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THE USE OF A PERMANENTLY CONTROLLED BILIARY FISTULA IN THE SHEEP TO STUDY THE EXCRETION OF CARBON TETRACHLORIDE IN THE BILE

by

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A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major Subject: Veterinary Surgery

Signatures have been redacted for privacy

Iowa State University
Of Science and Technology
Ames, Iowa

1966

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I. INTRODUCTION

Biliary fistulas are extensively used in physiological and pharmacological studies of bile and its constituents. In human medicine, biliary fistulas have been used in surgical conditions involving the gall-bladder and the extrahepatic biliary system. The majority of the biliary fistulas used in experimental work on animals were of a complicated nature, which required elaborate surgical procedures and special materials.

The purpose of this study was to establish a controlled biliary fistula in the sheep.

The choice of sheep as an experimental animal in surgery is growing rapidly. The economy of purchasing, feeding and maintaining of the sheep makes it a useful animal for many types of experimental biological studies. The temperament of the animal makes it easy to handle, feed, and maintain with a minimum of labor. Sheep tolerate implanted tubes or cannulas more than other animals, therefore, danger of mutilation is decreased.

Biliary fistulas were obtained by exteriorizing the papilla of Vater by three stages of operative procedures in dogs. Rubber tubes, Tygon tubes and polyethene tubes were also used in different animals with various operative techniques to establish a biliary fistula.

The biliary fistulas are utilized for the quantitative and qualitative studies of various bile constituents. The action of various cholagogues and choleretics can be evaluated by the utilization of this method. More information on the physiology of liver and bile formation could be obtained by modifications of the biliary fistulas.

Fascioliasis is one of the major parasitic problems in sheep all over the world. The need for study of this problem stimulated the author to develop a simple method in which the bile flow could be exteriorized by the use of two tubes which could be connected and disconnected whenever desired. This method could be utilized in further studies of this problem in regard to action of new drugs or the study of certain facets of the life cycle of the parasites in the bile ducts.

While carbon tetrachloride is extensively used in the treatment of fascioliasis, the author did not find information in the medical literature stating whether or not carbon tetrachloride is excreted in the bile of sheep. Thus after establishing the biliary fistula, the study of the excretion of carbon tetrachloride in bile could be commenced by administrating the agent and then obtaining bile samples for chemical analysis.

II. REVIEW OF LITERATURE

The use of a controlled biliary fistula in the sheep was intended for the study of excretion of carbon tetrachloride in bile in this experiment.

According to Rous and McMaster (1923), the use of a biliary fistula for the collection of bile from conscious animals goes back to 1844 when Schwann described the fistula method which is still employed in studies of bile. Also, he mentioned that Dastre in 1890 kept a tube in the fistulas of two dogs for more than two years with daily collection of bile into a bag suspended at the neck.

For the permanent sterile drainage of bile in dogs, Rous and McMaster (1923) ligated the cystic duct and cannulated the common duct with a black rubber tubing. The tube was exteriorized through the abdominal wall to collect bile into a rubber bag.

Since then, more complicated surgical procedures have been used for the establishment of biliary or pancreatic fistulas in animals and human beings. T-tubes were used and are still in use for the drainage of bile. The horizontal bar of the tube is placed into the bile duct, while the long arm is passed through the abdominal cavity and fixed to the skin (Judd and White, 1928).

John Baldwin _et al_. (1966) placed a specially designed T-tube in the common bile duct to insure the total collection of bile. This tube had a circular outer balloon and a small flap valve which could be inflated by the injection of air through a separate tube attached to the long arm of the T, thus obstruction of the distal end of the tube and common bile duct was accomplished. By this method the reverse flow of bile could be blocked whenever desired and bile could be collected through the long arm of the special tube.

Puestow (1931) in his studies on the discharge of bile into the duodenum of dogs, used three operative stages to exteriorize the papilla of Vater. First he dissected the pyloric end of the duodenum closing the entrance of the latter, and making an end-to-side anastomosis of the stomach to the jejunum. Three weeks later he isolated the pancreatic duct, incised it close to the duodenum, then the pancreas was separated from the duodenum to allow the latter to be brought under the skin. The third stage consisted of the dissection of the duodenum between the pancreatic papilla and the papilla of Vater. The sides of the duodenum were sutured to the skin, exposing the common bile duct.

In the study of collection of pancreatic juice in the conscious sheep, Taylor (1960) ligated the common bile duct proximal to the entrance of the pancreatic duct. The gallbladder was connected to the jejunum. An oblique-to-side anastomosis of the jejunum to the duodenum was performed and the latter was cannulated opposite the papilla of Vater, which was exteriorized through the body wall.

Two Tygon tubings were used by Cohen (1960) to establish a bile return system in the dog. The common bile duct was ligated, one tube was introduced into the gall-bladder, while the second was placed into the duodenum. Those tubes were exteriorized through the abdominal wall and connected.

Berci and Johnson (1965) in the functional studies of the extrahepatic biliary system in the dog inserted an acrylic button into the gall-bladder and to the button a polyethelene tube was attached. To the other end of the tube, an L shaped tube was connected and exteriorized through the abdominal wall. This external opening of the tube was sealed with a screw-cap.

Markowitz et al. (1964) in their experimental surgery book discussed the surgery of the biliary system in detail. Oberhelman (1957) described the surgical techniques and considerations of gall-bladder and extrahepatic bile ducts extensively.

Carbon tetrachloride is a colorless, clear, non-flammable heavy volatile liquid with a characteristic odor. It is prepared commercially by chlorinating carbon disulphide or methane. It was discovered by the French physician H. V. Regnaut in 1889 (Hardin, 1954).

Smith in 1867 used carbon tetrachloride as an analgesic and anesthetic on 52 patients suffering from headache, toothache, cancer pain, etc. However, the side effects and toxicity of carbon tetrachloride discouraged its use as an anesthetic.

Hall in 1921 published his work on the potency of carbon tetrachloride as an anthelmintic in dogs for hookworms.

Allen (1922) studied the efficiency of carbon tetrachloride against hookworms in the silver black fox and reported a 93 percent efficiency. One year after Hall's report, Leach (1922) used it extensively in the treatment of human hookworms. The anthelmentic use of carbon tetrachloride in human medicine did not last long due to the occasional mortalities reported in the literature (Hardin, 1954).

Montgomerie (1926) employed carbon tetrachloride in the treatment of liver rot (fascioliasis) of sheep. A single dose of one ml. in soft gelatine capsule was used for a 140 pound sheep affected with liver rot. Because of its efficacy and inexpensiveness, carbon tetrachloride has been the medicine of choice throughout the world for the treatment of fascioliasis in sheep, even though it is a toxic agent and may have as much as nine percent mortality associated with its use (Setchell, 1962). Carbon tetrachloride is still superior to the less toxic hexachloroethane in the treatment of fascioliasis, a world wide parasitic problem (Kuttler _et al_., 1963).

The fat solvent property of carbon tetrachloride has made it useful in dry cleaning. It is used extensively in industry in the production of freons which are used as refrigerants. Being nonflammable, it is used as a fire extinguisher.

In the United States, 11 million pounds of carbon tetrachloride was manufactured at 1923, this tripled by 1930 and in 1951, the production was 224 million pounds (Hardin, 1954).

