HEMOGLOBIN AND PLASMA PROTEIN

Simultaneous Production during Continued Bleeding as Influenced by Diet Protein and Other Factors*

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Simultaneous production of hemoglobin and plasma protein has never been studied carefully under the stress of depletion in dogs. This is of logical interest to workers in this laboratory where the output of hemoglobin in anemia and of plasma protein in hypoproteinemia has been studied for many years. We have found that certain food factors were especially suited for high hemoglobin output and others more effective for plasma protein output under the conditions of these published experiments. It seemed probable that one should be able to influence profoundly the ratio of hemoglobin and plasma protein by these familiar diet factors during simultaneous depletion. Contrary to expectation we note below that the depleted dogs always produced more hemoglobin than plasma protein, no matter what diet protein was used.

The conditions of the experiments are simple. A normal dog is put on a daily diet low in protein (for example 100 to 150 gm. of liver, plus accessories, plus fat and carbohydrate to supply caloric requirements). Iron (200 to 400 mg. a day) is given to make for an active hemoglobin regeneration in anemia. The dog is bled almost daily in considerable amounts to attain an anemia level of about 6 to 7 gm. per cent (one-third normal) and continued at this level. With the low protein intake this may bring about a real hypoproteinemia (Tables 5 and 6) sufficient to stimulate active new plasma protein regeneration (4). Other dogs on the same régime can hold the plasma protein almost at a normal level but there must be considerable stimulus to produce new plasma protein due to withdrawal of plasma (Tables 2 and 3).

In this emergency the dog uses available protein frugally but always produces more hemoglobin than plasma protein even when the stimulus for

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

hemoglobin and plasma protein is presumably maximal (Tables 5 and 6). The ratio of plasma protein to hemoglobin production in Table 5 runs from 38 to 55 per cent—that is about 2 to 3 times as much hemoglobin is produced as plasma protein.

In *plasma depletion experiments alone* when the dog gives its energies to producing new plasma protein with a generous protein intake we believe the dog can produce as much or even more plasma protein than it can form hemoglobin in anemia (9). It can produce 70 gm. new plasma protein a week (7) during plasma depletion when the protein *intake* is abundant. The ceiling for hemoglobin production in anemia with heavy liver feeding plus iron is about 70 gm. per week. However, when simultaneous production of plasma protein and hemoglobin is studied in the same type of dog we see preference being given to hemoglobin production no matter what type of protein is fed.

Methods

The general procedure relating to these dogs and the methods of these experiments are those described for the anemia colony (11). Plasma protein determinations are based on Kjeldahl nitrogen determinations (3). Unless otherwise noted the dogs are in a normal state.

In the following tables the periods run consecutively unless noted otherwise. The hemoglobin levels are the averages of the levels at the end of each week in the period. The plasma protein levels are the averages of the levels of each bleeding in the period.

Diets vary a good deal and for this reason are given under the clinical history of each dog. The liver used is freshly ground raw pork liver. The beef muscle is ground lean round steak. The yeast is dried (Standard Brands Inc., type 200-B). The "vitamin concentrate" is a liver powder containing per gram 150 to 200 micrograms riboflavin, 75 micrograms vitamin B_6, 850 to 1000 micrograms pantothenic acid, and other factors in liver concentrated to the same extent as the riboflavin. The salt mixture used is the McCollum-Simmonds formula without iron (11), except for dog 39-53, where the Wesson mixture (8) is used. Amino acids are given in a small portion of the food to insure complete intake. Iron is added to the entire quantity of food. Iron given by mouth was in the form of reduced iron or of ferric citrate. In some experiments colloidal iron was given by vein.[1] The lecithin used is said to contain at least 3.5 per cent choline.

EXPERIMENTAL OBSERVATIONS

The dogs used in these experiments vary in type. Some are a bull dog strain used in the standard anemia colony in this laboratory (11) and many had been anemic some time before these experiments were started (see clinical histories). Others are mongrels of various types. It will be

[1] We are indebted to Dr. David Loeser of the Loeser Laboratory, Inc., New York City, for the supply of colloidal iron.

noted that dogs vary in their capacity to use food protein advantageously. Some produce new hemoglobin and plasma protein up to 15 per cent of the food protein. Others can produce 30 to 40 per cent new protein from the food protein and approach the pig in efficiency of protein production. Some dogs produce plasma protein more readily than others and never show hypoproteinemia (Table 2). At present these differences appear to be individual and unexplained. We are familiar with similar variations among dogs in their abilities to regenerate hemoglobin.

TABLE 1

Production of Hemoglobin and Plasma Protein Due to Bleeding Anemia. Depressed Plasma Protein Levels and Gain in Weight

Dog 37-81

Period		Wt.	Iron added daily	Protein intake		Protein output				Production ratio plas. prot. to hemoglobin	Protein output to intake
						Hemoglobin		Plasma protein			
No.	Wks.			Type	Weekly	level	output per week	level	output per week		
		kg.	*mg.*		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*
1	7	11.0	0	Liver	147	6.4	12.1	5.6	7.0	58	13
2	4	12.8	200	Liver	147	7.2	24.0	5.5	16.1	67	27
3	8	—	—	Interval	—	—	—	—	—	—	—
4	13	15.2	0	Liver	224	6.6	22.7	5.7	15.5	68	17
5	14	15.7	200	Liver	228	7.6	33.3	5.8	21.0	63	24
6	2	14.2	200	Low protein	3	7.0	28.3	4.4	16.1	57	—
7	5	14.6	200	Liver	228	7.0	23.5	4.9	14.2	60	17
8	4	15.2	200	Salmon	208	7.6	31.0	5.2	19.4	63	24
9	5	14.9	200	Kidney	154	7.5	25.9	5.2	16.5	64	28
10	3	13.5	200	Salmon bread	155	6.9	25.0	4.8	12.6	50	24
11	4	13.6	200	Liver	193	7.7	30.7	5.5	18.0	59	25

Clinical Experimental History—Table 1.

Dog 37-81. Adult female coach.

