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Research Article

PLASMA PROTEIN AND HEMOGLOBIN IN THE PROTEIN-DEFICIENT RAT. A THREE-DIMENSIONAL STUDY[1]

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Laboratory evidence has indicated that low or inadequate protein diets usually result in partial inhibition of growth (1 to 3), depletion of tissue protein (4, 5), hypoproteinemia (4, 6 to 8), anemia (8 to 10), and variable edema (6, 11). Evidence derived from field observations of humans subsisting on protein-deficient diets has been equivocal. Growth inhibition and weight loss to the point of cachexia have been apparent (12, 13), but hypoproteinemia, definite anemia, and edema have not always been prominent (12, 14 to 16). In these latter instances, the concentrations of total protein and hemoglobin were determined. Since it is rather difficult to evaluate a three-dimensional function by means of a two-dimensional measurement (17), significant changes in the total quantity of hemoglobin and plasma protein may have been masked by measuring concentration without regard for the expanding or contracting blood volume. Consideration of the total circulating amounts of plasma protein (18 to 20) and hemoglobin (21, 22) presumably should afford somewhat better insight into the dynamic changes induced by protein deficiency. Four reports pertaining to total circulating protein (23) and total circulating hemoglobin (24 to 26) in the inadequately nourished rat have been found in the available literature. None of these was related to the effect of acute or chronic dietary protein deficiency in the growing animal which is the principal consideration of this report.

METHOD

Seventy-one male, Sherman-strain, weanling (35 to 40 grams), growing rats were placed in individual cages and fed a purified diet of the following composition: sucrose 73 per cent, casein (vitamin free) 18 per cent, corn oil (Mazola) 5 per cent, and Phillips and Hart salt mixture No. IV (27) 4 per cent. Thiamin chloride 200 μg., pyridoxine hydrochloride 200 μg., choline chloride 100 mgm., riboflavin 400 μg., niacin 2500 μg., and calcium pantothenate 1500 μg. were added per 100 grams ration. The fat-soluble vitamins, haliver oil, viosterol, and alpha-tocopherol, were fed in appropriate amounts by syringe *per os* biweekly. Water was allowed *ad lib.* The rats were weighed every other day. After an initial acclimatization week, concentrations of hemoglobin, erythrocytes (hematocrit), and plasma proteins were determined in all animals. The plasma protein concentration was estimated gravimetrically (28). Hemoglobin was measured in the Klett-Summerson photoelectric colorimeter, and the hematocrit was calculated from it. This relationship is based upon the assumption that cell gravity and hemoglobin concentrations within the cells are nearly constant, or that changes in cell gravity and cell hemoglobin are usually parallel. On such an assumption, other workers (29, 30) have shown that accurate estimations of cell volume and hemoglobin concentration could be made from the specific gravities of whole blood and plasma, since the weight of 100 ml. of blood is a function of the sum of the weights of its cells and plasma. The mean corpuscular hemoglobin and mean corpuscular volume of rat erythrocytes are proportionally decreased (31), and the anemia of protein deficiency in rats appears to be microcytic and hypochromic (8, 10); therefore, it would seem that the above gravimetric relationships probably are valid in this instance. The formulae for calculation of hemoglobin are dependent upon its oxygen capacity. Since the formulae for both hemoglobin and hematocrit are similar, the hematocrit may be calculated from the value for hemoglobin (in grams per 100 ml.) by substitution. Reduction of this substituted formula to its simplest terms results in the relationship, hematocrit = 2.95 × hemoglobin, which was used in this experiment. Such calculations, checked in the laboratory and against data reported in the literature (9, 32), result in an error of 1 to 2.5 per cent in the hematocrit as observed (33). The method is of some value in animals where macrocytic hypochromic anemias are not likely to be encountered because of simplicity, relative accuracy, and the small amount of blood needed.

Blood volume partition studies, using the dye T-1824 and a modified single sample technic (33), were done concurrently with the above concentration determinations in most instances. Total circulating hemoglobin and total circulating protein were readily calculated and

[1] This report represents part of a correlated study on the relation of dietary protein to resistance and immunity in respiratory infections. The work was aided in part by a grant from the William W. Wellington Memorial Fund.

adjusted to a unit value (grams per unit blood or plasma volume) by multiplying the hemoglobin and total protein concentrations by unit blood and plasma volume, respectively (34). Unit values for blood and plasma volume were obtained by adjusting the total volumes to a unit of surface area (ml. per 100 cm.^{2}) (33). Eight-tenths of a ml. of blood obtained by heart puncture was sufficient for all determinations. After these initial studies, the animals were arbitrarily divided into two groups in order to investigate acute and chronic protein deficiency.