Although determination of the pathogenicity and toxicity of carbon tetrachloride was not the purpose of this experiment, citation of a few important publications reviewing carbon tetrachloride toxicity seems pertinent at this point.

Lehmann and Flury (1943) and von Oettingen (1955) have given an excellent review of carbon tetrachloride toxicity in their books, while Hardin (1954) reviewed the medical literature on carbon tetrachloride thoroughly.

The pathological action of carbon tetrachloride, according to Gallagher (1961) is manifested in an increased permeability of the liver cell membranes, and decreased levels of intercellular respiratory co-enzymes. He also described mitachondrial injury as well as fatty degeneration in liver cells.

According to Nielsen and Larsen (1965) in addition to liver damage occurring with carbon tetrachloride poisoning, there is also renal damage manifested as degeneration of tubular epithelium causing acute renal failure.

Robbins (1929) stated that the absorption of carbon tetrachloride from the gastrointestinal tract increases with the ingestion of fat and alcohol. He found that the bone marrow absorbs five times the carbon tetrachloride as compared to the liver, pancreas or the brain. The majority of carbon tetrachloride absorbed was eliminated through the lungs. He did not detect elimination in the urine. McCollister _et al_. (1951) stated that monkeys inhaling a vapor containing 46 p.p.m. of C^{14} labeled carbon tetrachloride could absorb about 30 percent of the vapor. The highest concentration of the radioactive material deposited in the tissues was found in the fat which had a distribution ratio of 7.94 with blood taken as one. They also found that 50 to 60 percent is exhaled through the lungs during the first six hours after administration of carbon tetrachloride. Only a minor portion is excreted in the stool and urine.

Kondos and McClymont (1961) stated that blood levels of carbon tetrachloride in sheep will reach a peak of 15 to 20 μg./ml. when administered eight ml. of carbon tetrachloride intraruminally within 15 to 30 minutes, the level declines to undetectible levels of five μg./ml. in four to five hours. On intramuscular administration of the same dose of carbon tetrachloride, the peak blood level of ten μg./ml. was reached in four hours.

von Oettingen (1955) stated that carbon tetrachloride is readily absorbed by the lungs and more slowly from the gastrointestinal tract; he also stated that the amount of absorbed carbon tetrachloride was increased in the gastrointestinal tract with the ingestion of alcohol and fat.

Tikhonov and Ayunov (1964) stated that carbon tetrachloride injected intramusculary in a dose of 0.3 ml./kg. of body weight to sheep resulted in a decreased bile secretion for five hours. The maximum effect occurred during the third hour.

Bollmann and Mann (1936) reported that the formation but not the destruction of bile salts in the liver decreased with carbon tetrachloride administration.

Brednow and Jensen (1929) were unable to detect carbon tetrachloride in the bile of dogs and rabbits, while they were able to detect it in the liver.

Detection of carbon tetrachloride in biological materials is similar to the detection of most chlorinated hydrocarbons. Fujiwara (1916) described a method for the detection of chloroform in the blood, he stated that the reaction between chloroform and pyridine in an alkaline media gives a red colored pyridine layer which could be measured colorometrically.

The methods described for the detection of carbon tetrachloride by Habgood and Powell (1945), Alford (1947), Weber (1941), Powell (1945), Spain and Frey (1951), Burke and Southern (1958), Kondos and McClymont (1959), and Leibman and Hindman (1964) are all modifications of the Fujiwara's alkaline pyridine reaction.

Conway's microdiffusion technique was adopted by Burgen (1948) in estimation of chloroform in the blood. This method could be used also in the detection of carbon tetrachloride.

Gas chromotography was used by Curry _et al_. (1962) in determination of carbon tetrachloride from blood samples. This is a rapid and simple method in which the toxicologically significant concentration can be detected by comparison with control samples.

III. METHODS AND MATERIALS

A. Surgical Procedures

1. General considerations

The age, sex, breed and body weight of ten sheep used in this experiment were not taken into consideration. A physical examination was carried out on them to determine their state of health, and other than a mild parasitic infestation with gastrointestinal nematodes, they were in good condition. Thiabendazole[1] was used to deworm the animals. A suspension of two grams of the drug in one ounce of water was administered to each sheep individually by a standard dosing syringe.

The sheep were fed on good quality alfalfa hay prior to surgery. Fresh water was available at all times. They were kept indoors in groups of four in each pen. Each individual was kept alone after surgery to facilitate care and handling. In addition to alfalfa hay a concentrate containing corn, oats, soybean meal, linseed meal, dicalcium phosphate, trace mineral salts, vitamin A and vitamin D was fed to the animals undergoing study. After a few days of post surgical observation of the biliary flow and the general condition of the animals, carbon tetrachloride was administered and bile samples were obtained at hourly intervals for

[1]Thiazole. Merck Chemical Division, Merck & Co., Inc., Rahway, New Jersey.

chemical analysis.

2. Materials

Ten sheep were used in this experiment to establish a controlled biliary fistula to study the excretion of carbon tetrachloride in bile.

Two 25 cm. long size 10 French oxygen catheters[1] were used to exteriorize the flow of bile from the common bile duct (Figure 1). Each tube is provided with eight alternating perforations extending 2.5 cm. from a smoothly molded distal tip to assure uninterrupted flow of bile. On the other end, there is attached a solid multiple-fit connector which can be connected with larger size tubes or a syringe for aspiration or injection. The solid adaptor end has three rings which help in placing sutures around the tube to fix the latter to the skin.

A 12 gauge seven cm. long needle was attached to a one meter long plastic tube to be used in the evacuation of the accumulated gases from the rumen.

3. Anesthesia

The first five sheep used in this experiment were anesthetized with pentobarbital sodium.[2] Twenty minutes prior

