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INTRODUCTION

Although Wooten first described ligation of the deep dorsal vein for atonic impotence in 1902, \(^1\) penile vein operations remained unpopular until 1985. \(^2,3\) The approach was expanded from initial procedures involving single vessel ligation of the deep dorsal vein to more-elaborate techniques in which excision of the deep dorsal vein, cavernous vein, as well as the crural vein was described. \(^4-7\) The general consensus from these initial procedures was early success with few long-lasting cures. However, the outcomes we have achieved with venous stripping for impotence in 2241 patients over 16 years are gratifying. Many people have questioned us about the absolute, positive outcome of this surgery, since the clinical guidelines panel of the American Urological Association declared that venous and arterial surgeries are not justified in routine use, following a meta-analysis of literature reports which found that the procedure generally resulted in poor performance; venous surgery was subsequently discouraged in 1996. \(^8\) The venous surgery is very rarely indicated and should be performed only in highly selected patients because its high relapse rate. On the contrary, we are recommending our type of surgery which we developed in 1986, \(^5,9\) after repeated cadaveric studies and microsurgical training on rats. The incision is small and delicate. Neither a Bovie nor a suction apparatus is required during the entire operation. Our training of surgeons and assistants is second to none. Contrary to the deemed "high relapse rate," only a few of the 2241 patients, on whom the operation was performed, complained of recurrence. From June 1986 to May 1987, 23 of 31 patients who underwent venous stripping were available for follow-up. Today, 12 of them still enjoy a normal sexual life, although the operation was still based on the traditional illustrations. In the latter part of 1999, a new insight concerning erection-related veins was regarded as the blueprint for our venous surgery. \(^10,11\) Since a greater number of veins had to be removed, ligation sites vary from 76 to 125 ligatures. Following institution of the above procedure, our success rate improved from 75% to over 90%, which is effective in most patients with erectile dysfunction. We remain very excited about this operation. While the procedure bears the same name as the other procedures, it is an entirely different method. \(^12\) Some may say that our claim is too good to be true. It is, nevertheless, absolutely true. We urge any and all specialists, from any part of the world, to be present at and to witness the operations we perform, to examine the clinical results, and to determine for themselves the validity of our claims.

One reason for our success is that each surgeon must be deemed to have the prerequisite technical skills with microsurgical drills on rats before he/she initiates this operation on human patients. For practicing microsurgical techniques, fresh leaves, surgical gloves, and then live
rats, are good objects.\textsuperscript{13-18} Testicular autotransplantation in a rat is the most difficult phase, because of its anatomical complexity and the tiny size of the tissue. It is, however, an excellent organ for drill of acquiring surgeons' patience, perseverance as well as for tissue-layer recognition, moreover it is very helpful when performing surgery on patients, since the surgeon can enhance image his/her visual acuity to a 40-times magnification when using their naked eye.

This manual is mainly designed for surgeons who are interested in the field of vascular surgery for potency reconstruction. Microsurgery is a prerequisite in this field. It requires discipline, patience, and perseverance. But acquiring the necessary skills can bring great rewards and satisfaction to your career. This manual describes fundamental techniques from fresh leaf repair to advanced autologous testicular transplantation. The reader must master the basic steps before he/she can proceed to the next one. Frustration is common in beginners, yet it may be comforting to realize that most of the vessels in experimental animals are much more difficult to handle than those you will encounter in clinical situations because of their small anatomical size. Fortunately, you can practice and learn more because you are operating on an animal. Step by step, this booklet introduces you to the fascinating world of microsurgery.

Our manual describes the operation in simple terms. We are grateful to all of those who have assisted in creating this handbook. We would like to thank Drs. SS Wen, LJ Liu, YC Chen, and CW Chen for their technical support and for reviewing the text, Mss. TJ Kang, SY Hsu, and T Wang for their clerical assistance, and help with the photos and illustrations in the completion of this work. We are deeply indebted to Dan Chamberlin for correction of the English in the text.
CHAPTER 1
Microsurgery Skill Progression

If you are an urologist, a vascular surgeon, or a plastic surgeon with an interest in achieving a good command of microsurgical techniques, and are interested in male potency reconstruction, you are welcome to take a meticulous look at and carefully examine these operations: outpatient surgery for curvature correction of the penis, outpatient surgery for penile venous stripping, outpatient surgery for penile implantation, outpatient surgery for a varicocelectomy, outpatient surgery for penile enhancement, and outpatient surgery for penile enhancement in implant patients. All of these are performed in pure local anesthesia with neither a Bovie nor a suction apparatus being allowed. We offer a well-equipped laboratory, staffed with professionals whose technical skills far surpass the prerequisites for microsurgical drills. Meanwhile you are always welcome to observe any of the above operations clinically.

