

Time relationships between the elevation of the serum amino acid ratio and changes in liver composition in malnourished rats

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1. Changes in the serum amino acid ratio have been compared with alterations in liver composition in young rats growing on three different diets, control, low-protein and 'undernourished'. The rate of growth in the latter group was controlled to be the same as in the low-protein animals.
2. The amino acid ratio only became elevated in the protein-deficient animals but not to the degree found in protein-malnourished children.
3. Protein/g DNA was lost from the livers of the protein-deficient rats and this occurred at the same time as the serum amino acid ratio rose. The possibility that the two phenomena might be linked is discussed. Protein loss from the liver was not a feature in the undernourished animals.
4. Reduced serum total protein and albumin concentrations also developed in the rats fed the low-protein diet and this occurred as the amino acid ratio started to rise.
5. The liver did not become fatty in the low-protein animals until after the amino acid ratio had reached its maximal value. It was concluded, therefore, that the distorted pattern of serum amino acids could not be a result of this pathological condition.
6. There was no apparent relationship between liver RNA levels and the magnitude of the serum amino acid ratio.
7. The limitations of the rat as animal model for the study of chronic protein malnutrition in early childhood are discussed.

Although there have been many published reports describing the distortion which occurs in the pattern of the serum amino acids in chronic protein malnutrition, little is known about when the abnormality develops in relation to other metabolic and structural changes. This distorted pattern can be detected before the appearance of the typical clinical signs of kwashiorkor and was one of the reasons for suggesting it might be of value in assessing nutritional status in subclinically protein-malnourished children (Whitehead & Dean, 1964*a, b*). A semiquantitative screening method was developed to measure this abnormality and the degree of distortion in the amino acid pattern was expressed as an amino acid ratio (Whitehead, 1964).

Whitehead (1969) defined a biochemical test for malnutrition as one which had significance or potential significance in terms of changes in essential body structure and cellular function. To establish whether or not such relationships exist for various biochemical measurements based on serum and urine, a series of prospective longitudinal studies have been carried out in chronically malnourished animals. In a recent investigation (Grimble & Whitehead, 1969) pigs were fed diets containing progressively decreasing amounts of protein. It was found that the amino acid ratio started to

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rise when the quality of the diet had become so poor that the animals stopped growing. The ratio was also statistically correlated with the rate of growth, appetite, serum protein and albumin concentration and hydroxyproline excretion. The pig, however, is an expensive experimental animal and it was not possible to kill sufficient numbers at frequent enough intervals for serial tissue analysis. A parallel series of rat experiments was therefore carried out, so that these investigations could be performed. It was one of the aims of this study to find out whether the rat would provide a suitable model for this and more detailed metabolic investigations.

The animals were malnourished in two ways, some were deprived primarily of protein, since it was known that the amino acid ratio would become elevated under these conditions, and others were more generally deprived of food, a situation which does not result in this biochemical abnormality. Many have speculated that the serum amino acid ratio might be affected by the amount of 'labile protein reserves' in the liver and it was important that this possibility should be investigated experimentally. Liver RNA levels are also decreased in malnutrition, presumably because of a reduced rate of protein synthesis, and it was possible this measurement might provide information on the relation between the serum amino acid ratio and protein biosynthesis. For the same reason, the levels of the serum proteins were also measured, since these are important products of liver cell metabolism. A fatty liver is a major characteristic of severe primary protein malnutrition and Waterlow (1948) showed, in Jamaican children, that it developed at an early stage, before a reduction in serum protein levels. It was conceivable that this pathological condition might be the cause of abnormalities in amino acid metabolism resulting in the serum changes. It was the main aim of this investigation to study these possibilities.

