THE PATHOLOGY OF INTRAPERITONEAL BILE INJECTIONS IN THE RABBIT.*

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Sufficient clinical and experimental evidence has accumulated to demonstrate clearly that bile is a toxic secretion. From a casual review of literature bearing on this toxicity, there would seem to be a tendency on the part of investigators to assume that results obtained with the bile of one species of animal were valid when applied to the bile of all other species. Such an assumption would be justifiable if the sole toxic elements in the bile were the bile salts and the bile pigment, the only variation being the variation in concentration of these, with a further possible variation due to differences that may exist in the toxicity of bilirubin and biliverdin. However, if Meltzer and Salant[1] are correct in their conviction that the effect of the bile is not due to the sum of effects produced by the known components, generalizations in regard to bile would not seem valid, and the toxic effect of the bile of each species would constitute a distinct problem.

Certain experiments carried out in this laboratory would seem to indicate the truth of this latter contention, in demonstrating that rabbit bile is much more toxic to the rabbit than the bile of many other animals is to themselves or to the rabbit, if we may rely on the reports in literature. The experiments were undertaken originally to determine the part played by the various factors in the death of an animal, which, following a general *Bacillus coli* septicemia, had succumbed to a perforating suppurative cholecystitis. The results to be reported concern only the effect of the whole bile from the rabbit's gall bladder, when allowed to escape or when injected into the peritoneal cavity of the rabbit. We have

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[1] *Jour. Exper. Med.*, 1905, vii, 280.

not as yet attempted the separation of the bile into its components, nor have we pushed to completion the toxic effects of the bile when introduced intravenously or subcutaneously.

In the series of cases here reported, the abdominal cavity of the rabbits has, as a rule, been opened high in the mid-line, under strict surgical technique, and with ether anesthesia. The gall bladder has been drawn down, and either it has been opened by cutting off the tip or the contents have been aspirated through a fine needle. The bile thus obtained has been allowed to drip on to the peritoneal surfaces, in some cases in the mid-line, over the anterior surface of the stomach, and in other cases into either the right or the left half of the cavity. The wound has then been closed by separate suturing of the peritoneum and fascia and of the skin. The whole period of anesthesia has varied between twenty and thirty minutes. In the later cases, the aspiration method has been used in an attempt to control the dose of bile. The gall bladder may contain from 0.5 to 2.5 cubic centimeters, and simple incision of the bladder allows no opportunity to estimate the amount which escapes. In some cases, the bile of another rabbit has been injected either through the abdominal wall of the recipient, without anesthesia, or through a minute incision of the parietes with the slightest amount of ether anesthesia.

In considering the results of these experiments, there are two features to be noted: the general toxic action of the bile, and the direct pathological effect. It seems apparent from the series that the bile of all rabbits is not equally toxic. The perfectly clear light green bile has seemed somewhat more toxic than the dark bile with much precipitated pigment. We would not wish to imply, however, that our results necessarily show that the toxicity of the bile is dependent upon the amount of pigment in solution or in combination. While we have not, therefore, determined absolutely the minimal fatal dose of bile when injected into the peritoneal cavity, yet our experiments indicate that it lies between 0.25 and 0.50 of a cubic centimeter per kilogram, the latter dose constantly producing a fatal result in less than twenty-four hours. This seems to be about twice the dose necessary to kill when introduced intravenously. Death is apparently due to direct

action of the bile upon the myocardium. The local pathological effects may be very briefly summarized in the statement that bile apparently produces necrosis of every type of tissue with which it comes in contact. The details of this process will be brought out in a consideration of the individual cases.

It does not seem necessary to give complete protocols of all experimental animals. The delineation of the findings in a typical case, with a brief description of the distinctive features of the other cases, should satisfy scientific requirements. The following case may be taken as a type.

Rabbit F. N. xx.—Male, albino; weight 2,250 grams.

January 6, 1911. 2 P. M. Under ether, the gall bladder was aspirated and 1 c.c. of the clear green bile obtained was allowed to drip over the anterior surface of the stomach and into the left half of the abdominal cavity. The edge of the liver was also touched accidentally. Wound closed. Duration of anesthesia twenty-five minutes.

