Handbook of Animal Models in Transplantation Research

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Chapter 8

Liver Transplantation in Primates

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I. INTRODUCTION

The first description of experimental liver transplantation was by J. Cannon in 1956, although few details about the operation were supplied.[1] Concurrent developments of orthotopic liver transplantation were begun by Moore et al.[2] and Starzl et al.[3] By 1960, successful dog liver transplantation was achieved. This model allowed for technical and immunosuppressive developments which were essential to the eventual application in humans.[4] Nevertheless, a number of differences have been identified between canine and primate hepatic physiology, such as the presence of hepatic vein musculature in the dog which may act as a "throttle mechanism",[5] as well as the sensitivity of the canine liver to histamine-mediated vasoconstriction.

Myburgh and co-workers first described the use of non-human primates for experimental liver transplantation,[6] and independently by Fortner.[7] Non-human primates offer a number of advantages in the study of liver transplantation. Unlike the canine liver, the liver anatomy of non-human primates is similar to humans. Amongst the higher order primates, similarities exist between the major histocompatibility complex, as well as the cellular markers found in the immune system.[8] The blood groups are similar to the A and B blood types, although O blood types have only been reported in man. In addition, a number of human pathogenic virus, such as Hepatitis B, can be studied in certain of these primates.

Primates are comprised of two suborders, Prosimii and Anthropoidea.[9] Prosimian primates resemble squirrels or rats more than true monkeys. The Anthropoidea suborder can be further subdivided into five different families: new world monkeys, old world monkeys, lesser apes, great apes, and man. From an investigational standpoint, the most frequently used species are the old world monkeys. This family includes Rhesus monkeys *(Macaca mulata)*, Cynomologus monkeys *(Macaca fascicularis)* and baboons *(Papio cynocephalus)*. Great apes include the chimpanzee *(Pan troglodytes)* and gorillas *(Gorilla gorilla)*, but are not used in great numbers because of their endangered status.

The purpose of this chapter is to describe the techniques of liver procurement and transplantation in non-human primates, for purposes of experimental and perhaps eventual clinical applications.

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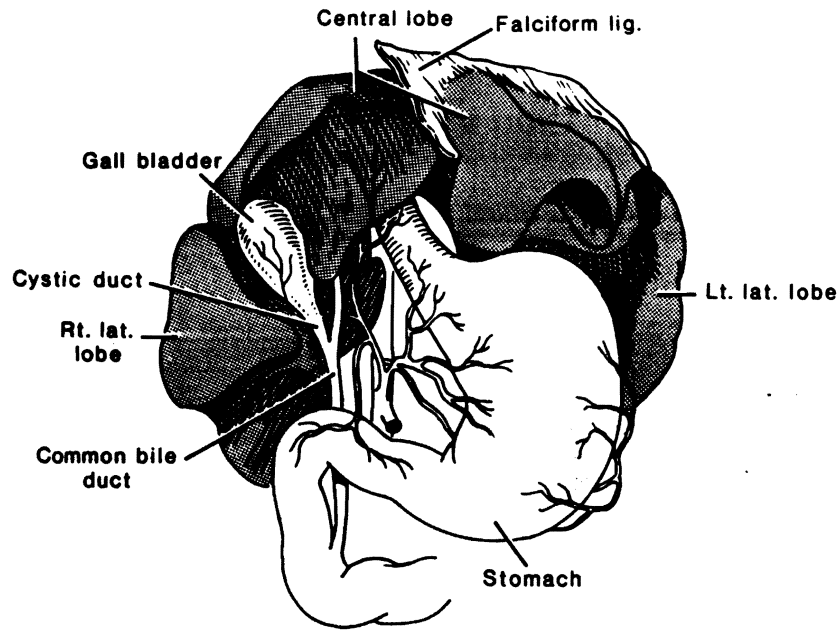

Figure 1 Non-human primate liver anatomy.

