BLOOD PLASMA PROTEIN PRODUCTION AS INFLUENCED BY VARIOUS DEGREES OF HYPOPROTEINEMIA AND BY AMINO ACIDS*

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Blood plasma proteins are an *active* part of the complex integrated system of body proteins. This thesis has been supported by various contributions coming from this laboratory. There is ample evidence that a constant ebb and flow exists between plasma and cell proteins, a *dynamic equilibrium.* We believe that this protein exchange may be at least as important in cell nutrition as the commonly accepted passage of amino acids from the digestive tract to the special cell proteins (2, 10, 14, 7).

This protein exchange, this ebb and flow between plasma and cell proteins is in large part dependent upon the *reserve store* of protein *within* various body cells (*e.g.*, in liver and in muscle cells). But this reservoir of protein material is in no sense an idle pool of protein. It participates in the constant steady state maintained between the plasma proteins on the one hand and the integral cell proteins on the other. It may be likened to a beaver pond beside a rapid stream, which rises and falls as does the stream and is further modified by drainage from and seepage into the adjacent banks.

As this reserve protein must be largely or wholly within the cell boundaries it is probably specific *cell protein*, yet in some way different from integral cell proteins because it can be withdrawn or supplemented on occasion. Our knowledge of cell proteins does not permit us to specify differences between these "reserve," "intermediate" labile cell proteins and the integral, relatively fixed cell proteins. In other papers (2, 9) we have used various terms to cover this labile protein material which flows in and out of body cells: "intermediates," or "large aggregates approximating proteins but not fixed tissue proteins." Perhaps the term "intermediate protein" may appear more suitable until the specifications can be more clearly written.

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This concept is in harmony with observations reported by Schoenheimer and Rittenberg (11) using a totally different approach.

The *production of new plasma protein* which can be removed from the body (by plasmapheresis) is controlled in a measure by the concentration of plasma protein in the circulation. Table 1 indicates that as the concentration of circulating plasma protein rises the amount of new plasma protein which can be removed falls rapidly. We believe that some (and

TABLE 1

Rate of Blood Plasma Protein Regeneration Decreases When a Low Plasma Protein Level Rises toward Normal

Dog 36-196.

Period 7 days	Diet	Protein intake Total for 7 days	Plasma protein removed Total for 7 days	Blood plasma Average concentration		R.B.C. hematocrit, average	Plasma volume
				Total protein	A/G ratio		
		gm.	*gm.*	*per cent*		*per cent*	*cc.*
	Kennel			5.81	1.30	45.0	
1	Fasting	0	35.5	5.28	0.93	51.5	491
2	Low protein	15	16.6	4.07	0.76	56.6	403
3	Liver basal	85	7.6	4.34	0.67	50.4	504
4	Liver basal	85	15.2	4.58	0.65	47.5	521
5	Liver basal	85	9.6	4.65	0.70	48.9	487
6	Liver basal	85	5.2	4.60	0.82	48.0	474
7	Liver basal	85	6.9	4.49	0.93	47.2	489
8	Liver basal	85	1.1	4.73	—	44.8	513
9	Liver basal	85	23.0	4.44	0.77	46.9	479
10	Liver basal	85	17.1	3.98	0.67	49.8	489
11	Liver basal	85	16.2	4.03	0.67	48.5	462
12	Liver basal	85	15.9	4.00	0.61	49.2	—

possibly all) of this apparent reduction in the formation of new plasma protein as the concentration rises may be accounted for by escape of plasma protein into the body cells.

The present study continues our research program relative to the factors controlling the production of new plasma protein. A normal adult dog is depleted by plasmapheresis (bleeding with return of red blood cells suspended in a saline solution) and is maintained hypoproteinemic by suitable plasmapheresis and a diet limited in its protein content. It is evident, in Tables 1 and 1-*a*, that much less plasma protein can be removed in maintaining a steady high hypoproteinemic level than in carrying a steady low hypoproteinemic level. If the rate of regeneration of plasma protein is the same at 4.6 per cent as at 4.00 per cent the rate of *disappearance*

of the new plasma protein into the body cells must be faster at the higher level. We have no measure of the true rate of regeneration of new plasma protein; we measure only what we can remove from the blood stream while maintaining comparable conditions. We realize that plasma protein can rapidly disappear from the blood stream but concerning the normal rate of this disappearance we have no measurement. This interesting problem has been discussed in a recent review (7).

