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The assessment of nutritional status in protein-malnourished children

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I have called my lecture 'The assessment of nutritional status in protein-malnourished children'. Under this title I include not only severely ill cases of kwashiorkor but also certain other children who have been living for a long period of time on an inadequate diet—that is, a diet commonly consumed by those showing clinical signs of disease. There is reason to believe that some of these children may be adversely affected, at a subclinical level, and it is the aim of my research programme to develop biochemical tests which can establish their state of malnutrition on an objective basis.

This subclinical condition is referred to by many names including pre-kwashiorkor, subclinical kwashiorkor, marginal protein malnutrition, and the very names reflect how little specific knowledge we have. Indeed to be strictly accurate we do not know whether such a condition exists at all. I believe, however, that the evidence justifies the concept that many children in the world pass through a phase in which, because of the limited amount of protein in their diet, certain essential aspects of cellular function become abnormal. It is from this condition that clinical kwashiorkor may precipitate. It is also possible that such children have a reduced resistance to disease and this could be one of the reasons for the high child mortality found in many developing countries.

Not all of the work I did in Uganda was directly associated with this subject but as time went on I became more and more obsessed with the problem and this lecture describes how this came about.

I first went to Uganda in 1959, to work with the late Professor R. F. A. Dean. Before this time, the work in the unit had concentrated on two main problems. The first of these was proving that the dietary cause of kwashiorkor definitely was primary protein deficiency, and not the lack of a specific amino acid or vitamin. Dean followed this up by studies designed to characterize the pathological changes of severe kwashiorkor with the ultimate aim of improving treatment, both dietary and other therapies. Dean was, however, already actively concerned with less severely malnourished children, and this led to his interests in the development

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of cheap protein supplements for public health preventive medicine projects. But we needed to know which children were in the most need, and what was equally important, how we could assess the efficiency of these therapeutic measures. Clearly if we could demonstrate a metabolic abnormality in these children which reflected their malnourished status we would have a valuable tool. It is sobering to realize that after 8 years we still have not produced a diagnostic test which is really suitable for this purpose.

One reason for this relative failure is the basic problem of studying an abnormality which is not well defined. Kwashiorkor itself, is, of course, characterized by the presence of various clinical signs but clearly this is not the case with subclinical kwashiorkor. The line of attack we adopted, and I think it still makes some sense, was to study metabolic changes in well-defined kwashiorkor and then to 'extrapolate back' in severity, through early kwashiorkor, to children known to be living on inadequate diets, but showing no definite clinical signs, to see if the abnormality was still present. At this time I considered it would be reasonable to diagnose a child as malnourished if he showed some of the metabolic derangements of severe kwashiorkor even if he exhibited none of the clinical signs. I am not sure I would accept this line of reasoning now, certainly not in such a simple form.

Abnormalities in the metabolism of histidine

The first metabolic abnormality we investigated which it seemed might be of diagnostic value was concerned with the metabolism of histidine. An unusual compound was detected in the urine of children with severe kwashiorkor, this was isolated and identified as imidazoleacrylic acid (Whitehead & Arnstein, 1961). The metabolism of this compound is shown in Fig. 1.

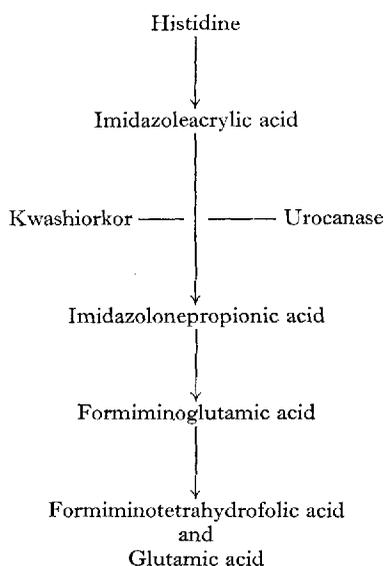


Fig. 1. A summary of the metabolism of histidine in kwashiorkor.

