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LIVER, BILIARY TRACT, AND PANCREAS

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The liver, biliary tract, and pancreas are often grouped together not only because of their anatomic proximity, but also because of their close functional interdependence. In particular, it is impossible to consider experimental procedures on the pancreas, pancreatic ducts, and extrahepatic biliary tree as single, independent problems, since the cross relationship of these anatomic systems is of the greatest interest. For this reason, experimental surgery of the liver will be considered separately, and the pancreas, extrahepatic biliary, and pancreatic ductal systems will be discussed together in the second section of this chapter.

THE LIVER

Clinical Liver Function Tests in Research

In acute or chronic preparations, various tests are frequently employed to evaluate liver function. Such determinations are useful, providing one has a clear understanding of what is being studied. Recent reviews of the limitations of the clinically employed liver function tests (74, 137) should be consulted before these tests are used in research.

In general, the normal values for an animal population should be checked in each laboratory. These may vary with different species (74, 120), or with the health and nutritional state of a given animal. For example, the cephalin flocculation test is normal by human standards only in primates. The serum proteins and albumin-globulin ratio have striking and specific species differences.(120) In dogs, the normal A/G ratio is 1:1.(69, 120) Spell and Hardy stated that the bilirubin, alkaline phosphatase, and thymol turbidity are the most reliable determinations for observing the course of obstructive disease of the canine biliary system, but were of very limited value in lesions affecting the blood supply of the liver.(158) In our laboratory, bilirubin, alkaline phosphatase, thymol turbidity, bromsulphalein excretion, and serum proteins were all reasonably good indices to follow changes in liver function providing that the dogs were dewormed and kept in the laboratory for several months before study.

Anatomic Methods

Although thorough knowledge of the structure of the liver is essential, the purely anatomic approach to experimental surgery has largely been exhausted.

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Nevertheless, special techniques described in recent years have clarified structural features of the liver in health and disease. Perhaps the most important of these is the three-dimensional histologic reconstructive technique developed by Elias from which an altered concept of the normal lobular structure has evolved (52, 53) as well as a new understanding of the intrahepatic vascular structure in cirrhosis. (132, 133)

Other techniques for studying the intrahepatic vascular distribution employ corrosion (26, 133) or radiographic (81, 133) procedures. These methods provide a permanent record of hepatic vasculature, but the specimen is ruined for histologic studies. Vessels smaller than 20 to 100 μ do not fill, so the methods are not suitable for study of sinusoids. With the corrosion technique, a 12.5 per cent solution of colored vinyl plastic is injected under pressure into the hepatic and portal veins and the hepatic artery. The liver is then digested from the cast with concentrated hydrochloric acid.(26) The radiographic record of the venous system can be obtained by injection of radiopaque material under pressure and by x-raying the specimen.(81, 133) Radiographic determinations of the hepatic vascular pattern can also be done by angiographic study during life, although the structural detail is less well defined.

Complete Extirpation

Information obtained from animals after hepatectomy is crude. Various measurements can be made and consequent alterations related to the absence of the liver. The exact mechanisms of the changes often cannot be clarified, since some of the observed phenomena may be secondary rather than specific results of removal of the liver.

Most experiments with total hepatectomy have been done in dogs. In this species, extirpation is difficult. The anatomic basis for this is the close approximation of the subdiaphragmatic inferior vena cava to the liver. The relationship is so intimate that it was formerly thought that removal of the liver was impossible without excision of a segment of the inferior vena cava. Provision for portal and vena caval venous return was done with multiple-stage operations designed to promote collateral venous return (72, 109, 115), or with the interposition of glass or plastic cannulas in replacement of the resected caval segment.(58, 98, 116) These methods all have serious disadvantages.

It is now known that total hepatectomy in dogs can be performed in a single stage with preservation of the inferior vena cava.(92, 159) The operation is done entirely through the abdomen. A side-to-side portacaval shunt is first constructed and the portal triad ligated and transected (Figure 9-1A). A temporary siliconized femoral-jugular polyethylene bypass is connected for decompression of the caval and portal venous systems (Figure 9-1B). The vena cava is next occluded above and below the liver, and the liver stripped from it (Figure 9-1C). Hepatic veins are ligated or sutured at their entry into the vena cava. At the conclusion of the procedure, the cephalad drainage of the portal and caval systems is unimpeded (Figure 9-1D). The polyethylene bypass is removed.

If it is desirable to have the animal awake after operation, any anesthesia which does not depend on the liver for detoxification can be used. Ether has been the most satisfactory agent in our hands. If pentobarbital is used, the dogs remain anesthetized until their death. Under optimum conditions, survival is from 24 to 48 hours following surgery. In order to prevent fatal hypoglycemia, glucose must be given postoperatively at the rate of 0.25 to 1 gm. per kg. per hour, either intermittently or with constant infusion. At first, the animals appear to be quite normal. Eventually they

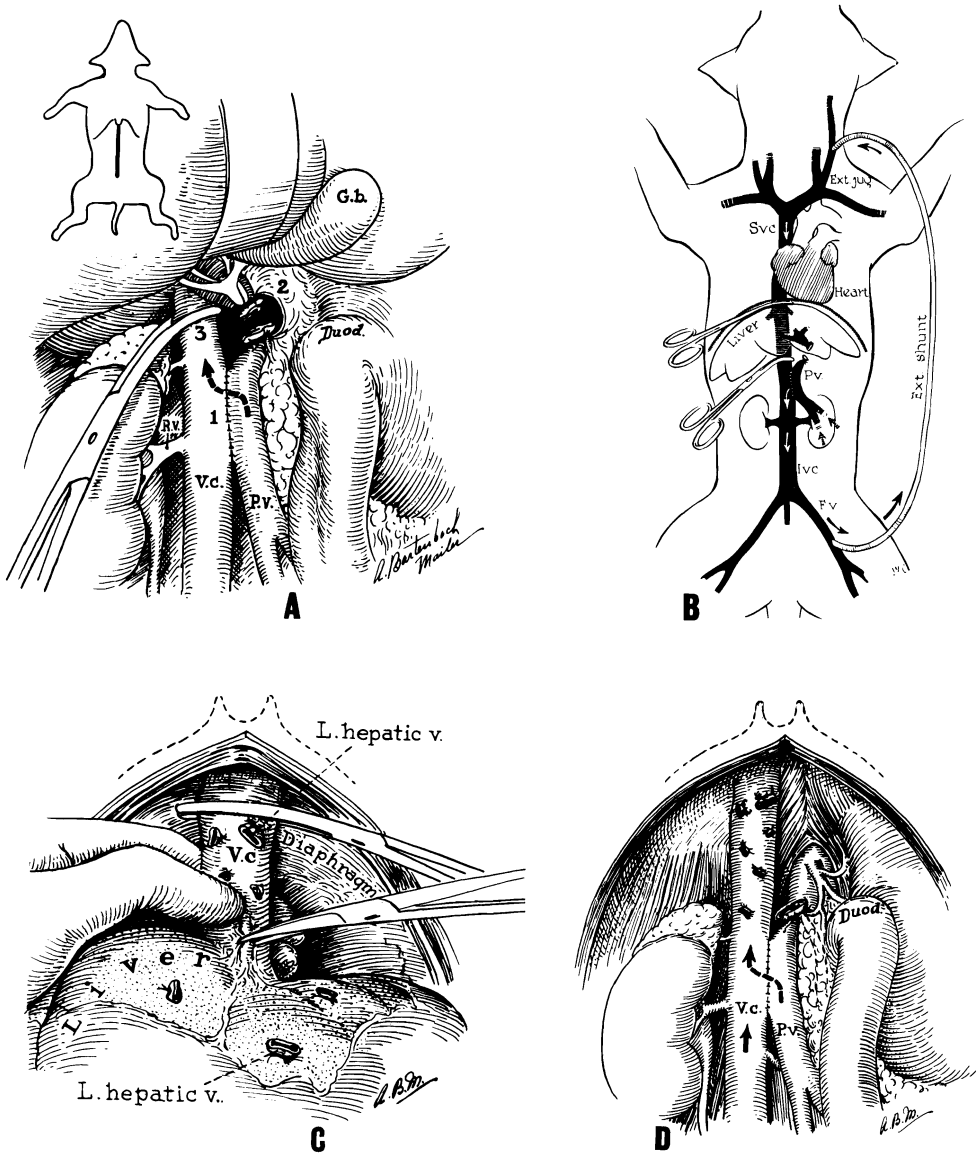

FIGURE 9-1.
Technique of total one-stage hepatectomy in the dog.(92, 159) A. Portal anastomosis has been completed and the portal triad ligated and divided. A noncrushing clamp has been applied. B. Polyethylene shunt from femoral to jugular vein which is used during the period of liver removal to decompress the portal and caval systems. C. During temporary occlusion of the suprahepatic vena cava, the liver is removed. Hepatic veins are ligated as encountered. D. Operative field after completion of hepatectomy. (By permission of Surgery)

develop terminal stupor, which is no longer responsive to intravenous glucose. Hemorrhage within the free abdominal cavity and from the wound invariably develops with all methods of hepatectomy, making difficult an exact analysis of the cause of death. Numerous measurable abnormalities develop, including rises in blood ammonia, bilirubin, alkaline phosphatase, and amino acids, and declines in urea, serum fats, fibrinogen, and prothrombin.

