SPLENECTOMY IN BILE FISTULA DOGS

BILE PIGMENT OVERPRODUCTION, ANEMIA AND INTOXICATION

By F. B. QUEEN, M.D., W. B. HAWKINS, M.D., and G. H. WHIPPLE, M.D.

(From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York)

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The essential features of the experiments tabulated below may be described in a few words. A *splenectomized* dog can be made anemic by bleeding and continued in this condition in our anemia colony for years and will remain in perfect health. Moreover the output of *hemoglobin* on various diets will be identical with that of a dog which retains his spleen and there is no icterus. A dog with a *bile fistula* opening into the renal pelvis will remain in perfect health, in weight equilibrium and without anemia or intoxication provided the diet intake is suitable. Such dogs in this laboratory have been under observation and live in health for several years.

When we *combine splenectomy with this bile fistula* we observe an unexpected physiological reaction. After a time (weeks or months) the dog begins to put out large surplus amounts of bile pigment and the hemoglobin falls but not in adequate measure to explain this large surplus of bile pigments which in some periods may reach 8–10 times control values. This large bile pigment excess output may appear in cycles of days or weeks with intermissions. Anemia may become severe and call for transfusions. Finally a tendency to hemorrhage may develop and cause death due to bleeding into the serous cavities, lungs or gastro-intestinal tract. There is inevitably a lethal outcome in weeks or months when one combines a bile fistula with splenectomy.

This is an intriguing riddle and its solution promises a better understanding of the complex internal metabolism of body pigments. That the spleen is essential to life in a bile fistula dog is not without interest and suggests some problematical contribution of the spleen to internal

metabolism. The bile salts may well be under suspicion as integrated in this reaction. It seemed obvious that a careful study of these cycles of pigment overproduction in splenectomized bile fistula animals would furnish valuable information related to the construction of pigments in the body.

Methods

The renal type of bile fistula was used as devised by Kapsinow, Engle and Harvey (3). This type of fistula has been utilized for several years in this laboratory and the care of these dogs with method of analysis of bile pigment has been described in detail elsewhere (5). We emphasize the fact that these dogs with bile flowing freely into the renal pelvis can be maintained in perfect health and weight equilibrium for years. It is necessary to give 50–75 cc. of bile daily, together with a balanced ration.

The spleen may be removed at the time of the bile fistula operation or subsequently. Dogs are kept in metal metabolism cages at all times and water is given by stomach tube about 3 hours before the 24-hour urinary collection is made. The dog usually empties the bladder between the water ingestion and urine collection which makes for uniformity in urine collection as obviously residual urine may give irregular values for daily bile pigment elimination. Catheterization would introduce infection and is never employed. Chloroform 5 cc. placed in the collection bottle acts as a preservative.

The dogs are weighed 3 times each week. Red cell, hematocrit and blood hemoglobin determinations are made at least once a week and often daily during periods of pigment overproduction and anemia (4). Occasional blood plasma volume determinations are done by the vital red method.

Diets are essentially the same in each experiment and consist of canned salmon, "Klim" (a commercial dried whole milk powder) and a bread prepared in this laboratory. Dog 29-353 received a daily diet of salmon bread 300 gm., canned salmon 50 gm., "Klim" 50 gm. and dog bile 50 cc. Dog 30-62 received a daily diet of salmon bread 400 gm., canned salmon 50 gm., "Klim" 30 gm. and ox bile 50 cc. Dog 31-73 received a daily diet of salmon bread 500 gm., canned salmon 100 gm., "Klim" 40 gm. and ox bile 50 cc. Water 400 cc. is added to the dried bread and the ingredients mixed into a mash. The bread is used in our anemia colony and is an adequate diet capable of maintaining dogs in health indefinitely. It contains wheat flour, bran, potato starch, canned salmon, sugar, cod liver oil, canned tomatoes, yeast and a salt mixture. Its preparation has been carefully described (7). On this diet the hemoglobin production of anemic healthy dogs has been carefully studied and is well understood. The obvious advantage of this diet in these bile fistula dogs for bile pigment study needs no comment. An output of 2–4 gm. of hemoglobin each week over and above the maintenance factor is to be expected on this diet.

The analysis of bile pigment in the urine is described elsewhere (5) and is done in duplicate.

EXPERIMENTAL OBSERVATIONS

Some years ago Hooper and Whipple (2) observed cycles of bile pigment overproduction in splenectomized bile fistula dogs. The *open bile fistula* was used in those experiments and the possibility of infection as responsible for a part of the reaction could not be excluded. In the experiments tabulated below we believe that infection is excluded as a causative or contributing factor. The experiments of Hooper and Whipple were also less complete as the daily bile collection period was but 6 hours while the present experiments cover all bile pigment eliminated from the body during 24 hours. The hemoglobin levels in the blood are also followed carefully with necessary blood volume measurements. The pigment forming capacity of the diet is also well understood and we can approximate a knowledge of the maximum hemoglobin pigment output of the anemic dog on this diet.