In the first group, 22 growing rats were used to ascertain the effect of the previous nutritional status upon changes induced by an acute protein deficiency. One-half of this group was initially fed at the control level of 18 per cent casein; the other, at a deficiency level of 8 per cent casein. This diet was essentially similar to the control diet, except that sucrose was substituted for the withdrawn protein to make up the caloric deficit. After 1 month on these diets, the casein fed in both the 18 and 8 per cent groups was restricted to a 2 per cent level. This diet contained the same elements as those previously mentioned, but since sucrose made up the caloric deficit, the allowance of synthetic water soluble vitamins added per 100 grams ration was doubled in the 2 per cent casein ration. The previous blood studies were repeated after 7 days on this very deficient diet.

The changes induced by chronic dietary protein deficiency were observed in the second group, composed of 49 growing rats. Of these, one group was fed casein at a control level of 18 per cent; the other, at a deficiency level of 8 per cent. Concentrations of hemoglobin, erythrocytes, and plasma protein were determined at the onset, after 3 to 6 weeks in all animals, and again after 10 to 14 weeks in 10 animals of each group. Blood volume partition studies were done concurrently in most instances. Unit circulating protein and unit circulating hemoglobin were estimated.

RESULTS

The comparative growth curves of the well-nourished and deficient rats are shown in Figure 1. For simplicity of discussion: "Well-nourished" = 18 per cent casein diet; "chronic deficiency" = 8 per cent casein diet; "previously deficient (with superimposed acute deficiency)" = 8 per cent casein acutely decreased to a 2 per cent level which was then maintained; "acute deficiency" = 2 per cent casein diet. The effects of acute marked protein deficiency on the hemoglobin and plasma protein of previously well-nourished and previously deficient animals are shown in Table I and Figures 2 and 3. The effects of chronic protein deficiency on the hemoglobin and plasma protein of growing rats are shown in Table II and Figures 2 and 3. The "t-test" (35) has been used to compare

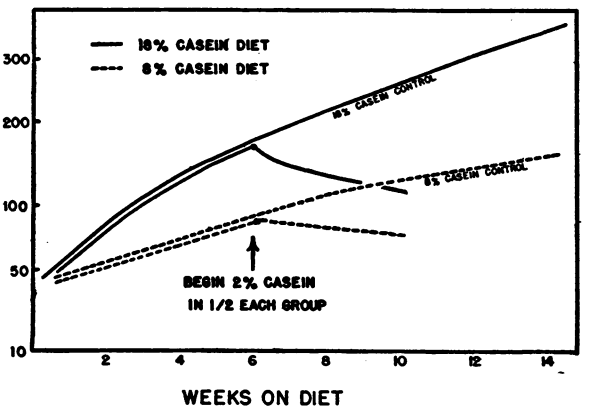


FIG. 1. GROWTH (WEIGHT IN GRAMS) EXPRESSED AS A SEMI-LOGARITHMIC FUNCTION OF TIME

The solid line represents growth of the control animals (18 per cent casein). The broken line illustrates growth of the chronically deficient rats (8 per cent casein). Later in the experiment, half the animals in each group were placed on a diet very deficient in protein (2 per cent casein). The previously well-nourished rats lost approximately 2.6 grams per day; the previously deficient, 1.0 gram per day.

means. The animals used as comparison controls were arbitrarily selected, according to weight, from the control groups. This procedure was used so that the distribution of individual weights and the average group weight would be similar to that of the deficient group with which they were being compared. Accordingly, these well-nourished body size controls were 2 to 3 weeks younger than the deficient animals. This comparison was made to obviate, insofar as possible, the effect of body size on the blood volume partitions and unit circulating protein and hemoglobin (33, 34).

