INFECTION AND INTOXICATION

Their Influence upon Hemoglobin Production in Experimental Anemia

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This paper deals with infection and a variety of intoxications which do or do not modify the production of hemoglobin and red cells in experimental anemia due to blood loss. When *clinical anemias* develop in association with infection the tendency is to explain this abnormal state of the blood on the basis of blood destruction or of lack of absorption from the intestine. This is touched upon briefly in a comprehensive review of anemias by Sturgis and associates (5). We believe that the experimental data given below indicate that the essential factor is a disturbance of the *internal metabolism* which is concerned with upbuilding of the large hemoglobin molecule. There is no evidence of any significant red blood cell destruction in certain experiments (Table 3) and strong evidence that the absorption of food constituents is normal.

These anemic dogs are perfect specimens to test in this fashion, as we have long fore periods preceding the experimental or accidental infection and long after periods for adequate base line control. In such dogs the continued anemia with adequate diet intake and constant body weight gives control of digestion and absorption of the factors which go to form body protein as well as new hemoglobin and red cells. We can use the simplest possible type of "infection"—a *sterile abscess*—which gives the clinical reactions of a bacterial abscess (leucocytosis, fever and elevated nitrogenous urinary excretion) but can be terminated in 4 days with prompt healing and no danger of continuation or complications.

Our interest in infection was first stimulated by a chance observa-

tion. One of the older anemic dogs developed an extensive subacute pyorrhea and at the same time a falling off in hemoglobin production was noted. The abnormal teeth were removed and the gums given proper treatment with prompt healing whereupon the production of hemoglobin returned to normal. We were inclined to attribute this to coincidence or to digestive disturbances but were lead to try some sterile abscesses in these same anemic dogs.

Our attention was finally sharply focussed on this question by a laboratory infection (*endometritis*) in one of the old anemic dogs whose history ran back 9 years. This dog retained its health and a normal food consumption but its capacity to form new hemoglobin and red cells began to fail slowly over a period of 5 months (Table 1 and clinical history Dog 24-45). Finally the dog could form no hemoglobin even on a liver diet. A vaginal discharge pointed to the uterus. Operation showed a greatly enlarged uterus with subacute inflammation of the mucosa and hysterectomy was promptly followed by a return to the normal hemoglobin production.

The crucial experiment is illustrated by Table 3 wherein an anemic dog during a fasting period in which a sterile abscess is present can produce no surplus of hemoglobin. It is well known (3) that the fasting anemic dog can produce a liberal excess of new hemoglobin (30–60 gm. per 2 weeks) which is formed obviously from body protein of the dog and in part by a *conservation* of nitrogenous material which in the normal fasting dog appears in the urea-ammonia fraction of the urine. This obviously is a function of the *internal metabolism* related to new hemoglobin production and this function is evidently disturbed and thrown out of balance by the intoxication related to the sterile abscess.

Dinitrophenol and *thyroid* medication can bring about an increased body metabolism. Various papers (2, 7) by Tainter and associates give clinical and experimental data dealing with dinitrophenol. We decided to test these substances in the anemic dog to ascertain whether this disturbance of body metabolism would in any measure modify the standard hemoglobin output under control conditions. It will be seen in Tables 6 and 7 that the fluctuations which do occur over various time intervals fall within the limits of physiological variations noted under control conditions. The doses used were large and definite clinical reactions were obtained indicating strong therapeutic effect

but we must admit that this type of metabolic disturbance does not modify hemoglobin production in the standard anemic dog.

Methods

The routine care of these anemic dogs has been described in earlier publications (8, 10, 11) and unless otherwise stated in the clinical history the given dog is active and clinically normal. Percentage food consumption is a valuable index of the clinical condition.

The "sterile abscess" is produced by the injection of 1 cc. of turpentine subcutaneously. The reaction is prompt and the abscess may rupture or be evacuated on the 3rd to 5th day. It contains 200–300 cc. of blood tinged pus and after escape of the pus the area heals promptly in 3–4 days. The dogs are clinically slightly indisposed and may refuse a part of a day's ration but this rarely lasts more than a day or two and the clinical recovery is very prompt. Leucocytosis and fever are noted.

All abdominal operations were done under surgical ether anesthesia with the usual aseptic technique. We are indebted to Dr. C. Arthur Elden of the Department of Obstetrics and Gynecology who did the two hysterectomies.

Thyroid (Armour's desiccated) and dinitrophenol (sodium 2.4—dinitrophenoxide—Eastman Kodak) were fed in large doses which produced the recognized therapeutic effect.

EXPERIMENTAL OBSERVATIONS

The first two experiments (Tables 1 and 2) dealing with *endometritis* are much like ordinary clinical infections of moderate severity. One followed catheterization and the other developed spontaneously. As in clinical conditions there are some confusing factors like loss of blood which cannot be accurately estimated but there can be little doubt about the influence of the infection upon the building of new hemoglobin and red cells in the standard anemic dog.

