TECHNICAL DETAILS FOR SAFER VENOUS AND BILIARY ANASTOMOSES FOR LIVER TRANSPLANTATION IN THE RAT

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Although orthotopic liver transplantation in the rat is a well-established experimental model, no detailed illustrated description of the procedure is available in the literature. As a result, achieving success in the technique without prolonged learning time requires training in specialized centers where the step-by-step details essential to success in the technique can be learnt. The aim of this paper is to provide beginners with the culminative experience of 15 years of improvements made to Kamada's original model, clearly illustrating the necessary steps and fine detail of each anastomosis to help beginners avoid unnecessary complications. We hope that this complete description of orthotopic liver transplantation in the rat, which remains the most difficult rodent transplant model to master, will greatly help microsurgeons acquire this demanding procedure.

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Despite several descriptions in the literature,[1–5] liver transplantation in the rat remains difficult for beginners to master because the rat's intolerance of portal venous (PV) clamping (maximum tolerated anhepatic time, 25 minutes) means that the anastomoses must always be performed quickly but perfectly. Both prolongation of the anhepatic time and any technical complication which results in bleeding lead to circulatory collapse and loss of the animal. This inevitably means that training should ideally take place in specialized laboratories, in which the procedure is established and that have the ability to support a long period of practice. When the procedure has been used de novo in trials by centers with no previous expertise, the results have invariably been poor. Previous published descriptions have been incomplete, as none have included illustrations, and they have omitted many important technical details vital to the acquisition of such a demanding technique.

To assist other surgeons to acquire this model, we present a fully illustrated description of the technique with several modifications added progressively by Kamada and his colleagues to the original model. This paper is based on recent personal experience of over 350 orthotopic rat liver transplants performed in Kamada's laboratory. Key points include continuous suturing for the suprahepatic vena cava (SHVC) anastomosis, the cuffed anastomotic technique for the infrahepatic vena cava (IHVC) and PV, and bile duct reconstruction using stents. The cuffed anastomotic technique was pioneered by Nitze[6] and Payr[7] and was introduced into microsurgical transplantation procedures by Heron[8] for rabbit cardiac transplantation. Kamada[1–3] introduced it into rat orthotopic liver transplantation for both IHVC and PV anastomoses in rat liver transplantation. Prior to Kamada's innovation all anastomoses had been performed with continuous sutures, as described by Sun Lee et al.[4,5]

MATERIALS AND METHODS

All rats used in the operations were adult males weighing between 250 and 350 g, with free access to food and water. Ether anaesthesia was used as it allows accurate control of the depth of anaesthesia which is crucial during the anhepatic phase of the operation. All procedures were performed and animals treated in accordance with the Australian and Belgian guidelines of animal care as assessed by the ethical committees of both the Queensland Institute of Medical Research and the University of Louvain Medical School.

Details of the donor hepatectomy have been described in a previous paper.[9] In this paper we address the performance of the anastomoses necessary for implantation. Most of the steps of the recipient hepatectomy are similar to the operation in the donor; where they differ, a detailed description will be given.

