CASEIN DIGESTS PARENTERALLY UTILIZED TO FORM BLOOD PLASMA PROTEIN

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The gist of this paper is that certain casein digests given by vein or subcutaneously are promptly used by the hypoproteinemic dog to produce needed plasma proteins. The digest of casein is approximately as effective by vein as is the casein digest or whole liver equivalents given by mouth. The digests tested are essentially non-toxic as used.

A considerable series of experiments on dogs done in this laboratory has been reported during the past several years (9, 15, 4, 11) to show that *normal dog plasma* given by vein to the protein fasting dog can supply all *protein requirements*. The dog has been kept many weeks in health, weight balance, and positive nitrogen equilibrium by suitable amounts of plasma by vein and sugar, fat, minerals, and accessories by mouth.

Normal dog plasma protein given by vein during such protein fasts is utilized much more effectively than protein by mouth. It is evident from these previous experiments, and others of Howland and Hawkins (10), that the plasma proteins are utilized in the body more directly, with less waste and with but slight cleavage into large aggregates when needed to supply the protein requirements of the fasting dog. This exchange of proteins between the blood plasma and body cells has been discussed in recent papers (13, 14).

Table 5 (period 17) also shows how effectively plasma protein is used in the fasting dog as compared with the casein digest. Note the low urinary nitrogen when plasma protein is given by vein—about one-half the amount recorded when equivalent amounts of casein digest are given by vein. However, the casein digest by vein is as effective in plasma protein formation as liver protein or casein digest given by mouth. This would support the current belief that practically all *food proteins* are reduced to amino acids and peptides before utilization for protein building in the normal body.

In the experiments tabulated below we assume that hypoproteinemia offers a strong continued stimulus to plasma protein regeneration. Daily

plasmapheresis and a constant basal diet enable the investigator to keep the plasma protein concentration close to 4 per cent. Maintenance of the plasma protein level near 4 per cent for many weeks is essential for steady maximal stimulation (13) and effects a relatively constant protein output, a product of the basal diet. In the early weeks a surplus is removed over and above the basal plasma protein output: this surplus is called the *reserve store* and varies with different dogs and different dietary régimes in effect before the plasmapheresis. The nature of this reserve store has been the subject of some discussion (14, 3, 2, 13).

By this type of experiment with carefully standardized dogs, we are able to determine *quantitatively* the worth of diet factors and this has been done for many diet proteins. The method is admirably suited to a study of protein digests designed for clinical use. It is possible to measure accurately the result in plasma protein removed and compare this result with the response to standard proteins by mouth.

Protein digests parenterally were tried years ago (8), and more recently Elman (5, 6) and others (16, 7, 1) have reported several favorable nitrogen balance studies with them. Elman notes rises in the plasma albumin level; Farr (7) finds no effect on the plasma protein concentration of nephrotic children.

Methods

All *dogs* were immunized against distemper. They were under close observation in metabolism cages.

The *diet materials* were analyzed for nitrogen: dried yeast (type 200-B, Standard Brands, Inc.), 7.88 per cent; liver powder (H-8083, Eli Lilly and Company), 9.66 per cent; commercial casein, 14.0 per cent; fresh pork liver, 3.20 per cent. Their protein content was assumed to be 6.25 times their nitrogen value. The salt mixture of Wesson (17) was used. The commercial lecithin contained 3.53 per cent choline.

We are aware that the vitamin supply of the oral diets, given as listed in the Experimental Histories, may not prove entirely adequate when more is known about the requirements of the individual members of the B complex, particularly in animals under the strain of plasmapheresis over long periods of time. To add more crude sources, such as yeast, is to complicate further the finer interpretations of the results of protein testing. We emphasize, however, the excellent clinical condition of these dogs.

The *casein digest L* used in these experiments was furnished us through the courtesy of the Research Laboratories of Eli Lilly and Company. We are particularly indebted to Mr. Elmer Stuart, Mr. George B. Walden, and Dr. G. H. A. Clowes. The material is prepared by papain digestion of commercial casein. The digestion goes on in an acid medium at a temperature of approximately 40° during several days. Final digest mixture is heated to boiling, cooled, filtered, and the filtrate evaporated to dryness in a vacuum. A golden-yellow, granular material is the end product. It has a nitrogen content of 12.5 per cent and an assumed protein equivalence of 78.1 per cent.

The *protein digest X*, the product of another laboratory, is said to be a combination of acid and alkali hydrolysates of protein, brought into a final solution containing 1 per cent nitrogen and 5 per cent dextrose. Its assumed protein equivalence is 6.25 gm. per 100 cc. solution.

The *procedures* used have been previously described for the most part (12). Aseptic precautions in the plasmapheresis technique and in the infusion of digest solutions were not taken in obtaining the data of Tables 4 and 5, nor in the first 8 periods of Tables 1 and 3. Aseptic methods were used elsewhere. Casein digest L and dextrose were brought into solution in previously boiled hot water and then filtered through a Seitz (EK) filter pad, with a final concentration of 5 per cent each. Dextrose was not added to the digest in the subcutaneous injection tests of Table 2. The rate of injection on different occasions varied from 4 to 8 cc. of the 5 per cent solution per minute. No change in the urinary nitrogen was associated with a change in rate within these limits. The daily quota of digest was given in 2 equal injections about 5 hours apart, except as noted in Tables 1 and 1-a and in Clinical History, dog 39-106. When plasmapheresis was performed, the first digest injection would immediately follow the return of the washed red blood cells. The oral diet was offered about 2 hours after the last digest injection for the day. When all the digest was given in one injection, the oral diet was offered about 6 hours later.