When practicing microsurgical techniques, fresh leaves, surgical gloves, and then live rats are good objects on which to practice the progressively more-difficult techniques for drilling a vasovasostomy, ureteroureterostomy, high ligation of the internal spermatic vein, nerve repair, venous patch of the rat penis, autotransplantation of a penis, autotransplantation of a kidney, and autotransplantation of a testicle. Among these, testicular autotransplantation in the rat is the most difficult phase, because of the anatomical complexity and the tiny size of the tissue. It is, however, very helpful when performing surgery on a patient, since the surgeon can image his/her visual acuity to a 25-fold magnification when using their naked eyes.

Advancing stepwise through the following procedures is mandatory for becoming an accomplished surgeon. The rat is an excellent model which allows any operator to wean him/herself from depending on a Bovie apparatus during surgery. It is second to none for obtaining indispensable skills with which to manage any bleeder using only ligation in which the animal surgery rather than any clinical operation is an ideal object in acquiring this technique. In deed, this can be exclusively caught up without any assistant.
Microsurgical Skill Progression

Familiarity with the microscope and microinstruments

\[\downarrow\]

Laboratory microsurgical instruments

\[\downarrow\]

Notes on focusing the microscope

\[\downarrow\]

Tying practice with a fresh leaf on a knot board

\[\downarrow\]

Tying practice with a surgical glove on a knot board

\[\downarrow\]

Preparation of rats

\[\downarrow\]

Vasovasostomy of rats

\[\downarrow\]

Ureteroureterostomy of rats

\[\downarrow\]

High ligation of the internal spermatic vein of the rat

\[\downarrow\]

Epineural nerve repair

\[\downarrow\]

Fascicular nerve repair

\[\downarrow\]

End-to-end anastomosis of femoral vessels

(<1 mm in diameter)

\[\downarrow\]

End-to-side anastomosis

\[\downarrow\]

Anatomical dissection of the rat penis

\[\downarrow\]

Autotransplantation of the rat kidney

\[\downarrow\]

Venous patch of the rat penis

\[\downarrow\]

Autotransplantation of the rat penis

\[\downarrow\]

Autotransplantation of the rat testicle
CHAPTER 2
Laboratory Microsurgical Instruments

Microsurgical instruments are generally not clinically suitable for potency reconstruction because of their sharpness; however, they are essential for animal experimentation. This implies that a surgeon is ready for clinical surgery once he/she masters these experimental exercises. Laboratory microsurgical instruments should include the following (when specific experimental procedures necessitate additional instruments, they should be added):

For general purposes
- A dozen shavers
- Several rolls of scotch tape
- A wooden board
- A bottle of normal saline
- Clean surgical gloves
- Several 4-0 or 5-0 silk sutures
- Vascular tape of green, yellow, and red colors
- A facemask if individually required for overcoming bad smells

For gross dissection
- Scalpels for cutting skin
- Small dissecting scissors
- Two baby mosquito hemostats
- At least 2 Adison forceps and one pair of ring-handled scissors
- Self-retaining skin retractor (for retraction with skin suturing)

General microsurgical instruments (Fig. 1):
- Double microclips of the Kleinert-Kutz type
- One microclip applicator
- Two sets of tying forceps
- Two sets of jeweler's forceps
- One needle holder of the Barraquer type
- One set of spring-loaded microscissors
- One lacrimal dilator of the hockey stick type made by Weck
- Two dozen of Weck-Cels (these are small sponges)
- A pack of 2 x 2 cm² sponges
- A box of Q-tips
Small stainless steel bowls
Normal saline for irrigation
Heparin when needed for the irrigation solution
A 2-cc syringe with a blunt-point needle for irrigation
A 1% Xylocaine solution for relief of vasospasms

Fig. 1.
Minimal requirements for experimental microsurgery.

Fig. 2.
Needle nomenclature.
The needle commensurate with its suture is so tiny that it is invisible if it is out of the field of view of the microscope. One has to be familiar with the nomenclature for the suture material, although the terminology differs among individual companies. Listed on the next page are each company's most commonly used microsurgical needles.