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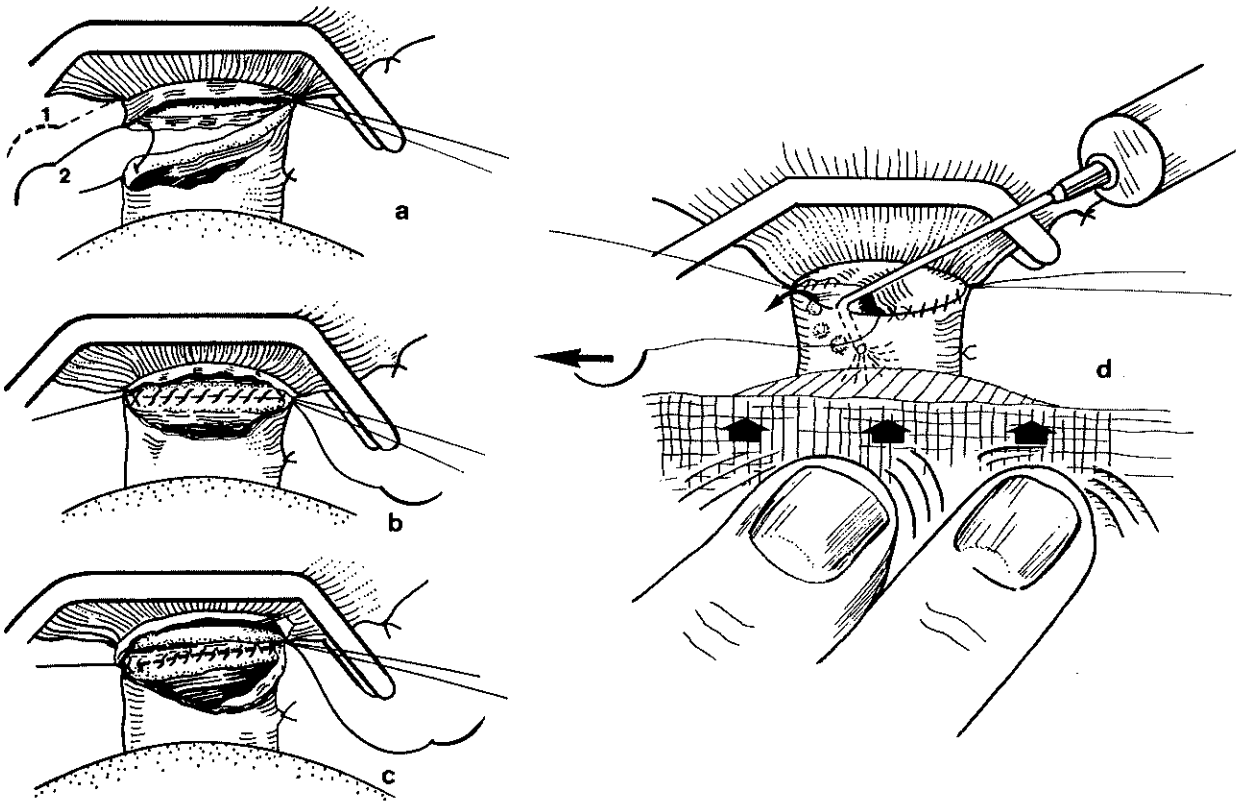

Figure 1. Supra-hepatic vena cava anastomosis. ***a:*** *Corner sutures (1) in the diaphragmatic ligament and (2) in the edge of the vein.* ***b:*** *Posterior wall anastomosis when performed on the diaphragmatic ligament.* ***c:*** *Posterior wall anastomosis when performed on the edge on the vein.* ***d:*** *Details of the technique for the anterior wall anastomosis. See text for complete description.*

Suprahepatic Vena Cava Anastomosis

This is the only remaining sutured anastomosis; it is essential that it be executed perfectly to avoid the complications of air embolism, haemothorax, bleeding, and stenosis. During the recipient hepatectomy, after clamping the vena cava and portal vein (see below), the liver lobes are returned to their anatomical position, and the SHVC is pulled toward the operator by traction on a sling previously placed around it during dissection. The left-hand side of the sling is grasped between the fourth and fifth fingers of the right hand whilst the right hand side is held between the first and second fingers. This ensures that retraction keeps the vein flat without diaphragm puckering. A diaphragmatic clamp (paediatric Satinsky; Lawton 23-1915, Tutlingen, Germany), held in the left hand, is introduced closed from the left, beneath the SHVC, and is opened under the liver against the diaphragm to tear any remaining adhesions. It is then reinserted to cross-clamp the SHVC; the exact position is crucial to ensure that the posterior ligament is seen by translucence through the anterior wall of the vein. It should clamp the diaphragm approximately 4 mm above the cut edge of the anterior wall of the SHVC. The clamp is secured with plasticine to ensure it doesn't spring open, to prevent movement during respiration, and to keep the clamp superficial so that the anastomotic area is not more than 1.5 cm below the level of the skin. The suprahepatic vein is cut as long as possible. Following hepatectomy, a 10 × 10 cm rolled swab is placed in the hepatic fossa to create a flat plane descending to the diaphragm; on the left side, a further swab is placed unfolded so that the graft may be wrapped and kept cold.