METHODS

The animals and their diets

The rats used in the experiment were of the black-hooded variety belonging to a well-established and highly inbred colony. It had been hoped, therefore, that biological variation between animals would be small, but this was not so. To overcome this problem the rats were selected as series of groups of three male animals, as near the same weight as possible, taken from the same litter. All animals were weaned at 21 days on to stock diet until day 28. They were then placed in separate cages and each triplet was fed on one of three different diets: (a) Control diet, which was 41 B rat food (Bruce, 1963); this contained 16% protein and was given *ad lib*. (b) Low-protein diet, in which 41 B was diluted with sucrose until the protein content was 6%; this also was fed *ad lib*. (c) 'Undernourished' diet; animals on this regimen were fed the same food as the controls but in amounts so reduced that they grew in weight at the same rate as the protein-deficient animals. The low-protein diet was supplemented with minerals and vitamins to levels similar to those in 41 B. The supplement contained potassium chloride, sodium chloride, sodium dihydrogen phosphate, tricalcium phosphate, magnesium sulphate, ferrous citrate, potassium iodide, manganese sulphate, sodium fluoride, vitamins A, B₁, B₆, B₁₂, C, D₃, E, riboflavine, nicotinic

acid, calcium pantothenate, biotin, folic acid, inositol and choline chloride. The animals were killed at 3, 7, 15, 30 and 60 days after starting the diets.

In man, studies on serum amino acid patterns are performed after an overnight fast. Preliminary studies indicated that such treatment in the rat might well have had a significant effect on the chemical composition of the liver, in particular on the labile protein fraction and probably also on the fat content. Consequently it was decided to study unfasted rats, but this left the problem of the effect of recently ingested food on the serum amino acid pattern. With the protein-deficient group, the protein content of the diet and the appetites of the animals eating it were low and thus the serum samples were collected under what were essentially fasting conditions. Invariably the undernourished animals had little or no food left when they were killed and these animals were also virtually fasted. Only in the control group might the effect of recently eaten protein have given rise to misleading results. To investigate to what extent the lack of a fasting period might affect the serum pattern a separate series of animals was investigated as already described but the amino acid ratios were measured after an 8 h fast.

Tissue analysis

Blood was collected after decapitation, and the serum, which was separated immediately, was stored at -20° before analysis. The whole of the liver was removed, lightly blotted and then weighed. Other studies demanded that the liver was homogenized in ice-cold physiological saline containing EDTA. The homogenate was stored at -20° before analysis.

Serum amino acid ratios were estimated by the method of Whitehead (1964) and serum total protein levels as described by Lowry, Rosebrough, Farr & Randall (1951). The protein content of the liver was also estimated by the same method. Liver fat was measured by a gravimetric method devised by Dr D. A. T. Southgate which will be published shortly, but essentially the analysis was as follows. A measured quantity of the liver homogenate was carefully evaporated to dryness at 105° . The residue was extracted with hot 2:1 chloroform-ethanol mixture and a portion was evaporated to dryness on a water bath. The residue was resuspended in light petroleum (boiling point $40-60^{\circ}$) and the mixture was dried with anhydrous sodium sulphate. A sample was again evaporated to dryness in a weighed bottle; the residue was the fat content of the liver sample. RNA and DNA were estimated according to Glock & McLean (1955). Yeast RNA and calf thymus DNA (British Drug Houses Ltd, Poole, Dorset) were used as standards. In a few serum samples albumin and globulin were analysed separately. These were measured as described by Grimble & Whitehead (1969).

Statistical analysis among litter-mates was done by the paired *t* test.

RESULTS

The mean weights of the animals, the weights of their livers and the total liver DNA are shown in Table 1. As was expected, feeding the 6% protein diet to 4-week-old rats virtually stopped their growth, although the diet did allow some increase in

weight in the later stages of the experiment. The table also illustrates the close matching of the weights of the protein-deficient and undernourished animals.

The sizes of the livers were markedly affected by both types of malnutrition, those of the protein-deprived rats were significantly smaller than those of the controls at the 0.1% level after only 3 days and this was true of the undernourished animals by day 7. On day 15 the liver weights of the animals fed the low-protein diet began to increase again and they were well above those of the undernourished animals by day 30 ($P < 0.01$) and remained so until the end of the experiment. Total liver DNA was measured as an index of cell number. In the control animals the values rose as the rats grew older but this did not occur in either of the deficient groups of animals and furthermore there was no significant difference in the liver DNA content of these rats. Thus the higher liver weights in the protein-deficient, compared with the undernourished, animals was probably not associated with a greater number of cells. The total liver DNA content at day 30 was higher than on day 60 in both the control and the two experimental groups of animals. Why this was so is not clear, but when the analysis was repeated the same results were obtained. It seems probable that the former batch of animals was in some way different from the others in the series, because studies on skin collagen carried out in the same animals also gave apparently spurious results (D. G. Coward, P. Meldrum & R. G. Whitehead, in preparation).