TABLE 1-*a*

Weight and Nitrogen Balance

Dog 36-196.

Period 7 days	Diet	Weight	Nitrogen balance					
			Intake		Output			Intake minus output
			in diet	in excess R.B.C. injected	in plasma	in urine	in feces	
		kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
	Kennel	13.6						
1	Fasting	12.1	0	+3.9	5.8	15.6	*	−17.5
2	Protein low	12.1	2.8	−3.7	2.7	10.7	*	−14.3
3	Liver basal	11.8	14.0	+0.1	1.3	10.5	5.1	−2.8
4	Liver basal	11.9	14.0	+1.2	2.6	9.9	2.8	−0.1
5	Liver basal	12.0	14.0	+0.2	1.6	9.4	3.2	0.0
6	Liver basal	12.0	13.8	−2.5	0.8	9.2	2.3	−1.0
7	Liver basal	12.1	13.8	+2.7	1.1	9.2	3.0	+3.2
8	Liver basal	12.1	13.9	−1.0	0.2	10.3	3.6	−1.2
9	Liver basal	12.1	13.9	+4.1	3.8	9.0	2.3	+2.9
10	Liver basal	12.3	13.9	+2.3	2.8	10.0	3.1	+0.3
11	Liver basal	12.3	13.9	+1.5	2.7	10.4	3.2	−0.9
12	Liver basal	12.4	13.9	+1.2	2.6	9.8	3.2	−0.5
Totals.....			141.9	10.0	28.0	124.0	31.8	−31.9

* Included in following period.

It is obvious that in such experiments with plasmapheresis it is necessary to maintain the plasma protein concentration at a uniform level if we would test accurately the effect of diet factors on plasma protein production—Table 2.

Methods

Previous papers in this series (5) have outlined the procedure employed in this present work. Attention should be called again to the figures given for blood plasma protein concentration. They are obtained by Kjeldahl nitrogen determinations on the plasma of the pooled daily bleeding, collected in saturated sodium citrate solution, 1 ml. per 100 ml. blood. They are lower than the levels of the plasma protein actually circulating in the dog immediately prior to such bleedings, for reasons noted in an earlier report (8).

They may be 10 to 15 per cent lower than these circulating levels but are within one per cent of the actual concentration of protein in the plasma removed.

The gelatin fed is of the same lot as that used previously (4). The naturally occurring forms of the amino acids were used except for racemic mixtures of isoleucine and methionine.

The amount of plasma protein removed on any day depends upon the concentration of protein in the plasma and the amount of plasma in the blood removed on that day. The amount of blood removed is adjusted to two factors: the amount the dog will tolerate without clinical disturbance, such as loss of appetite, and, second, the amount the workers estimate must be removed to achieve a certain level on a given régime. When the level is high and the aim is to reduce the protein mass rapidly, 25 per cent of the blood volume is a convenient and safe quantity to remove each day. When a significant hypoproteine-

TABLE 2

Blood Plasma Protein Regeneration

Favored When Certain Amino Acids Supplement Gelatin

Dog 36-196.

Period		Diet	Protein intake per 7 day period	Plasma protein removed per 7 day period	Blood plasma Average concentration		R.B.C. hematocrit, average
					Total protein	A/G ratio	
No.	*days*		*gm.*	*gm.*	*per cent*		*per cent*
12	7	Liver basal	85	15.9	4.00	0.61	49.2
13	4	Low protein	15	8.9	3.80	0.62	51.0
14	2	Low protein + gelatin + amino acids A	134	2.0	3.97	0.54	49.0
15	7	Low protein + gelatin + amino acids A	134	18.6	4.27	0.79	50.2
16	4	Low protein	15	8.7	4.02	0.79	52.0
17	3	Low protein + gelatin + amino acids B	127	11.0	4.13	0.72	48.9
18	7	Liver basal	85	16.5	4.20	0.78	50.2

mia is reached, 3.5 to 4.0 per cent, the size of subsequent bleedings may be based on experience. We have tried to maintain the concentration each day at the same constant hypoproteinemic level during weeks and months of testing of factors which would have varied this steady level were not the size of the daily bleedings properly adjusted. The effect of a test factor has been related to the quantity of plasma protein which had to be removed in order to maintain this level. We believe this measurement to be valid only if the beginning and ending concentrations of plasma protein are reasonably close and have each been maintained reasonably close by equivalent bleedings over a sufficiently long period of time. In other words a steady state must have been demonstrated to have preceded and followed any measurement of quantitative significance. Such qualifications, for example, are fulfilled in the experiments of Tables 2 and 2-*a*.

