THE PLASMA AMINO ACIDS IN MALNUTRITION:
PRELIMINARY OBSERVATIONS*

BY
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It is generally appreciated that malnutrition is the world's foremost paediatric problem. Directly or indirectly it makes the major contribution to child mortality. Nevertheless, it remains an entity which is not well defined, or, more accurately, a combination of entities only some of which are well defined. We recognize the effects of certain specific food factors, the accessory food factors or vitamins, and we have some knowledge of changes brought about by deficits of the inorganic components of diet. The effects of caloric deficits, of protein deficits and of possible deficits of specific amino acids have, however, been difficult to separate. Protein and caloric deficits are commonly associated and the existence of syndromes of specific amino acid deficiency has not been definitely established although there is some evidence that they exist.

It appears that the more exact definition of this area of deficiency will depend on chemical studies. We already know that plasma protein levels fall in protein deficiency, particularly the albumin fraction, and that blood urea is also reduced. We know that certain protein enzymes in the blood and the liver (Waterlow and Patrick, 1954; Burch, Arroyave, Schwartz, Padilla, Béhar, Viteri and Scrimshaw, 1957) are diminished. It has seemed to us that observations on the free amino acids of the plasma might be of help in delineating specific entities within this area. The free amino acid level of the plasma might prove to be a more significant index of protein adequacy than other criteria that have been applied and the plasma free amino acid pattern, even if it could not be correlated with symptoms of specific amino acid deficiency, might yet be of considerable value in pointing to limiting amino acids and thus providing a sounder basis for dietary supplementation than has hitherto been available.

Preliminary studies of urinary amino acids in kwashiorkor by members of our group (Cheung, Fowler, Norton, Snyderman and Holt, 1955), by Maggioni (1957) and by Hansen (1957) had shown abnormal amino acid excretion patterns, but since these might have been of renal origin it seemed preferable to explore the blood patterns rather than to extend the observation of urinary amino acids.

The observations reported here were made on four children admitted to the nutrition ward of the Hospital Infantil, Mexico City, with severe clinical evidences of malnutrition. There was no clinical evidence of vitamin deficiencies. They were all thought to be suffering from caloric deficiency as well as from protein deficiency, but the severity of the protein deficiency was much more marked in one of these subjects (M.M.C.) than in the other three. Only this patient exhibited the oedema and skin pigmenyary changes typical of kwashiorkor. The serum of these four patients was studied initially and at intervals during the phase of recovery by the technique of column chromatography. Individual case histories are given below.

Case Histories

Case 1. R.A.M. (231195), a boy aged 1 year 11 months, was admitted to the Hospital Infantil on June 11, 1957, suffering from diarrhoea. No reliable information was obtainable about his past history or present illness. He was grossly undernourished, though not dehydrated, and the panniculus adiposus was minimal. His weight was 6·7 kg., height 71 cm. The skin showed somewhat deeper pigmentation on the dorsal surfaces of the hands and feet than elsewhere, but there were no discrete lesions. The liver was palpable 2 cm. below the costal margin in the mammary line. No evidence of infection was discovered, and the tuberculin reaction was negative. The bone age corresponded to that of a child of 6 months, according to Todd's Atlas (1937).

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The diarrhoea proved not to be severe, only five to six stools a day being passed. No pathogens were isolated on culture, but a microscopic examination revealed ova of *Hymenolepis nana* and cysts of *Giardia lamblia*. The appetite was good and the patient took increasing amounts of the regular ward diet which consisted of whole cow's milk with supplements of bread, rice, banana, liver, lean beef and beans.

The samples of blood serum for amino acid assay were taken on the dates shown in Table 1.

During the period in hospital there was a gradual improvement in the child's general appearance. After the first four days he lost some weight, but after this there was a steady gain which reached 8·7 kg. on August 7, the day of discharge. Evidence of the 'recovery syndrome' (Gómez, Ramos Galván and Cravioto Muñoz, 1952) was first noted on June 20. The abdomen was enlarged with signs of free fluid in the abdominal cavity and prominent superficial abdominal veins. The liver edge at this time was felt 4 cm. below the costal margin. A blood examination at this time showed:

R.B.C. 3·3 millions, haematocrit 28%, haemoglobin 6·8 g., W.B.C. 18,000 (P. 57%, E. 4%, L. 35%, M. 4%). The urine was normal. The stools at this time showed only *Giardia* cysts. Total plasma proteins 7·43 g. %; albumin 3·82 g., total globulin 3·61 g. (α = 0·7, β = 1·59, γ = 1·32).

