Biochemical Assessment of the Nutritional Status of Pre-School Children in Kuala Trengganu

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Background and Purpose

WEST MALAYSIA launched its first applied nutrition programme on a pilot scale in August 1969, at Telok Datok, a rural district about 60 road miles south-west of the capital city of Kuala Lumpur.

This followed a baseline nutrition survey conducted some months earlier which covered clinical, anthropometric, biochemical and dietary assessments and the determination of the prevalence of stool and malaria parasites (Chong, 1970 & 1972; Jackson, 1970).

Action programmes aimed at raising the levels of community food production and its nutritional status have since been undertaken by various Government ministries including health, agriculture and education with the Ministry of National and Rural Development which receives support of the project from UNICEF, acting as the co-ordinating body.

The obvious and visible benefits of the above types of programmes to community health have prompted the authorities to consider extending similar activities to other rural parts of the country.

Subsequently, the district of Bukit Payong near the east coast town of Kuala Trengganu was selected for implementation of another applied nutrition programme. It must be pointed out here that its choice overruled that of a neighbouring inland district in Ulu Trengganu about 40-50 road miles south (nearest town, Kuala Berang), where moderately severe nutritional and health problems have been detected (McKay et al., 1971; Chong et al., 1972). On this basis, it would have seemed logical to make this district the next target area for an applied nutrition programme. However due to its poor accessibility including cut-off by floods during the annual north east monsoons and the lack of organised infra-structures deemed necessary for the successful implementation of applied nutritional activities, a neighbouring district, the site of the present survey was favoured instead.

The purpose of this paper is to report the results of a nutrition survey carried out on 399 Malay pre-school children from 26 villages in the proposed applied project area of Bukit Payong near the town of Kuala Trengganu during 1st-17th Sept. 1972, (see Fig. 1).

Population and Sampling

The area consisted of 26 kampongs (villages) with a population of 13,373 who were predominantly Malay in origin and living in 3,363 houses. Padi planting and rubber tapping were the principal means of livelihood. The number of pre-school children under 7 years was 2323 or 17.4% of the total population. The sample under survey included 399 children of both sexes below school age from 365 families comprising 17% of the total pre-school population. Their mean age was 3 years, with a range from 2 months to 6 years and 11 months.

Scope of Present Report

This report deals specifically with the biochemical aspect of the survey together with the prevalence of malaria and stool parasites. However, cognizant of the fact that a balanced account of

Fig. 1
Map of West Malaysia showing locality of survey.

the survey cannot be made entirely on these grounds, the text also makes reference to results of anthropometric measurements and abbreviated clinical examinations (the details of which are to be reported separately by Miss I. Coenigracht WHO consultant nutritionist) conducted simultaneously.

Methods

Specimens: Blood was obtained by finger prick. Usually it was possible to collect 5 heparinized capillary tubes from every child. (Clay Adams micro-capillary heparinized tubes; for haemoglobin determinations, one 0.02 ml "breakoff" heparinized tubes was used, Harshaw Chemicals Ltd., England.)

A thick blood film was also prepared on a microscopic slide for examination of malarial parasites.

Urine was collected into 30 ml capacity screw-capped bottles containing toluene and a few drops of concentracted hydrochloric acid as preservatives.

Stool specimens were collected similarly into screw-capped bottles containing polyvinyl alcohol. Specimens were available only from 50 children. The small number was due to discontinuation of collection owing to an isolated case of diarrhoea that was thought to be cholera.

Plasma was obtained by centrifuging blood specimens soon after collection. The former and the urine samples were stored in iced-containers for between 4-6 days and thereafter despatched to the laboratory where they were stored at 4°C and –15°C respectively until analysed.

All biochemical determinations were made as soon as the samples reached the base laboratory, beginning with plasma vitamin A determinations and followed by the other plasma parameters. Urine determinations were done soon afterwards. Despite these precautions and sense of priority, the last batch of urine analyses could only be completed after 4 months of storage at –15°C.

Laboratory and Biochemical techniques: Microhaematocrit was determined on an "International" microhaematocrit centrifuge while haemoglobin was estimated by the Cyanmethaemoglobin procedure on a Coleman Jr. II spectrophotometer. The foregoing two determinations were conducted in the field and where electricity was not available, power was supplied by a Honda generator.

Plasma amino acids ratio (the ratio of non-essential amino acids to essential amino acids) was done by the paper chromatographic method of Whitehead (1964) as modified by Prasana et al. (1971).

