HEMOGLOBIN PRODUCTION IN ANEMIA LIMITED BY LOW PROTEIN INTAKE

Influence of Iron Intake, Protein Supplements and Fasting

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Experiments in this laboratory indicate that the output of new hemoglobin in anemia due to blood loss can be kept at very low levels by limitation of the iron intake. A salmon bread diet low in iron (3.2 mg. per 100 gm. as fed) will limit the hemoglobin production of the anemic dog to about 2 gm. per week. The salmon bread (7) is a complete diet which maintains the dog in health indefinitely. A diet of white bread, salmon, milk powder and cod liver oil (5 mg. iron per day's ration) will limit the output of hemoglobin to very low levels and remove all excess stores of iron in the body (2). When the reserve stores of iron in the body are removed the *intake of iron is an absolute limiting factor* for new hemoglobin production in anemia due to blood loss.

Assuming that hemoglobin consists of iron, a pigment radicle and a protein factor (globin) we may then inquire about the pigment and the globin as possible limiting factors in the building of hemoglobin in the anemic dog.

The *pigment radicle* which is responsible for the color of the blood and bile and some other related body pigments is made up of four pyrrol rings. From experiments with bile fistula anemic dogs given hemoglobin by vein (3) it is clear that the dog can manufacture this pigment radicle in almost limitless amounts on any diet under the conditions of the cited experiments. It appears then that the pigment radicle does not serve as a limiting factor in hemoglobin production. It would seem that the dog might be able to form the pyrrol ring by closure of a straight chain compound since the diets studied would hardly contain the required material in the pyrrol form.

The *globin factor* makes up about 95 per cent of the hemoglobin molecule and would naturally require protein or protein split products for its production in the body. Limitation of protein intake presumably would make the fabrication of new hemoglobin more difficult and if carried to a sufficient degree should limit the production of hemoglobin in anemia even in the face of an excess intake of iron. This is precisely what is observed in the tables below.

Methods

Routine methods of caring for these anemic dogs have been described in detail elsewhere (7). Blood volumes were determined weekly by the brilliant vital red dye method (4). Hemoglobin determinations were done by the acid hematin method (5), 13.8 gm. per 100 ml. of blood being taken as 100 per cent. In calculating the hemoglobin produced per week the amount removed by bleeding was added algebraically to the gain in the *total circulating hemoglobin* (13.8 × per cent hemoglobin × blood volume). Periods were chosen for use in calculations such that the *total circulating hemoglobin* was approximately the same at the beginning and end. Failure to observe this may involve serious error, and under some circumstances may even lead to an apparent *negative* production of hemoglobin, an obvious impossibility. All of the diets used included 1 gm. per day of McCollum and Simmonds salt mixture without iron.

Iron when administered orally was given as the ferric citrate. Intravenously it was given as the colloidal ferric hydroxide.[1]

EXPERIMENTAL OBSERVATIONS

The four tables below show long (many months) periods of limited protein intake and the hemoglobin production in anemia related to this dietary régime. Other less complete experiments are not recorded but they are in harmony with those tabulated. It is not possible to carry through these experiments upon every dog as some animals will not tolerate this limited diet and will refuse food, resulting in loss of weight and poor general condition. We cannot place much emphasis upon experiments where dogs lose weight for various reasons (Table 3). It is important to note that Tables 1, 2 and 4 show satis-

[1] We are deeply indebted to Dr. David Loeser of the Loeser Laboratories, New York City, for the generous supply of colloidal iron necessary for use in this work.

TABLE 1

Hemoglobin Production Limited by Low Protein Intake in Spite of High Iron Intake

Dog 36-27. Spaniel mongrel, male, adult.

