HEMOGLOBIN PRODUCTION IN ANEMIA AS INFLUENCED BY THE BILE FISTULA

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Hemoglobin production in the anemic dog is profoundly influenced by the presence of a *bile fistula* and such fistula dogs will produce about half as much hemoglobin on standard diets as will control dogs. We may well investigate the reasons for this unusual reaction. One thinks at once of abnormalities of intestinal absorption but disturbance of liver function cannot be excluded.

A successful bile fistula in a dog excludes all bile from the intestinal tract and enables the investigator to collect the whole bile from a bag or in the urine. Dogs can be kept in a normal clinical state with weight equilibrium, normal appetite and activity if they are given a proper diet and whole bile by mouth daily (5). If bile is not given by mouth we observe after several weeks certain abnormalities which have been described in detail from this laboratory (5). The dog may show a *delayed blood coagulation* and even spontaneous bleeding which soon may be fatal. This delayed blood coagulation can be prevented or cured by whole bile by mouth and can be remedied temporarily by whole blood transfusion. This abnormal blood coagulation is due to a lack of prothrombin (3). The condition will not develop with prolonged complete biliary obstruction. Therefore the bile appears to contain something which contributes in some way to prothrombin formation.

Osteoporosis may develop in a bile fistula dog and be responsible for multiple fractures in the bony skeleton, especially in the ribs. A negative phosphorus and calcium balance will develop promptly when bile is excluded from the intestine. This osteoporosis will develop after several months (8 to 12) with a bile fistula given no

bile by mouth or in a dog with complete biliary obstruction. The osteoporosis develops because of lack of absorption of vitamin D and this state is corrected or prevented by the presence of bile salts in the intestine (1).

The osteoporosis seems to be due to faulty absorption. The abnormal *bleeding* of a bile fistula is not a simple matter of absorption as this condition does not develop in biliary obstruction. Substances formed in the liver, excreted in the bile and reabsorbed, appear to be necessary for normal prothrombin formation.

Likewise this *inadequate hemoglobin production* due to the presence of a bile fistula is not a simple matter of intestinal absorption related to the bile in the intestine. After the bile fistula is produced we may feed bile or not but this does not appreciably modify the hemoglobin production. One notes in the tables below that the output of hemoglobin is the same whether during bread periods the food is mixed with dog bile or given for a 10 week period with no bile at any time (dog 29-66, Table 3–continued). Iron by mouth given during periods with or without bile shows the same response.

One may choose to believe that once the *cycle* of bile salt secretion, intestinal absorption and resecretion by the liver is interrupted, certain abnormalities develop some of which are not corrected by bile feeding. This cycle of the bile salts may involve other fractions in the bile and the interruption of this cycle of secretion and reabsorption may disturb both intestinal mucosa and hepatic epithelium.

In the normal dog there is a more or less *continuous circulation* of bile salts and we have always felt that the *internal* part of this cycle was as important as the external (intestinal) portion. We may argue that the normal state of the liver cell is in part dependent upon this continuous bile salt cycle, and when this circulation is interrupted for a considerable part of each day, the liver epithelium suffers and is unable to assemble as skillfully as usual all the building stones which go eventually to form the complex hemoglobin molecule. There is no reason to suspect the *bone marrow* which is normal histologically.

Methods

The general methods used in the anemia experiments have been carefully described and various method controls are given in detail (10). The bile fistula

used in all the experiments tabulated below is designated as the renal bile fistula. The gall bladder is opened and fixed in the renal pelvis and the common bile duct ligated and cut. This fistula is described in a recent paper (5) which gives also the necessary control data mentioned in this paper. Renal bile fistulas are very useful for this type of experiment and can be kept in health for many years. We are indebted to Dr. John J. Morton of the Department of Surgery who performed two bile fistula operations. The other operative work was done by W. B. Hawkins.

The apricots used in these experiments were the dried apricots purchased on the open market. All fistula dogs received 20 gm. Klim (a skim milk powder) daily with the diet as given in the tables. Iron fed by mouth was given as a ferric citrate and the tables show the amount of the metal Fe in milligrams given each day. The colloidal iron given by vein, supplied through the courtesy of Dr. David Loeser, Loeser Laboratories of New York, has never caused any untoward results. "Lextron" is a term used to designate a mixture which contains liver-stomach concentrate (both primary anemia and secondary anemia liver fractions (11) being represented), iron ammonium citrate green and a vitamin B complex. This material was supplied by Eli Lilly and Company.

EXPERIMENTAL OBSERVATIONS

The first three experiments (Tables 1 to 3) are satisfactory as the dogs were standardized before the bile fistula operation. Therefore we are able to compare the hemoglobin production in anemia as influenced by iron, apricots and liver feeding before and after the bile fistula.

Table 1, Dog 32-1, shows that on the average this dog produced only 52 per cent as much hemoglobin after the bile fistula operation as it did in the normal fore period. Excepting the first 4 weeks after the operation, the dog received increasing amounts of bile by mouth daily as shown in Table 1. The second feeding of liver (200 gm. per day fresh weight) was given in less amount than usual because of incomplete consumption. The output of hemoglobin was the highest recorded in any experiment (Table 6), 52 gm. per 2 weeks, and if we correct for 300 gm. the figure would be 78 gm. compared with the pre-operative level of 97 gm. hemoglobin. We have no explanation for this unusual reaction.

Autopsy of this dog showed a rather extensive interstitial pneumonia and an acute pelvic peritonitis presumably related to the pneumonia. As there was a tendency to bleed (5) there was hemorrhage into the peritoneum and lungs which was the immediate cause of death.

TABLE 1

Hemoglobin Production in Anemia Depressed by Bile Fistula

Dog 32-1. Coach, male, adult.

