofuran. It is probably an F_1 cross between *M. aquatica* x wild-type *M. arvensis*. Genetic studies need to be done to establish this and thus determine the variety and, if necessary, even the form of polios. It is difficult to be certain of the origin of hybrid strains found in nature. Menthofuran, found in oil of *M. aquatica* L., would be expected in F_1 hybrids but only trace amounts have been reported in wild-type *M. arvensis*.



ACETATE-MEVALONATE PATHWAY TO 3-ISOPENTENYL PYROPHOSPHATE

Slide 22 — The biosynthesis of volatile oil components takes place through the well-known acetate-mevalonate pathway leading to the formation of the biological isoprene unit, isopentenyl pyrophosphate (Loomis, 1967; Banthorpe and Charlwood, 1972).

Slide 23 — This isomerizes to dimethylallyl pyrophosphate, then an isopentenyl pyrophosphate adds to the dimethylallyl pyrophosphate, to form a monoterpene geranyl pyrophosphate or its *cis* isomer, neryl pyrophosphate.



Slide 24 — A linear oxygenated monoterpene found in *Mentha* is citral, characteristic of lemon mint (M. *citrata* Ehrh.). Cyclization of neryl pyrophosphate may occur to form the spearmint types (oxygenated at position 2) or the peppermint types (oxygenated at position 3). Carvone is characteristic of *M. spicata* L. Piperitenone may be converted to piperitenone oxide [in *M. rotundifolia* (L.) Huds.], or to pulegone (in *M. pulegium* L.), or to piperitone which is epoxidized to piperitone oxide (in *M. longifolia* L.). Piperitone or pulegone may be converted to

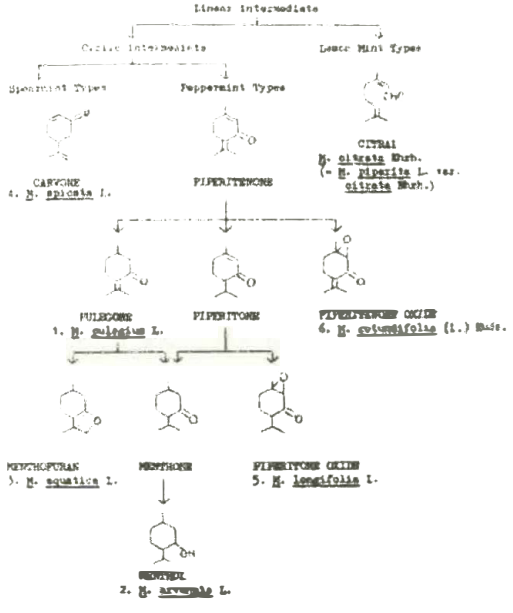
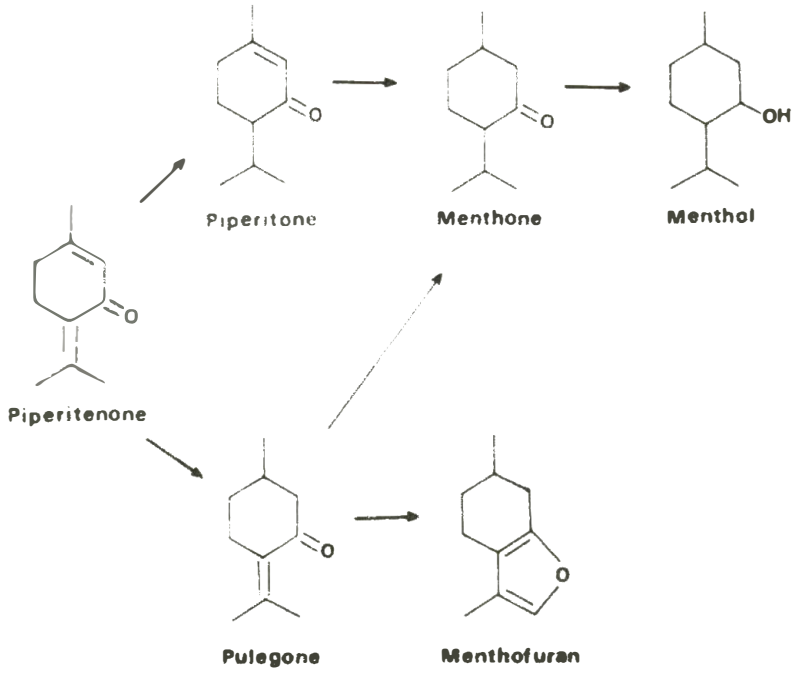


Fig. 26.1. Biosynthetic relationships of six *Mentha* species and one variety (after Reitsema, 1958).



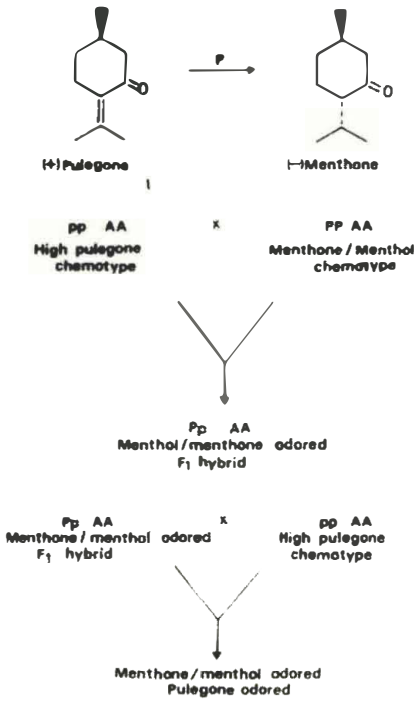
BIOSYNTHESIS OF OXYGENATED MONOTERPENES IN MINT

menthone and reduced to menthol (in *M. arvensis* L.). Pulegone may be converted to menthofuran in *M. aquatica* L. Thus, these biosynthetic relationships were postulated (Reitsema, 1958).

Slide 25 — Labeling studies using C-14-labeled acetate, mevalonate, and carbon dioxide in *M. piperita* L. var. *Mitcham* and *M. arvensis* L. var. *glabrata* Ray confirm the sequences piperitenone → piperitone → menthone → menthol; piperitone → pulegone → menthone and menthofuran (Hefendehl et al., 1967). Evidence suggesting the sequence: an unknown precursor → isomethone → menthone → menthol was obtained. In *M. piperita*, menthol is esterified to menthyl acetate.

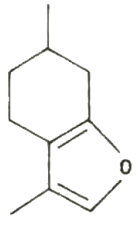
In young tissues of *M. piperita*, pulegone is the predominant terpene, accompanied by menthofuran. Menthone, found in older tissues, also gradually disappears and menthol accumulates, to be replaced in turn by the ester, menthyl acetate, as the plant ages. This explains why peppermint oil of good quality is obtained only from plant material containing a high proportion of mature tissues.

Slide 26 — The genetic basis for the conversion of pulegone to menthone has been examined (Lincoln and Murray, 1978). Clonally propagated individuals of the high pulegone chemotype of *M. arvensis*, *pp AA*, were hybridized with true breeding individuals of the menthone/menthol chemotype, *PP AA*. F_1 hybrids, *PP AA*, with a menthone/menthol odor were produced, confirming previous findings that the *P* gene allowing the conversion of pulegone to menthone is dominant. Backcrossing fertile F_1 hybrids, *Pp*, to the pulegone chemotype, *pp*, gave a 1:1 ratio, demonstrating the monogenic nature of the *P* gene that



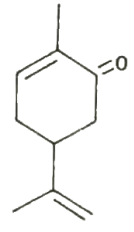
converts pulegone to menthone. The dominant *A* gene converts piperitenone to pulegone.

Slide 27 — Mentha piperita L. is a cross between *M. aquatica* x *spicata* (Clapham et al., 1952) and it is the former that may be present in peppermint oil. As a criterion for the identity of *M. piperita* oil, a characteristic color reaction which is due to the presence of menthofuran has been applied widely. This reaction has been incorporated in the NF monograph (USP XX — NF XV, 1980). With *M. arvensis* oil, the described sequence of color changes is not observed.



Menthofuran

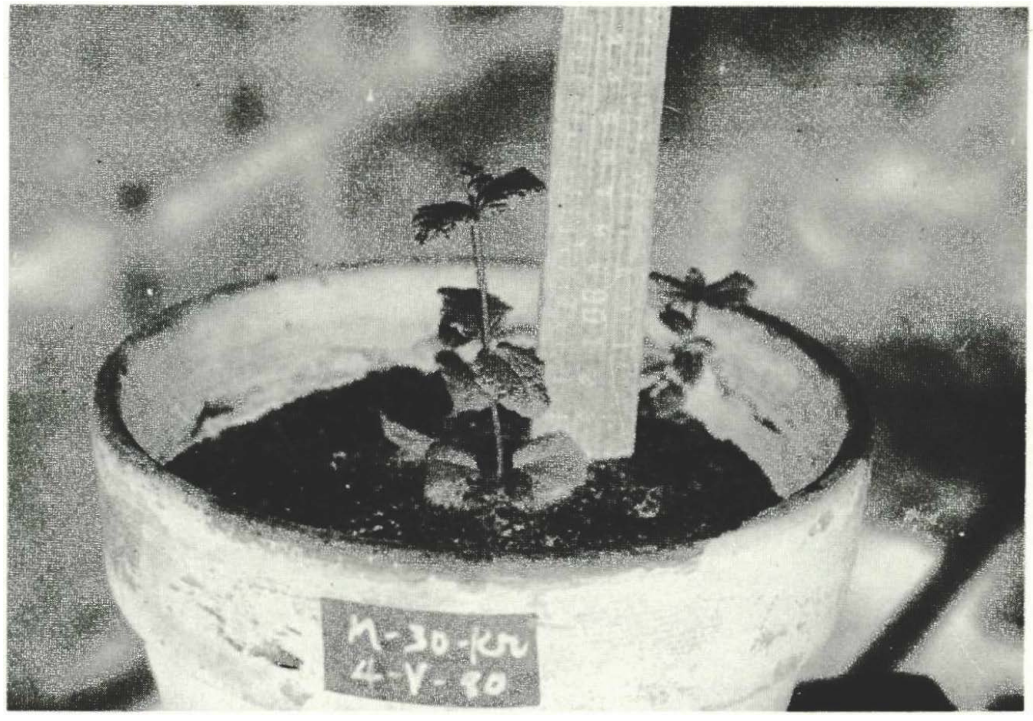
Mentha aquatica L.