The gross appearance of the livers of the protein-deprived animals during the first 7 days was not markedly different from that in the controls, apart from size. On day 15, however, the livers of the protein-deficient animals became much paler. The changes in chemical composition associated with these differences in gross appearance are given in Table 2. As was expected, there was no significant difference in the fat concentration in the livers of the three groups of animals during the 1st week of the experiment, but on day 15 the level had risen in the protein-deficient animals to values above those in both the control and undernourished animals. By day 30 the significance of these differences was greater than the 1% level. The pale appearance was probably not just due to the increased fat concentration of the liver cells but also to loss of protein. Relative to the amount of protein, the livers of the protein-deficient animals contained much more fat and water than those of the undernourished animals.

In contrast to the changes in fat, the protein content of the livers of the protein-deficient animals fell significantly below the control levels as early as day 3, and there was little change thereafter. The undernourished animals, however, did not react in the same way, protein concentration tended to be slightly higher than control values, although this was probably due to a reduction in the concentration of other liver constituents, principally glycogen (unpublished results).

Liver RNA/g DNA fell below control levels in both the undernourished and protein-deficient groups of animals. In the latter group the differences were significant from days 3 to 30 but on day 60 RNA concentration rose to control levels. In the undernourished animals the difference was not significant until day 7 and the values remained low even on day 60.

At no time was the water concentration of the low-protein and undernourished animals different from the control values.

Table 1. *Body-weights, liver weights and total liver DNA in young rats fed on three dietary regimes*

Days on diet	No. of animal triplets	(Mean values with their standard errors)							
		Body-weight (g)		Liver weight (g)					
		Control diet	Low-protein diet	Control diet	Low-protein diet				
3	6	63 ± 2	53 ± 2	2.76 ± 0.07	2.42 ± 0.09	2.28 ± 0.21	8.39 ± 0.69	7.46 ± 0.48	7.83 ± 0.57
7	7	80 ± 2	54 ± 1	3.71 ± 0.14	2.33 ± 0.09	2.13 ± 0.19	11.41 ± 1.16	8.07 ± 0.71	7.89 ± 0.80
15	5	116 ± 2	58 ± 3	5.36 ± 0.24	3.04 ± 0.10	2.40 ± 0.06	14.80 ± 2.31	8.24 ± 1.84	7.54 ± 0.98
30	5	170 ± 8	82 ± 2	7.15 ± 0.52	4.02 ± 0.17	2.72 ± 0.21	18.68 ± 1.52	10.82 ± 0.66	10.27 ± 0.60
60	8	244 ± 10	96 ± 6	8.92 ± 0.40	3.93 ± 0.32	3.27 ± 0.32	17.00 ± 1.57	6.80 ± 0.39	8.10 ± 0.87

Table 2. *Quantities of protein, fat, water/g wet weight and RNA/g DNA in the livers of young rats on three dietary regimes*