<table>
<thead>
<tr>
<th>Examples</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Davis and Geck</strong></td>
<td></td>
</tr>
<tr>
<td>TE-100</td>
<td>T tapering needle</td>
</tr>
<tr>
<td>TS-100</td>
<td>E 3/8 circle curvature</td>
</tr>
<tr>
<td>T-100</td>
<td>100 diameter of needle in microns</td>
</tr>
<tr>
<td></td>
<td>S straight needle</td>
</tr>
<tr>
<td></td>
<td>T (alone) tapered needle with 1/2 circle curvature</td>
</tr>
<tr>
<td><strong>Ethicon</strong></td>
<td></td>
</tr>
<tr>
<td>BV 75-3</td>
<td>BV blood vessel</td>
</tr>
<tr>
<td></td>
<td>75 diameter of the needle in microns</td>
</tr>
<tr>
<td></td>
<td>H chord length in millimeters</td>
</tr>
<tr>
<td></td>
<td>ST half-circle curvature (always 3/8 circle with no H)</td>
</tr>
<tr>
<td>BVH 100-3</td>
<td></td>
</tr>
<tr>
<td>ST 75-4</td>
<td>4 straight needle</td>
</tr>
<tr>
<td></td>
<td>4 needle length in millimeters (on a straight needle,</td>
</tr>
<tr>
<td></td>
<td>chord length equals needle length)</td>
</tr>
<tr>
<td><strong>S &amp; T</strong></td>
<td></td>
</tr>
<tr>
<td>7V43</td>
<td>7 70-micron needle diameter</td>
</tr>
<tr>
<td></td>
<td>4 vascular</td>
</tr>
<tr>
<td></td>
<td>3 chord length in millimeters</td>
</tr>
<tr>
<td>7VST</td>
<td>3 3/8-circle curvature</td>
</tr>
<tr>
<td>10V34</td>
<td>4 straight needle</td>
</tr>
<tr>
<td></td>
<td>4 chord length in millimeters</td>
</tr>
<tr>
<td></td>
<td>4/8 (1/2)-circle curvature</td>
</tr>
<tr>
<td><strong>Xomed</strong></td>
<td></td>
</tr>
<tr>
<td>MET, 70u, 105°, 4 mm, 3.5 mm</td>
<td>microedge taper</td>
</tr>
<tr>
<td></td>
<td>70u needle diameter in microns</td>
</tr>
<tr>
<td></td>
<td>105°C degrees of arc in needle</td>
</tr>
<tr>
<td></td>
<td>4 mm needle length</td>
</tr>
<tr>
<td></td>
<td>3.5 mm chord length</td>
</tr>
</tbody>
</table>

All manufacturers use U.S.P. (United States Pharmacopoeia) designations for suture diameter and some include metric gauge numbers.

<table>
<thead>
<tr>
<th>U.S.P.</th>
<th>Diameter range (mm)</th>
<th>Metric</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>9-0</td>
<td>0.030</td>
<td>0.039</td>
</tr>
<tr>
<td>10-0</td>
<td>0.020</td>
<td>0.029</td>
</tr>
<tr>
<td>11-0*</td>
<td>0.010</td>
<td>0.019</td>
</tr>
</tbody>
</table>

*Unofficial designation - U.S.P. has no size below 10-0.
CHAPTER 3
Notes on Focusing the Microscope

A microscope is a powerful tool for magnifying one's visual acuity. It enables you to discriminate tiny tissues because small objects appear huge and small areas seem as large as a basketball court. Therefore it is an indispensable tool for performing elaborate surgery on any small tissue. As the focal distance varies with the magnification power, each surgeon has to accustom him/herself to manipulating tissue and performing surgery through a microscope rather than using their own naked eye. The microscope (Fig. 3) is basically similar to the one used in medical student days, however its adjustment is more elaborate. One has to be aware that good command of these techniques is a prerequisite for mastering this experimental surgery. When adjusting the microscope, it is practical to use the surface of one's finger or the uneven surface of a surgical glove.

**OPERATION MICROSCOPE**

1. Microscopic Control Handle
2. Second Arm Balance Control Knob
3. Main Illumination Unit
4. Main Illumination Select Switch
5. Back Panel
   (Main Switch, Intensity Control Knobs)
6. Microscope Head Rotation Control Knob
7. 12.5 x Eyepiece
8. Inclination Control Knob
9. Magnification Control Drum
10. Objective Lens
11. Second Arm Rotation Control Knob
12. Up/Down Foot Switch
13. First Arm Rotation Control Knob

![Fig. 3. An operation microscope.](image-url)
CHAPTER 4
Tying Practice Using a Fresh Leaf on a Knot Board

This knot board consists of a piece of fresh green leaf (Fig. 4) of a broadleaf species fastened with white tape. A 2.5-cm incision is made on the leaf for repairing practice in passing the needle and in tying knots. As the texture of the fresh leaf is susceptible to ripping, the exit will match the inlet if the pulling direction of the suture is proper, and this is harmless to the fragile leaf. The suture will cut through the leaf surface if it is over-tightened, and the knot appears sloppy if it is under-tightened. This is an ideal material for surgeons to assess their adequacy with tying knots as well as the direction for pulling sutures.