Carvone

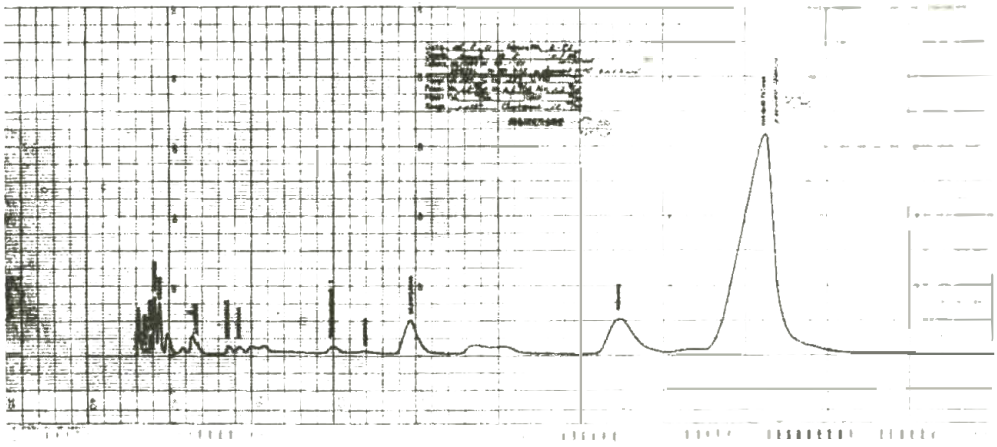
Mentha spicata L

Mentha piperita L.



Slide 28 — Irradiation of seeds of a hybrid generally gives rise to plants resembling the parent species. This has been noted in plants grown from seeds of yerba buena treated with 30 Kr of gamma radiation under moist conditions (Cantoria, 1972).

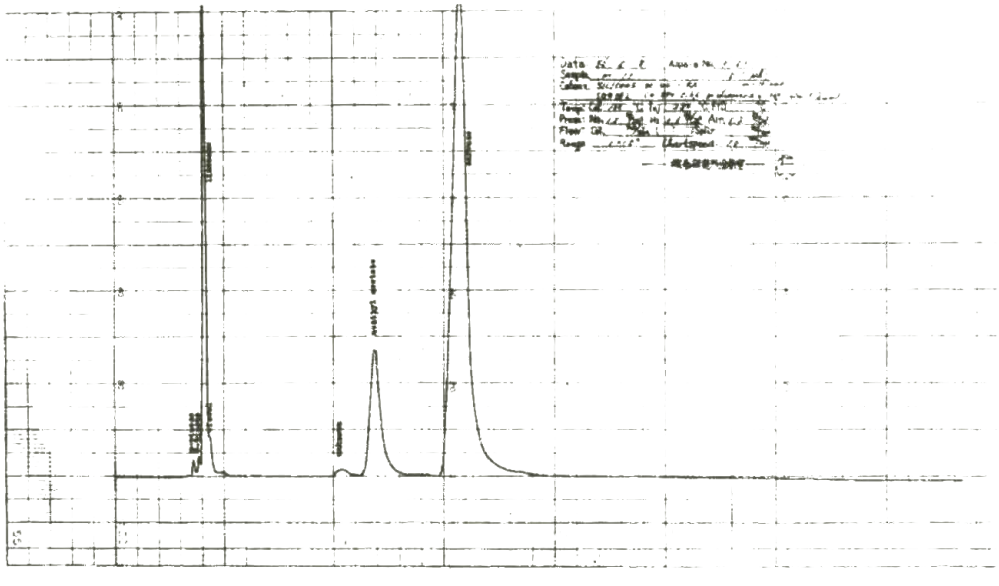
Slide 29 — The plant resembles *M. rotundifolia.* The leaves are broad and short so that they appear rounded and the plants are more hairy. Hairy mutations, however, are generally low oil yielders and the quality is most frequently undesirable (Landing, 1969).



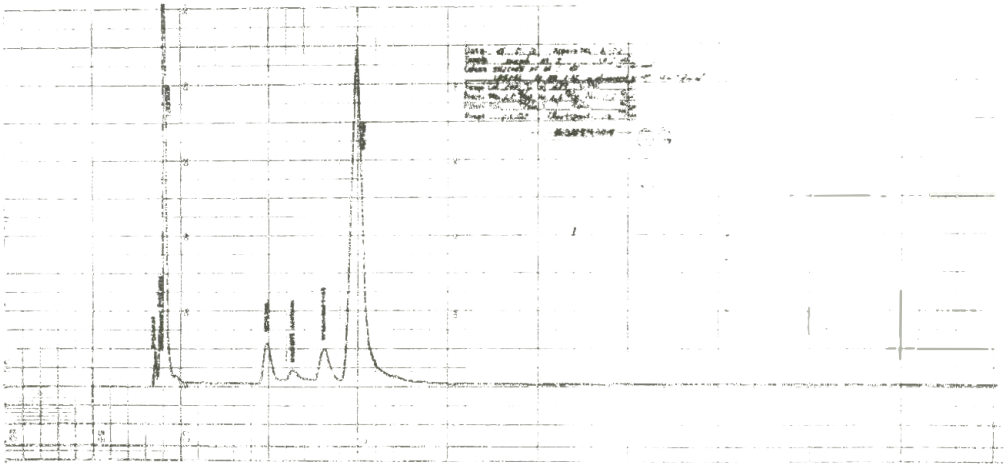
Mentha cardifolia Opiz (Philippine strain)
Oil from younger vegetative tops
Field-grown
July 1970.

Slide 30 — Gas chromatogram of oil from nonirradiated yerba buena.

Slide 31 — On an air-dry weight basis, the oil yield from vegetative tops of plants grown in the field from irradiated seeds was 0.75 to 1.31% while during the same period, the oil yield from vegetative tops of nonirradiated plants was 1.18 to 2.32% (Cantoria, 1972). The oil from the irradiated mutants resembles the oil from one of the parents, *M. spicata*, in that carvone is present and piperitenone oxide is absent (Handa *et al.*, 1964), although piperitenone oxide-containing *M. spicata* has been reported (Shimizu and Ikeda, 1961).



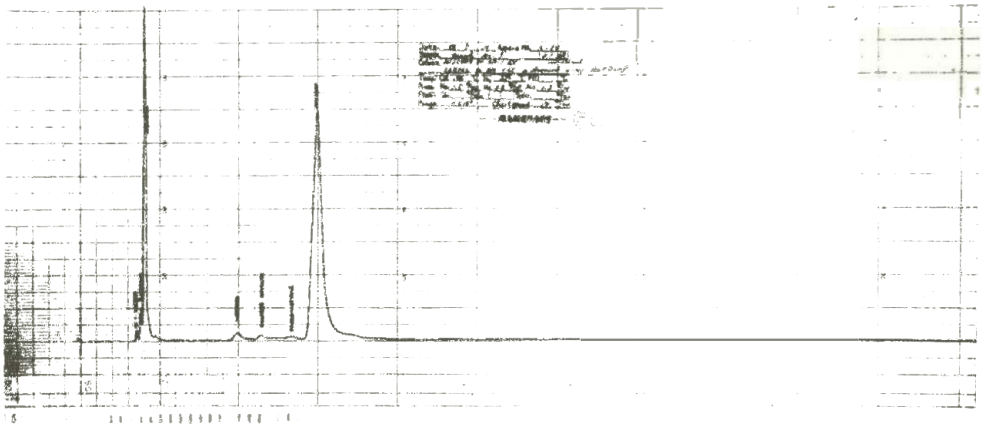
Mentha cardifolia Opiz (American strain)
Oil from younger vegetative tops
Greenhouse grown



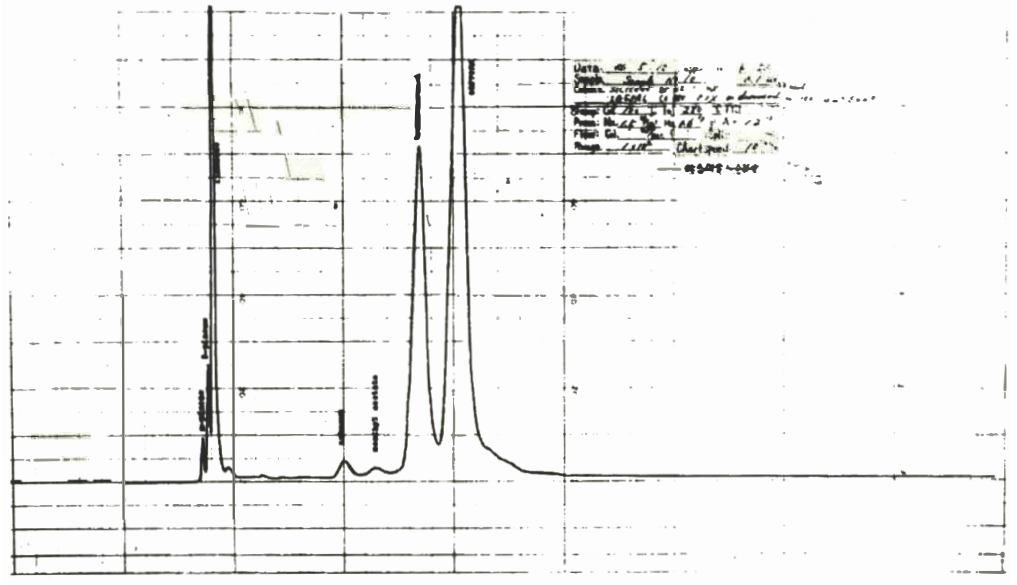
Plant No. 2 from irradiated seeds of Mentha cordifolia Opiz
Oil from vegetative younger tops
Field grown
April 1970

Slide 32 — Gas chromatogram of oil from Plant No. 2 grown from irradiated seeds of yerba buena, April, 1970.

