BLOOD PLASMA PROTEIN REGENERATION AS INFLUENCED BY FASTING, INFECTION, AND DIET FACTORS

VARIABLE RESERVE STORES OF PLASMA PROTEIN BUILDING MATERIAL IN THE DOG

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This paper continues the study of blood plasma protein production in dogs as influenced by various factors. Plasma protein production is measured by determination of the protein removed in daily plasma-pheresis (removal of whole blood and return of washed red cells suspended in a saline solution). The factors known to influence plasma protein production are controlled while this measurement is being made day after day, week after week. Some of these factors are the dietary intake of protein, the reserve store of plasma protein building material, the concentration of the circulating plasma protein, and the clinical state of health of the animal (freedom from infection, etc.). Data bearing on all these factors have been published in previous reports (3, 10, 7, 5) and further experiments particularly concerning the reserve store are presented in this communication. The details of the procedure used are reviewed below (Methods).

The presence of a *reserve store* of plasma protein building material in the normal dog is well established. Its amount varies in different dogs. It may be as low as 10 gm. or as high as 60 gm.—compared with about 30 gm. plasma protein, the mass circulating in this type of normal dog (10 to 13 kg.). To a great extent these wide variations are dependent upon the preceding dietary intake. After weeks on a diet low in protein the reserve store will be low, and following periods

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of liberal protein feeding a large reserve store will be accumulated. This reserve store may be even larger if a period of plasma depletion has preceded the period favorable to storage (Table 1, below).

The *time required for the depletion of this reserve store* varies directly with the amount of the store and is usually from 2 to 6 weeks (see Table 1). In the depletion of this reserve store the mere reduction of the circulating plasma protein concentration from a normal level to a concentration slightly above the edema level is no criterion of exhaustion of the store. Reference to Tables 2 and 4 below will show that such a reduction may occur from 1 to 4 weeks prior to complete removal of the reserve store. Therefore, it is essential that the hypoproteinemia be maintained a sufficient length of time (2 to 6 weeks) to demonstrate a *constant* minimal (basal) plasma protein production. To do this a diet must be chosen which will maintain the dog in an apparently normal state for an indefinitely long time. If the basal diet is inadequate no certain baseline can be established. This opinion relative to reserve stores differs from that held by others (8).

This *reserve store* of plasma protein building material we believe plays a rôle in protein metabolism which is only beginning to be understood. Under certain circumstances it can contribute to the building of other body protein, for example hemoglobin. It may participate in and modify the response to certain injuries involving body protein. For example, when a standard sterile abscess is produced in a dog which has been depleted of this reserve protein store the urinary nitrogen does not increase in the striking fashion noted in control normal dogs (Table 3-*a*) (1, 2). Evidently this reserve protein store contributes directly or indirectly to the surplus of eliminated urinary nitrogen observed with abscesses in normal dogs.

Methods

The *reserve store* in these experiments is and has always been (3, 10, 7, 5) determined as follows. After a week's fast the dog is fed a standard basal low protein diet daily as given in the clinical history of each animal. Daily plasmaphereses are done and the total protein removed each week recorded in the tables. An attempt is made to reduce the plasma protein concentration from the normal level of about 6.0 per cent to the depletion level of 4.0 per cent and to hold this depletion level at a very constant figure. At about 3.5 per cent plasma protein concentration edema and clinical disturbances may appear and

at 4.6 per cent some storage of protein in body tissues will occur. The bleedings are carefully estimated to keep the plasma protein concentration close to this optimum figure of 4.0 per cent and it is safe to assume that this level supplies a constant stimulus to the body to produce as much plasma protein as possible on the given diet intake. The red cell hematocrit is maintained at about 50 per cent by the return of suitable amounts of washed red cells. This plasmapheresis is continued steadily until the weekly plasma protein output is a constant or approximately so, and this usually requires 4 to 6 weeks of plasmapheresis (dogs 33-11 and 36-95). An actual example of the method used in calculation of the reserve store may now be given. In dog 36-95, Table 4, if the plasma protein weekly production attributable to the basal diet is assumed to be 20 gm. on the basis of its constancy in periods 4 and 5, the total basal output for the first 5 weeks (including the week of fasting) is 80 gm., but the *total plasma protein actually removed* is 105 gm. The difference equals a reserve store of 25 gm. To allow for that portion contributed by the mass of circulating protein during the reduction of its concentration from 5.34 to 4.04 gm. per cent we may deduct 7 gm. (assuming a constant plasma volume of 550 cc.) and obtain a *net reserve store* of 18 gm.

