BLOOD PLASMA PROTEIN REGENERATION CONTROLLED BY DIET

Systematic Standardization of Food Proteins for Potency in Protein Regeneration. Fasting and Iron Feeding

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One may properly ask three questions relating to plasma proteins. Where are these proteins formed? The finger of suspicion points to the liver although the proof is not yet absolute. From what materials are these proteins fabricated? Evidence given below shows that some food proteins are more potent than others in the regeneration of plasma proteins but one cannot group proteins into vegetable, grain, animal, complete or incomplete and relate their potency to such grouping. Each food protein must be tested and its potency measured. It will be of some interest to determine whether there are any key amino acids which are essential to this upbuilding of plasma protein. Are these plasma proteins static and only to be replaced when lost (hemorrhage, ascites, nephrosis) or are they dynamic and concerned with protein metabolism? This last question is answered at least in part in the second paper below in favor of an ebb and flow between plasma, organ and tissue protein—a dynamic concept.

Much evidence has been published relating to the potency of food factors for potency in the regeneration of new hemoglobin in experimental anemia in dogs (11). We note the widest range of potency in hemoglobin regeneration from a maximum due to a diet of liver to a minimum due to grains and fish. When it was found that plasma protein regeneration (3) like hemoglobin regeneration could be controlled by diet our first thought was that the food potencies for hemo-

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globin and plasma protein regeneration might run along parallel to each other. Like most "first thoughts" this was wrong. *Liver* to be sure was potent in both lists but *kidney* which is almost as potent as liver to regenerate new hemoglobin was found to be at the very bottom of the list of foods so far tested for the regeneration of new plasma proteins.

Beef serum stands at the head of our list of potent foods for new plasma protein regeneration. This perhaps is not surprising although hemoglobin when fed is poorly utilized for hemoglobin production in anemia. Feeding 2.6 gm. of *beef serum* protein will produce 1 gm. of new plasma protein. Under similar conditions to produce 1 gm. of new plasma protein we must feed about 20 gm. of kidney protein.

The general plan of these experiments is very simple although technical difficulties must be overcome by experience. The normal plasma protein level (6–7 per cent) is reduced by bleeding with return of washed red cells in Locke's solution (plasmapheresis) to plasma protein levels of 3.6–3.9 per cent which are just above the edema level for the dog under the conditions of these experiments. This low level of plasma proteins is maintained a constant by suitable bleedings and red cell replacement. The subnormal level of plasma proteins presumably acts as a *maximal stimulus* for the production of new plasma proteins and unless the plasmapheresis is continued almost daily the new formed plasma protein will bring the plasma protein concentration promptly back to normal.

When the plasma protein depletion is begun the weekly output of new plasma protein is quite high and gradually declines to the *basal output* within 4–6 weeks. After this initial period the basal output will continue indefinitely at a reasonably constant figure provided the basal diet and other factors are kept uniform. The initial high output may exceed the basal output of plasma protein by 30–120 gm. Allowing for the drop in plasma protein concentration the amount of this protein is far beyond any method errors and is designated a *reserve store* of protein building material. This reserve store varies depending upon the diet factors of the preceding weeks and must always be completely exhausted before the basal output becomes fairly constant. It is possible if not probable that some of this reserve store is held in the liver (7). Hemoglobin regeneration experiments in anemia

will show a similar reserve store of hemoglobin building material but often in larger amounts and it requires longer periods of depletion to exhaust this reserve. It is possible that the body holds a general reserve store of protein or protein building material which on demand can be used to manufacture either plasma protein or hemoglobin or tissue protein as occasion may require. Experiments are in progress which may throw light upon this point.

In a recent communication (3) we have mentioned some of the earlier papers dealing with plasma protein regeneration. Some of the recent papers may be mentioned briefly. Moschcowitz (9) reports clinical conditions associated with hypoproteinemia particularly nephrosis, colitis, ascites, and pernicious anemia. He gives an excellent review. Jones, Eaton, and White (5) produced edema in cats by a low protein diet together with excess fluid or salt intake or infection. Jürgens and Gebhardt (6) believe the liver and reticulo-endothelial system are the sources of plasma proteins but do not consider diet a factor except as a stimlus. Reimann, Medes, and Fisher (10) conclude that the liver is responsible for the elaboration of protein precursors of blood proteins which are incorporated in widely distributed cells. In their experiments on rabbits no data relating to diet are given.

Methods

The various methods used were described in considerable detail in a recent paper (3) and this need not be repeated. The basal ration consists of boiled peeled white potatoes, canned tomatoes, Post bran flakes, Karo corn syrup, and cod liver oil in amounts as given in each dog's clinical history. Each dog received daily 1 gm. of a salt mixture (8) added to the diet. The potato contains 2.2 gm. protein per 100 gm. and the bran flakes contain 15 gm. protein per 100 gm.

The various food substances as used from time to time in animal experiments were analyzed for nitrogen in triplicate or quadruplicate by the macro-Kjeldahl method. The protein content was calculated as 6.25 times the average nitrogen values. The food substances were added to the basal ration and fed as a hash. The kidney tissue was cooked in water and fed with the broth. Egg white was coagulated by boiling. Other meats were fed raw. Commercial canned salmon was used. The serum was obtained from defibrinated fresh beef blood, the red cells being removed by use of the centrifuge. The fresh serum was mixed with the basal diet.

Nitrogen loss (negative N balance) included the urinary N plus 1 gm. daily allowed for the feces (not measured in all cases but calculated from average figures) plus the nitrogen contained in blood plasma removed by plasmapheresis and hematocrit samples.