We realize that for maximum utilization the digest infusions should *follow* the ingestion of the non-protein portion of the meal of the day. They are practical objections to such a procedure. Moreover, no certain difference in utilization was noted when the second half of the digest and the diet were given only 2 hours apart (Tables 1 and 1-*a*).

EXPERIMENTAL OBSERVATIONS

The following five double tables include data obtained from testing protein digests in 5 dogs. In Tables 1 and 1-*a*, 2 and 2-*a*, 3 and 3-*a*, the casein digest L was given to dogs rendered hypoproteinemic by plasmapheresis. The same digest was given to 2 normal dogs as reported in Table 5. The protein digest X was given to a dog during plasmapheresis in Tables 4 and 4-*a* and to the 2 normal dogs of Table 5.

Casein digest L, given by vein or subcutaneously, is well utilized for plasma protein formation and for maintenance of nitrogen balance during hypoproteinemia. It promotes recovery from hypoproteinemia (Tables 1 and 2). It is utilized in normal dogs not subjected to plasmapheresis (Table 5) but insufficiently well to achieve nitrogen balance. This casein digest L appears to be just as effective in plasma protein regeneration when given by vein as when given by mouth (Table 3, periods 7 and 8), despite the fact that by vein it is always associated with an increase in urinary nitrogen, in other than the urea and ammonia fraction (Tables 1-*a*, 2-*a*, 3-*a*, and 5). This urinary nitrogen increase remains essentially the same and the utilization appears the same when the digest is given in one continuous dose rather than in 2 doses hours apart (Tables 1 and 1-*a*). The

utilization of this digest is not improved by the addition of tryptophane or of cystine or of both these amino acids (Tables 1, 3, and 5).

The *protein digest X* if supplemented by cystine and tryptophane is well utilized in plasma protein production and induces a positive nitrogen balance

TABLE 1

Casein Digest L Promotes Plasma Protein Production

Cysteine and Tryptophane Not Required as Supplements

Dog 39-223

Period 7 days	Diet	Protein intake Total for 7 days	Plasma protein removed Total for 7 days	Blood plasma Average concentration		R.B.C. hematocrit, average	Plasma volume	Weight
				Total protein	A/G ratio			
		gm.	*gm.*	*per cent*		*per cent*	*cc.*	*kg.*
	Kennel	—	—	5.92	1.26	40.9	366	8.9
1	Fasting	0	25.4	5.44	1.28	44.6	292	7.9
2	Low protein	15	14.9	3.95	.96	48.3	312	8.0
3	Liver basal	85	20.9	4.12	1.02	48.5	300	8.3
4	Liver basal	85	21.9	3.95	1.11	47.4	316	8.5
5	Liver basal	85	21.0	4.07	1.10	46.3	328	8.5
6	Liver basal	73	21.0	4.12	1.08	49.2	253	8.5
7	Low protein + *digest L* + *tryptophane* + *cysteine**	76	19.4	4.04	0.94	49.8	322	8.5
8	Low protein + *digest L* + *cysteine*	81	17.4	3.93	0.79	47.4	271	8.8
9	Low protein + *digest L*	79	22.8	4.27	0.68	45.0	—	8.5
10	Low protein + *digest L*	81	18.6	3.98	0.66	46.5	—	8.5
11	Low protein + *digest L*	81	3.4	4.54	0.69	46.1	—	8.7
12	Low protein + *digest L*	81	3.3	5.08	0.76	42.1	368	8.7
13	Low protein + *digest L*	81	1.6	5.18	0.76	43.5	368	8.9
14	Low protein + *digest L*†	81	1.7	5.29	0.83	46.0	—	9.0
15	Low protein + *digest L*†	81	3.3	5.56	0.90	46.9	—	9.2
16	Low protein + *digest L*†	81	1.8	5.59	0.87	45.8	—	9.2
17	Low protein + *digest L*†	81	23.5	4.54	0.83	54.8	—	9.4
18	Low protein + *digest L*†	77	17.6	4.07	0.75	52.2	—	9.3
19	Low protein + *digest L*†	76	12.9	4.04	0.69	45.6	—	9.3

* Items in italics given intravenously.

† Digest given in 1 injection daily.

(Tables 4 and 4-*a*). In striking contrast, if these amino acids are omitted, the plasma protein output drops and the urinary nitrogen excretion shoots up.

Experimental History.—Dog 39-233 (Tables 1 and 1-*a*). An adult female terrier, fasted during period 1, was given during period 2 a low protein diet consisting of cane sugar 95 gm.; corn starch 20 gm.; corn oil 10 gm.; crisco 10 gm.; salt mixture 5 gm.;

bone ash 10 gm.; cod liver oil 5 gm.; dried yeast 2 gm.; liver powder 2 gm.; nicotinic acid 0.05 gm.; choline hydrochloride 0.4 gm.