Chronic Hepatic Insufficiency

It is not consistently possible to produce chronic hepatic insufficiency by partial extirpation of the liver. If enough tissue is left to prevent immediate death, regeneration occurs so completely that liver function returns to normal.(111) If a preliminary Eck fistula is constructed, the capacity for regeneration is retarded.(32, 114) If subtotal hepatectomy is then performed in dogs (114), a state of chronic hepatic insufficiency can be achieved.

It is also possible to evoke diffuse damage of the hepatic parenchyma with toxins, immunologic techniques, infections, or dietary deficiencies.(138) The greatest use of these methods has been in the investigation of experimental hepatic cirrhosis (see Chapter 11).

Techniques suitable for the detailed study of subacute hepatic insufficiency have recently been developed, employing whole organ liver transplants in dogs.(122, 160, 161) Here the agent of injury is homograft rejection. Liver function is initially normal, but all measurable parameters progressively deteriorate from the fourth or fifth day until the death of the animal.(122, 161) Usual survival is about 10 days, but survival for as long as 32 days has been attained in our laboratory. Total hepatectomy is performed in the recipient animal, and the donor liver placed in the liver fossa (Figure 9-2A). The most important technical features are protection of homotransplant from effects of ischemia by hypothermic perfusion (Figure 9-2B), and decompression of the recipient splanchnic system during the period of implantation (see Figure 9-1B). The vascularity of the liver can be reconstituted in several ways, but the best results are obtained with as normal anatomic reconstruction as possible.(160) Biliary drainage is provided with cholecystoenterostomy (see Figure 9-2A). With practice, a useful preparation can be obtained in a high percent of experiments.

Hepatic Blood Flow

The best known of the older methods of blood flow measurement was that of Blalock and Mason (18), who measured hepatic blood flow directly. A double-ballooned cannula was inserted from the external jugular vein so that one balloon was below and the other above the entrance of the hepatic veins with fenestrations in the intervening cannula. With occlusion of the cava by means of inflation of the balloons, the efflux of blood through the cannula in a given time represented total hepatic flow. Occlusion of the hepatic artery with externalized ligatures could be done, and the reduction in flow was assumed to be the arterial component of total flow. The procedure was used in unanesthetized dogs. Their results for total hepatic blood flow were somewhat lower than the currently accepted figure of about 40 ml./kg./minute.

At present, various extraction techniques are in wide use for the estimation of hepatic plasma flow. Two general groups of compounds are used; colloid suspensions which are removed exclusively by the Kupffer's and other reticuloendothelial cells; and dyes which are removed by the parenchymal cells. The ideal

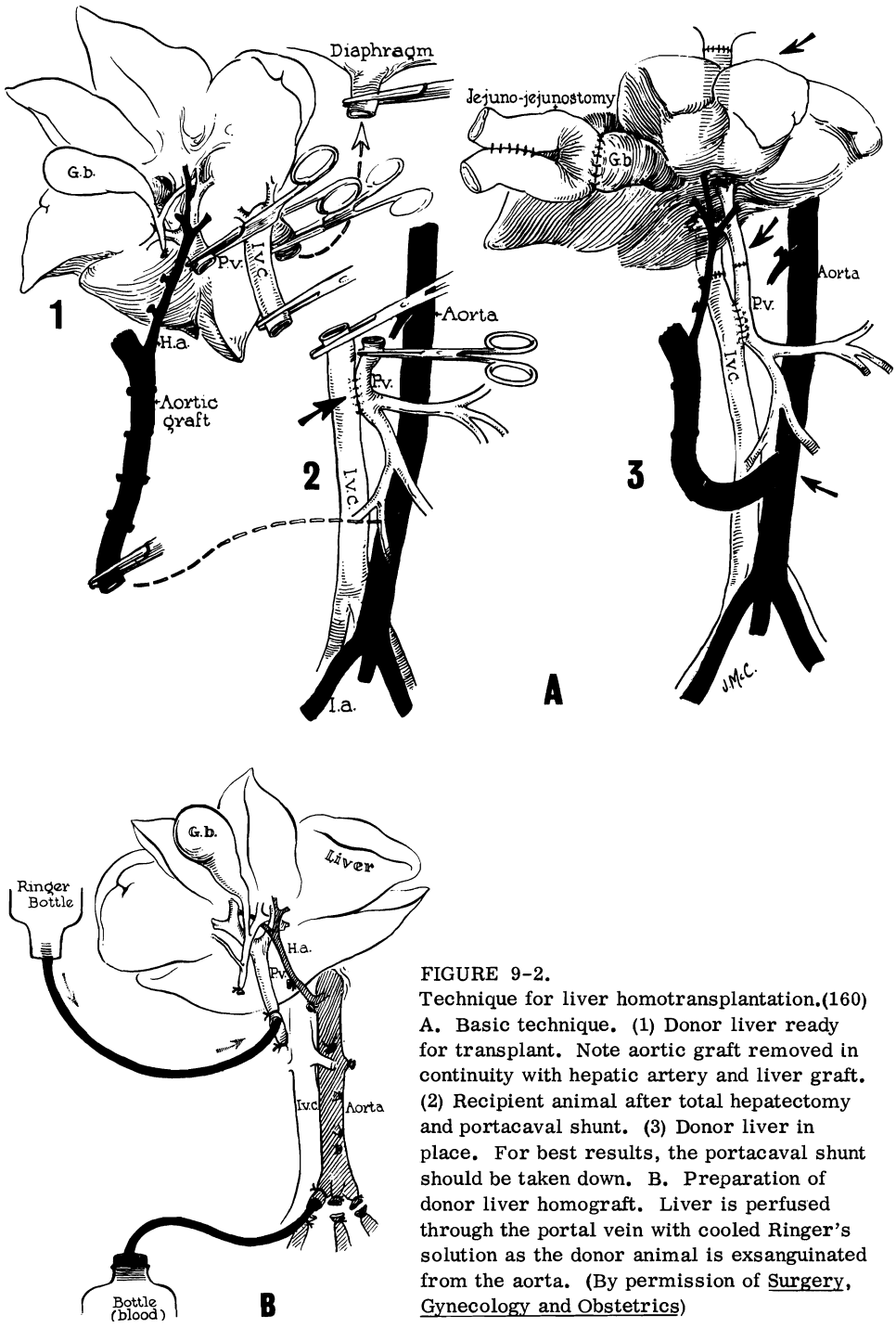

FIGURE 9-2.
Technique for liver homotransplantation.(160) A. Basic technique. (1) Donor liver ready for transplant. Note aortic graft removed in continuity with hepatic artery and liver graft. (2) Recipient animal after total hepatectomy and portacaval shunt. (3) Donor liver in place. For best results, the portacaval shunt should be taken down. B. Preparation of donor liver homograft. Liver is perfused through the portal vein with cooled Ringer's solution as the donor animal is exsanguinated from the aorta. (By permission of Surgery, Gynecology and Obstetrics)

substance is nontoxic, easily quantified in plasma, extracted exclusively by the liver, confined to the vascular compartment, and not redirected through the liver by enterohepatic circulation. Extraction methods are used either with constant infusion or with single injection. Constant-infusion techniques are based on the Fick principle (22) and most of the single-injection techniques are based on the Dobson-Jones (44) principle of flow calculation.

