Published May 1, 1946

PLASMA SUBSTITUTES*

HUMAN AND ANIMAL GLOBIN RELATED TO THE PRODUCTION OF HEMOGLOBIN AND PLASMA PROTEIN

DOG HEMOGLOBIN UTILIZATION IMPROVED BY METHIONINE BUT NOT BY ISOLEUCINE

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(Received for publication, January 17, 1946)

Globin makes up 95 per cent of the hemoglobin molecule. Its construction is of fundamental importance. It is a protein and its production in the body relates to the building stones of protein and to other body proteins. In fact plasma protein introduced into a fasting anemic dog will bring about a large output of new hemoglobin (globin). *Globin production* and the related hemoglobin can be controlled by the protein intake, once the protein reserve stores have been exhausted (6).

Globin utilization has been much studied in this laboratory. As red cells are constantly wearing out, the related globin must be a continuing factor in body protein metabolism. The body uses this globin waste material well. We have shown (10) that actually the globin (hemoglobin) nitrogen intake may balance the total urinary nitrogen output in protein fasting dogs receiving hemoglobin in considerable amounts intraperitoneally and carbohydrate, fat, and accessories by mouth. These observations suggest that globin might be useful as a plasma protein substitute and in fact in the dog it does have considerable usefulness in replacing protein wastage.

Studies of globin utilization are very much in order together with similar studies of hemoglobin (globin + iron + pigment radicle). We can safely assume that the iron is well conserved and the pigment radicle is excreted (10). In fact the dog uses the whole hemoglobin more effectively than the pure globin, probably because the globin as prepared is slightly or moderately toxic and this reaction may impair or delay the hemoglobin production in anemia or the protein utilization and conservation in protein fasting dogs.

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

We are indebted to Dr. M. M. Strumia (Bryn Mawr Hospital), Dr. M. P. Munro (Jefferson Medical College and Hospital), and the Difco Laboratories for the supply of the various globin preparations.

We are indebted to Merck and Co., Inc., for pure amino acids.

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Globin is a peculiar protein and among other things it contains less isoleucine and methionine than the usual body proteins. Supplementation of globin and hemoglobin with these amino acids as given to protein fasting dogs obviously needs investigation. Some experimental data are tabulated below to show that the metabolic response is favorable and a positive urinary nitrogen balance is attainable when hemoglobin is supplemented with methionine. The addition of *dl*-isoleucine in the two experiments is without effect.

Methods

The dogs used in the experiments in Table 1 were healthy mongrels maintained in the kennels for several months preceding the tabulated experiments, on a diet of table scraps supplemented by liver and dog biscuit. Tables 2 and 3 relate to dogs of a bull terrier and coach strain taken from the anemia colony (16). These dogs are continuously maintained at a hemoglobin and red cell level of about one-third normal by suitable bleeding and the standard diet of salmon bread. "Double depletion" (anemia and hypoproteinemia) is produced by blood removal and a low protein or non-protein diet plus abundant iron. When anemic dogs are used for double depletion experiments their plasma proteins are depleted usually within a 2 or 3 week period. Dogs previously non-anemic are occasionally subjected to either a week of fasting or a preliminary period of a low protein dietary intake before blood removal is begun. Protein depletion of these animals often necessitates a 4 to 6 week period. Non-protein diet periods with attendant weight loss cannot be continued indefinitely, therefore recovery periods are interspersed in the experimental program. The general technical procedures relating to these dogs and methods of these experiments are those described for the anemia colony and double depletion experiments (16, 13).

The *basal ration* concerns either the standard salmon bread during the anemia period which is described elsewhere (16) or the basal protein-free biscuit for double depletion experiments (13). In more recent experiments reduced iron 62 gm. and choline chloride 40 gm. are added to the total biscuit ingredients before baking, allowing a daily dose of approximately 600 mg. iron and 400 mg. of choline. In the experimental histories where iron addition to the diet is not mentioned the finished biscuit contains the daily dose of choline and iron.

Vitamin additions consist of a dried yeast (Standard Brands Inc., Type 200B) and a liver extract powder prepared from pig liver. In certain experiments—indicated in experimental histories,—a synthetic preparation (Eli Lilly and Company) was given in the daily diet (13). The concentrates (yeast, liver extract powder) contain nitrogen and the amount is added to the protein intake indicated in the tables. The salt mixture used represents the McCollum, Simmonds formula (16) without iron.

