LOW PROTEIN DIET AUGMENTS HYPERPROTEINEMIA PRODUCED BY REPEATED INJECTIONS OF HOMOLOGOUS PLASMA

Evidence for a Dynamic Equilibrium between Food, Plasma, and Tissue Proteins*

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During recent years the use of plasma transfusions has become widespread and increasingly so of late as a result of war conditions. More and more the need for really large amounts of plasma in treating certain types of cases is being appreciated. The data presented in this paper should be of interest to those engaged in studying the quantitative aspects of such therapy. Further these data have certain theoretical implications that bear upon the more general problems of plasma protein formation and protein metabolism.

Hyperproteinemia can regularly be produced in dogs, and presumably in other species of animals, by repeated injections of plasma obtained from homologous donors. Almost none of the injected protein escapes in the urine as protein, nor is it eliminated quantitatively as increased urinary or fecal N_2. While no direct measurements have been made in dogs, Addis' results following intraperitoneal injections of serum in rats (1) indicate that the protein content of all of the viscera and tissues is increased. The greatest increment is in the serum, next in the liver, but all organs and tissues show a definite increase in protein content.

Practically all of the experiments to date (2–6) have been carried out with the recipient of the plasma or serum injections maintained in a fasting state or receiving only sugar, or sugar and fat, by mouth. *A priori* one would expect that plasma injections in an animal maintained on a full diet or a high protein diet would yield summation effects and result in a more marked hyperproteinemia. In the experiments reported below this does not happen; in fact, a significantly higher hyperproteinemia is produced when the animals are maintained on a *low* protein diet than when a high protein diet is given. This seeming paradox is open to a number of interpretations but all of them seem to imply an equilibrium between food, plasma, and tissue proteins.

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Methods

All of the dogs were healthy adult mongrels. Small dogs (about 5.0 kilos) were chosen so that increase of the plasma protein level could be accomplished without using too great quantities of blood. They were kept in individual cages and had free access to water at all times. One group was fed a high protein diet, the other group a low protein diet.

The high protein diet consisted of lean beef—25 gm. per kg. per day—to which 1 gm. of the salt mixture (7) was added.

The low protein diet consisted of: calves' liver (raw wet weight) 32 parts, cane sugar 25 parts, corn starch 25 parts, butter 12 parts, and cod liver oil 6 parts. 1 gm. of salt mixture (7) and 5 gm. of kaolin were thoroughly mixed with each day's diet. Enough tomato juice was added to make a pasty mixture of which each gram contained 3 calories. The diet was fed in amounts to furnish 75 calories per kg. per day. Essentially this diet is a low protein diet with 7 per cent of its caloric value derived from protein, 50 per cent from carbohydrate, and 43 per cent from fat.

All of the dogs in both groups consumed 100 per cent of the diet each day.

The methods used in making the dogs hyperproteinemic have been published in detail (2, 8). Briefly these consisted of bleeding a donor—a sufficient number of large dogs (15 to 25 kg.) were used so that anemia did not develop—about 200 cc. into a flask containing 2.5 cc. of a saturated solution of trisodium citrate, centrifuging the citrated blood in 100 cc. centrifuge tubes at 3000 R.P.M. for 30 minutes, withdrawing the plasma (usually 100 to 110 cc.) with suction, warming it to 40°C., and injecting it into one of the external jugular veins (about 10 minutes being required for each injection). This procedure was repeated daily, 6 days per week, for 3 to 4 weeks.

Duplicate micro Kjeldahl analyses of total N, N.P.N. (the filtrate from 10 per cent trichloroacetic acid precipitation) and albumin plus N.P.N. (the filtrate from 22 per cent sodium sulfate precipitation by Howe's method) served as the basis for calculating the blood level studies. All of the recorded studies were made on hematocrit samples (using 2.0 cc. of 1.4 per cent sodium oxalate and 10 to 13 cc. of blood), and the "final samples" were taken at least 18 hours after the last injection of donor's plasma.

EXPERIMENTAL OBSERVATIONS

The experimental data are summarized in Tables I and II.

In dogs consuming a high protein diet (Table I) the intravenous injection of plasma obtained from healthy donor dogs, amounting in all to five or six times the quantity of plasma protein in circulation at the start, resulted in only a moderate increase in the concentration of the blood plasma proteins. The average increase was 20 per cent. As would be expected, there was a fall in the hematocrit reading but this was not great—ranging from about 5 per cent in dog 35-693 to about 28 per cent in dog 40-60 and averaging 18 per cent. "Final" blood and plasma volume studies made in isolated cases in this series agree with previous observations (2, 3) that these are increased

only about 15 per cent; and this increase—which is almost entirely in the plasma volume—accounts in part at least for this fall in hematocrit value. There were no significant changes in the albumin:globulin ratio, plasma N.P.N., or in body weight.