Slide 33 — Analysis of the oil from the plants grown from irradiated seeds of yerba buena shows that piperitenone oxide, menthone, and menthol are absent but carvone and *trans*-carveol are present. The ratio carvone:*trans-carveol* is highly variable (Cantoria and Vargas, 1972). The degree of irradiation and the age of the plant appear to affect this ratio.



Plant No 2 grown from irradiated seeds of Mentha cordifolia Opiz
Oil from younger vegetative tops
Field grown
May 1970



Plant No. 2 grown from irradiated seeds of Mentha cordifolia Opiz
Oil from younger vegetative tops
Field grown
July 1970

Slide 34 — Gas chromatogram of oil from Plant No. 2 grown from irradiated seeds of yerba buena, July, 1970.

Menthyl acetate previously found to be present in flowering tops and absent in vegetative tops of nonirradiated yerba buena is present in the oil from plants grown from irradiated seeds (Cantoria and Vargas, 1972). In both oils, menthyl acetate is a minor constituent. It would appear that the synthesis of menthyl acetate does not involve menthone or menthol in the plant from irradiated seeds.

Slide 35a — Twenty-one introduced plants representing six species, four hybrids, and two varieties were grown. An artificial

AN ARTIFICIAL KEY TO THE *MENTHA* SPECIES IN THE U.P. PHARMACOGNOSY GARDEN
(After Clapham et al., 1952)

1.	Leaves less than 1 cm long.	1. *pulegium* L.
	Leaves more than 1 cm long.	2
2.	Flowers in cymose or subglobose clusters, all axillary.	3
	Flowers in terminal crowded or interrupted spikes or subcapitate inflorescences, with or without some clusters in the upper axils.	5
3.	Calyx-teeth scarcely longer than broad; stamens normally exserted.	4
	Calyx-teeth much longer than broad; stamens included.	x *gentilis* L.
4.	Leaves ovate, less than 2 cm long, very shallowly serrate; stems hairy in the angles.	2. *arvensis* L.
	Leaves ovate to lanceolate, more than 2 cm long, serrate; stems very faintly hairy.	*arvensis* L. var. *piperascens* Malinvaud
5.	Flowers aggregated into terminal capitate spikes, often with	

axillary clusters below. 6
Flowers in interrupted spikes. 7

6. Pedicels and calyx hairy; leaves hairy; flowers in globose or subglobose spikes; stamens exserted. 3. *aquatica* L.
Pedicels and calyx glabrous or nearly so; leaves thinly hairy; flowers in dense capitate to ovoid spikes; stamens included. x *piperita* L. var. *citrata* (Ehrh.) Briq.

7. Leaves definitely petioled; spikes at first ovoid, lengthening, and becoming loose. x *piperita* L.
Leaves sessile or subsessile (petioles less than 3 mm); spikes slender and leafless. 8

8. Pedicels and calyx-tube glabrous; leaves glabrous or very thinly hairy. 9
Pedicels and calyx-tube hairy; leaves densely hairy at least below. 10

9. Leaves more than twice as long as broad, glabrous or nearly so. 4. *spicata* L.
Leaves less than twice as long as broad, rugose. x *cordifolia* Opiz

10. Leaves lanceolate, not or scarcely rugose. 5. *longifolia* (L.) Huds.
Leaves oblong, ovate, or suborbicular, rugose. 11

11. Leaves never suborbicular, cuspidate or acuminate, varying from green and hairy to white-tomentose below, serrate. x *niliaca* Jacq.
Leaves often suborbicular, rounded at apex or minutely cuspidate, densely white-tomentose below, crenate or dentate. 6. *rotundifolia* (L.) Huds.

In this key, in addition to the six species, four hybrids and two varieties are included. *M. gentilis* L. is a hybrid of *M. arvensis* x *spicata*, *M. piperita* L. is a hybrid of *M. aquatica* x *spicata*, *M. cordifolia* Opiz is a hybrid of *M. rotundifolia* x *spicata*, and *M. niliaca* is a hybrid of *M. longifolia* x *rotundifolia*. *M. arvensis* L. is complicatedly variable; several varieties and forms exist. The Wisconsin and Alaska strains do not grow well in the Philippines while Japanese

taxonomic key was prepared on the basis of the characteristics actually observed. (Cantoria, 1977).

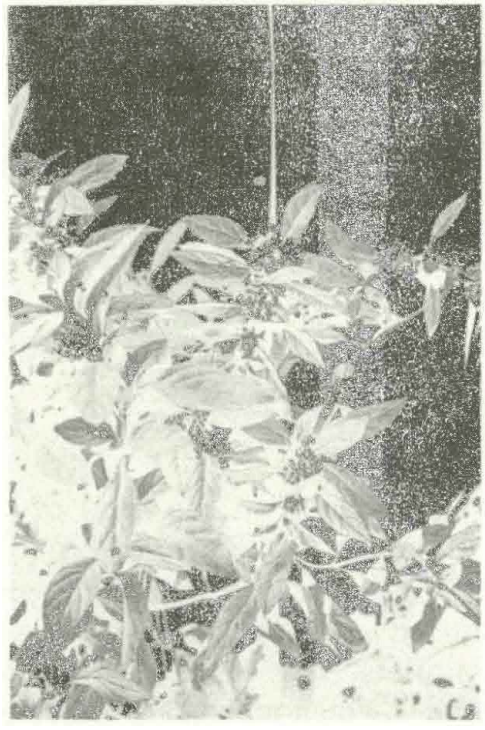
The family Labiatae is characterized by consisting mostly of herbs with four-angled stems, opposite leaves, bilabiate flowers, and fruit of four nutlets. The genus *Mentha* is distinguished by possessing four stamens which are usually exserted.

In the key prepared, the following characteristics were considered: (a) size of leaves, (b) location of inflorescence, (c) shape of calyx, (d) shape and margin of leaves, (e) degree of pubescence, (f) type of inflorescence, and (g) length of petiole.

Slide 35b — Lower half of taxonomic key.

Slide 36 — Among these introduced plants, the best growth was shown by Japanese mint or *M. arvensis* L. var. *piperascens* Malinvaud, the source of *l-menthol.*

Slide 37 — The flowers are in cymose clusters borne in the axils of leaves.



Slide 38 — Field studies were conducted on the oil yield of the plant under two levels of fertilizer and two levels of watering.[1] The plant thrives in Quezon City in well-watered soil during the months of September to April (dry season). Under the watering

Table 1. Dry weight production and oil yield of *Mentha arvensis* L. var. *piperascens* Malinvaud under two levels of fertilizer and two levels of watering*.

	1975						1976					
	W_1F_0	W_1F_1	W_1F_2	W_2F_0	W_2F_1	W_2F_2	W_1F_0	W_1F_1	W_1F_2	W_2F_0	W_2F_1	W_2F_2
Dry weight of 100 tops (g)												
Young tops	59.06	49.01	63.21	50.99	58.00	59.89	19.33	20.61	20.37	20.51	23.09	19.04
Old tops	96.78	99.74	106.54	85.95	92.83	90.31	27.70	29.02	30.19	24.90	28.10	24.67
Oil yield (% moisture-free weight												
Young tops	4.33	5.01	4.62	4.09	4.55	4.60	4.66	4.82	4.63	4.44	4.36	4.84
Old tops	3.33	3.91	3.15	3.37	3.92	4.19	4.12	4.27	4.30	3.91	3.91	4.31

*Means of six trials

W_1 6 to 9 gal/plot of 9 sq m

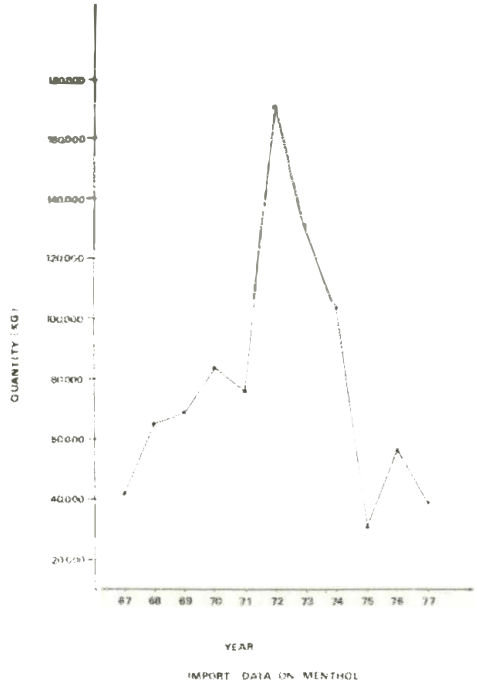
W_2 12 to 15 gal/plot of 9 sq m

F_0 0 g/plot of 9 sq m

F_1 60 g/plot of 9 sq m

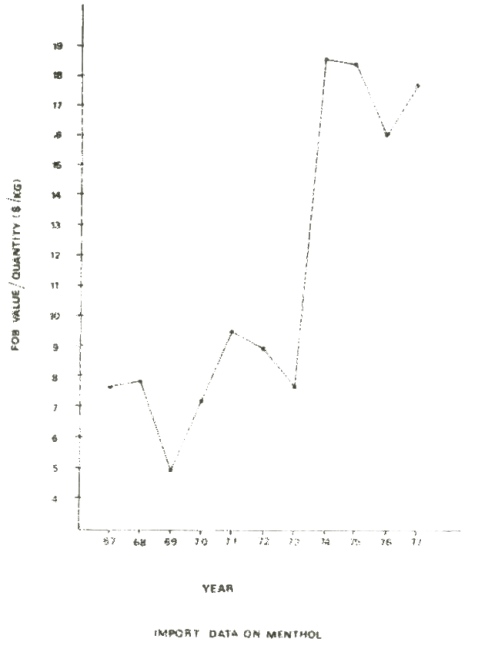
F_2 120 g/plot of 9 sq m

and fertilizing conditions employed, oil yield from young tops ranges from 4.09 to 5.01% and from older tops, 3.33 to 4.31% on a moisture-free basis. Differences in oil yield in response to the cultivation treatments were not significant except for the greater oil yield of old tops in response to the higher fertilizer level of 120 g per $9m^2$ than to the lower level of 60 g per $9m^2$. Total dry weight production of tops was 2.1 to 2.6 kg per 9 m^2.



