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# Simplified microvascular suture techniques for rat liver transplantation as a microsurgical model with arterial blood supply

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**Abstract.** The methods for liver transplantation in the rat mainly used do not include reconstruction of the arterial blood supply to the liver. Furthermore, to ensure a short anhepatic phase these methods almost all entail specially developed cuff anastomoses in the recipient operation instead of the conventional microvascular suture technique. Thus an acceptable survival rate can be attained in the experimental animals. This detailed description of simplified microvascular suture techniques is intended to present an alternative to the cuff anastomoses used almost exclusively. In the donor operation with this method, the liver is dissected with an arterial pedicle including the abdominal segment of the aorta, and the liver is flushed in situ not only via the portal vein, but also via the hepatic artery. The organ is implanted in the recipient animal using simplified microvascular suture reconstruction of the arterial blood supply to the liver. Use of telescopic spectacles with 2-fold magnification has proven to be adequate for the entire procedure. With mastery of this method of rat liver transplantation, the average duration of the anhepatic phase is about 20 min, substantially below the 30-min limit which is critical for the survival of the experimental animals. The donor operation requires about 60 min, and the recipient operation 70 to 80 min. With this method, the spectrum of investigations on liver transplantation which are possible in the rat is substantially extended in that clinical conditions can be reproduced very much more exactly by combination of portal and arterial in-situ flushing in the donor operation and rearterialization of the transplant in the recipient operation, as compared to the transplanted rat liver being supplied only with portal venous blood.

**Key words:** Rat liver transplantation – Arterial reconstruction – Microvascular suture techniques

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The necessity of keeping the anhepatic phase of the recipient operation in rat liver transplantation under 30 min led to the development of cuff techniques for anastomosis of vessels. Since rearterialization of the transplant is not crucial for survival of the rat, most experimental models for rat liver transplantation do not provide for a reconstruction of the arterial blood supply to the liver [1, 5, 6, 8–12].

The present paper describes in detail our method for orthotopic rat liver transplantation, which adheres to the principles of microvascular suture techniques for vascular anastomoses. For reconstructing the arterial blood supply of the transplant, it is necessary, during the donor operation, to prepare the liver with an arterial pedicle which includes the abdominal segment of the aorta. Furthermore, in situ perfusion is performed not only via the portal vein, but also via the hepatic artery. The organ is implanted in the recipient using simplified and thus rapidly executed microvascular suture techniques for all vascular anastomoses.

This method including portal and arterial in-situ flushing in the donor and rearterialization of the transplant in the recipient can be employed for a broad spectrum of investigations on liver transplantation, since clinical conditions are reproduced with greater accuracy in comparison to other methods.

## Materials and methods

The halogenated methyl-ethyl ether enflurane is used as the anesthetic agent, which is especially indicated for liver surgery. A pediatric Satinsky clamp, which is suitable for the suprahepatic vena cava, is an integral part of the microsurgical instruments. Small mosquito clamps are applied for the portal vein and infrahepatic vena cava, while microvascular clips are apt for the aorta. Ligatures are performed with 5–0 silk and vessels are sutured with 7–0 or 8–0 monofilament material.

### *Donor operation*

The preparatory steps of hepatectomy in the donor have been extensively described [1, 2, 5–8, 12]; therefore, only essential modifications

