PLASMA PROTEIN PRODUCTION AS INFLUENCED BY PARENTERAL PROTEIN DIGESTS, VERY HIGH PROTEIN FEEDING, AND RED BLOOD CELL CATABOLISM*

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The main objective of these experiments has been substitution for plasma. It is obvious that the supply of human plasma is not unlimited and will become less and less the further we get away from the acute war emergency. The need for plasma or plasma substitutes on the contrary will increase as their usefulness is better understood. The perfect plasma substitute may never be found but an approximation to perfection is to be anticipated. For perfection the plasma substitute should furnish materials out of which the body can build its own plasma proteins and in this respect certain protein digests do qualify.

The plasma-depleted dog properly standardized is an admirable test subject for such experiments and shows promptly whether any given digest will support plasma protein production. It is noted below that some digests are quite inert and pass out of the body without utilization. Other digests are as well used as good protein by mouth—that is, a return of 15 to 25 per cent of the injected nitrogen as new plasma protein nitrogen. The digests tested below are either not available commercially or have been modified for improvement.

Methods

The experimental procedure used has been described (4, 7). In the dog standardized by plasmapheresis during diet control, there were 5 protein digests tested.

Casein digest L (Lilly 36092) is a papain digest of commercial casein, 12.5 per cent nitrogen dry weight. Parenterally it was given as a 5 per cent solution sometimes in 5 per cent dextrose as stated in the experimental history, and was sterilized by Seitz filtration. Casein digest solution (Lilly 37767) is a similar papain digest, prepared by the manufacturer in 10 per cent solution, autoclaved, and filtered, 1.43 per cent nitrogen.

Serum digest (Lilly) is a papain digest of fresh beef serum, 12.9 per cent nitrogen dry weight. Intravenously it was given as a 5 per cent solution in distilled water. Serum digest solution (Lilly P 37766) is a specially prepared 10 per cent solution of serum digest, 1.43 per cent nitrogen. Another lot of serum digest solution (P 39114) contains 1.14 gm. nitrogen per 100 cc.

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Casein digest S (Stearns) is an acid hydrolysate of casein fortified with tryptophane, in 15 per cent solution, 2.2 per cent nitrogen, was given diluted with physiological saline to a 5 per cent solution.

Casein digest MJ (Mead Johnson) is an enzymatic digest of casein and pork pancreas, 12 per cent nitrogen dry weight. It was given as a 5 per cent solution in physiological saline.

TABLE 1

Plasma Protein Production from Protein Digests Given Parenterally

Catabolism of Red Blood Cells Enhances Plasma Protein Production

Dog 40–122

Period 7 days	Diet	Protein intake Total for 7 days	Plasma protein removed Total for 7 days	Blood plasma Average concentration		R.B.C. hemato-crit average	Weight
				Total protein	A/G ratio		
		gm.	*gm.*	*per cent*		*per cent*	*kg.*
	Kennel			6.00	1.40	55.0	10.8
1	Fasting	0	19.6	4.95	1.21	51.7	9.8
2	Low protein	15	13.6	3.99	1.07	47.3	9.4
3	Casein digest L*	97	12.2	3.66	0.84	46.0	9.5
4	Liver	85	13.9	4.20	0.77	43.6	9.9
5	Liver	85	15.8	3.95	0.81	43.5	9.7
6	Casein digest L*	95	11.3	3.82	0.73	45.8	9.9
7	Digest L, s.c.	97	16.9	4.08	0.62	45.2	10.3
8	Casein digest L*	97	18.3	4.06	0.56	43.9	9.9
9	Casein digest L,* 2 days; serum digest,* 5 days	99	13.2	3.83	0.66	44.9	10.0
10	Serum digest*	100	7.8	3.86	0.68	44.3	10.0
11	Serum digest*	100	11.5	4.01	0.57	43.0	9.9
12	Serum digest	100	19.1	4.00	0.72	42.6	9.8
13	Casein digest L*	97	11.7	3.83	0.70	41.4	9.9
14	Digest L + excess R.B.C.*	97	14.4	3.80	0.56	45.5	10.2
15	Casein digest L*	97	16.8	3.94	0.57	51.1	10.3
16	Casein digest L*	95	19.0	3.92	0.56	46.5	10.0
17	Casein digest L*	95	14.0	3.86	0.54	42.8	—
18	Casein digest L	88	19.2	4.05	0.54	42.4	10.4
19	Casein digest solution*	109	11.7	3.88	0.56	43.6	10.4
20	Casein digest solution*	108	10.9	3.74	0.56	44.1	10.2
21	Casein digest solution, i.p.	107	13.1	3.97	0.40	47.5	10.4
22	Serum digest solution*	107	10.3	3.77	0.41	47.7	10.4

* Given intravenously.