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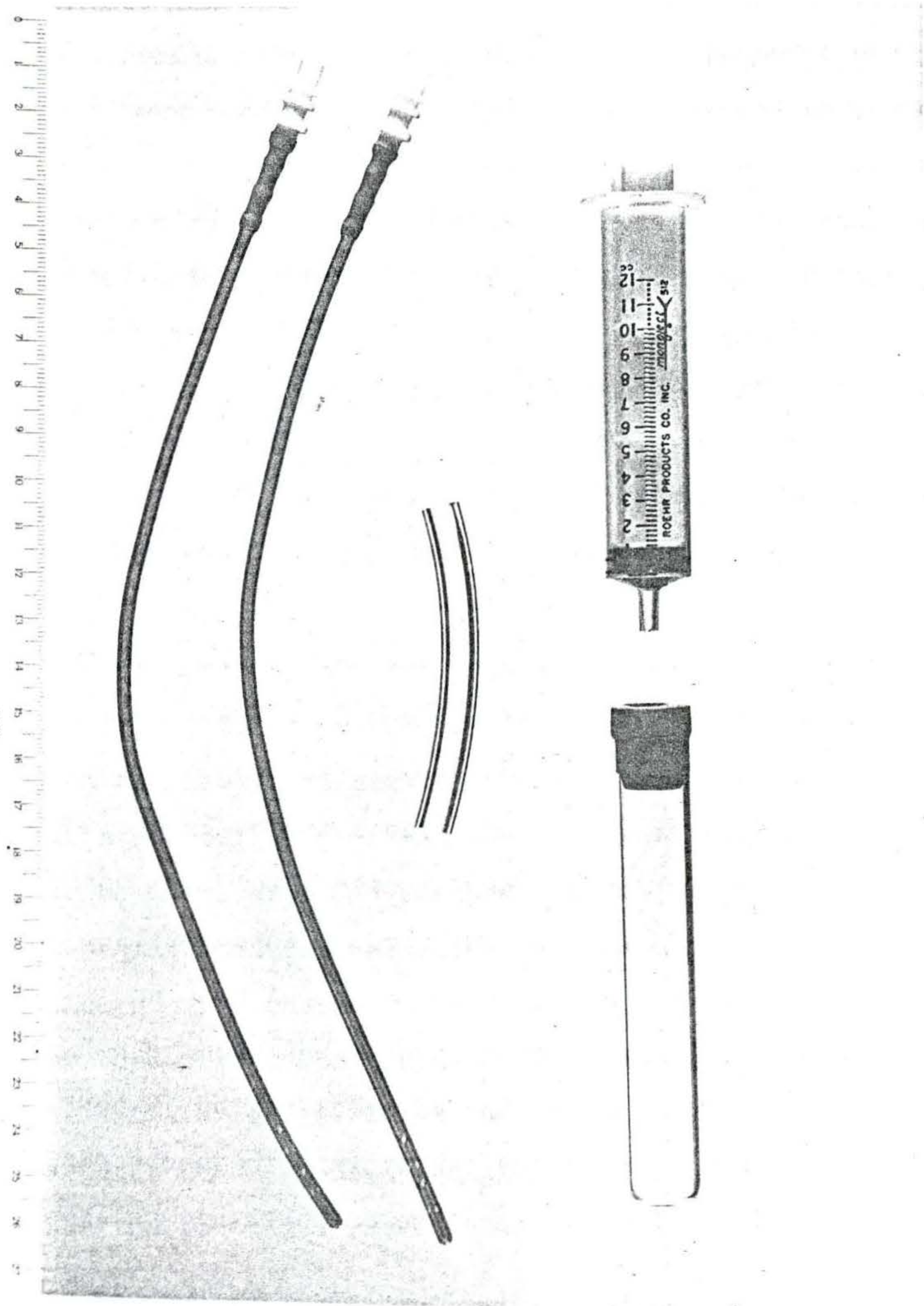
[1]Pharmaseal Laboratories, Glendale 1, California.

[2]Pento-sal: Aljen Veterinary Supply Company, Brooklyn, New York.

Figure 1. The two 25 cm. long tubes and the one seven centimeter long tube used to exteriorize the common bile duct in the sheep. The syringe and test tube are used in the collection of bile samples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ROEHR PRODUCTS CO. INC.

to surgery four mg. per 50 kg. body weight of atropine sulfate[1] was injected subcutaneously as a preanesthetic agent to decrease broncheal secretion and salivation.

Pentobarbital sodium was given in a rate of 25 mg. per kg. body weight intravenously, one-half of the calculated dose was given rapidly. After a pause for one minute, the second half was given slowly until satisfactory anesthetic level was obtained. Palpebral and pupillary reflexes were used as a criterion for the depth of anesthetic level. It is judicious to employ endotracheal intubation with general anesthetics in ruminants. It provides an open air passage and prevents asphyxia from inspiration of saliva or regurgitated ingesta.

Immediately after induction of anesthesia with pentobarbital sodium the sheep was placed on the operating table. When the jaw muscles were relaxed an assistant opened the mouth and pulled the tongue to one side. At the same time the head was extended backward as far as possible. A number 10 Magill endotracheal catheter was used to intubate the trachea. The catheter was held with the right hand and introduced into the mouth. The left hand was used to palpate the catheter as it passed into the larngeal region. The catheter has a tendency to glide easily into the esophagus, thus the esophagus was located and the catheter was pulled out

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[1]Atropine injectable, L. A.: Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

while compression was applied by the thumb and forefinger to the esophageal inlet to occlude it and in the same time to direct the catheter into the trachea. The catheter was gently rotated and pushed forward into the trachea. The soft nature of the trachea in the sheep facilitates palpation of the catheter inside.

The cuff of the catheter was inflated with a syringe, and the inflation tube was clamped with a hemostat. The passage of air through the catheter was checked by feeling the air current with the hand or a few fibers of wool were held in front of the opening of the catheter and the movement of the fibers with each inspiration or expiration was noticed. The catheter then was fixed to the upper jaw with adhesive tape.

There was no need for the use of an endoscope to provide vision nor was there any need to hold the epiglottis with a forceps to facilitate the intubation of the trachea.

The second group of five animals was anesthetised by a combination of an ataractic drug and a local anesthetic. Promazine hydrochloride[1] was used at a rate of 1.1 mg. per kg. body weight administered intravenously while the animal was prepared for surgery.

Two percent Xylocaine hydrochloride[2] was used as a local anesthetic. The agent was infiltrated subcutaneously in an

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[1]Sparine: Wyeth Laboratories, Philadelphia, Pennsylvania.

[2]Xylocaine Hydrochloride: Jensen-Salsbery Laboratories, Kansas City, Missouri.

inverted L pattern, starting from the cranial angle of the flank triangle and along the caudal margin of the costal arch toward the xyphoid cartilage. Then a ten cm. long horizontal line parallel to the lateral processes of the lumbar vertebrae was infiltrated to establish the desired local anesthesia in the site of the laparotomy incision.

4. Preoperative procedures

To facilitate operative procedures in the abdominal cavity of sheep, it was necessary to starve the animals at least for two days prior to surgery. Taking the animals off feed reduced the size of the abdominal contents considerably, which allowed a good exposure of the deep structures in the abdominal cavity. Water was available at all times to animals awaiting surgery.

Preoperative preparation of the surgical site consisted of clipping the wool from the right thoracoabdominal region with an electrical clipper with a number 40 clipper blade. The ventral aspect of the neck was also clipped to expose the site of the jugular veins for intravenous injections as well as collection of blood samples.

The animal was placed on the operating table in left lateral recumbency. The site of operation was scrubbed three times thoroughly with soap which was removed with a stream of water after each scrub. The area was dried with sterile gauze sponges and isopropyl alcohol applied. A few minutes later, the area was painted with 1:1000 Tincture of Merthiolate[1].

The surgical field was draped with sterile cloth drapes, leaving a sufficient opening to expose the operative field. The drapes were fixed with towel clamps. Aseptic technique was practiced during the entire surgical operation.

5. Anatomical considerations

The choice of surgical approach was governed by the need for good exposure of the bile duct. The sheep has a large rumen which occupies a considerable portion of the abdominal cavity making transperitoneal operations uncomfortable for the surgeon. The paramedian or flank laparotomy approach to bile duct surgery does not give good exposure for surgical manipulations. Therefore, the best approach was found to be at a line three cm. parallel to the right costal arch extending seven cm. from the xyphoid cartilage dorsally for about 20 cm. (Figures 2 and 3). The common bile duct in the sheep is formed from the union of the hepatic and cystic ducts, it is about nine cm. long and 0.4 cm. in diameter. The pancreatic duct opens into the bile duct three cms. caudad to the above mentioned union. It runs parallel with the duodenum for about one cm. before it opens into the papilla of Vater.