EXPERIMENTAL OBSERVATIONS

All tabulated experiments are of the same character and the tables present continuous periods of observation divided into 7 day units. Rarely a period may be 6 or 8 days or longer but in the tables the values are corrected as of 7 days. Each dog has at least two tables to give complete experimental data. Tables 1, 2, 3, and 4 give the necessary figures for complete understanding of the plasma protein regeneration and the related diet potency. Tables 1-*a*, 2-*a*, etc., give the figures on nitrogen balance, nutrition, and blood findings. Following the respective tables is found the clinical history of each dog.

TABLE 1

Blood Plasma Depletion and Regeneration
Kidney and Pancreas Compared with Beef Heart

Dog 32-394.

Period 7 days	Diet	Protein intake Total for 7 days	Protein removed			Protein removed above basal*	Potency ratio* Protein intake to protein output	Blood plasma Average concentration	
			Albumin	Globulin	Total			Total protein	A/G ratio
		gm.	*gm.*	*gm.*	*gm.*	*gm.*		*gm. per cent*	
1	Basal	98			44.7			4.57	
2	Basal	98			30.5			3.80	0.62
3	Basal	98			28.9			3.57	
4	Basal	98			26.4			3.36	
5	Salmon bread	210	7.9	26.8	34.7	49.9	4.2	3.85	0.30
6	Basal	98	11.6	21.5	33.1			3.74	0.54
7	Basal	98	9.0	17.1	26.1			3.69	0.52
8	Gelatin	?			21.4			3.78	
9	Albumin	?	3.1	8.9	12.0			3.50	0.35
10	Basal	98	8.8	17.8	26.6			3.77	0.50
11	Basal	98	3.1	8.0	11.1			3.18	0.39
12	Albumin	293	10.0	10.9	20.9			3.48	0.92
13	Basal	98	5.4	11.7	17.1			3.47	0.46
14	Kidney	265	13.6	19.4	33.0	9.4	20.8	3.97	0.70
15	Basal	98	7.2	16.2	23.4			3.62	0.44
16	Basal	98	6.6	12.4	19.0			3.40	0.53
17	Pancreas	578	16.4	21.0	37.4	30.0	19.0	4.10	0.79
18	Basal	98	16.3	19.1	35.4			3.72	0.85
19	Basal	98	11.0	12.2	23.2			3.50	0.90
20	Beef heart	481	23.2	22.0	45.2	47.9	8.0	4.07	1.05
21	Basal	98	24.6	19.8	44.4			3.82	1.24
22	Basal	98	11.3	13.0	24.3			3.49	0.87

* Estimated basal output equivalent to 22 gm. plasma protein per week.

Tables 1 and 2 present a continuous history lasting over 41 weeks during which period the plasma protein level was held at 3.5–3.9 per cent by frequent plasmaphereses (4–7 per week). The low initial blood plasma protein level of 4.57 per cent is explained by a 2 months' preliminary period on the basal ration which contains no animal protein.

TABLE 1-*a*

Nitrogen Balance, Blood Findings, and Clinical Condition

Dog 32-394.

Period 7 days	Diet	Diet supplement per period	Weight	Negative N balance	Urinary N	R. B. C. hematocrit	Plasma volume
		gm.	*kg.*	*gm.*	*gm.*	*per cent*	*cc.*
1	Basal		13.5			36.6	602
2	Basal		13.8			39.8	
3	Basal		13.6			41.7	
4	Basal		13.7	8.7	13.2	37.3	
5	Salmon bread	2100	13.4	0.8	21.8	35.2	
6	Basal		13.3	8.4	11.8	34.7	475
7	Basal		13.2	22.4		41.6	
8	Gelatin	?	13.2			40.3	720
9	Albumin	?	13.3			40.8	
10	Basal		12.7			33.0	594
11	Basal		12.7	1.7	8.6	39.4	561
12	Albumin	250	12.7	+26.1	10.5	35.6	562
13	Basal		12.4	5.9	10.6	37.3	541
14	Kidney	1283	12.6	+12.9	16.7	36.8	656
15	Basal		12.6	5.0	10.0	38.7	550
16	Basal		12.5	18.7		39.9	557
17	Pancreas	1360	12.9	+43.2	34.3	34.8	665
18	Basal		13.2	12.0	15.0	37.8	619
19	Basal		13.0	7.2	12.2	35.6	724
20	Beef heart	2100	13.7	+30.3	32.5	32.4	770
21	Basal		14.1	14.2	15.8	37.4	696
22	Basal		13.3	10.3	15.1	35.5	683

The estimated *basal output* for Tables 1 and 2 is given as 22 gm. plasma protein per week while the dog is on the basal ration after the reserve store has been exhausted. This is somewhat of an arbitrary figure but in all dogs so far tested it amounts to about 2 gm. plasma protein per kilo per week. One does not accept the lowest basal output for any given week but takes a general average of a number of

satisfactory low basal weeks where no heavy protein feeding has shortly preceded. If we averaged weeks 8–13 inclusive, the basal output would stand at 18 gm. plasma protein per week. However in two of these periods the dog was vomiting some of its diet and was clinically somewhat disturbed with some loss of weight. In view of the very low output of plasma protein during fasting (Table 4) it seems fair to place the *basal output* at 22 gm. Even if this figure is a gram or so too high it is on the conservative side and the potency ratio will be a bit larger.