Plasma albumin was determined by the bromcresol green dye method (Doumas et al,. 1971) using reconstituted commercial lyophilized sera (Monitrol, Dade) of known albumin content as standards instead of the recommended human albumin (Sigma) which gave unrealistically elevated plasma albumin values in our hands.

Urinary urea was determined by the diacetylmonoxime method (Wootton, 1964) and sulphate by the colorimetric procedure of Wainer and Koch (1962) which is based on the formation of colour between chloranilate and sulphate ions. The latter method employed 1 ml of urine and was found to correlate very closely (Ann. Report, IMR, 1971) with the macro-gravimetric procedure of Harding et al. (1967) requiring 50 ml urine.

Urinary hydroxyproline was estimated by the procedure of Kivirikko et al. (1967) and urinary creatinine by the picrate method as described in the ICNND Nutrition Manual (1963).

Plasma vitamin A was determined by the ultra micro-procedure of Neeld and Pearson (1963) using 50 ul of plasma. Readings were taken on a Coleman 44 spectrophotometer with an ultra-micro assembly and a flow-through cell. The use of the latter resulted in the loss of samples after taking readings; it was thus not possible to include a prior determination for carotene and allowance for its correction. The "vitamin A" values presented are therefore at best a crude indicator of the blood levels of this nutrient.

Parasite Studies: Malarial parasites were examined on a thick blood film (Giemsa stain). Helminth ova and protozoa in the stool were examined by the technique of direct smear on single specimens. (We wish to record here our grateful thanks to the Division of Medical Entomology and the Division of Parasitology of this Institute for conducting these examinations.)

Results

Detailed biochemical characteristics, their inter-relationships, association with anthropometric parameters and the parasite prevalence study are tabulated as follows:-

Table 1 portrays the percentage of children who were deemed to possess "unsatisfactory biochemical indices". Pronouncement of the latter was based on any one of the following criteria:-

Table 1
Number and percentage of pre-school children with unsatisfactory biochemical indices

	No. of children	% of total
Haematocrit < 33%	36/399	9
Haemoglobin < 11 g%	93/399	23
Plasma albumin < 3.5 g%	14/363	4
Plasma amino acid ratio > 2.5	27/101	27
Plasma vitamin A < 25 μg %	46/244	20
Urinary urea N/creatinine < 5.0	59/338	17
Urinary sulphate S/creatinine < 0.55	80/325	25
Urinary hydroxyproline index < 1.5	67/309	22

Anaemia was considered to exist when the haematocrit was less than 33% or when the haemoglobin was less than 11 g% (World Health Organisation, 1972).

Plasma albumin of less than 3.5 g% was regarded as low (Interdepartmental Committee on Nutrition for National Defence, 1963).

Plasma amino acid ratio of greater than 2.5 was regarded to reflect the potential development of kwashiorkor (Prasana et al., 1971).

Urinary urea N/creatinine of less than 5.0 was taken to indicate a recent history of unsatisfactory intake of dietary proteins (Dugdale, 1964 & Arroyave, 1966) and similarly a ratio of less than 0.55 for urinary sulphate S/creatinine. The latter criterion was adopted arbitrarily from the regression equation $y = 11.1x - 0.85$; $r = 0.70$ obtained between urinary urea N/creatinine and urinary sulphate sulphate S/creatinine where y (urinary urea) was taken as 5.0 (see Fig. 2).

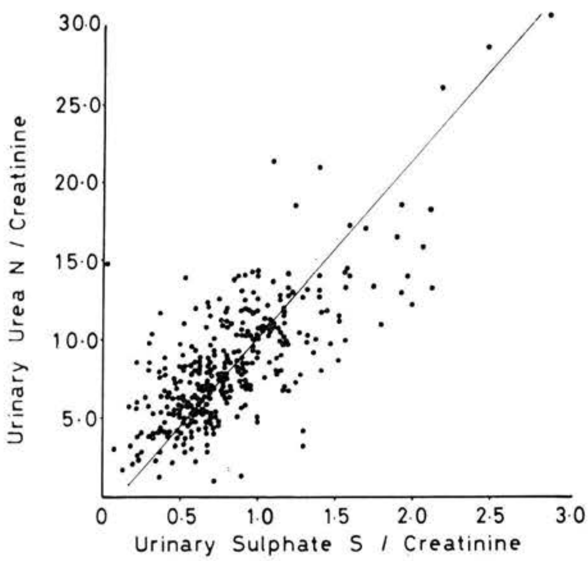

Fig. 2
The intercorrelation between urinary urea N/creatinine and urinary sulphate S/creatinine (random urine samples)
$y = 11.x + 0.85$; $r = 0.70$; $p < 0.001$