Born Sept., 1936. Continuous anemia history May 17, 1937, to July 15, 1940. Regular anemia experiments. Beginning weight 12.3 kilos.

Dec. 15, 1937, to May 10, 1938, banana basal low protein diet. Food consumption good. Plasma protein levels average 5.1 gm. per cent for this period.

May 10, 1938, to Mar. 14, 1939, salmon bread diet and various standard experiments dealing with hemoglobin production.

Mar. 14, 1939, experiments in Table 1 begun. Iron intake given in tables.

Period 1. Daily diet of raw pig liver 100 gm., sugar 125 gm., cornstarch 160 gm., tomato 50 gm., cod liver oil 20 gm., lard 30 gm., butter 35 gm., salt mixture 2 gm., bone ash 15 gm., and during the last 2 weeks of period, yeast 1 gm. Albumin:globulin ratio 0.75.

Period 2. Diet of liver 100 gm. plus usual accessories. A/G ratio 0.62.

Period 3. A series of hemoglobin injection experiments to be reported later. Anemia and plasma protein depletion continuous as before and after this period. Diet of liver 100 gm. plus usual accessories. A/G ratio varied from 0.56 to 1.1.

Periods 4 and 5. Diet of liver 150 gm. plus usual accessories. A/G ratio varied from 0.58 to 1.1.

Period 6. Diet of usual accessories plus vitamin concentrate 1 gm. but no added liver, total protein intake per week being only 3 gm. Food consumption was 92 per cent and 69 per cent for the 2 weeks. A/G ratio 0.93.

Period 7. Diet of liver 150 gm. plus usual accessories. A/G ratio varied from 0.69 to 0.72.

Period 8. Diet canned salmon 150 gm. plus usual accessories. Food consumption was 100 per cent for 3 weeks and 92 per cent for 1 week. A/G ratio varied from 0.66 to 0.89.

Period 9. Diet of pig kidney 150 gm. plus usual accessories. Food consumption varied from 85 per cent to 100 per cent. A/G ratio varied from 0.68 to 1.15.

Period 10. Diet of salmon bread 240 gm., sugar 50 gm., cornstarch 60 gm., butter 20 gm., lard 10 gm., cod liver oil 10 gm., tomato 50 gm., yeast 1 gm., vitamin concentrate 1 gm., bone ash 15 gm., salt mixture 2 gm. Food consumption varied from 62 per cent to 80 per cent. A/G ratio 0.99.

Period 11. Diet of liver 150 gm., sugar 125 gm., cornstarch 160 gm., butter 20 gm., lard 30 gm., cod liver oil 10 gm., tomato 50 gm., yeast 1 gm., vitamin concentrate 1 gm., bone ash 15 gm. Food consumption varied from 78 per cent to 100 per cent. A/G ratio varied from 0.67 to 0.77. Plasma volume varied during this period, Table 1, from 643 cc. to 997 cc.

Urine Dec. 6, 1938, contained albumin, few granular casts, and an occasional red blood cell.

Urine Oct. 4, 1939 (period 5), contained trace of albumin but no casts nor red blood cells.

Table 1 (dog 37-81) shows a long continued experiment of more than 60 weeks. The dog was in the anemia colony for about 2 years before Table 1 begins and presumably the reserves for hemoglobin building were pretty completely depleted. The dog gained weight and the food consumption was excellent. This experiment serves as a good example of average type of the subsequent experiments.

Period 1, Table 1, shows a 7 weeks experiment with a diet containing 100 gm. raw liver. The hemoglobin output is low and may be limited by the iron available in the liver only. The plasma protein level is slightly below normal and the plasma protein production is 58 per cent of the hemoglobin produced. The protein utilization is poor—only 13 per cent of the intake, but the dog gains weight which may indicate a much better protein utilization than is indicated by the figure for new hemoglobin and plasma protein removed.

Period 2, Table 1, shows the same diet continued plus 200 mg. iron daily.

There is a 100 per cent increase in hemoglobin and plasma protein output with no change in plasma protein levels and the protein utilization also doubles—27 per cent. It is not surprising that the hemoglobin production is doubled due to the iron but we were surprised to note the same increase in plasma protein with no change in plasma protein levels.

Periods 4 and 5 are to be compared. The diet is identical except for the presence of iron 200 mg. daily in period 5. The liver intake is 150 gm. and there is a slight gain in weight during these 27 weeks. The hemoglobin output is about 50 per cent higher in the iron feeding (period 5). This is what one would expect in the simple anemic dog. But the new plasma protein also increases 35 per cent and there is no change in the plasma protein levels which are again within low normal limits. This would lead us to suspect that this dog could have produced more plasma protein if it had been pushed (hypoproteinemia).

Period 6 is of much interest because during a diet practically protein free we note the expected large hemoglobin output (1) but also considerable new plasma protein production. This latter is to be explained in large part as related to the sharp drop in plasma protein levels indicating a strenuous depletion of circulating plasma protein and related reserve stores (4).

Period 7 is precisely like period 5 as to diet and iron intake but the hemoglobin and plasma protein outputs are distinctly less. We may choose to explain this on the basis of *repletion* of reserve stores exhausted during period 6 and we note gain in weight.

Period 8 shows that canned salmon supplemented with iron is well utilized to form both hemoglobin and plasma protein. In fact the output is almost equal to period 5 with liver replacing the salmon muscle. The utilization of protein in both periods is 24 per cent.

Period 9, Table 1, shows that kidney is also well used. The protein intake is about 50 gm. less than for salmon and the hemoglobin and plasma protein are correspondingly decreased in output. The ratio of plasma protein to hemoglobin remains unchanged as does the concentration of plasma protein in the circulation.

Period 10 shows that salmon bread (11) is used as well as the other proteins by this dog (bread protein = grain and salmon muscle). We felt confident that there would be an increase in the output of plasma protein as compared with hemoglobin but the reverse is true. We note a drop in the plasma protein levels and ratio of plasma protein to hemoglobin production. In the simple anemia experiments the salmon bread diet produces very little new hemoglobin unless there is a large iron supplement. In plasmapheresis the salmon bread is very well used to produce abundant new plasma protein. In this experiment the dog uses the proteins in salmon bread very well—protein output 24 per cent of the intake.