TABLE 1-*a*

Nitrogen Balance

Casein Digest L Well Utilized

Strongly Positive Nitrogen Balance after First Two Periods

Dog 39-223

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	3.6	4.2	—	12.6	—	—	−13.2
2	Low protein	2.4	0.1	2.5	‡	4.9	—	—	−4.9
3	Liver basal	13.6	2.0	3.4	3.5	8.8	—	—	−0.1
4	Liver basal	13.6	0.3	3.6	1.9	8.3	—	—	+0.1
5	Liver basal	13.6	0.1	3.4	2.2	7.8	—	—	+0.3
6	Liver basal	11.6	0.9	3.4	1.7	7.4	71	2.2	0.0
7	Low protein + *digest L* + *tryptophane* + *cysteine**	12.8	0.7	3.2	2.0	10.2	57	4.4	−1.9
8	Low protein + *digest L* + *cysteine*	13.4	1.7	2.8	1.9	10.4	56	4.6	0.0
9	Low protein + *digest L*	12.5	2.7	3.4	1.6	11.1	58	4.7	−0.9
10	Low protein + *digest L*	12.9	4.0	3.0	1.7	9.9	59	4.1	+2.3
11	Low protein + *digest L*	12.9	1.0	0.6	1.6	9.4	55	4.2	+2.3
12	Low protein + *digest L*	12.9	1.3	0.5	1.6	9.5	56	4.2	+2.6
13	Low protein + *digest L*	12.9	0.8	0.3	1.6	9.6	55	4.3	+2.2
14	Low protein + *digest L*†	12.9	0.8	0.3	1.8	10.1	54	4.6	+1.5
15	Low protein + *digest L*†	12.9	−0.5	0.5	1.8	9.9	51	4.8	+0.2
16	Low protein + *digest L*†	12.9	−1.5	0.3	1.7	9.4	51	4.6	0.0
17	Low protein + *digest L*†	12.9	5.3	3.8	1.8	9.7	54	4.5	+2.9
18	Low protein + *digest L*†	12.3	2.6	2.9	1.1	10.0	54	4.5	+0.9
19	Low protein + *digest L*†	12.2	2.4	2.1	0.9	9.2	55	4.2	+2.4
	Totals	221.2	28.3	44.2	30.4	178.2	—	—	−3.3

‡ Included in period 3.

* Items in italics given intravenously.

† Digest given in 1 injection daily.

In periods 3 to 6 the liver basal diet consisted of the above diet with the addition of raw pork liver, 50 gm., and the deduction of cane sugar, 15 gm. One day's diet was omitted during period 6.

In periods 7 to 19 the low protein diet was the same as that of period 2 except for the addition of thiamin chloride, 5 mg., and the deduction of cane sugar, 25 gm. During

these 13 weeks casein digest L, 12 gm., was given by vein every day, as described under Methods, except for 1 day in the 7th period when only 6 gm. was given. In period 7, *l*-tryptophane, 0.25 gm., and *l*-cysteine hydrochloride, 0.8 gm., were given daily, divided into 2 equal doses in the digest solution. In period 8, *l*-cysteine hydrochloride, 0.8 gm., was again injected daily in the digest solution. In period 9 the low protein diet was omitted by mistake on one day.

During periods 7 to 16, the low protein diet was entirely consumed every day and the behavior of the dog was normal except for slight disturbance associated with the infusions of digest. Salivation was noted with each injection and occasionally vomiting of small amounts of watery fluid occurred—7 times only during a total of 98 injections. Urination very commonly followed return of the dog to the cage, but aside from very slight depression of physical activity for a few minutes after many injections no other visible reaction to the procedure was observed. When single daily injections of digest were begun in period 14, vomiting occurred during each of the first 3, but only 5 times during the next 18 injections.

During periods 17 to 19 appetite for the low protein diet lagged. Consumption was 97 per cent in period 17, 76 per cent in period 18, and 70 per cent in period 19. Period 19 was only 6 days long but the data given in Tables 1 and 1-*a* are adjusted to a 7-day basis. During the following week, plasmapheresis became impossible through scarred jugular veins and the experiment was discontinued. Food consumption became 100 per cent immediately upon change of diet.

Tables 1 and 1-*a*, considered in more detail, present a most satisfactory demonstration of the replacement of oral diet protein by *casein digest L by vein* for a long period, *13 weeks*. The first 6 weeks of this experiment serve to standardize the biological testing machine, the dog. The 40.3 gm. plasma protein removed during periods 1 and 2 comes partly from reduction in the plasma protein level and partly from the protein of the vitamin supplements, but largely from reserve store—protein which extravascular tissues give up to the plasma under the stress of depletion. The constancy of the plasma protein weekly output (21 gm.) and of the plasma protein level (4± gm. per cent) of periods 3 through 6 indicates a direct relationship between the protein consumed and that produced. A similar direct relationship obtains during periods 7 through 10 when the *casein digest L*, equivalent to 66 gm. protein per week, replaced the liver yielding an average plasma protein production of 19.5 gm. per week. The addition of cystine and tryptophane is without effect.

