BLOOD PLASMA PROTEIN PRODUCTION AND UTILIZATION*

The Influence of Amino Acids and of Sterile Abscesses

By S. C. MADDEN, M.D., C. A. FINCH, W. G. SWALBACH, and G. H. WHIPPLE, M.D.

(From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York)

(Received for publication, December 21, 1939)

This research program on plasma proteins points toward a more complete understanding of these circulating proteins and their intimate relationship to food proteins and body cell (fixed) proteins. It is obvious from experiments below (Table 2) and others published earlier (5) that the plasma proteins given by vein can supply all protein needs of the body during a long protein fast. This indicates the importance of the plasma proteins in the internal protein metabolism of the body and supplies us with a valuable experimental approach to many problems relating to protein construction within the body.

Plasma protein production can be controlled by diet. In such dietary control some food proteins are potent and others relatively unsuitable for manufacture of new plasma protein. Such differences may in part be related to the different amino acid make-up of the different proteins. It is now apparent that certain amino acids are very important in the production of new plasma protein—cystine being of prime importance and probably also leucine and glutamic acid. Amino acids important in plasma protein formation are not necessarily those essential for total body growth and maintenance (17, 18). For body growth and maintenance *methionine can furnish* all the sulfur-containing amino acid needed. In plasma protein formation, however, it is indicated in the experiments below that *cystine better satisfies* the demand for sulfur-containing amino acids.

The normal dog has a *reserve store* of plasma protein building materials—insulation against the sharp winds of adversity—and depletion of this reserve renders the animal less resistant to infection (12) or intoxication (13, 14). Moreover the presence of an infection may disturb the plasma-forming body mechanism and favor hypoproteinemia—a vicious circle. The same observation has been made in experimental anemia in the dog

* We are indebted to Eli Lilly and Company for aid in this work.

where new hemoglobin formation on a favorable diet may be completely inhibited by infection (16).

In the following experiments it is assumed that hypoproteinemia offers a strong, steady stimulus to plasma protein regeneration. Daily plasmapheresis and the constant basal diet aim to keep the plasma protein concentration close to 4 per cent. After this level has been achieved for many weeks the amount of plasma protein removed each week is relatively constant, a product of the constant basal diet. In the initial weeks the amount of plasma protein removed is greater by the amount related to the previous dietary experience of the animal. This initial excess is called the *reserve store* of plasma protein building materials. Usually the reserve store is equivalent to one to two times the quantity of protein in the circulating plasma. The whole subject of plasma protein formation and utilization has been recently reviewed and discussed (9).

Methods

Plasmapheresis as used in this paper means the removal of whole blood (bleeding) and the return of washed normal red blood cells suspended in a saline solution. The procedures used in obtaining the data reported here are those fully described or referred to in a previous communication (7) with the following exception. The plasma given by vein to dog 36-211 was obtained from heparinized (20 to 30 mg. per 100 cc.) blood from healthy donor dogs. About 150 cc. of plasma were injected each day, divided into a morning and an afternoon dose. Kjeldahl nitrogen determinations were done on all plasma used to determine the protein content.

By macro Kjeldahl analysis and the conversion factors of Jones (6) the protein content is calculated for raw pork liver, 20 per cent; gelatin, 83.8 per cent; zein, 91.4 per cent; casein (according to Hammarsten), 87.9 per cent; casein, technical (Casein Manufacturing Company of America), 87.9 per cent; salmon, 19 per cent; salmon bread (21), 11 per cent. Liver extact (Lilly 343) contains 8.6 per cent nitrogen but less than half of it is thought to have protein-forming potentialities. The zein is from corn gluten and was prepared by the Harris Laboratories, Tuckahoe, New York, according to the method of Osborne and Vickery.

The crystalline amino acids used are the naturally occurring types except for racemic mixtures of methionine, isoleucine, and threonine.

EXPERIMENTAL OBSERVATIONS

The experiments represent continuous day after day observations on two dogs over long periods of time. For consecutive 7 day periods the daily determinations are totaled or averaged and recorded in the following tables. Pertinent data not included in the tables may be found in the accompanying Clinical Experimental Histories.

Interesting results from the *feeding of amino acid mixtures* are noted in Tables 1 and 1-*a* and are summarized in Table 1-*b*. Each of the six combi-

nations of amino acids induced a considerable excess production of plasma protein. In fact in each instance the excess plasma protein amounts roughly to a *doubling of the basal output.*

In comparing and contrasting the different amino acid mixtures it is noted that those with the incomplete proteins, zein and gelatin, are no more effective than those composed of pure amino acids. The relatively simple combination (D), of leucine plus cystine, glutamic acid, and glycine, is just as effective as the complex mixture A, of zein, cystine, tryptophane, lysine, glycine, and threonine. The latter combination (A), is just as effective when threonine is omitted, as in combination B. Furthermore, the addition to combination D of lysine, arginine, and isoleucine or of tyrosine does not significantly improve the value of that mixture (see combinations E and F). In view of the high leucine and glutamic acid content of zein, it is probably safe to consider *leucine, cystine, glutamic acid, and glycine as the common denominator* of all five combinations.

