Field evaluation of two biochemical tests which may reflect nutritional status in three areas of Uganda

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1. Serum amino acid ratios and urinary hydroxyproline indices have been studied in three groups of children from different parts of Uganda. The results of these biochemical investigations are compared with clinical assessments and anthropometric measurements made at the same time.

2. In Buganda, an area where protein deficiency is prevalent, a high incidence of both abnormal hydroxyproline indices and amino acid ratios was found. In Karamoja, where a drought had caused a widespread shortage of food, the hydroxyproline indices were particularly low but the amino acid ratios were relatively normal. In Bukedi the dietary pattern was changing and becoming more like that in Buganda; this was reflected by the biochemical results obtained which were similar in the two areas.

3. The biochemical results confirmed earlier investigations, carried out in more severely ill children in a metabolic ward, which had indicated that these two tests can differentiate between primary protein deficiency and total calorie deficiency.

4. The biochemical tests confirmed the anthropometric measurements in a quantitative way, and also indicated the reason for the nutritional difficulties which could otherwise only have been surmised from the food habits of the area.

Within the administrative country of Uganda are a number of different climatic and physical environments, as well as a large number of diverse tribal groups. Malnutrition is common among the children but the incidence varies considerably from area to area as does the aetiology, and therefore the type of malnutrition. It is well known (Scrimshaw & Béhar, 1961) that malnutrition in childhood expresses itself differently in different parts of the world in clinical appearance, laboratory findings and the presence of associated diseases. These differences, however, have not usually been correlated with the nature of the environment, and this omission has led to some confusion in the interpretation of the findings from different parts of the world.

Standard, Lovell & Garrow (1966) investigated the validity of a number of the so-called diagnostic clinical signs of malnutrition, and have found that, in general, these signs lacked specificity. The interpretation of anthropometric data, particularly in early malnutrition, is also not without difficulties (Jelliffe, 1966). In community surveys the main difficulties are generally the assessment of the child's age, and the possibilities of ethnic variation. As it is unlikely that these problems will be eliminated in the foreseeable future, in countries where childhood malnutrition is at present endemic, methods of assessment which are independent of age and ethnic variation could be of great value.

Some previous work from this Unit (Whitehead & Dean, 1964; Whitehead, 1965) indicated that the results of two biochemical tests might reflect nutritional status.

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Moreover, there was an indication that they may be able to distinguish between malnutrition in which the primary deficiency was of protein and that due to a lack of total calories. This work had, however, been confined to an area where protein deficiency was the main problem and it was felt that these tests should be evaluated in regions with a different nutritional background. The aim of the experiment was to see whether, in three separate groups of children living under different environmental conditions, the biochemical tests could provide information which could not be gained from clinical examination and anthropometric measurements alone.

MATERIAL AND METHODS

Subjects

Since the purpose of the investigation was not to perform a representative nutritional survey, the children studied were not randomly selected samples of each population, but children aged under 4 years whose parents volunteered to co-operate.

Buganda. Buganda stretches along the northern and western shores of Lake Victoria. The climate is equable with a temperature range of 56–86 °F and a rainfall of 40–60 in. There is little seasonal variation except for changes in rainfall, which is heaviest from March to May and from August to November.

The land is an eroded plateau at an average altitude of 4000 ft, consisting of flat-topped hills separated by broad open valleys floored extensively with papyrus. The vegetation is chiefly grassland with isolated forest and savanna trees. The soils are ferrallitic, mainly of the sandy clay loam variety, which under the prevailing conditions are productive. In consequence, food is almost always in plentiful supply. The main crop is plantain, which is a cookable banana with great prestige value as a food, and of almost religious significance, but it does not contain enough protein for weanling children. Sweet potatoes and cassava are also grown, but are of much less importance. Groundnuts and beans, often supplemented with green vegetables, are used as side dishes. Most of the population are subsistence farmers, and buy animal protein only occasionally, and kwashiorkor is common.

