BLOOD PLASMA PROTEIN PRODUCTION AS INFLUENCED BY AMINO ACIDS

Cystine Emerges as a Key Amino Acid under Fixed Conditions

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When one studies the production of plasma protein or hemoglobin or any other body protein inevitably there follows a consideration of the building stones of such protein. One always cherishes the hope (perhaps the illusion) that some key amino acid or group of amino acids may be found. A good many amino acids can influence the building of hemoglobin under uniform conditions in experimental anemia (13) but no one acid or small group of amino acids as yet appears to stand out above all others in this reaction. It appears that *cystine* in the experiments below nearly qualifies as a *key amino acid* in the complicated metabolic reaction which must be responsible for the construction of plasma protein in the depleted dog. Cystine alone as a supplement to gelatin does not render this incomplete protein a potent factor in plasma protein building. Cystine plus tyrosine or tryptophane as a supplement to gelatin makes this mixture as potent as beef serum itself for the building of new plasma protein in the hypoproteinemic dog.

This paper continues the study of blood plasma protein production in dogs as influenced by various amino acids alone or as supplements to the incomplete protein gelatin. *Plasma protein production* is measured by the determination of the protein removed by daily *plasmaphereses* (removal of whole blood by vein puncture and return of washed red cells suspended in a saline solution). Many factors which influence plasma protein regeneration have been discussed and the related data published in previous reports (3, 9, 7, 6, 5).

Methods

The procedures used in obtaining the data reported here are those fully described or referred to in a previous communication (5). It may be repeated here that the gelatin used in the experiments is a white, granular gelatin (Will Corporation) containing by Kjeldahl analysis 15.1 per cent nitrogen (83.8 per cent protein (4)). The pure crystalline amino acids used (Eastman Kodak Company) are the naturally occurring types except for racemic mixtures of phenylalanine and of methionine.

EXPERIMENTAL OBSERVATIONS

The data reported in this paper have been obtained from experiments with two dogs, one of which was observed for over a year and the other for 5 months. Tables 1 and 1-*a*, 2 and 2-*a* give these data for 54 consecutive weekly periods as recorded in dog 36-196. Tables 3 and 3-*a* record 23 similar periods for dog 37-6. Clinical details and the exact make-up of the diets given during each period are recorded in the Clinical Experimental Histories which follow. It is to be noted that raw *pork liver* was the *only protein given* these dogs except for the occasional test supplements of gelatin.

In the process of reducing the concentration of circulating plasma protein in dog 36-196 from a normal level of 5.70 per cent to the desired low level of 3.80 to 4.00 per cent, about 49 gm. plasma protein were removed during the first 3 periods of plasmapheresis (Table 1). It is estimated that the liver basal diet fed during 2 of these 3 periods furnished the materials to produce 24 of the 49 gm. The remaining 25 gm. must have come from materials (*a reserve store*) already present in the body and readily available for conversion into plasma protein. If we estimate the entire *reserve store* of plasma protein building material as *determined by the first 7 periods*, we note a total output of 116 gm. and a basal output for the 6 periods in which the basal diet was fed of 72 gm. (disregarding the gelatin feeding). This gives about 40 gm. as the reserve store. The size of this reserve store in any given dog depends largely upon the quantity and quality of dietary protein in the few weeks preceding plasmapheresis.

The *basal output of plasma protein* is determined by averaging those liver basal periods which are neither high nor low on account of some immediately preceding test. Thus, in dog 36-196 (Tables 1 and 2), the average of periods 15 to 19, 22, 26, 28, 30, 33, 35, 43, 46, 48, and 50 is 12 gm.

Of the 18 odd grams of protein removed above this 12 gm. basal output during periods 4 to 7, how much is reserve store and how much if any is due to the supplements of *tryptophane* and of *gelatin plus tryptophane* is not certain. In an earlier report (5) tryptophane alone produced no appreciable increase in plasma protein production but gelatin plus tryptophane was potent in a ratio of 3 gm. of gelatin protein to produce 1 gm. of plasma protein. No such potency is evident in this test, nor when repeated (period 10) nor when tried in another dog (Table 3, period 16).

The low production evident during periods 9 to 12 is not well explained. The animal appeared normal but may have been affected by the very hot summer weather. Period 13 gives confirmation of the observation previously reported (5) that the dog with exhausted reserve stores of plasma protein building material can make little if any new plasma protein while consuming a *protein-free* otherwise adequate diet. The one gram listed as "protein" intake (period 13) is probably only in part true protein suitable for utilization in nutrition. The expected fall in urinary nitrogen is noted (Table 1-*a*). It is important to note that when the liver basal diet was again fed in the following week (period 14) the urinary nitrogen did not return to the basal level nor did the plasma protein output rise to the basal level. It may be proper to argue that the fasting week depleted body protein whose repair or replacement took precedence over the manufacture of new plasma protein.

Gelatin is lacking in *tryptophane* and *tyrosine* and is very poor in *cystine* (1). When it is supplemented with these three amino acids, as in period 20, the whole mixture becomes a potent source of materials for plasma protein production—slightly less than 3 gm. gelatin protein being required to produce 1 gm. plasma protein. This combination is practically as potent in plasma protein production as any substance yet examined—2.6 gm. beef serum yielded 1 gm. plasma protein (9). It should be noted that often there is a *carry over* of the effect of supplementary feeding upon plasma production into the week (period 21) or two following such feeding.