Adult dogs immunized (Laidlaw-Dunkin) against distemper were used. Their care and feeding have been previously described (5). Certain changes in procedure are here enumerated.

1. Fecal nitrogen determinations were done. As collected the feces were suspended in water and charred by the addition of an equal volume of concentrated sulfuric acid. The quantities thus treated for a complete week were then diluted to volume and nitrogen determined on a pipetted aliquot.

2. Boric acid, 4 per cent aqueous solution, was used to hold the distilled ammonia in solution in performing the Kjeldahl nitrogen determination (11).

3. No glucose was added to the modified Locke's solution used in suspending the washed red cells for injection into the dogs. Previously in this work glucose to a 5 per cent concentration has been used, but such hypertonicity induces crenation of the cells. The donor cells are always freshly prepared and injected within less than 3 hours after their removal from the donor dogs.

4. Large negative nitrogen balances have been recorded for the dogs subjected to plasmapheresis (3, 10, 7, 5). Part of this deficit has been calculated on the assumption in some instances of a fecal nitrogen output of 1 gm. per day. A larger part of the deficit has arisen from failure to account for the nitrogen in *excess* red blood cells injected for the purpose of maintaining a constantly normal hematocrit. All the work reported in this paper was done prior to attempts at exact measurement of red cells injected, and the *recalculation* that has been done probably includes considerable error.

As in previous work we have intended to feed the dogs basal diets containing the minimal protein consistent with a normal existence during prolonged plasmapheresis. The exact diets used are given in the clinical histories. The percentage protein of the various diets is calculated from the following figures: pork liver, 20 per cent, pork kidney, 16.2 per cent; canned tomatoes, 1.2 per

cent; gelatin, granular (Will Corporation), 83.8 per cent (15.1 per cent nitrogen); "protein-free" diet of Cowgill, 0.05 gm. protein (but 0.019 gm. nitrogen) per kilo dog. About 95 per cent of the nitrogen in this latter diet is present in the liver extract vitamin supplement. The protein-free diet was given exactly as described by its originators (8). Except in this latter, the salt mixture used in all diets was that of McCollum and Simmonds (6) without the iron. Plasma volume determinations were done at the end of each week as tabulated.

EXPERIMENTAL OBSERVATIONS

Plasma protein building stores may vary in the same dog under different conditions. It is known that long periods on a low protein diet or short periods of fasting will deplete this reserve. As illustrated

TABLE 1

Reserve Store of Plasma Protein Building Material Determined in 3 Successive Years in Dog 33-11

Period	Diet before depletion	Weight		Plasma protein concentration		Time of depletion*	Total reserve protein
		Initial	Final	Initial	Final		
		kg.	*kg.*	*gm. per cent*	*gm. per cent*	*wks.*	*gm.*
1935 (7)	Low protein 9 wks.	11.6	11.2	5.04	4.10	2	11.1
1936 (5)	Kennel 16 wks.	12.5	11.5	6.09	4.13	3	34.1
1937 (Table 2)	Kennel 20 wks.	13.1	11.4	6.50	4.05	6	67.0

* One week of fasting precedes each depletion period—see Table 2.

in Table 1, the first period shows a very low protein store, due in part to the preceding 9 weeks period of potato-bran basal ration (7)—a low protein diet containing only vegetable and grain proteins.

Table 1—the second and third periods are comparable as the dog in 2 successive years had been on the kennel diet (hospital table scraps) for 16 and 20 weeks, had been fasted for 1 week, and then put on the same kidney basal diet for plasma depletion. The protein reserve store in the second period was 34 gm. and one year later 67 gm. This observation suggests that a dog subjected to plasma depletion may subsequently pile up a larger protein reserve store when a favorable diet offers the opportunity for storage.