Period 11 with a shift back to liver shows an increase of hemoglobin in proportion to the protein fed but even more increase in plasma protein output.

TABLE 2

Production of Hemoglobin and Plasma Protein Due to Bleeding Anemia. Normal Plasma Protein Levels. Gain in Weight

Dog 36-11

Period		Wt.	Iron added daily	Protein intake		Protein output				Production ratio plas. prot. to hemoglobin	Protein output to intake
						Hemoglobin		Plasma protein			
No.	Wks.			Type	Weekly	level	output per week	level	output per week		
		kg.	*mg.*		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*
1	10	15.1	200	Liver	192	7.2	15.2	5.2	—	—	—
2	10	14.4	200	Liver	192	7.0	24.2	5.4	15.1	62	20
3	1	14.4	200	Liver + phenylalanine	192	6.4	1.3	—	0	—	—
4	5	14.5	200	Liver	192	6.7	21.3	5.4	13.3	62	18
5	14	—	—	Interval	—	—	—	—	—	—	—
6	14	17.3	0	Liver	218	7.0	20.7	5.8	13.0	63	15
7	8	17.0	200	Liver	217	6.8	22.0	6.1	16.3	74	18
8	2	16.9	200	Liver + cystine + leucine	218	7.5	25.0	6.1	20.0	80	21
9	4	16.6	200	Liver	218	6.6	15.3	6.1	11.7	76	12
10	5	—	—	Interval	—	—	—	—	—	—	—
11	6	15.0	0	Liver	218	6.7	15.5	6.5	12.0	77	13
12	7	15.4	200	Liver	196	7.1	27.8	6.1	18.9	68	24

Clinical Experimental History—Table 2.

Dog 36-11. Adult male bull.

Born Apr., 1936. Continuous anemia history Oct. 22, 1936, to Feb. 2, 1940. Regular anemia experiments. Beginning weight 18.5 kilos.

July 16, 1938, liver basal diet without iron. Aug. 13, iron was added.

Oct. 15, 1938, experiments in Table 2 begun.

Periods 1 and 2. Daily diet of raw pig liver 125 gm., sugar 125 gm., cornstarch 150 gm., canned tomato 50 gm., cod liver oil 40 gm., butter 20 gm., bone ash 15 gm., salt mixture without iron 2 gm., kaolin 20 gm. Albumin:globulin ratio during periods 1 and 2 ranged from 0.42 to 0.60.

Period 3. *dl*-Phenylalanine (2 gm. daily) was added to the diet of liver 125 gm., sugar 150 gm., cornstarch 160 gm., canned tomato 50 gm., cod liver oil 15 gm., butter 30 gm., lard 30 gm., salt mixture 2 gm., bone ash 15 gm., kaolin 20 gm.

Period 4. Same liver basal ration plus usual accessories as in period 3.

Period 5. A series of hemoglobin injection experiments to be reported later. Anemia and plasma protein depletion continuous as before and after this period. Diet of liver 150 gm. plus usual accessories and iron. A/G ratio ranged from 0.55 to 0.61.

Period 6. Diet of liver 150 gm. plus usual accessories. A/G ratio ranged from 0.54 to 0.88.

Period 7. Diet of liver 150 gm. plus usual accessories. A/G ratio 0.50 average.

Period 8. 1 gm. *l*-cystine and 3 gm. *l*-leucine were added to the diet of liver 150 gm., butter 40 gm., lard 20 gm., cod liver oil 10 gm., and other usual accessories. A/G ratio 0.55.

Period 9. Diet of liver 150 gm. plus usual accessories. A/G ratio 0.70.

Period 10. A low protein intake experiment with poor food consumption. Anemia and plasma depletion continuous as before and after this experiment. A/G ratio ranged from 0.40 to 0.46. Loss of weight.

Period 11. Diet of liver 150 gm. plus usual accessories. A/G ratio 0.46 to 0.54.

Period 12. Diet of liver 150 gm. plus usual accessories. A/G ratio 0.47 to 0.68. Plasma volume varied during this period (Table 2) from 786 cc. to 1164 cc.

Urine Mar. 15, 1938, contained small amount of albumin, no casts, no red blood cells.

Urine Nov. 29, 1938 (period 7), contained trace of albumin but no casts, no red blood cells.

Table 2—dog 36-11 is in many respects like Table 1. Dog 36-11 was in the anemia colony 2 years before the experiments of Table 2 began. Food consumption is good and the anemia level is constantly maintained. The plasma protein level is a little below normal in the first 4 periods but normal in the last 6 periods in spite of active bleeding.

Amino acids were tested in this dog as a supplement to the liver diet as given. Phenylalanine, leucine, and cystine all have an influence upon hemoglobin production in anemia due to blood loss (12). Cystine has a strong influence on plasma protein production under certain conditions with experimental hypoproteinemia (3). Period 3 shows a negative effect if anything—*phenylalanine* appears temporarily to inhibit hemoglobin production. The dog was normal, no lack of appetite, no diarrhea. If we compare period 2 as control with periods 3 and 4 combined, the output of hemoglobin and plasma protein is definitely decreased. It is hard for us to believe this effect is directly related to phenylalanine and the experiment must be repeated in other dogs.

Cystine and *leucine* (period 8) do show an increase in hemoglobin production as compared with control periods before and after. The plasma protein production increases also and the plasma protein level remains unchanged. One can scarcely avoid the conclusion that this dog could have produced much more plasma protein at this time.

The high hemoglobin output in period 6 with no iron added is to be explained by iron reserves related to the injections of hemoglobin during period 5.

It is interesting that in period 11 when the hemoglobin production is decreased by withholding the iron, the plasma protein level does increase to 6.5 per cent and returns to 6.1 per cent when more blood is removed in period 12 as the iron is replaced in the diet. The utilization of protein runs from 12 to 24 per cent—protein removed as a percentage of protein fed.