Human globin (Table 1) consisted of three separate lots, all prepared from human erythrocytes. Lot E-2154 was a grayish white powder (dog 43-431), lot E-2644 was in liquid form (dog 43-304), dog 43-250 received a powder form of a different lot. Table 1, dog 43-431 was given a daily dose (six doses per week) of 12 gm. globin dissolved in 200 cc. of sterile distilled water, filtered with aseptic precautions, and slowly injected. Dog 43-304 received the liquid material as such. This contained 10 gm. of modified human globin in 300 cc. of a 0.9 per cent NaCl solution and was osmotically equivalent to 285 cc. of normal human plasma. Dog 43-250 was given a powdered form beginning with a dose of 0.88 gm. and increasing the amount to 3 gm. per dose. The method of preparation of the powdered globin for injection was the same as that used for lot E-2154. The tryptic digest of globin (Table 2) was prepared from dog globin separated from dog erythrocytes according to the method of Anson and Mirsky (2) which is essentially a precipitation by a solution of acetone and hydrochloric acid. A

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tryptic digest was prepared from this dog globin by the Difco Laboratories. The digest powder was dissolved in sterile distilled water, filtered with aseptic precautions, and slowly injected intravenously. The horse globin relating to Table 3 was prepared from horse erythrocytes according to the method of Anson and Mirsky. The globin powder was dissolved in 300 cc. water, 75 gm. of dextrose were added, and the mixture was fed daily by stomach tube.

Details concerning urinary nitrogen studies have been described elsewhere (13). Other methods used in the experiments of Tables 4 and 5 in which hemoglobin as laked red blood cells was given intraperitoneally, are identical with those previously described (10). The basal diet used in these experiments has also been described in detail (10) and is again referred to as low protein diet I.

In periods in which *dl*-isoleucine and *dl*-methionine were used, the amino acids were dissolved in 30 to 40 cc. of hot distilled water (20 to 25 cc. when methionine alone was used), and the clear solution gently boiled for 5 to 8 minutes. It was then cooled to 30–40°C. and added to the laked red cells.

During the periods of hemoglobin injection, the first urines passed after the injection almost invariably contained a small amount of hemoglobin.

As indicated in the experimental history of dog 43-31, Table 5, about 30 cc. of blood were drawn at stated intervals, oxalated, and the plasma obtained was subjected to electrophoretic analyses which were carried out on the day after the blood sample was drawn. Veronal buffer of pH 8.5 and ionic strength 0.1 was used. Other details are described in a previous paper (17).

In the following tables for any given dog the periods run consecutively. Hemoglobin levels (Tables 1, 2, and 3) 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

Table 1 gives three satisfactory experiments with *human globin*. These dogs were well standardized and maintained a satisfactory level of anemia and hypoproteinemia (double depletion). The production of new plasma protein and hemoglobin in all three experiments totals an average of 76 gm. return for 100 gm. globin given. In the first experiment (dog 43-431) the largest amount of globin was injected with the smallest percentage return (37 per cent). It is probable that slight intoxication due to the foreign globin was responsible. We have found that dog globin is toxic (14) and the human globin gave no anaphylactoid response. It is also noted that there is a rather rapid loss of weight—2 kilos or more during the 3 week period of injection. The nitrogen figures for intake and urinary output show a definite negative balance. When hemoglobin is given intraperitoneally under like conditions (10) there is less loss of weight, more blood protein production, and better nitrogen balance.

For comparison note that a favorable diet protein (periods 1 and 2) in the

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first experiment of Table 1 shows a gain in weight and a return of only 43 gm. new blood protein for 384 gm. diet protein (11 per cent return). This is lower than the average response to this diet but the accretion of body protein is a part of the protein utilization.

TABLE 1

Human Globin Parenterally in Anemic and Hypoproteinemic Dogs

Period 1 wk.	Weight	Protein intake		Protein output				Production ratio plasma protein to hemoglobin	Total nitrogen	
		Type	Weekly	Hemoglobin		Plasma protein			Intake	Urinary output
				Level	Output per wk.	Level	Output per wk.			
	kg.		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*gm.*	*gm.*
Dog 43-431—globin intraperitoneal and vein										
1	12.8	Salmon 120 basal	192	9.1	16.4	4.4	7.6	46	30.7	
2	13.3	Salmon 120 basal	192	7.1	13.7	4.2	5.1	37	30.7	
3	13.2	Globin 66 + basal	76	5.7	10.8	4.9	7.6	70	12.2	11.7
4	12.4	Globin 54 + basal	58	6.5	19.9	5.5	12.1	61	9.3	12.0
5	11.1	Globin 48 + basal	52	6.5	7.0	4.9	4.7	67	8.3	9.0
6	10.7	Basal	8	6.9	9.1	4.4	4.9	54	1.2	6.6
		Total globin intake = 168 gm. Total blood protein output = 62 gm.								
Dog 43-304—globin by vein										
1	19.2	Basal	40	8.5	2.3	4.0	0		6.6	
2	18.6	Globin 38 + basal	49	11.7	2.4	3.7	0		7.8	13.4
3	17.9	Globin 47 + basal	59	10.8	49.4	4.7	21.9	44	9.4	14.2
4	17.1	Globin 20 + basal	29	8.4	17.3	3.9	5.7	33	4.6	9.9
5	16.9	Basal	21	8.4	1.6	3.6	0		3.3	9.9
		Total globin intake = 105 gm. Total blood protein output = 97 gm.								
Dog 43-250—globin by vein										
1	16.6	Basal	14	8.6	11.8	4.4	6.0	51		
2	15.8	Globin 8 + basal	22	8.2	24.6	5.3	13.9	57		
3	14.2	Globin 18 + basal	28	6.9	13.3	5.0	8.6	65		
4	13.4	Globin 18 + basal	23	6.0	14.2	5.9	12.2	86		
5	11.7	Globin 6 + basal	14	6.0	1.2	5.6	0			
		Total globin intake = 50 gm. Total blood protein output = 87 gm.								

