HEMOGLOBIN AND PLASMA PROTEIN

Simultaneous Production during Continued Bleeding As Influenced by Amino Acids, Plasma, Hemoglobin, and Digests of Serum, Hemoglobin, and Casein*‡

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The immediate objective of these experiments is the best possible substitute or supplement for plasma or whole blood as used in the clinic to combat shock, post-operative difficulties, and lack of absorption from the gastro-intestinal tract. The long term objective is a better understanding of the utilization and replacement of the blood proteins and their interrelation with other body proteins. We prefer to think of a *body protein pool* which includes blood proteins, cell proteins, and reserve store proteins, a pool within which there is constant interchange, the plasma protein acting as an active intermediary.

It is well established (2, 7) that plasma protein as whole plasma given by vein to the dog can supply all the protein requirements of the body over considerable periods of time. Therefore, it is logical to test these anemic and hypoproteinemic dogs with plasma by vein and with serum digests (Table 1). Assuming that whole plasma is the very best material to supply protein to the human body tissues whether normal or abnormal (shock, tissue injury, etc.), this human plasma is limited as to supply and is costly. It should be possible to substitute various materials for this optimum material (plasma) and *digests* have been rather extensively tested. Digests at times may give disturbing reactions, but the perfect digest may be forthcoming and will be of great value. *Amino acid mixtures* as tested resemble digests in general but are non-toxic and can be given (dogs and human beings) in large amounts by vein, subcutaneously, or intraperitoneally without reaction (8).

The dog can make large amounts of hemoglobin or plasma protein when given a large intake of suitable proteins by mouth, in fact the maximal output for a depleted 10 kilo dog approximates 10 gm. of either protein per day—that is one-third the normal circulating plasma protein per 24 hours. Following the usual administration of digests by vein (Table 1), the output of plasma protein may be only a fraction of the maximal plasma protein output (1/5 to 1/10 the circulating plasma protein), but this addition is valuable to the animal and

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might be life saving for a human patient under certain conditions. Given larger amounts of digest material presumably more plasma proteins would be produced.

This paper shows that digests of serum, hemoglobin, and casein are well utilized to build new plasma protein and hemoglobin. More important perhaps is the fact that the *amino acids necessary for growth* (Rose) (14) (see Table 4) supply the material out of which abundant plasma protein and hemoglobin can be produced. Some digests are improved by amino acid supplements and cystine is an important amino acid for this purpose. Amino acids other than the 10 necessary for growth may be proved to accelerate plasma protein or hemoglobin building and therefore be valuable supplements to digests or to such amino acid mixtures as promote growth. All these questions can be answered in time by means of suitable physiological experiments. The complete answer means much in the therapy of shock, tissue injury, post-operative states, and cachexia due to intestinal or other abnormalities.

Methods

The dogs used in these experiments (2 exceptions) were taken from the anemia colony (18). This strain has been maintained in this laboratory for 20 years—predominantly a white bull terrier strain with some coach and terrier blood—and these animals thrive on a cage régime. They are conditioned to a zero protein diet which is prepared in the form of a biscuit. On a low protein diet plus abundant iron, bleeding alone will reduce the normal hemoglobin and plasma protein levels, thus supplying the stimulus to form both of these proteins. Abundant iron intake means that hemoglobin production is limited by the protein intake (5). This double stress of anemia and hypoproteinemia is a severe strain on the dog and can not be continued indefinitely in contrast to simple anemia due to blood loss (18) or to simple hypoproteinemia alone due to plasmapheresis (9).

The *basal ration* biscuit is composed of the following ingredients: corn starch 4800 gm., dextrin 800 gm., baking powder 200 gm., bone ash 700 gm., salt mixture 150 gm., sugar 2400 gm., crisco 1450 gm., corn oil 300 cc., cod liver oil 150 cc., water 1300 cc. These are mixed with the water to make a firm cookie mixture, are lightly rolled out, cut into squares, and baked. The resulting biscuit is similar to a commercial dog biscuit in hardness. The amino acid-containing biscuit represents the same ingredients as the basal ration, to which the pure crystalline amino acids are added (formula V*c* and VI*a*—Table 4), the whole thoroughly mixed, rolled out, and baked. Any amino acid-containing biscuit which has not been consumed is spoon fed to the dog to insure 100 per cent food consumption. Diets are indicated in the clinical history of each dog.

Vitamin additions to the diet consist of dried yeast (Standard Brands Inc. type 200B) and a vitamin B_2 complex prepared from pig liver which contains per gm. 150 to 200 μg. riboflavin, 75 μg. pyridoxine, 750 to 1000 gammas of nicotinic acid, 850 to 1000 μg. pantothenic acid, and other factors in liver concentrated to the same extent as the riboflavin. These concentrates contain nitrogen. The amount is shown in the tables, by the figures expressed as nitrogen or protein intake. In certain experiments—indicated in tables—synthetic preparations (Eli Lilly) were used daily, composed of vitamin A 5000 U.S.P. units, thiamin HCl 3 mg., riboflavin 2 mg., pyridoxine HCl 1 mg., pantothenic acid 1 mg., nicotinamide 20 mg., ascorbic acid 50 mg., vitamin D 500 U.S.P. units, natural tocopherols 50 mg., choline hydrochloride 100 mg. These vitamins are administered in capsule form and fed separately immediately before diet feeding. The salt mixture used is the McCollum-Simmonds formula (11) without iron.

Protein digests[1] are either enzymatic using papain (P 39114; P 36092; KB 81341; KB 42241; KB 2-47; P 36996) or acid hydrolysates (KB 2-23).

For intraperitoneal hemoglobin administration either dog or sheep blood, collected in heparin solution, is centrifugalized, the cells washed three times with normal salt solution, then laked with an equal volume of distilled water. The mixture is centrifugalized again and the supernatant hemoglobin solution filtered through sterile gauze, cotton pads, and filter paper, and injected intraperitoneally with aseptic precautions.

For urinary nitrogen studies the animals are kept in metabolism cages; the urine is collected and preserved with toluene and refrigeration. Total urinary nitrogen is estimated by macro Kjeldahl analysis. Total urea and ammonia nitrogen is determined by aeration after treatment with urease. The latter figures are not given in the tables but are mentioned in experimental observations.

In the following tables for any given dog the periods run consecutively. Hemoglobin levels are those obtained by sampling 48 hours following the removal of the hemoglobin indicated in the adjacent columns. The plasma protein levels represent the average of samples of each bleeding during the week. "Total output net" in various tables means the amounts of plasma protein and hemoglobin removed, plus or minus the amounts related to *change* in hemoglobin levels or plasma protein concentration at the beginning and at the end of any given period which includes the 2 weeks of specific intake plus the 2 or 3 weeks of after period. For example if the hemoglobin level rose from 6 to 9 gm. per cent and the blood volume was 1000 cc., then 30 gm. hemoglobin would be *added* to any hemoglobin removed by bleeding during the experiment to give the "total hemoglobin output net."

EXPERIMENTAL OBSERVATIONS

These doubly depleted dogs (anemic and hypoproteinemic) are very susceptible to *infection*. In the early experiments endocarditis, pneumonia, and septicemia were frequent fatal complications. In these experiments which continue over many weeks and months without interruption, great care is taken to prevent infection. The dogs are vaccinated against distemper and dysentery, are isolated from other dogs, and careful attention is given to their feeding. Aseptic technique is used to withdraw blood or in the administration of digests, hemoglobin, or amino acids parenterally. Rooms are air conditioned and a

[1] We are greatly indebted to G. B. Walden, Dr. G. H. A. Clowes, A. L. Caldwell, Otto K. Behrens, and E. Stuart of Eli Lilly and Company for the preparation of the digests of casein, serum, and hemoglobin and for the vitamin materials.

thermostat maintains temperature between 65° and 70°. Unless otherwise stated these dogs used in the tabulated experiments were clinically normal.