II. TECHNIQUE

A. ANATOMY

Liver anatomy among primates is similar, with a right and left lateral lobe placed dorsally and a single large ventral central lobe.[10,11] The liver of the *Macaca* and *Papio* is notable for lobation, with four identifiable lobes. In the higher order primates, the central lobe fuses with the right and left lateral lobes. The quadrate lobe is much more narrow in non-human primates than in man, and the caudate lobe may encircle the circumference of the inferior vena cava. The ligamentous attachments are similar to those described in man (Figure 1).

The blood supply to the liver is similar in the Anthropoidea, with a great variation in the arterial blood supply, as noted by several authors.[7-13] The portal venous system is essentially identical with that of the higher order primates. The hepatic venous drainage is similar to man, with small short hepatic venous tributaries draining the right and central lobes, and two large hepatic venous branches, one right and one left.

In all primates, the gallbladder lies closely attached to the right or central lobe. The arterial supply is usually from a branch from the right hepatic artery. The cystic duct joins the common hepatic duct a variable distance to form the common bile duct, before emptying into the duodenum.

B. GENERAL CONSIDERATION

Generally, the donor should be smaller in size than the recipient. Blood type compatibility should be established and the animals should be fasted the evening prior to the operation.

Animals are tranquilized by the use of intramuscular injection of ketamine (10 mg/kg). Using intravenous induction with 25 to 35 mg/kg sodium thiopental, a cuffed endotracheal tube is inserted and attached to a respirator. Ventilation is with a mixture of oxygen and nitrous oxide, with inhalation anesthesia using isofluorane at a concentration between 0.5 and 2.0%.

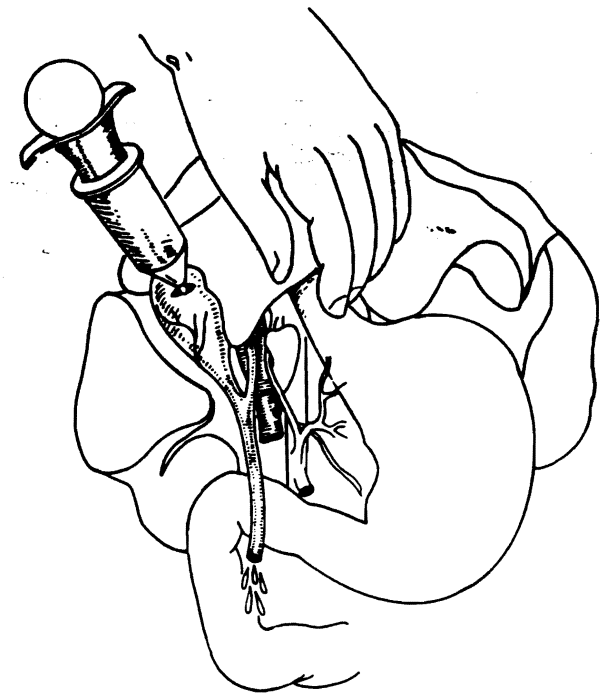

Figure 2 Procurement technique. Common bile duct is being flushed through gallbladder incision.

Arterial pressure monitoring is accomplished by canalization of the radial artery and central venous monitoring utilizes a catheter in the jugular vein. Low-dose dopamine or phenylephrine can be used to maintain adequate systolic blood pressure. Measurement of blood gas and electrolytes should be done frequently, and correction of abnormalities of ionized calcium and in acid-base balance should utilize calcium chloride and sodium bicarbonate.

Blood can be taken from the donor during the organ procurement, prior to heparinization, for immunologic studies or for transfusion into the recipient to maintain adequate oxygen carrying capacity.

C. DONOR PROCUREMENT

Little has changed in the procurement technique since described in detail by Starzl for human organ procurement[14] and applied to baboons by Myburgh and co-workers.[15]

Allowing adequate time for hydration and stabilization of donor blood pressure and oxygenation, the abdomen and chest are thoroughly prepped and draped. A long midline incision from the sternal notch to the pubis is made, and adequate exposure maintained. Care must be taken in handling the liver, since it is very soft and more friable than that of man. The ligamentous attachments of the liver are incised, first cutting the ligamentum teres, followed by the falciform and left triangular ligaments. The left phrenic vein can now be visualized and ligated. The hepatogastric ligament is examined for the presence of an accessory left hepatic artery from the left gastric artery. The hilar structures are identified, starting in the right side of the porta hepatis. The common bile duct is ligated as inferior as possible. The gallbladder is incised and flushed through the cut common bile duct (Figure 2).