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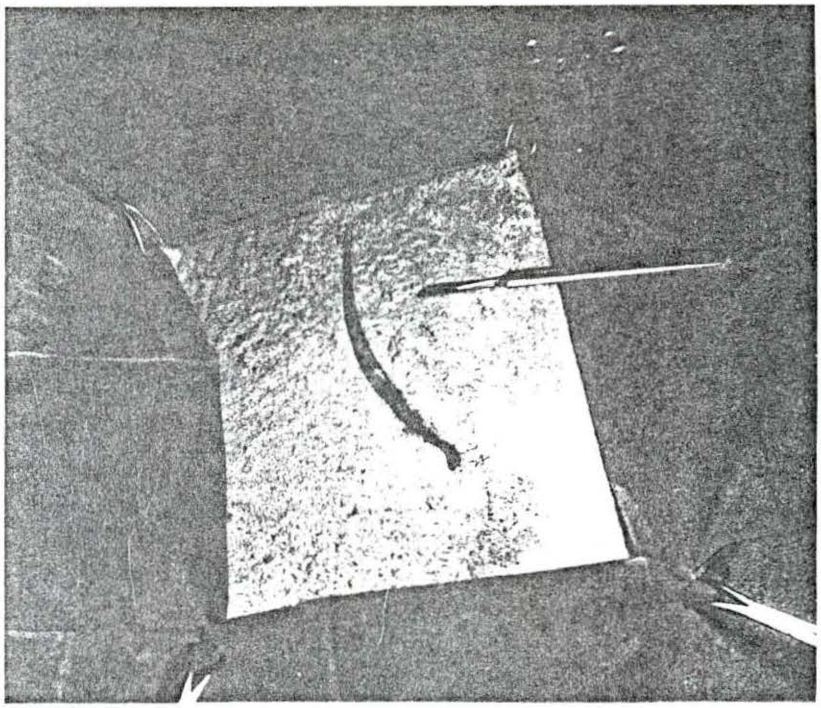
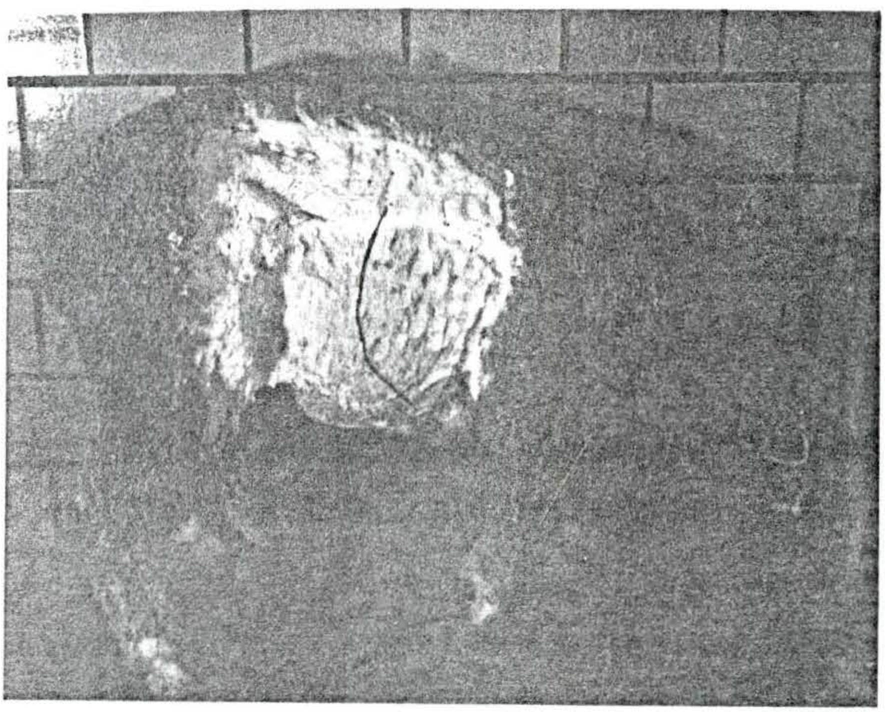
[1]Corvel, Inc., 1124 Harney Street, Omaha, Nebraska.

Figure 2. The skin incision was made parallel to the costal arch, to which the scalpel is pointing

Figure 3. The costal arch was marked to orient the site of skin incision





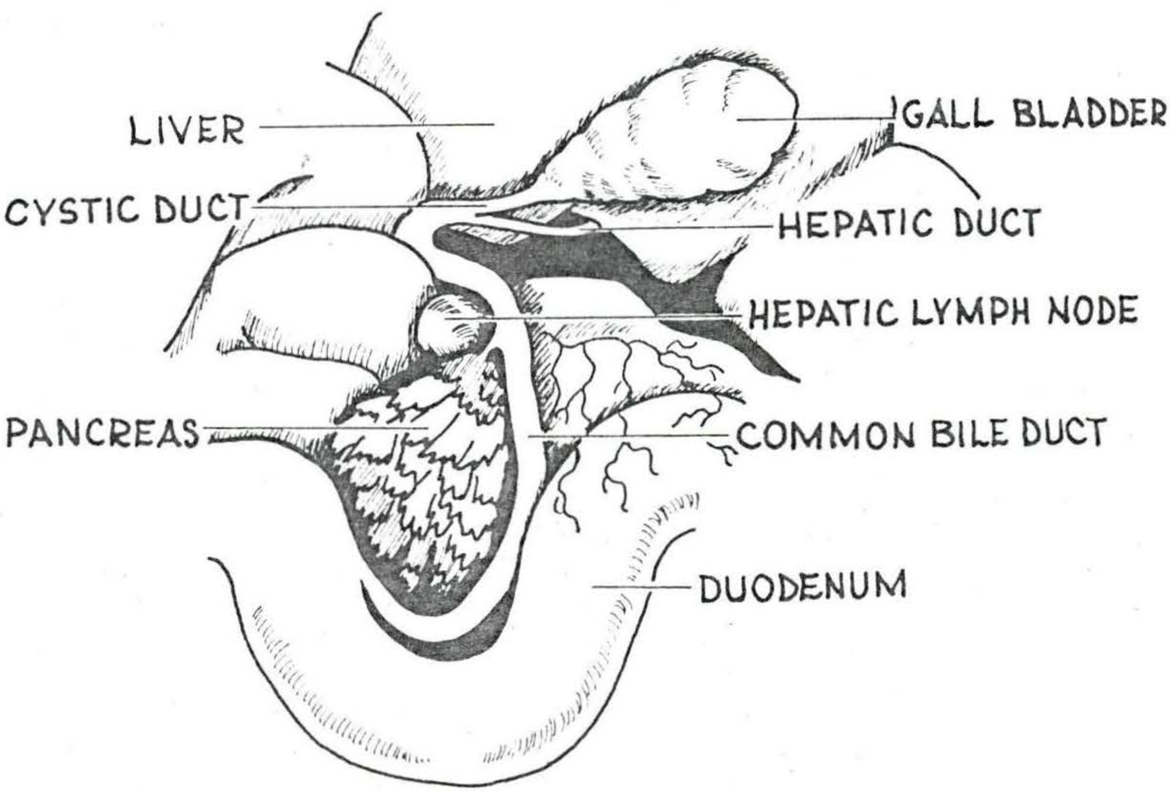
6. Operative technique

A 20 cm. long skin incision was made three cm. parallel to the right costal arch. The external and internal oblique abdominal muscles were incised and the transverse abdominal muscle was separated along its muscular fibers. The peritoneum was then incised and the abdominal cavity exposed.

