Changes in Body Fluid Distribution in Experimental Protein Malnutrition

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Abstract

The sequential changes taking place in the plasma proteins, blood volume and body fluids in rats during the condition simulating "pre kwashiorkor" is described.

Protein malnutrition was induced in growing rats by feeding them a diet containing **0.5**% *protein. At weekly intervals the total plasma protein and albumin concentrations, hematocrit and the volumes of the different fluid compartments were determined.*

During the eight weeks of study, there was a marked loss in body weights, fall in total plasma protein and albumin concentrations, lowering of hematocrit levels and a slight increase in the plasma and extracellular fluid volumes. The changes in total body water and blood volumes were not significant.

Hypoproteinemia with hypoalbuminemia and an increase in the extracellular fluid volume were not associated with clinical edema, which probably is a late manifestation of protein malnutrition.

Introduction

PROTEIN MALNUTRITION is a problem of major concern to many developing countries in the world today. It is most often due to inadequate consumption of proteins or intake of proteins of poor quality. In protein malnutrition there is no sharp line between health and disease. The scientific study of protein malnutrition in experimental animals has contributed considerably to our understanding of biochemical changes taking place in protein malnutrition. For ethical and humanitarian considerations it is not possible to study the serum protein and body fluid changes during the development of protein malnutrition in man and so an animal model becomes extremely essential. Protein malnutrition in children is invariably complicated by deficiencies of calories, vitamins, minerals and perhaps trace elements, as well as by intercurrent infections and infestations. These alter in endlessly different patterns the basic clinical and metabolic picture seen in the child suffering from protein malnutrition. It is now possible to reproduce in animals the features of the protein deficiency syndrome known as Kwashiorkor (Kirsh, Brock and Saunders, 1968 and Edozien, 1968). In the animal model it is possible to keep to a minimum the individual variations and study the development of fluid changes under carefully controlled conditions.

One aspect in which there is a paucity of information and no general agreement is the fluid and electrolyte changes during the development of protein malnutrition (Gopalan, Venkatachalam and Srikantia, 1953; Picou and Waterlow, 1962; Davidson, 1964). The sequence of changes taking place in serum proteins, blood volume and body fluids have usually been reported after the development of gross edema or manifestations of advanced protein calorie malnutrition. The present study was designed to investigate the sequential changes in serum proteins and body fluids during the early stages in the development of protein malnutrition in rats simulating the condition "pre kwashiorkor".

Experimental

Male Sprague Dawley rats weighing 130 – 160 g were fed freely a diet containing 0.5% lactalbumin. A control group was fed freely a diet containing

18% lactalbumin. The diets were formulated after Edozien (1968). At weekly intervals batches of six rats from each group were weighed and the following determinations carried out:- Total body water, extracellular fluid volume, plasma and blood volumes, total plasma protein and albumin concentrations, and hematocrit values.

Total body water and thiocyanate space (ECF):

An accurately weighed amount (about 1 ml) of 2.5% thiocyanate solution containing 10 μCi of tritiated water was injected intraperitoneally. The accuracy of the amount injected was checked by weighing the syringe before and after injection in a sensitive balance. All food and water were withdrawn from the animals after the injection. Equilibrium of the thiocyanate and tritiated water is known to be complete by about four hours (Smith, 1960), and a sample of plasma can be assumed to be representative of the general body concentration of the labelled water and thiocyanate in the extracellular compartments of the body. Plasma was obtained after the injection of Evans Blue (see plasma volume below).

For total body water determination, the proteins were precipitated from 0.5 ml of plasma by adding 2.5 ml of absolute ethanol and the precipitate removed by centrifugation. One ml of the supernatant was pipetted into 20 ml of Bray's solution (1960) and the activity of tritium counted for 10 minutes in a scintillation counter (Hayes and Gould, 1953). The average of three such counts was taken for calculation of total body water.

The extracellular fluid volume was calculated by the method described by Huang and Bondurant (1955).

The intracellular fluid volume was obtained from the difference between the total body water and the extracellular fluid volume.

Plasma and blood volume:

Exactly four hours after the injection of tritiated water and thiocyanate, the rats were anaesthetized with ether and laparotomy performed. An accurately weighed amount of (about 0.5 ml) a 0.1% solution of Evans Blue was injected directly into the inferior vena cava. Here again this was made possible by weighing the syringe containing the dye before and after injection. After five minutes, as much blood as possible was withdrawn from the inferior vena cava in heparinised syringes and the animal sacrificed. Blood and plasma volumes were calculated using the formula of Loring (1954).

