THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

II. The Relation of Hemoglobinemia to Hemoglobinuria and Jaundice in Normal and Splenectomized Animals.*

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This publication is the second of a series on the general subject of the influence of the spleen on blood degeneration and regeneration and the production of hemolytic jaundice, and has for its object the establishment of a satisfactory theory to explain, on the one hand, the quantitative relations of hemoglobinuria and jaundice, and, on the other, the relative importance of the liver and kidneys in removing free hemoglobin from the circulating blood.

The main investigation, to which the present study bears only an incidental relation, was undertaken for the purpose of determining the relation, if any, which may exist between the peculiar (spodogenous) enlargement of the spleen and the jaundice so characteristic of acute destruction of the blood by hemolytic agents.

In early experiments in which hemolytic serum was administered to dogs shortly after splenectomy, jaundice not infrequently failed to appear. The first, and perhaps natural conclusion was that the spleen is in some way concerned in the preparation of hemoglobin for action by the liver cells, but after repeated attempts had been made to explain the phenomenon it became evident that the problem was not easy of solution and that fundamental observations on the fate of free hemoglobin in the circulating blood were necessary as

* Aided by a grant from The Rockefeller Institute for Medical Research. Received for publication, June 3, 1912.

a basis for further investigation. The study of the fate of known quantities of hemoglobin injected into normal and splenectomized animals was therefore undertaken, and it is the results of this study which are here presented. The points studied were: (1) the degree of hemoglobinemia necessary in order to recognize free hemoglobin in the serum; (2) the degree of hemoglobinemia necessary for the escape of hemoglobin through the kidneys; (3) the percentage of hemoglobin eliminated by the kidneys; (4) the degree of retention of hemoglobin necessary to cause jaundice; (5) the influence of the absence of the spleen on the elimination or retention of hemoglobin.

METHODS.

Defibrinated dog blood was hemolyzed with distilled water, sodium chlorid was added to render the solution isotonic with dog blood, the diluted blood was centrifugalized, and the hemoglobin content was determined by Fleischl's method. Definite amounts of the hemoglobin solution, always freshly prepared, were allowed to flow from a burette into a small branch of the femoral vein. The first appearance of hemoglobin in the urine was determined by a catheter in the bladder only or by a second catheter in one ureter. In order to aid the flow of urine each dog received 300 cubic centimeters of water by stomach tube. From time to time the skin was punctured and the blood was drawn into capillary tubes to determine how early free hemoglobin appeared in the serum.

The elimination of hemoglobin in the urine was estimated by rendering the urine acid with hydrochloric acid to about N/10 and comparing this solution of acid hematin, suitably diluted, with a 1 per cent. solution made according to the Sahli method from blood containing 100 per cent. of hemoglobin by the Fleischl scale. A Duboscq colorimeter was used for making the comparison.

Quantities of hemoglobin are designated throughout the paper in grams calculated on the assumption, for the sake of convenience, that blood giving a reading of 100 per cent. by the Fleischl scale contains 14 per cent. of hemoglobin. This figure is, of course, only approximately correct, but as relative quantities are of importance in our work, an approximate determination of the absolute quantities of hemoglobin is sufficient.

It is of the utmost importance that all urine containing hemoglobin be placed promptly in the refrigerator until the colorimetric estimation is made. If such urine is allowed to stand a few hours at room temperature a green precipitate of a hemoglobin derivative is formed which, when treated with sodium chlorid and glacial acetic acid, gives the hemin test.

The experiments were made almost exclusively on female dogs, in order that urine obtained by catheterization might be available for the bile tests. This has been found to be most important on account of the small amounts of bile pigments occurring in the urine under the condition of these experiments. In all experiments ether anesthesia was employed.

The earlier experiments, which are given below, were made with the object of determining approximately (a) how much free hemoglobin is necessary to stain the serum, (b) how much must be introduced to cause its appearance in the urine, and (c) to gain some idea of the time relations.

Experiment I.—Normal dog, female; weight 8,360 gm. Ether anesthesia; cannula in left femoral vein; catheter in bladder; 300 c.c. of water by stomach tube.

Feb. 21, 1912. 3.20 P. M. Urine normal.

3.22 P. M. Blood serum clear.

3.25 P. M. 10 c.c. of hemolyzed blood injected (equal to 0.32 gm. of hemoglobin).

3.30 P. M. Blood serum clear.

3.32 P. M. 10 c.c. of hemolyzed blood injected.

3.37 P. M. Blood serum clear.

3.39 P. M. 10 c.c. of hemolyzed blood injected.

3.40 P. M. Much albumin in urine.

3.44 P. M. Blood serum faintly tinged with hemoglobin.

3.51, 3.58, 4.05, 4.12, 4.19, 4.26 P. M. Blood serum deeply stained with hemoglobin.

3.46, 3.53, 4.00, 4.07, 4.14, and 4.21 P. M. 10 c.c. of hemolyzed blood injected.

3.55, 4.15, 4.21 P. M. Much albumin in urine.

4.22 P. M. Hemoglobin in urine.

5.23 P. M. Hemoglobin in urine decreasing in amount. Total elimination of hemoglobin = 0.18 gm.

8.00 P. M. Urine free of hemoglobin.

Feb. 22–29, 1912. Urine free of hemoglobin; no bile pigment; slight trace of albumin on 22d and 23d; none after this.

