Functional Studies of the Extrahepatic Biliary System in the Dog by Use of a Controlled Biliary Fistula *

GEORGE BERCI, M.D., NEIL JOHNSON, M.D., F.R.A.C.S.

From the Department of Surgery, University of Melbourne, Royal Melbourne Hospital, Melbourne, Australia

SINCE THE first removal of the gallbladder, the recurrence of pain following this operation has stimulated interest in functional studies of the extrahepatic biliary system.

Although Oddi investigated the function of the sphincter which bears his name as long ago as 1887, and although many aspects of its innervation have been considered since, there are still many questions that remain unanswered.

Most of the experimental methods which have been used in investigations on this subject involve the introduction of a T-tube or cannula into the hepatic duct, common bile duct or the cystic duct or, alternatively, the isolation of a segment of one or other of these structures.

It became apparent to us that if we planned to make studies at will in the conscious or unconscious animal without interfering in any material way with the physiology of the biliary drainage system, we would need to develop a new technic. We also felt that we should avoid any of the complicated procedures which involve refeeding or the re-injection of bile. The method should also make it easy to: 1) tap bile at different intervals in time for biochemical investigation and 2) perform x-ray examinations.

Method

Eleven mongrel dogs were used, and under general anesthesia an acrylic button was inserted into the fundus of the gallbladder and to this a polythene tube with an inside diameter of 2.5 mm. was attached. The button was secured by a double purse string suture and omentum was wrapped around the tube to avoid leakage. The polythene tube was then tethered by a few stitches inserted through a convenient fold in the parietal peritoneum. The other end of the tube was connected to an L-shaped acrylic tube, the long limb of which was brought through the parietes close to the vertebral column and fixed on the skin by means of a plastic washer and two plastic nuts. The external opening of this artificial fistula was sealed with a screw-cap (Fig. 1, 2). The removal of this screw-cap afforded immediate access to the extrahepatic biliary system. In practice this appliance did not interfere with the animal's normal activities. During observations, the screw-cap was removed and the end of the tube was joined by means of a Y-piece to a perfusion syringe containing saline and also to a manometer. Perfusions were then carried out at a controlled rate of 7, 15 and 35 ml./minute (Fig. 3).

Observations were always made when the dog was conscious and fasting. After the base-line resting pressure had been determined, the perfusion was started, and several observations were made at each session. The same procedure was repeated on another occasion in the anesthetized animal. (No premedication was used and narcosis was achieved by intravenous nembutal.)

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Between the examinations, the fistula was kept patent by washing it daily with normal saline.

Results

In a serial fashion, 280 perfusions were carried out on 11 dogs, 100 in the conscious and 118 in the anaesthetized state. As soon as the perfusion commenced, the pressure rose gradually and then flattened off at a constant level. This plateau level was taken to represent the pressure at which the perfusate had begun to leak through the sphincter and was called the "opening pressure."

We found many extraneous fluctuations in the pressure tracings which were produced by events such as changes of intra-abdominal pressure and by slight movements of the animal itself. Since these minor changes did not appear to be of any significance as far as the present experiment was concerned, the mean pressure only was recorded (Fig. 4). The normal intraluminal pressure (resting pressure) was found to vary from 3–10 mm. Hg.

Marked variations in pressure were initiated by changes in posture. There were, for example, considerable differences in the values as recorded in the sitting or lying positions. Even slight head movements were important, and one of our greatest problems was to keep the conscious animal still during the periods of study (Fig. 5).

In some experiments the opening pressure fell after a short interval and remained at the lower level until the conclusion of the perfusion, suggesting that as soon as the initial tone of the sphincter was overcome, it was not able thereafter readily to recover its early contraction (Fig. 6). This phenomenon was usually reproduced on

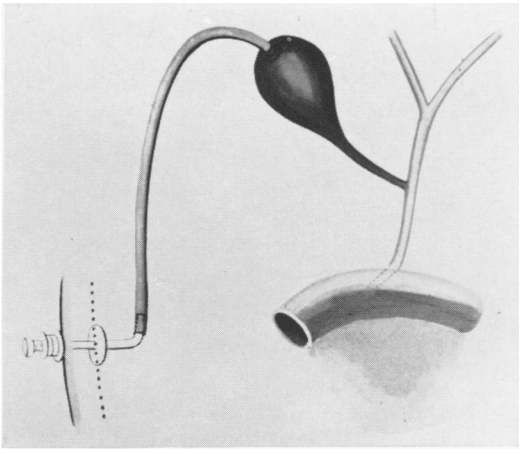

FIG. 2. This schematic diagram represents the positioning of the controlled fistula.