TABLE 3

Production of Hemoglobin and Plasma Protein Due to Bleeding Anemia. Plasma Protein Levels Nearly Normal. Weight Loss

Dog 37-01

Period No.	Period Wks.	Wt.	Iron added daily	Protein intake Type	Protein intake Weekly	Protein output Hemoglobin level	Protein output Hemoglobin output per week	Protein output Plasma protein level	Protein output Plasma protein output per week	Production ratio plas. prot. to hemoglobin	Protein output to intake
		kg.	*mg.*		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*
		12.1		Kennel		21.0		6.1			
1	4	10.6	252*	Beef, liver, salmon	97	9.6	50.9	5.9	19.9	39	73
2	7	9.9	400	Salmon-liver	198	8.9	27.1	5.3	12.5	46	20
3	4	10.0	400	Salmon-liver	203	6.9	27.9	5.6	17.7	63	22
4	7	10.0	400	Salmon-liver	188	6.7	18.3	5.8	12.0	66	16
5	1	—	0	Fast	0	6.6	6.4	5.6	4.3	67	—
6	2	10.0	400	Salmon-liver	198	6.2	15.3	5.2	10.8	71	13
7	10	—	—	Interval	—	—	—	—	—	—	—
8	1	—	112*	Fast	0	7.0	16.9	5.9	12.0	71	—
9	3	10.0	400	Salmon-liver	200	7.6	25.1	5.5	14.2	57	20
10	11	11.1	400	Salmon-liver	203	—	—	—	—	—	—
10	1	12.0	400	Salmon-liver	203	19.1	—	—	—	—	—
11	3	11.3	400	Salmon-liver	138	10.2	46.3	5.4	19.7	43	48
12	4	—	400	Salmon-liver	149	7.0	11.6	5.7	8.8	76	14
13	5	10.3	400	Salmon-liver	148	7.6	18.4	5.7	12.2	66	21

* Iron by vein, total for period.

Clinical Experimental History—Table 3.

Dog 37-01. Adult male mongrel short-haired terrier.

Oct. 7, 1938, experiments in Table 3 begun.

Period 1. Diet varied considerably to find one acceptable to dog.

Periods 2 to 4. Diet of liver 50 gm., salmon 100 gm., sugar 100 gm., lard 30 gm., cod liver oil 5 gm., salt mixture 2 gm. In period 4 food consumption decreased from maximum weekly average of 203 gm. protein but dog was well and of constant weight.

Period 5. No food intake.

Period 6. Diet as in period 2.

Period 7. Included experiments with hemoglobin injection by vein. Depletion steadily maintained.

Period 8. The fasting was accompanied by the daily injection by vein of colloidal iron, 16 mg.

Period 9. Diet as in period 2.

Period 10. Diet as before but no bleeding done.

Periods 11 to 13. Diet modified to attain better food consumption. Added dried yeast 1 gm., and in period 13, tomatoes 50 gm. During most of these 3 periods diet protein from salmon and liver, each 75 gm.

Viviperfusion under ether done at end of period 13.

Autopsy: Hyperplasia of bone marrow and spleen. Other organs normal.

Between periods 3 and 9 the albumin:globulin ratio varied from 1.38 to 1.04. Aside from the initial determination of 329 cc. the plasma volume varied between 420 cc. and 559 cc. during the entire 63 weeks of the experiment.

Table 3—dog 37-01 shows a different type of dog (mongrel terrier) which started from normal in period 1 and was rapidly depleted. There were periods of incomplete food consumption which make the experiments less significant but they are in harmony with others given below (Table 4).

This dog was *subnormal* in *hemoglobin production* as indicated by period 4 of 7 weeks with uniform weight and large iron intake—see also periods 12 and 13. The plasma protein output is 60 to 70 per cent of the hemoglobin output and the plasma protein level is close to the low normal range (5.7 per cent). During fasting periods the hemoglobin production is also much below expected levels. Period 5 shows only 6.5 gm. and period 8 with some intravenous iron 16.9 gm. hemoglobin.

The reserve store of hemoglobin producing material is much below the expected reserve—compare Table 5. Note also rest period 10 of 12 weeks and depletion from a normal hemoglobin level of 19.1 gm. in the short period 11.

The ratio of protein produced to food protein in satisfactory periods ranges from 16 to 22 per cent. The high figures of protein utilization in period 1 (73 per cent) and period 11 (48 per cent) obviously are related to the *reserve* stores for hemoglobin and plasma protein production.

Clinical Experimental Histories—Table 4.

Dog 37-242. Normal adult male chow mongrel completely consumed throughout this experiment the following diet: liver 75 gm., cane sugar 130 gm., lard 25 gm., cod liver oil 5 gm., salt mixture 2 gm., reduced iron 400 mg., bone ash 10 gm. In period 2 urine examination was completely negative. In periods 3 and 4 the albumin:globulin ratio was 1.09 and 0.93 respectively. An accidental air embolus suddenly terminated the experiment. Autopsy revealed hyperplasia of bone marrow and spleen. The initial plasma volume was 442 cc. Subsequent plasma volumes varied from 411 cc to 530 cc.

Dog 34-146. Adult male bull. Born Dec., 1933.

June 24, 1937, Eck fistula operation. Recovery rapid.

Sept. 28, 1937, to Apr. 12, 1938, continuous anemia history. Beginning weight 16.4 kilos. Routine anemia experiments.

Apr. 12, 1938, to May 10, 1938, banana basal diet (low protein). Beginning plasma protein level 6.8 per cent, at end of diet period 6.0 per cent.

May 10, 1938, salmon bread diet and various standard anemia experiments dealing with hemoglobin production.