Most techniques using the Fick principle depend upon the infusion of dye, although any substance exclusively removed by the liver can be used. To apply the Fick principle, it is necessary to know the amount of a substance being removed per unit time, and the difference between the concentration of substance entering the organ and the concentration of that leaving the organ (A-H). With this information, plasma flow can be calculated from the formula:

$$\text{Hepatic plasma flow} = \frac{\text{Substance removed (per minute)}}{\text{A-H}}$$

The hepatic blood flow can then be calculated from the formula:

$$\text{Hepatic blood flow} = \frac{\text{Hepatic plasma flow}}{\text{one - Hematocrit}}$$

In using these techniques, constant infusion is usually used to provide a measure of the rate of removal.(19, 182) A loading dose of dye is given to raise the plasma concentration to a desired level. It is then necessary to achieve an infusion rate at which the systemic dye concentration neither rises nor falls rapidly.(19) Under these circumstances, the amount of dye given is equal or nearly equal to the amount of dye removed and provides the numerator of the Fick equation. Minor changes in systemic plasma concentration can be corrected to give the true dye removal.(19, 182) Bromsulphalein (19,152), indocyanine green (97), and rose bengal (37), among other substances, have been used with this method. Modifications of the Fick principle, using a single injection of galactose (178, 182), or using clearance of endogenous urea (101), have been described.

The proper choice and use of dye is important in hepatic blood flow measurements with the Fick principle. Although indocyanine green (31, 85, 185, 187) and rose bengal (156) are both theoretically preferable, BSP has been in use for the longest time. The criticisms of this dye have been summarized by Sapirstein (148) and Waldstein.(182) These are that the dye is removed extrahepatically, that it undergoes enterohepatic circulation, and that it escapes from the intravascular compartment. Shoemaker (152), in a careful study, pointed out that these artifacts are largely related to the use of excessive plasma levels of BSP (more than 1.5 mg./100 ml.). In our laboratory, it has been a reliable method if careful attention is paid to the details enumerated by Shoemaker. It is probable, however, that other test substances, such as rose bengal and indocyanine green, will gradually replace BSP as a test dye.

The Dobson-Jones principle (44) has received wide attention in recent years as a means of estimating hepatic plasma flow following single injection. The physiologic requirements of the substances used are the same as with the Fick method, with the additional requirement that the extraction efficiency must remain constant through a much wider range of plasma concentration. If a material with such characteristics is injected intravenously as a single dose, its disappearance from the plasma (13, 31, 45, 85, 163, 185) follows a simple exponential function (straight line on semilog paper). With knowledge of the plasma volume of the animal and the extracti

ratio of the test substance used flow can be calculated from the slope of the disappearance curve.(13, 44, 45, 141, 187)

The mathematics of the Dobson-Jones calculations must be carefully studied before applying this principle. In oversimplified form, however, an example of the Dobson-Jones principle, using a test drug which is completely extracted with one passage through the liver (extraction ratio of 1), could be stated as follows: After an animal is given a single injection of a known quantity of the test drug, peripheral arterial or venous samples are obtained for analysis every two or three minutes. The disappearance curve is plotted on semilog paper, and by extrapolating the resultant straight line back to zero time, the plasma volume is obtained. If the plasma volume is found to be 1000 ml., and the disappearance curve indicates that 20 per cent of the drug present at any time on the slope is removed in the ensuing minute, it is evident that 20 per cent of the plasma volume has passed through the liver during that minute. The hepatic plasma flow would then be 20 per cent of 1000 ml. or 200 ml./minute. If the extraction ratio were 0.5 instead of 1.0, the amount of plasma flow necessary to clear 20 per cent of the dye would be 400 ml./minute.

A number of substances have been used with the Dobson-Jones technique. These include colloidal chromic phosphate (45), colloidal gold (144, 179), and heat-denatured radioactive iodinated serum albumin (16), which are cleared by the reticuloendothelial system, and rose bengal (156) and indocyanine green (13, 141, 187), which are removed by the parenchymal cells. Since the extraction ratio of each of these substances has been determined in normal animals, it is possible to obtain a crude estimate of hepatic plasma flow without catheterization of the hepatic vein. However, the extraction ratio varies considerably from animal to animal, so that for accurate investigative work the extraction ratio must be determined by hepatic vein catheterization in every experiment.

Additional developmental techniques of hepatic flow measurement are based on the Stewart-Hamilton (94, 121) indicator-dilution method. This technique is based on the fact that the rate of dilution of a known amount of indicator placed in a moving stream is dependent on the flow in that stream. The requirements for this method are substantially different than for the Fick or Dobson-Jones methods. The test substance should not be picked up by the liver (142, 154), it must be injected directly into the hepatic vascular inflow, it must mix completely within the liver, and it must not leave the vascular system. Continuous rapid sampling of the flow of blood as it exits from the area of intrahepatic mixing has been possible by the use of external suprahepatic surface scintillation counting, using nondenatured RISA (142) or chromate-tagged red cells.(154) Substances that are extracted by the liver, such as indocyanine green, provide falsely high plasma flows.(11)

In recent years, other techniques have been developed for measuring hepatic blood flow. The most promising of these employ electromagnetic flowmeters. The electromagnetic flowmeter operates on the principle that if an electrical conductor moves through a magnetic field cutting the lines of force, a voltage will be generated. The voltage is proportional to the velocity of the conductor, strength of the magnet, and the length of the conductor.(42) Sine-wave (25), square-wave (47), and trapezoidal-wave (190) flowmeters have been described. The major advantage of these devices is that flow can be continuously recorded and interpreted, and that the fractional contribution of hepatic arterial and portal venous blood can be measured. The major disadvantages are that surgical installation of the recording electrode requires major preliminary surgery, and that the electronic equipment is complex. Drapanas et al. (47), using a square-wave electromagnetic flowmeter, have shown an almost absolute check with hepatic flows determined simultaneously with the BSP Fick method. His studies also indicate that the hepatic artery

contributes about one-third of total flow to the liver, in contrast to the older estimate of one-fifth.

Multiple-Catheter Techniques

Although the multiple-catheter techniques have not been widely used in experimental laboratories, they are precise and versatile methods for studying various aspects of moment-to-moment hepatic function. These techniques are most applicable to larger animals and have been applied primarily in the dog. In principle, they all involve the simultaneous sampling of blood coming to and leaving the liver. BSP, rose bengal, or indocyanine green is administered to the animal during the experiment, as described under the section on hepatic blood flow. The samples are analyzed for dye, as well as for the metabolite under study. Knowing the metabolic gradients and the flow rates across the liver, the turnover of various substances in the liver can be computed.

The most successful multiple-catheterization technique for use in the anatomically normal dog is that of Shoemaker.(155) Preliminary laparotomy is necessary, at which time catheters are placed in the portal vein, a branch of the celiac axis, and the left main hepatic vein (Figure 9-3). A few days later the desired

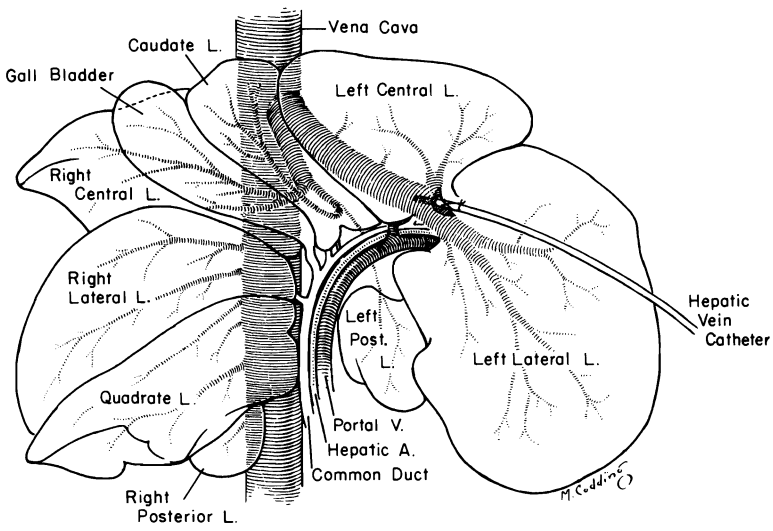

FIGURE 9-3.
Method for multiple-catheterization study of hepatic function in normal dog.(155) Hepatic vein, portal vein, and hepatic arterial catheters are inserted at preliminary laparotomy. The nomenclature is based on a special anatomic study of the liver lobes. (By permission of the American Journal of Physiology)

studies are carried out. The main disadvantages of this method are that preliminary operation is necessary shortly before the actual experiment. Problems with clot formation in the chronically implanted catheters are troublesome. The presence of

catheters in the blood stream eventually leads to fever and deterioration of the animal. Finally, the fractional contribution of the arterial and venous inflow may vary during an experiment. Although there is reason to believe that these changes are usually not profound (18), Knisely (95) has described complex sphincter mechanisms within the liver which, conceivably, could radically alter the relative contribution of the two sources of flow. It is thus possible that the assumption that inflow fractionation is relatively stable may lead to serious errors in computation of hepatic metabolic processes.