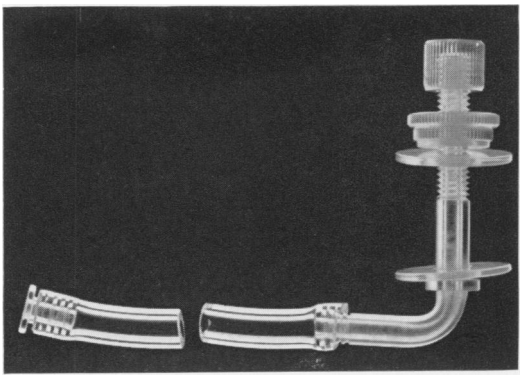

FIG. 1. The acrylic L-shaped tube which is passed through the parietes, paravertebrally and is sealed with a screw cap. Inside diameter is 2.5 mm. A connected polythene tube leads to the acrylic button which is inserted into the fundus of the gallbladder and secured with a double purse string suture.

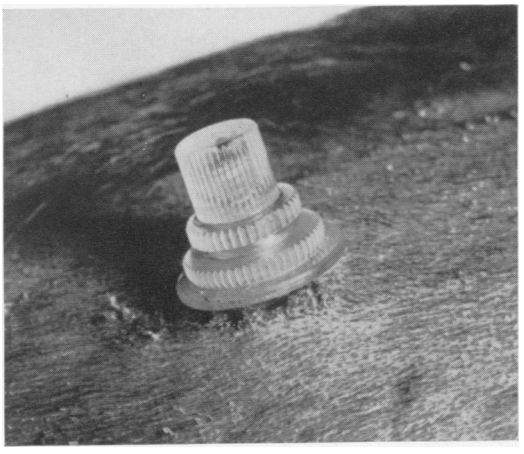

FIG. 2a. This procedure does not interfere with the animal's normal life.

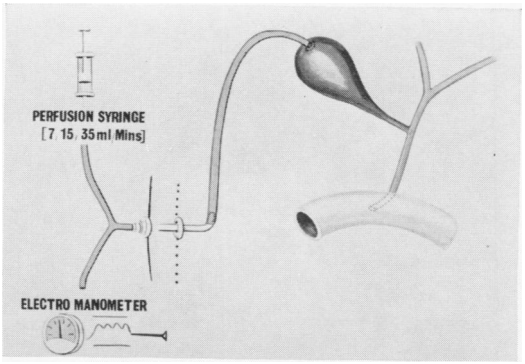

FIG. 3. During observation the cap is removed and the external opening of the fistula is connected to a perfusion syringe and an electromanometer.

subsequent experiments in these particular animals.

Morphine, given in doses of 15 mg. to the unconscious and 30 mg. to the conscious animal, caused a striking increase in the tone and resistance of the sphincter. The effect was prompt and occurred within 5 minutes of an intramuscular injection. The response to a morphine injection was, however, not the same in all of the animals tested. The results of morphine administration in the 11 dogs in the conscious and in the anesthetized state are shown qualitatively in Table 1. This was evident in three animals only. In four animals under test no effect was observed on the tonus of the sphincter following morphine injection on several occasions.