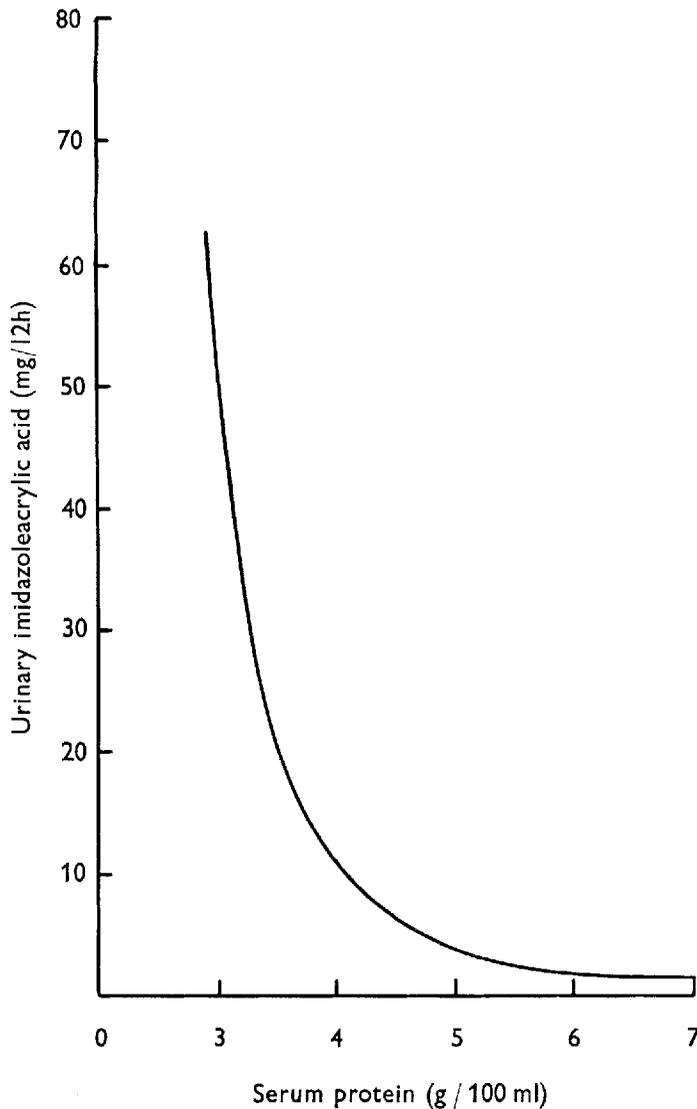


Fig. 2. The relationship between urinary imidazoleacrylic acid excretion and serum protein levels in protein-malnourished children.

It was concluded that imidazoleacrylic acid accumulated because of a deficiency of liver 'urocanase', and this has been confirmed by investigations in protein-malnourished rats (Rao, Deodhar & Hariharan, 1965). Imidazoleacrylic acid can easily be detected, following paper chromatography, either by photography in ultraviolet light, or by the colour formed on coupling the compound with diazotized sulphanilic acid. If it is present in quantity it can be estimated merely by scanning a diluted sample of urine through the ultraviolet range: imidazoleacrylic acid has a maximum absorption at 270 nm at pH 1. We had then at least one valuable charac-

teristic for a diagnostic test, an easy method of analysis. Unfortunately, subsequent work proved that the metabolic abnormality was confined to severely ill children (Whitehead, 1964a). When we 'extrapolated back' to less severely malnourished children we were unable to detect the compound. Fig. 2 shows imidazoleacrylic acid excretion plotted against serum total protein. The latter is a rough index of the severity of primary protein malnutrition and little imidazoleacrylic acid was excreted in children with protein levels above 4.5 g per 100 ml. Similar findings have been reported in Ethiopia (Björnesjö, Belew & Zaar, 1966).

Although the compound might not be present in fasting samples of urine collected from marginally malnourished children we wondered if it could be induced by a test load of histidine. In severely ill children this test dose did produce a greatly increased excretion of imidazoleacrylic acid, but unfortunately the same load had no effect in either early kwashiorkor or on suspected protein-malnourished cases. This approach has recently been repeated in Guatemala with similar results (Halbricht, private communication).

I have described this experiment in some detail because it illustrates well the 'extrapolation' principle on which our investigations were based. Although this work did not fulfil my wider hopes of a test for marginal malnutrition, it did confirm that major changes in intermediary metabolism do occur in protein malnutrition. This investigation has not completely failed to find practical application, however, and this, strangely enough, has been in England. Neale, Antcliff, Welbourn, Mollin & Booth (1967) at the Hammersmith Hospital have studied imidazoleacrylic acid excretion, after a histidine test dose, in the kwashiorkor-like state which occurs in certain adult patients after partial gastrectomy. They found it a useful index for measuring progress during treatment and it is reassuring to know that biochemical work developed in Africa can be of value in a London hospital.

The metabolism of phenylalanine and tyrosine

After these histidine studies, we went on to investigate abnormalities in phenylalanine and tyrosine metabolism (Whitehead & Milburn, 1962). It was already suspected that there might be a reduced ability to convert phenylalanine into tyrosine and we were able to confirm that this was so. The metabolism of phenylalanine is summarized in Fig. 3. A test dose of phenylalanine led to the excretion of phenylpyruvic acid and phenylalanine in cases of severe kwashiorkor, but not in the same children after treatment. The catabolism of tyrosine was also abnormal and *p*-hydroxyphenylpyruvic, *p*-hydroxyphenyllactic and *p*-hydroxyphenylacetic acids were also excreted (Whitehead & Milburn, 1964). In the serum the ratio of phenylalanine to tyrosine was also abnormally high, although this was mainly due to low tyrosine values. Various workers have in fact suggested that the measurement of serum tyrosine might be a useful index of nutritional status. Unfortunately, as Table 1 shows, when we followed our 'extrapolation procedure' we found that this abnormality also was confined to severely malnourished children. Even a test dose of phenylalanine failed to reveal any abnormalities in less severe cases.