By July 7, hepatomegaly and ascites had further increased as had the prominence of the abdominal vein, and examination of the blood showed the following:

R.B.C. 3·1 millions, haematocrit 26%, haemoglobin 7·3 g., W.B.C. 8,000 (P. 46%, E. 3%, B. 1%, L. 48%, M. 2%). Total protein 7·60 g., albumin 3·63 g., total globulin 3·97 g. (α = 1·55, β = 1·15, γ = 1·27).

Between July 7 and August 7 evidence of the recovery syndrome diminished markedly, but did not altogether disappear, and a blood study on July 25 showed:

R.B.C. 3·4 millions, haematocrit 29%, haemoglobin 7·3 g., W.B.C. 6,500 (P. 23%, E. 5%, L. 69%, M. 3%).

Case 2. E.F.A. (231172), a boy of 2½ years, was admitted to the Hospital Infantil on June 10, 1957, with malnutrition which had developed since weaning at 2 months of age. For a time he had been fed on a mixture of dilute cow's milk and tea, but on this he had not thrived and had suffered attacks of diarrhoea associated with fever and loss of weight. These attacks had recurred at intervals up to the time of admission. After the age of 1 year he had received almost no milk, his diet consisting of corn meal, beans, bread and noodle soup.

On admission the patient was found to be markedly undernourished. His weight was 5·7 kg., height 71 cm. The anterior fontanelle was still open (2 × 2 cm.). The skin showed no oedema, but there was a striking loss of subcutaneous fat. There was some excessive diffuse pigmentation, particularly on the extremities, but no discrete lesions. The liver edge was not palpable. Bone age corresponded to that of a child of 6 months (Todd, 1937).

The patient was given the regular ward diet. For the first two days some weight was lost but thereafter there was a steady gain, reaching 6·3 kg. on June 21, 6·8 kg. on July 10 and 7·1 kg. on August 7, the day of discharge. He had improved steadily up to June 25 when he developed bronchopneumonia which necessitated the use of antibiotics and oxygen. This attack was, however, brief and did not interrupt his weight gain. By June 28 the pulmonary signs had virtually disappeared. Evidence of the recovery syndrome was noted on July 8: the abdominal veins were engorged and the liver was now palpable 1½ cm. below the xyphoid. By August 1 these signs were receding.

Routine studies of the blood morphology and of the plasma proteins were made on three occasions:

On June 21: R.B.C. 3·6 millions, haematocrit 31%, haemoglobin 8·9 g., W.B.C. 12,000 (P. 63%, E. 2%, L. 30%, M. 5%). Total plasma proteins 6·9 g. %, albumin 3·31 g., total globulins 3·59 g. (α = 0·44, β = 1·99, γ = 1·11). Urine was normal. Stools showed cysts of *Giardia lamblia*.

On July 8: R.B.C. 3·1 millions, haematocrit 26%, haemoglobin 8·2 g., W.B.C. 6,000 (P. 36%, E. 8%, L. 54%, M.2%). Total protein 6·35 g. %, albumin 3·63 g., globulin 2·72 g. (α = 0·73, β = 0·72, γ = 1·57).

On August 2: R.B.C. 4·7 millions, haematocrit 40%, haemoglobin 12 g., W.B.C. 9,700 (P. 56%, E. 4%, L. 38%, M. 2%). Total plasma protein 6·07 g. %, albumin 3·09, total globulin 2·98 (α = 1·54, β = 0·36, γ = 1·08).

Case 3. F.C.G. (231194), a boy of 3 years, was admitted to the Hospital Infantil on June 11, 1957, with a history of bouts of diarrhoea since the age of 8 months. The attack for which he was admitted had lasted 15 days, and was precipitated by measles. The diarrhoea was not severe, only five to six stools a day being passed.

