BLOOD PLASMA PROTEIN REGENERATION CONTROLLED BY DIET

I. Liver and Casein as Potent Diet Factors

By RUSSELL L. HOLMAN, M.D., EARLE B. MAHONEY, and GEORGE H. WHIPPLE, M.D.

(From the Department of Pathology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.)

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We have ample evidence to show that blood plasma protein regeneration in the dog can be modified at will by diet factors. The evidence in this paper indicates that liver and casein feeding are very potent in effecting rapid plasma protein regeneration. Vegetable diets may be less effective as shown below and reported in the publications from other laboratories. Possibly other proteins in the diet may prove to be even more efficient than liver in bringing about plasma protein regeneration. We are testing systematically other proteins. No one can group proteins into complete or incomplete, vegetable or animal, and by that grouping indicate the value of any protein for the regeneration of plasma protein. Individual proteins must be tested in the fire of metabolism. It will be of some interest to determine in what protein fractions or split products this potency may reside.

Eventually all this information will be of interest to the physician who deals with human cases showing hypoproteinemia or blood protein loss. The maintenance of blood proteins above the edema level has a practical bearing in the treatment of certain human cases of nephrosis and nephritis.

The general experimental plan is extremely simple but a certain number of technical difficulties had to be overcome. Dogs are reduced to a blood plasma protein level of approximately 4.0 per cent just above the edema level (3.3–3.8 per cent) and held continuously at this level by suitable bleedings combined with replacement of red cells suspended in Locke's solution (plasmapheresis). This low plasma protein level acts as a stimulus for the production of new plasma proteins presumably in maximal amounts. It was soon found that a normal dog has a considerable amount of material stored in the body out of which plasma proteins can be fabricated. The same thing (23) is true for hemoglobin in the dog. When this reserve is exhausted with the dog on a basal diet the animal can still produce 15–30 gm. of new plasma protein each week. The basal diet is poor in vegetable protein and contains no animal protein, yet the dog can be kept in nitrogen equilibrium with actual gain in weight if the diet is eaten over periods of many months (Dog 32-58).

When the basal diet is replaced by cooked liver or supplemented by casein we note a large output of new plasma protein, several times the basal diet output (Table 3). The excess output due to liver or casein feeding for 1 week may amount to 60–70 gm. plasma protein over and above the basal output.

Space will not permit a complete review of earlier work in this field. Bloomfield (3) has recently reviewed much of the literature dealing with edema and blood plasma protein concentration. He points out that the "loss and lack theory" does not seem adequate to explain all the observations on serum proteins. We believe that the *reserve store* of protein or protein building material shown in the tables below will clear up some of the discrepancies in the literature. The specific action of various dietary proteins will help still more in the solution as will the observations in the second paper which indicate that *plasma proteins may be utilized in the body economy.* We do not refer to the many papers reviewed by Bloomfield but list a few of the important ones in the bibliography.

Henriques and Klausen (7) using rabbits with phosphorous poisoning and dogs with bile duct ligation record a decrease in plasma albumin but not in globulin. Edema developed in some instances. They claim this is proof that albumin is formed in the liver and globulin elsewhere. They did not control the diet. Compare Dog 32-394, Table 1 below, where the same change is noted as due to many weeks of the "basal ration" and nothing else.

All investigations by means of plasmapheresis have been done with the dog. Kerr, Hurwitz, and Whipple (10) first pointed out the influence of diet factors on plasma protein regeneration after plasmapheresis. The dog regenerated these proteins much faster on a mixed diet than during a fasting period. There was evidence of an emergency *reserve store of proteins.* Liver injury impaired this reaction and indicated that the liver might be concerned with maintenance of the blood plasma protein concentration. Whipple, Belt, and Smith (22) confirmed and extended the above observations. The liver was believed to be concerned with plasma protein regeneration. Barker and Kirk (1) were particularly interested in edema produced in the dog by plasmapheresis. The diet used was not given but must have been rich in protein. Leiter (13) was interested especially in edema produced by plasmapheresis. A bread and meat diet of unknown amount was used. Shelburne and Egloff (18) used a limited diet of potatoes, cream, turnips, butter, and lactose in dogs with and without plasmapheresis. They note a change in the albumin-globulin ratio and fall in total protein. Edema was readily produced by an administration of saline by stomach tube after 83 days on this diet. Barnett, Jones, and Cohn (2) showed that on a liberal meat diet the dog could regenerate large amounts of plasma protein removed by plasmapheresis. They doubt the emergency storage of protein. Darrow, Hopper, and Cary (4) made similar observations. Weech (19, 20) and his collaborators used dogs to study the effect of a low protein diet alone and with plasmapheresis. They noted a fall in albumin and a rise in globulin as edema developed. They studied the nitrogen balance and gave small amounts of serum intravenously to combat the edema without result. Lepore (14) using a milk and bread diet combined with plasmapheresis in dogs was able to reduce the total plasma proteins to 3.5 per cent, and observed edema when fluids were forced.