Diet, daily average intake	Bile given daily	Experimental period	Food consumed av.	Weight av.	Blood Hb. level av.	Hb. removed per wk. av.	Total net Hb. output 2 wks.
gm.	*cc.*	*wks.*	*per cent*	*kg.*	*per cent*	*gm.*	*gm.*
Bread 260, salmon 117	0	6	99	13.0	46	10.4	
Apricots 100, br. 225, salm. 125	0	2	100	12.1	52	27.8	34.2
Bread 263, salmon 125	0	2	100	12.3	47	10.1	
Fe 40 mg., bread 300, salmon 100	0	2	100	12.4	59	42.3	68.7
Bread 325, salmon 100	0	2	100	12.5	46	12.9	
Pig liver 300, bread 300	0	2	100	13.1	59	51.3	97.0
Bread 375, salmon 92	0	3	100	13.5	47	15.5	
Renal Bile Fistula Operation							
Bread 375, salmon 125	0	4	92	13.4	44	4.1	
Pig liver 300, bread 300	40	2	80	12.3	48	22.2	26.2
Bread 288, salmon 200	40	2	74	12.1	44	0	
Fe 40 mg., bread 225, salmon 200	70	2	100	12.0	50	15.7	21.6
Bread 225, salmon 175	70	2	91	11.9	46	3.2	
Bread 336, salmon 139	100	9	95	13.0	48	8.5	
Fe 40 mg., bread 350, salmon 150	100	2	90	13.2	54	17.4	18.1
Bread 350, salmon 150	100	2	95	12.9	54	8.7	
Apricots 100, br. 300, salm. 125	100	2	100	13.5	50	16.4	18.7
Bread 350, salmon 100	100	2	89	13.3	45	10.0	
Fe 400 mg., bread 300, salmon 200	50	2	78	12.8	66	46.0	59.1
Bread 225, salmon 200	91	3	97	12.6	51	6.0	
Pig liver 200, bread 263	80	2	77	11.2	57	26.1	51.6
Bread 225, salmon 200	80	3	88	10.7	41	6.8	
Bread 225, salmon 200	80	6	92	11.0	41	4.2	
Fe 40 mg., bread 300, salmon 175	115	2	100	11.7	50	16.5	27.7
Bread 300, salmon 150	125	2	96	12.0	43	5.8	

Clinical History.—Table 1. Dog 32-1. Adult male coach. Born Apr., 1932. Raised on beef muscle diet from weaning until 1 year of age. Experimental anemia from Apr., 1933, to Feb., 1934. Routine anemia experiments. Dog returned to normal blood hemoglobin level for bile fistula operation.

May 31, 1934. Renal bile fistula operation. Uneventful recovery.

Sept. 6, 1934. Hemoglobin reserve again exhausted. Continuous experimental anemia until June 15, 1936. During entire anemia period 40 to 125 cc. dog bile and 20 to 70 cc. ox bile daily were added to food. Sodium taurocholate 1 to 2 gm. by mouth administered intermittently. Clotting time of fresh plasma was determined weekly.

Mar. 27, 1935. Food consumption began to drop during a liver feeding test (not included in Table 1) for a 3 week period ranging from 53 per cent to 65 per cent on the average per week. Bile was given by stomach tube. Dog was put on kennel diet.

Apr. 15. Trace of bile pigment in plasma. Eye grounds slightly yellow tint Probable obstruction by precipitates in renal pelvis.

Apr. 16. Urine: Bile pigment for 24 hrs. 32 mg.

Apr. 20. Urine: Bile pigment for 24 hrs. 22 mg.

Apr. 26. Urine: Bile pigment for 24 hrs. 88 mg.

Apr. 29. Food consumption increased to 83 per cent average for the week. Kennel diet gradually replaced by salmon bread diet.

June 18. Complete salmon bread diet. Dog was kept anemic by blood removal during entire period.

July 23. Restandardization for hemoglobin regeneration begun. Anemia history uneventful until June 17, 1936.

June 18, 1936. Left half of food but animal is active and lively during morning. Death within 2 hours.

Autopsy showed peritoneal cavity filled with unclotted blood. Pelvic tissues posterior to bladder are infiltrated with fresh blood.

Heart shows nothing of interest. *Lungs* show rather extensive patches of *pneumonia.* Much of this change is interstitial with the exudate of wandering cells in the alveolar walls. Some alveoli do contain exudate of red and white cells. It is remarkable that this pneumonia caused minimal clinical disturbance. Related to the pneumonia we believe is the peritoneal hemorrhage and acute inflammation of the bladder wall and pelvic tissues. Bladder mucosa normal. The exudate here is largely polymorphonuclear cells with much fresh blood. The dog probably had some tendency to bleeding because of the long standing renal fistula but the acute inflammation was an important factor. *Spleen* normal in gross. Histological sections show megakaryocytes and marrow cells in the spleen pulp (marrow metaplasia) and phagocytes filled with fine granules of a yellow pigment (6) like that observed in the muscle coats of the small intestine and in the pancreas. *Liver* is practically normal in gross and in histological sections. The

common duct is occluded and the gall bladder is not distended but opens freely into the renal pelvis. There are no calculi. The renal pelvis shows many mononuclears in the stroma just below the epithelial covering which is normal and intact. *Kidneys* are normal but for the scars related to the fistula tract. Histological sections show normal structures. Gastro-intestinal tract is normal except for brown pigmentation of the muscle coats of the small intestine. Histological sections show a conspicuous deposit of fine grains of yellow pigment (6) within the smooth muscle cells. Pancreas normal in all respects but for a deposit of similar pigment in phagocytes and acinar epithelium.

Bones show no osteomalacia and a normal structure. Fat marrow is abundant in ulnae, radii and tibiae. The humeri and femora show a brick red cellular hyperplastic marrow as do the ribs and vertebrae. Histological sections show normal marrow cells and the usual picture of marrow hyperplasia in long continued anemia (7).

Iron analyses of various tissues show the expected low values found in long continued anemia (2), liver 1.5 mg. per cent, spleen 4 mg. per cent, kidney 2.5 mg. per cent, ribs 2 mg. per cent, vertebrae 4.5 mg. per cent.

Dog 32-2 (Table 2) is a very satisfactory animal—a litter mate of dog 32-1 above. This dog remained active and healthy for 3 years after the fistula operation. A mixture of dog and ox bile was given daily in liberal amounts. This dog since the fistula operation produced about one-half as much hemoglobin on standard diets as during the normal control fore period. It is to be noted especially that food consumption at all times is 100 per cent and the loss of weight during the greater part of this period is less than 1 kilo. All these factors make this almost a perfect experiment and the values recorded deserve especial emphasis.