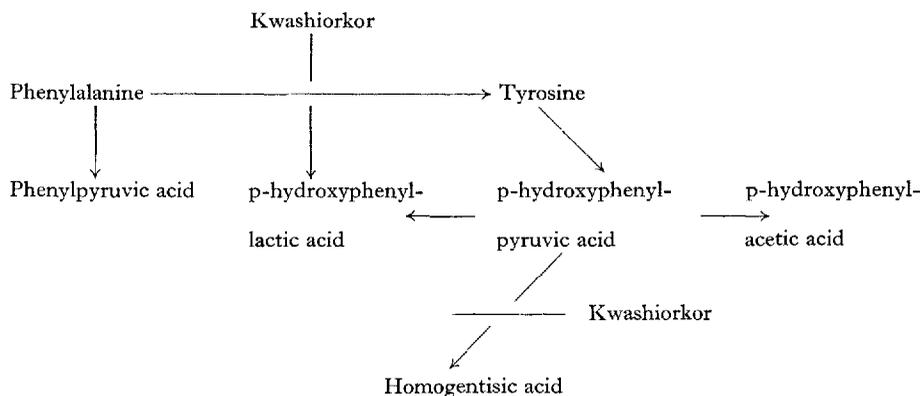


Fig. 3. A summary of the metabolism of phenylalanine and tyrosine in kwashiorkor.

Halbricht has come to the same conclusion in Guatemala (private communication).

It is inevitable that people should have speculated on the relationship between this biochemical abnormality and the pale hair and mental changes which are common to both kwashiorkor and phenylketonuria. It is most unlikely that the pale hair in kwashiorkor is a result of a fault in this mechanism since hair changes are apparent long before the biochemical abnormality can be detected. It is not so easy to answer the question of the mental changes, but apathy is described in very early cases and again it is unlikely that this could be related to an abnormality in phenylalanine metabolism. It is of course possible that when this abnormality does appear it could produce some superimposed effect.

Table 1. Serum tyrosine levels and phenylalanine to tyrosine ratios in children malnourished to different degrees

Severity	Serum protein (g/100 ml)	Serum tyrosine (μ M/L)	Ratio, phenylalanine: tyrosine
Severe kwashiorkor	<4.5	20	2.6
Early kwashiorkor	5.6-6.0	72	0.6
'Subclinical' malnutrition	6.5-6.8	61	0.7
Treated cases	6.5-7.5	83	0.7

Serum amino acid patterns

So far, then, we had failed to find any metabolic defect in severe kwashiorkor which could also be found in earlier cases of malnutrition. During some previous investigations I had noticed that the relative proportions of various amino acids in the serum were abnormal in children with kwashiorkor. This was not a new finding; Holt, Snyderman, Norton, Roitman & Finch (1963), in particular, have studied this phenomenon in serum samples sent to them from various countries. Our interest in this metabolic defect, however, was greatly stimulated when I applied our policy of 'extrapolation' to the problem. We found that not only was the pattern distorted in severe kwashiorkor, and in early cases, but also in children whom our

clinicians regarded as potential cases of kwashiorkor (Whitehead & Dean, 1964*a,b*). At last we had something to work with.

This distorted pattern of serum amino acids has often loosely been described as the imbalance caused by abnormally low concentrations of the essential amino acids in the presence of normal or even elevated levels of the non-essential ones. This is not quite true, as Table 2 shows. The fact that phenylalanine and histidine concentrations are not depressed could possibly be due to the abnormalities in their catabolism described above.

Table 2. *Changes in the concentrations of certain free amino acids in the serum of children with kwashiorkor*

Greatly reduced levels	Slightly reduced	Little change
Leucine	Lysine	Histidine
Isoleucine		Phenylalanine
Valine		
Methionine		
Threonine		
Tryptophan		

If this abnormal pattern was to be investigated in large numbers of children it was obviously necessary to develop a screening method suitable for analysing serum samples collected under rural conditions. Only small quantities of blood could be collected if the co-operation of the mothers was to be maintained. The analytical technique had to be so designed that it required only simple apparatus; simple apparatus was all that was available. I developed a paper chromatographic method (Whitehead, 1964*b*), which separated two groups of amino acids: one consisted mainly of the branched-chain amino acids, whose concentration is particularly reduced in protein malnutrition; the other group contained glycine, serine, glutamine, and taurine. A diagrammatic representation of a chromatogram is shown in Fig. 4. The results were expressed as a ratio of the extinctions of the salmon pink, copper-ninhydrin derivatives: the more distorted the amino acid pattern, the higher is the ratio. African children who had been successfully treated for kwashiorkor had a mean ratio of 1.9 and the scatter of values showed a normal distribution, with a range between 1.0 and 2.9. European children had somewhat lower values with a mean of 1.5, and a maximum ratio of 2.0.

The amino acid ratios that we obtained for children attending our clinic are shown in Fig. 5. These values are obviously skewed to the right and about 40% of the ratios are above 3.0. It is of interest that an almost identical distribution of amino acid ratios has been found by G. Arroyave in statistically sampled children in rural Honduras (private communication).

We related these amino acid ratio values to other measurements, carried out in the same children. Linear regression analysis demonstrated a correlation with the weight deficit, when body-weights were expressed as percentages of the Boston standards (Stuart & Stevenson, 1954).