Important tests with human patients are reported by Liu, Chu, Wang, and Chung (15). They studied the effect of different levels of animal and vegetable protein intake on nitrogen balance, plasma proteins, and edema in two cases of nutritional edema. They believe animal protein is twice as effective as vegetable protein for plasma protein regeneration.

Methods

Dogs were used in all experiments and the basal ration was fed usually some days or weeks to make sure that the animal would take it freely. Dogs were protected against distemper with the Laidlaw-Duncan vaccine and unless otherwise noted were in normal health.

The *basal ration* is a result of our experience and that of others. It contains no animal and but little vegetable protein—about 7 per cent of its caloric value being protein. This ration does not appeal to all dogs but many will consume adequate quantities over months and remain in nitrogen equilibrium with even some gain in weight. The basal ration consists by weight of 40 parts boiled white potatoes, 20 parts canned tomatoes, 10 parts Post bran flakes, 10 parts karo corn syrup, and 5 parts cod liver oil. Each dog received daily, with this diet, 1 gm. of the McCollum-Simmonds salt mixture (16). The weighed basal ration was placed in the cage in the afternoon and the amount consumed recorded in the tables. Water was available in the cages at all times.

Plasmapheresis in these experiments means the removal of 25–40 per cent of the blood volume as estimated by the dye method (Hooper, Belt, Smith, and Whipple (8)). Immediately following the bleeding and sometimes during the last part of the bleeding there is injected intravenously a mixture of normal red cells suspended in Locke's solution. The red cells for injection were obtained from healthy donors. For this purpose large dogs were used and the amount of bleeding was regulated so that they should show no anemia. A donor was bled 300–400 cc. in the morning and the red cells separated from the plasma by centrifugalization—3,000 R.P.M. for 35 minutes in 100 cc. tubes. The plasma was removed by suction, the cells washed in modified Locke's solution and recentrifugalized. The constituents of the Locke's solution were mixed each morning, the calcium salt being left out because of its tendency to cause coagulation. The washed red cells were then resuspended in Locke's solution containing 5 per cent glucose and heated to 37°C. for injection. The volume was maintained at about 10 per cent more than that originally bled. It was found that this single washing as described removed more than 99 per cent of the plasma protein. The blood was usually removed from the femoral artery and the washed red cells injected into the jugular vein by means of a gravity bottle. 1 cc. of concentrated Na citrate per 50 cc. of blood was used as an anticoagulant. The dilution of the plasma due to the citrate was corrected for in calculating the total protein. Daily hematocrits were taken, using 2 cc. of 1.4 per cent sodium oxalate, and these are recorded as an indication of the dog's hemoglobin level. An excess of red cells was always injected as the trauma of the centrifugalization injures many of the introduced red cells and they disintegrate with the appearance of some hemoglobin in the blood plasma.

During the initial period and liver or casein periods it is necessary to bleed and replace maximal amounts to reduce the plasma protein levels. Five or six bleedings a week are necessary. It was thought best not to attempt to maintain the red cell hematocrit at 50 per cent, or normal, but Dog 32-30 was maintained at approximately 30 per cent at which anemia level dogs will show no clinical disturbance. Moreover the gross bleeding required will be less to remove a given amount of plasma protein.