Diet periods	Experimental period	Weight Average	Blood Hb. level Average	Hb. output per week Average	Hb. above basal* per week Average	Net Hb. expected standard salmon bread replacing banana diet†
	wks.	*kg.*	*per cent*	*gm.*	*gm.*	*gm. per wk.*
1. Banana diet + iron 400 mg. daily	4	9.2	57	17	—	45
2. Banana diet + iron 400 mg. daily + vitavose 50 gm. daily 3 days	1	9.4	60	17	—	45
3. Banana diet + iron 400 mg. daily	1	9.1	67	22	5	45
4. Banana diet + iron 400 mg. daily + liver extract‡	1	9.1	63	23	6	65
5. Banana diet + iron 400 mg. daily	1	9.1	59	17	—	45
6. Banana diet + iron 400 mg. daily + salmon 200 gm. daily	1	9.7	55	16	—	45
7. Banana diet + iron 400 mg. daily	1	9.8	47	16	—	45
8. Banana diet + iron 400 mg. daily + pig liver, 100 gm. daily, 1 wk.	2	10.0	58	25	8	52
9. Banana diet + iron 400 mg. daily + salmon 200 gm. daily	1	10.0	63	27	10	45
10. Banana diet + iron 400 mg. daily	1	9.9	61	19	2	45
11. Banana diet + iron 400 mg. daily	4	10.0	71	28	11	45
12. Banana diet + iron 400 mg. daily + thymus, 180 gm. daily, 1 wk.	3	10.7	66	29	12	51
13. Banana diet + iron 400 mg. daily	3	10.7	58	23	6	45
14. Banana diet + iron 400 mg. daily + pig liver, 200 gm. daily, 1 wk.	3	11.6	56	26	9	56
15. Banana diet + iron 400 mg. daily	1	11.4	52	24	7	45
16. Banana diet + iron 400 mg. daily + nucleic acid, 4 gm. daily, 5 days	1	11.0	55	23	6	45
17. Banana diet + iron 400 mg. daily	1	11.0	48	19	2	45

Banana diet daily = Banana 200 gm. + canned salmon 75 gm. + Karo syrup 75 cc. + cod liver oil 20 cc.

Total calories 716; protein calories 10.2 per cent of total.

Protein intake about 17 gm. per day on banana diet.

* Basal hemoglobin output on banana diet + 400 mg. iron, about 17 gm. per week.

† Basal hemoglobin output on salmon bread diet, 2 to 5 gm. per week.

‡ Liver extract (No. 55) equivalent to 300 gm. fresh liver for 3 days.

factory experiments in which the dogs were in excellent clinical condition, ate their diet regularly, and gained weight.

Table 1 gives a very satisfactory experiment of 30 weeks with the low protein (banana-salmon) diet. The dog gained considerable weight, was active and clinically normal. The right hand column of Table 1 shows the expected output of hemoglobin per week under anemic conditions had the dog received the standard salmon bread plus the iron plus the supplements as noted. These are figures drawn from large experience with standard anemic dogs and are not high values but average figures. It is obvious at a glance that the anemic dog produces about one-half as much new hemoglobin on the *limited protein diet* plus iron and supplements as a similar dog would produce on the standard salmon bread (liberal protein intake) plus the same supplements. It is difficult to escape the conclusion that the limited protein intake limits the capacity of the dog to produce *globin* and therefore hemoglobin.

It should be noted in Table 1 that the first 7 periods show clearly a basal hemoglobin production of 17 gm. per week and therefore a potency ratio of 7 (it requires 7 gm. of diet protein to produce 1 gm. of new hemoglobin). Periods 8 to 15 inclusive in Table 1 show a larger hemoglobin output and it is fair to assume that a part of this increase is due to the supplements (liver and thymus). We can explain at least 65 gm. (refer to last column, Table 1) of the new hemoglobin produced as related to the supplements as these reactions of the anemic dog to liver feeding are well established. On the basis of 17 gm. per week as the fixed basal hemoglobin output, we have a surplus of 160 gm. hemoglobin instead of 65 gm. to explain. These extra 95 gm. hemoglobin probably are related in some measure to these supplements of liver and thymus and we may argue that the absorption is improved or that the utilization of the protein is much improved.

In addition to the hemoglobin produced on this limited protein diet the dog gains some 2.4 kilos in weight. This must mean some gain in body protein coming also from the limited intake.

Bleedings remove not only hemoglobin but *plasma protein* which must be replaced from the protein factors coming into the body during this long period. The amounts of plasma protein lost in these bleedings are not recorded in these experiments but from data available we are confident that the plasma levels rarely fell below a low normal. If we say as a rough estimate that 1/3 to 1/2 as much plasma protein was removed as the hemoglobin removed and recorded we see that this totals a good many grams each week.

