ICTERUS.

A RAPID CHANGE OF HEMOGLOBIN TO BILE PIGMENT IN THE CIRCULATION OUTSIDE THE LIVER.*

BY G. H. WHIPPLE, M.D., AND C. W. HOOPER.

(From the Hunterian Laboratory of Experimental Pathology, Johns Hopkins Medical School, Baltimore.)

The object of this communication is to submit evidence that hemoglobin can be transformed into bile pigment in the circulation when the liver has been excluded from participation in the reaction. It has been shown (Whipple and Hooper) that normal and Eck fistula dogs react essentially in a similar manner to an intravenous injection of hemoglobin, indicating that the liver is not the essential factor in this rapid change of hemoglobin to bile pigment which is completed within a few hours. Obviously the next step in this problem is the exclusion of the liver from any part in this chemical change. The earlier workers performed experiments with frogs (Löwit), pigeons (Stern), and geese and ducks (Minkowski and Naunyn), but did not report successful work with mammals. Their conclusions were that jaundice and bile pigment formation could not develop without the liver (Stadelmann). Löwit claimed that in the frog bile pigments were produced without the agency of the liver, but other observers did not confirm his findings.

Widal and his co-workers have recently claimed that human cases present a form of icterus in which the bile pigment formation is hematogenous, or extrahepatic. They grant that bile pigment may be formed from hemoglobin which remains for a long time in the body tissues or serous cavities, but they state that in the group of cases under consideration there is a retention of bile pigment in the blood but not of bile salts and cholesterin. Stadelmann has shown that when hemoglobin was injected intravenously it increased the

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output of bile pigment, but decreased the output of bile acids. Widal argues that the absence of bradycardia speaks against the accumulation of bile salts in these cases, attributing the slowing of the heart-beat to the bile salts. It has been shown by King and Stewart that the bradycardia is due to the bile pigments, so Widal's observation indicates only the absence of a great amount of bile pigment in the blood, as compared with obstructive jaundice.

The cases show no disturbance of fat metabolism and on these grounds Widal claims that all the bile salts must gain admission to the intestine. It is not claimed by most workers that there is complete obstruction in these cases, but only a plugging of the canaliculi with dense tenacious bile and a partial capillary obstruction. The observations of Widal do not establish the point that some of the bile pigment production in these cases may be truly hematogenous and thus extrahepatic.

It might seem that removal of the liver would be the best way out of this difficulty, but there are many objections to this procedure. The operation is associated with much hemorrhage, laceration of the liver, and escape of fluids from the liver tissue (bile pigments, etc.), which can be rapidly absorbed from the body surfaces. This absorption of bile pigments has been mentioned in criticism of the experiments of Minkowski and Naunyn. The operation causes great surgical shock and lowers the blood pressure to a grave degree, which militates against an experiment of long duration. It is difficult to be sure that every bit of liver tissue is removed from the region of the vena cava and root of the diaphragm.

By means of the Eck fistula and ligation of both branches of the hepatic artery, almost all blood can be shut out of the liver and the animal dies in four to six hours from hepatic insufficiency. In this type of experiment a very small amount of blood can, of course, trickle into the liver by way of tiny collaterals in the suspensory ligament and liver capsule. It is possible, too, that there may be a certain amount of to and fro movement of blood in the larger hepatic veins associated with respiration. However, scarcely as much as 1 per cent. of the blood could come in contact with the perishing liver cells in this way. The experiments given below show that hemoglobin is changed to bile pigment in the circulation and
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that the pigments appear in the urine in the normal interval. The question therefore arises as to whether the liver is the essential factor in the transformation.

METHOD.

The operative procedure is the same as that already described. Huppert's test was used for the bile tests in the urine. In testing the plasma or serum the fluid was made definitely alkaline with the addition of sodium carbonate and calcium chloride. The mixture stood over night causing the calcium bile pigment compound to settle as a yellowish precipitate. After centrifugalization at high speed the supernatant fluid was poured off, and the precipitate was washed free from other coloring matter and collected again by centrifugalization. Finally the precipitate dissolved in acid alcohol gave the familiar blue green color when bile pigment was present. Fat and other tissues were first washed free from blood and then incubated at 40° C. in contact with two or three volumes of acid alcohol. After twelve to twenty-four hours the bile pigment when present appears in the alcohol, may be filtered off, concentrated, and tested in other ways.

ECK FISTULA. HEPATIC ARTERY LIGATED. HEMATOGENOUS JAUNDICE.

Dog C-95.—Large mongrel, female; weight, 36 pounds.

June 18. Dog is in excellent condition. 80 c.c. of urine give a negative test for bile pigments.

June 19, 9 A. M. Clear amber urine (50 c.c.) gives a negative test for bile pigments. Given 300 c.c. of water by stomach tube. 9.45 A. M. Ether anesthesia and bleeding from the jugular vein to obtain blood corpuscles for use later. Plasma (25 c.c.) gives a negative test for bile pigments. 10.30 A.M. Hepatic artery ligated. 10.55 A.M. Eck fistula is completed in the usual way and a ligature tied on the portal vein at the hilum of the liver. 11 A.M. 25 c.c. of red blood cells washed and laked with an equal volume of distilled water are given intravenously, followed by 400 c.c. of 0.9 per cent. salt solution. 11.13 A.M. Hemoglobin appears in the urine, and ether anesthesia is removed. Bile pigments first appeared in the urine at 12.30, when 60 c.c. gave a faintly positive test for bile pigments. Bile pigments and hemoglobin were constantly present until 4.20 P. M., when the dog is bled from the carotid. 25 c.c. of this plasma give a positive test for bile pigments. The plasma was a light claret color and showed oxyhemoglobin bands. Urine (14 c.c.) obtained from the bladder at autopsy gives a strong test for bile pigments.

Autopsy.—Performed at once. All the subcutaneous fat, which is abundant, shows obvious jaundice staining, the fat having a faint olive yellow tint, especially in the epicardium, perirenal, and omental fat. The heart and thymus are normal. The lungs contain a few small, purplish patches at the bases. The bronchi contain some vomitus. The spleen is engorged. The peritoneal cavity shows a few small clots around the site of operation and contains a little blood-stained fluid. The bladder is normal. The kidney shows definite chocolate staining of the cortex.

1 Whipple, G. H., and Hooper, C. W., Jour. Exper. Med., 1913, xvii, 593.
The liver is mottled, reddish gray in color, and rather soft. On section it is very juicy and is obviously undergoing autolytic digestion. The Eck fistula is perfect. The ligature on the portal vein obstructed the lumen completely, but one thread-like collateral opens into the portal vein above the ligature leading into the gastrohepatic omentum. Careful dissection showed that this collateral was included by the upper ligature on the hepatic artery so that no blood could come into the portal vein from this source. The ligatures on the hepatic artery closed this vessel completely, and occluded the greater number of the lymphatics running in the gastrohepatic omentum. This careful dissection shows that all arterial and portal blood was shut out of the liver, as far as the supply entering the hilum of the liver is concerned.

### Dog C-95.

| Time        | Urine | Color | Bile pigment test | Pulse | Respiration | Temperature, C. | Remarks                                      |
|-------------|-------|-------|-------------------|-------|-------------|-----------------|---------------------------------------------|
| June 18     | 80    | Amber |                   | 0     |             |                 | 300 c.c. of water by stomach tube.          |
| June 19     | 50    | Amber |                   | 0     |             |                 |                                             |
| 9.55 to 10.55 A.M. | Eck fistula and hepatic artery ligated |       |                   |       |             |                 |                                             |
| 10.50 A.M.  | 7     | Amber |                   | 0     |             | Plasma (25 c.c.) negative for bile pigments. |
| 11.00 A.M.  | Laked red cells intravenously |       |                   |       |             | 400 c.c. of salt solution.                  |
| 11.10 A.M.  | 180   |       | 120               | 37.0° |             | 11.13 A.M. Oxyhemoglobin +                  |
| 11.30 A.M.  | Pink  | o     | 180               | 90    | 37.5°       |                                              |
| 12.00 M.    | 35    | Claret|                   | 140   | 86          | 38.0°           |                                              |
| 12.30 P.M.  | 62    | Claret|                   | 120   | 80          | 39.0°           |                                              |
| 1.00 P.M.   | 85    | Claret|                   | 120   | 96          | 39.2°           |                                              |
| 1.30 P.M.   | 42    | Claret|                   | 120   | 100         | 39.0°           |                                              |
| 2.00 P.M.   | 68    | Claret|                   | 90    | 100         | 39.0°           |                                              |
| 2.30 P.M.   | 50    | Claret|                   | 140   | 100         | 39.0°           |                                              |
| 3.00 P.M.   | Pink  | ++    | 140               | 100   | 39.0°       | 200 c.c. of salt solution.                  |
| 3.30 P.M.   | Pink  | ++    | 130               | +     | 39.0°       | 200 c.c. of salt solution.                  |
| 4.00 P.M.   | Cherry| +++   | 100               | 100   | 38.8°       | Muscular tremors.                           |
| 4.15 P.M.   | ++    |       |                   | 52    |             |                                              |
| 4.20 P.M.   | Bled into oxalate |       |                   |       |             |                                              |
| Autopsy     | 14    | Cherry|                   | ++    |             | Plasma (25 c.c.) positive for bile pigment.|

Dog C-80.—Mongrel, female; weight, 28 pounds. This dog had been tested on a previous date (June 3) for its reaction to laked red blood cells injected intravenously.

