TRAINING WORKSHOP

Hands-on

RECONSTRUCTIVE MICROSURGERY

BASICS OF MICROVASCULAR SURGERY

Organized by:

Department of Plastic Surgery

Jinnah Burn & Reconstructive Surgery Center

Allama Iqbal Medical College

Lahore

CONTENTS

EQUIPMENT

The Operating Microscope

THE cost of operating microscopes is now so high that it is often difficult to decide whether to buy new and purchase the most basic equipment, or to look around for second-hand microscopes and buy a more sophisticated machine with the funds available. Non-motorised, hand-operated microscopes on a bench stand are adequate for learning, but if the laboratory expects in future to be involved in training large numbers of surgeons then it should try to reproduce clinical conditions as closely as possible and purchase a microscope with a double head (diploscope) and foot-operated controls for motorised zoom focus and magnification. A mobile stand adds convenience but must be rigid. Most important of all, the quality of optics and illumination must be excellent. The depth of focus and breadth of field should be adequate even at high magnification: 10 or 12.5 × eyepieces are most comfortable for long operations and a range of magnifications from 4x up to 25x is adequate for even the finest work. Some knowledge of the optical and illumination systems is valuable when selecting the most suitable microscope.

Optical system: First, consider the optical system consisting basically of objective lens, binocular tubes and eyepiece lenses. Objective lenses can be varied for focal length with important consequences for working distance and brightness of the image; those suitable for microsurgery are available in focal lengths ranging from 150mm to 400mm, but the standard is 200mm (marked f = 200mm). The working distance is the distance between the front of the objective lens and the plane of the operating field and is approximately (less than 5% deviation) equal to the focal length of the objective. Hence, working distance increases with greater focal length. However, when an objective is changed for another of different focal length the amount of light entering the observation path also changes — as the working distance increases so the amount of light reaching the operator is *diminished*.

Binocular tubes can also be varied as required; they can be either straight, inclined or tiltable. In the first, the tubes are parallel with the microscope axis and offer a straight line view of the operating field whereas inclined tubes are set at an angle of 45° to the microscope axis but still provide a straight view of the operating field. To cope with changes in microscope position during a surgical procedure and to allow the surgeon and assistant to change places, a tiltable binocular tube with a focal length of 160mm is valuable. This can be used in the straight or inclined position, and adjusted to intermediate positions in between. In addition, it has been equipped with an adjustor which can be operated with one hand to move the eyepieces to the correct interpupillary distance (PD) for each individual.

Binocular tubes can be supplied in different focal lengths, the most commonly used in microsurgery being 125mm, 160mm or 180mm (f = 125, 160 or 180 imprinted on tube). These differences have 4 main consequences:

(i) Overall lengths can be selected to suit the individual surgeon.

(ii) Magnification will be different. The base magnification (V_m) is calculated by multiplying the ratio of tube focal length (f_t) to objective focal length (f_o) by the magnification power of the eyepieces (V_e) hence:

$$V_m = \frac{f_t}{f_o} \times V_e$$

For example,
if f_t = 125, f_o = 200 and V_e = 12.5
then

$$V_m = \frac{125}{200} \times 12.5 = 7.8$$

If f_t is increased to 160 and f_o to 200 then with the same eyepieces, a magnification of 10 can be obtained.

(iii) The field of view (i.e. the operating field visible through the operating microscope will vary in diameter with focal length of the tube. If, for example, the eye-piece tubes have an internal diameter of 20mm, and f_t = 125 and f_o = 200 then the field-of-view diameter D_o can be calculated as

$$D_o = \frac{20mm}{f/f_o} = \frac{20mm}{0.625} = 32mm$$

Hence, if we increase the length of the tube, the diameter of the field-of-view will *decrease*.

(iv) Image brightness — the longer the tube, the *lower* the brightness of the microscope image.

From this knowledge, it is possible to select combinations of objective lens and focal tubes to obtain optimum operating conditions for the individual.

Eyepieces are designed to magnify the intermediate image produced in the binocular tubes by the microscope objective. As demonstrated above, the intermediate image is generally reduced. So long as the eyepieces are pushed fully home into their mounting, the distance between eyepieces and the intermediate image will be automatically established. Different magnifications are only obtainable by eyepieces of different focal length. Again, when choosing them, the total magnification required and the field-of-view have to be considered. Eyepieces are described by their magnifying power (eg. V_e = 10x, 12.5x = 20x). Now to establish the overall magnification (V_m):

$$V_m = \frac{f_t}{f_o} : V_e$$

$$\text{e.g. } V_m = \frac{125}{200} \times 12.5 = 7.8$$

The diameter of the field-of-view can be obtained from this information by dividing the objective focal length by the total magnification
$\left(\text{e.g. } \frac{200}{7.6} = 25.6mm\right)$