The blood from the dog on basal diet was centrifugalized at 3,000 R.P.M. for 35 minutes and the plasma drawn off as completely as possible by suction. All analyses were run on this sample. The total volume of plasma removed was accurately measured and checked against the total amount bled and the hematocrit. By allowing 1 cc. for each centrifuge tube used as the amount of plasma that could not be drawn off without contaminating the plasma with red cells, it was possible to measure amounts that checked within 2 per cent of the calculated yield. Since the single most important figure in these studies is the *total grams of plasma protein removed*, particular attention has been paid to all factors which might alter this figure. It is recognized that injecting washed donor's corpuscles before completion of bleeding, by dilution, lowers the protein concentration of the plasma removed, that during a large bleeding some dilution may normally occur in an attempt to restore the blood volume, and that the use of hypertonic citrate certainly dilutes the plasma by taking fluid out of the red cells. For these reasons too much emphasis is not placed on the column listed "Blood plasma average concentration." But none of these factors alter the actual number of grams of plasma protein removed, since this is figured as the product of the cubic centimeters of plasma removed and the concentration of that removed. And since any dilution is accomplished by fluid that is presumably very nearly protein-free, it is believed that the figures for the fractionation into albumin and globulin are absolute within the limits of error of the method.

This citrated blood was centrifugalized and the plasma accurately measured. The *plasma* was then analysed for total nitrogen by the macro-Kjeldahl method, using 1 cc. of plasma. Non-protein nitrogen was determined by duplicate macro-Kjeldahl analyses of 50 cc. of Folin-Wu filtrate, 200 cc. of which were prepared with 20 cc. of plasma. The analysis of albumin and globulin was done according to Howe's method as described by Peters and Van Slyke (17) using 22 per cent sodium sulfate at 37°C.; triplicate analyses were carried out. The dogs were kept in clean metabolism cages during the entire experiment. The weekly *urinary output* was collected in large bottles, using 20 cc. of concentrated H_2SO_4 as a preservative. The volume was measured and the urine analysed for total nitrogen, using 1 cc. aliquots with the macro-Kjeldahl procedure. Thus the macro-Kjeldahl method was employed in all analyses and in all determinations duplicate or triplicate analyses were made and repeated if they did not check to within 1 part in 60. This reduces the probable error to less than 1 per cent.

Fecal nitrogen was not determined routinely but in many experiments the fecal nitrogen was calculated for 1–2 weeks and this figure used throughout the experiment. Obviously the nitrogen balance is not absolutely accurate but the error probably is not large. In some experiments (Dog 32-30) the fecal nitrogen was figured as 1 gm. per day and not analysed. The figure is too high and increases the negative balance as given.

Total protein removed as given in the table is probably about 5 per cent too low as the washings from the centrifugalized red cells contain about this amount of protein and this correction is not made. However the error is relatively constant and makes the reaction to diet factors a little less conspicuous than would the corrected figures.

EXPERIMENTAL OBSERVATIONS

The first thing to determine was the behavior of a normal dog on the basal ration. Some dogs will not eat this diet in sufficient amount to maintain weight.

Basal Ration and Normal Dog

Dog 32-58.—A small adult female mongrel; received the basal diet 425 gm. per day plus 1 gm. salt mixture, the composition as described under Methods. This amounted to about 100 calories per kilo body weight. The dog ate 75 to 100 per cent of this diet every day for 27 weeks while under observation, and was in positive nitrogen balance. The final weight was 7.1 kg. as compared with 5.8 kg.

at the start. The dog was in perfect health throughout. The plasma proteins were followed at intervals during this long period. The control level was unusually high—7.8 per cent total protein. After 1 week on the basal diet this high value fell to 6.1 per cent total protein in blood plasma. For the next 10 weeks there

TABLE 1

Blood Plasma Protein Depletion and Regeneration
Initial Reserve of Normal Dog

Periods 7 days	Diet	Total protein removed	Estimated basal output	Total protein removed above basal			Blood plasma Average concentration		
				Total protein	Albumin	Globulin	Total protein	Albumin	Globulin
Dog 32-394. Basal ration for 2 mos. before beginning depletion									
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*
1	Basal	44.7	25	19.7	7.5	12.2	4.82	1.76	3.05
2	"	30.5	25	5.5	—	—	3.80	—	—
3	"	28.9	25	3.9	—	—	3.57	—	—
4	"	25.0	25	0.0	0.0	0.0	3.30	—	—
Total reserve above basal.............				29.1					
Dog 32-168. Basal ration for 1 wk. before beginning depletion									
Control							4.80	2.81	1.99
1	Basal	85.8	35	50.8	—	—	4.09	—	—
2	"	69.0	35	34.0	17.2	16.8	3.75	1.89	1.86
3	"	57.9	35	22.9	13.2	9.7	3.35	1.51	1.84
4	"	32.7	—	—	—	—	3.72	—	—
Total reserve above basal.............				107.7					
Dog 32-290. Basal ration for 1 wk. before beginning depletion									
Control							6.62	3.53	3.09
1	Basal	86.7	30	56.7	36.7	20.0	5.42	3.44	1.98
2	"	69.5	30	39.5	23.1	16.4	4.61	2.69	1.92
3	"	55.2	30	25.2	12.3	12.9	4.01	1.97	2.04
Total reserve above basal.............				121.4	72.1	49.3			