Colloidal iron given intravenously (Table 2–continued) shows a return of 86 gm. hemoglobin compared with the theoretical return of 88 gm. assuming that all the injected iron reappeared in the new formed hemoglobin. In other words this dog uses iron by vein just like a normal dog but produces much less than normal when the iron is fed. This is strong evidence that the absorption of iron is seriously disturbed. A similar experiment is recorded in Table 3–continued.

The last liver feeding experiment (Table 2–continued) shows a large intake of bile spread over 12 hours in an attempt to show whether this would modify the output of new hemoglobin. In this

experiment the new hemoglobin produced is 53.6 gm. which is more than the preceding liver experiment, 38 gm., but about the same as the experiment just after the production of the bile fistula, 57.2 gm. Other experiments (Table 3) of similar type are negative.

A liver-iron mixture (Lextron) described under Methods gives very high values before the bile fistula operation, 129.1 gm. hemoglobin, but relatively low values after the operation, 39 gm. hemoglobin.

Clinical History.—Table 2. Dog 32-2. Adult female coach mongrel. Born Apr., 1932. Raised on liver diet from weaning until 1 year of age. Experimental anemia from Apr., 1933, to Feb., 1934. Routine anemia experiments (Table 2). Dog returned to normal blood hemoglobin level for bile fistula operation.

June 4, 1934. Renal bile fistula operation. Uneventful recovery. Kennel diet.

Sept. 6, 1934. Hemoglobin reserve exhausted. Experimental anemia continuous. Salmon bread diet. During entire anemic period 25 to 70 cc. ox bile and 20 to 100 cc. dog bile were added to food. Sodium taurocholate 1 gm. by mouth administered intermittently. Clotting time of fresh plasma was determined weekly.

Jan. 30, 1935. Restandardization for blood regeneration begun (Table 2).

Uneventful anemia history from Jan., 1935, to Apr., 1937. Weight uniform and food consumption 100 per cent. During April the dog lost weight and became clinically sick. This period is not included in Table 2. Death Apr. 23, 1937.

Autopsy, Dog 32-2. The general picture is much like that of the other bile fistula dogs. The pigmentation of the liver, pancreas and intestine is well marked. Heart is normal. Lungs show edema and bronchopneumonia of moderate grade. Spleen normal in gross. There is little or no marrow metaplasia in spite of the long anemia history.

Gastro-intestinal tract is normal but for pigmentation (6). The bile is completely excluded from the intestinal tract. Pancreas is normal but for moderate pigmentation.

Liver is not abnormal in gross but sections show a widespread acute *cholangitis* with many wandering cells in the small bile ducts and adjacent stroma. The larger bile ducts are slightly dilated but there are no calculi. The bile fistula opens freely into the renal pelvis. Urinary *bladder* contains blood clots, shows hemorrhages and tiny miliary abscesses in its wall—a *severe cystitis* is probably the cause of the recent ascending cholangitis.

Bones show the usual picture of marrow hyperplasia and there is no osteomalacia.

TABLE 2

Hemoglobin Production in Anemia Depressed by Bile Fistula

Dog. 32-2. Coach, female, adult.

Diet, daily average intake	Bile given daily	Experimental period	Food consumed av.	Weight av.	Blood Hb. level av.	Hb. removed per wk. av.	Total net Hb. output 2 wks.
gm.	*cc.*	*wks.*	*per cent*	*kg.*	*per cent*	*gm.*	*gm.*
Bread 320, salmon 96	0	6	100	14.1	48	2.0	
Apricots 75, br. 200, salm. 100	0	2	100	13.7	53	28.8	57.6
Bread 263, salmon 100	0	2	100	14.0	43	4.0	
Fe 40 mg., bread 275, salmon 100	0	2	100	14.0	57	36.6	71.1
Bread 300, salmon 100	0	2	96	13.8	52	3.0	
Pig liver 300, bread 350	0	2	100	14.0	60	44.5	102.0
Bread 367, salmon 100	0	3	100	14.2	46	7.7	
Lextron, bread 375, salmon 75	0	2	100	14.1	63	51.1	129.1
Bread 400, salmon 75	0	2	100	14.2	49	17.5	
Renal Bile Fistula Operation							
Bread 450, salmon 75	70	4	100	14.5	44	4.2	
Fe 40 mg., bread 450, salmon 75	70	2	100	14.4	56	23.0	53.9
Bread 450, salmon 75	65	2	100	14.2	44	12.2	
Pig liver 300, bread 300	70	2	100	13.8	50	29.7	57.2
Bread 375, salmon 75	70	3	100	14.0	47	6.1	
Bread 400, salmon 75	70	14	100	13.6	47	10.0	
Lextron, bread 400, salmon 75	70	2	100	13.4	56	28.2	39.0
Bread 400, salmon 75	70	3	100	13.4	51	13.6	
Apricots 100, br. 337, salm. 75	70	2	100	13.3	55	20.7	20.3
Bread 400, salmon 75	70	2	100	13.0	46	9.5	
Fe 400 mg., bread 400, salmon 75	100	2	100	13.1	53	31.9	47.7
Bread 400, salmon 75	70	3	100	12.9	50	17.0	
Bread 400, salmon 75	70	3	100	12.4	43	7.4	
Pig liver 300, bread 350	70	2	100	12.5	51	26.1	33.5
Bread 400, salmon 75	70	3	100	12.4	43	7.4	
Bread 450, salmon 100	100	5	100	12.2	49	8.6	
Fe 21 mg.,* bread 400, salmon 75	70	2	100	12.3	54	37.4	86.0
Bread 400, salmon 75	70	3	100	12.0	54	18.1	

* Iron given intravenously—86 gm. Hb. is 97 per cent of theoretical return.

TABLE 2—*Continued*

Dog 32-2 after renal bile fistula operation.