TABLE 4

Production of Hemoglobin and Plasma Protein Due to Bleeding Anemia. Plasma Protein Levels Depressed. Food Protein Well Utilized

Period		Wt.	Iron added daily	Protein intake		Protein output				Production ratio plas. prot. to hemoglobin	Protein output to intake
						Hemoglobin		Plasma protein			
No.	Wks.			Type	Weekly	level	output per week	level	output per week		
Dog 37-242											
		kg.	*mg.*		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*
		12.3		Kennel		20.2		5.6			
1	3	11.7	400	Liver	95	9.7	57.8	5.3	19.9	34	82
2	4	11.0	400	Liver	105	7.7	24.3	4.7	11.1	46	34
3	3	11.0	400	Liver	105	7.8	27.5	4.9	13.9	50	39
4	3	11.0	400	Liver	105	6.9	20.1	4.9	11.3	56	30
Dog 37-146, Eck fistula											
1	7	17.5	400	Liver	226	8.9	46.5	4.8	23.4	50	31
2	6	16.8	400	Liver	226	8.7	40.1	4.9	19.6	49	26
3	4	16.3	200	Liver	226	8.2	44.0	4.6	21.2	48	29
Dog 32-4											
1	9	Depletion of reserve—total output					456.8		210.5	46	
2	5	12.7	400	Liver	207	8.2	30.9	5.7	19.1	62	24
3	5	12.8	400	Liver	202	7.3	29.3	5.2	18.3	62	24

Mar. 5, 1940, experiments in Table 4 begun.

Period 1. Diet of raw pig liver 150 gm., sugar 125 gm., potato starch 150 gm., lard 40 gm., cod liver oil 20 gm., yeast 1 gm., vitamin concentrate 1 gm., tomato 50 gm., salt mixture 2 gm., bone ash 15 gm., kaolin 20 gm. A/G ratio 0.88.

Period 2. Diet of liver 150 gm. plus usual accessories. A/G ratio 0.68 to 0.75.

Period 3. Diet of liver 150 gm. plus usual accessories. A/G ratio 0.79 to 0.87. Plasma volume varied during this period (Table 4) from 887 cc. to 986 cc.

Dog 32-4. Adult female coach. Raised from weaning to adult age on liver and salmon bread. Continuous anemia history Apr. 24, 1933, to Feb. 23, 1934. Regular anemia experiments. Blood donor and normal rest period to Feb. 17, 1940.

Apr. 29, 1940, experiments in Table 4 begun. Beginning weight 12.2 kilos.

Period 1. Diet of liver 75 gm., soy bean 50 gm. fed as soy bean mush, sugar 135 gm., potato starch 160 gm., lard 30 gm., cod liver oil 15 gm., salt mixture 2 gm., bone ash 10 gm., yeast 1 gm., vitamin concentrate 1 gm. Feb. 28, 1940, to Apr. 29, 1940, depletion of hemoglobin and plasma protein reserve.

Period 2. Diet of liver 150 gm., sugar 125 gm., potato starch 150 gm., yeast 1 gm., vitamin concentrate 1 gm., lard 30 gm., butter 20 gm., cod liver oil 15 gm., salt mixture 2 gm., tomato 50 gm., bone ash 10 gm.

Period 3. Diet of liver 150 gm. plus usual accessories. Plasma volume varied during this period (Table 4) from 582 cc. to 701 cc.

Table 4 shows 3 dogs of very different type in which the general response is very similar. The plasma protein levels are somewhat depressed below normal which should have served as a considerable stimulus for plasma protein formation.

Dog 37-242 (Table 4), a chow, was taken from a normal level of hemoglobin and plasma protein almost to the depletion levels in 3 weeks. Removal of the reserve stores explains the high figures for hemoglobin and plasma protein removal. During periods 2 to 4 this dog was in weight equilibrium, the anemia level was satisfactory, there was definite hypoproteinemia and the ratio of plasma protein to hemoglobin removed was 50 per cent. The ratio of protein production to protein intake was 30 to 39 per cent which is distinctly above the average.

Dog 34-146 (Table 4), an Eck fistula, is included because it behaves in all respects in this type of experiment as a normal dog. This bull dog had been anemic over 2 years before this experiment started but the general response (protein and production ratios) is very like that of dog 37-242 just above.

Dog 32-4 (Table 4) had been in the anemia colony and then used as a blood donor for years. This dog was 8 years old and would come in the group of *old animals*. The hemoglobin and plasma protein reserve stores were considerable. In spite of vigorous bleeding and a large hemoglobin output the plasma protein levels are held above 5 per cent. The utilization of food protein is 24 per cent in contrast to the other 2 dogs in this table.

Clinical Experimental Histories—Table 5.

Dog 39-8. Young adult male coach. Born Dec., 1938.

Dec. 29, 1939, experiments in Table 5 begun. Beginning weight 11.7 kilos.

Period 1. Diet during first 2 weeks of bleeding consisted of raw pig liver 100 gm., sugar 100 gm., cornstarch 125 gm., lard 30 gm., cod liver oil 20 gm., salt mixture (8) 3 gm., yeast 1 gm., bone ash 10 gm., tomato 30 gm. During the balance of the depletion period the diet consisted of varying amounts of soy bean meal dependent upon food consumption plus the usual accessories plus iron.

Period 2. Diet of soy bean meal 80 gm., potato starch 150 gm., sugar 100 gm., lard 20 gm., cod liver oil 20 gm., yeast 1 gm., vitamin concentrate 1 gm., tomato 50 gm., salt mixture (8) 3 gm.

Period 3. Diet of soy bean meal 80 gm., sugar 125 gm. plus usual accessories. Albumin:globulin ratio 0.90.

Period 4. Diet of raw pig liver 150 gm., potato starch 180 gm., sugar 125 gm., tomato 50 gm., yeast 1 gm., vitamin concentrate 1 gm., lard 30 gm., butter 20 gm., cod liver oil 20 gm., salt mixture (8) 3 gm., bone ash 10 gm. Plasma volume varied during this period (Table 5) from 525 cc. to 583 cc.