Table 1 indicates clearly that *dog plasma* is well utilized *to form new hemoglobin*—a total of 125 gm. during the 2 weeks of plasma injection and 2 weeks following (dog 37-23). It must be obvious that the plasma contributes readily to the fabrication of the *globin*, the large protein part of hemoglobin. In spite of all plasma injected, the blood plasma levels (dog 37-23) rise to only 7.1 per cent, an indication of the speed with which the plasma proteins are utilized for body protein needs. This dog shows the highest plasma protein to hemoglobin ratio recorded in any experiment (87 per cent) which means that almost as much plasma protein as hemoglobin was produced or removed. One might anticipate complete withdrawal of the introduced plasma protein and this would probably occur were the plasma protein not used promptly to build hemoglobin and for other body protein requirements. The "plasma protein output" heading in Table 1 might be designated as "plasma protein withdrawal," where whole plasma was given by vein. The ratio of protein output to protein intake (dog 37-23) is 58 per cent.

Dog 34-146 (Table 1), an Eck fistula, is somewhat different from a normal dog but shows that much new hemoglobin (76 gm.) is formed from the introduced plasma. Total nitrogen figures show nitrogen retention. Urinary nitrogen partition shows in general only 56 per cent as ammonia—urea with continued low undetermined nitrogen indicating good use of the protein constituents in general body metabolism.

Table 1 also gives details indicating that *serum digests* are well utilized to form hemoglobin and plasma proteins, but not so effectively as whole plasma. Contrary to what might be expected, the *serum digests* form more hemoglobin than plasma proteins (about 3 to 1). Again the Eck fistula (dog 34-146) is less efficient in using the digest, probably in part due to the smaller liver with its limited blood supply—the Eck fistula gives a by-pass for all the portal blood which enters the inferior vena cava direct. In general we may say that the *Eck fistula dog* at this time behaved much like a normal dog but was able to use plasma protein and serum digest with approximately two-thirds the efficiency of the normal control—ratios for protein output compared with intake of 58 per cent (normal dog) and 41 per cent (Eck) given whole plasma; 30 per cent (normal dog) and 19 per cent (Eck) given serum digest (Table 1).

Clinical Experimental History—Tables 1, 5.

Dog 37-23. Male (white bull) coach. Born Sept., 1936. Continuous anemia history Apr. 27, 1938, to Oct. 14, 1941. Regular anemia experiments. Beginning weight 17.9 kilos. Average blood volume 1300 cc. Feb. 1, 1939—Urine contains trace albumin but neither casts nor red cells. Apr. 14, 1942—Urine contains albumin, occasional hyaline and granular casts, and few red cells. Apr. 23, 1942—General

TABLE 1

Production of Hemoglobin and Plasma Protein Due to Dog Plasma and Beef Serum Digest in Same Dogs

Period 1 wk.	Weight	Protein intake		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake	Total nitrogen	
		Type	Weekly	Hemoglobin		Plasma protein				Intake	Urinary output
				Level	Output per wk.	Level	Output per wk.				
Dog No. 37-23. Whole fresh dog plasma by vein											
	kg.		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*
1	16.8	Basal	76	10.1	12.4	4.1	5.4	—	—	—	—
2	16.3	Dog plasma	154	8.3	50.0	7.1	33.4	67	—	—	—
3	16.5	Dog plasma	164	7.8	41.1	6.8	31.9	78	—	—	—
4	16.2	Basal	38	7.6	30.5	5.7	22.8	75	—	—	—
5	16.0	Basal	38	7.4	22.6	4.8	15.1	67	58	—	—
Total output net					125		109	87			
Dog No. 37-23. Beef serum digest KB 2-47 by vein											
1	16.1	Serum digest	74	7.5	1.6	4.8	0	—	—	—	—
2	15.4	Serum digest	117	7.7	45.2	5.1	22.1	51	—	—	—
3	15.5	Serum digest	117	7.7	1.6	4.8	0	—	—	49.4*	29.4*
4	14.5	Basal	19	8.8	17.0	4.5	7.2	42	30	—	—
5	14.0	Basal	19	8.8	1.6	4.6	0	—	—	6.8‡	9.0‡
Total output net					77		27	35			
Dog No. 34-146. Eck fistula—whole fresh dog plasma by vein											
1	20.4	Dog plasma	134	5.8	13.3	6.1	9.8	74	—	—	11.6
2	20.1	Dog plasma	115	7.3	1.5	6.4	0	—	—	39.9‡	14.2
3	19.4	Basal	20	7.9	37.5	6.1	26.8	71	—	—	—
4	18.5	Basal	38	6.9	22.5	4.9	17.8	79	41	9.3‡	17.0‡
Total output net					76		52	68			
Dog No. 34-146. Beef serum digest P39114 by vein											
1	18.4	Basal	55	8.0	1.7	4.3	0	—	—	—	—
2	17.8	Serum digest	196	7.6	41.2	4.6	17.1	42	—	—	—
3	17.9	Serum digest	201	7.5	9.8	4.2	4.4	45	19	63.5‡	49.8‡
4	16.7	Basal	39	9.1	1.9	4.1	0	—	—	—	5.7
Total output net					61		20	33			

* Combined figures for 3 periods.

‡ Combined figures for 2 periods.

condition normal. Blood removal begun. Diet of protein-free-basal biscuit 350 gm., cod liver oil 10 cc., dried yeast 10 gm., vitamin B complex 5 gm., reduced iron 400 mg.

May 7—*Dog plasma* experiment (intravenous) begun. Table 1. Daily diet of basal protein-free biscuit 375 gm., cod liver oil 10 cc., dried yeast 5 gm., vitamin B complex 5 gm. Food consumption 100 per cent. Dog plasma from normal animals collected in heparin. Blood volume 1244 cc. Weight 16.8 kilos. May 15—A/G ratio 0.99. June 4—A/G ratio 0.72.

June 8—*Beef serum digest* (KB 2-47—intravenous). Table 1. Daily diet of basal protein-free biscuit 375 gm., synthetic vitamins 5 times regular standard amount. Food consumption during experiment 80 per cent. July 9 to Aug. 20—Rest period and kennel diet of table scraps. Aug. 20—Weight 20 kilos. General condition excellent. Blood volume 1512 cc. Daily diet of basal protein-free biscuit 400 gm., cod liver oil 10 cc., dried yeast 5 gm., vitamin B complex 3 gm., reduced iron 400 mg.

Sept. 10—*Beef serum digest* (KB 2-47, oral). Table 5. Daily diet of basal protein-free biscuit 400 gm., cod liver oil 10 cc., dried yeast 5 gm., vitamin B complex 3 gm., reduced iron 400 mg. Food consumption 100 per cent. Plasma protein 4.7 gm. per cent. Sept. 16—A/G ratio 0.40. Sept. 24—A/G ratio 0.57. Dog in good condition.

Clinical Experimental History—Table 1.

Dog 34-146. Male bull. Born Dec., 1933. *Eck fistula* operation June 24, 1937. Continuous anemia history Oct. 4, 1937, to Mar. 5, 1940. Regular anemia experiments. Mar. 5, 1940—Plasma protein studies begun. Weight 19.4 kilos. Blood volume 1474 cc. Oct. 30, 1941—Daily diet of sugar 150 gm., cornstarch 150 gm., canned tomato 50 gm., crisco 40 gm., cod liver oil 20 cc., corn oil 20 cc., bone ash 15 gm., salt mixture 2 gm., dried yeast 5 gm., vitamin B complex 4 gm., choline 100 mg., pyridoxine 1 mg., reduced iron 200 mg.