Diet, daily average intake	Bile given daily	Experimental period	Food consumed av.	Weight av.	Blood Hb. level av.	Hb. removed per wk. av.	Total net Hb. output 2 wks.
gm.	*cc.*	*wks.*	*per cent*	*kg.*	*per cent*	*gm.*	*gm.*
Beef muscle 300, bread 313	150	2	100	12.6	54	26.4	17.0
Bread 400, salmon 100	150	3	100	12.7	45	2.4	
Bread 400, salmon 100	150	5	100	13.3	47	3.7	
Beef heart 300, bread 250	150	2	99	13.2	44	15.9	28.9
Bread 400, salmon 100	150	3	100	13.3	45	5.2	
Pig kidney 300, bread 350	150	2	100	13.0	44	28.5	41.0
Bread 400, salmon 100	150	3	100	13.4	42	2.9	
Bread 400, salmon 100	150	10	100	13.2	46	5.2	
Pig liver 300, bread 325	150	2	99	12.8	54	27.9	38.0
Bread 400, salmon 100	150	2	99	13.2	45	1.3	
Fe 40 mg., bread 400, salmon 100	150	2	100	13.1	50	19.1	41.0
Bread 400, salmon 100	150	2	100	13.3	47	11.3	
Bread 410, salmon 95	150	13	99	12.9	46	4.3	
Pig liver 300, bread 325	†200	2	92	11.7	53	31.1	53.6
Bread 450, salmon 75	†200	3	97	11.5	44	1.3	

† 50 cc. bile given at 9 a.m., 12 m., 4 and 8 p.m.

Dog 29-66 (Tables 3 and 3–continued) carried a renal bile fistula for 4 years and 5 months. During almost all of this period the dog was in a normal state of health, weight equilibrium and activity. Food consumption was practically 100 per cent and for about 4 years the weight fluctuations were no greater than those observed in the standard anemia dogs on this identical régime. The fore period gives a complete control base line to show the hemoglobin production in anemia on standard diets before the bile fistula operation. This experiment then is almost perfect and gives in much detail the effect of the bile fistula upon the output of new hemoglobin in anemia. On the average this dog showed but 43 per cent as much capacity to

TABLE 3

Hemoglobin Production in Anemia Depressed by Bile Fistula

Dog 29-66. Bull terrier, male, adult.

Diet, daily average intake	Bile given daily	Experimental period	Food consumed av.	Weight av.	Blood Hb. level av.	Hb. removed per wk. av.	Total net Hb. output 2 wks.
gm.	*cc.*	*wks.*	*per cent*	*kg.*	*per cent*	*gm.*	*gm.*
Bread 400, salmon 75	0	6	100	15.3	49	2.0	
Fe 40 mg., bread 400, salmon 75	0	2	100	14.9	54	26.3	52.0
Bread 400, salmon 75	0	2	100	15.0	47	3.8	
Pig liver 300, bread 350	0	2	100	15.6	58	45.5	104.7
Bread 400, salmon 75	0	3	100	15.7	46	7.2	
Apricots 100, bread 350, salmon 75	0	2	100	16.0	50	17.0	46.7
Bread 400, salmon 75	0	2	96	16.2	51	10.3	
Bread 400, salmon 82	0	10	99	16.2	46	11.0	
Bread 383, salmon 125	0	6	99	16.6	49	8.6	
Fe 40 mg., bread 375, salmon 125	0	2	100	16.3	55	28.9	38.0
Bread 375, salmon 125	0	2	100	16.2	41	7.3	
Pig liver 300, bread 275	0	2	95	15.7	65	45.6	70.2
Bread 400, salmon 125	0	3	100	15.6	51	7.4	
Dog liver 300, bread 280	0	2	100	15.4	61	46.0	99.0
Bread 400, salmon 100	0	3	100	15.3	49	17.0	
Renal Bile Fistula Operation							
Bread 425, salmon 115	50	11	99	14.3	47	7.0	
Apricots 100, bread 350, salmon 150	50	2	100	14.6	51	15.1	28.6
Bread 400, salmon 75	50	2	100	14.5	53	13.1	
Fe 40 mg., bread 400, salmon 75	50	2	100	14.7	49	19.6	21.7
Bread 400, salmon 75	50	2	100	14.2	45	5.2	
Pig liver 300, bread 350	50	2	100	13.7	56	23.8	34.4
Bread 450, salmon 75	50	3	96	14.4	45	7.2	
Pig kidney 300, bread 375	40	2	100	13.8	44	11.4	30.1
Bread 400, salmon 130	45	3	97	13.9	48	14.1	
Pig spleen 300, bread 300	40	2	100	13.1	59	25.0	20.6
Bread 375, salmon 125	40	2	100	13.0	50	0	

TABLE 3—*Continued*

Dog 29-66 after renal bile fistula operation.

Diet, daily average intake	Bile given daily	Experimental period	Food consumed av.	Weight av.	Blood Hb. level av.	Hb. removed per wk. av.	Total net Hb. output 2 wks.
gm.	*cc.*	*wks.*	*per cent*	*kg.*	*per cent*	*gm.*	*gm.*
Pig liver 300, Fe 40 mg., bread 325	40	2	100	12.2	50	21.5	41.0
Bread 375, salmon 125	60	3	100	13.4	48	11.0	
Pig liver 300, bread 335	80	2	94	13.4	61	21.7	39.2
Bread 450, salmon 75	70	3	100	13.8	48	10.3	
Fe 40 mg., bread 450, salmon 75	70	2	99	14.2	48	16.8	12.2
Bread 450, salmon 75	70	2	100	14.3	50	15.9	
Bread 470, salmon 75 (no bile)	0	10	100	14.6	48	12.4	
Bread 475, salmon 75	100	11	100	15.5	48	14.2	
Fe 400 mg., bread 450, salmon 100	100	2	98	16.5	63	43.4	47.2
Bread 475, salmon 75	100	3	100	16.8	48	8.5	
Fe 19 mg.,* bread 400, salmon 100	75	2	100	14.8	60	39.5	69.0
Bread 400, salmon 100	75	3	100	14.8	49	16.5	
Pig spleen 250, bread 350	125	3	76	14.3	63	24.1	0
Bread 360, salmon 125	125	3	100	14.6	44	0	
Beef heart 300, bread 350	150	2	87	14.9	57	36.7	13.1
Bread 375, salmon 100	125	3	78	14.1	49	0	
Bread 294, salmon 100	150	10	100	13.8	46	9.7	
Pig liver 300, bread 238	150	2	90	13.3	70	43.0	48.5
Bread 250, salmon 150	125	3	93	12.7	45	2.6	
Fe 400 mg., bread 275, salmon 125	150	2	100	13.1	51	34.3	58.2
Bread 300, salmon 125	150	3	100	13.0	42	12.7	
Fe 400 mg., bread 275, salmon 125	0	2	100	12.7	64	42.8	57.0
Bread 300, salmon 125	0	3	100	12.5	46	7.0	
Fe 19 mg.,† bread 375, salmon 125	0	2	100	13.2	57	31.3	86.0
Bread 375, salmon 125	0	3	100	13.2	58	24.4	
Pig liver 300, bread 275	‡200	2	90	12.2	68	42.4	77.0
Bread 315, salmon 130	‡200	3	96	12.7	47	14.0	

* Iron given intravenously—69 gm. Hb. is 80 per cent of theoretical return.

