Projects H.2 - H.5
Roport No. 8

SOUTH PACIFIC COMMISSION

PROJECTS H.2 - H.5

NUTRITION AND ALIMENTATION

REPORT ON THE ACTIVITIES OF

Mr. F.E. PETERS

BIOCHEMIST

FOR THE PERIOD 10th MAY - 31st DECEMBER, 1951

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### INTRODUCTION

The South Pacific Commission at its Sixth Session (Noumea, New Galedonia, 23rd October - 2nd November, 1950) considering project H.2: Infant Feeding and Nutrition, and project H.5: Alimentation and Nutrition, adopted the recommendations of the Research Council (Second Meeting, Sydney, Australia, 7th - 18th August, 1950) for the continuation of those projects.

One of the recommendations submitted was:

"that funds be reserved for the employment of a biochemist at any existing (adequate) laboratory, when such an individual becomes available, to determine the nutritive value of various native foods, with a special view to completion of project H.2".

Immediately after the Sixth Session, the Executive Officer for Health brought to the notice of several Organisations the vacancy of a position for a biochemist and started investigations for locating an adequate laboratory where the biochemist could carry out his (or her) researches.

Several applications were received for the position. In the mean time, Dr. A.J. Metcalfe, Director General of Health, Australia, and Dr. E.H. Hipsley, Medical Officer-in-Charge, Australian Institute of Anatomy, Canberra, agreed to make available the facilities and equipment of the Institute for a period of one year.

Dr. Hipsley interviewed some of the selected applicants and recommended the appointment of Mr. F.E. Peters, Bachelor of Science with Honours in Biochemistry from the Sydney University. Dr. Hipsley also accepted to advise Mr. Peters and supervise his research work in Canberra.

Mr. Peters accepted an appointment from the 10th May, 1951 to the 31st December, 1952.

It was stated that Mr. Peters' duties would be:

"In general, to determine the nutritive value of various native foods with a view to completion of research on foods suitable for infants during the weaning period and to carry out such other biochemical work as may be directed, specific instructions being given by the Executive Officer for Health. The work will be in close relation with the nutritionist already appointed by the Commission under projects H.2 and H.5".

Details of Mr. Peters' programme were worked out by Dr. Hipsley and the Executive Officer for Health. Dr. Hipsley expressed the following opinion with which the Executive Officer for Health fully agreed:

"These are some serious disadvantage about using a biochemist on research in the preparation, from the usual staples and accessories, of an infant food suitable for the weaning period. The ingredients of such foods will vary greatly and will depend on the availability in the locality. Therefore the problem resolves itself really into an analysis of the basic foodstuffs used. Although I confess there are gaps in our knowledge of the nutrients in the tropical foodstuffs, we do possess enough

"nutritional programmos. We can, of course, go on collecting more and more figures, trying to iron out the previous discrepancies where they occur, but I cannot see how the results would make a significant contribution to the nutrition of the South Pacific peoples for many years. A further disadvantage is that for the work to be of a sufficiently high standard, it would need to be carried out in a properly equipped laboratory in Australia. There are difficulties in transporting foodstuffs for analysis, as most are perishable."

In 1949, Miss Carey D. Miller, Professor, Foods and Nutrition, University of Hawaii, had expressed the same opinion.

It was therefore decided that Mr. Peters would especially investigate:

- a) the nutritive value of the coconut for humans, a major foodstuff of the people in the South Pacific Commission area;
- b) the composition of autochtonous women's breast milk, in conjunction with Miss S. Malcolm's researches on nutrition in the New Hebrides; and
- c) any others matters of interest which might arise.

The establishment at the Australian Institute of Anatomy, Camberra, the organisation and pursuance of his work, obliged Mr. Peters to perform a certain amount of administrative work which was carried out prior to, or in the course of, the implementation of his research programme.