Dog 24-45 (Table 1) presents an interesting story. The anemia was begun February, 1924, and was uneventful until March, 1933, when a metabolism experiment was done with several catheterizations. The dog had been permitted to return to a normal hemoglobin level (139 per cent) at this time but during April it was noted that the hemoglobin level fell slowly to 80 per cent. There was no bleeding and no blood withdrawal except for small samples. From May to August the hemoglobin level varied between 70 and 100 per cent and the dog was clinically normal and active, with a normal weight record. A little bleeding from the vagina was noted Aug. 10 which was thought to be

TABLE 1

Infection—Endometritis

Dog 24-45. Bull, female, adult Table begins Aug. 11, 1933

Diet periods 1 wk. each	Food consumed	Weight	Plasma volume	R.B.C.	Blood hemoglobin level	Hemoglobin removed per week
Food, gm. per day	*per cent*	*kg.*	*cc.*	*mil.*	*per cent*	*gm.*
Bread 450, salmon 100, Klim 40	100	24.4	1120	5.9	93	1.4
Bread 450, salmon 100, Klim 40	89	23.4	1129	5.2	72	2.1
Bread 450, salmon 100, Klim 40	100	23.1	1320	4.9	69	2.0
Bread 450, salmon 100, Klim 40	100	23.1	1285	4.7	55	1.4
Secondary anemia extract + Fe*	100	23.7	1248		49	2.1
Secondary anemia extract + Fe*	100	23.6	1275	4.6	77	2.1
Pig liver 300, bread 350, Klim 40	100	23.6	1406	4.2	62	2.6
Pig liver 300, bread 350, Klim 40	96	23.5	1505	3.4	46	3.3
Hysterectomy—Transfusions						
Pig liver 300, secondary anemia extract + Fe†	100	22.3	1460	3.9	51	3.8
Pig liver 300, secondary anemia extract + Fe†	100	22.8	1359	4.0	56	3.6
Bread 400, salmon 150, Klim 50	100	22.4	1195	4.7	64	1.6
Bread 400, salmon 125, Klim 50	100	23.1	1200	5.0	62	42.1
Bread 450, salmon 125, Klim 50	100	23.1	1284		62	52.7
Bread 450, salmon 100, Klim 40	100	22.9	1325	4.8	58	70.8
Bread 450, salmon 100, Klim 40	100	22.8	1322		48	47.2
Bread 450, salmon 100, Klim 40	100	22.6	1370	3.7	48	1.4
Bread 450, salmon 100, Klim 40	100	22.6	1291	4.7	54	62.3
Bread 450, salmon 100, Klim 40	100	23.0	1350	4.0	48	47.1
Bread 450, salmon 100, Klim 40	100	23.0	1395	3.5	45	1.7
Bread 450, salmon 100, Klim 40	100	23.2	1304	4.2	55	36.7
Bread 450, salmon 100, Klim 40	100	23.5	1421	3.9	43	1.3

* Bread 450, salmon 100, Klim 40.

† Bread 350, Klim 40, cod liver oil 10.

Clinical History—Endometritis—Table 1.

Dog 24-45. Adult female bull. Born Nov., 1922. Experimental anemia from Sept., 1924, to Feb., 1933. Dog returned to normal blood hemoglobin level for metabolism experiment.

Mar. 6, 1933—Metabolism experiment begun. Catheterization Mar. 15, 22, 29, Apr. 1, 1933 (3).

Mar. 28—Hemoglobin 139%, plasma volume 661 cc. Salmon bread basal ration throughout.
Apr. 3— " 110% " " 827 "
10— " 103% " " 972 "
17— " 80% " " 936 "

Apr. 17–May 29. Hemoglobin range 80–84%, plasma volume back to normal 1169 cc. (May 29).

June 5—Hemoglobin 69% No blood removal since metabolism experiment.
12— " 101%

Salmon bread diet since metabolism experiment Apr. 1.

June 12–July 24—Hemoglobin range 88–104%. Dog clinically active and normal since metabolism experiment. Food consumption 100%, no weight loss.

Aug. 10—Noticed very slight bleeding from vagina—dog believed to be in heat. See Table 1 for details of anemia and diet. There was a little vaginal bleeding as in heat during these 6 weeks.

Sept. 6—Feces examined for ova, none found. Urine: Trace albumin, moderate number of red blood cells, *pus cells*, few hyaline casts.

8—Transfusion of 15 gm. hemoglobin. Considerable bleeding 100 cc. ±, external genital tract sensitive, labia swollen. Possible abortion suspected. Ergot 15 drops twice daily.

9—Bleeding less. Ergot continued.

11—No noticeable bleeding. Ergot discontinued.

13—Tablespoonful milky bloody fluid discharged from vagina. Dog acts normal. Food consumption 100% throughout. Leucocytes ranging from 8000–10,000.

14—Transfusion 14 gm. hemoglobin.

15—Diet 300 gm. pig liver plus secondary anemia extract plus iron. Dog very lively. Food consumption 100%. Bleeding from vagina slightly increased 25–50 cc. ±.

20—Bleeding increased to 50 cc.

22—Transfusion 13 gm. hemoglobin.

25—Over Sunday bled considerably 100 cc. +. Left 23% food on *one* day only. Leucocytes 12,400. Transfusion 18 gm. hemoglobin. No weight loss during entire period—June 30, 23.4 kg., Sept. 25, 23.6 kg.

26—*Operation* (Dr. C. A. Elden). Hysterectomy, uterus greatly enlarged. One ovary removed. Anesthesia 1 hr. 45 min. Transfusion 18 gm. hemoglobin.

27—Condition fair. Temperature 39.4°. Glucose intravenously. Leucocytes *high*. Dog stands up when spoken to. Small amount food given and all consumed.

28—Leucocytes 12,500, dog more active.

Oct. 2—Wound healing satisfactorily. Hemoglobin 51%, transfusion 18 gm. hemoglobin. Liver and secondary anemia extract plus iron diet continued.

3—Full food ration completely consumed; marked improvement. Acts well. Total hemoglobin given in all transfusions before and after operation = 96 gm.

10—Put on salmon bread diet—recovery complete. Hemoglobin 56%—see Table 1.

23—Hemoglobin steadily rising 71%, healing of wound complete. *Blood removal begun.* Total amount removed—Oct. 24–Dec. 21—365 gm. hemoglobin.