Considering hemoglobin and plasma protein removed plus some gain in total body protein we see that the dog to a remarkable degree is able to conserve for frugal use the small amount of protein coming from banana and salmon muscle.

Clinical History.—Table 1. Dog 36-27. Dog in excellent condition at end of period 17 (July 1, 1937). Plasma depletion experiments done during following month. Aug. 2 dog not well. Death Aug. 13, 1937.

Autopsy: Heart normal. Lungs bronchopneumonia. Organs show nothing of interest relating to the above experiment. The abnormalities were all acute (duration of 2 weeks or less).

Table 2 (dog 33-329) shows several points of interest. Carrots are not well taken by many dogs but this dog ate the carrot diet avidly during the long period of continuous observation, a total of 72 weeks, and maintained a normal condition and weight equilibrium. The bulky diet passed rather rapidly through the intestinal tract but there was no diarrhea. The poor absorption of iron given by mouth (Table 2) is possibly due to this fact.

Table 2 shows a strong contrast between the effects of iron by mouth (periods 1 to 6 inclusive) and by vein (periods 7 to 13 inclusive) where the output of new hemoglobin is very greatly increased. The basal hemoglobin output for this dog is well established at about 9 gm. per week. Supplements of iron by mouth are poorly utilized as compared to standard reactions of dogs on a salmon bread ration.

Period 7 begins a long sequence of weeks in which amounts of *colloidal ferric hydroxide were given by vein*. Throughout the whole period (periods 7 to 13 inclusive) the amount of new hemoglobin above the basal hemoglobin output of 9 gm. per week totals about 54 per cent of the amount of hemoglobin expected for the same iron injections in anemic dogs receiving a standard salmon bread diet. As the reaction of the standard anemic dog on the salmon bread diet is so uniform to iron given by vein we feel much confidence in this period of 23 weeks (periods 7 to 13 inclusive) when the observed hemoglobin production on the carrot diet was 174 gm. in contrast to the expected hemoglobin

TABLE 2

Hemoglobin Production in Anemia Limited by Low Protein Intake
Carrot Diet and Salmon Bread Diet Contrasted
Iron Given by Mouth and by Vein

Dog 33-329. Beagle, male, adult.

Diet periods	Experimental period	Weight Average	Blood Hb. level Average	Hb. output per week Average	Hb. above basal* per week Average	Net Hb. expected standard salmon bread replacing carrot diet†
	wks.	*kg.*	*per cent*	*gm.*	*gm.*	*gm. per wk.*
1. Carrot diet (1)	8	13.2	60	10	—	—
2. Carrot diet + iron 40 mg. daily orally, 10 days	4	12.4	60	14	4	10
3. Carrot diet, no iron	4	12.6	61	9	—	—
4. Carrot diet (2)	2	12.3	60	9.5	—	—
5. Carrot diet + iron 40 mg. daily orally, 14 days	5	12.3	65	17.6	8	11
6. Carrot diet + iron 400 mg. daily orally, 10 days	3	12.4	65	12	3	22
7. Carrot diet + iron 15 mg. daily by vein	3	12.3	79	18	9	31
8. Carrot diet + iron 24 mg. daily by vein	3	12.8	68	37	28	50
9. Carrot diet + iron 24 mg. daily by vein‡	3	13.6	72	44	35	60
10. Carrot diet + iron 20 mg. daily by vein	6	14.3	69	40	31	42
11. Carrot diet + iron 18 mg. daily by vein‡	3	12.8	63	30	21	48
12. Carrot diet + iron 20 mg. daily by vein + thymus, 183 gm. daily for 2 wks.	3	12.4	65	34	25	56
13. Carrot diet + iron 16 mg. daily by vein	2	13.5	73	34	25	33
14. Carrot diet, discontinued iron	9	14.0	65	20	11	—
15. Carrot diet, discontinued iron	3	13.9	62	18	9	—
16. Carrot diet + iron 16 mg. daily by vein	2	14.0	70	42	33	33
17. Carrot diet + iron 18 mg. daily by vein	5	14.0	76	39	30	38

Carrot diet (1) = Carrots 500 gm. + Klim 40 gm. + canned salmon 75 gm. + Karo syrup 60 cc. + cod liver oil 25 cc.