- I Several commercial firms were requested to furnish quotations for the complementary laboratory apparatus and for the necessary chemicals.
- II Contacts were established and continued with several Administrations, Organisations, Institutions and individuals for obtaining technical information and documentation. Amongst them the following should be mentioned:

### a) General information:

- 1) UNESCO, Manila, supplied a copy of the list of scientific periodicals published in South East Asia. Later on a supplement to the first list was received.
- 2) The United States Information Office, Sydney, was unable to make available a copy of the U.S. Department of Agriculture Year Book for 1950-1951.
- 3) The Department of Science, Thailand, supplied a list of publications.
- 4) The Nutritional Observatory, Pittsburg, U.S.A., sent pamphlets on tropical foods.
- b) Information on Goconut and coconut products:
  - 5) The High Commissioner for India in Australia, sent a copy of the Indian Central Coconut Committee Annual Report for 1948-1949.
  - 6) The Philippines Legation in Australia furnished several addresses of Institutions and individuals engaged on work on the coconut and coconut products in the Philippines.
  - 7) The Philippines Institute of Science kindly circulated a letter requesting information on the nutritive value of coconut and of coconut products.
  - 8) The Fhilippines Institute of Nutrition furnished information on the nutritive value of coconuts and of coconut products.
  - 9) Professor Brown of the Department of Chemistry, University of Philippines, circulated a letter requesting information on the nutritive value of eccount and of eccount products, passed on all the information he had and mentioned the "lava" patent. Inter on Professor Brown sent more information on the subject, especially on the "lava" method and mentioned another process patented by E.R. Inzuriaga who was contacted.
  - 10) The Cewlon Coconu+ Research Scheme supplied all

- 11) Mr. Lloyd of the New South Wales Department of Agriculture supplied information on margarine and on coconut products, sent copies of articles he had written on these subjects and suggested a direct contact.
- · 12) The Malayan Department of Agriculture replied that no work had been done in Malaya on the nutritive value of coconut and of coconut products.
  - 13) Professor Roche, Paris, sent a reprint of his paper on the "Amino-acid composition of the proteins of the copra press cake and the variations of its methionine content".
- 14) The Food and Agriculture Organization of the United Nations Organization, Rome, supplied approximately a dozon references on the nutritive value of coconut and of coconut products.

### c) Information on milk composition:

- 15) The South African Institute of Medical Research accepted to forward, when available, the results of their analyses of Bantu mothers' breast milk.
- d) Information on other foods;
  - 16) Dr. Vicenty of Puerto-Rico sent a reprint of an article on "Yeast as a human foodstuff in the Tropics".
  - 17) Dr. Kamath, Bombay, forwarded a copy of his paper on "The utilization of oil seed cake".
  - 18) Dr. Sure, Arkansas, U.S.A., sent a reprint of his paper on "The nutritional improvement of cereal grains with small amounts of foods of high protein content".
- III Several other Institutions, Organizations and individuals, whose names appeared in publications related to the present researches were contacted with various results. The Bollowing may be mentioned:
  - 19) Dr. Velho, Angola.
  - 20) Dr. Escudero, Argentine.
  - 21) Dr. Cromer, Brazil.
  - 22) Dr. Child, Ceylon.
  - 23) Dr. Cramer, Dr. Overboek and the Instituto of Tropics, Holland.
  - 24) Dr. Moorjani, India.
  - 25) Dr. Tokimager, Dr. Tani, Japan.
  - 26) O.S.R. Publisher, Java.
  - 27) Dr. Anderson, Lebanon.

- 30) Dr. Urbanski, Poland.
- 31) Dr. Karnitskaya, Russia.
- 32) UNESCO, Cairo and New Delhi.
- 33) Professor van Veen, Dr. Harris and the Iowa State College, U.S.A.
- 34) Dr. Pinto, Venezuela.

The technical work carried out by Mr. Poters comprises:

- A. The preparation of technical documents and of drafts of research projects, and
- B. Biochemical investigations.

### A - Technical documentation.

A report on "The biological value of the coconut as a human foodstuff" was prepared in June 1951 and submitted to the Executive Officer for Health. This report which was published in the South Pacific Commission's Quarterly Bulletin Vol. 2 no. 1, January 1952, pp. 29-33, is reproduced in Annex I to the present report. It covers the available literature up to May 1951. It is now intended to prepare an annotated bibliography on the subject.