June 10. Dog is in perfect condition and urine is free from bile pigments.

June 13. Dog has been starved for twenty-four hours; weight, 27½ pounds. 9.40 A. M. Ether anesthesia and bleeding from the jugular vein. The red blood cells were washed and prepared for use later. 10 A. M. Ligation of the hepatic artery as usual. 10.40 A. M. Eck fistula is complete and the ligature is tied on the portal vein above the fistula. There was but little bleeding into the abdominal cavity. Urine is clear amber colored and negative for bile pig-

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1 Whipple, G. H., and Hooper, C. W., loc. cit., p. 594.
ments. 25 c.c. of washed red blood cells laked with an equal volume of boiled distilled water are introduced into the external jugular vein together with 100 c.c. of 0.9 per cent. salt solution. Ether is then removed. 11.05 A.M. Hemoglobin appears in the urine and absorption bands of oxyhemoglobin are conspicuous on examination with the spectroscope. 11.40 A.M. Bile pigments are positive in the urine and hemoglobin is very abundant. 2 P.M. Muscular tremors and convulsions appear and the flow of urine ceases at once. 2.30 P.M. Dog is bled from the carotid and the blood pressure is quite low. Heart-beats are irregular in force and rhythm. Blood plasma and blood serum are cherry red in color and give positive tests for bile pigment.

Autopsy.—Performed at once. The abdominal cavity contains a little blood-stained fluid and no clots. The thymus is large. The heart is normal. The lungs show a few small, purplish areas in the bases. The spleen, liver, kidneys, and gastro-intestinal tract are normal. The Eck fistula is large and wide open. There are no clots on the edges. The ligature on the portal vein is just at the bifurcation in the hilum of the liver, above all collateral branches. The three ligatures on the hepatic artery are effective, and the ligature in the gastro-hepatic omentum above the arch of the hepatic artery includes most of the lymphatics draining the liver. The body fat everywhere, particularly in the intercostal spaces and over the heart, shows a delicate jaundice tinge, a faint olive yellow color.

Dog C-80.

| Time          | Urine in c.c. | Color       | Bile pigment test. | Pulse | Temperature, C. | Remarks                                      |
|---------------|---------------|-------------|--------------------|-------|-----------------|----------------------------------------------|
| June 10       | 10            | Amber       | 0                  |       |                 |                                              |
| June 13       | 9.40 A.M.     | Amber       | 0                  |       |                 |                                              |
| 9.40 A.M.     |               | Eck fistula and hepatic artery ligated |               |       |                 |                                              |
| 10.04 A.M.    | 100 c.c.      | Pink        | —                  | —     | 37.7°           |                                              |
| 11.04 A.M.    | Laked red cells given intravenously | Cherry red | +                  | + 180 | 38.2°           |                                              |
| 11.40 A.M.    | 45            | Claret      | ++                 | 200   | 39.3°           | Starved for 24 hours. Operation completed. |
| 12.10 P.M.    | 55            | Claret      | ++                 | 200   | 39.0°           |                                              |
| 12.10 P.M.    | 55            | Claret      | ++                 | 200   | 39.0°           |                                              |
| 12.30 P.M.    | 70            | Claret      | ++                 | 200   | 39.0°           |                                              |
| 1.10 P.M.     | 33            | Claret      | ++                 | 240   | 39.2°           |                                              |
| 1.40 P.M.     | 22            | Claret      | ++                 | 250   | 39.0°           |                                              |
| 2.10 P.M.     | 39            | Claret      | ++                 | 250   | 39.0°           |                                              |
| 2.30 P.M.     |               | Death; bled from carotid into oxalate |               |       |                 | Plasma (25 c.c.) and serum (25 c.c.) positive for bile pigment. |

Eck Fistula. Hepatic Artery ligated. Spleen removed. Hematogenous Jaundice.

Dog C-98.—Large mongrel, female; weight, 34 pounds.
June 24. Urine (25 c.c.) is negative for bile pigments.
June 25. Dog is active and strong; has been starved for twenty-four hours.
9.20 A. M. Urine (30 c.c.) gives a negative test for bile pigments. Ether anesthesia and bleeding from the jugular vein to obtain red blood cells for use below. Blood plasma (30 c.c.) gives a negative test for bile pigments. Laparotomy is performed and the hepatic artery ligated as usual. 10.30 A. M. Urine (55 c.c.) gives a negative test for bile pigment. Eck fistula is completed and the portal vein above the fistula ligated as usual. 10.40 A. M. 25 c.c. of red blood cells washed and laked with distilled water are introduced intravenously, followed by 200 c.c. of Ringer solution. During this time the spleen is extirpated and omental fat (15 gm.) removed. This gives a negative test for bile pigments and is of the usual appearance. 10.45 A. M. Ether discontinued. 10.55 A. M. Hemoglobin appears in the urine. Diuresis has not yet begun. 11 A. M. 100 c.c. of Ringer solution given intravenously. 11.10 A. M. Given ¼ gr. of morphia intravenously. The urine first shows bile pigments at 1 P. M., after which time they are constantly present in the urine, together with oxyhemoglobin. Death took place at 3.30 P. M. after violent convulsions. Blood is obtained at once. Plasma (50 c.c.) gives a negative test for bile pigments.

**Autopsy.—**Performed at once. All the subcutaneous fat shows a definite jaundice tinge. The omental, pericardial, and subcutaneous, as well as the epicardial fat show a delicate olive yellow tint. 15 gm. of fat gave a strong test for bile pigments. The peritoneal cavity contains about 200 c.c. of blood-stained fluid together with a few clots. The viscera in general are normal. The liver is definitely softened, mottled grayish red in color, soft and juicy on section, and is evidently undergoing autolysis and digestion.

**Dog C-98.**

| Time          | Urine in c.c. | Color | Bile pigment test | Pulse | Respiration | Temperature, C. | Remarks                                      |
|---------------|---------------|-------|-------------------|-------|-------------|-----------------|----------------------------------------------|
| June 24       | 25            | Amber | 0                 | 160   | 96          | 39.1º           | Dog starved for 24 hrs.                       |
| June 25       | 30            | Pink  | 0                 |       |             |                 | Omental fat (15 gm.) negative for bile pigments. |
| 9.20 A.M.     |               |       |                   |       |             |                 |                                               |
| 10.30 A.M.    | 55            | Amber | 0                 | 160   | 96          | 39.3º           | Oxyhemoglobin + 100 c.c. Ringer solution and 1/8 gr. morphia. |
| 10.40 A.M.    |               |       |                   |       |             |                 |                                               |
| 10.55 A.M.    | 15            | Claret| 0                 | 140   | 24          | 38.9º           |                                               |
| 11.15 A.M.    | 55            | Claret| 0                 | 140   | 26          | 38.5º           |                                               |
| 12.00 M.      | 80            | Claret| 0                 | 132   | 28          | 38.0º           |                                               |
| 12.30 P.M.    | 55            | Claret| 0                 | 140   | 56          | 38.3º           |                                               |
| 1.00 P.M.     | 50            | Cherry| + (Faint)         | 120   | 26          | 38.0º           |                                               |
| 1.30 P.M.     | 50            | Cherry| + +               | 120   | 38          | 38.0º           |                                               |
| 2.00 P.M.     | 54            | Cherry| + +               | 134   | 36          | 38.7º           |                                               |
| 2.30 P.M.     | 48            | Cherry| + +               | 120   | 48          | 30.0º           | Muscular tremors.                             |
| 3.00 P.M.     | 25            | Claret| ++                | 160   | 100         | 39.0º           | Muscular tremors increasing.                  |
| 3.30 P.M.     | 13            | Claret| ++                | 200   | 70          | 39.0º           | Convulsions; death. Plasma (50 c.c.) negative for bile pigment; fat (15 gm.) positive for bile pigment. |
The Eck fistula is perfect. There are no clots. The ligature on the portal vein above the fistula closed the vessel completely and no collaterals are found. The portal vein above the ligature shows a soft, flabby clot, indicating complete absence of circulation in this region. Ligatures on the hepatic artery are efficient and blood clots are found in this vessel as well. It is obvious that all blood entering the hilum of the liver had been shut out and that the liver was undergoing autolysis.