With zoom or step magnification built into the operating microscope, these basic functions are slightly altered. For example, where the basic magnification V_m is expressed by the formula:

$\frac{f_t}{f_o} \cdot V_e$ it is $\frac{f_t}{f_o} \cdot y \cdot V_e$

e.g. if f_t = 125mm, f_o = 200mm,

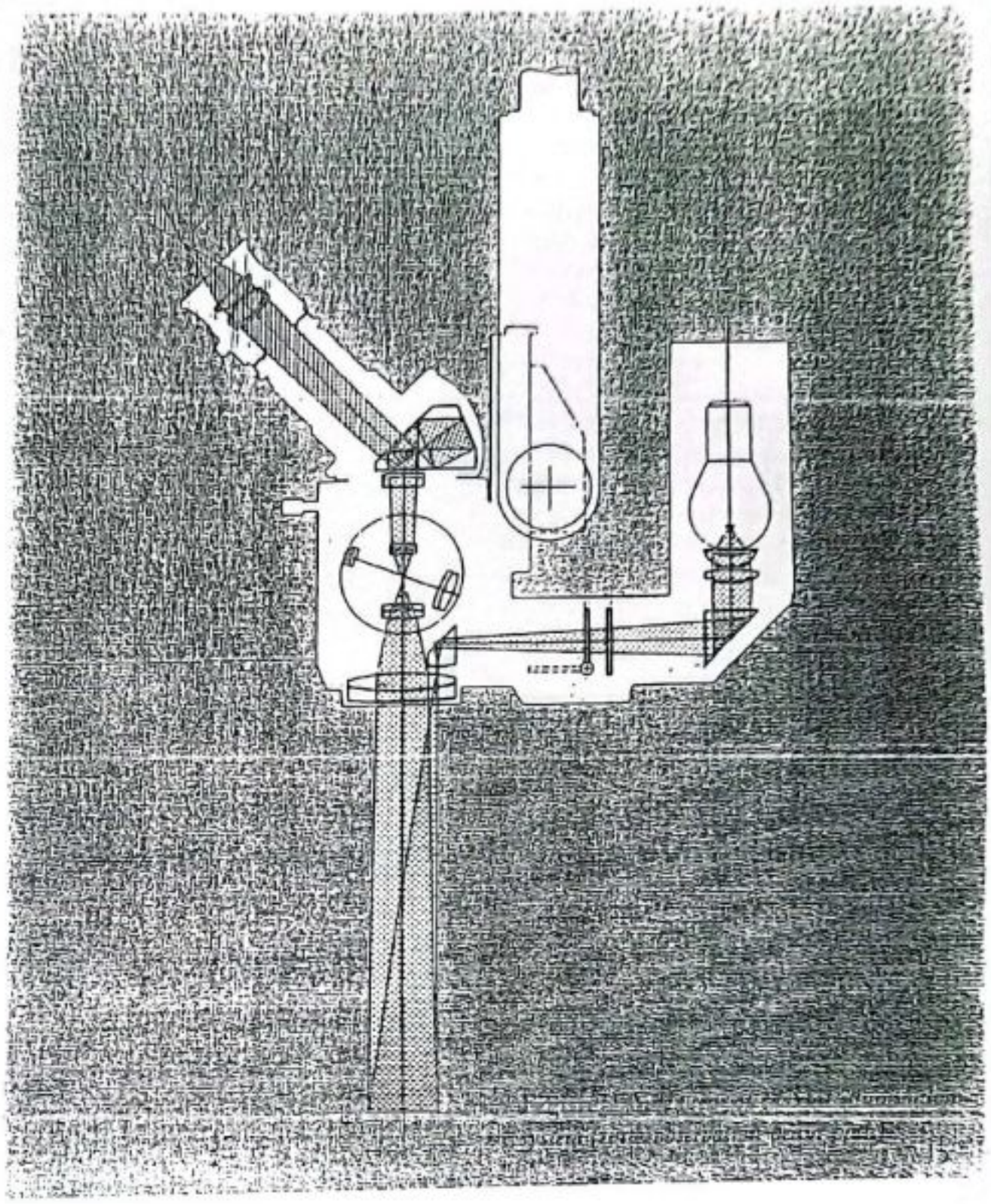
V_e = 12.5 x and y = 1.6 then the total magnification will be 12.5. From this can be calculated the field-of-view diameter of the complete microscope

$$D_m = \frac{200mm}{12.5} = 16.0mm$$

Illumination: The illumination system is also of prime importance. Several different types of light source are available for illumination, most nowadays, incorporated as an integral part of the microscope. Ideally this would produce minimal heat and would be shock proof, fail-safe and simple to operate; it would be readily replaced during an operation if it failed; it should provide a light intensity which does not fluctuate during an operation and which illuminates the operating field uniformly at a high intensity; and it should be economical in use.