was no change in the level of plasma protein. There was no change in the red cell hematocrit at any time. During the 16th and 17th week the total blood plasma protein is recorded as 4.9 per cent and 4.7 per cent. In the 26th and 27th weeks the plasma protein values are recorded as 4.7 and 5.2 per cent. Evidently on this basal diet this dog could maintain a protein concentration in its blood within normal limits. On several occasions the albumin-globulin ratio was determined and found to be normal. This is in contrast to Dog 32-394, Table 1, where there is a reversal of the albumin-globulin ratio after 2 months' feeding of the basal ration.

Table 1 shows the amounts of plasma protein which must be removed in normal dogs to exhaust the *reserve storage* of plasma proteins or their

TABLE 2

Bleeding, Nitrogen Balance, and Clinical Condition

Periods 7 days	Food consumption	Weight	Edema	Negative N balance	Total bleeding	R.B.C., hematocrit
Dog 32-394. Basal ration 525 gm. daily						
	per cent	*kg.*		*gm.*	*cc.*	*per cent*
Control	100	13.4	0	—	1,500	36.6
1	100	13.7	0	—	1,255	39.7
2	100	13.8	0	—	1,400	41.8
3	100	13.7	0	—	1,120	37.6
Dog 32-168. Basal ration 850 gm. daily						
Control	100	21.6				
1	55	21.5	0	20.0	3,300	33.8
2	43	21.0	0	35.4	2,875	30.5
3	33	20.5	+++	24.0	2,575	25.2
4		20.0	+++	13.3	1,325	20.5
Dog 32-290. Basal ration 420 gm. daily						
Control	100	16.6				
1	100	15.2	0	35.4	2,560	34.6
2	95	14.5	+	44.9	2,110	24.0
3	67	13.3	0	41.4	1,970	30.1

parent substances. Compare Table 3 below where the periods of observation are longer and the basal level more convincing because determined on two different occasions after long periods of plasmapheresis.

The basal output per week on this diet is close to 2 gm. plasma protein per kilo per week. There is a tendency for this value to fluctuate a little as the experiments run on for several months (Table 3) but the amount seems reasonably constant under the conditions of these experiments.

The *reserve storage* amounts to quite a respectable figure but appears to be lower in dogs which have been on the basal diet for some time (Dog 32-394). There may be other factors which determine the amount of this reserve storage but in these dogs it varies from 30 to 120 gm. total plasma protein—2 to 7 gm. per kilo body weight (Table 1—Total protein removed above basal).

Albumin appears as a conspicuous feature in this reserve store and usually makes up one-half or more of the total protein removed. This is in conspicuous contrast to the protein removed subsequently where globulin makes up more than twice the amount of albumin removed (Table 3).

Clinical History, Dog 32-394.—An adult female bull mongrel weighing 15.0 kg. This dog had been on the basal ration for 2 months. The plasma protein level following this basal period was 4.82 per cent, albumin 1.76 per cent, and globulin 3.05 per cent. The blood volume was 920 cc., the plasma volume 560 cc. Throughout the periods of observation here recorded the dog was in excellent condition. This dog is being used for diet experiments and plasma regeneration at the present time (November, 1933) and is in excellent condition.

Clinical History, Dog 32-168.—An adult shepherd mongrel male weighing 21.8 kg. The initial level of plasma protein was 4.8 per cent, albumin 2.81 per cent, and globulin 1.99 per cent. The blood volume was 1,600 cc., the plasma volume 760 cc. Plasmapheresis was done with exchanges of 500 cc. of blood. At the end of the 4th week there was difficulty in entering the jugular vein to inject the washed cells following a large bleeding. The dog went into shock and died in 3 hours. Autopsy showed viscera normal but for anemia.