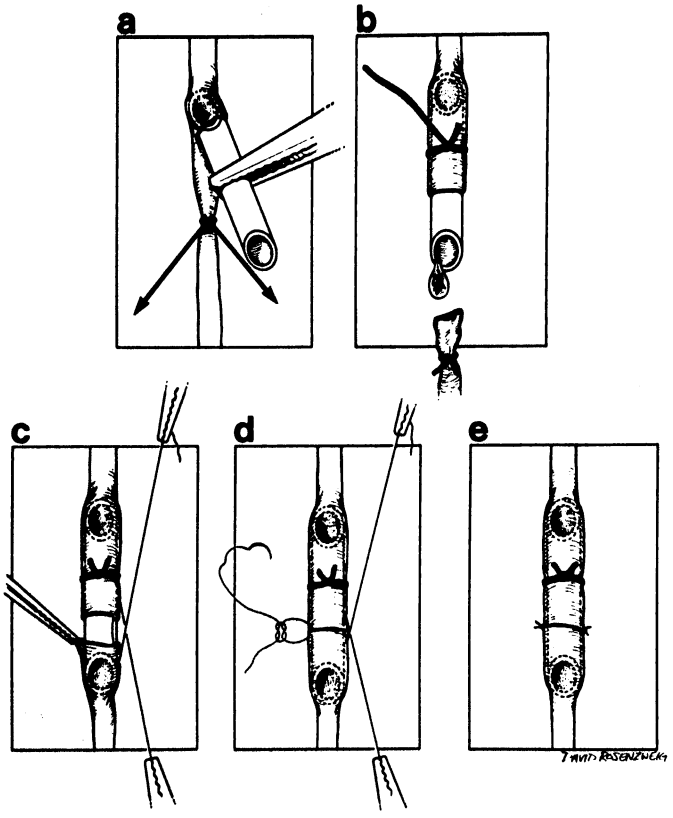


**Fig. 1a–e.** Reconstruction of the bile duct. **a, b** Introduction and fixation of a splint in the donor operation. **c–e** Choledocho-choledochostomy with splint technique and additional suturing in the recipient operation, with use of traction sutures

are reported. The liver is prepared with an arterial pedicle which includes the abdominal aorta. All branches of the aorta between the diaphragm and the bifurcation into the common iliac arteries are ligated and cut, while the celiac trunk is preserved. Additionally, the hepatic artery is isolated in its course to the hilus after the splenic artery and the left gastric artery have been ligated and dissected.

An angiocatheter, (about 8 mm long, beveled at both ends) is introduced with over two thirds of its length in the lumen at a distance from the liver and fixed with one ligature, followed by the complete separation of the duct (Figure 1a, b).

Antecedent to the beginning of in-situ perfusion of the liver two mosquito clamps are applied to the infrahepatic vena cava above the right renal vein. Division of the infrahepatic vena cava is followed by clamping of the portal vein at the junction of the splenic vein. Flushing begins with the introduction of the perfusion catheter (14-gauge angiocatheter) into the transversely incised portal vein. The perfusion pressure equals the physiologic pressure in the portal vein of the rat [1]. For simultaneous arterial perfusion, 2 to 3 ml of the perfusate are injected into the caudal end of the aorta.

Application of two traction sutures (7–0) for the later anastomosis of the suprahepatic vena cava during the recipient operation represents the last step of the donor operation; these sutures are fixed to the vessel wall with a surgical knot (Figure 2a). The liver is removed at the clamp on the intrahepatic vena cava and is placed in the preservation bath without removing this clamp.

## *Recipient operation*

After the steps preparing for the removal of the recipient's liver, which are basically analogous to the procedure for donor hepatectomy [1, 2, 5, 8–12] the anhepatic phase begins when the infrahepatic vena cava is clamped at the junction of the right renal vein and the portal vein at the junction of the splenic vein (with mosquito clamps from the right side of the rat); and finally, the pediatric Satinsky clamp is applied to the suprahepatic vena cava. A diaphragm cuff is included in the clamp. Removal of the liver begins with the circular excision of the suprahepatic vena cava at its entry to the liver. Next, the portal vein is cut at the bifurcation into its two main branches, followed by the dissection of the infrahepatic vena cava at its juncture with the liver. After the liver has been removed and before the transplant is introduced, the dissection board is rotated through 180°. The donor organ is placed in the anatomically correct position by means of the clamp on the infrahepatic vena cava.