Cystine and tyrosine together with gelatin have increased the basal output in this same dog 191 per cent on one occasion and 121 per cent on another (8). *Methionine,* in replacing cystine in this combination, was a poor substitute on the first attempt (8), increasing the basal output only 57 per cent. In its second trial (combination C, Table 1-*b*), twice as much methionine was given and the basal output was increased 96 per cent, still *considerably below* the better and somewhat less than the poorer of the cystine tests.

The quantities of methionine and cystine given should be compared. A total of 10.5 gm. *dl*-methionine contains about 4 times as many molecules as 4.2 gm. *l*-cystine and of course twice as many sulfur atoms. Rose states that the *d*- form of methionine is an effective substitute for the natural *l*- form in growth (17). Therefore, *if* this substitution holds for plasma protein production, in the quantities given methionine should be twice as effective as cystine in any reaction in which they are interchangeable. Obviously this is not the case.

Zein is notably and totally ineffective in plasma protein formation, even when given to a dog on a non-deficient diet (period 15, Tables 1 and 1-*a*). A similar inadequacy has been shown previously for gelatin (8). The extra nitrogen ingested as zein (8.8 gm.) is rather completely accounted for in excess urinary and fecal nitrogen. It appears from the fecal nitrogen increase that about 35 per cent of the zein protein fed was not digested sufficiently to be absorbed from the intestinal tract. This is also true *when amino acids supplemented the zein* in periods 9 and 12, but here it appears that the absorbed nitrogen from zein, about 6.6 gm., has been at

TABLE 1

Certain Amino Acids Increase Plasma Protein Production
Zein Is Ineffective

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.*
	Kennel				6.26	1.22	41.5	495
1	Fasting	0	26.3		5.97	1.16	45.7	454
2	Liver basal	70	30.0		4.65	1.12	47.8	488
3	Liver basal	70	22.7		4.08	0.92	49.0	488
4	Liver basal	70	18.5		4.05	0.78	49.3	443
5	Liver basal	70	18.4		3.96	0.82	50.9	477
6	Liver basal	68	15.2		3.93	0.70	52.0	—
7	Liver basal	70	15.0	68.0	3.98	0.76	52.7	425
8	Liver basal	68	12.3		4.07	0.81	51.1	466
9	Liver basal + combination A†	133	18.5	14.1	4.16	0.90	49.2	478
10	Liver basal	70	19.2		4.10	0.90	49.9	462
11	Liver basal	70	15.4		3.95	0.86	50.1	447
12	Liver basal + combination B†	133	19.9	18.4	4.25	0.92	49.5	506
13	Liver basal	70	21.4		4.14	0.90	49.0	485
14	Liver basal	70	16.1		3.97	0.91	50.6	445
15	Liver basal + zein	124	10.5		3.98	0.96	49.6	464
16	Liver basal	70	14.3		4.09	0.86	49.7	462
17	Liver basal + combination C†	129	17.6	12.5	4.10	1.00	50.4	476
18	Liver basal	70	19.0		4.16	0.91	49.8	413
19	Liver basal	70	14.9		4.04	0.84	48.7	—
20	Liver basal + combination D†	70	19.1	13.9	4.07	1.08	48.9	420
21	Liver basal	70	20.2		4.02	0.83	50.2	—
22	Liver basal	70	14.6		3.86	0.71	49.5	446
23	Liver basal	70	12.0		3.88	0.70	49.7	460
24	Liver basal + combination E†	70	16.6	15.2	3.98	0.81	50.4	456
25	Liver basal	70	22.9		4.09	0.81	49.6	—
26	Liver basal	70	14.7		3.89	0.77	49.5	—
27	Liver basal + combination F†	70	19.0	15.8	3.96	0.98	49.9	—
28	Liver basal	70	21.9		3.93	0.75	48.8	489
29	Liver basal	70	13.9		3.82	0.83	47.7	462

* Estimated basal output per week equals 13 gm. plasma protein.

† See Table 1-*b*.

least three-fourths conserved. This statement presumes that the amino acid nitrogen (2.0 to 2.5 gm.) is completely retained and that the nitrogen eliminated relative to the basal diet remains the same. Actually we vis-

TABLE 1-*a*

Weight and Nitrogen Balance

Dog 36-196.