Slide 39. — Graphs of importation figures of menthol into the Philippines during the period 1975-77 based on quantity in kg.

Slide 40 — The graph based on FOB value/quantity ($/kg)^2 show a general increase in FOB value although there was a general



decrease in quantity from 1972 to 1977. The principal sources are Brazil, Japan, and the United States.

The major domestic menthol-consuming industries are the (a) pharmaceutical, (b) cigar and cigarette, (c) confectionery and candy-making, and (d) flavoring industries.

A market study[3] shows that there is no local *l*-menthol production so that a virtually competition-free market exists. A plant has been designed to produce *l*-menthol from Japanese mint which will have a capacity of 200,000 lb/year. It is to operate 24 hours a day for 300 days a year, requiring a fixed capital of ₱5,634,369. With a unit cost of ₱160 per 300 grams and an added income from livestock feed (the cooked leaves) of ₱1.00 per kg, total annual sales will amount to ₱57,086,246. Annual return of investment is 76.5% and pay-out time is one year. The project appears economically feasible, but a more thorough analysis is needed.

Summary

Slide 41 — 1. Yerba buena is identified as *Mentha cordifolia* Opiz. It is a long-day plant, developing flowers on a terminal slender spike which branches below. The principal component of the oil of the Philippine strain is piperitenone oxide which is characteristic of the oil of *M. rotundifolia* L. Huds., one of the parents of *M. cordifolia*. The American strain resembles the Philippine strain in appearance but the principal component of the oil is carvone, characteristic of *M. spicata* L., the other parent of *M. cordifolia*.

Slide 42 — 2. Polios is *Mentha arvensis* L. and is possibly an F_1 cross between *M. aquatica* L. x wild-type *M. arvensis.* It belongs to the high pulegone chemotype of *M. arvensis*, which is a highly variable species with a wide distribution. Further genetic studies are needed to establish the relationships of polios and its variety and form have to be determined.

3. In the identification of Mentha species, morphological characteristics have to be considered together with genetic and chemical studies. This is usual in plants which hybridize readily or which have unstable chromosomes.

4. Studies of the genetic control of mint monoterpenoid biosynthesis have both scientific and commercial values. There is considerable interest in the chemosystematics of *Mentha.* Developmental patterns in the biosynthesis of the components of the oil are important in the peppermint and spearmint oil industries.

Slide 43 — Japanese mint, *Mentha arvensis* L. var. *piperascens* Malinvaud, the source of *l*-menthol, grows well in the Philippines. Considering the domestic market for *l*-menthol, the development of a menthol-producing industry appears economically feasible.

Acknowledgements

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A.M. Todd Company, Kalamazoo, Michigan, and of Dr. Akira Komatsu, director of research of the Takasago Perfurmery Co., Ltd. of Japan are gratefully acknowledged. The gas chromatography work in these studies was done in the laboratory of Dr. Komatsu with the assistance of Dr. Shigeru Muraki. The guidance of Dr. Mariano D. de Ramos, Professor of Statistics UPLB, in the statistical design and analysis of data is deeply appreciated. The author thanks Dr. Donoso B. Mabasa, Mrs. Ma. Vicenta C. Gacutan, Miss Nerissa S. Vargas, Dr. Florian M. Orejana, and Miss Gloria R. Querijero for their able technical assistance. The photographs and slides were very kindly prepared by Mr. Honorio T. Palarca.

References

1. Banthorpe, D. V., and B. V. Charlwood. 1972. Biogenesis of terpenes. In: Newman, A. A., ed., Chemistry of Terpenes and Terpenoids, Academic Press, London and New York. Pp. 337-411.

2. Burkill, I. H. 1935. A Dictionary of the Economic Products of the Malay Peninsula, Vol. 2, Published on behalf of the Governments of the Straits Settlements and Federated Malay States by the Crown Agents for the Colonies, London.

3. Cantoria, Magdalena. 1970. Physiological and chemical studies for the identification of Philippine mint. *Philippine J. Sci.* 97:277-87.

4. Cantoria, Magdalena. 1972. Research on mint in the Philippines. *Anais da Academia Brasiloira de Ciencias* 44 *(Suppl.)*: 64-7.

5. Cantoria, Magdalena. 1974. Studies on the physiology of Philippine mint *(Mentha cordifolia* Opiz.). III. Variation in oil yield. *Philippine J. Sci.* 103: 67-7.

6. Cantoria, Magdalena C. 1977. Pharmacognosy in Action. National Research Council of the Philippines, Bicutan, Tagig, Metro Manila.

7. Cantoria, Magdalena, and Ma. Vicenta T. Cuevas-Gacutan. 1974. Studies on the physiology of Philippine mint *(Mentha cordifolia* Opiz). II. Effect of two different light intensities on the vegetative growth and oil yield. *Philippine J. Sci.* 103: 13-9.

8. Cantoria, Magdalena, and D. B. Mabasa. 1965. Studies on the physiology of *Mentha arvensis* L. I. Effect of two different daylengths on the vegetative growth. *Plant and Cell Physiol.* 6:561-4.

9. Cantoria, Magdalena, and Nerissa S. Vargas. 1972. The chemical constituents of the volatile oils from irradiated and nonirradiated Philippine mint plants. *J. Philippine Pharm. Assoc.* 58: 38.

10. Clapham, A. R., T. G. Tutin, and E. F. Warburg. 1952. Flora of the British Isles. The University Press, Cambridge.

11. Gill, L. S., B. M. Lawrence, and J. K. Morton. 1973. Variation in *Mentha arvensis* L. (Labiatae). I. The North American populations. *Bot. J. Linn. Soc.* 67: 213-32.

12. Handa, K. L., . M. Smith, I. C. Nigam, and L. Levi. 1964. Essential oils and their constituents. XXIII. Chemotaxonomy of the genus *Mentha. J. Pharm. Sci.* 53:1407-9.

13. Hefendehl, F. W., E. W. Underhill, and E. von Rudloff. 1967. The biosynthesis of the oxygenated monoterpenes in mint. *Phytochemistry.* 6:823-35.

14. Landing, J. E. 1969. American Essence. A. M. Todd Foundation, Kalamazoo, Michigan.

15. Lincoln, D. E., and M. J. Murray. 1978. Monogenic basis for reduction of (+)-pulegone to (—)-menthone in *Mentha* oil biogenesis. *Phytochemistry* 17: 1727-30.

16. Loomis, W. D. 1967. Biosynthesis and metabolism of monoterpenes. In: Pridham, J. B., ed., Terpenoids in Plants, Academic Press, London and New York. Pp. 59-82.

17. Merrill, E. D. 1923. An Enumeration of Philippine Flowering Plants. Vol. 3. Bureau of Printing, Manila.

18. Reitsema, R. H. 1956. A new ketone from oil of *Mentha rotundifolia*. *J. Am. Chem. Soc.* 78: 5022-5.

19. Reitsema, R. H. 1958. A biogenetic arrangement of mint species. *J. Am. Pharm. Assoc., Scient. Edn.* 47: 267-9.

20. Shimizu, S. 1956. Studies of the essential oil of *Mentha rotundifolia*. Part I. Isolation of rotundifolone, a new terpenic ketone. *Bull Agr. Chem. Soc. Japan* 20:84-8.

21. Shimizu, S., and M. Ikeda. 1961. Isolation of (+)-piperitenone oxide or (−)-piperitone oxide from *Mentha spicata* L. with 24 chromosomes in the somatic cells. *Perfumery and Essent. Oil Record 52: 708-13.*

22. Stewart, Sara R. 1944. *Mentha arvensis* and some of its North American variations. *Rhodora* 46: 331-5.

23. United States Pharmacopeial Convention, Inc. 1980. The United States Pharmacopeia, Twentieth Revision, and the National Formulary, Fifteenth Edition. The USP Convention, Inc., Rockville, Md.

William Padolina, Ph.D., Discussant

I would like to give my compliments to the two papers that had been delivered this afternoon. They are prime arguments against brain-drain. We see examples of two dedicated scientists which have been able to do their work in the Philippines in spite of all the claims of those people who live and say that they can not do the work here.

Secondly, I like to cite also the work of Dr. Cantoria. If you will examine the way she presented it, you will see the interdisciplinary nature of the work. It involves the knowledge of botany, chemistry and I think that is the trend that we should follow at this time. We can do much if we struggle in the two fields because there is a lot of information that we can gain in both, in this type of studies. I would like to confine my comments on one portion of her work and that is on the keymost systematics portion because I think that is were I am more able to make my comments.

As you know the area of keymost systematics or more acceptably biochemical systematics was developed only during the early fifties (50's) by Alston and Cornell and also Smith and Levin and they used as their keymost systematic markers, the flavonoids. Now, their studv was quite crude but they were able to show that the flavonoids from the parents would be inherited in the hybrids either through qualitative or quantitative complementation. In other words, let us say you have flavonoids, A, B and C in one parent and C, D and E in other parent, the hybrid will contain A,B,C and E. Some work in this one like what was done on volatile terpenes and this involves the genus Idioma. And this kind of inheritance where you have qualitative or quantitative complementation was tested quantitatively by Meroff, Hanover and Zabarin. They found that if you get the parents of Hideyoma and hybridize them, you find that the hybrids although varying in fertility, display a complete complementary or additive inheritance pattern of the parental monoterpene products. In other words if one finds terpenes A, B and C in one parent and C, D, E and F in the other parent, the hybrid contains the monoterpene A to F. And along

this line I'd like to point out that in one of the slides of Dr. Cantoria, she showed that the chemotype of *Mentha cordifolia* from the United States has a different. . . I think carbon is the main terpene there, while the Yerba Buena that we get here has piperitenone oxide as the main terpene. But we do not find the piperitenone oxide in *Mentha cordifolia* from the States. I don't know if my interpretation of the slide was correct. I think it is quite difficult for me to accept if we follow the pattern of complementation of inheritance because they should have the same complement of terpene but they can have varying quantities of the terpenoids expressed.

