

THE CONSTITUENT OF THE BILE CAUSING PANCRE-
ATITIS AND THE EFFECT OF COLLOIDS
UPON ITS ACTION.

By SIMON FLEXNER, M.D.

(From the Rockefeller Institute for Medical Research, New York.)

The origin of many cases of acute inflammation of the pancreas in a diversion of the biliary secretion from the intestine into the pancreatic duct has been established by Opie's¹ observation and experiments. His experiments did not, however, take into account the nature of the biliary constituent which excites the inflammatory and other changes, neither did they undertake to discover whether varying effects were ever produced by samples of bile of different composition. While Opie's discovery threw much light upon acute hæmorrhagic pancreatitis it did not illuminate particularly the less acute or the chronic forms of pancreatic disease. It is noteworthy that the association of cholelithiasis with pancreatic disease² is more frequent than the mere acute affections of the gland, and the coincidence of cholelithiasis with sclerosis of the pancreas is so common as to arouse a suspicion of a possible pathological relationship of these conditions. The experiments which form the subject of the present communication are believed to show, first, the nature of the constituent of bile which acts injuriously upon the pancreatic tissue, and, second, the mechanism by which the injurious action of this constituent is modified and controlled and hence rendered capable of bringing about widely diverse effects. The experiments made upon the dog are believed not to be without application to similar pathological conditions found in man.

The bile contains two sets of constituents of highly different chemical composition: crystalline principles, and colloids. The

¹ "The Etiology of Acute Hæmorrhagic Pancreatitis." *Bull. of the Johns Hopkins Hospital*, 1901, xii, 182.

² Opie, *Diseases of the Pancreas*, 1903.

former consist chiefly of the biliary salts and coloring matter, the latter of nucleoalbumin. The biliary salts are known to act injuriously upon cells, for which a measure may be found in the dissolving effects upon red-blood corpuscles³; while no such direct cellucidal property is known either for the biliary coloring matter or the mucin.

Bile is, however, a secretion of much complexity and hence it is possible that it contains uniformly, or at times, still other substances of less defined nature which may act injuriously upon cells. The experiments to be related would seem to make unnecessary the assumption in bile of any hypothetically injurious substance upon which depends the power to set up inflammation of the pancreas.

The first experiments were made with a sample of sodium taurocholate as representing the main bile salt of the dog's bile. The specimen was brownish in color and gave on solution a yellowish-brown fluid. The salt was dissolved in a proportion approximating the biliary salt strength of dog's bile, namely, 0.650 gram of the salt in five cubic centimeters of water or saline solution.⁴ The injection was made directly into the pancreatic duct as it passed into the intestinal wall. The duct was frequently tied after the injection. The later experiments with bile salts were made with samples of the total salts prepared directly from dog's bile. The method was to treat gall-bladder bile with absolute alcohol in large excess, separating the precipitated mucin and in part the coloring matter. The alcoholic extract was evaporated to dryness on a water bath with animal charcoal. The dried mass was repeatedly extracted with absolute alcohol, and the alcoholic extract after moderate concentration was precipitated with ether. The salts were quickly separated by filtration with suction and dried over sulphuric acid. They were obtained in a white state. The muciginous residue from the first alcoholic extraction was dried and used in certain experiments in which the elements of the bile were recombined.

³ Rywosch, *Inaugural Dissertation*. Dorpat, 1901.

⁴ Shafer, *Text-Book of Physiology*, I, p. 370.

Experiment 1. 3. 15. 05. Brown mongrel. 11 A.M., injected into main pancreatic duct 5 c.c. solution of 0.650 grm. white bile salts in water. Duct tied. Loop of intestine exposed quickly became congested. Pancreas at place of injection became immediately congested, and in 2 or 3 minutes stasis or hæmorrhage occurred. 5 P.M., dog lying in farthest corner of cage; very sick. 3. 16. 05., very sick; dead, 2 P.M. Autopsy: No excess of fluid in the peritoneal cavity; serosa, except for fat necroses to be mentioned, smooth and glistening. These fat necroses are typical in appearance and are scattered in the omentum and mesentery. The pancreas, except a small fraction of the duodenal end, shows swelling, hæmorrhage, and necrosis. The hæmorrhagic areas occupy spaces in the pancreatic substance and reach an extent of 2 x 3 cm. Fat necrosis also occurs in the interstitial pancreatic tissue.