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	10.9	0.0	5.4	4.3	15.7	0.0	−14.6
2	Liver basal	10.5	11.2	3.6	4.9	13.4	3.2	−6.7
3	Liver basal	10.5	11.2	3.5	3.8	11.7	2.3	−3.1
4	Liver basal	10.1	11.2	3.8	3.0	10.5	2.2	−0.7
5	Liver basal	10.1	11.2	3.7	3.0	9.7	2.4	−0.2
6	Liver basal	10.3	10.9	2.2	2.5	9.7	2.2	−1.3
7	Liver basal	10.0	11.2	3.1	2.5	9.3	2.2	+0.3
8	Liver basal	9.5	10.9	0.5	2.0	11.0	2.8	−4.4
9	Liver basal + combination A†	9.9	24.0	4.1	3.0	10.2	6.4	+8.5
10	Liver basal	9.8	11.2	1.1	3.2	8.5	2.8	−2.2
11	Liver basal	9.9	11.2	1.1	2.5	8.5	2.3	−1.0
12	Liver basal + combination B†	10.0	23.5	2.7	3.3	10.0	6.0	+6.9
13	Liver basal	9.9	11.2	2.3	3.5	8.7	2.3	−1.0
14	Liver basal	9.9	11.2	1.8	2.7	8.9	2.4	−1.0
15	Liver basal + zein	9.8	19.9	0.2	1.7	13.2	6.8	−1.6
16	Liver basal	9.9	11.2	3.0	2.3	7.8	2.7	+1.4
17	Liver basal + combination C†	10.0	23.1	1.0	2.9	15.9	2.5	+2.8
18	Liver basal	10.0	11.2	1.8	3.1	8.4	2.3	−0.8
19	Liver basal	10.1	11.2	−0.4	2.4	9.1	3.9	−4.6
20	Liver basal + combination D†	10.2	15.8	1.9	3.1	8.4	2.7	+3.5
21	Liver basal	10.2	11.2	2.3	3.3	9.1	2.4	−1.3
22	Liver basal	10.3	11.2	0.6	2.6	8.5	2.8	−2.1
23	Liver basal	10.3	11.2	2.0	2.0	9.5	2.3	−0.6
24	Liver basal + combination E†	10.4	18.3	2.0	2.7	9.5	2.1	+6.0
25	Liver basal	10.4	11.2	−1.0	3.8	8.4	2.3	−4.3
26	Liver basal	10.5	11.2	1.7	2.4	9.7	2.6	−1.8
27	Liver basal + combination F†	10.7	16.4	0.6	3.1	9.8	2.6	+1.5
28	Liver basal	10.7	11.2	−1.4	3.6	8.1	2.6	−4.5
29	Liver basal	10.6	11.2	0.4	2.3	8.9	2.3	−1.9
	Totals.....		375.6	53.6	85.5	290.1	82.4	−28.8

† See Table 1-*b*.

ualize the complicated process as involving the conservation of some amino nitrogen from the diet liver, as well as from zein and the added amino acids. It may be that none of the zein nitrogen is conserved for plasma protein

production and that the liver of the basal diet furnishes all of the amino acids needed to combine with those fed and produce excess plasma protein, as is apparently true for the experiments with combinations D, E, and F.

The *basal output* for dog 36-196 in the experiments reported in Tables 1 and 1-*a* is estimated at 13 gm. plasma protein per week, slightly higher than the 12 gm. basal in a previous report (8). This estimate is based

TABLE 1-*b*

Amino Acid Combinations Double Plasma Protein Production

Cystine, Leucine, and Glutamic Acid of Prime Importance

Dog 36-196.

Amino acid combinations with zein or gelatin	Amount fed		Increase above basal output	Amino acid combinations	Amount fed		Increase above basal output
	Total	Nitrogen			Total	Nitrogen	
	gm.	*gm.*	*per cent*		*gm.*	*gm.*	*per cent*
A				D			
Zein	70.0	10.30		*l*-Leucine	21.0	2.24	
l-Cystine	4.2	0.49		*l*-Cystine	7.0	0.82	107
l-Tryptophane	2.1	0.29	108	*d*-Glutamic acid	8.4	0.80	
d-Lysine $(HCl)_2$	6.3	0.81		Glycine	4.2	0.78	
Glycine	2.1	0.39					
dl-Threonine	4.2	0.50		E			
				l-Leucine	21.0	2.24	
B				*l*-Cystine	7.0	0.82	
Zein	70.0	10.30		*d*-Glutamic acid	8.4	0.80	
l-Cystine	4.2	0.49		Glycine	4.2	0.78	117
l-Tryptophane	2.1	0.29	142	*d*-Lysine $(HCl)_2$	7.0	0.90	
d-Lysine $(HCl)_2$	6.3	0.81		*d*-Arginine HCl	4.2	1.12	
Glycine	2.1	0.39		*dl*-Isoleucine	4.2	0.45	
C				F			
Gelatin	70.0	10.57		*l*-Leucine	21.0	2.24	
dl-Methionine	10.5	0.99	96	*l*-Cystine	7.0	0.82	
l-Tyrosine	4.2	0.33		*d*-Glutamic acid	8.4	0.80	121
				Glycine	4.2	0.78	
				l-Tyrosine	7.0	0.54	