Another interesting observation that she has pointed out is in the case again of the *Mentha cordifolia* US chemotype and *Mentha cordifolia* (yerba buena) Philippine chemotype. Although, they appear the same morphologically, the terpene pattern are different and we can see here what they termed as the independent segregation of morphology and terpene expression. I should like to point out also that in the biogenetic trends that she has proposed for the chemosystematics of Mentha species she is proposing that the more mature species seem to have reduced functionalities like when we go from menthone to menthol where the ketone was reduced to an alcohol. This seems to be a very interesting observation because we find that also in other plant species. If you look at the chemistry of the primitive species and the more advance species, you find that the primitive species are more oxygenated than the more advanced species.

The last comment I like to make is an appeal that we intensify our study on not only the Mentha but all the spice plants in the Philippines. We need to know their chemistry because I am sure we are being short-changed by people who are importing them. I, for one, have directly experienced that many of the perfume companies in Europe are now looking for new essences. And they are not looking at the major components, they are looking at the minor components because these are the things we can not extract and they duplicate it by synthesis and they sell it to us as a new essence and the rich people always want to smell different from the other. And they make a premium out of it. Not only that, I think these are also important in studies for breeding and improvement of spice plants, spice and flavor plants that we have. That is all that I like to say.

Adoracion T. Aranez, Ph. D., Discussant

The paper of Dr. M. Cantoria is multidisciplinary in scope. It includes taxonomy, morphology, genetics, physiology, and biochemistry. The writer has not only studied the physiology and biochemistry of the volatile oils of *Mentha* species, but also has elucidated the conflict in the true identity of the Philippine *Mentha*. Through her knowledge of physiology, she is able to induce flowering in yerba buena which usually remain vegetative under ordinary Philippine conditions. The structure of the flower is one of the most dependable parameters in the identification of flowering plants. The main composition of the volatile oils determined by biochemical processes, confirms the identification made by using floral structure.

Dr. Cantoria mentioned that yerba buena is *Mentha cordifolia* Opiz, a result of a cross between *M. rotundifolia* (L.) Huds. and *M. spicata* L. The Philippine strain of *Mentha cordifolia* has piperitenone oxide as the principal constituent resembling that of *M. rotundifolia* parent, while the American strain has carvone as the main component of the volatile oil which is similar to that of *M. spicata* parent. However, the two strains when grown side by side cannot be distinguished from each other. Mutation which affects the gene or genes that are involved in the biosynthesis of volatile oil in one of the strains must have taken place. The hypothesis that mutation is the one responsible for the difference in the composition of volatile oil of the Philippine and the American strains is supported by the predominance of carvone and trans-carveol in the volatile oil of the mutant yerba buena plant grown from irradiated seeds.

Seeds of hybrids exposed to radiation give rise to plants resembling the parent species. When seeds of yerba buena were subjected to radiation plants with leaves resembling *M. rotundifolia* in shape are produced. Oil analysis from the plants grown from irradiated seeds shows carvone and trans-carveol as main components and in this respect, the irradiated progeny resembles *M. spicata*. The untreated yerba buena has leaves that are similar to *M. spicata* and oil that is similar to *M. rotundifolia* which is piperi-

tenone. The character manifested by the hybrid is usually controlled by the dominant gene. When the hybrid is irradiated, it is possible that the dominant allele may be converted to a recessive gene, thus the plant will become homozygous for this gene and its phenotypic effect will be manifested.

Hermes Gutierrez, M.S., Discussant

I would like to thank Dr. Cantoria for having done this work and my part here is to discuss in a limited way the taxonomy and systematics of the Mentha group.

The work of the biochemist or the phytochemist in particular has always been of great help to plant chemotaxonomists in delineating the position of the species through the use of known chemical components found to occur among the individuals. It is also being widely used by Plant taxonomists/systematists, who are basically dependent on morphological characteristics, as an added information to support the authenticity of the identity of the species especially when the delineation of such species becomes complicated and difficult in terms of gross morphology.

The paper presented to us, while it is primarily aimed at elucidating the physiology and biochemistry of the volatile oils or essential oils of *Mentha* species of the family *Labiatae* introduced to and grown in the Philippines, also attempts to determine the correct identity of these species by using and interpreting the results of this research work.

It might be worthwhile to mention at the outset, that the taxonomy and systematics of the genus *Mentha* (after the Greek name of the nymph menthe) is indeed complicatedly difficult because the component species hybridizes readily in nature producing enormous intergrading forms. So much so that there are more than 1,067 scientific names attributed to the group of plants of which only 25 to 30 species are recognized as valid. The species of *Mentha Arvensis* L. described by Linnaeus in 1753, in his Species Plantarum, has been re-described and renamed by botanists for more than 124 times. All these names, however, have been invalidated through the efforts of modern day taxonomists/systematists, assisted in part by the works of phytochemist and other branches of botanical endeavor, in delimiting the position of the species.

Of these 25 to 30 or so species, only 4 species, 1 being represented by a varietal form, have found a place of great economic importance to man, they are namely:

1. *Mentha piperita* L., commonly known as Peppermint, extensively used in confectionary and in medicine for the production of menthol or more properly pipmenthol.
2. *Mentha spicata* L., or Spearmint, also extensively used in medicine, flavoring, in making the seductive and intoxicating drink known as "mint julep" and as an ingredient of the famous American "mint sauce."
3. *Mentha aquatica* L., or Bergamot Mint, from which the fragrant lemon-scented Bergamot oil is distilled for use in making perfume. *Mentha citrata* Ehr. is considered a synonym of this species.
4. *Mentha arvensis* L. var. piperascens Malinvaud, or Japanese Mint, although the oil is inferior to that of *Mentha piperita* L., it is reported to contain a higher percentage of crystalizable menthol. It is extensively cultivated in Japan for commerial purposes.

Of lesser importance but equally useful to man, particularly among Asians are:

1. *Mentha arvensis* L., or Field Mint or Corn Mint, getting its latter name from the Irish's practice of placing cut sprigs of the plant in corn stacks to keep away the mice. The former is from the Greek word "arvens" for "arable field" or "cultivated land." Among Asians it is used in folk or traditional medicine as carminative, expectorant, emmenagogue, tonic to the kidney, diseases of the liver and spleen, asthma, pains in the joints, refrigerant, stomachic, diuretic, sudorific, antispasmodic, diaphorecti, anti-pyretic, indigestion, cephalalgia, and the juice of the leaves for the sting or bites of poisonous insects.
2. *Mentha rotundifolia* L. or Apple Mint or Wooly Mint which also contains a similar oil, but not as much, and not as extensively used as *Mentha piperita* Linn.
3. *Mentha pulegium* L. known as Pennyroyal or European Pennyroyal as differentiated from American Pennyroyal which is *Hedeoma pulegioides* producing an oil similar to this species. Among the *Menthas*, it is the only species whose essential oil contains toxic constituents. It is reported to cause violent irritation of the entire gastro-intestinal tract with consequent pain, vomitting and diarrhea. It is stated further that the

hyperaemia may spread to the peritonium and to all neighboring parts, among others, in the female, the genital organs, where the congestion may find expression in hemorrhage and abortion. It is sometimes used, therefore, as an emmenagogue and as an abortifacient.

Going back, therefore, to what has been presented to us, we find nothing to cause us to differ as far as the physiological and biochemical studies of the different species are concerned. It has also provided us, plant taxonomists/systematists, a better picture and understanding of the *Mentha* species grown in our country. However, we find a number of problems that have to be resolved botanically that has resulted from this work in relation to our knowledge and observation with this particular group of plants.

Based from the report, and considering the widely acknowledged taxonomic complexity of the group but which is otherwise classifiable, the common name "Yerba Buena" could have referred to more than one species or to any of such species growing in the country. The common name "Polius" in Bicol could also mean for the same "Yerba Buena," if at all there were two individuals which the Bicolanos could refer to separately.

But it is apparent from the report that there are two natural species that have been growing in our. country (there could be more) whose identity were never exactly known until they were made to flower in the laboratory.

One of the species belongs to the Piperita group supposed to be represented by *Mentha rotundifolia* L. whose flowers are in terminal crowded spikes, while the other belongs to the Arvensis group represented by *Mentha arvensis* L. whose flowers are subglobose clusters, borne on the axils of the leaves. The latter species, however, which was never known to flower under Philippine climatic conditions have been found flowering naturally in Parañaque.

The species *Mentha cordifolia* Opiz which is known to be a hybrid between *Mentha rotundifolia* L. "Apple Mint" and *Mentha spicata* L., "Spearmint", which was only recently mentioned in the Literature (Clapham, et al. 1952, Flora of the British Isles) could not possibly be the "Yerba Buena" that has been spoken of and introduced to the country 400 to 500 hundred years ago. Furthermore, a known hybrid does not take precedence nor is ever used taxonomically for interpreting and delimiting the position of natural species such as *Mentha rotundifolia* L. and *Mentha arvensis* L. as it is in this paper being considered at hand.

Mentha cordifolia Opiz, therefore, remains as a hybrid recently introduced to the Philippines which resembles one of its parents, that is *Mentha rotundifolia* L.

Whichever way we use the common names, whether we refer to one or to many species, the *Mentha rotundifolia* L. and *Mentha arvensis* L. as accepted natural species will remain as such.

While it is true that we have to consider the morphological characteristics, the genetics, the chemical constituents of the plants for the proper identification of the *Mentha* species, the proper interpretation and the exact delimitation of the species may find the physiologist, the phytochemist and the plant taxonomists/systematist a reason to agree or to disagree.

While this may be true, as in all scientific endeavor, we cannot deny the fact that the work of Dr. Cantoria has brought us closer to the truth. This information and other data from other areas, such as cytology, genetics, etc. can provide a solid foundation for taxonomic/systematic decisions.

We can postpone this decision, but we can no longer postpone our decision to produce tangible, ordinary, day-to-day necessities capable of easing the tremendous cost-of-living burdens of the masses of our people. The members of the National Academy of Science and Technology must assert its superiority in knowledge and influence, not only as Academicians, but also, if I may say, as "technologicians."

SUNFLOWER RESEARCH IN THE PHILIPPINES

By Filomena Campos, Ph.D.

Sunflower (*Helianthus annus* L.) is a member of the family Compositae. The inflorescence is a head in which the disk flowers upon fertilization bear the oil seed. The main product derived from the seeds is an edible oil of superior quality highly poly-unsaturated, relatively cholesterol free and contains no trypsin-inhibitory factors.