The most commonly used light source for operating microscopes has been the 6 volt 30 watt tungsten *incandescent lamp*. The average life of these bulbs is 200 hours but if the light is used frequently at maximum intensity this life-span will be drastically reduced. Unfortunately, all incandescent bulbs emit a maximum of only 4% of the supplied electrical energy as visible light and most is emitted in the infra-red range as heat. Intensity is varied by means of the voltage control and a simple potentiometer.

More recently, 12 volt 100 watt *halogen lamps* have been used to gain improvements. They have a tungsten filament and iodine vapour within the bulb, and conversion of electrical to light energy is far more efficient than incandescent bulbs. They too emit a high proportion of infra-red radiation but the light emitted has a higher percentage of blue light than incandescent lamps and therefore appears brighter. This also increases the contrast between objects of different colours. The 12 volt 100 watt halogen lamp can either be built into the microscope or used as a light source for fibre optic transmission. The average life of these bulbs is 50 service hours. Although halogen lamps are small, the lamp housing is large because heat has to be removed and a ventilator with high air throughput/unit time is needed to keep the illumination system cool.



The light transfer system can be either an integral built-in part of the microscope body or alternatively a fibre optic system. The former consists of an illumination beam relayed from the bulb through a lamp condenser, deflecting prism, colour filter, heat filter and a further deflecting prism (illuminating prism) thence through the objective lens to the operating field (Figure 1). The illuminating prism is sited next to the two observation beam paths and off-centre, hence, although this is generally referred to as coaxial illumination it is not strictly so. The main thing is that the illumination beam must coincide completely with the observation beams in the object plane. In fibre-optic systems, the light energy is transmitted along flexible glass fibre bundles from a halogen lamp. They have several advantages: first, the lamp housing can be sited on a stand at some distance from the microscope body, and so avoids heat transfer to the microscope; they permit both coaxial and oblique illumination; and they can be fitted with two lamps to allow switch-over if one fails during the middle of an operation. However they also have disadvantages: they are more expensive; the fibres can break if the cable is bent sharply; the light

intensity can be reduced by 50% even in a perfect cable at the point of entry to the fibres; and light losses due to absorption increase with the length of cable. Each fibre is circular in cross-section so there are spaces in between each where light transmission cannot take place. Once the number of fibres broken reaches about 25% of the total the transmission of light becomes very inefficient. Hence it is most important not to drop or bend the cable around sharp corners.

Care: Microscopes are easily damaged and a few simple rules should be followed in their care:

(i) Never move the mobile stands over rough surfaces on their own castors and always remove and carry the microscope head separately.

(ii) Clean the eyepiece and objective lenses with lens tissue every time the microscope is used.

(iii) Always cover the whole microscope head in a plastic bag after use to prevent dust getting inside the instrument.

(iv) Do not run the light sources at high intensity for longer than 5 minutes as their life span is otherwise shortened dramatically.

(v) Treat fibre-optic cables gently - the fibres are easily fractured if dropped or bent around sharp angles. Furthermore, it is essential that these are fitted to conduct light in the correct direction, otherwise they deteriorate rapidly.

Magnifying Ocular Loupes

Loupes are often more convenient than the microscope for preliminary dissection and for anastomosis of vessels greater than 3mm in diameter. A simple model with an elasticated headband gives a magnification of 1.8x whilst more sophisticated binocular loupes fitted to spectacles can provide up to 4x magnification without operator fatigue. In our experience, loupes with a higher magnification have too small a field of vision and depth of focus to be comfortable.

Instruments

Purchase of a basic set: It is a waste of time and money trying to skimp on instruments for the laboratory: if they are not good enough for use on patients then they are not good enough to learn microsurgery in a rat. If possible, it is best for each surgeon to have their own set which should then be looked after with care and never lent to anybody else. Many variations on the basic microsurgical set described below are available from different manufacturers but a few features to look for when making a purchase are common to all: they should have a satin finish to avoid glare under the microscope; handles should be long enough to rest in the thenar web of the hand; and where these are spring-loaded the closing tension should be sufficiently gentle to avoid fatigue.

(i) Jeweller's or watchmaker's forceps (hereafter called microsurgical forceps): Dumont forceps (Nos. 3, 5 or 7) are basic to any set and are used in the left hand for tying sutures and handling tissues. Their tips must meet evenly over a length of 2mm so that 10/0 and 11/0 nylon thread can be picked up easily without damage. They can be either straight (**Figure 2a, 2b**) or curved (**Figure 3**).

(ii) Vessel dilator: Dumont No. D-5a are microsurgical forceps modified at the tip so that they are rounded and polished. The closed tips can be inserted into the end of a divided vessel and then gently opened to provide counter pressure whilst suturing (**Figure 4**).