Children under the age of 5 years make up about 15% of the population of Buganda which is mainly of bantu origin. In a survey of paediatric admissions to the largest hospital in the district (Musoke, 1961) gastro-intestinal diseases, malnutrition and malaria were the three most numerous causes for admission, followed closely by respiratory diseases and anaemia. The adults are usually well nourished, and although they tend to be shorter than Africans of nilotic and nilohamitic origin in Uganda (Oschinsky, 1954; Coles, 1957) they are strong and well built. Buganda babies are, however, known to be small at birth (Allbrook & Sibthorpe, 1961). The children studied were 133 of those attending the Unit's Rural Child Welfare Clinic at Namulonge (Burgess, 1960), about 15 miles from Kampala, in March 1966.

Karamoja. Karamoja lies in the north-east of Uganda and is, for the most part, an arid barren plain at an altitude of 3500–4000 ft. A rocky watershed, rising in places to 8000 ft, runs north–south in the eastern part of the district and the plain is broken here and there by groups of inselbergs.

Rainfall, except on high ground, is between 20 and 40 in. and often irregular. Temperatures range between 59 and 95 °F with a daily variation of 25–30°. There is an intense dry, hot season from November to March when the streams dry up and the scrub vegetation is subject to extensive fires which produce a marked smoke haze. The rainy season is from April to August with most rain generally falling in May and July.

The soils over most of the plain are vertisols ('black cotton soils') but in the areas of high ground there are also some ferruginous tropical soils. Under conditions of low rainfall these require careful usage to maintain their productivity. The natural vegetation is tree and grass steppe on the plains and dense shrubby thicket on the higher ground.

The Karamojong way of life is dictated by the availability of water and this is the reason why most of their permanent settlements are in the vicinity of the higher ground where there is a little more rain. They are a nilohamitic tribe of cattle herdsmen, the number of cattle greatly exceeding the human population (Jelliffe, Bennett, Jelliffe & White, 1964). The cattle and men lead a largely nomadic existence in search of water and grazing, while the women and children and some of the smaller stock, together with a few cows, remain at the permanent settlements where small plots of sorghum beans and maize are cultivated. The adult diet in the settlements consists largely of sorghum porridge, maize, beer and milk, both fresh and sour, and the nomadic diet of milk and blood. Infants receive small amounts of sorghum, fresh and soured cow's milk and butter while still on the breast, and by the age of 2 years a child is usually consuming the diet of an adult, but at the time of the survey there was a widespread shortage of food, owing to drought.

Children under the age of 5 years make up 22% of the population and, in a survey of childhood disease in the area (Jelliffe *et al.* 1964), the most common findings in this age group were malaria, intestinal parasites and conjunctivitis. The adults are tall and muscular, but light, and obvious clinical trachoma is common among them. Two hundred and ninety-eight children were examined at two centres in the district in January 1966.

Bukedi. Bukedi is situated in the south-eastern part of Uganda at an altitude of 3000–4000 ft. The region is largely flat with few hills and a considerable area of papyrus swamp. The temperature range is 60–87 °F and the rainfall about 50 in., less rain falling in the northern than in the southern part of the district. The wet season extends from April to October with peaks in April, May and August. Vegetation is of the wooded savanna type and the soils are ferrallitic, but mainly of the sandy loam variety and generally of low productivity. The main food crops are millet in the north and plantains in the south. Sorghum, cassava, maize and sweet potatoes are also grown, as well as groundnuts and beans. Cotton is the chief cash crop, but the majority of people in Bukedi are still on a subsistence economy, and consume only small amounts of meat, milk and fish. In the west of Bukedi where the investigation was made, the traditional cereal staple, millet, is being increasingly replaced by the plantain.

The area is intensely malarious and in a child health survey 88% of infants and preschool children were found to have malarial parasites (D. B. Jelliffe, personal communication). The incidence of intestinal parasites was also high (mainly hookworm) and scabies and 'sores' were common.

In Bukedi 19% of the population is under the age of 5 years. The 241 children studied, in May 1965, were of the Padhola tribe which is of nilotic origin. The adults of this group are tall and heavily built and their skin colour, like that of the Karamojong, is somewhat darker than that of the Ganda.

Assessments

Each child was examined clinically, and the results were recorded on a pre-arranged form by circling the appropriate item or filling in the necessary information. This was satisfactory in most instances, but the categories for changes in the colour of the hair and skin gave trouble, and the results must be regarded as subjective. Unfortunately it was not possible for the same clinician to take part in all three surveys, but each used the definitions given by Jelliffe (1966) as the basis for his clinical assessment of the signs of malnutrition.