† Iron given intravenously—86 gm. Hb. is 98 per cent of theoretical return.

‡ 50 cc. bile given at 9 a.m., 12 m., 4 and 8 p.m.

form hemoglobin in anemia after the fistula operation in comparison to pre-operative periods (Table 6).

This dog shows interesting figures during long periods of bread feeding with and without bile. Table 3–continued during a bread feeding period of 10 weeks *without any bile* shows an average weekly output of 12.4 gm. hemoglobin. The period of 11 weeks immediately following with the same bread diet and food intake *plus* 100 cc. bile and 2 gm. of sodium taurocholate daily indicates an average weekly output of 14.2 gm. hemoglobin. Some months later (Table 3–continued) during a 10 weeks' diet period of the same character *plus* 150 cc. of bile, the dog shows an average weekly output of 9.7 gm. hemoglobin. During another 11 week period shortly after the bile fistula operation (Table 3) with a similar diet *plus* 50 cc. bile, the dog shows an average weekly output of 7.0 gm. hemoglobin. It is obvious therefore that the actual presence of bile in the food given daily does not appreciably modify the hemoglobin production of a dog on the standard bread and salmon diet.

When bile in large amounts is given by mouth during the day time (9 a.m., 12 m., 4 and 8 p.m.) during standard liver feeding experiments (Table 3–continued) we note some increase in this dog (77 gm. hemoglobin total net output) as compared with many earlier feeding experiments (48.5—39.2—41.0—34.4 gm. hemoglobin). In another experiment (Table 2) another dog shows little if any increase in hemoglobin production under similar conditions of bile feeding.

Iron given by *mouth* (Table 3–continued) whether with or without bile shows the same output of new hemoglobin per week—58.2 and 57.0 gm., respectively. These figures are approximately two-thirds the normal average return of 90 gm. hemoglobin in the anemic control dog (see Table 6).

Iron given by *vein* (Table 3–continued) gives 80 and 98 per cent of the theoretical return if all the injected Fe is returned quantitatively within new hemoglobin.

Clinical History.—Table 3. Dog 29-66. Adult male bull terrier. Born Sept. 7, 1928. Experimental anemia from Sept., 1930, to Feb., 1932. Routine anemia experiments (Table 3). Dog returned to normal blood hemoglobin level for bile fistula operation.

Apr. 18, 1933. Renal bile fistula operation. Uneventful recovery.

May 16, 1933. Hemoglobin reserve exhausted. Continuous experimental anemia.

Aug. 16, 1933. Restandardization for hemoglobin regeneration begun (Table 3). 40 to 50 cc. dog bile and 10 to 25 cc. ox bile were added to daily food until Oct. 24, 1934. Sodium taurocholate 1 to 2 gm. by mouth administered intermittently. Clotting time of fresh plasma was determined weekly.

May 24, 1934. Slight bleeding from puncture wound in jugular vein. Dog bile by mouth increased to 100 cc.

Oct. 24, 1934, to Dec. 20, 1934. All bile and sodium taurocholate omitted from food (Table 3–continued).

Dec. 20, 1935. Slight bleeding from puncture wound in jugular vein during night. 15 to 20 cc. blood lost. Bile by mouth 100 cc. daily. Transfusion of 45 cc. normal blood. Bleeding stopped. Uneventful anemia history to Aug., 1937.

The few abnormal periods do not appear in the Tables 3 and 3–continued which record the reactions to various diet periods when the dog was clinically normal. Some experiments are excluded from Table 3 for lack of space but net total figures of all experiments appear in Summary Table 6. During August and September, 1937, dog began to fail. Vomiting was noted on several occasions and there was loss of weight. It was decided to terminate the experiment and the dog was killed with ether Sept. 20, 1937.

Autopsy done at once. Pigmentation of the intestine and pancreas is conspicuous and the intestines present a deep buff color. *Heart* and *lungs* normal in gross and in histological sections. *Spleen* normal in gross and shows some marrow metaplasia in sections. *Liver* normal in gross, bile ducts in liver clear. Bile was excluded from the duodenum. Sections show clear bile ducts and canaliculi. Liver cells are normal but contain a good deal of lipochrome pigment. *Gall bladder* contains one large gall stone consisting largely of pigment and soft coagulum measuring $1\frac{1}{4}$ cm. in diameter. Two smaller similar stones 2 to 3 mm. in diameter are found in the renal pelvis continuous with the gall bladder. Gall bladder and renal pelvis in sections show evidence of chronic inflammation but no ulcers and no acute inflammation. Gastro-intestinal tract normal but for the deep pigmentation noted also in sections (6). *Kidneys* except for the operative scars about the bile fistula are normal in gross and in histological sections. Urinary *bladder* shows a cystitis cystica with some associated chronic and acute inflammation obvious in sections. Pancreas normal except for pigmentation (6). Thyroid shows scattered adenomas.

Bones: All bones are of normal hardness. The cellular marrow in general presents the usual picture found in these anemic dogs (7) as to hyperplasia and cell detail in gross and in histological sections.

Table 4 (dog 26-19) adds many points of interest to the general discussion of the long standing renal bile fistula. This was one of the first renal bile fistulas in our group and much was learned from this

TABLE 4

Hemoglobin Production Much below Normal Due to a Bile Fistula

Dog 26-19. Bull terrier, male, adult.