In past experiments we have not recognized any significant change in the quantity of plasma required to be removed in maintaining a constant level at any point between 3.7 and 4.3 gm. per cent. Below this range our past

data are inconclusive since clinical disturbances are frequent and stop the experiment. Above this range, the experiments in Tables 1 and 1-*a* indicate that the quantity to be removed decreases sharply.

EXPERIMENTAL OBSERVATIONS

The data presented in Tables 1 and 1-*a*, 2 and 2-*a*, are all obtained from one continuous 16-week experiment in a dog, 36-196, additionally valuable because of the similar experiments previously performed with its excellent cooperation (6, 4). In Tables 1 and 1-*a* the data are given as the totals or averages of those obtained on the individual days of the consecutive

TABLE 2-*a*

Weight and Nitrogen Balance

Dog 36-196.

Period		Diet	Weight	Nitrogen balance, per 7 day period					
				Intake		Output			Intake minus output
				in diet	in excess R.B.C. injected	in plasma	in urine	in feces	
No.	*days*		*kg.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
12	7	Liver basal	12.4	13.9	+1.2	2.6	9.8	3.2	−0.5
13	4	Low protein	—	2.7	+0.2	1.5	12.1	3.0	−13.7
14	2	Low protein + gelatin + amino acids A	12.2	27.5	−2.4	0.3	16.6	3.8	+4.4
15	7	Low protein + gelatin + amino acids A	12.2	27.5	+2.9	3.1	17.5	3.8	+6.0
16	4	Low protein	12.3	2.7	+1.1	1.4	10.6	3.0	−11.2
17	3	Low protein + gelatin + amino acids B	12.4	24.6	0.0	1.9	13.1	3.7	+5.9
18	7	Liver basal	—	13.9	+2.5	2.7	9.2	3.7	+0.8

weekly periods. In Tables 2 and 2-*a* the data are also given as totals or averages of weekly periods but in 4 instances for convenient comparison these represent expansions of shorter periods as indicated.

In Tables 1 and 1-*a* is depicted a cycle of protein depletion and partial repletion all occurring during the first 4 weeks. From an initial level of 5.81 per cent the plasma protein was reduced by fasting and plasmapheresis and then by low protein diet and more plasmapheresis to 3.79 per cent by the end of 2 weeks. Only a small fraction (at most 5 gm.) of the fifty-odd grams of plasma protein removed can have come from materials in the 15 gm. of crude vitamin accessories. The bulk of this protein represents a reduction in the reserve store of protein (at least 35 gm.) and the balance is yielded by a reduction in the mass of plasma protein in the circulation (about 10 gm.). No steady level of hypoproteinemia was attempted at the close of the second period, so that it cannot be clearly demonstrated that

the reserve store was in nice balance with the briefly maintained low plasma concentration of protein. Rather the plasma level was allowed to rise during the 3rd week and reached 4.71 gm. per cent on the last day, while a liver basal diet was being instituted and plasmapheresis was limited. It was desired to keep the level between 4.6 and 4.7 per cent so that more protein was removed during the fourth period. The level promptly fell to 4.38 per cent. It became apparent that 15 gm. plasma protein could not be removed if this higher level were to be maintained. Note that 16 gm. appears to be the amount which can be removed weekly in maintaining the level near 4 per cent (periods 10, 11, 12, and 18).

In periods 5 to 8 plasmapheresis was decreased. The removal of 6.9 gm. protein during period 7 dropped the level of plasma protein from 4.64 at the end of period 6 to 4.41 at the end of period 7. And with complete cessation of plasmapheresis during period 8 the concentration rose only to 4.71 gm. per cent. This rise of 0.3 gm. per 100 ml. plasma in plasma volumes as measured on those 2 days of 489 ml. and 513 ml. represents an increase in the mass of circulating protein of only 2.6 gm. If 16 gm. of new plasma protein were produced during this 8th week, a quantity within the capacity of this dog when maintained at a level of 4 per cent, then at least 13 gm. of it has moved out of the circulation. We have no means of determining whether new plasma protein was converted into tissue protein or whether the tissue protein was formed directly from digestion products. We do believe that approximately this quantity of protein was formed in or stored in the tissues, in that the urinary nitrogen figures are comparable after the third period whether the plasma protein concentration is high or low.