On admission the patient was markedly wasted, his weight being 8·0 kg., height 80 cm. The skin showed general pigmentation but no discrete lesions. There was no oedema. The liver was not palpable. Laboratory examinations on admission showed a normal urine. No pathogenic bacteria were cultivated from the stools, but cysts of *Giardia lamblia* were present. The blood showed:

R.B.C. 3·6 millions, haematocrit 31%, haemoglobin 10·3 g., W.B.C. 2,000 (P. 38%, L. 60%, M. 2%). Total protein 5·35 g. %, albumin 3·12, globulin 2·23 (α = 0·64, β = 0·32, γ = 1·27).

For the first five days in hospital the patient was given a diet of corn meal and fish flour; for a second five-day period he was fed with corn and beans supplemented with glycine; after this he received the regular ward diet, similar to that given to the two patients previously described. Loss of weight was confined to the first 24 hours after admission. After this he gained steadily, reaching a weight of 9·6 kg. on August 6, the day of discharge. The diarrhoea proved to be mild, subsiding after the first few days, and the general appearance improved steadily.

The signs of the recovery syndrome, enlargement of the abdomen with hepatomegaly and engorgement of the superficial abdominal veins, were first noted on
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July 8. The liver edge was felt 5 cm. below the costal margin and there was some evidence of free fluid in the abdominal cavity. On August 1 these signs, though still detectable, were definitely on the wane.

A routine blood examination on July 5 showed the following: R.B.C. 3-8 millions, haematocrit 32%, haemoglobin 9·1 g., W.B.C. 6,700 (P. 44%, E. 1%, L. 53%, M. 2%). Total proteins 7·02 g., %, albumin 3·94, total globulin 3·08 (α = 0·91, β = 0·52, γ = 1·65).

Case 4. M.M.C. (231196), a girl of 1 year 7 months, was admitted to the Hospital Infantil on June 11, 1957, with a history of diarrhoea and vomiting for two months. For the preceding two weeks she had been weak and apathetic, and there was loss of appetite and oedema of the legs. Her diet had consisted largely of beans and noodle soup, but some milk had been given two to three times a week, possibly as much as 10 oz. on the days it was given. She was markedly undernourished, weight being 7·4 kg., height 77 cm. Discrete skin lesions, hyperpigmented and scaly, and typical of kwashiorkor, were present on the arms and legs and to some extent on the trunk. The hair was dry and relatively depigmented; it was easy to pull out. The tongue was red with hyper trophy of the fungiform and atrophy of the filiform papillae. There was tremor of the arms and a definite carpopedal spasm.

The patient was given calcium intravenously with prompt disappearance of the tetanic symptoms. The routine ward feeding was given from the start. Diarrhoea and vomiting were not observed in the hospital, and no parasites were found in the stools. The appetite and general well-being improved steadily. Weight was lost during the first two weeks in hospital as the oedema disappeared, the lowest point, 6·6 kg., being reached on June 25. After this the weight steadily increased to 8·6 kg. on August 9, the day of discharge. On June 27 it had increased and engorgement of the abdominal veins was observed. There was evidence of free fluid in the abdominal cavity on July 8. Defervescence of all these manifestations was clearly evident during the first days of August.

Routine blood studies were made on four occasions with the following results:

<table>
<thead>
<tr>
<th>Date</th>
<th>R.B.C. (millions)</th>
<th>Hct. (%)</th>
<th>Hb (g.)</th>
<th>W.B.C. (P.)</th>
<th>Polys. (%)</th>
<th>Total Protein (g-%)</th>
<th>A/G</th>
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<th>β</th>
<th>γ</th>
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<td>—</td>
<td>9·5</td>
<td>15,600</td>
<td>83</td>
<td>5·03</td>
<td>2·34/2·69</td>
<td>0·71</td>
<td>0·14</td>
<td>1·24</td>
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<tr>
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<td>4·5</td>
<td>38</td>
<td>11·2</td>
<td>15,600</td>
<td>28</td>
<td>6·75</td>
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<td>1·03</td>
<td>1·17</td>
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<tr>
<td>July 31</td>
<td>4·5</td>
<td>38</td>
<td>11·2</td>
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<td>3·75/3·00</td>
<td>1·03</td>
<td>1·17</td>
<td>0·80</td>
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Experimental Procedure

Blood samples were taken at four different times from each patient, the first soon after admission, another sample about eight to nine days later, a further sample at the 25-30 day period and a final sample at eight to nine weeks. Approximately 20 ml. of blood, taken at least three hours after the last meal, was drawn with a heparinized syringe from the jugular vein. The sample was centrifuged and 10 ml. was shaken with 50 ml. of 1% picric acid following precisely the details described by Moore and Stein (1954) for deproteinizing the plasma and removing the excess picric acid. The conditions used for the column chromatographic analyses again followed closely those described by Stein and Moore (1954) except that Dowex 50 × 5% cross-linked resin was used instead of a blend of the 4 and 5% cross-linked resin recommended by the above authors.