Clinical History, Dog 32-290.—An adult female terrier whose normal weight was 16.5 kg. The initial plasma protein level was 6.62 per cent, albumin 3.53 per cent, and globulin 3.09 per cent. The blood volume was 1,283 cc., the plasma volume 690 cc. At the beginning exchanges of 450 cc. were performed. At the beginning of the 2nd week the dog started vomiting and developed hemoglobinuria. Subsequent exchanges did not exceed 350 cc. of blood. Pitting edema developed during the 3rd week and persisted during the dog's life. The dog was found dead in the cage at the end of the 3rd week following an exchange of 350 cc. on the preceding day. Autopsy showed bronchopneumonia, pleural effusion left, acute tricuspid and mitral endocarditis.

Table 2 gives more data related to the experiments outlined in Table 1. Dog 32-394 is of particular interest because it is obvious that a dog eating all the basal ration can maintain its weight during a period of plasma depletion. This dog showed no edema at any time in spite of a rather low plasma protein level. The red cell hematocrit in this dog was held close to 40 per cent, a low normal figure.

Dog 32-168 is a satisfactory experiment in which the dog did not eat the basal ration well and lost weight. Edema developed in the last 2 weeks and the hematocrit was too low: this perhaps helps to explain the edema appearing at plasma protein levels of 3.7 per cent. The dog died as the result of an accident and all viscera were normal at autopsy.

Dog 32-290 is the least satisfactory of these three experiments in Table 2. This dog in the last week or two of life developed an acute infection of the lungs and endocardium. There was conspicuous loss of weight in spite of a good food consumption. This unusually high output of plasma protein may be due in part to infection which is known to stimulate a great overproduction of fibrinogen. It is significant however that the albumin remained high in all this reserve output.

The Effects of Liver or Casein Feeding

Table 3 shows a successful experiment lasting 19 weeks in which the output of blood plasma proteins due to *liver* or *casein* feeding is in conspicuous contrast with the reaction to the basal diet. This surplus production due to either liver or casein is about 70 gm. plasma protein per week over and above the basal level while the reaction to the basal diet is about 20 gm. plasma protein per week.

The ratio of food protein intake to plasma protein production is of considerable interest. With liver feeding the total liver protein intake amounted to 893 gm. while the plasma protein regeneration above the basal level for the 2 week period is recorded as 131 gm. Each 6.8 gm. of liver protein was responsible for the production of 1 gm. of plasma protein surplus. The reaction to casein is not quite as striking but of the same order. Approximately 700 gm. of commercial casein as food intake were responsible for a total plasma protein surplus of 70 gm. (Table 3) or a ratio of 10 gm. casein to 1 gm. of produced plasma protein. It is to be kept in mind that the *liver* ration *replaced* the basal ration while the *casein* was *added to* the basal ration. A more accurate experiment would show the *addition* of the liver to the basal ration.

TABLE 3

Blood Plasma Protein Depletion and Regeneration
Liver and Casein Compared with Basal Diet

Dog 32-30.

Periods 7 days	Diet	Total protein removed	Total protein removed above basal*			Blood plasma Average concentration			N.P.N.
			Total protein	Albumin	Globulin	Total protein	Albumin	Globulin	
		gm.	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*mg. per 100 cc.*
1	Low	39.6	19.6	10.1	9.5	4.08	2.09	1.99	17
2	Basal	30.1	10.1	5.3	4.8	4.00	2.11	1.89	20
3	"	37.2	17.2	9.0	8.2	3.38	1.76	1.62	18
4	"	34.3	14.3	7.0	7.3	3.65	1.79	1.86	20
Total reserve above basal*..............			61.2	31.4	29.8				
5	Basal	3.6		1.3	2.3	4.11	1.50	2.61	16
6	"	28.1		9.8	18.3	3.60	1.26	2.34	19
7	"	10.8		4.0	6.8	2.98	1.14	1.84	16
Average basal*...........		14.1		5.0	9.1				
8	Liver	27.7	27.7	11.7	16.0	4.61	1.94	2.67	23
9	"	53.5	53.5	26.4	27.1	4.61	2.27	2.34	35
10	Basal	27.0	7.0	3.0	4.0	4.62	1.97	2.65	20
11	"	42.4	22.4	7.9	14.5	4.23	1.49	2.74	18
12	"	31.0	11.0	3.7	7.3	4.06	1.37	2.69	19
13	"	30.0	10.0	3.2	6.8	3.79	1.22	2.57	20
Total regeneration due to liver..........			131.6	55.9	75.7				
14	Basal	25.4		8.1	17.3	3.48	1.10	2.38	17
15	"	16.6		4.9	11.7	4.19	1.23	2.96	12
16	"	21.1		7.1	14.0	3.46	1.15	2.31	15
Average basal*...........		21.0		6.6	14.4				
17	Casein	53.0	33.0	13.9	19.1	5.24	2.21	3.03	38
18	Basal	48.1	28.1	11.0	17.0	5.02	1.81	3.21	20
19	"	29.0	9.0	3.9	5.1	3.78	1.64	2.14	20
Total regeneration due to casein.........			70.1	28.9	41.2				

* Estimated basal output equivalent to 20 gm. plasma protein per week.