The *potency ratio* means the grams of protein which must be fed to produce 1 gm. of new plasma protein above the basal output. Obviously the lower the ratio the more potent is the given diet factor (Table 1)—kidney protein (20 gm.) or beef heart protein (8 gm.) will each produce 1 gm. new plasma protein. In the tables this *potency ratio* is calculated from the total protein intake minus the basal protein intake divided by the total plasma protein removed over and above the basal plasma protein output per week.

When a potent diet factor is added to the basal ration after a control period there is usually no change for 2 days but on the 3rd day of feeding the plasma proteins begin to rise and call for larger and more frequent plasmaphereses to maintain the usual depletion level of plasma protein concentration. The plasma protein output reaches a peak on the 7th day of feeding when the dog is returned to the basal ration. The accelerated plasma protein output continues for many days depending upon the potency and amount of the diet factor but sinks gradually to the basal level, usually by the end of the 2nd week of the basal ration. We assume that material has been accumulated and the stored reserve again built up during the favorable diet period and this store is called out and finally exhausted during the after period of 2 weeks. This we term the "carry over" and the picture is like that described for the regeneration of new hemoglobin in anemia on a favorable diet.

The *reserve store* of this dog (Table 1) is found to total 43 gm. plasma protein which represents the total output (131 gm.) for the first 4 weeks minus 88 gm., the standard basal output for this period. This dog consumed 100 per cent of its rations at all times except in three

periods as noted in the tables where the protein or food intake is not given. The basal ration amounted to 525 gm. per day or about 66 calories per kilo.

It has been noted (3) that *albumin* may make up about half of the total plasma protein contributed from the reserve store and this figure will vary with the diet which precedes the depletion period. It will be observed in all the tables that the *globulin* makes up a considerable part of the total protein removed during basal control periods in which no animal proteins are fed—the usual ratio is 2 parts globulin to 1 part albumin. It was suggested (3) that vegetable and grain proteins might favor the production of globulin in contrast to animal protein which might favor albumin production. The *salmon bread* period (Table 1) gives some evidence to indicate that grain protein may favor globulin production. During the week of salmon bread feeding there was removed 7.9 gm. albumin and 26.8 gm. globulin which is associated with the lowest A/G ratio observed (0.30). The bulk of protein (about 60 per cent) in the salmon bread is from wheat. Salmon bread (12) contains wheat flour, potato starch, bran, sugar, tomatoes, cod liver oil, canned salmon, yeast, and a salt mixture and its preparation has been carefully described. Salmon bread in anemia will permit a minimum of hemoglobin regeneration but with plasma depletion it will produce abundant globulin and some albumin with a potency ratio of 4.2 to 1. The potency ratio for salmon bread is of necessity figured somewhat differently as the salmon bread *replaced* the basal diet. Period 5, Table 1, shows an intake of 210 gm. protein in the salmon bread. The plasma protein output in period 5 should be all credited to the salmon bread diet = 34.7 gm. plus the "carry over" in periods 6 and 7 or 33.1 − 22 = 11.1 and 26.1 − 22 = 4.1. Total credit to the salmon bread diet is 49.9 on an intake of 210 gm., a potency ratio of 4.2. This corresponds closely to the potency ratio of the basal diet which for this dog is 4.5—that is the basal diet protein intake (98 gm.) divided by the basal protein output (22 gm.). The potency ratio for salmon is given below (Table 4) as 15.2 which compares unfavorably with these grain proteins and beef muscle proteins.

Albumen (Table 1, period 12) represents a feeding of "Egg Albumen, Impalpable Powder, Soluble, Merck." It is probable that most of

this material was not absorbed or digested as there is no increase in the urinary N. There is a slight increase of the albumin plasma fraction at the expense of the globulin—compare Table 3 below.

Kidney (Table 1, period 14) presented a great surprise because this tissue is so potent in building new hemoglobin in anemia. Kidney shows the lowest potency ratio 20.8 and we must think of 21 gm. of kidney as required to form 1 gm. new plasma protein. It pushes the A/G ratio up toward unity but there is no comparison with beef heart in the same dog where the potency ratio is 8 and the A/G ratio 1.24.

Pancreas (Table 1, period 17) stands close to the kidney and its potency ratio is 19.0. It would appear that nucleoproteins which are abundant in the pancreas are not particularly concerned with plasma protein regeneration.

Beef heart (Table 1, period 20) is a meat protein much used in laboratory studies. Its potency ratio is 8.0 and it corresponds with liver in this respect. Beef heart however produces a very large output of albumin and pushes the A/G ratio well above unity (1.24). In this particular dog (Tables 1 and 2) beef heart shows the highest output of albumin, even more than skeletal muscle and gizzard (smooth muscle) both given in Table 2. This question of the specificity of diet proteins for the production of plasma albumin in more abundance than plasma globulin deserves further study.

Blood volume figures deserve some attention in this dog (Tables 1-*a* and 2-*a*). With animal protein feeding there is always an increase (100 cc. average) in plasma volume. One notes also in spite of heavy withdrawal that the plasma protein concentration rises above 4.0 per cent. These two facts would point to a considerable outpouring of new protein into the circulation and an actual volume response as well.

Red cell hematocrit figures in this dog are uniformly satisfactory and average about 35–40 per cent only somewhat below the normal of 50 per cent for the dog. Iron feeding shows a rise of red cell hematocrit to 50 per cent but this is a familiar response.

Weight curve in Tables 1-*a* and 2-*a* is satisfactory and indicates good nutrition and adequate diet intake. The urinary nitrogen figures are a bit irregular but are to be explained in part on the basis of urine retained in the bladder. These dogs are never catheterized and their urination is irregular.