These problems are largely circumvented when the multiple-catheterization technique is used in animals with an Eck fistula.(108) Here, there is no need for preliminary operation. The catheters can be placed under fluoroscopic control on the day of the experiment, using peripheral cutdowns. The vascular inflow to the liver is confined to the hepatic artery, so that analysis of the arterial blood is representative of all the flow to the liver. This method of study has been widely used in the field of carbohydrate metabolism, particularly by Madison and his associates.(108) There is, however, one serious disadvantage to this technical approach. The liver of the Eck fistula dog is functionally abnormal.

In our laboratories, multiple-catheter techniques have been most useful in dogs with modified portacaval transposition. Several months before an actual experiment, transposition is performed (see Chapter 11), ligating in addition all vena caval tributaries between the inguinal ligament and the venous anastomotic sites with the exception of the renals.(162) On the day of the actual experiment, catheters can be introduced into the hepatic vein, the aorta and the transposed portal vein through peripheral cutdowns (Figure 9-4). An additional catheter can be placed from a cutdown in the neck into the distal splanchnic bed (see Figure 9-4).

The actual technique of catheterization in the dog with a transposition is a simple one and can be done in an unanesthetized trained dog. The hepatic catheter is inserted through a cutdown of the external jugular vein. The most satisfactory position for this catheter is in the left main hepatic vein. It is easiest to insert the catheter with the dog in a lateral position. When the catheter enters the left main hepatic vein, it can be passed for 3 to 4 inches in a left-anterior direction. The distal splanchnic catheter is also inserted from the neck and passed through the portacaval anastomosis (see Figure 9-4). The position of the arterial and vena caval catheters inserted through branches of the femoral vessels is shown in Figure 9-4. The experiment may be started within a half-hour or so after catheter placement.

With this technique, it is possible to obtain simultaneously both arterial and venous gradients across the liver, as well as gradients in the nonhepatic splanchnic bed and the hindquarters plus kidneys. The hepatic plasma flow provides an indirect measure of the blood flow through the hindquarters and kidneys since these areas provide the venous component of the hepatic blood flow. Finally, the preparation offers the opportunity to compare the effect of drugs given into the systemic circulation (via the forelimb) or directly into the liver via the inferior vena cava (see Figure 9-4).

With careful technique, particularly in avoiding wedging the hepatic vein catheter, the samples from one hepatic vein are reasonably representative of contents of all the lobes (152), although Sapirstein and Reininger (149) have shown that under less than ideal circumstances the composition from different venous outflow areas may differ widely. In experiments where it is desirable to obtain a mixed hepatic venous sample from the entire liver, it is necessary to construct a common hepatic vein. This is accomplished by occlusion of the infrahepatic inferior vena cava. Occlusion is performed by staged ligation (157), by the insertion of plastic bypasses

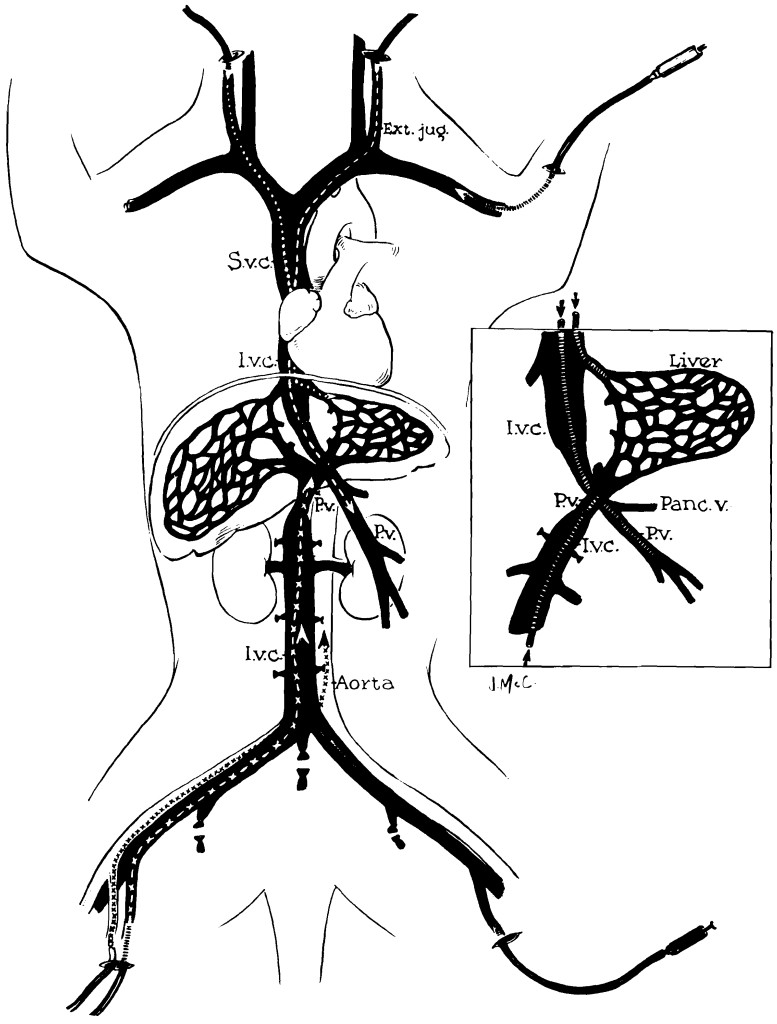

FIGURE 9-4.
Multiple-catheter technique in dogs with portacaval transposition.(162) Note that all cutdowns are in the extremities or the neck. Insertion shows relation of catheter tips to liver. Note that blood entering and leaving liver is accessible for sampling. In addition, drugs can be given directly into the hepatic venous inflow if desired. (By permission of Surgery)

which clot slowly over days or weeks, permitting development of collaterals (12, 180), or by the performance of right nephrectomy to reduce the vena caval flow

combined with ligation of the infrahepatic inferior vena cava.(149) The last method is the simplest and quickest technique for creating a common hepatic vein. With any of these methods a catheter can subsequently be inserted from the neck into the blind common hepatic vein for sampling.

Isolated Perfusion of the Liver

The use of isolated perfusion has many theoretical advantages in the study of hepatic metabolism. The volume of blood infused through the hepatic artery and portal vein can be exactly controlled. Chemical constituents coming to and passing from the liver can also be controlled and determined with great precision. The influence of independent or counterregulatory physiologic mechanisms in other parts of the body is eliminated. Despite these advantages, the techniques are so difficult and the circumstances of the experiments are so different from those obtaining in life that perfusion has enjoyed a limited role in most laboratories. Isolated liver perfusion has been accomplished in many species, including the cat, monkey, rabbit (6), and rat.(20) It has only been recently, however, that perfusion techniques have been consistently satisfactory in larger animals such as the cow or calf (29, 30), the pig (51), and the dog.(93)

Although the dog is probably the most convenient and cheapest animal for perfusion studies, the canine liver has peculiarities which deserve special comment. A variety of experimental traumas produce a characteristic liver injury in which large quantities of blood are entrapped in the parenchyma. This occurs with ischemia, anaphylaxis and peptone shock, temporary devascularization of the liver, histamine injection, endotoxin shock, hemorrhagic shock (160), and with alterations in blood pH and CO_2.(93) The inability of blood to escape from the liver is probably due to constriction of small sublobular and intraparenchymal hepatic veins demonstrated by Deysach (43) and Thomas and Essex.(177) These vessels have been demonstrated by Arey (8) to have extraordinarily well-developed muscular coats in the dog but not in other animals or in man. The phenomenon of hepatic congestion which follows constriction of these vessels is termed "outflow block." The quantity of blood leaving the liver is suddenly reduced. The organ swells and becomes cyanotic. Sinusoids are intensely congested with blood, with rupture of many of the vascular channels. Serous fluid exudes from the surface. Function is immediately and seriously impaired. Although outflow block can be ameliorated with epinephrine, its appearance terminates the value of a perfusion experiment.