Correct positioning of the graft greatly facilitates the performance of the anastomosis. It should be placed on the swab so that the SHVC is 2.5–3 cm from the cut edge of the recipient SHVC. To ensure accurate positioning of the corner sutures, these are inserted during the graft preparation whilst the liver is in the cold preservation fluid. It is important when moving the liver that the bulldogs and suture on the SHVC be held by the left hand and the liver grasped between the first and second fingers of the right hand. The left-sided 6/0 Prolene stay suture is passed through the left-hand corner of the recipient SHVC, including a reasonable amount of the diaphragmatic ligament, avoiding the left diaphragmatic vein. This is gently tied with two throws and placed on traction using a rubber-shod mosquito. The same procedure is repeated on the right (7/0 Prolene); the needle can be passed either through the diaphragmatic ligament (Fig. 1a, 1) or through the edge of the vein (Figs. 1a, 2). One strand is placed under traction, and the left side of the swab

is folded over the liver and kept moist with 10 ml of cold preservation solution. The liver is pulled down by traction on the swab.

If these steps are closely adhered to, the SHVC of graft and recipient are presented closely opposed and lying superficially, allowing a rapid, efficient, and easy anastomosis. The 7/0 suture is passed inside at the right-sided posterior corner of the donor SHVC and locked. A running suture is then performed on the posterior wall of the anastomosis using either the diaphragmatic ligament or the venous wall (Figs. 1b,c) depending on which was used for the corner suture. Kamada originally used the ligament; the second option was developed to avoid haemothorax due to the sutures being placed too deeply and damaging the intrathoracic cava. The gap between sutures should not be greater than 1.5 mm and must not tear the vein as immediate haemostasis is essential for success. If the anterior wall of the cava obscures the suture line, it can be gently pushed downward using a wet cotton bud. On completion of the posterior wall, the suture must be locked to prevent narrowing of the anastomosis by overtightening when the suture is tied. The suture is then passed to the outside of the left corner on the recipient side of the anastomosis, tied to the stay suture, and maintained on traction using a rubber-shod mosquito forceps. The corner is strengthened by two further locked sutures as this is the site of most frequent bleeding. The graft is moistened with 10 ml of cold preservation solution.

The anterior wall is then sutured in the same manner avoiding any anterior traction on the suture to prevent air being introduced into the lumen. It is essential that this suture be placed as delicately as possible, as the thin-walled vein is easily torn. At the mid-point of the anterior wall, the suture should be locked and the inside of the vein flushed with cold fluid. Whilst the fluid is injected, the median lobe is gently squeezed to extract deeply placed air bubbles (Fig. 1d). If the anastomosis is superficial the bubbles are easy to see and remove. To avoid further air introduction, the final sutures are placed horizontally with no vertical lifting of the caval edges. The corner suture is repeated, locked, and tied to the stay suture. The hepatectomy and this anastomosis should take 10–15 minutes of the anhepatic phase. On reperfusion there should be no bleeding from the SHVC anastomosis. If there is bleeding, the PV and diaphragm should be reclamped. If the bleeding point is on the anterior wall, a hemostatic 8/0 prolene suture can be attempted; if it is posterior, hemostasis can be attempted by pressure with a cotton bud after application of fibrin powder.

Cuff Anastomoses on the PV and IHVC

Preparation of the recipient infrahepatic vena cava and portal vein during hepatectomy. Details of the donor hepatectomy are given in our previous paper.[9] Certain additional steps are required during the recipient hepatectomy. After mobilization of the IHVC from surrounding tissue, the right kidney is pulled downward and separated from the adrenal gland. A right-angle forceps is used to free the posterior wall of the IHVC at the confluence of the right renal vein and the right renal pedicle from the lumbar muscle; the lumbar and adrenal veins are ligated and divided.