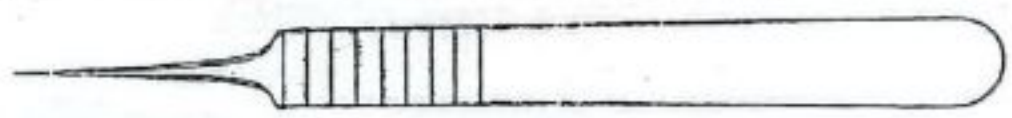


Figure 2a. *Dumont No. 5 straight, 11cm microsurgical forceps.*

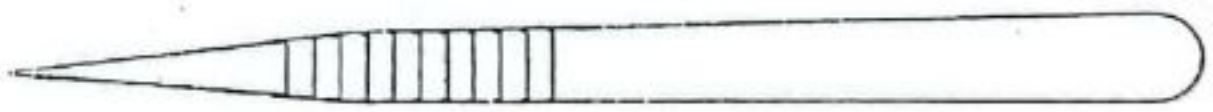


Figure 2b. *Dumont No. 3 straight, 13.5cm microsurgical forceps.*

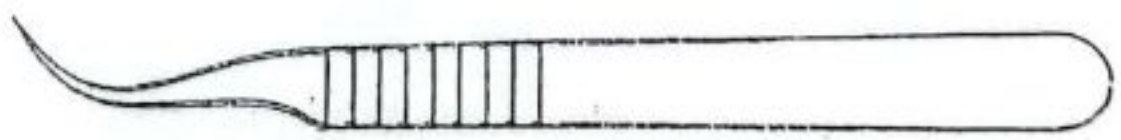


Figure 3. *No. 7 curved, 12cm microsurgical forceps (also useful as needleholders).*