Kestens and his colleagues have shown that isolated perfusion of dog liver can be done with consistent success employing a skilled team and elaborate apparatus which allow accurate control of blood chemistry.(93) With Kestens' technique, a key to success is protection of the liver from trauma during the dissection which precedes the actual removal of the liver and minimization of the ischemic period during transfer. In the past, with perfusion of small animals, vascularization was done only via the portal vein. With most modern techniques, both arterial and venous inflow are provided.

Shoemaker and his associates (153) have described an alternative procedure for perfusion of the canine liver. The liver is denervated and the various vessels cannulated and encircled with chokers. When the chokers are tightened, the catheters become, in effect, cannulas. The hepatic circulation is instantly and completely divorced hemodynamically from the systemic circulation and controlled by a pump.

EXTRAHEPATIC BILIARY SYSTEM AND PANCREAS

Important Anatomic Interrelationships

In many studies of the biliary tract, pancreas, or pancreatic ducts, the relationships of these structures to each other is crucial. For this reason, it is important to review the comparative anatomy of the species to be used before embarking on a study. The species differences have been tabulated by Mann, Foster, and Brimhall.(112) The ductal drainage systems can be arbitrarily divided into three groups. The first type has completely separate openings into the duodenum of the common bile and pancreatic ducts, as exemplified by the rabbit and the guinea pig. In the second type, exemplified by the dog, cat, and primate, there are two distinct ducts, one of which usually empties into the duodenum by a common channel. In the dog, the accessory pancreatic duct which empties independently is the duct of major pancreatic drainage (Figure 9-5), making the situation quite different from that in man. The last group is that in which the pancreatic duct or ducts empty directly into the choledochus. The mouse, rat, goat, and sheep fall into this category. The rat occupies a special position in this last group. There are two major pancreatic ducts and eight to fifteen minor ducts, all of which empty into the common bile duct.(151)

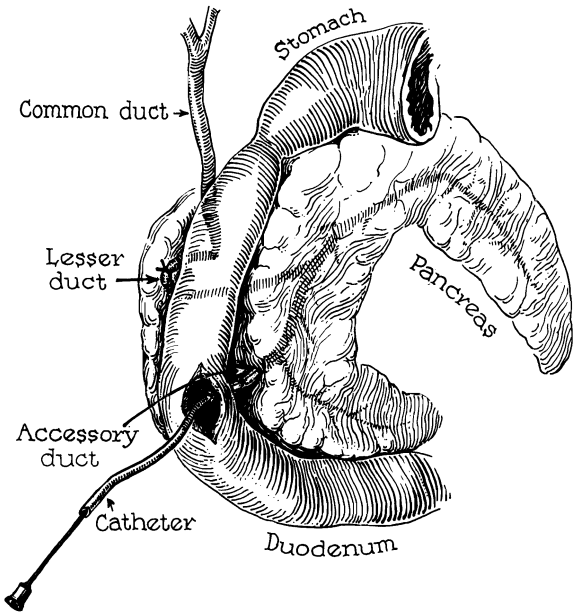

FIGURE 9-5.
Prototype of pancreatic ductal injection techniques used to produce experimental pancreatitis in the dog.(4) Note that the lesser pancreatic duct joins the common duct. The major (or accessory) duct empties more distally. In the experiment shown, the minor pancreatic duct is ligated to prevent uncontrolled escape of injectate. (By permission of Surgery, Gynecology and Obstetrics)

Cholecystitis and Cholelithiasis

Calculi do not develop spontaneously in most animals, the sheep and goat being exceptions.(17) In dogs, low-grade cholecystitis without stones occurs spontaneously with sufficient frequency that controls within an individual laboratory are necessary for proper evaluation of histologic results from experiments.(40)

Cholecystitis and/or cholelithiasis has been produced in a variety of ways which can be loosely grouped under either local or systemic chemical factors. Murphy and Higgins produced hemorrhagic cholecystitis in dogs with intravenous injection of an acid solution of hypochlorite.(126) The lesions were completely reversible, but were valuable for studies of motility during the acute reaction. Mossbach, Bevans, et al. (15, 124, 125) have produced both cholecystitis, choledochitis, and bile acid-cholesterol stones in rabbits following chronic oral administrations of cholesterol precursor substances. These observations were extended to guinea pigs by Caira et al.(24) Christensen and his associates (34) produced cholesterol stones in golden hamsters by means of a cholesterol-free, low-fat diet, supplemented with large doses of vitamins A and D. Later, it was shown that these cholesterol stones were reabsorbed by placing the animals on a nonlithogenic diet.(33) Imamoglu and his associates have produced gallstones in rabbits with estradiol and progesterone.(87) Eighty per cent of females and about one-half of the males developed stones.

A variety of chemical irritants can produce cholecystitis if injected into the obstructed gallbladder. Because of their potential relation to human disease, the greatest attention has focused on bile and pancreatic secretions. A number of authors (9, 40, 80, 84, 173, 189) have instilled bile, concentrated bile, pure bile acids, and activated or inactive pancreatic juice into the gallbladder of rabbits or dogs. In general, these experiments indicate that any or all of these substances can cause acute cholecystitis providing that the gallbladder does not have free drainage. A number of experimental preparations, described in the section on pancreatitis, have been devised to drain pancreatic secretions artificially through the gallbladder or biliary system. An example used by Reid (143) in the dog is shown in Figure 9-6A. When excretory flow is unobstructed, there is little effect on the biliary system.(3, 39, 186) With distal obstruction, converting the pancreatic and biliary drainage into a common undrained pool, the major changes are cholecystitis, choledochitis, and cholangitis.(3, 17, 113, 184)

The role of infection and stasis in extrahepatic biliary disease has been subjected to considerable scrutiny in the experimental laboratory. Studies by Morris et al. (123) indicated that intraluminal injection of bacteria did not injure the normal gallbladder in dogs but resulted in empyema when the cystic duct was ligated. Similar results were obtained by Wilkie (188) in rabbits, using the intramural injection of pure strains of streptococcus. Of great interest was the fact that intravenous infusions of bacteria given to rabbits with obstructed cystic ducts resulted in selective injury and empyema of the gallbladder.

The above studies emphasized the combined role of infection and biliary stasis. Recent decisive studies show that biliary stasis alone is an etiologic factor of great importance. Bisgard and Baker (17) occluded the distal common duct in five goats. In all animals, cholecystitis developed, and in three animals stones formed. In a classic study, Imamoglu and associates (86, 88) produced both cholecystitis and cholelithiasis in rabbits, dogs, and monkeys. Partial obstruction was produced by wrapping the terminal common duct with cellophane that had been sprinkled with dicetyl sodium phosphate. Jaundice did not develop. Bile cultures were sterile in the majority of animals. These observations were confirmed by Murphy et al. (127)