largely on the outputs of the periods immediately prior to the feeding of test supplements. The supplements given do not *carry over* their effects beyond a 2 weeks' after period, as is evident in the test periods 20 to 23. On this basis it is logical to assume that of the 146 gm. plasma protein removed during the first 7 periods, 78 gm. arises from the basal diet and the balance of 68 gm. is related to the *reserve store*. Comparison with the previous depletion data for this dog (8) shows the present experiment to give a

larger reserve store and a higher initial plasma protein concentration, in accordance with our original observations (7). The size and nature of the reserve store has been more fully discussed elsewhere (9).

Weight was well maintained after the initial fasting period. If one regards the negative nitrogen balance of the first 2 periods as related to the establishment of equilibrium under a lower intake level, the animal was in good balance for the remaining 27 periods.

Clinical Experimental History.—Dog 36-196 (Tables 1, 1-*a*, and 1-*b*). An adult female beagle hound used in similar experiments previously (8) was in good condition and weighed 11.6 kg. after an interval of 19 weeks on a diet of hospital table scraps. The daily liver basal diet consisted of raw pork liver, 50 gm.; cane sugar, 120 gm.; lard, 25 gm.; cod liver oil, 5 gm.; salt mixture (11), 2 gm.; bone ash, 15 gm. The test supplements recorded in Table 1-*b* were divided into seven equal daily feedings added to the basal diet during the periods noted in Tables 1 and 1-*a*. When gelatin was added the basal cane sugar was reduced by 9 gm. 1 gm. of sodium bicarbonate was fed each day with combination E, period 24. Consumption of diet and supplements was complete except on one day during period 6, when 20 per cent was spilled, and one day during period 8, when slight diarrhea occurred and 20 per cent was left. The zein supplement of period 15 amounted to only 59.8 gm. and while consumption was complete, the appetite for the mixture lagged noticeably. Appetite was good during the gelatin feeding and continued so. The animal was in excellent condition at the termination of the experiment.

Tables 2 and 2-*a* record the data obtained during the plasma *depletion* of dog 36-211, the test weeks during standard hypoproteinemia, and the subsequent *repletion* of plasma protein. At the end of the period 3, a steady hypoproteinemia (about 4 per cent) was reached and subsequently maintained. In accomplishing this standardization, 72 gm. of plasma protein were removed, about 50 gm. more than can be credited to the protein fed. This 50 gm. is the *reserve store* of plasma protein building material. The weekly basal output attributable to the liver diet approximates 13 gm., as estimated from periods 4, 5, 6, 8, 9, 10, 12, 13, and 15. This represents the same efficiency of utilization of the liver basal protein exhibited by dog 36-196, Table 1, that is, about 19 per cent.

Casein replaced the liver in the basal diet during period 7 and was somewhat more efficiently used than liver protein, producing plasma protein equal to 33 per cent of the casein fed. In a previous test (4), 8 times as much casein was added to an already very ample basal diet, and only 12 per cent utilization was obtained. The results of testing a great variety of proteins in this laboratory and elsewhere have been recently summarized and discussed (9).

An *infection* of the eye smoldered and flared from period 2 to period 8

(see Clinical Experimental History, dog 36-211) but no appreciable alteration in plasma protein production is recorded. In addition, surgical wounds in the neck and groin were produced under ether anesthesia during the 6th

TABLE 2

Plasma Protein Production and Utilization

Fasting and Abscesses

Dog 36-211.