A considerable number of splenectomized bile fistula animals have been observed. We report in detail the pigment studies on 3 of these dogs in which the data on pigments, diet, hemoglobin level and anatomical conditions are reasonably complete. The general pigment reaction is uniform and the anatomical findings very similar which gives one confidence that this is not a physiological curiosity of rare occurrence but a uniform reaction under these fixed conditions.

The *first dog* (29-353) presents a number of cycles of pigment overproduction —in all a continuous period of observation of 190 days. This dog was an adult male mongrel collie. For 7 months following the operation (bile fistula and splenectomy) this dog was used for dietary experiments and hemoglobin injection. A hematoma developed at the site of an injection with softening so that pigment observations were discontinued for several weeks. The precise date at which the bile pigments showed a rise is not known. Regular observations were begun 7 months after the splenectomy and continued until death 190 days later. During this period of observation 6 cycles of pigment overproduction were observed of which 3 are given in Tables 1–3. The shortest cycle was 14 days and the longest 51 days in duration. After the first cycle the hemoglobin never rose to the control levels nor did the pigment output ever fall to normal for more than a few days at a time.

Table 1 shows daily observations of bile pigment output during a short control period—average 82 mg. bile pigment per 24 hours.

TABLE 1

Cycles of Pigment Overproduction

Dog 29-353.

Date	Bile pigment output per 24 hrs.	Hb. level	Urine-bile collected 24 hrs.	Food consumed	Wt.	Remarks
1931	*mg.*	*per cent*	*cc.*	*per cent*	*kg.*	
Jan. 22	104	137	520	100	15.6	Splenectomy Sept.
23	74		520	100		17, 1930
24	74		1100	100		
25	75		930	100		Control period
26	81		900	100	16.2	
27	75		1000	100		
28	76		1300	100	16.0	Blood volume 1100
29	99	136	1100	100		cc.
Average....	82					
May 7	690	45	480	66	14.1	Maximum pigment
8	567		710	66		output
9	704		580	100		
10	284		470	62		
11	615		625	100	13.3	
12	397		570	74		
13	620		665	75		
14	494	44	465	74	13.3	
Average....	546					
May 21	199	45	755	100		
22	196		620	100	12.4	
23	211		540	100		
24	222		760	100		End of 1st cycle of
25	163		640	100		pigment overpro-
26	127		610	100	12.7	duction
27	113		650	100		
28	94	72	640	100	12.7	
29	80		655	100		
30	77		740	100		
31	62		575	100		
June 1	71		660	100	13.4	
2	63		670	100		
3	110		630	100		
4	206	75	735	100	13.5	
Average....	133					

During a cycle of bile pigment overproduction, this average value rises to 546 mg. per 24 hours or about seven times normal. The blood hemoglobin has fallen meanwhile and there was some icteric staining of the tissues. The last part of Table 1 shows the end of this particular

TABLE 2

Short Cycle of Pigment Overproduction

Dog 29-353 (continued).

Date	Bile pigment output per 24 hrs.	Hb. level	Urine-bile collected 24 hrs.	Food consumed	Wt.	Remarks
1931	*mg.*	*per cent*	*cc.*	*per cent*	*kg.*	
June 18	86	86	500	100	14.6	
19	90		780	100		
20	76		600	100		
21	66		435	100	14.3	
22	91		900	100		
23	110	93	875	100		
24	150		685	100		
25	257		660	100	14.0	
26	422		840	100		
27	484		690	100		
28	662		660	100		
29	631		700	100	13.9	
30	523	28	585	100		
July 1	405		860	100		
2	403		790	100		
3	294		585	100		
4	165		640	100		
5	219		630	100		
6	138		1235	100		
7	110	66	660	100	14.6	Blood volume 1100 cc.
Average....	269					

cycle of pigment overproduction. The fall to normal is of but short duration and the bile pigment values begin to rise again. There is some loss of appetite and weight during this cycle and this is noted in almost all dogs. Spontaneous recovery from the anemic level took place in all the cycles recorded. This dog did receive one transfusion at a time when his hemoglobin level was dangerously low.

Table 2 shows a short cycle of bile pigment overproduction with a

TABLE 3

Cycles of Pigment Overproduction

Dog 29-353 (continued).