The interestitial fluid volume was obtained from the difference between the extracellular fluid volume and the plasma volume.

Hematocrit:

Hematocrit was determined by transferring heparinised blood to a Wintrobe tube with a Pasteur pipette and centrifuging at 1500 $\times$ g for 30 minutes. The level of packed cells was expressed as a percentage of the total volume of blood.

Plasma proteins:

The total plasma protein concentration was calculated from the micro-Kjeldahl determinations of plasma nitrogen using a factor of 6.54 (Sunderman, Sunderman, Flavo and Kallick, 1958). Electrophoresis of the plasma on cellulose acetate was performed and the percentage distribution of albumin calculated. The absolute amount of albumin was obtained by multiplying the total plasma protein concentration by the relative percentage of albumin (Chandrasekharan, 1969). The total amount of protein and albumin in the circulation was calculated.

Results and Discussion

The absolute volumes of the different fluid compartments and the total circulating plasma proteins are given in Table I.

The changes in the body weights, plasma proteins and hematocrit are illustrated in fig. I.

The changes in fluid volumes are shown in fig. 2.

Body weights:

The animals on the 0.5% lactalbumin diet lose weight progressively, the loss being greatest during the first week (25%) and at the end of eight weeks the rats had lost 45% of their initial weights. The overall severity of malnutrition may be assessed from the loss in body weights. During the same period of time the control animals gained over 125% in body weights (Fig. 1a). The experimental animals looked emaciated, but there was no evidence of edema or accumulation of body fluids during the two month period of experimentation. In Enwonwu's study (1971) 5% of the animals fed the low protein diet developed edema after six weeks and by 10 weeks 18 – 25% were grossly edematous.

Plasma proteins:

There was a gradual diminution in the total plasma protein concentration especially after the fourth week (Fig. 1b). With low protein levels edema formation is to be expected according to the

Table 1

Changes in body fluid volumes and plasma protein content in rats during protein malnutrition*

Duration: (Weeks)		1	2	3	4	5	6	7	8
Experimental rats (0.5% Lactalbumin diet)									
Plasma vol. (ml)	M.	3.69	3.79	3.45	3.83	3.97	4.32	3.84	3.82
	S.D.	0.33	0.14	0.36	0.36	0.53	0.34	0.40	0.22
Blood vol. (ml)	M.	8.31	7.39	5.95	7.13	6.73	6.97	5.99	6.28
	S.D.	0.68	0.48	0.61	0.42	0.74	0.18	0.65	0.28
Total body water (ml)	M.	73	82	75	70	62	74	58	54
	S.D.	7	5	7	5	5	3	4	3
Extracellular water (ml)	M.	23.44	18.27	22.51	17.80	19.46	19.25	18.05	19.11
	S.D.	2.83	2.08	1.45	1.92	1.46	1.43	1.08	1.20
Total circulating plasma protein (mg)	M.	165	192	166	161	169	166	159	154
	S.D.	35	12	24	25	13	23	23	11
Total circulating albumin (mg)	M.	84	102	91	88	90	104	91	76
	S.D.	22	17	10	25	14	10	25	11
Control rats (18% Lactalbumin diet)									
Plasma vol. (ml)	M.	5.62	7.06	7.45	8.76	9.76	12.17	12.49	12.18
	S.D.	0.44	0.67	0.43	0.91	1.02	0.89	1.38	1.21
Blood vol. (ml)	M.	12.03	12.11	14.55	17.86	18.84	23.83	24.11	24.01
	S.D.	1.01	1.27	0.99	2.01	1.76	0.98	2.33	2.22
Total body water (ml)	M.	107	144	176	196	174	214	260	214
	S.D.	12	11	5	11	42	12	15	24
Extracellular water (ml)	M.	36.77	27.78	43.48	52.82	42.91	62.86	56.82	65.51
	S.D.	2.85	0.84	4.67	2.20	9.77	6.96	4.00	6.37
Total circulating plasma protein (mg)	M.	338	420	487	558	660	840	890	822
	S.D.	16	57	56	52	37	90	72	90
Total circulating albumin (mg)	M.	187	236	247	307	400	431	506	417
	S.D.	17	27	35	37	39	85	42	103