With discontinuance of plasmapheresis during periods 11 through 16, the plasma protein level showed an initial rapid rise and then a slower steady climb. With the first 6 days it reached 4.91 gm. per cent but another 29 days were required to reach 5.65 gm. per cent (period 16). The peak recorded in period 17 was 5.70 gm. per cent. This is the characteristic *curve of repletion* obtained in a plasma depleted dog when the low level of

4 gm. per cent is the starting point. The albumin:globulin ratio rose also. When depletion was resumed (period 17), the protein output returned to approximately the previous level in period 18. There was, however, during periods 18 and 19 the first disinclination to eat the oral diet. In addition, accumulated trauma to the veins made the proper bleedings difficult. There is some reserve protein evident in the 23.5 gm. of period 17 and the weight increase persists to the end of period 19. If the dietary and the technical difficulties had not supervened, further extraction of reserve protein might have occurred (12, 13).

Experimental History.—Dog 39-316 (Tables 2 and 2-*a*). An adult female beagle hound, used first in the experiment of Table 5, had a rest interval of 17 weeks before the observations of Table 2 were begun. After fasting during period 1, the dog was given during period 2 a low protein diet containing cane sugar 115 gm.; corn starch 20 gm.; corn oil 15 gm.; crisco 15 gm.; salt mixture 5 gm.; bone ash 10 gm.; cod liver oil 5 gm.; dried yeast 2 gm.; liver powder 2 gm.; choline hydrochloride 0.4 gm.; nicotinic acid 0.05 gm.; thiamin chloride 0.005 gm. Consumption was 100 per cent.

During periods 3 to 6 casein digest L, 15 gm., was given intravenously in conjunction with the low protein diet, from which 15 gm. cane sugar had been deducted. There was vomiting after 1 dose on the first day but not thereafter, and the transient disturbances noted in dogs 39-324 and 39-233 were even less conspicuous in this dog.

Even the introduction of single daily injections (on 2 days in period 6 and 2 in period 7) instead of divided doses did not result in vomiting (see Experimental History, dog 39-223). When the digest was given subcutaneously, 15 gm. daily in period 8 and 27 gm. in period 9, it was rapidly absorbed and left no recognizable tissue injury.

Consumption of the low protein diet was less complete than by the other dogs. In period 3 it was 70 per cent, in the next period 44 per cent. The vitamin supplements were then given separately and excessive doses of thiamin chloride by vein (15 to 20 mg.) were tried. The vitamins were eaten 100 per cent but consumption of the caloric portion remained between 49 and 71 per cent for periods 5 to 9.

Because the jugular and the leg veins would no longer permit plasmapheresis it was discontinued after period 6. At the close of period 9 the plasma protein level had reached 5.63 gm. per cent.

The data of Tables 2 and 2-*a* demonstrate plasma protein production from digest L given intravenously and also when given subcutaneously. The plasma protein level rose from 3.93 to 4.41 gm. per cent during period 7. We assume this rise was due partly to utilization of materials carried over from the intravenous digest of the previous period and partly to materials from the yeast-liver vitamin supplements. In period 8 the plasma protein concentration rose to 4.89 gm. per cent, and in period 9 a more brisk rise to 5.63 gm. per cent probably was occasioned by the increase in the amount of digest given (from 15 to 27 gm.). This rise should be compared with that seen in Table 1, periods 11 through 16.

Experimental History.—Dog 39-234 (Tables 3 and 3-*a*). An adult female mongrel dachshund, fasted during period 1, was given during period 2 a low protein diet consisting of cane sugar 110 gm.; corn starch 20 gm.; corn oil 10 gm.; crisco 15 gm.; salt mixture 5 gm.; bone ash 10 gm.; cod liver oil 5 gm.; dried yeast 2 gm.; liver powder 2 gm.; nicotinic acid 0.05 gm.; choline hydrochloride 0.4 gm.

During periods 3 to 6 the liver basal diet consisted of the above diet with the addition of raw pork liver, 50 gm., and the deduction of cane sugar, 15 gm. One day's diet was omitted during period 6.

During periods 7 to 9 the low protein diet was the same as that of period 2, except for the addition of thiamin chloride, 5 mg., and the deduction of cane sugar, 25 gm.

TABLE 2

Casein Digest L Subcutaneously as well as by Vein Promotes Plasma Protein Production

Dog 39-316

Period 7 days	Diet	Protein intake Total for 7 days	Plasma protein removed Total for 7 days	Blood plasma Average concentration		R.B.C. hematocrit, average	Plasma volume	Weight
				Total protein	A/G ratio			
		gm.	*gm.*	*per cent*		*per cent*	*cc.*	*kg.*
	Kennel	—	—	5.35	1.43	56.1	425	10.7
1	Fasting	0	25.6	4.85	1.53	52.1	—	9.7
2	Low protein	15	15.7	3.68	1.37	45.7	390	10.5
3	Low protein + *digest L**	93	12.5	4.14	0.58	46.2	—	9.9
4	Low protein + *digest L*	88	26.1	4.60	0.57	46.1	—	9.7
5	Low protein + *digest L*	95	18.9	4.34	0.57	45.6	—	9.8
6	Low protein + *digest L*	97	15.6	4.04	0.60	48.3	—	9.6
7	Low protein	15	1.5	4.36	0.59	46.0	—	9.5
8	Low protein + digest L†	97	1.5	4.59	0.68	41.7	—	9.5
9	Low protein + digest L†	153	2.9	5.42	0.68	42.6	—	9.4

* Items in italics given intravenously.

† Digest given subcutaneously.