Date	Bile pigment output per 24 hrs.	Hb. level	Urine-bile collected 24 hrs.	Food consumed	Wt.	Remarks
1931	*mg.*	*per cent*	*cc.*	*per cent*	*kg.*	
July 22	429	43	625	100	14.6	
23	385		835	100		Maximum bile pigment output
24	485		640	100	15.1	
25	422		560	100		
26	606		730	100		
27	462		550	100	14.4	
28	760		730	100		
29	878		630	100	14.7	
30	798	51	835	100		
Average....	581					
Sept. 24	130	71	745	100	14.5	
25	102		850	100		
26	85		695	100		Low pigment output
27	90		615	100		
28	101		785	100	14.5	
29	82		515	100		
30	88		830	100		
Oct. 1	94	95	825	100	14.5	
Average....	96.5					
Oct. 7	181	99	710	100	15.0	
8	266		760	100		Blood volume 1057 cc.
9	221		700	100	15.3	
10	230		960	100		
11	202		740	100		
12	220		1150	100	15.0	
13	193		445	100		
14	230		850	100	15.0	
15	127	94	790	100		
Average....	208					

Death Oct. 24, 1931. Autopsy given below.

short fore period of normal output. There is a sharp drop in blood hemoglobin but spontaneous recovery took place and food consumption and the weight curve remained constant. Icterus was observed during this cycle.

Table 3 shows some very high values for bile pigment for 24 hours—an average of 581 and one day of 878 mg. bile pigment—more than ten times the control level. During this period there is actually a gain

TABLE 4

Unexplained Pigment Surplus in the Splenectomized Bile Fistula

Dog 29-353. Basal bile pigment output 78 mg. per day.

Days of experiment	Total bile pigment output			Blood Hb. level		Pigment lost gm. Hb. equivalent		Pigment gained gm. Hb. equivalent		"X" pigment surplus
	Total	above control	expressed as Hb.	start	end	in urine	from blood	in blood	trans-fusion	
	mg.	*mg.*	*gm.*	*per cent*	*per cent*					*gm.*
45	19512	16002	400	60	72	400		18	36	382
5	353	−37	−1	72	75	−1		4		3
15	4474	3304	83	75	86	83		17		100
5	409	19	0.5	86	93	0.5		11		12
15	4973	3803	95	93	66	95	41			54
51	20915	16937	423	66	79	423		20		443
13	2041	1027	26	79	90	26		17		43
13	2587	1573	39	90	71	39	29			10
9	822	120	3	71	95	3		36		39
19	2906	1424	36	95	89	36	9			27
190	58992	44172	1104.5			1104.5	79	123	36	1113
Per day	311	233	5.8			5.8	0.42	0.65	0.19	5.8

in blood hemoglobin—truly a remarkable pigment output. Food consumption and the weight curve remained normal. There was some icterus. The middle period of low pigment output does not fall to the control level.

Table 4 is a condensed table giving a summary of pigment data throughout the entire period of observation. The various periods run from 5 days to 51 days and represent arbitrary divisions in which conditions were relatively similar. Some periods show high or low or intermediate pigment output. The high periods are obvious from a

consideration of the total bile pigment overproduction. This total production of bile pigment above the control level is also expressed in column 4 as grams of hemoglobin obtained by calculating 40 mg. of bile pigment as equivalent to 1 gm. hemoglobin.

Pigment lost to the body is expressed in columns 7 and 8 in terms of hemoglobin as coming from the urine bile pigment above the control levels—columns 4 and 7 are equivalent. The pigment lost from the blood (column 8) is estimated from the fall in blood hemoglobin levels during any given period—the blood volume x 13.8 x per cent hemoglobin lost. Hemoglobin 100 per cent = 13.8 gm. per 100 cc. whole blood.

Pigment gained for the body is given as grams of hemoglobin in columns 9 and 10. Column 10 records the gain from transfusions. Column 9 records the gains in the hemoglobin level in the blood during any given period and the grams hemoglobin estimated exactly as were the grams hemoglobin lost. This new formed hemoglobin is presumably derived at least in part from the diet intake.

Unexplained "X" pigment surplus is given in the last column expressed as hemoglobin grams equivalent whether coming from hemoglobin or bile pigment. It is the sum of the surplus bile pigment expressed in hemoglobin equivalents (column 4 or 7) plus any hemoglobin gained in the blood by rise of hemoglobin per cent (column 9) *less* any hemoglobin given by transfusion (column 10) and less any hemoglobin lost from the blood stream (column 8) as presumably this would go direct to form bile pigment and therefore is "explained."

The total *unexplained pigment surplus* amounts to 1113 gm. hemoglobin equivalent whether appearing as hemoglobin or bile pigment. This amounts to 5.8 gm. hemoglobin equivalent per day or 40.6 gm. per week.

A standardized anemic dog on this ration will put out 2–4 gm. hemoglobin per week over and above the unknown maintenance factor. This output of 40.6 gm. hemoglobin equivalent per week approximates closely the high levels of hemoglobin production in anemic dogs when fed liver or kidney diets.

The *second dog* (30-62) was a young female mongrel setter about 1 year of age. Biliary occlusion was incomplete which necessitated a second operation. She began a cycle of pigment overproduction 5 weeks after the second operation and 4 months following the splenectomy. She lived 89 days after the pigment overproduction began. The dog went through 4 cycles of pigment overproduction of 13 to 27 days duration with short periods of low bile pigment output between cycles. She showed icterus during the last 2 months of life. Appetite, food consumption and weight were not disturbed. Two transfusions were given during periods of dangerously low blood hemoglobin levels.