Fig. 4. Photo of a fresh leaf on a knot board. A) Three stitches were made (Reduction from x 10). B) The magnification was adjusted to 16-times.

There are two ways to tie micro-square knots that have useful clinical applications. Method I (Fig. 5): After a suture is made (A) and positioned (B) properly. The long tail with sufficient length is caught using the needle holder (C), it is then wrapped twice around the pick-up
forceps in which the short tail is held. (D). The first knot is finished when these two tails exchange their positions and are fastened. Thus the first tie is made by pulling the short tail through the double loops of the long tail. Note that this should be gently tightened with appropriate tension (E). Immediately after the pick-up forceps release the short tail, it is wrapped again once more (F) using the needle holder which holds the long tail through the entire procedure (tying). Pull the short tail through the loop of the long tail to make the second knot (first tie) while the first tie is firmly done (G). i.e. The second knot is made when the two tails exchange positions and are fastened again. A third knot (H) is performed in the same manner to complete the tying procedure. Method II: This method is the same as described for method I except that the roles of the needle holder and the pick-up/forceps are switched. These are ideal methods to create a firmly fastened square knot. In making the first knot, use two loops if the suture material is monofilament, but one loop is sufficient for a polyfilament suture such as chromic catgut. Clinically we recommend each surgeon to use finger's palm to hold the long tail and a needle holder to catch the short tail in making each knot.

Fig. 5. A) The needle is introduced into the cleft of the fresh leaf. B) The suture together with its needle is positioned properly. C) The long tail is caught by the needle holder with sufficient length in order to wrap it around the pick-up/forceps (D) which subsequently grasp the short tail.
Fig. 5. E) The first knot is made by pulling the short tail through the two loops of the long tail. Thus the first knot is finished when these two tails exchange their positions and are fastened properly. F) Immediately after the pick-up forceps release the short tail, it is wrapped once again by the needle holder, which holds the long tail through the entire procedure. G) The second knot is made by pulling the short tail through the loop of the long tail. Thus this first tie is made when these two tails exchange their positions and are fastened again. During tying, the needle holder holds the long tail all the time, while the pick-up forceps release the short tail immediately before each knot is started. H) A third knot is performed.

CHAPTER 5

Tying Practice Using a Surgical Glove on a Knot Board

The knot board consists of a rectangular piece of surgical glove fastened with white tape over a blue background, both of which are affixed to a flat cardboard base. An incision 2-3 cm long is made in the glove material for practice in passing the needle and in tying knots. With the elastic nature of the glove, the focus of the microscope will be altered if a pulling force is applied. This is very helpful for experimenting with differences of pulling a suture at various angles and velocities. It is an ideal material to practice drilling before a surgeon advances to experimentation with live rats, since the viscosity of the blood can hamper the smooth motion of the suture.
CHAPTER 6
Preparation of Rats

A male rat weighing 250 to 300 g should be handled with a glove that is sufficiently thick in order to prevent the handler from being bitten by the rat. Then the animal is anesthetized with intraperitoneal sodium pentobarbital (5.0 mg/100 g body weight). Once anesthetized, it is placed on a dissecting board in the usual fashion such that its extremities as well as its tail are fastened firmly, but without over-tightening to prevent any ischemic change (Fig. 6). A booster anesthesia is given whenever necessary. Because of the hair of the rat, the proper region should be shaved before it is opened. Since the animal has high immunity to bacterial infection, it is not necessary to apply any antibiotics unless for special purposes.

Fig. 6. An anesthetized rat on a dissecting board. A) illustration. B) photo, an endotracheal tube may be recommended, in our experience however it is not necessary.
CHAPTER 7
Vasovasostomy of the Rat

Once a rat is prepared as discussed in chapter 6, the scrotal region is shaved. A longitudinal scrotal incision is made, which is then deepened with each layer being tagged with 4-0 silk sutures. The diameter of the vas deferens is as large as 3-5 mm, and it is susceptible to bleeding unless its blood vessels are traumatized; Operating with one's naked eye is allowed until the actual vasovasostomy is performed. At least a 0.5-cm segment of the vas deferens is skeletonized from its accompanying vessels and nerves. A microanastomosis is meticulously made after it is transected with a pair of microscissors. A spatula of the vas stump in which a longitudinal cut of 2 mm is made is helpful in preventing the anastomotic site from experiencing stricture after the operation. Using an 8-0 ethicon suture, the needle is placed the same distance from the cut end of the vas deferens as the thickness of the deferential wall (Fig. 7). Blunt microforceps are inserted in the lumen for the initial pass of the needle because it protects the back wall, while providing counterpressure on the anterior wall, which facilitates placing the exit bite. The first two tag sutures are placed at the 3 and 9 o'clock positions followed by two intermediate sutures which are placed in-between while microforceps are inserted in the lumen to receive the needle and to protect the back wall. These two tag sutures are then exchanged. Likewise, the two intermediate sutures are performed. During the suturing procedure, counterpressure from the forceps is very helpful. Over-tightening of the suture can cut through the deferential wall because of its fragility. Another method allows the lumen to be preset with a small catheter at the beginning of suturing. In practice, the vas deferens can receive several anastomoses at different positions. Sperms of the rats, which are thinner and slenderer than that of human's can be observed through a cover glass if semen is collected from the vas deferens.