Measurements were made of the weight of the body, standing height or recumbent length according to age, circumference of the head, chest and mid upper arm, and skinfold thickness over the triceps. All the measurements, with the exception of weight and length, were made by the same observer. Weight was measured with an Avery platform scale with steel-yard indicator weighing to 50 g, height with a Gneupel anthropometric rod and length with a lengthboard graduated in mm. The circumferences of the head and chest were measured with a narrow flexible steel tape, and that of the mid upper arm with a cloth tape. Harpenden callipers were used for triceps skinfold thickness.

For statistical purposes, children without birth certificates were grouped according to the age given by the parents, except when this conflicted strongly with the stage of dental development and the height of the child. Nine of the Karamojong children were regrouped in this way into an older age group.

For the purposes of the regression analysis each anthropometric measurement for each individual child was expressed as a percentage of a standard of reference. In the case of weight, standing height, recumbent length and head and chest circumference the figures given by Stuart & Stevenson (1954), for children of north European descent, were used. The standard of reference used for triceps skinfold was that of Tanner & Whitehouse (1962) for British children, and for the circumference of the mid upper arm the values of Wolansky, for Polish children quoted by Jelliffe (1966).

Weight, height, head and chest circumference measurements were expressed as a percentage of standard values only for the children whose ages were known accurately. For triceps skinfold and the circumference of the mid upper arm, however, the measurements of all children were used as after the 1st year of life, when age assessment may become a problem, until the 5th year the change in the standard of reference for triceps skinfold is not more than 0·6 mm per annum, involving a maximum possible error of 6%, and that for the circumference of the mid upper arm is not more than 0·5 cm per annum, involving a maximum possible error of 3%. The normal range of the anthropometric measurements has been taken as the range between the 97th and 3rd percentile.

The concentration of albumin and the amino acid ratio were determined, on finger-prick samples of blood collected into heparinized capillary tubes, by the method of Reinhold (1953) and Whitehead (1964) respectively. The method of Prockop & Undenfriend (1960), as organized by Howells & Whitehead (1967) was used for the hydroxyproline index in the urine.

The amino acid ratio is a semiquantitative estimate of the relative concentrations of two groups of amino acids in the serum:

$$\text{Amino acid ratio} = \frac{\text{glycine} + \text{serine} + \text{glutamine} + \text{taurine}}{\text{leucine} + \text{isoleucine} + \text{valine} + \text{methionine}}.$$

The hydroxyproline index is a measurement of the excretion of hydroxyproline relative to creatinine. The introduction of the weight factor results in a constant normal range independent of age between 1 and 6 years.

$$\text{Hydroxyproline index} = \frac{\mu\text{moles hydroxyproline} \times \text{body-weight in kg}}{\mu\text{moles creatinine}}.$$

The normal range of values of the hydroxyproline index in children aged up to 6 years is from 2·0 to 5·0 with a mean value of 2·9 (Howells & Whitehead, 1967). Control values of the amino acid ratio for African children were obtained from 195 children who had been successfully treated for kwashiorkor and marasmus. The mean value was 1·9 with a standard deviation of $\pm 0{\cdot}5$. The normal range has been taken as the mean ± 2 SD; thus values of 3·0 or above are regarded as abnormal. These values are higher than those found in healthy European children, in whom the mean value was 1·5 (Whitehead & Dean, 1964).

RESULTS

The various clinical, anthropometric and biochemical measurements are compared separately in the three areas studied. There was no statistical sampling of the population since the aims of the experiment did not make it necessary. There is no suggestion that the same kinds of children were seen in each area and therefore no comparison is made, one district against another. Neither the environmental conditions nor the general clinical picture are constant throughout the year; the results merely describe the situation at the time of the investigations.

Clinical examination

There were important differences in the appearance of the children from the three samples as well as wide variations in the clinical assessments and anthropometric measurements within each group, especially at different ages. It is in the light of these differences that the biochemical results must be interpreted and for this reason the children are described in some detail in Tables 1 and 2.

Table 1 gives details for all three groups of the incidence of clinical kwashiorkor and marasmus and of four clinical signs associated with protein-calorie malnutrition.