Mr. Peters attended as observer the First Conference of the Cereal Group of the Royal Australian Institute held in Sydney from the 17th to the 19th October, 1951. Mr. Peters report on this Conference is reproduced in Annex II to the present report.

### B - Biochemical investigations.

I - General work on the nutritive value of the coconut and coconut products.

Most of the work to date on the nutritive value of the coconut has been confined to copra press cake.

Samples of purified coconut protein have been prepared from both fresh Queensland coconuts and copra press cake. These protein preparations are white, odourless and almost tasteless powders. It is hoped to have these samples analysed for amino-acid composition and content.

In collaboration with the dictition of the Australian Institute of Aratomy, palatability experiments have been carried out using copra press cake, in verious combinations in "Sea Islands" biscuit. In these experiments crude copra press cake and copra press cake that had been exhaustively extracted with other - petroleum ether were used. The results showed that solvent extracted copra press cake could be incorporated into the biscuits to give a palatable product.

II - Analyses of breast milk of autochtonous mothers from the Port Vila area, New Heleides.

As an adjunct to the New Hebrides research of Miss

The report on these investigations appears in Annex III to the present report.

### III - Thiamin analyses of Fijian rice samples.

Four samples of Fijian rice milled locally were assayed for thiamin at the request of the South Pacific Hoalth Service. These samples were obtained from the villages of Raki-Raki, Navoua and Nausori.

Method: The thiamin evaluation method of Slater, described in Australian Journal of Experimental Biology and Medical Sciences, 19, 29, (1941), was employed. In this method the thiamin is oxidized by potassium forricyanide to thiochrome and the fluorescence produced was determined using a Klett Fluorimeter.

Results: The results are set out in the following table and represent the mean value of three sets of duplicates performed on different days.

Rice	Unwashed	Washed
Raki-Raki A	1.40 g/g	0.73 g/g
Raki-Raki B	1.10	
Navoua	1.20	0.70
Nausori	1.10	. 0.65
American rice *	0.84	0.37

It will be seen that these Fijian rices milled locally contain more thiamin than the American milled rice.
Washing tends to remove about 50% of the thiamin.

If it is assumed that rice gives 360 calories per 100 grams, then washed Fijian milled rice would contain about 0.2 milligrams thiamin per 1,000 calories. In 1948 the National Research Council of the U.S.A. quoted the figure of 0.23 milligrams per 1,000 calories as being the lower limit of safety for the prevention of beri-beri. Therefore, should the wash or cooking water be utilized, e.g. in a soup or stew, these rices would in themselves contain sufficient quantities of thiamin.

<sup>\*</sup> Figures for American milled rice were published by Kik and Willians "The nutritional improvement of white rice "National Research Council Bulletin, no. 112, (1945).

ANNEX I

to Report No. 8

THE BIOLOGICAL VALUE

OF THE COCONUT

AS A HUMAN FOODSTUFF

BY

Mr. F.E. PETERS

BTOCHEMIST

SOUTH PACIFIC COMMISSION

A preliminary survey of the literature between 1915-1950 leads one to the conclusion that the coconut is a neglected food from the nutritional point of view. This is somewhat surprising when one considers that coconut, in one form or another, constitutes a major item of diet throughout the Pacific and other tropical areas.

The majority of the work which has so far been carried out on the coconut has been confined almost exclusively to copra and the coconut oil industries. Much information is readily available on the fatty acid content of coconut oil, and on comparisons between oleomargarine and butter. Much work has also been carried out on the suitability of coconut press-cake for the feeding of stock. Most of these latter results, however, are comparative and qualitative rather then quantitative.

### General Composition:

Most of the work reported has been carried out on the mature nut (copra), and to date only one paper containing a comprehensive analysis of coconuts at various stages during their growth has been available (by F.T. Adriano and M. Manaham - "Philippines Agriculturist and Forester" - 1931).