Nov. 6—*Dog plasma* experiment (intravenous). Table 1. Daily diet same as that of Oct. 30. Food consumption 96 per cent and 87 per cent. Nov. 12—A/G ratio 0.70. Dec. 12, 1941—Kennel diet of table scraps and rest period. Jan. 15, 1942—Blood removal begun. Daily diet of basal protein-free biscuit, vitamin accessories, and reduced iron. Routine plasma protein studies. Apr. 23, 1942—Daily diet of basal protein-free biscuit 375 gm., cod liver oil 15 cc., dried yeast 10 gm., vitamin B complex 5 gm., reduced iron 400 mg. Apr. 30—A/G ratio 1.0. Blood volume 1340 cc.

May 8—*Beef serum digest* (P 39114, intravenous). Table 1. Daily diet same as that of Apr. 23, but with synthetic vitamins in 5 times standard amount in place of dried yeast and vitamin B complex. Food consumption 96 per cent and 93 per cent, periods 2 and 3. Dog in good condition.

Tables 2 and 3 are of particular interest because they show the ready *availability and use of pure amino acids*[2] given by mouth or vein. Hemoglobin

[2] Crystalline amino acids supplied by Merck & Co., Inc. Dr. J. M. Carlisle and Dr. D. F. Robertson gave valuable advice.

and plasma proteins are formed promptly and the 10 amino acids (Rose) essential for growth in the rat are used to better effect in the *manufacture of hemoglobin* than of plasma proteins, as compared with the usual diet proteins.

Table 2 shows that the growth mixture (Rose) of amino acids (Table 4, V*c* mixture) favors new hemoglobin construction to a surprising degree. In fact, the hemoglobin-producing capacity of the dog is not given a maximal test as the usual grade of anemia cannot be attained even by bleeding sufficient to produce a dangerous hypoproteinemia. In each experiment on 3 dogs (Table 2) there is a striking *rise in hemoglobin levels* which we know decreases the stimulus to new hemoglobin production (13). The stimulus for plasma protein production is assumed to be maximal. One dog could produce very little plasma protein (dog 39-2, Table 2), but the hemoglobin piled up to high levels (11 gm. per cent) giving a fantastic ratio of 20 to 1 in favor of the hemoglobin production.

When we calculate the theoretical protein equivalent of the amino acid mixture and compare it with the produced hemoglobin and plasma protein, we note production ratios of 23, 32, and 46 per cent—comparing favorably with the best diet proteins.

Table 2 shows also that the amino acids are well utilized and yield a positive nitrogen balance. The single experiment with amino acids given *by vein* (dog 40-32) shows results similar to those recorded by mouth. We have other observations to support this experiment (8). The albumin globulin ratio is held at high levels rising from 0.97 at the start to 1.81 during the amino acid feeding (dog 40-32). This compares favorably with the reactions noted in meat feeding experiments.

Clinical Experimental History—Table 2, 5.

Dog 40-32. Male bull. Born Aug., 1940. Continuous anemia history. Dec. 2, 1941, to May 26, 1942—Regular anemia experiments. Beginning weight 16.5 kilos. Blood volume 1400 cc. Apr. 22, 1942—Urine examination shows faint trace albumin but no casts nor red cells. May 26 to June 17—Diet of salmon bread 150 gm., protein-free basal biscuit 300 gm., reduced iron 400 mg. June 17—A/G ratio 1.1. Blood volume 1047 cc. Salmon bread omitted from diet.

June 23—*Amino acid mixture Vc* (methionine—oral). Table 2. Daily diet of amino acid biscuit 150 gm. Synthetic vitamins (5 times standard intake) fed separately. Basal protein-free biscuit 250 gm. fed later during day. Food consumption 100 per cent. July 8—A/G ratio 1.81. Blood volume 959 cc.

July 10—*Amino acid mixture Vc* (methionine—intravenous) for 1 week. Table 2, period 4. Amino acids were dissolved in 300 cc. distilled water and given by drip method with aseptic precaution. Daily diet of protein-free biscuit 400 gm., synthetic vitamins (5 times standard intake), reduced iron 400 mg. Food consumption 96 per cent. July 20—A/G ratio 0.80. Blood volume 1009 cc. July 23 to Sept. 10—Rest period. Sept. 10—Diet of basal protein-free biscuit 400 gm., dried yeast 3 gm., vitamin B complex 3 gm., reduced iron 400 mg. Blood removal begun.

Oct. 1—*Beef serum digest* (KB 2-47, oral). Table 5. Daily diet of basal protein-free biscuit 400 gm., dried yeast 3 gm., vitamin B complex 3 gm. A/G ratio 0.84.

TABLE 2

Production of Hemoglobin and Plasma Protein Due to Amino Acids Necessary for Growth (Rose)

Period 1 wk.	Weight	Protein intake		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake	Total nitrogen	
		Type	Weekly	Hemoglobin		Plasma protein				Intake	Urinary output
				Level	Output per wk.	Level	Output per wk.				
Dog No. 40-32. Amino acid mixture Vc daily											
	kg.		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*
1	13.9	Basal	34	6.8	1.7	4.7	0	—	—	—	—
2	13.8	Amino acid—oral	177	8.8	18.5	4.8	12.4	67	—	28.3	15.8
3	12.9	Amino acid—oral	177	7.5	38.0	4.9	19.8	52	—	28.3	15.8
4	12.8	Amino acid—*vein*	177	8.8	15.8	4.7	7.7	49	23	28.3	16.7
5	12.6	Basal	0	8.8	1.6	3.9	0	—	—	0	4.03
Total output net					89		34	38			
Dog No. 39-2. Amino acid mixture Vc daily											
1	14.1	Basal	40	5.4	7.6	4.6	4.8	63	—	—	—
2	13.5	Amino acid—oral	100	7.3	1.7	4.6	0	0	—	—	—
3	13.2	Amino acid—oral	77	9.8	12.3	4.3	6.6	54	32	28.3*	16.7*
4	12.6	Basal	0	9.8	2.1	4.3	0	0	—	—	—
5	12.3	Basal	0	11.0	2.1	4.0	0	0	—	0	15.2*
Total output net					59		3	5			
Dog No. 37-82. Amino acid mixture Vc daily											
1	12.6	Basal	40	7.7	7.1	4.6	4.3	61	—	—	—
2	11.8	Amino acid—oral	117	9.1	16.8	4.8	7.2	43	—	—	—
3	11.1	Amino acid—oral	60	9.1	22.4	4.7	12.2	55	—	—	—
4	10.6	Basal	0	8.1	10.5	4.2	3.3	32	46	—	—
5	9.8	Basal	0	9.4	1.9	4.2	0	0	—	—	—
Total output net					64		20	31			

* Combined figures for 2 periods.

Oct. 7—A/G ratio 1.3, 1.1 during periods 1 and 2. Oct. 14—Food consumption 100 per cent. Blood volume 850 cc. Oct. 21—A/G ratio 0.63. Oct. 29—Blood volume 1130 cc. Dog in good condition.

Clinical Experimental History—Table 2.