During the 9th week only 7 gm. plasma protein were removed above the basal output of 16 gm. In a previous experiment (5) it appeared that protein produced during 2 weeks of rest from plasmapheresis was almost quantitatively removed in again establishing a steady level at 4 per cent. In this experiment 38 gm. plasma protein were removed during periods 4 to 8 and at 16 gm. per period a total of 80 gm. might have been produced, yet only 7 gm. of the 42 gm. difference were removed. It is reasonable to argue that this longer period allowed the body cells to modify some of the reserve proteins toward integral or immobilized protein. There was also a gain in weight.

It is to be emphasized that Tables 1 and 1-*a* approximate a perfect metabolic experiment. After the initial periods 1 and 2, the dog was in total nitrogen balance, in weight equilibrium (actually a slight gain 0.3 kilos in 10 weeks), in perfect clinical condition with 100 per cent intake of a rather

unpalatable food mixture. These factors are essential for a clean-cut experiment and the conclusions have sound basis of fact. In contrast Tables 2 and 2-*a* show in period 17 that refusal to eat will terminate a promising experiment.

Tables 2 and 2-*a* present another experiment in the search for the specific construction materials in plasma protein synthesis. In our previous experiments (6, 4) amino acids and incomplete proteins plus amino acids have been added to already adequate basal diets. In periods 14 and 15 gelatin, 20 gm. per day, and the mixture called "amino acids A" were added to a low protein diet consisting of sugar, starch, fat, salts, and accessories (see Experimental History, dog 36-196).

"Amino acids A," per day	
	gm.
l-cystine	1.5
dl-isoleucine	1.0
l-tyrosine	1.0
d-valine	0.5
dl-methionine	0.3
l-tryptophane	0.3
ethyl β-hydroxybutyrate	1.0

In the preliminary 2 days, period 14, only samples of blood for chemistry were removed but in the test period 15, more than 18 gm. plasma protein were removed and the average concentration was significantly higher than in the control period 13. This experiment indicates a definite production of new plasma protein from materials contained in the gelatin, the amino acids, and the small amounts of protein of the yeast and the liver fraction in the basal diet. This mixture of proteins and amino acids yielded only 14 per cent return as plasma protein, and less if the 32.2 gm. of amino acids were considered as protein, as compared with a 19 per cent return of plasma protein from the protein of the liver basal diet.

The after period 16 is comparable to period 13 on the low protein diet. The mixture of period 17 was refused by the dog on the 3rd day and to avoid difficulties the animal was returned immediately to the liver basal diet, responding with a normal basal production of plasma protein, period 18.

"Amino acids B," per day	
	gm.
l-cystine	1.5
l-tryptophane	0.3
dl-methionine	0.3

The experiment with this mixture "B" is too short to warrant conclusions regarding its effectiveness, but there is some evidence that new plasma protein was being produced.

We may say that these experiments (Tables 2 and 2-*a*) in general support our theory that *cystine* approximates a *key amino acid* in the production of plasma protein (4).

Experimental History.—Dog 36-196 (Tables 1 and 1-*a*, 2 and 2-*a*). An adult female beagle hound used in similar experiments previously (6, 4) was in good condition after an interval of 12 weeks on a diet of hospital table scraps. The *low protein diet* contained only that protein present in the vitamin supplements, about 2 gm. per day. The diet consisted of cane sugar 90 gm.; cornstarch 20 gm.; lard 10 gm.; butter fat 20 gm.; lecithin (3.5 per cent choline) 5 gm.; cod liver oil 5 gm.; liver powder (Lilly H-8083) 2 gm.; dried yeast (Standard Brands, Inc.—type 200B) 2 gm.; nicotinic acid 25 mg.; salt mixture (13) 5 gm.; bone ash 10 gm. When raw pork liver, 50 gm., was added in period 3 the cane sugar was reduced to 75 gm. After period 7, choline hydrochloride 0.4 gm.; corn oil 15 gm.; and crisco 20 gm., replaced the lecithin, lard, and butter fat. The diet was completely consumed each day. During periods 13 to 18 the diet was also completely consumed each day except for the 3 days of period 16 when consumption fell to 95 per cent, including slight vomiting on the 3rd day. During these periods the low protein diet consisted of all that of period 12 except the liver, and the gelatin and the amino acids were mixed in just before the feeding. The quantities of these materials are given in the Experimental Observations. The dog was in excellent condition at the close of the experiment.