Glycinin digest (Cutter) is a mixed acid and alkali hydrolysate of glycinin from soy bean, in 5 per cent solution, 0.7 per cent nitrogen.

EXPERIMENTAL OBSERVATIONS

Tables 1 and 1-*a*, 2 and 2-*a* represent large portions of a continuous 64 week study of plasma protein production in dog 40-122. The data for periods 23 to 35 and 56 to 61 have been reported in a paper concerned with amino acids and

plasma protein production (2). About 15 gm. of the protein intake per week as listed in the intake column is a credit to the nitrogen of the vitamin sources (see experimental history). We believe this nitrogen contributes little if any to

TABLE 1-*a*

Nitrogen Balance Maintained by Protein Digests Given Parenterally

Dog 40-122

Period 7 days	Diet	Nitrogen balance							
		Intake		Output					Intake minus output
		in diet	in excess R.B.C. injected	in plasma	in feces	in urine			
						Total	Urea + NH_3	Undetermined	
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*
	Kennel								
1	Fasting	0.0	−1.0	3.2	*	9.9	83.0	0.8	−14.1
2	Low protein	2.4	1.0	2.2	3.1	14.3	73.7	3.8	−16.2
3	Casein digest L‡	15.5	4.0	2.0	3.1	13.7	63.7	5.0	+0.7
4	Liver	13.6	2.3	2.3	3.1	8.2	65.9	2.8	+2.3
5	Liver	13.6	4.3	2.6	2.8	8.5	64.4	3.0	+4.0
6	Casein digest L‡	15.2	2.4	1.8	2.5	13.4	62.6	5.0	−0.1
7	Digest L, s.c.	15.5	5.6	2.7	2.8	13.2	66.7	4.4	+2.4
8	Casein digest L‡	15.5	4.9	3.0	2.5	15.7	67.0	5.2	−0.8
9	Casein digest L,‡ 2 days; serum digest,‡ 5 days	15.8	1.3	2.2	2.3	13.9	65.1	4.8	−1.3
10	Serum digest‡	15.9	1.6	1.3	2.4	15.2	65.7	5.2	−1.4
11	Serum digest‡	15.9	1.9	1.9	3.1	15.9	65.3	5.5	−3.1
12	Serum digest	15.5	3.6	3.1	3.6	10.6	75.5	2.6	+1.8
13	Casein digest‡	15.5	0.9	1.9	3.6	14.2	63.5	5.2	−3.3
14	Digest L + excess R.B.C.‡	15.5	16.2	2.4	3.0	14.1	61.4	5.4	+12.2
15	Casein digest L‡	15.5	0.2	2.7	2.8	15.6	64.0	5.6	−5.4
16	Casein digest L‡	15.2	3.1	3.1	2.3	15.8	68.3	5.0	−2.9
17	Casein digest L‡	15.2	2.8	2.3	2.1	13.3	60.2	5.3	+0.3
18	Casein digest L	14.0	5.5	3.1	4.0	12.3	80.7	2.4	+0.1
19	Casein digest solution‡	17.3	0.8	1.9	2.0	16.0	63.9	5.8	−1.8
20	Casein digest solution‡	17.2	5.7	1.8	2.7	15.9	60.3	6.3	+2.5
21	Casein digest solution, i.p.	17.0	4.1	2.1	2.4	16.1	61.7	6.2	+0.5
22	Serum digest solution‡	17.1	−1.0	1.7	2.5	17.3	66.5	5.8	−5.4
	Totals.....	313.9	70.2	51.3	58.7	303.1			−29.0

* Included in following period.

‡ Given intravenously.

plasma production and it is disregarded in considering the effect of the test material.