Summary.—The total amount of hemoglobin injected was 2.91 gm. (0.35 gm. per kilo); the total elimination by the kidneys was 0.18 gm. The hemoglobin

retained was 2.91 — 0.18 = 2.73 gm., or 0.33 gm. per kilo. Hemoglobin was demonstrated in the blood serum after a lapse of nineteen minutes when 30 c.c. of the solution (0.97 gm. of hemoglobin) had been injected, and appeared in the urine after fifty-seven minutes when 90 c.c. of the solution (2.91 gm. of hemoglobin) had been introduced. Despite the great retention of hemoglobin, bile pigments did not appear in the urine.

Experiment II.—Normal dog, female; weight 11,090 gm. Ether anesthesia; cannula in branch of right femoral vein; catheter in bladder; 300 c.c. of water by stomach tube.

Feb. 29, 1912. 3.57 P. M. Urine normal.

4.01 P. M. Blood serum clear.

4.02–4.07 P. M. 20 c.c. of hemolyzed blood injected, equal to 0.49 gm. of hemoglobin.

4.07 P. M. Blood serum clear.

4.09–4.15 P. M. 20 c.c. of hemolyzed blood injected.

4.10 P. M. Some albumin in urine.

4.14 P. M. Blood serum clear.

4.16–4.18 P. M. 20 c.c. of hemolyzed blood injected.

4.20 P. M. Much albumin in urine.

4.21 P. M. Blood serum showed a trace of hemoglobin.

4.23–4.26 P. M. 20 c.c. of hemolyzed blood injected.

4.28 P. M. Blood serum deeply colored by hemoglobin.

4.30 P. M. 10 c.c. of hemolyzed blood injected.

4.32 P. M. Large amount of albumin in urine.

4.33 P. M. Hemoglobin present in urine.

4.35 P. M. Blood serum deeply colored by hemoglobin.

March 1, 1912. The cage urine showed a trace of albumin, no bile pigment, and no hemoglobin. Urine by catheter at 10 A. M. showed no albumin.

Summary.—The total amount of hemoglobin injected was 2.19 gm. (0.20 gm. per kilo); the total elimination by the kidney was 0.05 gm. of hemoglobin. The amount retained was, therefore, 2.19–0.05 = 2.14 gm. of hemoglobin, or 0.19 gm. per kilo. Hemoglobinuria was evident after a lapse of nineteen minutes when 60 c.c. of the solution (1.46 gm. of hemoglobin) had been injected, and hemoglobin appeared in the urine after thirty-one minutes when 90 c.c. of solution (2.19 gm. of hemoglobin) had been injected. Although nearly all the hemoglobin was retained, bile pigment did not appear in the urine.

Experiment III.—Normal dog, female; weight 11,040 gm. Ether anesthesia; cannula in branch of femoral vein; catheter in bladder; 300 c.c. of water by stomach tube.

March 1, 1912. 2.30 P. M. Urine normal.

2.34 P. M. Blood serum clear.

2.35–2.38 P. M. 20 c.c. of hemolyzed blood injected (equal to 0.80 gm. of hemoglobin).

2.40 P. M. Blood serum showed faint trace of hemoglobin.

2.42–2.45 P. M. 10 c.c. of hemolyzed blood injected.

2.47 P. M. Blood serum showed faint trace of hemoglobin.

2.49–2.51 P. M. 10 c.c. of hemolyzed blood injected.

2.52 P. M. Hemoglobin present in urine.

2.54 P. M. Serum showed much hemoglobin.

March 2, 1912. 9.00 A. M. Urine obtained by catheter showed no hemoglobin, no bile pigment, and no albumin. Cage urine contained 0.15 gm. of hemoglobin.

12.00 M. Urine obtained by catheter showed no hemoglobin, no bile pigment, and no albumin.

March 3, 1912. Urine normal.

Summary.—Hemoglobin appeared in the serum five minutes after the beginning of the injection of 20 c.c. of the solution (0.80 gm. of hemoglobin), and was present in the urine after seventeen minutes, when 40 c.c. (1.60 gm. of hemoglobin) had been injected. The total amount of hemoglobin introduced was 1.6 gm. (0.14 gm. per kilo). The total amount eliminated was 0.15 gm. The amount retained was, therefore, 1.45 gm. (0.13 gm. per kilo). As in the other experiments, the retained hemoglobin did not appear in the urine as bile pigment.

In order to determine precisely the moment when the kidney begins to excrete hemoglobin, the following experiments, in which a single large injection of hemoglobin was given, were undertaken.

Experiment IV.—Normal dog, female; weight 7,100 gm. Ether anesthesia; cannula in left external jugular vein; catheter in bladder; cannula in left ureter projecting into pelvis of kidney; 300 c.c. of water by stomach tube.

March 2, 1912. 3.29–3.33 P. M. 106 c.c. of hemolyzed blood injected (= 0.34 gm. of hemoglobin.

3.39½ P. M. Hemoglobin appeared simultaneously in both vesical catheter and ureteral cannula.

Experiment V.—In another experiment of the same type a hemoglobin solution containing 0.32 gm. of hemoglobin was injected into the jugular vein between 4.04 and 4.06 P. M. Hemoglobin appeared in the ureteral cannula at 4.14½ P. M., and in the vesical catheter a few seconds later.

In these last experiments, if we take experiment IV for purposes of comparison, it is obvious that the hemoglobin concentration of the blood, determining the threshold of the kidney for hemoglobin, was reached at some time betwen 3.29 and 3.33 P. M. The detection of hemoglobin in the ureter and bladder was possible at 3.39½ P. M. It is evident from this that hemoglobin can be detected in the bladder about eight minutes after the threshold value of the kidney is reached. We may assume, therefore, that in experiments I, II, and III the threshold of the kidney for hemoglobin was reached eight minutes before hemoglobin appeared in the urine; that is, in experiment I at 4.14 P. M., at which time 2.59 grams of hemoglobin had been injected; in experiment II, at 4.25 P. M., at which time 1.95 grams of hemoglobin had been injected; and in ex-

periment III, at 2.44 P. M., at which time 1.2 grams of hemoglobin had been injected.