Experimental History—Table 1.

Dog 43-431. Male adult spaniel. No early history available. Dog has been in laboratory kennels for several months under standard experimental conditions. October, 1944, daily diet of basal protein-free biscuit 450 gm., canned squash 350 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Plasma protein and hemoglobin depletion begun. Beginning plasma volume 822 cc., weight 17.5 kilos. Regular double depletion experiments.

Apr. 13 to June 2, 1945—*human globin* experiment—Table 1.

Periods 1 and 2—daily diet of canned salmon 120 gm., basal protein-free biscuit 400 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Average plasma volume 693 cc. Food consumption 100 per cent. May 11—periods 3 to 6—human globin intraperitoneal injection begun. Because of slow absorption dose of globin was occasionally decreased to one-half and given by vein. Daily diet of basal protein-free biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Plasma volumes 778 cc., 809 cc., and 736 cc. during injection periods. A/G ratios 1.1, 0.96, and 0.80. Food consumption 54 per cent, 21 per cent, and 22 per cent. No other unfavorable reaction. Period 6—daily diet of basal, protein-free biscuit 300 gm., dextrose 30 gm., yeast 5 gm., liver extract powder 2 gm., reduced iron 600 mg. Dog in good condition.

Experimental History—Table 1.

Dog 43-304. Female adult hound. No previous history available. Dog has been in laboratory kennels for several months under standard experimental conditions. October, 1944, daily diet of basal protein-free biscuit 450 gm., canned squash 300 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Plasma protein and hemoglobin depletion begun. Beginning plasma volume 829 cc., weight 20.1 kilos.

Oct. 26 to Nov. 20, 1944—*human globin* experiment—Table 1.

Period 1—Above daily diet. Periods 2 to 5, human globin intravenous injection, beginning with 100 cc. (3.3 gm.) and increasing to 250 cc. daily except Sunday. Basal daily diet as above. Period 4—three doses globin only. Plasma volumes 952 cc., 869 cc., and 878 cc. during injection periods. A/G ratios 1.0, 1.5, and 1.1. Food consumption 56 per cent, 64 per cent, and 49 per cent. Occasional edema around eyes, red blotches on skin of inner surfaces of hind legs. Symptoms gradually disappeared toward end of injection period. Period 5—daily diet of basal protein-free biscuit 300 gm., canned squash 100 gm., yeast 3 gm., liver extract powder 2 gm. Complete recovery from skin symptoms during subsequent week on whole egg diet.

Experimental History—Table 1.

Dog 43-250. Male adult hound. No early history available. Dog has been in laboratory kennels for several months under standard experimental conditions. February, 1944, daily diet of basal protein-free biscuit 450 gm., yeast 5 gm., reduced iron 600 mg. Plasma protein and hemoglobin depletion begun. Beginning plasma volume 758 cc., weight 18.0 kilos. Regular double depletion experiments.

Aug. 24 to Sept. 24—*human globin* experiment—Table 1.

Period 1—daily diet of basal protein-free biscuit 350 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Food consumption 79 per cent. Periods 2 to 5 inclusive, human globin intravenous injection beginning with 0.9 gm., and increasing to 3 gm. daily except Sunday. Daily diet of basal protein-free biscuit 400 gm., synthetic vitamin mixture 10 cc., reduced iron 600 mg. Food consumption 85 per cent, 65 per cent, and 55 per cent during injection periods. Plasma volumes 790 cc., 879 cc., and 720 cc. A/G ratios 1.3, 1.7, and 0.9. Rest and recovery period follows experiment.

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Table 2 gives the response of the anemic dog to a *tryptic digest* of dog globin. In these experiments we measure accurately the production of hemoglobin by means of standardized anemic dogs. The toxicity of this material was very slight, the dogs' appetites unimpaired, and there was no loss of weight. If we average all four experiments we find a total return of 65 gm. hemoglobin for a globin digest intake of 175 gm.—a return of 37 per cent. This is about the expected return for the feeding of globin (note Table 3—34 per cent and 24 per cent). Digests of hemoglobin, serum proteins, and casein are notoriously uncertain as to their toxicity, some being almost completely non-toxic and others too toxic to use in any significant amounts. When one comes to evaluate the responses to intravenous injections of anemic dogs receiving considerable protein by mouth there will be differences of opinion. These reactions are recorded and one person will believe that the response is of a general nature due to threshold metabolic disturbances—another, that the specific digest is responsible for the new globin formation. Isotope studies using heavy or radioactive elements incorporated in blood plasma proteins (5) will clear this point.