Tests of 2 digests of casein and 2 digests of beef serum are given in Table 1. Of added interest is the effect of the catabolism of a large excess of red blood cells (periods 14 to 17).

Casein digest L by vein produced in this dog (periods 3, 6, 13, 17) about 15 gm. plasma protein per 100 gm. (digest nitrogen X6.25) injected, a little less than in previous tests in other dogs (7). For comparison the liver tested in periods 4 and 5 favored the production of about 21 gm. per 100 gm. liver protein fed. The digest was very well used when fed in period 18 (25 gm. plasma protein produced per 100 gm. digest fed). In previous tests utilization of this digest for plasma production has been equally good by vein or by mouth (7).

Of some interest is the reduction in the undetermined nitrogen fraction with oral feeding evident in periods 4, 5, 12, and 18, Table 1-*a*. Total urinary nitrogen is also less but fecal nitrogen is increased. This better conservation of oral nitrogen has been found previously (7).

Digest L by subcutaneous route (period 7) provided more plasma protein production than by vein and the effect lasted into the following week. Edema of the leg and abdomen appeared at the close of the week and promptly disappeared with resumption of intravenous injections. Slower absorption of amino nitrogen might account for the better utilization.

Casein digest solution while better tolerated (periods 19 and 20) was slightly less potent than digest L. It was a little better used upon intraperitoneal injection (period 21).

Serum digest (periods 10 to 12) was a little better tolerated by vein than digest L but not so well used. By mouth it was about equal to the casein material.

Serum digest solution produced allergic reaction and was poorly used both for plasma production and nitrogen balance.

Measurement of the effect of return of a *large excess of red blood cells* was attempted in periods 14 to 17. A total of 254 cc. packed washed red cells (nitrogen content 16.2 gm.) was returned during the plasmaphereses of period 14 over and above the volume of red cells removed. This quantity was at least half the total mass of cells in the circulation of this dog. The hematocrit was raised from 40.2 per cent at the beginning of period 14 to 50.8 per cent at the end. It remained high during period 15, then fell during period 16 from 51.3 to 41.7 per cent despite the giving of 49 cc. excess cells. This fall coincides with a considerable excess production of plasma protein.

If one expects about 12 gm. plasma protein per period from an intake of 82 gm. casein digest L then the excess of plasma protein of 16.2 gm. during periods 14 to 17 is great enough to be at least partially attributable to red blood cell catabolism. If the recapture is as great as the figures indicate, that is 16.2 gm. red blood cell nitrogen accounting for 2.6 gm. plasma protein nitrogen, then the nitrogen is as well utilized as that of most proteins—16 per cent return. Another test (3) has shown no measurable supplementation by the products of hemoglobin catabolism but the quantities involved were rather small. Plasma protein may be produced during feeding of hemoglobin digests (1) but usually there is simultaneous loss of weight. A recent metabolism study (9) shows that

dog hemoglobin given intraperitoneally in a protein-fasting dog will be used effectively to supply the protein requirements of the body. Under proper conditions nitrogen balance may be maintained for 20 days.

Throughout the 22 weeks weight was well maintained, and, discounting the initial 2 protein depletion periods, nitrogen equilibrium was obtained.

Tables 2 and 2-*a* present tests of additional protein digests and an attempt to determine the maximal capacity of the dog to produce plasma protein.

Period 36 followed some strenuous experiments with amino acid feeding. On liver and salmon feeding in periods 38 and 39, the dog further recuperated and gained weight.

The *highest production of plasma protein* we have ever measured occurred in periods 40 and 41. The actual peak was not measured even in this experiment. More protein was being produced than could be removed in 2 plasmaphereses daily. In period 41 about 0.9 gm./kilo/day was removed and still the dog had a large positive nitrogen balance of 17 gm., had gained weight, and the plasma protein level was high. The dog can make, it appears, at least 1 gm. plasma protein/kilo/day when amply supplied with building material. This rate, however, cannot be reached in a day. The feeding today of 75 gm. of protein to a 10 kilo plasma-depleted dog will not permit removal tomorrow of 10 gm. new plasma protein. A few days are required before maximum production is reached. Perhaps the lag is as little as 2 to 3 days. Protein consumed in periods 41 and earlier continued to contribute to the production of periods 42 and 43, the familiar carry-over or lag in nitrogen metabolism upon change in its level.