It is instructive to contrast periods 6 and 10 in which there is gelatin plus tryptophane added to the basal diet, with periods 20 and 23 (Table 1) in which gelatin is supplemented with cystine and tyro-

TABLE 1

Blood Plasma Protein Regeneration

Gelatin Becomes Very Efficient upon Addition of Cystine, Tyrosine, and Tryptophane

Dog 36-196.

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: Total protein	Blood plasma Average concentration: A/G ratio	R.B.C. hematocrit, average	Plasma volume
		gm.	*gm.*	*gm.*	*per cent*		*per cent*	*cc.*
	Kennel				5.70	1.59	41.7	—
1	Fasting	0	4.8		5.68	1.33	41.1	490
2	Liver basal	70	26.4		4.90	1.12	43.9	460
3	Liver basal	70	18.0		4.13	0.76	47.6	464
4	Liver basal + tryptophane, 2.0 gm.	70	16.6		4.10	0.94	50.3	450
5	Liver basal	70	15.7		4.11	1.01	50.0	439
6	Liver basal + gelatin, 70 gm. + tryptophane, 2.8 gm.	129	17.8	2.0±	4.15	1.02	48.1	474
7	Liver basal	70	16.5		4.05	0.97	50.3	441
8	Liver basal	70	11.9		3.95	0.85	51.7	467
9	Liver basal	70	7.3		4.03	0.97	53.6	385
10	Liver basal + gelatin, 70 gm. + tryptophane, 2.8 gm.	129	11.8	0.0	4.05	0.93	54.3	456
11	Liver basal	70	9.9		3.84	0.83	49.7	478
12	Liver basal	70	9.1		4.02	0.80	46.2	496
13	Protein-free	1±	1.7		3.95	—	45.4	444
14	Liver basal	70	4.7		3.95	0.70	41.7	474
15	Liver basal	70	12.2		4.06	0.74	46.5	437
16	Liver basal	70	10.5		3.87	0.79	50.4	447
17	Liver basal	70	10.3		4.20	0.74	45.5	—
18	Liver basal	70	14.5		3.99	0.84	45.7	424
19	Liver basal	70	10.0		3.81	0.73	47.2	452
20	Liver basal + gelatin, 70 gm. + tryptophane, 2.8 gm. + cystine, 4.2 gm. + tyrosine, 4.2 gm.	129	25.0	20.1	4.15	0.91	47.6	465
21	Liver basal	70	20.5		3.86	0.81	49.2	460
22	Liver basal	70	10.6		3.80	0.75	47.0	448
23	Liver basal + gelatin, 70 gm. + tyrosine, 4.2 gm. + cystine, 4.2 gm.	129	18.1	23.0	4.08	0.91	45.6	471
24	Liver basal	70	22.4		4.15	0.67	49.4	438
25	Liver basal	70	18.6		3.88	0.80	52.6	492
26	Liver basal	70	11.9		3.79	0.73	50.9	492
27	Liver basal + gelatin, 70 gm. + tyrosine, 4.2 gm.	129	15.1	3.0	3.99	0.79	49.3	449
28	Liver basal	70	11.9		3.97	0.74	48.9	419

* Estimated basal output per week equals 12 gm. plasma protein.

TABLE 1-*a*

Weight and Nitrogen Balance

Dog 36-196.