**ECK FISTULA. HEPATIC ARTERY PARTIALLY LIGATED. HEMATOGENOUS JAUNDICE.**

**Dog C-83.—Mongrel, female; weight, 16 pounds.**

May 28. Dog is tested with an intravenous injection of laked red cells. *Whipple, G. H., and Hooper, C. W., loc. cit., p. 595-

July 18. Weight, 14½ pounds. Dog is in excellent condition and has been starved for twenty-four hours. 9:30 A. M. Urine contains no bile pigment. 10:35 A. M. Ether anesthesia. ½ gr. of morphia given subcutaneously. 10:40 A. M. Bled from carotid to obtain red blood cells used below. Plasma is water clear. 16 c.c. give a negative test for bile pigment. 11:10 A. M. Laparotomy.

### Dog C-83.

| Time          | Urine in c.c. | Color | Bile pigment test. | Pulse | Respiration | Temperature, °C | Remarks                        |
|---------------|---------------|-------|--------------------|-------|-------------|-----------------|--------------------------------|
| July 18       |               |       |                    |       |             |                 |                                |
| 11:40 A.M.    | 10            | Amber |                    | 0     | 30          | 36.0°           | Starved for 24 hrs.            |
| 11:50 A.M.    | 15            | Amber |                    | 0     | 140         |                 |                                |
| 11:55 A.M.    |               |       |                    |       |             |                 |                                |
| 12:40 P.M.    | 11            | Claret |                    | 0     | 120         | 36.0°           |                                |
| 1:10 P.M.     | 9             | Cherry |                    | 0     | 80          | 37.4°           |                                |
| 1:40 P.M.     | 10            | Cherry |                    | 0     | —           | 38.7°           |                                |
| 2:10 P.M.     | 12            | Cherry | (Faint)            | 60    | 20          | 38.5°           | Pulse irregular.               |
| 2:40 P.M.     | 11            | Cherry |                    | 72    | 20          | 39.6°           | 1/8 gr. morphia.               |
| 3:10 P.M.     | 9             | Claret |                    | 60    | 20          | 39.4°           | 100 c.c. salt solution.        |
| 3:40 P.M.     | 13            | Claret |                    | 60    | 20          | 39.0°           |                                |
| 4:10 P.M.     | 24            | Cherry |                    | 60    | 18          | 38.8°           | Pulse irregular.               |
| 4:40 P.M.     | 27            | Cherry |                    | 60    | 18          | 38.1°           |                                |
| 5:10 P.M.     | 23            | Cherry |                    | 60    | 18          | 38.1°           |                                |
| 5:40 P.M.     | 20            | Cherry |                    | 60    | 18          | 38.3°           |                                |
| 6:10 P.M.     | 17            | Pink   | (Faint)            | 94    | —           | 38.6°           | Pulse irregular.               |
| 6:40 P.M.     | 20            | Pink   |                    | 94    | —           | 39.0°           |                                |
| 7:10 P.M.     | 20            | Pink   |                    | 120   | 70          | 38.9°           | Polyneza.                      |
| 7:40 P.M.     | 21            | Pink   |                    | 96    | 30          | 38.5°           | 100 c.c. salt solution.        |
| 8:10 P.M.     | 23            | Pink   |                    | 96    | 30          | 38.3°           |                                |
| 8:40 P.M.     | 17            | Pink   |                    | 92    | —           | 38.3°           |                                |
| 9:10 P.M.     | 16            | Pink   | (Faint)            | 92    | —           | 38.4°           |                                |
| 9:40 P.M.     | 16            | Pink   | (Faint)            | 108   | 30          | 38.4°           |                                |
| 10:10 P.M.    | 10            | Pale pink |                    | 88    | 30          | 38.5°           | 100 c.c. salt solution.        |
| 10:40 P.M.    | 20            | Pale pink |                    | 120   | 30          | 38.6°           | Dog drowsy.                    |
| 11:10 P.M.    | 10            | Pale pink |                    | 104   | —           | 38.7°           | Feces passed.                  |
| Autopsy       | 37            | Brown  | (Faint)            |       |             |                 | Omental fat (28 gm.) and cardiac fat (7 gm.) negative for bile pigment. |

* Whipple, G. H., and Hooper, C. W., loc. cit., p. 595.
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otomy and ligation of the hepatic artery in the usual way. 11.40 A.M. Eck fistula is completed as usual, and the portal vein above the fistula carefully ligated. 11.50 A.M. Ether anesthesia removed. Urine (15 c.c.) is negative for bile pigments. 11.55 A.M. 20 c.c. of red blood corpuscles washed with salt solution and laked with an equal volume of distilled water are given intravenously, followed by 200 c.c. of 0.9 per cent. salt solution. It is probable that death occurred between 12 and 1 o'clock during the night, judging from the amount of urine which collected in the bladder after the last observation at 11.10 P.M.

Autopsy.—July 10, 8 A.M. The heart contains blood clots. The lungs are normal. The spleen is enlarged and soft. The liver shows beginning post-mortem change. The peritoneal cavity contains about 75 c.c. of blood-tinged fluid which contained no clots. The Eck fistula is large. It shows one small clot adherent to the upper end of the fistula on the side toward the vena cava. The hepatic artery shows an anomalous branch given off close to the origin of the hepatic artery from the celiac axis, running in a thin fold of the gastro-hepatic omentum to the right portion of the right lobe. This branch was not included in the ligatures, and probably carried about one third of the volume of blood furnished by the arterial system to the liver. The blood supply was insufficient for the functional activity of the liver. It will be noted that the curve of appearance and excretion of bile pigments in the urine is practically identical with that of the normal dog. Omental fat (28 gm.) and cardiac fat (7 gm.) give negative tests for bile pigments.

ECK FISTULA. HEPATIC ARTERY LIGATED. HEMATOGENOUS JAUNDICE.

Dog C-85.—Fox terrier, female; weight, 15 pounds.

May 30. Urine negative for bile pigments. Ether anesthesia and Eck fistula established in the usual way.

June 1. Animal will not eat. Urine is clear amber and negative for bile pigments.

June 2, 9.30 A.M. Ether anesthesia and bleeding from the jugular vein to obtain the red blood cells used below. 10.45 A.M. Hepatic artery ligated as usual. There is considerable bleeding from the recent adhesions about the site of the previous operation. At the completion of the operation the red cells (27 c.c.), washed and laked with an equal volume of distilled water, are given intravenously, followed by 50 c.c. of normal salt solution. Urine (20 c.c.) is dark and concentrated and contains no bile pigments and no hemoglobin. Ether removed. 11.15 A.M. Hemoglobin appears in urine. 11.25 A.M. 200 c.c. of normal solution given intravenously. 11.30 A.M. Urine is deep red in color and contains abundant oxyhemoglobin. 40 c.c. are negative for bile pigments. 12 M. Urine cherry red in color. 80 c.c. give a positive test for bile pigments. 12.30 P.M. Urine is cherry red in color. 30 c.c. give a positive test for bile pigments. Muscular tremors are noted in the neck and legs. 12.55 P.M. Animal died.

Autopsy.—Performed at once. The organs in general are negative. The heart contains blood which clots slowly, requiring two to three hours for complete coagulation. There is an excess of antithrombin present. The peritoneal
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cavity contains about 300 c.c. of blood-stained fluid. There are clots present and there has been a good deal of oozing from the adhesions broken at the time of the second operation. The Eck fistula is perfect and the ligature on the portal vein above the fistula closes the vessel completely and there are no collaterals above it. One of the ligatures on the arch of the hepatic artery was inefficient allowing a certain amount of blood coming from the collaterals through the pancreaticoduodenal branch to return and pass into the liver.