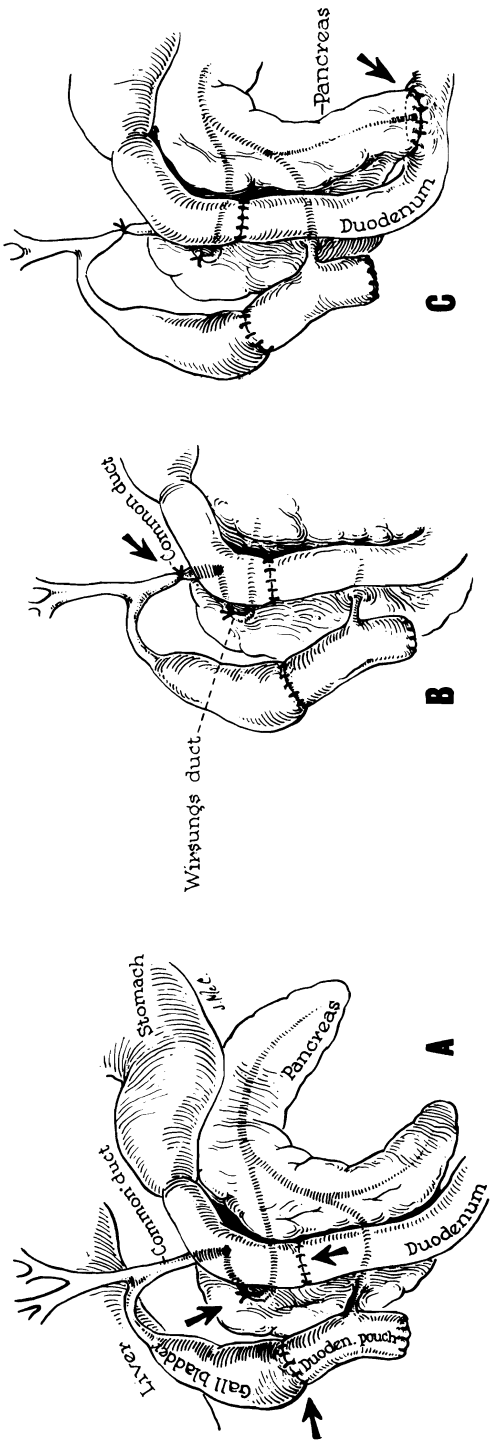

FIGURE 9-6.
A. and B. Method of Reid (143) and Anderson (3) for producing a closed continuous system between pancreas and biliary tract of dog. C. Diversion of closed system into the duodenum via the pancreatic duct system by anastomosis between pancreatic head and duodenum. (By permission of Annals of Surgery)

who partially occluded the distal common duct with a cotton ligature. In Bisgard and Baker's method (17), the role of reflux of pancreatic juice into the biliary tree may have been important, since the obstruction was below or at the entry of pancreatic drainage. With Imamoglu's technique the pancreatic factor is minimized, since the obstruction is above the entrance of the pancreatic ducts.

Total and Partial Biliary Tract Occlusion

Little need be said concerning the surgical technique involved in complete obstruction to all or part of the biliary excretory system. Complete division between ligatures is necessary in an attempt to secure permanent obstruction. With simple ligation, the suture sometimes cuts through over a period of time and at least partial flow can be inadvertently reconstituted. The various parameters of hepatic function following complete biliary obstruction in the dog have been well studied by MacGregor.(102)

The sequelae of segmental ductal obstruction have been described in the rabbit (146), cat (75, 164), dog and monkey.(106) In all species mentioned, the obstructed portion of the liver undergoes cirrhotic changes and eventual atrophy. The remaining hepatic parenchyma which is sharply demarcated from the obstructed segment tends to hypertrophy. It is, therefore, felt that intercommunication between the ductal systems of anatomically separate portions of the hepatic parenchyma does not exist or is minimal. The completion of this sequence of events is fairly rapid in the rabbit, requiring four to six weeks, and slower in the other species, taking from twelve to fifteen months. There is little or no evidence of hepatic functional impairment during this time.

Intraductual Pressure Measurement

The problems of pressure measurement in the common bile and pancreatic ducts are inseparable in animals with a common channel. In planning an experiment, one must determine whether obstructed- or free-flow pressures are desired in the pancreatic or bile ducts or both. Measurements should always be taken with a standard baseline point.

Free-flow pressure measurement requires not only that flow be unobstructed, but usually also that the ductal sphincters not be damaged. The technique used by Grindlay and his associates (71, 119, 129) is well suited to this kind of experiment (Figure 9-7). The biliary catheter is inserted through an intrahepatic or the cystic duct and fed retrograde into the common duct. The pancreatic duct catheter is placed through the tail of the pancreas. For long-term observations, Brown, Earley, and Eiseman (21) have described an imbedded cutaneous stopcock which is connected to a T-tube in the common duct. At the time of study, the manometer is connected to the stopcock and at other times the stopcock is closed

In obstructed-flow measurements, the ducts can be simply ligated around an indwelling tube which is exteriorized. However, the best methods involve the use of chronically altered dogs. Mann and Giordano (113) used Mann's (110) preparation of biliary fistula for this purpose. The duodenum is sutured to the abdominal wall, and a duodenal-cutaneous fistula created at the second stage. Obstructing catheters can be introduced through the fistula into the ducts for pressure measurement. Alternatively, a Thomas cannula (see section on pancreatic fistulas) can be employed as the window through which the catheters are inserted with direct vision.(54) The method using the Thomas cannula is probably the most suitable one at present.

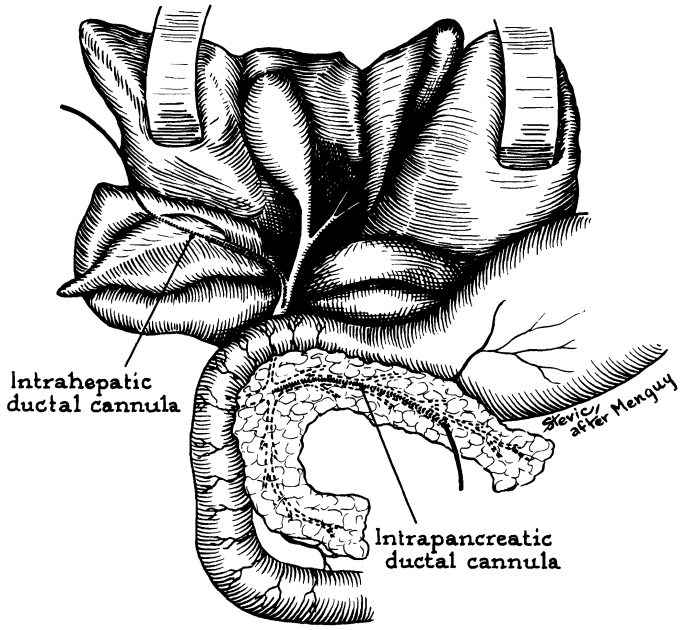

FIGURE 9-7.
Method of Menguy and his associates (118) for free-flow pressure measurement in the pancreatic and common ducts. The catheters are led out through the tail of the pancreas and from intrahepatic biliary radicals.

Biliary Fistula

Results from studies of biliary and pancreatic fistulas must be considered against the background of the abnormal physiology imposed upon the animal by the particular technique used. In acute experiments, anesthesia and surgical manipulation profoundly influence results. In the chronic preparation, infection and loss of metabolites, fluid, and electrolytes may lead to deterioration of the animal. Most of the techniques for the construction of fistulas have attempted to minimize these factors.

Biliary fistulas have been studied extensively in mice and rats, which have no gallbladder. Bile can be drained into specially constructed glass bulbs placed either in the peritoneal cavity (36) or subcutaneously in the abdominal wall.(76) A more useful technique for studying biliary secretion in the rat is that of tunneling polyethylene tubing subcutaneously following cannulation of the bile duct, and bringing it out either on the back (59) or through the plantar foot pad.(63) With this latter method, the foot is secured to the bottom of the cage and the tubing is brought through the wire mesh into an appropriate collecting apparatus. By this maneuver, a continuous collection of bile can be obtained in an unanesthetized animal who is able to move about, although in somewhat limited fashion.

Extensive studies of biliary and pancreatic physiology using external fistulas have been carried out in the rabbit. The regional anatomy and exact technique for catheterization are well described.(96)

The dog has been studied more frequently than other animals. As with other species, ascending infection has been a troublesome problem with permanent external fistulization. However, McMaster and Rous (107), utilizing tubing purposely left long within the peritoneal cavity, were able to collect sterile bile from dogs for up to three months. Later, this technique was modified to allow intermittent diversion of the biliary stream back into the intestinal tract.(105) Catheters were placed proximally and distally in the common duct, and these were connected when collections were not being done. A method of intermittent collection of biliary drainage via the gallbladder after division of the common duct was recently reported.(35) This method is essentially a cholecystostomy performed with a long collecting tube, in dogs with ligation of the distal common duct. The authors stated that the bile remained sterile for from three to eighteen days, providing that systemic antibiotics were given.