General data for the forms in which coconut is used are presented in Table I. These results represent the mean of what may be considered reliable published data. Some definitions are necessary to avoid confusion:

- (1) Green soft pulp and green firm pulp represent the meat of the unripe nut at various stages.
- (2) Coconut water is the liquid found in the non-mature nuts.
- (3) Wet meal or wet kernel is the meat as it stands in the ripe nut.
- (4) Coconut milk or cream (if concentrated) is an emulsion obtained by pressing together with water the grated kernel in a fresh stage.
- (5) Copra is the kernel in the dried condition.
- (6) Desiccated coconut is the desiccated kernel in which the moisture content is lower than that of copra and the oil content is slightly higher.
- (7) Coconut press-cake is what remains after extraction of the oil of the copra.
- (8) Coconut flour is obtained by grinding to powder the residue left after the extraction of oil from desiccated coconut.
- (9) Toddy comes from the flower shoots of the coconut palm.

### The Protein Content:

There would appear to be only one major paper on the value of the coconut protein in human diet. This is a paper by B.C.P. Jansen, "Coconut Press-cake as Protein Food for Man", published in 1920 in Med. Geneesk. Lab. Weltevreden (Java).

The protein of the coconut kernel was identified fairly early as being a globulin, and percentage figures for the amino-acid content of this globulin were published between 1919 and 1924 by C.O. Johns and D.B. Jones in the Journal of Biological Chemistry. Since then, however, work on the amino-acid content of the coconut protein has apparently stagnated. Most of the work reported has been carried out on the mature nut and on copra.

Several methods were used for the determinations. Adriano and Manaham arrived at the protein value by multiplying the total nitrogen by the factor 6.35. Johns and Jones isolated each amino acid and determined each as a proportion of the total protein. E.S. Pradera (1942) gave figures for amino acids in the coconut water, using the method of analysis suggested by R.G. Block and D. Belling in "Amino-acid Composition of Proteins and Foods".

Recently Pr. J. Roche and N. Baudouin (1951) published a paper on the composition in amino acids of the protein of the coconut press-cake and its variation in methionine under several conditions.

Although the kernel of the coconut contains a globulin, it would appear that the water has its amino acids bound as polypeptides, peptones or some other smaller protein moieties. In Table II are shown the amino-acid compositions of coconut water and of coconut (press-cake) in comparison with cow's milk (from available papers).

F.M. Fronda and Dales (1939) claim that fresh coconut kernel compares more than favourably with corn as food for chicks. It has been reported by Johns and co-workers (1919) that the purified coconut globulin caused normal growth to occur in rats fed it as their only protein source.

Frof. Rouche and N. Baudouin consider that the amino acid composition of coconut press-cake exhibits features of special interest; the relatively high amino-acid content necessary for growth suggests that the addition of coconut press-cake to peanut and soya press-cakes would not only be particularly suitable for fowl feeding but also would be valuable as a foodstuff for mammals.

Coconut press-cake is richer in glycocolle (5.% against 2.6%) than the peanut and soya press-cakes which in turn contain higher quantities of arginine (respectively 10.8% and 8.5%) and are richer in thistidine and lysine. Therefore the proteins of coconut press-cake are an interesting source of nitrogen for the preparation of composite foodstuffs.

### Fat Content:

Because of its commercial applications, considerable work has been carried out on coconut oil. This oil is made up of glycerides of fatty acids containing 6-22 carbon atoms. The majority of these acids are saturated. Table III lists the percentage content of fatty acids in coconut oil and butterfat. Because of its content of unsaturated fatty acids (about 8%), coconut oil will tend to rancify but not to the same extent as butterfat, unsaturated fatty acid content of which is about 35-40%.

It has been claimed that when fed to rats coconut oil compares favourably with butterfat in eliciting the growth response reaction. No harmful effects were noted when rats were fed diets containing 25% coconut oil for 90 days, except for a slight fatty infiltration into the liver. This reaction, however, was also observed when rats received 25% butterfat for 90 days. It has been said that butterfat gives a better response than coconut oil because of the higher unsaturated fatty acid content.