Buganda. In Buganda 74% of the children had no clinical signs attributable to malnutrition. One child was seen who had kwashiorkor but the most common finding was hair changes which were present in 24% of the children. These changes mainly took the form of slight dispigmentation, but not to the same degree as found in clinical kwashiorkor. Few children had respiratory or malarial infections at the time of the survey but about 10% of the children had infections of the skin, mainly scabies.

Table 1. *Incidence, in children in Uganda, of kwashiorkor and marasmus and of four clinical signs associated with protein-calorie malnutrition*

Group	Age range (months)	No. in group	Kwashior-kor	Maras-mus	Hair changes	Skin changes	Moon-face and/or oedema	Wast-ing	Total no. of children without clinical signs
Buganda	0–6	25	0	0	0	0	0	0	25
	7–12	36	0	0	13	2	0	0	23
	13–24	43	1	0	12	3	2	1	29
	25–36	29	0	0	7	1	2	1	21
	Total	133	1	0	32	6	4	2	98
Karamoja	0–6	56	0	0	5	1	1	2	49
	7–12	51	0	2	9	1	0	7	37
	13–24	85	0	1	15	6	0	24	55
	25–36	72	0	1	2	3	0	23	49
	37–48	34	0	0	4	1	0	16	17
	Total	298	0	4	35	12	1	72	207
Bukedi	0–6	32	0	0	13	3	0	0	19
	7–12	37	0	0	26	15	4	0	11
	13–24	69	0	0	55	36	11	0	14
	25–36	53	0	0	31	23	7	0	22
	37–48	50	0	0	33	16	3	0	17
	Total	241	0	0	158	93	25	0	83

Karamoja. In Karamoja 70% of the children had no obvious clinical signs of malnutrition although nearly all the children appeared to be a little thin. Four children with very severe wasting were diagnosed as marasmic but a very much larger number (24%) were clinically wasted. Only 12% of the children had hair changes, and skin changes were even less frequent. Conjunctivitis was found in 80% of the children, but only 6% of the children had a palpable spleen. Nearly one-third of the children (29%) had a history of diarrhoea and 20% of upper respiratory tract infections.

Bukedi. In Bukedi no clinical kwashiorkor or marasmus was seen, but 66% of the children had hair changes, again mainly reduced pigmentation, and 39% showed slight skin changes. A 'moonface' appearance was described in 10% of the children but none were found to be clinically wasted. In all children with a 'moon face' oedema was also suspected. Only 34% had no clinical signs associated with malnutrition but it is emphasized that never were these changes serious enough for a clinical diagnosis of malnutrition. The spleen was palpable in 16% of the children.

Infections of the skin, mainly scabies and impetigo, were diagnosed in 20% of the children and 16% of the children had signs of eye infection. Seventeen per cent of the children had a history of abdominal pain but very few a history of diarrhoea. There was little respiratory infection.

Anthropometric measurements

Table 2 gives the mean values and standard deviations of the anthropometric measurements in various age groups in the three populations. As accurate ages were not available for all the children, the mean age for each group has not been calculated but has been taken as the mid point of the age range.

Table 2. *Mean values and standard deviations for the various anthropometric measurements made on children in Uganda*