Dog 39-2. Male bull coach. Born Oct., 1938. Continuous anemia history Oct. 24, 1940, to May 22, 1942. Regular anemia experiments. Nov. 13—Urine examination showed faint trace albumin but no casts nor red cells. General condition excellent. Weight 15.7 kilos. Blood volume 1159 cc. May 1, 1942—No albumin in urine. May 22 to June 25, 1942—Diet of basal protein-free biscuit 250 gm., dried yeast 5 gm., vitamin B complex 5 gm., reduced iron 400 mg. Average food consumption 92 per cent. A/G ratio 0.50.

June 25—*Amino acid mixture Vc* (methionine—oral). Table 2. Daily diet of amino acid mixture (incorporated into biscuit and fed first). Food consumption 100 per cent. Synthetic vitamins fed separately. Basal protein-free biscuit 250 gm., given later in the day. Food consumption 66 per cent. Blood volume 1068 cc. July 1—A/G ratio 0.93. July 3—A/G ratio 0.84. July 23—Kennel diet of table scraps. Blood volume 977 cc. Weight 12.3 kilos.

Clinical Experimental History—Tables 2, 3, 5, 9.

Dog 37-82. Male white bull coach. Born Aug., 1936. Continuous anemia history May 25, 1937, to Sept. 23, 1941. Regular anemia experiments. Beginning weight 14.2 kilos. Average blood volume 1250 cc. Sept. 24, 1941—Daily diet of potato starch 100 gm., sugar 100 gm., dextrin 100 gm., canned tomato 50 gm., crisco 40 gm., corn oil 20 cc., cod liver oil 10 cc., salt mixture 2 gm., dried yeast 5 gm., vitamin B complex 3 gm., choline 100 mg., pyridoxine 1 mg., reduced iron 200 mg. Oct. 2—Plasma protein 5.78 per cent.

Oct. 8 to Oct. 22—*Casein digest* (P 36092—intravenous). Table 9. Daily diet same as that of Sept. 24. Food consumption 92 per cent and 63 per cent. Average blood volume 950 cc. Nov. 5—Salmon bread diet and recovery period 6 weeks. Nov. 17—Plasma protein level 6.84 per cent. Dec. 4, 1941, to Mar. 4, 1942—Regular anemia experiments. Dec. 9—Plasma protein level 7.02 per cent. Average blood volume 1150 cc. Mar. 4, 1942—Protein-free basal biscuit diet. Plasma protein 6.59 per cent.

Mar. 27—*Amino acid mixture VI a* (cystine—oral). Table 3. Daily diet of amino acid-containing biscuit 150 gm., basal protein-free biscuit 150 gm., cod liver oil 10 cc., synthetic vitamins 2 times standard amount, reduced iron 400 mg. Food consumption 100 per cent. Blood volume 972 cc. Apr. 1—A/G ratio 0.86. Apr. 16—A/G ratio 0.71. Blood volume 904 cc. Apr. 17—Urine examination negative for albumin, sugar, blood, and casts. May 7 to 21—Diet of basal protein-free biscuit 375 gm., plus usual vitamin accessories and reduced iron 400 mg. A/G ratio 1.10. Average blood volume 950 cc.

May 21—*Amino acid mixture Vc* (methionine—oral). Table 2. Daily diet of amino acid biscuit 150 gm., plus basal protein-free biscuit 140 gm., cod liver oil 10 cc., reduced iron 400 mg., synthetic vitamins 5 times standard amount. Food consumption 100 per cent. A/G ratio 0.79. June 18 to Aug. 20—Food consumption 96 to 100 per cent. Average blood volume 1250 cc. July 23—Front feet slightly swollen. Aug. 20—Diet of basal protein-free biscuit 400 gm., reduced iron 400 mg., plus usual vitamin accessories.

Sept. 10—*Serum digest* (P 36996—oral). Table 5. Daily diet of basal protein-free biscuit 400 gm., dried yeast 3 gm., vitamin B complex 3 gm., reduced iron 400 mg. Food consumption 88 per cent.

Table 3 emphasizes the fact that *cystine* is an important amino acid related to the production of plasma protein. This has been observed previously in hypoproteinemic non-anemic dogs (9), and cystine under certain circumstances was designated as a "key amino acid" for plasma protein production. Table 3 shows that *cystine + choline alone* can be associated with considerable hemoglobin and plasma protein production. This response probably would not continue for more than 7 to 10 days, and further tests will be made.

Table 3, dog 37-82, gives an admirable contrast with the same dog in Table 2. The growth mixture (Rose) of amino acids (Table 2) gives predominance to hemoglobin production over plasma protein (ratio 31 per cent), but when *cystine replaces methionine* (Table 3) the ratio changes to 50 per cent which means more plasma protein production related to the cystine. It is probable that this output cannot be continued indefinitely, as cystine cannot replace methionine adequately in internal metabolism; but the experiment is in harmony with other observations (9) in hypoproteinemic dogs.

Dog 40-155, Table 3, is not a satisfactory experiment but does indicate that the *cystine* amino acid mixture (Table 4) does initiate some production of hemoglobin and plasma protein and supports the observations in dog 37-82. The experiment was discontinued because the clinical condition of the dog became abnormal.

Clinical Experimental History—Tables 3, 7, 9.

Dog 40-155. Male tan and white hound. Apr. 2, 1941—Total plasma protein 5.24 per cent. A/G ratio 1.55. Blood volume 1157 cc., weight 18.2 kilos. Blood removal begun. May 2 to June 4—Daily diet of canned salmon 150 gm., potato starch 100 gm., dextrin 200 gm., sugar 50 gm., lard 30 gm., protein-free butter 20 gm., cod liver oil 10 cc., canned tomato 50 gm., bone ash 15 gm., salt mixture 3 gm., dried yeast 2 gm., vitamin B complex 2 gm., reduced iron 200 mg. June 4—A/G ratio 0.95.

June 12—*Casein digest* (P 36092—intravenous). Table 9. Daily diet of potato starch 100 gm., dextrin 150 gm., sugar 50 gm., crisco 40 gm., corn oil 20 cc., cod liver oil 20 cc., canned tomato 50 gm., bone ash 15 gm., salt mixture 3 gm., reduced iron 200 mg., synthetic vitamins in standard amounts. Food consumption 52 per cent and 72 per cent. June 18—A/G ratio 0.75. July 4—A/G ratio 0.77. July 10 to Sept. 24—Rest period and kennel diet of table scraps. Sept. 24, 1941—Daily diet of canned salmon 200 gm., potato starch 100 gm., sugar 75 gm., dextrin 150 gm., crisco 40 gm., corn oil 20 cc., cod liver oil 20 cc., canned tomato 50 gm., bone ash 15 gm., salt mixture 3 gm., yeast 4 gm., vitamin B complex 3 gm., choline 100 mg., reduced iron 200 mg. Blood removal begun. Oct. 10—A/G ratio average 0.83. Nov. 6—Canned salmon decreased to 100 gm. Nov. 27—A/G ratio 1.27. Weight 18.6 kilos. Blood volume 1338 cc. Dec. 5 to 12—Fast. Water *ad libitum*.

Dec. 12—*Hemoglobin digest* (KB 42241—intravenous). Table 7. Daily diet of potato starch 100 gm., sugar 75 gm., dextrin 150 gm., crisco 40 gm., corn oil 20 cc., cod liver oil 20 cc., canned tomato 50 gm., bone ash 15 gm., salt mixture 3 gm., yeast 4 gm., vitamin B complex 3 gm., choline 100 mg., reduced iron 200 mg. Food consumption 50 per cent and 49 per cent.