Period 7 days	Diet	Weight	Nitrogen balance: Intake: in diet	Nitrogen balance: Intake: in excess R.B.C. injected	Nitrogen balance: Output: in plasma	Nitrogen balance: Output: in urine	Nitrogen balance: Output: in feces	Intake minus output
		kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
	Kennel	10.5						
1	Fasting	9.1	0.0	0.0	0.8	14.8	0.0	−15.6
2	Liver basal	9.2	11.2	8.0	4.2	12.6	3.1	−0.7
3	Liver basal	9.2	11.2	4.3	2.9	11.3	3.1	−1.8
4	Liver basal + tryptophane, 2.0 gm.	9.2	11.5	3.5	2.7	11.1	2.0	−0.8
5	Liver basal	9.0	11.2	−2.3	2.5	9.6	1.8	−5.0
6	Liver basal + gelatin, 70 gm. + tryptophane, 2.8 gm.	9.0	22.2	5.2	2.8	19.5	2.3	+2.8
7	Liver basal	9.2	11.2	2.9	2.6	10.3	1.7	−0.5
8	Liver basal	9.3	11.2	1.6	1.9	12.3	2.3	−3.7
9	Liver basal	9.3	11.2	−1.6	1.2	9.6	2.0	−3.2
10	Liver basal + gelatin, 70 gm. + tryptophane, 2.8 gm.	9.5	22.2	4.4	1.9	18.3	2.3	+4.1
11	Liver basal	9.5	11.2	1.4	1.6	10.6	2.3	−1.9
12	Liver basal	9.6	11.2	−0.6	1.5	9.5	3.3	−3.7
13	Protein-free	9.4	0.3	0.0	0.3	6.5	1.6	−8.1
14	Liver basal	9.5	11.2	1.6	0.8	7.8	1.0	+2.8
15	Liver basal	9.6	11.2	4.1	2.0	8.0	2.5	+2.8
16	Liver basal	9.7	11.2	−0.7	1.7	8.8	2.0	−2.0
17	Liver basal	9.6	11.2	2.2	1.7	9.8	3.1	−1.2
18	Liver basal	9.6	11.2	2.6	2.3	9.0	2.1	+0.4
19	Liver basal	9.5	11.2	4.5	1.6	9.3	2.7	+2.1
20	Liver basal + gelatin, 70 gm. + tryptophane, 2.8 gm. + cystine, 4.2 gm. + tyrosine, 4.2 gm.	9.7	23.0	3.4	4.0	12.5	2.8	+7.1
21	Liver basal	9.5	11.2	3.7	3.3	9.2	2.7	−0.3
22	Liver basal	9.5	11.2	1.5	1.7	9.8	2.6	−1.4
23	Liver basal + gelatin, 70 gm. + tyrosine, 4.2 gm. + cystine, 4.2 gm.	9.3	22.6	1.5	2.9	14.4	3.3	+3.5
24	Liver basal	9.3	11.2	3.8	3.6	8.1	2.2	+1.1
25	Liver basal	9.3	11.2	1.5	3.0	9.0	1.8	−1.1
26	Liver basal	9.3	11.2	1.7	2.0	8.6	—	—
27	Liver basal + gelatin, 70 gm. + tyrosine, 4.2 gm.	9.3	22.1	4.7	2.5	15.5	2.4	+6.4
28	Liver basal	9.2	11.2	2.4	2.0	9.1	2.1	+0.5

sine. In the first two experiments (periods 6 and 10) there is no excess production of new plasma protein and there is a large excess of urinary nitrogen (19.5 and 18.3 gm.). In the second two experiments (periods 20 and 23) there is a large surplus of new plasma protein (20 to 23 gm.) but a decrease in urinary nitrogen (12.5 and 14.4 gm.). It seems inevitable to conclude that the gelatin incompletely supplemented presents a mélange of amino acids which cannot be utilized to build plasma protein or other body protein and are thrown away in the urine. When cystine and tyrosine or tryptophane are added to the gelatin, the body is then able to utilize the gelatin split products in an extraordinarily efficient manner so that less nitrogen appears in the urine and much more plasma protein is manufactured.

During periods 23 to 28 (Table 1) and 29 to 35 (Table 2) 5 different groupings of these amino acids with gelatin were tried. It appears that the addition to gelatin of *cystine* and either or both *tryptophane* and *tyrosine* gives a mixture highly efficient for plasma protein production. Without added cystine the gelatin is inefficient; yet cystine alone does not appreciably improve the efficiency. Corroboration of some of these findings is given in another dog (Table 3, periods 14 to 21) and the results of all experiments are summarized in Table 4.

Tomatoes were added to the basal diet in period 36 (Table 2) in an attempt to duplicate the conditions under which tryptophane and gelatin produced a large amount of new plasma protein (29 gm.) in the earlier experiment referred to above (5). No significant production of protein above the basal output was obtained. The shock reaction occurring on the 4th day of the 37th period prevented accurate measurement of any hypothetical carry over but no evidence of any such impending reaction was given in those first 4 days.

The *anaphylactic shock* reaction is part of another problem not considered in this report. The dog recovered from the severe effects in two days but in order to insure a full return to a normal state the protein intake in the diet was doubled and plasmapheresis was discontinued (periods 38 to 40, see Clinical History). Plasmapheresis was resumed in the 41st period and it required 2 weeks to reestablish basal conditions as obtained in the 43rd period. It is obvious that protein had been stored during periods 38 to 40—a weight gain of 0.9 kg. and a large positive nitrogen balance speak for this. Also, during the 6 periods 38 to 43 only 78.8 gm. protein were removed to achieve basal conditions, whereas had there been no call for protein to repair shock injury it would probably have been necessary to remove 108 gm. (5).

Cystine and tyrosine were again added with gelatin to the liver basal in period 44. While the response in plasma protein production was not so large as in period 23, it compares favorably and indicates consistent performance of this biological test machine.

Laked red blood cells afford little if any material for plasma protein formation when introduced into the blood stream. This conclusion had already been reached from experiments in normal dogs (10) and is confirmed in this plasma depleted dog in period 47. Slightly less than 25 gm. of hemoglobin containing about 23 gm. protein was responsible for the production of 1 gm. of plasma protein, a quantity well within the limits of error in this type of experiment. These experiments do not support recent inferences by Melnick, Cowgill, and Burack (8) to the effect that hemoglobin may be utilized to build new plasma protein.

Methionine adds no more to the efficiency of gelatin than cystine alone and cannot replace cystine in its potent combination with tyrosine (periods 49 to 52). Of considerable interest is the apparent inability of phenylalanine to act effectively with cystine and gelatin in the way that tyrosine does (period 53).