Group	Age range (months)	Weight (kg)		Height (cm)		Head circumference (cm)		Chest circumference (cm)		Triceps skinfold (mm)		Mid upper arm circumference (cm)	
		No.	Value	No.	Value	No.	Value	No.	Value	No.	Value	No.	Value
Buganda	0–6	25	5·5 ± 1·1	25	57·7 ± 3·8	23	39·9 ± 3·1	23	38·3 ± 4·6	23	7·4 ± 1·9	22	12·3 ± 2·1
	7–12	36	7·9 ± 1·3	36	67·9 ± 3·8	29	44·4 ± 1·4	29	43·3 ± 2·4	29	8·2 ± 1·5	29	14·0 ± 1·4
	13–24	43	9·7 ± 1·5	43	75·2 ± 4·5	17	46·5 ± 1·7	17	45·5 ± 2·6	17	7·8 ± 1·4	17	14·0 ± 1·1
	25–36	29	11·3 ± 1·3	29	82·5 ± 6·0	11	48·5 ± 1·5	11	50·2 ± 1·9	11	8·3 ± 1·5	11	14·9 ± 0·7
Karamoja	0–6	56	6·1 ± 1·4	56	61·6 ± 5·2	56	41·7 ± 3·4	56	39·2 ± 3·4	56	9·1 ± 1·8	56	13·5 ± 1·8
	7–12	51	7·3 ± 1·0	51	69·6 ± 4·6	51	44·8 ± 1·2	51	42·2 ± 2·5	51	7·8 ± 1·8	51	13·3 ± 1·3
	13–24	85	10·4 ± 1·7	85	81·1 ± 6·1	85	47·2 ± 1·8	85	45·9 ± 2·9	84	7·5 ± 1·5	85	13·6 ± 1·1
	25–36	72	11·6 ± 1·7	72	91·3 ± 7·1	72	48·2 ± 1·4	72	47·9 ± 2·2	72	6·9 ± 1·7	72	13·9 ± 1·3
	37–48	34	13·5 ± 1·9	33	99·1 ± 6·6	34	49·4 ± 1·3	34	49·8 ± 3·6	33	6·3 ± 1·7	34	14·0 ± 1·0
Bukedi	0–6	32	5·9 ± 1·0	32	59·3 ± 2·8	32	41·2 ± 3·4	32	41·2 ± 4·6	32	8·0 ± 1·6	32	13·7 ± 1·8
	7–12	37	7·7 ± 1·3	37	67·3 ± 3·5	37	45·1 ± 2·2	37	44·0 ± 2·8	37	8·6 ± 3·2	37	14·2 ± 1·3
	13–24	69	9·6 ± 1·0	69	76·7 ± 3·6	69	47·0 ± 2·1	69	48·3 ± 2·2	69	8·6 ± 2·6	69	14·6 ± 1·1
	25–36	53	11·9 ± 1·4	53	84·9 ± 4·5	53	48·3 ± 2·6	53	50·8 ± 3·4	52	9·6 ± 2·6	53	15·5 ± 1·3
	37–48	50	13·7 ± 1·9	50	92·0 ± 5·4	50	49·4 ± 1·4	50	52·4 ± 2·8	49	9·6 ± 2·5	50	15·9 ± 1·0
Standard (sexes combined)	3		5·7		60·0		40·4		40·2		8·1		13·0
	9		8·9		70·7		45·3		45·7		10·1		15·5
	18		11·3		81·4		47·9		49·1		10·2		15·9
	30		13·5		91·8		49·5		51·4		9·8		16·4
	42		15·5		99·7		—		52·8		9·6		16·4

* For source of standard values see p. 4.

Buganda. In Buganda the mean values for all the anthropometric measurements were below the standard values in all age groups. Expressed as a percentage of the standard values, the mean values, however, decreased with age for all measurements except head and chest circumference. The greatest fall was in weight from 96% for the 0–6 months age group to 84% for the 25–36 months age group. Over the same age range height fell from 96 to 90%, triceps skinfold from 91 to 85% and mid upper arm circumference from 95 to 91%.

Karamoja. In Karamoja the mean values for all anthropometric measurements, except chest circumference, were above the standard values in the 0–6 months age group, but in the older age groups they were always less than the standard. The mean values, expressed as a percentage of the standard values, decreased with age except in the case of head and chest circumference. The most striking fall was in triceps skinfold from 112% in the 0–6 months age group to 66% in the 37–48 months age group. Weight and mid upper arm circumference fell from 107 to 87% and 104 to 85% respectively, but the change in height was only relatively small, from 103 to 99%.

Bukedi. In Bukedi, in the 0–6 months age group, the mean values for all measurements, except height and triceps skinfold, were greater than the standard values, but, again, in the older age groups the mean values for all measurements were below these values. In this area the greatest change with age, was in weight which fell from 103% in the 0–6 months age group to 88% in the 37–48 months age group. Over the same range, mid upper arm circumference fell from 105 to 97% and height from 101 to 94%. Head, chest and triceps skinfold values, as a percentage of the standard values, however, altered little with age.

Biochemical measurements

Table 3 gives the means and standard deviations for the biochemical findings at various ages in each of the three communities.