TABLE 5

Cycles of Pigment Overproduction

Dog 30-62.

Date	Bile pigment output per 24 hrs.	Hb. level	Urine-bile collected 24 hrs.	Food consumed	Wt.	Remarks
1931	*mg.*	*per cent*	*cc.*	*per cent*	*kg.*	
Aug. 13	72		715	100	10.6	
14	76		745	100		
15	84		725	100		
16	107		1050	100		Control period
17	66		960	100	10.6	
18	68		670	100		
19	70		640	100	10.7	
20	56	103	425	100		Blood volume 1081 cc.
Average....	75					
Sept. 24	430	41	630	100		
25	363		485	100	11.1	
26	362		670	100		
27	365		1180	100		
28	352		500	100	10.8	High pigment output
29	341		575	100		
30	405	35	665	100		
Oct. 1	529		625	100		
2	550		615	100	11.1	
3	414		615	100		
4	444	25	800	100		
Average....	414					
Oct. 22	412	43	640	100		
23	425		720	100	11.5	
24	430		920	100		High pigment output
25	438		955	100		
26	391		1000	100	11.5	
27	357		750	100		
28	434		800	100	11.6	
29	379	58	810	100		
Average....	409					

Death Nov. 28, 1931. Autopsy given below.

Table 5 shows the control level of bile pigment output as 75 mg. per 24 hours. The control hemoglobin level is lower than normal and is probably related to the second operation. Following this operation there was a small stitch abscess and some drainage from the wound.

The periods of high pigment output average 414 and 409 mg. per 24 hours—about six times the control level. The blood hemoglobin levels show considerable decrease.

Table 6 is a summary of the pigment output of the second dog (30-62). This type of table is explained under Table 4. The base line

TABLE 6

Unexplained Pigment Surplus in the Splenectomized Bile Fistula

Dog 30-62. Basal bile pigment output 72 mg. per day.

Days of experiment	Total bile pigment output			Blood Hb. level		Pigment lost gm. Hb. equivalent		Pigment gained gm. Hb. equivalent		"X" pigment surplus
	Total	above control	expressed as Hb.	start	end	in urine	from blood	in blood	trans-fusion	
	mg.	*mg.*	*gm.*	*per cent*	*per cent*					*gm.*
16	2810	1658	41	107	86	41	31			10
21	7992	6480	162	86	27	162	88		25	49
18	6183	4887	122	27	43	122		24		146
19	5834	4466	112	43	38	112	8			104
9	1187	539	13	38	81	13		64	34	43
6	1147	715	18	81	72	18	13			5
89	25153	18745	468			468	140	88	59	357
Per day	283	211	5.3			5.3	1.6	1	0.66	4

bile pigment output is the average of all control observations—72 mg. per 24 hours. The surplus of bile pigment above the control level is expressed as hemoglobin equivalent in columns 4 and 7. This amounts to 211 mg. bile pigment per day or 5.3 mg. hemoglobin equivalent. When we allow for the hemoglobin destroyed to account for the fluctuations in the blood hemoglobin levels we find the "unexplained X" pigment surplus as 357 gm. equivalent of hemoglobin or 4 gm. per day.

When we recall that the standard anemic dog on this diet can produce only 2–4 gm. of hemoglobin a week, this figure of 28 gm. of hemoglobin equivalent becomes a conspicuous figure, not as high as the

TABLE 7

Cycles of Pigment Overproduction

Dog 31-73.

Date	Bile pigment output per 24 hrs.	Hb. level	Urine-bile collected 24 hrs.	Food consumed	Wt.	Remarks
1931	*mg.*	*per cent*	*cc.*	*per cent*	*kg.*	
Nov. 25		119		100	15.1	
26				100		
27	111		920	100	15.8	Splenectomy Nov. 5, 1931
28	153		870	100		
29	165		780	100		
30	173		970	100	15.7	Control observation
Dec. 1	77		850	100		
2	228		1280	100	15.5	
3		116		100		
Average....	151					
Dec. 31		51		100	17.2	
1932						
Jan. 1	785		980	100		
2	526		1100	84		
3	950	29	900	95	17.5	Transfused 4 times. Total 108 gm. hemoglobin
4	950	37	700	90		
5	1096	27	920	48		
6	1147	40	820	100	17.1	
7	782	54	910	100		
8	580	55	810	100	17.6	Blood volume 1410 cc.
Average....	852					
Feb. 7	523	89	770	100		
8	441	78	960	100	18.6	
9	290	80	1020	100		
10	225	78	960	100	18.5	
11	211	77	840	100		
12	233	65	980	100	18.9	High pigment output
13	189	69	840	100		
14	349	73	750	100		
15	449	60	880	100	18.7	
16	400		800	100		
17	615	48	860	100	19.2	
18	837	52	780	100		
19	795	46	900	100	19.3	
20	849	44	890	100		
Average....	458					

Death, Mar. 20, 1932. Autopsy given below.

figure of Table 4 but far beyond any possibility of error in observation or technique.

