HEMOGLOBIN AND PLASMA PROTEIN

THEIR RELATION TO INTERNAL BODY PROTEIN METABOLISM*

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Hemoglobin and plasma proteins have been studied in this laboratory for many years. We have investigated *construction* factors as well as the result of *destruction*, conservation, wastage, wear and tear of these proteins. This paper integrates the *disintegration of hemoglobin* with the reutilization of globin and its split products to establish nitrogen balance and build other body proteins (plasma protein).

Hemoglobin is a peculiar basic protein having a low content of some of the essential amino acids (isoleucine and methionine) (1) and it contains a pigment radicle and attached iron. It is generally agreed that iron is conserved in the body but recent studies (6) with radio-iron have emphasized the frugal handling of iron within the body and that iron absorption is determined largely by the need for iron. After hemoglobin destruction the pigment radicle of 4 pyrrol rings is thrown away in the bile as the body prefers to make new pigment radicles rather than to save the old pigment.

Globin makes up about 95 per cent of the hemoglobin molecule. Its catabolism and conservation by the body is not well understood, to put it mildly. The experiments given below are concerned with the study of *globin* as it is broken down in the body. It is clear that under the conditions of these various experiments the breakdown products of globin do contribute effectively to internal body protein metabolism. Under favorable circumstances the *globin* by parenteral route may maintain the dog in *positive nitrogen balance* for 2 or 3 weeks, the globin being practically the sole source of intake nitrogen (Tables 1 and 2).

Plasma proteins in plasma are used as controls for the hemoglobin injections as it is well established (2, 11, 17) that plasma proteins as the sole source of nitrogen can maintain the dog in positive nitrogen balance for several weeks—Table 1 shows well that there is no surplus nitrogen escape in the *afterperiod* of 8 days following plasma injections. This nitrogen retention and utilization was questioned by Elman (3, 4) but it is clear that the nitrogen wastage in his experiments was due to *citrated* plasma and the related citrate intoxication. In

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

all our experiments heparinized plasma was used and the only untoward results were due to specific hemolysins in certain blood donors. Sensitization of this general character has been described by Wright (22).

Methods

All dogs used in the experiments of Tables 1, 2, 3, and 4 were active, healthy adult mongrels. They were maintained in the animal house for at least several months on a diet of mixed table scraps before the start of the experiments, details of which are given in the experimental history of each table. The dogs used in the experiments of Tables 5 and 6 were taken from the anemia colony as described elsewhere (18, 21). Dog 40-41 of Table 6 was a renal bile fistula dog of the type described by Kapsinow, Engle, and Harvey (13).

The *diet* referred to in the experimental histories as low protein diet I consists of sucrose 72.2 per cent, Wesson salt mixture (20) 4.6 per cent, calcium phosphate 4.6 per cent, lard 14.9 per cent, Mazola oil 6.5 per cent, cod liver oil 1.4 per cent, yeast powder (Fleischman's Type 200-B) 0.7 per cent, a powdered liver extract, "vitamin B_2 complex" (Eli Lilly and Company) 0.7 per cent, nicotinic acid 13.9 mg. per cent, and choline chloride 111 mg. per cent. The diet referred to in the experimental histories as low protein diet II consists of the following ingredients: cornstarch 4800 gm., dextrin 800 gm., baking powder 200 gm., bone ash 700 gm., salt mixture 150 gm., sugar 2400 gm., lard 1450 gm., corn oil 300 cc., cod liver oil 150 cc., and water 1300 cc. These are mixed with the water to make a firm cookie mixture, are lightly rolled out, cut into squares, and baked.

Vitamin additions to low protein diet II consist of dried yeast (Fleischman's Type 200-B) and a vitamin B_2 complex prepared from pig liver (liver extract powder) 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 and Company) 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 300 mg. These vitamins are administered in capsule or liquid form and fed separately immediately before diet feeding.

The dog blood used as the source of plasma and red cells was obtained by bleeding members of the donor colony and collecting the blood in heparin with precautions for asepsis. The plasma was obtained by centrifugalization of the blood and drawing off the supernatant plasma. The remaining red cells were then thoroughly washed by mixing with an equal volume of normal salt solution, and subsequent centrifugalization. For the preparation of hemoglobin solution (laked red cells), the packed, washed red cells were thoroughly mixed with an equal known volume of sterile distilled water, and after standing at room temperature for not more than 30 to 45 minutes, the resulting solution was injected intraperitoneally. For the preparation of the whole red cell suspension, the above centrifugalized red cell mass was mixed with an equal known volume of normal saline and injected intraperitoneally with aseptic precautions.

After pronounced *hemolytic reactions* were produced in two dogs (Table 2) by the repeated injection of heparinized plasma, the serum of each dog in the donor dog colony was tested against the red cells of the next recipient (Table 1, Dog 43–141) using the method described by Wright (22). This revealed that the sera of two dogs in the donor colony were strongly agglutinative for the red cells of the recipient. These two dogs were not used as plasma donors and the plasma injections of Table 1 proceeded uneventfully.

The *hemoglobin digest* used in the experiments of Table 4 was an enzymatic papain digest

of beef red cells (lot KB 42241).[1] A weighed amount of the dry powder (15.0 gm.) was dissolved in about 150 cc. of boiling normal salt solution. The resulting hazy solution was passed through a Seitz filter, and the clear, amber filtrate cooled and injected intravenously as described in the experimental histories.