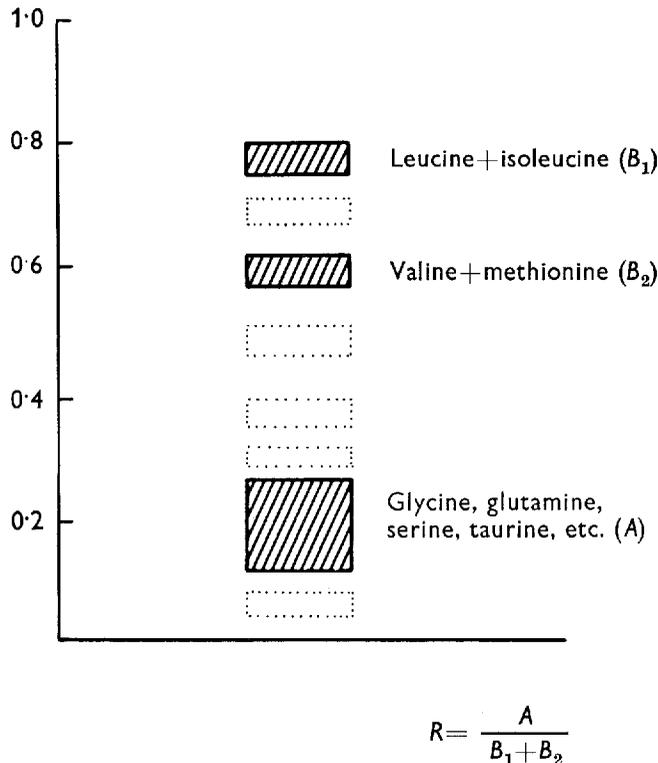


Fig. 4. A diagrammatic representation of the chromatographic separation of the amino acids used in the calculation of the amino acid ratio. The scale shows the approximate R_f values.

Whilst none of the children showed any definite pathological signs, our clinicians expressed the opinion that certain features were not absolutely normal. Statistical analysis demonstrated that children with abnormal amino acid ratios did tend to have more discoloration of hair, and children diagnosed as potential cases of kwashiorkor also had statistically higher ratios, but as might be expected when a subjective impression is correlated with a biochemical measurement, the scatter of results was wide.

Although these investigations indicated that the distorted pattern of amino acids in the serum was related to malnutrition they revealed nothing of the metabolic cause of the abnormality nor did they provide information about what the appearance of an elevated amino acid meant in terms of cell and tissue metabolism and whole body function. Clearly these are questions which must be answered, and this is partly what I have been trying to do in Cambridge.

The method for estimating the amino acid ratio was first published as a preliminary communication. We hoped it would result in this metabolic abnormality being investigated in many parts of the world where protein malnutrition was a problem but where apparatus and skilled technicians were at a premium. This has certainly occurred and the results have been most interesting. Not all people have

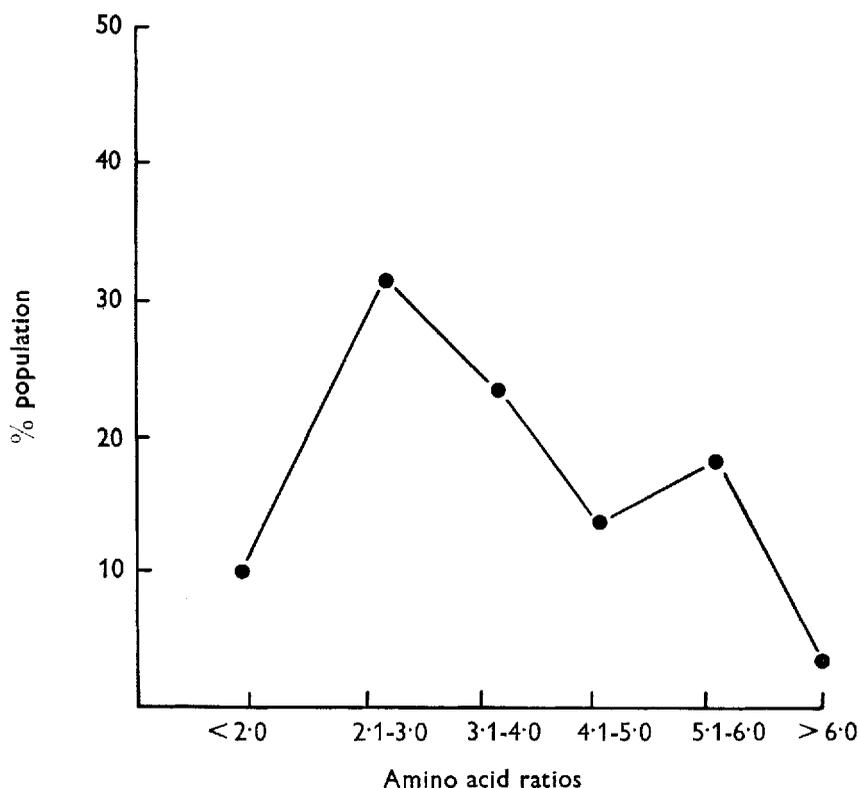


Fig. 5. The distribution of amino acid ratios in children attending a child welfare clinic near Kampala, Uganda.

been able to confirm our findings in Uganda, and whilst it is pleasant when people do agree with us, it is the conflicting results which have proved the most instructive. Table 3 shows some of the countries where the amino acid ratio has been measured. The list is by no means complete. The United Kingdom appears because of the work at the Hammersmith hospital on adults developing a kwashiorkor-like condition, following partial gastrectomy. Elevated amino acid ratios appeared as the clinical signs were developing and disappeared with successful therapy, just like they do in children.