January 7. Animal died during night. Post mortem examination shows a marked ventral subcutaneous, dark, slightly bile-stained, and hemorrhagic effusion extending from the groins to high up on the thorax. Upon opening the peritoneal cavity, the serous surfaces are found smooth and glistening and free from exudate except for a few small flecks of fibrin near the gall bladder wound. There is a considerable amount of bile-stained serous exudate in the cavity. Both pleural cavities also show a serous exudate, that on the right being bile-stained.

The *heart* is very pale and widely distended, both ventricles being filled with soft, dark clots. The *lungs* are air-containing throughout, but both show congestion and edema of the lower lobes, especially the right lung which is also somewhat bile-stained. (The right half of the diaphragm is bile-stained throughout its thickness.) The *spleen* is noticeably enlarged, is of a dark purplish color, and shows opaque greenish spots on its capsule. The *liver* is large, and pale and has, especially on its under surface, large areas of dead opacity, slightly bile-stained. The cut surface is cloudy, with a subcapsular, narrow, opaque line. On the convex surface of the *stomach* there are opaque greenish spots. The splenic end of the *pancreas* is injected, swollen, and opaque in appearance. In the omental, mesenteric, and especially in the peripancreatic fat, there are small opaque dots of from 1 to 2 mm. in diameter. The *kidneys* are large, dark in color, and somewhat mottled. The cortex is increased in width and has an opaque greyish color with a brownish cast. The vessels are injected. The *thyroid* is pale and greyish. The individual acini are not distinguishable.

Microscopical Examination. Heart.—Throughout the left ventricle wall, there are scattered groups and isolated muscle fibers which are hyaline, often vacuolated, with pyknotic nuclei but with no trace of striation to be made out. There is some fragmentation and segmentation of fibers. The vessels are injected and there is slight edema.

Lungs.—Section through the lower right lobe shows congestion, extensive edema, and necrosis of the pleura with a beginning leukocytic reaction.

Liver.—The liver shows in general marked swelling of the cells with fatty degeneration in the mid and central zones of the lobule. The peritoneal epithelium and the three or four rows of liver cells immediately below the capsule show complete necrosis, with beginning leukocytic invasion.

Spleen.—The spleen shows areas of capsular necrosis covered by flecks of fibrin. The organ itself gives the picture of an acute splenic tumor of the hyperemic type, with pigment present in many pulp cells. There is much fragmentation of nuclei in the splenic cells.

Pancreas.—The pancreas shows interlobular edema, some hemorrhage, and slight necrosis of surface acini, with leukocytic reaction. In the section, there are several areas of typical fat necrosis, the fatty acid crystals appearing as sheaves of fine needles in the necrotic cells. In some areas of fat necrosis, a yellowish pigmentation is apparent.

Kidneys.—The convoluted tubules show necrosis of the epithelium, the nuclei failing entirely to stain. The collecting tubules and glomeruli show nuclear stain. Hyaline casts are present and many tubules show fine yellowish granules which give the Prussian blue reaction for iron. There is edema and congestion of the kidney.

Stomach.—The stomach shows necrosis of the outer layers of muscle.

Diaphragm.—The right side shows complete necrosis of the surface and of the muscle. The muscle fibres are much smaller and hyaline and without nuclear stain.

The muscle of the abdominal wall shows a similar necrosis, and to some extent a disintegration of the fibers. The subcutaneous tissue shows edema, with a very slight leukocytic content.

Thyroid.—The alveoli are small and in both lobes are almost entirely free from colloid. Some of the larger alveoli show, on the contrary, a fibrin network and a finely granular albuminous precipitate.

With the formalin fixation, the blood collections in the section show the brownish granules of hemochromogen and hematin.