The ratio of food protein (vegetable) in the basal diet to plasma protein production is 88 gm. food protein per week and 20 gm. basal output per week—or 4.4 gm. vegetable protein are responsible for the production of 1 gm. plasma protein. Obviously this vegetable protein is utilized to very good advantage by the dog under these conditions

TABLE 4

Bleeding, Nitrogen Balance, and Clinical Condition

Dog 32-30.

Periods 7 days	Diet	Food consumption	Weight	Edema	Negative N balance	Total bleeding	R.B.C., hematocrit
		per cent	*kg.*		*gm.*	*cc.*	*per cent*
1	Low	80	9.3	0	21.2	1,615	33.9
2	Basal	89	9.2	0	20.2	1,290	28.3
3	"	50	9.6	0	20.4	1,905	28.1
4	"	50	9.9	+++	16.9	1,460	26.3
5	"	88	9.5	++	9.1	155	29.1
6	"	73	7.7	0	11.6	1,220	29.4
7	"	78	8.8	+++	8.2	575	34.8
8	Liver	100	8.2	0	+94.2	990	37.7
9	"	100	8.5	0	+82.5	1,925	35.9
10	Basal	95	8.9	0	0.7	935	37.1
11	"	100	8.8	0	13.6	1,440	30.0
12	"	98	8.7	0	11.4	1,240	38.1
13	"	98	8.6	++	9.4	1,190	31.1
14	"	97	8.6	+++	9.1	1,105	37.2
15	"	100	9.1	++	5.3	620	35.7
16	"	91	8.3	++	9.2	950	34.0
17	Casein	90	8.7	0	+51.8	1,500	29.1
18	Basal	58	8.3	0	36.4	1,500	32.8
19	"	75	7.8	0	14.6	1,220	26.1

Basal ration 425 gm. daily.

and we cannot say that the liver protein per 100 gm. ingested is more or less efficiently utilized. It is possible that the liver protein if given in such small amounts would be as completely utilized. Nor can we support the thesis of the Chinese investigators (15) that animal protein is worth twice as much as vegetable protein. Much more work is needed and from what is known at present we can only say that each protein must be tested in the animal economy to give the answer to this question.

The *reserve storage* (Table 3) amounts to 61 gm. plasma protein over and above the basal production on the standard basal diet—or 6.7 gm. per kilo body weight. It is of interest that albumin and globulin in about equal amounts (31.4 and 29.8 gm.) are represented in this reserve store.

The *basal output* per week (5th to 7th weeks) averages 14.1 gm. plasma protein but at a later period (14th to 16th weeks) averages 21 gm. plasma protein. During both these periods the globulin removed amounts to twice the amount of albumin. Evidently on this basal

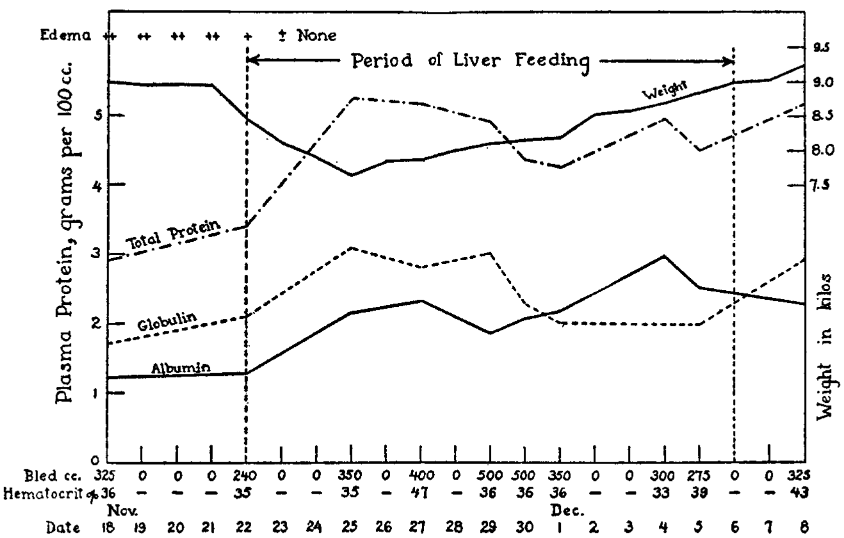

CHART A

ration the dog can form twice as much globulin as albumin. It is possible that the vegetable and grain proteins favor the formation of globulin more than do the animal proteins.