Diet, daily average intake	Bile given daily	Experimental period	Food consumed av.	Weight av.	Blood Hb. level av.	Hb. removed per wk. av.	Total net Hb. output 2 wks.
gm.	*cc.*	*wks.*	*per cent*	*kg.*	*per cent*	*gm.*	*gm.*
Bread 450, salmon 100	0	2	84	14.1	42	1.6	
Spinach 110, bread 283, salmon 100	0	3	80	13.1	41	14.8	
Bread 363, salmon 50, meat 50	0	4	100	14.4	47	8.8	
Peas 100, bread 325, salmon 50	0	2	99	13.4	52	13.5	17.5
Bread 385, salmon 50	0	2	100	13.4	45	12.9	
Spinach 150, salmon 50, meat 50	0	2	94	13.5	46	27.2	34.2
Bread 400, salmon 100	0	1	89	13.5	43	3.2	
Cabbage 125, bread 350, salmon 100	0	2	100	13.8	44	10.0	2.5
Bread 425, salmon 100	0	2	100	13.9	44	8.9	
Bread 394, salmon 100	40	4	94	14.2	41	3.9	
Beef kidney 160, bread 291	75	3	95	13.4	51	8.6	28.6
Bread 350, salmon 100	75	3	100	14.2	46	8.7	
Liver extract,* bread 325, salmon 100	50	2	100	14.8	43	7.0	11.3
Bread 325, salmon 100	40	2	100	14.9	46	6.5	
Fe 25 mg., bread 325, salmon 100	40	4	100	15.0	52	14.3	24.1
Bread 325, salmon 100	40	3	100	15.0	48	6.1	
Pig liver 300, bread 300	40	2	98	13.7	60	27.2	26.0
Bread 400, salmon 100	40	3	99	13.8	46	0	
Beef muscle 375, bread 300	40	2	100	14.0	61	29.0	53.1
Bread 400, salmon 100	40	3	100	14.6	51	7.7	
†Salt mixture 6, bread 400, salm. 100	40	2	100	14.4	47	7.0	0
Bread 400, salmon 100	40	2	100	14.8	46	6.5	
Salt mixture 6 + Fe 38 mg., br. 400, salm. 100	40	2	100	15.2	51	16.5	34.2
Bread 400, salmon 100	40	2	100	15.3	47	14.2	
Fe 40 mg., bread 400, salmon 100	40	2	100	14.9	52	25.2	43.6
Bread 400, salmon 100	40	2	100	15.2	41	10.3	

* Liver fraction potent in pernicious anemia—given in equivalent for 500 gm. liver daily.

† Salt mixture (McCollum and Simmonds (10)) without iron 6 gm. daily.

dog about the factors of bleeding and osteomalacia which may be troublesome.

The clinical history of dog 26-19 shows many periods of bleeding and delayed blood coagulation which necessitated some transfusions. It was found that calcium salts by mouth or subcutaneously had no influence upon this abnormality of blood coagulation. The feeding of liver or kidney in the amounts given had no significant effect upon the bleeding. Bile salt alone and ergosterol did not cure the bleeding but whole bile did hold it in check when given by mouth daily. As bile was not given in sufficient amounts this dog did continue to show bleeding tendencies and a moderate grade of osteomalacia.

The true hemoglobin regenerative power of this dog was not adequately tested but we see that the reaction to liver feeding was only about one-fourth of normal. The reaction to iron feeding was much more favorable but not adequately tested. Periods of bleeding and related transfusions interrupted and spoiled many experiments not listed in Table 4.

Autopsy shows the characteristic findings. The brown pigmentation of the intestinal muscle coats, pancreas, lymph glands, spleen and liver is well shown. The fistula shows two small calculi and a little chronic inflammation of the submucosa of the renal pelvis but the bile ducts, bile canaliculi and liver cells are quite normal. The bone marrow shows the usual hyperplasia due to long continued anemia (7) but is normal. The ribs show a moderate grade of osteomalacia. Detailed autopsy findings are very like those recorded in other dogs above.

Bile pigment excretion in the urine was followed daily for 8 months. The daily output was quite uniform month by month and averaged for the total period 29 mg. per 24 hours. The bearing of this observation on pigment metabolism is discussed below.

Clinical History.—Table 4. Dog 26-19. Adult male bull terrier. Born Sept., 1925.

Dec. 13, 1926. Renal bile fistula operation. Uneventful recovery.

Jan. 8, 1927, to July 8, 1929. Experimental anemia. Routine anemia experiments. No bile added to food during the first 20 weeks of the anemia period.

July 25, 1927. Spontaneous bleeding from venous puncture of jugular vein. Two transfusions. Calcium lactate 2 per cent solution 30 cc. subcutaneously and 3 gm. added to diet.

July 26. Bleeding continues. Calcium chloride 15 gm. given by stomach tube.

July 27. Bleeding continues. Transfusion 100 cc. normal blood. Calcium chloride 15 gm. by stomach tube. Liver 100 gm. added to diet. Food consumption good.

July 28. No bleeding, liver diet continued.

Aug. 11 and 12. Ox bile 50 cc. by stomach tube. Salmon bread diet. Calcium chloride given intermittently.

Nov. 21. Slight bleeding from venous puncture wound. Diet contains 150 gm. liver.

Nov. 28. Spontaneous bleeding from venous puncture wound. Transfusion 50 cc. normal blood. Liver increased to 300 gm. daily.

Dec. 1. Slight bleeding from venous puncture wound. Dog bile 50 cc. given by stomach tube. Bleeding continues. Transfusion 120 cc. normal blood. Beef kidney diet instead of liver.

Dec. 5 to 21. Dog bile 75 cc. added to food daily. Salmon bread diet.

Jan. 10, 1928. Sodium taurocholate 1 gm. added to daily diet.

Jan. 16. Bile omitted from diet.

Jan. 18. Dog bled about 100 cc. during the night. Sodium taurocholate omitted from diet. Dog bile 75 cc. given by stomach tube. Transfusion 130 cc. normal blood.

Jan. 19. Dog bile 50 cc. added to daily diet.

Feb. 7. Irradiated ergosterol 4 mg. daily added to food. Bile omitted.

Feb. 17. Spontaneous bleeding from venous puncture wound. Transfusion 110 cc. normal blood. Dog bile 50 cc. and calcium chloride 10 gm. by stomach tube.

Feb. 21. Dog bile 40 cc. daily added to food.

Uneventful anemia history until July 26, 1928.