*Anastomosis of the suprahepatic vena cava (running suture with 7–0, Figure 2b–f).* With traction sutures already having been applied to the transplant at the end of the donor operation, it is considerably easier to approximate the suprahepatic stumps of the vena cava by placing two corner sutures. We begin to perform the running suture with the thread of one of the corner sutures, from the inside of the posterior wall. As in the venous anastomoses that follow, there is no need for also sewing the suture from the outside to the inside. At the opposite corner this suture is brought to the outside of the recipient vena cava. Without tying a knot with the opposite corner suture, we continue the running suture on the anterior wall, applying a single interlocking stitch which maintains tension on the posterior wall until the anterior line is completed. For the elimination of air bubbles, the vessel lumen is flushed before completing the anastomosis.

*Anastomosis of the portal vein (running suture with 8–0, Figure 3) and reperfusion of the transplant.* The dissection board is rotated back into its original position. The corner sutures for the anastomosis of the

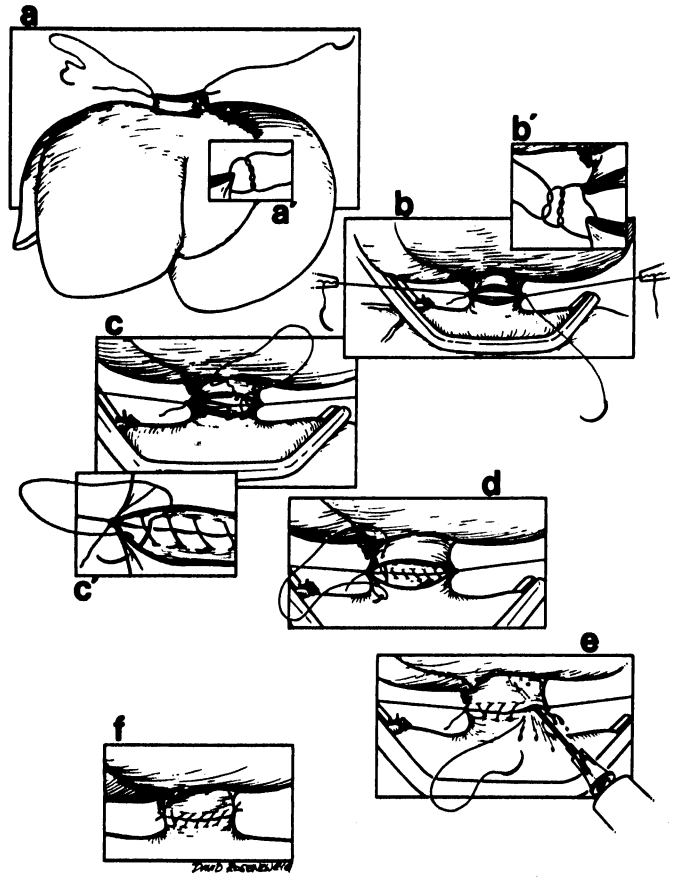


**Fig. 2a–f.** Anastomosis of the suprahepatic vena cava. **a** Application and tying of traction sutures at the end of the donor operation. **b** Application of corner sutures. **c** Suture of posterior wall from inside. **d** Interlocking stitch at start of suturing of anterior wall. **e** Flushing of lumen of the anastomosis. **f** Completed anastomosis