Tables 2 and 2-a show 18 consecutive weeks of plasma depletion in an active dog, clinically normal. The following 12 weeks are recorded

in Tables 3 and 3-*a*. Details relating to diet intake are given under clinical histories below. The first seven periods show a large *reserve store* of plasma protein building material—about 67 gm. plus the unknown amount used up during the first period of protein fasting. From other experiments in preceding years we estimate the basal output as 12 gm. plasma protein each week but even if we place the basal output at 13 to 14 gm. plasma protein per week, the surplus protein store is 50 to 60 gm. and requires 7 weeks for removal. If experimental observations with gelatin or other materials had been begun at the end of 2 weeks initial depletion, the errors due to undepleted reserve would have been large. It is noted that the albumin-globulin ratio falls from 1.1 at the start of the rapid plasma depletion to 0.7 at the end of the 3rd week but rises again as the plasma depletion is less vigorous at the end of the 6th period. This reaction to plasma depletion has been noted before.

Liver basal ration was then used and the liver contains practically all the ingested protein except for small amounts present in the canned tomatoes. Dogs tolerate the liver basal well and remain clinically normal even when plasma depletion is continued for 30 weeks.

Gelatin added to the basal ration during the 11th period causes very little increase in production of plasma protein, a total of 7.7 gm. as the result of feeding 105 gm. gelatin. It is noted (Table 2-*a*) that the *intake* in food nitrogen is increased about 16 gm. and the *output* of urinary nitrogen likewise is increased about 16 gm. giving a positive nitrogen balance of only 1.7 gm.

Gelatin plus tryptophane added to the basal ration during the 16th period gives a very different picture and an increase of plasma protein production of more than 28 gm. or about 4 times the figure for gelatin alone. Evidently gelatin is supplemented by tryptophane and the basal diet so that it is readily built into plasma protein after digestion. In fact the *potency ratio* for this gelatin plus tryptophane is 3.0 as compared with a ratio of about 12 for gelatin alone. This is compared with the potency ratio of the liver in the basal diet of 4.3—that is 4.3 gm. of liver protein as fed will produce 1 gm. of plasma protein. The positive nitrogen balance during period 16 amounts to 8.2 gm. *Tryptophane alone* added to the basal diet in the 23rd period (Table 3) shows no response.

The protein-free diet during period 14 gives a plasma protein output of 8.8 gm.—refer to Table 4, periods 6 and 7, for more complete observations.

TABLE 2

Blood Plasma Protein Production as Influenced by Gelatin and Tryptophane
Dog 33-11.

Period 7 days	Diet	Protein intake Total for 7 days	Plasma protein re-moved Total for 7 days	Pro-tein re-moved above basal*	Blood plasma Average concentration		R.B.C. hema-tocrit, average	Plas-ma vol-ume
					Total pro-tein	A/G ratio		
		gm.	*gm.*	*gm.*	*per cent*		*per cent*	*cc.*
	Kennel				6.50	1.2	47.2	485
1	Dextrose, 350 gm.	0	3.2		5.72	1.1	45.0	468
2	Kidney basal	64	30.0		4.92	1.0	46.5	—
3	Kidney basal	64	27.9		4.63	0.7	48.8	468
4	Kidney basal	55	24.1		4.04	0.8	52.2	—
5	Kidney basal	64	20.5		3.93	0.8	54.0	398
6	Kidney basal	64	13.8		4.06	0.9	52.6	390
7	Kidney basal	64	17.8	67.0	4.05	0.9	51.7	459
8	Liver basal	108	21.2		4.18	0.9	49.8	411
9	Liver basal	109	24.2		4.14	0.9	48.4	450
10	Liver basal	109	23.6		4.27	1.0	50.0	—
11†	Liver basal + gelatin, 105 gm.	197	26.8	7.7	4.26	1.1	52.2	441
12‡	Liver basal	109	26.0		4.19	1.0	52.8	504
13	Liver basal	109	26.9		4.05	1.0	52.0	438
14	Protein-free	4	8.8		3.97	0.9	50.4	461
15	Liver basal	109	22.4		4.08	0.9	50.2	484
16	Liver basal + gelatin, 105 gm. + tryptophane, 2.7 gm.	197	28.3	28.7+	4.35	1.0	52.5	459
17	Liver basal	109	33.2		4.30	1.0	53.6	462
18	Liver basal	109	39.2		4.35	0.9	53.4	—

* Estimated basal output on kidney basal diet = 12 gm. plasma protein per week and on liver basal = 24 gm. per week.

† 8 day period recalculated on basis of 7 days.

‡ 6 day period recalculated on basis of 7 days.

