Metabolism of Albumin and Body Fluid Compartments in Protein Deficiency—an Experimental Study in the Rhesus Monkey

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ABSTRACT   The influence of protein deficiency on the catabolism of serum albumin and body fluid compartments was investigated in a group of Rhesus monkeys tube-fed a protein-deficient diet for 9 weeks. Animals fed similarly an isocaloric diet rich in protein for the same duration served as controls. The data indicate that protein deficiency resulted in a marked reduction in the amount of albumin and its catabolism. The reduction was more marked in the extravascular than in the intravascular albumin pool. In spite of a reduction in the serum albumin there were no significant alterations in plasma volume and extra cellular fluid. These findings suggest that factors other than hypoalbuminemia may be responsible for the edema of protein–calorie malnutrition. J. Nutr. *104:* 858–864, 1974.

INDEXING KEY WORDS   protein deficiency   ·   rhesus monkey   ·   albumin metabolism   ·   body composition   ·   fluid compartments

Edema associated with hypoproteinemia is a characteristic feature of kwashiorkor, a syndrome of protein–calorie malnutrition, affecting young children (1, 2). Protein metabolism which is markedly disturbed in this syndrome has been investigated by several groups of workers both in human and experimental protein malnutrition (3–12). Protein synthesis may be differentially affected in different organs. Thus synthesis is lowered in muscle but is relatively unaffected in the liver (4). Although there is general agreement that catabolism of albumin is decreased (3, 6–10), this is not universally accepted (11, 12).

The mechanism of edema which is a characteristic of kwashiorkor is not properly understood (2, 13–16). Increases in the total body and extracellular fluids have been reported both in human and experimental protein malnutrition (14–19). However, there is no unanimity of opinion about the alterations observed in plasma volume (6, 20, 21). It has been suggested that retention of fluid may be due to a lowering of colloid osmotic pressure, consequent upon a reduction in plasma albumin (14). Other factors such as hor-

monal disturbances (22, 23) and depletion of potassium (24) have also been incriminated in its pathogenesis. Also, in malnutrition there are marked alterations in cardiac and renal functions (25, 26).

Human protein malnutrition is often complicated by multiple dietary deficiencies, concurrent infections and infestations. Moreover, studies in the human syndrome are influenced by the urgent need for therapy. These factors can alter the metabolic picture in kwashiorkor. We have been able to successfully induce a kwashiorkor-like syndrome in rhesus monkeys by feeding them a diet deficient only in proteins and adequate in all other nutrients (27, 28). Using this model, we have investigated the metabolism of albumin and body fluid compartments in protein deficiency.

MATERIALS AND METHODS

Thirteen young growing male rhesus monkeys were used. They were housed separately and fed a stock diet consisting of whole wheat chapati, gram, seasonal

green vegetables and fruits. Water was provided ad libitum throughout the study. The stock diet provided 15% protein and supported good growth. The animals were observed for a period of 3 to 4 weeks before being used for the experiments. Two animals used in group 2 could be observed only for 1 week. They had to be used at that stage since the specially prepared labeled albumin had arrived from Bombay.

The animals were divided into two dietary groups. Group 1 consisted of nine animals tube-fed a diet containing negligible protein (low protein diet) for 9 weeks. The other four animals, group 2, were tube-fed a protein-rich diet containing 16% casein for an equal period of time. Except for the protein content, the two diets were identical. Each animal, in both of the groups, was tube-fed a diet which provided 100 kcal/kg/day. The composition of the diets including that of vitamin and mineral mixtures, techniques of their preparation and feeding were the same as those followed in this laboratory and described earlier (29).

Investigations

Measurements of plasma proteins, albumin turnover, sodium space and plasma volume were carried out by the techniques mentioned below before the start and again after 9 weeks of experimental dietary regime.