TABLE 3

Production of Hemoglobin and Plasma Protein Due to Amino Acids—Cystine Replacing Methionine

Period 1 wk.	Weight	Protein intake		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake
				Hemoglobin		Plasma protein			
		Type	Weekly	Level	Output per wk.	Level	Output per wk.		
Dog No. 37-82. Amino acid mixture VI*a* daily by mouth									
	kg.		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*
1	12.4	Amino acids	174	9.9	13.2	5.2	7.3	55	—
2	12.1	Basal	0	10.5	10.8	4.5	4.4	41	—
3	12.0	Basal	0	7.2	39.0	4.8	19.2	49	45
4	11.6	Basal	0	6.3	1.3	4.1	0	0	—
Total output net................					54		27	50	
Dog No. 40-155. Amino acid mixture VI*a* daily by mouth									
1	12.5	Basal	19	7.3	1.7	4.7	0	0	—
2	11.8	Amino acids	123	6.6	1.8	4.4	0	0	—
3	11.0	Amino acids	52	6.5	10.9	4.6	5.9	54	7
Total output net................					7		6	86	
Dog No. 36-14. Cystine 1.6 + choline 0.4 daily by mouth									
1	16.5	Basal	23	5.9	0	4.6	0	—	—
2	15.7	Cystine + choline	32	7.2	11.6	5.1	7.1	68	—
3	15.1	Basal	17	6.9	14.0	4.9	9.6	76	—
4	14.7	Basal	10	5.5	12.7	5.1	6.3	55	103
Total output net................					35		27	77	

Jan. 14, 1942—*Casein digest* (P 36092—oral). Table 9. Daily diet of basal protein-free biscuit 300 gm., yeast 3 gm., vitamin B complex 2 gm., bone ash 15 gm., choline 100 mg., natural tocopherols 50 gm., pyridoxine 1 mg., reduced iron 200 mg. Food consumption 90 per cent average. Jan. 21—A/G ratio 0.50.

Feb. 12—*Hemoglobin digest* (KB 2-23—oral). Table 7. Daily diet of basal protein-free biscuit 200 gm., protein-free butter 20 gm., synthetic vitamins in standard amounts, reduced iron 200 mg. Food consumption 56 per cent and 52 per cent in periods 1 and 2. A/G ratio 0.73 and 0.74 during periods 1 and 3. Mar. 4—Blood volume 888 cc.

Mar. 5—*Amino acid mixture VI a* (cystine—oral). Table 3. Daily diet of amino acid-containing biscuit 190 gm. plus basal protein-free biscuit 20 gm., synthetic vitamins in standard amounts, reduced iron 200 mg. Average food consumption 63 per cent. Superficial skin lesions—head, joints of legs, and back. Mar. 22—Rest period. Weight 11 kilos. Blood volume 830 cc.

Clinical Experimental History—Tables 3, 8.

Dog 36-14. Male bull. Born Apr., 1936. Continuous anemia history Oct., 1940, to Dec., 1941. Beginning weight 21.6 kilos. Regular anemia experiments. Average blood volume 1600 cc. Dec. 12—Daily diet of dextrin 100 gm., potato starch 100 gm., sugar 75 gm., canned tomato 50 gm., crisco 40 gm., corn oil 20 cc., cod liver oil 10 cc., bone ash 15 gm., salt mixture 2 gm., dried yeast 4 gm., vitamin B complex 3 gm., natural tocopherols 50 mg., pyridoxine 1 mg., reduced iron 200 mg. Average blood volume 1275 cc. Average weight 18 kilos.

TABLE 4

Amino Acid Mixtures VI a and V c

Mixture	VI *a*	V *c*
	gm. per day	*gm. per day*
dl-Threonine	2.0	2.0
dl-Valine	2.0	2.0
dl-Leucine	5.0	5.0
dl-Isoleucine	2.0	2.0
dl-Lysine HCl	5.0	5.0
l(−)-Tryptophane	0.5	0.5
dl-Phenylalanine	1.3	1.3
l(+)-Histidine HCl	2.0	2.0
l(+)-Arginine HCl	1.0	1.0
Glycine	5.0	5.0
l(−)-Cystine	1.5	—
dl-Methionine	—	2.5

Dec. 29—*Hemoglobin digest* (KB 81341—intravenous). Table 8. Daily diet same as that of Dec. 12. Food consumption 75 per cent, 78 per cent, and 40 per cent. Jan. 15, 1942—Kennel diet of table scraps and rest period. Feb. 15 to Mar. 13—Diet of protein-free basal biscuit 375 gm., cod liver oil 15 cc., dried yeast 5 gm., vitamin B complex 3 gm., choline 100 mg. Average plasma protein 5.5 gm. per cent. Average blood volume 1275 cc. Average weight 17.3 kilos.

Mar. 13—*Cystine plus choline*—oral. Table 3. Daily diet of cystine 1.6 gm., choline 400 mg., basal protein-free biscuit 200 gm., synthetic vitamins in twice the standard amount, reduced iron 400 mg. Amino acid was added to small portion of basal biscuit and fed first to insure 100 per cent consumption. Feeding of balance of basal diet followed. Food consumption 69 per cent.

Table 5 gives evidence that *serum digests* are well used to form plasma protein and hemoglobin when given *by mouth*—compare in Table 1 the effect of the same serum digest on the same dog when given *by vein*. Dog 37-23 uses the digest as effectively by mouth as by vein, the ratio of protein output to protein

TABLE 5

Production of Hemoglobin and Plasma Protein Due to Beef Serum Digests by Mouth

Period 1 wk.	Weight	Protein intake		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake
				Hemoglobin		Plasma protein			
		Type	Weekly	Level	Output per wk.	Level	Output per wk.		
Dog No. 37-23. Digest KB2-47 oral									
	kg.		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*
1	17.9	Basal	29	11.6	16.2	4.5	8.1	—	—
2	17.8	Serum digest	149	10.3	26.0	4.8	12.8	50	—
3	17.4	Serum digest	149	10.2	28.9	4.7	12.2	42	—
4	16.8	Basal	20	9.9	23.6	4.6	10.8	46	30
5	16.1	Basal	30	9.9	2.1	4.3	0	—	—
Total output net................					69		34	49	
Dog No. 40-32. Digest KB2-47 oral									
1	16.8	Serum digest	170	7.6	48.3	4.9	22.4	46	—
2	16.4	Serum digest	170	9.0	23.3	5.1	15.3	65	—
3	16.2	Basal	24	7.3	26.7	4.5	14.6	55	33
4	15.6	Basal	24	7.3	1.5	4.4	0	—	—
Total output net................					73		52	71	
Dog No. 37-82. Digest P36996 oral									
1	16.7	Basal	30	6.4	18.0	4.9	13.9	—	—
2	16.4	Serum digest	182	6.4	1.4	4.9	0	—	—
3	15.9	Serum digest	188	9.1	1.7	4.4	0	—	—
4	15.4	Basal	23	10.5	34.4	4.8	16.5	48	—
5	14.5	Basal	30	8.7	26.8	4.4	10.3	38	21
Total output net................					81		21	26	

intake being 30 per cent in both experiments (Tables 1 and 5). When given by vein, this dog (37-23) used the digest to produce more hemoglobin than plasma protein (ratio 77 to 27); but by mouth the ratio was 69 to 34. It is noteworthy that in all experiments (Table 5) the ratio of plasma protein to hemoglobin ranged from 26 to 71 per cent—that is for every gram of new plasma protein produced there was formed 1.5 to 4 gm. of new hemoglobin.