Growth (Figure 1). Rats fed casein at an 18 per cent level had a growth increment of 3.5 to 4 grams per day, more than tripling their initital body weight in 1 month. The rate of increase was somewhat more exponential thereafter. This may be considered a good increment of growth for this strain and diet. Rats fed casein at an 8 per cent level had a growth increment of approximately 1 gram per day, failing to double their initial body weight in 1 month. Except for obvious stunting, there was no marked difference in the appearance of the deficient group.

Altering the levels of casein from 18 per cent and 8 per cent to 2 per cent, after 1 month on

the respective diets, caused a weight loss of approximately 2.5 grams per day in the previously well-nourished rats and slightly less than 1.0 gram per day in the previously deficient rats. No gross edema was observed in either group. There were no deaths from intercurrent infections.

Plasma volume and plasma protein (Figures 2 and 3). Acute protein deficiency (Figure 2) in previously well-nourished rats produced no significant change in the plasma protein concentration. The unit circulating protein, however, was significantly decreased,[2] associated with contraction of the unit plasma volume.[3]

Acute protein deficiency in previously deficient animals with normal total protein concentration induced a significant decrease in the plasma protein concentration.[4] This decrease was not apparent if the acutely deficient animals were compared with well-nourished body size controls. The unit plasma volume and the unit circulating protein, however, were significantly decreased in the acutely deficient group. This decrease, accordingly, was apparent in comparison with both the deficient and body size controls (Table I).[5]

[2] Unit circulating protein (acute deficiency *vs.* control): $\sigma = 0.0234$, $t = 7.55$, $n = 19$, P = less than 0.01; therefore significant.

[3] Unit plasma volume (acute deficiency *vs.* control): $\sigma = 0.343$, $t = 5.90$, $n = 19$, P = less than 0.01; therefore significant.

[4] Total protein concentration (acute deficiency *vs.* chronic deficient): $\sigma = 0.522$, $t = 3.89$, $n = 20$, P = less than 0.01; therefore significant.

[5] Unit plasma volume (acute deficiency *vs.* chronic deficiency): $\sigma = 0.664$, $t = 2.18$, $n = 21$, P = less than 0.05; therefore probably significant. Unit plasma volume (acute deficiency *vs.* size control): $\sigma = 0.368$, $t = 6.65$, $n = 22$, P = less than 0.01; therefore significant. Unit

TABLE I

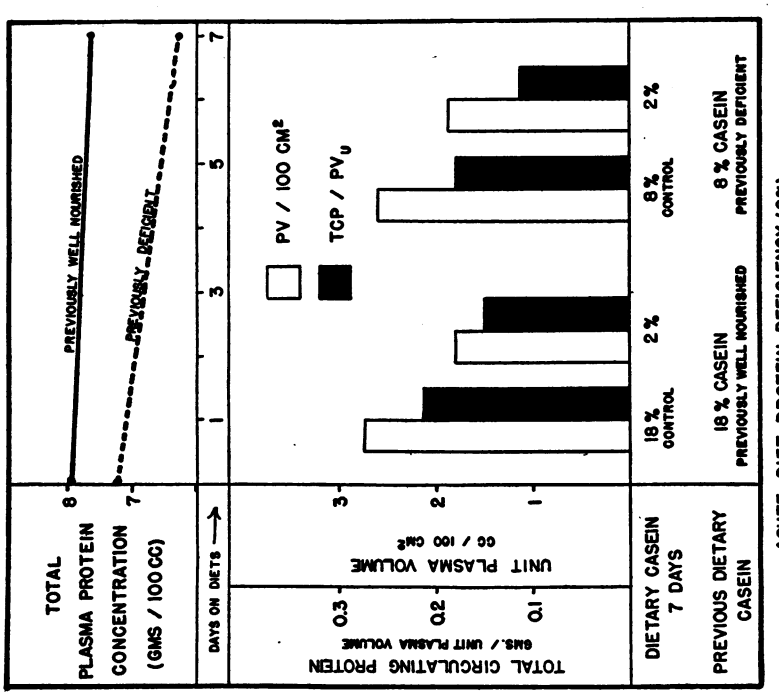
Two per cent protein in diet—acute deficiency (7days)