The *nitrogen* content of the hemoglobin digest, the plasma and hemoglobin (laked red cells) injected daily, and the nitrogen content of the urines passed during the metabolism studies were determined by macro-Kjeldahl analysis. The urines were collected at the same time daily from the metabolism cages, and preserved with toluene and refrigeration. Total urinary urea and ammonia nitrogen was determined by aeration and titration after incubation with urease.

The *bilirubin* analysis of the urine of the renal bile fistula dog (Table 6) was done by using the van den Bergh reaction as described by Malloy and Evelyn (15), on aliquots of each 48 hour urine collection shortly after the close of each period. Urines were preserved with toluene and refrigeration until analysis. Samples of blood were drawn in isotonic sodium oxalate for *hematocrit* determinations, and plasma protein levels were determined by macro-Kjeldahl. The icterus index of the plasma (Table 2) was estimated by comparison with the potassium dichromate standards of Farahaugh and Medes (5).

EXPERIMENTAL OBSERVATIONS

The first experiments (Tables 1, 2, and 3) are much alike. There is no anemia although dog 43-141, Table 1, shows hematocrit levels below what we consider normal—that is 50 per cent red cells. A preliminary period of fasting followed by low protein intake (see experimental histories) removes much of the reserve store of protein building materials and there is a tendency for a moderate fall in the levels of circulating plasma proteins. Presumably dogs whose protein stores are depleted will use all available material out of which the needed protein can be produced.

When hemoglobin given intraperitoneally is utilized by the body to approximate nitrogen balance and maintain body weight, it is obvious that this incomplete protein must be supplemented by its lacking essential amino acids to produce plasma proteins and to supply needed proteins to body cells. That some dogs tolerate this experiment and show a better nitrogen balance may depend in part upon available reserves of these amino acids known to be low in hemoglobin.

Basal diet contains about 0.86 gm. nitrogen per period but probably less than half is protein nitrogen. This nitrogen comes from commercial yeast powder and liver extract powder given to supply diet accessories. In some experiments pure synthetic vitamins have been used and the basal ration then (Table 5) contains no nitrogen. The response of the dog and the nitrogen balance is not significantly modified by one or the other basal ration.

Experimental History—Table 1.

Dog 43-141. Male short-haired hound. Maintained for months on a good mixed diet, fasted 1 week, and at start of and throughout the experiment placed on low protein diet II 200

[1] We are indebted to Eli Lilly and Company for the preparation of this digest.

TABLE 1

Plasma and Laked Red Cells Intraperitoneally—Nitrogen Balance

Dog 43-141—Mongrel

Period No.	Blood proteins injected Total N	Total urinary N	Urea N + NH_3-N	Total undetermined urinary N	Circulating plasma protein level	A/G ratio Tiselius	R.B.C. hematocrit	Weight
48 hrs.	*gm.*	*gm.*	*per cent*	*gm.*	*gm. per cent*		*vol. per cent*	*kg.*
Basal diet contains little protein (0.86 gm. N per period)								
1		3.31	76.7	0.77	5.72		41	9.8
2		2.55	73.4	0.68				
3		2.08	68.7	0.55				
4		2.34						
Basal diet + whole blood plasma intraperitoneally								
5	4.60	1.80	75.5	0.44				9.4
6	4.17	2.53	83.5	0.42	7.64		38	
7	3.43	1.48	71.4	0.42				
8	1.86	1.30	65.8	0.45	8.90		45	9.3
9	3.80	1.61	67.4	0.52	9.70		40	
10	3.77	2.19	73.9	0.57				
11	2.07	2.70	69.9	0.82	9.98		41	9.4
12	3.81	2.52	72.3	0.70				
13	4.03	2.38	68.2	0.76				
14	4.06	2.14	69.3	0.66	9.32		35	9.5
Total.....	35.6	20.7						
Basal diet								
15		2.14	69.3	0.66		0.8	29	
16		2.00	63.7	0.73			30	
17		2.05	67.2	0.67	7.72		32	
18		1.69	66.0	0.57				
Basal diet + laked red blood cells intraperitoneally								
19	4.31	2.00	61.5	0.77	6.64	1.0	36	9.2
20	4.62	2.56	72.0	0.72	6.61		43	9.2
21	4.10	2.74	70.6	0.80	6.50		47	
22	1.80	2.40	66.7	0.80				
23	4.09	2.06	71.5	0.59				
24	4.30	3.39			6.93		51	
25	2.25	2.46						9.1
26	3.62	3.34					46	
27	3.80	1.86				0.5		
28	4.63	2.79						9.1
Total.....	37.5	25.6						
Basal diet								
29		2.88						
30		1.63						
31		1.60						9.0

gm. daily plus 3 gm. yeast powder, 2 gm. liver extract powder, and 200 mg. choline chloride per day. Periods 1 through 4—Ate 100 per cent diet.

Periods 5 through 14—Heparinized dog *plasma* given intraperitoneally (160 to 185 cc. in 5 to 10 minutes) daily except Sunday without reaction. Diet eaten 100 per cent with occasional forced feeding; this resulted in some vomiting on one occasion. Periods 15 through 18—Diet eaten 100 per cent.