Total calories 905; protein calories 16 per cent of total. Daily protein intake 34 gm.; daily iron intake 10 mg.

Carrot diet (2) = Carrots 500 gm. + Klim 40 gm. + canned salmon 100 gm. + Karo syrup 100 cc. + cod liver oil 40 cc. Total calories 1230; protein calories 14 per cent of total. Daily protein intake 41 gm.; daily iron intake 14 mg.

* Basal hemoglobin output on carrot diet, about 9 to 10 gm. per week.

† Basal hemoglobin output on salmon bread diet, 2 to 5 gm. per week.

‡ + *l*-Histidine, 1 gm. per day, 1 week.

production on standard salmon bread of 320 gm. hemoglobin—a ratio of 54 per cent of the standard reaction which presumably is due to protein diet limitation. It is inconceivable that errors of omission or commission could account for this large difference between the 174 gm. hemoglobin observed and 320 gm. hemoglobin expected on standard salmon diet plus the iron given.

Supplements added to the carrot diet plus iron by vein are not of significance. Histidine 7 gm. during a week in periods 9 and 11 probably gave no significant response although in anemic dogs on a standard salmon diet it does give an excess hemoglobin production. Thymus given in period 12 shows no significant response.

Intravenous iron was discontinued for 12 weeks during periods 14 and 15 but a considerable surplus of hemoglobin was produced over and above the basal output—11 and 9 gm. hemoglobin per week. This indicates that this dog had stored considerable excess of iron during the injection periods, which excess was called upon to produce the observed surplus. This also shows that during periods 7 to 13 the dog was receiving more iron than it could use because of limited protein available to build globin and hemoglobin.

Clinical History.—Table 2. Dog 33-329. Dog was in perfect clinical condition during and at the end of the experiment (Table 2). Dog came to autopsy 11 months subsequent to these observations. All organs were normal.

Table 3 shows a 33 week period on a banana-salmon diet with and without iron by vein. There are however some variables (infection) which may confuse the picture. The first 3 periods of Table 3 include 28 weeks during which the basal hemoglobin output on the banana diet is about 11 gm. per week. It cannot be denied that a low grade endocarditis may have been active during some of these periods and it has been shown recently that infection will limit hemoglobin production in standard anemic dogs (6).

Iron given by vein (periods 4, 5 and 6, Table 3) should produce 90 gm. hemoglobin on a standard salmon bread diet but actually does produce 39 gm. on the banana diet—a substantial limitation due to the low protein diet. Period 7, Table 3, shows a low output of 5 gm. hemoglobin and we believe this is due to the acute infection which 5 weeks later caused death. The rapid loss of weight in periods 6 and 7 gives more evidence of this infection.

Clinical History.—Table 3. Dog 34-220. This dog was under observation several years in the laboratory. A Thiry-Vella fistula was done in April, 1935. Banana basal diets were begun in October, 1936. February, 1937, the dog had some skin lesions which may have been in part due to diet. There were periods of leucocytosis which may have been due to the skin lesions or to a smoldering endocarditis (see Autopsy). The albumin-globulin ratio was found to be as low

TABLE 3

Basal Hemoglobin Output in Anemia on Banana Diet
Limited Reaction to Iron by Vein

Dog 34-220. Foxhound mongrel, female, adult.

Diet periods	Experimental period	Weight Average	Blood Hb. level Average	Hb. output per week Average	Hb. above basal* per week Average	Net Hb. expected standard salmon bread replacing banana diet†
	wks.	*kg.*	*per cent*	*gm.*	*gm.*	*gm. per wk.*
1. Banana diet	15	12.0	64	14	—	—
2. Banana diet	11	11.5	67	11	—	—
3. Banana diet	2	10.6	52	11	—	—
4. Banana diet + 16 mg. iron by vein daily	1	10.6	52	13	2	33
5. Banana diet + 9 mg. iron by vein daily	2	11.0	63	22	11	19
6. Banana diet + 9 mg. iron by vein daily	1	9.8	66	26	15	19
7. Banana diet + 9 mg. iron by vein daily	1	8.9	68	5	—	—

Banana diet daily = Banana 300 gm. + canned salmon 100 gm. + Karo syrup 120 cc. + cod liver oil 40 cc.