*Each result is the mean of six rats

classical hypothesis of Starling (Landis and Pappenheimer, 1963), as a result of the reduced colloid osmotic pressure of the plasma. A deficiency of colloid osmotic pressure would lead to escape of fluids into the tissues. In infants with protein malnutrition edema is almost invariably accompanied by hypoproteinemia (Waterlow, Cravioto and Stephen, 1960). Enwonwu (1970) observed that the development of edema was always preceded by a large drop in the serum protein concentration. He also suggested that "the association between hypoproteinemia and edema does not in any way imply a cause and effect relation." In the present study there was a decrease in the total circulating plasma proteins inspite of an increase in the plasma volume and this is probably due to a greater fall in the plasma protein concentration (Fig. 1c). However, it is observed that in protein malnutrition, even when there is no hypoproteinemia, there may be a decrease in plasma volume and so in the total circulating plasma protein (Waterlow, *et al,* 1960).

The plasma albumin concentration in rats fed the low protein diet was consistently lower than the control group, and so was the total amount of circulating albumin. However the relative amount of albumin remained rather unchanged. It is interesting to note that a significant fall in the concentration of albumin occurred after one week on the low protein diet and decreased further only towards the end of the study. It is possible that mobilisation of albumin from the extravascular compartments prevent marked dimunition in the albumin concentration in animals fed a low protein diet in the intervening period (Fig. 1d and 1e).