Liver and casein diet periods show a greater amount of albumin production and during the 2nd week of liver feeding the albumin output practically equals the globulin production (Table 3 and Chart A).

It is to be noted that this Dog 32-30 with a blood plasma volume of approximately 450 cc. had in circulation normally about 23 gm. total plasma protein. The production per week on the basal ration (20 gm. plasma protein) is almost as much as the circulating proteins while on the liver diet this figure per week may be 65 gm. plasma protein total output. The reserve of 61 gm. amounts to roughly three times the normal amount of plasma protein in circulation.

Clinical History, Dog 32-30.—A young adult female about 1 year of age, initial weight 10.1 kg. The plasma protein was 5.2 per cent, albumin 3.3 per cent, and globulin 1.9 per cent. The blood plasma volume during the long period of observation (5 months) varied but little and averaged about 450 cc. During the edema periods the blood plasma volume showed only slight change and averaged about 420 cc. The 1st week of the experiment the dog was on a low protein diet in which rice or white bread supplied the only protein. After this for the remaining 19 weeks the dog was on the basal ration used in the other experiments. The food ration was given as described 425 gm. per day and the percentage consumption indicated in Table 4. The 1st day's bleeding amounted to 750 cc. whole blood in three separate exchanges done under ether anesthesia by placing cannulas in femoral vein and artery (September 30, 1932) (22). Following this the dog was bled by needle puncture of jugular vein or femoral artery. Red blood cells suspended in glucose Locke's solution were injected into jugular or femoral veins. From December 15, 1932 to February 21, 1933, blood was removed by cardiac puncture. On many occasions after several days of bleeding quite marked hemolysis followed by icterus would be noted. Coincident with this hemolysis, diarrhea and vomiting might ensue and the dog would refuse a part of its diet. Bleeding would be discontinued for a day or two, the clinical condition would become normal, and the food intake return to the usual level.

The *liver diet period* of 2 weeks means a diet of cooked whole pig liver 300 gm. (fresh weight) daily.

The casein diet period means 100 gm. commercial casein per day *added to the basal ration* of 425 gm. per day.

During the last week of life the dog showed signs of infection with fever, icterus, prostration, and loss of appetite. This week was not included in Tables 3 and 4. Autopsy—Bilateral bronchopneumonia and acute pleuritis. Acute mitral and aortic and subacute tricuspid endocarditis (cardiac punctures).

Table 4 gives other experimental data on Dog 32-30 and supplements Table 3. It is seen that the food consumption is excellent but there is a slight loss of weight. There is a continuous negative nitrogen balance but the figures are somewhat high as the fecal nitrogen was not analysed but figured as 1 gm. per day. The plasma nitrogen removed by plasmapheresis is included in the nitrogen balance. The conspicuous positive nitrogen balance during liver and casein feeding is well shown. The red cell hematocrit is too low to be considered normal but it is known from anemia work with dogs in this laboratory that this degree of anemia does not cause any recognizable clinical or physiological disturbances.

Edema appears when the total protein level approaches 3.5 per cent whether the albumin-globulin ratio is 1/1 or 1/2. The total protein level would seem more important than the albumin-globulin ratio or the total albumin concentration. Ascitic fluid obtained on one occasion showed 0.93 per cent total protein of which 0.26 per cent was albumin and 0.67 per cent globulin.

Chart A illustrates beautifully the reaction of Dog 32-30 on the basal ration to a diet of liver. The dog showed a very low plasma protein level (plasmapheresis) with tissue edema and ascites. At first the albumin-globulin ratio is reversed but after a week of liver diet the albumin fraction returns toward normal and even shows the normal preponderance of albumin. As the edema vanishes the loss of weight is shown with a subsequent gain in weight. The enormous output of plasma protein is shown in Table 3 and it was found impossible to keep the usual low plasma protein level during the liver period. When the basal diet is again resumed the usual preponderance of globulin promptly appears.