Total calories 1170; protein calories 8.4 per cent of total. Protein intake about 23 gm. per day on banana diet.

* Basal hemoglobin output on banana diet, about 11 gm. per week.

† Basal hemoglobin output on salmon bread diet, 2 to 5 gm. per week.

as 0.3 on one occasion, total protein = 5.2 per cent. During the last weeks of life the total plasma proteins ranged from 4.3 to 7.9 per cent, the lowest figures being noted in period 5, Table 3, and the highest the week before death. Table 3 begins Mar. 18, 1937, and ends Nov. 18, 1937. Death Dec. 23.

Autopsy: Heart—there were old calcified vegetations in the left auricle as is not uncommon in dogs with a past history of distemper. Lungs—there were large recent thrombi blocking the pulmonary arteries. The pulmonary arteries

TABLE 4

Hemoglobin Output in Anemia on Banana Diet plus Abundant Iron by Mouth
Influence of Fasting and Protein Supplements

Dog 36-96. Spitz mongrel, female, adult.

Diet periods	Experimental period	Weight Average	Blood Hb. level Average	Hb. output per week Average	Hb. above basal* per week Average	Net Hb. expected standard salmon bread replacing banana diet†
	wks.	*kg.*	*per cent*	*gm.*	*gm.*	*gm. per wk.*
1. Banana diet + iron 400 mg. daily	3	9.4	62	19	—	45
2. Banana diet + iron 400 mg. daily	2	9.6	56	19	—	45
3. Banana diet + iron 400 mg. daily	3	9.8	52	14	—	45
4. Banana diet + iron 400 mg. daily	2	10.0	62	13	—	45
5. Hospital table scraps, no extra iron	2	10.4	59	23	9	—
6. Banana diet + iron 400 mg. daily	1	10.6	64	22	8	45
7. Banana diet + iron 400 mg. daily	1		65	16	3	45
8. Banana diet + iron 400 mg. daily + 200 gm. pig liver, 1 wk.	3	10.8	64	23	10	56
9. Banana diet + iron 400 mg. daily	3	10.8	62	10	—	45
10. Fasting	1	9.9	61	7	—	40
11. Banana diet + iron 400 mg. daily	1	10.7	54	10	—	45
12. Fasting	1	9.8	63	16	6	40
13. Banana diet + iron 400 mg. daily	1	10.2	63	9.6	—	45

Banana diet daily = Banana 200 gm. + canned salmon 75 gm. + Klim 40 gm. + cod liver oil 20 cc.

Total calories 715; protein calories 10 per cent of total. Protein intake about 17 gm. per day on banana diet.

* Basal hemoglobin output on banana diet + 400 mg. iron daily, about 14 gm. per week.

† Basal hemoglobin output on salmon bread diet, 2 to 5 gm. per week.

showed areas of calcification in their walls and areas of recent necrosis showing an acute inflammatory reaction. The thrombi here showed some large masses of bacteria. The lungs in gross showed interstitial pneumonia and histologically the alveolar changes plus many capillary thrombi and some necrosis. Kidneys showed glomerulo-nephritis and degeneration involving the great majority of the glomeruli. Spleen negative. Marrow shows acute edema and some fibrin deposit —changes related to the blood stream infection. Liver normal but for a little fat and some pigment. Gastro-intestinal tract was normal.

Table 4, dog 36-96, shows a satisfactory experiment of 24 weeks. During periods 1 to 4 (10 weeks) the banana diet is supplemented by heavy iron feeding (400 mg. iron daily). The basal output of about 13 gm. hemoglobin per week is one-third of the standard output of the anemic dog receiving salmon bread plus the same amount of iron. Hospital table scraps give an increase due to a larger protein intake and this extends through periods 5, 6 and 7. The supplement of pig's liver in period 8 gives an increase of 30 gm. hemoglobin which is about the same as the expected increase from this same amount of liver added to the salmon bread (33 gm.) in a standard anemia experiment.