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TABLE 2

Dog Globin

Tryptic Digest by Vein and Hemoglobin Production in Anemia

Diet periods 1 wk. each	Food consumption	Weight	Plasma volume	Blood hemoglobin level	Hemoglobin removed bled
	per cent	*kg.*	*cc.*	*gm. per cent*	*gm.*
Dog 35-6					
Bread 450, salmon 50, Klim 20 (basal)	100	18.5	1118	6.1	1.3
Total globin digest 11 gm. + basal	100	18.5	1085	6.6	1.4
Total globin digest 18 gm. + basal	100	18.8	1087	7.2	13.1
Total globin digest 18 gm. + basal	100	18.4	1019	5.8	23.0
Basal diet	100	18.6	1237	5.2	1.1
Total globin intake = 47 gm. Total net hemoglobin output = 25 gm.					
Dog 34-149					
Bread 300, salmon 75, Klim 20 (basal)	100	16.3	1035	6.3	1.3
Total globin digest 14 gm. + basal	100	16.4	962	6.1	11.6
Total globin digest 18 gm. + basal	100	16.0	984	6.1	1.3
Total globin digest 15 gm. + basal	100	16.2	950	6.1	1.3
Basal diet	100	16.1	976	6.2	1.2
Total globin intake = 47 gm. Total net hemoglobin output = 7 gm.					
Dog 34-148					
Bread 450, salmon 50, Klim 20 (basal)	100	16.6	946	6.6	1.4
Total globin digest 12.5 gm. + basal	100	16.6	1038	5.8	20.1
Total globin digest 15.0 gm. + basal	100	16.9	1074	5.7	9.0
Total globin digest 18.0 gm. + basal	100	16.8	1018	6.6	1.3
Basal diet	100	17.2	1089	5.4	1.1
Total globin intake = 46 gm. Total net hemoglobin output = 15 gm.					
Dog 32-5					
Bread 300, salmon 50, Klim 20 (basal)	100	15.7	858	5.8	1.3
Total globin digest 9 gm. + basal	100	15.7	870	5.5	18.9
Total globin digest 18 gm. + basal	100	15.5	793	5.9	1.3
Total globin digest 8.5 gm. + basal	100	15.6	889	6.1	1.2
Basal diet	100	15.8	900	6.2	1.3
Total globin intake = 36 gm. Total net hemoglobin output = 18 gm.					

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Experimental History—Table 2.

Dog 35-6. Male adult bull. Born November, 1934. Continuous anemia history July 11, 1936, to Sept. 19, 1940. Regular anemia experiments. Beginning weight 19.7 kilos. Average plasma volume 1125 cc.

June 10 to July 2, 1938—*dog globin* digest experiment—Table 2.

Daily basal diet of salmon bread 450 gm., canned salmon 50 gm., skim milk (Klim) powder 20 gm., 2nd to 4th week, inclusive, globin digest intravenous injection beginning with 0.25 gm. and increasing to 3 gm. per dose, daily except Sunday. No unfavorable reaction. Daily basal diet as above, and for week following injection period. Dog in excellent condition.

Experimental History—Table 2.

Dog 34-149. Male, white bull. Born December, 1933. Continuous anemia history, Mar., 1937, to July, 1940. Regular anemia experiments. Beginning weight 17.2 kilos. Average plasma volume 950 cc.

June 16 to July 7, 1938—*globin digest* experiment—Table 2.

Daily basal diet of salmon bread 300 gm., canned salmon 75 gm., skim milk powder 20 gm., 2nd to 4th week, globin tryptic digest by vein beginning with 0.50 gm. and increasing to 3 gm. per dose, daily except Sunday. No unfavorable reaction. July 7—basal bread ration as above. Dog in good condition.

Experimental History—Table 2.

Dog 34-148. Female, adult bull. Born December, 1933. Continuous anemia history, March, 1937, to date. Regular anemia experiments. Beginning weight 15.6 kilos. Average plasma volume 1050 cc.

Feb. 8 to Mar. 2, 1939—*dog globin tryptic digest* experiment—Table 2.

Daily basal diet of salmon bread 450 gm., canned salmon 50 gm., skim milk powder 20 gm. 2nd to 4th week, globin digest by vein beginning with 0.5 gm. and increased to 3 gm. per dose, daily except Sunday. No unfavorable reaction. Daily diet as above and for week following injection experiment. Dog in excellent condition.

Experimental History—Table 2.

Dog 32-5. Female, adult coach. Born April, 1932. Continuous anemia history April, 1933, to date. Regular anemia experiments. Beginning weight 14 kilos. Average plasma volume 750 cc.

Feb. 26 to Mar. 15, 1938.—*dog globin tryptic digest* experiment—Table 2.

Daily diet of salmon bread 300 gm., canned salmon 50 gm., skim milk powder 20 gm. 2nd to 4th week, globin digest by vein beginning with 0.5 gm. and increasing to 3 gm., per dose, daily except Sunday. No unfavorable reaction. Daily diet as above and for week following injection period. Dog in excellent condition.

Table 3 supports published data (14) to show that globin by mouth is associated with a significant production of new hemoglobin in the anemic dog. Globin was the only protein material given by mouth during the 2 week experiments. The net return of new hemoglobin was 34 and 24 per cent. In our standard experiments 40 per cent represents maximal utilization of any food protein to produce new hemoglobin or plasma protein in depleted dogs. Some

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of the new blood proteins in these experiments (Table 3) may come from body stores.