To position the clamps and stay sutures on the PV and IHVC, the median and left lateral lobes are retracted upward against the diaphragm and covered with a wet swab. The left branch of the PV is ligated distal to the emergence of the branch supplying the left caudal lobes using a 6/0 prolene suture. The median and left lateral lobes receive no further blood; it is important that the SHVC not be clamped until they have changed color to ensure that as little blood as possible is lost on removing the liver. The flow through the other lobes is enough to avoid intestinal congestion. A length of the suture is left as a retractor. The IHVC is clamped just above the confluence of the right renal vein, introducing the clamp from the right, and the PV is occluded 2 mm from the PV bifurcation. The right branch of the PV is then ligated using 6/0 prolene suture which is also cut long to use as a retractor. After division of the SHVC, the median and left lateral lobes are retracted to the right and the PV branch to the left caudal lobes, well seen in this position, divided. The left and right branches of the portal vein are cut on the hepatic side of the ligature and the PV separated from the liver ensuring that the posterior wall is not damaged. We always ensure that the IHVC is incised anteriorly 1 mm from the liver, the incision being extended obliquely first laterally and then posteriorly which facilitates the placement of the corner sutures as the vein remains open and accessible. The liver is then removed.

Portal vein anastomosis. Positioning the liver correctly greatly facilitates the performance of this anastomosis. Using a wet cotton bud, the liver is pushed into the right hypochondrium and covered with a wet swab. The lobes are positioned as follows: the median in contact with the diaphragm, the left lateral lobe beneath the lateral edge of the diaphragmatic clamp to lie on the spleen and the stomach, the superior right caudal lobe beneath the center of the diaphragmatic clamp (which is elevated to make this easier), the right inferior caudal lobe anatomically, and the left caudal lobes beneath the lesser curve of the stomach. The PV clamp is retracted to expose the vein fully, and the two sutures on the branches are placed under traction with bulldog clamps so that the donor's PV cuff is in front of the bifurcation of the recipient PV. The vena cava and bile duct sutures are retracted to the right and left, respectively, so they are not in the anastomotic field. The PV clamp is moved proximally to the convergence of the gastroduodenal vein (Fig. 2a), preventing blood loss by occluding the vein with forceps. Blood from this segment escapes through the orifice of the left caudal lobe branch and facilitates the