The question at once arises as to the reason for the apparent differences in these threshold values, experiments II and III being on the same animal. It is to be found, we believe, in the rate of injection. Thus in experiment I, the lapse of time from the beginning of the injection to the time the threshold of the kidney was reached was forty-nine minutes (3.25 to 4.14 P. M.); in experiment II, this interval was twenty-three minutes (4.02 to 4.25 P. M.), and in experiment III, it was nine minutes (2.35 to 2.44 P. M.).

It is suggested at once that even while we are injecting, the hemoglobin is being removed at a definite rate by the tissues (liver?) as well as by the kidney, and that when the threshold value is reached we have actually less hemoglobin present in the serum than the above figures would indicate. Thus, if in experiments II and III (which were on the same animal and are, therefore, comparable) we assume that from the moment the injection began, tissues other than the kidneys were removing the hemoglobin at the rate of 0.054 of a gram per minute, then in experiment II at the end of twenty-three minutes there will have been removed by the tissues 1.24 grams (23 times 0.054 of a gram) of the 1.95 grams injected, leaving 0.71 of a gram (0.06 of a gram per kilo) present in the blood serum at 4.25 P. M. when the kidney threshold was reached; and in experiment III at the end of nine minutes there will have been removed 0.49 of a gram (9 times 0.054 of a gram) of the 1.2 grams injected, leaving again 0.71 of a gram (0.06 of a gram per kilo) present in the blood serum at 2.44 P. M. when the threshold was reached. That this rate is not constant for all animals is shown in experiment I, in which the absorption took place more slowly. That the hemoglobin is nevertheless removed from the serum at about the rate of 0.05 of a gram per minute is borne out by the fact that after the injection of 2.8 to 4 grams of hemoglobin, we found the serum almost clear in about an hour or a little more. We therefore conclude that elimination of hemoglobin by the kidneys does not occur until it is present in the serum in a concentration of about 0.06 of a gram per kilo of body weight.

Concerning the amount of hemoglobin eliminated by the kidneys,

it is probable that were it possible to stop injecting at the moment the renal threshold is reached, the quantity of hemoglobin eliminated would be a mere trace that could not be estimated. To do this, however, is practically impossible since one must wait for about eight minutes in order to detect the hemoglobin in the urine after the threshold is reached; but if very small amounts of hemoglobin are injected at such long intervals the hemoglobin will be entirely removed by tissues other than the kidneys during the eight minute intervals and the threshold will never be reached.

In each of the experiments quoted it happened that one injection of hemoglobin was given after the renal threshold was reached. Thus in experiment I, at 4.21 P. M., 0.32 of a gram of hemoglobin had been injected; in experiment II, at 4.30 P. M., 0.24 of a gram of hemoglobin had been injected; and in experiment III, at 2.49 to 2.51 P. M., 0.39 of a gram of hemoglobin had been injected. The total amounts eliminated in the urine were in experiment I, 0.18 of a gram; in experiment II, 0.05 of a gram; and in experiment III, 0.15 of a gram (table I). Thus these quantities bear, as we should expect, a relation to the amount of blood injected above the threshold value; the amount eliminated being about half (or somewhat less), in excess over the threshold value. There is, of course, no relation between the total amount injected and the amount recovered in the urine, nor should we expect such a relation since the kidney is active only when a concentration has been reached which is above the threshold value for the kidney.

In none of these experiments was jaundice observed, and, as a matter of fact, we have found that in all normal animals, when injections are made slowly, jaundice is less readily produced than when the injections are made rapidly, as in the experiments to be described later.

The next problem for consideration was that concerning the degree of retention of hemoglobin necessary to cause jaundice. In order to study this question quantitatively and accurately in the shortest possible time, decreasing quantities of hemolyzed blood were injected intravenously into a series of normal dogs; in each case the percentage elimination by the kidney and occurrence or non-occurrence of bile pigments in the urine were noted.

TABLE I.

Details of Injection of Hemoglobin.

Experiment No.	Remarks.	Injection time.		Hemoglobin injected.		Hemoglobin eliminated.		Hemoglobin retained.			Choluria.
		Duration in min.	Gm. of hemoglobin per kilo per min.	Total in gm.	Amount in gm. per kilo.	Total in gm.	Per cent.	Total in gm.	Amount in gm. per kilo.	Per cent.	
I	Normal	56	0.006	2.91	0.35	0.18	6.1	2.73	0.33	93.9	None.
II	Normal	28	0.007	2.19	0.20	0.05	2.3	2.14	0.19	97.7	None.
III	Normal	16	0.009	1.60	0.14	0.15	9.6	1.45	0.13	90.4	None.
VI	Normal	13	0.045	3.36	0.58	1.09	32.5	2.27	0.39	67.5	Marked.
VII	Normal	8	0.039	2.38	0.31	0.64	26.8	1.74	0.23	73.2	Faint trace.
VIII	Normal	11	0.024	2.21	0.26	0.38	17.1	1.83	0.22	82.9	Very faint trace.
IX	Normal	4	0.072	2.03	0.29	0.73	35.9	1.30	0.18	64.1	None.
X	Before splenectomy	27	0.030	5.03	0.81	0.85	16.0	4.18	0.68	84.0	Marked.
X	After splenectomy	40	0.013	3.36	0.54	0.63	18.8	2.73	0.44	81.2	Marked.
XI	Before splenectomy	32	0.012	3.94	0.38	1.06	26.5	2.88	0.28	73.5	Faint trace.
XI	After splenectomy	8	0.046	3.92	0.37	0.66	16.8	3.26	0.31	83.2	Faint trace.
XII	Splenectomy 28 days	10	0.032	3.08	0.32	0.65	21.1	2.43	0.25	78.9	Present.
XIII	Splenectomy 9 mos.	10	0.038	6.57	0.38	1.81	27.5	4.76	0.28	72.5	Trace.
XIV	Splenectomy 61 days	5	0.056	2.09	0.28	0.15	7.2	1.94	0.26	92.9	Present.
XV	Splenectomy 60 days	7	0.039	3.46	0.27	0.64	18.4	2.82	0.22	81.6	Trace.
XVI	Splenectomy 110 days Hemolytic jaundice	10	0.025	3.92	0.25	1.12	28.6	2.80	0.18	71.4	Marked.
XVII	Splenectomy 23 days Obstructive jaundice	7	0.036	2.23	0.25	0.59	26.4	1.67	0.19	73.6	Marked.