Construction of a Thomas fistula with intermittent catheterization of the choledochus is probably the most useful physiologic method for intermittent sampling of the biliary secretion. This will be discussed in greater detail under pancreatic fistulas.

Complete diversion of the biliary stream without collection of bile can be accomplished by external drainage, transplanting the common duct and a halo of duodenum to the skin.(140) This method has some inherent disadvantages. First, ascending infection of the biliary tree is inevitable, and second, the extensive multistaged operation interferes with the normal anatomy and nerve supply in this region.

For complete, sterile biliary diversion, the gallbladder can be anastomosed to the renal pelvis either without (91) or with concomitant nephrectomy.(50) Freeman (62) has connected the gallbladder directly to the urinary bladder by means of a plastic conduit. With all these methods, the bile is diverted into the urinary system after ligation of the common duct.

Pancreatic Fistulas

Complete pancreatic fistulas are attended by complications which make them unsuitable for the prolonged study of pancreatic physiology. Within a few days, the secretion becomes continuous and increases in amount, and its composition changes.(175) The animals, if untreated, live 5 to 8 days, but with replacement of lost electrolytes their lives may be prolonged.

In general, two types of pancreatic fistulas can be constructed — those in which pure inactive pancreatic juice is collected, and those in which the pancreatic ferments are activated by contact with duodenal mucosa. The original and most widely known method of collecting inactive pancreatic juice was that described by Elman and McCaughan (55), and later modified by Baggenstoss.(10) The duct of major pancreatic drainage is catheterized transduodenally following avulsion of the minor duct. The tube purposely left long within the peritoneal cavity is then exteriorized via a stab wound. Difficulties with this method have been clogging and extrusion of the tube. A useful modification of this method, described by Routley et al. (147), provides for adequate fixation of the cannula within the pancreatic duct by means of plastic collars (Figure 9-8) and return of pancreatic juices to the intestinal tract via a Witzel gastrostomy when collections are not being made.

The most physiologic method of pancreatic juice collection employs the Thomas fistula.(174) In principle, this preparation provides a window from the anterior abdominal wall into the duodenum (Figure 9-9). The window can be opened at will by uncapping the fistula, providing access to the interior of the duodenum. The bile or pancreatic ducts can be cannulated under direct vision for obstructed- or free-flow

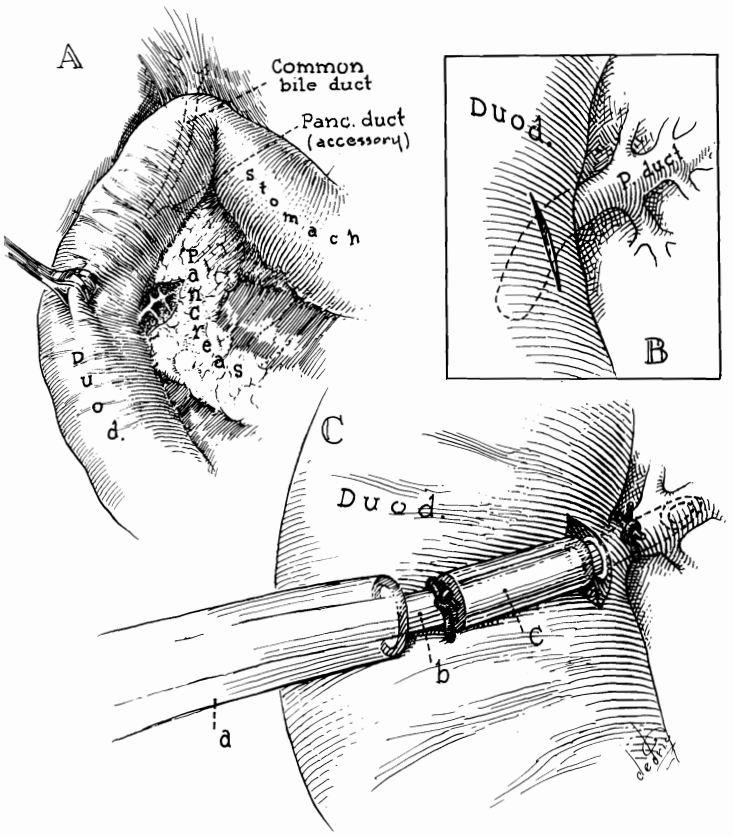

FIGURE 9-8.
Routley (147) type of pancreatic fistula. The externalized tube can be connected to a Witzel gastrostomy to return secretions to the stomach when they are not being collected. Note the polyvinyl collar which provides proper fixation of the cannula tip. (By permission of Surgery [Vol. 48, p. 854, 1960])

secretions, or for pressures. Secretions can be collected with a soft rubber funnel pressed around the duct opening.(176) Alternatively, the duct can be directly cannulated.(77) Between experiments the fistula is capped, thereby preventing loss of fluids or infection. If total collection of pancreatic juice is desired, the minor pancreatic duct is avulsed or resected.

A technique employing retrograde drainage of the pancreas has been described.(103) All pancreatic ducts are ligated and divided, and the uncinate process of the pancreas is mobilized and exteriorized. The tip is amputated and the exposed pancreatic duct is cannulated when specimens are required. Inlow (89) has described a method for collecting inactive pancreatic juice by the direct transplantation of the duct to the skin. A multistaged operation is necessary. The chief disadvantages of these latter methods are continuous loss of pancreatic secretion,

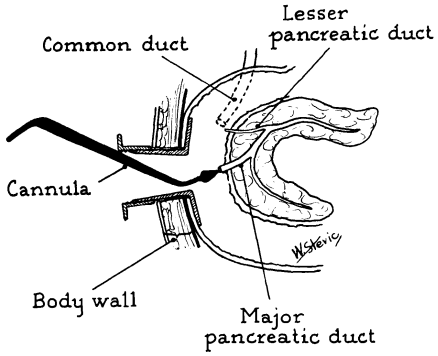

FIGURE 9-9.
Schematic representation of Thomas fistula. Note cannula provides access to structures in interior of duodenum.

alkali dermatitis of the skin surrounding the fistula, and closure of the transplanted duct due to scarring.(150)

Activated pancreatic juice is most easily collected by the method of Dragstedt (46), later modified by Archambeau.(7) A blind segment of duodenum containing the major pancreatic duct but excluding the common duct is constructed, the intestinal tract being reconstituted by end-to-end anastomosis of the duodenal segments. The blind segment of duodenum is anchored to the anterior peritoneal surface and cannulated. The pancreatic juice is returned to the animal via a catheter in the distal duodenum when specimens are not being obtained. A concomitant gastrojejunostomy is constructed to prevent clogging of the distal cannula by food.

Analysis of Pancreatic Secretions

In various of the foregoing and subsequently described methods, it may be important to analyze pancreatic products in the bowel, ducts, serum, feces, urine, or tissues. Pancreatic juice contains enzymes capable of digesting proteins, fats, carbohydrates, and nucleic acids. Internal secretions include insulin, glucagon, and possibly other hormones. The methods of laboratory analysis of various exocrine and endocrine pancreatic secretions have been described in several recent monographs, books and papers.(23, 135, 165) For comparison of techniques or for a comprehensive review of the subject, the reader is referred to these sources. Briefly, it may be said that more or less well-standardized techniques are available for the determination of amylase, lipase, esterases, trypsin inhibitors, leucine aminopeptidase, insulin or insulin-like substances, and glucagon. Contents can be determined in various body fluids, some by direct measurement and others by bioassay methods. Indirect appraisal of various aspects of pancreatic function is possible with the glucose tolerance, fecal fat, secretin, and numerous other tests.

Acute Pancreatitis

The production of experimental pancreatitis is usually undertaken for one of two reasons — either to evaluate the effectiveness of some form of therapy, or to learn more of the pathophysiology of this disease. In either case, it must be

recognized that there are multiple factors that may contribute to the inception and development of pancreatitis, amongst which are the anatomical interrelations of the common bile and pancreatic ducts (see page 106).