Coconut oil is the most readily digested of all the fats in general use in the world. Miss Hartwell says - "It seems possible that if margarines contain this fat (coconut oil) they may be more rapidly digested than butter... Coconut oil may prove a more valuable food than has hitherto been supposed." The oil has been reported to contain traces of vitamins A and E; it is, however, not a good source of vitamins. The oil furnishes about 9.500 calories of energy per gram, and is the most concentrated and sustaining of all food materials.

### Carbohydrate Content:

A careful study of the carbohydrates in coconut has not been made. At least 7% of ordinary sugar is present in the fresh dried kernel (copra or desiccated coconut). The bulk of the carbohydrate is in the form of cellulose, and much of this is digestible. Sweet toddy contains about 16% of sugar.

### Mineral Content:

Potash is the principal mineral constituent of coconut products. They do not contain notable quantities of calcium.

### Vitamins:

Coconut is not regarded as a good source of vitamins, except for vitamin E. Coconut water and cream do contain small quantities of vitamin C, but not sufficient to cover the requirements. It has been reported that there is a rise in vitamin C content of coconut water from 2.5mg/ in the green nut without kernel to 3.7mg/ in the green nut with semi-hard kernel, and then a falling off again to 2.5mg/ in the brown-green nut with a hard kernel.

Vitamin A, if present, occurs only in traces. An analysis is reported to have revealed that five of the B vitamins are present in

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As a food, the fresh coconut is rather unbalanced because of its high fat content and relatively low protein and carbohydrate content. However, after extraction of the fat, the residual meal does constitute a good source of apparently high-grade proteins.

15 9 3 fr It is desirable, nevertheless, that further investigations be carried out on the biological value of this protein and on its amino acid composition. The low vitamin content could be overcome were coconut meal produced commercially for human consumption by the simple expedient of adding vitamin concentrates, although in tropical areas it is doubtful whether this would be necessary.

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All Sections of the Control of the C MEAN ANALYTICAL FIGURES (FROM VARIOUS SOURCES)

	Coconut water %	Green soft pulp	Green firm pulp	Cream %	Kernel wet %	Copra %	flour %
Fat Protein Carbohydrates Calories	1.0 0.4 5.0 20 30 mg 7-37 mg: 0.7	17 mg	2.67 0.7 2.66	52 27 4.0 18.0 320 10 mg 15 mg 1.08 absent 3-4 mg 0.2 mg/s		6.8 63.7 7.6 16.1 2.0 3.8	5.7 7.2 20.4 5.4 9.2

TABLE II

AMINO-ACID COMPOSITION OF COCONUT WATER,
COCONUT PRESS CAKE AND COW'S MILK

	Coconut Water	Coconut Press Cake	Cow's Milk
Alanine Arginine Aspartic acid Cystine Glycocoll Glutamic acid Histidine Leucine Lysine Methionine Proline Phenylalanine Serine Tryptophan Tyrosine Valine	2.41 12.75 3.60 0.97 - 1.17 9.76 - 14.5 1.95 - 2.05 1.75 - 4.4 1.95 - 4.57 1.21 - 4.12 1.23 0.59 - 0.91 2.83 - 3.00 (% of	2.9 - 4.11 7.0 - 15.92 5.12 1.08 - 1.44 5.9 19.07 2.42 - 2.7 5.96 - 11.3 4.8 - 5.2 0.4 - 1.9 5.54 2.05 - 5.2 1.76 1.25 - 1.6 3.18 2.4 - 3.57 total protein)	1.89 3.75 4.87 0.57  20.36 2.51 10.64 7.94  7.04 3.5 0.69 2.2 5.81 7.20

TABLE III

FATTY ACID CONTENT OF COCONUT OIL AND BUTTERFAT

A <b>c</b> id	Coconut Oil %	Butterfat %
Entyric Caproic Coprylic Capric Lauric Myristic Palmitic Stearic Arachioic Oleic Linoleic Decenoic Tetradicenoic Hexadecenoic Arachidonic	0.5 9.0 7.0 48.0 18.0 7.5 2.3 0.4 5.0 1.0 - 2.6	2.4 1.1 0.7 3.6 2.3 12.6 28.5 12.1 - 30.5 3.6 0.2 1.1 3.3 1.6

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to Report No. 8

# REPORT ON THE CEREAL GROUP CONFERENCE OF THE ROYAL AUSTRALIAN CHEMICAL INSTITUTE SYDNEY 17th - 19th OCTOBER, 1951.

The first conference of the Cereal Group of the Royal Australian Chemical Institute was held from Wednesday 17th to Friday 19th October at the New South Wales University of Technology. About 30 - 40 persons attended and although most of them were connected with the bread, biscuit, wheat and flour industries, several other interests were present, for example, Dr. White of the Queensland Department of Agriculture and Dr. Franklin of the Division of Animal Health of the C.S.I.R.O.