Once the vasovasostomy is completed, the abdominal wall is closed layer by layer. The rat is then used for examination of the surgical result in another step of the experiment.
Fig. 7. Illustration of vaso-vasostomy: A) The vas deference is transected. B) Using scissors it is carefully spatulated on a cut end. C) Similarly the spatulation is performed on the other end in order to widen the anastomotic area. D) The first suture is made with 10-0 ethicon suture. E) The fashion is virtually finished.
CHAPTER 8
Ureteroureterostomy of the Rat

After a rat is prepared as described in chapter 6 and the abdominal region is shaved, a medial laparotomy is made, which is deepened until the ureter is encountered. An impressive peristalsis which mimics the gentle swimming motion of an eel can clearly be seen if the surgeon is patient enough to observe for 5 min. The ureter is transected obliquely after it is freed from the surrounding tissues. The anastomosis is made in the fashion as described in chapter 7, but it may be completed in two to three layers. It is permissible to perform several ureteroureterostomies on one ureter without the necessity of an internal stent if the technique is perfectly performed. One week after the operation, the anastomosis site should be difficult to find.

Fig. 8. An illustration of ureteroureterostomy. A) An impressive peristalsis which mimics the gentle swimming motion of an eel can clearly be seen if the surgeon is patient enough to observe for 5 min. After careful dissection and free from surrounding tissue, the ureter is transected. B) A spatulation might be meticulously made if the surgeon intends, the first suture is being performed. One-layer or two-layer suture is permissible. C) Subsequently the fashion is made. Great care shall be taken not to over-tighten them to prevent from being cut-through.
CHAPTER 9
High Ligation of the Internal Spermatic Vein of the Rat (Fig. 9)

After a rat is prepared as described in chapter 6 and the abdominal region is shaved, a low medial laparotomy is made, which is then deepened, and each layer is tagged to facilitate the repair. The spermatic cord is dissected extraperitoneally and is tagged with atraumatic vascular tape above the internal ring. The components of the cord can be well studied. The transparent lymphatic vessel can readily be identified if the pampiniform plexus of the ipsilateral testicle is squeezed. Impressive pulsation of the testicular artery can be seen. The vein which is blue and engorged in response to the squeezing action can be discriminated from the pulsatile and pinkish artery. There is one pair of vessels accompanying the vas deferens. They are the deferential artery and vein, and it is best if they are preserved. The internal spermatic veins are ligated and its abdominal wall is repaired layer by layer.

Fig. 9. Ligation of internal spermatic vein: A) Isolate right spermatic cord (x16). The spermatic cord is dissected extraperitoneally and is tagged with atraumatic vascular tape if available above the internal ring. The components of the cord can be well studied. B) Ligate the internal spermatic veins (x16). The transparent lymphatic vessel can readily be identified if the pampiniform plexus of the ipsilateral testicle is squeezed. Impressive pulsation of the testicular artery can be seen. The vein which is blue and engorged in response to the squeezing action can be discriminated from the pulsatile and pinkish artery.
Chapter 10
Epineural Nerve Repair

After a rat is prepared and the femoral region is shaved, a transverse incision is made on the ventral surface of the thigh exposing the femoral vessels and femoral nerve.

Before the transection is performed, surface features of the epineurial texture should be carefully studied to properly match and align the nerve stumps. During suturing, the first bite (Fig. 10A) is taken 1 mm from the cut surface and is immediately leveled off to prevent fascicular damage.

The needle is inserted into the opposite stump (Fig. 10B) just beneath the epineurium. The needle should remain parallel to the long axis of the nerve until the exit bite is taken 1 mm from the cut surface. Sutures are tied loosely for nerve repair, functioning only to maintain alignment and prevent rotation. Tight sutures create fascicular buckling and lateral bulging.

The second suture is made 120° from the first one on the circumference of the epineurium (Fig. 10C). The nerve should be flipped over, and the third suture inserted (Fig.10D).

It is important to note that in epineural nerve repair, three sutures are used on a 1-mm nerve to maintain its orientation while minimizing scarring.