In terms of its production potential in relation to other oil crops, it is a higher yielder per unit area per unit time. The oil content ranges from 33-45 percent by weight depending on the variety, the biotic and agro-climatic conditions prevailing in the production area.

Aside from the oil, the meal after oil-extraction has been used for human food and to a great extent as animal feed. Other by-products such as chip-boards from the stalks, green fodder for animal feed and certain chemical extracts have been derived from various parts of the plant.

Research on sunflower in the Philippines has been motivated by the Philippine Refining Co. some nine (9) years ago where it tapped Central Luzon State University to spearhead the development of a package of production technology in order to introduce sunflower in the mainstream of Philippine agriculture.

An integrated and multidisciplinary research program "Sunflower Breeding and Production Under Tropical Conditions" was submitted by Central Luzon State University to the NSDB which was immediately approved and funded.

This paper highlights the significant research accomplishments; discuss the major problems and constraints apertain to the development of the technology pack and its adoption and focus attention on the research thrust for the immediate future.

A. *Major Areas of Research*

It is worth emphasizing that the research program was multidisciplinary in approach and total integration of research results was the target concern.

Listed herewith are the project/studies undertaken over a period of nine years.

A. Sunflower Researches Conducted at Central Luzon State University through NSDB Funding, October 1971 to December 1979.

Title	*CY Conducted*
I. Varietal Improvements	
1.1 Introduction and Maintenance of Sunflower Varieties	1971-1972
1.2 Trial Planting of Different Varieties of Sunflower Under CLSU Condition	1971-1972
1.3 Performance of Different Lines Within Varieties of Sunflower	1971-1972
1.4 Varietal Evaluation and Maintenance of Sunflower Varieties	1972-1979
1.5 The Use of Ethrel in the Induction of Male Sterility in Sunflower	1973
II. Cultural Management	
2.1 Planting Density	
2.1.1 Interaction of Plant Population, Time and Rate of Nitrogen Fertilization on the Yield of Sunflower	July-Sept. 1971
2.1.2 Variation in the Response to Planting Density in Sunflower	Jan.-March 1972
2.1.3 Interaction of Distance of Planting and Number of Plants Per Hill on the Growth and Yield of Large Seeded Variety of Sunflower	Nov. 1971-Feb. 1972
2.1.4 Response of Sunflower to Different Row and Hill Spacings	1974
2.1.5 Interaction of Seeding Rate Per Hill and Levels of Nitrogen	1974
2.1.6 Effect of Different Rates of Seeding and Distance Between Hills on the Growth and Yield of Sunflower	1974-1975
2.1.7 Seedset in Sunflower as Affected by Agents of	

	Pollination and Time of Planting	1975-1976
2.2	Fertilizer Studies	
2.2.1	Sunflower Seed Production as Influenced by Levels of Fertilizer and Plant Population	Feb.-May 1971
2.2.2	Effect of Different Grades of Fertilizer on Sunflower	Nov. 1971-June 1972
2.2.3	Response of Sunflower to Different Levels of N, P, K	Nov. 1971-June 1972
2.2.4	Response of Sunflower to Various Sources and Rates of Fertilizer	June-April 1972
2.2.5	Rate and Time of Nitrogen Application on Sunflower	Oct. 1972-Jan. 1973
2.2.6	Yield Performance of Sunflower on Different Levels of Nitrogen Fertilizer	Oct. 1972-Jan. 1973
2.2.7	Effect of P and N with Constant K on Sunflower	Oct. 1972-Jan. 1973
2.2.8	Response of Sunflower to different grades of P and K with Fixed N	Oct. 1972-Jan. 1973
2.2.9	Response of Sunflower to Different Levels of Nitrogen	Oct. 1972-Jan. 1973
2.2.10	Response of Sunflower to Plant Density and Fertilizer on Cultivated and Uncultivated Riceland	Jan.-April 1973
2.2.11	Effect of Different Levels of Complete Fertilizer	1974
2.2.12	Effect of Different Sources and Levels of N	1974
2.2.13	Time and Method of Nitrogen Fertilizer Application	1974

2.2.14	Response of Sunflower to Different Grades of Liquid Fertilizer	1974
2.2.15	Effect of Varying Levels of Urea as Liquid or in Granular Form on the Growth and Yield of Sunflower	1974-1975
2.2.16	Study on the Use of Chicken Manure and Sagana 100 as Supplement to Inorganic Nitrogen in Sunflower Production	1975-1976
2.2.17	Response of Sunflower to Different Level Combination of Nitrogen and Phosphorus	1975-1976
2.2.18	Effect of Nitrogen from Vitasoil and Urea on the Yield of Sunflower	1975-1976
2.2.19	Effect of Liquid Fertilizer Combined with Insecticide on Sunflower Production	1975-1976
2.2.20	Effect on Cytozyme's Crop on the Growth and Yield of Sunflower	1979
2.3	Cultural Management	
2.3.1	Response of Different Time of Cultivation and Method of Fertilizer Application	1972
2.3.2	Growth and Yield Performance as Affected by Five Practices of Land Preparation Using Hand Tractors	1975
2.4	Irrigation Studies	
2.4.1	Preliminary Study on the Effect of Different Systems of Planting and Irrigation on the Growth and Yield of Sunflower	1974-1975
2.4.2	Consumptive Use of Water by Sunflower	Nov. 1971-Feb. 1972
2.4.3	Irrigation Timing on Sunflower as Used on Available Soil Moisture	Nov. 1971-Feb. 1972

2.4.4	Study on the Proper Time of Terminating Irrigation on Sunflower	1973
2.4.5	Evapotranspiration of Sunflower as a Function of Climatic Factors, Soil Moisture Content and Plant Age	1975
2.4.6	Further Study on the Tolerance of Sunflower to Waterlogging	1975
2.4.7	The Effects of Different Irrigation Schemes and Methods of Land Preparation on the Growth and Yield of Sunflower	1976
2.4.8	The Viability of Sunflower Seeds as Affected by Different Water Management Practices, Initial Moisture Content and Storage	1976
2.4.9	Further Study on the effect of Different Systems of Planting and Irrigation on the Growth and Yield of Sunflower	1976

2.5 Cropping Systems

2.5.1	Trial Planting of Sunflower Under Dry and Wet Season	June-Aug. 1971 Jan.-April 1971
2.5.2	Monthly Planting of Sunflower Under CLSU Condition	Oct. 1972-Sept. 1973
2.5.3	Comparative Study of Three Legumes as Intercrop With Sunflower	1973-1974
2.5.4	Study on Intercropping and Mixed Cropping	1975
2.5.5	Feasibility Study of Intercropping Sunflower With Upland Rice	1974-1975
2.5.6	Study on Intercropping Sunflower With Peanut	1974-1975

Species Associated With Sunflower and Other Upland Crops	1974-1976
3.12 Effect of Weeding and Intercropping Within Rows on the Growth and Yield of Sunflower and Peanut	April-July 1975
3.13 Phenology and Life Span Observation of Weeds Associated with Peanut, Sunflower and Sesame	April-Aug. 1975
3.14 Effect of Duration of Weed Control on Growth and Yield of Sunflower	April-July 1975
3.15 Effect of the Duration of Weed Competition on the Growth and Yield of Sunflower	April-June 1975
3.16 Study on Weed Control	1975
3.17 Effect of Different Positions of Weeds on the Growth and Yield of Sunflower	April-July 1975
3.18 Fungicide Screening for the Control of Sunflower Wilt	1975-1976
3.19 Field Evaluation of Fluometuron for Weed Control of Sunflower	Nov. 1975-Feb. 1976
3.20 Field Evaluation of Diuron for Weed Control in Sunflower	Nov. 1975-Feb. 1976
3.21 Insecticide Recommendation for Sunflower Production	1976-1977
3.22 Comparison in Yield Between Sprayed and Unsprayed Area of Sunflower	Nov. 1976-May 1977
3.23 Screening of Different Insecticides for the Control of the Major Insect Pests of Sunflower Under Laboratory and Field Conditions	Nov. 1978-June 1979
3.24 The Biology and Population Dynamics of the Major Insect Pests of Sunflower Under Central Luzon Conditions	1979
3.25 Survey and Identification of Sunflower Diseases in Sunflower	1979

3.26 Leafhopper Damage Studies on Sunflower 1979

IV. Utilization

4.1 Sunflower Flour in Bread Rolls 1972
4.2 The Use of Sunflower Seed Flour in the Preparation of Bread Rolls 1972
4.3 Utilization of Unprocessed Sunflower Seed as Component of Broiler Ration (Starbro Strain) 1972
4.4 Performance of SCWL Cockerels Fed With Unprocessed Sunflower Seeds as Part of the Ration 1972
4.5 Effect of Sunflower Leaf Meal on the Performance of Broilers 1972
4.6 Comparative Study on Sunflower Seed and Sorghum Grain as Scratch Feed on SCWL Layers 1972
4.7 Effect of Varying Proportion of Soybean Oil Meal and Sunflower Seed on Weanling Rabbits 1972
4.8 Performance of Broiler Chicks (Arbor Acre Strain) With Commercial Mash Supplemented With Unprocessed Sunflower Seeds 1973
4.9 The Possibility of Utilizing Sunflower (*Helianthus annus* L.) as Source of Furfural 1975
4.10 Suitability of Sunflower Seed in the Preparation of Brittle 1976
4.11 Acceptability of Sunflower Seeds as a Substitute for Coffee 1976
4.12 Acceptability of Sunflower Butter Stabilized at Different Levels of Hydrogenated Fats 1976
4.13 Utilization of Sunflower Seeds in the Preparation of Kropeck 1976
4.14 The Use of Sunflower Seed Flour in the Preparation of Siopao 1976
4.15 Effect of Sunflower Seed Meal and Rice Bran as Concentrate Feeds on the Growth of Weanling Rabbits 1976
4.16 Sunflower Seed Flour in Butter Cake 1976

V. Processing

5.1 Design and Construction of an Engine Driven Sunflower Thresher 1972

5.2	Study on the Time of Harvesting As Affecting Seed Yield and Germination	1973
5.3	Design and Construction of a Self-Feeding Power Driven Sunflower Thresher	1973
5.4	Development for a Technology for Processing Sunflower Seeds As "Cracked Seeds."	1975

VI. Economics

6.1	Cost and Return Analysis Established that Sunflower can be Developed As One of the Major Cash Crop Which can Command Higher Returns Per Unit Area Per Unit Period of Time	1975
6.2	Establishment of the Profitability of Intercropping/Mixed Cropping Sunflower With Other Cash Crops	1976
6.3	Evaluating the Economic Feasibility of the Package of Technology on Sunflower Production Under Philippine Farm Conditions	1976
6.4	Cost and Returns Study of Sunflower Under Commercial Scale Operations	1976
6.5	Sunflower Production Under Different Levels of Input and Management	1979

B. Significant Accomplishments

1. Introduce sunflower in the stream of Philippine agriculture.
2. Developed a variety, CLSUN-1 which is comparable in yield to other foreign standard varieties; adopted to the country's agro-climatic conditions and relatively resistant to the major insect pests and diseases locally attacking sunflower.
3. Package of technology which is ready for adoption by farmers (Appendix A).
4. Identified the problems and constraints both technical and from the view point of economics.
5. Succeeded in developing a technology for utilization other than for oil. This is the product known as "Sunflower Crackseeds" which has been developed as a cottage-industry in some localities.