A hydroxyproline index of less than 1.5 was regarded as indicative of poor physical growth (Whitehead, 1965)

With particular reference to the present study, a plasma vitamin A of less than 25 ug % is regarded as low. The adoption of this slightly higher cut-off point is necessary as the vitamin A determination did not exclude the colour contribution due to carotene. In arriving at the foregoing guide for vitamin A, it was assumed that plasma carotene contributed no more than the equivalent of 5 ug vitamin A, i.e. a plasma carotene level of no greater than 50 ug %. Such a correction appeared justifiable based on the known low carotene and vitamin A intakes of children in the neighbouring district of Ulu Trengganu (McKay, 1971; Chen, 1971).

Table 2 depicts the valid statistical correlations between biochemical and anthropometric measurements.

Table 3 shows the biochemical characteristics of children below and above 75% ideal weight. Ideal weight was computed from a nomogram on the basis of height, weight and age and 75% ideal weight has been suggested by McLaren (1972) as the cut-off point below which severe protein-calorie malnutrition is supposed to exist.

Table 4 shows the correlations between hydroxyproline index and % Ideal weight achievements.

Table 5 shows the intercorrelations of some biochemical parameters.

Table 4
The correlation of urinary hydroxyproline index with % ideal weight achievements (up to 5 years old only)

% Ideal weight	Mean hydroxyproline index
65 – 74% ideal weight	1.6 *(22)
75 – 84% ideal weight	1.8 (100)
85 – 94% ideal weight	2.2 (75)
> 95% ideal weight	2.4 (32)

*Figures in parentheses refer to the number of individuals

Table 6 shows differential detection of protein and calorie deficiencies – children with poor hydroxyproline indices possessing concurrent high (unsatisfactory) or low (satisfactory) plasma amino acid ratios.

Tables 7 shows the prevalence of malarial and stool parasites.

Table 8 shows the means and standard deviations of the various biochemical parameters according to age group. Overall means and standard deviations are also included.

Table 2
Some statistically valid correlations between anthropometric measurements and biochemical indicators

% Ideal weight	vs.	packed cell volume	$r = 0.35 : p < 0.001$
% Ideal weight	vs.	haemoglobin	$r = 0.25 : p < 0.001$
% Ideal weight	vs.	urine hydroxyproline index	$r = 0.40 : p < 0.001$
Mid-arm circumference	vs.	packed cell volume	$r = 0.19 : p < 0.05$
Mid-arm circumference	vs.	haemoglobin	$r = 0.27 : p < 0.001$
Mid-arm circumference	vs.	plasma albumin	$r = 0.23 : p < 0.001$
Triceps skinfold	vs.	plasma albumin	$r = 0.15 : p < 0.05$

Table 3
Biochemical characteristics of children below or above 75% ideal weight (up to 5 years old only)

	No. of Children	PCV %	HB g%	Plasma Albumin g%	Urine -OH proline index	Urine Urea N/ creatinine	Urine SO_4 S/ creatinine
Children *75% ideal weight or below	39	36.8	11.36	3.95	1.57	8.7	0.89
Children above 75% ideal wight	265	37.0	11.84	4.01	2.10	8.5	0.84
probability		n.s.	$p < 0.05$	n.s.	$p < 0.01$	n.s.	n.s.

*% Ideal weight is computed from a nomogram on the basis of height, weight and age of the child (17). Severe protein-calorie malnutrition is deemed to exist when ideal weight is below 75%.