Protein metabolism. Blood was collected from the dorsal vein in the popleteal fossa. Heparin was used as the anticoagulant. Total plasma proteins were estimated by the biuret technique and the different proteins by paper electrophoresis (30). The albumin turnover studies were carried out by using ^{131}I-labeled monkey serum albumin. The technique of Lajtha was modified to suit monkeys (31). Each animal was administered intravenously 20 μCi of ^{131}I-albumin (sp. activity 126 μCi/100 mg) dissolved in normal saline. In order to block the thyroidal uptake of ^{131}I released during catabolism of the albumin, the animal was given orally 6 mg of KI twice daily for 3 days, prior to administration of the label. Administration of KI was continued throughout the period of study. The first blood sample, taken 10 minutes after injection of ^{131}I-albumin, was used

to estimate plasma volume by the isotope dilution formula. As labeled albumin continuously diffuses out into extravascular space at a rate of 10% per hour, a correction factor of 1.015 was applied to the observed activity at 10 minutes to get the activity at zero time (31). In the baseline study blood samples were taken twice daily for 3 days and then once daily for 7 days. In the albumin turnover study carried out at the end of the experiment, in addition to the 10-minute sample, only one blood sample containing less than 2 ml of blood was collected every day for 10 days; 0.5 ml of plasma was used to measure the radioactivity. The plasma proteins were estimated from the plasma samples pooled throughout the study period. As it is difficult to accurately collect urine from the animals, the albumin turnover was calculated by extrapolating the plasma decay curve. The stools contained negligible amounts of radioactivity, and hence were not collected. The total exchangeable, the extravascular and intravascular albumin pools, and albumin turnover rate were calculated as measured by Sterling from plasma decay curve and plasma volume (32).

Body fluid compartments. Body fluid compartments were estimated by using isotope dilution techniques (33). Plasma volume was measured using ^{131}I-labeled albumin as mentioned above.

Extracellular space was measured using [24]NaCl (sp. ac. 89 mCi/g Na) (33); 20 μCi of the isotope was injected intravenously after overnight fasting. Results of the pilot study in which repeated plasma samples were collected at intervals, indicated that the isotope reached equilibrium with the extracellular space (sodium space) by 10 hours after its administration. In subsequent studies, therefore, only one plasma sample, withdrawn at 12 hours after the injection of the isotope, was used; 0.5 ml of the sample and 0.5 ml of 1:1000 diluted standard solution were used for counting. Urine was collected during the 12-hour interval and a 5-ml aliquot of this was used for counting using a gamma scintillation counter. The sodium space was measured by using the isotope dilution formula after making correction for the amount excreted in the urine. Radioactivity

TABLE 1

Plasma proteins in protein-deficient animals

| | Group I (9)[1] | | Group 2 (4) | |
	Stock diet	Low protein	Stock diet	High protein
Total plasma protein (g/100 ml)	7.12±0.49[2]	5.04±0.50 b[3]	6.61±0.65	7.71±0.31 a
Plasma albumin (g/100 ml)	2.55±0.61	1.40±0.39 b	1.92±0.37	3.69±0.38 b
Globulins (g/100 ml)				
Total	4.57±0.61	3.64±0.41 b	4.69±0.73	4.02±0.23
Alpha	0.96±0.15	0.51±0.21 b	0.97±0.06	0.86±0.16
Beta	1.35±0.47	1.20±0.90	1.25±0.34	1.05±0.39
Gamma	2.26±0.56	1.93±0.38	2.46±0.45	1.90±0.70

[1] Figures in parentheses denote the number of animals used.   [2] Mean ±Standard deviation.   [3] a: Difference due to diet ($P < 0.05$) within the respective group based on Student's paired t test (34). b: Difference due to diet ($P < 0.01$) within the respective group.

was measured using a well scintillation detector [1] attached to a spectrometer.

Histological techniques.   All the animals in both groups were killed at the end of the study. Sections from different organs were fixed in 10% neutral buffered formaline. Paraffin sections cut at 5 μ were stained routinely with Hematoxylin and Eosin.

RESULTS

The animals fed the low protein diet maintained their body weights during the first 2 weeks, but thereafter lost weight gradually. However, the weight loss even at the end of the study was not more than 20%. Except for the weight loss the animals were otherwise healthy and alert throughout the study.

At autopsies, there was no evidence, in the protein-deficient animals, of infection or abnormal accumulation of fluid in any of the cavities. The morphological alterations were similar to those described in detail earlier (27, 28), hence they are mentioned only briefly in this communication. The animals fed the deficient diet showed periportal fatty liver, atrophy of exocrine pancreas, salivary glands, gastrointestinal tract, spleen and skeletal muscle including the heart. There was a marked diminution in endochondral bone formation as evidenced by a reduction in zones of resting, proliferating, maturing and calcifying cartilage cells. The metaphyseal bony trabeculae were thin, stunted and reduced in number. The endocrine organs did not

show any morphologic alterations and were considered to be within normal limits.