Determination	Previously deficient with superimposed acute deficiency (11)*	Controls		Previously well nourished with superimposed acute deficiency (11)	Control
		Chronic deficiency (12)†	Well nourished (13)‡		Well nourished (10)
Weight (*grams*)	61.8–119.6	88.0–129.1	56.3–148.7	136.9–209.9	137.2–231.4
Surface area (*cm.*²)	149.0–221.3	184.2–231.7	140.9–252.2	240.1–310.1	240.4–328.8
Hemoglobin (*grams per 100 ml.*)	16.3 ±1.61	14.7 ±1.08	12.4 ±0.96	16.8 ±0.60	14.7 ±1.13
Hematocrit (*ml. per 100 ml.*)	48.1 ±1.63	43.2 ±2.93	36.8 ±2.84	49.5 ±1.57	43.4 ±3.42
Total protein (*grams per 100 ml.*)	6.30 ±0.53	7.21 ±0.49	6.76 ±0.75	7.61 ±0.32	7.99 ±0.46
Total plasma volume (*ml.*)	3.48 ±0.77	5.02 ±1.63	5.21 ±1.01	5.20 ±0.79	7.72 ±1.43
Unit plasma volume (*ml. per 100 cm.*²)	1.90 ±0.34	2.50 ±0.86	2.86 ±0.41	1.90 ±0.18	2.78 ±0.46
Unit blood volume (*ml. per 100 cm.*²)	3.55 ±0.53	4.53 ±1.60	4.58 ±0.59	3.79 ±0.36	4.92 ±0.73
Unit cell volume (*ml. per 100 cm.*²)	1.65 ±0.34	1.96 ±0.77	1.72 ±0.26	1.88 ±0.20	2.09 ±0.037
Unit circulating protein (*grams per unit blood volume*)	0.119 ±0.026	0.181 ±0.045	0.193 ±0.027	0.145 ±0.014	0.221 ±0.037
Units circulating hemoglobin (*grams per unit blood volume*)	0.575 ±0.099	0.670 ±0.254	0.572 ±0.079	0.638 ±0.071	0.727 ±0.105

* Numbers in parentheses refer to number of animals.

† Weights similar to experimental rats before acute deficiency initiated; therefore chronic deficiency control.

‡ Weights similar to those of acutely deficient rats; therefore body size control but (2 to 3 weeks) younger.

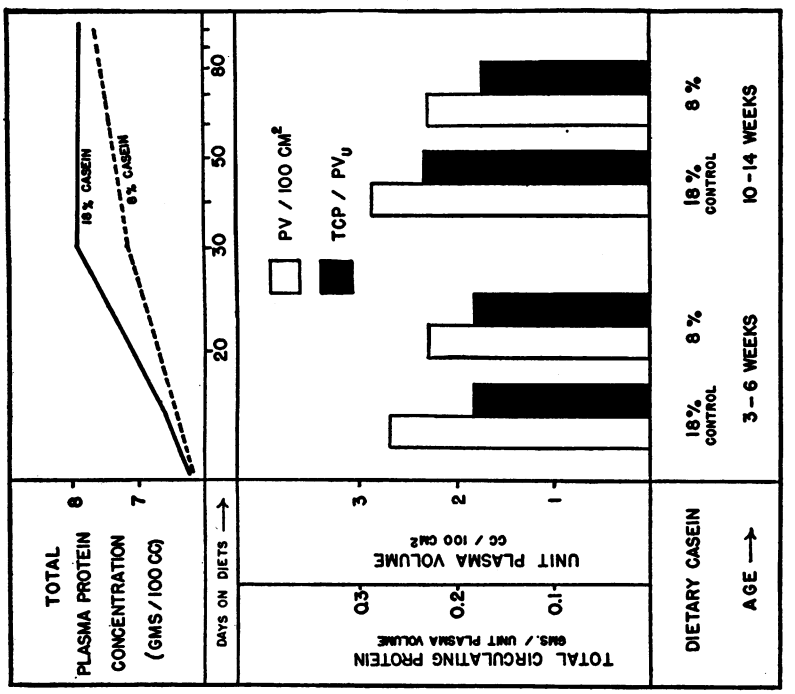


ACUTE DIET PROTEIN DEFICIENCY (2%)

FIG. 2. THE EFFECT OF ACUTE PROTEIN DEFICIENCY (2 PER CENT CASEIN FOR 7 DAYS) ON THE PLASMA PROTEINS

The upper portion of the figure illustrates the decrease in plasma protein concentration with restriction of dietary protein in previously well-nourished and previously deficient animals. A significant, but not marked decrease is apparent only in the previously deficient animal.