July 26, 1928. Convulsions, dog appears weak—hemoglobin is 51 per cent. Transfusion 100 cc. normal blood a.m. and p.m.

July 30. Condition improved.

Dec. 7, 1927, to Aug. 20, 1928. Bile pigment output in urine per 24 hour period average. On the few occasions when transfusions were given the bile pigment figures for the 4 days subsequent to the transfusion were omitted from the averages as listed.

Dec. 7, 1927	to	Jan. 7, 1928	18 mg.
Jan. 12, 1928	"	Feb. 12	27 "
Feb. 15	"	Mar. 15	24 "
Mar. 16	"	Apr. 16	33 "
Apr. 18	"	May 18	28 "
May 18	"	June 18	31 "
June 20	"	July 20	35 "
July 20	"	Aug. 20, 1928	34 "
Average 9 months			29 mg. per 24 hours

June 3, 1929. Slight bleeding from venous puncture. Bile 60 cc. added to daily diet.

June 18. Bleeding from venous puncture wound. Bile 80 cc. added to daily diet. Transfusion 105 cc. normal blood.

June 25. Bile intake increased to 90 cc.

TABLE 5

Hemoglobin Production below Normal Due to a Bile Fistula
No Bile Given at Any Time

Dog 24-9. Beagle mongrel, female, adult.

Diet, daily average intake	Experimental period	Food consumed av.	Weight av.	Blood Hb. level av.	Hb. removed per wk. av.	Total net Hb. output 2 wks.
gm.	*wks.*	*per cent*	*kg.*	*per cent*	*gm.*	*gm.*
Bread 258, salmon 90	12	97	8.0	43	3.4	
Cabbage 115, bread 300, salmon 75	2	100	8.0	50	10.8	8.0
Bread 285, salmon 75	2	98	8.1	44	0	
Fe 6 mg., bread 275, salmon 75	2	92	8.1	42	4.3	3.6
Bread 265, salmon 100	2	94	8.2	39	4.8	
Pig liver 150, bread 200	2	89	7.8	47	13.4	22.7
Bread 275, salmon 100	2	87	8.3	46	4.8	
Pig kidney 100, bread 265	2	93	7.8	44	5.9	7.7
Bread 225, salmon 75	2	100	7.9	47	4.8	
Apples 65, bread 225	2	96	7.4	44	6.1	8.6
Bread 225, salmon 75	2	100	7.6	48	7.1	
*Hemoglobin 1.1, bread 225, salmon 75	2	100	7.5	40	0	0
Bread 250, salmon 75	2	100	7.9	47	6.9	
Liver sausage 150, bread 200	2	100	7.8	51	16.6	27.0
Bread 250, salmon 75	3	100	8.0	45	3.6	

* Hemoglobin 15.2 gm. given intravenously during 2 weeks.

July 8. Considerable spontaneous bleeding from venous puncture wound. Transfusion 120 cc. normal blood. The duration of this fistula was 2 years, 7 months. Death July 9.

Dog 24-9 (Table 5) is the first of our renal bile fistula experiments and is of special interest because the dog received no bile nor bile salts at any time and was given no blood transfusions. The dog

lived a long time considering the absence of bile feeding—a period of about 15 months. The anemia period lasted 10 months and we note the reaction to liver and kidney feeding was one-half normal hemoglobin production or less. It should be noted that this dog was much smaller and of a totally different breed from that used in our standard anemia experiments. This dog was given 15 gm. hemoglobin by vein during a 2 weeks' period and showed no return in new red cell output. This is totally different from the usual reaction (4) and we have no explanation to offer.

Clinical History.—Table 5. Dog 24-9. Adult female beagle mongrel. Renal bile fistula operation done by Dr. Morton approximately Apr. 1, 1926. The dog was used by Dr. Sperry for studies in lipid excretion (8).

Sept. 25, 1926, to July 20, 1927. Experimental anemia. Routine anemia experiments. No bile nor bile salts given at any time. No transfusions.

June 2, 1927. Moderate bleeding during night from venous puncture. Stopped spontaneously.

June 22. Slight bleeding from venous puncture wound.

July 6. Slight bleeding from venous puncture wound.

July 21. Marked bleeding from two venous puncture wounds. Hematoma formed.

July 22. Slight oozing of blood still noticeable. X-ray films of lower extremities and pelvis indicate normal bone findings. Blood calcium 9 mg. per 100 cc. blood. Dog was given ether and killed. Blood clotted very slowly—40 min. No jaundice.

July 22, 1927. *Autopsy* done at once. Heart and lungs normal. *Spleen* normal in gross. Histological sections show some marrow metaplasia with many megakaryocytes in spleen pulp. Fine granules of a yellow pigment are abundant in phagocytes. Lymph glands show much of the same pigment within phagocytes in the gland pulp. *Liver* is normal in gross. Histological sections show a few granules of yellow pigment in the liver cells. A few mononuclear cells are found in the periportal stroma. The liver is essentially normal. *Fistula* is patent and the common duct occluded. There are a few silk sutures in the wall of the gall bladder and they are crusted with salts and pigment. The gall bladder wall is slightly thickened and the renal pelvis normal in gross. Histological sections show an essentially normal gall bladder mucosa. An occasional mononuclear cell is seen in the wall of the gall bladder and right renal pelvis. Small renal scars adjacent to the fistula are noted. *Kidneys:* Left kidney is slightly hypertrophied but otherwise normal. Right kidney shows some atrophy and scars. There are casts in the tubules close to the scars related to the fistula. In general the histological sections show normal glomeruli and tubules. Small *intestine* shows a deep

brown pigmentation of its muscle coats but is otherwise normal. Histological sections show the usual fine granules of yellow pigment (6). *Pancreas* normal except for the same pigmentation and deposit of yellow granules in acinar cells and phagocytes. Bladder and pelvic organs normal.

Bones show no osteomalacia and present the usual hard structures to the knife or saw. The red marrow is abundant and cellular showing more than average hyperplasia. Fat marrow is observed only in the extremities of radii, ulnae and tibiae. Elsewhere the bones show a deep red hyperplastic marrow. Histological sections show the usual marrow hyperplasia due to long continued anemia (7). The marrow cells appear to be normal in all respects.