The abdominal viscera were inspected for any abnormalities. In cases of distended rumen, a sterile 12 gauge needle to which a one meter long plastic tube attached was used to evacuate the accumulated gases in the rumen. A purse-string suture was placed around the puncture of the needle in the rumen to prevent leakage of ruminal contents and contamination of the operative area. The bile duct was located and identified (Figure 4), the viscera adjacent to the liver, namely the duodenum, abomasum and rumen were retracted and packed with a sterile towel which was soaked in warm sterile physiological saline. An assistant gently retracted the liver and the viscera allowing a good exposure of the bile duct and gall-bladder. The appearance of both structures were noted followed by careful palpation to determine the contents and differentiate it from the portal vein. The gall-bladder was massaged gently and the bile flow was noted in the bile duct.

A stay suture was passed around the common bile duct between the junction of the cystic and hepatic ducts and the

Figure 4. A general view of the structures related to the common bile duct as it is seen through the laparotomy incision


LIVER
GALL BLADDER
CYSTIC DUCT
HEPATIC DUCT
HEPATIC LYMPH NODE
PANCREAS
COMMON BILE DUCT
DUODENUM

pancreatic duct which helped in holding the duct which is situated relatively deep in the abdominal cavity from this particular approach. A small longitudinal slit was made in the common bile duct, a flap of the wall was held by a forceps to facilitate the insertion of the perforated end of the polyvinyl tube which was passed towards the origin of the duct. Number 000 black braided surgical silk[1] was used to fix the tube in the bile duct, a ligature passed through the walls of the bile duct and the tube to hold the latter in position. Two more ligatures were passed around the bile duct distal to the first ligature to prevent leakage of bile into the peritoneal cavity. The bile flow through the tube was noted. The second tube was passed into the common bile duct in an opposite direction and fixed in place in the same manner (Figure 5).

Physiological saline was injected into the second tube and the flow of saline was noticed in the common bile duct running towards the duodenum.

Two stab incisions were made in the middle of the right flank triangle four centimeters apart and the adapter ends of the tubes were exteriorized through the abdominal wall. The tubes were held in close contact to the abdominal wall internally by a few sutures placed through the peritoneum and abdominal muscles and around the tubes, thus, interference

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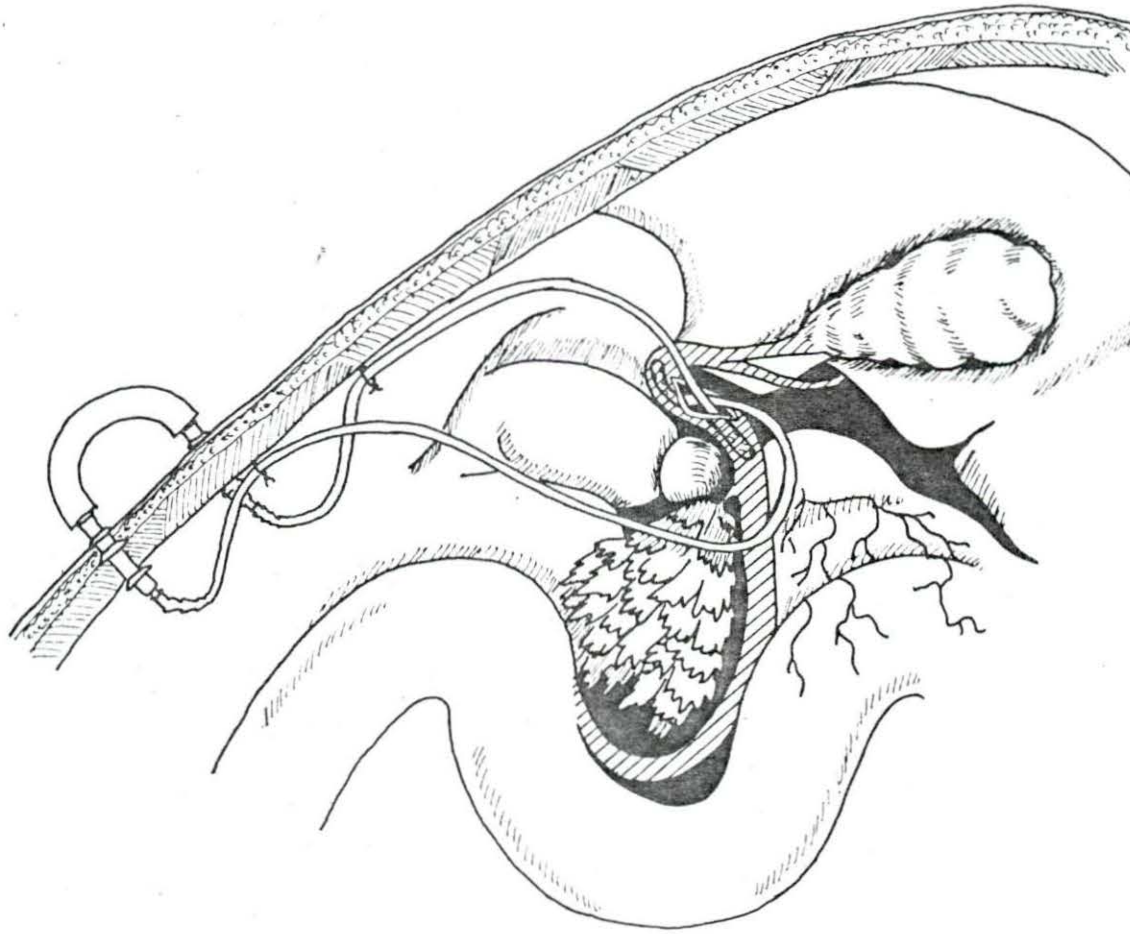
[1]Ethicon, Inc., Semerville, New Jersey.

Figure 5. The polyvinyl tubes are placed into the common bile duct each in an opposite direction. The tubes are anchored to the peritoneal surface of the abdomen and the connector heads are fixed to the skin. The exteriorized portions are connected with a polyvinyl tube



of abdominal viscera with tubes was minimized.

The sterile towel used to pack the abdominal viscera was removed. The laparotomy incision was closed in the following manner: Continuous suturing of the peritoneum and transverse abdominis muscle with number one catgut. The oblique abdominal muscles and the subcutaneous fascia were closed with a second row of continuous sutures. The skin incision was closed by a continuous interlocking suture using Vetafil[1]. The tubes penetrating the abdominal wall in the right flank region carried a four centimeter long rigid adaptor which prevents occlusion of the tubes, meanwhile, the three rings at the end of the tube served as a device to give a more accurate hold to the purse-string suture used in the skin around the tubes. Both ends were checked again carefully by aspiration of bile from the first tube and injection of physiological saline into the second tube. Then both tubes were connected with a seven centimeter long plastic tube to allow normal bile circulation (Figure 6).