The fasting periods are discussed below. The periods 11 and 13 which follow the fasting periods are of interest as they show a hemoglobin production below the basal level of 13 gm. It is possible that the depletion of body protein due to the fasting may mean that food protein when available may be used in part to replace body protein and less to build new hemoglobin. Fluidity and exchange between various body protein stores are coming to be recognized.

Clinical History.—Table 4. Dog 36-96. Dog in perfect condition at end of the experiment and is normal today, 6 months after this experiment.

DISCUSSION

Fasting periods in anemic dogs have received much study in this laboratory. Two fasting periods in Table 4 deserve some further comment. It would seem that these anemic dogs maintained on a low protein intake with a surplus of iron should have in *reserve stores* considerable iron but little if any protein building material. The anemic dog maintained on the standard salmon bread when fasted 2 to 3 weeks will produce 50 to 75 gm. new hemoglobin as a result of this fast (1). During this fasting period the urea-ammonia fraction of the urinary nitrogen decreases greatly ("reaction of conservation") suggesting that the precursors of the urea-ammonia material have been conserved to build new hemoglobin.

The observations in Table 4 indicate that this dog (fasting) can produce less new hemoglobin (7 to 16 gm. per week) than the standard dog which has been on salmon bread régime (25 to 40 gm. per week) during one week of protein fasting. We may reason that the 7 to 16 gm. hemoglobin per week (Table 4) is due wholly to conservation of nitrogenous factors related to protein wear and tear. As this dog can scarcely have any appreciable protein reserve stores we may properly claim that the considerable excess of new hemoglobin produced in the dogs on the salmon bread régime reported elsewhere (1) is related to protein reserve stores retained during the salmon bread feeding periods.

It is possible to argue on the contrary that these dogs with protein reserves exhausted may use some of the material conserved from protein wear and tear to repair body proteins and only a part for the fabrication of new hemoglobin due to the anemia stimulus. Nitrogen partition studies of dogs like 36-96 in Table 4 should help to settle the question.

Utilization of diet protein to make new hemoglobin is well shown in Table 1. On a protein intake of 17 gm. per day (banana diet) the output of new hemoglobin is about 17 gm. per week—that is it requires 7 gm. of diet protein to make 1 gm. of new hemoglobin—a potency ratio of 7. Table 4 shows the same protein intake, 17 gm. per day, but a lower output of new hemoglobin, 14 gm. per week. It requires 8 gm. of diet protein to make 1 gm. of new hemoglobin, a potency ratio of 8.

We may compare these values with the utilization of liver protein in the anemic dog to make new hemoglobin and find the potency ratio to be about 9, which indicates that the banana diet in these dogs is very effectively utilized under these conditions,—that in these experiments the proteins of the banana and salmon muscle are as well utilized to form new hemoglobin in anemia as is true for liver protein. It should be noted that as a general rule small amounts of protein in these limited diets are somewhat more completely utilized than are the larger intakes.

It has been claimed by several workers that the potency of the liver feeding in anemia is wholly due to the contained iron. The experiments in Tables 1 and 4 would seem to prove that the liver contains potent materials (presumably proteins in part) which give an increase in hemoglobin production above the basal output. In the presence of a large excess of iron by mouth the effect of the relatively small amount of iron in the liver could hardly influence the hemoglobin output. Moreover the increase in hemoglobin output on liver feeding (Table 4) is of the order of that observed in standard anemic dogs fed salmon bread plus liver.

SUMMARY

A low protein intake will cause limited hemoglobin production in standard anemic dogs. It appears that the dog on a limited protein intake is unable to produce the usual amount of globin and therefore of hemoglobin even in the presence of a large excess of iron.

Iron given by mouth or by vein shows the same result—the dog made anemic by blood withdrawal cannot produce the expected new hemoglobin related to the iron intake when the *protein intake* is held at low levels. These dogs can be kept in perfect health and weight equilibrium during these long periods of limited diet intake and anemia.

Under the stress of protein limitation the proteins of salmon muscle, banana and carrot are well utilized and it requires only 7 to 8 gm. of these food proteins to produce 1 gm. of new hemoglobin.

These experiments show clearly that the iron content of liver is not wholly responsible for its potency in anemia due to hemorrhage.

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