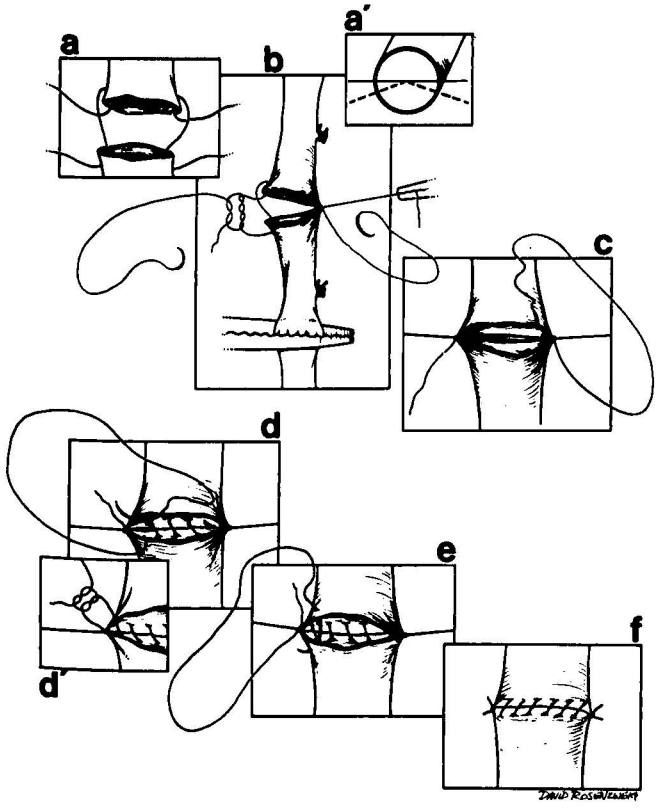


**Fig. 3 a–f.** Anastomosis of the portal vein. **a, b** Application of corner sutures. **c, d** Suture of posterior wall from inside. **e** Suture of anterior wall. **f** Completed anastomosis

portal vein are placed at about 120° and thus the anterior wall is flaccid, while the posterior one is stretched. It is advisable to place the corner stitches on both stumps from the inside toward the outside. Due to an indistinct view of the collapsed venous tissue, this procedure not only proves to be time-saving, but also makes it possible to locate the opposite corners with greater accurary, without affecting adversely the quality of the anastomosis. The ligated junction of the gastroduodenal vein which is located on the left side of both recipient and donor vessels serves as an orientation and helps to avoid any twisting. Three to four stitches are performed on the posterior wall from the inside with the thread of one of the corner sutures (Fig. 4); this suture is then brought to the outside and tied to the short end of the second corner suture, and then continues to be used for suturing the anterior wall (Fig. 4). Immediately after the completion of the anastomosis of the portal vein, the anhepatic phase ends with removal of the clamps from the portal vein and suprahepatic vena cava.

*Anastomosis of the infrahepatic vena cava (running suture with 8–0).* The infrahepatic vena cava is sutured, after the two clamps have been placed in parallel, with a technique analogous to the anastomosis described for the portal vein (Fig. 5). However, in contrast to the portal anastomosis the corner stitches should be applied in the conventional way; i.e., the end of the vein on the transplant should be sutured from the outside to the inside, and the recipient end, from the inside to the outside.

*Anastomosis of the infrarenal aorta of the recipient and of the arterial pedicle of the transplant (side-to-end, running suture with 8–0).* Following isolation, clamping and longitudinal incision of the aortal segment lying directly caudal to the ventrally crossing left renal vein, a side-to-end anastomosis with the arterial pedicle of the transplant is performed. For the aortotomy, application of corner sutures, and suturing of the left lateral wall, it is required to place the dissection board sideways, with the head of the rat near the right hand of the operator; for suturing of the right lateral wall, it is turned in the opposite direction (with the head of the rat on the left).

*Choledocho-choledochostomy (with use of a splint, two single button sutures with 8–0, Fig. 1 c–e).* After the ligature on the bile duct of the recipient has been cut, a traction suture on the left side of both stumps is applied to approximate them. While introducing the free end of the splint into the recipient's duct, simultaneously applied tension to the left corner suture prevents the separation of the stumps, so that the right corner suture can be placed and tied. By tying the left corner suture the choledocho-choledochostomy is completed. The recipient operation is concluded with flushing the abdominal cavity and closing the abdominal wall.