Dog C-85.

| Time.          | Urine in c.c.          | Color. | Bile pigment test. | Remarks.         |
|----------------|------------------------|--------|--------------------|-------------------|
| May 30         | Eck fistula produced   | 10     | Amber              |                   |
| June 1         |                        |        |                    |                   |
| 10.45 A.M.     | Hepatic artery ligated | 10     | Amber              | 300 c.c. salt solution. |
| 10.50 A.M.     | Laked red cells given intravenously | 20 | Brown              | Ether discontinued. |
| 11.00 A.M.     |                        | 40     | Claret             | 11.15 A.M. Oxyhemoglobin + 200 c.c. salt solution. |
| 11.30 A.M.     |                        | 30     | Cherry             |                   |
| 12.00 M.       |                        | 30     | Cherry             |                   |
| 12.30 P.M.     |                        | Death  |                    |                   |
| 12.55 P.M.     |                        |        |                    |                   |

Dog C-85.—Mongrel, female; weight, 16½ pounds.

May 30. Dog is in excellent condition. Ether anesthesia and Eck fistula produced in the usual manner.

May 31. Dog is in excellent condition. During the next month the animal was in fair condition except for a slight but steady loss in weight.

July 11. Weight, 13½ pounds. Dog is active and vigorous. 9.30 A.M. Urine is concentrated and dark brown. 17 c.c. give negative tests for bile pigments. 10.00 A.M. Ether anesthesia and bleeding to obtain red blood corpuscles to be used later. Blood plasma (30 c.c.) is negative for bile pigments. 10.40 A.M. Laparotomy and ligation of the hepatic artery in the usual way. 10.45 A.M. Red blood cells (15 c.c.) washed and laked with an equal volume of distilled water are introduced intravenously, followed by 270 c.c. of 0.9 per cent. salt solution. 10.55 A.M. Given ½ gr. of morphia and 1/300 gr. of atropin subcutaneously. Urine (22 c.c.) is dark, concentrated, and negative for bile pigments. 10.57 A.M. Hemoglobin appears in the urine. 11.20 A.M. Given 100 c.c. of 0.9 per cent. salt solution intravenously. 11.25 A.M. Urine is dark red in color. The oxyhemoglobin spectrum was characteristic. 12 c.c. give a negative test for bile pigments. 11.55 A.M. Urine is deep claret red in color. 17 c.c. give a faintly suspicious test for bile pigments. 12 M. Muscular tremors are noted in the neck and legs. 100 c.c. of 0.9 per cent. salt solution are given intravenously. 12.07 P.M. Injection of ½ gr. of morphia and 1/300 gr. of atropin intravenously. 12.20 P.M. Urine is deep claret red and contains a large amount of oxyhemoglobin. 10 c.c. give a faint but positive test for bile pigments. 12.20 P.M. The heart stops beating. The thorax is opened immediately and blood drawn from the heart with a pipette. The blood plasma is a deep claret red. 50 c.c. give a faint but positive test for bile pigments.
Autopsy.—Performed at once. The subcutaneous fat is scanty and of normal appearance. The heart shows numerous ecchymoses in the myocardium and subendocardial tissue. The spleen, pancreas, kidneys, and intestine are negative. The liver is pale yellow. Bile can be squeezed easily into the duodenum. The Eck fistula is perfect, but above the ligature on the portal vein are found two tiny collaterals just visible, leading from the gastrohepatic omentum into the portal vein at the hilum. The ligature on the portal vein occludes the lumen completely, but evidently a little collateral supply could gain entrance above the ligature. The hepatic artery was completely shut off from the liver.

Microscopical Examination.—The liver shows considerable atrophy particularly of the central parts of the lobules where the liver cells contain some pigment, and the blood vessels are dilated. There is practically no fat and only a small increase in connective tissue about the portal spaces. The myocardium shows no evidence of inflammation.

Dog C-S1.

| Time      | Urine color | Bile pigment test | Pulse | Respiration | Temperature, C. | Remarks                      |
|-----------|-------------|------------------|-------|-------------|-----------------|------------------------------|
| May 30    |             |                  |       |             |                 |                              |
| July 11   |             |                  |       |             |                 |                              |
| 8.30 A.M. | Brown       |                  | 0     |             |                 |                              |
| 10.40 A.M.|             |                  |       |             |                 |                              |
| 10.45 A.M.| Laked red cells given intravenously | 22 Brown | 0     |             |                 |                              |
| 10.55 A.M.|             |                  |       |             |                 |                              |
| 11.15 A.M.|             |                  | 80    | 30          | 37.0°           | 10.57 A.M. Oxyhemoglobin in urine + |
| 11.25 A.M.| Claret      |                  | 0     |             |                 |                              |
| 11.55 A.M.| Claret (?)  |                  | 120   | 24          | 38.4°           | Muscular tremors.             |
| 12.29 P.M.| Claret      |                  | (Faint) |             |                 | Death. Blood plasma (50 c.c.) faintly positive for bile pigment. |

The six preceding experiments are uniform in many respects. After an Eck fistula and hepatic artery ligation with the introduction of laked red cells intravenously, bile pigments appear in the urine within two hours, and at times within one hour. This reaction is similar to that noted in the preceding contribution in normal dogs or in animals with a simple Eck fistula. Removal of the spleen does not modify this experiment. The dogs die within a varying period, the maximum being six hours; death is preceded by muscular tremors which become more intense and merge into violent tetanic convulsions. The pulse, respiration, and temperature show interesting variations. When the operation for Eck fistula is performed and the hepatic artery is ligated after an interval, the dogs do not
Icterus.

survive as long as when the two operations are done at once, but the results are the same. When the Eck fistula is performed several days or weeks previously there is an opportunity for the formation of collateral blood vessels in the adhesions around the operative area. In one experiment due to an anomaly of the hepatic artery, one branch was not secured. The dog lived for twelve hours, but reacted normally to hemoglobin injected intravenously and began to show clearing of the urine which contained only traces of the pigment during the last hours of life.

LIVER EXCLUDED. HEMATOGENOUS JAUNDICE INCREASING.

Dog C-89.—Large bull dog, female; weight, 23 pounds.

June 27, 10.30 A. M. Ether anesthesia and bleeding from the jugular vein. The plasma after repeated centrifugalization was clear, but of a faint, pinkish tinge, and showed oxyhemoglobin bands when examined by the spectroscope. The leucocytic layer was several times thicker than normally. Urine (22 c.c.) is highly colored and concentrated, and gives a positive test for bile pigment. 11.05 A. M. Hepatic artery ligated as usual. 11.35 A. M. The Eck fistula is completed and the ligature tied on the portal vein. Omental fat (10 gm.) is negative for bile pigments. 11.40 A. M. Ether discontinued. 11.45 A. M. 250 c.c. of Ringer solution is introduced intravenously, and shortly afterwards ½ gr. of morphia dissolved in 50 c.c. of Ringer solution. 6 P. M. Animal is bled from the carotid into the oxalate. The plasma is a pale clear red and oxyhemoglobin bands are conspicuous. Plasma (50 c.c.) gives a positive test for bile pigments.

Autopsy.—Performed at once. The heart shows tiny vegetations on the mitral valve associated with hemorrhages 1 or 2 mm. in diameter on the valves above these vegetations and over the tips of the papillary muscles. The lungs are normal. The peritoneal cavity contains 30 c.c. of blood-tinged fluid. The spleen shows a few very small infarcts below the capsule and is somewhat swollen. The liver shows a mottled, reddish gray color. It is definitely softened and undergoing autolysis. The gall bladder and duodenum contain bile, and there is no obstruction in the course of the common bile duct. Ligatures on the hepatic artery shut off the arterial blood completely. The Eck fistula is large and patent. There were two small clots on the edge toward the vena cava. The ligature on the portal vein above the fistula closed the vessel completely and was placed just at the bifurcation of the vein at the hilum of the liver. No collaterals entered the portal vessels above this point. The kidneys are negative.