This is adequate proof together with the evidence in Table 1 that plasma protein contributes freely to the manufacture of hemoglobin. It is always amazing to us that these dogs receive these serum products (or whole plasma) and rather than make the new plasma for which these materials are perfectly suited always produce more new hemoglobin. It may be argued that these serum digests do go into new plasma protein which is promptly turned over to form the needed hemoglobin. The levels of plasma proteins in the circulation (4.3 to 4.5 per cent) guarantee adequate stimulus for new plasma protein formation. The clinical histories of the dogs in Table 5 are given above.

Table 6 holds particular interest for students of hemoglobin metabolism. In simple anemia in standard dogs, hemoglobin given by vein (dog, sheep, goose hemoglobin) (16, 17) will yield 100 per cent return as new hemoglobin; yet it may be argued that the dog uses only the contained iron and not the globin. The pigment radicle is thrown out quantitatively as bile pigment (6). Moreover, one cannot give much hemoglobin by vein as the renal threshold is low—therefore the chances for error are larger.

When *hemoglobin* is given *intraperitoneally* there is no significant hemoglobinuria even with the large doses in Table 6. There is a large surplus of iron by mouth in the basal diet, so that the few milligrams of iron contained in the introduced hemoglobin cannot be a determining factor. The large output figures of hemoglobin and plasma protein leave no doubt that the introduced hemoglobin is conserved and used to make new hemoglobin and plasma protein under these conditions. In addition to the plasma protein removed there is made more new hemoglobin than was given into the peritoneum. It is safe to assume complete conservation of the introduced *globin* plus the manufacture of new hemoglobin from the basal diet protein and protein reserves (body weight loss). Nitrogen balance studies in this type of experiment will be of interest.

When hemoglobin is given by vein to the plasma depleted non-anemic dog there is no evidence that the hemoglobin contributes to the formation of new plasma protein (9). When a hemoglobin digest is given intravenously to protein-depleted dogs (long continued low protein diets with fasting periods), they can be kept very close to nitrogen balance (20), indicating that globin contributes to the nitrogen or protein pool under such conditions. The experiments in Table 6 give definite evidence that the hemoglobin contributes to plasma protein production in these doubly depleted dogs (anemic and hypoproteinemic). Sheep hemoglobin is utilized as effectively as dog hemoglobin. We believe that the contribution of the introduced hemoglobin is to the *body protein pool* from which emerges some of the needed plasma protein.

Clinical Experimental History—Table 6.

Dog 35-6. Male, white bull. Born Nov., 1934. Continuous anemia history July 11, 1936, to September 19, 1940. Regular anemia experiments. Beginning weight 19.7 kilos. Average blood volume 1450 cc. Sept. 19, 1940, to May 15, 1941—diet of potato starch 150 gm., sugar 100 gm., dextrin 100 gm., canned tomato 50 gm., crisco 40 gm., corn oil 20 cc., cod liver oil 20 cc., bone ash 15 gm., salt mixture 2 gm., dried yeast 5 gm., vitamin B complex 3 gm., pyridoxine 1 mg., reduced iron 200 mg.

TABLE 6

Production of Hemoglobin and Plasma Protein Due to Dog and Sheep Hemoglobin Given Intraperitoneally

Period 1 wk.	Weight	Protein intake		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake
		Type	Weekly	Hemoglobin		Plasma protein			
				Level	Output per wk.	Level	Output per wk.		
Dog No. 35-6. Dog hemoglobin in peritoneum									
	kg.		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*
1	15.8	Fast	0	12.0	16.4	5.7	12.2	74	—
2	16.1	Hb 24.8 gm.	33	12.3	44.6	4.9	18.2	41	—
3	15.8	Hb 31.9 gm.	39	9.1	60.2	4.6	23.1	38	—
4	14.9	Hb 21.5 gm.	30	8.8	4.6	4.0	2.6	57	—
5	14.8	Basal	7	10.2	9.8	4.4	3.8	39	123
Total output net................					102		38	37	
Dog No. 40-156. Dog hemoglobin in peritoneum									
1	15.1	Hb 37.6 gm.	45	9.9	49.8	6.3	29.2	59	—
2	14.6	Hb 40.2 gm.	71	9.7	62.3	5.3	33.2	53	—
3	13.6	Basal	23	8.0	37.6	4.9	19.9	53	—
4	12.5	Basal	16	6.9	17.1	5.1	10.4	61	148
Total output net.................					150		86	57	
Dog No. 40-29. Sheep hemoglobin in peritoneum									
1	10.6	Basal	36	8.1	1.7	4.6	0	—	—
2	10.4	Hb 37.3 gm.	36	8.0	45.3	5.2	20.0	44	—
3	9.7	Hb 14.1 gm.	14	8.0	1.8	4.4	0	—	150
4	9.4	Basal	0	9.9	2.0	3.8	0	—	—
Total output net................					62		16	26	

Regular anemic and hypoproteinemic experiments. May 15, 1941—1 week fast. Weight 15.6 kilos. Blood volume 1100 cc.

May 22—*Dog hemoglobin* (intraperitoneal). Table 6. Daily diet of potato starch 150 gm., sugar 100 gm., dextrin 100 gm., canned tomato 50 gm., crisco 40 gm., corn oil 20 cc., cod liver oil 20 cc., bone ash 15 gm., salt mixture 2 gm., synthetic vitamins (twice the standard amount). Food consumption average 96 per cent.

Clinical Experimental History—Table 6, 8.

Dog 40-156. Male tan hound. Beginning weight 17.2 kilos. Blood volume 1480 cc. Feb. 28, 1941—Daily diet of casein 40 gm., potato starch 100 gm., dextrin 200 gm., sugar 50 gm., lard 30 gm., butter 20 gm., cod liver oil 10 cc., canned tomato 50 gm., bone ash 15 gm., dried yeast 5 gm., vitamin B complex 3 gm., salt mixture 3 gm., no iron. Apr. 2—Blood removal begun. Plasma protein 5.64 gm. per cent. Apr. 11—Canned salmon 150 gm. replaces casein 40 gm. in daily diet. May 22—Reduced iron 200 mg. is added to daily diet. June 11—Average A/G ratio 0.88. Average blood volume 1200 cc.

June 12—*Dog hemoglobin* (intraperitoneal). Table 6. Daily diet of potato starch 100 gm., dextrin 100 gm., sugar 100 gm., protein-free butter 60 gm., corn oil 20 cc., cod liver oil 10 cc., bone ash 15 gm., salt mixture 3 gm., dried yeast 4 gm., vitamin B complex 3 gm., natural tocopherols 50 mg., reduced iron 200 mg. Food consumption 28 per cent during 1st week. Stomach tube feeding of corn syrup, yeast, and milk during 2nd week, also 20 gm. of sugar intravenous daily. Trace of hemoglobin pigment in urine for 3 days. Food consumption 3rd week of experiment 75 per cent. Average blood volume 1050 cc. June 24—A/G ratio 0.80. July 16 to Sept. 25—Kennel diet of table scraps with additional liver and meat. Rest period 11 weeks. Sept. 25 to Dec. 12—Average A/G ratio 0.85. Average food consumption 83 per cent. Average blood volume 1200 cc. Dec. 12—Fast 1 week. Dec. 19—Daily diet of potato starch 125 gm., sugar 75 gm., dextrin 100 gm., butter 40 gm., cod liver oil 10 cc., bone ash 20 gm., salt mixture 3 gm., dried yeast 4 gm., vitamin B complex 3 gm., choline 100 mg., pyridoxine 1 mg., reduced iron 200 mg. Food consumption 65 per cent. A/G ratio 0.81.

Dec. 27—*Hemoglobin digest* (KB 81341—intravenous). Table 8. Diet same as that of Dec. 19. Food consumption 52 per cent and 48 per cent. A/G ratio 0.90.