The animal was removed from the table and a many-tailed bandage was applied to protect the operative site.

Postoperative care of the animals consisted of daily

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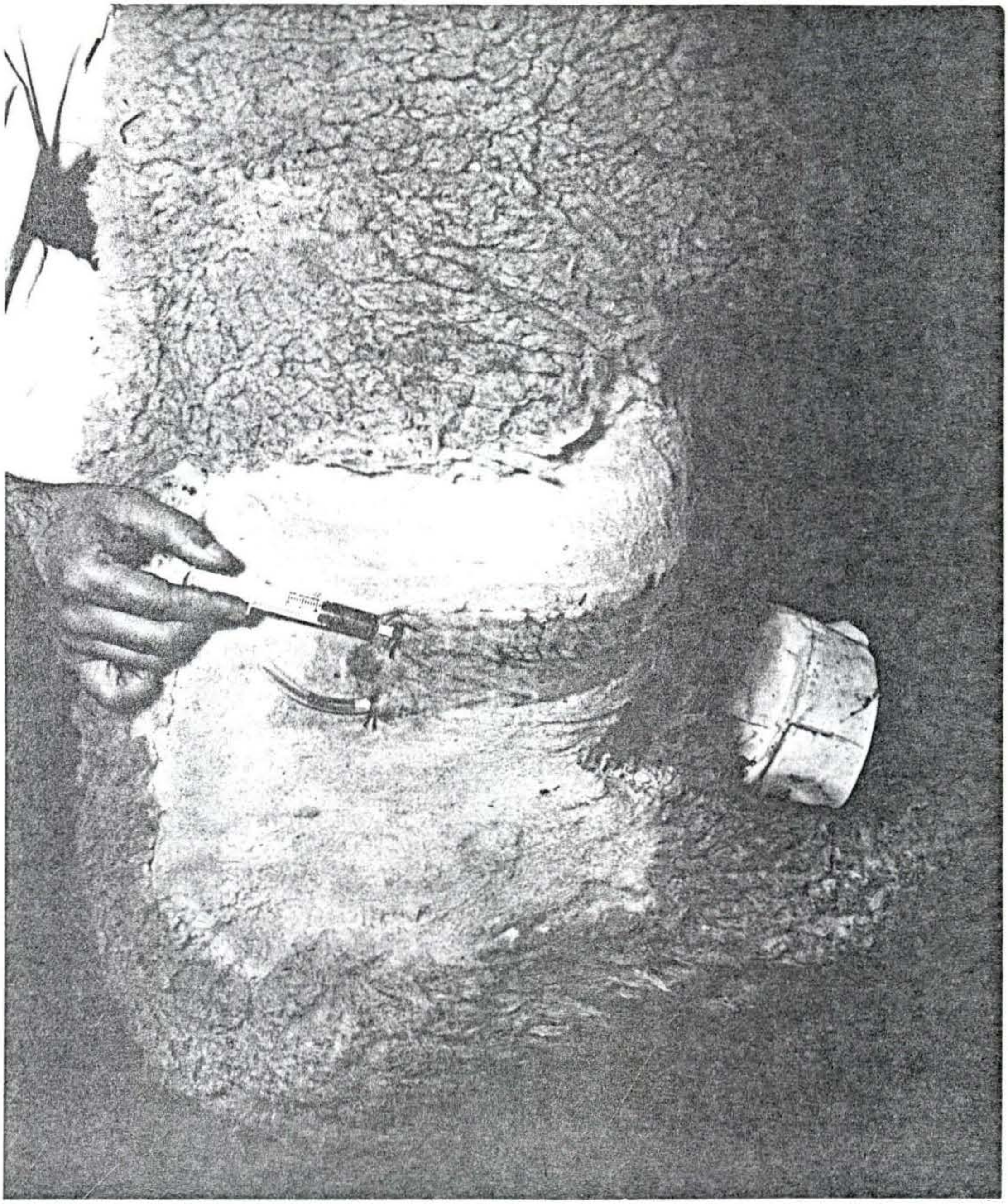
[1]Vetafil: Bengen, Bengen and Co., GmbH., 8-12 Dreyer Street, Hanover, West Germany.

Figure 6. Collection of bile samples from the sheep. The syringe is attached to the tube which is draining from the common bile duct



checking of body temperature and general condition of health. The bile flow was checked several times daily by removing the connection tube and aspiration of bile with a sterile syringe and injecting it into the second tube.

B. Chemical Procedures

1. General procedures

The biliary fistulas which were established in ten sheep were utilized in a study of the excretion of carbon tetrachloride in bile. After surgery each sheep was fed a concentrate ration for four days and when each recovered from the stress of the surgery the experiment was started.

To the first six sheep two ml. of carbon tetrachloride (A. R. grade) was administered in a gelatine capsule orally. (Two ml. is twice the standard dose used for the treatment of fascioliasis in sheep.) Bile samples were collected before administration of carbon tetrachloride to be used as controls then more samples were collected at 1, 2, 4, 8, 16 and 32 hour intervals. The samples were collected with separate sterile syringes into sterile ten milliliter rubber stoppered test tubes. The quantity of bile collected at each time ranged between 2.5 ml. and seven ml. To prevent evaporation of carbon tetrachloride from the samples air was injected into each tube to create a positive pressure and minimize evaporation. The samples were immediately refrigerated. At intervals of four hours a spectrophotometric determination of carbon tetrachloride was done on the bile samples.

The last sheep from the first group and five sheep from the second group were administered ten ml. carbon tetrachloride by an intraruminal injection. The same procedures in collecting and handling of bile samples were employed.

2. Procedure and technique

The method described by Kondos and McClymont (1959) was adopted for the detection of carbon tetrachloride in bile. An appropriate calibration graph was prepared by adding known quantities of carbon tetrachloride to bile and analyzing the solution for the detection of carbon tetrachloride, a range of 10-150 ug. per ml. was obtained.

Reagents: (1) Acetone - A. R.

(2) Pyridine - A. R.

(3) 20% W/V sodium hydroxide

(4) Absolute ethanol

To 3.0 ml. of bile in a 15 ml. test tube 7.0 ml. of acetone was added, stoppered and shaken. The sample was centrifuged for five minutes (between 2000-5000 r.p.m. 12 cm. radius) while 7.0 ml. of pyridine was pipetted into a test tube. Two ml. of the clear centrifuged supernatent fluid was added to the pyridine and mixed. Then 3.5 ml. of 20% W/V sodium hydroxide was added and gently shaken. The test tube was stoppered and heated in a boiling water bath for exactly two and one-half minutes. The test tube was cooled in tap water for two minutes.

Five milliliters of pyridine layer was transferred immediately into another test tube. To remove turbidity 1.5 ml. of absolute ethanol was added, shaken and the color intensity was measured at 535 millimicrons with a spectrophotometer[1].

In positive cases the pyridine reacts with chlorinated hydrocarbons in an alkaline media and gives a light red color which can be measured colorimetrically.

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[1]Spectronic 20 colorimeter. Bausch and Lomb Optical Company, Rochester, New York.