## Results

With increasing practice, our most recent perioperative mortality was 12,9% (this figure applied to the most recent 186 transplantations). With mastery of this method of rat liver transplantation, the average duration of the an-

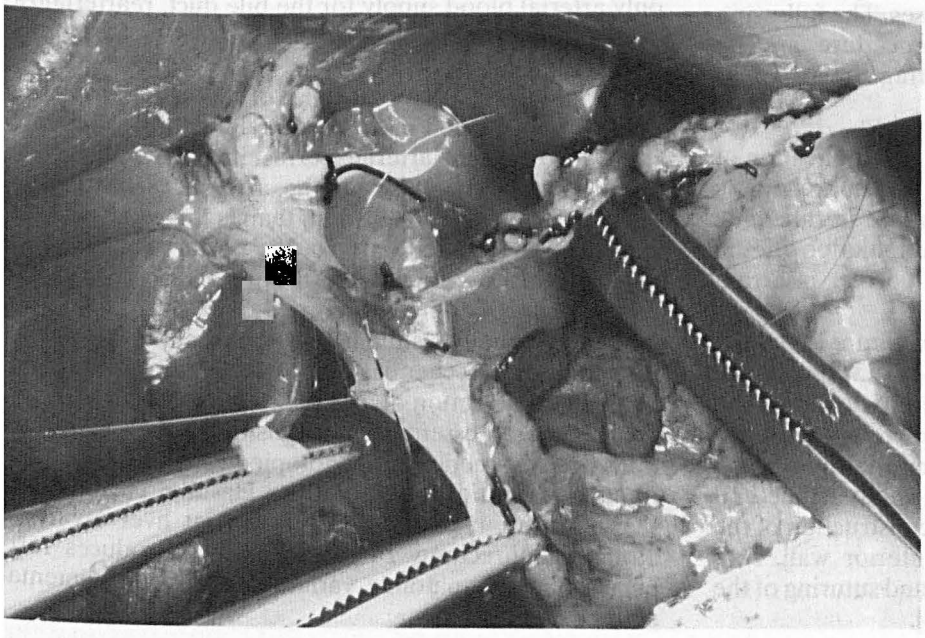

**Fig. 4.** Anastomosis of the portal vein. Completed suture of posterior wall, first stitch on the anterior wall. On the *right* in the figure, arterial pedicle, which includes the abdominal segment of the donor aorta, for arterial reattachment of the transplant

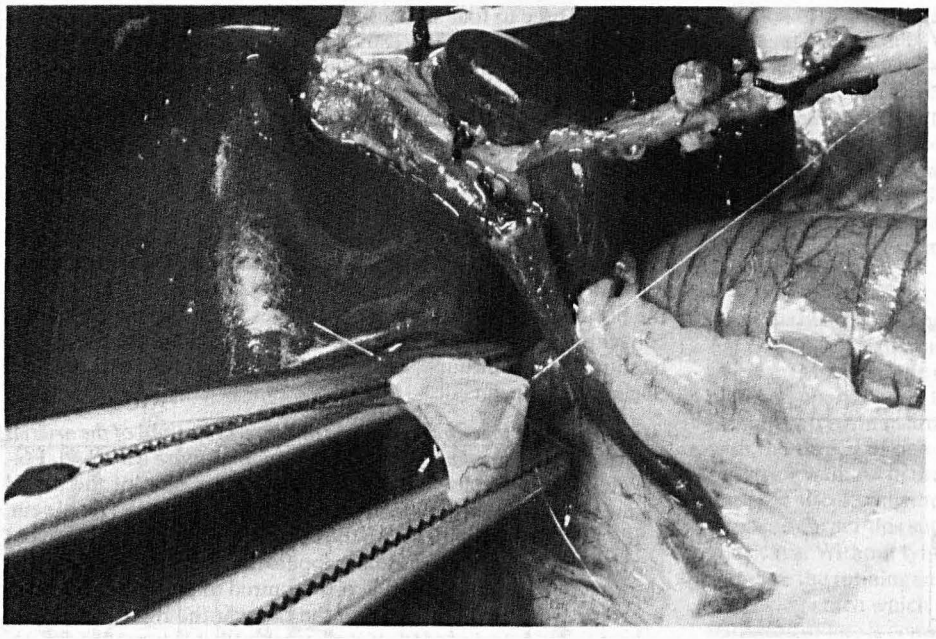

**Fig. 5.** Anastomosis of the infrahepatic vena cava. On the *right* in the figure, reperfused transplant with completed anastomosis of portal vein