TABLE I

Plasma Injections in Dogs on High Protein Diet

Dog No.	Body weight		No. of injections	Total amount injected	Plasma protein concentration		Albumin/globulin		N.P.N.		Hematocrit reading	
	I.*	F.‡			I.	F.	I.	F.	I.	F.	I.	F.
	kg.	*kg.*		*cc.*	*gm./100 cc.*				*mg./100 cc.*		*per cent*	
35-693	12.7	12.7	19 in 24 days	4355	6.6	8.2			27	27	41	39
40-59	6.3	5.8	18 " 21 "	1595	7.5	8.9	1.2	1.3	36	36	58	44
40-60	6.5	6.3	24 " 28 "	2665	7.2	8.7	1.7	1.4	30	30	57	41
40-67	4.8	4.3	20 " 24 "	2080	7.2	8.3	0.9	0.8	40	32	53	39

* Initial value—before first plasma injection.

‡ Final value—18 to 24 hours after last plasma injection.

TABLE II

Plasma Injections in Dogs on Low Protein Diet

Dog No.	Body weight		No. of injections	Total amount injected	Plasma protein concentration		Albumin/globulin		N.P.N.		Hematocrit reading	
	I.*	F.‡			I.	F.	I.	F.	I.	F.	I.	F.
	kg.	*kg.*		*cc.*	*gm./100 cc.*				*mg./100 cc.*		*per cent*	
39-28	6.7	5.1	24 in 28 days	2225	7.2	9.9	1.3	0.7	41	23	36	32
39-34	4.5	4.4	22 " 28 "	2625	6.5	9.4			30	41	43	40
39-40	6.3	6.5	17 " 21 "	2010	7.0	8.8	1.3	1.0	33	32	51	42
39-45	7.1	7.3	22 " 26 "	2210	7.1	9.2	1.2	0.9	36	31	54	43
40-50	4.7	4.8	24 " 28 "	1880	6.6	10.0	1.9	0.9	41	25	55	49
40-63	8.1	9.2	24 " 28 "	2960	6.0	8.0	1.2	1.1	28	28	48	36
40-80	5.7	5.8	18 " 21 "	1840	6.7	9.4	1.3	1.0	31	24	48	38

* Initial value—before first plasma injection.

‡ Final value—18 to 24 hours after last plasma injection.

In dogs consuming a low protein diet (Table II) the intravenous injection of comparable amounts of plasma—obtained in most instances from the same donor dogs—resulted in a more marked increase in the concentration of the plasma proteins. The average increase was 40 per cent, or twice as great as in the group of dogs maintained on high protein diet. Changes in the hematocrit readings were in the same direction and of the same order of magnitude in

both groups. In the group on the low protein diet (Table II) the albumin: globulin ratio fell in every instance and in some of the dogs (*e.g.* 40-50) this change was marked. The average reduction in this ratio in the six dogs on which it was determined was about 30 per cent. There were no significant changes in plasma N.P.N. or in body weight.

Following cessation of the daily injections, the plasma protein level returned to approximately normal in about 2 weeks regardless of diet.

DISCUSSION

A *dynamic equilibrium* between food, plasma, and tissue proteins was postulated by Holman, Mahoney, and Whipple in 1934 (2). The evidence which has accumulated since that time has lent support to this view. The subject has recently been reviewed by Madden and Whipple (9) and by Whipple (10).

Briefly stated this concept implies that food protein, absorbed from the gastrointestinal tract as amino acids, can be fabricated into units (or aggregates) in one portion of the body for utilization in another part of the body. During transport these units (or aggregates) constitute part of the plasma proteins. It is probable that a large portion of this synthesis takes place in the liver, but other tissues are undoubtedly involved, *e.g.* antibody (globulin) formation by lymph nodes and insulin production by the islets of Langerhans, and it is not improbable that much of this equilibrium is maintained in the blood which as an organ is about four times the size of the liver. This concept also implies that neither the capillary endothelium nor the cell membrane is impermeable to these units or aggregates which by the usually employed methods are classed as proteins. Direct measurement of lymph protein in all parts of the body and the rapid rate of restoration of plasma protein following acute severe hemorrhage or plasmapheresis lend support to both of these assumptions. This view in no way invalidates the Starling hypothesis, but merely adds an adaptive mechanism that makes it more dynamic.