The hepatic artery is identified and traced back, ligating the right gastric and gastroduodenal artery. An aberrant right hepatic artery, if present, can be felt as a pulsatile

structure posterior to the portal vein. Care must be taken during the arterial dissection, since the vessels are fragile, and overmanipulation may result in intimal dissection and subsequent thrombosis of the artery. The celiac axis is mobilized and the celiac ganglion is divided. The portal vein can be seen at this time. Careful mobilization to the junction of the splenic and superior mesenteric vein allows the portal vein to be cannulated, either through the superior mesenteric vein or the splenic vein. The aorta is mobilized at the aortic bifurcation and cannulated.

The connective tissue attached to the retrohepatic vena cava is bluntly dissected by lifting the caudate lobe. The liver is retracted to the left and the right triangular ligament is divided and the vena cava is freed circumferentially. The pleural spaces are opened and the intrathoracic aorta is mobilized and clamped. The preservation solution is allowed to perfuse the aortic and portal vein cannulas. The intrathoracic vena cava is incised to allow venting of the flush solution. The liver is allowed to cool *in situ* with 750 ml aortic flush and 500 ml portal vein flush. The liver is then removed and taken to the back table in an ice-filled basin.

Stitches are applied to the corners of both the suprahepatic and infrahepatic vena cava. A tongue of hepatic tissue is carefully dissected from the lower vena cava to obtain an adequate length of lower vena cava. The right adrenal vein is ligated. The diaphragm surrounding the suprahepatic vena cava is trimmed. A Carrell patch around the celiac axis is then prepared and the liver is ready for implantation.

D. RECIPIENT OPERATION

The initial steps in the dissection of the recipient liver are identical to those described in the donor operation, except for the incision. The porta hepatis is approached by ligating the common hepatic duct as high as possible. The hepatic artery is ligated at the bifurcation into the right and left hepatic arteries and mobilized to the level of the gastroduodenal artery. The portal vein is skeletonized from the connective tissue. The suprahepatic and infrahepatic vena cava are mobilized circumferentially and Potts clamps are applied. A vascular clamp is applied to the portal vein and the liver is removed. While some investigators have used the veno-venous bypass described by Shaw and co-workers,[16] other have not found this necessary, especially if the venous anastomosis can be completed in a short time frame (30 min). The vascular anastomoses are performed in an end-to-end manner using continuous non-absorbable monofilament polypropylene sutures. The sequence of anastomosis is generally the upper vena cava followed by the lower vena caval anastomosis. The portal vein is usually connected prior to the hepatic artery. The technique used for all vascular anastomoses utilizes corner stitches and a continuous everting over and over suture, completing the posterior wall prior to starting the anterior wall (Figure 3).

In smaller caliber vessels, such as the portal vein, but also with small vena cavas, a "growth factor" is utilized to prevent stenosis following expansion of the circumference with reperfusion (Figure 4). The growth factor is usually the length of one diameter of the portal vein, while it is much shorter for the vena cava. Once the venous anastomosis are completed, the clamps are released to allow for timely decompression of the mesentery.

The hepatic artery anastomosis is performed between the proper hepatic artery with a Carrell patch from the donor, and the common hepatic artery at the level of the takeoff of the gastroduodenal artery of the recipient (Figure 5).

There are a number of techniques available for the biliary reconstruction. The preferred method for reconstruction is with an end-to-end choledochocholedochostomy using a silastic stent (Figure 6), although the side-to-side choledochocholedochostomy (Figure 7) described by Neuhaus can be utilized. Choledochojejunostomy, choledochoduodenostomy, or the Weidell-Caine gallbladder conduit (Figure 8) can also be utilized.