Tables 3 and 3-*a* continue the experimental life history of dog 33-11. Period 19 presents some interesting departures from normal and we cannot give a satisfactory explanation as yet. Possibly experiments in progress may make clear some of these obscure points. On the 2nd day of the 19th period, the donor cells were probably damaged

by overheating. When introduced into dog 33-11 there was *prompt
hemolysis*, the dog was clinically abnormal but not seriously shocked
and there was hemoglobinuria for about 24 hours. 3 days later the

TABLE 2-*a*

Weight and Nitrogen Balance

Dog 33-11.

Period 7 days	Diet	Weight	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	13.1						
1	Dextrose, 350 gm.	11.7	0.0	−2.5	0.5	17.7	0.5	−21.2
2	Kidney basal	11.7	10.2	8.1	4.9	13.4	2.5	−2.5
3	Kidney basal	11.7	10.2	2.1	4.6	12.9	2.2	−7.4
4	Kidney basal	11.5	8.8	5.3	4.0	12.5	2.1	−4.5
5	Kidney basal	11.4	10.2	2.9	3.4	11.5	2.0	−3.8
6	Kidney basal	11.4	10.2	3.1	2.3	10.4	1.8	−1.2
7	Kidney basal	11.4	10.2	−1.6	2.9	10.7	1.8	−6.8
8	Liver basal	11.3	17.3	3.1	3.5	9.6	3.1	4.2
9	Liver basal	11.4	17.5	2.1	4.0	9.7	2.5	3.4
10	Liver basal	11.5	17.5	3.1	3.9	10.9	2.4	3.4
11*	Liver basal + gelatin, 105 gm.	11.6	33.4	2.3	4.4	26.6	3.2	1.5
12†	Liver basal	11.6	17.5	0.9	4.5	11.2	2.7	0.0
13	Liver basal	11.7	17.5	0.8	4.4	10.5	3.5	−0.1
14	Protein-free	11.5	1.4	−0.7	1.4	7.2	1.1	−9.0
15	Liver basal	11.5	17.5	6.1	3.7	10.3	2.2	7.4
16	Liver basal + gelatin, 105 gm. + tryptophane, 2.7 gm.	11.6	33.7	5.2	4.6	24.4	1.7	8.2
17	Liver basal	11.7	17.5	2.0	5.4	11.8	4.3	−2.0
18	Liver basal	11.8	17.5	4.0	6.5	13.0	2.4	−0.4
Totals			268.1	46.3	68.9	234.3	42.0	−30.8

* 8 day period recalculated on basis of 7 days.
† 6 day period recalculated on basis of 7 days.

dog was back to normal, although the plasma was still icteric. Food
consumption was normal and there was no significant fall in red cell
hematocrit as extra red cells were given by vein. Excess urinary
nitrogen amounted to 5 or 6 gm.

This whole reaction is of interest because there is a large excess of

plasma protein produced during periods 19 to 21—an excess of 52 gm. plasma protein over and above the basal output of 24 gm. per week. This *excess* of plasma protein may be related to one or more of several factors—the large surplus of globin released from hemoglobin in the circulation; the clinical shock and related tissue or cell injury; a "carry over" from the period of very abundant plasma protein production

TABLE 3

Blood Plasma Protein Production as Influenced by Hemolysis and by Abscesses Dog 33-11.

| Period 7 days | Diet | Protein intake Total for 7 days | Plasma protein re- moved* Total for 7 days | Blood plasma Average concentration | | R.B.C. hema- tocrit, average | Plas- ma vol- ume |
				Total pro- tein	A/G ratio		
		gm.	*gm.*	*per cent*		*per cent*	*cc.*
19	Liver basal (hemolysis)	109	51.1	4.29	0.8	51.0	484
20	Liver basal	109	43.1	4.22	0.8	50.5	488
21	Liver basal	104	30.5	3.98	0.8	49.5	457
22†	Liver basal	109	26.3	3.92	1.0	49.5	511
23	Liver basal + tryptophane, 4.2 gm.	109	21.0	4.38	0.8	50.9	482
24	Liver basal	109	17.6	4.22	0.9	50.2	486
25	Liver basal	109	19.2	4.27	0.9	51.3	505
26	Fasting + abscesses	0	15.5	4.76	0.6	51.1	—
27	Liver basal	108	17.1	4.95	0.6	49.5	452
28	Liver basal	108	23.5	4.52	0.8	54.3	428
29	Liver basal	109	19.7	4.24	0.9	52.7	497
30	Liver basal	107	15.8	4.21	0.8	50.4	458

* Estimated basal output on liver basal diet = 24 gm. plasma protein per week.
† 8 day period recalculated on basis of 7 days.

due to gelatin plus tryptophane 3 weeks previously. More experiments are required to give a complete answer.