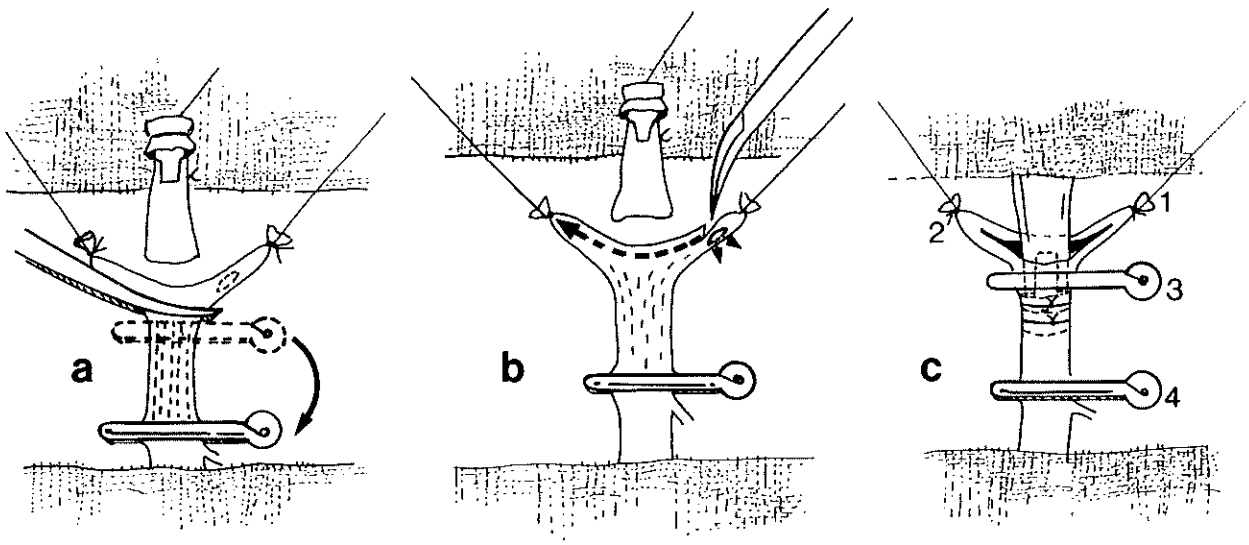

Figure 2. Portal vein anastomosis. ***a:*** *Isolation using a forceps and clamp of the trunk of the portal vein.* ***b:*** *Incision of the roof of the portal vein division colored by blood—some blood escapes from the portal branch coming from the left caudal lobe.* ***c:*** *After the introduction of the cuff it is secured in position by two ligatures; the retracting sutures and the clamps are removed successively in the order indicated. See text for complete description.*

opening of the PV bifurcation because the presence of blood makes identification of the PV edges easier. A small incision is made in the left branch and the superior edge of the bifurcation opened with scissors from left to right (Fig. 2b). Any blood and thrombus in the PV is removed by flushing with heparinized saline.

After ensuring that the donor PV is not twisted by checking that the gastroduodenal tie is on the left, the cuff extension is grasped with nontoothed forceps in front of the recipient right branch suture, the cuff ligature cut, and the cuff introduced into the recipient vein. Both the vein and cuff are filled with fluid to eliminate the risk of air embolism using a needle to retract the anterior wall of recipient PV forward. The cuff is introduced as far as possible, held in position with a further arterial clamp, and then secured in position with a 6/0 silk tie which is only tightened when perfectly positioned. It is very important that this tie does not slip as removal can be dangerous. Ideally, it should lie below the ligature which holds the donor vein in position in the cuff; otherwise a further tie is necessary in this position to ensure endothelial apposition which reduces the risk of thrombosis (Fig. 2c). Again, this ligature requires meticulous placing to prevent it from slipping off the cuff. All retracting sutures are cut (Figs. 2c, 1 and 2), followed by removal of the securing clamp (Figs. 2c, 3); the vein is then straightened by cotton bud manipulation and the portal clamp removed (Figs. 2c, 4), allowing liver reperfusion. The clamp on the diaphragm/SHVC is removed. The SHVC anastomosis is examined to ensure that no bleeding is present. If there is blood loss, an attempt is made to achieve hemostasis, as detailed previously on the section on the SHVC anastomosis.

Infrahepatic vena cava anastomosis. The IHVC anastomosis has been totally redesigned from that originally described by Kamada, to facilitate the removal of any clot formed in the IHVC during its occlusion. The liver is retracted with a wet swab against the diaphragm and the IHVC area exposed with the clamps easily visible. The suture on the IHVC cuff is pulled upward and placed on the hepatic swab. Stay sutures (7/0) are placed in the two corners of the recipient IHVC and held under tension by bulldogs; the renal pedicle is clamped, a right-angled clamp is passed behind the recipient IHVC just beneath the right renal vein convergence and a clamp is placed across the IHVC just above the left renal vein convergence (Fig. 3a). The clamp placed on the recipient IHVC at the time of hepatectomy is removed and any blood and thrombus removed easily, a procedure that is assisted by flushing the isolated segment of the vein with heparinized saline (Fig. 3b). This allows adequate flushing to be performed without blood loss. The cuff is grasped with microforceps and any twisting of the vein checked by ensuring that the right renal vein ligature is in the correct position. The cuff and the vein are washed and filled with fluid. Cuff introduction and anchorage are identical to those of the PV. Once secured, the retracting sutures are cut (Figs. 3c, 1 and 2) and the clamps removed, firstly that on the donor IHVC (Figs. 3c, 3), secondly the securing clamp on the cuff extension (Figs. 3c, 4), thirdly the clamp on the renal pedicle (Figs. 3c, 5), and finally that on the recipient IHVC (Figs. 3c, 6). Immediately after full circulation is restored, a penile vein injection of 2.5 ml (2 ml of colloid and 0.5 ml of 8.4% bicarbonate) is performed as rapidly as possible. The unimpeded blood flow through the vena cava cuff is verified by lack of re-