DISCUSSION

The evidence appears overwhelming that large protein molecules may pass readily from cell to plasma and from plasma to cell. For example, fibrinogen is formed in the liver cell and promptly appears in the plasma when there is need for it; again, the dog can be kept in nitrogen equilibrium by plasma given intravenously as the sole source of nitrogen, which fact indicates that as the body cells need protein they can take it in from the plasma. We know of no explanation of this fundamental reaction by which large protein molecules can pass through cell membranes or borders as a need develops on one side or the other of such cell borders. It is obvious that these large protein molecules pass through endothelial cells as well as through organ cell borders. It would appear that the rules relating to protein molecules and membrane passage must be fundamentally different from the rules relating to cell membranes and electrolytes.

We believe that much plasma protein is produced in the liver. When a liver cell produces various globulins and albumin on demand we cannot visualize a variety of such proteins stored in the liver cell like canned goods in an ice box, but suggest that the reserve protein in the liver cell is modified slightly by cleavage into large aggregates with recasting into the special

protein needed. Conversely when the dog is kept in protein equilibrium by plasma given by vein we cannot imagine that the body cells take in this protein and hold it as such—rather that the body cells modify slightly the incoming protein, recasting it into suitable cell protein.

This change in the identity of protein within the cell without fundamental cleavage to amino acids is a process which would naturally relate to enzyme action (Bergmann (1)). If this cleavage went to the amino acid stage we would expect some excess urinary nitrogen but the experiments of Howland and Hawkins (3) exclude this reaction. It is possible that this same type of enzyme reaction may be responsible for cell *border*, *boundary*, or *surface modification* and resultant passage of these large protein molecules.

We have always assumed that the stimulus to plasma protein regeneration was greatest when the blood plasma concentration was just above the edema level, that is when the concentration lay between 3.5 and 4.2 gm. per cent. The experiment recorded in Tables 1 and 1-*a* adds weight to that opinion. At what point in the rise above this range the *apparent* rate of regeneration declines our experiment does not show. It does indicate that the *apparent* rate of regeneration is very much reduced when the level reaches 4.6 gm. per cent, cut in half at least. The experiments of Weech (12) indicate that following dietary depletion the *serum albumin* regenerates at a constant rate between the levels of 2.8 and 3.7 gm. per cent, a range entirely above the highest *albumin levels* reached by dog 36-196 (Table 1). Weech also notes that the globulin concentration varies little in his regeneration studies. It should be noted that his experimental approach is totally different from that used in our experiments.

Shock is an abnormal state of immediate interest to everybody at the moment. It is being treated by plasma injections together with other surgical procedures. It is obvious that anything bearing on the production and utilization of plasma and plasma proteins in the body bears on these problems of shock, hemorrhage, and protein wastage. Not only does the plasma supply the normal environment of the body cells and help to stabilize the osmotic balance but the plasma proteins supply the proteins needed by the injured tissue and protein depleted cells. If one could enable body cells to produce much new plasma protein without the aid of the digestive tract, this procedure would be of great practical value to replace or supplement the intravenous use of plasma which may be difficult to obtain, prepare, and preserve.

SUMMARY

When blood plasma proteins are depleted by bleeding with return of the washed red blood cells (plasmapheresis) it is possible to bring dogs to a

steady state of hypoproteinemia and a uniform plasma protein production on a basal low protein diet. These dogs are clinically normal but their resistance to infection is distinctly below normal. Introduction of variables into this standardized existence gives information relative to plasma protein production.

Plasma protein production under these conditions with a plasma protein concentration of 3.5 to 4.2 gm. per cent is relatively constant. As the plasma protein concentration rises the plasma protein removed falls rapidly (Table 1). At 4.6 gm. per cent the protein removed is less than 50 per cent of the amount removed at a plasma protein level of 4.0 gm. per cent.

Cystine appears to be an important amino acid for plasma protein formation. This shows in Table 2 and is supported by data coming from published experiments.

These experiments related to the factors which control *plasma protein production* bear on the problems of *shock*, *hemorrhage*, and *protein wastage* and their treatment by *plasma injections* which hold the attention of surgeons and physiologists at the moment.

Again we would emphasize the fluidity of body protein including plasma protein—an ebb and flow between protein depots and plasma protein—a "dynamic equilibrium" of body protein. A discussion of the passage of large protein molecules through cell borders is submitted.

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