Plasma proteins.   There was a marked decrease in plasma proteins chiefly due to a fall in albumin in the group 1 animals. The gamma globulins, however, did not show any alterations. Feeding a protein-rich diet to the animals (group 2) resulted in a rise in plasma proteins and albumin. However, in these animals, as in those of group 1, the gamma-globulin level was not altered (table 1).

Albumin metabolism.   The mean basal exchangeable albumin pool in the group 1 animals was 3.063 g. This was reduced to almost half after 9 weeks of protein deficiency. Although a reduction was observed in both the extravascular and circulating albumin pools, the change was not uniform. The circulating pool was relatively less affected as evidenced by a higher ratio of circulating/exchangeable albumin pool. Protein deficiency resulted in prolongation of half-life and decreased turnover of albumin in the group 1 animals (table 2).

Albumin pools showed a uniform rise in the control animals upon feeding the protein-rich synthetic diet. The half-life and turnover, expressed as percentage renewal of albumin, did not change. However, there was a 40% rise in the absolute turnover when it was expressed in grams/day.

Body compartments.   The mean plasma volumes in group 1 animals before and after 9 weeks of protein deficiency

[1] Tracer Lab. detector P-20-D and spectrometer SC-81.

TABLE 2

Albumin metabolism in protein-deficient monkeys

| | | Group 1 (9)[1] | | Group 2 (4) | |
		Stock diet	Low protein	Stock diet	High protein
Albumin pool g/kg body weight	Total exchangeable	3.063 ±0.691[2]	1.389 ±0.430 b[3]	2.555 ±1.04	3.980 ±0.659b
	Circulating	1.320 ±0.350	0.593 ±0.202 b	1.017 ±0.197	1.612 ±0.194 a
	Extravascular	1.932 ±0.553	0.796 ±0.285 b	1.538 ±0.926	2.368 ±0.597 a
	Circulating/ Exchangeable	0.37 ±0.048	0.42 ±0.044 b	0.42 ±0.109	0.41 ±0.064
Albumin turnover	T/2 (days)	7.11 ±0.47	9.82 ±1.68 b	6.77 ±0.45	6.82 ±0.74
	% day	9.93 ±0.73	7.24 ±1.28 b	10.26 ±0.66	10.24 ±1.09
	g/day	0.813 ±0.175	0.229 ±0.072 b	0.647 ±0.286	1.069 ±0.118 a

[1] Figures in parentheses denote the number of animals used.   [2] Mean ±Standard deviation.   [3] a: Difference due to diet (*P* < 0.05) within the respective group based Student's paired *t* test (34).   b: Difference due to diet (*P* < 0.01) within the respective group.

were 120 and 99 ml. A similar reduction was recorded in group 2 animals. However, when expressed as a percentage of body weight, statistically significant differences were observed in the plasma volume only in the latter group (table 3). Sodium space expressed in absolute value did not alter in either group, but when expressed as a percentage of body weight there was a small but statistically significant rise in it, in the deficient animals (table 3).

DISCUSSION

Animals fed the protein-deficient diet developed all essential features of kwashiorkor including a reduction in plasma proteins and albumin. In the present study, however, unlike the earlier observations in rhesus monkeys (35) and recent observations in humans with kwashiorkor and in

baboons (36), no alterations were noted in γ-globulins in the protein-deficient animals. The reasons for this discrepancy are not clear.