TABLE 3

Horse Globin by Mouth

Well Utilized to Produce Hemoglobin in Anemia

Diet periods 1 wk. each	Food consumption	Weight	Plasma volume	Blood hemoglobin level	Hemoglobin removed bled
	per cent	*kg.*	*cc.*	*gm. per cent*	*gm.*
Dog 27-238					
Bread 375, salmon 100, Klim 40 (basal)	100	16.1	907	5.8	1.3
Total globin 52.5 gm.—dextrose 75 gm. daily		14.5	794	7.2	23.1
Total globin 52.5 gm.—dextrose 75 gm. daily		13.3	768	5.7	9.0
Basal diet	100	15.2	936	5.7	1.1
Basal diet	100	15.8	940	5.8	13.9
Basal diet	100	15.9	946	6.2	1.3
Total globin intake = 105 gm. Total net hemoglobin output = 36 gm.					
Dog 27-236					
Bread 375, salmon 100, Klim 50 (basal)	100	16.7	982	5.7	1.2
Total globin 52.5 gm.—dextrose 75 gm. daily		15.1	784	6.3	12.4
Total globin 52.5 gm.—dextrose 75 gm. daily		13.6	698	5.9	11.6
Basal diet	100	13.9	852	5.9	1.2
Basal diet	100	14.9	876	6.1	13.0
Basal diet	100	15.6	942	5.8	1.2
Total globin intake = 105 gm. Total net hemoglobin output = 25 gm.					

Experimental History—Table 3.

Dog 27-238. Female coach. Born February, 1927. Continuous anemia history, Nov., 1928, to Aug., 1937. Beginning weight 13.5 kilos. Average plasma volume 850 cc. Diet at no time contained potent animal protein substances in an effort to produce dietary anemia. Experiments pertained to testing drugs, minerals, amino acids, fruits, and vegetables.

Feb. 17 to Mar. 3, 1934—*horse globin* experiment—Table 3.

Horse globin 7.5 gm., dextrose 75 gm., and water 300 cc. given by stomach tube daily. No unfavorable reaction. March 3—basal diet as indicated in table. Dog in good condition at end of experiment.

Experimental History—Table 3.

Dog 27-236. Male bull. Born Feb., 1927. Continuous anemia history, Mar., 1930, to Aug., 1935. Beginning weight 13.0 kilos. Average plasma volume 800 cc. Regular anemia experiments.

Feb. 12 to Feb. 26, 1934—*horse globin* experiment—Table 3.

Horse globin 7.5 gm., dextrose 75 gm., water 300 cc. given by stomach tube daily. No unfavorable reaction. Basal diet of salmon bread 375 gm., canned salmon 100 gm., skim milk powder 50 gm. Dog in good condition at end of experiment.

The data of Tables 4 and 5 are mutually supporting. In periods 3 to 7 of Table 4 hemoglobin given intraperitoneally to dog 43-346 is well retained and utilized for the maintenance of weight and urinary nitrogen balance in a manner similar to that previously reported (10). The addition of a daily supplement of *dl*-methionine and *dl*-isoleucine to the injected hemoglobin (periods 8 to 12) results in a very rapidly manifested fall in the total urinary nitrogen with a corresponding decrease in the urea-ammonia fraction. There is maintenance of weight and well-being.

The omission of the small supplement of *dl*-methionine is followed rapidly by increased total urinary nitrogen excretion and a rise in the urea-ammonia fraction to levels similar to those seen during the injection of unsupplemented hemoglobin. This indicates that *dl*-isoleucine as a supplement is ineffectual in improving nitrogen retention.

The prompt return to basal nitrogen excretion levels in periods 18 and 19 supports our previous conclusion that parenterally administered hemoglobin is rapidly metabolized and that carry-over excretion of urinary nitrogen is correspondingly minimal.

The experiments of Table 5 were designed to test further the validity of conclusions drawn from Table 4. The nitrogen excretion figures per period are lacking in uniformity partially because 48 hour metabolism periods were not terminated by catheterization.

In periods 5 to 9 hemoglobin supplemented with *dl*-isoleucine was given intraperitoneally; the poor nitrogen retention seen here is in contrast to a previously reported hemoglobin injection experiment in the same dog (10) in which unsupplemented hemoglobin given intraperitoneally was better utilized. Vomiting of small amounts of mucus noted after injections of periods 6 and 9 suggests a disturbing element of intoxication; however food consumption was spontaneous and complete. In spite of this, there is some small weight loss.

dl-Isoleucine is replaced by *dl*-methionine in periods 10 to 14, and a prompt fall in total urinary nitrogen excretion is seen, with approximate urinary nitrogen balance and small weight gain. The restoration of *dl*-isoleucine in addition to the methionine (periods 15 to 19) contributes only slightly and to the extent of its own nitrogen content toward nitrogen balance. There is very slight weight loss. There is no evidence that the *dl*-isoleucine was toxic to this dog as one might suspect from periods 5 to 9 above.