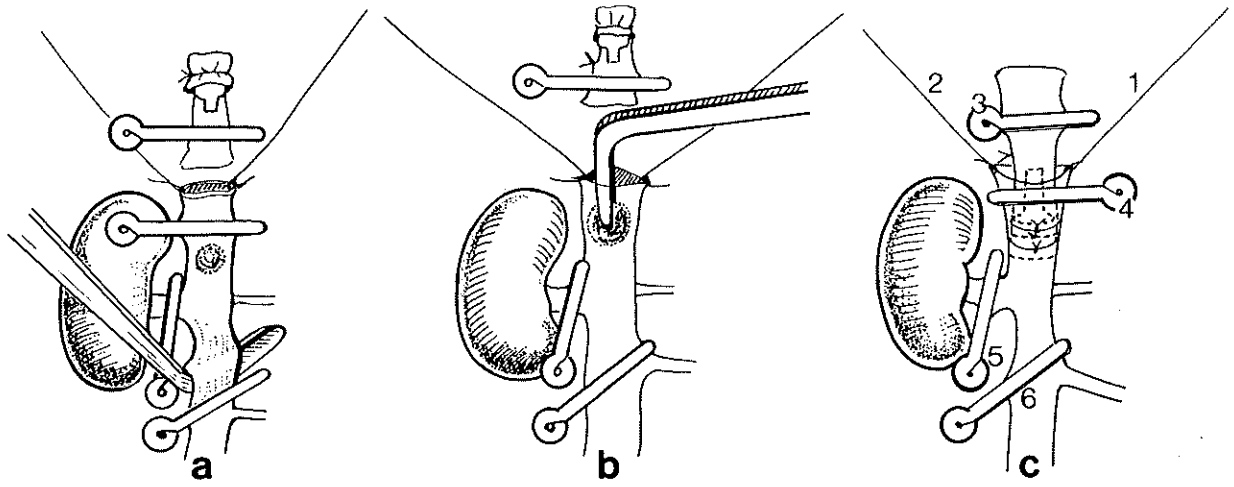

Figure 3. Infra-hepatic vena cava anastomosis. ***a:*** *Position of the clamps applied on the IHVC.* ***b:*** *Removal of the thrombus after flushing the vein.* ***c:*** *After the cuff has been introduced and secured in position by two ligatures, the retracting sutures and the clamps are removed successively in the order indicated. See text for complete description.*

sistance during the fluid injection. Any remaining blood is removed and the duodenum placed back in its anatomical position to cover the IHVC anastomosis.

Bile Duct Reconstruction

The donor bile duct is cannulated during the hepatectomy (for full details see ref. 9). The recipient bile duct is prepared during the recipient hepatectomy. The initial preparation is identical to that of the donor operation. After division of the hepatic artery the intestine is retracted to the left and inferiorly using a swab exposing the hepatic duct confluence. In cases in which the branch from the left caudal lobes joins below the confluence of the two main branches, this branch has to be ligated separately. The bile duct is separated from the portal vein for approximately 2 mm, the confluence of the left and right hepatic ducts dissected, and the anterior surface of the left branch opened as far as the right branch. A 4F prosthesis (intravenous cannula 4G, Portex, England) is introduced after slight dilatation of the bile duct, and the prosthesis is prepared in the same way as for the donor bile duct apart from slightly stretching one end into which the donor duct prosthesis will be inserted. The other end is spatulated, gently introduced into the common hepatic duct, taking care that the biliary epithelium is not damaged, and then secured with a ligature whose right-hand strand is left 5 cm long and used to place traction on the duct whilst the hepatic branches are divided.