Casein digest S (periods 45 to 47) was apparently an inadequate or even an inhibiting material for plasma protein production. Most likely it was deficient in an essential amino acid. Cysteine in period 47 did not change the response. The high urinary nitrogen excretion reflects the deficient quality of the nitrogen intake.

Casein digest MJ (periods 48 to 51) brought return of plasma protein production but was not adequate to maintain weight. The nitrogen from the excess red blood cells injected was large in periods 42 and 49. If recapture of some of it contributed to plasma protein production in periods 50 and 51 as appears to be true in periods 15 and 16, Table 1, then the utilization of digest MJ is definitely poorer than of digest L. It should be pointed out, however, that the vitamin intake was probably deficient from period 44 on (1). This deficiency in unidentified factors may contribute to poor utilization of protein. Cysteine appears to have been of no additional value in period 51 when given with digest MJ.

Glycinin digest (period 53) appears to be useless for nitrogen metabolism. The decline in plasma protein level from 4.63 to 3.31 accounts for the apparent production of the 7.6 gm. plasma protein removed. The excess urinary

nitrogen suggests virtually complete excretion of the glycinin nitrogen. If no protein had been given in period 53, the urinary nitrogen excretion would have

TABLE 2

Plasma Protein Production Related to Protein Digests

Maximal Production of Plasma Proteins

Dog 40-122

Period 7 days	Diet	Protein intake Total for 7 days	Plasma protein removed Total for 7 days	Blood plasma Average concentration		R.B.C. hemato-crit average	Weight
				Total protein	A/G ratio		
		gm.	*gm.*	*per cent*		*per cent*	*kg.*
36	Liver	56	14.4	4.04	0.62	40.7	9.2
37	Liver-salmon	162	25.5	4.30	0.70	40.5	9.9
38	Liver-salmon	194	32.8	4.43	0.71	43.8	10.2
39	Liver-salmon	203	33.9	4.37	0.72	47.4	—
40	Liver-salmon-beef	519	55.3	4.47	1.20	44.6	11.8
41	Liver-salmon-beef	519	68.5	4.43	1.08	41.0	11.4
42	Liver	70	29.3	4.18	0.90	50.2	10.1
43	Liver	69	19.6	4.30	0.78	56.6	10.9
44	Protein-free	0	9.8	4.05	0.65	48.8	10.5
45	Casein digest S*	97	8.4	3.92	0.71	43.5	10.6
46	Casein digest S*	97	1.4	3.62	0.71	40.9	10.0
47	Digest S + cysteine*	97	1.9	3.61	0.80	38.5	10.4
48	Casein digest MJ*	79	1.6	3.77	0.81	42.0	10.0
49	Casein digest MJ*	132	10.3	4.05	0.74	52.6‡	9.3
50	Digest MJ + cysteine*	113	9.9	4.11	0.69	46.0‡	9.9
51	Digest MJ + cysteine*	129	13.5	3.96	0.80	47.3	9.5
52	Protein-free	0	4.3	4.63‡	0.67	46.0	9.1
53	Glycinin digest*	112	7.6	3.31‡	0.71	41.5	8.9
54	Glycinin digest,* 2 days; casein digest L,* 5 days	90	4.9	4.35‡	0.65	43.9	8.5
55	Casein digest L*	82	9.1	3.99	0.57	44.6	8.5
62	Serum digest solution*	149	5.1	3.85	0.76	48.4	8.4
63	Serum digest solution*	149	4.0	3.85	0.52	42.2	8.2
64	Serum digest solution + methionine + tryptophane*	149	15.1	4.01	0.50	44.5	8.0

* Given intravenously.

‡ Level at end of period.

reached a low figure probably little greater than the difference between the actual output of 23.4 gm. and the actual intake of 19.6 gm. With casein digest L (periods 54 and 55) recovery in protein production and utilization began.