Table 3. *A list of some of the countries in which the amino acid ratio has been measured, with details on the agreement of the findings with Uganda results*

General agreement	Disagreement	Authors
Ethiopia		Bjönesjö <i>et al.</i> (1966)
Guatemala		Arroyave & Bowering (1968)
Honduras		G. Arroyave (1968, private communication)
India		Ittyerah, Pereira & Dumm (1965)
United Kingdom		Neale <i>et al.</i> (1967)
Indonesia		Hin, Rose, Muhilal & Zuraida (1967)
Kenya		Simmons (1968, private communication)
	South Africa	Truswell <i>et al.</i> (1966)
	Jordan	McLaren <i>et al.</i> (1965)

The investigations of Truswell, Wannenburg, Wittmann & Hansen (1966) in Capetown and McLaren, Kamel & Ayyoub (1965) in Jordan are important because both workers have attempted to explain why their results differ from ours in Uganda. In the area around Kampala, the dietary aetiology of kwashiorkor is fairly simple since the type of food eaten varies little and is largely based on the low-protein food, matooke, or steamed plantain. In Capetown, and in Jordan, the diet is by no means so constant, and an additional complication is that kwashiorkor is often preceded by an outbreak of infective diarrhoea and vomiting. This would bring with it an acute episode of calorie deficiency and under these circumstances an elevated amino acid ratio would not be expected, since studies in man and in animals have shown that the distorted pattern of amino acids occurs to a very much greater extent in primary protein malnutrition than it does in undernutrition caused by too few calories (Widdowson & Whitehead, 1966). This explanation may not be the reason for all conflicting results but it does emphasize an important point. The development of kwashiorkor is rarely, if ever, a simple process of chronic protein malnutrition. The child could be subjected to any number of infections during this period, any of which might distort the biochemical pattern found in simpler forms of protein malnutrition.

It is now about 4 years since I developed the amino acid ratio and, whilst it is undoubtedly of considerable theoretical interest, there are many fundamental problems to be solved before it can be interpreted with confidence.

Hydroxyproline metabolism

Part of the virtue, but also the limitation, of the amino acid ratio is that it only becomes abnormal in the type of malnutrition which develops into kwashiorkor. This is of course only part of the spectrum of protein-calorie malnutrition. I wanted to develop a test which would be of wider sensitivity.

A general feature of protein-calorie malnutrition is a reduced rate of growth. It is well known that in hormonal dwarfism there occurs a reduced excretion of hydroxyproline peptides in the urine and I wondered if the same might be true in nutritional dwarfism. A similar idea had occurred to Picou, Alleyne & Seakins (1965) in Jamaica, and to Anasuya & Rao (1966) in India, and we were all able to confirm this supposition, both in children with severe kwashiorkor, and in those with marasmus. In Uganda, we followed up these investigations by our now customary practice of studying the abnormality in less severely malnourished children. This work indicated that a reduction in hydroxyproline excretion occurred at an early stage (Whitehead, 1965).

If this abnormality was to be investigated in large numbers of children like the amino acid ratio had been, some way of overcoming the need for 24 h samples had to be devised; it would be impossible to collect timed samples, even for a shorter period, from young children living under rural conditions. It was natural to consider relating hydroxyproline excretion to urinary creatinine content. We found however that a simple ratio of hydroxyproline to creatinine fell progressively with

increasing age; for example, in normal children, the value was 0.6 at 6 months and only 0.2 at 4 years. The reason is that creatinine excretion rises with increasing size and musculature whilst hydroxyproline excretion shows much less marked changes over this age range. A value which varied with age was undesirable since age would rarely be known accurately and so we decided to introduce a body-weight factor into our calculation to counteract the effect of increasing creatinine excretion. This produced a value which, in normal children, was virtually constant over the age range of 6 months to 5 years. We called it the hydroxyproline index.

$$\text{Hydroxyproline index} = \frac{\mu\text{M hydroxyproline/ml}}{\mu\text{M creatinine/ml per kg body-weight}}$$

Values found in well-nourished European, African and Asian children ranged between 2.0 and 5.0 and the mean value was 2.9.

When the test was performed on children in our outpatient clinic many values were found below 2.0. We related these values to the weight deficit, as shown in Fig. 6, and we found a significant linear correlation. Since our clinic was in a