In Tables 1-*a* and 2-*a* it should be noted that throughout the entire year the animal's weight varied little—between 8.8 and 9.7 kg. except for the loss during the anaphylactic shock episode. Moreover, for the entire 54 weeks a positive nitrogen balance of 14 ± gm. was obtained. The urinary nitrogen figures are of some special interest. For the 15 basal periods listed above in determination of the *basal protein output* the average weekly urinary nitrogen excretion was 8.8 gm., with limits of 7.4 and 9.8 gm. If we compare these figures (8.8 gm. nitrogen per week) during the *basal diet periods* with the figures observed during *gelatin feeding* there are striking differences. When gelatin plus tryptophane or tyrosine or both were added to the basal ration the urinary nitrogen per week averaged 17.6 gm. and the plasma protein output 1 to 2 gm. In striking contrast when gelatin plus cystine and tyrosine or tryptophane were added to the

TABLE 2 (*continues Table 1*)

Methionine and Phenylalanine Ineffective Compared with Cystine and Tyrosine Hemoglobin by Vein Not Utilized to Build Plasma Protein

Dog 36-196.

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. hematocrit, average	Plasma volume
					Total protein	A/G ratio		
		gm.	gm.	gm.	per cent		per cent	cc.
29	Liver basal + gelatin, 70 gm. + cystine, 4.2 gm.	129	15.9	5.7	4.07	0.80	49.5	442
30	Liver basal	70	13.8		3.98	0.69	49.5	425
31†	Liver basal + gelatin, 70 gm. + cystine, 4.2 gm. + tryptophane, 2.8 gm.	129	21.5	18.8	4.29	0.88	49.3	428
32	Liver basal	70	22.5		4.13	0.79	50.5	389
33	Liver basal	70	10.8		3.78	0.67	49.9	431
34	Liver basal + gelatin, 70 gm. + tyrosine, 4.2 gm. + tryptophane, 2.8 gm.	129	12.4	0.7	3.88	0.62	48.8	434
35	Liver basal	70	12.3		3.87	0.58	46.6	447
36	Liver basal + gelatin, 70 gm. + tryptophane, 2.8 gm. + tomatoes, 245 gm.	132	13.7	1.7+	3.91	0.55	47.4	376
37	Liver basal (anaphylactic shock)	58	8.0		3.95	0.55	46.5	434
38	Liver basal + liver, 350 gm.	140	2.8		4.65	0.57	43.2	470
39	Liver basal + liver, 350 gm.	140	17.5		5.05	0.67	44.5	452
40	Liver basal + liver, 350 gm.	140	5.0		4.97	1.41	43.8	469
41	Liver basal	70	26.1		4.32	0.77	43.6	471
42	Liver basal	70	14.8		3.69	0.56	46.0	484
43	Liver basal	70	12.6		3.85	0.55	46.9	450
44	Liver basal + gelatin, 70 gm. + tyrosine, 4.2 gm. + cystine, 4.2 gm.	129	20.5	14.6	4.06	0.68	47.2	442
45	Liver basal	70	17.3		3.74	0.72	45.0	461
46	Liver basal	70	12.8		3.84	0.62	45.5	460
47	Liver basal + hemoglobin by vein, 24.8 gm.	93	13.1	1.0	3.89	0.49	44.9	419
48	Liver basal	70	11.9		3.74	0.60	44.1	486
49	Liver basal + gelatin, 70 gm. + methionine, 5.2 gm.	129+	14.4	4.0	3.92	0.69	46.1	477
50	Liver basal	70	13.6		3.90	—	47.4	437
51	Liver basal + gelatin, 70 gm. + methionine, 5.2 gm. + tyrosine, 4.2 gm.	129	15.6	6.8	3.97	0.70	46.2	483
52	Liver basal	70	15.2		3.86	0.65	45.7	471
53	Liver basal + gelatin, 70 gm. + cystine, 4.2 gm. + phenylalanine, 4.2 gm.	129	15.4	3.5	3.93	0.64	46.2	473
54	Liver basal	69	12.1		3.85	0.72	47.7	429

* Estimated basal output per week equals 12 gm. plasma protein.

† Fed 60 gm. gelatin—recalculated on basis of 70 gm.

TABLE 2-*a* (*continues Table 1-a*)

Weight and Nitrogen Balance

Dog 36-196.