Table 5
Intercorrelation of some biochemical characteristics

Hb	vs.	pcv	$r = 0.39 : p < 0.001$
Urine -OH proline index	vs.	plasma albumin	$r = 0.29 : p < 0.001$
Urine -OH proline index	vs.	urine urea N/creatinine	$r = 0.47 : p < 0.001$
Urine -OH proline index	vs.	urine SO_4 S/creatinine	$r = 0.46 : 0 < 0.001$
Urine urea *N* creatinine	vs.	urine SO_4 S/creatinine	$r = 0.70 : p < 0.001$

Table 6
Differential detection of protein or calorie deficiency on the basis of hydroxyproline index, plasma amino acid ratio and plasma albumin

Children with Hydroxyproline index of < 1.5	Plasma amino acid ratio < 2.5	Plasma amino acid ratio > 2.5
1.2	—	2.8 (4.1)*
0.5	—	3.0 (4.0)
1.0	—	2.7 (4.0)
0.6	—	2.7 (4.3)
1.1	—	2.5 (3.8)
1.4	—	2.6 (3.8)
1.4	—	2.8 (4.4)
1.3	2.1 (4.1)	—
0.3	1.6 (3.2)	—
1.3	2.4 (4.0)	—
0.8	2.0 (4.0)	—
1.2	1.0 (4.1)	—
0.7	1.7 (3.6)	—
1.3	2.0 (4.0)	—
1.0	2.1 (3.8)	—
1.2	2.4 (4.0)	—
1.3	1.9 (3.9)	—
1.1	2.1 (3,7)	—
1.2	2.1 (4.0)	—
1.4	2.1 (4.2)	—
1.0	1.9 (3·7)	—
1.2	2.1 (3.7)	—
0.7	2.1 (3.9)	—
Mean	2.0 (3.9)	2.7 (4.1)

*Figures in parentheses refer to plasma albumin in g per 100 ml.

Table 7
***Prevalence of parasites**

	No. of children infected	Percent of children infected
Malaria parasites	5/399	1%
Ascaris only	10/56	20%
Trichuris only	6/56	9%
Hookworm only	Nil	Nil
Ascaris and trichuris	23/56	41%
Hookworm, ascaris and trichuris	1/56	1%
Stools negative for helminth ova	17/56	30%

*(All specimens were negative for protozoa)

Discussion

Recent years have witnessed the development of numerous biochemical tests aimed at assessing the nutritional status of communities (Interdepartmental Committee on Nutrition for National Defence, 1963; Committee Report, 1970).

Many of these tests have been designed to detect early or sub-clinical protein-calorie malnutrition, the severe forms of which still contribute largely to the high mortality of children between 1 to 4 years old of technologically backward countries.

Table 8
Biochemical characteristics according to age groups (mean values and standard deviations)

Age in years	PCV	Hb g%	Plasma albumin g%	Plasma amino acid ratio	Urine sulphate *S* creatinine	Urine urea *N* creatinine	Urine -OH proline index	Palsma vitamin A ug %
0 - 0.99	33.3 ± 2.0	10.7 ± 1.0	3.9 ± 0.3	2.1 ± 0.4	0.82 ± 0.5	7.7 ± 3.4	2.5 ± 0.9	41 ± 17
1 - 1.99	36.5 ± 2.8	11.2 ± 1.3	4.0 ± 0.3	2.3 ± 0.4	0.82 ± 0.4	8.2 ± 2.3	1.9 ± 0.6	37 ± 12
2 - 2.99	38.4 ± 3.0	12.1 ± 1.1	4.0 ± 0.3	2.3 ± 0.5	0.99 ± 0.4	10.0 ± 4.7	2.0 ± 0.9	39 ± 16
3 - 3.99	37.7 ± 2.8	12.1 ± 1.3	4.1 ± 0.3	2.2 ± 0.3	0.83 ± 0.3	8.0 ± 2.8	1.8 ± 0.5	35 ± 16
4 - 4.99	37.3 ± 2.7	12.0 ± 1.1	4.0 ± 0.3	2.3 ± 0.4	0.78 ± 0.3	8.3 ± 3.8	1.8 ± 0.8	36 ± 20
5 - 5.99	37.7 ± 2.7	12.1 ± 1.1	4.0 ± 0.3	2.4 ± 0.3	0.92 ± 0.5	9.7 ± 6.2	2.3 ± 1.2	38 ± 11
6 - 6.99	37.9 ± 3.1	12.2 ± 1.5	3.9 ± 0.2	2.1 ± 0.2	0.76 ± 0.3	7.2 ± 2.5	2. 0± 0.6	37 ± 18
All age groups	36.9 ± 3.2 *(399)	11.8 ± 1.3 (399)	4.0 ± 0.3 (363)	2.3 ± 0.4 (101)	0.84 ± 0.4 (325)	8.5 ± 4.0 (388)	2.1 ± 0.9 (309)	38 ± 16 (244)

*Figure in parentheses refer to the number of specimens determined

Amongst such tests are the ratio of the non-essential to essential amino acids in plasma, the urinary excretion of hydroxyproline peptides, the ratio of urinary urea N to creatinine, the creatinine/height index and the ratio of urinary inorganic sulphate S to creatinine (Committee Report, 1970) Added to these is the recent innovation for the measurement of plasma albumin by dye binding which obviates the need for a separate total protein determination (Doumas et al., 1971).