Casein digest, 12 gm., *l*-cysteine hydrochloride, 0.8 gm., and *l*-tryptophane, 0.25 gm., were injected daily by vein in period 7 and consumption of the low protein diet was 99 per cent, except on the first day of this period. On this day the digest (6 gm.) was given as a 10 per cent solution; salivation was marked, hyperpnea was moderate, and vomiting of watery fluid occurred 3 times during the 30-minute injection. The second 6 gm. of digest were not given. On this day activity was subdued but the dog ate the low protein diet 100 per cent. For the remaining 6 days of period 7, the digest was given as a 5 per cent solution with the same reactions in the dog on each injection but of very mild nature. Vomiting occurred once with 9 of the 12 injections. The reactions were transient and a few minutes after the injection the activity returned to normal.

In period 8, casein digest, 12 gm., *l*-cysteine hydrochloride, 0.8 gm., and the low protein diet were offered daily by mouth and 97 per cent consumed.

In period 9, casein digest, 12 gm., and the low protein diet were offered by mouth

and the average daily consumption was only 77 per cent. The dog appeared in good condition but throughout the period showed a trace of albumin in the urine and rare red blood cells. The jugular veins were so scarred by this time that adequate bleedings were impossible and by the end of the period the plasma protein concentration had reached 5.27 gm. per cent.

TABLE 2-*a*

Nitrogen Balance

Nitrogen Equilibrium Maintained after First Two Periods

Dog 39-316

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	−2.1	4.2	—	13.3	83	2.2	−19.6
2	Low protein	2.4	2.5	2.6	2.2	10.1	72	2.8	−10.0
3	Low protein + *digest L**	14.8	1.8	2.1	1.8	14.4	63	5.4	−1.7
4	Low protein + *digest L*	14.0	4.7	4.2	1.7	14.3	61	5.6	−1.5
5	Low protein + *digest L*	15.1	2.9	3.1	1.7	12.7	55	5.7	+0.5
6	Low protein + *digest L*	15.5	3.7	2.5	1.4	13.0	59	5.3	+2.3
7	Low protein	2.4	0.7	0.2	1.5	7.1	66	2.4	−5.7
8	Low protein + digest L†	15.5	1.3	0.2	2.1	12.8	64	4.6	+1.7
9	Low protein + digest L†	24.6	−0.5	0.5	1.2	18.1	66	6.2	+4.3
	Totals	104.3	15.0	19.6	13.6	115.8	—	—	−29.7

* Items in italics given intravenously.

† Digest given subcutaneously in 2 equal injections daily.

The *casein digest L* is as effective in plasma protein production by vein as by mouth (Table 3) despite the fact that more of its nitrogen is eliminated in the urine when given by vein. The reduced output of plasma protein in period 9, Table 3, is explained in the Experimental History. The negative nitrogen balance of period 9 is largely the result of difficulty in reinjecting red blood cells.

Experimental History.—Dog 39-106 (Tables 4 and 4-*a*). An adult male beagle hound, fasted during period 1, was given during period 2 a low protein diet consisting of cane sugar 70 gm.; lard 30 gm.; butter fat 20 gm.; cod liver oil 5 gm.; lecithin 5 gm.; dried yeast 1 gm.; nicotinic acid 0.025 gm.; salt mixture 4 gm.; bone ash 10 gm. In period 3, liver, 50 gm., replaced sugar, 20 gm., of the above diet, and in periods 4 and 5 casein, 15 gm., replaced the liver. These diets were completely eaten every day.

In periods 6 and 7, the protein digest X was given intravenously, 200 cc. daily, in 4 equal portions, 2 to 3 hours apart, at a rate of 2.5 to 3 cc. per minute, without reaction. The low protein diet, as described above except for reduction in sugar to 40 gm., was completely consumed in period 6 but in period 7 the appetite and the activity of the dog decreased and a total of 50 per cent of one day's diet was rejected. The diet was changed during the last 4 days of the period by adding canned tomatoes, 50 gm., and by increasing the sugar to 100 gm. and decreasing the lard to 20 gm., the butter to 10 gm.

In period 8, protein digest X, 200 cc., was given daily mixed with the low protein diet of the 6th period and supplemented by *l*-cysteine, 0.8 gm., and *l*-tryptophane, 0.3 gm.

TABLE 3

Casein Digest L by Mouth or by Vein

Shows Same Plasma Protein Production as Liver Protein by Mouth

Dog 39-234

Period 7 days	Diet	Protein intake Total for 7 days	Plasma protein removed Total for 7 days	Blood plasma Average concentration		R.B.C. hematocrit, average	Plasma volume	Weight
				Total protein	A/G ratio			
		gm.	*gm.*	*per cent*		*per cent*	*cc.*	*kg.*
	Kennel	—	—	5.87	1.23	43.2	438	10.7
1	Fasting	0	31.8	5.22	1.46	45.5	296	9.5
2	Low protein	15	15.3	3.85	0.95	48.3	317	9.2
3	Liver basal	85	22.1	3.99	0.76	49.4	366	9.3
4	Liver basal	85	20.8	3.93	0.71	47.2	381	9.3
5	Liver basal	85	20.6	4.17	0.78	45.7	384	9.4
6	Liver basal	73	22.0	4.10	0.82	48.4	364	9.3
7	Low protein + *digest L + tryptophane + cysteine**	76	20.3	4.12	0.78	46.8	—	9.2
8	Low protein + digest L + cysteine by mouth	81	19.6	4.07	0.64	46.0	372	9.5
9	Low protein + digest L by mouth	62	10.7	4.48	0.53	44.5	—	9.1

* Items in italics given intravenously.