Surgical Specimen

Dog 24-45 (Table 1). *Uterus* greatly enlarged and elongated probably 3–4 times normal length and more than 3 cm. in diameter. Its serous surfaces are injected but show no peritonitis. The mucosa is thick and velvety, specked with ecchymoses, and covered with thin, pinkish, purulent exudate and fibrin-like material.

Histological sections show some hypertrophy of the muscle coats but the great change is in the mucosa which is greatly thickened and the submucosa is filled with great masses of plasma cells and mononuclears. In some areas there is loss of the covering epithelium (ulcers) and the underlying tissue is a mass of plasma cells mixed with polymorphs and fibrin. Phagocytes are numerous and some contain blood pigment. Small hemorrhages are seen here and there in the inflamed submucosa. Here is conclusive evidence of a long continued inflammation, both chronic and acute reactions being represented.

due to a period of heat. The hemoglobin level from this date on is shown in Table 1 and we note a steady fall in hemoglobin level from 93 to 55 per cent with a normal food intake and but little blood withdrawal for samples and blood volume determinations. The vaginal blood loss continued and on Sept. 8 was considerable—100± cc. bloody fluid. This suggested an abortion in spite of a clear history of isolation from males.

Transfusions of whole blood containing 60 gm. hemoglobin were given during the preoperative period and this amount probably covered adequately the vaginal bleeding. Transfusions containing 36 gm. hemoglobin were given after the operation and probably swell the total of hemoglobin removed when the dog was put back on the anemia routine blood withdrawal (Table 1).

Removal of the greatly *enlarged uterus* showing acute and chronic inflammation of the mucosa was followed promptly by recovery and a

return to a normal state of blood production. This *endometritis* obviously began in March, 1933, and lasted with progressive increase up to September 26, the date of the operation—a period of more than 5 months. The dog steadily lost its normal capacity to form hemoglobin and red cells until it could scarcely maintain the usual anemia level on a liver diet but there is the uncertain factor of vaginal bleeding.

The postoperative period is one of great regenerative activity and enormous hemoglobin production—a total of 365 gm. hemoglobin during 11 weeks. The normal base line hemoglobin output per week for this dog is 15 gm. and the base line output for 11 weeks is 165 gm. leaving a 200 gm. excess. If we deduct 36 gm. for the postoperative transfusions and 140 gm. hemoglobin for the postoperative feeding of whole liver, liver extract and iron we have explained about all the surplus hemoglobin production. The dog is well at the present date—age 13 years—and the standard reaction at present to the basal ration, iron or liver feeding is just the same as in normal periods before the operation. There is no evidence that this infection had left any permanent impairment of the mechanism which carries on the manufacture of hemoglobin.

Table 2, Dog 27-240, shows a more satisfactory experiment with few confusing factors and the indication is clear that this infection (endometritis) lowers the capacity of this standard anemic dog to produce the usual output of new hemoglobin and red cells under standard conditions. This dog's history reveals evidence that the endometritis was active as early as March when the reaction to liver feeding was found to be about 60 per cent of normal. In May the basal bread output per week had dropped from 16 gm. hemoglobin to 9 gm. In July (Table 2) pig kidney feeding had no positive effect upon the hemoglobin production and an exploratory laparotomy (August 23) showed a very large uterus (endometritis) which was removed.

No blood transfusions given in the weeks before operation confused the picture of blood regeneration (Table 2) and the vaginal bleeding was trivial. Transfusions just preceding and after operation containing 42 gm. hemoglobin appear in the blood withdrawn in the weeks following the operation.

It is probable that this endometritis of unknown origin began about 5 months before the uterus was removed but it was not as severe as

TABLE 2

Infection—Endometritis

Dog 27-240. Bull, female, adult Table begins June 26, 1935

Diet periods 1 wk. each	Food consumed	Weight	Plasma volume	R.B.C.	Blood hemoglobin level	Hemoglobin removed per week
Food, gm. per day	*per cent*	*kg.*	*cc.*	*mil.*	*per cent*	*gm.*
Bread 275, salmon 125, Klim 20	89	13.3	884	4.5	43	12.1
Bread 275, salmon 150, Klim 20	91	13.5	780	4.2	47	2.2
Bread 275, salmon 150, Klim 20	79	12.9	820	5.6	52	20.5
Bread 225, salmon 200, Klim 20	85	13.5	806	4.1	44	1.2
Pig kidney 300, bread 225	71	12.3	742	3.9	47	1.4
Pig kidney 300, bread 225	93	12.6	826	4.5	52	11.2
Bread 225, salmon 200, Klim 20	82	12.6	800	3.8	49	1.3
Bread 225, salmon 200, Klim 20	95	12.2	763	3.7	46	1.8
Bread 200, salmon 200, Klim 20	96	12.0	713	4.2	47	1.5
Hysterectomy—Transfusions						
Bread 250, salmon 200, Klim 20	100	11.7	699	4.2	55	2.0
Bread 300, salmon 200, Klim 20	100	12.0	713	5.4	60	40.5
Bread 300, salmon 200, Klim 20	100	12.0	683	4.9	58	27.5
Bread 300, salmon 200, Klim 20	100	12.2	718	4.5	56	12.5
Bread 350, salmon 125, Klim 20	100	12.7	755	4.8	68	22.9
Bread 350, salmon 125, Klim 20	100	12.7	779	5.5	62	46.6
Bread 350, salmon 125, Klim 20	100	13.1	780	4.8	55	34.0
Bread 350, salmon 125, Klim 20	100	13.0	800	4.6	43	23.7
Bread 350, salmon 125, Klim 20	98	13.2	800	4.6	51	14.0
Bread 375, salmon 100, Klim 20	100	13.2	822		55	26.4
Bread 375, salmon 100, Klim 20	100	13.3	760	5.5	54	34.3

Clinical History—Endometritis—Table 2.