Egg white is well utilized and shows a potency ratio of 5.8 which is practically identical with lactalbumin, gizzard, and skeletal muscle (Table 2). Egg white feeding causes almost as much albumin as globulin production (period 23, Table 2).

Liver extract (Table 2, period 26) indicates a combination of two liver fractions[1]—No. 55 (13) which contains much of the material effective in hemoglobin regeneration in experimental hemorrhagic anemia in dogs and No. 343 which contains much of the material effective in the therapy of pernicious anemia. This material has been described elsewhere (13) and both fractions amount to 7 per cent of the fresh liver weight. Its potency for new hemoglobin production is very great and corresponds to 300 gm. fresh liver given daily for a week. Its potency for the regeneration of new plasma protein is negligible and corresponds to the small amount of nitrogenous material which the two fractions contain. The amount of new plasma protein is only 7.7 gm. above the basal level but as the calculated protein in the liver fraction fed amounts to only 67 gm. the potency ratio is 8.6 which is close to the potency ratio (6.5) for whole liver (Table 3).

Lactalbumin (Table 2, period 28) gives a liberal response and we note that a good deal more globulin than albumin is produced so that the A/G ratio does not rise above 0.72. The potency ratio is 5.5 and obviously the material is completely digested and utilized.

Ferric citrate fed in large amounts (2 gm. daily) gives a reaction which is distinct but not adequately explained. This dose of 2 gm. corresponds to 360 mg. of Fe which in an anemic dog will give a maximal regeneration of hemoglobin, approximately 50 gm. new hemoglobin from 1 week iron administration. This dog put out 14.4 gm. new plasma protein above the basal level. The same dog at this time had a plasma volume of 700 cc. and a protein concentration of 3.76 per cent or a circulating mass of plasma protein of 26 gm. Iron administration caused the production of plasma protein amounting to 55 per cent of the total proteins in the circulating plasma. This amount is far beyond any possible experimental error or known physiological fluctuation. The proper explanation will be of considerable significance.

Gizzard (Table 2, period 36) means that the smooth muscle only was

[1] Valuable material was supplied by Eli Lilly and Company.

fed and the lining mucosa with the tendinous parts were cut away. Its reaction is very much like the skeletal muscle response and the potency ratios are about the same.

TABLE 2

Blood Plasma Depletion and Regeneration
Lactalbumin, Gizzard, and Striated Muscle All Potent
Iron Reaction Unexplained

Dog 32-394.

Period 7 days	Diet	Protein intake Total for 7 days	Protein removed			Protein removed above basal*	Potency ratio* Protein intake to protein output	Blood plasma Average concentration	
			Albumin	Globulin	Total			Total protein	A/G ratio
		gm.	*gm.*	*gm.*	*gm.*	*gm.*		*gm. per cent*	
23	Egg white	295	18.7	23.7	42.4	33.5	5.8	3.87	0.78
24	Basal	98	16.7	17.6	34.3			3.65	0.95
25	Basal	98	8.9	13.9	22.8			3.53	0.64
26	Liver extract	165	10.3	17.8	28.1	7.7	8.6	3.57	0.58
27	Basal	98	7.8	15.8	23.6			3.61	0.49
28	Lactalbumin	389	18.1	25.1	43.2	52.5	5.5	4.17	0.72
29	Basal	98	15.8	23.5	39.3			3.61	0.67
30	Basal	98	11.3	20.7	32.0			3.68	0.55
31	Basal	98	10.9	15.1	26.0			3.67	0.72
32	Ferric citrate	98	10.7	20.3	31.0	14.4		3.76	0.53
33	Basal	98	7.1	20.3	27.4			3.87	0.35
34	Liver residue	?			26.1			3.95	
35	Basal	98	8.2	13.8	22.0			3.43	0.59
36	Gizzard	408	22.1	27.8	49.9	58.5	5.3	4.10	0.79
37	Basal	98	20.9	21.7	42.6			3.78	0.96
38	Basal	98	12.3	19.7	32.0			3.66	0.62
39	Skeletal muscle	475	25.5	31.2	56.7	66.4	5.7	4.17	0.82
40	Basal	98	17.6	20.5	38.1			3.96	0.86
41	Basal	98	13.5	24.1	37.6			3.70	0.56

* Estimated basal output equivalent to 22 gm. plasma protein per week.

Skeletal muscle (Table 2, period 39) shows an active production of albumin as well as globulin and the A/G ratio comes back toward normal. It may be argued properly that the gizzard and skeletal muscle periods should have been followed by 3 weeks basal diet to exhaust completely the "carry over." This probably would give potency ratios slightly smaller than those recorded.

Clinical History, Dog 32-394.—An adult, female, bull mongrel weighing 13 kg. This dog had been on basal diet for 2 months before depletion was begun. The basal daily diet consisted of 300 gm. white potato (6.6 gm. protein); 100 gm. tomato; 50 gm. Post's bran flakes (7.5 gm. protein); 50 gm. Karo syrup; 25 cc. cod liver oil; and 1 gm. salt mixture. 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. From periods 1 to 41 the

TABLE 2-*a*

Nitrogen Balance, Blood Findings, and Clinical Condition

Dog 32-394.