The mixture was completely consumed. For the final 2 days of the period tomatoes, 50 gm., and thiamin chloride, 5 mg., were added to the diet.

In period 9, casein, 15 gm., sugar, 10 gm., and B_2-liver concentrate, 1 gm., were added to the protein-low diet as of period 6.

In the 10th period the diet casein of the preceding period was replaced by protein digest X by vein as in periods 6 and 7 plus the daily addition to the digest of *l*-cysteine hydrochloride, 1.04 gm., and *l*-tryptophane, 0.3 gm. The digest and supplements were given on only the last 6 of the 7-day period. Consumption of the low protein diet was complete except for 20 per cent on the 6th day and 93 per cent on the 7th day. There was no obvious explanation for this decline in appetite although one soon developed. The dog appeared generally normal except for slight reduction in activity.

Throughout this experiment, including the beginning of the 10th week, the urine

was normal in the gross and microscopically, but at the end of period 10 the urine contained a slight amount of albumin, a few white blood cells and red blood cells, and occasional granular casts. In the 11th week appetite further declined, the dog appeared definitely sick after a few days and was sacrificed one week after the close of the 10th period. Blood culture the day before death yielded *Streptococcus viridans* and *B. alcaligenes*, and at autopsy similar organisms were recovered from fresh vegetations on the mitral valve. Also found were an acute focal embolic nephritis; infected infarcts of

TABLE 3-*a*

Nitrogen Balance

Nitrogen Balance Maintained after First Two Periods

Dog 39-234

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	6.1	5.1	—	17.2	—	—	−16.2
2	Low protein	2.4	0.8	2.5	3.0	12.3	—	—	−14.6
3	Liver basal	13.6	1.6	3.6	2.6	10.7	—	—	−1.7
4	Liver basal	13.6	0.2	3.4	3.4	9.1	—	—	−2.1
5	Liver basal	13.6	−0.8	3.4	2.5	8.8	—	—	−1.9
6	Liver basal	11.6	1.3	3.6	2.6	9.1	—	—	−2.4
7	Low protein + *digest L + tryptophane + cysteine**	12.8	1.6	3.2	2.3	9.7	59	4.0	−0.8
8	Low protein + digest L + cysteine by mouth	13.3	3.6	3.2	2.7	8.1	70	2.4	+2.9
9	Low protein + digest L by mouth	10.0	−5.8	1.8	2.0	8.3	72	2.3	−7.9
	Totals.....	90.9	8.6	29.8	21.1	93.3	—	—	−44.7

* Items in italics given intravenously.

heart, spleen, and kidneys; hemorrhages in serous membranes and gastro-intestinal tract. From histological examination the apparent age of the vegetations agrees with the clinical onset of the illness about 10 days before death, no doubt a consequence of the multiple intravenous injections without aseptic precautions and the low resistance of the protein depleted dog.

It is obvious from the data of Tables 4 and 4-*a* that *cystine and tryptophane* are effective supplements for *protein digest X*, a digest which is not utilized by vein unless supplemented.

Of some interest is a clear demonstration of the retention of the added

cystine (or cysteine) sulfur. The urinary total sulfur during periods 5, 6, and 7 was 125 mg., 136 mg., and 129 mg., respectively, a satisfactory base line. When cystine sulfur of 1495 mg. was added in period 8, the sulfur excretion rose only to 186 mg. The added *cystine sulfur* was more than *95 per cent retained.* Plasma protein production increased sharply. One might point out that the quantity of plasma protein removed during period 8 must have contained only about 160 mg. of cystine sulfur. The urinary

TABLE 4

Protein Digest X by Mouth or by Vein
Promotes Rapid Plasma Protein Formation
Only When Supplemented with Cystine and Tryptophane

Dog 39-106

Period 7 days	Diet	Protein intake Total for 7 days	Plasma protein removed Total for 7 days	Blood plasma Average concentration		R.B.C. hematocrit, average	Plasma volume	Weight
				Total protein	A/G ratio			
		gm.	*gm.*	*per cent*		*per cent*	*cc.*	*kg.*
	Kennel	—	—	6.43	0.93	—	—	11.5
1	Fasting	0	17.6	5.89	0.87	49.2	474	10.2
2	Low protein	3	30.4	4.49	0.75	50.7	356	10.2
3	Liver basal	73	28.8	4.13	0.64	49.0	429	10.0
4	Casein basal	96	20.4	3.86	0.54	47.4	429	10.2
5	Casein basal	96	18.4	4.06	0.67	50.3	424	10.2
6	Low protein + *protein digest X**	91	12.1	3.70	0.60	49.9	327	10.0
7	Low protein + *protein digest X*	93	8.4	4.02	0.51	47.8	323	9.7
8	Low protein + protein digest X + cystine + tryptophane by mouth	92	22.1	4.21	0.64	47.4	449	10.2
9	Casein basal	100	25.2	4.33	0.64	48.8	373	10.1
10	Low protein + *protein digest X + cysteine + tryptophane*	94	23.8	4.15	0.50	47.8	435	10.0

* Items in italics given intravenously.

sulfur of period 9 was 146 mg. and rose during period 10 to 176 mg. when cysteine sulfur, 1478 mg., was injected by vein. Again, the added sulfur was more than 95 per cent retained. The conservation of this sulfur is associated with a large production of plasma protein. Sulfur retention has been demonstrated under many other circumstances.