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
				Total protein	A/G ratio		
		gm.	*gm.*	*per cent*		*per cent*	*cc.*
	Kennel			6.06	1.38	46.3	371
1	Protein-free	2±	23.4	5.14	1.01	49.2	300
2	Protein-free, 3 days	1±					
	Liver basal, 4 days	40	24.9	4.47	0.99	50.8	—
3	Liver basal	70	23.6	4.23	1.05	51.3	295
4	Liver basal	70	11.8	3.83	0.71	50.3	307
5	Liver basal	70	11.6	4.01	0.66	49.0	299
6	Liver basal	67	13.4	4.21	0.62	50.0	333
7	Casein basal	74	24.3	4.42	0.50	51.1	315
8	Liver basal	70	13.4	4.03	0.62	53.3	242
9	Liver basal	67	13.9	4.02	0.61	52.2	299
10	Liver basal	70	13.4	3.89	0.55	48.4	—
11	Fasting, 2 days	0	3.0	3.85	0.55		
	Fasting + abscesses, 5 days	0	3.0	4.11	0.55	43.6	386
12	Liver basal	70	13.6	4.11	0.49	42.0	343
13	Liver basal	69	15.5	4.07	0.53	42.9	326
14	Fasting, 2 days	0	3.7	3.97			
	Fasting, 5 days	0	3.6	4.16	0.52	44.5	336
15	Liver basal	70	12.6	3.95	0.53	46.6	256
16	Protein-free + plasma by vein	62	4.4	5.47	0.99	42.3	382
17	Protein-free + plasma by vein	68	3.9	7.51	1.14	38.0	489
18	Liver basal + liver	210	2.7	6.86	0.95	35.8	338
19	Liver basal + liver	210	3.0	6.33	0.72	34.7	—
20	Liver basal + liver	210	2.9	6.03	0.84	37.0	377
21	Liver basal + liver	210	2.5	6.22	0.83	40.9	347

Estimated liver basal output per week equals 13 gm. plasma protein. Reserve store removed in periods 1 to 6 amounts to 49 gm.

week without noticeably influencing plasma regeneration. A slight increase in urinary nitrogen is observed (Table 2-*a*, period 6).

None of these injuries were of type or extent sufficient to promote the generalized reaction and impaired protein production previously noted with

turpentine inflammation and abscess formation (10). In the non-hypoproteinemic dog, the subcutaneous injection of turpentine produces the complete clinical picture of infection except for bacterial invasion—inflammation and abscess formation, fever, leucocytosis, and increased urinary

TABLE 2-*a*

Weight and Nitrogen Balance

Dog 36-211.

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.*
1	Protein-free	7.35	0.9	−0.1	3.8	9.6	—	−12.6
2	Protein-free, 3 days							
	Liver basal, 4 days	6.88	6.6	5.6	4.1	6.7	1.0	+0.4
3	Liver basal	6.99	11.2	1.3	3.9	11.5	1.5	−4.4
4	Liver basal	6.82	11.2	1.5	1.9	8.2	1.9	+0.7
5	Liver basal	6.82	11.2	−0.1	1.9	8.2	2.0	−1.0
6	Liver basal	6.85	10.7	0.7	2.2	10.0	1.6	−2.4
7	Casein basal	6.70	11.7	2.5	4.0	9.2	1.8	−0.8
8	Liver basal	6.82	11.2	0.3	2.2	7.7	1.5	+0.1
9	Liver basal	6.82	10.7	1.2	2.3	9.0	2.2	−1.6
10	Liver basal	7.02	11.2	1.3	2.2	8.7	2.5	−0.9
11	Fasting, 2 days	6.65	0.0	1.7	0.5	3.4		−2.2
	Fasting + abscesses, 5 days	6.53	0.0	0.6	0.5	8.2	*	−8.1
12	Liver basal	6.59	11.2	3.1	2.2	6.0	2.5	+3.6
13	Liver basal	6.59	11.0	6.8	2.6	8.7	2.5	+4.0
14	Fasting	5.85	0.0	6.6	1.2	10.0	*	−4.6
15	Liver basal	6.31	11.2	3.4	2.1	8.6	2.6	+1.3
16	Protein-free + plasma by vein	6.31	10.7	4.5	0.7	7.2	1.0	+6.3
17	Protein-free + plasma by vein	6.25	11.3	2.2	0.6	8.8	0.8	+3.3
18	Liver basal + liver	6.54	33.6	1.8	0.4	24.6	4.0	+6.4
19	Liver basal + liver	—	33.6	−1.5	0.5	33.0	4.6	−6.0
20	Liver basal + liver	7.18	33.6	0.6	0.5	20.7	4.3	+8.7
21	Liver basal + liver	7.33	33.6	0.5	0.4	25.7	4.1	+3.9
	Totals.....		286.4	44.5	40.7	253.7	42.4	−5.9

* Included in following period.

nitrogen (2). Moreover, it can be promptly terminated on the 3rd or 4th day with subsequent rapid healing. In the first observation on a hypoproteinemic dog (10), the systemic response to inflammation was marked by much depression of plasma protein production despite adequate intake of protein in the diet.

In a subsequent report (7), the influence of *infection upon plasma protein production during fasting* was studied, but, as then noted, the interpretation of the increased production was confused by the probability of the storage of protein immediately prior to the abscess period. The abscess experiment has been repeated in a very satisfactory manner in periods 8 to 15, Tables 2 and 2-*a*. The protein production during fasting and turpentine abscesses in period 11 is almost identical with that during the later fasting period 14. The periods before and after these tests are as perfectly base line as could be hoped for in such experiments. The urinary nitrogen during the abscess period 11 is only slightly above that of the strictly fasting control period 14. Figures are given for the first 2 days and the subsequent 5 days to indicate the extremely low level of production after much of the carry-over from the preceding basal period has been removed (in the first 2 days).