Days on diet	No. of animal triplets	(Mean values with their standard errors)				RNA/g DNA(g)	
		Protein (g)		Fat (g)			
		Control diet	Low-protein diet	Control diet	Low-protein diet		
3	6	18.0 ± 1.0	13.6 ± 1.1**	4.57 ± 0.30	4.33 ± 0.21	3.74 ± 0.31	2.93 ± 0.38*
7	7	16.7 ± 1.2	14.9 ± 1.4	4.76 ± 0.20	4.59 ± 0.18	2.94 ± 0.08	2.35 ± 0.14*
15	5	16.8 ± 1.9	11.9 ± 0.3*	5.28 ± 0.73	6.81 ± 0.76	2.71 ± 0.15	1.99 ± 0.18*
30	5	15.7 ± 0.5	12.1 ± 0.2***	4.14 ± 0.50	6.80 ± 0.43††	2.83 ± 0.09	2.10 ± 0.19*
60	8	16.4 ± 0.3	12.5 ± 0.5***	5.37 ± 0.19	8.20 ± 0.76††	2.60 ± 0.20	2.50 ± 0.23
Low-protein diet							
3	6	18.7 ± 1.8	13.6 ± 1.1**	4.51 ± 0.19	4.33 ± 0.21	3.74 ± 0.31	2.93 ± 0.38*
7	7	18.5 ± 0.5	14.9 ± 1.4	4.50 ± 0.26	4.59 ± 0.18	2.94 ± 0.08	2.35 ± 0.14*
15	5	17.8 ± 0.3	11.9 ± 0.3*	4.37 ± 0.57	6.81 ± 0.76	2.71 ± 0.15	1.99 ± 0.18*
30	5	16.6 ± 0.3	12.1 ± 0.2***	3.66 ± 0.26	6.80 ± 0.43††	2.83 ± 0.09	2.10 ± 0.19*
60	8	18.0 ± 0.6	12.5 ± 0.5***	5.58 ± 0.10	8.20 ± 0.76††	2.60 ± 0.20	2.50 ± 0.23
Undernourished							
3	6	18.7 ± 1.8	13.6 ± 1.1**	4.51 ± 0.19	4.33 ± 0.21	3.74 ± 0.31	2.93 ± 0.38*
7	7	18.5 ± 0.5	14.9 ± 1.4	4.50 ± 0.26	4.59 ± 0.18	2.94 ± 0.08	2.35 ± 0.14*
15	5	17.8 ± 0.3	11.9 ± 0.3*	4.37 ± 0.57	6.81 ± 0.76	2.71 ± 0.15	1.99 ± 0.18*
30	5	16.6 ± 0.3	12.1 ± 0.2***	3.66 ± 0.26	6.80 ± 0.43††	2.83 ± 0.09	2.10 ± 0.19*
60	8	18.0 ± 0.6	12.5 ± 0.5***	5.58 ± 0.10	8.20 ± 0.76††	2.60 ± 0.20	2.50 ± 0.23

Significantly lower than control values by the paired *t* test: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. Significantly greater than control values by the paired *t* test: †† *P* < 0.01.

Table 3 shows changes in the serum amino acid ratios which were occurring at the same time as the changes in the chemical composition of the liver; values are given only when results for all three animal triplets were available. The mean ratios in the control rats were essentially the same during the whole of the experiment. In the protein-deficient animals, however, the ratios were significantly greater than the control values after only 3 days, but the maximum values were not reached until day 7, after which time there was no further change. The ratios in the undernourished animals did tend to be slightly higher than the control values but the differences were never statistically significant. At all stages the ratios in the protein-deprived animals were greater than those of their undernourished litter-mates, the difference being significant at day 7 and in subsequent samples.

Although these rats had not been fasted there was no difference between their ratios and those of a similarly treated group of animals who had been fasted for 8 h before they were killed. The mean ratio and standard error for ten fasted control rats was 1.9 ± 0.2 , the values for four rats who had previously been fed the low-protein diet for 15 days was 3.2 ± 0.1 and the corresponding value for four undernourished rats was 1.9 ± 0.3 . Thus it may be assumed that the lack of a fasting period has had no misleading effect on the interpretation of the results for the amino acid ratios.

The changes in total serum protein concentrations are also shown in Table 3. In the control and undernourished rats the levels gradually increased as the animals grew older. This was very similar to the findings in normal and undernourished pigs (Grimble & Whitehead, 1969). This rise did not occur at the same rate in the protein-deficient animals, and even on day 3 the levels were significantly lower than in both the control and undernourished animals. By days 30 and 60 the serum protein levels had risen in the protein-deprived animals but they were still below the levels in the other two groups.

It was considered that such early changes in serum protein concentration in the low-protein animals might have been due to an expansion of the plasma volume. Unfortunately insufficient serum was available for serum protein fractionation, but a subsequent animal experiment, carried out for a different purpose, but which had the same experimental design, enabled these estimations to be performed in rats protein malnourished and undernourished for 3 and 7 days. The results are summarized in Table 4. The changes in serum protein concentration were due almost entirely to a reduction in albumin levels, the concentrations of total globulin were virtually unchanged. It was concluded, therefore, that the fall in total protein concentration was a genuine metabolic effect of protein depletion and was not due to haemodilution.