TABLE 5

Production of Hemoglobin and Plasma Protein Due to Bleeding Anemia. Hypoproteinemia Marked. Soy Bean and Liver Compared

Period		Wt.	Iron added daily	Protein intake		Protein output				Production ratio plas. prot. to hemoglobin	Protein output to intake
						Hemoglobin		Plasma protein			
No.	Wks.			Type	Weekly	level	output per week	level	output per week		
Dog 39-8											
		kg.	*mg.*		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*
1	8	Depletion of reserve—total output					346.5		165.4	48	
2	4	10.8	400	Soy bean	232	8.8	24.0	4.3	11.6	48	15
3	4	10.0	400	Soy bean	199	8.9	19.6	4.1	7.4	38	14
4	3	10.3	400	Liver	199	8.5	18.9	4.8	10.2	54	15
Dog 39-6											
1	9	Depletion of reserve—total output					301.0		173.0	58	
2	6	12.9	0	Liver	138	6.6	14.9	4.5	7.7	52	16
3	4	12.7	400	Liver	134	8.4	13.8	4.2	7.3	53	16

At termination of this experiment dog died because of pulmonary emboli. The femoral punctures were responsible. Autopsy showed the pulmonary veins filled with emboli which derived from the femoral veins. The heart, lungs, gastro-intestinal tract, spleen, pancreas, liver, kidneys were all normal. The marrow showed the usual hyperplasia.

Dog 39-6. Young adult male coach. Born Dec., 1938, a litter mate of dog 39-8. Oct. 4, 1939, experiment in Table 5 begun. Beginning weight 12.3 kilos.

Period 1. Diet of raw pig liver 100 gm., sugar 120 gm., lard 50 gm., cod liver oil 10 gm., butter 10 gm., cornstarch 100 gm., yeast 1 gm., bone ash 10 gm., Wesson salt mixture (8) 3 gm.

Period 2. Same liver basal ration plus usual accessories.

Period 3. Diet of liver 100 gm., sugar 130 gm., cornstarch 120 gm., butter 30 gm., lard 30 gm., tomato 50 gm., salt mixture (8) 3 gm., cod liver oil 10 gm., yeast 1 gm., vitamin concentrate 1 gm., bone ash 10 gm. Plasma volume varied during this period (Table 5) from 605 cc. to 744 cc.

Urine Oct. 12, 1939, contained a trace of albumin, no casts, and no red blood cells.

Dog was in apparent health during all of the time recorded in Table 5. The only abnormality observed was occasional hemoglobinemia of moderate grade which may be apparent and not real—due to technical slips in blood removal and collection. 3 days after the experiment was concluded the dog was found dead.

Autopsy: Icterus was present and a rare finding of diffuse acute hepatitis. This probably was of only 2 to 3 days' duration—cause unknown. The heart showed old calcified vegetations in pulmonary artery, mitral and aortic valves. There was no acute endocarditis. The lungs showed a recent bronchopneumonia. The bone marrow showed the usual hyperplasia (6).

Table 5 shows 2 interesting experiments with litter mates. The dogs were a year old when the experiments in Table 5 were begun. The smaller dog has the greater reserve store of material out of which hemoglobin is made.

Dog 39-8 (Table 5) shows that soy bean meal is utilized just as well as raw liver to form hemoglobin and plasma protein. It is significant that these dogs used the food protein to poor effect and produced only 14 to 16 per cent of new protein. Also there was a definite and continued hypoproteinemia which should furnish a strong stimulus to produce new plasma protein but the ratio of plasma protein to hemoglobin produced remained close to 50 per cent. In period 3, Table 5, dog 39-8, we note a little decrease in plasma protein production and the ratio falls to 38 per cent. At this time the food intake was decreasing which may have been a factor. One would expect soy bean meal to favor the production of plasma protein rather than hemoglobin and this experiment must be repeated in a dog which will tolerate a full diet of soy bean meal over long periods.

Dog 39-6 shows a response to the liver feeding very like that of its litter mate in the same Table 5. The hemoglobin production is subnormal and we find nothing in the autopsy to explain this response. This dog died of a hepatitis probably of recent origin and the litter mate of a pulmonary embolism due to vein trauma. Dog 39-6 does show the same hemoglobin output when *iron* was *not included* in the daily diet. We believe this is explained by the presence of iron reserve stores which were not exhausted by the depletion period 1.

Clinical Experimental History—Table 6.

Dog 39-53. Adult male hound.

Plasma depletion 9 weeks by plasmapheresis. Discontinued because of hemolysins.

Jan. 3, 1940, experiments in Table 6 begun. Dog in good condition, hemoglobin 14.7 gm. per cent and plasma protein 4.5 gm. per cent.

Period 1. Diet of liver 50 gm., sugar 70 gm., cornstarch 30 gm., lard 30 gm., cod liver oil 5 gm., yeast 2 gm., vitamin concentrate 1 gm., salt mixture (8) 5 gm., bone ash 10 gm. Liver replaced by beef muscle 50 gm., because of poor food consumption before end of period 1, making total protein intake for this period: liver 48 gm., beef 61 gm., yeast 7 gm.

TABLE 6

Production of Hemoglobin and Plasma Protein during Blood Loss Anemia and Hypoproteinemia

Dog 39-53

Periods 1 wk.	Wt.	Protein intake		Protein output				Production ratio plas. prot. to hemoglobin	Protein output to intake
				Hemoglobin		Plasma protein			
		Type	Weekly	level	output per week	level	output per week		
No.	*kg.*		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*per cent*	*per cent*
1	9.4	Liver; beef	76	11.1	72	4.31	20.2	28	121
2	9.1	Beef muscle	80	9.4	46	3.91	13.5	28	74
3	9.1	Beef muscle	87	9.8	31	3.81	8.8	28	46
4	9.1	Casein	183	11.0	47	4.01	12.0	26	32
5	9.1	Casein	192	8.8	67	4.14	23.4	35	47
6	8.9	Casein	104	8.9	37	4.12	11.3	31	46
7	8.4	Casein; salmon; fasting	38	9.4	20	4.20	8.0	40	74
8	8.4	Fasting; casein	138	9.1	31	4.22	11.3	36	31
9	8.6	Liver	147	7.6	29	4.30	12.8	44	28
10	8.9	Beef muscle	154	8.0	31	4.41	13.2	43	29
11	9.1	Salmon	140	7.2	25	4.22	11.5	46	26
12	9.4	Salmon	140	6.5	24	4.43	13.0	54	26
13	9.3	Casein	159	7.3	25	4.49	11.5	46	23
14	9.3	Casein + cystine	161	7.4	38	4.68	17.6	46	35
15	9.2	Casein	150	8.1	34	4.72	16.9	50	34
16	9.2	Casein; liver	139	7.3	41	4.84	19.0	46	43
17	9.6	Salmon	140	6.3	28	4.47	16.1	57	32
18	9.6	Salmon	140	6.8	28	4.37	15.0	54	31
19	9.6	Salmon + cystine	110	6.9	20	4.38	10.9	54	28
20	9.5	Beef + hemoglobin and cysteine by vein	154	8.1	28	4.43	12.8	46	27
21	9.6	Beef muscle	154	7.1	44	4.56	20.7	47	42
22	9.6	Beef muscle	147	7.6	26	4.71	14.3	55	27