The following experiments are illustrative.

Experiment VI.—Normal dog, female; weight 5,780 gm. Ether anesthesia; cannula in small branch of saphenous vein; catheter in bladder; 300 c.c. of water by stomach tube.

March 27, 1912. 3.20 P. M. Urine normal. Blood serum clear.

3.32–3.45 P. M. 150 c.c. of hemoglobin solution (3.36 gm. of hemoglobin) injected.

3.47 P. M. Blood serum deeply stained with hemoglobin.

3.48 P. M. Urine contained hemoglobin in small amount.

3.49 P. M. Urine deeply stained with hemoglobin.

12.00 M. Urine collected at this time contained 1.09 gm. of hemoglobin. Bile pigment present.

March 28, 1912. 8.00 A. M. The cage urine contained much bile pigment; no hemoglobin, no albumin.

10.00 A. M. Urine obtained by catheter contained bile pigment in lessened amount.

March 29, 1912. Faint trace of bile pigment in urine.

Summary.—The total amount of hemoglobin injected was 3.36 gm. (0.58 gm. per kilo). The total amount eliminated by the kidney was 1.09 gm. The amount retained was, therefore, 3.36–1.09 gm. = 2.27 gm. (0.39 gm. per kilo). Bile pigments appeared in the urine in considerable amounts.

Experiment VII.—Female dog; weight 7,700 gm. Ether anesthesia. Injections of hemoglobin solution through hypodermic needle into superficial vein of lower part of leg.

March 28, 1912. 2.00 P. M. No albumin and no bile in urine.

3.59–4.07 P. M. Injection into vein of 96.5 c.c. of hemoglobin solution (2.38 gm. of hemoglobin).

4.12 P. M. Catheterized. Hemoglobinuria was marked.

March 29, 1912. The cage urine contained much hemoglobin, a trace of bile, but no albumin. Total hemoglobin eliminated = 0.64 gm.

10.30 A. M. Urine obtained by catheter contained no hemoglobin, and no albumin, but a trace of bile.

March 30, 1912. No albumin and no bile in the urine.

Summary.—The total amount of hemoglobin injected was 2.38 gm. (0.31 gm. per kilo). The total amount eliminated by the kidney was 0.64 gm. The amount retained was, therefore, 1.74 gm. (0.23 gm. per kilo). For about twenty-four hours bile pigments occurred in traces in the urine.

Experiment VIII.—Female dog; weight 8,435 gm. Ether anesthesia. Injections through hypodermic needle into superficial vein of lower part of leg.

March 29, 1912. No bile in urine.

3.38–3.49 P. M. Injection into vein of 90 c.c. of hemoglobin solution (.21 gm. of hemoglobin).

11.50 P. M. The cage urine (31 c.c.) contained 0.38 gm. of hemoglobin. There was a light cloud of albumin and the faintest possible trace of bile.

March 30, 1912. 10.00 A. M. The cage urine contained no hemoglobin, and no bile, but a trace of albumin.

March 31, 1912. The cage urine contained no bile.

Summary.—The total amount of hemoglobin injected was 2.21 gm. (0.26 gm. per kilo). The total amount eliminated by the kidney was 0.38 gm. The amount retained was, therefore, 1.83 gm. (0.22 gm. per kilo). Bile pigments occurred in the urine for about eight hours in the faintest possible traces.

Experiment IX.—The same dog as in experiment VII; weight 7,010 gm. Ether anesthesia. Injections through hypodermic needle into superficial vein of lower part of leg.

April 2, 1912. No albumin and no bile in urine.

2.50–2.54 P. M. Injection into vein of 71.5 c.c. of hemoglobin solution (2.03 gm. of hemoglobin).

April 3, 1912. 8.00 A. M. Urine contained 0.73 gm. of hemoglobin, no bile, and no albumin.

April 4, 1912. Urine contained no bile and no albumin.

Summary.—The total amount of hemoglobin injected was 2.03 gm. (0.29 gm. per kilo). The total amount eliminated by the kidney was 0.73 gm. The amount retained was, therefore, 1.30 gm. (0.18 gm. per kilo). No bile pigments appeared in the urine.

A comparison of these four experiments on normal dogs shows that the retention of 0.39 of a gram of hemoglobin per kilo caused marked choluria; of 0.23 of a gram, slight choluria for twenty-four hours; of 0.22 of a gram, a very faint choluria for eight hours; and of 0.18 of a gram, no choluria. The threshold for jaundice by this method in the normal dog lies apparently, therefore, between about 0.18 of a gram and 0.22 of a gram of hemoglobin per kilo of body weight.