Newer biochemical procedures designed to detect nutritional deficiencies other than that of proteins have also made their appearance. They include erythrocyte transketolase and glutathione reductase assays for thiamine and riboflavin nutriture respecitvely and the micro-spectrofluorometric assay of serum vitamin A. (Schouten et al, 1964; Sauberlich et al, 1972 & Selvaraj, 1970).

Used in conjunction with simple haematology, (haematocrit and haemoglobin determinations), the above battery of biochemical tests should yield invaluable and specific information on the nutritional status of a population particularly when considered in relation to data obtained by clinical examination and anthropometry.

The reasons why these tests have not found wider usage are that many of them are recent in origin and their usefulness remain to be evaluated. Besides, performance of these tests require a moderately specialized nutrition laboratory, the availability of trained personnel, equipment, resources and last, but not the least in importance, the accessibility of samples – blood and urine.

Although this laboratory has the potential to perform all the aforementioned tests, we were hampered mainly by the impracticability of obtaining venous blood specimens which would have allowed the complete range of the above tests to be attempted. This difficulty of obtaining venous blood samples necessitated blood collection by finger prick which narrowed down the range of tests that could be performed and restricted them only to those based on ultra-microprocedures. Similar difficulties however did not apply to urine collection.

Despite these limitations, the tests selected in this study were those known to be able to throw light on nutritional problems related to protein-calorie malnutrition, nutritional anaemia and xerophthalmia which are believed to be the major deficiency diseases amongst rural Malaysian children and children of city dwellers from the lower socio-economic strata.

On the present survey, the evidence presented in Table 1 indicates that 23% of the children may be regarded as anaemic on the basis of their low haemoglobin levels, 22% were retarded in physical growth judging from their poor hydroxyproline index and between 17 and 25% of the children were probably not eating a diet that met their daily protein requirements, as judged by their unsatisfactory urinary excretion of urea and sulphate, 27% of the children had poor plasma amino acid ratios and 4% had a plasma albumin value of below 3.5 g%.

The following statistically significant associations were found between some of the laboratory parameters and indices of physical growth (Table 2):–

% ideal weight with haematocrit, haemoglobin and urinary hydroxyproline index,

mid-arm circumference with haematocrit, haemoglobin and plasma albumin and

triceps skinfold with plasma albumin.

Similar associations were however not observed between urinary urea and sulphate with the indices of physical growth or between the latter and plasma amino acids.

The association of some of the laboratory parameters with physical growth is also evident from Table 3 which shows that children with ideal weights of 75% and below had significantly lower levels of haemoglobin and hydroxyproline indices than those whose % ideal weights exceeded 75%. Further evidence for such an association is again apparent in Table 4 which shows a direct relationship between the urinary hydroxyproline indices with the ideal weight achievements of the children.

It must be pointed out that although 39 out of 304 children up to 5 years old i.e. 9% had ideal weight of 75% and below (Table 3), only one case of clinical marasmus was observed. This child's ideal weight was 68% and his expected mid-arm circumference and triceps skinfold were 70% and 60% respectively according to the standards cited by Jelliffe (1966); his palsma albumin was 2.6 g %, haematocrit 39% and haemoglobin 11.1 g%. Other biochemical parameters were unfortunately not available.

None of the remaining children, who numbered 398 examined physically showed any evidence of clinical kwashiorkor or its accompanying cardinal sign of oedema. We wish to emphasise this in view of McLaren's proposal that children with an ideal weight of 75% and less should be classified as sufferring from severe protein-calorie malnutrition (McLaren, 1972).

It is noteworthy that the biochemical index of physical growth i.e. the hydroxyproline index correlated with other protein parameters like plasma albumin, urinary urea and inorganic sulphates (but not plasma amino acids) and that significant intercorrelations were also observed between the ratios of urinary urea N/creatinine and urinary sulphate S/creatinine. (Table 5).

The latter observation seems particularly pertinent in view of the fact that the determination of urinary sulphate has rarely been recorded in field nutrition surveys and that this index is known to have the possible advantage of reflecting the recent dietary intake of proteins of high biological quality (Committee Report, 1970).