DISCUSSION

The experiment with malnourished young pigs (Grimble & Whitehead, 1969) demonstrated that the serum amino acid ratio started to rise when the quality of the diet became so poor that the animals stopped growing. This was the situation from the beginning in this rat experiment and the ratios started to rise immediately, although they did not reach their final plateau level until day 7. The percentage rise in the ratio, however, was small compared with that found in malnourished children, in

Table 3. Serum amino acid ratios and total protein concentrations in young rats fed on three dietary regimes

Days on diet	No. of animal triplets	Amino acid ratio			Serum total protein (g/100 ml)		
		Control diet	Low-protein diet	Under-nourished	Control diet	Low-protein diet	Under-nourished
		(Mean values with their standard errors)					
3	5	1.7 ± 0.1	2.3 ± 0.1***	2.0 ± 0.1	5.7 ± 0.2	4.7 ± 0.1††	5.5 ± 0.1
7	6	1.9 ± 0.2	3.4 ± 0.4***	2.2 ± 0.1	6.3 ± 0.3	5.6 ± 0.4	6.0 ± 0.1
15	5	1.6 ± 0.1	3.2 ± 0.3*	2.2 ± 0.1	6.0 ± 0.2	5.1 ± 0.3†	5.5 ± 0.3
30	5	1.9 ± 0.1	3.1 ± 0.2**	2.1 ± 0.1	7.6 ± 0.2	5.9 ± 0.1††	7.0 ± 0.3
60	6	1.8 ± 0.1	3.3 ± 0.2***	2.2 ± 0.2	7.6 ± 0.2	5.6 ± 0.3††	7.2 ± 0.1

Significantly higher than control values by paired *t* test: * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.
Significantly lower than control values by paired *t* test: † *P* < 0.05; †† *P* < 0.01.

Table 4. Serum total protein, albumin and total globulin concentrations (g/100 ml) in young rats fed on three dietary regimes

Days on diet	No. of animal triplets	Total protein						Albumin						Total globulin					
		Control diet		Low-protein diet		Under-nourished		Control diet		Low-protein diet		Under-nourished		Control diet		Low-protein diet		Under-nourished	
		(Mean values with their standard errors)																	
3	6	5.51 ± 0.33	4.43 ± 0.18**	5.66 ± 0.30	3.16 ± 0.26	2.32 ± 0.17***	3.11 ± 0.25	2.36 ± 0.12	2.11 ± 0.06	2.55 ± 0.15	2.36 ± 0.12	2.36 ± 0.14	2.35 ± 0.13	2.45 ± 0.16	2.36 ± 0.12	2.36 ± 0.14	2.35 ± 0.13	2.45 ± 0.16	
7	8	5.75 ± 0.31	4.74 ± 0.17*	5.95 ± 0.23	3.13 ± 0.23	2.38 ± 0.11**	3.19 ± 0.10	2.36 ± 0.10	2.36 ± 0.14	2.35 ± 0.13	2.45 ± 0.16	2.36 ± 0.12	2.36 ± 0.14	2.35 ± 0.13	2.45 ± 0.16	2.36 ± 0.12	2.36 ± 0.14	2.35 ± 0.13	2.45 ± 0.16

Significantly lower than control values by paired *t* test: * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

whom values may be 400–800 % above normal. Even in the pig experiment the protein-deficient animals eventually had values 200 % greater than the controls. The levels in the protein-deficient rats were barely twice those of the control and undernourished animals.

In the pigs the ratios had continued to rise, as protein malnutrition was prolonged, but in the rat this did not occur. The reason for this is not clear, but there was evidence that the 6 % protein diet did not create the same degree of nutritional stress as the animals became older. Growth, for example, completely stopped during the first 2 weeks of the experiment, but in the second 2 weeks the surviving animals started to grow again and this continued during the final month of the study. The rate of growth, however, was well below that of the controls. The appetite, an important behavioural characteristic, also improved and this no doubt contributed to the renewed growth.

There is another, more basic, reason why the rat did not prove a more suitable model for the study of this metabolic abnormality. Growth and development in the rat is both quantitatively and qualitatively different from that in the child. The rat, like most other mammals, has a much more rapid rate of growth in early life. As was the normal practice, the rats in this experiment were weaned at 3 weeks of age, but by 6 weeks the animals were responding to puberty changes, as indicated by a rise in the weight of the seminal vesicles. Since the feeding experiment could not be started until week 4, only 2 weeks' malnutrition before puberty was possible, and the equivalent of the 'preschool child' period could only have been a few days. These facts constitute a fundamental limitation in the use of the rat as an animal model for the study of malnutrition in early childhood, the stage of development during the period of chronic malnutrition is completely different. The same problem existed in the pig but it was not so acute, and this could have been why the elevation of the amino acid ratio was different in these two animals. Under the experimental conditions used, however, neither the rat nor the pig produced the required animal model and future investigations will be carried out in the baboon, an animal with a growth and development pattern qualitatively similar to that of man.