This experiment shows a cause for jaundice which may be present in dogs apparently in good health. The animal had a recent verrucose mitral endocarditis with a leucocytosis and definite hemolysis, the plasma showing definite bands of oxyhemoglobin and a faint
Dog C-89.

| Time       | Urine in c.c. | Color | Bile pigment test | Pulse | Respiration | Temperature, ° | Remarks                                                                 |
|------------|---------------|-------|-------------------|-------|-------------|----------------|-------------------------------------------------------------------------|
| June 26    | 20            | Brown | 0                 |       |             |                |                                                                         |
| June 27    | 22            | Brown | +                 | 200   | 60          | 36.8           | Omental fat (10 gm.) negative for bile pigments. 300 c.c. Ringer solution and 1/8 gr. morphia. Ether discontinued. |
| 10.30 A.M. | Brown         | +     | 160               | 30    |             | 37.5           |                                                                         |
| 11.00 A.M. | Amber         | +     | 140               | 85    |             | 38.0           |                                                                         |
| 11.30 A.M. | Amber         | +     | 120               | 48    |             | 38.8           |                                                                         |
| 12.00 A.M. | Amber         | +     | 160               | 42    |             | 38.1           |                                                                         |
| 12.30 A.M. | Amber         | +     | 160               | 38    |             | 38.0           |                                                                         |
| 1.00 A.M.  | Amber         | +     | 160               | 42    |             | 38.2           |                                                                         |
| 1.30 A.M.  | Amber         | +     | 160               | 42    |             | 38.1           |                                                                         |
| 2.00 A.M.  | Amber         | +     | 150               | 40    |             | 37.0           |                                                                         |
| 2.30 P.M.  | Amber         | +     | 140               | 38    |             | 37.5           |                                                                         |
| 3.00 P.M.  | Amber         | +     | 140               | 32    |             | 37.6           |                                                                         |
| 3.30 P.M.  | Amber         | +     | 140               | 24    |             | 37.4           |                                                                         |
| 4.00 P.M.  | Amber         | +     | 120               | 28    |             | 37.3           |                                                                         |
| 4.30 P.M.  | Amber         | +     | 120               | 28    |             | 37.3           |                                                                         |
| 5.00 P.M.  | Amber         | +     | 120               | 28    |             | 37.3           |                                                                         |
| 5.30 P.M.  | Amber         | +     | 120               | 28    |             | 37.3           |                                                                         |
| 6.00 P.M.  | Amber         | +     | 120               | 28    |             | 37.3           |                                                                         |
| 6.30 P.M.  | Amberg        | +     | 120               | 28    |             | 37.3           |                                                                         |
| 7.00 P.M.  | Bled from carotid |     |                   |       |             |                |                                                                         |

Pink color. It has been recognized that spontaneous jaundice might occur in dogs, and in the spring it is not uncommon, especially associated with a mild grade of distemper. This dog after the Eck fistula and hepatic artery ligation showed an increase in bile pigments in the urine, body fluids, and tissues. It is probable that there was increased hemolysis and absorption of hemoglobin from the peritoneum around the area of operation, as the plasma at autopsy was of a deeper pink than at the beginning of the experiment.

**Eck Fistula. Hepatic Artery Ligated. Control.**

*Dog C-47.—Mongrel terrier, female; weight, 18 pounds.*

July 16. Dog is in excellent condition. Urine (20 c.c.) is amber clear and contains no bile pigment.

July 17. Dog has been starved for twenty-four hours. 8.15 A.M. Urine (15 c.c.) contains no bile pigments. 10.45 A.M. Ether anesthesia. Bleeding from the jugular vein into the oxalate. The plasma after centrifugation was water clear with a faint amber tinge. 24 c.c. give a negative test for bile pigments. This clear plasma gives no oxyhemoglobin bands when examined with
the spectroscope. The dog is given \( \frac{1}{2} \) gr. of morphia and \( \frac{1}{300} \) gr. of atropin subcutaneously.

The operation was done with more difficulty than usual and a small Eck fistula was made. There was considerable bleeding into the peritoneal cavity at this time, but it was readily checked and most of the blood mopped out. The hepatic artery was ligated in three places as usual after the Eck fistula had been completed and finally the ligature on the portal vein above the fistula was tied. This gave an opportunity for beginning thrombus formation on the edges of the fresh fistula and the color of the intestine was rather bluish, indicating considerable obstruction to the venous flow.

11.45 A.M. 280 c.c. of 0.9 per cent. salt solution given intravenously. 11.55 A.M. Urine (30 c.c.) is rather turbid, amber yellow, and bile pigments are negative. Ether anesthesia discontinued. 12 M. 100 c.c. of 0.9 per cent. salt solution are given intravenously. Diuresis is not active. 12.30 P.M. 100 c.c. of 0.9 per cent. salt solution given intravenously. 12.45 P.M. Urine (5 c.c.) is concentrated and turbid, and negative for bile pigments. 4.45 P.M. Animal dies in severe convolution. The heart beats vigorously for one minute after the respirations ceased. The blood is removed immediately after death with a clean pipette from the auricle. Plasma after centrifugation shows a pale pink tinge, and the spectroscope shows oxyhemoglobin bands. 50 c.c. of plasma give a negative test for bile pigments.

**Autopsy.**—Performed at once. The peritoneal cavity contains unclotted blood, which is to be explained undoubtedly by the violent convolution just before

| Time       | Urine in c.c. | Color  | Bile pigment test | Pulse | Respiration | Temperature, °C | Remarks                                      |
|------------|---------------|--------|-------------------|-------|-------------|-----------------|----------------------------------------------|
| July 16    | Brown         | 0      |                    | 110   | 64          | 37.4            | Dog starved for 24 hrs.                      |
| July 17    | Brown         | 0      |                    | 140   | 18          | 37.3            |                                              |
| 10.45 A.M. | Salt solution intravenously, 280 c.c. | Amber | 0      | 140   |             | 37.7            |                                              |
| 11.45 A.M. | Beck fistula and hepatic artery ligated | Brown | 0      | 124   | 18          | 38.9            |                                              |
| 11.55 A.M. | Amber         | 0      |                    | 120   | 26          | 38.1            |                                              |
| 12.30 P.M. | Amber         | 0      |                    | 120   | 26          | 38.1            |                                              |
| 12.45 P.M. | Amber         | 0      |                    | 120   | 26          | 38.1            |                                              |
| 1.15 P.M.  | Amber         | 0      |                    | 120   | 26          | 38.1            |                                              |
| 1.45 P.M.  | Amber         | 0      |                    | 120   | 26          | 38.1            |                                              |
| 2.15 P.M.  | Amber         | 0      |                    | 120   | 26          | 38.1            |                                              |
| 2.45 P.M.  | Amber         | 0      |                    | 120   | 26          | 38.1            |                                              |
| 3.15 P.M.  | Amber         | 0      |                    | 120   | 26          | 38.1            |                                              |
| 3.45 P.M.  | Amber         | 0      |                    | 120   | 26          | 38.1            |                                              |
| 4.15 P.M.  | Amber         | 0      |                    | 120   | 26          | 38.1            |                                              |
| 4.45 P.M.  | Death; convulsions | Amber | 0      | 120   | 26          | 38.1            |                                              |
| Autopsy    | Amber         | (?)    |                    | 120   | 26          | 38.1            |                                              |

**Dog C-41.**

Plasma (50 c.c.) and peritoneal fluid (4 c.c.) pale pink in color and negative for bile pigment.
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death. There are also a few blood clots to be seen. The thorax, heart, and lungs are normal. The intestine shows a moderate congestion of the serous surface, but a deep red velvety mucosa indicating considerable obstruction to the portal outflow. The Eck fistula shows partial occlusion by a fresh thrombus adherent to one margin. The ligature on the portal vein above the Eck fistula completely occluded the lumen and careful dissection revealed no collaterals above it opening from the gastrohepatic omentum into the portal vein. The subcutaneous, perirenal, and mediastinal fat are all normal.

**Dog C-105.**—Mongrel hound, female; weight, 24 pounds.

July 12. Urine is concentrated. 25 c.c. are negative for bile pigments.

July 13. Dog has been starved for thirty-six hours. 8:30 a.m. 25 c.c. of concentrated urine are negative for bile pigments. 9:45 a.m. Ether anesthesia with bleeding from the jugular vein. Plasma is water clear, and the spectrum is negative for oxyhemoglobin. 25 c.c. of plasma give a negative test for bile pigments. 11:06 a.m. The Eck fistula is completed with much bleeding and injury to the tissues because of a band which had been previously placed upon the left renal vein. This old operation had been associated with much inflammatory reaction and thickening of the inferior vena cava. There was a slight amount of laceration of the liver and about 30 c.c. of blood escaped into the peritoneal cavity. This was mopped out carefully. The fistula was of small size. 11:10 a.m. The hepatic artery is ligated securely as usual. 11:15 a.m. 250 c.c. of 0.9 per cent. salt solution are introduced into the jugular vein. 1/8 gr. of morphia and 1/300 gr. of atropin are given subcutaneously. 11:25 a.m. 200 c.c. of salt with 1/100 gr. of hirudin are given intravenously, the hirudin to prevent any thrombosis in the neighborhood of the Eck fistula. 4:30 p.m.