Periods 19 through 28—Laked dog *red cells* given intraperitoneally (65 to 85 cc. of solution in 10 to 12 minutes) without reaction, daily except Sunday. Diet eaten 100 per cent with occasional forced feeding; this resulted in vomiting of 20 to 30 per cent of food once in periods 19 and 20. Periods 29 through 31—Diet eaten 100 per cent.

Table 1 (dog 43-141) approximates the perfect experiment. The dog was in perfect physiological condition and ate the basal diet throughout. There is but little weight loss except in the 8-day foreperiod, a slight gain in weight during the plasma period, and slight loss during the subsequent injection of laked red cells.

A plasma protein intake of 35.6 gm. of nitrogen and a urinary excretion of 20.7 gm. nitrogen gives an adequate test period of 20 days. The average urinary nitrogen of 2.07 gm. nitrogen per 48 hour period during plasma injection is as low as the lowest period in the 8-day foreperiod—that is, no rise in nitrogen excretion accompanies the plasma injection. The 8-day control midperiod shows no surplus excreted nitrogen. The rise in plasma protein circulating levels due to plasma injection subsides in the afterperiod.

Albumin-globulin ratios were determined by the electrophoresis technique of Tiselius[2] at the end of each long injection period. After plasma injection the albumin-globulin ratio (Tiselius) was 0.8 and by the usual chemical method (Howe) was 1.1. After the control midperiod albumin-globulin ratio was 1.0 (Tiselius) and 1.3 (Howe). After the long hemoglobin injection the albumin-globulin ratio (Tiselius) was 0.5. The first observations were read as normal for the dog and the last observation showed a moderate decrease in albumin with some general increase in the several globulins. If any one plasma protein was used by the body for general metabolic purposes rather than some others we would expect a marked change in the albumin-globulin ratio after plasma injections. As it does not follow we feel that the evidence points to general use of *all plasma proteins* for the diverse protein needs of the body.

Hemoglobin (laked red cells) was given intraperitoneally (Table 1) in about the same amounts as for plasma—a total of 37.5 gm. nitrogen during 20 days with a total urinary nitrogen of 25.6 gm. There is a distinct rise in urinary nitrogen of 0.5 gm. per period above control and plasma injection periods. The conservation of globin nitrogen is not as good as for plasma protein nitrogen but it is surprisingly good in this dog. The urea-ammonia urinary fraction (60 to 80 per cent) is compatible with a normal protein utilization (Table 1).

There seems to be no escape from the conclusion that the *plasma proteins*

[2] We are indebted to Dr. L. J. Zeldis and Dr. E. L. Alling for these analyses.

TABLE 2

Nitrogen Retention during Intraperitoneal Injection of Laked Red Cells and Plasma—Hemolytic Reactions to Plasma

Dog 43-31

Period No.	Blood proteins injected Total N	Total urinary N	Circulating plasma protein level	R.B.C. hematocrit	Icterus index	Weight
48 hrs.	*gm.*	*gm.*	*gm. per cent*	*vol. per cent*		*kg.*
Basal diet contains little protein (0.86 gm. N per period)						
1		4.39				
2		3.61				10.3
3		2.57	5.18	58		
Basal diet + laked red blood cells intraperitoneally						
4	5.76	3.99				
5	2.49	4.04				
6	6.06	4.85				
7	4.60	3.91				
8	5.25	3.78				
9	2.30	3.20				9.8
10	5.39	5.17				
Total.....	31.9	28.9				
Basal diet						
11		3.74				
12		2.65				9.8
13		2.81	5.44	64		
14		2.10				9.6
15–20			4.91			
(Average)		2.11				
Basal diet + whole blood plasma intraperitoneally						
21	3.74	2.17				9.7
22	4.33	1.94				
23	1.86	1.76	5.90	48		
24	4.09	2.03				9.9
Hemolytic reaction. Basal diet						
25		11.16		21	160	
26		8.66			88	
27		4.33			60	Recovery

TABLE 2—*Concluded*

Dog 43-33

Period No.	Blood proteins injected Total N	Total urinary N	Circulating plasma protein level	R.B.C. hematocrit	Icterus index	Weight
48 hrs.	*gm.*	*gm.*	*gm. per cent*	*vol. per cent*		*kg.*
Basal diet						
1		2.35			0	
2		2.81				
3		2.12				12.0
Basal diet + whole blood plasma intraperitoneally						
4	3.76	2.47	6.19	50	0	
5	3.72	1.56				
6	1.94	1.67				12.2
7	3.68	6.16		39	6	
8			8.54	13	100+ (Hemolysis)	Death

are used without loss of nitrogen to cover the body protein needs and that *globin* from hemoglobin is also *well utilized* to maintain protein balance. A little surplus nitrogen does escape in the urine during hemoglobin injections but the urinary nitrogen balance is still positive and there is no significant weight loss. Undetermined nitrogen is unchanged.

Experimental History—Table 2.

Dog 43-31. Male hound mongrel. Maintained for months on good mixed diet, fasted 6 days, and placed on low protein diet II 305 gm. daily plus 3 gm. yeast powder and 2 gm. of liver extract powder per day throughout the experiment.

Periods 1 through 3—Ate 100 per cent of diet. Periods 4 through 10—Laked dog *red cells* given intraperitoneally daily except Sunday (80 to 90 cc. in 8 to 10 minutes)without reaction. Ate average of 83 per cent of diet. Periods 11 through 20—Ate 100 per cent of diet.