Tables 3 and 3-a (period 26) show an unexpected reaction with plasma protein production much above the 4 to 8 gm. usually obtained during fasting (10, 7). We believed from previous experiments (5) that *abscesses* lessened the output of plasma protein during periods of liver feeding and we suspected that the fasting output with abscesses might be zero or even show a rapid fall in plasma protein con-

centration. Therefore the plasma protein concentration was not held at the usual level of 4.0 per cent but permitted to rise as high as 4.76 per cent. This rise confused the picture and we cannot argue about the surplus plasma protein (15.5 gm.) observed in period 26. There may have been some storage during periods 24 and 25. We suspect also that under these conditions there has probably been

TABLE 3-a

Weight and Nitrogen Balance

Dog 33-11.

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.*
19	Liver basal (hemolysis)	11.9	17.5	12.0	8.7	18.1	3.0	−0.3
20	Liver basal	11.8	17.5	1.8	7.1	13.9	2.6	−4.3
21	Liver basal	11.8	16.6	6.3	5.1	13.8	2.2	1.8
22*	Liver basal	11.8	17.5	6.4	4.4	13.8	2.7	3.0
23	Liver basal + tryptophane, 4.2 gm.	11.5	18.1	1.3	3.5	11.6	2.9	1.4
24	Liver basal	11.6	17.5	2.6	2.9	11.7	3.1	2.4
25	Liver basal	11.8	17.5	1.2	3.2	12.1	2.4	1.0
26	Fasting + abscesses	10.7	0.0	4.1	2.5	11.6	1.8†	−11.8
27	Liver basal	11.1	17.2	2.7	2.7	9.9	3.2	4.1
28	Liver basal	11.3	17.4	3.0	3.9	10.0	3.1	3.4
29	Liver basal	11.6	17.5	1.1	3.2	10.0	3.1	2.3
30	Liver basal	11.5	17.1	−0.1	2.6	10.3	4.3	−0.2
Totals............................		191.4	42.4	49.8	146.8	34.4		2.8

* 8 day period recalculated on basis of 7 days.

† Nitrogen in pus from abscesses, fecal nitrogen included in following period.

some *conservation* of the split products produced by the abscesses with production of new plasma protein. There is little or no rise in urinary nitrogen which is a very different response from the reaction with abscesses in the undepleted dog.

Red cell hematocrits in this experiment (Tables 2 and 3) were kept at a level above the initial level for this dog of 47 per cent. It is possible that this favored red cell destruction and removal by the

normal mechanism and accounts in part for the heavy intake values for red cell nitrogen. The figures for this dog are about 3 times as high as for dog 36-95 (Table 4-a) which received about 1 gm. of nitrogen a week from excess red cells injected.

Clinical Experimental History.—Dog 33-11 (Tables 2 and 2-a; 3 and 3-a). An adult female mongrel, born Nov. 25, 1932, this dog has been the subject of two periods of plasmapheresis previously reported (7, 5). Between the end of the latter period and the beginning of the present observations 20 weeks elapsed. During the 1st week (Tables 1 and 1-a) the dextrose was given by stomach tube. The *kidney basal* ration contained 50 gm. (raw weight) cooked pork kidney; canned tomato, 25 gm.; Vitavose (Squibb), 5 gm.; cane sugar, 95 gm.; butter fat, 10 gm.; lard, 30 gm.; cod liver oil, 10 gm.; bone ash, 5 gm.; salt mixture, 1 gm.; and furnished 9.15 gm. protein and 908 calories. By mistake one day's diet was omitted in the 4th week.