The percentage of hemoglobin eliminated by the kidney appears to be a variable quantity. Thus, in experiment VI, 32.5 per cent. of the hemoglobin injected was eliminated by the kidney; in experiment VII, 26.8 per cent.; in experiment VIII, 17.1 per cent.; in experiment IX, 35.9 per cent.

In these four experiments the hemoglobin solution was rapidly injected during a period of from four to thirteen minutes. When the solution was introduced slowly a much larger amount could apparently be cared for in the liver without the production of jaundice. Thus, if we refer again to experiment I, in which the solution was introduced at intervals throughout a period of fifty-six minutes, we find that an amount of hemoglobin was retained equal to 0.33 of a gram per kilo, without bile pigments occurring in the urine.

DISCUSSION.

These experiments seem definitely to establish the mechanism by which free hemoglobin is removed from the blood serum under normal conditions. Our conception of this mechanism is as follows. The kidney does not eliminate hemoglobin until its concentration in the blood serum reaches a certain level. This concentration we conclude from experiments I, II, and III is about that produced by the presence of 0.06 of a gram of free hemoglobin per kilo of body weight. As soon as the concentration of the hemoglobin in the serum is above this point, the hemoglobin passes through the kidneys and we have hemoglobinuria, but as soon as it falls below this amount, the hemoglobinuria ceases. However, other tissues, of which presumably the liver is the most important, appear to take up hemoglobin as soon as mere traces are present in the serum and continue to remove it from the serum whether the renal threshold is exceeded or not. Therefore, whenever the kidney is removing hemoglobin from the serum these other tissues are also removing it. The kidneys apparently remove 17 to 36 per cent., and the liver (and other tissues?) 64 to 83 per cent.

The hemoglobin which the liver removes is changed into bile pigment, which, if it is not produced in too large amounts, or if the hemoglobin is not taken to the liver too rapidly, passes out as bile pigment in the usual manner through the bile passages. On the other hand, if the hemoglobin is taken up by the liver in larger quantities, and especially if this occurs rapidly, the bile is formed faster than the bile capillaries can remove it and it is reabsorbed into the circulation and appears in the urine. Obviously if this hypothesis is correct, the larger the amount of bile pigment already present in the liver, the smaller will be the amount of hemoglobin necessary to call forth a choluria, and *vice versa.*

Whether the accumulation of bile pigment or of hemoglobin in the liver influences the rate at which the liver removes hemoglobin from the serum has not been determined with certainty, but some of the experiments (to be presented later) apparently show a greater elimination of the hemoglobin by the kidney when the liver is presumably more or less saturated, as in spontaneous and obstructive jaundice, and the natural explanation for this is, that accumulation

in the liver diminishes the rate at which the liver takes up the hemoglobin from the serum, and, consequently, more hemoglobin is left for the kidneys to remove.

The Effect of Splenectomy.—Having determined these facts concerning the elimination of hemoglobin and the production of jaundice in normal dogs, we next studied the effect of splenectomy.

In two experiments hemoglobin solution was injected into normal dogs, after which these animals were splenectomized and later the hemoglobin injections were repeated.

The experiments follow.

Experiment X.—Normal dog, female; weight 6,190 gm. Ether anesthesia; cannula in small vein of left leg; catheter in bladder; 300 c.c. of water by stomach tube.

Feb. 26, 1912. 3.25 P. M. Urine normal.

3.28–3.55 P. M. 140 c.c. of hemolyzed blood injected (5.03 gm. of hemoglobin).

4.10 P. M. Urine contained hemoglobin.

Feb. 27, 1912. 1.00 A. M. Urine contained much hemoglobin (0.80 gm. of hemoglobin). No bile pigment.

8.13 A. M. Urine obtained by catheter contained a small amount of hemoglobin (0.05 gm.). Faint but definite positive test for bile pigment.

3.00 P. M. Urine free of hemoglobin; bile test strongly positive.

4.00 P. M. Splenectomy.

Feb. 28, 1912. Moderate amount of bile pigment in urine.

Feb. 29, 1912. Trace of bile pigment in urine. Animal prepared for test as on Feb. 26. Injection into small vein of right leg.

2.30–2.42 P. M. Faint trace of bile pigment.

2.42–3.22 P. M. Slow injection of 140 c.c. of hemolyzed blood (3.36 gm. of hemoglobin).

3.05 P. M. Urine contained hemoglobin (at this time, 2.49 gm. of hemoglobin had been injected).

3.42 P. M. Urine contained much hemoglobin.

March 1, 1912. 8 A. M. The cage urine was slightly tinged with hemoglobin. The total amount eliminated was 0.63 gm. There was a faint bile reaction.

9.00 A. M. Urine obtained by catheter was free from hemoglobin, but gave a well marked bile reaction.

Summary.—Before splenectomy the amount of hemoglobin injected was 5.03 gm. (0.81 gm. per kilo), the amount eliminated by the kidneys was 0.85 gm., and the amount retained was 4.18 gm. (0.68 gm. per kilo). Bile pigment was abundant in the urine. The hemoglobin eliminated through the kidneys was 16 per cent. of the total amount injected. After splenectomy the amount of hemoglobin injected was 3.36 gm. (0.54 gm. per kilo); the amount eliminated by the kidneys was 0.63 gm., and the amount retained was 2.73 gm. (0.44 gm. per kilo). Bile pigment was abundant in the urine. The percentage of hemoglobin eliminated through the kidneys was 18.8 per cent. of the total amount injected.

In the second experiment of the same type smaller amounts of hemoglobin were used.

Experiment XI.—Normal dog, female; weight 10,500 gm. Ether anesthesia; catheter in bladder; cannula in small vein of left leg; 300 c.c. of water by stomach tube.

March 4, 1912. 4.10 P. M. Urine normal.