Basal levels of nitrogen excretion are rapidly attained in follow-up periods 20 and 21.

TABLE 4

Laked Red Cells Intraperitoneally

Nitrogen Retention Improved by dl-Methionine but Not by dl-Isoleucine

Dog 43-346

Period	Hemoglobin N injected	Urinary total N	Urea N + NH_3-N	Urea N + NH_3-N	Undetermined N*	Weight	Plasma protein	R.B.C. hematocrit
48 hrs.	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*kg.*	*gm. per cent*	*vol. per cent*
Basal diet (0.46 gm. N per period)								
1		2.82	2.00	70.7	0.52	10.1	6.02	50
2		2.95	2.21	75.0	0.56	10.0		
Basal diet. Intraperitoneal hemoglobin								
3	4.44	3.98	3.00	75.5	0.73			
4	2.00	3.38	2.76	81.8	0.37	9.9		
5	4.95	4.24	3.06	72.3	0.96	10.0		
6	4.77	4.08	2.86	70.1	1.11			
7	2.28	3.81	2.68	70.7	1.07			
Total	18.64	19.49 (20.94)‡						
Basal diet. Intraperitoneal hemoglobin plus methionine and isoleucine								
8	4.40	3.27	1.87	57.2	1.18	9.9		
9	4.16	2.63	1.33	50.7	0.62	9.9	6.87	62
10	3.59	2.88	1.96	68.2	0.74	9.9		
11	2.08	1.95	1.20	61.4	0.59			
12	4.01	2.70	1.32	49.0	1.17	9.9		
Total	18.24	13.43 (22.00)‡						
Basal diet. Intraperitoneal hemoglobin plus isoleucine								
13	2.61	3.56	2.55	71.7	0.83			
14	1.57	3.12	2.29	73.4	0.64			
15	2.97	3.14	2.37	75.5	0.62	10.0		
16	3.16	3.22	2.30	71.5	0.74		6.75	62
17	3.59	3.52	2.37	67.3	1.05	9.9		
Total	13.90	16.56 (17.28)‡						
Basal diet								
18		1.77	1.09	61.8	0.55			
19		1.84	1.34	72.8	0.35			

* Undetermined N is calculated *not* to include creatine, creatinine, and uric acid N, which were determined but are not shown in table.

‡ Totals in parentheses include nitrogen of injected hemoglobin, basal diet, and amino acid supplements.

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TABLE 5

Laked Red Cells Intraperitoneally

Nitrogen Retention Improved by dl-Methionine but Not by dl-Isoleucine

Dog 43-31

Period	Hemoglobin N injected	Urinary total N	Urea N + NH_3-N	Urea N + NH_3-N	Undetermined N*	Weight	Plsma protein	R.B.C. hematocrit
48 hrs.	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*kg.*	*gm. per cent*	*vol. per cent*
Basal diet (0.54 gm. N per period)								
1		4.71	3.69	78.3	0.72		6.87	66.2
2		5.76	4.41	76.5	0.90			
3		3.45	2.27	65.8	0.82			
4		3.71	2.51	67.7	0.85	14.8	5.22	
Basal diet. Hemoglobin intraperitoneally plus isoleucine								
5	3.82	4.12	2.02	49.2	1.92			
6	4.01	6.87	5.53	80.5	0.95			
7	2.37	6.06	4.32	71.3	1.36			
8	3.24	3.95	2.60	65.8	1.04			
9	3.86	4.47	3.65	81.7	0.54	14.2	5.56	64
Total	16.80	25.47 (20.57)‡						
Basal diet. Hemoglobin intraperitoneally plus methionine								
10	4.18	3.90	3.12	80.1	0.50	14.0		
11	1.30	2.77	1.86	67.3	0.60			
12	3.34	2.65	2.02	76.3	0.36			
13	3.73	4.35	2.81	64.7	1.18	14.1		
14	3.90	3.11	2.27	72.9	0.47	14.3	5.95	
Total	16.45	16.78 (19.48)‡						
Basal diet. Hemoglobin intraperitoneally plus isoleucine and methionine								
15	3.59	2.47	1.29	52.2	0.92			
16	3.71	4.21	2.78	66.1	1.11		5.75	63
17	3.73	3.17	1.51	47.5	1.37			
18	2.03	3.79	2.88	76.6	0.66	14.2		
19	3.69	3.09	1.88	60.7			5.18	
Total	16.75	16.73 (20.91)‡						
Basal diet								
20		2.44		59.3				
21		1.94		51.4		14.0		

* Undetermined N is calculated *not* to include creatine, creatinine, and uric acid N, which were determined but are not shown in tables.

‡ Totals in parentheses include nitrogen of injected hemoglobin, basal diet, and amino acid supplements.

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Experimental History—Table 4.