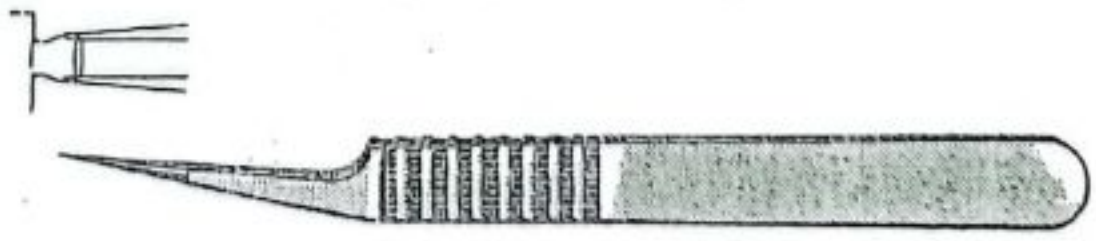


Figure 4. *No. D-5a vessel dilating forceps, 11cm.*

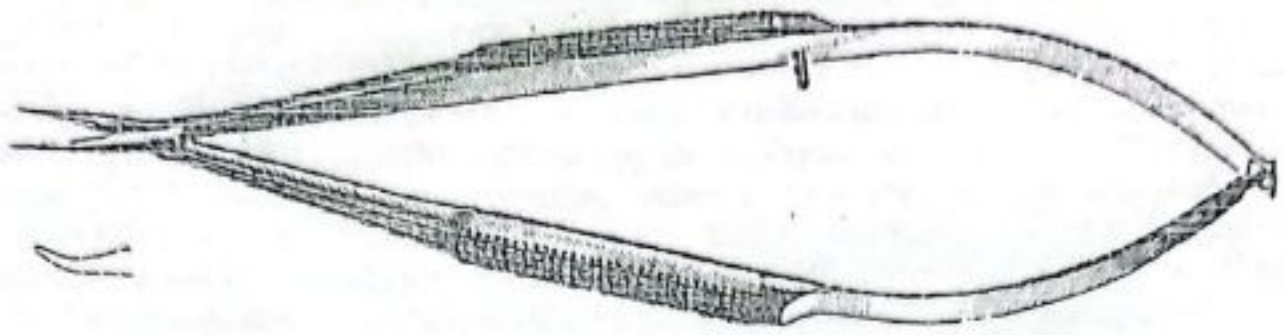


Figure 5. *Round handled, microsurgical needleholder, 13cm, without lock.*

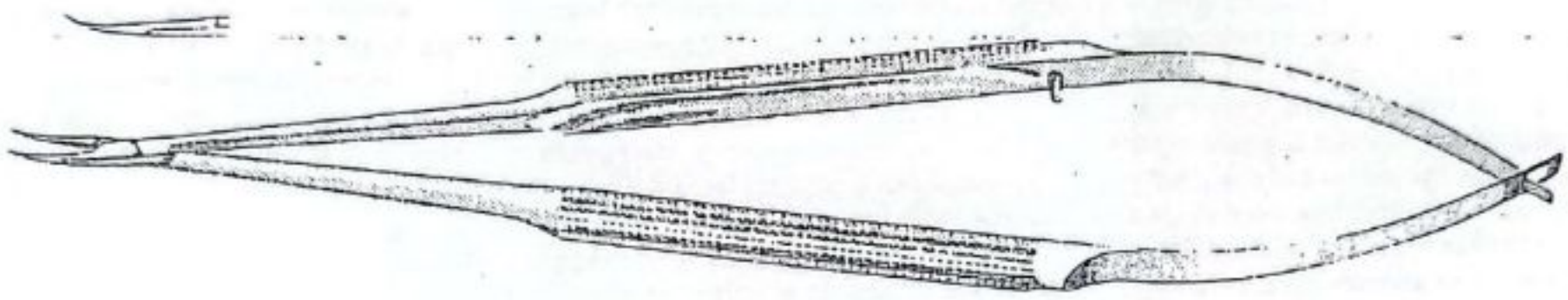


Figure 6. *55C needleholder, round handled, 18cm, without lock.*

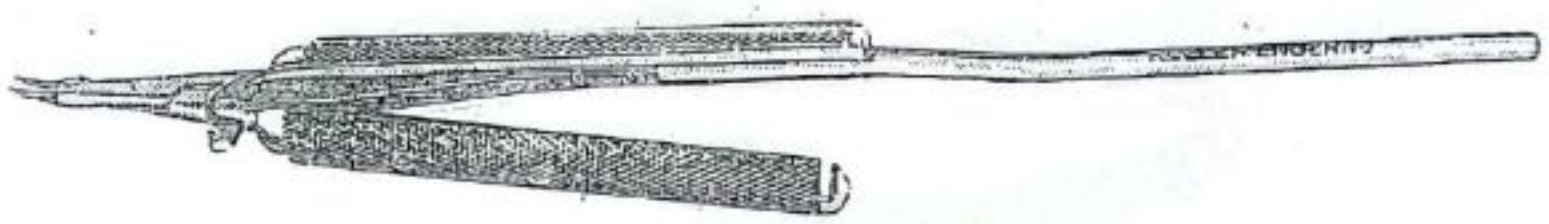


Figure 7. *Vickers microsurgical needleholder, fine jaws curved.*

Figure 8. *Needleholding forceps No. 5, angled 45°, 11cm.*

Figure 9. *Dissecting scissors, curved, 15cm long blade.*

Figure 10. *Adventitia scissors, straight, 15cm long blade.*

(iii) Needle-holders: purpose-designed models (Figure 5, 6, 7) have spring-loaded handles which can have a round or flat grip and fine tips which can be curved, angled or straight. Some of those on the market are fitted with ratchets for arming the needle but these should not be used because release of the lock is impossible to control and the needle can be easily damaged or lost. The tips should be fine enough both to hold the needle without distorting its designed curve *and* for tying knots. However, some surgeons prefer to use 45° angled microsurgical forceps (Figure 8) as needle-holders because they are less expensive, have no hinges to corrode or snag the thread and because they open and close easily.

(iv) Dissecting scissors: these should be spring handled and the curved blades can be short or long according to personal preference. The blade tips should be rounded so that tissues adjacent to the vessels can be dissected without damaging them (Figure 9).

(v) Adventitia scissors: these are identical to dissecting scissors but the blades are straight (Figure 10).

(vi) Vanna's scissors: these are again spring handled scissors with very sharp pointed tips on fine straight or curved blades (Figure 11). They are used for removing the adventitia from vessels and for cutting stitches. Sharply-angled Vanna's scissors are useful for interfascicular nerve dissections.

(vii) Vessel clamps: these again come in many shapes and sizes which appeal to personal preference. Whatever design is chosen, it is best to have at least two single clamps available in each set of instruments and a double approximator clamp in which the two clamps, or clips, slide along a bar. The following can be recommended:

(a) Acland clamps — single 11mm (CC1A or V) which can be purchased spring loaded for artery or vein (Figure 12).

Acland clamps — double approximator clamp (ACC1) with a suture holding frame and cleats so that stay sutures can be placed.

(b) Kee's clamps which have the advantage that they grip the vessel right along the length of the jaws and which are heavy enough to attach to stay sutures and hold these out under tension when it is impossible to use approximating clamps and frame.