Periods 21 through 24—Heparinized dog *plasma* given intraperitoneally (170 to 185 cc. daily except Sunday) without reaction. Ate 100 per cent of diet. Period 25—Urine very dark. Strongly positive for hemoglobin and bile pigment. Left 75 per cent of food. Jaundiced. Acts sick. Periods 26 and 27—Dog jaundiced, weak, but complete recovery ensued.

Dog 43-33. Male bull terrier. Maintained for months in animal house on good mixed diet, fasted for 6 days, and then placed on low protein diet II 200 gm. plus 3 gm. yeast powder and 2 gm. liver extract powder per day for 2 days preceding start of experiment and during the experiment. Periods 1 through 3—Ate 100 per cent of diet.

Periods 4 through 6—Dog *plasma* given intraperitoneally (185 cc. in 5 to 10 minutes) without reaction. Ate 100 per cent of diet. Period 7—After second plasma injection of this period, dog had malaise, passed very dark urine, and refused to eat. Period 8—Dog very weak, grossly jaundiced, vomited mucus. Found dead. Autopsy showed the changes expected due to extensive hemolysis plus extreme pulmonary edema.

Table 2 (dog 43-31) in general supports Table 1 to show that globin contributes to the body nitrogen requirements. During 7 periods (14 days) laked red cells were given intraperitoneally (total nitrogen 31.9 gm.) and the total urinary nitrogen was 28.9 gm.—an average urinary nitrogen per period of 4.1 gm. This is to be compared with the control midperiod of 20 days with 2.1 gm. as the control basal urinary nitrogen per period of 48 hours. There is some surplus urinary nitrogen which appears in the control midperiod (periods 11 to 13, Table 2). There is some loss of weight.

Plasma injections show the usual nitrogen conservation in periods 21 to 24 and then comes a hemolytic crisis similar to that previously described from this laboratory by Wright (22). The reaction terminates the experiment and recovery follows.

Dog 43-33 (Table 2) shows a similar hemolytic crisis during the fourth period of intraperitoneal injection of plasma. Up to that time the pattern of the experiment followed that of Table 1 and we note the usual low urinary nitrogen and evidence of complete plasma protein conservation and utilization.

Experimental History—Table 3.

Dog 42–618. Male mongrel hound. Fasted 1 week then placed on low protein diet I 150 gm. plus 200 mg. choline chloride daily for *7 weeks before* and during experiment. Periods 1 and 2—Ate 100 per cent of diet.

Periods 3 through 11—Laked dog *red cells* given intraperitoneally (45 to 80 cc. of solution in 8 to 10 minutes) daily except Sunday. Ate only about 30 per cent of diet in periods 3 and 4 and 50 per cent in period 11; otherwise ate 100 per cent of diet. Periods 12 through 14—Ate 100 per cent of diet.

Table 3 (dog 42–618) shows again that hemoglobin (laked red cells) intraperitoneally can approximate nitrogen equilibrium, hemoglobin nitrogen 21.8 gm. injected and total urinary nitrogen output 21.6 gm. The basal urinary nitrogen in the control periods is about 1.7 gm. and during the hemoglobin injection periods averages 2.4 gm. nitrogen. There is some weight loss. The urea-ammonia fraction of urinary nitrogen is compatible with normal protein utilization.

This dog was fasted a week and then given the low protein diet for 7 weeks before the experiment to insure low protein reserve stores. Plasma protein levels were low at the start and remained definitely subnormal. This indicates that with moderate hypoproteinemia alone the hemoglobin injection does not contribute to the production of obvious amounts of new plasma protein. This is similar to the response noted in standard plasma-depleted dogs (14) in which no evidence of hemoglobin utilization to form new plasma protein was observed. When anemia and hypoproteinemia are combined the reaction is quite different (Table 5) and new plasma protein in considerable amounts is produced.

TABLE 3

Laked Red Cells Intraperitoneally—Nitrogen Retention but No Change in Circulating Plasma Proteins

Dog 42-618

Period No.	R.B.C. injected Total N	Total urinary N	Urea N + NH_3-N	Total undetermined urinary N	Circulating plasma protein level	R.B.C. hematocrit	Weight
48 hrs.	*gm.*	*gm.*	*per cent*	*gm.*	*gm. per cent*	*vol. per cent*	*kg.*
Basal diet							
1		1.48	56.3	0.64	4.90	47	10.4
2		1.80	66.9	0.60			
Basal diet (0.36 gm. N per period) + laked red blood cells intraperitoneally							
3	2.32	1.96	61.0	0.76			
4	4.48	3.78	80.5	0.74	5.12	51	
5	1.74	2.67	75.3	0.66			10.2
6	1.85	2.33	65.1	0.81			10.1
7	2.16	2.25	75.8	0.54			
8	1.29	1.95	72.8	0.53	4.97	48	
9	2.30	1.88	76.9	0.43			
10	2.83	2.22	74.3	0.57	4.78	45	9.9
11	2.79	2.56	78.6	0.55			
Total.....	21.8	21.6					
Basal diet							
12		2.12	74.8	0.53			
13		1.91	70.3	0.57			
14		1.68			4.79	49	

Experimental History—Table 4.

Dog 39-251. Male mongrel hound. Maintained for *7 weeks* on low protein diet I 200 gm. daily plus 10 gm. commercial casein daily. Casein supplement discontinued 3 days before start of experiment during which only low protein diet I was fed and eaten 100 per cent during the whole course of the experiment.