Experiment 2. 3. 23. 05. Pug. 10.30 A.M. Same injection as in previous experiment. Almost immediate extreme congestion and hæmorrhage into injected portion of the organ. Dead, 3. 25. 05., during night. Autopsy: Peritoneal cavity contains much bloody fluid; serosa covered with fine, granular exudate. Fat necroses occur in the omentum, mesentery, and fat about operation wound. Adhesions occur between the liver, pancreas, and colon, which on separation disclose a cavity the size of a small orange containing dark bloody and slightly offensive fluid. The walls, composed of pancreatic tissue, are necrotic and covered with fat necroses and hæmorrhages. In other parts the pancreas shows softened areas of varying size (reaching 3 to 4 cm.), which prove to communicate with the larger cavities. The substance of the gland contains large, unsoftened necrotic foci of pancreatic tissue and fat.

The power to set up inflammation of the pancreas of high degree is proven for the bile salts by these experiments. The fatal nature of the inflammation could be further emphasized by citing other experiments, but these should suffice. In one experiment with bile salts death occurred in eight hours, by which time the entire pancreas was swollen and diffusely infiltrated with blood. The fact should be emphasized that besides the typical picture of experimental pancreatitis in the dog the bile salts have produced in one instance (Experiment 2), in the brief space of one and a half days, a condition of hæmorrhagic and gangrenous inflammation of the gland.

The next experiments relate to the action of the alcohol-insoluble or muciginous residue, in the stating of which an account is also given of a third series of experiments, in which the two separated portions of the bile were used after recombination.

Experiment 3. 3. 15. 05. 10 A.M. Brown Skye terrier. 5 c.c. of an aqueous suspension of the alcohol-insoluble residue injected. The suspension was pale brownish-yellow in color and of muciginous consistence. Duct tied.

No discoloration of pancreas except from injection; no congestion. 5 P.M. Dog bright; walks about. No subsequent symptoms. 3. 20. 05. Chloroformed. No lesion of pancreas except, possibly, slight sclerosis from ligation of the duct. No general increase in consistence of the organ.

Experiment 4. 3. 15. 05. White and brown cur. 10.30 A.M. 5 c.c. of the bile-residue suspension of Experiment 3 plus 0.650 grm. bile salts. Duct tied. Immediate discoloration followed by stasis or hæmorrhage of the pancreas in injected area. The duodenal loop of intestine became greatly congested. 5 P.M. Dog appears bright and is up. 3. 20. 05. Dog looks well. Chloroformed. General peritoneal cavity normal in appearance. The pancreas in part is swollen and hæmorrhagic. The duodenal tail is the most normal in appearance, but the tissue immediately adjacent to this is firmer than normally and of a roughened appearance. This pale, firm tissue passes into the acutely swollen part of the gland, which, on section, shows hæmorrhagic and necrotic foci. In this process the entire mid- and splenic-portions are involved.

The absence of all inflammatory power in the alcohol-insoluble residue is brought out by these experiments, which also point to a positive property of this residue, namely, that of reducing in activity the biliary salts. Since this is a property of some importance still another set of experiments will be given.

Experiment 5. 3. 1. 05. Black mongrel. 11 A.M. Injected 5 c.c. of dissolved alcohol-insoluble residue. Duct not tied. Chloroformed, 3. 4. 05. Pancreas appears normal except at the point of separation from the intestinal wall in exposing the duct. A few small necroses in the fat at this place.