Fig. 10. Epineural nerve repair. A) The initial bite is taken 1 mm from the cut surface and leveled off [like an aircraft taking off / very gradually] to prevent fascicular damage. B) The needle is inserted into the opposite stump just beneath the epineurium. The needle should remain parallel to the long axis of the nerve until the exit bite is taken 1 mm from the cut surface. C) The second suture is made 120° from the first one on the circumference of the epineurium. D) The nerve should be flipped over and the third suture inserted.
CHAPTER 11
Fascicular Nerve Repair

After a rat is prepared and the femoral region is shaved, a transverse incision is made on the ventral surface of the thigh exposing the femoral vessels and femoral nerve.

The cut surface of the severed fascicles should be carefully studied and the fascicles matched up after a short segment of the epineurium is removed (Fig. 11A). A 50- or 70-μ needle is essential for fascicular repair.

For the entrance bite (Fig. 11B), the needle is inserted 0.5 mm from the cut surface at a 45° angle to the perineurium. As soon as the perineurium is penetrated, the needle should be leveled off parallel to the long axis of the fascicle, which minimizes the possibility of intraneural damage. The exit bite is subsequently taken 0.5 mm from the cut edge using similar precautions.

Sutures are preferably tied loosely (Fig. 11C) rather than tightly in order to prevent scarring. Only one suture is needed for fascicles 1 mm in diameter or smaller. One or more additional sutures (Fig. 11D) may sometimes be required for larger central fascicle.

Fig. 11. Fascicular nerve repair. A) For fascicular repair, a short segment of the epineurium is removed. B) The needle is inserted 0.5 mm from the cut surface at a 45° angle to the perineurium. As soon as the perineurium is penetrated, the needle should be leveled off parallel to the long axis of the fascicle, which minimizes the possibility of intraneural damage. The exit bite is subsequently taken 0.5 mm from the cut edge using similar precautions. C) Sutures are preferably tied loosely rather than tightly. D) Only one suture is needed for fascicles 1 mm or smaller in diameter. One or more additional sutures may sometimes be necessary.
CHAPTER 12

End-to-end Anastomosis of Femoral Vessels (Fig. 12-I & Fig. 12-II)

After a rat is prepared and the femoral region is shaved, a transverse incision is made on the ventral surface of the thigh to expose the femoral vessels. The artery, vein, and nerve should be separated from the surrounding muscular bed and connective tissue via blunt dissection. The deep muscular branches of the femoral artery and vein should be ligated to allow better mobilization. Once adequate length is exposed and mobilized, a suitable background material (blue, red, yellow, or green) should be placed underneath the vessel to be anastomosed.

A standard microvascular double clamp is expanded to its maximum spacing and atraumatically applied to the vessel. A quick, sharp transection is performed midway between the two clamp jaws at 90° to the axis of the vessel. The deep muscular branch may be excised if desired. The two cut ends are irrigated to remove any blood using heparinized saline delivered with a blunt-pointed needle or a small cannula attached to a syringe.

To prevent interposition of adventitia in the anastomosis, the adventitia is slightly stretched and excised with scissors before it is fashioned. The thickest layer of the vein is the adventitia, which cannot be stripped away from the vessel as it can from the artery. Not surprisingly, preparation of the vein requires a gentler technique for tearing away the excessive adventitia and trimming (Fig. 12-I A) it with microscissors.

The lumen is then possibly dilated with jeweler's forceps or a lacrimal dilator. Next, the distance between the clamp jaws is adjusted to approximate the ends without applying undue tension.

During the anastomosis, the first two guided sutures are placed at the 10 and 2 o'clock positions (Fig. 12-I B), 120° apart on the circumference of the vessel end. This allows direct visualization and easy placement of both sutures. Traction on these sutures can be easily applied with a suture tying frame to avoid inconvenience of using an assistant. One or two intermediate sutures are completed (Fig. 12-I C). The clamp approximator is turned over, and the third suture is placed such that it bisects the angle defined by the first two sutures (Fig. 12-I D). The posterior wall suture bisects the angle between the first two sutures. Intermediate sutures five and six are placed using the same biangulation technique (Fig. 12-I E). With increasing diameter, an additional number of intermediate sutures will need to be placed in between the three guided sutures.
The needle is placed in the same distance from the cut end of the artery as the thickness of the arterial wall during the arterial anastomosis, while the distance from the end of the vessel to the entrance of the needle should be more than twice the wall thickness in the venous anastomosis.

Blunt microforceps (tying or jeweler's) are inserted in the lumen for the initial pass of the needle. The instrument protects the back wall while providing counterpressure on the anterior wall. This facilitates placement of the exit bite.