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### Dog C-105

| Time          | Urine in c.c. | Color | Bile pigment test | Pulse | Respiration | Temperature, °C | Remarks                                |
|---------------|---------------|-------|-------------------|-------|-------------|-----------------|----------------------------------------|
| July 12       | 25            | Brown | 0                 | 188   | 42          | 39.0            | Dog starved for 36 hrs.                |
| July 13       | 25            | Brown | 0                 | 160   | 40          | 38.9            |                                        |
| 9:45 to 11:10 |               |       |                   |       |             |                 |                                        |
| A.M.          |               |       |                   |       |             |                 |                                        |
| 11:15 to 11:30|               |       |                   |       |             |                 |                                        |
| A.M.          | 450 c.c. salt solution given intravenously |       |                   |       |             |                 |                                        |
| 11:40 A.M.    | 4             | Brown | 0                 | 160   | 40          | 39.3            | 1/8 gr. morphia, 1/300 gr. atropin.    |
| 12:10 P.M.    | 4             | Brown | 0                 | 160   | 40          | 38.9            | 100 c.c. salt solution.                |
| 12:40 P.M.    | 10            | Amber | 0                 | 160   | 40          | 39.0            | 100 c.c. salt solution.                |
| 1:10 P.M.     | 15            | Brown | 0                 | 160   | 36          | 38.6            | Panting respiration.                  |
| 1:40 P.M.     | 22            | Brown | (?)              | 160   | 36          | 39.4            | Muscular tremors.                     |
| 2:10 P.M.     | 25            | Brown | (?)              | 160   | 40          | 39.3            |                                        |
| 2:40 P.M.     | 18            | Brown | (?)              | 154   | 160         | 39.3            |                                        |
| 3:10 P.M.     | 17            | Brown | +(Faint)          | 160   | 70          | 39.3            |                                        |
| 3:40 P.M.     | 23            | Red   | +                 | 200   | 80          | 39.4            |                                        |
| 4:10 P.M.     | 18            | Brown | +(Faint)          | 160   | 28          | 39.0            |                                        |
| 4:30 P.M.     | 24            | Brown | (?)              |       | —           |                 |                                        |
Death in convulsions. The thorax is opened immediately and blood taken from the heart with a clean pipette. Plasma obtained after repeated centrifugalization is a definite pink color showing conspicuous oxyhemoglobin bands. 50 c.c. give a negative test for bile pigments.

**Autopsy.**—Performed at once. The peritoneal cavity contains about 50 c.c. of claret colored fluid which clots in three or four minutes. The serum squeezed out is of a cherry red color. 20 c.c. give a negative test for bile pigments. The heart, lungs, intestine, and spleen are all normal. The liver shows a mottled reddish gray color, and is soft and juicy. On section the liver is evidently undergoing autolysis. The gall bladder is flabby and collapsed. The common duct is normal. The Eck fistula is perfect. One small clot was found on the edge but the lumen is clear. The ligature tightly occludes the portal vein above the fistula and no collaterals open in above the ligature. The portal vein above the ligature shows a definite clot adherent to the intima injured by clamping and tying the vessel. The hepatic artery is completely occluded by ligatures.

**Eck Fistula. Control.**

*Dog C-99.*—Fox terrier, female; weight, 14 pounds.

July 3, 9,30 A.M. Dog is in good condition. 60 c.c. of concentrated urine give a negative test for bile pigments. 11.15 A.M. Ether anesthesia. Operation and production of Eck fistula in the usual way. There was some bleeding (20 c.c.) at the time of operation, but this was controlled and the abdomen mopped out. The abdominal cavity contains a little blood when closed. 12.15 P.M. The fistula is complete and the ligature tied on the portal vein above the fistula. 12.40 P.M. Given 200 c.c. of Ringer solution in which was dissolved ½ gr. of morphia. Bile pigments in small amounts appear in the urine after two hours, but disappear before the end of the observation. 3 P.M. Dog is bled from the jugular vein into the oxalate in the usual way. The plasma shows a
definite pink tinge and the spectrum shows the presence of oxyhemoglobin bands. This plasma was obtained after two separate centrifugalizations with pipetting off of the supernatant fluid, and indicates that there must be more or less absorption of hemoglobin from the site of operation.

July 4. Urine is negative for bile pigment.

July 19. Ether anesthesia and bleeding from the carotid. The plasma and fat obtained at autopsy give negative tests for bile pigments. The heart contains many filariae. The lungs, spleen, and intestine are normal. The liver is somewhat pigmented and pale brown in color. The Eck fistula is perfect. The ligature present at the hilum of the liver completely occludes the portal vein and there are no collaterals opening in above this ligature.

The three preceding experiments serve as controls on the operative procedure. Bile pigments in small amounts may appear in the urine following the operation, but the appearance of the pigments is late and the duration short. Blood examination shows the presence of a definite amount of hemoglobin in the plasma from which the bile pigments in very small amounts are formed. The presence of this hemoglobin in the plasma is probably due to absorption from the field of operation, especially the peritoneal cavity, but may be due in part to the long anesthesia and the introduction of large amounts of normal saline or Ringer solution to promote a brisk diuresis. The fluid at autopsy obtained from the peritoneal cavity gives a negative test for bile pigments, so it is unlikely that any preformed bile pigments were absorbed from this region following the operative manipulations.

LIVER AND ABDOMINAL VISCERA EXCLUDED. HEMATOGENOUS JAUNDICE.

Dog C-97.—Strong brindle bull, female; weight, 32 pounds.

July 16. Dog is in excellent condition. 20 c.c. of urine give a negative test for bile pigments.

July 17, 8.30 A. M. 20 c.c. of urine give a negative test for bile pigments. 10.45 A. M. Given ½ gr. of morphia subcutaneously. 11 A. M. Ether anesthesia with bleeding from the external jugular vein. The plasma is slightly turbid due to lipemia, but the spectrum is negative. Bile pigment test (25 c.c.) is negative. 11.30 A. M. Artificial respiration is established and the mammary vessels ligated through the thorax. 11.40 A. M. The thoracic aorta just below the subclavian artery is closed securely by a screw clamp. 11.45 A. M. The abdominal aorta is clamped at the celiac axis and the inferior mesenteric artery is clamped securely. The aorta is clamped again just above its bifurcation. The portal vein at the hilum of the liver is securely ligated. The vena cava is left intact. 11.50 A. M. After these vessels are secured, 25 c.c. of washed laked red blood cells are introduced intravenously. The animal is kept under
light anesthesia during the entire observation. 12.20 P. M. The color of
the tongue and mucous membrane is a bright vivid pink, and pressure shows
that the capillary circulation is excellent. The heart pulsation is strong
and regular. 12.45 P. M. Many of the smaller vessels which were severed
at the time of operation through the thorax begin to bleed, evidently due
to rise in blood pressure. They are carefully secured by clamps. 1 P. M.
Dog is in excellent condition and was given 50 c.c. of 0.9 per cent. salt
solution containing 0.1 per cent. dextrose. 1.49 P. M. The color of the
tongue and mucous membranes is excellent. 2.15 P. M. Muscular tremors
are noted in the neck and forelegs. The heart pulsations are strong and regular.
Given 50 c.c. of 0.9 per cent. salt solution containing 0.1 per cent. dextrose and
½ gr. of morphia. 2.25 P. M. Muscular tremors are becoming more violent.
The dog is given ½ gr. of morphia subcutaneously in the neck. 3.15 P. M.
The color of the tongue and mucous membranes is good and the circulation is
active. Muscular tremors have ceased. 3.30 P. M. Given 50 c.c. of 0.9 per cent.
salt solution with 0.1 per cent. dextrose. 4 P. M. Given 50 c.c. of a
similar solution intravenously. 4.15 P. M. The color of the mucous mem-
branes is bright pink. 4.40 P. M. The heart stops beating. There are no
tetanic convulsions. The thorax is opened at once and blood is drawn from the
heart with a clean pipette. The blood plasma after centrifugalization is pale
claret red. The spectrum shows conspicuous oxyhemoglobin bands. 50 c.c. of
plasma give a strong positive test for bile pigments. 25 c.c. of serum likewise
give a strong test for bile pigments.