The general features of this description will apply to some ten other rabbits of the experimental series, which either died within twenty-four hours after the injection or were killed a short time thereafter. The bile-stained peritoneal effusion and subcutaneous edema have been constant and extensive, especially in the acutely fatal cases. The latter has been due either to bile-staining of the edges of the wound at the time of operation or to leakage of bile along the sutures. Necrosis of the muscle fibres of the abdominal wall has been shown by all. The pale, widely dilated heart with hyaline degeneration and necrosis of muscle fibres in its wall has been constant. The surface necrosis of the liver has occurred whenever bile has touched it and has usually been most marked around the gall bladder leak. Fat necrosis has been a constant

finding, usually in association with hemorrhagic necrosis of the pancreas. The kidneys in all cases have shown signs of toxic action. In them there has been constantly marked congestion, minute hemorrhages, degeneration and necrosis of the tubular epithelium, and casts and hemoglobin within the tubular lumina. From the large amount of pigment precipitated from the blood cells by the formalin fixation, and from the hemoglobin staining of tissues, there has evidently been blood destruction which is further indicated by the acute splenic tumor and the pigment accumulations in the splenic pulp cells.

The special features of the post mortem findings may best be brought out by the condensed protocols. The findings here recorded were confirmed by microscopical examination.

Rabbit F. N. 1.—Female, albino. March 17, 1910. Under ether, the bile duct was tied and the gall bladder opened by a wide incision.

March 18, 1910. Rabbit died during night. Post mortem findings in general as in rabbit xx. Degeneration of heart muscle; necrosis of under surface of liver; edema, necrosis, and slight leukocytic invasion of pancreas; fat necrosis.

Rabbit vi.—Male, Belgian; weight 2,500 grams. March 18. Under ether, 0.65 c.c. of bile was aspirated from the gall bladder and allowed to drip over the anterior surface of the stomach in the mid-line.

March 19. Animal found dead. Dilation and degeneration of heart. Congestion of all viscera. Necrosis of under surface of liver; acute hemorrhagic necrosis of pancreas; peripancreatic fat necrosis; hemorrhagic infarction of a loop of intestine due to thrombosis of mesenteric vessels secondary to bile necrosis of walls of vessels; degeneration of kidney; casts; hemoglobinuria.

Rabbit vii.—Male, Belgian; weight 3,000 grams. March 18. During an attempt to aspirate the gall bladder, it was torn and the bile escaped into the peritoneal cavity in the neighborhood of the gall bladder.

March 19. Animal found dead in morning. Cardiac dilatation. Necrosis of liver. Acute pancreatitis in descending limb; bile-stained fat necrosis; necrosis of wall of mesenteric artery; necrosis of kidney cortex.

Rabbit viii.—Female, Belgian; weight 3,000 grams. (Shown at autopsy to be pregnant.)

March 24. The gall bladder was opened under ether anesthesia and bile was allowed to escape into the peritoneum.

March 25. Animal died during night previous. Most extensive edema and ascites; necrosis of liver and of capsule of spleen. Pronounced degeneration of kidneys; hemoglobinuria.

Rabbit ix.—Female, Belgian; weight 2,500 grams. (Also pregnant.)

March 24. Treated as previous rabbit.

March 25. Animal found dead. Cardiac degeneration and dilatation. Pronounced edema and ascites. Necrosis of liver; acute hemorrhagic pancreatitis; marked degeneration of kidneys; hemoglobinuria; casts.

Rabbit x.—Male, black; weight 2,000 grams.

April 1, 1910. 3 P. M. Ether anesthesia of twenty minutes duration. The gall bladder was aspirated, and 0.5 c.c. of light green watery bile was allowed to flow down over the anterior surface of the stomach.

April 2. Animal found dead in morning. Marked exudation in all serous cavities and in subcutaneous tissue. Dilated heart; necrosis of surface of liver; acute hemorrhagic pancreatic necrosis; fat necrosis; degeneration and necrosis of cells of renal cortex.

Rabbit xi.—Male, black; weight 2,000 grams.

April 1. 3:30 P. M. Operation as in rabbit x, except that 0.5 c.c. of dark green bile was obtained, and was allowed to flow into the right side of the abdominal cavity.

April 2. Animal died between 6 and 8 P. M. Marked exudation; dilated heart; acute hemorrhagic pancreatic necrosis in descending limb of pancreas; fat necrosis; necrosis of liver; necrosis of stomach wall.

Rabbit xxi.—Male, albino; weight 2,250 grams.

January 6, 1911. 3:30 P. M. Under ether, the gall bladder was aspirated and 1 c.c. of clear green bile was allowed to flow into the right side of the abdominal cavity.