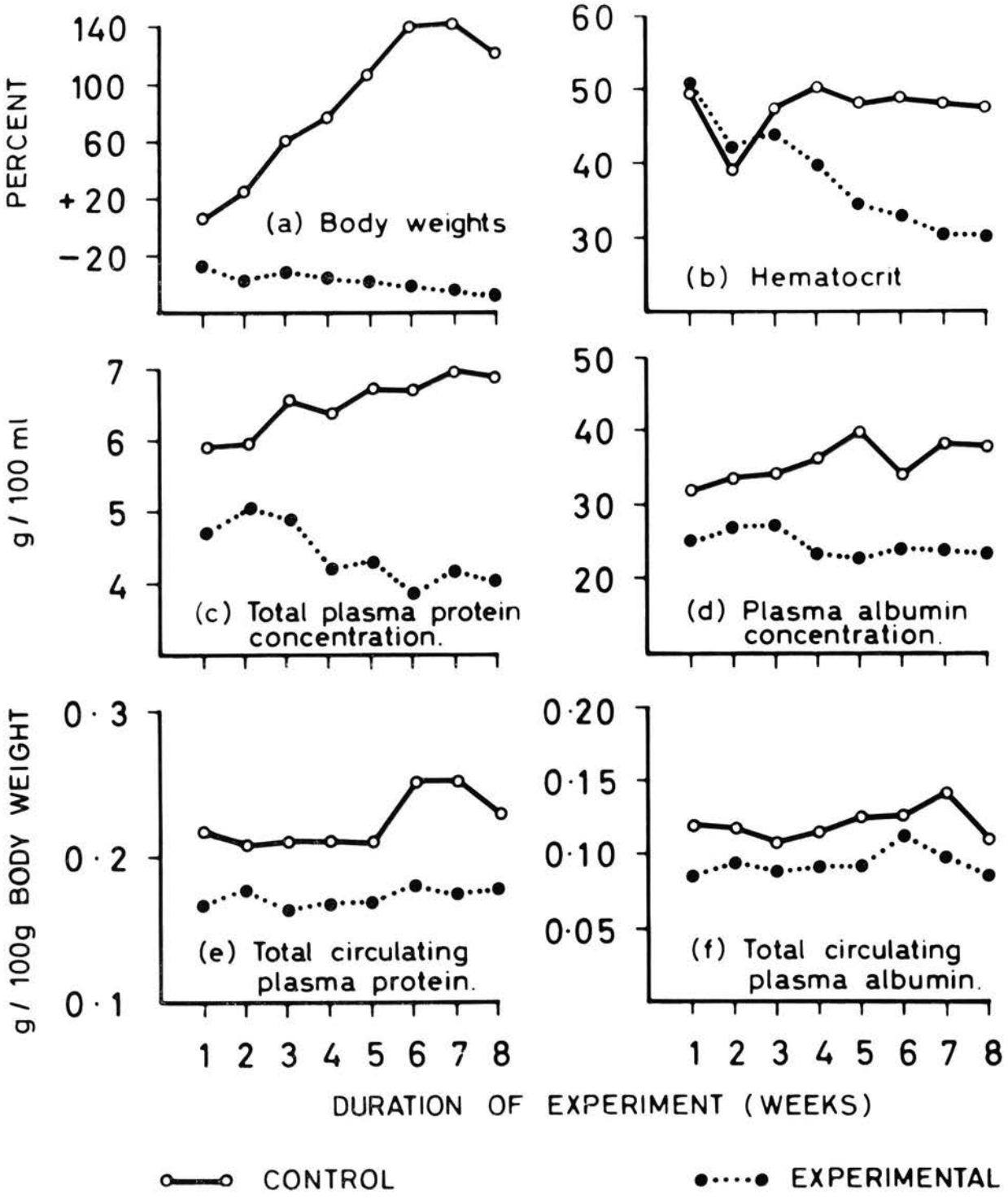

Fig. 1

Changes in body weights, hematocrit, and plasma proteins during experimental protein malnutrition in rats. The results for body weights are expressed as percent change from initial body weights. Each point is the mean of six rats. The lines joining the points are not meant to represent the changes in the intervening period, but merely to indicate general trends.