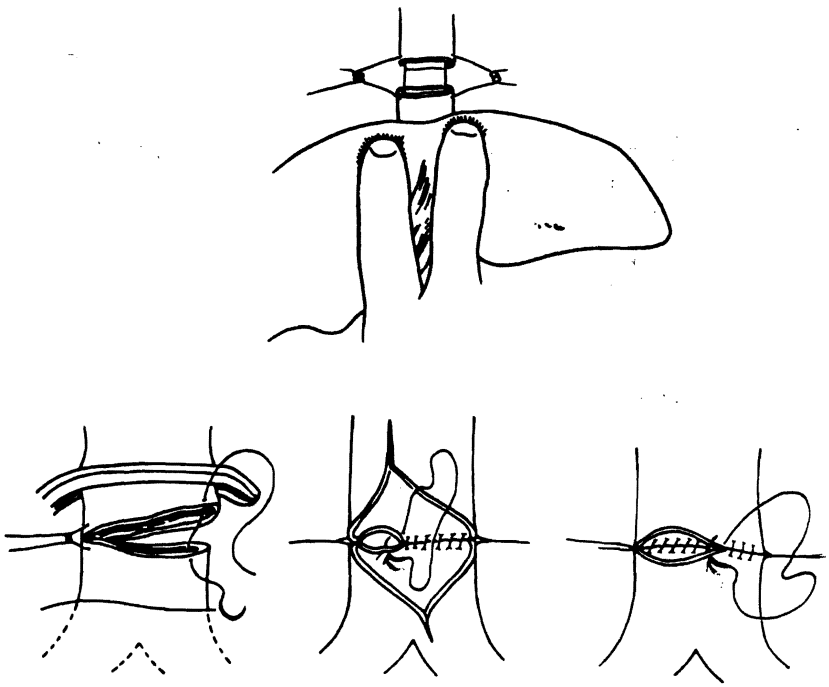

Figure 3 Vascular anastomosis: (A) both ends are approximated with corner stitches; (B) corner stitches have been placed; (C) continuous everting suture of posterior wall; and (D) continuous everting suture of anterior wall.

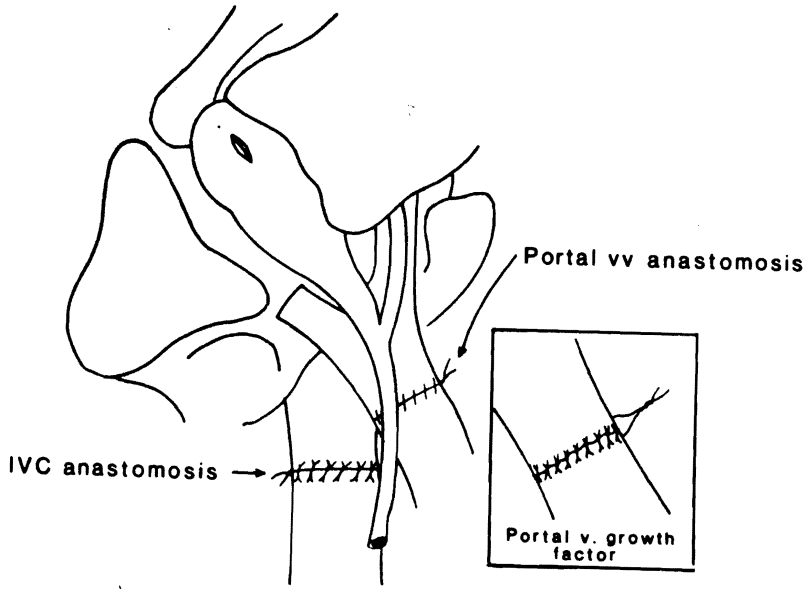

Figure 4 Growth factor before unclamping (picture), and final result after expansion of the circumference.