Following completion of the guide sutures, intermediate sutures are placed with the aid of microforceps which are inserted in the lumen to receive the needle and to protect the back wall.

A second method is particularly useful for placing intermediate sutures in thin-walled veins. This requires putting traction on the guide sutures while simultaneously dilating the lumen with saline, thereby separating the anterior and posterior walls.

To minimize the operative time, an experienced microsurgeon can place intermediate sutures by penetrating both vessel ends in one pass, which is a good indicator for self-assessing whether one has mastered the technique.

Sometimes if stay sutures are inserted under tension at the anastomosis, surgeon's knots (double loop) should be used as the initial two sutures. Three square knots are sufficient for each suture placement. Over-tightening is as bad as under-tightening of the sutures, because it causes overlapping of the endothelium and produces an uneven inner surface.

When tying knots, a suture circle should be visible indicating coaptation and not bunching of the intima.

Suture ends should be cut 0.2 to 0.3 mm in length since nylon sutures hold knots poorly. Usually one tail is left longer to serve as a "handle" in providing traction for placing subsequent ones. Six stitches are sufficient to fashion the vessel, but more sutures can be added if necessary.
Fig. 12-I. Illustration of an end-to-end anastomosis: A) A little segment of the adventitia of the vessel end is trimmed off. B) Using a 10-0 ethicon suture, two guided sutures are placed 120° apart on the circumference of the vessel end. C) One or two intermediate sutures are completed. D) Subsequently, the clamp approximator is turned over, and the third suture is placed such that it bisects the angle defined by the first two sutures. E) Similarly one or two intermediate sutures may be used in each 120° sector.
Fig. 12-II. Photo of an end-to-end anastomosis: A) The right femoral vessels are prepared, and the vein is sufficiently freed (x10). B) The vein is managed. Note the anatomical relationship of the femoral vessels to their tributary ones (x10). C) A double microclips is applied (x10). D) The vein is transected using a microscissors (x10). E) The cut ends are identified and prepared (x10). F) The first suture is made and the first tie has been tying (x10). G) The second suture is placed 120° apart on the circumference of the vessel end (x10). H) Two intermediate sutures are completed (x16).
CHAPTER 13
End-to-side Anastomosis

Once a rat is prepared as in chapter 6 and its abdominal region is shaved, a low medial laparotomy is made, which is deepened, while each layer is tagged in order to facilitate the subsequent repair. The internal iliac vein and the common iliac vein are identified and adequately mobilized from the surrounding tissues. The internal iliac vein is ligated proximally close to the entrance to the common iliac vein; distally it is clamped with one microclip and then is transected perpendicular to its longitudinal axis. The common iliac vein is cross-clamped proximal and distal to the site of the anastomosis. Using microscissors, an elliptic venotomy (Fig. 13A) is performed which is a little larger than the size of the cross-section of the internal iliac vein. It is made at an angle of 30° to the venous wall of the common iliac vein (Fig. 13B). Two guided sutures are placed at the 12 and 6 o'clock positions (Fig. 13C), 180° from each other. Using 10-0 ethicon sutures, an intermediate suture (Fig.13D) is begun counterclockwise from the 12 o'clock position, maintaining the distance between the two guide sutures that were placed previously. The internal iliac vein is then rotated to the opposite side (Fig. 13E), and the intermediate sutures (Fig. 13F) are begun from the 12 o'clock position clockwise. It is essential that there is sufficient slack in the vessel to allow such mobility. In our experience, it is practical to begin the suture from the highest point because of the antigravity of blood, the blood will naturally drain by gravity.

Fig. 13. End-to-side anastomosis. A) Using microscissors, an elliptic venotomy is performed. B) The window is completed. C) Two guided sutures are placed at the 12 and 6 o'clock positions, 180° from each other. D) Using 10-0 ethicon sutures, an intermediate suture is begun counterclockwise from the 12 o'clock position, while maintaining the distance between the two guide sutures that were placed previously. E) The internal iliac vein is then rotated to the opposite side. F) The intermediate sutures are begun from the 12 o'clock position clockwise.
CHAPTER 14
Anatomical Dissection of the Rat Penis (Fig. 14)

After a rat is prepared as described in chapter 6, the region of the exogenitalia is well shaved. Dissection of the rat penis is begun by denuding the overlying skin which is peeled back until the pubic region is encountered. The neurovascular bundle can be observed. The deep dorsal vein should be identified and stripped. The bleeding stump is tied with 8-0 ethicon. A milking motion in which squeezing pressure is delivered by the assistant's hand is very helpful in enhancing the visibility of the vein. Hydropressure produced by several milliliters of normal saline injected in between the tunica albuginea and Buck's fascia is helpful in tissue dissection. The neurovascular bundle is tagged with vascular tape after it is freed from the tunica albuginea. During the procedure, the bony structure (baculum) of the penis provides adequate counterpressure that may be present in human cadavers. The outer longitudinal layer as well as the inner circular layer of the tunica albuginea is meticulously dissected. The corpus spongiosum is freed from the corpora cavernosa. Hydropressure followed by blunt dissection might be the only way of facilitating its separation. The corpora cavernosa is gently detached from the periosteum of the pubic arch.