April 7. Animal found dead. Lesions almost identical with those in rabbit xx (given in full above), but more extensive. Extensive fat necrosis along descending limb of pancreas and in fat above right kidney. Necrosis of kidney tubules.

Rabbit xxv.—Male, albino; weight 2,700 grams.

January 17. The gall bladder was aspirated and 1.75 c.c. of bile were obtained, of which 0.8 c.c. was allowed to flow into the right side of the abdominal cavity.

January 19. Animal killed. General serous and subcutaneous effusion; dilated heart; congestion and edema of lungs; extensive fat necrosis above right kidney; slight fat necrosis along pancreas; necrosis of surface of liver.

Rabbit xxvi.—Male, albino; weight 2,500 grams.

January 17. Through a small median incision made under ether, 0.75 c.c. of bile obtained from rabbit xxv was allowed to drip into the intestinal surface.

January 19. Animal killed. Beginning fibrino-purulent peritonitis, evidently secondary to the extension from the intestine which showed numerous areas of necrosis extending through the whole thickness of the wall and varying from 3 to 5 mm. in diameter. These lay beneath the point of injection. The pancreas was swollen and injected; surface necrosis of liver.

Rabbit iii.—Male, black and white; weight 1,400 grams.

February 26. 1 c.c. of bile was injected hypodermically through the abdominal wall at about the level of the ensiform. Some escaped into the subcutaneous tissue upon withdrawal of the needle.

February 27. Animal found dead. Subcutaneous effusion; necrosis of muscle of abdominal wall; necrosis of under surface of liver; slight serous effusion; dilated, pale heart.

Rabbit A vi.—Male. April 18. (An accidental experiment.) Under ether, the bile duct was ligated just above the entrance into the duodenum.

April 19. Animal found dead. All the signs of bile intoxication; extensive

effusions; dilated heart; acute pancreatitis; fat necrosis; liver necrosis. Examination showed that the ligature had cut through the duct; bile was escaping at that point.

These cases illustrate well the pathological changes following upon the peritoneal injection of a fatal dose of bile. No type of tissue appears immune to its direct necrotizing effect, and even at a distance, as in the heart, the kidney, and the blood cells, we have evidence that after absorption and dilution by the blood, the toxic action of the bile is still powerful. The death of the animal is apparently due to the effect of the bile upon the heart. This remote effect of the bile is rather surprising, as inspection of the peritoneal cavity has given the impression that the marked pathological effects are more or less sharply localized in the region where the bile was injected or where it acted in concentrated form. The bile appears to be very quickly diluted by the serous exudate to a degree at which its direct toxic action is almost lost. The effect of dilution seemed most marked in animals which received sublethal doses of bile. These animals, killed several days or a week later, often gave little evidence of any injury, unless the bile happened to have been brought into direct contact with a very susceptible organ, such as the pancreas or the liver. In that case, scarring and regenerative phenomena gave evidence of the primary injury.

No organ appears more susceptible to the direct action of the bile than the pancreas, as in practically every case in which the bile has come into direct contact with it, we have found the typical picture of an acute hemorrhagic necrosis of the gland. This is perhaps not surprising in view of the important part played in the causation of acute pancreatitis in man by regurgitation of bile into the pancreatic duct. Bile applied externally to the thin, spread out pancreas of the rabbit is brought into almost immediate relation to all parts of the gland.

The relation of the bile to the fat necroses is not so clear, and possibly these experiments do not prove whether the necroses are due to a combined action of bile and the lipase normally found in the fat cell; or whether it is a combined action of bile and pancreatic lipase, liberated by the injury to the pancreas; or even further, whether the bile is at all responsible except in producing a primary

pancreatic injury. Theoretically there seems to be no reason why the first suggestion might not be true. Bile is toxic to the cell and is further an accelerator of lipase. In the experimental animals, it is true, the fat necrosis was most marked near the pancreas. Yet in several cases it occurred in less than twenty-four hours at points more remote from the pancreas than it is found in rabbits in which the pancreatic duct has been incised and the juice allowed to escape into the peritoneal cavity over a period of several days. In other cases, the necroses were found when the injury to the pancreas, if present, escaped both gross and microscopical examination, although in an organ spread over so great an area, microscopical injuries might escape detection. The question is one which further investigation should clear up.