The conference was opened by Professor Le Fevre of the Chemistry Department of Sydney University, who gave a short survey of the present relationship between food production and the increasing world population.

Professor A.E. Alexander of the New South Wales University of Technology presented an extremely interesting paper on some of the colloidal aspects of proteins and starches. In this paper he discussed the nature of the bindings between the constituents of colloidal particles and the forces of attraction etc. between the particles themselves. He then discussed the physical properties, as far as they are known, of the colloidal constituents of wheat flour, viz. the gliadin, glutonin and the starches. It would appear that the phosphate linked to the starches, and also the free fatty acids present play an important role in the maintenance of particle configuration.

In the discussion that developed after the presentation of the paper the hypothesis was advanced that a knowledge of the gliadin/glutenin ratio should give an indication of the possible baking qualities of flours from various strains of wheat. It was, however, pointed out that at the moment there did not exist any reasonably accurate standard method for the letermination of this ratio.

Mr. Farror of the Kraft Walker Cheese Co. discussed the thermal stability of the B vitamins, especially during manifacturing processes. He outlined the various assay methods for each vitamin, and then went on to discuss their thermostability under varying conditions of pH, hydration, ion concentration etc. The majority of B vitamins are highly thermostable under normal food processing conditions, although biotin, folic acid and pantothenic acid are somewhat susceptable to heat and pH variations. Thiamin is the least stable although thiamin nitrate would appear to be more stable than the thiamin chloride hydro chloride.

The properties and distribution of the cereal lipases was discussed by Dr. Watley of the Biochemistry Department of Sydney University. The cereal lipases differ from most other lipases in not being activated by Ca<sup>++</sup> and Mg <sup>++</sup> ions. The distribution and activity of the lipases, and the distribution of fats varies during the germination and growth of the grain. Besides a very active lipase cereals, especially wheat germ, contain an active lipoxidase.

The suggestion was made during the discussion that cereal lipoxidase could be used in the manufacture of ghee from Australian

Mr. Jones of Kimpton and Sons, flour millers of Melbourne, discussed the heat resistance of some thermophilic organisms present in wheat and flour. He derived a mathematical expression for sterilization requirements of foods in terms of thermal death times, and discussed the principles involved therein. Clostridium botulinum spores have been reported in tinned bread made in the United States although no actual cases of botulism have been traced to this source. Sterilization requirements were discussed at length after the paper as tinned breads are now appearing on the local market.

Dr. R.A. Bottomley of the Weston Research Inboratories in Adelaide presented a very interesting paper on the biochemical and microbiological changes that occur during the storage of grains. This paper was largely based on as yet unpublished work that Dr. Bottomley had been doing at the University of Minnesota. It would appear that some fungi, mainly Cephalosporium, are capable of growing within the grain even the CO<sub>2</sub> concentration of the storage bin exceeded 30%. As these fungi grow within the grain, deterioration is hard to detect from inspection, and a criterion of deterioration due to microbiological factors is hard to establish. Dr. Bottomley is of the opinion that the amount of non reducing sugars present (sucrose etc.) provide a better index of deterioration than does the free fatty acid content.

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### THE CHEMICAL COMPOSITION OF THE MILK

### OF NEW HEBRIDEAN MOTHER'S

The peoples of the New Hebrides, in common with those of most Pacific areas, do not wean their infants until quite a late age. In these islands it is common to see children of 18 - 24 months of age still suckling, and the normal procedure would appear to be to wean the child some time during the 12 - 24 month period, unless another pregnancy intervenes, in which case the child is weaned immediately.