Buganda. In Buganda plasma albumin was measured in only fifty-three of the children. The mean values in all age groups were similar and at the upper limit of the

Table 3. *Mean values and standard deviations for the biochemical measurements* made on children in Uganda*

Group	Age range (months)	Plasma albumin (g/100 ml)		Amino acid ratio		Hydroxyproline index	
		No.	Value	No.	Value	No.	Value
Buganda	0–6	14	5·6 ± 0·6	25	1·9 ± 0·5	23	3·1 ± 1·3
	7–12	18	5·8 ± 0·6	35	2·3 ± 0·8	35	2·8 ± 1·2
	13–24	12	5·3 ± 1·0	38	2·9 ± 0·9	41	2·1 ± 0·8
	25–36	9	5·7 ± 0·7	26	3·2 ± 1·0	29	2·1 ± 0·7
Karamoja	0–6	46	6·1 ± 1·4	51	2·0 ± 0·5	53	2·7 ± 1·3
	7–12	45	6·5 ± 1·1	49	2·0 ± 0·6	50	1·4 ± 0·8
	13–24	66	5·8 ± 1·2	76	2·2 ± 0·7	82	1·6 ± 0·9
	25–36	62	6·3 ± 1·4	53	2·3 ± 0·8	71	1·6 ± 0·8
	37–48	28	6·1 ± 1·3	31	2·1 ± 0·7	33	1·4 ± 0·6
Bukedi	0–6	29	3·6 ± 1·3	31	2·3 ± 0·8	30	3·1 ± 1·3
	7–12	35	3·6 ± 1·3	37	2·6 ± 0·9	29	2·2 ± 1·1
	13–24	58	3·1 ± 0·9	69	2·4 ± 0·8	54	2·1 ± 1·1
	25–36	46	3·3 ± 1·1	36	2·6 ± 1·1	51	1·9 ± 0·7
	37–48	42	3·0 ± 0·9	37	2·8 ± 1·0	48	1·9 ± 0·9

* For standard values see p. 5.

normal range. The high plasma albumin values found both in Buganda and Karamoja are difficult to explain, but it can only be stressed that the determinations were carried out by the same person in the three areas with frequent, concurrent analysis of control sera. The amino acid ratio, however, altered with age. In the 0–6 months age group it was similar to the control value, but thereafter it was significantly higher than this, rising to over 3·0 in the 3rd year ($P < 0·001$). Of the children aged over 1 year, 47% had an amino acid ratio greater than 3·0. The mean values for the hydroxyproline index were normal in the 1st year, but in the 2nd and 3rd years they were significantly lower than the normal ($P < 0.001$). Thirty-six per cent of all the children studied had an index less than 2·0.

Karamoja. In Karamoja plasma albumin values were a little higher, even, than in Buganda, but did not alter with age. The amino acid ratio was essentially normal and significantly greater than the control value only in the 3rd year ($P < 0.01$). Only 14% of the children over 1 year of age had an amino acid ratio greater than 3·0. The hydroxyproline index, however, was normal only in the children aged 0–6 months. In the older age groups it was significantly below the normal value ($P < 0.001$). Seventy-one per cent of all the children, in fact, had an index of less than 2·0.

Bukedi. In Bukedi the mean albumin values in all age groups were at the lower limit of the normal range and 40% of all the children had albumin values of less than 3·0 g/100 ml. The amino acid ratio increased with age and was significantly higher than the control value in all age groups ($P < 0.01$–$P < 0.001$). Thirty-two per cent of the children aged over 1 year had an amino acid ratio greater than 3·0. The mean hydroxyproline index for the children aged 0–6 months was normal, but in all the older groups it was significantly lower than the normal ($P < 0.001$). In 46% of all the children studied the index was less than 2·0.

Correlations between anthropometric and biochemical findings

Linear regression analyses of the biochemical results on the anthropometric measurements have been carried out. There were also statistically significant interrelationships between the various anthropometric measurements, but these are not described, since they lie outside the scope of this paper. Table 4 gives the coefficient of correlation (r) and the level of significance (P) for the associations between the amino acid ratio and the hydroxyproline index and some anthropometric and biochemical measurements in each of the three survey areas.

Buganda. In Buganda there were significant correlations between the hydroxyproline index and each of the anthropometric measurements, but the highest degree of association was with weight for age and mid upper arm circumference. There was also a significant correlation between the hydroxyproline index and plasma albumin. The only significant correlations between the amino acid ratio and the various measurements were with the hydroxyproline index and the triceps skinfold thickness.