Dog 27-240. Adult female bull. Born Feb., 1928. Continuous experimental anemia Apr., 1930, to date.

Mar., 1935—Liver feeding experiment 300 gm. daily for 2 weeks results hemoglobin output of 52 gm. in contrast to last liver experiment (Dec., 1933) with 93 gm. hemoglobin output. Lextron experiment including Fe 300 mg. on May 16, 1935, resulted in 69 gm. hemoglobin output in contrast to similar test Jan. 31, 1935, when output was 78 gm. hemoglobin.

Salmon bread basal hemoglobin output—16 gm. per week, Jan., 1935
" " " " " 12 " " " Mar., 1935
" " " " " 9 " " " May, 1935

Food consumption fell off from 91% to 79% on the average.

July 17—Pig kidney feeding experiment (300 gm. daily for 2 weeks). Negative hemoglobin output (Table 2).
24—Leucocytes 15,000—previous average 9,500.
Aug. 1–12—Food consumption 100%.
11—Slight vaginal bleeding noticed. Small amount of blood on floor of cage. Dog is active and does not appear ill.
14—Leucocytes 20,400. Temperature 100.8°.
14–21—Slight vaginal bleeding continues, temperature ranging from 100.8–101.2°. Leucocytes 10,600.
23—Bloody vaginal discharge increasing. Endometritis suspected. *Operation.* Uterus removed (Dr. C. A. Elden). Transfusion before and after operation—total 42 gm. hemoglobin.
Weight loss from July 19–Aug. 23, 0.7 kg.
Food consumption Aug. 14–23 (date of operation) averages 96%.
During entire bleeding period dog did not appear acutely ill, only noticeable symptoms were leucocyte increase, temperature rise and slight bleeding.
Aug. 24—Clinical condition good. Food consumption 100%, dog began to gain weight. Note hemoglobin output—Table 2.
Sept. 5—Recovery complete and wound healed.

Surgical Specimens

Dog 27-240 (Table 2). Uterus is greatly enlarged as in Dog 24-45. It measures about 3–5 cm. in diameter and is about three times longer than normal. Both horns contain blood tinged purulent material and the mucosa is covered with purulent exudate. The lumen is greatly dilated but the walls are thickened. Studies made by Dr. L. Ackerman of the Department of Bacteriology showed pure cultures of *B. coli.*

Histological sections show evidence of a chronic inflammation with great numbers of mononuclears and plasma cells in the interglandular stroma of the mucosa. The crypts of the glands of the mucosa are greatly dilated and contain numbers of polymorphonuclears and much cell debris. Here are found colonies of bacteria. The acute change in this case predominates but there are no definite ulcers in the mucosa. The muscle coats are thickened but show no significant extension of the inflammation. No hemorrhages are seen and no phagocytes containing blood pigment.

seen in the other dog—Table 1. There were no ulcers, no phagocytes containing pigment and the bleeding from the vagina was of no significance. *Bacillus coli* was probably largely responsible but we have been unable to cause this type of endometritis by the introduction of bacteria into the normal dog's uterus.

Hemoglobin production after hysterectomy is of considerable interest.

If we omit the 1st postoperative week because of wound healing and take the next 10 weeks period, the total hemoglobin production is 282 gm. If we assume that the hemoglobin production due to basal salmon bread feeding is the same after as before the endometritis—namely 16 gm. per week, we can account for 160 gm. hemoglobin plus the 42 gm. hemoglobin given in transfusions = 202 gm. hemoglobin. This deducted from 282 gm. hemoglobin actually removed leaves a total of 80 gm. hemoglobin unexplained. We are inclined to attribute this 80 gm. hemoglobin to the favorable *kidney diet* given shortly *before* the operation. If this is true an important point is established—that infection under such conditions does not interfere with *absorption* and *storage* of products going to form hemoglobin but does interfere with the orderly *production* of new hemoglobin within the body (see also Table 3—fasting). This experiment would answer the question about *absorption* of hemoglobin building stones in this type of infection—the trouble is not with absorption but somewhere along the line of *protein anabolism* within the body. It must be admitted that hypothetical blood destruction might explain a part of this 80 gm. surplus as the products of the destroyed red cells would be stored in speen and liver to be utilized at a later date. However we can control this factor in the next experiment (Table 3).

Table 3 gives two important experiments to show clearly that *intoxication* (sterile abscess) disturbs the production of hemoglobin during a fasting period. Obviously the disturbance cannot influence any *absorption* and must act on some phase of *internal protein metabolism.* The contrast (Table 3) in the same dog fasting with and without the sterile abscess is striking. Dog 24-45 produces 76 gm. new hemoglobin as the result of a 2 weeks fast and at another time in the presence of these sterile abscesses can produce no hemoglobin above the basal output. It is well known (1) that there is an increased protein breakdown and increased urinary nitrogen caused by a sterile abscess but the body cannot use this nitrogenous material. Under simple fasting conditions the anemic dog can make large amounts of new hemoglobin and actually conserves for hemoglobin construction some of the nitrogenous material which otherwise in the non-anemic dog would be wasted and appear in the ammonia-urea fraction in the urine (3).