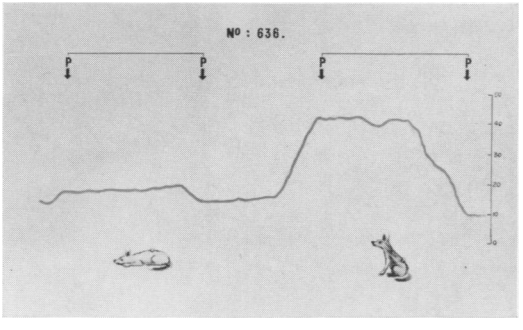

FIG. 5. The differences recorded of the "opening pressure" in the sitting and lying position. (Conscious animal, perfusion period marked by arrows.)

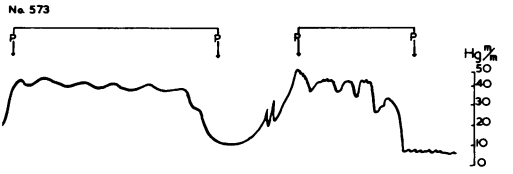

FIG. 4. Many extraneous fluctuations were found (seen on the right), produced by respiratory movement, changes of intra-abdominal pressure and slight movements of the animal itself. Therefore the mean pressure only is registered (seen on the left). The period of perfusion is marked by arrows (P–P).

The Effect of Perfusion Flow Rate on the "Opening Pressure:" Most of our observations were made at a perfusion rate of 15 ml. per minute, but in five animals the experiments were repeated with pressures observed at perfusion rates of 7, 15 and 35 ml. per minute, respectively. The total volume of perfusate (35 ml.) was the same in every instance; only the rate at which it was instilled varied from one observation to the next.

The results indicated that higher opening pressures were obtained with faster flow

TABLE 1. *Effect of Morphine Administration in Conscious and Anaesthetized State. ++ = Elevation of Pressure Exceeding 10 Hg mm. + = Pressure Change Less Than 10 mm. Hg. No Effect Observed in 4 Animals Even on Repeated Investigations.*

Conscious	Anaesthetized	No. Dogs
++	++	3
0	0	4
++	+	2
++	0	1
+	++	1

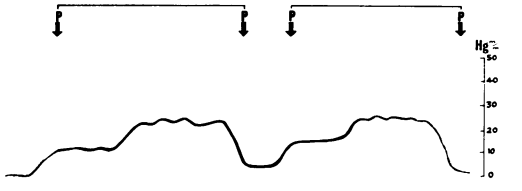

FIG. 6. The opening pressure fell after an interval and remained at the lower level until the conclusion of the perfusion. Changes of the initial tone of the sphincter.

rates. It was interesting to note, for example, that in some animals, if we perfused at 35 ml. per minute, the peak of the curve was achieved as soon as 6 seconds after the commencement of the perfusion (after 3.5 ml. of fluid had been injected). If, however, in the same animal we changed the rate to 7 ml. per minute, 30 seconds was required for the injection of the same 3.5 ml. of perfusate. But in this case we still had not reached the plateau of the pressure curve or opening pressure (Fig. 9).

We found so many factors that influenced our records that we thought that it might be instructive to combine the pressure measurements with a simultaneous x-ray investigation to confirm visually whether or not the pressure values as recorded and interpreted were accurately related in time to the observed passage of a dyestuff through the sphincter into the duodenum. For this reason we substituted as our perfusion fluid a 35 per cent water-soluble radiopaque dye and performed cholangiography monitored by a 5-inch image-intensifier coupled to a television camera and screen linkage. We proved by preliminary testing that the dynamics of the experimental system were not significantly altered by substituting the dye for the saline used

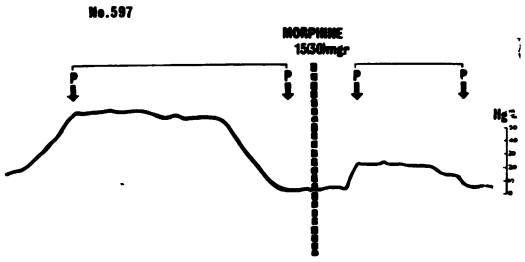

FIG. 7. The effect of morphine administration (15 mg. to the unconscious and 30 mg. to the conscious animal). Dotted line demonstrates the time of administration. The curve has to be read from right to left. The perfusion period is marked with arrows.

in our other experiments. These x-ray investigations performed only in the anesthetized animal.