The reason for this late weaning appears to be nutritional. There is a lack of suitable weaning foods, although from a very early age the children have food, such as pre-masticated tare, placed in their mouths. It is hard to assess the relative importance of these supplements, but it would appear that they could play an important role in the food pattern of the infants. It is also hard to fix a definite time when suckling actually ceases altogether, for, as Hipsley (1) points out, toddlers up to perhaps three years of age are allowed to suckle should they become unhappy.

As Miss Sheila Malcolm, Nutritionist to the South Pacific Commission, was visiting the New Hebrides, it was decided to carry out a biochemical survey of the breast nilks, as an adjunct to her work, and also to try to determine

- a) whether there were any marked variations in the proportions of milk constituents between European and Melanesian women, and
- b) whether there were any variations in the constituents during these latter lactation periods.

In all, 54 samples were collected, but as one was lost in transit and two were too meagre to use, the results presented are for 51 samples. Each sample was examined for fat, lactose, ash, protein, calcium and phosphorus.

### METHODS

### Material:

The milk was collected from lactating mothers in the Port Vila area of the New Hebrides, and air-freighted to Canberra for examination. As it is the local practice to allow the infant to suckle whenever he feels inclined, and as twenty-four hour samples would be almost impossible to obtain from "bush" peoples, it was decided to take four-hour samples and to standardize the technique of collection.

The recommended method of collection was as follows:

- a) Empty both breasts completely.
- b) Allow no feeding for four hours.
- c) Collect all the milk from both breasts at the

- d) Measure and record the quantity collected.
- e) Agitate thoroughly and take a sample of at least 60 mls. if possible.
- f) Cork, label and seal the containers by dipping them in molten paraffin wax.

As the milk had to be transported from Port Vila to Canberra, a journey occupying 2 days, 4 - 5 drops of 40% aqueous formaldehyde were added to each 60 ml. bottle. The bottles were made light-proof by coating the outsides with black "Dulux" paint. On their arrival in Canberra, the samples were placed in a refrigerator. In no case did any sample show signs of souring or ropiness. A group of seven milk samples were obtained from Canberra women whose infants ranged from six weeks to seven months old, and were analysed concurrently with the New Hebridean milks.

### Chemical Analysis:

The methods used for the analysis of the various constituents were:

- i) Fat. The fat content was determined using the solvent extraction method of Rose-Gottlieb, described in the A.O.A.C. (2).
- ii) Lactose. The Lactose was determined by a modification of Somogyi's (3) method for blood glucose.

To 0.5ml of milk were added 0.5ml of 10% Zn SO4.7H2O, 0.5ml of 0.5 N.NaOH and 3.5ml of water. The precipitate formed was filtered off. Duplicates using 0.1ml and 0.2ml of the filtrate (measured with a standard capillary pipette) were diluted to 5 ml with water and 5 ml of the Somogyi copper solution added. The solutions were kept in boiling water for 25 minutes, cooled to 35 - 40°G, acidified with 1 ml of 5N.H2SO4 and titrated against 0.005N.Na2S2O3, using a starch indicator.

A curve was constructed using known quantities of lactose monohydrate, and the amount of anhydrous lactose in the milk samples was determined from this curve.

- iii) Protein. The total nitrogen content of the milk was determined using Conway's microdiffusion technique (4). The total nitrogen figure was multiplied by 5.1 to give the percentage protein. This factor 5.1 takes into account to 20% non protein nitrogen quoted by Macy (5).
  - iv) Ash. The amount of ash was determined by the direct weighing of the residue left after 10 ml of milk had been ignited at 650° C for 2 hours.
  - v) Calcium and Phosphorus. These elements were estimated by the methods of Winikoff (6).

### RESULTS

The samples examined were obtained from women whose lactation period ranged from 2 - 24 months. In order to determine whether there were any gross variations in the composition of the milk with the length of lactation, the samples were divided into three groups, 2 - 5 months containing 18 samples, 6 - 11 months containing 15 samples and 12 - 24 months containing 18 samples.