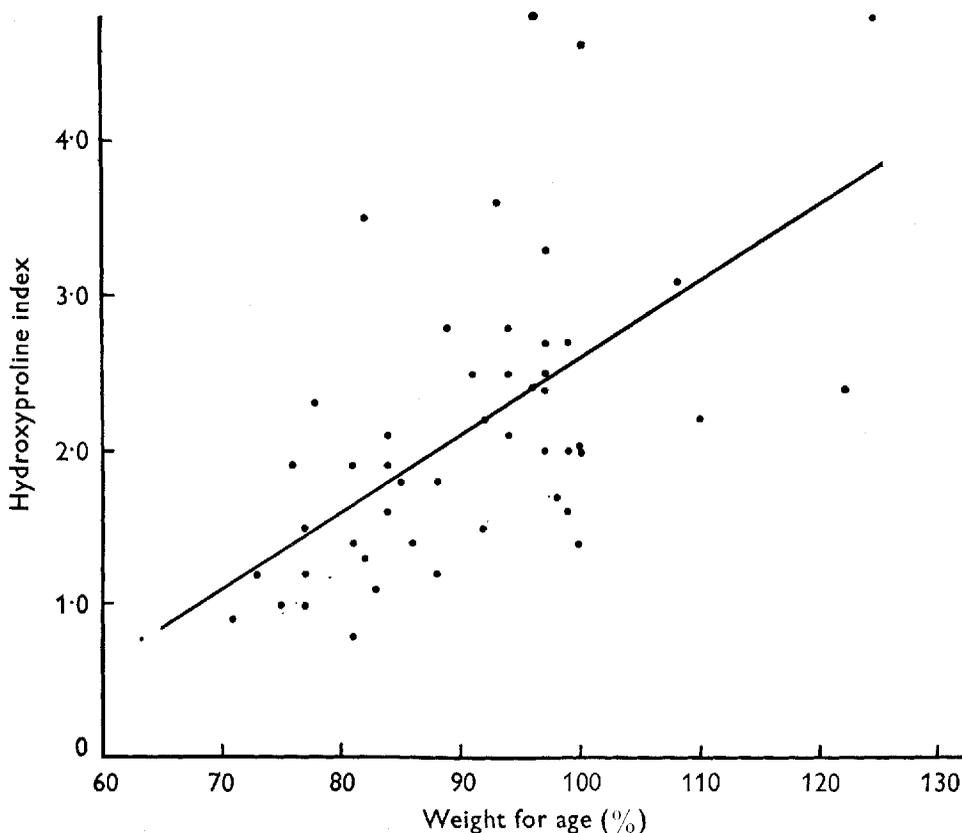


Fig. 6. The relationship between hydroxyproline indices and weight deficit in a group of children attending a child welfare clinic near Kampala, Uganda ($P < 0.001$).

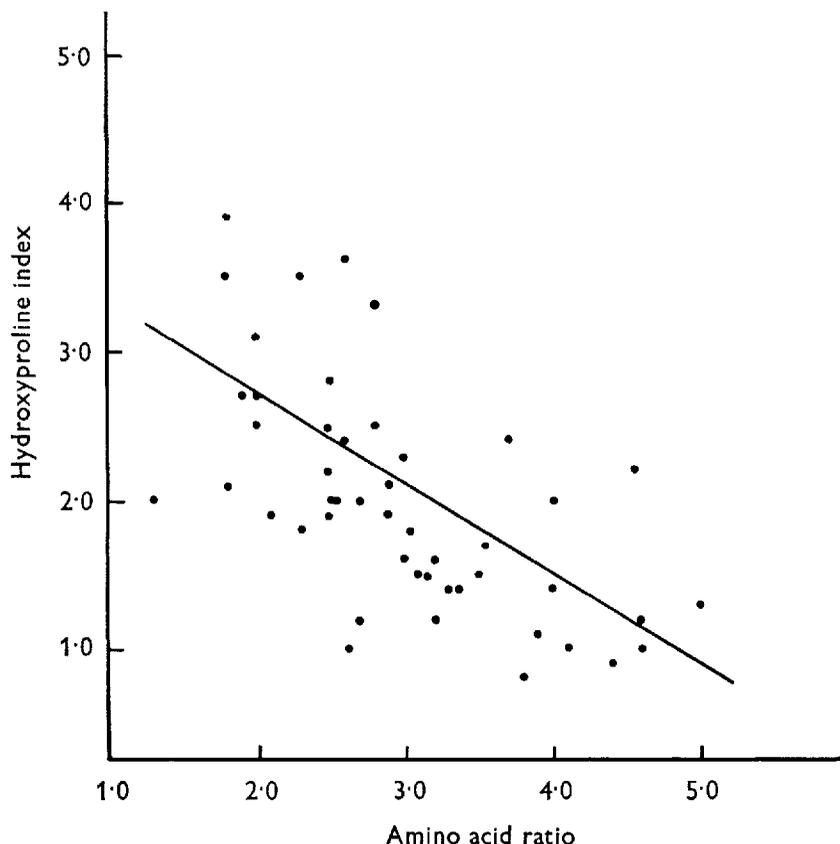


Fig. 7. The relationship between hydroxyproline indices and amino acid ratios in a group of children attending a child welfare clinic near Kampala, Uganda ($P < 0.001$).

kwashiorkor area we expected there should also be a relationship between the hydroxyproline index and the amino acid ratio, and Fig. 7 substantiates this assumption.

More recently we have carried out field trials of this and other tests in three areas of Uganda, and the results are given in Table 4. Buganda is a province in which primary protein deficiency is the common problem; Karamoja is a semi-desert area and, during droughts, a lack of total calories is the main deficiency; Bukedi is a district in which the dietary patterns are changing and the aetiology of malnutrition is far from simple. The hydroxyproline index was compared with various other biochemical and anthropometric parameters using linear regression analysis (Rutishauser & Whitehead, 1969).

There was a statistically significant correlation between the index and the anthropometric values in all three areas with the exception of triceps skinfold thickness in Bukedi. No analyses are given for weight and height in Karamoja because the parents did not know the ages of their children.