Iron 200 mg. daily in all but first two periods.

Period 2. Beef muscle diet consumed 100 per cent.

Period 3. Diet of beef 75 gm., sugar 40 gm., cornstarch 30 gm., lard 20 gm., butter 20 gm., cod liver oil 5 gm., plus usual accessories. Leucocytes 10,250.

Periods 4, 5, and 6. Diet of casein 30 gm., lard 15 gm., lecithin 5 gm., plus carbohydrates, other fats, and accessories as in period 3. Food consumption poor in period 6, dog in good condition.

TABLE 6-*a*

Nitrogen Balance and Albumin:Globulin Ratio

Dog 39-53

Period 1 wk.	A/G ratio of plasma	Protein intake Type	Nitrogen balance					
			Intake	Output				Intake minus output
				in urine	in feces	in R.B.C.	in plasma	
			gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	0.91	Liver, beef muscle	12.6	9.0	3.6	12.1	3.3	−15.4
2	1.19	Beef muscle	13.5	8.7	3.3	7.9	2.8	−9.2
3	1.27	Beef muscle	14.5	8.1	3.3	5.7	1.5	−4.1
4	1.02	Casein	30.2	8.9	2.8	8.3	2.0	+8.2
5	1.02	Casein	31.7	9.8	3.0	11.8	3.9	+3.2
6	—	Casein	16.3	8.1	3.3	5.1	1.9	−2.1
7	0.95	Casein, 1 day; salmon, 2 days; fasting, 4 days	6.7	8.3	*	3.7	1.3	−6.6
8	0.87	Fasting, 1 day; casein, 6 days	22.5	7.8	5.2	5.4	1.9	+2.2
9	1.10	Liver	24.2	8.6	4.6	5.4	2.1	+3.5
10	1.14	Beef muscle	25.3	9.6	2.5	5.5	2.1	+5.6
11	1.23	Salmon	23.1	10.9	3.0	4.5	1.9	+2.8
12	1.00	Salmon	23.1	11.7	3.8	4.5	2.1	+1.0
13	1.08	Casein	26.0	10.3	4.1	4.7	1.9	+5.0
14	1.44	Casein + cystine	26.3	8.9	3.2	7.0	2.9	+4.3
15	1.02	Casein	24.5	8.5	3.6	6.4	2.8	+3.2
16	1.14	Casein, 4 days; liver, 3 days	25.4	12.0	3.6	7.3	3.4	−0.9
17	1.09	Salmon	25.8	10.8	3.3	5.3	2.6	+3.8
18	1.10	Salmon	25.8	12.3	4.3	5.2	2.5	+1.5
19	0.92	Salmon + cystine	21.5	13.3	3.9	4.0	1.8	−1.5
20	0.81	Beef muscle + hemoglobin and cysteine by vein	31.4	10.3	3.3	5.2	2.1	+10.5
21	1.06	Beef muscle	28.2	11.3	3.4	8.3	3.4	+1.8
22	—	Beef muscle	27.0	12.1	3.1	4.7	2.4	+4.7
		Totals..................	505.6	219.3	74.2	138.0	52.6	+21.5

* Included in following period.

Period 7. Because of poor food consumption (6.6 gm. casein protein first day and 29 gm. salmon protein next 2 days), 5 days of fast imposed and well tolerated.

Period 8. Following one day of fasting, diet of casein 25 gm., sugar 60 gm., cornstarch 20 gm., lard 10 gm., butter 20 gm., plus usual accessories.

Period 9. Liver 100 gm. replaced casein in diet of period 8.

Period 10. Beef muscle 100 gm. replaced casein in diet of period 8.

Periods 11 and 12. Salmon 100 gm. replaced casein in diet of period 8.

Period 13. Diet as in period 8. Food consumption good. Ulceration of skin (superficial, moist, at times coated with dried serum or blood) persisted during the rest of the experiment. Urine, previously normal on several occasions showed a faint trace of albumin. Except for one occasion urine remained free of albumin subsequently. Kidneys normal at autopsy.

Period 14. *l*-Cystine 1.2 gm. added to casein diet of period 8 with corn oil 10 gm., crisco 20 gm. replacing the lard and butter.

Period 15. Diet as in period 14 without the cystine.

Period 16. Diet of liver 100 gm. replaced casein, vitamin concentrate increased to 5 gm.

Periods 17 and 18. Diet of salmon 100 gm. as in period 11.

Period 19. *l*-Cystine 2 gm. added to diet of period 18. Poorly eaten until after one day's fast.

Period 20. Diet of beef muscle 100 gm. replaced salmon in diet of period 18, while laked red blood cells, containing total of 17.5 gm. hemoglobin, were injected by vein together with total of 4.2 gm. *l*-cysteine hydrochloride. The red blood cells, from donor dogs, were washed once with modified Locke's solution, laked with distilled water, filtered through 16 layers of gauze. The injected solutions, freshly prepared each day, contained 0.90 to 1.43 gm. hemoglobin in volumes of 9 to 13 cc. and were given 3 cc. per minute. Cysteine hydrochloride, 0.3 gm., was given in dilute solution immediately following the hemoglobin. Sodium lactate, 50 cc. of 1/6 molar solution, was given immediately prior to each hemoglobin infusion. The dog tolerated the régime without recognizable disturbance. Hemoglobin, 0.99 gm., escaped into the urine. This hemoglobin was measured in the photoelectric colorimeter after conversion to cyanmethemoglobin (5). Nicotinic acid intake increased to 200 mg. from this period on.