Whitehead proposed that the hydroxyproline index and the plasma amino acid ratio could be used to differentiate physical retardation due to a primary protein or calorie deficiency; where protein deficiency was prevalent a high incidence of both abnormal hydroxyproline indices and amino acid ratios was found. Whereas in areas of food shortage (calorie deficiency), the amino acid ratios were relatively normal with the hydroxyproline indices remaining low (Whitehead, 1967).

Amongst the 67 children in the present survey who were found to have hydroxyproline indices of less than 1.5, 23 also had determinations made on their plasma amino acid ratios. Of these 23 children, 16 possessed satisfactory plasma amino acid ratios whereas 7 had unsatisfactory ratios of above 2.5 (Table 6). This seems to suggest the possibility of a calorie deficiency existing with a protein deficiency and appears to be strengthened by the finding of the triceps skinfold data (I. coenigracht, unpublished) which showed that 20% of the children measured in this survey had triceps skinfold below 70% of the expected standard of Jelliffe.

The biochemical assessment of vitamin A status, crude though it was, suggests that 20% of the children had low levels of this nutrient in their plasma (Table 1). But a slightly higher percentage of children (35%) was found clinically to have dryness of the conjunctiva (I. Coenigracht, unpublished); this clinical sign however was uncommon amongst the younger children.

The present finding of the existence of a vitamin A deficiency problem agrees well with two recent studies conducted on pre-school Malay children in the neighbouring districts of Kuala Berang (Chen, 1972) and inland villages of Ulu Trengganu (Chong et al, 1972; McKay, 1971). The former reported conjunctival xerosis in 10 out of 27 pre-school children whose diets were also found to contain negligible amounts of carotenes and vitamin A, while the latter studies reported a 10% prevalence for xerophthalmia and mean serum vitamin A levels in the "deficient" range.

There was no clear cut trend in the biochemical characteristics with age except for the slightly lower haematocrit, haemoglobin and higher hydroxyproline indices of children below 1 year old (Table 7).

Both ascaris and trichuris infestations were common either singly or in combination and no protozoa were found in the stool specimens examined. Malaria was not a major health problem. Only 5 of the 399 children (1%) were found to have parasites in their blood films. Only one child was found to have hookworm infestation (Table 8).

Although a malaria eradication programme was already launched in the survey area at the time of the study, it is known that houses in this district had not received more than a single cycle of indoor spraying with DDT. The Ministry of Health reported a parasite prevalence rate of 1% for malaria in school children of the same district prior to the launching of the malaria eradication project (unpublished). This is similar to the parasite rate found presently. The low prevalence for malaria therefore should not be associated with the existence of a malaria eradication programme.

Conclusions and Summary

A baseline nutrition survey involving biochemical assessment, nutritional anthropometry and abbreviated clinical examination was conducted on 399 pre-school children of Malay origin in Bukit Payong, a rural district near the east coast town of Kuala Trengganu during September 1972.

The evidence derived from the biochemical evaluation considered in conjunction with nutritional anthropometry suggests that between 20-25% of the children examined were suffering from some moderate to severe degree of malnutrition which were related to deficiencies in protein, calories and vitamin A and to anaemia. The latter is presumed to be dietary in origin since the prevalence of both malaria and hookworm was minimal.

Acknowledgement

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Addendum

The above report was completed in June 1973. Since then, a WHO Assignment Report by Miss I. Coenigracht dated 21st December, 1973 has become available. The results of abbreviated clinical examination and nutritional anthropometry of the children surveyed are found in this WHO Assignment Report.