In Buganda the index was statistically related to both serum albumin and the amino acid ratio but in Karamoja no such correlation existed. Both these results

Table 4. *The relationship between the hydroxyproline index and various anthropometric and biochemical parameters in malnourished children in different areas of Uganda*

Index compared with:	Buganda			Karamoja			Bukedi		
	No.	<i>r</i>	<i>P</i>	No.	<i>r</i>	<i>P</i>	No.	<i>r</i>	<i>P</i>
Weight for age	95	0.56	<0.001	—	—	—	82	0.41	<0.001
Height for age	95	0.26	<0.02	—	—	—	82	0.33	<0.01
Weight for height	95	0.46	<0.001	226	0.36	<0.001	82	0.28	<0.01
Mid upper arm circumference	50	0.62	<0.001	226	0.41	<0.001	82	0.41	<0.001
Triceps skinfold thickness	50	0.43	<0.01	226	0.40	<0.001	82	0.14	NS
Serum albumin	50	0.34	<0.02	226	0.03	NS	82	0.20	NS
Serum amino acid ratio	57	0.39	<0.01	142	0.01	NS	57	0.10	NS

r = coefficient of correlation; *P* = level of significance; NS = not significant.

are as one would expect since low serum albumin levels and high amino acid ratios typify primary protein deficiency, not calorie insufficiency.

The Bukedi results are instructive since they show the type of values which are obtained in districts in which the dietary background is not so clear-cut. The hydroxyproline indices are less easy to interpret. The complex situation in Bukedi is probably more typical of the general situation in most parts of the world than is the area around Kampala, and this could be why our results differ sometimes from those of other workers. This emphasizes the need for scientists like myself to work occasionally in unaccustomed areas, and this was why these field trials were carried out.

Hydroxyproline excretion and infection

These results from our clinic children were very promising, but we were made to think again by some unexpected results obtained from some critically ill children in our ward.

During the time Professor Dean was Director it had been our policy to avoid studying kwashiorkor in children with complicating factors such as hook-worm infestations and malaria. This was to ensure we were measuring metabolic abnormalities due to malnutrition and not to parasite infestations. For various reasons we had become interested in the clinically more complicated type of child, and it was when they were admitted to our ward that we started to get anomalous results (Whitehead, 1967). These children, instead of showing a reduced excretion of hydroxyproline, had normal or even slightly elevated levels. They were different from the previous children we had studied in other biochemical respects as well: for example, blood sugar levels were not nearly so low. Clinically the children were not completely typical of kwashiorkor; in particular the hair colour changes were only slight, which indicated their illness might be of acute rather than chronic origin. This theory was supported by parasite investigations which showed a very much higher incidence of hook-worm and malarial parasite infestations in the dark-haired cases, and haemoglobin levels were also lower.

These results again illustrate how different the metabolic response to malnutrition can be when the aetiology of the disease is made more complex by intercurrent

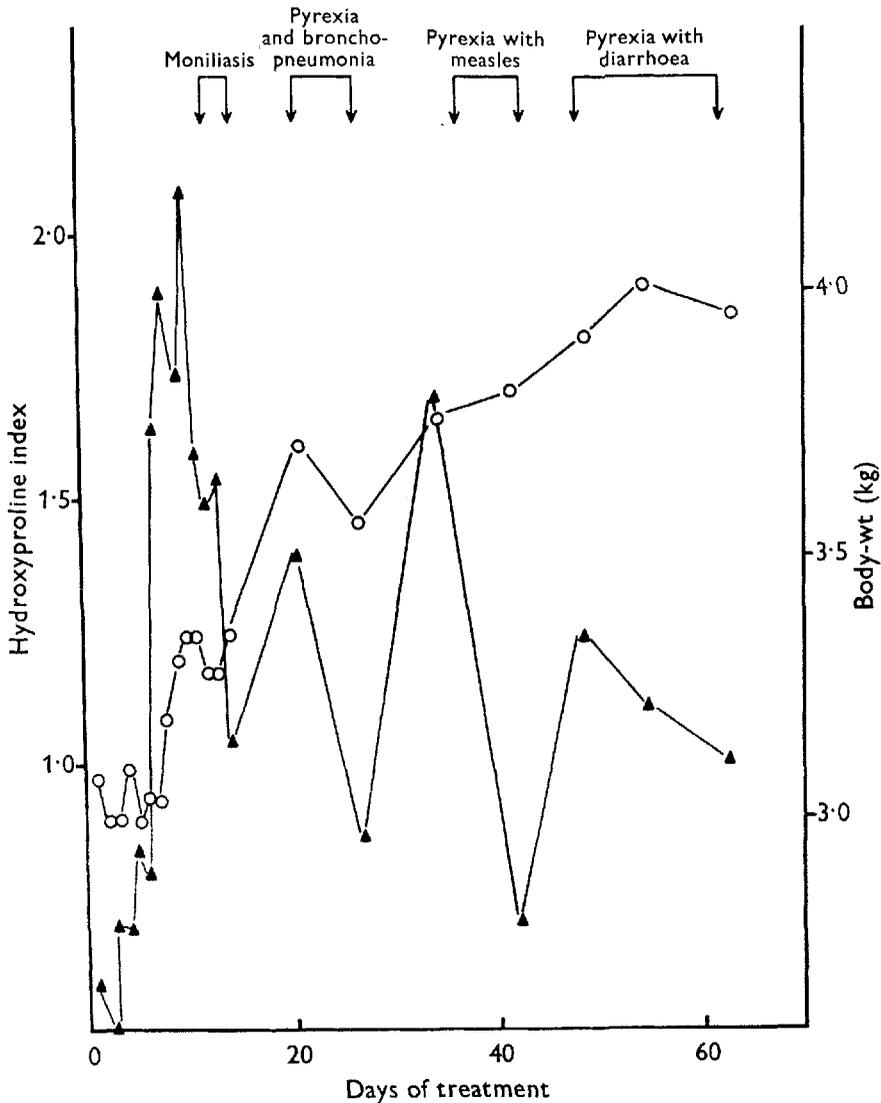


Fig. 8. The effect of various infections on the hydroxyproline index during recovery from nutritional marasmus. ▲—▲, hydroxyproline index; ○—○, body-weight.

factors. The effect of infection on the metabolism of malnourished children was again demonstrated by some other studies, this time in children recovering from kwashiorkor and marasmus (see Fig. 8).