The *third dog* (31-73) was an adult male mongrel bull dog. The clinical story of this dog is the shortest of the three. He went into an acute cycle of bile pigment overproduction less than 7 weeks after operation and lived only 79 days subsequently. The periods of anemia developed very acutely and called for transfusions. Icterus was not noted until a few days before death. Food consumption was good and there was some gain in weight. In all 11 transfusions were given and the total grams of hemoglobin so introduced were 286 (Table 8).

TABLE 8

Unexplained Pigment Surplus in the Splenectomized Bile Fistula

Dog 31-73. Basal bile pigment output 154 mg. per day.

Days of experiment	Total bile pigment output			Blood Hb. level		Pigment lost gm. Hb. equivalent		Pigment gained gm. Hb. equivalent		"X" pigment surplus
	Total	above control	expressed as Hb.	start	end	in urine	from blood	in blood	transfusion	
	mg.	*mg.*	*gm.*	*per cent*	*per cent*					*gm.*
20	11063	7983	200	51	66	200		29	108	121
14	1700	−456	−11	66	101	−11		69		58
17	8110	5492	137	101	32	137	135			2
9	4315	2929	73	32	73	73		80	65	88
19	16422	13496	337	73	36	337	72		113	152
79	41610	29444	736			736	207	178	286	421
Per day	527	373	9.5			9.5	2.6	2.3	3.6	5.5

Table 7 shows a control period which is not adequate as probably the rise in bile pigments due to splenectomy had already started. The appearance of the pigment overproduction was unusually early and acute. We give as a base line bile pigment production 154 mg. per day but have a conviction that the true control level was nearer 100 mg. Even with this high base line the excess of bile pigment output is extreme and on two occasions exceeds 1 gm. per day. There was a sharp drop in the blood hemoglobin which called for transfusions but the dog remained in excellent condition.

Table 8 gives the usual summary for Dog 31-73. This type of table is explained under Table 4. The daily excess of bile pigment output above control is recorded as 373 mg. or the hemoglobin equivalent as

9.5 gm. When we allow for the transfused blood and the loss of circulating blood hemoglobin, we find 5.5 gm. hemoglobin equivalent per day as the "unexplained" pigment surplus. This amounts to 38.5 gm. hemoglobin equivalent per week and we recall that anemic dogs on this diet can regenerate only 2–4 gm. hemoglobin per week above the maintenance factor. This is a very large excess of bile pigment or hemoglobin to explain.

AUTOPSY PROTOCOLS

First dog (29-353)—Tables 1–4 above.

The final period of intoxication was associated with bleeding from puncture wounds of veins. Whole blood given intramuscularly failed to check it. Bloody stools appeared and transfusions had little effect.

Autopsy done immediately after death. All tissues showed definite icteric staining. There was no fluid or blood in the serous cavities.

Heart: Shows extensive hemorrhage under endocardium of left ventricle. *Histological sections* normal. The hemorrhagic areas in vein walls and fat show no white cells and no evidence of infection.

Lungs: Are crepitant and show many small hemorrhagic spots. *Histological sections*: Many alveoli contain red blood cells, a little fibrin and some polymorphs. There is definite edema. Bronchi are clear. Many alveoli are normal. There is evidently a slight amount of inflammation in some areas.

Gastro-intestinal tract: Stomach normal. Jejunum and ileum show red injected tips of villi and there is partly digested blood in the lumen. There are numerous pinpoint hemorrhages in the colon. *Histological sections*: Stomach normal. Jejunum shows normal mucosa. No evidence of inflammation. There are fresh red cells in the crypts. At the base of the mucosa are many mononuclear phagocytes which contain a yellow granular pigment. These same phagocytes are found also in the tips of the villi. They are a conspicuous feature of the section. Iron stains show no reaction in the majority of these phagocytes but in the tips of some of the villi some of this pigment gives a positive stain for iron. Colon normal.

Liver: In gross the color is a dark reddish brown. The lobules are rather indistinct. There is no evidence of scar tissue. *Histologically* the outer half of the lobules presents normal liver cells. The central part of each lobule shows much atrophy of liver cells and an accumulation of large phagocytes packed with granular yellow pigment, some of which gives a positive stain for iron. The mid-zone of each lobule shows conspicuous bile canaliculi within the liver cells filled with dark brown colloid material. Kupffer cells are numerous in all parts of the lobule and enlarged. Fat stains show no fat droplets anywhere. Bile ducts are clear and normal. No evidence of infection. In the portal stroma are mononuclears and pigmented phagocytes of the same nature as those noted in the center of the lobule.