Period 7 days	Diet	Diet supplement per period	Weight	Negative N balance	Urinary N	R. B. C. hematocrit	Plasma volume
		gm.	*kg.*	*gm.*	*gm.*	*per cent*	*cc.*
23	Cooked egg white	1750	13.6	+7.7	25.7	37.8	745
24	Basal		13.4	16.1	19.3	40.5	753
25	Basal		13.0	12.6	17.6	38.6	682
26	Liver extract	150	13.0	2.7	17.6	44.8	557
27	Basal		13.3	9.0	13.9	44.4	582
28	Lactalbumin	350	13.4	+25.9	21.2	45.6	721
29	Basal		13.7	16.2	18.6	44.2	695
30	Basal		13.4	11.6	15.2	36.7	685
31	Basal		13.1	13.2	17.7	40.1	647
32	Ferric citrate	14	12.9	11.6	15.3	47.5	707
33	Basal		12.8	9.6	13.9	51.2	559
34	Liver residue	?	12.5			46.5	
35	Basal		12.4			42.8	408
36	Gizzard	1400	12.7	+24.7	25.6	40.2	625
37	Basal		13.1	13.6	15.5	41.9	580
38	Basal		13.1	12.5	16.1	43.7	565
39	Skeletal muscle	1400	13.3	+35.0	25.9	38.1	647
40	Basal		13.7	16.4	19.0	44.6	629
41	Basal		13.4	15.5	18.0	47.3	627

non-protein nitrogen of the blood varied from 12 mg. to 30 mg. per cent, reaching the latter level only once and that during period 40. During the weeks of initial depletion (periods 1–4), the dog maintained its body weight and remained in excellent clinical condition. During period 5 the dog was given salmon bread 300 gm. daily to replace the basal ration. During period 8 the dog refused gelatin mixed with the basal diet, and vomited the gelatin when it was given separately by stomach tube. On this account the gelatin feeding was discontinued after 4 days. During the following 8 days the dog was fed the basal ration and consumed 100 per cent of the diet. This 12 day period is recorded as a 7 day period, the

proper correction being made. In the last 3 days of period 9 the dog became lethargic, refused food, and developed hemoglobinuria. The attempt to feed powdered egg albumen was discontinued and the dog put back on the basal ration. Dextrose was given by stomach tube During the two following periods on the basal ration (periods 10–11), the dog was again in good health, the hemoglobinuria had ceased, the dog was active, and consumed all food. Periods 10–33 were without mishap. The diet ration in each particular experiment during that time was consumed 100 per cent. Liver residue feeding was attempted during the first

TABLE 3

Blood Plasma Depletion and Regeneration
Liver and Serum Potent Factors

Dog 32-130.

Period 7 days	Diet	Protein intake Total for 7 days	Protein removed			Protein removed above basal*	Potency ratio* Protein intake to protein output	Blood plasma Average concentration	
			Albumin	Globulin	Total			Total protein	A/G ratio
		gm.	*gm.*	*gm.*	*gm.*	*gm.*		*gm. per cent*	
1	Basal	114	22.6	14.3	36.9			5.34	1.58
2	Basal	114	16.6	23.3	39.9			4.28	0.71
3	Basal	114	10.9	18.9	29.8			3.99	0.58
4	Basal	114	14.2	19.9	34.1			3.89	0.71
5	Albumin	407	12.4	18.5	30.9	?	?	3.78	0.67
6	Basal	114	12.9	19.7	32.6			3.80	0.65
7	Basal	114	8.3	17.6	25.9			3.57	0.47
8	Liver	534	25.2	29.6	54.8	65.2	6.5	4.44	0.85
9	Basal	114	27.2	31.8	59.0			3.99	0.85
10	Basal	114	13.6	18.8	32.4			3.79	0.72
11	Ox serum	335	28.3	26.1	54.4	83.0	2.6	3.97	1.08
12	Basal	114	32.1	33.4	65.5			3.98	0.96
13	Basal	114	18.7	25.4	44.1			3.83	0.74
14	Skeletal muscle	533	34.9	39.2	74.1	47+		4.53	0.89

* Estimated basal output equivalent to 27 gm. plasma protein per week.

2 days of period 34. The dog refused the residue mixed with the basal diet, and vomited the mixture when it was spoon fed. During the remaining 5 days of this period the basal ration was fed, which was entirely consumed. Periods 35–41 were uneventful. During the 41 weeks 55.58 liters of blood were removed in 202 exchanges (plasmaphereses). Following period 41 beef serum concentrated by drying was given. After 3 days of this feeding, the dog refused food and developed hemoglobinuria and marked conjunctivitis. Petechiae were present on the mucous membranes. The sclerae were icteric. The dog was returned to the

basal ration and given blood transfusions and dextrose by vein. Three days later the animal was found dead. Autopsy revealed: Central necroses of liver involving perhaps two-thirds of the liver substance. Hemorrhagic bronchopneumonia. Acute bacterial endocarditis, mitral valve. Acute focal nephritis. Hemorrhage into large bowel. Petechiae on mucous membranes and pleurae. Microscopical sections showed considerable iron containing pigment in spleen, liver, kidneys, lymph nodes, and bone marrow.

Beef serum and *liver* are adequately standardized in this dog (Table 3, periods 8 and 11). Beef serum stands at the head of the list of

TABLE 3-*a*

Nitrogen Balance, Blood Findings, and Clinical Condition

Dog 32-130.