The *liver basal* diet contained liver, 75 gm.; canned tomato, 50 gm.; cane sugar, 100 gm.; lard, 30 gm.; cod liver oil, 15 gm.; bone ash, 20 gm.; salt mixture, 1 gm.; and furnished 15.6 gm. protein and 920 calories. About 5 per cent of this diet was vomited one day during the 1st week it was given. When gelatin 15 gm. was added to this ration in the 11th and the 16th weeks the cane sugar was reduced by 14 gm. to maintain a constant daily caloric intake. The granular gelatin was mixed into the basal diet without addition of water. The "protein-free" diet (calculated for a 11.7 kilo dog) contained cane sugar, 102.8 gm.; dextrin, 33.9 gm.; lard, 31.1 gm.; butter, 12.2 gm.; bone ash, 4.4 gm.; salt mixture, 2.2 gm., giving a total of 920 calories. The vitamin supplements given in conjunction were the same as those chosen by the original users of this diet (8): 4 tablets of cod liver oil concentrate and 8.2 cc. of the liver extract-rice polishing extract suspension. This diet was not well taken and small regurgitations during the 7 days amounted to a total of 80 per cent of a single day's diet. In the 16th period, *l*-tryptophane was added, 0.1 gm. per day for the first 3 days and 0.6 gm. per day for the last 4 days.

On the 2nd day of the 19th period the 136 cc. donor red cells given to replace the 119 cc. cells withdrawn from the dog in the day's bleeding were evidently injured by heat. The hematocrit dropped and the plasma was red with hemoglobin the next day; hemoglobinuria occurred and the animal was clinically upset. On the 5th day of the period the animal appeared largely recovered, the urine was pale yellow, but the plasma was slightly icteric. By the 7th day the plasma was clear. Throughout the entire episode the animal ate all of its diet. In the 21st period 30 per cent of one day's diet was vomited for no apparent reason. The dog was mildly upset in the first days of the 23rd period: ate all of its food but with less speed, and had slight diarrhea, probably slight edema. The latter could be demonstrated only by firm pressure of the tissue about the Achilles tendons. The plasma protein concentration on the last day of the 22nd period

reached the low point for the entire course of this experiment of 3.56 gm. per 100 cc.

For production of acute *sterile inflammation*, turpentine, 0.8 cc. was injected subcutaneously on the left side on the last day of the 25th period. The following day the rectal temperature was 39.7°C. and the leucocyte count 42,200. The next day the temperature was 37.4°C. but the leucocytosis 62,800. The following day (3rd of the 26th period) a fluctuant *abscess* was present on the left side, and turpentine 0.8 cc. was injected on the right. Next day temperature was 39.4°C. and leucocyte count 40,600. Both abscesses were drained 3 days later (7th day of 26th period), the left yielding 35 cc. and the right 100 cc. of blood-stained pus. Leucocytosis persisted (to 60,000) in the first of the following (27th period) but had dropped to 13,200 by the end of this period. The right abscess cavity drained another 45 cc. pus during this time. Pitting edema of moderate degree appeared in neck and legs at the beginning of this 27th period, despite the high plasma protein concentration (note low A/G ratio). The edema gradually disappeared and was scarcely perceptible in the 28th period.

Tables 4 and 4-*a* show that the reserve of plasma protein building material was depleted by one week of fasting plus 2 weeks of depletion on the liver basal diet. This reserve protein store in this dog amounted to 25 gm. plus the amount used from this store during the fasting week. About 7.0 gm. is accounted for by the drop in plasma protein concentration from 5.25 to 4.02 gm. per cent. The dog gained weight on this liver basal in spite of a week's fast and 2 weeks of protein-free diet.

Protein-free diet (periods 6 and 7) gives valuable information and shows clearly that when the protein reserve store is exhausted the dog can produce very little plasma protein indeed. The first protein-free week (period 6) shows an output of 8.4 gm. as compared with 3.4 gm. protein output for the 2nd week (period 7). This *surplus* of 5 gm. plasma protein in period 6 probably relates to a "carry over" from the preceding week of liver basal diet and represents materials from that diet in "process of production" from the food amino acids to the finished product (plasma protein). During the 2nd week of protein-free diet the dog could furnish to the plasma only 3.4 gm. protein. The plasma protein level was kept very constant and there was the usual fasting *shrinkage* of the blood *plasma volume* which would account for 1.4 gm. of the plasma protein removed. This brings the actual protein produced in period 7 to a mere 2 gm. which indicates how difficult the body finds its task of producing plasma protein when

all reserves are exhausted and only traces of "protein" are coming in through the digestive tract.

On the first day of the 8th period (Table 4) 24 hours after the resumption of the liver basal the circulating plasma protein concentration had jumped from 4.06 per cent, on the last day of the 7th period,

TABLE 4

Blood Plasma Protein Production as Influenced by Protein-Free Periods and by Interruption of Plasmapheresis

Dog 36-95.