IV. DISCUSSION

The mechanism of action of medicines used in the treatment of fascioliasis of sheep is one of the least investigated facets of this condition. The need for further information in this regard stimulated the author to develop a simple method for the surgical exteriorization of the bile duct.

The emphasis was on the use of materials readily available and simple surgical procedures which could be performed to establish the desired biliary fistula.

Pentobarbital sodium has been used as a general anesthetic agent in sheep in operative procedures involving the extrahepatic biliary system (Pahneuf 1957 and 1961, Taylor 1960, and Harrison 1962).

In ruminants a few precautionary measures should be taken when using a general anesthetic in order to minimize regurgitation and asphyxia. Sheep should be fasted 48 hours prior to pentobarbital sodium administration and given atropine at the time of surgery to reduce salivation and bronchial secretions.

Endotracheal catheters are recommended by Borrie and Mitchell (1960) to prevent aspiration of saliva or regurgitated ingesta. The endotracheal catheter is also helpful in cases where oxygen is needed. This method of induction of general anesthesia was used on five sheep with good results.

The combination of an ataractic and a local anesthetic agent was used on the second group of five sheep. This combination gave very satisfactory anesthesia, there were no signs of pain or discomfort in animals treated with this method. Only very slight sensation to pain was noticed when excessive traction was applied to organs during retraction. The advantage of this method was that the animals were very active and alert as soon as the surgery was completed and there was no need for the intubation of the trachea.

Having not eaten for 48 hours, an excellent appetite was noticed in these animals as soon as they were back in the pens.

The laparotomy approach in intra-abdominal surgery is governed by the anatomical locations of the organs involved. In hepatic or extrahepatic biliary system surgery, the best approach is a paracostal one (Glenn, 1965). In the sheep the liver, abomasum, colon and a portion of the rumen occupy the right cranial quadrate of the abdominal cavity. The size and contents of the abomasum and the rumen interfere to a great extent with the operative procedures in the abdominal cavity.

Taylor (1960) recommended a ruminal fistula to be done a few days prior to the main surgery for the collection of pancreatic juice in the sheep. The fistula was used to evacuate the ruminal contents which were stored in a warm place and returned to the rumen after surgery.

Rumenotomy or ruminal fistula was not necessary in this study. Fasting the sheep for 48 hours prior to surgery reduced the contents of the rumen and abomasum considerably. The excessive amounts of ruminal gases were evacuated by a 12 gauge needle attached to a plastic tube (Moore et al., 1954). To prevent any possible contamination a purse-string suture was used on the rumen and around the puncture of the needle prior to the insertion of the latter.

By adopting this method there was no need for an extra surgical procedure which would cause further stress on the animals health. Fasting has also a favorable effect on decreasing regurgitation which minimizes possibilities of asphyxia during general anesthesia.

The portal vein is very close to the common bile duct in the portal fissure and should be carefully considered and protected from any unnecessary trauma. The origin of the common bile duct in the abdominal cavity is deeply seated when the animal is in left side recumbency. Proper and sufficient retraction of the liver, abomasum and the small intestine are required to provide vision of the field and space for the operative manipulations.

A small branch of the hepatic artery accompanies the common bile duct and should be avoided while making the longitudenal slit incision in the duct for the insertion of the tubes. Considerable hemorrhage was noticed when the duct was transversly sectioned which obscured the operative field.

The terminal portion of the common bile duct is readily accessible, thus, if the pancreatic juice or a mixture with the bile are needed it can be more easily obtained.

The anatomical location of the common bile duct in the abdominal cavity is close to the right flank region, therefore two 25 cm. long polyvinyl tubes were sufficient to exteriorize the bile flow. The length of the tubes gave flexibility to move along with the natural movements of the abdominal viscera. The solid connector heads of the second end prevented collapse of the tubes due to muscular contractions or postoperative scar tissue.

The interior diameter of the solid connector pieces were suitable for the attachment of a syringe to aspirate bile samples whenever needed. The seven centimeter long plastic tube was a convenient device to connect the two ends of the exteriorized tubes to continue the circulation of bile.

Gentle aspiration of bile samples prevented irritation to the lumen of the bile duct, otherwise flakes of cells and debris were observed in the bile. Obstruction of the tubes was also found to be due to the deposition of bile sediment. The thickness of the tubes and their tensile strength were sufficient to overcome kinking.

In one sheep the tubes were pulled out three weeks after surgery. During four weeks of observation the animal was in good condition and no sign of sickness or obstructive jaundice was noticed. This was an indication of self reconstruction of the common bile duct to fulfill its natural function. The animal was euthanised and on necropsy the gall-bladder was atrophied and collapsed. The common bile duct at its proximal half was extremely enlarged and its diameter was about 20 mm. The wall of the duct was thickened and fibrosed.

The findings in this sheep indicate that even if the tubes pull out or become obstructed, the animal can survive by reconstruction of a fistulus tract which could function as a normal common bile duct.

The interior diameter of the tubes used in establishment of the biliary fistulas were about 2.5 mm, while the corresponding diameter of the common bile duct is about four millimeters. When this size tube was used it was believed that the difference between the two diameters could not cause significant changes in bile flow. Eventually, in three sheep hypertrophy of the gall-bladder and bile stasis in the liver were observed. As far as the collection of bile samples were concerned no difficulties were encountered. A larger size tube in this regard might have given better bile flow and the pathologic changes in the related organs might have been less severe.

T-tubes are very convenient to drain bile from the common bile duct, they can be removed after a considerable length of time. The special T-tube designed by John Baldwin et al. (1966) is an ideal tube for such studies relative to bile or biliary system. The inflatable balloon on the distal short limb of the T has a dual purpose. From one side it occludes the space between the tube and the common bile duct while from the other side it closes the valves inside the T-tube to prevent seepage or back flow of bile.

At the time this study was commenced such T-tubes were not available, otherwise this tube could have replaced the two tubes employed in the exteriorization of the bile flow.

Simplicity was intended in this study in regard of materials and methods used in the establishment of a biliary fistula. The plastic tubes used are available from any hospital supply agency, they can be utilized for various short term experimental studies on the biliary or the pancreatic secretions.

Daily checking of the tubes for patency, aspiration of bile with a sterile syringe and reinjection of bile back into the second tube can maintain a continuous bile flow for at least three weeks, which is sufficient for any short term study.

A bile sample was collected prior to the administration of carbon tetrachloride to be used as a blank control in the chemical analysis (Kondos and McClymont, 1959). Separate dry sterile syringes were used to collect subsequent samples of bile at 1, 2, 4, 8, 16, and 32 hour intervals. The samples were immediately transferred to stoppered test tubes to minimize possibilities of air contact as much as possible.

Tikhonov and Ayunov (1964) reported that the amount of bile secretion decreased after the intramuscular injection of carbon tetrachloride. The secretion decreased for five hours, the maximum being at three hours post administration. Their findings were noticed in this study also, the bile samples obtained during the first four hours decreased considerably, the minimum being two and one-half milliliters. The maximum was about seven milliliters.

The bile samples being not sufficient to fill the test tubes, a simple physical theory was employed to overcome this difficulty. Air was injected into the tube under pressure to reduce evaporation.