Experiment 6. 3. 1. 05. White mongrel. 12 M. 0.650 grm. sodium taurocholate (Merck's) in 5 c.c. aqueous suspension of alcohol-insoluble residue. Injected portion of pancreas became immediately congested, possibly hæmorrhagic. 3. 3. 05. 10 A.M. Dog recovered from operation; looks well. Chloroformed. Peritoneal cavity shows nothing abnormal except a small quantity of bloody fluid in the pelvis. Pancreas, except duodenal end, greatly swollen, and the serous surface covered with bloody exudate. Very few areas of fat necrosis, limited to region about pancreas. On section, the swollen pancreas permits much blood-stained serum to escape. The hæmorrhagic and œdematous areas are somewhat separated, the former being in the parenchyma, the latter in the interstitial tissue chiefly. The duodenal extremity alone has escaped.

These experiments leave no reasonable doubt of the modification by other biliary constituents of the effects upon the pancreas of bile salts. The modification is expressed by, first, the less fatal character, and, second, less acute and destructive nature of the inflammation of the gland. The latter changes, as compared with the severer fatal inflammations, do not differ so much in extent as in intensity, and while hæmorrhage and œdema com-

monly exist marked necrosis is not so prominent. Since, however, the natural full bile is able to cause an acutely fatal inflammation we should also expect the recombined bile to do the same unless the chemical processes have permanently altered the constituents. The next experiment bears on this subject.

Experiment 7. 3. 23. 05. Brown dog. 10.45 A.M. 5 c.c. resuspended bile residue plus 0.650 grm. bile salts injected. The suspension was thinner than previous ones; mixture kept in thermostat at 60° C. for $\frac{1}{2}$ hour. 3. 24. 05. 6 P.M. Dead. Autopsy: The peritoneal cavity congested, and contains excess of fluid. The pancreas is softened, congested, and hæmorrhagic. The section of the gland shows intermingled areas of necrotic parenchyma, hæmorrhages, and fat necrosis; moderate number of peritoneal fat necroses.

It was, of course, desirable to determine the nature of this inhibitive action upon the bile salts. Two possibilities presented themselves: the first, that a directly antagonistic substance exists in the bile which tends to inhibit the salts; the second, that the restraining action is bound up with the physical properties of the medium, since the proteid constituents of the bile give a colloid quality to the mixture.

Experiment 8. 3. 22. 05. Brown mongrel. 11 A.M. 5 c.c. semi-solid mixture, at room temperature, of agar-agar and gelatine was melted and in it was dissolved 0.650 grm. bile salts. Mixture kept at 60° C. for 30 minutes, cooled to 40° C. and injected. Duct not tied. 3. 30. 05. Chloroformed. Dog has lost considerably in weight. Autopsy: General peritoneum normal. Firm adhesions about pancreas, liver, stomach, and intestine. On exposing the pancreas it appears shrunken and is firmer and more nodular than normally. Only the duodenal end retains its normal appearance. The main part of the gland is grayish-white in color and infiltrated, here and there, with blood. An area of necrosis the size of a bean and extending to the surface occurs in the mid-duodenal portion. On section, the whole gland, except a superficial sheath or armor of pancreatic tissue below the capsule, is necrotic, grayish, and soft, and gas bubbles in small number slowly disengage themselves from the cut surface. Odor not offensive. On cover slips bacteria are not numerous, but of varied morphology.

That a bland colloidal solution can restrain the activity of the otherwise very highly destructive and certainly fatal bile salts is shown conclusively by this experiment. Several experiments were next made with the whole bile in which the colloid strength was increased by the addition of bland colloidal substances or diminished by diluting the bile with saline solution.

Experiment 9. 11. 13. 05. Two dogs of equal size. 12-1 P.M. Brown mongrel: 2 c.c. bile plus 2 c.c. 5 per cent. agar-agar. Mixture cooled to 40° C. Duct tied. No immediate effect on pancreas. White cur: 2 c.c. bile plus 2 c.c. salt solution. Duct tied. No immediate action on pancreas. 11. 14. 05. Both dogs still sick; the brown less prostrated than the white. 11. 15. 05. Both on feet; chloroformed, 11 A.M. Autopsies Brown (bile plus agar-agar). Peritoneum clear; no fat necrosis except spot or two just beside seat of operation. Corresponding to injected area of gland and extending slightly beyond, swelling and hæmorrhage of slight degree. No visible necrosis. The remainder of the organ appears normal. White (bile plus salt solution). Peritoneum normal except for fat necrosis in mesentery. Marked fat-necrosis about the pancreas. The mid-portion of the gland, except 2 cm. of splenic end, shows marked swelling, œdema, hæmorrhage, and fat necrosis.