TABLE 3

Turpentine Abscess and Fasting

Dog 24-45. Bull, female, adult

Diet periods 1 wk. each	Food consumed	Weight	Plasma volume	R.B.C.	Blood hemoglobin level	Hemoglobin removed per week
Food, gm. per day	*per cent*	*kg.*	*cc.*	*mil.*	*per cent*	*gm.*
Bread 450, salmon 50	100	21.2	1211	3.6	42	1.2
Turpentine—1 dose, fasting		19.1	1075	4.3	41	1.2
Turpentine—2 doses, fasting		17.2	980	5.6	53	13.9
Bread 450, salmon 50	100	17.6	1065	3.9	41	1.2
Bread 450, salmon 50	100	18.7	1170	3.7	35	1.0
Basal output—5 gm. Hb. per week. Total net Hb. output—0						Total = 17.3
Bread 600, salmon 50	100	23.1	1140	5.6	58	1.9
Fasting		20.6	1082	6.1	60	21.1
Fasting		18.3	901	6.4	59	18.4
Bread 400, salmon 50	100	20.0	1108	4.9	44	18.4
Bread 350, salmon 50	100	19.9	1118	5.5	49	36.6
						Total net Hb. output—76 gm. Total = 94.5

Clinical History—Sterile Abscess and Fasting—Table 3.

Dog 24-45. Adult female bull. Born Nov., 1922. Experimental anemia begun Sept., 1924, and a normal anemia history to Mar., 1927.

Mar. 22, 1927—Fasting begun, turpentine 1 cc. subcutaneously.

25—Abscess draining bloody purulent thick material. 2nd abscess, 1 cc. turpentine subcutaneously.

28—Abscess draining.

Apr. 3—3rd abscess, 1 cc. turpentine subcutaneously.

5—Returned to salmon bread diet with prompt healing and recovery.

Dog 24-46 (Table 4) gives equally convincing data to show that the sterile abscess inhibits the production of new hemoglobin during the *2nd week* of a fast and the 2 following weeks. The usual reaction due to simple fasting is noted in the 1st week when 24 gm. hemoglobin are removed. This is to be compared with the simple fasting reaction (Dog 24-45, Table 3).

TABLE 4

Turpentine Abscess and Fasting

Diet periods 1 wk. each	Food consumed	Weight	Plasma volume	R.B.C.	Blood hemoglobin level	Hemoglobin removed per week
Dog 27-234. Bull, male, adult						
Food, gm. per day	*per cent*	*kg.*	*cc.*	*mil.*	*per cent*	*gm.*
Bread 325, salmon 75, Klim 20	100	16.9	992	4.3	46	1.4
Turpentine—2 doses, fasting		15.0	870	4.9	45	11.0
Turpentine—1 dose, fasting		14.4	873	3.7	41	1.2
Bread 325, salmon 75, Klim 20	100	13.7	818	4.6	48	1.3
Bread 325, salmon 75, Klim 20	100	13.8	756	4.8	50	22.0
Basal output—17 gm. Hb. per week. Total net Hb. output—6 gm. Total = 35.5						
Dog 24-46. Bull, female, adult, splenectomy						
Bread 400, salmon 75	100	19.5	907	4.0	46	1.3
Fasting		17.4	902	3.9	41	24.7
Turpentine—1 dose, fasting		15.5	886	3.1	41	1.1
Bread 400, salmon 75	100	15.9	950	3.5	38	2.0
Bread 400, salmon 75	100	16.4	965	4.1	44	1.4
Basal output 3 gm. Hb. per week						

Clinical Histories—Sterile Abscess and Fasting—Table 4.

Dog 27-234. Male, adult, bull, born Jan., 1928. Continuous uneventful anemia history Oct., 1929, to date.

Jan. 16, 1936—Fasting begun, turpentine 1 cc. subcutaneously.

21—Abscess drained, second dose of turpentine 1 cc. subcutaneously.

22—Leucocytes 16,000. Temperature 101°.

23—First abscess filled again, opened, 200 cc. bloody pus withdrawn. Urine contained no bile pigment.

24—Dog is not clinically well. Two intravenous injections of 200 cc. normal salt solution. Temperature 101.7°.

25—Leucocytes 24,000. Temperature 100°. No bile pigment in urine. Abscess draining. Normal salt solution injected intravenously. Dog improved.

26—One injection of normal salt solution. No bile pigment in urine.

Jan. 27—Third dose of turpentine subcutaneously. Leucocytes 12,600. Temperature 102°.
28—Leucocytes 16,600. Temperature 102.2°. Normal salt solution intravenous. No bile pigment in urine.
29—No bile pigment in urine. Leucocytes 30,400.
30—Leucocytes 23,000. Temperature 101°. Given ⅓ usual ration of salmon bread diet.
31—Given ½ usual ration of salmon bread.
Feb. 1—Abscess draining. Leucocytes 11,600. Temperature 100°. Full bread ration given.
5—Healing complete. Leucocytes 9,500.

Dog 24-46. Female bull, young adult, born 1923. Continuous uneventful anemia history Sept., 1924, to Aug., 1927. Splenectomy June 16, 1925.
Mar. 29, 1927—Fasting begun.
Apr. 5—Fasting continued, 1 cc. turpentine subcutaneously.
11—Abscess draining.
12—Returned to salmon bread diet. Rapid recovery.

Dog 27-234, Table 4, gives additional evidence on the important point that the sterile abscess will stop the production of hemoglobin. This dog has a high base line production of 17 gm. hemoglobin per week on salmon bread but this animal shows no significant production of hemoglobin during the fasting period or in the 2 weeks after period. Compare Dog 24-45, Table 3, with a lower base line hemoglobin production (5 gm. per week) and yet 76 gm. hemoglobin output due to fasting alone. Dog 27-234, Table 4, showed no bile pigment in urine or blood plasma during the abscess period.

A final test of possible blood destruction due to the sterile turpentine abscess may be briefly outlined.