Dog 43-346. Female Doberman mongrel. Kennel diet for 6 months followed by 10 days' fast with water *ad libitum*. Placed on low protein diet I, 150 gm. with choline chloride 400 mg. and nicotinic acid 25 mg. daily. *Periods 1 and 2*, diet eaten 100 per cent. *Periods 3 to 7*, laked red blood cells 75 to 87 cc. given intraperitoneally daily on 8 of 10 days without reaction; diet eaten 100 per cent. *Periods 8 to 12*, laked red blood cells 63 to 83 cc. supplemented with *dl*-isoleucine 1 gm. and *dl*-methionine 0.40 gm., given daily on 9 of 10 days without reaction; small amounts of food left were force fed to insure 100 per cent consumption. *Periods 13 to 17*, laked red blood cells 58 to 75 cc. supplemented with 1 gm. *dl*-isoleucine, given intraperitoneally without reaction on 9 of 10 days. Peritoneal puncture in period 17 released about 200 cc. of faintly turbid straw-colored fluid, with specific gravity 1.004, 0.87 gm. per cent protein, and only occasional mesothelial cells from which bacteria were grown on culture. However dog appeared well, temperature was normal, and food consumption was spontaneous and complete in periods 13 through 18; a small food residue in period 19 was force fed. Returned to kennel and condition has remained normal.

Experimental History—Table 5.

Dog 43-31. Short-haired female hound. After long continued maintenance on kennel diet, fasted for 3 days and then placed on low protein diet I, 180 gm. plus 400 mg. choline chloride and 25 mg. nicotinic acid daily. *Periods 1 to 4*, diet eaten 100 per cent. At close of period 4, drew 30 cc. blood for electrophoretic study. *Periods 5 to 9*, 56 to 80 cc. of laked red blood cells with 1 gm. *dl*-isoleucine given intraperitoneally daily on 9 of 10 days without immediate reaction. Diet eaten 100 per cent. Dog vomited a little mucus after injections in periods 6 and 9. At close of period 9, drew 30 cc. of blood for electrophoretic study. *Periods 10 to 14*, 56 to 74 cc. of laked red blood cells supplemented with 0.40 gm. *dl*-methionine given daily intraperitoneally on 9 of 10 days without reaction. Diet eaten 100 per cent. Drew 30 cc. of blood for electrophoretic study at close of period 14. *Periods 15 to 19*, 62 to 74 cc. of laked red blood cells given intraperitoneally with 0.40 gm. methionine and 1 gm. *dl*-isoleucine on 9 of 10 days. Food consumption 100 per cent with some force feeding. At close of period 19, 30 cc. of blood drawn for electrophoretic study. Periods 20 and 21, ate 100 per cent of diet.

Table 6 shows the results of electrophoretic analysis of the plasma of dog 43-31. The first and last analyses are on plasma obtained at the end of 8 days of basal low protein diet only. The three intervening analyses are at the ends of 10 day periods during which the dog received hemoglobin intraperitoneally together with small amounts of either *dl*-methionine, *dl*-isoleucine or both (Table 5). At the end of the first 10 day period of intraperitoneal hemoglobin there is a marked fall in the albumin concentration, a rise in the α-globulin, and a very marked rise in the β-globulin and "fibrinogen." During the next two periods of 10 days each during which hemoglobin was given intraperitoneally, the albumin level rises slightly to remain fairly constant and the β-globulin and "fibrinogen" do likewise. It must be remembered that in the normal dog only about 40 per cent of the area of the "fibrinogen" peak is due to fibrinogen. At the end of the final 8 day period during which the dog received only the basal

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low protein diet, the concentrations of β-globulin and "fibrinogen" declined sharply.

TABLE 6

Intraperitoneal Hemoglobin Supports Electrophoretic Albumin Level and Increases Concentration of Proteins Migrating with Mobilities of Beta Globulin and Fibrinogen

(*Compare with Table 5 for Other Experimental Data*)

Dog 43-31

Period*	Electrophoretic concentrations, gm. per cent					
48 hrs.	Total protein	Albumin	α‡	β	ϕ§	γ
Basal diet—very low protein						
1–4	5.11	2.34	1.63	0.40	0.31	0.43
Basal diet + hemoglobin and *dl*-isoleucine intraperitoneally						
5–9	5.41	1.72	1.96	0.66	0.57	0.50
Basal diet + hemoglobin and *dl*-methionine intraperitoneally						
10–14	5.14	1.97	1.34	0.61	0.88	0.34
Basal diet + hemoglobin and *dl*-isoleucine and *dl*-methionine						
15–19	5.13	1.87	1.46	0.59	0.71	0.50
Basal diet						
20–23	4.90	1.90	1.59	0.34	0.57	0.50

* Periods correspond to those of Table 5.

‡ α refers to the sum of the concentrations of all four α-globulins.

§ Only about 40 per cent of the normal ϕ peak is due to fibrinogen.

DISCUSSION

These experiments add support to our previous contention that hemoglobin given parenterally, like plasma proteins, enters into the body protein economy and undoubtedly contributes to the body protein pool. Hemoglobin may thus provide nitrogen adequate for maintenance of weight and nitrogen balance.