TABLE 6—*Summary*

Hemoglobin Production before and after the Bile Fistula Operation
Net Hemoglobin in Grams per 2 Week Period

Diet	Average for standard anemic dog	Dog 32-1		Dog 32-2		Dog 29-66		Dog 26-19	Dog 24-9
		Before operat.	After operat.	Before	After	Before	After	After	After
Apricots	45	34	19	58	20	47	29		
Fe 40 mg.	50	69	22, 18	71	54, 41	52, 38	22, 12	44	
			28				21		
Fe 400 mg.	90		59		48		47, 58		
							57		
Beef heart	45				29		13		
Pig kidney	75				41		30		23
Pig spleen	85						21, 0		
Pig liver	102	97	26, 78	102	54, 34	105	34, 39	26	46
							77, 49		
					38, 57	99, 70	58, 49		
Average of normal, *per cent*.			52		50		43		

DISCUSSION

The bile pigment output of dog 26-19 (clinical history) during a period of 8 months averaged 29 mg. per 24 hours. This figure is somewhat lower than the average of other dogs given elsewhere (4). Some work soon to be published will give evidence that in these bile fistula dogs the life cycle of the red cells approximates 120 days (a destruction of 0.83 per cent of the circulating hemoglobin per day). Therefore in this dog it is of interest to speculate about the pyrrol

metabolism as related to hemoglobin destruction and bile pigment production. This dog's plasma volume varied from 810 to 960 cc. during this period—total blood volume from 1100 to 1250 cc. If we take the blood volume as 1100 cc. and the hemoglobin level at 50 per cent, the dog had 75.9 gm. hemoglobin in circulation. With a destruction of 0.83 per cent a day (or 0.65 gm. hemoglobin) if quantitatively changed to bile pigment (1 gm. hemoglobin = 40 mg. bile pigment) we should expect 26 mg. bile pigment per 24 hours. Likewise with a blood volume of 1200 cc. we should expect an output of 28 mg. bile pigment per 24 hours. The actual average value for this dog is 29 mg. bile pigment per 24 hours.

We must not forget the probability that the wear and tear of *muscle hemoglobin* will yield some bile pigment as muscle hemoglobin introduced into the blood promptly appears as bile pigment (9). This figure for the normal wear and tear of muscle hemoglobin is an unknown. It may even be argued that the muscle hemoglobin as a part of the matrix of the striated muscles is repaired *in situ* without any loss of the pigment radicle by waste—a rather unusual reaction in body metabolism. One may choose to believe that the slight difference between the expected bile pigment output per 24 hours of 26 to 28 mg. and the actual measured output of 29 mg. bile pigment represents a contribution from the wastage of muscle hemoglobin.

Autopsy findings in the dogs reported in this paper and others reported elsewhere (5) deserve some emphasis as they give convincing evidence that the bone marrow is structurally normal. The liver lobules are normal and there is no evidence for long continued infection or bile stasis within the biliary system of ducts. The spleen may show a little marrow metaplasia as is seen frequently in the anemia colony without fistula and reported elsewhere (7). The gastro-intestinal tract is normal histologically. Yet in spite of this normal structural picture this body machine with an established bile fistula can form only about one-half normal amounts of new hemoglobin under the stress of severe anemia (refer to Table 6).

Iron absorption obviously is seriously impaired in an anemic bile fistula dog which returns about one-half the expected hemoglobin as the result of standard doses of iron by mouth. *Iron given by vein* on the contrary will return the expected amount of new hemoglobin

assuming that the Fe is quantitatively utilized for incorporation in the hemoglobin molecule. Feeding of bile in large amounts does not correct this faulty absorption of the bile fistula dog. The continuous cycle of bile and bile salt secretion and absorption apparently is necessary for a normal absorption of iron salts from the intestinal tract.

Protein digestion and absorption obviously is much more complex than iron absorption and we must not jump to the conclusion that impaired absorption is solely responsible for the fact that the bile fistula dog cannot utilize liver to form hemoglobin in anemia as effectively as does the control non-fistula anemic dog.

A glance at the tables above shows that these fistula anemic dogs can be kept in satisfactory weight equilibrium for years just as is true for the anemic non-fistula control dogs. This means adequate protein metabolism and absorption of protein building stones from the intestinal tract. It is rather rash to say that a bile fistula dog can metabolize protein sufficient for all its protein needs in the body *except* for the manufacture of new hemoglobin—a selective absorption of protein building stones. Possibly one may argue that accelerated hemoglobin production in anemia subjects the building mechanism to the stress of overload in contrast to the normal body protein maintenance and therefore in this emergency (anemic bile fistula) the hemoglobin building mechanism shows partial failure in production.

We refer again to the bleeding tendency (3) noted in these bile fistula dogs which is not a matter of absorption but presumably a disturbed liver function related to the production of prothrombin. Liver feeding in these bile fistula anemic dogs produces only about one-half the expected amount of new hemoglobin found in non-fistula controls yet the other protein needs of these dogs are adequately met. Unfortunately we cannot test the absorption of protein and organic materials to produce hemoglobin as we can do in the case of iron. Taking all these various factors into consideration, we suspect that a disturbed liver function cannot be excluded—that the disturbed (bile fistula) liver is unable adequately to assemble the protein building materials which form the globin fraction of the large hemoglobin molecule.

SUMMARY

The presence of a renal bile fistula with escape of all bile into the urinary tract seriously impairs the capacity of an anemic dog to form new hemoglobin on standard diets.

These bile fistula dogs will produce about one-half as much hemoglobin in anemia on standard diets as during earlier control periods without a bile fistula.

Iron given by mouth to an anemic bile fistula dog will effect the production of about one-half the amount of new hemoglobin as in control periods.

Iron given by vein to an anemic bile fistula dog will approximate the theoretical 100 per cent return of new hemoglobin. Obviously absorption is a very important factor in the utilization of iron by these dogs.

The reaction to liver feeding is much like the reaction to iron feeding but we have no proof of inadequate protein digestion and absorption in these bile fistula dogs.

In fact the uniform body weight and normal clinical state over periods of years speak for adequate absorption of protein digestion products. Evidence cited above supports our belief that inadequate hemoglobin production (protein formation) noted in these bile fistula dogs may be related to a disturbed liver function.

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