The striking difference in the lesions caused, on the one hand, by bile of high colloidal quality, and, on the other, by the same bile in which the normal colloidal strength was reduced, is well brought out by this experiment. The protection afforded by colloidal substances may extend even further, so that the pancreas may entirely escape injury from a quantity of bile salts which, without such protection, is capable of causing severe lesions.

Experiment 10. 11. 17. 05. Two dogs of equal size. 11-2 A.M. Male: 2.5 c.c. bile + 2.5 c.c. 5 per cent. agar-agar. Duct not tied. Female: same mixture injected; duct tied. 11. 20. 05. Both dogs appear well. Female chloroformed. Peritoneum normal. No fat necrosis. Mid-portion of gland, receiving injection, moderately swollen and slightly œdematous, and on section slight hæmorrhage in tissue. No necrosis of parenchyma or fat. 11. 25. 05. Male chloroformed. The injection passed into the duodenal half and very little into the splenic parts of the gland. The peritoneum, excepting pancreas, normal. No fat necrosis anywhere. The injected end of the pancreas is white and glistening, the surface and section being granular. The granules include lobules and areas below lobules in size. The duct is patent. No evidence of hæmorrhage or necrosis.

It is clear from this experiment that by increasing sufficiently the colloidal strength of the mixture the bile can be made to act slightly upon, or be entirely prevented from causing acute lesions in, the pancreas. This experiment also suggests that, possibly, when the action is greatly diminished so that acute lesions fail to appear, the gland is yet sufficiently injured to respond with chronic productive lesions. Some years ago I showed⁵

⁵ Flexner, *Johns Hopkins Hosp. Reports*, 1900, ix, 743; Flexner and Pearce, *Univ. Penna. Med. Bulletin*, 1901, xiv, 193.

that in the dog chronic pancreatitis, especially a focalized kind about acute lesions, easily develops. But the present experiment does not prove that the bile modified and reduced by agar-agar sets up alone the chronic lesions, for should the agar-agar not be easily eliminated from the ducts it would act as a foreign obstructive body and in this way itself cause chronic inflammation. Indeed, I have found that if a three-per-cent. suspension of agar-agar is injected into the duct in sufficient quantity it will cause, at the end of a week, a well-marked sclerosis. On section of the gland in these cases remains of the agar-agar can be found in the tissues.

That the biliary constituents, suspended in agar-agar, can pass out and come into relation with the gland tissue is shown to the naked eye by the loss of color which the agar suspension undergoes in the substance of the pancreas. This interchange of fluid between the gland and the agar suspension is a slow one relatively; and upon the rate of diffusion and the consequent degree of concentration of the biliary salts at any one time in contact with the parenchyma will depend, probably, the extent of the lesions.

Experiment 11. 11. 27. 05. Two dogs of equal size. 11-12 A.M. Brown: 2 c.c. bile plus 3 c.c. agar-agar. Injected duodenal half of gland. Black and white: 2 c.c. bile plus 6 c.c. agar-agar. Injected largest part of gland. 12. 4. 05. 11 A.M. Chloroformed. Brown dog: The injected portion of the gland is swollen and shows diffuse hyperæmia and punctiform hæmorrhages. The consistence is increased in this part, and the surface is granular. On section, the surface shows small mottled areas of hæmorrhage, fine yellowish, intra-acinar points of degeneration, and two larger softened areas of necrosis. The splenic half of the gland is kinked and at the angle an extraglandular cyst the size of a marble, containing clear fluid, has formed. The duct is patent until the bend is reached. It can be followed no farther. Beyond and about the cyst the gland is sclerosed. Black and white dog. Entire organ granular, sclerosed, and contracted. No acute lesions or fat necrosis. Main duct dilated slightly. In some parts the agar-agar has not been removed. The bile staining of the agar-agar has wholly disappeared.