Table 2 shows also a rapid fall in the albumin:globulin ratio, which when observed in these experiments always is an index of potential trouble—infection or intoxication which all too frequently terminate a given experiment. In fact this dog did develop a panophthalmitis in periods 7 and 8 when the albumin:globulin ratio was close to 0.5. Note the rapid return of the albumin:globulin ratio to normal when no protein other than plasma protein by vein was given in periods 16 and 17.

At the close of the hypoproteinemia experimental period, the *repletion* mentioned above was accomplished by the injection of plasma by vein (see Methods). Thus in period 16 the plasma protein concentration was returned to normal (6.21 per cent) after 4 days and reached 6.80 per cent at the end of the week. The albumin:globulin ratio rose sharply and the nitrogen balance was positive in both injection periods. The animal remained in excellent clinical condition.

Nitrogen balance in non-hypoproteinemic (normal fasting) dogs has been repeatedly achieved *by plasma injection* (5, 15, 3) but in the present experiment the following observation not apparent from the tables is of particular interest. After 3 days and the injection of 35 gm. of plasma protein, the concentration had risen from 4.19 per cent to 5.71 per cent and the circulating mass of plasma protein from about 11.5 gm. to about 20 gm. About 26 gm. of plasma protein had vanished from the circulation. Obviously *plasma protein passes out of the normal capillary bed even during hypoproteinemia.* No single injection was sufficient to produce even momentary hyperproteinemia nor to raise the blood volume significantly above normal. It has been recorded previously (7) that plasma protein *building materials* may be formed from dietary protein and stored in preference to

removing an existent hypoproteinemia. This present observation is further evidence of the *importance of plasma protein in body nutrition* (9). Its physical rôle in the plasma has long been recognized.

Clinical Experimental History.—Dog 36-211 (Tables 2 and 2-*a*). An adult female beagle hound, weighing 7.35 kg., was started on a régime of daily plasmapheresis and given a "protein-free" diet consisting of cane sugar, 93 gm.; lard, 18 gm.; butter fat, 9 gm.; salt mixture (19), 1.5 gm.; bone ash, 2 gm.; cod liver oil concentrate (White's), 2 tablets; liver extract (Lilly 343), 1.6 gm.; thiamin chloride, 0.133 mg. This diet was well consumed for the first week but was refused during the second and replaced by one consisting of fresh pork liver, 50 gm.; cane sugar, 45 gm.; corn starch, 45 gm.; lard, 6 gm.; cod liver oil, 15 gm.; salt mixture (11), 2 gm.; bone ash, 10 gm.

A severe conjunctivitis of the left eye occurred spontaneously during this 2nd period but cleared very well by the 4th period. In the 6th period under ether anesthesia the right femoral artery was canulated, in order to make some blood pressure recordings, and post-operatively the wound healed cleanly. The conjunctivitis of the right eye, however, flared up again, developed into a panophthalmitis in the 7th period, but was fairly well healed in the 8th period, leaving a shrunken eyeball and opaque cornea. During the 7th period casein (prepared according to Hammarsten), 12 gm. per day, was substituted for liver in the basal diet and was completely consumed.

There was fasting for the entire 11th period and on its 2nd day a sterile inflammation was started in the left lateral thoracic region by the *subcutaneous injection of turpentine*, 0.8 cc. During the 5 days prior to this injection the rectal temperature ranged between 38.8°C. and 39.0°C. and the leucocyte count between 10,000 and 14,600. On the day following the injection the temperature was only 38.9°C. but the leucocyte count was 22,000. On the 2nd day following the injection the swelling began to soften, and a 2nd and similar injection of turpentine was made over the right thorax. During the week following the first injection, the leucocyte count varied between 22,000 and 26,600 but the temperature never rose above 39.3°C. 4 days after its inception each abscess was drained of thick sanguinopurulent material, 60 cc. and 25 cc. respectively. 10 days after the first turpentine injection the leucocyte count was 10,500 and the temperature 38.6°C. Clinically the dog withstood the fast and the abscesses well.

Plasmapheresis was largely discontinued after the 15th period. During periods 16 and 17 the "protein-free" diet given above was again fed, this time in conjunction with the daily intravenous injection of dog plasma (see Methods). The total plasma protein given by vein in period 16 was 60.2 gm. and in period 17 was 65.9 gm. The dog tolerated this régime very well. By qualitative test a trace of albumin was the most ever found in the urine during these 2 weeks. By Esbach's method less than 0.2 gm. of the weekly urinary nitrogen was protein nitrogen. The liver content of the basal diet was tripled to 150 gm. a day during periods 18 to 21, the corn starch reduced to 30 gm., and the lard completely omitted. The dog ate this diet well.