Period 7 days	Diet	Weight	Nitrogen balance: Intake, in diet	Intake, in excess R.B.C. injected	Output, in plasma	Output, in urine	Output, in feces	Intake minus output
		kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
29	Liver basal + gelatin, 70 gm. + cystine, 4.2 gm.	9.2	22.2	3.7	2.6	13.5	3.1	+6.7
30	Liver basal	9.2	11.2	1.1	2.3	8.4	2.5	−0.9
31*	Liver basal + gelatin, 70 gm. + cystine, 4.2 gm. + tryptophane, 2.8 gm.	9.2	22.6	2.2	3.5	12.6	2.4	+6.3
32	Liver basal	9.1	11.2	0.6	3.7	10.6	1.7	−4.2
33	Liver basal	9.1	11.2	0.8	1.8	9.7	2.3	−1.8
34	Liver basal + gelatin, 70 gm. + tyrosine, 4.2 gm. + tryptophane, 2.8 gm.	8.9	22.5	1.7	2.1	17.7	3.0	+1.4
35	Liver basal	8.9	11.2	2.2	2.0	9.3	2.4	−0.3
36	Liver basal + gelatin, 70 gm. + tryptophane, 2.8 gm. + tomatoes, 245 gm.	8.8	22.7	2.4	2.3	17.0	2.3	+3.5
37	Liver basal (anaphylactic shock)	8.2	9.3	1.1	1.3	13.0	1.1	−5.1
38	Liver basal + liver, 350 gm.	8.6	22.4	0.7	0.5	10.2	3.3	+9.1
39	Liver basal + liver, 350 gm.	8.9	22.4	3.7	2.8	11.6	2.7	+9.0
40	Liver basal + liver, 350 gm.	9.1	22.4	0.5	0.8	11.3	2.7	+8.1
41	Liver basal	8.9	11.2	6.0	4.3	11.0	2.5	−0.6
42	Liver basal	9.1	11.2	1.9	2.5	9.5	2.8	−1.7
43	Liver basal	8.9	11.2	1.9	2.0	9.1	2.9	−0.9
44	Liver basal + gelatin, 70 gm. + tyrosine, 4.2 gm. + cystine, 4.2 gm.	8.8	22.6	−0.7	3.4	13.3	3.4	+1.6
45	Liver basal	8.8	11.2	1.3	2.9	8.2	2.8	−1.4
46	Liver basal	9.0	11.2	1.4	2.1	7.4	2.8	+0.3
47	Liver basal + hemoglobin by vein, 24.8 gm.	8.9	11.2	3.6†	2.2	9.8	2.9	−0.1
48	Liver basal	9.0	11.2	1.0	2.0	8.7	2.8	−1.3
49‡	Liver basal + gelatin, 70 gm. + methionine, 5.2 gm.	9.0	22.2+	2.0	2.4	13.4	3.6	+4.8
50	Liver basal	9.1	11.2	−2.0	2.3	7.7	3.5	−4.3
51	Liver basal + gelatin, 70 gm. + methionine, 5.2 gm. + tyrosine, 4.2 gm.	9.2	22.5	1.3	2.6	13.4	3.2	+4.6
52	Liver basal	9.0	11.2	0.1	2.5	8.5	2.9	−2.6
53	Liver basal + gelatin, 70 gm. + cystine, 4.2 gm. + phenylalanine, 4.2 gm.	9.2	22.6	1.9	2.6	13.4	3.2	+5.3
54	Liver basal	9.2	11.2	0.3	2.0	9.8	4.5	−4.8
	Totals for 54 periods (Tables 1-*a* and 2-*a*)..		762.1	106.0	123.5	593.0	134.4	+14.3

* See footnote to period 31, Table 2. The figures for nitrogen balance are correspondingly adjusted.

† Includes nitrogen in laked red blood cells injected intravenously.

‡ The dog consumed additional protein—see Clinical Experimental History.

basal ration the urinary nitrogen per week averaged 13.2 gm. and the plasma protein output 19 gm. We may say that about 4.4 gm. of nitrogen was conserved from wastage in the urine which might account for about 26 gm. of new body protein and actually 19 gm. appeared as plasma protein. It should be noted also that when gelatin was supplemented with cystine or methionine the urinary nitrogen averaged about 13.4 gm. per week although the plasma protein output was only 4 to 7 gm. per week. This may suggest some amino acid conservation in this dog with the production of some body protein but only a modest amount of plasma protein.

Clinical Experimental History.—Dog 36-196 (Tables 1 and 1-*a*; 2 and 2-*a*). An adult female beagle hound, weighing 10.5 kg., was fasted for one week. It was then given a liver basal diet which it consumed daily for the next 54 weeks except as herein noted. The diet consisted of raw pork liver, 50 gm.; cane sugar, 50 gm.; cornstarch, 50 gm.; cod liver oil, 25 gm.; salt mixture, 2 gm.; bone ash, 10 gm.; kaolin, 5 gm. When this basal diet was supplemented by gelatin the basal caloric intake was maintained by reducing the cane sugar given to 41 gm. The "protein-free" diet fed during the 13th period contained cane sugar, 77 gm.; dextrin, 25 gm.; lard, 23 gm.; butter fat, 9 gm.; salt mixture, 2 gm.; bone ash, 3 gm.; "Ryzamin B" concentrate, 0.4 gm. (*i.e.*, 32 international units of vitamin B_1); liver extract, parenteral,[1] 1 cc.; White's cod liver oil concentrate, 3 tablets (*i.e.*, about 9400 units of vitamin A and 940 units of vitamin D). Consumption of these diets was uniformly complete, except during period 37. The dog was sensitized to horse serum and when the shocking dose was given in the 37th period a severe characteristic reaction occurred. 3 weeks (38 to 40) were allowed for recovery from this disturbance, during which time the intake of liver in the diet was doubled to 100 gm. daily. Segments of *Tenia pisiformis* appeared in the feces and arecoline hydrobromide, 18 mg., was given in period 38, yielding 7 scolices and their bodies. Plasmapheresis, resumed in the 38th week after a 10 days' rest, was not performed in the 40th week. The clinical condition of the dog was good.