In the lower half of the figure, unit circulating protein and plasma volumes of the acutely deficient animals are compared with appropriate controls. PV/100 cm.^{2} = unit plasma volume, and TCP/PV$_u$ = unit circulating protein. The relative magnitudes of the changes may be compared with the somewhat equivocal concentration changes above.



CHRONIC DIET PROTEIN DEFICIENCY (8%)

FIG. 3. THE EFFECT OF CHRONIC PROTEIN DEFICIENCY (8 PER CENT CASEIN) ON THE PLASMA PROTEINS

The upper portion of the figure illustrates the increase of plasma protein concentration with growth in both deficient and control animals. The difference is not marked.

In the lower half of the figure, unit circulating protein and plasma volumes of the deficient animals are compared with appropriate controls. The difference in unit circulating protein after 10 to 14 weeks of partial protein restriction is statistically significant. The relative magnitude of this change may be compared with the rather equivocal concentration changes above.

TABLE II
Eight per cent protein in diet—chronic deficiency

Determination	3 to 6 weeks		10 to 14 weeks	
	Deficient (25)*	Control (24)	Deficient (10)	Control (10)
Weight (*grams*)	58.5–129.1	52.3–194.4	107.8–204.9	69.1–194.1
Surface area (*cm.*²)	144.2–231.7	134.5–296.1	207.8–305.6	185.5–296.1
Hemoglobin (*grams per 100 ml.*)	14.9±0.24 ±1.03	13.2±0.31 ±1.47	14.0 ±1.28	14.5 ±1.26
Hematocrit (*ml. per 100 ml.*)	44.0±0.72 ±3.04	39.0±0.96 ±4.78	41.3 ±3.94	42.8 ±3.79
Total protein (*grams per 100 ml.*)	7.20±0.12 ±0.57	6.95±0.20 ±0.97	7.65 ±0.51	7.88 ±0.77
Total plasma volume (*ml.*)	4.15±0.32 ±1.60	4.43±0.48 ±2.34	5.69 ±1.38	7.48 ±1.50
Unit plasma volume (*ml. per 100 cm.*²)	2.28±0.14 ±0.70	2.71±0.079 ±0.54	2.17 ±0.37	2.85 ±0.46
Unit blood volume (*ml. per 100 cm.*²)	4.05±0.231 ±1.39	4.50±0.132 ±0.90	3.69 ±0.59	4.98 ±0.77
Unit cell volume (*ml. per 100 cm.*²)	1.78 ±0.65	1.77 ±0.43	1.54 ±0.31	2.08 ±0.43
Unit circulating protein (*grams per unit plasma volume*)	0.170±0.011 ±0.054	0.192±0.006 ±0.042	0.166 ±0.030	0.220 ±0.040
Unit circulating hemoglobin (*grams per unit blood volume*)	0.620 ±0.216	0.602±0.022 ±0.153	0.517 ±0.113	0.722 ±0.107

* Numbers in parentheses refer to number of animals.

Chronic protein deficiency (Figure 3) of 10 to 14 weeks' duration did not produce a significant change in the plasma protein concentration. Unit plasma volume was decreased significantly in 3 to 6 weeks.[6] The decrease in unit circulating protein, suggestive at 3 to 6 weeks, was not statistically significant until a later time. The observed decrease at 10 to 14 weeks was significant (Table II).[7]

Blood volume and hemoglobin (Figures 4 and 5). Acute protein deficiency (Figure 4) resulted in hemoconcentration as indicated by the increased hemoglobin concentration and decreased plasma volume. The blood volume was decreased largely at the expense of the plasma, since the cell volume was not significantly altered. The suggestive decrease in unit circulating hemoglobin was statistically significant only in the case of the previously well-nourished group (Table I).[8]

Chronic dietary protein deficiency (Figure 5) did not appear to alter the hemoglobin concentration. The unit circulating hemoglobin, like the unit blood and cell volumes, was significantly decreased [9] after 10 to 14 weeks on the 8 per cent casein diet.

circulating protein (acute deficiency *vs.* chronic deficiency): $\sigma = 0.038$, $t = 3.90$, $n = 21$, P = less than 0.01; therefore significant. Unit circulating protein (acute deficiency *vs.* size control): $\sigma = 0.0258$, $t = 7.12$, $n = 22$, P = less than 0.01; therefore significant.