Results

The plasma amino acid concentrations in mg./100 ml. of plasma of the four patients, initially and during recovery, are given in Table 1. A range of normal values is also included. These normal values are derived partly from an analysis of plasma amino acids of normal children of similar age, which we have performed in the Pediatric Department of Bellevue Hospital, New York, and partly from the few figures available from published reports (Huisman, 1954; Stein and Moore, 1954; Iber, Rosen, Levenson and Chalmers, 1957). Throughout we were unable to detect any tryptophan in the plasma of the four children in this study nor was this amino acid found in the plasma of our normal children of this age group. We found it in older children and Stein and Moore reported a well-defined peak in their diagram of adult plasma. However, the latter authors reported that some loss of tryptophan can occur during the analytical procedure. We can only assume, therefore, that in children of 1 to 3 years the plasma concentration of tryptophan is so low that its presence is masked by the small losses inherent in the method.

The figures for ammonia and urea are also given since these are also determined by this method. The ammonia figure does not represent true ammonia present in plasma at the time of sampling since it is well known that ammonia is liberated and the concentration builds up during the deproteinization procedure. The figure for total amino acids represents the sum of the amino acids which were determined individually.

The changes observed in the plasma of the three patients who showed neither oedema nor appreciable skin changes were fairly consistent. They were least conspicuous in R.A.M. who showed the least reduction in weight and who during treatment failed to increase his total amino acid concentration.
in the plasma. The general findings were as follows:

R.A.M., the least affected of the group, showed only a slight reduction in total amino acids although the urea concentration was low. Some of the amino acids were just within normal range, but significant reductions were present in the concentrations of threonine, taurine, lysine, leucine, ornithine and arginine. The eight-day specimen showed approximately the same picture except that aspartic acid was absent. In the two later specimens a fall in urea was noted, a phenomenon seen in the other subjects in association with the recovery syndrome.

F.A.E. and F.C.G. showed the same changes as R.A.M. and, in addition, several others. Their total amino acid concentrations were substantially reduced and several other individual amino acids were distinctly low in the initial specimen: alanine, aspartic acid, isoleucine, methionine, phenylalanine, tyrosine and valine. In the second specimen, the total amino acids had returned to normal as had the individual amino acids with the exception of aspartic acid and tyrosine. The tyrosine was slow in returning to normal and aspartic acid was absent in the eight-day specimen but returned later. Again the later specimens showed a reduction in urea.

M.M.C., the one who exhibited oedema and the typical kwashiorkor skin changes, showed the most profound changes in the plasma amino acids. The blood urea was extremely low and the total free amino acids were roughly one third of the normal value. Virtually all the amino acids were reduced to some extent with the exception of glycine, histidine and isoleucine. The most striking reductions were observed in the case of tyrosine, valine and cystine (to one tenth or less of the normal level). Substantial reductions (to one third or less of the normal level) were noted also in the concentrations of ornithine, threonine, taurine, leucine, alanine and phenylalanine. The levels of the remaining amino acids were reduced but less spectacularly so. The specimen taken nine days later showed a marked rise in the total amino acid figure to a level somewhat above normal. The increase was largely brought about by an abnormally high concentration of alanine and serine but at the same time the levels of the majority of the amino acids, which had been low, had returned to normal. Exceptions were phenylalanine and cystine and, most markedly, tyrosine. The subsequent specimens remained normal in respect of the amino acid concentration but tyrosine was particularly slow in reaching a normal level. Again, blood urea was low in the final specimen.