4.18–4.23 P. M. 50 c.c. of hemolyzed blood injected (1.97 gm. of hemoglobin).

4.45–4.50 P. M. 50 c.c. of hemolyzed blood injected (1.97 gm. of hemoglobin).

4.55 P. M. Urine contained 0.11 gm. of hemoglobin.

March 5, 1912. 8.00 A. M. The cage urine contained 0.95 gm. of hemoglobin. Bile test was faintly positive.

5 P. M. Urine obtained by catheter contained no hemoglobin and no bile.

March 6, 1912. 2.00 P. M. Urine obtained by catheter contained no hemoglobin, but had a very faint trace of bile.

4.00 P. M. Splenectomy.

March 8, 1912. The animal was prepared for test as on March 4; injection into small vein of right leg.

4.30 P. M. Urine normal.

4.32–4.40 P. M. Injection of 100 c.c. of hemolyzed blood (3.92 gm. of hemoglobin).

4.47 P. M. Urine contained hemoglobin.

March 9, 1912. Urine contained much hemoglobin and a trace of bile.

The total elimination of hemoglobin was 0.66 gm.

March 10–11, 1912. Urine showed faint trace of bile.

March 12–13, 1912. Urine free of bile.

Summary.—Before splenectomy the amount of hemoglobin injected was 3.94 gm. (0.38 gm. per kilo). The amount eliminated by the kidneys was 1.06 gm., and the amount retained was 2.88 gm. (0.28 gm. per kilo). Bile pigments appeared in the urine in traces. The percentage of hemoglobin eliminated through the kidneys was 26.5. After splenectomy the amount of hemoglobin injected was 3.92 gm. (0.37 gm. per kilo), the amount eliminated by the kidneys was 0.66 gm., and the amount retained was 3.26 gm. (0.31 gm. per kilo). Bile pigments were present in the urine in traces. The percentage of hemoglobin eliminated through the kidneys was 16.8 per cent.

In this experiment, therefore, the spleen did not have any influence on the elimination or the retention of hemoglobin, or on the retardation of the appearance of bile pigments in the urine.[1] However, as these experiments were done only a few days after splenectomy and with quantities of hemoglobin larger than the minimum

[1] Recently it has been shown by Gilbert, A., Chabrol, E., and Bénard, H. (Recherches sur la biligénie consécutive aux injections expérimentales d'hémoglobine, *Presse méd.*, 1912, xx, 113) that absence of the spleen does not influence the power of the liver to transform into bile the hemoglobin furnished in the form of laked blood.

necessary to produce jaundice in normal animals, it was thought wise to make the same test at longer periods after operation and with smaller quantities of hemoglobin.

Experiment XII.—Female dog.

March 13, 1912. Splenectomy.

April 10, 1912. Weight 9,740 gm. Ether anesthesia; needle in small vein of leg. No albumin and no bile in urine.

4.04–4.10 P. M. Injected 76 c.c. of a solution containing 2.66 gm. of hemoglobin.

April 11, 1912. 8.00 A. M. The cage urine contained 0.15 gm. of hemoglobin and also a quantity of precipitated and altered hemoglobin that could not be accurately measured; no bile pigment was present.

10.00 A. M. Urine obtained by catheter contained no hemoglobin and no bile.

4.00–4.10 P. M. Injected 122 c.c. of a solution containing 3.08 gm. of hemoglobin.

April 12, 1912. 8.00 A. M. The cage urine contained 0.65 gm. of hemoglobin, a trace of bile, and a light cloud of albumin.

9.30 A. M. Urine obtained by catheter showed moderate bile test; no albumin.

April 13, 1912. No bile in urine.

Summary.—At the first injection, 2.66 gm. of hemoglobin (0.27 gm. per kilo) were introduced; the amount eliminated by the kidneys was uncertain; no bile pigment appeared in the urine. A second injection of 3.08 gm. of hemoglobin (0.32 gm. per kilo) was made one day later. The amount eliminated by the kidneys was 0.65 gm., and the amount retained was 2.43 gm. (0.25 gm. per kilo). Bile pigment was present in the urine. The amount of hemoglobin eliminated by the kidneys was 21.1 per cent.

Experiment XIII.—July 8, 1911. Splenectomy.

April 2, 1912. (Nine months after splenectomy.) Weight 17,220 gm. Ether anesthesia; needle in small vein of leg. Urine contained no albumin and no bile.

3.17–3.27 P. M. Injected 167.5 c.c. of solution containing 6.57 gm. of hemoglobin.

April 3, 1912. 8.00 A. M. The cage urine contained 1.81 gm. of hemoglobin and had a faint trace of bile.

April 4, 1912. Urine contained no hemoglobin, no albumin, and no bile.

Summary.—The amount of hemoglobin injected was 6.57 gm. (0.38 gm. per kilo), the amount eliminated by the kidneys was 1.81 gm., and the amount retained was 4.76 gm. (0.28 gm. per kilo). Bile pigments were present in the urine in traces. The hemoglobin eliminated by the kidneys equalled 27.5 per cent. of the total amount injected.

Experiment XIV.—February 10, 1912. Splenectomy.

April 11, 1912. Weight of animal 7,520 gm. (Sixty-one days after splenectomy.) Ether anesthesia; needle in small vein of leg.

Urine contained no albumin and no bile.

3.33–3.38 P. M. Injected 59.8 c.c. of a solution containing 2.09 gm. of hemoglobin.

7.20 P. M. The cage urine contained 0.15 gm. of hemoglobin, no albumin, and no bile.

April 12, 1912. 8.00 A. M. The cage urine was free from hemoglobin; bile pigment present; no albumin.