Bilary reconstruction is the last manoeuvre of the transplant operation; by the time this stage is reached there should be evidence of bile production. The securing sutures on the two bile ducts are pulled together to bring the two prostheses close to each other; they are then flushed to remove any remaining blood clots (Fig. 4a) and carefully checked to ensure that neither is twisted. Using microforceps the donor prosthesis is inserted as far as possible into the recipient one until a tight fit is obtained; the sutures are then tied together under tension to ensure anastomotic impermeability (Fig. 4b). A portion of the greater omentum is freed, wrapped around the anastomotic area, and secured with the same ligatures (Figs. 4c,d).

DISCUSSION

This paper describes rat liver transplantation in detail, giving the technical steps necessary to perform the procedure safely and facilitate its use by microsurgeons learning the technique. Our aim has been to provide all necessary details to reduce the incidence of complications during the learning curve when someone is acquiring the technique.

The SHVC anastomosis is the most difficult part of the procedure; following the steps outlined above should considerably reduce complications such as bleeding, air embolism, haemothorax, and stenosis. Prevention of bleeding is dependent on precise suturing, avoiding tears in the venous wall, leaving only small gaps between sutures and adding supplementary corner sutures. Immediate hemostasis must be the aim since further suturing after reperfusion is very risky and often unsuccessful. Deaths due to bleeding usually occur shortly after the operation. The animal sometimes wakes up but then rapidly weakens and dies. Air embolism is caused by the same problems as bleeding and by failure to evacuate trapped air bubbles completely before finishing the anterior wall of the anastomosis. Death by massive air embolism is immediate. Haemothorax is usually an unrecognized complication, and autopsy must always include opening the chest if no blood is seen in the abdomen. It is usually caused by damage to the intrathoracic vena cava

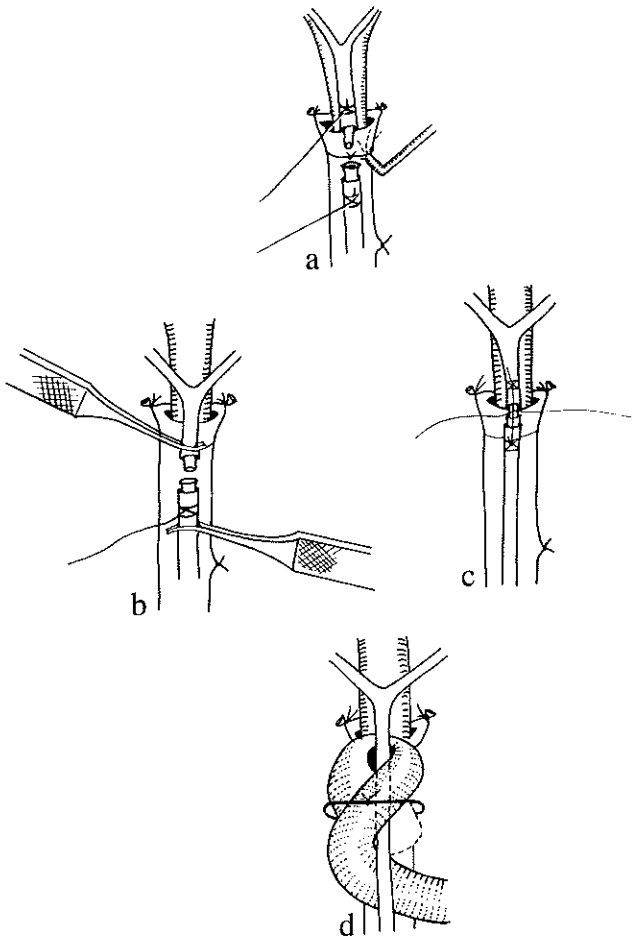

Figure 4. Bile duct reconstruction. ***a:*** *Biliary stents are brought together after the recipient duodenum is replaced in its anatomical position.* ***b:*** *The stents flushed to remove thrombi.* ***c:*** *After insertion of the donor stent in the recipient stent, the securing ligatures of the stents are tied together.* ***d:*** *Omentum is wrapped around the stented anastomosis and secured in position. See text for complete description.*