Karamoja. In Karamoja it was not possible to carry out a regression analysis for the hydroxyproline index with weight for age or height for age but with the remaining anthropometric measurements there was a highly significant degree of association. There was no significant correlation between the hydroxyproline index and plasma albumin. The only significant correlations with the amino acid ratio were with serum albumin and triceps skinfold.

Bukedi. In Bukedi there was a significant correlation between the hydroxyproline index and all anthropometric measurements except triceps skinfold, the highest degree of association was with weight for age and mid upper arm circumference. There was no correlation between the hydroxyproline index and plasma albumin, and there was also no correlation between the amino acid ratio and any of the other measurements.

Table 4. *Coefficients of correlation* (r) *and level of significance* (P) *for the association between the hydroxyproline index and amino acid ratio, and some anthropometric and biochemical measurements in each of the three samples of children in Uganda*

	Buganda			Karamoja			Bukedi		
Association	No.	*r*	*P*	No.	*r*	*P*	No.	*r*	*P*
Hydroxyproline index									
Weight for age	95	0·5569	< 0·001	—	—	—	82	0·4067	< 0·001
Height for age	95	0·2597	< 0·02	—	—	—	82	0·3349	< 0·01
Weight for height	95	0·4580	< 0·001	226	0·3637	< 0·001	82	0·2872	< 0·01
Mid upper arm circ.	50	0·6133	< 0·001	226	0·4070	< 0·001	82	0·4181	< 0·001
Triceps skinfold	50	0·4291	< 0·01	226	0·4028	< 0·001	82	0·1401	NS
Albumin	50	0·3372	< 0·02	226	0·0363	NS	82	0·1986	NS
Amino acid ratio									
Weight for age	57	0·2322	NS	—	—	—	57	0·0181	NS
Height for age	57	0·1725	NS	—	—	—	57	0·0097	NS
Weight for height	57	0·2170	NS	142	0·0888	NS	57	0·0033	NS
Mid upper arm circ.	21	0·2897	NS	142	0·0084	NS	57	0·1411	NS
Triceps skinfold	21	0·4745	< 0·05	142	0·1753	< 0·05	57	0·0242	NS
Albumin	21	0·3416	NS	142	0·2610	< 0·01	57	0·0817	NS
Hydroxyproline index	57	0·3916	< 0·01	142	0·0147	NS	57	0·0983	NS

NS = relationship not significant, $P > 0·05$.

DISCUSSION

The districts of Buganda, Karamoja and Bukedi were chosen for the investigation because it was already known that there were likely to be differences in nutritional status between them which might be placed on a quantitative basis by the biochemical tests at our disposal. This has turned out to be the case.

Buganda

The nature of the food and the traditional pattern of the diet in Buganda are known to be associated with protein malnutrition in children aged from 1 to 3 years. The clinical examination, however, gave no indication of protein deficiency in the majority of children investigated. The anthropometric findings showed that the Buganda children were smaller than the reference children, but because the children in Buganda are known to be somewhat small during the first 6 months of life, when dietary intake is good, it was difficult to know whether the low weights found in the older age groups were due to a different genetic potential for growth or to growth retardation resulting from a deficient diet. When the Boston standards were used as the basis of reference, 27% of the children had weights below the normal range, but with the growth curve for healthy Buganda children (Rutishauser, 1965) only 12% of the weights would be considered abnormal. These facts illustrate well the difficulty in interpreting anthropometric results.

The biochemical findings, however, supported the interpretation that malnutrition was present at a subclinical level in many of the children. The large number of amino acid ratios which fell outside the normal range indicated the existence of protein deficiency, and the significant correlation between the hydroxyproline index and all anthropometric measurements lent support to these latter values being caused by growth retardation, presumably as a result of nutritional deficiency. The correlation between the hydroxyproline indices and plasma amino acid ratios is also typical of primary protein malnutrition (Whitehead, 1965). Plasma albumin values gave no indication of protein deficiency, but it is known that they may be little, if at all, reduced in marginal malnutrition (Waterlow, 1963).

A statistical relationship between the amino acid ratio and the anthropometric measurements was only proven in the case of triceps skinfold thickness, but the correlation coefficients indicate that had more children been investigated then a significant association would have been found. Recent, and as yet unpublished, studies in protein-deficient rats and pigs have indicated, however, that changes in the amino acid pattern in the serum are not directly linked with growth mtaebolism, as the excretion of hydroxyproline is, and hence any statistical correlation which might be found would be purely fortuitous.