The *hemoglobin* given intravenously during period 47 was prepared from fresh washed red blood cells (dog) laked in distilled water. The three injections given each day were at least 6 hours apart and the exact quantities of hemoglobin given, as determined by the acid hematin method, varied from 0.97 to 1.66 gm. After 5 of the 19 injections there was evidence of slight hemoglobinuria both grossly and by benzidine test; during and after the other 14 injections the benzidine test was negative. Clinically the dog gave not the slightest reaction.

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[1] We are indebted to Eli Lilly and Company for this material.

During period 49 the dog consumed about 5 gm. additional protein in ground beef and salmon bread inadvertently available. At most this quantity of these proteins could produce only 1 gm. plasma protein, and such an allowance has been made in calculating the percentage utilization of gelatin plus methionine given in Table 4.

In Tables 3 and 3-*a* observations are recorded on another dog (37-6) tested under similar conditions and with some of the same amino acid and gelatin combinations as noted in the experiments tabulated above. The experiments in the two dogs are in complete agreement. This dog (37-6, Tables 3 and 3-*a*) had a rather large reserve store of plasma protein building material which required 11 weeks for complete removal. During the 11 periods including the first period of fasting, the dog put out 270.7 gm. of plasma protein and if we deduct 190 gm. for the 10 weeks of basal diet intake we record 80 gm. as the reserve, using 19 gm. as the basal weekly plasma protein output. During these periods (2 to 11 inclusive) the diet intake was 84 gm. of protein and the basal diet was determined on subsequent periods on a protein intake of 70 gm. (Table 3). If we correct for this larger protein intake the basal output for periods 2 to 11 should have been 22.8 gm. plasma protein. Using this figure the reserve store of plasma protein building material would be 43 gm. but the anaphylactic shock comes into the picture. With anaphylactic shock the body proteins suffer injury and there is an excess of nitrogen eliminated in the urine (not shown in Table 3-*a* but refer to Table 2-*a*). This may well signify removal of protein material from the reserve stores and to explain an increase in urinary nitrogen of 4 gm. would require 24 gm. of body protein. The true figure of plasma protein reserve store probably falls between 43 and 80 gm. for this dog.

The estimate of the *basal output* of plasma protein in dog 37-6 on a protein intake of 70 gm. per week is an average of periods 12, 13, 15, and 20 or 18.8 gm.

Protein utilization was very efficient in dog 37-6 (Tables 3 and 3-*a*), much more so than in the other dog (36-196). On the same diet and the same protein and caloric intake this dog (37-6) was able to gain slightly in weight, produce more new plasma protein, and eliminate less urinary nitrogen. Both dogs were in nitrogen equilibrium. This dog (37-6) required only 4 gm. of diet protein to produce 1 gm.

TABLE 3

Blood Plasma Protein Regeneration

As Modified by Gelatin with Tryptophane, Cystine, and Phenylalanine

Dog 37-6.

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. hematocrit, average	Plasma volume
					Total protein	A/G ratio		
		gm.	gm.	gm.	per cent		per cent	cc.
	Kennel				5.69	1.71	44.8	478
1	Fasting	0	29.2		5.24	—	48.7	—
2	Liver basal	84	28.4		4.16	0.95	49.3	414
3	Liver basal	84	23.1		3.95	1.02	46.8	438
4	Liver basal	84	22.1		4.03	0.98	47.6	423
5	Liver basal (anaphylactic shock)	72	19.5		4.22	1.19	46.1	557
6	Liver basal	84	29.1		4.30	0.78	45.0	434
7	Liver basal	84	27.4		4.08	0.84	47.4	419
8	Liver basal	84	24.0		3.83	0.81	47.5	433
9	Liver basal	84	24.5		3.95	0.91	45.6	468
10	Liver basal	84	19.2		3.99	0.82	46.8	472
11	Liver basal	84	24.2		4.02	0.84	47.3	442
12	Liver basal	70	19.9		3.88	0.85	48.7	434
13	Liver basal	70	16.9		3.90	0.71	48.3	410
14	Liver basal + gelatin, 69 gm.	127	16.9	1.0±	3.85	—	45.6	444
15	Liver basal	70	18.8		3.93	0.79	46.8	460
16	Liver basal + gelatin, 70 gm. + tryptophane, 2.8 gm.	129	18.7	2.0	3.97	0.86	47.4	484
17	Liver basal	70	21.3		4.03	0.76	44.6	477
18	Liver basal + gelatin, 70 gm. + cystine, 4.2 gm. + tryptophane, 2.8 gm.	129	25.7	24.0	4.32	1.16	46.1	—
19	Liver basal	70	31.0		4.51	0.72	47.2	431
20	Liver basal	70	23.5		3.99	0.89	46.0	462
21	Liver basal	70	19.8		4.03	0.81	46.8	434
22†	Liver basal + gelatin, 70 gm. + cystine, 4.2 gm. + phenylalanine, 4.2 gm.	129	22.5	7.5	4.01	0.61	47.6	—
23	Liver basal	70	23.0		4.11	—	46.6	—

* On a protein intake of 70 gm. the estimated basal output of plasma protein equals 19 gm. per week.

† One day's diet was omitted from this period. For comparative purposes, the figures given for protein intake and output are 7/6 of those obtained.