Periods 3 through 9—15.0 gm. of *hemoglobin digest* (lot KB 42241) in about 150 cc. physiological saline given intravenously (2.18 gm. nitrogen daily). Injections were invariably associated with severe urticaria, and occasionally with tenesmus and defecation but no vomiting. Periods 10 and 11—Ate 100 per cent of diet.

Dog 40-401. Male mongrel hound. 10 days fast, followed by *7 weeks* maintenance on low protein diet I 200 gm. per day. Non-protein diet supplemented with 10 gm. commercial casein daily for 2 weeks immediately preceding the start of the experiment. Only non-protein diet was fed during the experiment. Period 1—100 per cent food eaten.

TABLE 4

Hemoglobin Digests Intravenously—Nitrogen Balance
Hemoglobin and Plasma Protein Levels

Period No.	Hemoglobin digests injected Total N	Total urinary N	Urea N + NH_3-N	Total undetermined urinary N	Circulating plasma protein level	R.B.C. hematocrit	Weight
48 hrs.	*gm.*	*gm.*	*per cent*	*gm.*	*gm. per cent*	*vol. per cent*	*kg.*
Dog 39-251							
Basal diet (0.50 gm. N per period)							
1		1.48	59.5	0.60			
2		1.78	55.5	0.80	5.56	49	10.9
Basal diet + hemoglobin digest intravenously							
3	4.36	5.10	59.5	2.07			
4	4.36	3.55	60.3	1.41			10.8
5	4.36	5.33	66.8	1.77			
6	4.36	5.28	65.5	1.72			
7	4.36	4.08	70.5	1.20			
8	4.36	4.53	72.1	1.26			11.1
9	4.36	4.50	67.8	1.45	5.37	46	
Total.....	30.5	32.4		10.88			
Basal diet							
10		1.92	64.1	0.69			
11		1.76	60.4	0.70			11.0
Dog 40-401							
Basal diet (0.50 gm. N per period)							
1		2.03	70.3	0.60			
Basal diet + hemoglobin digest intravenously							
2	4.36	4.34	67.8	1.41	4.75	41	10.3
3	4.36	4.64	75.9	1.12			
4	4.36	4.35	77.8	0.96			
5	4.36	3.98	70.6	1.17			10.1
6	4.36	4.27	68.8	1.34			
7	4.36	4.70	75.4	1.12			
8	4.36	3.61	71.1	1.04			10.2
Total.....	30.5	29.9		8.16			
Basal diet							
9		1.85	71.9	0.52	4.86	36	
10		1.35	56.9	0.58			
11		1.53	63.2	0.56			

Periods 2 through 8—*Hemoglobin digest* (lot KB 42241) 15.0 gm. in physiological saline given intravenously (2.18 gm. nitrogen per day) in 45 to 75 minutes. Injections associated with hyperpnea and vomiting of a little mucus almost daily. Food consumption averaged 50 per cent during injection periods. Periods 9 through 11—Ate average of 50 per cent of food daily.

Table 4 shows two experiments with *digests of hemoglobin* given by vein. Hemoglobin digests are often very toxic when given by vein but these digests were only mildly intoxicating, causing some vomiting or subcutaneous edema not unlike urticaria. This intoxication may be in part repsonsible for some of the excess urinary nitrogen. The *undetermined* nitrogen is about double the control periods and the surplus presumably represents amino acids or aggregates derived from the digest which pass the kidney barrier. When hemoglobin is given intraperitoneally there is no excess of undetermined urinary nitrogen (Table 1).

Both these dogs were maintained 7 weeks on a low protein diet to reduce the protein reserve stores before the start of intravenous injections. Presumably this would insure utilization of all available materials suited to furnish nitrogen requirements. In spite of the intoxication and increase of undetermined nitrogen the nitrogen balance is close to equilibrium. Dog 39-251 shows an intake of 30.52 gm. nitrogen and output of 32.37 gm. nitrogen. Dog 40-401 shows an intake of 30.52 gm. nitrogen and an output of 29.89 gm. nitrogen. This response is not as good as the nitrogen balance noted with hemoglobin given intraperitoneally (Table 1). The weights in the two dogs show little change. The circulating levels of blood plasma proteins show no increase. The hemoglobin blood levels show a slight fall. It is possible that the hemoglobin digests actually caused slight hemolysis when given daily in these amounts. We would expect a rise rather than a fall in hemoglobin levels with all this intake of material perfectly suited for hemoglobin production.

Experimental History—Table 5.

Dog 37-23. Male coach. Born September, 1936. Continuous anemia history April 10, 1938, to Dec. 3, 1941. Low protein diet composed of sugar, starch, fat, salts, and vitamin accessories. *Double depletion* experiments begun. Standard tests of various protein factors interspersed with recovery periods. Table 5, Jan. 14, 1943—Periods 1, 2—Daily diet of basal protein-free biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 400 mg. Depletion of hemoglobin and plasma protein. Feb. 4—Periods 3, 4, 5—*Hemoglobin injection* intraperitoneal. During periods 3, 4, 5, the yeast and liver extract were replaced by synthetic vitamins. Blood volumes 1520, 1460, 1386 cc. and plasma volumes 1112, 946, 972 cc. in respective periods. Food consumption 78–, 55, 76 per cent. Dog in good condition.