According to Powell (1945) the distribution coefficient (which is the ratio between the concentration of the substance in the liquid and concentration in the gas) of carbon tetrachloride is twice higher in 20° C. than it is in 37° C. in blood. Therefore, bile samples were refrigerated immediately to decrease any evaporation and escape of carbon tetrachloride.

When detecting carbon tetrachloride in blood samples that had been refrigerated and stored overnight, six to eight percent lower results were reported by Kondos and McClymont (1959). The bile samples obtained from sheep were analyzed within a maximum time of four hours, to decrease errors in the detection of carbon tetrachloride (Table 1).

Sheep number two died seven hours after an oral administration of two milliliters of carbon tetrachloride. On necropsy, nutmeg liver, bile stasis in the liver, hypertrophy of the gall-bladder and adhesions of the omentum to the common bile duct were noticed. Sections from the liver and kidneys were preserved in ten percent formalin for histopathologic study, which revealed heavy accumulation of bile in hepatic cells, particularly in the centrilobular areas; individual cellular necrosis of hepatic cells with early accumulation of neutrophils was also prominent in this area. Mild mixed inflammatory cell accumulation in the portal triads was also observed. The kidneys showed toxic tubular nephrosis. Many calcified casts in medullary collecting ducts were also noticed.

The necropsy and histopathologic findings in this sheep are characteristic of carbon tetrachloride poisoning (Woods 1946, Smith and Jones 1961, Ford and Lawrence 1965).

The normal dosage of carbon tetrachloride as an anthelmentic in sheep ranges between four and ten milliliters, one milliliter is used in the treatment of fascioliasis

Table 1. Administration of carbon tetrachloride to sheep and analysis of bile samples for carbon tetrachloride

Sheep	Days between surgery and administration	ml. carbon tetrachloride (oral)	ml. carbon tetrachloride (intraruminal)	Number of bile samples[a]	Carbon tetrachloride detected in bile
1	5	2		5	0
2	5	2		4[b]	0
3	6	2		6	0
4	4	2		5	0
5	5	2[c]	10	7	0
6	5	2[c]	10	7	0
7	5		10	6	0
8	7		10	7	0
9	6		10	7	0
10	6		10	7	0

[a]Bile sample #1 obtained before administration of carbon tetrachloride. Sample numbers 2, 3, 4, 5, 6, and 7 were obtained after 1, 2, 4, 8, 16, and 32 hours after administration.

[b]Sheep died seven hours after administration.

[c]One week later administered ten ml. of carbon tetrachloride by the intraruminal route.

(Jones, 1962).

There are great variations in susceptibility of sheep to carbon tetrachloride poisoning. Setchell (1962) reported a nine per cent mortality in 123,113 sheep treated with one and two milliliters of carbon tetrachloride mixed with four and three milliliters of liquid paraffin which were administered orally. Three milliliters were also used intramuscularly. Gallagher et al. (1962) studied the susceptibility of sheep to carbon tetrachloride toxicity under different experimental conditions. Fifty milliliters of the agent were administered to 49 adult sheep, and the authors reported a mortality rate ranging between 25 and 100 per cent.

One sheep out of ten used in this experiment died with carbon tetrachloride toxicity which limited the use of larger doses. Sheep number nine was very weak after 48 hours from the administration of ten milliliters of carbon tetrachloride. The animal was euthanized and on necropsy hypertrophy of the gall-bladder and bile stasis in the liver was observed. The histopathological examination of the liver revealed moderate accumulation of inflammatory cells in the portal triads; marked congestion and foci of neutrophils were seen around some bile ducts. The gall-bladder showed chronic cholecystitis which was characterized by multiple foci of lymphocytes and hyperemia in the lamina propria. The characteristic lesions of carbon tetrachloride poisoning were not clear in this case. The cause of the chronic cholecystitis is probably due to contamination during collection of bile samples.

Adhesions of the omentum to the common bile duct at the site of insertion of the tubes were a constant finding on necropsy of all animals. In two cases proliferations from the omentum surrounded the tubes. This indicated that the polyvinyl tubes used had a mild irritant effect on the tissues.

The death of one sheep with two milliliters of carbon tetrachloride discouraged the use of higher doses than ten milliliters.

Trials to determine the presence of carbon tetrachloride in the bile samples with the method described in the chemical procedures in both groups of sheep administered two and ten milliliters of the agent gave negative results. This means that either carbon tetrachloride is not excreted in the bile in a detectable level when administered ten milliliters intraruminally or two milliliters orally, or the amount administered was distributed in the various tissues of the body, and what reached the liver was acted upon the hepatic cells as a direct fat solvent agent. Thus the liver was not able to detoxify or excrete carbon tetrachloride in the bile.

A qualitative detection of carbon tetrachloride was used on blood samples obtained from two sheep in the second group which had received ten milliliters of the agent via the intraruminal route, and the results in both cases were positive. The levels of carbon tetrachloride had been studied by Kondos and McClymont (1961), therefore, repetition was not required.

V. CONCLUSIONS AND SUMMARY

1. Surgical exteriorization of the bile flow by a controlled biliary fistula was accomplished on ten sheep. Two polyvinyl tubes (2.5 mm. in diameter and 25 cm. in length) were introduced into the common bile duct between its origin and the entrance of the pancreatic duct. The second end of the tubes were passed through the abdominal wall and fixed to the skin. The ends were connected with another piece of polyvinyl tubing to allow bile circulation.

2. No ruminal fistula was required prior to the major surgery on the common bile duct. Fasting the animals for 48 hours facilitated surgery by reducing the size of the rumen and abomasum.

3. The use of a combination of an ataractic and a local anesthetic agent gave satisfactory anesthesia, the animals were alert and very active soon after surgery.

4. The right paracostal approach to the laparotomy incision was very suitable for the exposure of the common bile duct.

5. Collection of bile samples was achieved by aspiration with a syringe.

6. The biliary fistula could be utilized for physiological and pharmacological studies relative to the bile and liver.

7. Carbon tetrachloride was administered to two groups

of animals, two milliliters orally to the first group and ten milliliters intraruminally to the second group. Bile samples were collected at regular intervals, and analyzed within four hours to determine the presence of carbon tetrachloride by the method described by Kondos and McClymont (1959).

8. The analytical results in all bile samples were negative for carbon tetrachloride. This is an indication that either carbon tetrachloride is not excreted in the bile of sheep in a significant level to be detected, or the liver holds it temporarily and releases it into the blood circulation rather than the bile.

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VII. ACKNOWLEDGMENTS

The author wishes to express his profound appreciation and many thanks to Dr. Wallace M. Wass for his constructive guidance, encouragement and judicious advice throughout the entire course of this study.

To Dr. Neal Cholvin for his helpful suggestions; to Drs. R. Getty, R. L. Lundvall and E. C. Jensen for their invaluable advice.

To Mrs. V. M. Marshall and the staff of the Iowa Veterinary Medical Diagnostic Laboratory for providing the laboratory facilities used in the chemical analysis used in this work. To Mrs. R. Loomis for making the drawings; to Mr. C. Deutsch for photographic work; to Mr. P. Debaecke for assisting in surgery.

To the ministry of Agriculture of the Republic of Iraq for providing the scholarship.

To Drs. W. Adams and R. Bristol and to all my colleagues whose continuous encouragement was stimulating, and to my wife Suhaila, for her inspiration and help.