C. Problems/Constraints

1. The readiness among small farmers to adopt the sunflower production technology are constrained by:
   - 1.1 Ready market at a margin equal to other crops that the farmer grows.
   - 1.2 "The variability in yield (ranges from 750 kgs — 1,450 kgs) due to unreasonable use of insecticides for other crops. It is well to state that sunflower is a cross-pollinated crop which depends mainly on insects as pollinators. Indiscriminate use of insecticides in adjoining areas prejudices the yield of sunflower.
   - 1.3 There is need therefore to develop apiculture (bee-keeping) as an adjunct to sunflower production in order to insure high yields. However, apiculture is an added investment for a farmer which he might not be able to afford not to say that it needs less maintenance during off-production periods.
   - 1.4 Hidden apprehension among coconut producers that sunflower oil might compete with coconut oil.
   - 1.5 Availability of good-quality seeds for a relatively big area of production. There needs to encourage certified seeds producers to be involved in this concern.

D. Looking Forward

The CLSU sunflower researchers have now focused their attention to the following activities:

1. Pilot testing the package of technology in potential sunflower growing areas of the country.
2. Expand and continue research activities in varietal improvement, crop protection especially in screening pesticides which are effective but not detrimental to the beneficial insect pollinators.
3. Further refinement of the package of technology towards increasing yields to offset the increase in the cost of inputs.
4. Strengthen the research on cropping system as this is one method to increase farm income and also cushion the effect of crop loss.
5. Intensify research on apiculture as an adjunct to sunflower production.
6. Lobby to authorities concern for credit/market support for sunflower as part of the cropping system.

Acknowledgment

Due acknowledgment and appreciation are bestowed to: The National Science Development Board (NSDB) for the moral and financial support it has afforded the research program.

To the Philippine Refining Company (PRC) for challenging CLSU to undertake research on sunflower and to my dear colleagues in CLSU for their untiring efforts and commitment to sunflower research.

APPENDIX "A"

Input-Output Analysis of Sunflower Seed Production for One Hectare as of July 1980

Item of Expenditure	*Man-Hr/Hrs.*	*Value (₱)*	*Percent*
I. Non-Cash Cost			
A. Land rental (3-month period)		210.95	6.94
B. Interest on operating capital investment[1]		87.22	2.87
II. Cash-Cost			
A. Labor[2]			
1. Land Preparation			
a. plowing & harrowing	120	225.00	7.41
b. furrowing	25	46.87	1.54
2. Field Labor			
a. planting	63	118.12	3.89
b. thinning & replanting	63	118.12	3.89
c. fertilization	59	110.62	3.64
d. irrigation (4x, 7 water tenders)	103	193.12	6.36
e. 2x cultivation & weeding	50	93.75	3.09
f. spraying (3 men, at most 4x)	96	180.00	5.93
g. harvesting & shelling	66	123.75	4.07
B. Use of Machineries (hrs.)			
1. Use of 6" water pump at ₱8.50/in/hr.	4.07[3]	207.57	6.84
2. Use of 4" water pump at ₱6.50/in/hr.	15.87[3]	412.62	13.58
C. Production Inputs			
1. 12 kg sunflower seeds at ₱4.00/kg		48.00	1.58
2. Fertilizer (Urea 45% N) ₱94.00/bag		501.02	16.50
3. Insecticides			
a. Sevin 85 WP (0.3 box at ₱27.27/box)		8.18	0.3

b. Azodrin 168 (1.6 quart at ₱58.00/qrt)	92.80	3.05
c. Thiodan (5.8 qrt. at ₱44.63/qrt)	258.85	8.52
TOTAL	₱3,036.56	100.00

III. Net Returns

1,300 kgs. of seeds at ₱4.00/kg	₱5,200.00
Total cost of production	3,036.56
Net returns	₱2,163.44

IV. Net Return Per Peso Invested ₱ 0.79

Net income	2163.44
Cash expenses	2738.39

V. Net Return on Investment (ROI) Includes Non-Cash Costs ₱ 0.71

Net income	2163.44
Total expenses	3036.56

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[1]Computed at 12% per annum straight-line interest

[2]₱15.00 per 8-hr/day

[3]Computed based on 1977 figures:
a) Use of 6" water pump at ₱6.00/in/hr. at ₱146.66
b) Use of 4" water pump at ₱4.00/in/hr. at ₱254.00

Eduvigis Pantastico, Ph.D., Discussant

Thank you Dr. Velasquez and of hand I would like to congratulate the speaker, a former colleague in the Department of Botany at UP Los Baños.

I have three points to talk about the paper, I would like to look at sunflower as an example of the "brand" of CLSU. Second, I would like to take the sunflower as a biological material and therefore I'll talk about the physiological requirement. Thirdly, I look at the sunflower as discussed, that is, as a tool for development.

As a "brand" of CLSU I think this is one commodity among the others, we used to hear before of cotton, from CLSU. They did a lot of studies about cotton and now it is taken in by a corporation and it is an ongoing business in the country. Another is probably you have heard of rice-fish culture at CLSU and is being adopted again by the ministries and by other regions of the country, CLSU the rice-fish culture, inland fisheries and so on.

As a brand of CLSU, this crop commodity among others can be identified only with that University. The University tends to select only few commodities to study and making good at them. For example, their researchers have specialized in cotton, carabeef, rice-fish culture and inland fisheries. This kind of specialization may lead to agro-climatic zonification of growing crops in the country, if only other agricultural universities and research agencies will follow the same type of approach.

Sunflower as a biological material. Sunflower belongs to the C_3 — pathway of photosynthesis and two groups are known to exist: one, requiring low temperature (15-20°C) for maximum photosynthesis and the second, requiring high temperature (25-30°C) for maximum photosynthesis. For our purpose, the high temperature requiring varieties of sunflower should be preferred, particularly in the breeding program. Besides, the high temperature requiring varieties can also convert higher amounts of CO_2 into dry matter than the low temperature requiring ones.

Sunflower as a tool for development. As yet, sunflower industry is not existing in the country. But we can consider this crop

as one with potential, in similar category as winged bean and apple. All these crops can be the future tools for economic development.

I would like to cite at this point what happened in cotton. CLSU did a lot of studies in cotton and now cotton production is an ongoing industry in the country. Whether or not the same will happen to sunflower, I don't know. It may be used as feeds, oil for industry or for its aesthetic value but one thing is sure, it is a potential tool for development.

Juan Carlos, Jr. Ph.D. Discussant

I would like to congratulate CLSU with Dr. Campos for the leadership having pioneered in this project and they have completed about 103 experiments in five years and by one agency and that I think it deserves a very good commendation. Another thing is that I notice in your analysis that they're getting about 79% return on investment. This is not bad. In fact, this is even higher than rice if you are not getting about 90 cavans/per hectare these days — even better than corn. So it may not really be that preferential treatment if the market is there and the market problem is I think a very serious one. And because of this then, the classifical uses of sunflower in the Philippines may not be that strong yet. Probably, one direction of research that you may want to take at this point, is the development of other users than what is now practically known. This is one way. The other is probably there are actual big users locally who are still importing. That we don't know. And some can produce this now in the Philippines. The Central Luzon Sun No. 1 is a big achievement. I'm just wondering whether this is a selection or a hybrid. If it is a selection then probably, it is now stable and probably ready for mass production. We have solved one problem with it but it is still needed to produce a few more varieties because it is dangerous to maintain one variety for any commodity because once we are hit by one problem then we are hit one hundred percent (100%). Maybe another problem is on seed technology. I didn't see any of your experiments geared towards the production of quality seed and probably this is one research direction that you can take in the future. Once the pilot, with this experiences and the time that you had put on to the project I think you have to be encouraged by everyone to go on the pilot. It may not be that expensive but I wonder what the Philippine Refining Company would say after having catapulted the CLSU to go into this. Maybe we can ask the PCARR, the NSDB or private agencies to help us in this direction.

Cesar Bautista, Discussant

Although I have a science orientation, both academically and professionally, I would like to comment this afternoon on the business side of the paper.

As the managing director of the Philippine Refining Company I have the happy association with Dr. Campos of CLSU on this project. Because we are all anxious to partake of the salted "bungang araw" I will make this comment short and sweet.

Our interest of course here stems from the fact that sunflower seeds produce oil and that is what you call soft quality oil. In the country today there are only three crops that give commercial oil, namely coconut, palm and corn. Soft oil is only available from corn. And corn is, in fact, in very limited supply. This is mainly used more as feeding stuff directly for either human consumption or for animals. You are aware of the relationship between fat consumption, quality of the fat with cardio-vascular diseases. Recent newspaper reports say that reduced calorie intake together with the high polyunsaturates will reduce blood cholesterol level which reduce the risk of cardiovascular diseases notwithstanding the stand of thousands of research institutions.