Discussion

A definite interpretation of these preliminary findings must await confirmative studies. At the most, the present data can be regarded only as suggestive. As regards the most limiting amino acid
in the indigent Mexican diet, as far as it can be judged from these results which do not permit an evaluation of tryptophan in this respect, arginine, ornithine and threonine would seem to be consistently affected even in mild cases, and leucine, valine, lysine and tyrosine thereafter. Urea is also consistently low. Methionine, phenylalanine and alanine, although not so affected in the milder cases, were markedly affected in the more severe case with typical kwashiorkor symptoms. Here also, the cystine and tyrosine concentrations were particularly low. From the above data it would be difficult to incriminate any one particular amino acid as being limiting in the Mexican diet.

With regard to imbalance of amino acids in the plasma of these patients, it follows that some imbalance must exist since the levels of some amino acids are more affected than others. However, there was certainly no abnormally high concentration of any one amino acid and the only general comment is that the glycine concentration was consistently higher than the alanine level in the initial samples whereas the reverse ratio applies in all normal plasmas.

We are, however, impressed with the possibility that the low protein diet, particularly in the case of the severely affected patient, M.M.C., interfered with the development and activity of enzymes concerned with the metabolism of amino acids. The low concentrations of arginine and ornithine suggest some interference with the urea cycle. The low concentration of cystine seen in M.M.C. suggests a deficiency in its synthesis from methionine. Even more striking in this patient is the reduced level of tyrosine as compared with phenylalanine which supports the suggestion previously made on excretion data (Cheung et al., 1955) that there is this condition, a deficiency of the enzyme which converts phenylalanine into tyrosine, phenylalanine hydroxylase.* A deficiency of this enzyme had been postulated by an increased excretion of phenylalanine in the urine, a phenomenon also noted by Maggioni (1957). The slow recovery of the blood tyrosine level of our patient is also worthy of comment. It may be that assays of this enzyme will prove useful in characterizing protein deficiencies.

The marked drop in the plasma urea concentration two months after treatment began puzzled us greatly, coinciding as it appeared to do with the presence of the recovery syndrome (Gómez et al., 1952). Conceivably this might be due to impaired deamination. It might also be due to some re-utilization of urea although why this should occur so late is not easy to understand. A more likely explanation would be that the recovery growth spurt causes an extremely high utilization of protein and consequently little nitrogen is available for excretion. This argument can be supported to some extent by the low level of plasma taurine in the three patients with low blood urea at this time. Dent (personal communication) points out that in a number of disease states where the utilization of protein is poor and the subject is also in negative nitrogen balance owing to excessive enzymic breakdown of body protein, the excretion of taurine, which is derived from cystine or cysteine, is high. He believes also that the converse is true and that with high utilization of protein there is little wastage of cystine and consequently a low excretion of taurine. Unfortunately, in this series of cases we were unable to cope with equivalent analyses of urine but it is now obvious that in subsequent studies it would be desirable to carry out amino acid analyses on the urine as well as the plasma.

In considering the levels of the various amino acids in the initial samples of plasma we can say without doubt that they are, in general, much below normal. To go beyond that we find ourselves at a loss as to what further inferences can be drawn as we lack the basic knowledge. For instance, in the absence of an adequate protein intake at what level of plasma amino acid concentration does the body call upon endogenous proteins to reinforce the plasma amino acids? Further, once the endogenous protein available is used up how far can the plasma amino acid level fall before the vital organs are affected? It is difficult to see how this knowledge can be adequately answered by work on experimental animals, particularly as in kwashiorkor we are dealing with children in a narrow age group. An extensive study of kwashiorkor patients, including those with the mildest and the severest forms, would, in time, give us the answer to these questions.

Summary

A study is reported of the free amino acids of blood plasma in four patients suffering from severe malnutrition. Three of these showed no specific clinical symptoms of protein deficiency, whereas the fourth exhibited oedema and skin lesions typical of kwashiorkor, and can hence be regarded as a well defined type of protein deficiency. A tendency to aminoacidopenia was found in all four patients, involving certain essential as well as certain unessential amino acids. It is suggested that

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* Since this paper was written we have had the opportunity of studying the blood of three other patients with the frank kwashiorkor syndrome, one from French West Africa and two from Nigeria. The findings, which will be reported elsewhere, showed certain differences from those reported here, but the exceptionally low tyrosine value was consistently found.
these changes are best interpreted on the basis of
defective enzyme systems.

Particular attention is called to evidence pointing
to a deficiency of the enzyme phenylalanine hydroxy-
lase in the frank kwashiorkor syndrome.

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