Autopsy.—Performed at once. The subcutaneous, intercostal, supraclavicular,
mediastinal, and epicardial fat is of a canary yellow color suggesting a definite
jaundice, and gives a positive test for bile pigments. 6 gm. of epicardial fat
give a strong positive bile pigment test; 8 gm. of intercostal fat give a fainter
positive test; 13 gm. of mediastinal fat give a faintly positive test; 27 gm.
of supraclavicular and subcutaneous fat give a strong positive test for
bile pigment. Fat from the omentum and about the kidneys is normal in
appearance. 18 gm. of this fat give negative tests for bile pigments. The
heart is dilated and shows a few ecchymoses below the intima of the left
ventricle. The lungs and viscera in general are normal. The intestine, stomach,
and spleen show definite post-mortem changes, with much gas formation in the
lumen of the small intestine. The liver is small and contains less blood than

| Bile pigment tests. | Urine. | Blood. | Fat. |
|--------------------|--------|--------|-----|
| Before operation   |        |        |     |
| After operation    |        |        |     |
|                    | 20 c.c. negative | 25 c.c. negative |
|                    | Liver and abdominal viscera excluded for 5 hours |
|                    | Plasma (50 c.c.) ++ | Epicardial (6 gm.) ++ |
|                    | Serum (25 c.c.) ++ | Intercostal (8 gm.) + |
|                    | Mediastinal (13 gm.) +  |
|                    | Supraclavicular (27 gm.) ++ |
|                    | Omental (18 gm.)— |
|                    | Perirenal (18 gm.)— |
normally and shows no gross evidence of autolysis. The gall bladder is flaccid and the common duct is patent. The portal vein shows a trifling amount of congestion but no marked engorgement, indicating the absence of any collateral supply to this area. The peritoneal cavity contains about 5 c.c. of blood-tinged fluid.

The preceding experiment answers most of the objections that can be brought forward in criticism of the Eck fistula hepatic artery ligation group of experiments. In this experiment the portal and arterial blood is shut out from the liver and abdominal viscera in general, and with a clamp upon the aorta below the subclavian artery there can be no active circulation through any of the diaphragmatic and ligamentary collaterals to the liver. This practically excludes all seepage through the capsular collaterals of the liver and means that the liver is cut off from all active association with the circulating blood. Because of the respiratory movement, a to and fro motion of the blood in the hepatic veins and vena cava may take place and bring a certain amount of blood in contact with liver tissue. That this could be sufficient for any active participation of the liver cells in the transformation of hemoglobin into bile pigments is unlikely. The series of experiments given below answers this question. The vena cava was left intact so that it might return the small amount of blood which escapes by collaterals along the costal margin, in the vertebral column, and attachments of the diaphragm.

**HEMORRHAGIC JAUNDICE.**

*Dog C-106.*—Large bull dog, female; weight, 38 pounds.  
July 15. Dog is in excellent condition; starved for twenty-four hours.  
9 A. M. 25 c.c. of concentrated urine are negative for bile pigments. 10.20 A. M. Given ½ gr. of morphia and 1/300 gr. of atropin subcutaneously.  
10.30 A. M. Ether anesthesia and bleeding from the external jugular vein. The plasma is water clear. 35 c.c. of plasma give negative tests for bile pigments. 10.35 A. M. Given ½ gr. of morphia and 1/300 gr. of atropin subcutaneously. 11 A. M. Artificial respiration begun. Mammary vessels ligated.  
11.05 A. M. The thoracic aorta is clamped just below the subclavian artery.  
11.10 A. M. The abdominal aorta is ligated at the celiac axis. Wire ligatures are placed through the recti muscles and along the costal margin to control the collateral vessels. 11.15 A. M. The vena cava is ligated above the diaphragm and 25 c.c. of laked corpuscles are introduced intravenously. 11.30 A. M. The color of the tongue and mucous membranes is good. The heart pulsation is strong and regular. 11.45 A. M. 50 c.c. of normal salt solution containing 0.1
per cent. dextrose are given intravenously. 12.25 P. M. The condition is uniformly excellent. 25 c.c. of salt solution are given intravenously as before. 1 P. M. The color of the tongue is excellent. The heart pulsates regularly. 25 c.c. of 0.9 per cent. salt solution given intravenously. 1.15 P. M. Muscular tremors are noted in the neck. 1.30 P. M. The heart stops pulsating but the circulation had been vigorous and active up to within five minutes before this time. Blood taken at once from the heart clotted in the normal way and the serum is a cherry red color. 50 c.c. of this serum give a strong test for bile pigments.

_Autopsy._—Performed at once. The fat of the thorax, mediastinum, and epicardium shows a definite jaundice tinge as compared with that below the diaphragm. The supraclavicular fat (27 gm.) and mediastinal fat (16 gm.) give positive tests for bile pigments; the epicardial fat (12 gm.) gives a strong positive test for bile pigments; the omental fat and the subcutaneous fat below the diaphragm are normal in color. The omental fat (22 gm.) gives a negative test for bile pigments, the inguinal fat (30 gm.), and retroperitoneal fat (38 gm.) likewise give negative tests. The heart is greatly dilated. The lungs are normal. The spleen is considerably engorged. The liver is distended with blood. The vena cava and branches together with the kidneys are also engorged. All the ligatures at the places indicated are effective and close the lumen of the vessels completely.

_Dog C-106._

| Bile pigment tests. | Urine | Blood | Fat |
|--------------------|-------|-------|-----|
| Before operation    |       |       |     |
| 25 c.c. negative    | 35 c.c. negative | Serum (50 c.c.) ++ + |
| Head and thorax     | circulation, 21/4 hrs | Supraclavicular (27 gm.) + |
| After operation     |       |       |     |
| 25 c.c. negative    | 35 c.c. negative | Mediastinal (16 gm.) + |
| Head and thorax     | circulation, 21/4 hrs | Epicardial (12 gm.) ++ |
| 25 c.c.             |       |       |     |
| Serum (50 c.c.)     |       |       |     |
| ++                  |       |       |     |

_Dog C-12._—Large pointer, male; weight, 44 pounds. June 22. Dog is in excellent condition; starved for twenty-four hours. 9.30 A. M. Ether anesthesia with bleeding from the femoral artery. Plasma is water clear. 25 c.c. give a negative test for bile pigments. 10 A. M. Given ½ gr. of morphia dissolved in 50 c.c. of 0.9 per cent. salt, intravenously. Artificial respiration begun. 10.15 A. M. The aorta ligated with wire just beyond the subclavian artery. 10.20 A. M. The mammary vessels are ligatured and wire ligatures placed across the recti muscles and along the costal margin to control the collateral circulation. 10.30 A. M. The vena cava is ligated above the diaphragm and immediately afterwards 25 c.c. of red blood cells laked with distilled water are given into the jugular vein, followed by 85 c.c. of 0.9 per cent. salt solution. 11.10 A. M. 50 c.c. of salt solution is given into the jugular vein. 12.15 P. M. The pulse is strong and regular. The tongue is bright red. 25 c.c. of salt solution are given intravenously. 12.50 P. M. The color of the
tongue has become bluish.  1 P. M. The heart stopped beating. The thorax is opened and blood is taken from the heart with a clean pipette. Plasma after centrifugalization is of a deep claret red. 30 c.c. of serum give a positive test for bile pigments. 50 c.c. of serum in the second test give a strong positive test for bile pigment.

**Autopsy.**—Performed at once. The fat over the thorax in the intercostal spaces, in the supraclavicular fossa, in the mediastinum, and the epicardium shows a definite jaundice tinge. 4 gm. of this material extracted with hot acid alcohol give a pale green color indicating the presence of bile pigment. The fat in the omentum and abdomen is normal in appearance. The line of demarcation between the normal and abnormal fat is along the costal margin, one fading gradually into the other. 4 gm. of omental fat treated in a similar way give no color reaction test. The heart and lungs are negative. The spleen is engorged, purple, and very soft. The liver is acutely engorged and deep purple. The ligature on the vena cava occludes the lumen completely and there are soft purple clots in the vein below the ligature extending into the hepatic veins. The intestinal mucosa shows a good deal of engorgement, giving it a pinkish color. The kidneys are a deep purple color and show great vascular engorgement. The ligature on the aorta had cut into the wall with considerable injury to the vessel, and it is possible that a little blood could pass the ligature.