Dog 32-2. A *renal bile fistula* anemic for 21 months.
Feb. 1—Urine 24 hrs. 520 cc.—bile pigment content 55 mg.
2— " " " 500 "— " " " 43 "
3— " " " 530 "— " " " 42 "
Turpentine 1 cc. given subcutaneously caused the usual abscess reaction.
Feb. 4—Urine 24 hrs. 960 cc.—bile pigment content 56 mg.
5— " " " 545 "— " " " 84 "
6— " " " 675 "— " " " 92 "
Abscess incised and drained of about 120 cc. pus.
Feb. 7—Urine 24 hrs. 670 cc.—bile pigment content 70 mg.
8— " " " 600 "— " " " 50 "
Abscess healing—no urobilin at any time. Dog in excellent clinical condition.

It is well known (4) that in an anemic bile fistula dog of this type the destruction of red cells or the injection of hemoglobin will be followed promptly by an elimination in the urine of bile pigment in amounts close to the theoretical equivalent (40 mg. bile pigment = 1 gm. hemoglobin). This dog has a base line bile pigment output per 24 hours of about 50 mg. and this output may be somewhat increased to a peak of 92 mg. bile pigment just before rupture of the abscess. This is probably due to the acute inflammatory reaction in the abscess wall with capillary red cell extravasation. In all the dog put out about 100 mg. bile pigment above its normal base line as the result of the presence of this sterile abscess or the equivalent of not more than 3 gm. hemoglobin, presumably broken down in the area of inflammation. This is a trivial amount of hemoglobin when we are talking about a difference of more than 70 gm. of hemoglobin (Table 3) which represents the difference in hemoglobin production in the same dog with and without a sterile abscess during and related to a 2 weeks fast. This striking effect of a sterile abscess is due therefore not to infection and blood destruction and not to lack of absorption but to some reaction related to internal protein metabolism and hemoglobin construction.

Table 5, Dog 25-97, shows that a sterile abscess will impair hemoglobin production when a favorable diet (liver) is given. Not only is there less hemoglobin formed but there is a conspicuous *delay in hemoglobin production* as though materials had been absorbed but not promptly utilized to make new hemoglobin as is seen in the control experiment on the same dog without the abscess. The food consumption was decreased by the abscess reaction and this diminished food intake will explain about one-half of the diminished hemoglobin production.

A second experiment (Dog 27-233) is not tabulated here but the reaction was identical with that in Table 5.

Table 6 shows three satisfactory experiments on different dogs indicating that with this low level of hemoglobin production on the basal salmon bread ration, there is no significant change attributable to the sterile abscesses. Possibly the *low hemoglobin output* puts less demands on the hemoglobin producing body mechanism which can carry this low output in spite of the intoxication related to the sterile

TABLE 5

Turpentine Abscess and Liver Feeding

Dog 25-97. Bull, male, adult

Diet periods 1 wk. each	Food consumed	Weight	Plasma volume	R.B.C.	Blood hemoglobin level	Hemoglobin removed per week
Food, gm. per day	*per cent*	*kg.*	*cc.*	*mil.*	*per cent*	*gm.*
Bread 450, salmon 75, Klim 25	100	17.6	1133	4.9	49	1.2
Turpentine—2 doses*	85	17.1	1140	5.5	52	15.0
Turpentine—1 dose*	72	16.8	1067	5.6	54	17.2
Bread 450, salmon 75, Klim 25	100	17.2	1073	4.7	52	20.1
Bread 450, salmon 75, Klim 25	100	17.2	935	5.0	50	18.3
Bread 450, salmon 75, Klim 25	100	17.1	1086	5.1	42	28.6
Basal output 5 gm. Hb. per week.	Total net Hb. 67 gm.				Total = 99.2	
Bread 450, salmon 75, Klim 25	100	18.0	1177	5.2	49	1.4
Liver 300, bread 400	100	18.3	1058	6.9	67	49.1
Liver 300, bread 400	100	18.4	1034	6.4	67	47.4
Bread 450, salmon 75, Klim 25	100	18.6	1075	5.4	52	29.4
Bread 450, salmon 75, Klim 25	100	18.6	1124	4.2	45	1.2
	Total net Hb. output 103 gm.				Total = 127.1	

* Liver 300, plus bread 350.

Clinical History—Sterile Abscess and Liver Feeding—Table 5.

Dog 25-97. White bull, female, adult, born Feb. 22, 1926. Continuous uneventful anemia history. Jan. 28, 1927, to Nov., 1929.

Apr. 26, 1927—Urine examination showed normal findings.

Nov. 16, 1929—Dog had diarrhea accompanied by slight vomiting. Urine examination: Considerable albumin with moderate number of granular casts. No blood cells. Food consumption for that week averaged 58%.

Dec., 1929—Recovery.

Apr. 12, 1930—Salmon bread diet plus 300 gm. pig liver daily. First sterile abscess produced by 1 cc. turpentine subcutaneously.

14—Left 51% food.

15—Second dose of 1 cc. turpentine subcutaneously.

16—Left 40% food. First abscess drained, 100 cc. bloody pus.

Apr. 18—Second abscess draining.
22—Third abscess produced by 1 cc. turpentine subcutaneously.
23—Left 85% food.
24—Left 58% food. Urine examination: Trace of albumin. No casts, no red blood cells.
25—Left 36% food.
26—Abscess draining.
May 2—Healing complete. Dog clinically normal.
8 weeks later the liver feeding control (Table 4) was carried out as indicated.