Period 7 days	Diet	Diet supplement per period	Weight	Negative N balance	Urinary N	R. B. C. hematocrit	Plasma volume
		gm.	*kg.*	*gm.*	*gm.*	*per cent*	*cc.*
1	Basal		14.6			43.3	
2	Basal		14.2			34.1	
3	Basal		14.4	9.5	16.0	34.4	702
4	Basal		13.6	10.2	16.0	39.1	711
5	Albumen	350	13.3		13.9	36.9	623
6	Basal		12.9	6.0	12.1	40.5	690
7	Basal		12.5	7.3	14.4	39.5	693
8	Liver	2100	13.1	+33.8	35.8	37.9	755
9	Basal		13.6	25.5	27.3	35.6	707
10	Basal		13.4	12.5	18.6	29.7	623
11	Ox serum	2800	13.4	4.8	33.1	33.7	743
12	Basal		13.4	28.6	29.3	24.8	
13	Basal		13.3	20.6	24.8	26.3	760
14	Skeletal muscle	2100	13.7			25.2	

potent materials most favorable for new plasma protein regeneration. Beef plasma protein (2.6 gm.) will produce 1 gm. of new plasma protein in the depleted dog—a potency ratio of 2.6. Moreover there is a large output of albumin and the A/G ratio rises to 1.08. The A/G ratio of the beef serum as *fed* reads 1.07. It is probable that not all of the "carry over" was removed as the 2nd week on the basal diet shows a total protein output of 44 gm. (Table 3, period 13) and the total plasma protein level is 3.83 per cent. Had this experiment been continued through a third control week a little more of the "carry

over" might have been removed which would place the potency ratio slightly below the recorded figure. A repetition of this experiment using smaller amounts of serum is to be desired.

It is of interest that 100 gm. of liver compare almost exactly with 100 cc. of beef serum when tested for their potency to regenerate new plasma protein. The liver has 2.5 times as much protein as the beef serum. The potency ratio for the liver is 6.5. The potency ratio for liver previously reported (3) was 6.8.

Albumen as used in Table 3, period 5, is a chemical preparation ("Egg Albumen, Impalpable Powder, Soluble, Merck") and appears to be inert physiologically.

The *basal output* for this dog was estimated to be 27 gm. plasma protein per week and the basal diet contained 114 gm. protein per week giving a potency ratio of 4.2 which compares with 4.5 for the basal diet in the preceding experiments (Tables 1 and 2).

The *reserve store* of protein building material amounted to 40 gm. estimating the basal output at 27 gm. plasma protein per week. It required 6 weeks to exhaust this reserve which was found to be highest on meat diets and lowest after long diet periods unfavorable to plasma protein regeneration. The kennel diet of hospital table scraps has a large factor of uncertainty but is more favorable as a rule for plasma protein regeneration than the basal ration as given this dog.

Table 3-*a* shows a slight loss of weight and a negative nitrogen balance. The red cell hematocrit fell below a safe figure and this may have been a factor in the terminal bronchopneumonia. The large plasmaphereses which equalled one-third the blood volume each day, during the last week were important in causing the hemoglobinuria which is always a danger sign.

Clinical History, Dog 32-130.—An adult male hound weighing 14.5 kg. was placed on a basal diet consisting of 400 gm. potato (8.8 gm. protein); 100 gm. tomato; 50 gm. Post's bran flakes (7.5 gm. protein); 50 gm. Karo syrup; 25 cc. cod liver oil; 1 gm. salt mixture; and plasmapheresis was started at once. During the preceding month the dog was on the kennel diet of hospital table scraps. The initial plasma protein level was 6.30 per cent; albumin 3.12 per cent; and globulin 3.18 per cent. The blood volume was 1215 cc., the plasma volume 638 cc. The non-protein nitrogen throughout the experiments varied from 13–24 mg. per cent.

The dog's clinical condition was excellent throughout the periods of observation until the 12th week when the red cell hematocrit ranged between 23 and 30 per cent. Two transfusions of red cells suspended in Locke's solution were given during this week. The clinical condition was otherwise quite good. In the 14th week however, the dog refused its diet and appeared to be very weak. The hematocrit was 23 per cent, rectal temperature 39.8°C. The plasma was quite red due to hemolysis and there was hemoglobinuria. Dextrose (100 cc. of a 10 per cent solution) was given by stomach tube and the animal's condition improved only slightly. The following day the dog was killed by gas anesthesia in order to study the pathological lesions. Autopsy showed: Bronchopneumonia. Central necroses of liver. Blood pigment in spleen, kidneys, and lymph glands. Chronic myocarditis.

Dog 33-238 (Table 4) is an extremely interesting animal. Even after 10 weeks on the basal ration without depletion, the A/G ratio was unity and during the 14 weeks of plasma depletion the dog produced surprisingly large amounts of albumin on the basal ration so that the A/G ratio was usually close to unity. Moreover the utilization of the food protein was unusually complete. The dog received 66 calories per kilo and 52 gm. protein each week—15.4 gm. protein from potato and 36.7 gm. protein from the bran flakes. The potency ratio for this diet is 2.7 or to produce 1 gm. plasma protein only 2.7 gm. protein in the given diet mixture need be fed. This is about as favorable a diet reaction as is seen with beef serum (Table 3). Whether the potato protein or bran protein is responsible we cannot say with certainty but the basal diets in which a larger protein proportion is fed as potato do not give as favorable a potency ratio. However these factors have not yet been tested on the same dog to eliminate individual metabolic capacities.

Salmon (a commercial canned product) has a low potency for regeneration of plasma protein—a potency ratio of 15.2. This test is wholly satisfactory in all respects and the 2nd basal diet week shows a return to the basal output (19 gm. protein).

Liver residue (Table 4, period 7) contains most of the liver protein and is the residue after extraction and removal of the fractions potent in anemia described above (Table 2, period 26). It is seen that the potency ratio for liver residue is 7.4 and for whole fresh liver is 6.5 (Table 3, period 8). Evidently the potency for this material resides largely in the protein fraction and is not much disturbed by the preparation methods—acid and heat.