At first glance it might seem that the data presented in this paper do not support the hypothesis of an equilibrium. It might be contended that the more protein going into the body by whatever route, the greater should be the concentration in the plasma and in the tissues. Within narrow limits this is true, for by high or low protein feeding the concentration of plasma protein can be raised or lowered by 5 to 10 per cent of the normal. It must be remembered, however, that the nitrogen balance can be established with widely varying intakes, and that under these wide variations in the intake the body holds tenaciously to a rather limited zone of concentration of plasma protein (5.5 per cent to 7.0 per cent). Marked reductions in diet or great physical removals (plasmaphereses) must be carried out in order to lower significantly the plasma protein concentration. The same is true of hyperproteinemia—relatively large quantities of donor's plasma totalling several times the amount

actually in circulation at the start of the experiment must be injected before significant elevations in the plasma protein concentration are effected. During the first week of such injections the increment in the concentration of the plasma protein is slight, and it is only during the later weeks that significant amounts of the injected protein pile up in the circulation. These amounts are greater with low protein feeding, presumably because less of the necessary "chemicals" to maintain the normal equilibrium is supplied. High protein feeding on the other hand maintains the normal equilibrium better by supplying more of these necessary "chemicals."

Qualitative differences in the utilization of the injected protein under varying conditions of dietary protein are indicated by the fact that in the animals maintained on a low protein diet the albumin:globulin ratio is decreased; whereas there is no significant change in this ratio in the animals maintained on a high protein diet. Most of the difference in the extent of the hyperproteinemia in the two groups can be accounted for by the greater increase in globulin in the group maintained on a low protein diet. It could be argued that the more marked hyperproteinemia in this group is necessary to maintain the same osmotic pressure relationships. This is a teleological argument and is not supported by available data, for albumin is not decreased and there is no need for a further increment in globulin. Rather the data point to an upset in the normal equilibrium with the accumulation of an excess of globulin. Whatever the true explanation happens to be, it seems definite from the experiments here reported that the hyperproteinemia following repeated plasma injections is *greater* in animals maintained on a *low* protein diet than in animals maintained on a *high* protein diet.

The promptness with which the plasma proteins return to normal level after cessation of injections or withdrawals despite high or low protein feeding serves to emphasize the abnormality that must be present in certain cases of Bright's disease in which "optimum" feeding fails to influence materially the hypoproteinemia, and in some cases of multiple myeloma in which extreme hyperglobulinemia persists even during inanition and fasting. The comparative rareness of these abnormalities serves in turn to emphasize the stability of the normal mechanism which must be of the nature of an equilibrium between food, plasma, and tissue proteins.

The data in this paper do not embrace the tissue proteins except indirectly in the figures on body weight. In previous publications (2–4, 8, 9) the part played by the tissue proteins in this equilibrium has been discussed.

The experimental findings in this paper and the general thesis of an equilibrium have received partial confirmation by other workers using the opposite approach to the problem, namely treatment of hypoproteinemia occurring in human cases of "nephrosis" or induced in experimental animals by plasmapheresis or extremely low protein feeding. Liu and Chu (11), Keutmann

and Bassett (12), and Farr (13) have all found an "optimum" intake of about 3.0 gm. of protein per kg. per day. Greater intake resulted in less retention. Whipple and his coworkers (10) found, "In general, food proteins are better used when given alone and in moderate amounts. Larger protein intake yields a lower per cent return of plasma protein."

The "ideal" protein to combat hypoproteinemia has not yet been defined, but it is reasonable to predict on the basis of the findings reported in this paper that when it is defined quantitative as well as qualitative factors will play an important part in the utilization of that protein. It is possible that this "ideal" protein may prove to be a more or less specific substance that controls the normal equilibrium between food, plasma, and tissue proteins. Possibly the repeatedly confirmed observation (9, 14, 15) that serum protein is the most potent of all the proteins that have been tested thus far for combating hypoproteinemia means that more of this hypothetical substance is present in serum.

SUMMARY

1. In 4 dogs maintained on a *high* protein diet (lean meat) repeated intravenous injections of plasma obtained from healthy donor dogs (18 to 24 injections during the course of 3 to 4 weeks, totalling 1595 to 4355 cc.—averaging 1800 cc. when figured on the basis of a 5 kg. dog) resulted in a mean increase in the plasma protein concentration of 20 per cent (from 7.1 per cent to 8.5 per cent).

2. In 7 dogs maintained on a *low* protein diet (only 7 per cent of total caloric value derived from protein) almost identical injections of donor's plasma caused an average increase in the plasma protein concentration of 40 per cent (from 6.7 per cent to 9.4 per cent).

3. The albumin:globulin ratio in the group on the low protein diet showed an average fall of 30 per cent (from 1.4 to 0.9) while in the group on the high protein diet the change in this ratio was insignificant (from 1.3 to 1.2).

4. In all dogs in both groups there was a consistent fall in the hematocrit value of about 15 to 20 per cent (from 49 to 40, or 18 per cent) which can be explained in part at least by the increase in plasma volume of about 15 per cent.

5. There were no significant changes in body weight or in plasma N.P.N.

CONCLUSIONS

1. Hyperproteinemia produced by repeated daily injections of homologous plasma is more marked in dogs maintained on a low protein diet than it is when comparable amounts of plasma are injected into dogs maintained on a high protein diet.

2. This seeming paradox is interpreted as additional evidence for a dynamic equilibrium between food, plasma, and tissue proteins.

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