All experiments concerned with the influence of amino acids in plasma protein formation are recorded in Table 3. It should be noted that (*a*) *cystine*, with tryptophane or tyrosine, adds much plasma protein producing power to gelatin; (*b*) *cystine*, with tryptophane and the other amino acids

indicated, adds much plasma protein producing power to zein; (*c*) *cystine*, with glycine, glutamic acid, and leucine adds much potency to the liver

TABLE 3

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				Average percentage increase*
Reference	(8), Table 1	(8)	(7)	(10)	
Dog No.	36-196	37-6	33-11	34-152	
Basal Diets					
Liver	17	27	22		
Kidney				22	
Supplements					
Gelatin + cystine + tyrosine + tryptophane	34				168
Gelatin + cystine + tyrosine	39, 25				156
Gelatin + cystine + tryptophane	32	41			142
Gelatin + cystine	10				48
Gelatin + tyrosine	5				25
Gelatin + tryptophane	3±	3	33?		14
Gelatin + tyrosine + tryptophane	1				6
Gelatin + cystine + phenylalanine	6	13			34
Gelatin + methionine + tyrosine	12, 21				77
Gelatin + methionine	5				25
Zein + cystine + tryptophane + lysine + glycine + threonine	22				108
Zein + cystine + tryptophane + lysine + glycine	28				142
Tryptophane	0*		0*		0
Lysine				0*	0
Histidine + lysine + arginine				26*	26
Cystine + glycine + glutamic acid				50*	50
Cystine + glycine + glutamic acid + leucine	107*				107
Cystine + glycine + glutamic acid + leucine + isoleucine + arginine + lysine	117*				117
Cystine + glycine + glutamic acid + leucine + tyrosine	121*				121

* These figures represent the percentage increase in the basal plasma protein output induced by the supplements.

basal diet. Under certain conditions, therefore, cystine qualifies as a key amino acid in plasma protein regeneration. Leucine and glutamic acid are probably also of prime importance.

DISCUSSION

There is little to say and much to be done relative to amino acid feeding experiments. Rose is contributing the basic information which may ultimately lead to the determination of the specific biologic functions of each amino acid essential to life. He has shown (17, 18) that 9 or 10 specific amino acids will furnish all the nitrogen required by the rat and the dog. No doubt some or all of these will satisfy all nitrogen requirements for plasma protein synthesis. It is probable that those essential amino acids demonstrated by chemical analysis to be present in plasma protein molecules are essential for plasma protein formation.

This last belief was questioned in 1936 by Alcock (1). He felt that no known evidence adduced the commonly held conclusion that protein synthesis required specific amino acids for building stones. We believe that such evidence has subsequently been furnished. Previous experiments from this laboratory together with those presented in this paper, all summarized in Table 3, show that *quantitative differences in plasma protein synthesis are based on qualitative differences in the available amino acids.* It, therefore, appears probable that the synthesis of tryptophane or any other essential amino acid present in plasma protein is just as difficult for the body as its synthesis for any non-protein body need. Amino acids are probably not stored as such. *To make new plasma protein the body must have available or be able to synthesize promptly all the constituent amino acids.*

Certain amino acids found in protein no doubt can be synthesized by the body and then into protein. It may be, however, that protein synthesis proceeds more favorably when these certain amino acids are available preformed. Cystine may be such an amino acid. Methionine is apparently able to furnish the sulfur amino acid needs for bodily growth and maintenance, and cystine incapable (17). It is probable, however, that cystine itself better satisfies a large part of the sulfur amino acid requirement for plasma protein formation.

That abscesses appear to have a negative effect upon plasma protein production during fasting (Table 2, period 11), may not be wholly according to the expected pattern. One might argue that the abscess intoxication would *slow up* plasma protein production, or on the other hand that the excess of protein split products set free as the result of the abscess would make available material from which an *excess* of plasma protein could be formed. We may choose to believe that the algebraic sum of these two reactions may give the true explanation for the negative response observed in period 11, which corresponds to the control fasting period 14.

We have written much about that portion of the body protein which, upon its conversion into plasma protein, can be withdrawn during the initial stages of a plasmapheresis-induced hypoproteinemia. We call it the *reserve store* of plasma protein building materials and think of it as part of the more generally recognized (9) protein stores of the body. The evidence is mounting that this *reserve store is a bulwark against infection and some poisons*. When this reserve store is depleted, resistance to infection is lowered (12) and susceptibility to chloroform (14) and arsphenamine (13) poisonings may be greatly enhanced. Moreover, *in the absence of this reserve store* little or no new plasma protein is produced if sufficient infection (experimental turpentine inflammation) is present, whether the dog be given an ample protein diet (10) or be fasted (Table 2). If *reserve stores* of plasma protein producing materials *are present* in the body at the time of infection (7) production of plasma protein apparently may proceed as usual. This availability of the reserve store despite infection is further evidence of the readiness with which a certain portion of the body protein can be shifted to the site of body need (20). Such fluidity of body protein is further noted above in the plasma injection experiment.