Serum digest solution (periods 62 to 64) was ineffective until supplemented with methionine and tryptophane. Even this plasma protein production of

TABLE 2-*a*

Positive and Negative Nitrogen Balances with Various Protein Digests Given Parenterally and Protein Feeding

Dog 40-122

Period 7 days	Diet	Nitrogen balance							
		Intake		Output					Intake minus output
		in diet	in excess R.B.C. injected	in plasma	in feces	in urine			
						Total	Urea + NH_3	Undetermined	
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*
36	Liver	9.0	−0.2	2.3	4.0	7.5	70.6	2.2	−5.0
37	Liver-salmon	26.0	5.1	4.2	4.0	18.1	75.3	4.5	+4.8
38	Liver-salmon	31.0	5.4	5.3	3.7	23.2	85.8	3.3	+4.2
39	Liver-salmon	32.5	3.9	5.6	4.1	21.0	75.1	5.2	+5.7
40	Liver-salmon-beef	83.1	5.0	9.1	7.6	44.8	81.7	8.1	+26.6
41	Liver-salmon-beef	83.1	4.7	11.3	6.5	52.3	84.5	8.1	+17.7
42	Liver	11.2	16.1	4.8	2.9	18.3	79.5	3.7	+1.3
43	Liver	11.0	2.5	3.2	2.6	15.0	77.6	3.4	−7.3
44	Protein free	0.1	−0.3	1.6	1.7	10.3	78.9	2.2	−13.8
45	Casein digest S*	15.4	−1.5	1.4	1.6	17.9	74.8	4.5	−7.0
46	Casein digest S*	15.4	−1.5	0.3	1.4	15.8	76.5	3.7	−3.6
47	Digest S + cysteine*	16.0	2.4	0.3	1.5	17.3	70.4	5.1	−0.7
48	Casein digest MJ*	12.6	−1.8	0.3	0.7	10.3	62.5	3.9	−1.5
49	Casein digest MJ*	21.0	13.1	1.7	1.2	17.4	64.6	6.2	+13.8
50	Digest MJ + cysteine*	18.4	−5.3	1.6	2.1	13.6	71.7	3.8	−4.2
51	Digest MJ + cysteine*	21.5	1.7	2.2	2.0	17.3	71.3	5.0	+1.7
52	Protein free	0.1	−0.7	0.7	2.0	6.1	74.3	1.6	−9.4
53	Glycinin digest*	19.6	2.0	1.2	2.0	23.4	68.7	7.3	−5.0
54	Glycinin digest,* 2 days casein digest L,* 5 days	15.0	3.1	0.8	2.2	15.6	67.3	5.1	−0.5
55	Casein digest L*	13.1	−0.8	1.5	2.2	10.3	60.5	4.1	−1.7
	Totals	455.1	52.9	59.4	56.0	375.5			+16.1
62	Serum digest solution*	24.1	−1.0	0.8	3.0	25.9	66.0	8.8	−6.6
63	Serum digest solution*	24.1	1.3	0.7	1.9	22.6	69.3	5.9	+0.2
64	Serum digest solution + methionine + tryptophane*	25.6	6.2	2.5	2.0	24.1	70.8	7.0	+3.2
	Totals	73.8	6.5	4.0	6.9	72.6			−3.2

* Given intravenously.

10 per cent of the protein intake in period 64 is mediocre. There was an allergic reaction to this digest in period 62 as was also found with a different lot in period 22, Table 1, but definite improvement in the condition of the dog occurred (see experimental history).

Experimental History—Tables 1 and 1-*a*, 2 and 2-*a*.

Dog 40-122, an adult female beagle hound, was the subject of plasma protein production studies for 64 consecutive weekly periods, a portion of which has been reported (2).

Period 1 of complete fasting was followed by period 2 with feeding of a diet without protein except for that in the vitamin supplements. It contained in grams sucrose 115, cornstarch 20, corn oil 15, Crisco 15, cod liver oil 5, salt mixture 5, bone ash 10, yeast powder 2, liver powder 2, thiamin chloride 0.005, nicotinic acid 0.05, choline chloride 0.4. The yeast, liver, and salt mixture are those used previously (7).

In period 3, casein digest L 15 gm. in 300 cc. solution was given in 1 injection daily, usually at a rate between 2 and 3 mg. nitrogen/kilo/minute. Vomiting occurred with 2 of the injections. The basal diet of period 2 minus 15 gm. sucrose was fed daily.