TABLE 3-*a*

Weight and Nitrogen Balance

Dog 37-6.

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	11.6						
1	Fasting	10.3	0.0	−0.3	4.8			
2	Liver basal	10.4	13.4	−0.1	4.7			
3	Liver basal	10.7	13.4	2.8	3.8			
4	Liver basal	10.7	13.4	0.8	3.6			
5	Liver basal (anaphylactic shock)	10.6	11.5	2.6	3.2			
6	Liver basal	10.8	13.4	2.4	4.8	6.7	2.9	+1.4
7	Liver basal	10.9	13.4	1.4	4.5	8.3	1.2	+0.8
8	Liver basal	11.1	13.4	−2.0	3.9	6.3	3.8	−2.6
9	Liver basal	11.3	13.4	1.0	4.0	8.3	3.0	−0.9
10	Liver basal	11.4	13.4	0.7	3.2	7.7	1.4	+1.8
11	Liver basal	11.6	13.4	−0.2	4.0	7.4	1.9	−0.1
12	Liver basal	11.7	11.2	−1.9	3.3	6.6	1.7	−2.3
13	Liver basal	11.8	11.2	−1.1	2.8	6.8	2.0	−1.5
14	Liver basal + gelatin, 69 gm.	12.0	21.4	−0.8	2.8	15.5	2.7	−0.4
15	Liver basal	12.0	11.2	−0.6	3.1	8.3	2.8	−3.6
16	Liver basal + gelatin, 70 gm. + tryptophane, 2.8 gm.	12.3	22.2	2.6	3.1	15.8	1.9	+4.0
17	Liver basal	12.5	11.2	2.5	3.5	9.1	2.1	−1.0
18	Liver basal + gelatin, 70 gm. + cystine, 4.2 gm. + tryptophane, 2.8 gm.	12.5	22.6	7.8	4.2	13.3	2.8	+10.1
19	Liver basal	12.7	11.2	3.3	5.0	10.0	3.4	−3.9
20	Liver basal	13.1	11.2	4.6	3.8	8.7	2.6	+0.7
21	Liver basal	12.7	11.2	1.6	3.2	9.8	2.4	−2.6
22†	Liver basal + gelatin, 70 gm. + cystine, 4.2 gm. + phenylalanine, 4.2 gm.	12.4	22.6	2.3	3.7	19.1	3.1	−1.0
23	Liver basal	—	11.2	1.4	3.8	8.8	2.3	−2.3
	Totals, periods 6 to 23................		258.8	25.0	66.7	176.5	44.0	−3.4

† See footnote to period 22, Table 3. The figures for nitrogen balance are 7/6 of those obtained.

plasma protein—a potency ratio of 4 and an efficiency percentage of 27. Dog 36-196 required 6 gm. of diet protein to produce 1 gm. plasma protein under identical circumstances (Table 4). This dog (37-6) during basal periods put out an average of 7.9 gm. urinary nitrogen while dog 36-196 eliminated 8.8 gm. urinary nitrogen per week under identical conditions. Dog 37-6 which conserved its proteins to better advantage was a quiet, calm, inactive dog, but dog 36-196 was very active at all times. It is possible that this difference in activity was a factor but we do not wish to stress the point. We have no further comments to make relative to such individual differences except that they exist. It has been noted that some dogs can produce more new hemoglobin during experimental anemia than other dogs under identical conditions (14) and it is a commonplace that some dogs can run faster than others. Undoubtedly many unknown factors are involved in these individual differences.

The gelatin amino acid supplements gave responses similar to those recorded in Tables 1 and 2. Gelatin plus cystine and tryptophane gave a large production of plasma protein (24 gm.) in period 18, Table 3. It is significant that the albumin/globulin ratio showed a sharp rise to 1.16 indicating presumably a sharp increase in albumin production. This is a familiar response (7) to the feeding of proteins favorable for plasma protein production (usually meat proteins). In contrast, this dog with gelatin feeding alone showed no increased output of plasma protein (period 14, Table 3) and when the gelatin was supplemented with tryptophane the increase was scarcely recognizable (period 16, Table 3).

Phenylalanine obviously cannot effectively replace tyrosine in the potent cystine, tyrosine, gelatin mixture (Table 1, period 23). The phenylalanine, cystine, gelatin mixture showed an increase of 7.5 gm. plasma protein (period 22, Table 3) or about one-third the amount to be expected from the tyrosine, cystine, gelatin supplement. The urinary nitrogen figures are completely out of line with all other observations, and we have no adequate explanation.

The urinary nitrogen figures in periods 14, 16, and 18, Table 3-*a*, are in harmony with those recorded above (Tables 1-*a* and 2-*a*). With gelatin alone or gelatin plus tryptophane the urinary nitrogen is higher than is observed with the cystine, tryptophane, gelatin mixture, favorable for plasma protein production.