TABLE 6

Turpentine Abscess and Bread Feeding

Diet periods 1 wk. each	Experimental period	Food consumed	Weight average	Blood hemoglobin level average	Hemoglobin removed per week average
Dog 30-114. Coach, male, adult					
Food, gm. per day	*wks.*	*per cent*	*kg.*	*per cent*	*gm.*
Bread 450, salmon 75, Klim 20	10	100	14.1	44	12.6
Turpentine 1 cc.—4 doses*	4	98	15.8	47	9.7
Bread 450, salmon 50, Klim 20	6	100	16.3	44	10.4
Dog 30-121. Coach, male, adult					
Bread 430, salmon 55, Klim 20	10	100	13.9	46	12.1
Turpentine 1 cc.—4 doses†	3	100	14.7	43	15.7
Bread 450, salmon 50, Klim 20	5	100	14.4	42	9.5
Dog 24-45. Bull, female, adult					
Bread 400, salmon 50, Klim 20	6	100	24.6	46	12.0
Turpentine 1 cc.—4 doses‡	3	100	24.3	50	6.0
Bread 400, salmon 50, Klim 20	4	100	24.0	42	16.0
Bread 400, salmon 50, Klim 20	8	100	24.1	46	16.5

* Bread 450, salmon 75, Klim 20.
† Bread 350, salmon 75, Klim 20, cod liver oil 10.
‡ Bread 400, salmon 50, Klim 20.

abscesses. All dogs were in good condition, food consumption complete and recovery rapid from the abscess reaction.

Tables 7 and 8 show that the accelerated metabolism due to thyroid feeding or dinitrophenol does not modify the hemoglobin output in these standard anemic dogs.

TABLE 7

Desiccated Thyroid—Negative Influence on Hemoglobin Production

Diet periods 1 wk. each	Experimental period	Food consumed	Weight average	Blood hemoglobin level average	Hemoglobin removed per week average
Dog 33-14. Coach, female, adult					
Food, gm. per day	*wks.*	*per cent*	*kg.*	*per cent*	*gm.*
Bread 375, salmon 75, Klim 20	17	99	11.3	52	22.2
Thyroid 15, bread 375, salmon 125, Klim 20	4	99	11.4	47	10.0
Bread 375, salmon 100, Klim 20	8	100	11.9	49	13.0
Dog 33-13. Coach, male, adult					
Bread 390, salmon 75, Klim 20	18	100	17.4	46	14.4
Thyroid 10, bread 425, salmon 75, Klim 20	4	100	16.4	45	19.4
Bread 425, salmon 75, Klim 20	11	100	17.8	48	15.9
Dog 30-114. Coach, male, adult					
Bread 450, salmon 75, Klim 25	18	100	14.2	47	11.0
Thyroid 15, bread 450, salmon 75, Klim 20	5	100	13.5	47	19.3
Bread 450, salmon 75, Klim 20	10	100	14.1	44	12.6

Clinical History—Dinitrophenol and Thyroid—Tables 6, 7 and 8.

Dog 30-114. Coach, mongrel, male. Born Sept., 1930. Continuous anemia history Mar. 1, 1932, to date. Bread basal hemoglobin output—9 gm. average in 1933.

Dec. 12, 1933—Sodium dinitrophenol feeding begun. Dose 16 mg. daily, weight 14.7 kg. Increasing doses up to 280 mg. daily added to salmon bread diet during 21 weeks. Temperature range 38.8–39.4°.

June 2—Temperature 40.1°, dog is very warm to touch but does not appear ill. Dinitrophenol feeding stopped with last dose of 280 mg. Salmon bread diet for 18 weeks thereafter.

Oct. 2, 1934—Desiccated thyroid 15 gm. daily for 5 weeks (Armour). Salmon bread diet. Thyroid to Nov. 7, 1934. Weight 14.9 kg. at start—at end of thyroid feeding period 12.7 kg. Maximum temperature 38.9°. No unfavorable symptoms.

Clinical History—Thyroid Feeding—Table 7.

Dog 33-13. Coach, male, adult, born Nov., 1932 (littermate to 33-14). Anemia history Dec. 29, 1933, to date. Uneventful.

Mar. 2, 1935—Desiccated thyroid daily feeding 15 gm. with salmon bread diet. Following giving of 11 doses dog became ill, sudden collapse. Thyroid omitted for 2 days, complete recovery.

Mar. 15—Thyroid 5 gm. for 8 days, increased to 7 gm. daily for 2 days.

29—Dog not acting well, thyroid reduced to 5 gm. for 3 days. Because dog appeared sick thyroid feeding was stopped. Food consumption 100% in spite of upsets. Weight at beginning of thyroid feeding 17.6 kg. at end 15.7 kg. Uneventful anemia history thereafter to date.

Clinical History—Thyroid and Dinitrophenol—Tables 7 and 8.

Dog 33-14. Coach, female, adult, born Nov., 1932. Anemia history Dec. 22, 1933, to date. Uneventful.

July 16, 1934—Began daily feeding of sodium dinitrophenol with 66 mg. increasing to 210 mg. during a period of 17 weeks with salmon bread diet. Food consumption 100%. Temperature range from 38.6–39.5°. Total weight loss 1.7 kg. Anemia history continuous.

Feb. 5, 1935—Desiccated thyroid feeding 15 gm. daily for 4 weeks with salmon bread diet. No symptoms. Food consumption 100%. Clinically normal. Weight at beginning of thyroid feeding 12.8 kg. at end 10.6 kg. Loss 2.2 kg.