during the formation of the posterior wall anastomosis of the SHVC. When the diaphragmatic clamp is removed, the vein can tear in the chest which is not obvious unless the area is carefully examined. Stenosis of the anastomosis is avoided by taking small steps and bites during suturing and placing securing corner sutures. Any stenosis results in a Budd-Chiari-like syndrome with a congested liver, intestine and vena cava; the animal dies a few hours following the graft.

The cuff technique for the portal vein and IHVC anastomoses greatly facilitates the execution of the procedure, but certain pitfalls need to be recognized and avoided. These include twisting or tearing of the veins, immediate thrombosis due to failure to remove all blood clots, air embolism, and bleeding because of cuff slippage. Twisting can be avoided by carefully checking the vein and position of ligatures on either the gastroduodenal or renal veins and tearing of the vein by being very careful with all manipulations, and suturing accurately. Immediate thrombosis due to in situ blood clots can be minimized by moving the PV clamp and modifying the positioning of the clamps on the IHVC. Careful flushing of the PV should be performed, especially if portal clamping time is greater than 26 minutes. As with the SHVC, air embolism is prevented by meticulous filling of the vein and cuff before and during anastomosis; slippage of the cuff is avoided by a double ligature, giving tight endothelium-to-endothelium contact.

Despite the details given above, beginners may also benefit from the ability to prolong the permitted anhepatic time beyond 25 minutes whilst familiarizing themselves with the different steps of the procedure. One method of achieving this is to establish a portosystemic shunt which allows anhepatic times to up to an hour without prejudicing the recovery of the rat. This shunt can be achieved by subcutaneous transposition of the spleen[10] which results in a natural shunt after approximately 3 weeks.

Biliary reconstruction has been described as the Achilles' heel of human liver transplantation[11]; the same is equally true for the rat. In rat orthotopic liver transplantation it is not difficult to perform the stented anastomosis, but many details described here need to be applied to avoid common complications. Incomplete drainage of the liver is usually the result of malposition of the prosthesis in the donor bile duct and its entry into one of the hepatic ducts, causing partial occlusion. This can become total occlusion if a calculus develops in the anastomosis. The position of the prosthesis must also be checked after completion of the anastomosis because manipulation of the cuffs during their insertion into each other can easily result in displacement of the cannulae into one of the hepatic ducts. Removal of any blood clot from the bile ducts helps to prevent rapid stone formation. Twisting of the anastomosis is avoided by careful positioning of the ligatures on the anterior side of the duct and routine preservation of the right-hand thread of the securing suture. The restoration of the anatomical position of the duodenum following anastomosis is also important. Delayed leakage and biliary peritonitis are prevented by the impermeability of the anastomosis. This is ensured by tying the ligature holding each prosthesis securely, making sure that the prostheses are tightly fitting and that pressure is applied by tying the retraction ligatures together and an omentoplasty using the greater omentum. The long-term permeability of the anastomosis remains a problem, as most rats kept long-term develop at least partial occlusion of the prosthesis by a stone. Whilst in some rats the liver remains normal, secondary biliary cirrhosis or biliary distension (biloma) can occur, followed by infection and the death of the animal.

The procedure described here does not include rearteri-

alization of the graft, the importance of which remains open to debate. Recent studies have shown that arterialization is an important step to avoid long-term biliary damage,[12–14] improve graft microcirculation,[15] and diminish graft expression and liberation of soluble class I and II antigens.[16,17] In the nonarterialized model, long-term success may also depend on the rapidity by which the rearterialization is obtained through collaterals to the liver from the omentum applied to the biliary anastomosis.[18] Our laboratory does not yet use arterialization routinely, and technical details can be found in the papers cited above.

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