Clinical Experimental History—Table 6, 7.

Dog 40-29. White female bull. Born Jan., 1940. Mar. 9 to Apr. 9, 1942—Diet of salmon bread 450 gm., canned salmon 50 gm., klim 20 gm. Apr. 8—Plasma protein 5.9 gm. per cent. Blood volume 1027 cc. Weight 13.7 kilos. Blood removal begun. Apr. 9 to May 6—Daily diet of basal protein-free biscuit 350 gm., cod liver oil 10 cc., dried yeast 10 gm., vitamin B complex 5 gm., reduced iron 400 mg. Food consumption 98 per cent.

May 6—*Sheep hemoglobin* (intraperitoneal). Table 6. Daily diet of basal protein-free biscuit 300 gm., protein-free butter 20 gm., cod liver oil 10 cc., synthetic vitamins and choline three times regular standard amount. Food consumption 71 and 73 per cent. May 14—A/G ratio 1.30. Rest period. July 23 to Aug. 27—Daily diet of basal protein-free biscuit 400 gm., dried yeast 5 gm., vitamin B complex 3 gm., cod liver oil 15 cc., canned salmon 30 gm., reduced iron 400 mg. Average food consumption 93 per cent. Aug. 26—Blood volume 873 cc.

Aug. 27—*Hemoglobin digest* (KB 81341—oral). Table 7. Daily diet of basal protein-free biscuit 300 gm., reduced iron 400 mg., synthetic vitamins 5 times regular standard amount. Food consumption 61 and 96 per cent. Sept. 21—A/G ratio 0.56. Blood volume 684 cc. Dog in good condition.

Table 7 shows that *hemoglobin digests* by mouth are well used by these doubly depleted dogs. The ratios of protein output to protein intake, 26 and 15 per cent, compare favorably with the common diet proteins. It was reported recently from this laboratory (19) that hemoglobin fed as fresh red cells was readily digested and the resultant rise in blood non-protein nitrogen was due to the *globin* digestion—the pigment radicle remaining in the intestine. This hemoglobin digest does not favor the production of new hemoglobin any more than do the usual diet proteins—ratios of plasma protein to hemoglobin being

TABLE 7

Production of Hemoglobin and Plasma Protein Due to Hemoglobin Digests by Mouth

Period 1 wk.	Weight	Protein intake		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake
				Hemoglobin		Plasma protein			
		Type	Weekly	Level	Output per wk.	Level	Output per wk.		
Dog No. 40-29. Hemoglobin digest KB-81341 by mouth									
	kg.		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*
1	11.2	Basal, salmon	47	8.7	1.8	4.6	0	—	—
2	10.6	Digest	97	9.8	11.9	5.1	6.7	56	—
3	10.1	Digest	107	8.5	26.3	4.9	11.1	42	—
4	9.7	Digest	77	8.5	12.2	4.8	6.0	49	26
5	9.4	Basal	22	8.5	1.8	4.5	0	—	—
Total output net................					51		24	47	
Dog No. 40-155. Hemoglobin digest KB 2-23 by mouth									
1	13.0	Digest	72	6.2	1.3	4.7	0	—	—
2	12.5	Digest	111	7.5	11.5	4.5	5.6	51	15
3	12.5	Basal	15	7.3	1.7	4.7	0	—	—
Total output net..................					23		6	26	

47 and 26 per cent. Table 7 should be compared with Table 5 (serum digests by mouth). The serum digests are used more effectively (ratio of protein output to intake is higher), but the dogs produce little if any more plasma protein on the serum digest than on the hemoglobin digest.

It may be possible to make use of the discarded red cells in various blood banks by proper digestion technique. Such digestion products perhaps supplemented with certain amino acids might be of considerable value given by mouth or by vein.

Table 8 is to be compared with Table 7. The digests of hemoglobin give a response very similar whether given by mouth or by vein. The digest is used to form new hemoglobin and plasma protein more promptly when given by mouth, but the net return and ratios of plasma protein to hemoglobin are very similar. The utilization of the digest given by vein (Table 8) is delayed, probably due to the slight reactions caused by the intravenous daily injection. Dog 40-156 (Table 8) is a particularly satisfactory experiment as the hemoglobin and plasma protein levels are very uniformly maintained, insuring constant maximal stimuli for new production of these proteins.

TABLE 8

Production of Hemoglobin and Plasma Protein Due to Hemoglobin Digests by Vein

Period 1 wk.	Weight	Protein intake		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake
				Hemoglobin		Plasma protein			
		Type	Weekly	Level	Output per wk.	Level	Output per wk.		
Dog No. 40-156. Hemoglobin digest KB-81341 by vein									
	kg.		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*
1	13.9	Digest	151	6.2	1.4	4.1	0	—	—
2	13.6	Digest	181	6.6	11.5	4.5	6.3	57	—
3	12.8	Basal	10	7.0	24.9	4.7	13.4	56	—
4	12.6	Basal	15	7.7	8.0	4.3	4.3	56	—
5	11.8	Basal	14	6.5	20.1	4.3	9.8	51	26
6	11.3	Basal	12	6.5	1.4	4.1	0	—	—
Total output net.................					67		30	45	
Dog No. 36-14. Hemoglobin digest KB-81341 by vein									
1	14.8	Digest	180	6.5	1.4	4.6	0	—	—
2	14.5	Digest	180	7.5	11.2	4.9	6.9	65	—
3	13.0	Basal	6	8.5	34.1	5.3	21.4	66	24
Total output net.................					62		28	45	
Dog No. 40-155. Hemoglobin digest KB-42241 by vein									
1	16.8	Digest	129	6.6	1.4	5.6	0	—	—
2	16.2	Digest	143	5.2	1.9	5.4	0	—	—
3	15.5	Basal	16	8.0	13.2	5.2	9.9	78	—
4	14.8	Basal	15	7.5	29.1	5.2	17.3	62	—
5	13.9	Basal	12	7.5	21.7	5.4	11.6	56	35
Total output net.................					75		39	52	

Casein digests (Table 9) are of immediate interest because various digests have been tested clinically (1, 3, 4, 15). Reports on the utilization of these digests in plasma-depleted non-anemic dogs have been published from this laboratory (10). The response of these anemic and hypoproteinemic dogs is very similar to the response of the hypoproteinemic *non-anemic* dogs. There is a positive nitrogen balance whether the digests are given by vein or by mouth. When given by mouth (dog 37-81), the urea ammonia value of 63