References

1. *Annual Report, Institute for Medical Research, Kuala Lumpur, Malaysia* 1971, 110.
2. Arroyave, G. – (1966) Biochemical characteristics of malnourished children. *Proc. West Hemisphere Nutr. Congr.* Am. Med. Assn. Chicago, U.S.A. p. 30.
3. Chen, P. C. Y. – (1972) Sociocultural influences on vitamin A deficiency in a rural Malay community. *J. Trop. Med. and Hyg.* 75: 231.
4. Chong, Y. H. – (1970) A report on the biochemical assessment of nutritional status of pre-school children in an applied nutrition programme pilot area – Telok Datok, Kuala Langat. *Nutrition Division Report of the Institute for Medical Research, Kuala Lumpur.*
5. Chong, Y. H., and McKay, D. A. – (1971) Urinary urea and hydroxyproline as indicators of marginal protein deficiency. *Proc. of the 6th SEAMEO – TROPMED Seminar: the 1st S.E. Asian Regional Seminar on Nutrition, Djakarta, Oct.*, 1969. Central Co-ordinating Board, SEMEO Regional Tropical Medicine and Public Health Project, 420/6 Rajvith. Road, Bangkok 4, Thailand. p. 53.
6. Chong, Y. H., D. A. McKay and Ruth, K. H. Lim – (1972) Some results of recent nutrition surveys in West Malaysia. *Bull. Pub. Hlth. Soc. (Malaysia)* 6: 55.
7 *Committee Report* – (1970) Assessment of protein nutritional status. *Am. J. Clin. Nutr.* 23: 807.
8. Doumas, B. T., W. A. Watson and H. G. Biggs – (1971) Albumin standards and the measurement of serum albumin with bromcresol green. *Clin. Chim. Acta.* 31: 87.
9. Dugdale, A. E. and E. Edkins – (1964) Urinary urea/creatinine ratio in healthy and malnourished children. *Lancet.* 1: 1062.
10. Harding, R. A., G. A. Leville, E. M. Baker III, Z. Z. Ziporin and H. E. Sauberlich – (1967) Gravimetric determination of inorganic sulphates in urine. *Biochemical Procedures* U.S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado 1: 67.
11. Interdepartmental Committee on Nutrition for National Defence Manual for Nutrition Surveys – (1963) Washington, D.C. U.S. Govt. Printing Office, 2nd Ed.
12. Jackson, J. – (1970) Applied Nutrition in Malaysia. *Assignment report of WHO (Western Pacific Regional Office).* WPR/393/70 – (Mimeographed).
13. Jelliffe, D. B. – (1966) The Assessment of the nutritional status of the community *WHO Monogr. Ser. No.* 53.
14. Kivirikko, K. I., O. Laitinen and D. J. Prockop – (1967) Modifications of a specific assay for hydrooxyproline in urine. *Anal. Biochem.* 19: 249.
15. McKay, D. A. – (1971) Food, illness and folk medicine: Insights from Ulu Trengganu, West Malaysia. *Ecol. of Food and Nutrition.* 1: 67.
16. McKay, D. A., R. K. H. Lim K. H. Notarney and A. E. Dugdale – (1971) Nutritional assessment by comparative growth achievement in Malay children below school age. *Bull. Wld Hlth Org* 45: 233.
17. McLaren, D. A., and W. W. C. Read – (1972) Classification of nutritional status in early childhood. *Lancet* 2: 146.
18. Neeld, J. B., and Pearson, W. H. – (1963) Macro- and micromethods for the determination of serum vitamin A using trifluoroacetic acid. *J. Nutrition* 79: 454.
19. Prasana, H. A., B. L. M. Desai and M. Narayana Rao – (1971) Detection of early protein-calorie amlnutrition in population groups. *Br. J. Nutr.* 26: 71.
20. Sauberlich, H. E., J. H. Judd, G. E. Nichoalds, H. P. Browuist and W. J. Darby – (1972) Application of the erythrocyte glutathione reductase assay in a high school population. *Am. J. Clin. Nutr.* 25: 756.
21. Schouten, H., L. W. Status Van Eps and A. M. Struyker Boudiet (1964) Transketolase in blood. *Clin. Chim. Acta.* 10: 474.
22. Selvaraj, R. J., and T. P. Susheela – (1970) Estimation of serum vitamin A by a microfluorometric procedure. *Clin. Chim. Acta.* 27: 165.
23. Wainer, A. and A. L. Koch – (1962) Inorgranic sulphates in urine. *Anal. Biochem.* 3: 457.
24. Whitehead, R. G. – (1964) Rapid determination of some plasma amino acids in subclinical kwashiorkor. *Lancet,* 1: 250.
25. Whitehead, R. G. – (1965) Hydroxyproline creatinine ratio as an index of nutritional status of growth. *Lancet.* 2: 567.
26. Whitehead, R. G. – (1967) Biochemical test in differential diagnosis of protein and calorie dificiencies. *Arch. Dis. Childh.* 42: 479.
27. Wootton, I. D. P. – (1964) *Mirco-Analysis in Medical Biochemistry* 4th Edition, J. and A. Churchill, London.
28. World Health Organisation – (1972) *Tech. Rep. Ser. No.* 503.