The scale of the electromanometer was projected with the help of a mirror onto the top of the TV monitor screen and both phenomena—radiologic appearance of the extra-hepatic biliary system and the intraluminal pressure—were filmed together, with the help of a synchronized 16 mm. movie camera (Fig. 10). In this way it was possible to relate the differing appearances of sphincteric function with complete accuracy with the corresponding pressures in the duct system. From these investigations three interesting points emerged:

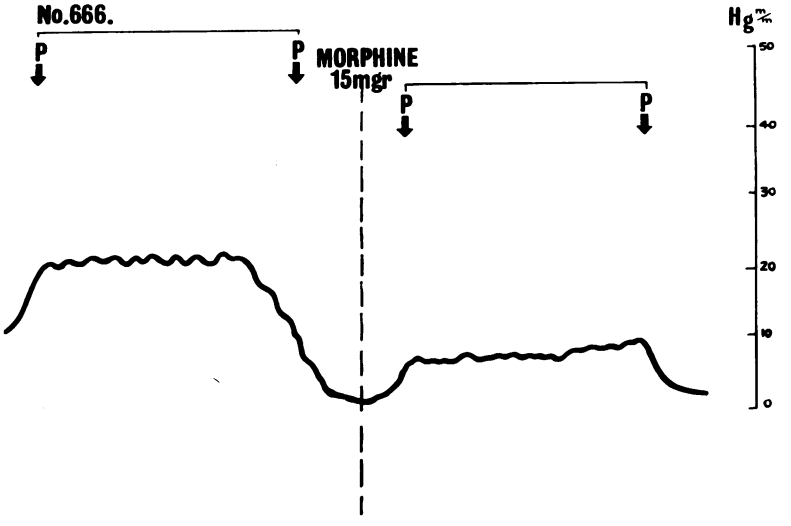

FIG. 8. Shape of curve varied but after administration of morphine a typical plateau recording was achieved, suggesting that tonus of sphincter had been increased and sustained.

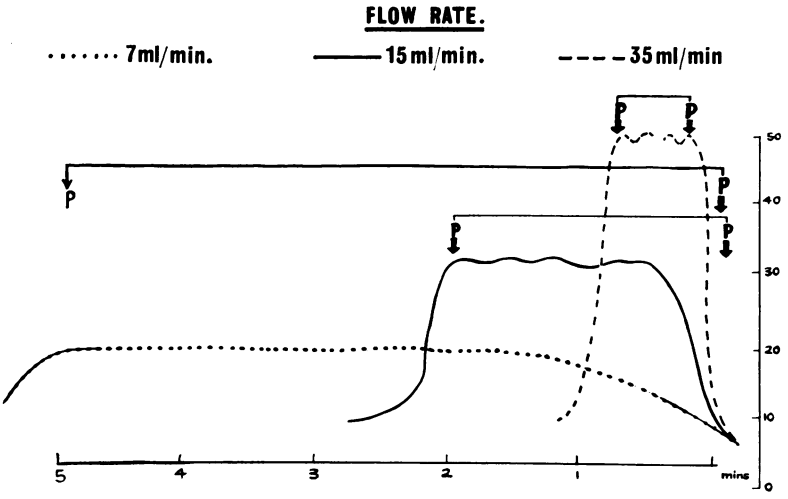

FIG. 9. The effect on the sphincter tonus of differing perfusion flow rates. ---- = 35 ml./minute. —— = 15 ml./minute. .... = 7 ml./minute. Higher opening pressures were obtained with faster flow rates.

1. *Open sphincter at low pressures.* In two animals the duct system was perfused at 15 ml. per minute. The contrast medium began to appear in the duodenum while the pressure was still rising. In other words, the dye stuff was already running freely into the duodenum at a level of pressure considerably lower than that which would have been determined had we based our observations on a pressure curve alone.