Karamoja

The severe shortage of food in Karamoja at the time of the survey was evident from both the clinical examination and the anthropometric measurements. A quantitative assessment of the growth retardation, in terms of weight, was, however, not possible in the absence of accurate information on age, but about one-third of the children had triceps skinfold and mid upper arm circumference measurements which were below the normal range. The large numbers of abnormally low hydroxyproline indices and the significant correlation with the anthropometric measurements supported the view that the children were being adversely affected by their diet. The relatively normal amino acid ratios and their lack of correlation with the hydroxyproline indices is typical of nutritional marasmus (Whitehead, 1965). Plasma albumin values were high, higher sometimes than values found in healthy children, but similar values for serum protein levels have been found in quite advanced nutritional marasmus (Whitehead, 1967).

Bukedi

The dietary background in Bukedi was not clear cut and in many ways is representative of the conditions developing in other parts of the world where the traditional diet is being replaced by a prestige food. The clinical appearance of the children suggested protein deficiency and the anthropometric information indicated a slower rate of growth than in the reference children, but again it was difficult to make a quantitative assessment of growth retardation because ages were known in only half of the children.

Biochemically the picture was similar to that in Buganda except that nearly half of the children had low plasma albumin levels. The significant correlation between the hydroxyproline index and all the anthropometric measurements, except triceps skinfold thickness provided evidence that the growth metabolism in these children was abnormal. The reason for the absence of statistically significant correlations between the hydroxyproline index and the plasma albumin and the amino acid ratio is not clear but it could be related to a difference in the aetiology of the protein deficiency between this area and Buganda. For example, in Bukedi the incidence of hookworm infection is extremely high (D. B. Jelliffe, personal communication), in Buganda it is only moderate and in Karamoja low (Jelliffe *et al.* 1964). Thus in Bukedi, parasitic infestations may be an important factor in the protein deficiency, whereas in Buganda it is likely to be primarily dietary. Unfortunately, when these evaluation studies were planned, the need for information about parasite infestations and the haematological pattern, in the interpretation of the biochemical findings was not appreciated. This omission does emphasize, however, the value of field trials such as these in revealing the extent of the information which is required.

The difference in plasma albumin concentration between the Buganda children and those from Bukedi may shed some light on the controversy of whether or not the amino acid ratio is an earlier indicator of protein malnutrition than serum protein measurements. In Buganda this certainly seems to be so and this is in agreement with results obtained in rat experiments at present in progress. In Bukedi there was no evidence to suggest that the elevation of the amino acid ratios had preceded a fall in serum albumin, but this might again be due to the suspected difference in nutritional aetiology in the two areas. Reduced serum protein levels are found in children with hookworm infestations, and in kwashiorkor, complicated by hookworm anaemia, the values are particularly low (Whitehead, 1967). The interpretation of the serum albumin levels is complicated by the high values which were found in Buganda and Karamoja, but these were measured under carefully standardized conditions and there is no reason to doubt their authenticity.

The effects of infection and parasite infestation on the metabolic response to malnutrition have been discussed elsewhere (Whitehead, 1966, 1967). They represent a limitation to the use of biochemical tests in the assessment of nutritional status. Work is in progress to try and clarify the position. The present results do indicate, however, that the hydroxyproline index can amplify the clinical and anthropometric information, and in addition, the amino acid ratio can provide extra information about the nature of the dietary deficiency. The tests must not be used blindly, however, and any results need careful interpretation in relation to all environmental factors prevalent in the area being studied. The aim of the experiment was to see whether the biochemical tests would provide information which the clinical and anthropometric measurements did not. This was the case in all the three areas studied. In general there was a good statistical relationship between the hydroxyproline index and the anthropometric measurements but the individual values showed a wide distribution. This scatter is inevitable since biochemical tests reflect metabolic abnormalities at the time the sample is collected whilst anthropometry measures changes which have been developing for weeks.

These field trials have indicated the type of problems which might be encountered in more representative surveys of nutritional status. It is clear there are many difficulties to be resolved before the biochemical tests can be interpreted with absolute confidence, but this is equally true of the clinical examinations and anthropometric measurements, and it must be concluded that all three types of investigation are of value only if their limitations are clearly understood.

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