Previous reports show (10) that hemoglobin as the principal source of nitrogen intake contributes to the production of both red cells and plasma protein in the *doubly depleted dog* under conditions of constant weight loss. In the simple hypoproteinemic dog we have observed excellent nitrogen retention and maintenance of weight without production of enough plasma protein to raise the lowered plasma protein levels present (10). A recent report from this laboratory (7) indicates that massive infusion of red cells in plasmapheresis of a simple

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hypoproteinemic dog receiving a casein digest may be followed by new plasma protein production. This new plasma protein is derived, in part at least, from the catabolism of injected red cells.

The work of Devlin and Zittle was interpreted by them (4) as showing that human globin is inadequate to support growth in rats unless supplemented with isoleucine; unfortunately the very poor food consumption of the rats receiving the unsupplemented globin was not controlled by paired feeding, and the failure to grow may be simply the result of the very low food intake reported. Other workers (12) have controlled the dietary intake and shown that rats on a human or beef globin diet become anemic and fail to grow unless fed a supplement of isoleucine. It has also been found necessary to add *l*-isoleucine to an acid digest of hemoglobin before it will support growth in rats (1).

However, *dl*-isoleucine supplementation of dog hemoglobin fails to cause a significant improvement in nitrogen retention in our studies. The isoleucine content of dog red cells (hemoglobin) as determined microbiologically has not yet been reported. This may resolve the apparent discrepancy in our observations on *maintenance* in the dog as compared with those on *growth* in the rat. In any event, it must be kept in mind that the amino acid requirement for growth is much greater than that for maintenance.

A small supplement of *dl*-methionine on the contrary, results in significant improvement of nitrogen retention. The amino acid composition of hemoglobin as reported by Block and Bolling (3) indicates that hemoglobin is low in its content of isoleucine, and low in its content of methionine as compared with dietary proteins of known high biologic value. Using the method of McCarthy and Sullivan (8) we have found human and dog red blood cells to contain 1.28 and 1.36 gm. methionine per cent red cell protein respectively.

Methionine supplementation of a protein dietary low in methionine has been known to improve its biologic value (15). We have also recorded experiments showing increased efficiency of utilization of body protein stores in the dog fed methionine with a very low protein diet (9). Nitrogen sparing by methionine during hemoglobin injection may be the result of both improved biologic value and sparing of body protein stores.

From the practical point of view it is of interest to compute the amount of hemoglobin nitrogen liberated and available for metabolism as a result of red cell breakdown from ordinary wear and tear. A normal dog weighing 12 kilos with a blood volume of 1 liter and 18 gm. of hemoglobin per cent will have a total of 180 gm. of hemoglobin in circulation. If approximately 1 per cent of the red cells are broken down per day, 1.8 gm. of hemoglobin or approximately 0.3 gm. of nitrogen daily would derive from this source. Under conditions of greater red cell breakdown correspondingly more hemoglobin nitrogen would become available.

The exact fate of the protein moiety of hemoglobin in intermediary metab-

olism is not clear. Is globin split directly from the pigment radicle and can globin be detected in the peripheral circulation after large infusions of hemoglobin? The prompt increase in the areas of the β-globulin and "fibrinogen" peaks after the intraperitoneal injection of hemoglobin and the subsequent decrease in these same peaks after discontinuing the giving of hemoglobin suggest the possibility that globin may enter the circulation and be responsible for these increases. If the isoelectric point of dog globin is similar to that of human globin, 7.5 according to Munro and Munro (11), one would expect that at a pH of 8.5 the mobility would be low. We determined the mobility of a sample of "modified human globin" in veronal buffer of pH 8.5 and ionic strength 0.1 and found it to lie between the mobilities of human fibrinogen and β-globulin. (This modified human globin is the same as that used in the experiments of Table 1.)

It is unlikely that there was an actual increase in fibrinogen as a result of peritoneal irritation because there was no rise whatever in the α 3 globulin peak. In our experience a rise in fibrinogen produced by tissue injury is always accompanied by a marked rise in α 3 globulin. We plan further work to determine whether or not globin appears in plasma after intraperitoneal administration of hemoglobin.

SUMMARY

Hemoglobin and globin alone, supplemented, or modified in various ways are seriously considered as plasma substitutes.

Human globin given to doubly depleted (anemic and hypoproteinemic) dogs by vein contributes to the production of new hemoglobin and plasma protein, but there is some toxicity and weight loss. Dog hemoglobin given intraperitoneally is better tolerated and somewhat more completely utilized with more blood proteins formed and less weight loss.

Dog globin (tryptic digest) given by vein in anemic dogs is associated with a moderate production of new hemoglobin.

Horse globin by mouth contributes to the formation of new hemoglobin in the standard anemic dog.

Dog hemoglobin given intraperitoneally in protein fasting, non-anemic dogs is well utilized to maintain nitrogen and weight balance. A *dl*-isoleucine supplement fails to improve this utilization of hemoglobin for *maintenance* in the dog. A small supplement of *dl*-methionine greatly improves the utilization of dog hemoglobin for maintenance in the dog and further addition of isoleucine is without effect.

The intermediary metabolism of dog hemoglobin is not yet worked out. Electrophoretic analyses (Table 6) suggest that globin appears in the peripheral circulation after intraperitoneal injections of hemoglobin.

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