Periods 21 and 22. Beef muscle diet as in period 20. Last 3 days of period 22, dog was not so active as usual. Food consumption dropping to 70 per cent on last day of period. Viviperfusion at end of period.

Autopsy: Hyperplasia of bone marrow and spleen. Other organs normal. Plasma volume varied from 361 to 535 cc. during entire experiment. The condition of the skin and hair was subnormal during the last half of the experiment. The ulcers noted in period 13 persisted and 2 new ulcers appeared on the scrotum. The hair generally became thinner and more of a dark brown than the original black.

Table 6—dog 39-53 illustrates the fact that during periods of fixed iron intake the hemoglobin production can be controlled by the protein intake as has been shown in another publication (2), see periods 6 to 10. During the first 8 periods (Table 6) we note a steady hypoproteinemia (3.8 to 4.2 per cent) which would cause a maximal stimulus to form plasma protein, yet during this period the dog consistently put out at least 3 times as much hemoglobin as plasma protein. If this dog could have formed more plasma protein in this emergency, one would expect to see the plasma protein levels rise close to 6 per cent or the low normal level. The utilization of the diet protein is very good and remains close to 40 per cent or the maximal utilization for the dog. The high figures for protein utilization in periods 1, 2, and 7 are obviously due to a depletion of reserve protein stores.

This dog did not tolerate these low protein diets well and often refused food and this explains the frequent shifts in amount and protein type in the weekly periods. In spite of all this, the body weight was relatively constant except during periods of partial fasting (periods 7 and 8). There was a positive nitrogen balance of 21.5 gm. or an average of about 1 gm. per week (Table 6-*a*).

Cystine was used as a supplement in periods 14 and 19. Cystine is not well represented in the amino acid analysis of casein so it was thought that as a supplement to the casein diet it might have a positive influence. This appears to be a fact and we note a sharp rise in period 14 in hemoglobin and plasma protein output and also in the following period—a "carry-over" due to lag in the manufacture of the new hemoglobin. The plasma protein levels also rise, indicating an effect on the plasma protein output, not merely a passive removal of plasma protein. *Cystine* as a supplement to salmon causes no increase in hemoglobin or plasma protein output.

Cystine given as a supplement to casein and beef muscle (periods 14 and 20, Table 6-*a*) causes a distinct retention of urinary nitrogen which suggests effective supplementary effect. Cystine added to salmon, however, causes no retention of urinary nitrogen and indicates that it is not an effective supplement to salmon muscle protein.

Hemoglobin given by vein in period 20 appears to be utilized to form new hemoglobin in red cells as is true in simple anemia (10). The hemoglobin given (17.5 gm. less 1.0 gm. escaped into urine) was almost quantitatively returned during the next period 21. At the same time *cystine* was given along with the hemoglobin by vein and we note an increase in the plasma protein removed but also a rise in the plasma protein levels which may indicate an excess plasma protein output due to the cystine supplement.

During periods 14 to 22, the ratio of protein output (hemoglobin plus plasma protein) to diet protein intake is consistently high (30 to 40 per cent) which is close to the maximum of 40 per cent. The ratio of plasma protein to hemoglobin output in the same interval is very close to 50 per cent. The albumin-globulin ratio in this dog is constantly close to 1 or above which is in contrast to previous experiments (see clinical histories) where the albumin: globulin ratio is often close to 0.50. We have no explanation for these observed differences.

DISCUSSION

Normally the ratio of hemoglobin to plasma protein in the dog's circulation is about 6 to 1 and in the anemic state about 2 to 1 (50 per cent ratio plasma protein to hemoglobin). From Table 2 with normal plasma protein levels during the long periods of bleeding and depletion one might argue that the plasma protein removed was merely a fixed fraction of the blood removed to keep the fixed anemia level of 7 gm. per cent—that the dog could produce much more plasma protein if the stimulus was maximal.

But in Tables 5 and 6 we see dogs which are unable to keep the plasma protein levels normal and definite hypoproteinemia is constantly present—an obvious stimulus to produce plasma protein in this emergency. Here we believe there are strong stimuli acting to drive the dog to maximal hemoglobin and plasma protein production yet the ratio is about the same. The dog continues to produce more hemoglobin than plasma protein which may indicate that in this emergency the mechanism within the body is set to turn out more hemoglobin than plasma protein. This ratio of production (plasma protein to hemoglobin) can be modified within limits in various animals and it will be worth while to study factors which can change this ratio or influence hemoglobin production perhaps at the expense of plasma protein production and *vice versa.* Incomplete proteins, protein digests, and amino acids should be thoroughly tested as to their response under these conditions.

SUMMARY

Given healthy dogs, fed abundant iron and a limited protein diet, with sustained anemia due to simple bleeding, we can study the capacity of each animal to produce new hemoglobin and plasma protein.

Some dogs can produce much hemoglobin and enough new plasma protein to maintain the plasma protein concentration at approximately a low normal level. It is probable that their plasma protein producing capacity is not fully extended (Table 2).

Other dogs (Table 5) can produce the same amount of hemoglobin but a hypoproteinemia develops and continues which should mean a maximal stimulus to produce new plasma protein. In such dogs we have strong stimuli to produce simultaneously new hemoglobin and new plasma protein. The ratio of plasma protein to hemoglobin varies from 40 to 60 per cent.

The total new formed blood protein may amount to 30 to 40 per cent of the total diet protein intake which shows that some dogs have remarkable capacity to conserve and use diet protein.

In this emergency of simultaneous depletion of hemoglobin and plasma protein levels, the dog gives preference to hemoglobin manufacture no matter what one of the listed food proteins is tested.

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