hepatic phase is about 20 min, and thus substantially below the 30-min limit which is critical for the survival of the experimental animals [2, 5]. The donor operation requires about 60 min, and the recipient operation, between 70 and 80 min. Since rearterialization of the liver is not essential for survival of the animals, the technique described here can be applied alternatively without restoring the arterial blood supply; in this case particularly the donor operation is shortened considerably.

## Discussion

This technique of orthotopic rat liver transplantation represents a further development of the technique described by Lee et al. [7, 8] and is based on the principle of microsurgical suturing for vascular anastomoses. The following technical details, which simplify the procedure, are the basis for a short anhepatic phase during the recipient operation:

1. The donor operation is finished with the application of traction sutures for anastomosis of the suprahepatic vena cava. By keeping the clamp on the infrahepatic vena cava in place ex-situ, the manipulation of the transplant during the anatomically correct insertion into the recipient site, is simplified.
2. In all vessel anastomoses, the circular suture is applied with only one of the corner sutures. For all venous anastomoses, the posterior wall is sewn from the inside; the corner suture used does not need to be brought from the outside to the inside in addition when the running suture is begun. An apparently more cumbersome and time-consuming alternative is the reconstruction of the portal vein with suturing of the anterior wall, subsequent rotation of the anastomosis, and suturing of the posterior wall from the outside [1, 12].
3. The corner sutures in the anastomosis of the portal vein are sewn from the inside to the outside on both ends, contrary to the conventional procedure. Taking the collapse of the venous tissue into consideration, which interferes with visibility, this procedure not only saves time, but also makes it possible to locate the corners more precisely without affecting adversely the quality of the anastomosis.

Choledocho-choledochostomy with the aid of a splint was originated by Zimmermann et al. [12]. The traction-suture technique is a useful addition, because it prevents the recipient bile duct from sliding back from the splint.

Postoperative morbidity and mortality after liver transplantation in the rat, to the extent that they are caused by the operative procedure, are due mainly to complications in the bile duct; since the hepatic artery represents the only arterial blood supply for the bile duct, rearterialization of the transplant lowers considerably the rate of bile duct complications [2–4].

Furthermore, the conditions of clinical transplantation are reproduced more accurately with the combination of portal and arterial perfusion in situ during the donor operation, and with the arterial reconstruction during the recipient operation, than is the case when the transplant is supplied only via the portal vein. Elimination of rejection phenomena by means of a syngeneic donor-recipient combination renders rat transplantation models especially valuable for experimental investigations on organ ischemia, reperfusion and preservation. However, restoration of a physiological blood supply appears to be an essential prerequisite for such investigations on a rat model for orthotopic liver transplantation. A cuff anastomosis proposed by Hasuike et al. [3] for rearterialization of the transplanted rat liver involving a right nephrectomy of the recipient introduces further stress and anatomic variation in the experimental animals.

By providing a detailed description of simplified microvascular suture techniques, an alternative to the almost exclusively used cuff anastomoses is presented. Although the cuff methods can be learned somewhat more quickly, the disadvantages outweigh the advantages from our point of view. Cuff anastomoses differ from the clinical procedure, require relatively long vessel stumps, can be used only for end-to-end attachments, and necessitate additional ex-situ preparation of the vessel ends of the donor organ. It would appear that the cuff technique [10, 11] is more demanding and more subject to complications than is the suture technique especially for anastomosis of the suprahepatic vena cava.

In conclusion, physiologic conditions for the graft are restored extensively with the technique described. Furthermore, high survival rates can be attained. We consider this model to be appropriate for most studies involving liver grafting in the rat.

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