TABLE 8

Sodium Dinitrophenol—Negative Effect on Hemoglobin Production

Diet periods 1 wk. each	Experimental period	Food consumed	Weight average	Blood hemoglobin level average	Hemoglobin removed per week average
Dog 30-114. Coach, male, adult					
Food, gm. per day	*wks.*	*per cent*	*kg.*	*per cent*	*gm.*
Bread 450 gm., salmon 75 gm., Klim 40 gm.	3	100	14.7	46	8.6
Dinitrophenol 71 mg.*	7	100	15.0	49	14.2
Dinitrophenol 144 mg.*	5	100	15.3	44	9.0
Dinitrophenol 235 mg.*	4	100	15.0	45	13.5
Dinitrophenol 280 mg.*	5	100	13.7	48	9.9
Bread 450 gm., salmon 75 gm., Klim 25 gm.	18	100	14.2	47	11.0
Dog 33-14. Coach, female, adult					
Dinitrophenol 75 mg.†	8	98	10.7	47	10.1
Dinitrophenol 138 mg.†	6	100	10.4	49	9.6
Dinitrophenol 210 mg.†	3	100	9.7	58	17.1
Bread 375 gm., salmon 75 gm., Klim 20 gm.	17	99	11.3	52	22.2

* Bread 450, salmon 75, Klim 40 gm.

† Bread 325, salmon 75, Klim 20 gm.

Thyroid (Table 7) was given in large doses as we learned from Dr. Wm. S. McCann that these doses were necessary to produce a definite physiological response in dogs. There was noted a definite loss of weight in all dogs (clinical histories). One dog shows a slight fall in hemoglobin output and two dogs show a slight increase but no significance is attached to moderate changes of this type as they come within the limits of unexplained physiological variations.

Sodium dinitrophenol (Table 8) was given in increasing doses and the amount noted each week is the daily average for that particular week. No modification of hemoglobin production is noted as due to this drug and we must conclude that like thyroid under these conditions it has no effect upon the hemopoietic tissues. Sufficient dinitrophenol was given to cause loss of weight and some temperature change (see clinical histories). The work of Tainter and associates (6) indicates that even with large doses of this drug no change is noted in the liver and other viscera.

DISCUSSION

There are certain difficulties in the use of the terms infection and intoxication. *Infection* obviously is appropriate to designate the disturbances noted in the two dogs with endometritis. Moreover in four dogs with this sort of endometritis pure cultures of *B. coli* were isolated from the uterus in three. The *sterile abscess* introduces a difficulty as it is not an infection in the strict sense of the word and yet this type of abscess (turpentine) gives the exact clinical picture of a bacterial abscess—fever, leucocytosis, accumulation of pus and increased nitrogenous excretion in the urine. Bacteria and turpentine both kill tissue at a local spot in the body and the reactions are obviously due to disintegration of the host protein with escape of split products (1). The term *intoxication* may be best for this condition (sterile abscess) but this term is so vague and has been used so loosely as to be in bad repute. At any rate whether we term it "infection" or intoxication the *sterile abscess* has a very clear cut clinical picture and a positive inhibiting effect upon the internal metabolism of hemoglobin construction during a fast.

We note (Table 5) that the sterile abscess depressed the hemoglobin output due to a liver diet but does not appreciably modify the basal output on the standard bread basal ration (Table 6). We may choose to believe that the dog on a liver diet is producing hemoglobin in far

greater amounts than on a bread diet, that the physiological machine is working under greater stress and therefore is more susceptible to toxic agencies which disturb the internal metabolism related to hemoglobin production. The disturbance due to a sterile abscess is greatest during a fasting period and at this time it would seem that the body must be using every agency and all available material working under great stress to produce new hemoglobin. At any rate the physiological mechanism related to hemoglobin production during a fast is profoundly disturbed by the intoxication related to a sterile abscess.

Absorption of food products from the intestine is of fundamental importance and may be an important factor in the anemia of experimental or human infections. Appetite may be impaired and less food be consumed. When food consumption is 100 per cent, digestion normal and body weight maintained we believe that *absorption* is not a factor of importance as it would be necessary to postulate a selective interference with absorption of hemoglobin building material. One experiment (Table 2) gives evidence that hemoglobin building materials may be absorbed and stored during a period of infection and impaired hemoglobin production to appear later as new hemoglobin after removal of the infected uterus.

Blood destruction is an important factor to have clearly in mind in evaluating the experiments tabulated above. In the endometritis experiments (Tables 1 and 2) blood destruction may be an important factor although the evidence is against this explanation but when we deal with the sterile abscess in a fasting period this hypothetical blood destruction can be excluded as the freed hemoglobin would be promptly turned over by the anemic dog to appear in the subsequent weeks as new hemoglobin (9). These dogs with sterile abscesses show no bile pigment in blood plasma or urine. Moreover the anemic bile fistula dog given a sterile abscess shows only a slight increase in bile pigment elimination due to red cell extravasation in the area of inflammation—perhaps the equivalent of 3 gm. hemoglobin destroyed within the body. This is a trivial amount of hemoglobin when we are seeking an explanation to cover a difference of 50–70 gm. hemoglobin—the difference in the same dog (Table 3) between the fasting hemoglobin output with and without a sterile abscess.

SUMMARY

Infection in human cases is often believed to be responsible for anemia. It is generally believed that lack of absorption and definite blood destruction are responsible for the anemia.

Accelerated metabolism due to thyroid or dinitrophenol does not modify hemoglobin production in these standard anemic dogs.

Endometritis lasting over many weeks will profoundly reduce the production of hemoglobin in the standard anemic dog.

A *sterile abscess* also will diminish the production of new hemoglobin in the anemic dog when liver is being fed but particularly during *fasting periods* when the usual abundant production of new hemoglobin is reduced to zero.

Impaired absorption can be excluded as a factor of any significance in certain experiments given above.

Destruction of red cells can likewise be excluded as of any significance in certain experiments given above.

These experiments point to a *disturbance of internal metabolism* related to hemoglobin building in the body as responsible for the inhibition of hemoglobin production under these conditions. We believe this same factor is often of importance in human disease.

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