This experiment calls for no discussion since it shows that with a sufficiency of colloid present the acutely injurious action of the bile can be prevented. It does not, however, settle the question of the relation of the chronic lesions to the diminished

action of bile salts since the agar-agar itself remained, in the one case, to act as a foreign body. Hence in the next experiment colloids were chosen which were free of the objection that they were capable of congealing in the ducts.

Experiment 12. 12. 2. 05. Two dogs of equal size. 12 M. to 1 P.M. Buff mongrel: Bile 2 c.c. plus mixture of mucin and nucleoproteid⁶ 4 c.c. Injection entered duodenal half of gland. Hairy mongrel: Bile 2 c.c. plus 20 per cent. gelatine 4 c.c. Injection entered splenic half of gland. Ducts not tied. The first dog quickly recovered, the second never really recovered, from the operation. Chloroformed, 12. 9. 05. Autopsy: Buff dog: The entire duodenal half of the gland and a small part of the middle portion are swollen, white in color, and firm in consistence. The remainder of the splenic portion of the pancreas is normal in consistence and appearance except for a vivid congestion. The line of demarcation between the swollen and normal gland is sharp. No evidence of acute lesion and no fat necrosis exist. Duct not dilated. Hairy dog: At seat of operation fat necrosis in the pancreas. The splenic portion of the gland except the tip is swollen moderately and firm. The serosa of the tip contains small hæmorrhages, and the injected portion, on section, is the seat of marked necroses in the parenchyma and interstitial tissue. This part and the duodenal end are both somewhat sclerosed.

From these experiments the conclusion can be drawn that the suspension of bile in a bland mixture of high colloidal strength protects the pancreas from the immediate and acutely injurious action of the biliary salts. But besides this conclusion another can be drawn, namely, that a relation exists between the nature of the suspending colloid and the reaction excited in the pancreas. If, for example, the colloid is readily attacked by the pancreatic juice and the density of the mixture reduced, then the injurious salts may still be brought into such mediate relation with the gland as to produce moderately severe acute lesions: this would appear to be the case with gelatine. But with agar-agar and the mucin-nucleoproteid mixture, which are acted upon little if at all by the pancreatic secretion, the protection is so effective as to eliminate wholly gross injury to the gland tissues.

That this explanation is the correct one is further proved by the next experiment, in which gum arabic was employed as the diluent.

⁶ A syrupy suspension of tendo-mucin and spleen nucleo-proteid was made in water by careful neutralization with $\frac{1}{10}$ N. sodium hydrate by Dr. Levene.

Experiment 13. 12. 14. '05. Two medium-sized dogs. 3.30 P.M. Hairy mongrel: Bile 2.5 c.c. plus gum-arabic suspension 2.5 c.c. Black terrier: Bile 2.5 c.c. plus saline 2.5 c.c. Ducts not tied. Both dogs up early next morning, but the first still very sick. About noon he refused to rise in his cage; dead, 4 P.M. Autopsy. The injection entered the duodenal half of the gland, which showed no marked inflammation, and no hæmorrhage or fat necrosis. There was slight swelling of the gland and a few necroses in the fat of the mesentery about the ducts. The cause of death of this dog is therefore not wholly clear. The intestinal contents were blood-stained, and a few small broncho-pneumonic foci existed in the lungs. The terrier was chloroformed. Disseminated fat necrosis existed throughout the peritoneal cavity; it was very marked about the pancreas. The injection entered the mid-portion and splenic end of the gland. Fully two-thirds of the gland was the seat of marked acute inflammation associated with hæmorrhage. A blood clot of considerable size existed in the pancreatic and peripancreatic tissues about the middle of the gland.

This experiment is convincing in showing that by the addition of a bland colloid the action of bile on the pancreas can be greatly reduced or even entirely prevented; and it is also useful in showing that by reducing the colloid strength of normal bile by the addition of salt solution its injurious action can be considerably enhanced.