Albumin metabolism studies have revealed a marked reduction in albumin pools although the reduction was not uniform. The intravascular pool when expressed as a fraction of exchangeable pool increased, suggesting a tendency of the body to conserve intravascular albumin in protein depletion states. These results are in agreement with those of other workers, both in human and experimental protein malnutrition (6–9). Results of the experiments in which hypoalbuminia was induced by plasmapheresis or bleeding of animals, also suggest that the intravascular albumin mass is kept constant by the transfer of albumin from the extravascular pool (37, 38). This may be a compensatory

TABLE 3

Sodium space and plasma volume in protein-deficient and control animals

| | Group I (2)[1] | | Group II (4) | |
	Stock diet	Low protein	Stock diet	High protein
Weights (kg)	2.69± 0.22[2]	2.33± 0.26 b[3]	2.60± 0.19	2.65± 0.10
[24]Na-space				
Total (ml)	915.0 ±64.44	876.0 ±48.32	912.2 ±59.72	936.7 ±32.37
% body weight	34.1 ± 1.40	37.9 ± 2.92 b	35.6 ± 2.20	35.4 ± 0.59
Plasma volume				
Total (ml)	120.0 ±14.20	99.0 ±12.67 b	138.0 ± 6.27	116.0 ± 7.14 a
% body weight	4.48± 0.42	4.28± 0.30	5.35± 0.32	4.37± 0.10 b

[1] Figures in parentheses denote the number of animals used.   [2] Mean ±Standard deviation.   [3] a: Difference due to diet (*P* < 0.05) within respective group based on Student's paired *t* test (34). b: Difference due to diet (*P* < 0.01) within respective group.

mechanism to maintain plasma colloid osmotic pressure in protein depletion states. For the same reasons, the degree of hypoalbuminimia in protein malnutrition provides an underestimate of the extent of albumin depletion. This may be true of kwashiorkor.

Protein deficiency in rhesus monkeys like in humans and other experimental animals results in marked prolongation of the half-life and turnover of albumin (6–10). This appears to be a compensatory mechanism to conserve proteins in the face of protein depletion. It is not certain if the decreased catabolism is due to the protein deficiency state of the body (6, 39) or to low protein intake (8). James and Hay (8) have shown that alterations in albumin metabolism occur very early at a time when there are no changes in plasma proteins. On the other hand, reduced catabolism has been reported in patients with proteinurea (40) and cirrhosis (41), as well as in experimental animals during plasmapheresis (7, 38). In these conditions which are associated with hypoalbuminimia, there is no dietary deficiency of proteins. Waterlow (42) has put forward evidence that catabolism is affected both by plasma protein level and by the dietary protein intake. In the present study, an interesting finding, although on a limited number of animals, was seen. Two animals in group 2 had very low plasma albumin at the start of the experiment, but were getting an adequate protein diet. These animals in spite of hypoalbuminimia had a normal rate of catabolism of albumin. These findings which support the contention of James and Hay (8), emphasize the importance of dietary protein in modifying protein catabolism. They may explain the discrepancy in the findings on albumin catabolism in kwashiorkor, since the human picture is often modified by an urgent need for energetic therapy.

Plasma volume. There was no appreciable change in the plasma volume, when expressed as a percentage of body weight, in the protein-deficient animal. There is no unanimity of opinion about the changes in the plasma volume both in human and experimental malnutrition (6, 20, 21). Some workers have found an increase in the plasma volume during the early phase of the treatment (43, 44). In the present study a consistent reduction in the plasma volume was observed in group 2 animals. The reasons for this are not clear.

Extracellular space. The extracellular water (sodium space) in the animals fed stock diet in the present study was found to be far greater than the values reported by Srikantia and Gopalan (19) in monkeys using thiocyanate. This may be due to differences in the methods used, for it is known that sodium space tends to be greater than actual extracellular water, as some of the tracer exchanges rapidly with sodium in the bone and penetrates into the cells.

None of the animals in the present study showed signs of edema formation at the time of estimation of sodium space. The increase seen in the extracellular fluid, when expressed in terms of body weight, is due to a reduction in the weight rather than accumulation of excess fluid. This is surprising as markedly low levels of albumin were seen, indicating a very low osmotic pressure of plasma, and yet no edema appeared. There was no correlation between the plasma albumin level and the degree of change in the extra-cellular space. Similar lack of correlation was seen by Keys et al. (13) in human volunteers subjected to protein deficiency and by Srikantia and Gopalan (19) in experimentally produced protein malnutrition in monkeys. The role of other factors like antidiuretic hormones, ferritin and aldosterone, disturbances of electrolyte balance and impaired renal and cardiac functions which have been incriminated in the pathogenesis of edema of protein malnutrition (22–26) cannot be elucidated in the present study.

ACKNOWLEDGMENT

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