Kidneys: The left is somewhat hypertrophied and a deep brown color. The right is small and somewhat scarred. In the right pelvis there are a few soft bile pigment concretions. There is no evidence of obstruction. *Histological sections*: The right kidney shows mononuclears in the scar tissue but no evidence of acute infection. The left kidney shows normal glomeruli, collecting tubules and stroma. The convoluted tubules appear normal except for the presence of a coarse granular pigment within the epithelium. This pigment is yellow and highly refractile but gives no stain for iron or fat.

Bladder shows many small hemorrhagic spots in the mucosa. *Histologically* bladder wall shows some fresh hemorrhage and some pigmented phagocytes. This is probably related to the hemorrhagic condition as described above and not to infection.

Lymph glands: Those about the liver and portal veins are enlarged and many measure as much as 2 cm. in length. They are deeply pigmented and contain some blood. *Histological sections* show that the sinuses contain great numbers of phagocytes packed with yellow pigment. These are obviously related to similar phagocytes described in liver sections and in intestinal mucosa. This pigment does not give any positive test for iron.

Bone marrow: In the femora and humeri is dark red and cellular. In the radii and tibiae it shows a good deal of fat with scattered islands of red marrow. Vertebral marrow is red and cellular. Everywhere the bone tissue is dense and hard. There is no sign of softening. *Histological sections* in areas of greatest hyperplasia within the long bones show a good many normal fat cells but the hyperplasia is well advanced. The marrow cells look normal in all sections both those of the red and white cell chains. The megakaryocytes are normal. Scattered through all sections in moderate numbers are large phagocytes containing granular brown pigment. Some of this pigment gives a positive stain for iron.

Pancreas, aorta, testis are normal in gross and histologically.

Blood coagulation was studied in material obtained immediately after death and shows delayed coagulation which appeared to be due to some anticoagulant. Within the test tube after a long interval normal tough clots formed. Extracts of muscle, heart and intestines showed no inhibiting substance having effect on blood coagulation.

Second dog (30-62)—Tables 5 and 6.

Death occurred in this animal without any external evidence of hemorrhage. *Autopsy* was done immediately after death. About 280 cc. of unclotted blood was found in the peritoneal cavity. There was granulation tissue beneath the operative wound in the region of the liver and it appeared that this peritoneal blood oozed from this granulation tissue. Obviously no large vessel had ruptured. Except for this, the serous cavities were all normal. Icterus was obvious in all tissues.

Heart normal in gross and histologically.

Lungs crepitant and normal throughout in gross and histologically.

Gastro-intestinal tract normal throughout in gross. *Histological sections* show normal tissue. There are no large phagocytes containing yellow pigment in this case as observed in the preceding dog 29-353.

Liver presents a dark brown color and a normal architecture. *Histological sections* show evidence of some subacute inflammation about the bile ducts where many mononuclear and polymorphonuclear cells are seen. Large phagocytes are also present. Some of the smaller ducts show evidence of infection. The liver cells in the center of the lobule show conspicuous atrophy. There is no evidence of necrosis. Many liver cells show conspicuous canaliculi filled with brown hyalin material. These are most conspicuous in the central zone. Many phagocytes and Kupffer cells containing granular pigment are found in all parts of the lobules, particularly in the central portions. The liver cells in the outer half of the lobule show relatively normal protoplasm. Fat stains are negative. Iron stains show iron-containing pigment in the Kupffer cells in the central half of the liver lobule. No iron-containing pigment is noted in the liver cells.

Kidneys: Right kidney is slightly scarred at point of insertion of gall bladder. Otherwise both kidneys show no abnormalities. There is some brownish staining. *Histological sections* in general show normal kidney tissue. The glomeruli, stroma and collecting tubules are normal. In the convoluted tubules one finds a good deal of yellow granular refractile pigment. There are also some finer grains of pigment which give a positive stain for iron. The coarse granules are negative for iron. Fat stains show a few fat droplets in the epithelium of the convoluted tubules but the pigment granules are negative for fat. There is no evidence of infection within the kidney tissue.

Bladder: Shows nothing of interest. The small *sinus tract* in the upper angle of the laparotomy wound shows the usual granulation tissue and some fresh hemorrhage into this tissue. Old ligatures are observed. It is obvious that this dog had a focus of infection here which involved the wound and a part of the biliary tree. From this area the hemorrhage occurred into the peritoneal cavity.

Lymph nodes are enlarged and rather cellular looking and moderately pigmented. *Histologically* they show granular brown and yellow pigment in moderate amount deposited in phagocytes within the sinuses. Some of this pigment gives a positive stain for iron and some of it is negative for iron.