Dog 37-82. Female coach. Born August, 1936. Continuous anemia history May 10, 1937, to Mar. 4, 1942. Regular anemia experiments. Mar. 4, 1942—Low protein diet and double depletion experiments begun. Weight 15.6 kilos. Blood volume 1154 cc., plasma volume 942 cc. standard tests of various protin factors, interspersed with recovery periods. Mar. 4, 1943—Period 1—Diet of basal protein-free biscuit 350 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Blood volume 1345 cc. plasma volume 1025 cc. Mar.

12—Periods 2, 3, 4—*Hemoglobin injection* intraperitoneal. Diet of basal protein-free biscuit 350 gm., synthetic vitamin preparation 8 cc., reduced iron 600 mg. Food consumption 56 per cent, 46 per cent, and 57 per cent. Blood volumes 1200, 1177, 1116 cc., plasma volumes

TABLE 5

Intraperitoneal Hemoglobin and Whole Red Cells Contribute to Plasma Protein and Hemoglobin Production in Anemia and Hypoproteinemia

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 37-23		Dog hemoglobin—intraperitoneal								
1	21.9	Basal	22	8.0	62.0	4.9	32.2	52	3.5	11.9
2	20.9	Basal	20	6.7	19.5	4.7	11.0	56	3.2	10.9
3	19.4	Hb—31.9 gm.	30.7	9.4	57.3	5.4	25.8	45	5.2	8.2
4	19.2	Hb—42.9 gm.	40.8	8.1	48.2	4.8	21.7	45	6.9	8.3
5	18.9	Hb—44.0 gm.	42.2	9.4	12.9	4.5	5.8	45	7.1	8.7
6	17.9	Basal	15	8.2	25.5	4.3	11.1	44	2.4	6.2
7	17.0	Basal	11	8.2	1.6	4.4	0	—	1.8	6.3
Dog 37-82		Dog hemoglobin—intraperitoneal								
1	17.1	Basal	18	7.7	16.2	4.6	9.5	59	2.9	8.4
2	16.4	Hb—38.6 gm.	37	10.9	12.9	4.5	5.8	45	6.2	7.1
3	15.9	Hb—60.7 gm.	58	10.7	45.5	4.7	19.7	43	9.8	6.8
4	15.3	Hb—44.2 gm.	51	11.7	18.3	4.3	7.3	40	8.6	6.4
5	14.7	Basal	9	11.7	1.7	3.4	0	—	1.4	5.3
Dog 40-32		Whole dog red cells—intraperitoneal								
1	18.6	Basal	17	9.9	1.4	4.3	0	—	—	—
2	18.1	Hb—52.5 gm.	61	13.6	45.7	4.7	15.5	34	9.8	5.68
3	17.9	Hb—94.7 gm.	101	12.7	69.2	4.4	18.4	27	16.0	6.64
4	18.0	Hb—15.8 gm.	24	9.6	39.8	4.3	11.3	28	3.8	6.05
5	17.6	Basal	9	8.3	29.9	4.3	10.8	36	1.3	5.03

850, 824, 803 cc. in respective periods. Dog in good condition other than weight loss at end of injection experiment.

Dog 40-32. Male white bull. Born August, 1940. Continuous anemia history December, 1941, to May 26, 1942. Regular anemia experiments. May 26—Low protein diet, double depletion (anemia and hypoproteinemia) begun. Standard tests of various protein factors interspersed with recovery periods. Weight 16 kilos. Apr. 18, 1943—Period 1—Daily diet of basal protein-free biscuit 450 gm., yeast 2 gm., liver extract powder 2 gm. Apr. 24—Periods 2, 3, 4—Intraperitoneal injections of *whole red cells* begun. Plasma volume 886 cc.

Daily diet of basal protein-free biscuit 450 gm., yeast 2 gm., liver extract powder 2 gm., choline 300 mg., rice polishings 500 mg., aminobenzoic acid 30 mg., inositol 50 mg., reduced iron 600 mg. Food consumption 100 per cent during injection period. Dog in excellent condition. May 5—blood volume 1107 cc., plasma volume 639 cc. May 7—No bile pigment in urine sample of 2nd week's collection. May 12—Blood volume 1259 cc., plasma volume 789 cc. May 13—End of experiment. Dog in excellent condition.

Table 5 shows three experiments, two with hemoglobin and one with whole red cells given intraperitoneally. This type of experiment has been described previously (18, 19). Anemia and hypoproteinemia are produced in the dogs (double depletion) by bleeding frequently, together with the continuance of a low protein diet containing adequate carbohydrate, fat, accessories, and 400 to 600 mg. iron daily. After an interval of weeks the dog approaches an anemia level of 7 to 8 gm. hemoglobin and a hypoproteinemia level of 4 to 5 gm. per cent, which levels insure a strong stimulus to produce both new hemoglobin and plasma protein.

We have published two experiments (18) to show that hemoglobin intraperitoneally will produce much hemoglobin in new red cells and new plasma protein in doubly depleted dogs as noted in Table 5. The amounts of injected hemoglobin are greater in Table 5 than in the earlier experiments (18) and nitrogen balance figures are available to show maximal conservation of protein nitrogen.