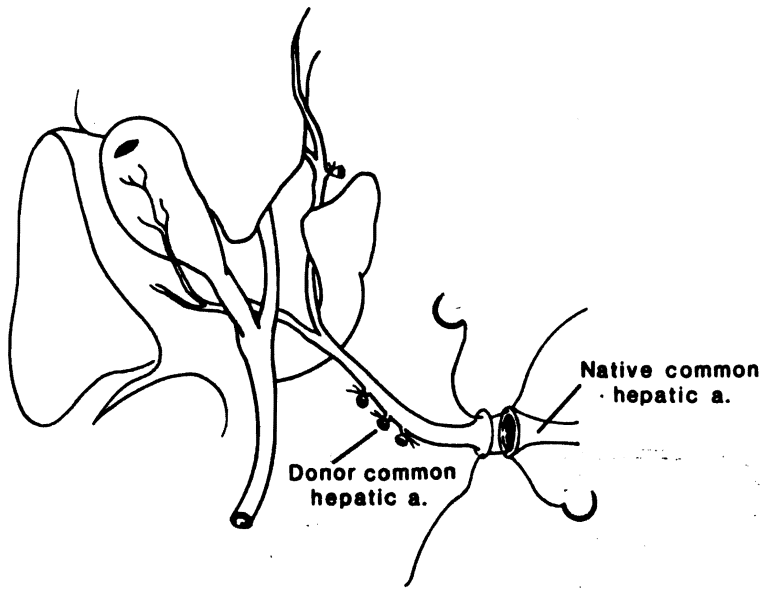

Figure 5 Hepatic artery anastomosis (see text).

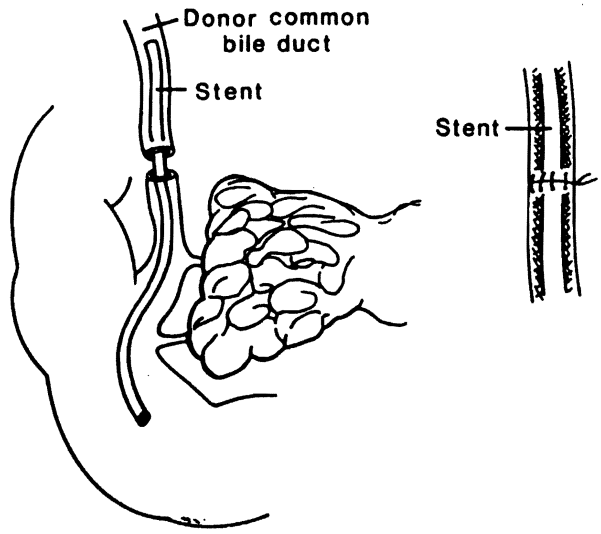

Figure 6 End-to-end choledochocholedochostomy using a silastic stent as a factor.

Incisions in the skin must be closed by an intradermal suture, otherwise metal clips are apt to be scratched off.

E. POSTOPERATIVE CONSIDERATIONS

In a number of non-human primates, including the rhesus monkey and baboon, the males have large canine teeth. These can be vicious weapons, and it is recommended to either extract them or to shorten them prior to, or at the time of surgery.