2. *High resistance of the sphincter.* In two animals perfused at a rate of 15 ml. per minute it was noted that the pressure suddenly increased to over 50 mm. Hg and remained at this level even after the sphincter had opened and the contrast material had entered the duodenum. As soon as the perfusion was stopped the pressure fell back, as promptly as it had risen, to 30 mm. Hg. It was also noted that in the same animals we had recorded a higher resting pressure of more than 10 mm. Hg. The same result was found in these animals when the experiment was repeated on several occasions. Care was taken to ensure that the fistula and the extrahepatic biliary system was patent at all points.

3. *Duodenal peristalsis and intra-luminal pressure.* To investigate duodenal function we changed to a perfusion rate of 7 ml. per minute so as to keep at a minimum any rise in pressure in the extrahepatic biliary system and to avoid, as far as possible, interference with the peristaltic activity of the duodenal wall. The peristaltic waves of the duodenum pass the choledochoduodenal junction without significant pressure changes in the extrahepatic biliary system.

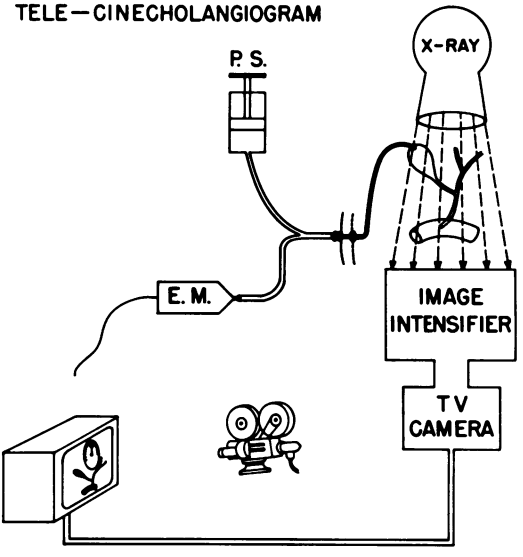

FIG. 10. The arrangements for television-cholangiography. The pressure values from a meter are projected in the upper part of the television monitor screen and both procedures are filmed together with a synchronized 16 mm. movie camera. (Flow rates 15 and 7 ml./min.) P.S. = perfusion syringe. E.M. = Electro-manometer.

Discussion

The functional studies of the extrahepatic biliary system in the dog have hitherto been made by cannulation of the common bile duct or common hepatic duct. In this study a controlled biliary fistula was established by means of a polythene tube tied into the gallbladder at one end and anchored at the other end to the skin of the back of the animal. This enabled us to have easy access to the extrahepatic biliary system as often as was deemed necessary and over a period of some months. Using this experimental system it was found that morphine did not increase the tone of the sphincter in all animals, and in 4 of the 11 it had no effect whatsoever, even on repeated examinations. The shape of the curves varied a great deal, and we found so many factors affecting it that it was exceedingly difficult to determine from an analysis of the pressure tracings precisely what was happening in the sphincteric region. For this reason we decided to combine radiologic and pressure measurement investigations, projecting the pressure data on the top of the television monitor and recording the two images on film together. In this way, and with the help of analytic projector, we were able to determine that during perfusion:

1. The sphincter may be open at a low pressure and even when the pressure in the system is still rising,

2. The sphincter seems to have a high natural resistance in some animals for no obvious reason,

3. Duodenal peristalsis does not appear to have any significant effect on the intraluminal pressure of the extrahepatic biliary system.

However, the flow rates used in these experiments were grossly in excess of those which would be encountered in the dog under physiologic conditions and pressure measurements, and observations of sphincter activity made under these circumstances may bear very little relationship to those exhibited by the normally functioning sphincter.

Summary

1. A semipermanent biliary fistula has been developed to facilitate perfusion of the biliary tree at a controlled rate and to allow simultaneous pressure measurements to be made. These studies were made on 218 occasions in the conscious and unconscious animal.

2. A method has been contrived of relating pressure measurements to sphincter function as studied with the aid of a telecine (kine-record) cholangiogram.

3. The results of these studies suggest that the interpretation of pressure changes in the extrahepatic biliary system are vulnerable to criticism.

4. The method of investigation here elaborated has been found very useful in facilitating studies in the experimental animal over a long period.

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