The first dog (37-23) in Table 5 was not depleted of protein reserves as indicated by the large output of urinary nitrogen in periods 1 and 2. Removal of 60 gm. hemoglobin and 32 gm. plasma protein in the first period indicates a large reserve store of protein building material in this dog. During the hemoglobin injection periods in this dog and dog 37-82 synthetic vitamins were used so that the basal diet contained no appreciable nitrogen. Dog 37-23 is not in nitrogen balance and this amount of protein is scarcely a contributing factor to nitrogen balance as more blood proteins must be removed than were injected to maintain the anemia and hypoproteinemia. In all 119 gm. hemoglobin were given intraperitoneally and there were removed 156 gm. hemoglobin plus 64 gm. plasma protein, correction being made for difference in circulating blood protein levels before and after the injections of hemoglobin. There is a rapid loss of weight and evidently there was available a surplus of material from which the doubly depleted dog made new hemoglobin and plasma protein. This might be in part a protein building reserve not exhausted or the resultant of tissue wastage and related weight loss.

The final two periods (dog 37-23) show maximal conservation of nitrogen and urinary nitrogen output of less than 1 gm. per day. Obviously all the injected hemoglobin was well utilized in this doubly depleted dog and emphasis should be placed on the 60 gm. of plasma protein which were removed—presumably hemoglobin contributing to the production of this needed plasma protein.

Dog 37-82 (Table 5) is much like that of the first experiment but perhaps more convincing. A total of 144 gm. hemoglobin was given intraperitoneally and a net production of 107 gm. hemoglobin plus 30 gm. plasma protein recorded. There is weight loss but the urinary output is less than the nitrogen intake. The urinary nitrogen in the last two periods shows a very careful conservation of nitrogen. All the evidence would indicate complete utilization of the introduced hemoglobin—to make new hemoglobin and new plasma protein and to participate in the internal protein metabolism. We assume a protein pool which is supplemented by the injected hemoglobin from which pool come the needed hemoglobin and plasma protein and needed tissue protein.

The last experiment in Table 5 (dog 40-32) shows the response to large injections of *washed red cells*. A total of 163 gm. hemoglobin is given and a net production of 173 gm. hemoglobin plus 56 gm. plasma protein is recorded. The total nitrogen intake exceeds the urinary nitrogen and the weight loss is much less than in the two other experiments in Table 5. When smaller amounts of red cells are given intraperitoneally, a rapid escape of these red cells from the peritoneum into lymphatics and into the circulation is readily demonstrated (7) by means of red cells labeled by radio-iron. When very large amounts of washed red cells are given intraperitoneally, the amount of red cell destruction, hemoglobin solution, and absorption plus phagocytosis is certainly considerable and is a part of the response in Table 5, dog 40-32. The amount of red cells removed compared with removed plasma protein (production ratio of plasma protein to hemoglobin) in this dog indicates a removal from the blood of some of the cells which were given into the peritoneum. The production of 56 gm. of plasma protein is not negligible and indicates the same general response in all three experiments in Table 5.

Experimental History—Table 6.

Dog 40-41. Female bull. Born 1942. Renal *bile fistula* operation Feb. 25, 1943. May 25—Hemoglobin depletion begun. Blood volume 1134 cc., hemoglobin 18.2 gm. per cent. Weight 15 kilos. Continuous anemia history to July 14, 1944. Maintained on a diet of salmon bread 350 gm., salmon 200 gm., Klim 20 gm., bile 80 cc., and vitamin K solution 1 cc. per day for 6 weeks before start of experiment. Changed to low protein diet II 350 gm., plus yeast powder 3 gm., liver extract powder 2 gm., and dog bile, 80 cc. per day throughout the experiment. Periods 1 through 3—Ate 100 per cent of diet. Periods 4 through 9—Laked dog red cells given intraperitoneally daily except Sunday (65 to 85 cc. of solution in 8 to 10 minutes) without reaction. Ate 100 per cent of diet in periods 4 and 5, 45 to 88 per cent in periods 6 through 9 (average 61 per cent). Periods 10 through 12—Ate 55 to 75 per cent of diet (average 63 per cent). Condition good at close of experiment.

Table 6 (dog 40-41) shows the response of a renal bile fistula dog to intraperitoneal injection of laked red cells. The dog at the start was moderately depleted, hypoproteinemic, and anemic. The primary objective of the experiment was to determine whether the depleted dog which needs every type of

protein split product would show any conservation of the pigment radicle. Many published reports (9) from this laboratory show that under normal circumstances when hemoglobin is injected the pigment radicle of the hemoglobin is quantitatively excreted as bile pigment in the bile. The observations in this table give no evidence for any conservation of the pigment radicle even under the urgent demands of anemia and hypoproteinemia. The injected

TABLE 6

Bile Pigment Excretion in Bile Fistula Dog Increased by Laked Red Cell Injections—Anemia and Hypoproteinemia