Clinical Experimental History.—Dog 37-6 (Tables 3 and 3-*a*). An adult female mongrel pointer, weighing 11.6 kg., was fasted for one week. For the succeeding 10 weeks it was fed daily a basal ration consisting of raw pork liver, 60 gm.; cane sugar, 120 gm.; lard, 15 gm.; cod liver oil, 15 gm.; salt mixture, 2 gm.; bone ash, 10 gm. In the 5th week horse serum antigen given intravenously to the previously sensitized animal, as an aside to the main experiment, produced a severe characteristic shock. Within 24 hours the dog had recovered for the most part and appeared entirely normal within 3 days. Beginning with period 12 the amount of liver in the basal diet was reduced to 50 gm. daily and the cane sugar was increased to 124 gm. During the periods in which gelatin supplement was given the quantity of cane sugar in the diet was reduced to 115 gm. The appetite for the basal diet lagged by the 21st period and during the 22nd week a total of exactly one day's diet was not eaten. Since a considerable gain in weight had occurred the lard was deleted from the diet beginning with the last 2 days of the 21st period; and the carbohydrate was reduced to cane sugar, 60 gm., cornstarch 30 gm. during the 23rd period. The experiment was discontinued at the close of this period.

DISCUSSION

Plasma protein is not to be looked upon as static material whose sole function is related to the osmotic balance of circulating and tissue fluids. This plasma protein material has a *turn over* of unknown value but the body can be kept in nitrogen equilibrium on a non-protein diet plus adequate plasma given intravenously (2). In other words the plasma protein can replace the body protein wear and tear and in this sense is active in nutrition under such conditions. It is obvious therefore that information as to plasma protein production is of significance relating to body protein exchange and metabolism (12).

In a comprehensive review (11) Rose shows that for *growth* the amino acids phenylalanine and methionine are indispensable but tyrosine and cystine are dispensable. The experiments above, summarized in Table 4, indicate that for *plasma protein building* methionine cannot take the place of cystine as a supplement to gelatin and phenylalanine cannot replace tyrosine in the same type of reaction.

It would seem that the urge to produce plasma protein in these depleted dogs was maximal and the dog uses the liver protein in the basal diet quite efficiently—4 to 6 gm. of liver protein being required to produce 1 gm. of plasma protein. Gelatin supplemented with cystine and tyrosine is used as efficiently as beef serum itself to build new plasma protein, 3 to 4 gm. of gelatin protein being needed to produce 1 gm. of plasma protein. When gelatin is supplemented with cystine and phenylalanine or methionine and tyrosine, it is utilized poorly and it requires about 9 gm. of gelatin protein to produce 1 gm. of new plasma protein (Table 4). Gelatin alone does not contribute to the building of new plasma protein.

TABLE 4

Summary of Experiments on the Influence of Certain Amino Acids in Plasma Protein Formation

	Efficiency in plasma protein formation: Protein output per cent of protein intake				
Reference	Tables 1, 2	Table 3	(5)	(6)	(9)
Dog No.	36-196	37-6	33-11	34-152	32-130
Basal Diets					
Liver	17	27	22		
Kidney				22	
Potato-bran					24
Supplements					
Beef serum (best protein yet tested)					38
Gelatin + cystine + tyrosine + tryptophane	34				
Gelatin + cystine + tyrosine	39, 25				
Gelatin + cystine + tryptophane	32	41			
Gelatin + cystine	10				
Gelatin + tyrosine	5				
Gelatin + tryptophane	3±	3	33		
Gelatin + tyrosine + tryptophane	1				
Gelatin + cystine + phenylalanine	6	13			
Gelatin + methionine + tyrosine	12				
Gelatin + methionine	5				
Gelatin		2±	9		
Tryptophane	0*		0*		
Lysine				0*	
Cystine + glycine + glutamic acid				50*	
Histidine + lysine + arginine				26*	

* These figures represent the percentage increase in the basal plasma protein output induced by the amino acid supplement.

Valine should be mentioned even if we have no experiments with this amino acid to report at this time. Valine is listed by Rose (11) as indispensable for growth. What its significance may be relating to plasma protein production we cannot say but certainly it does not appear to be indispensable as a gelatin supplement. Gelatin supplemented with cystine and tyrosine is as potent as beef serum in the building of new plasma protein in these depleted dogs and it is hard to imagine that valine would add to the potency of this gelatin mixture. However, it must be put to the biological test.

SUMMARY

When blood plasma proteins are depleted by bleeding with return of the washed red blood 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. By the introduction of variables into their standardized existence insight into the formation of plasma proteins can be obtained.

The liver basal diet maintains health in such hypoproteinemic dogs during periods as long as a year. 17 to 27 per cent of its protein content (entirely liver protein) is presumably converted into plasma protein.

Gelatin alone added to the liver basal diet causes very little if any extra plasma protein production.

The addition to gelatin of cystine, or tyrosine, or tryptophane, or of both tyrosine and tryptophane has little or no effect on its potency for plasma protein production.

When gelatin is supplemented by cystine and either tryptophane or tyrosine, 25 to 40 per cent of the protein content of the combination is converted into plasma protein—an efficiency equaling that of any protein hitherto tested.

Preliminary experiments indicate that methionine cannot substitute for cystine nor can phenylalanine substitute for tyrosine in the efficient combination of gelatin plus cystine plus tyrosine.

Laked red blood cells given by vein afford little or no material for plasma protein formation.

When the *reserve stores* of plasma protein building material are exhausted the dog can form little if any plasma protein during protein-free diet periods.

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