Period 7 days	Diet	Protein intake Total for 7 days	Plasma protein removed Total for 7 days	Protein removed above basal*	Blood plasma Average concentration		R.B.C. hema-tocrit, average	Plasma volume
					Total protein	A/G ratio		
		gm.	*gm.*	*gm.*	*per cent*		*per cent*	*cc.*
	Kennel				5.34		47.4	603
1	Dextrose, 350 gm.	0	0.8		5.25	1.4	50.5	486
2	Liver basal	111	38.6		4.63	1.3	51.8	524
3	Liver basal	111	25.1	24.9	4.04	1.2	49.6	546
4	Liver basal	111	20.3		4.02	1.1	48.6	507
5†	Liver basal	111	20.1		4.04	1.1	48.8	533
6‡	Protein-free	4	8.4		4.03	1.1	51.4	526
7	Protein-free	4	3.4		4.00	1.0	50.8	489
8	Liver basal	111	25.1		4.22	0.9	49.0	526
9§	Liver basal	111	3.9		4.45	1.0	46.3	524
10§	Liver basal	111	4.0		4.56	1.2	45.3	570
11	Liver basal	111	28.4		4.33	1.1	50.1	546
12	Liver basal	111	31.8		4.26	0.9	51.8	565
13	Liver basal	111	29.8	−2.1	4.12	0.9	50.2	567

* Estimated basal output on liver diet = 20 gm. plasma protein per week.

† 9 day period recalculated on basis of 7 days.

‡ 5 day period recalculated on basis of 7 days.

§ No plasmapheresis.

to 4.45 per cent, a quick response to the ingestion of protein. Plasmapheresis was over-vigorously employed so that by the first day of the 9th period the circulating protein level had been reduced to 3.87 per cent. This explains in part at least the slightly greater plasma protein output in period 8.

Plasmapheresis was *interrupted* completely during periods 9 and 10

(Table 4). The plasma protein removed represents plasma with-drawal associated with the necessary sampling and blood volume determinations.   During the 5 weeks (periods 9 to 13) in Tables 4 and 4-*a* the basal diet was continued and the *expected* plasma protein out-put was 100 gm.   *Actually* during the last 3 weeks 97.9 gm. were re-

TABLE 4-*a*

Weight and Nitrogen Balance

Dog 36-95.

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	13.1						
1	Dextrose, 350 gm.	11.7	0.0	−1.0	0.1	12.8	0.7	−14.6
2	Liver basal	11.8	17.8	0.4	6.3	9.7	8.3*	−6.1
3	Liver basal	11.8	17.8	1.0	4.1	12.6	4.2	−2.1
4	Liver basal	12.2	17.8	1.0	3.4	11.1	3.8	0.5
5†	Liver basal	12.2	22.9	3.3	4.3	15.3	4.2	2.4
6‡	Protein-free	12.4	1.2	0.0	1.2	5.3	1.0	−6.3
7	Protein-free	12.2	1.6	−0.7	0.6	6.4	1.1	−7.2
8	Liver basal	12.3	17.8	1.4	4.1	10.5	2.9	1.7
9§	Liver basal	12.8	17.8	−2.2	0.6	8.7	3.2	3.1
10§	Liver basal	13.2	17.8	−0.4	0.6	9.1	3.2	4.5
11	Liver basal	13.5	17.8	6.1	4.5	10.0	2.6	6.8
12	Liver basal	13.3	17.8	0.5	5.2	12.7	4.0	−3.6
13	Liver basal	13.6	17.8	2.6	5.0	12.9	2.5	0.0
Totals...........................			185.9	12.0	40.0	137.1	41.7	−20.9

* Includes some urinary nitrogen.
† 9 day period.
‡ 5 day period.
§ No plasmapheresis.

moved to return the dog to its true depletion level.   This indicates clearly that during the 2 weeks of no plasmapheresis the dog stored 32 gm. plasma protein building material, while the plasma protein concentration did not rise above 4.75 per cent.   Subsequently the dog withdrew from this store all of the material which was contributed to

the circulating plasma and removed in the last 3 weeks of plasma-pheresis (periods 11 to 13). The maximum concentration of plasma protein during the period of no plasmapheresis was attained within 4 days after cessation of plasmapheresis and ample storage took place at or below this level. All of this emphasizes the ease with which the dog can shift protein from the circulating plasma to organs or tissues and subsequently reverse the process. More data on this mechanism have just been published (2, 4).