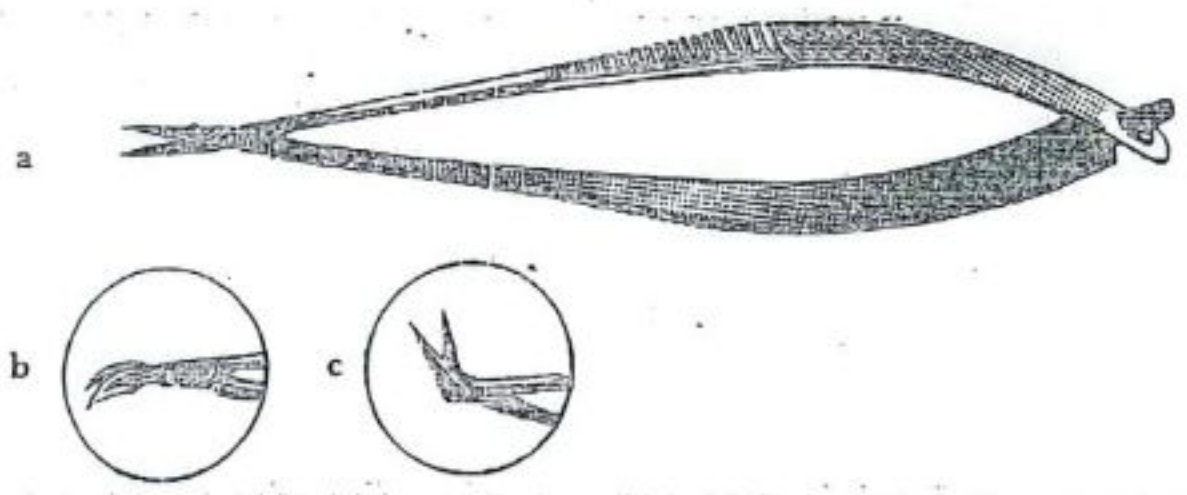


Figure 11. *Miniature spring scissors: Vanna's type* a *short straight blades, 8cm,* b *short curved blades 8cm,* c *short fine blades, angled on side 7.5cm.*

Figure 12. *Single Acland clamps: A designed for arteries, V for veins.*

In general, the Acland clamps are best for very small vessels but the tiniest amount of blood or dirt in the joint renders them inefficient: the jaws only meet for part of their length and it is very easy for a vessel to partially slip and allow blood to leak from the cut end. The cleated frame is ideal for most microsurgical exercises in animals but is often not suitable in clinical situations. Kee's clamps are then sometimes more useful. Clamp applying forceps (**Figure 13**) are mandatory for the Acland clamps: the Rizutti clamp applier is particularly useful as it can be used for single clamp application and removal or for simultaneous application of both microclips in the Acland clamp.

(viii) Tubal clamps: Winston modified Acland clamps for oviduct and vas deferens (**Figure 14**).

(ix) Instrument case: this is not absolutely essential but is an insurance against damage to these delicate instruments. The best design is constructed of metal lined inside with rubber spigots which grip the instruments and prevent them from moving during autoclaving.

(x) Basic dissection set: all that is needed for microsurgical exposure of the operative site are fine round-tipped bow scissors, fine-toothed fixation forceps (**Figure 15**) and No. 10 or 15 scalpel blades on a No. 3 handle.

(xi) Bipolar coagulator or disposable coagulators: a single function unit with a good pair of bipolar forceps is useful for haemostasis; alternatively, disposable coagulators which are battery operated and provide just sufficient heat to coagulate small vessels without risk of damaging surrounding tissues are useful in the laboratory setting. In either case it is essential to isolate the tissue to be coagulated from adjacent structures before application.

Care of instruments: Microsurgical instruments are so delicate at the tips that they are easily damaged by contact with hard objects or surfaces. Whilst concentrating under the microscope it is easy to put them down carelessly. Rather than keep them on a metal tray with other instruments, it is worth getting into a habit of laying them out