[6] $\frac{\bar{M}c - \bar{M}d}{\text{diff.}} = 3.7$, more than 2; therefore significant.

[7] Unit circulating protein (3 to 6 weeks): $\sigma = 0.487$, $t = 1.53$, $n = 44$, P = more than 0.05; therefore not significant. Unit total circulating protein (10 to 14 weeks): $\sigma = 0.0306$, $t = 3.80$, $n = 17$, P = less than 0.01; therefore significant.

[8] Unit circulating hemoglobin (acute deficiency *vs.* control): $\sigma = 0.0913$, $t = 2.18$, $n = 18$, P = less than 0.05; therefore probably significant. The lack of statistical significance relative to the diminution in hemoglobin of the previously deficient group is probably in part the result of distribution of the data in the deficient group with which comparison was made.

[9] Unit circulating hemoglobin (deficiency *vs.* control); $\sigma = 0.113$, $t = 3.83$, $n = 16$, P = less than 0.01; therefore significant.

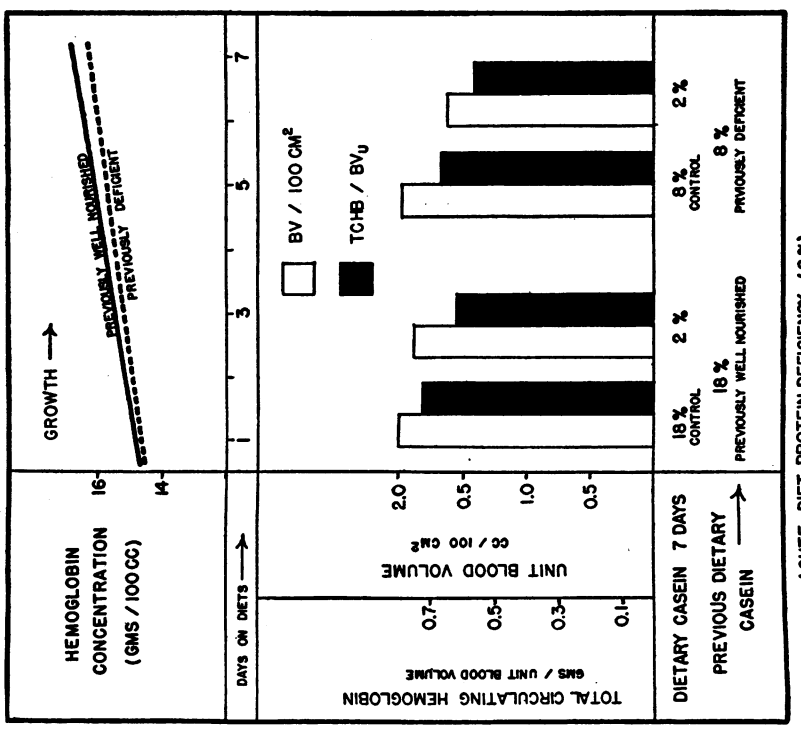


FIG. 4. THE EFFECT OF ACUTE PROTEIN DEFICIENCY (2 PER CENT CASEIN FOR 7 DAYS) ON HEMOGLOBIN

Some indication of the degree of hemoconcentration induced by acute protein deficiency is evident in the upper portion of the figure. The three-dimensional changes in unit circulating hemoglobin may be compared with the concentration change. BV/100 cm.² = unit blood volume, and TCHb/BV_u = unit circulating hemoglobin.