In periods 4 and 5 raw pork liver 50 gm. daily replaced the digest of period 3.

Periods 6 to 8 provided digest L again. Because vomiting occurred during 4 of the first 7 injections, the next 7 days' injections were given subcutaneously. There was no vomiting but edema of the legs and abdominal wall appeared on the last day so that intravenous infusions were resumed for period 8, with vomiting on 4 days. For periods 6 through 22 the basal diet was given as in period 2 minus sucrose 30 gm.

Periods 9 to 12 serum digest 15 gm. daily was given without vomiting.

Period 13 casein digest induced vomiting 6 of the 7 days.

Periods 14 to 17 vomiting still occurred frequently during digest injections.

Periods 19 and 20 passed with vomiting during only 1 of 14 rapid injections, 4 to 5 mg. nitrogen/kilo/minute.

In period 21 the digest solution was given *intraperitoneally* without obvious reaction although the dog became apprehensive of injection by the end of the week. Each day's total of 150 cc. was given in about 10 minutes.

Period 22 of serum digest solution (P 37766) provided sharp vomiting reactions when given as rapidly as the serum digest of periods 9 to 12 and on the 7th day an urticarial reaction developed during the injection. Erythema, edema, and urticaria disappeared within a few hours.

Periods 23 to 35 have been reported (2).

Period 36 provided some recuperation from strenuous experiments, during which skin ulcers and weight loss occurred. The liver diet was eaten readily but vomiting occurred frequently. The basal diet contained in grams sucrose 85, cornstarch 20, corn oil 20, Crisco 15, salt mixture 5, bone ash 10, and vitamins A 5000 U.S.P. units, D 500 U.S.P. units, and in milligrams thiamin chloride 3, riboflavin 2, pyridoxine hydrochloride 1, calcium pantothenate 1, nicotinamide 20, ascorbic acid 50, natural tocopherols 50, choline chloride 100, 2-methyl-1,4-naphthaquinone 1, rice polish concentrate 500. This basal diet was continued through period 47.

In period 37 salmon 100 gm. daily was added to the liver 50 gm. but vomiting and some diarrhea continued. In period 38 this subsided.

Periods 40 and 41 liver 50 gm., salmon 100 gm., and lean beef 200 gm. were fed, and plasmapheresis was performed twice daily about 5 hours apart.

Periods 45 to 47 casein digest S produced vomiting during every injection except one. Injection time was 150 minutes for the first 5 injections (1.4 mg. nitrogen/kilo/minute) with vomiting 3 or 4 times during each. When reduced to 60 and then 45 minutes (4.6 mg. nitrogen/kilo/minute) vomiting usually occurred only once during an injection. *l*(—)-Cysteine·HCl, 1 gm., was added to the daily injection of period 47.

Period 48 casein digest MJ, 15 gm., produced no vomiting at the daily rate of 4 mg. nitrogen/kilo/minute. The basal diet was changed (periods 48 to 64) to the basal cake (7) for calories and salts, but the vitamin supplements remained as in periods 36 to 47, except for addition of choline chloride 300 mg. during periods 58 to 64.

Periods 49 to 51 had casein digest MJ increased to 25 gm. daily and vomiting occurred during 5 of 20 injections at rates less than 3 mg. nitrogen/kilo/minute. No digest was given on the middle day of period 50 and (—)-cysteine·HCl, 1.5 gm., was added to the daily injection thereafter, also with the addition of 0.8 gm. sodium bicarbonate.

In period 53 the glycinin digest was given 2.5 mg. nitrogen/kilo/minute, always with vomiting. It was continued for 2 days in period 54 but because the condition appeared poor, casein digest L replaced it for the remaining 5 days and all of period 55. Digest L was given at the same rate as the glycinin with vomiting on only 1 occasion when the rate was increased.

Periods 56 to 61 have been reported (2).

Periods 62 to 64 serum digest solution (P 39114) produced immediate allergic reaction of urticaria as occurred in period 22. Vomiting also occurred but after 3 days the allergic and vomiting reactions subsided although vomiting occurred twice again in period 64. Injections were done at about 5 mg. nitrogen/kilo/minute. In period 64 *dl*-methionine 1.5 gm. and *l*(—)-tryptophane 0.5 gm. were added to the digest solution. Skin lesions which appeared during periods 56 to 61 (2) improved considerably.