Now sunflower is a major source of soft oil internationally. Corn oil is not, in fact, as good as sunflower in the supply of linoleic. For example we know that corn oil has only 40% linoleic while sunflower has got 78%. Some pragmatics among us will say who cares about the linoleic when the Philippines' problems is lack of food. This is where we talk now about international situation because in the world market, sunflower oil commands a premium price over all the other kinds of oil. I don't think we have to be parochial about any of our research activities. One must look not in the country as the market, but in the world in general. This happily enough is getting to be an official statement from the Philippines as well — that export of Philippine products must be a primary issue in any of our undertakings. Now, our main mother company which is Unilever in fact has got research laboratories and they have had similar projects in Turkey and Mexico and some of the African countries.

Therefore we thought sunflower development was a relevant situation for the Philippines as well. With this in mind, we knew that CLSU was going to be the right agency to interest the growers to take sunflower production as part of their projects. The techni-

cal details of course will be competently handled by Dr. Campos and her staff, supply her with research information and some seeds. And we bought from the complete production from her 50 hectare lot and we helped in gathering growers' conferences so that they can exchange views and ask questions. I'm happy to say a general interest has been stimulated by the work and from the results of her discussion we feel and we agree that sunflower production for a farmer is in fact very profitable. You will notice that this conclusion is valid inspite of the fact that the price she quoted was a bit lower when you talk about the world market price. The economics of fats and oils is really quite difficult to finalize because if the discrepancy between local prices and the world market i.e. coconut oil which is currently depressed but local corn oil is artificially expensive here.

On the other hand, the meal that you get out of this will be compared and in fact should be better than the meal that one gets from other sources because of the higher protein content. In fact I don't think it was brought out by anyone before that the protein content of sunflower meal after removing the oil in the factory is 40% and it has a very high level of essential amino acid which meets in fact the minimum standard for human consumption, 48% of the total protein is essential amino acid. Having said that therefore one can feel optimistic about the technical aspect and the business aspect of sunflower seed growing. It will be a case of selling the idea to farmers so that one can consider a big scale undertaking to merit an equivalent action from the industry. With that I hope that the future projects of CLSU on sunflower will continue and continued success will be achieved.

ABOUT THE AUTHORS

PAULO C. CAMPOS, Academician. Doctor of Medicine, University of the Philippines, 1946. President, National Academy of Science and Technology. A man who is used to being first in any endeavor he pursues, Dr. Campos topped the first Medical Board Examination given after World War II (1946) with a grade of 90.85%. He established the first and best known Radioisotope Laboratory in the U.P. and in the country for that matter as well as the first Research Laboratory in the College of Medicine UP-PGH.

President Ferdinand E. Marcos bestowed on him a Presidential Commendation last December 30, 1968 for bringing out a comprehensive Community Health Program. Among his other awards are: the 1960 and the 1968 Philippine Medical Society Basic Award and an outstanding scientist awardee by the Philippine Association for the Advancement of Science, Gregorio Y. Zara Award in 1969. His name is found in the World Who's in Science from Antiquity to the present (1967), Who's who in Atoms of England, and in the Philippine Men of Science.

Noting the prevalence of goiter among Filipinos, he established the Tyroid Clinic at the UP-PGH Medical Center. He gets first hand knowledge of medical developments by post graduate studies (which he did in three renowned schools in the United States), observation studies, fellowships, and grants as well as seminars, conferences and congresses, mostly international. Every inch a professional, Dr. Campos is professor, consultant, researcher-scientist, executive, editor-writer and civic leader in addition to overly-tight timetable as a leading physician-surgeon.

MELECIO S. MAGNO, Academician. Ph.D. in Physics, 1958, John Hopkins University, Minister, National Science Development Board and currently Assemblyman, Interim Batasang Pambansa. He holds a B.S. in Mining Engineering, *cum laude*, class '44 from U.P., and M.S. in Physics and a Ph.D. in Physics in 1959-both of which he completed from John Hopkins U. In 1974, he finished his post-doctoral at the University of Colorado and National Oceanic and Atmospheric Administration, U.S.A. Right after graduation from college, he started out as assistant instructor in Physics at the College of Arts and Sciences, U.P. During the war, from 1943 to '44, he served as seismologist at the Weather Bureau, now PAGASA. After which, he went to his hometown to teach for two years. With the war over, in 1946 he returned to U.P., this time as Physics instructor and rose to be the vice-president for Academic Affairs until 1975 — when he assumed the chairmanship of NSDB.

Getting into print were some of his researches, among which were "Absorption and Flourescence Spectra of Hexagonal $SmCl_3$ and their Zeeman Effects" which he authored with G.H. Dieke in the Journal of Chemical Physics (USA), 1962; and a college textbook titled "University Physics" 1969, co-authored by J.F. Asperilla and Science Deputy Minister S.V. Roxas.

ENCARNACION ALZONA, Academician. Ph. D. Columbia University. She has become specialized in Philippine History. Dr. Alzona has received several national awards in recognition of her dedication to historical writings that brought about Philippine history in a nationalistic perspective. Among these awards are: Republic Cultural Heritage Award for Historical Writing in 1966, Rizal Pro-Patria Award and Apolinario Mabini Memorial Award. The Philippine Women's University had conferred on her a degree of Doctor of Laws, *honoris causa.* She is engrossed on researches involving important people and events in Philippine past, one of these is about T. H. Pardo de Tavera. With these series of works, she initiated the translation of several works of our national heroes from Spanish to English. She sees a fair chance of getting these published in the future.

BIENVENIDO FLORENDO NEBRES, Ph.D. Mathematics, Standford University, California, 1970. A Jesuit, he is the Dean of the School of Arts and Sciences, Ateneo de Manila University where he has been a faculty member since 1963. Father Nebres served as a visiting Professor of both the Department of Mathematics, Monash University in Australia as well as the Department of Mathematics, Sophia University in Tokyo. A noted Mathematician, he has researched and published extensively on the subject. He is the president of the Mathematics Society of the Philippines from 1973 up to now and of Southeast Asia (1977-1978). The roster of the Mathematics Society of America includes him.

LUZ B. OLIVEROS-BELARDO, Academician. Ph.D. in Pharmacy, University of Connecticut, 1957. A science educator, school administrator, researcher, wife, mother and big sister rolled into one, she is still actively and attentively engaged professionally at 74. Her main interest is phytochemistry. De la Salle University has her as visiting Professor in Chemistry, though she conducts researches there since 1979 — specifically on essential oils. The Philippine Women's University had conferred on her a Doctor of Science, *honoris causa* degree, among the many awards given her by said university. For a time she was a visiting professor at Texas Southern University. She has a variegated professional experiences: head, chairman, vice-president, consultant, and professor of various universities and/or research agencies. Because of the zeal and dedication to her profession, she has been an awardee of various plaques, diploma of merits, and citations.

ALFREDO C. SANTOS, Academician. National Scientiest. Dr. Phil. While serving as professor in the University of the Philippines, Dr. Santos concentrated his research on *Phaenthine*, dealing mostly on chemical structure elucidation. His researches on herbal plants were guiding lights to the career of many of his students who are now, like him, accomplished scientists/researchers. One of his earlier works, "The Isolation and Chemical Characterization of the Alkaloid *Phaentine* from *Phaentus ebracteolus* (Presl.) Merr., made him win the Anacleto del Rosario gold medal in 1931. He also isolated several other alkaloids, such as *anonaine* from *Anona reticula* and suaveoliness. Because of his efforts, he was cited for his "Outstanding Achievements in the Field of Chemistry of Alkaloids" by the late President Ramon Magsaysay in 1954. Twenty years later, he was selected the "Most Outstanding Scientist" by the Philippine Association for the Advancement of Science.

JOSE RAMIREZ VELASCO, Ph., D., University of California, 1949. Field of specialization: Agricultural Chemistry, Plant Physiology, and Comparative Biochemistry. Appointed in 1969, he was the Commissioner of the National Institute of Science and Technology for eight years. He has an extensive research work in agriculture especially those dealing with the problem of Cadang-cadang, a grave disease of coconut plantations specifically in the Bicol Region way back in the 50's. His membership with various science organizations stems from the fact that he is an active scientist-researcher and an award winner at that.

CLARA Y. LIM-SYLIANCO, Academician. Ph.D. in Biochemistry and Organic Chemistry, University of Iowa, 1957. She teaches at the Department of Chemistry College of Arts & Sciences and Professorial Lecturer, Department of College of Medicine, both of the University of the Philippines. Her present research trust is on the "Mutagenicity Potential of Philippine Medicinal Plants" and "Antimutagenic Effects of Some Vitamins." At this stage, it has already attracted many camps like food nutritionists and ecologists. Adding to her achievements are several chemistry books which she had written, namely: *Principle of Organic Chemistry; Modern Series of Molecular Biochemistry: Nucleic Acids, Protein, Carbohydrates, Lipids; Laboratory Manual in Organic Chemistry* and *Laboratory Manual in Biochemistry*. She has been a recipient of various awards, fellowships, travel grants and citations.

GEMINIANO T. DE OCAMPO, Academician. Doctor of Medicine, University of the Philippines, 1932. Professor Emeritus at the University of the Philippines, he is the Director and Ophthalmologist of De Ocampo Eye Hospital. He has received no less than 29 awards in medical researches, mostly first prize in various contests. Conferred with 22 honors, among which are the "Republic Cultural Heritage Award for Filipino, Life Scientist, 1965; Jose Rizal Award for Excellence in Ophthalmology, Asia-Pacific Academy of Ophthal-

FILOMENA FORTICH CAMPOS, Ph.D. in Cytogenetics, University of Maryland, 1955. She is Professor and Director of the Research and Development Center, Central Luzon State University, Muñoz, Nueva Ecija, since April 1970. Prior to this assignment, she was Professor and Director of Research at the Central Mindanao University, November 1966 to April 1970. A University scholar and *magna cum laude* graduate from the University of the Philippines, she has been a consistent scholar throughout college. Among her awards are the Presidential Golden Plow Award for Public Service, 1976, the Ayala Award as one of the Outstanding Scientists, 1974 and the Gregorio Y. Zara Scientist Award, 1973. Through the CLSU, she pioneered sunflower research in the Philippines some years back. Funded by the National Science Development Board, the project is multi-disciplinary and total in approach, covering all the important aspects relevant to the development of a package of sunflower technology.