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. Introduction of variables into their standardized life gives insight into the production of plasma protein.

Casein retested as the basal protein in the ration may show high yield of plasma protein, equal to 33 per cent of the protein fed. This equals the potency of liver protein (17 to 33 per cent) and approaches the utilization of plasma protein by mouth (40 per cent).

Zein has no effect upon plasma protein regeneration but when it is supplemented with cystine, tryptophane, lysine, and glycine, there is a doubling of the liver basal plasma protein production and a retention of the fed protein nitrogen. Threonine does not modify the above reaction.

Liver protein supplemented with cystine, leucine, glutamic acid, and glycine in the basal diet yields *double* the amount of new formed plasma protein compared with liver alone. This combination is then as potent as plasma protein itself when given by mouth—40 per cent utilization. Tyrosine or lysine, arginine, and isoleucine do not modify the above responses.

Methionine is not as effective as cystine in supplementing gelatin and tyrosine to produce plasma protein.

Cystine, leucine, and glutamic acid appear to be of primary importance in the building of new plasma protein in these experiments.

Plasma protein formation is dependent upon materials coming from the body reserve and from the diet. Given an exhaustion of the reserve store there is very little plasma protein produced during a protein fast (3 to 6 gm. per week). A turpentine abscess does not modify this fasting plasma protein reaction.

Homologous plasma given by vein will promptly correct experimental hypoproteinemia due to bleeding. It will maintain nitrogen equilibrium and replenish protein stores. Even during hypoproteinemia plasma protein may promptly pass out of the circulation to supply body needs for protein.

Perhaps the most significant concept which derives from all these experiments is the *fluidity* of the body protein (including plasma protein)—a ready give and take between the protein depots—a "dynamic equilibrium" of body protein.

BIBLIOGRAPHY

1. Alcock, R. S., *Physiol. Rev.*, 1936, **16,** 1.
2. Cooke, J. V., and Whipple, G. H., *J. Exp. Med.*, 1918, **28,** 223.
3. Daft, F. S., Robscheit-Robbins, F. S., and Whipple, G. H., *J. Biol. Chem.*, 1938, **123,** 87.
4. Holman, R. L., Mahoney, E. B., and Whipple, G. H., *J. Exp. Med.*, 1934, **59,** 251.
5. Holman, R. L., Mahoney, E. B., and Whipple, G. H., *J. Exp. Med.*, 1934, **59,** 269.
6. Jones, D. B., *U. S. Dept. Agric., Bureau Chem. and Soil, Bull. 183*, 1931.
7. Madden, S. C., George, W. E., Waraich, G. S., and Whipple, G. H., *J. Exp. Med.*, 1938, **67,** 675.
8. Madden, S. C., Noehren, W. A., Waraich, G. S., and Whipple, G. H., *J. Exp. Med.*, 1939, **69,** 721.
9. Madden, S. C., and Whipple, G. H., *Physiol. Rev.*, 1940, **20,** No. 2, in press.
10. Madden, S. C., Winslow, P. M., Howland, J. W., and Whipple, G. H., *J. Exp. Med.*, 1937, **65,** 431.
11. McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, 1918, **33,** 55.
12. McNaught, J. B., Scott, V. C., Woods, F. M., and Whipple, G. H., *J. Exp. Med.*, 1936, **63,** 277.
13. Messenger, W. J., and Hawkins, W. B., *Am. J. Med. Sc.*, 1940, **199,** 204.
14. Miller, L. L., and Whipple, G. H., *Am. J. Med. Sc.*, 1940, **199,** 216.
15. Pommerenke, W. T., Slavin, H. B., Kariher, D. H., and Whipple, G. H., *J. Exp. Med.*, 1935, **61,** 283.
16. Robscheit-Robbins, F. S., and Whipple, G. H., *J. Exp. Med.*, 1936, **63,** 767.
17. Rose, W. C., *Physiol. Rev.*, 1938, **18,** 109.
18. Rose, W. C., and Rice, E. E., *Science*, 1939, **90,** 186.
19. Wesson, L. G., *Science*, 1932, **75,** 339.
20. Whipple, G. H., *Am. J. Med. Sc.*, 1938, **196,** 609.
21. Whipple, G. H., and Robscheit-Robbins, F. S., *Am. J. Physiol.*, 1936, **115,** 651.