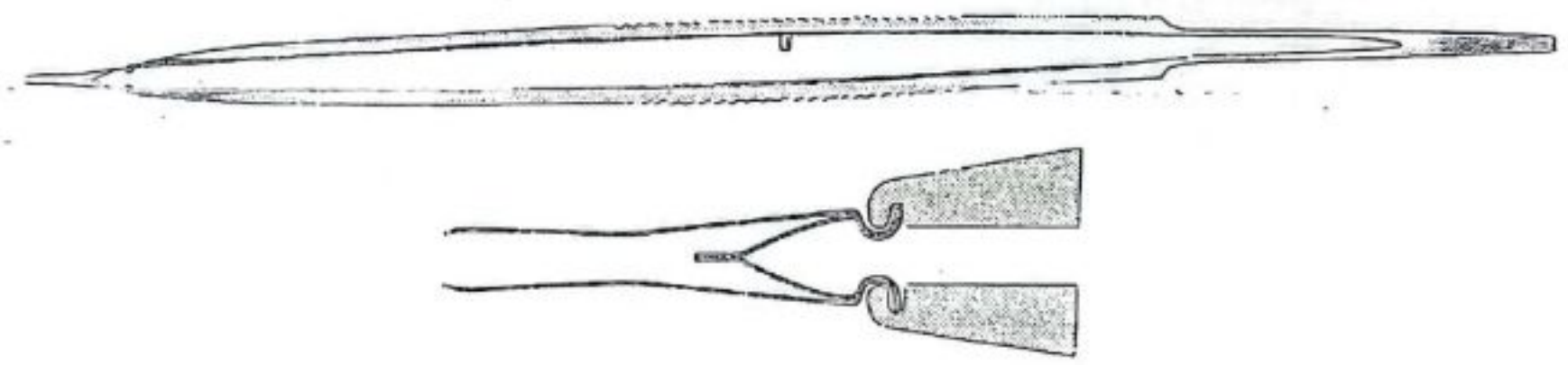


Figure 13. *Meyer clamp applying forceps, straight 16cm, without lock.*

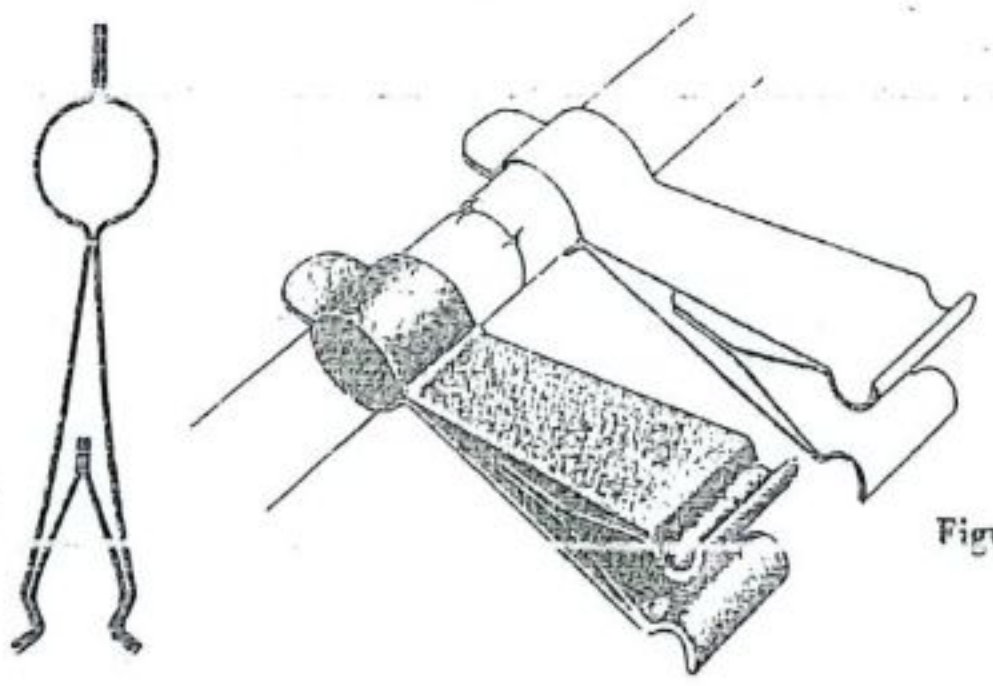


Figure 14. *Winston modified Acland clamps for tubal surgery.*

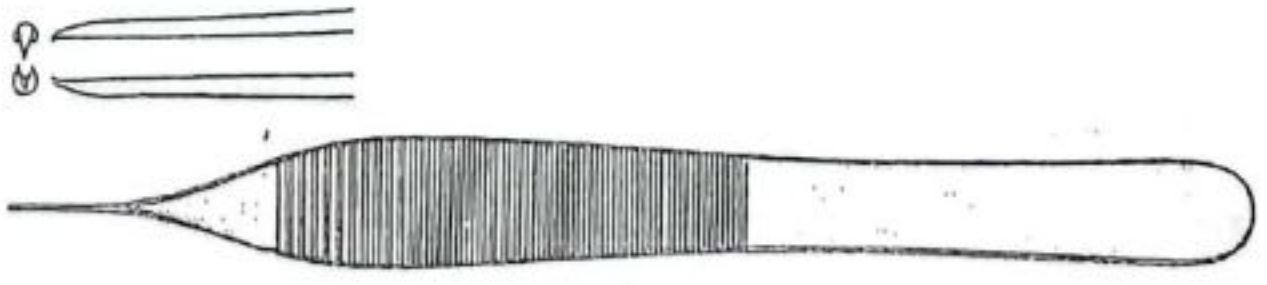


Figure 15. *Fixation forceps, 12cm.*

in a semi-circular pattern on a soft drape in a set order and replacing them in the same order each time they are used: in time, it will become second nature to pick them up slowly without having to look up from the microscope. If the tips of microsurgical forceps do get damaged, it is essential that they are straightened and filed with an Arkansas stone or fine emery paper before they are used again otherwise the hooked tip will damage tissues and rough-edges may cut fine sutures. The microsurgical instruments should be laid out away from the bulkier dissecting set. Then whenever they are not in immediate use, the tips should be protected with rubber tubing. Clamps should be placed in a plastic dish of isotonic heparinised saline solution and kept there whilst not in use. It is important to repeatedly wash blood and serum from the clamps and other microsurgical instruments throughout the procedure and wipe them with a damp gauze to remove debris as well as to demagnetise the metal.