The results obtained are set out in Table I. This table shows the mean value, range and standard deviations for the constituents examined for each group and also for the total number of samples. Also included for comparison is the group of seven "Canberra" milks.

Table II compares the mean results and range of values obtained with those previously published.

### DISCUSSION

A statistical analysis of the data indicates that there is a significant dedine with time for ash and protein, and that over the period of lactation of 2 - 24 months there is no evidence of curvilinearity for any of the measures in the trend. The regression of lactose, calcium and phosphorus to time are also negative but are not statistically significant.

The ratios of the regression coefficients to their respective means were taken in pairs and the differences texted for significance. The ratios do not differ significantly and this suggests that a dilution of the non-fat solids takes place with time, the ratio of the constituents to one another remaining constant.

It will be seen from the tables that the lactose, calcium and ash figures are low, although they do fall within the range of minimum-maximum values published.

The lactose figures are particularly low. This may be due to the method used, although all the results were computed from a curve obtained using pure lactose monohydrate as a standard.

The results for fat vary over an extremely wide range. This is probably due in the main to a faulty collection technique. It is well known that the "strippings" contain a very high fat content, and so milk which has not been fully stripped will contain less fat than if all the milk in the breasts is obtained.

### CONCLUSIONS

Although there appear to be some variations between the milk of Melanesians and Europeans, insufficient results are available from which to draw concrete conclusions as to the realness or otherwise of these variations. Many more samples would have to be obtained, and the technique of collection would have to be rigidly adhered to, before any definite conclusions could be drawn. The indications are, however, that Melanesian milk is lower in lactose and calcium than European milks.

The decline in constituents with the length of the lactation period follows a trend already observed by other workers. Whether the dilution effect is real or not, cannot be deduced from the number of results obtained.

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TABLE I - MEAN RESULTS OF THE MAJOR CONSTITUENTS

OF NEW HEBRIDEAN MOTHERS MILK SAMPLES

Inctation period	No. of sample:		Lactose	Protein	Fat	Ash	Calcium	Phosphorus
2 - 5		Mean	5.05	1.17	: 3 <b>.</b> 8	188	27.4	15.9
<b>ζ ¬</b> )	<b>1</b> 8	Range	4.2-5.8	0.86-1.42	1.8-6.4	170-210	20.8-34.0	13 <b>-1</b> 8
months		S.D.	0,453	0.141	1.71	13.03	4.14	1.61
6 - 11		Moan	5.00	1,09	3,80	178	25.1	15.1
	15			0.86-1.26				12-19
months	······································	S.D.	0.519	0.141	1,36	13.31	4.92	2,39
12 - 24		Mean	ر. 4- 91	1.05	; ; ; 3_ <b>\$</b> 0	176	! 24•9;	14.7
13.4	18			0.81-1.26	i		1	
months	٠,			0.118				2.11
2 - 24	51		5.00 4.1-6.0	1,10			25.8 18.6-34.0	
months		S.D.	0.432	0.140	1.53	16.37	4.71	2.06
				! 	: 	!		· · · · · · · · · · · · · · · · · · ·
2 - 7		Mean	6.35	0.96	· 4.1	219	31.1	15.6
•	7	Range	5 <b>.</b> 2 <b>-</b> 7 <b>.</b> 4	0.82-1.10	2.6-7.1	210-240	26.5-34.5	13-18
months Canberra mothers		S.D.	1.517	0,99	1.54	13.64	3.14	1.92

TABLE II - COMPARISON BETWEEN NEW HEBRIDES AND EUROPEAN MILKS

:		Lactose	Protein	Fat	$\Lambda$ sh	Calcium	Phosphorus
, , , , , , , , , , , , , , , , , , ,	Moan	5.00	1.10	3.8	181	25.8	15.6
New Hebrides	Range	4.1-6.0	0.81-1.42	1.8-6.4	155-210	19 <b>-</b> 34	12-19
мъс	Mean	7,00	1,10	3.8	210	34.0	16

## (有数数) は、数数は対象の数数はない数のは、重要(力数) は、対象の数据を対象を対象を対象

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