9.30 A. M. Urine obtained by catheter showed distinct reaction for bile.

April 13, 1912. No bile.

Summary.—The amount of hemoglobin injected was 2.09 gm. (0.28 gm. per kilo), the amount eliminated by the kidneys was 0.15 gm., and the amount retained was 1.94 gm. (0.26 gm. per kilo). Bile pigments were present in the urine in considerable amount. The hemoglobin eliminated by the kidney equalled 7.2 per cent. of the total amount injected.

Experiment XV.—February 10, 1912. Splenectomy.

April 10, 1912. Weight 12,680 gm. (Sixty days after splenectomy.) Ether anesthesia; needle in small vein of leg.

No albumin and no bile in urine.

4.22–4.29 P. M. Injected 99 c.c. of solution containing 3.46 gm. of hemoglobin.

April 11, 1912. 8.45 A. M. The cage urine contained 0.36 gm. of hemoglobin in solution and about 0.28 gm. of precipitated hemoglobin; the total amount eliminated was about 0.64 gm. No albumin; faint trace of bile.

9.30 A. M. Urine obtained by catheter contained a trace of bile; no hemoglobin.

April 12, 1912. No bile.

Summary.—The amount of hemoglobin injected equalled 3.46 gm. (0.27 gm. per kilo), the amount eliminated by the kidneys was about 0.64 gm., and the amount retained was about 2.82 gm. (0.22 gm. per kilo). A trace of bile appeared in the urine. The amount of hemoglobin eliminated by the kidneys was about 18.4 per cent. of the total amount injected.

These six experiments on splenectomized animals, in all of which bile pigments appeared in the urine for a short time and in small quantities after the retention of 0.44, 0.31, 0.25, 0.28, 0.26, and 0.22 gm. per kilo, respectively, indicate that the threshold for jaundice in splenectomized dogs is approximately 0.22 of a gram per kilo, the same as in the experiments (VI to XI) with normal dogs, in which the threshold was found to be between 0.18 and 0.22 of a gram per kilo.

When we examine the percentage of hemoglobin eliminated by the kidneys in the six splenectomized animals, we find that it runs a trifle lower than the limits determined for normal animals, being 18.8, 16.8, 21.1, 27.5, 7.2 (?), and 18.4 per cent. (average, excluding the fifth figure, 20.5 per cent.), as compared with 32.5, 26.8, 17.1, 35.9, 16, and 26.5 per cent. (experiments VI to XI), with an average of 25.8 per cent. We can, therefore, conclude that splen-

ectomy has no influence in increasing the elimination of free hemoglobin by the kidneys nor does it, as is shown by the occurrence of choluria in each of the experiments, alter the ability of the liver to form bile pigments from hemoglobin, or interfere with the elimination of these pigments. Thus one of the possible explanations for the failure of jaundice to follow the administration of a hemolytic serum in splenectomized animals, as suggested in the first paper[2] of this series, is shown to be untenable.

Our next experiments were undertaken for the purpose of determining whether a liver saturated with bile pigments would take up hemoglobin from the serum less rapidly than a normal liver and thus lead to the elimination of a larger percentage of hemoglobin through the kidneys. For this purpose we used (1) a dog that, after splenectomy, had developed a spontaneous choluria, and (2) a dog with obstructive jaundice. The observations on the former follow.

Experiment XVI.—November 15, 1911. Splenectomy.

March 4, 1912. Weight of animal 15,550 gm. (110 days after splenectomy). Ether anesthesia; needle in small vein of leg.

Urine intensely stained with bile pigment.

Injection during a period of ten minutes of 104 c.c. of a solution containing 3.92 gm. of hemoglobin.

March 5, 1912. 8 A. M. The cage urine contained 1.12 gm. of hemoglobin. Urine was deeply bile-stained.

5.00 P. M. The cage urine was free from hemoglobin, but bile pigment was still abundant.

Summary.—The amount of hemoglobin injected equalled 3.92 gm. (0.25 gm. per kilo), the amount eliminated by the kidneys was 1.12 gm., and the amount retained was 2.80 gm. (0.18 gm. per kilo). Bile pigments continued to be as abundant as before the experiment. The hemoglobin eliminated by the kidneys equalled 28.6 per cent. of the total amount injected.

The second experiment was on a splenectomized dog in which we ligated the common bile duct two days before the injection of the hemoglobin.

Experiment XVII.—Female dog.

March 6, 1912. Splenectomy.

March 27, 1912. Common bile duct doubly ligated. Severed between ligatures.

March 28, 1912. Large amount of bile in urine.

[2] Pearce, R. M., Austin, J. H., and Krumbhaar, E. B., I. Reactions to Hemolytic Serum at Various Intervals after Splenectomy, *Jour. Exper. Med.*, 1912, xvi, 363.

March 29, 1912. Much bile and no albumin in urine. Weight 8,945 gm.

3.17–3.24 P. M. Ether anesthesia; needle in small vein of leg. Injected 90.5 c.c. of solution containing 2.23 gm. of hemoglobin.

11.50 P. M. The cage urine contained 0.59 gm. of hemoglobin; trace of albumin; intense bile reaction.

March 30, 1912. The cage urine contained no hemoglobin; trace of albumin; intense bile reaction.

March 31, 1912. Urine the same as on March 30.

Summary.—The amount of hemoglobin injected equalled 2.23 gm. (0.25 gm. per kilo), the amount eliminated was 0.59 gm., and the amount retained was 1.67 gm. (0.19 gm. per kilo). Bile pigments continued to be abundant in the urine. The hemoglobin eliminated by the kidneys equalled 26.4 per cent. of the total amount injected.