These results are typical of many of the children we investigated. Practically all infections, regardless of their nature, affected hydroxyproline excretion. There was also a close relationship between the rate of weight increase and the magnitude of the index. Weight is of course a factor in the calculation of the index, but it plays only a very minor part in these index fluctuations which are due almost completely to changes in hydroxyproline excretion.

We have as yet no complete answer to the cause of the elevated hydroxyproline levels found in the severely malnourished children with hook-worm and malarial infestations. They could be due to an increased catabolism of structural collagen, as occurs in burns. Alternatively they might be related to changes in metabolism along the synthetic pathway towards new collagen; our more recent studies in protein-malnourished rats have demonstrated a close relationship between the amount of skin collagen soluble in 0.15 M-sodium chloride solution and the quantity of hydroxyproline peptides excreted in the urine (Whitehead & Coward, 1968).

Ideally any test for malnutrition should specifically reflect metabolic changes, resulting from a defective diet and nothing else. Clearly both the amino acid ratio and the hydroxyproline index fail on this score. It seems unlikely however that this ideal will ever be achieved, since both malnutrition and infection affect intermediary metabolism, often in a closely related way. The problem of spurious results caused by intercurrent factors is of course nothing new to clinical biochemistry. The tests I have developed, like most other biochemical diagnostic tests, should not be applied and interpreted without due attention to their limitations.

Ideas for the future

What about future investigations to develop more effective ways of assessing nutritional status? Although our approach, of investigating abnormalities in severe kwashiorkor first and then performing the same investigations in less severely affected children, has provided valuable results, I do not think it is the most satisfactory way. Results obtained by this method are difficult to interpret since they shed little light, in terms of cellular function, on the significance of biochemical abnormalities measured in serum and urine. Probably a more effective approach is to study systematically the progressive metabolic changes that occur in various organs during chronic malnutrition and from this information to devise tests, based on easily obtainable material such as serum and urine, that reflect the cellular changes.

I think it is necessary that when new diagnostic procedures are proposed we distinguish clearly between dietary status and nutritional status. The former is merely related to the quantity and quality of food eaten, whilst the latter is concerned with the effect the diet has had on the body. Biochemical tests designed to detect a state of malnutrition should not just measure a physiological response to an inadequate diet. Even if a change in tissue composition is found this does not by itself, in my opinion, afford justification for calling a child malnourished. Any abnormality must first be shown to have significance or potential significance in terms of essential body function. In other words, subclinical malnutrition can only be diagnosed in terms of malfunction. I have stressed these points because I feel we have paid too little attention to them in our past work. Both in the field of anthropometry and biochemistry we have concentrated too much on the actual detection of abnormalities and too little on what they really mean.

If these relationships between cellular function and serum and urine biochemistry are to be established, part of the investigations must be performed in controlled

nutritional experiments in animals. Although we are concerned with characterizing the subclinical effects of chronic protein malnutrition, I think it is imperative that the animal model we use should be planned in such a way that, in the closing stages of the experiment, a clinical state develops that resembles kwashiorkor so closely that an experienced paediatrician would have no difficulty in diagnosing the condition. Perhaps this is stating the obvious, but I have spent a very sobering 2 years in England trying to reproduce this human deficiency state in experimental rats and pigs. Whilst I believe many of our results are of theoretical value, their relationship to human malnutrition must be regarded as tenuous. The clinical signs in kwashiorkor, gross mental apathy, oedema, skin lesions and hair changes, are so characteristic it is essential they are reproduced as otherwise we cannot possibly understand the subclinical changes which precede them.

During this talk I have laid stress on the requirement that biochemical tests intended for use in surveying populations should be based on material, such as blood and urine, that can be sampled without undue trauma either to the child or the parent. This is important, and I know from my own experience how easy it is to lose the co-operation of people living in a rural African community. In the past I have also recommended the development of tests that can be performed by semi-skilled technicians with a minimum of apparatus. The reasons for this are obvious but now I feel that this approach is too limiting. The automation of biochemistry opens up new possibilities, and our future work will be planned assuming the availability of such equipment. This will mean that the component to be analysed must be stable enough to allow transport from the collection site to a central laboratory.

I am fortunate to be able to return to Uganda with an enthusiastic team and a generous supply of equipment. I hope that we will successfully apply the principles I have mentioned in improving our knowledge of protein malnutrition, in particular the subclinical aspects. I believe this is a most important scientific, technical and humanitarian challenge; it is certainly an exciting time to be a nutritionist.

I wish to acknowledge the help given to me during the past years by the late Professor R. F. A. Dean, Dr E. M. Widdowson, Professor J. C. Waterlow and Sir Harold Himsworth.

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