DISCUSSION

The ratio of albumin to globulin in the total plasma protein concentration is of considerable interest and invites speculation. Few authors can resist this urge and too much space has been wasted on this elusive ratio. The normal ratio is about 2 parts albumin to 1 part globulin so it was logical to assume that these substances might be produced in about this same ratio. Experiments by many workers soon showed that as these substances were depleted the ratio might even be reversed so that the total plasma protein would show 1 part albumin and 2 parts globulin.

Because some workers recorded sudden shifts in this ratio due to relatively simple procedures, it was assumed by certain writers that albumin could be changed readily to globulin or *vice versa*. This did not seem to be reasonable because of known chemical differences between albumin and globulin and it is now believed that these earlier observations were in error due to method inaccuracies.

However it is well recognized that there may be a slow change in the albumin globulin ratio due to plasma depletion or even to diet alone (Table 1—Dog 32-394). It would appear that as the plasma proteins are used up their replacement may result in a more prompt output of globulin as compared with albumin. Certain diets may favor globulin production (vegetable protein) and others (liver) may favor albumin production (see Table 3 and Chart A).

Why should liver feeding favor new plasma protein regeneration and especially serum albumin production? There may be several reasons for this observation (Table 3). It was shown by Kerr, Hurwitz, and Whipple (10) that there was a *reserve storage* of material which could be thrown into the circulation after a short severe plasmapheresis and that a previous liver injury impaired this reaction. In Table 3 it is noted that the initial *reserve storage*, which is exhausted by continued plasma depletion, is made up of albumin and globulin in about equal amounts. It is possible that this *reserve store* may be in large part in the liver and that albumin or its parent substances are well represented.

Furthermore it is generally accepted that fibrinogen, the plasma globulin, is formed in the liver (9) and by no other body tissues. It is not too much to suspect that the liver is also largely concerned with maintenance of the total plasma protein concentration (22). Also the liver protein in the food is known to favor the production of another important protein—hemoglobin. All this argument then is in harmony with the recorded observations (Table 3) that liver feeding exerts a potent influence on the regeneration of plasma proteins and in particular on the production of the albumin fraction.

When a dog with depleted plasma protein level is placed on a favorable diet (liver or casein) there is a 3–4 day interval before the rapid regeneration and production of plasma protein is evident. After the favorable diet period terminates and the dog is put back on the basal ration the active production of plasma protein continues briskly for 5–7 days and tapers off slowly to the basal diet level. This we term the "carry over" from the potent diet.

Exactly the same reaction is noted in the study of hemoglobin regeneration in dogs on various diets (21). Evidently it takes time for the body mechanism to elaborate these proteins from the various food factors and certain materials are stored in reserve during such a favorable diet period, which stores are used up or exhausted in the subsequent basal diet period.

SUMMARY

When blood plasma proteins are depleted by bleeding and return of the washed red cells (plasmapheresis) the regeneration of new plasma proteins can be controlled at will by diet. The amount and character of protein intake is all important.

Liver protein and *casein* are efficient proteins to promote rapid regeneration of plasma proteins but some vegetable proteins are also efficient.

The blood plasma proteins are reduced by plasmapheresis close to the edema level (3.5–4.0 per cent) and kept at this level by suitable exchanges almost daily. The amount of plasma protein removed is credited to the given diet period.

A *basal ration* is used which is poor in vegetable protein (potato) and contains no animal protein. The dog on this ration can be kept in nitrogen balance but can produce only about 2 gm. plasma protein per kilo body weight per week. With liver or casein feeding this production can be increased three- or fourfold.

A *reserve* of protein building material can be demonstrated in the normal dog when its plasma proteins are depleted. In the first 3 weeks of depletion this reserve in excess of the final basal output may amount to 30–120 gm. protein. This may be stored at least in part in the liver. As much as 50 per cent of this reserve may be albumin or albumin producing material.

A reversal of the *albumin-globulin ratio* may be observed on the basal diet alone. The reversal will always follow plasmapheresis with the dog on the basal diet and the total plasma protein output will consist approximately of 2 parts globulin and 1 part albumin. Liver diet will raise the production and output of albumin and bring the ratio back toward normal. Albumin production may actually exceed the globulin output during liver diet periods. The change is less conspicuous with casein but in the same direction.

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