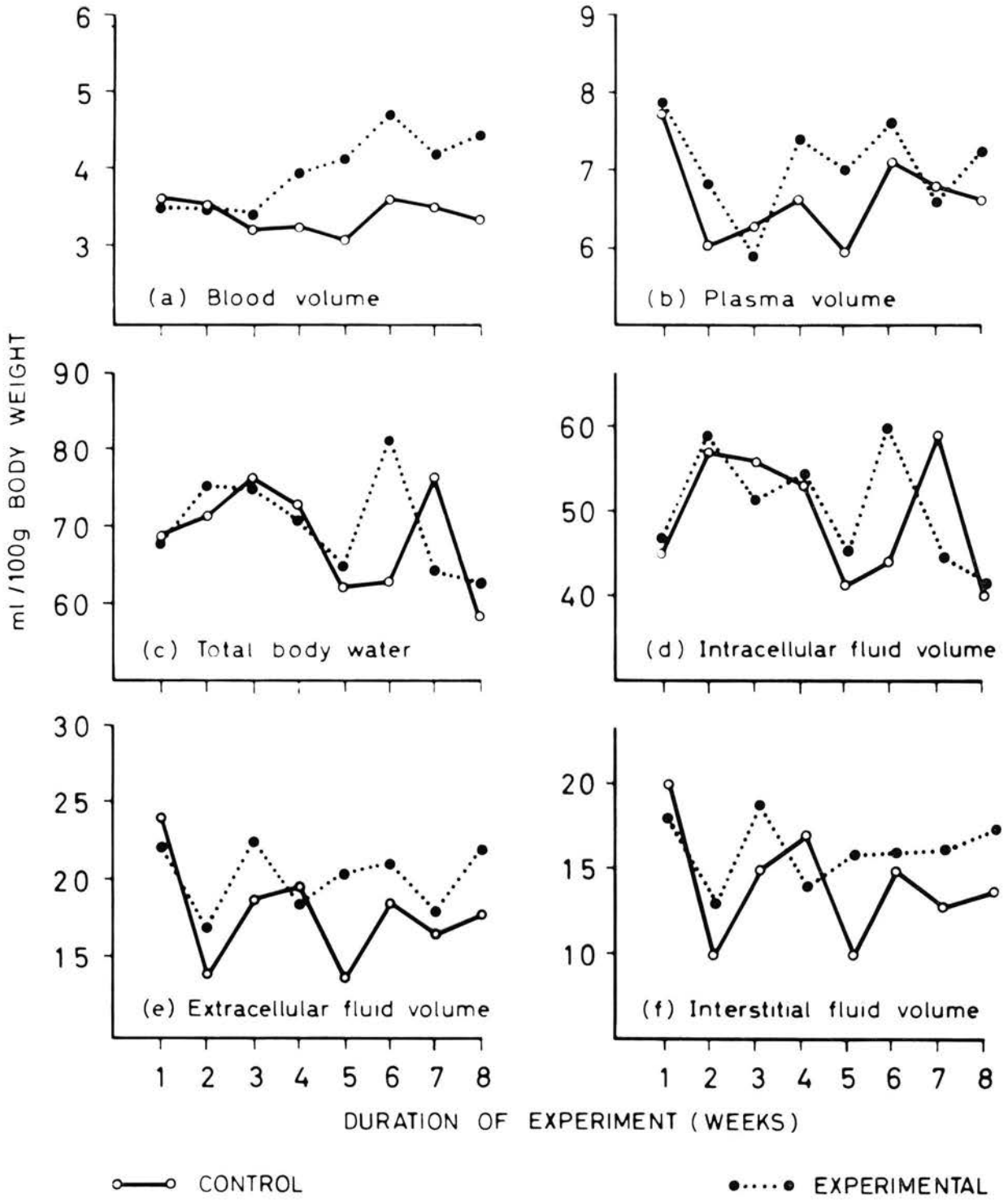

Fig. 2

Changes in the blood and plasma volumes, total body water and intracellular, extracellular and interstitial fluid volumes during experimental protein malnutrition in rats. Each point is the mean of six rats. The lines joining the points are not meant to represent the changes in the intervening period, but merely to indicate general trends.

Hematocrit:

There was a gradual fall in the hematocrit value as the duration on the low protein diet increased (Fig. 1f). This is most likely due to the development of anemia and an increase in the plasma volume.

Plasma volume:

The plasma volume per unit of body weight increased and was relatively greater, the higher the loss in body weight. In the present study the increase was evident from the 4th week onwards (Fig. 2a). There are many conflicting reports, some workers claiming that in nutritional edema the plasma volume is actually increased (Waterlow *et al,* 1960; McLaren, 1969), while others report that it is stationary (Keys, Bronzek, Henschel *et al.*; 1950). In nutritional edema at the height of the disease there is an absolute diminution in plasma volume (Gopalan *et al,* 1953).

Blood volume:

There was a fall in the relative blood volume towards the end of the 7th and 8th weeks of the experiment (Fig. 2b). Blood volume varies, on the whole, directly with the body weight, especially to the metabolically active body mass. During growth total plasma and blood volumes increase slowly according to the surface area until adult volumes are reached. After cessation of growth the blood volume remains unchanged in the rat. A decrease in plasma protein concentration may influence the blood volume through colloid osmotic pressure. In cases of malnutrition the blood volume seems to decrease at the same rate as the serum proteins (Walters, Lehman, and Rossiter, 1947). The fall in blood volume towards the end could possibly be explained by the development of anemia and this is substantiated by the low hematocrit findings in the present study.

Total body water:

There were no significant changes in the total body water during the first six weeks. Towards the end there was a very slight increase in the total body water (Fig. 2c). In malnutrition the water content of the body tends to be increased even without clinical edema. Clinical edema, as occurs in kwashiorkor, appears only after a considerable degree of water retention (Dean, 1962). In Edozien's study (1968) edema developed only about the 4th month. Perhaps the onset of edema marks a transition from the mild to the severe state of the disease (Enwonwu, 1970).

Intracellular water:

The intracellular fluid volume did not show any significant alterations, except for one observation each in the control and experimental group. It is suggested that protein malnutrition does not affect the intracellular fluid volume at least during the early stages.

Extracellular water:

There was a slight increase in the extracellular water content (thiocyanate space) after the 4th week (Fig. 2d). The volume of extracellular fluid is often high in malnutrition, and a consequence of this is the appearance of edema. Many explanations have been brought forward to account for the increased extracellular fluid volume in malnutrition (Widdowson, 1968). None of them explains all the facts, there are probably several causes. The more important ones seemtobe:-a) Fall in the oncotic pressure of the plasma and the resulting flow of fluid to the extracellular compartment (Picou and Waterlow, 1962). b) Alterations in the relative proportions of the three great components of the body due to malnutrition. The replacement by water of body space previously occupied by fat and cellular tissues is probably an important cause. Diminution is total body solids by providing space for the accumulation of excessive extracellular fluids may tend to mask the severity of the edema (Waterlow *et al,* 1960). c) Protein malnutrition can also result in diminished renal blood flow and glomerular filtration resulting from diminished cardiac output. This would presumably lead to sodium retention and edema (Davidson, 1964). d) Increased concentration of a substance with anti diuretic activity (Srikantia and Mohanram, 1969).