Bone marrow of femora, humeri, tibiae and radii, ribs and vertebrae all a deep red color. The bone cortex everywhere is hard and strong, no evidence of softening. *Histologically* the marrow hyperplasia is well marked, much like that observed in animals anemic over many years as seen in the anemia colony in this laboratory. Some fat cells remain in sections from the radii and humeri. All marrow cells are normal and it looks like a simple hyperplasia associated with anemia. There seems to be a normal ratio between the red and white parent cells and megakaryocytes. Very occasional phagocytes are observed but they have to be looked for carefully. They give a strong positive stain for iron.

Third dog (31-73)—Tables 7 and 8.

This dog ran a very acute course with severe anemia requiring a considerable number of transfusions. Death obviously was due to anemia associated with blood destruction of unknown origin. There was no evidence of bleeding internally or externally.

Autopsy was done immediately after death. Well formed clots were present in heart chambers. All the serous cavities were smooth and glistening. No hemorrhages. Tissues show only a slight icteric tinge.

Heart: Normal in gross. *Histological sections* show a few cellular scars. Heart muscle cells are normal.

Lungs: Normal in gross and histologically.

Gastro-intestinal tract: Normal throughout in gross as well as histologically. No phagocytes containing yellow pigment observed in any sections of the intestinal tract.

Liver: Is dark brown in color, shows a regular architecture. *Histologically* the central part of the lobules shows atrophy of liver cells and here we notice many large phagocytes which contain granular yellow pigment. Some of this pigment gives a positive reaction for iron. The liver cells in the outer half of the lobule are relatively normal. Between these two zones the liver cells in the central zone show conspicuous bile canaliculi filled with dark brown hyalin material. Kupffer cells are numerous and large in all parts of the lobule. Bile ducts are all clean and normal looking. There is no evidence of any infection. Portal tissues show a few mononuclears, some of which contain pigment obviously related to the phagocytes present in the liver lobule and in the hepatic lymph glands and represent a transport of material from the liver through the lymphatics to the hepatic lymph glands. No polymorphs are seen anywhere. No fat droplets can be stained within the liver lobule.

Kidneys: The left kidney is somewhat hypertrophied and pigmented. The right is scarred at the site of the operative incision and about half normal size. *Histological sections*: The *gall bladder* epithelium within the renal pelvis is quite normal and retains its familiar characteristics. No evidence of infection. Some phagocytes contain old blood pigment. The kidney parenchyma is normal histologically. The convoluted tubules within their epithelium show coarse yellow pigment as in the other cases. It gives no stain for iron nor for fat. An occasional cast is seen in a collecting tubule. Glomeruli normal. Urinary bladder normal.

Lymph nodes: The hepatic lymph nodes are enlarged and deep brown in color, rather soft and moist. The retroperitoneal nodes are moderately enlarged and light brown in color. Other lymph nodes are normal. The pigmented lymph glands histologically show many phagocytes in the sinuses which contain pigment granules, some of which stain for iron. No marrow cells are observed but some phagocytes contain red cells.

Bone marrow of the femora and humeri is dark red throughout. The tibiae contain a good deal of fat along with the red marrow. The bony substance is

hard and strong. *Histological sections* of the humerus show almost complete cellular hyperplasia replacing all fat. We see a moderate number of phagocytes containing a coarse brown pigment which gives a positive stain for iron. All marrow cells appear normal. Megakaryocytes appear in normal numbers. Femur sections show a good deal of fat remaining, making up about half the section. Tibiae show more than half of the section made up of fat. Wherever the marrow cells occur they are normal. Phagocytes are scattered about uniformly in all sections including those of the vertebrae. This is a moderate grade of hyperplasia, not as extreme as the preceding case.

DISCUSSION

Death in these splenectomized bile fistula animals is due to anemia, usually associated with bleeding into the tissues, intestines or from vena punctures. The blood clots slowly whether in the test tube or vascular system or combined with normal blood. This suggests the presence of an anticoagulant in this abnormal blood. We have not been able to demonstrate that these tissues contain any substance which inhibits the clotting of normal blood plasma.

Blood destruction is an important factor in this abnormal condition and obviously is related to some interrelation of a spleen factor and a bile factor. The bile salts certainly deserve careful scrutiny as we are accumulating evidence that certain of the intoxications related to bile fistulas can be prevented by a proper intake of bile salt by mouth. The spleen factor can be tested by the use of various spleen fractions given by mouth or by vein. We hope to report on this point in the near future.

We expect to study factors which may be related to the blood destruction. It may be suggested that the red cells are produced with inherent defects because of faulty stroma. The marrow looks hyperplastic and is normal as to cell detail. The plasma will be studied to reveal the presence of any factor capable of laking normal red cells. Because transfused normal red cells do not persist in the circulation of these dogs, some hypothetical hemolysin may be suspected.

Muscle hemoglobin does not seem to enter into the picture. There is no conspicuous loss of muscle hemoglobin during the course of this pigment overproduction in these dogs. The striated muscle is normal in gross and histologically at autopsy.