Fig. 14. Rat penis. A) Gross anatomy of a sagittal section of rat penis. The os penis (baculum) positions between the glans penis and the corpora cavernosa. It looks like the lateral aspect of the knee joint. The penile cruri are not included. B) Detailed description of anatomical components: The cartilaginous part (black arrowhead) as well as the bony portion (white arrowhead) of baculum, urethra (black arrow), sinusoids of corpus cavernosum (white arrow) are arrayed. C) Cross section of proximal portion of rat penis: The dorsal nerves (white arrowheads), dorsal artery (white arrow), deep dorsal vein (black arrow) and urethra (black arrowhead) are indicated.
CHAPTER 15
Autotransplantation of the Rat Kidney (Fig. 15 & 16)

After a rat is prepared as described in chapter 6, the abdominal region is shaved. A medial laparotomy is performed, and it is deepened until the ureter is encountered; it is identified by observation of peristalsis. The perirenal tissue is preferably dissected out. The vascular pedicle is well freed and readied for removal. A traumatic vascular clamp is applied to the proximal stump of the renal artery. The renal vessels are transected one by one while the ureter is transected 1.5 cm distal to the ureteropelvic junction, with the distal stump being tagged with a 4-0 silk suture.

The renal vein is first re-anastomosed with 8-0 ethicon or proline. The renal artery is similarly treated. The renal capsule may be fixed to the abdominal cavity with 4-0 silk suture. The ureter is fashioned as described in chapter 6.

Fig. 15. Illustration of kidney transplantation. A) The kidney is implanted to its original position. B) The kidney is auto-transplanted to the femoral vessels.
Fig. 16. Renal transplantation: A) Left kidney was harvested (x10). B) Renal perfusion with Collin's solution via renal artery (x10). C) Spatulation of renal vein (x16). D) An arteriotomy (arrow) was made on the femoral artery (a battery of arrowheads) which served as the recipient artery (x10). E) End-to-side anastomosis (arrow) of renal artery to femoral artery (x16). Note the bright appearance of femoral vein (arrowheads). F) End-to-side anastomosis of renal vein to femoral vein (x25).
CHAPTER 16
Venous Patch of the Rat Penis

After a rat is prepared as described in chapter 6, the region of exogenitalia is well shaved. A circumferential incision is made, and the foreskin is degloved to the pubic region. The deep dorsal vein is stripped down to the infrapubic angle. The removed vein is immersed in saline solution for at least 30 min and is then detubularized. The neurovascular bundle and the corpus spongiosum are freed and well protected via application of the hydropressure technique. A circumferential incision is carefully made on the middle tunica preferably with a new, sharp scalpel. The incision should not be too deep, or else massive bleeding is unavoidable. The detubularized vein is sutured to the corporotomy defect with the serosal side facing outward using an 8-0 ethicon suture.

CHAPTER 17
Autotransplantation of the Rat Penis

After a rat is prepared as described in chapter 6, the region of the exogenitalia is well shaved. A circumferential incision is made and the foreskin is degloved to the pubic region. The penile shaft is transected at its mid-portion, while the corpus spongiosum associated with the bulbourethral vein, the dorsal arteries, and the deep dorsal vein are temporarily clamped. Anastomosis of the penis begun with the tunica albuginea by initiating the tag sutures at the 12 and 6 o’clock positions, and then spreading to the 9 and 3 o’clock positions, respectively. The deep dorsal vein is repaired, followed by repair of each dorsal artery and nerves. Subsequently the corpus spongiosum is anastomosed.

CHAPTER 18
Autotransplantation of the Rat Testicle

This surgery might be the most difficult phase in this manual.
After a rat is prepared as described in chapter 6 and the scrotal region is shaved, a longitudinal scrotal incision wound is made. The wound is deepened until the process vaginalis is opened. The tissue is tagged with 4-0 silk suture layer by layer. The spermatic cord is well freed. All vascular stumps are temporarily clamped with microclips on one side arbitrarily, whereas the opposite testicle is managed with an orchietomy. A proper anastomosis is performed on each corresponding tissue. During vascular anastomosis, 1-2 μg of PGE 1 is helpful in encouraging vessel dilatation.
REFERENCES