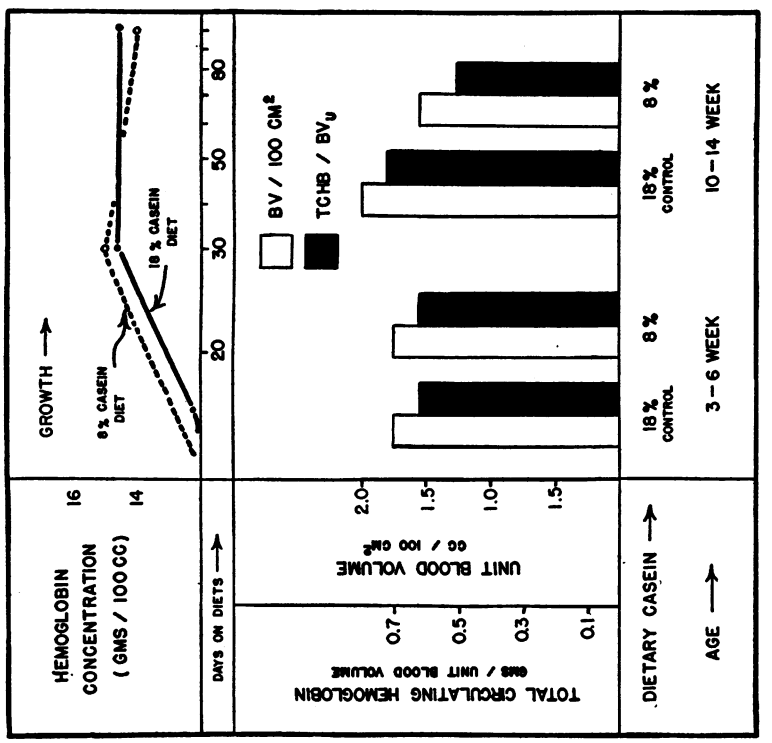


FIG. 5. THE EFFECT OF CHRONIC PROTEIN DEFICIENCY (8 PER CENT CASEIN) ON HEMOGLOBIN

The change in hemoglobin concentration with growth in both the control and protein deficient animals is illustrated in the upper portion of the figure. The difference, after 14 weeks on the respective diets, is not marked.

In the lower half of the figure, unit circulating hemoglobin and blood volume of the chronically deficient animals are compared with controls. The diminution of unit circulating hemoglobin may be compared with the apparent lack of concentration change above.

DISCUSSION

The observed data on blood volume partitions, unit circulating protein, and unit circulating hemoglobin are *relative* rather than *absolute* owing to limitations in the technics employed. The results remain proportional, however, since the error is systematic.

In the consideration of plasma protein concentration, the absence of significant change with dietary protein deficiency (Tables I and II; Figures 2 and 3) is not an unusual finding. It is probably dependent upon the masking effect of hemoconcentration (4, 14, 18, 19). Plasma protein concentration in the acutely protein-deficient rat (Table I; Figure 2) is similar to that observed in rats starved for 7 days (23), or fed a carrot diet for that period of time (36). In the chronically protein-deficient rat, no significant change was noted at 11 weeks (Table II; Figure 3). Inanition *per se* does not produce a low plasma protein concentration (8). Although low plasma protein levels have been observed in 2 to 3 months (6), it has been suggested that they may not be apparent after 4 months of depletion (37) unless large quantities of fluid and a diet deficient in calories as well as protein are consumed (36). These qualifications probably do not apply to the conditions of this experiment.

In the consideration of the unit circulating protein, significant decreases were noted with both the acute 2 per cent and chronic 8 per cent casein diets (Tables I and II; Figures 2 and 3). The proportionate depletion in the acutely deficient animals was somewhat greater than that observed in rats starved for 7 days (23). In the chronically deficient rats, the proportionate depletion was slightly less than that observed in dogs on a protein deficient diet for a similar length of time (9). This depletion was, in part, a manifestation of the contracted plasma volume. It has been suggested that the decrease in plasma volume associated with hypoproteinemia is the result of decreased plasma albumin concentration (38, 39) if the total cell volume is constant (40). No fractionation of the proteins was done, but the majority of evidence from other sources indicates that diet protein deficiency principally depletes the albumin fraction (5, 6, 9, 14, 17, 18). Unit cell volume was not constant in the protein deficient rats; it was diminished (Table II). Other factors influence the plasma volume; their consideration is beyond the scope of this report.

In the consideration of hemoglobin concentration, the acute increase in rats on the 2 per cent protein diet was probably the result of hemoconcentration (Table II; Figure 4). Chronic protein deficiency did not markedly alter the hemoglobin concentration during the 10 to 14 weeks of observation. This finding is somewhat at variance with another report (10), in which a decrease in hemoglobin concentration from 15.8 to 11.5 per cent over a similar period of time was observed. The two experiments are not strictly comparable in that this other deficient diet contained 3.5 per cent protein as lactalbumin. There is some evidence that casein is more effective in the prevention of anemia than is lactalbumin (41).