The oldest methods for inducing acute pancreatitis, dating from Claude Bernard's experiments of the mid-nineteenth century, involve injecting various substances under pressure into an occluded pancreatic duct. Results with these techniques in dogs have been reviewed by Anderson et al. (4), who emphasized the desirability of controlling the volume of injectate, the pressure of injection, and the avoidance of loss of injectate into the duodenum through the lesser pancreatic duct (see Figure 9-5). Despite these precautions, the variability of the resultant pancreatitis is great with bile, bile acids, and enzymes, or combinations of these substances.(4) In addition, the pressures necessary to cause the lesion far exceed those thought to obtain normally and must be great enough to rupture the duct system.(4, 54, 145) Similar techniques, using staphylococcal exotoxin, cause a severe hemorrhagic pancreatitis (38, 172), but the requisite intraductal pressures are not nearly so great.

A step forward in standardization of ductal injection techniques was the preliminary incubation of injectates containing trypsin. Elliot (54) demonstrated that bile and trypsin, if incubated for 24 hours, had a greatly increased potency which suggested that bile participated in the activation of trypsin. Nemir and Drabkin (128) demonstrated that whole blood incubated with pancreatic enzymes uniformly produced experimental pancreatitis, using lower injection pressures than had been previously possible. Anderson and Bergan (2) have shown that the reaction is not due to bacterial activity in the incubate, and that full toxicity occurs only when whole blood is used. At present, Nemir's method appears to provide the most uniform and reproducible lesion for testing the efficacy of experimental therapy. The ramifications of this technique have also furnished increased understanding of the role of activated trypsin in conjunction with bile and blood in the production of pancreatitis.

The multiplicity of factors which contribute to the development of pancreatitis have led to numerous experimental preparations designed to isolate a single factor under controlled conditions. In species in which pancreatic drainage is separate from biliary drainage, complete occlusion of the pancreatic duct does not cause severe pancreatitis.(14, 73, 183) There is a rapid cessation of exocrine secretion and atrophy.(48, 90, 183) If, however, ligation is carried out immediately after a meal (99, 118), or, if secretin or pilocarpine is given shortly after ligation (64, 99, 134, 184), varying degrees of pancreatitis ensue. In the method of Pfeffer (104, 130, 131), pancreatitis consistently results which is due to the reflux of activated pancreatic juice under pressure. A blind duodenal loop is created into which only the pancreatic ducts have egress. The common bile duct is ligated above the duodenum. Within a few hours, pressure rises in the blind intestinal sac, with regurgitation into the duct system. Death occurs in 24 to 48 hours.

Efforts to evaluate the common-channel theory of pancreatitis in animals have resulted in many ingenious experiments either to drain bile into the pancreatic duct or pancreatic juice into the bile duct. Transpancreatic continuous drainage of bile has been achieved in goats by ligation of common-channel with retrograde pancreatic decompression (186), and in dogs by operations which reroute the biliary drainage through the pancreatic duct system.(3, 39, 140) An example of such an operation in the dog (3) is shown in Figure 9-6. In general, these experiments suggest that bile passage through the pancreas is not harmful in animals with an unobstructed duct system.(3, 39) Even with obstructed and freely communicating pancreatic-biliary systems in the goat (17), cat (113, 184), and dog (3, 113), the major injury is usually to the hepatobiliary tree rather than to the pancreas. This has led to a

search for other factors in addition to the common channel. The important work on alteration of bile and blood by incubation with activated trypsin has already been mentioned.

In recent years, the role of vascular factors in pancreatitis has received much attention. Key observations were reported by Popper, Necheles, and Russell in 1948.(136) These authors observed that the mild edematous pancreatitis caused by pancreatic duct ligation and exocrine stimulation could be converted to hemorrhagic pancreatitis by occluding the vascular supply to the pancreas for 15 minutes. Later, Adams and Musselman (1) showed that venous occlusion provoked a similar violent pancreatitis. Other authors have elucidated the role of vascular injury in pancreatitis provoked by more traditional methods. Rich and Duff ascribed the major injury with trypsin and bile injection to vascular injury.(145) Thal, using quartz-rod illumination of the pancreas, demonstrated vascular stasis after injection of bile into the pancreatic parenchyma.(166) Vascular injury is thought to contribute to the pancreatitis evoked with staphylococcal exotoxin (38, 172), with blood-trypsin incubates (2), and with hypersensitivity reactions.(167, 168)

One of the newer concepts of pancreatitis concerns the role of auto-immune mechanisms in the production and perpetuation of pancreatitis. This arose from Thal's observations that pancreatic necrosis could be produced experimentally with local hypersensitivity reactions (167, 168), and with his later observation that antibodies reactive against the pancreas could be induced with injection of pancreatic tissue extracts.(169, 171) Evidence that auto-immune mechanism might be of significance in human pancreatitis has been presented by Thal (169-171), and Fonkalsrud and Longmire.(61)

Experimental pancreatitis has also been produced by metabolic derangements or systemic drugs. Changes have been invoked with a high-fat, low-protein diet in dogs (100), and ethionine in dogs.(56, 65)

Chronic Pancreatitis

Methods for producing chronic pancreatitis are less satisfactory and controllable than for acute pancreatitis. The most commonly used methods involve stimulation of pancreatic exocrine function with secretin or fatty meals in conjunction with intermittent (79, 181), partial (79), or slow (60) progressive occlusion of the pancreatic duct. The recently described method of Thal (169) in which autoantibodies to the pancreas are induced is a promising technique. Anderson et al. have produced chronic pancreatitis in dogs by injecting the cell-free fraction of whole blood incubated with trypsin.(5)

Pancreatectomy

Despite its importance in the experimental laboratory, pancreatectomy is a straightforward technical exercise and will not be described in detail here. Rather, the peculiar problems in different species will be alluded to, and full details can be acquired from the appropriate references.

Total pancreatectomy was first studied extensively in dogs by von Mehring and Minkowski in 1889.(117) Virtually 100 per cent of the gland can be removed with preservation of the common duct and duodenum. The major and minor pancreatic ducts are ligated and the pancreas, which is attached by a mesentery, teased away from surrounding tissue. Details of the operation have been summarized by Markowitz et al.(115) In experiments in which 100 per cent pancreatectomy is essential, it is advisable to perform pancreaticoduodenectomy, with reimplantation of the common duct into the bowel, as is performed clinically.(27, 83)

It was formerly thought that rats developed late and mild diabetes after total pancreatectomy. Scow (151) has shown that this resistance to diabetes was due to inadequacy of the pancreatectomy. The major technical problem in the rat is its diffuse ductal drainage system, in which two large and eighteen to fifteen small ducts empty into the common duct. With care, and with the use of a dissecting microscope, total pancreatectomy is followed by fulminating diabetes.

Rabbits present a different problem. The pancreas is diffusely distributed in the upper abdomen. Extirpation has been said to be a shocking operation in which complete pancreatectomy is often not accomplished. Greeley (70) has described a staged operation in which the entire gland is removed with a low mortality. With modern knowledge of pancreatic surgery, the staging may be omitted.

Monkeys and other primates have not been widely used for experimental diabetes. For laboratory studies, the techniques developed for man (27, 83) should be useful.

The primary postoperative problems involve control of the diabetic state. Exocrine pancreatic replacement therapy is not essential providing the animals receive a high-protein, high-carbohydrate, high-vitamin diet (28), but the ease of care and the state of health are improved when pancreatic enzymes are provided.

Selective Destruction of Pancreatic Components

Selective destruction of the pancreatic acini with relative preservation of the islets was demonstrated by Banting and Best in 1923.(14) Subsequently, preferential injury to either the alpha or beta cells has been achieved with chemical cytotoxins. Goldner and Gomori (67) introduced alloxan, a beta cell toxin, for the production of experimental diabetes. A variety of chemicals, including cobalt chloride (68), synthalin (41, 57) and P-aminobenzenesulphonamidoisopropylthiodiazole (IPTD) (57, 82), have been used with varying success to injure alpha cells.

It should be realized that the islet-cell poisons are not completely specific. All except perhaps IPTD are also nephrotoxic and hepatotoxic. Goldner (66) has pointed out that the alloxan effect is prevented if either the kidney or the liver is devascularized for a few minutes at the time of intravenous drug administration. The relation to human disease of the altered metabolic states induced by these various drugs is not known.

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