Dog 40-41—Bile fistula

Period No.	R.B.C. injected Total N	Total urinary N	Bile pigment excretion	Circulating plasma protein level	Hemoglobin	Weight
48 hrs.	*gm.*	*gm.*	*mg.*	*gm. per cent*	*gm. per cent*	*kg.*
Basal diet (0.86 gm. N per period)						
1		4.53	63	5.50	8.9	16.4
2		3.53	58			
3		2.73	68			
Basal diet + laked red blood cells intraperitoneally						
4	2.09	2.71	83			14.9
5	4.03	3.75	386		12.7	
6	2.54	4.97	525	5.50		
7	3.66	3.82	459			
8	2.26	3.30	222			14.1
9	4.63	3.07	391			
Total.....	19.2	21.6				
Basal diet						
10		3.51	192	4.83	15.4	13.6
11		3.19	104			
12		1.61	96			

hemoglobin could produce theoretically 3400 mg. bile pigment. A recovery of 2056 mg. is recorded and the urinary collection and analysis is recognized as less than 100 per cent. This is true because the van den Bergh reagent reacts quantitatively only with bilirubin and not with other bile pigments and because bile pigment produced by hemoglobin breakdown following intravenous injection of hemoglobin may be retained in part by the reticulo-endothelial system and released slowly (16). We conclude that there is no evidence that the pigment radicle was conserved under these conditions.

The total hemoglobin nitrogen injected was 19.2 gm. and total urinary nitrogen 21.6 gm.—an approximation to a balance. As a drain on available nitrogen the production of pigment for new hemoglobin is obviously insignificant since the pigment nitrogen is only approximately 0.3 per cent of the hemoglobin. Otherwise the experiment is much like that in Table 3. There was considerable weight loss. There was a rise in hemoglobin levels and no bleeding was done. Plasma protein levels showed no rise.

Dogs with long standing bile fistulas are not normal and these abnormalities have been discussed elsewhere (8, 10). There is evidence that liver function is somewhat abnormal and intestinal absorption is diminished. This may explain a part of the weight loss. The utilization of the nitrogen of the injected hemoglobin is good and compares with the experiment in Table 3.

DISCUSSION

Globin from hemoglobin is broken down in the body continually due to red cell obsolescence and is obviously perfectly suited to build new hemoglobin with the addition of iron and the pigment radicle. These experiments show that globin contributes to the "protein pool" material which serves well the protein requirements of the body. *Nitrogen balance* can be attained in dogs with *normal blood* when abundant laked red cells are given intraperitoneally and a basal ration supplies the needed carbohydrates, fats, minerals, and vitamin accessories. Globin is not as well used as is plasma protein and there is a larger nitrogen output in the globin experiments (Tables 1, 2, and 3) but these dogs remain close to nitrogen and weight balance. It is surprising that an incomplete protein (globin) can contribute so effectively to the protein pool as it must obviously be supplemented by the amino acids inadequately represented in globin drawn from some reserve store to produce cell protein or plasma protein. The whole globin in some dogs is more effectively used than the hemoglobin digests (Table 4) suggesting that some of the globin may be used without much breakdown and loss of nitrogen as is so conspicuous in the body use of plasma proteins where there is no loss of nitrogen (12).

When dogs are depleted of plasma proteins by plasmapheresis and present a continuing hypoproteinemia, the injection of hemoglobin does not result in an increased output of plasma protein (14). Table 3 likewise shows a subnormal plasma protein level while hemoglobin is being given in large amounts intraperitoneally. Under such circumstances it is probable that the injected globin is not used to make much plasma protein but to supply general body protein needs. When *anemia* is added to the hypoproteinemia we note an active output of hemoglobin in new red cells and abundant new plasma protein. These observed facts are difficult to explain. About all one can say at the moment is that globin can contribute to the body protein pool and maintain approximate nitrogen balance and when there is need for much new hemoglobin

and plasma protein there is a definite contribution from the protein pool as supplemented by globin. We can offer no good explanation as to why the needs of body cells or blood production have priority at one time and not at another. The rules of internal body protein metabolism are obscure but probably very flexible.

SUMMARY

Hemoglobin (presumably its essential protein globin), given intraperitoneally to a protein-fasting dog, will be used effectively to supply the protein requirements of the body. Nitrogen balance may thus be maintained for 20 days under favorable conditions. New hemoglobin and plasma protein will be formed related to hemoglobin injections in depleted dogs where there is urgent need for these proteins (anemia and hypoproteinemia). Obviously this calls for supplementary amino acids which in globin are low and we assume these amino acids must be contributed from body protein stores.

Plasma proteins (in plasma) tested in the same manner are completely utilized with no loss of nitrogen, positive nitrogen balance, weight balance, and no change in the albumin-globulin ratios.

Hemoglobin (globin) is less effectively utilized as compared with plasma protein given parenterally and there is some increase in urinary nitrogen above control periods. The albumin-globulin ratio may be somewhat modified by hemoglobin injections intraperitoneally. Hemoglobin (globin) *digests* contribute effectively to body maintenance of nitrogen equilibrium. These digests are about as effective as whole hemoglobin in maintaining nitrogen balance but cause a rise in undetermined nitrogen not seen when hemoglobin alone is given intraperitoneally.

Pigment radicles derived from hemoglobin given intraperitoneally are thrown away and appear as surplus bile pigment even when there is urgent need for all available nitrogenous material—given protein fasting, anemia, and hypoproteinemia in a bile fistula dog. The body evidently prefers to make rather than conserve the pyrrol aggregate (pigment radicle).

We assume that the injected hemoglobin (globin) or hemoglobin digests contribute to the *body protein pool* and from this pool various proteins emerge to supply protein requirements of tissue or organ cells or to produce new hemoglobin or plasma protein if needed. We have no explanation as to what determines the pattern of this protein flow but new hemoglobin is very high on the priority list.

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