TABLE 9

Production of Hemoglobin and Plasma Protein Due to a Casein Digest

Period 1 wk.	Weight	Protein intake		Protein output				Production ratio plasma protein to hemoglobin	Ratio protein output to intake	Total nitrogen	
		Type	Weekly	Hemoglobin		Plasma protein				Intake	Urinary output
				Level	Output per wk.	Level	Output per wk.				
Dog No. 40-155. Casein digest P36092											
	kg.		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*
1	15.1	Digest—vein	182	5.1	41.1	6.6	20.0	51	—	—	25.1
2	14.8	Digest—vein	188	6.9	20.5	6.5	16.7	84	—	59.2*	25.2
3	14.0	Basal	26	6.8	10.0	6.0	8.2	85	—	—	14.7
4	13.3	Basal	30	5.9	10.7	5.9	7.4	72	25	8.8*	10.7
Total output net.........					62		45	73			
1	14.1	Digest—oral	144	6.3	27.5	5.0	17.8	67	—	—	—
2	13.7	Digest—oral	136	6.3	1.3	5.5	—	—	—	—	—
3	13.8	Digest—oral	131	7.7	13.9	5.3	8.1	61	—	—	—
4	13.5	Basal	18	6.2	22.5	4.8	12.2	57	25	—	—
Total output net.........					73		38	52			
Dog No. 37-82. Casein digest P36092											
1	14.9	Digest—vein	188	10.5	62.7	5.1	29.2	49	—	—	23.3
2	13.7	Digest—vein	181	8.7	38.0	5.5	17.0	47	—	59.0*	19.3
3	12.7	Basal	20	8.0	28.6	5.5	14.4	—	—	—	12.3
4	12.1	Basal	27	5.7	22.2	5.1	12.2	—	44	7.5*	13.0
Total output net.........					116		71	61			
Dog No. 37-81. Casein digest P36092											
1	15.1	Digest—oral	153	8.8	23.8	5.1	14.5	63	—	—	15.2
2	15.0	Digest—oral	178	8.1	45.2	4.8	21.5	50	—	53.0*	15.8
3	14.4	Basal	12	7.2	18.7	4.7	11.0	62	—	1.9	11.8
4	14.0	Digest—vein	178	5.7	34.7	5.6	24.2	73	35	28.5	16.1
Total output net.........					109		75	69			

* Combined figures for 2 periods.

per cent and the low undetermined urinary nitrogen indicates good utilization of the fed nitrogen. When given by vein (dog 37-81, dog 37-82), the urea ammonia nitrogen values of 50 per cent and 52 per cent are associated with a twofold output in undetermined urinary nitrogen. The utilization of the nitrogen is therefore less efficient by vein but still good enough to give positive nitrogen balances. These digests are essentially the equivalent of the very best proteins given by mouth and the ratio of protein output to intake is correspondingly high.

Casein digests (Table 9) favor the production of plasma protein as much as any digest or protein tested—compare Table 5, serum digests by mouth. The ratio of plasma protein to hemoglobin is high in all experiments in Table 9 (50 to 70 per cent) and the plasma protein levels are high suggesting that even more plasma protein could have been produced had the stimulus been maximal.

Clinical Experimental History—Table 9.

Dog 37-81. Female, white bull coach. Born Aug., 1936. Continuous anemia history May 24, 1937, to Mar. 14, 1939. Regular anemia experiments. Beginning weight 12.3 kilos. Average blood volume 1100 cc. Mar. 14, 1939, to Nov., 1940—Regular anemia and hypoproteinemia experiments. Nov. 5, 1940—Daily diet of sugar 150 gm., cornstarch 100 gm., lard 20 gm., protein-free butter 40 gm., canned tomato 60 gm., bone ash 15 gm., cod liver oil 10 cc., salt mixture 2 gm., dried yeast 1 gm., vitamin B complex 2 gm., reduced iron 200 mg. Blood volume 1063 cc.

Nov. 12—*Casein digest experiment* (P 36092—oral). Table 9. Daily diet as of Nov. 5. Food consumption 93 per cent average for periods 1 and 2, and 100 per cent during period 4.

DISCUSSION

When these doubly depleted dogs (anemic and hypoproteinemic) were first observed we could not believe our figures which showed that invariably more hemoglobin was produced than plasma protein. Under all circumstances on all diets tested this was found to be true. Our first published experiments (12) dealt with food proteins—liver, casein, meat, soy bean, salmon—and the production ratio of plasma protein to hemoglobin ranged from 30 to 60 per cent.

The experiments tabulated above show clearly that these dogs continuously produce more hemoglobin than plasma protein even when given plasma or serum digests by vein (Table 1). Further (Table 2) the growth *amino acid mixture favors hemoglobin production* more than any other materials tested—even hemoglobin itself or the digests (Tables 6 to 8). Modification of the amino acid mixture should enable us to change this ratio of production within limits—at times to favor plasma protein production, but whether one can ever drive the production of plasma protein above the hemoglobin production in a doubly depleted dog remains to be seen.

When one asks why the body favors hemoglobin production over plasma production—each protein being essential for life—the answer is pure speculation. Perhaps the best argument is that in the normal dog's circulation hemoglobin (18 to 20 gm. per cent) is about three times the concentration of plasma protein (6.0 to 6.5 gm. per cent), and when there is a deficiency in both proteins the production flow of protein-building factors favors hemoglobin because of this normal concentration ratio. Further, one may assume that much of the globin requirements for new hemoglobin may come from plasma protein, that any surplus of plasma protein would flow naturally toward hemoglobin (given adequate iron) under the stimulus of anemia. The use of plasma protein containing lysine labeled with heavy nitrogen should give valuable information on this point, and we hope to complete this experiment.

It seems obvious that plasma protein can contribute to the building of hemoglobin and *vice versa* when a need exists. It is known that plasma protein by vein can supply all body protein requirements in the dog (2, 7). All of these observations support our belief that there is a *dynamic equilibrium* between blood proteins and tissue or organ proteins. Therefore any non-toxic digests or combination of amino acids which will produce plasma protein and hemoglobin will serve body protein needs in an emergency—tissue injury, replacement, and repair.

SUMMARY

Given healthy dogs, fed abundant iron and protein-free or low protein diets, with sustained anemia due to bleeding, we can study the capacity of these animals to produce simultaneously new hemoglobin and plasma protein.

The reserve stores of blood protein producing materials in this way are very largely depleted, and levels of 6 to 8 gm. per cent for hemoglobin and 4 to 5 gm. per cent for plasma protein can be maintained for considerable periods of time. These dogs are very susceptible to infection and to injury by many poisons.

Under such conditions, these anemic and hypoproteinemic dogs will use very efficiently a variety of *digests* (serum, hemoglobin, and casein) and the growth mixture (Rose) of pure *amino acids*. Nitrogen balance is maintained and considerable new blood proteins are produced.

Dog plasma by vein is used freely in these doubly depleted dogs to make new hemoglobin in abundance (Table 1). *Serum digests* by vein are well utilized to make new hemoglobin and plasma protein in the same dogs (Table 1). Serum digests by mouth are effectively used to make new blood proteins (Table 5).

Dog or *sheep hemoglobin* given in large amounts intraperitoneally are remarkably well utilized to form hemoglobin and plasma protein (Table 6). It must be obvious that the globin of the hemoglobin is saved in these protein-depleted dogs and used to make large amounts of hemoglobin and plasma protein.

Hemoglobin digests are also well utilized whether given by mouth (Table 7) or by vein (Table 8) and *liberal amounts of plasma protein* are manufactured from digests presumably ideally suited for hemoglobin production.

Casein digests are well used (Table 8) and form as much new plasma protein as any material tested—even serum digests.

Amino acid mixtures are of especial interest. The growth mixture of 10 amino acids (Rose) is well utilized by mouth or by vein and favors new hemoglobin production more than any material tested (Table 2). *Cystine* replacing methionine in the amino acid mixture increases the plasma protein—hemoglobin output ratio, that is it favors plasma protein production.

Digests of various sorts and amino acid mixtures or combinations of digests and amino acid mixtures can be used rapidly and effectively to build new hemoglobin or plasma protein, to maintain nitrogen equilibrium, and to replete reserve protein stores. These experiments point to clinical problems.

The unexplained preference given to hemoglobin production in these hypoproteinemic dogs is observed under all conditions, even when whole plasma or serum digests are given by vein. In general, 2 to 4 gm. of hemoglobin are formed for every gram of plasma protein.

This all adds up to a remarkable fluidity in the use of plasma protein or hemoglobin which can contribute directly to the *body protein pool* from which are evolved, without waste of nitrogen, the needed proteins, whether hemoglobin, plasma protein, or tissue proteins.

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