Can we explain all this excess bile pigment overproduction on the

basis of blood hemoglobin destruction? We believe some other factor comes into the equation. Although the internal metabolism of pigments is complex we venture to express our beliefs as follows: that all bile pigment is derived solely from destroyed blood hemoglobin is common belief but we have given adequate reasons to show that this simple thesis is inadequate and inaccurate (6, 1). Were we to assume that all the excess of bile pigment came from blood hemoglobin matured by the usual building up from diet factors we must assume that these bile fistula dogs can utilize this standard ration to produce 10 times as much new hemoglobin as healthy anemic dogs under similar conditions. There is every reason to object to this assumption.

Figures to show the enormous production of hemoglobin equivalent as bile pigment are given in Table 3 in the first period of maximum bile pigment output. The daily output is 581 mg. bile pigment, about 7 times the control level. This is equivalent to 14.5 gm. hemoglobin to be destroyed daily to produce this amount of bile pigment. The blood hemoglobin also is rising during this period which means other pigment is being formed. This dog (29-353) with a blood volume of 1050 cc. and a hemoglobin of 50 per cent should have in circulation about 72 gm. hemoglobin. To explain these high periods of bile pigment output we must then believe that this circulating hemoglobin is completely turned over by destruction and repair in 5 days. This is hard to believe and invites speculation as to other possibilities.

Observations published recently (1) from this laboratory may have some bearing on this point. Given a standardized anemic bile fistula dog with injection of hemoglobin intravenously it was found that practically a quantitative hemoglobin conservation followed with reconstruction of the same amount of new red cells and hemoglobin. In addition the usual amount of excess bile pigment appeared in the urine, 70–100 per cent of the injected hemoglobin calculating 1 gm. hemoglobin = 40 mg. bile pigment (5). There is a large excess of pigment produced under these conditions. It was suggested that from the injected hemoglobin the pyrrol aggregate is split off to form bile pigment and that from the *globin fraction* is formed the new hemoglobin. This too suggests that the body can synthesize the pyrrol aggregate (four pyrrol rings) in this emergency.

It may be argued in like manner that the anemic, splenectomized

bile fistula dog breaks down red cells and from the released hemoglobin splits off the pyrrol aggregate to form bile pigment while the globin fraction is utilized over and over again to expedite subsequent red cell and hemoglobin production. This proposal likewise assumes that the body can produce a very large surplus of the pyrrol aggregate (four pyrrol rings). Another suggestion which has some support from experiments yet unpublished is that the liver can build up bile pigment direct from various building stones and release it from the liver without any breaking down of mature hemoglobin. In our understanding of liver metabolism and pigment production, there should be little difference between the direct production of bile pigment which contains four pyrrol rings and the synthesis of the pyrrol aggregate. We are forced to one or the other conclusion by these experiments and perhaps both reactions occur.

SUMMARY

A splenectomized dog can be kept anemic for months or years in perfectly good condition.

A renal bile fistula dog on a suitable diet can be kept in perfect health and activity with normal weight for years.

When we combine splenectomy and the bile fistula, after a latent period, we invariably observe a striking reaction with enormous overproduction of bile pigment, a definite anemia and finally death from anemia or tissue hemorrhage.

The spleen is essential for life in the bile fistula animal and this suggests some contribution from the spleen to body internal metabolism. The spleen and bile together are essential for the normal metabolism of pigment.

The bile salts are suspected of playing some obscure rôle in this reaction.

It seems difficult to explain all this great excess of bile pigment as coming from hemoglobin built up from the usual diet factors which in these experiments are well standardized.

It is suggested that the body can synthesize the pyrrol aggregate (four pyrrol rings). There is some evidence that the liver can build up bile pigment direct from "building stones." It seems necessary to postulate one or the other mechanism and they do not seem unlike in

the final analysis as both bile pigment and the pyrrol aggregate contain four pyrrol rings. Possibly both reactions may take place under these conditions.

BIBLIOGRAPHY

1. Hawkins, W. B., Sribhishaj, K., Robscheit-Robbins, F. S., and Whipple, G. H., *Am. J. Physiol.*, 1931, **96,** 463.
2. Hooper, C. W., and Whipple, G. H., *Am. J. Physiol.*, 1917, **43,** 275.
3. Kapsinow, R., Engle, L. P., and Harvey, S. C., *Surg. Gynec. and Obst.*, 1924, **39,** 62.
4. Robscheit, F. S., *J. Biol. Chem.*, 1920, **41,** 209.
5. Sribhishaj, K., Hawkins, W. B., and Whipple, G. H., *Am. J. Physiol.*, 1931, **96,** 449.
6. Whipple, G. H., *Physiol. Rev.*, 1922, **2,** 440.
7. Whipple, G. H., and Robscheit-Robbins, F. S., *Am. J. Physiol.*, 1925, **72,** 395.