In the consideration of unit circulating hemoglobin, the decrease with both acute and chronic protein deficiency was probably a function of altered blood volume (9) and induced hypochromia (8, 10). The apparent differential change in the acute deficiency was complicated by the relative contractions of unit cell and plasma volumes in the previously well-nourished and previously deficient rats. The contraction of the plasma volume was greater in the former, but the cell volumes decreased a small, statistically insignificant amount in both (Table I). The decrease in chronic deficiency involved both the unit cell and plasma volumes (Table II). Others have observed increased (25), unchanged (26), or decreased (24) blood volumes in malnourished anemic rats.

The changes in the protein economy of the growing rat imposed by dietary protein deficiency are, as yet, speculative. The concept of dynamic equilibrium between blood and tissues, however, suggests a possible interpretation of the data. Moderate chronic protein deficiency supports suboptimal growth. Presumably the quantity of protein diverted to tissue would appear to be limited by that required to maintain a normal amount in the circulation. In the diet-deficient animal, therefore, the unit circulating protein seems to be initially defended at the expense of body growth. Concentration is maintained by contraction of the plasma volume

(42). In time, this adaptation appears to fail, and the anabolic needs result in withdrawal of some of the circulating protein. Further contraction of the plasma volume maintains an oncotic homeostasis since the plasma protein concentration appears to be within the normal range. Withdrawal of more protein from the circulation would ultimately result in low plasma protein concentrations and diminished oncotic relationships resulting in edema. This eventual state was not observed during this experiment.

When the dietary protein is inadequate to maintain positive nitrogen balance, as in acute deficiency, the tissues ordinarily contribute the major portion of the nitrogen required in metabolism (9, 43). This was evident in the proportionately more rapid weight loss of the previously well-nourished animals, and possibly accounts for the paradoxical observation that previously deficient animals make an apparently better adjustment, as measured by weight loss, to acute protein deficiency. In neither case does weight loss alone seem to be sufficient for maintaining nitrogen balance since both groups show depletion in the amount of circulating protein, apparently after homeostatic mechanisms become inadequate.

The mechanism of anemia resulting from protein deficiency is not well understood. Dietary protein nitrogen is less readily accepted by hemoglobin than by other tissues (44). In addition, the exchange of iron in erythrocyte hemoglobin with that of the tissues is negligible (45). The "tissue reserves," therefore, are probably not the primary factor involved in protein deficiency anemia. Immature rats on diets lacking in protein can synthesize new protein combinations (44). Inadequate synthesis of hemoglobin, therefore, does not seem to be solely responsible. Altered caloric intake or inanition alone apparently does not result in anemia (8, 10). In the realimentation of animals made anemic by bleeding, protein preferentially restores the hemoglobin to normal levels; therefore, the mechanism of anemia is apparently not irreversible (10, 46). The observations of this report are in accord with the suggestion that a diminution in unit cell volume is a prominent factor in the anemia of chronic protein deficiency.

SUMMARY

The effect of acute and chronic dietary protein deficiency upon the total circulating amounts of plasma protein and hemoglobin was investigated in growing rats fed purified diets. The effect of growth and body size was considered in the analyses. The following results were observed:

1. The rate of growth of rats on an 8 per cent casein diet was less than one-third of that noted on an 18 per cent casein diet.

2. Acute protein deficiency (2 per cent casein) imposed upon animals on an 8 per cent casein diet and animals on an 18 per cent casein diet caused a relatively greater weight loss in the latter group.

3. Acute protein deficiency resulted in significant contraction of the plasma volume and depletion of unit circulating protein; whereas only a slight lowering of the plasma protein concentration was noted.

4. The acute protein deficiency was associated with hemoconcentration; however, since plasma and blood volumes were significantly decreased and cell volume slightly diminished, hemoglobin depletion was evident on a three-dimensional basis.

5. Chronic protein deficiency (8 per cent casein) did not alter the plasma protein or hemoglobin concentration; however, plasma volume, cell volume, unit circulating protein, and unit circulating hemoglobin were significantly decreased.

Thus alterations of plasma protein and hemoglobin in both acute and chronic dietary protein deficiency are appreciated only if the total circulating amounts, adjusted to a unit of surface area, are considered.

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