An attempt was made in a few animals to increase the nutritional stress after the first 2 weeks by reducing the protein content of the diet from 6 % to 4 %. This was a failure, mainly because of the reduced appetite which resulted. This illustrates another major problem in studying primary protein malnutrition in experimental animals, striking the delicate balance between protein lack without affecting, unduly, the total calorie intake.

It is clear that the abnormalities in serum amino acid pattern did not arise because of impaired liver function resulting from the accumulation of fat. In this experiment the ratio had reached its maximum level before the liver became fatty.

Some of the experimental findings did support the view that the distortion of the serum amino acid pattern might be associated with a fall in the labile protein fraction of the liver. The serum amino acid ratio and protein concentration in the liver, calculated either per g wet weight or per mg DNA, both became abnormal only in the protein-deficient animals. There were, however, important differences in their subsequent developmental patterns which cast doubt on there being any relation between

the labile protein levels and the amino acid ratio. There was no further fall in protein concentration in the liver after day 3 whilst the amino acid ratio was much more elevated on day 7 than on day 3. Furthermore if one calculates the total protein present in the livers of the low-protein and undernourished rats it can be demonstrated that the difference in amino acid ratio is much greater than the differences between the amounts of liver protein. Certainly, the present results provide no conclusive evidence to suggest that the elevation of the amino acid ratio is caused by a loss of liver protein, or, vice versa.

The investigation failed to reveal any time relationship between changes in the RNA content of the liver cell and the elevation of the amino acid ratio; RNA/g DNA fell by the same amount in both the protein-deficient and undernourished animals. The increase in liver weight and protein content of the protein-deficient rats, which occurred at the end of the experiment, was also associated with an increase in RNA content, but this failed to be reflected in a change in serum amino acid pattern. If it is assumed that the RNA content of the cell is related to protein synthesis it must be concluded that the distortion in the serum amino acid pattern does not solely reflect changes in protein synthesis.

The present results do confirm, however, that an elevated amino acid ratio and a reduction in concentration of serum total protein and albumin are closely related in primary protein malnutrition, but not in undernutrition. In both the pig and the rat these measurements became abnormal at the same time, but once again there is evidence that the ratio is not directly associated with a failure in protein synthesis because serum protein and albumin concentrations were no lower on day 7 than on day 3 although the ratio was much higher.

Further proof is provided that the elevation of the amino acid ratio is confined to malnutrition caused by low-protein, high-carbohydrate diets. The abnormality does not develop in simple calorie deprivation. This has now been shown in man, rat, pig and dog (Whitehead, 1965; Widdowson & Whitehead, 1966; Grimble & Whitehead, 1969; Heard, Kriegsmann & Platt, 1968). It is obvious, therefore, that factors other than an inadequate protein intake are necessary for the elevation of the amino acid ratio and these investigations all indicate that the amount of dietary carbohydrate is of fundamental importance. Presumably this is because of the protein-sparing effect of carbohydrate, not only on dietary protein but also on endogenous tissue proteins. In animals who are growing slowly on a balanced diet, but one limited in total calorific content, it can be postulated that during short periods when extra energy metabolites are required, these are furnished by the tissue proteins. This process would also provide a source of essential amino acids for the free amino acid pools and hence the serum. If extra carbohydrate were eaten this metabolic response would not occur to the same extent, which might explain the failure of the animal to maintain a normal amino acid pattern in the serum. The carbohydrate might have an additional effect in diverting, under the influence of insulin, blood amino acids to muscle rather than to liver and other tissues. Current ideas on the regulation of protein metabolism in this way have been reviewed by Munro (1964). These mechanisms merit further investigations in chronically malnourished children.

It may be concluded that the serum amino acid pattern starts to become distorted at an early stage in chronic primary protein malnutrition, before the development of a fatty liver and at about the same time as protein is lost from the liver and the synthesis of albumin is impaired. Because of inadequacies in the animal model no information was provided about the significance of the grossly distorted amino acid pattern found in severe primary protein malnutrition.

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