Clinical Experimental History.—Dog 36-95 (Tables 4 and 4-*a*). An adult male mongrel weighing 13.1 kg. was fasted for a week except for daily administration of 50 gm. dextrose by stomach tube. While completely consuming a daily diet of liver, 75 gm.; cane sugar, 50 gm.; corn starch, 50 gm.; lard, 25 gm.; cod liver oil, 25 gm.; canned tomatoes, 75 gm.; bone ash, 20 gm.; salt mixture, 1 gm.; kaolin, 15 gm., the *protein stores* were depleted by plasmapheresis. This diet was replaced in the 6th period by a "protein-free" diet (see Methods), calculated for a 12.2 kilo dog, and having the same caloric value, 960 calories, as the one described above. It contained cane sugar, 107.3 gm.; dextrin, 35.4 gm.; lard, 32.5 gm.; butter, 12.7 gm.; bone ash, 4.6 gm.; salt mixture, 2.3 gm.; and had the vitamin supplements of 4 tablets cod liver oil concentrate and 8.5 cc. rice polish-ings-liver extract suspension. This diet was readily consumed and retained, except for the vomiting of about 20 per cent of one day's intake in the 7th period. From the 8th through the 13th periods the liver diet described above was con-sumed completely. No plasmapheresis was performed during the 9th and 10th periods except for daily sampling for analysis and the return of washed red cells on one occasion in each period to maintain the hematocrit, 36 cc. and 60 cc. respectively.

DISCUSSION

Interesting experiments have been reported by Melnick and Cowgill (8, 9) which are obviously related to the problems involved in the experiments tabulated in this paper. A detailed discussion of the differences between their work and ours would not seem appropriate in this place. There are many differences in methods, experimental procedure, and research approach which are responsible for divergent opinions. We have no quarrel with divergent opinions as they usually stimulate work which eventually uncovers the truth.

Their belief (8) that a *single week* of rapid plasma depletion will exhaust the reserve store of plasma protein building material, we cannot share. Tables above and in other papers indicate our belief

that 2 to 6 weeks are required to exhaust this plasma protein reserve store and that dogs differ in this respect. These tables demonstrate that a plasma protein concentration of 4 per cent is usually reached and maintained for 1 to 4 weeks prior to complete exhaustion of the reserve store and is therefore no indicator of the exhaustion of this store. If this protein reserve is not exhausted the subsequent picture is confused as it relates to new protein production. We also believe that when the reserve protein store has been completely exhausted by plasmapheresis and continued low protein diet the dog can produce *very little new plasma protein* (2 to 4 gm. a week) on a "protein-free" diet. This small amount of new formed protein *may* be related to the wear and tear of tissue or organ protein and subsequent conservation of end products.

SUMMARY

When blood plasma proteins are depleted by bleeding, with return of the washed red 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 with normal appetite, no anemia and normal nitrogen metabolism. These dogs become test subjects by which various factors relating to plasma protein production may be tested.

The normal dog (10 to 13 kg.) has a substantial reserve store of plasma protein building material (10 to 60+ gm.) which requires 2 to 6 weeks plasmapheresis for its complete removal. After this period the dog will produce uniform amounts of plasma protein each week on a fixed basal diet.

Dogs previously depleted by plasmapheresis and then permitted to return to normal during a long rest period of many weeks, may show much higher reserve stores of protein building material in subsequent periods of plasma depletion (see Table 1).

Under uniform conditions of low protein diet intake when plasmapheresis is discontinued for 2 weeks the plasma protein building material is *stored* quantitatively in the body and can subsequently be recovered (Table 4) in the next 2 to 3 weeks of plasmapheresis.

Given *complete* depletion of plasma protein building reserve stores the dog can produce very little (2± gm. per week) plasma protein on

a protein-free diet.   This *may* be related to the wear and tear of body protein and conservation of these split products.

Abscesses produced in a depleted dog during a fast *may* cause some excess production of plasma protein which is probably related to products of tissue destruction conserved for protein anabolism.

Gelatin alone added to the basal diet causes very little plasma protein production but when supplemented by *tryptophane* gives a large protein output, while tryptophane alone is inert.

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