**Dog C-zo2.**—Bull dog, female; weight, 27 pounds.

July 9, 9 A. M. 20 c.c. of urine give a negative test for bile pigments. 10.15 A. M. Dog is given 600 c.c. of water by stomach tube. 11.10 A. M. Ether anesthesia and bleeding from the jugular vein. Plasma is water clear. 50 c.c. give a negative test for bile pigment. 11.20 A. M. Given ½ gr. of morphia dissolved in 25 c.c. of Locke's solution, intravenously. 11.35 A. M. Artificial respiration started. 11.40 A. M. Ligation of mammary vessels. 11.46 A. M. The aorta is ligated with wire just below the subclavian artery. 11.50 A. M. A clamp is put across the aorta including the celiac axis. Heavy wire ligatures are put across the recti muscles and along the costal margin to occlude collaterals. 12 M. The vena cava inferior is ligated just above the diaphragm and 25 c.c. of washed, laked red blood cells are introduced intravenously with 50 c.c. of Locke's solution. 1 P. M. Condition is very good. The tongue and mucous membranes are a bright pink color. The heart-beat is strong and regular. Given 50 c.c. of Locke's solution intravenously. 1.45 P. M. Muscular tremors are noted in the neck muscles. 1.55 P. M. The heart stops beating. The thorax is opened at once and blood taken from the heart. The plasma after centrifugalization appears claret red. Oxyhemoglobin bands are conspicuous. 65 c.c. of plasma give a very faint but positive test for bile pigments.

**Autopsy.**—Performed at once. Fat in the supraclavicular, intercostal, and epicardial tissue has a definite canary yellow color as compared with the fat in the groin and omentum. 20 gm. of supraclavicular fat give a positive test for bile pigments; 5 gm. of epicardial fat give a positive suspicious test for bile pigments; 25 gm. of fat from the groin give a negative test for bile pigments. Examination of the vessels shows that all the ligatures are effective. The abdominal viscera as usual show engorgement.
HEAD AND THORAX CIRCULATION. JAUNDICE NOT POSITIVE.

Dog C-100.—Large male; weight, 36 pounds.

July 5. Dog has been starved for twenty-four hours and is in excellent condition. 9 A. M. Given 800 c.c. of water by stomach tube. 10.20 A. M. Ether anesthesia. A large amount of urine is collected while the animal is going under the anesthetic. 50 c.c. of this urine are negative for bile pigments. 10.25 A. M. Dog given intravenously ¾ gr. of morphia dissolved in a little salt solution, after bleeding from the jugular vein. Plasma after centrifugation is water clear. 25 c.c. give a negative test for bile pigment, and the spectrum shows no oxyhemoglobin bands. 10.40 A. M. Artificial respiration begun. 11 A. M. The aorta occluded by a strong clamp is placed just below the subclavian artery and the mammary vessels are ligated. 11.10 A. M. The vena cava is tied above the diaphragm. 11.15 A. M. Red blood cells (25 c.c.) laked with an equal volume of distilled water are given intravenously, followed by 50 c.c. of Ringer solution. 11.45 A. M. The color of the mucous membranes and tongue is bright pink. The heart-beat is regular and strong. 12.15 P. M. Condition continues the same. 1.15 P. M. Muscular tremors are noted in the neck and the color of the tongue is poor. 1.50 P. M. Death. The thorax is opened and blood is taken directly from the heart. After centrifugation the plasma is claret red in color. The tests made with 25 c.c. of plasma were not positive but give a suggestive reaction for bile pigments.

Autopsy.—Performed at once. The fat in the abdominal cavity is normal in appearance, except for congestion. The fat in the supraclavicular fossa has a faint suggestion of bile tingeing, but it was not definite. The epicardial fat also

**Bile Pigment Tests. Dog C-13.**

|                      | Urine. | Blood. | Fat. |
|----------------------|--------|--------|------|
| Before operation     | 25 c.c. |        |      |
| After operation      |         | Serum (30 c.c.) + | Epicardial (4 gm.) + |
|                      |         | Serum (50 c.c.) ++ | Omental (4 gm.) -- |

**Dog C-102**

|                      | 20 c.c. | 50 c.c. |      |
|----------------------|--------|--------|------|
| Before operation     |        | Head and thorax circulation for 2 hrs. | |
| After operation      | 65 c.c. + (faint) | SuprACLAVICULAR (20 gm.) + |
|                      |         | Epicardial (5 gm.) + (?) |
|                      |         | Ingual (25 gm.) -- |

**Dog C-100**

|                      | 50 c.c. | 25 c.c. |      |
|----------------------|--------|--------|------|
| Before operation     |        | Head and thorax circulation for 2½ hrs. | |
| After operation      | 25 c.c. |        |      |
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had a suggestive color, but this was not striking. This fat was tested in the usual way for bile pigments with negative result (26 gm. of supraclavicular, 10 gm. of cardiac, and 32 gm. of omental fat, in separate specimens). The aorta is completely occluded by the clamp and the vena cava by the ligature. The spleen and viscera below the diaphragm show a good deal of engorgement.

The four preceding experiments give the final proof that the liver is not essential to a rapid change of hemoglobin into bile pigment. Of the four experiments one was not definitely positive but the others were positive in every respect and demonstrate that hemoglobin circulating actively in the head and thorax for a space of two hours will be in part changed to bile pigment. Not only the blood serum from the heart, but also the fat in the epicardial, mediastinal, intercostal, and supraclavicular regions contain readily demonstrable amounts of bile pigment. The zone of demarcation shows in the gradual change from the canary yellow fat of the thorax to the normal abdominal and peritoneal fat. No objections can be brought against these experiments on the ground of trauma and operative manipulation of the liver, for the organ was not disturbed at any time and the bile pigments cannot be derived from injured liver parenchyma.

It will be seen from this and the preceding paper that hemoglobin has been introduced into the circulation of dogs, first normal, second with Eck fistula, third with Eck fistula and hepatic artery ligation, fourth with exclusion of the liver, spleen, and intestines, and lastly with head and thorax circulation. This gives a series in which the liver circulation diminishes from normal to zero and the same ratio must hold for its physiological activity and participation in the systemic reactions. The change of hemoglobin to bile pigment goes on in much the same way and in about the same time under these different experimental conditions, from one to two hours being necessary for the elaboration of bile pigment. It must be remembered that it is easier to detect bile pigment in the urine than in the body fluids and tissues.

What tissues or cells may be concerned in this reaction? In the head and thorax circulation experiments one may imagine the change to be effected by (1) endothelium, (2) bone marrow, or (3) supporting tissues or muscle. There is evidence that the endothelium
may be the active factor. It is known that endothelium is capable of engulfing and digesting red cells and in certain diseases this activity is striking. The bone marrow cannot be ruled out as a factor. One possible way of settling this point is by means of a heart and lung circulation, and we hope to report further on this subject.

Those who believe that the liver is necessary for the formation of bile pigment may support the theory that the liver elaborates a ferment, present in the blood, which brings about the change of hemoglobin to bile pigment. Experiments with blood serum, hemoglobin, and various organ extracts in vitro do not bear out this theory, but cannot be said definitely to disprove it.

**SUMMARY.**

The intravenous injection of red cells obtained from the same animal and laked by distilled water is similar to certain types of hemolysis which result in hematogenous jaundice. This procedure cannot be criticized on the grounds of introducing toxic substances. The hemoglobin circulating in the blood stream is rapidly changed, in part at least, to bile pigment. The change goes on with practically the same rapidity in a normal circulation, in an Eck fistula animal, and in a dog with Eck fistula and hepatic artery ligation. Moreover, the bile pigment formation goes on in a dog whose liver, spleen, and intestines have been shut out of the circulation, and in those with a head and thorax circulation. In the last experiments there had been no operative manipulation of the liver and the bile pigment could not have escaped from the liver and have been absorbed by the circulation above the diaphragm; for example, by the thoracic duct. It is possible that the endothelium of the blood vessels is the agent which brings about the rapid change of hemoglobin to bile pigment. This mechanism probably comes into play when there has been a destruction of many red cells with much hemoglobin free in the plasma. We may conclude that in dogs, at least, hemoglobin can be rapidly changed into bile pigment in the circulating blood without participation of the liver.
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