DISCUSSION

Protein digests as prepared to date do not have the freedom from clinical disturbances upon intravenous injection that most physicians desire. We have shown that the glutamic acid content of digests is responsible for most of the disturbance characterized by vomiting (6). The aspartic acid content may also be responsible for vomiting (1). Protein digests given by vein, subcutaneously, or intraperitoneally, may result in nitrogen balance, weight gain, and production of much new plasma protein and hemoglobin, if the digests can be given in large enough quantity with freedom from obvious disturbance. In our hands mixtures of pure amino acids can be given parenterally with less disturbance and good physiological response—in other words a better product (5). Moreover we may learn to use various mixtures of the pure amino acids which can be modified according to body needs. The demands of the body resulting from a burn may be somewhat different from those following prolonged gastrointestinal disease or injury—tissue suppuration, bone fractures, prematurity, and many other abnormal states.

The normal dog has a very efficient mechanism for the production of new plasma protein when hypoproteinemia demands it and adequate protein intake is available. The plasma production is so rapid that plasmapheresis cannot keep down the level to 4 per cent even when performed twice daily. Table 2 shows a maximal output of 68.5 gm. per week in period 41 which is about 1 gm. per kilo per day or about one-half of the total circulating plasma protein of a plasma-depleted dog produced each day. This observation shows how jealously the body guards its level of functioning plasma protein concentration, and suggests the continuing use of plasma proteins. In earlier experiments similar observations (10) were recorded in dogs, to show a ceiling of 65 to 75 gm. plasma protein production per week due to similar diets rich in animal protein.

Albumin-globulin ratios are higher on animal protein diets. We have noted

(8) that vegetable and grain proteins favor a lower ratio and it was suggested that the animal proteins favored albumin production (see Table 2). Digests whether obtained from casein or serum when given over many weeks in the amounts indicated favor a low-albumin-globulin ratio. When we see the ratio go to 0.3 we have learned that the dog is in a dangerous state and usually the change is made to an animal protein diet. The reasons back of these changes are not understood, but vomiting and slight physical disturbance may have been concerned.

Whether hemoglobin can contribute to the production of new plasma protein has been debated. In one paper (3) it was concluded that the evidence was against this hypothesis but the amounts of hemoglobin given were rather small. Table 1, periods 14 to 17, may be used to support the thesis. It appears that 254 cc. red cells (16.2 gm. nitrogen) did increase the output of new plasma protein by about 16 gm. (2.6 gm. nitrogen). A simpler type of experiment in which hemoglobin in considerable amounts is given intraperitoneally, gives a definite response (9). There is nitrogen and weight balance for at least 20 days during which time the hemoglobin contributes effectively to the protein needs of the body during protein fasting. We assume that plasma proteins must participate in these needs and in hemoglobin utilization. Finally in dogs both anemic and hypoproteinemic (9) where there is acute need for both hemoglobin and plasma protein production the dog uses abundant hemoglobin given intraperitoneally in the formation of both hemoglobin and plasma protein. It would seem that under certain conditions hemoglobin may contribute effectively to body protein needs including the formation of much new plasma protein. We have used the term *body protein pool* and believe that hemoglobin contributes to this pool from which various proteins are produced to meet body requirements.

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 constant level of plasma protein production if the diet nitrogen is controlled and limited. Such dogs are outwardly normal but have a lowered resistance to infection and to certain intoxications.

Certain protein digests given by vein may favor good production of plasma protein, as well as nitrogen and weight equilibrium, over long periods in these standardized dogs. These digests may be equally effective when given subcutaneously or intraperitoneally and more effective orally (one dog). Certain other digests may not be well utilized.

The total nitrogen of the protein digests is better retained upon oral feeding than parenteral injection. Most of the excess nitrogen excretion is not in the urea and ammonia fraction of the urine.

The rate of plasma protein production may reach as high as 1 gm./kilo/day in the dog when ample protein of good quality is fed.

The products of catabolism of red blood cells *in vivo* may add to the production of plasma protein, at least during the administration of casein digest by vein.

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