Experimental History.—Dog 39-316 (Table 5). An adult, female beagle hound was fasted 2 days (period 1). The low protein diet fed the remaining 16 two-day periods consisted of cane sugar 146 gm.; lard 34 gm.; butter fat 14 gm.; salt mixture 6 gm.; bone ash 6 gm.; cod liver oil 10 gm.; dried yeast 1 gm.; choline hydrochloride 0.2 gm.; nicotinic acid 0.025 gm. This diet was eaten completely through period 4.

During periods 4 and 5, casein digest L by vein was given, 30 gm. per period in 3 equal doses as an 8 or 9 per cent solution, also containing glucose 4 per cent and sodium chloride 0.9 per cent. In periods 6 and 7, the digest was increased to 40 gm. per period, given in 4 equal doses. In periods 8 and 9, *l*-tryptophane, 1.2 gm., was added to 40 gm. digest per period. In periods 10 and 11, *l*-cysteine hydrochloride, 2.0 gm. per period,

TABLE 4-*a*

Nitrogen Balance

Nitrogen Retention Only When Protein Digest X Is Supplemented with Cystine and Tryptophane

Dog 39-106

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	2.9	†	19.3	—	—	−22.1
2	Low protein	0.8	−0.5	5.0	3.9	14.2	—	—	−22.8
3	Liver basal	12.1	−0.3	4.7	1.5	14.5	—	—	−8.9
4	Casein basal	15.6	2.8	3.4	2.1	14.7	—	—	−1.8
5	Casein basal	15.6	1.5	3.0	1.8	15.6	77.8	3.6	−3.3
6	Low protein + *protein digest X**	14.6	1.3	2.0	1.9	22.9	74.2	6.0	−10.9
7	Low protein + *protein digest X*	14.7	5.5	1.3	2.0	22.5	71.6	6.3	−5.6
8	Low protein + protein digest X + cystine + tryptophane by mouth	15.6	8.0	3.6	3.3	11.9	72.7	3.2	+4.8
9	Casein basal	16.3	7.2	4.2	2.2	14.7	82.8	2.5	+2.4
10	Low protein + *protein digest X + cysteine + tryptophane*	16.0	7.4	3.9	2.3	14.2	74.7	3.6	+3.0
	Totals.....	121.3	33.0	34.0	21.0	164.5	—	—	−65.2

† Included in following period.

* Items in italics given intravenously.

was added to this combination. Reaction to these injections was minimal; a little mucus was vomited on only 2 occasions. In periods 5 through 9 the oral diet was consumed 95 per cent, but in periods 10 and 11 half of the diet had to be spoon fed each day.

In periods 12 and 13, casein digest L, 20 gm., *l*-tryptophane 0.6 gm., and *l*-cystine, 0.8 gm., were mixed daily into the low protein diet and fed. Appetite improved and spoon feeding was unnecessary in period 13.

In periods 14 and 15, protein digest X, 500 cc., with added tryptophane, 1.2 gm., and cysteine hydrochloride, 2.0 gm., was injected intravenously, in 4 equal doses in period 14 and in 8 equal doses in period 15. Vomiting of mucus occurred with several

of the injections, even when given at a rate of 2 to 3 cc. per minute. Spoon feeding achieved 100 per cent consumptions of the oral diet. Period 16 was of only 1 day's duration but the data in the table are adjusted to a basis of 2 days.

During period 17, *heparinized dog plasma* was given by vein in 2 injections daily, totaling 5.08 gm. nitrogen. The oral diet had to be entirely spoon fed.

TABLE 5

Casein Digest L Utilized by Normal Dogs

Period 2 days	Diet	Total N Intake	Dog 39-316—Urine				Dog 39-307—Urine			
			Total N	Urea + NH_3 N		Undetermined N	Total N	Urea + NH_3 N		Undetermined N
		gm.	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*
1	Fasting	0	9.49	8.12	85.6	1.37	6.80	5.91	86.8	0.89
2	Low protein	0.16	4.79	3.44	71.8	1.35	4.82	3.67	76.1	1.15
3	Low protein	0.16	3.37	2.50	74.1	0.87	4.63	3.50	75.6	1.13
4	Low protein + *digest L**	3.90	5.85	3.63	62.2	2.22	6.62	4.47	67.7	2.25
5	Low protein + *digest L*	3.90	5.52	3.70	67.0	1.82	8.50	6.42	72.9	2.08
6	Low protein + *digest L*	5.16	5.87	3.53	60.2	2.34	8.97	5.92	66.0	3.05
7	Low protein + *digest L*	5.16	6.30	3.98	63.1	2.32	7.28	4.82	66.2	2.36
8	Low protein + *digest L* +	5.33	6.04	3.86	63.9	2.18	7.07	4.41	62.4	2.66
9	*tryptophane*	5.33	6.60	4.14	62.7	2.46	7.01	4.35	62.0	2.66
10	Low protein + *digest L* +	5.51	7.00	4.54	64.9	2.46	6.14	3.71	60.5	2.43
11	*cysteine* + *tryptophane*	5.51	5.92	3.54	59.8	2.38	6.27	3.81	60.8	2.46
12	Low protein + digest L +	5.51	3.40	2.24	65.9	1.16	3.40	2.27	66.7	1.13
13	cystine + tryptophane	5.51	3.96	2.76	69.7	1.20	3.58	2.42	67.5	1.16
14	Low protein + *digest X* +	5.51	6.60	4.22	64.0	2.38	6.56	4.17	63.7	2.39
15	*cysteine* + *tryptophane*	5.51	5.00	3.03	60.5	1.97	6.00	3.84	64.0	2.16
16	Low protein	0.16	2.48	1.54	61.8	0.96	3.16	2.04	64.6	1.12
17	Low protein + *plasma protein*	5.22	2.45	1.54	62.9	0.92	3.46	2.50	72.2	0.96