The percentage of hemoglobin eliminated by the kidney in these two experiments, namely 28.6 and 26.4 per cent., is distinctly higher than in the six other experiments on splenectomized dogs (with the single exception of experiment XIII), although well within the limits established for normal dogs. With the increased elimination, there was a corresponding slight decrease in the amount retained, as compared with both normal and splenectomized dogs. We conclude, therefore, that saturation of the liver with bile pigments may diminish, but only to a very slight extent, the relative amount of hemoglobin removed from the serum by the liver with a concomitant very slight increase in the amount removed by the kidneys.

SUMMARY.

The results of this study may be stated as follows.

1. Rapid injection of more than 0.06 of a gram per kilo of hemoglobin intravenously into a normal animal is followed by the appearance of hemoglobin in the urine (pelvis of kidney) within eight to ten minutes.

2. After rapid injection of more than 0.012 of a gram per kilo per minute of hemoglobin, 16 to 36 per cent. of the total amount, if this equals 0.25 of a gram per kilo, is eliminated in the urine and is accompanied by choluria.

3. If the injection of not more than 0.35 of a gram per kilo is made slowly (less than 0.01 of a gram per kilo per minute), the amount eliminated in the urine is only 2.33 to 9.5 per cent. of the total amount injected, and choluria does not occur.

4. The concentration of free hemoglobin in the blood which constitutes the threshold value of the kidneys for hemoglobin is approximately 0.06 of a gram of hemoglobin per kilo of body weight. When about this concentration is reached, hemoglobin appears in the urine.

5. The amount of hemoglobin per kilo of body weight which, after rapid injection, may be retained without jaundice, is approximately 0.18 of a gram. When 0.22 or 0.23 of a gram is retained bile pigments appear in the urine. The threshold of the liver for jaundice in point of hemoglobin saturation lies, therefore, between 0.18 and 0.22 of a gram per kilo of body weight. With slow injections a greater amount may be retained without choluria.

6. The absence of the spleen does not alter greatly the percentage of hemoglobin eliminated by the kidney, nor does it raise the threshold of the liver for jaundice.

7. In the presence of jaundice, either hemolytic or obstructive, the amount of hemoglobin retained by splenectomized animals is slightly diminished and that eliminated by the kidneys is correspondingly increased.

Upon these data may be based the following explanation of the mechanism by which free hemoglobin is removed from the blood serum. Hemoglobin is not removed by the kidney until its concentration in the blood serum reaches a certain level (0.06 of a gram of free hemoglobin per kilo of body weight). This constitutes the threshold value of the kidneys for hemoglobin and when it is reached hemoglobin appears in the urine. When the concentration is lower, hemoglobinuria ceases; at the same time, however, the liver, and possibly other tissues, take up hemoglobin as soon as mere traces are present in the serum and they continue this removal whether the renal threshold is exceeded or not. The two processes go on simultaneously, the rate of removal, when the renal threshold is exceeded, being for the kidneys 17 to 36 per cent., and for the liver and other tissues 64 to 83 per cent, of the total amount introduced. The hemoglobin which is removed by the liver is transformed into bile pigments. If the amount reaching the liver is small and is received slowly, the amount of bile formed is not increased above the excretory capacity of the liver, and it is removed by the bile passages

without the occurrence of choluria. This is shown in our experiments in which injections of hemoglobin were made more slowly than 0.01 of a gram per kilo per minute. On the other hand, if the hemoglobin is taken up by the liver rapidly and in large amounts, the bile capillaries are overtaxed and the bile cannot be rapidly removed, but is reabsorbed into the blood, and choluria develops.

If this theory is correct we have an explanation of those instances of blood destruction in man characterized by jaundice, but not accompanied by hemoglobinuria. In a slow, gradual destruction of the red blood cells, the liver removes the hemoglobin from the serum so rapidly that the concentration of hemoglobin in the serum does not reach the threshold value of the kidneys and hemoglobinuria, therefore, cannot occur. The constant absorption of large amounts of hemoglobin by the liver and the increase in bile formation which results does, however, overtax the bile passages and jaundice occurs.

In the same way may be explained the continuance of jaundice after the disappearance of a transient hemoglobinuria. A rapid destruction of a large amount of blood raises the concentration of hemoglobin in the serum so quickly that the threshold value of the kidney is quickly exceeded and hemoglobin appears in large amounts in the urine. When an amount of hemoglobin sufficient to reduce the concentration of the serum below the threshold value of the kidney has been removed, a considerable amount of hemoglobin may still remain in the serum, and it is the slow elimination of this through the liver that causes the choluria to continue.

The demonstration that the absence of the spleen has no important influence on the elimination of hemoglobin by the kidney, on its transformation into bile pigments, or on the removal of such pigments, is of interest in connection with an observation made in the first paper of this series. This was concerning the frequent failure of jaundice to follow the administration of hemolytic serum during the early period following splenectomy.[3] Among the possible explanations was the suggestion that the spleen is in some way concerned in the disintegration of free hemoglobin or in the elaboration of its derivatives. The present investigations demonstrate that such an explanation is without experimental basis, though it does not

[3] Pearce, R. M., Austin, J. H., and Krumbhaar, E. B., *loc. cit.*

controvert the possibility of the spleen being concerned in liberating hemoglobin from the red cells and suggests that the failure of jaundice is due to some other factor or factors. Evidence to indicate that the changes in the blood that follow splenectomy are important factors is offered in the third paper[4] of this series.

[4] Pearce, R. M., Austin, J. H., and Musser, J. H., Jr., III. The Changes in the Blood Following Splenectomy and Their Relation to the Production of Hemolytic Jaundice, *Jour. Exper. Med.*, 1912, xvi, in press.