Fasting (Table 4, periods 10 and 11) gives information of great interest. The experiment was wholly satisfactory and the dog was in excellent condition at all times. 60 gm. of dextrose in 200 cc. water were given daily by stomach tube. The fecal N was not determined but estimated from other similar experiments to be about 0.4 gm. per day. There was loss of 1.4 kilograms in weight and the usual

TABLE 4

Blood Plasma Depletion and Regeneration
Fish and Liver Residue Compared with Fasting

Dog 33-238.

Period 7 days	Diet	Protein intake Total for 7 days	Protein removed			Protein removed Above basal*	Potency ratio* Protein intake to protein output	Blood plasma Average concentration	
			Albumin	Globulin	Total			Total protein	A/G ratio
		gm.	*gm.*	*gm.*	*gm.*	*gm.*		*gm. per cent*	
1	Basal	52	17.2	17.7	34.9			3.98	0.97
2	Basal	52	11.7	11.8	23.5			3.83	0.99
3	Basal	52	10.7	11.8	22.5			3.78	0.91
4	Salmon	331	13.8	16.7	30.5	18.3	15.2	4.03	0.83
5	Basal	52	12.1	13.3	25.4			3.75	0.91
6	Basal	52	10.1	9.3	19.4			3.66	1.08
7	Liver residue	222	16.1	15.4	31.5	23.1	7.4	3.95	1.05
8	Basal	52	13.2	16.7	29.9			3.66	0.79
9	Basal	52	7.1	11.6	18.7			3.58	0.61
10	Dextrose	0			3.5			3.45	1.00
11	Dextrose	0			5.4			3.84	0.67
12	Basal	52	8.5	10.6	19.1			3.43	0.80
13	Skeletal muscle	265	16.7	14.9	31.6			4.32	1.12
14	Basal	52			27.2			3.72	

* Estimated basal output equivalent to 19 gm. plasma protein per week.

shrinkage of plasma volume from 465–373 cc. The dog had been very carefully standardized and the basal plasma protein output (19 gm. per week) is observed in the week preceding and following the sugar periods. The total plasma protein output for these 2 weeks is 9 gm. About 3 gm. of this amount can be accounted for by the shrinkage of plasma volume so that the total output amounts to about 3 gm. per week. This is close to physiological limitations. Evidently this dog can produce little if any new plasma proteins

during fasting periods—a very different story from that relating to new hemoglobin production in anemia or new liver cell production after injury.

The low N output in the urine after the sugar periods (Table 4-*a*, period 12) is of interest and shows distinct conservation of protein products.

TABLE 4-*a*

Nitrogen Balance, Blood Findings, and Clinical Condition

Dog 33-238.

Period 7 days	Diet	Diet supplement per period	Weight	Negative N balance	Urinary N	R.B.C. hematocrit	Plasma volume
		gm.	*kg.*	*gm.*	*gm.*	*per cent*	*cc.*
1	Basal		9.8			48.7	417
2	Basal		9.7			43.1	
3	Basal		9.7	18.9	16.6	47.3	
4	Salmon	1050	9.7	+15.8	25.3	48.5	451
5	Basal		9.8	17.8	15.1	51.5	413
6	Basal		9.6	13.9	12.1	53.5	
7	Liver residue	252	10.0	+5.4	18.2	55.3	398
8	Basal		11.1	15.9	12.4	51.0	416
9	Basal		9.9	11.6	9.9	51.0	465
10	Dextrose		9.3	11.0	7.6	44.7	452
11	Dextrose		8.5	9.6	5.9	43.3	373
12	Basal		9.2	9.3	7.6	48.1	353
13	Skeletal muscle	1050	9.9	+13.7	16.6	52.0	430
14	Basal		10.1		20.6		

Table 4-*a* shows a highly satisfactory condition. The weight is uniform except during the sugar feeding period. The red cell hematocrit is maintained at practically a normal level throughout. The food consumption was 100 per cent in all periods.

Clinical History, Dog 33-238.—An adult male terrier weighing 9.8 kg. This dog had been on the basal ration consisting of 100 gm. potato (2.2 gm. protein); 60 gm. tomato; 50 gm. Karo syrup; 35 gm. Post's bran flakes (5.25 gm. protein); 25 cc. cod liver oil; and 1 gm. salt mixture, for 10 weeks prior to the beginning of plasmapheresis. The *reserve store* in this dog amounts to only 24 gm. of potential protein building material. The protein level following this basal period was 4.5 per cent; albumin 2.26 per cent; globulin 2.25 per cent. The blood volume was 872 cc. and the plasma volume 417 cc. The non-protein nitrogen ranged between

15 and 20 mg. per cent throughout the periods of observation. The dog was in excellent clinical condition up to the day of its death which was due to a mistake in the plasmapheresis—a hypertonic solution having been used. Autopsy showed: Pulmonary edema and organs which were grossly normal but for congestion. Microscopical sections of organs showed normal tissues. There was a slight excess of brown pigment in the spleen pulp and Kupffer cells of the liver.

Table 5 shows all the potency ratios for comparison. The *potency ratio* means the number of grams as protein which must be fed to re-

TABLE 5

Summary of Diets and Potency Ratios

Dietary factors	D/P or potency ratio		
	Dog 32-394	Dog 32-130	Dog 33-238
Beef serum		2.6	
Basal ration	4.5	4.2	2.7
Salmon bread	4.2		
Gizzard	5.3		
Lactalbumin	5.5		
Skeletal muscle	5.7		
Egg white	5.8		
Liver		6.5	
Liver residue			7.4
Liver extract	8.6		
Beef heart	8.0		
Salmon			15.2
Pancreas	19.0		
Kidney	20.8		

generate 1 gm. of plasma protein in depleted standard dog. It may be called the D/P ratio—diet to plasma protein.