Interestitial fluid:

There was a small increment in the interstitial fluid volume after the fifth week, but not significant enough to manifest itself as clinical edema. This finding is consistent with the absence of clinical edema during the early stages of protein malnutrition.

Conclusion

The severity of protein malnutrition as gauged by gross alterations in body weights are not paralleled by equally significant alterations in the volumes of the fluid compartments of the body during the early stages. The classical edema so often associated with protein malnutrition is not evident during the early stages in the development of protein deficiency.

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References

Bray, G.A. (1960). A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem., 1: 279.

Chandrasekharan, N. (1969). Change in serum protein levels in the rat following laparotomy. Far East Med. J., 5: 25.

Davidson, C.S. (1964). Undernutrition, Starvation, Hunger edema and anorexia nervosa: In Cecil Loeb Text Book of Medicine. (Beeson P.B. and McDermott, W. ed.) W.B. Saunders, Ltd. London. p. 1198.

Dean, R.F.A. (1962). Production and control of edema. In symposium on mild and moderate forms of protein calorie malnutrition. Swedish Nutrition Foundation, Stockholm. p. 60.

Edozien, J.C. (1968). Experimental kwashiorkor and marasmus. Nature, 220: 917.

Enwonwu, C.O. and Sreebny, L.M. (1970). Experimental protein calorie malnutrition in rats – biochemical and ultrastructural studies. Exp. Mol. Pathol., 12: 332.

Enwonwu, C.O. and Sreebny, L.M. (1971). Studies on hepatic lesions of experimental protein calorie malnutrition in rats and immediate effects of refeeding an adequate protein diet. J. Nutr., 101: 501.

Gopalan, C., Venkatachalam, P.S. and Srikantia, S.G. (1953). Body composition in nutritional edema. Metabolism, 2: 335.

Hayes, F.N. and Gould, R.G. (1953). Liquid scintillation counting of tritium labelled water and organic compounds. Science, 117: 480.

Huang, K.C. and Bondurant, J.H. (1955). Simultaneous estimation of plasma volume, red cell volume and thiocyanate space in unanaesthetised normal and splenectomised rats. Amer. J. Physiol., 185: 441.

Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H.H. Biology of human starvation, vol. I and II. (University of Minnesota Press, Minneapolis, 1950).

Kirsh, R.E., Brock, J.F. and Saunders, S.J. (1968). Experimental protein calorie malnutrition. Amer. J. Clin. Nutr., 21: 820.

Kirsh, R.E., Saunders, S.J. and Brock, J.F. (1968). Animal models and human protein calorie malnutrition. Amer. J. Clin. Nutr., 21: 1225.

Landis, E.M. and Pappenheimer, J.R. (1963). Exchange of substances through the capillary walls. Handbook of physiology (ed. Hamilton) vol. 2., p. 961. Washington, American Physiological Society.

Loring, W.E. (1954). A rapid simplified method for serial blood volume determination in the rat. Proc. Soc. Exp. Biol. Med., 85: 350.

McLaren, R.S. (1969). In Diseases of Metabolism. (ed. P.K. Brondy) Saunders, U.K. p. 1245.

Picou, D. and Waterlow, J.C. (1962). The effect of malnutrition on the metabolism of plasma albumin. Clin. Sci., 22: 459.

Smith, R. (1960). Total body water in malnourished infants. Clin. Sci., 19: 275.

Srikantia, S.G. and Mohanram, M. (1969). Antidiuretic hormone values in plasma and urine of malnourished children. J. Clin. Endocr., 31: 312.

Sunderman, F.W. Jr., Sunderman, F.W., Falvo, E.A. and Kallick, C.J. (1958). Studies of the serum proteins. II. The nitrogen content of purified serum proteins separated by continuous flow electrophoresis. Amer. J. Clin. Path., 30: 112.

Walters, J.H., Lehman, H. and Rossiter, R.J. (1947). Blood volume changes in protein deficiency. Lancet, 1: 244.

Waterlow, J.C., Cravioto, J. and Stephen, J.M.L. (1960). Protein Malnutrition. Adv. Protein Chem., 15: 131.

Widdowson, E.M. (1968). Biological implications of body composition. In Symposium on body composition in animals and man. National Academy of Sciences, Washington, p. 71.