* Items in italics given intravenously.

Weight varied little after the start of the experiment: 9.8 kg. in period 5, 9.6 kg. in period 13, 9.8 kg. in period 17. This dog was the subject of later experiments, Tables 2 and 2-*a*.

Experimental History.—Dog 39-307 (Table 5). An adult female, mongrel, long-haired terrier was fed the same diet and given the digest and amino acid mixtures in the same amounts and in the same fashion on the same days as dog 39-316. Since the oral diet was eaten only 10 to 30 per cent during periods 2 to 4, it had to be spoon fed for complete

consumption during the remainder of the experiment. In the course of the first casein digest L injection, there was some restlessness, weakness of pulse, and vomiting. Some mucus was vomited following the injection of protein digest X, period 14. Small amounts of the low protein diet were vomited following spoon feedings on 3 occasions, periods 14, 16, and 17. The amount of dog plasma given in period 17 was practically the same as that given dog 39-316 (5.04 gm. nitrogen). Weight varied little after the start of the experiment: 11.3 kg. in period 6 and 11.1 in period 17.

In Table 5, both digests (casein L and protein X) were tested in 2 normal dogs not subjected to plasmapheresis. Casein digest L was well utilized by mouth, periods 12 and 13. It was utilized by vein but not as well as is demonstrated above in the plasma depleted dogs. For example, in dog 39–316, for 16 days during digest injection (periods 4 through 11) the nitrogen intake was 39.8 gm., and the nitrogen output (assuming fecal nitrogen of 0.5 gm. per period) was 53.1 gm. This negative balance of 13.3 gm. or 5.8 gm. per 7-day period may be compared with negative balances obtained in this dog when no digest was being given. For period 2, Table 2-*a*, the negative balance was 10.0 gm. per 7-day period. It would appear that some of the nitrogen of the intravenous digest had been retained. Cysteine and tryptophane obviously do not improve this retention. The protein digest X is similarly ineffective (periods 14 and 15). The slower rate of injection in period 15 (see Experimental History) did not improve nitrogen retention. This difference in the digest nitrogen retention of plasma depleted dogs as compared with that of non-depleted dogs is being investigated.

Intravenous plasma protein again proves its nutritional value (period 17). Its nitrogen is completely retained in both dogs. The response to both digests is the same in dog 39-307 as in dog 39-316, except that dog 39-307 may not have been entirely normal during the early part of the experiment (see Experimental History, dog 39-307). If some intoxication existed, it seems proper to refer it to the factors responsible for the poor food consumption prior to digest injections and to the higher concentration of digest used, unfiltered and unsterile, not the water-clear solution of the 5 per cent concentration.

DISCUSSION

Perhaps it is not for us to debate clinical problems but we may mildly suggest that the experiments designed to study plasma protein production do have a bearing on the many-sided problem of *shock* as well as clinical *hypoproteinemia*. If the body can be aided in producing new plasma proteins this procedure may be as valuable as the administration of plasma by vein. Plasma protein by vein is most effective to correct emergency hypo-

proteinemia and to furnish protein to depleted or injured body cells. But casein digests by vein or subcutaneously are as effective as protein by mouth in building new plasma protein and when protein cannot be eaten the digest can wholly replace food protein for many weeks. It may be that *casein digests* can be used with profit to *supplement plasma injections* or even to replace the intravenous plasma when the acute emergency is passed. The advantages of the casein digest for clinical use scarcely need mention. It is non-toxic and can be stored in concentrated form. It is inexpensive and obtainable in unlimited amounts and can be readily sterilized.

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 diet limited in protein. Such dogs are clinically normal but have a lowered resistance to infection and certain intoxications.

Casein digests given by vein or subcutaneously to such plasma depleted dogs are effective in promoting abundant new plasma protein production. Casein digest L by vein is equivalent to whole liver of like protein equivalence by mouth. The ratio of new plasma protein production to protein intake is 20 to 25 per cent in both instances.

Casein digest L by vein gives the same response in plasma protein output as the same digest by mouth. Protein digest X by vein requires addition of tryptophane and cysteine to be effective in plasma protein production. The added cysteine sulfur is more than 95 per cent retained by the dog.

The speed of digest injection has no effect on its utilization, within the range tested.

Casein digest L given by vein to non-depleted dogs is less well utilized than in dogs depleted of plasma protein.

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