It would seem as if these conditions might be simulated in human pathology in instances in which the composition of the bile was modified by obstructive and inflammatory processes. The changes which imprisoned bile undergoes are well known. The loss of the diffusible salts and increase in muciginous constituents, which occur on obstruction of the ducts, and the accumulation of albuminous products, which takes place in inflammation, would provide exactly the theoretical conditions for the production of subacute or chronic lesions of the pancreas, rather than acute ones, provided this bile had been diverted into the pancreatic duct. If the conditions of these experimental lesions occur in human beings it seems not impossible that in this way may arise certain instances of partial or independent sclerosis of the pancreas which up to this time have received no adequate explanation.⁷

⁷ Perhaps it may be worth while to indicate that a mechanism exists in nature, comparable with, if not the equivalent of, the syringe, for driving a colloidal suspension of considerable thickness into the pancreas. This mechanism is the

That biliary salts should act as poisons for pancreatic tissue and blood-vessels is, in view of the established cytolytic property of the salts for various kinds of cells⁸—blood-corpuscles, amoebæ, infusoria, muscle, nerve, ciliated epithelium, liver cells,—not remarkable. Since this power is so great and can be exerted against the liver itself some restraint would seem to be required in nature. This restraint, apparently, can be supplied by the colloids in and about the cells. It is, however, probable that all parenchymatous cells are not equally susceptible to injury by the bile salts, and the liver cells are stated by Rywosch to be especially resistant.

When one reflects upon the mechanism of the restraining action of colloidal substances upon bile salts, one naturally turns to the consideration of physico-chemical notions for an explanation of the effects observed.⁹ Bile salts exhibit a low surface tension and hence diffuse readily. They tend therefore to pass quickly into a surrounding fluid medium; and in the case of the pancreas soon find themselves in contact with tissue cells and blood-vessels. The latter immediately respond to their presence by hyperæmia and extravasation; and the injurious action is soon extended to the pancreatic cells. The colloids, on the other hand, are composed of large molecules of high surface tension. When the two sets of substances are mixed in solution, the molecules of the salts are spread out over those of the colloid, whereby mechanical conditions producing decrease in diffusion velocity of the salts are brought about.¹⁰ In order that severe injury may be inflicted upon the pancreas, a certain concentration of the salts must exist in the fluids in immediate relation with the tissues; and as these fluids are in process of constant change, this concentration must be reached in a unit of time. Any

gall-bladder and ducts, the muscular contraction of which might easily suffice for this purpose under conditions in which the biliary and pancreatic ducts had been, through pathological conditions, made continuous

⁸ See Rywosch, *Inaugural Dissertation*. Dorpat, 1891.

⁹ In view of the union of bile salts with proteids perhaps some account should be taken of this property.

¹⁰ I am indebted to Professor Ostwald for this explanation. It was transmitted to me by the kindness of Dr. C. A. Herter.

marked reduction in diffusion velocity will, therefore, tend to reduce the action of the salts. Should the injury fall below a certain degree, and the action be prolonged, conditions favoring the production not of acute but of subacute or chronic lesions might be inaugurated. Moreover, the colloidal substances themselves while bland may not be without effect in causing new growth of connective tissue. For if the intrapancreatic pressure is too low to remove readily the colloid because of the higher friction which it exerts on the ducts, obstructive atrophy and sclerosis would result. But while this factor must be considered both in the experiments on dogs, and under similar hypothetical conditions in man, it can have no part in explaining the absence of the acute experimental lesions.

The rate of diffusion of crystalloids of low molecular weight into colloids (agar-agar) would appear, from Voightländer's¹¹ experiments, to be little lower than in water. Whether the same holds true for such large molecules as compose the bile salts I do not know; and I do not know, by direct experiment, whether a large molecular crystalloid, enclosed in a colloidal solution, would diffuse readily into a solution as complex as lymph. The experiments related in this paper would tend to speak against such ready diffusion. But it will be necessary to make the conditions of study simpler than those of these experiments before a final comparison of all the facts mentioned can be made.¹²

¹¹ *Zeitsch. f. physik. Chemie*, 1889, iii, 316.

¹² Experiments relating to these questions are being pursued.