DISCUSSION

The unexpected reaction of these dogs with plasma protein depletion to fasting and iron feeding calls for discussion. *Fasting* during anemia periods has been studied in this laboratory and in a recent report (1) it was shown that the dog can produce a good deal of new hemoglobin during fasting or sugar feeding periods and the reaction is striking if large doses of iron are given with the sugar. The evidence points to *conservation* of nitrogenous end products which are built up into new

hemoglobin. Furthermore during sugar feeding periods the dog can regenerate a considerable mass of new liver cells to repair a liver injury caused by chloroform (2). But in a similar emergency with depleted plasma proteins the dog can produce little if any new plasma protein.

We cannot say that one of these substances is any more urgently needed than the other. Moreover there is much evidence that plasma protein when present in excess can be utilized in the body to replace other proteins (4). With plasma depletion to levels below 4.0 per cent it is apparent that there is a strong stimulus to form new plasma protein from any satisfactory diet material. Moreover when the dog (Table 4, period 12) is put back on the basal ration the production of new plasma proteins begins immediately and progresses at the usual pace showing the normal basal output for that week. One might anticipate a subnormal plasma protein output owing to the demand coming from other depleted body stores of protein. There is a conspicuously low figure for urinary N on this first week after fasting, indicating very careful conservation of all nitrogenous material. There is a gain in weight of 0.7 kg.

We may state that in an emergency the plasma protein may contribute to body protein but the current will not flow easily in the opposite direction and the body proteins may be said to stand by helpless to aid while vital plasma proteins are depleted even to a lethal point. This is the only possible conclusion to be drawn from Table 4 but repeated observations must determine whether this is a constant reaction. Evidently in this emergency the plasma protein is very largely if not wholly dependent on materials coming in from the gastro-intestinal tract. Here again the finger of suspicion is placed on the liver, as these materials must be assembled into the complex structure of albumin or globulin.

The *reserve store* of plasma protein building material represents insurance for the normal dog against dangerous depletion of plasma proteins during a fasting period. This reserve store may amount to 30–120 gm. of potential plasma protein material—twice or three times as much on the average as this type of dog has in its circulation. We may well ask as to the nature of this *reserve store*. Although some of it may be in mature protein form the inability of body proteins during fasting to contribute to the plasma proteins speaks against this and

we are inclined toward the belief that this emergency *reserve store* is tucked away in the liver and perhaps other tissues as some "intermediate" substance—not as small aggregates like polypeptides but as large aggregates approximating proteins but not fixed tissue proteins. Probably such reserve store of protein building material can be used for other purposes than plasma protein regeneration—for example tissue or organ repair or red cell and hemoglobin regeneration.

Why should *iron feeding* stimulate plasma protein production—an excess output (Table 2) of 14.4 gm. above basal levels? We cannot say that iron enters into the reaction as is the case with its effect on the production of new hemoglobin. It is not known that iron modifies absorption from the intestinal tract; in fact iron is not readily absorbed even when in anemia due to blood loss there is an urgent need for it in internal metabolism.

The surplus plasma protein due to iron feeding probably comes from the basal food intake, as during fasting periods in the depleted dog the body proteins cannot contribute appreciably to the formation of new plasma protein. Let us argue that iron modifies internal metabolism so that new hemoglobin can be formed and further that as a result of this modified internal metabolism more materials emerge which can be built into new plasma protein. This is of the nature of a catalytic reaction. Fasting experiments with iron feeding and plasma depletion should give evidence bearing on this point.

SUMMARY

When blood plasma proteins are depleted by bleeding, with return of washed red cells (plasmapheresis) it is possible to bring the dog to a steady state of low plasma protein and uniform plasma protein production on a basal diet. Such dogs are excellent test subjects by which the potency of various diet factors for plasma protein regeneration can be measured.

To regenerate plasma proteins in any significant amount the depleted dog requires food protein. Some proteins are very potent for new plasma protein production and others are utilized poorly.

Beef serum is very potent and its proteins (2.6 gm.) will produce 1 gm. of new plasma protein in the depleted dog—a potency ratio of 2.6.

Kidney protein stands at the bottom of our list and the dog needs 21 gm. of kidney protein to regenerate 1 gm. of plasma protein—a potency ratio of 21.0.

Some grain proteins approximate the potency of beef serum and may show potency ratios of 2.7 to 4.6. Some of these grain proteins appear to favor the production of globulin more than albumin in the plasma.

Skeletal muscle, gizzard (smooth muscle), lactalbumin and egg white fall into a favorable group with a potency ratio of 5.3 to 6.0.

Whole liver, liver fractions, casein, and beef heart are a little less potent and present potency ratios of 6.5 to 8.0. Many of these food substances favor the production of albumin more than globulin.

Pancreas and salmon muscle show less favorable potency ratios of 19.0 and 15.0 respectively.

Fasting periods indicate that these depleted dogs can produce little if any new plasma protein.

Iron feeding in some unexplained manner will influence body metabolism so that an excess of plasma protein will be produced.

These observations have a bearing on clinical conditions associated with hypoproteinemia and give suggestions for diet aid or control in some of these abnormal states. The make-up of the diet is obviously of great interest and it is possible that protein combinations may be more potent than a single protein or that food potency ratios may differ in health and disease.

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