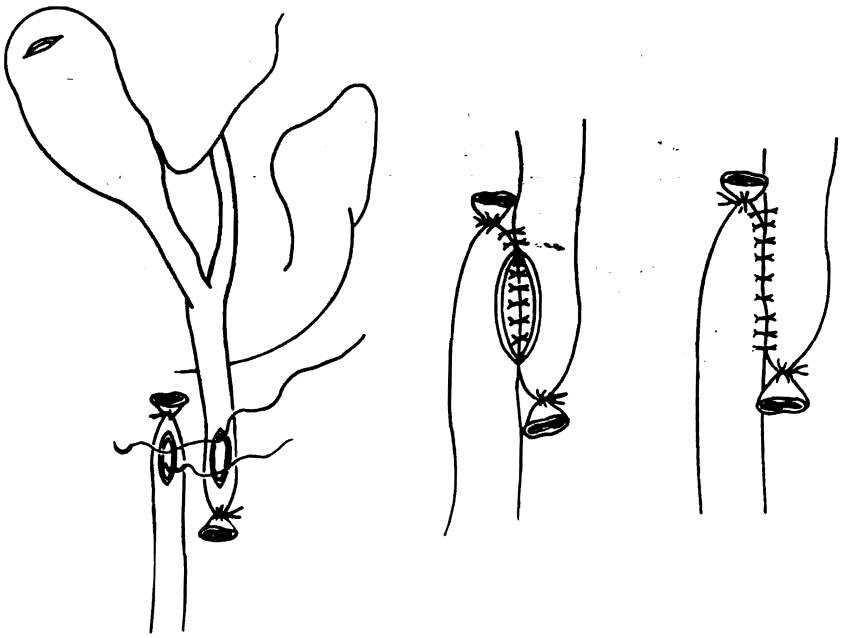

Figure 7 Side-to-side choledochocholedochostomy.

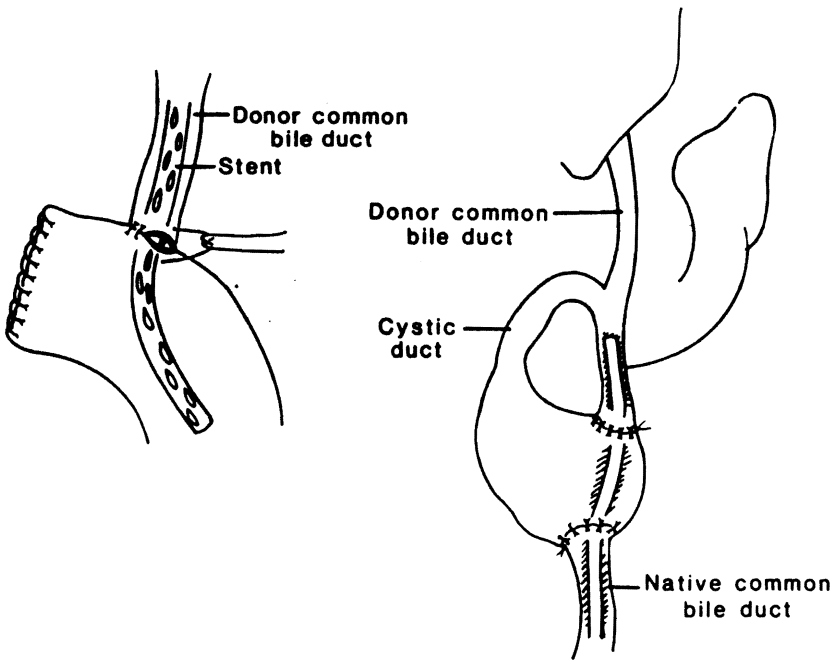

Figure 8 (A) Choledochojejunostomy. (B) Biliary reconstruction using the gallbladder as a conduit (Weidell-Calne).

Primates are quite strong. For animals weighing less than 15 lb, manual restraint is often satisfactory, while larger animals should be sedated with ketamine. Administration of sedatives is greatly facilitated by keeping the animals in a "squeeze-back" type cage.

Shortly after transplantation, animals should be monitored in a recovery setting, with warming lamps. Animals may be extubated shortly after transplantation. Intravenous lines cannot be used once the animals are awake, and any intravenous medications must be completed, or the animal will require further sedation. Antibiotics are given to the animals at the initiation of surgery and once again at the completion of the transplant.

Animals are allowed to drink ad libitum for the first day, and a solid diet is given by the third day following transplantation. Immunosuppressive drugs can be given either as an intramuscular injection or by mixing it with food.

Tests, such as phlebotomies and liver biopsies, require sedation. These studies should be done with a brief fasting period, to avoid aspiration during sedation.

III. CONCLUSIONS

Non-human primates are an important resource in biomedical research. Because of their similarity to humans anatomically and immunologically, refinements in the understanding of liver transplantation may well be derived from studying non-human primates. In addition, this resource may also prove to be an answer to the growing problem of organ shortage. Concordant xenotransplantation has been shown to be successful, both in animal models and in a few human cases.[19-22] While ethical issues surround the application of concordant xenotransplantation, responsible investigations using non-human primates must continue.

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