After the operation, the instruments should be cleaned with wet gauzes, then soaked in an enzymic solution for 45 minutes to dissolve any clots trapped in the hinges and joints. They should then be rinsed with a jet of water again taking care to get into the hinges and are placed in an ultrasonic agitator for 3 minutes. Finally, they must be thoroughly dried before storing them away.

Sundries

Electric clippers: the operating site in both rats and rabbits is best clipped closely with high quality electrical clippers (Oster A5-00) with the 40 grade cutting head. Depilation can then be completed with an aerosol depilating foam.

Operating boards: cork boards covered with disposable plastic sheeting are most convenient for rats and rabbits (about 12cm × 25cm and 25cm × 50cm) respectively. Adhesive tape is useful for holding the animal in position during the operation and ordinary office paper clips attached to rubber bands which can be pinned out with plastic headed mapping pins make adequate tissue retractors.

Visibility background material: strips of blue, green or yellow plastic sheeting are essential to put behind the vessels whilst they are being anastomosed. The strips (3mm × 1.2mm) serve both as an aid to visualising structures during suturing and to isolate the vessels from surrounding tissues.

Rubber practice cards: these are used for the very first microsurgical exercises and can be made up from surgical gloves stretched over 2cm diameter holes cut in strong cardboard. It is important to wash the gloves first otherwise the powder attached to the rubber snags on sutures and sticks to instruments.

Microsurgical Sutures

(i) Suture materials: Monofilament polyamide (nylon) is the material most commonly used in microsurgical sutures. It is noted for its smooth flow through tissue characteristics, its strength and its relatively inert behaviour in tissue. It knots reasonably well and can be dyed black to render it clearly visible under the microscope.

Other non absorbable sutures are now available including monofilament polypropylene and monofilament polyester. Polypropylene sutures are inert, retain their strength in tissue indefinitely and knot extremely well as the material is softer than nylon. Their main advantage is that they pass easily through delicate tissue because of their extremely smooth surface finish. They do have slight disadvantages however in that their light colour make them more difficult to see under the microscope and special care is needed when handling them to avoid damaging the material with surgical instruments.

Monofilament polyester is the latest in the line of microsurgical suture materials. It is as inert as monofilament polypropyelene and polyamide in tissue but has a higher coefficient of friction than either of the other materials and this helps to prevent knots from untying.

All these materials are available in different sizes described as 8/0, 9/0 and 10/0 gauge sizes or in the new metric gauging of 0.4 metric, 0.3 metric and 0.2 metric respectively. Nylon is also available in 11/0 gauge (0.1 metric) size. Both systems represent a specification band into which a material must fall, eg to be called 0.2 metric or 10/0 the material must be between 0.020mm and 0.029mm in diameter (20-29 microns). 11/0 has a mean diameter of 18μ, 10/0 of 25μ, 9/0 of 35μ and 8/0 of 45μ.

(ii) Needles: The monofilament material is swaged into one end of atraumatic ⅜ circle needles. The following information is needed in selecting the best needle for the job:

(a) Needle length — this is the size of the needle in mm measured from point to butt around the outside curvature of the needle.

(b) Needle profile — this is invariably curved but occasionally half circle needles are used for end-to-side anastomoses.

(c) Needle diameter — this is measured in microns and is the critical measurement for micro needles. Until quite recently 130 micron needles were the finest available but diameters as fine as 30 microns are now manufactured.

(d) Cord length — this is the length in a straight line between the point and butt of the needle.

(e) Cross section — the vast majority of needles used in micro surgery have a tapered atraumatic round bodied tip with a flattened rear end to make them easier to grip with needle holders. Micro cutting needles are also available for nerve repair and tubal surgery.

(iii) Packaging: Handling micro sutures presents difficulties not found with normal suture dispensing so all micro needles 100 microns diameter and finer are packaged in a transparent envelope. The needles are inserted into a foam needle park which can be removed from the sterile pack with the needle and suture enabling the needle holder to be armed easily under the microscope. Non-sterile packs are now available for training purposes.

USING THE OPERATING MICROSCOPE

General Hints

THE lowest comfortable level of illumination should be used as this is less fatiguing for the operator and ensures maximum life for the light bulbs. Similarly, the lowest level of magnification appropriate to each stage of the operation should be employed: far too many beginners believe that high magnification is necessary for success and then run into problems. In general, high magnification (10x to 16x) is needed for preparing the ends of vessels and for passing the needle through the vessel wall, low (4x to 6x) is adequate for pulling the thread through and for vessel dissection, and intermediate (6x to 10x) magnification is used for actually tying the knots and for securing stay sutures to cleats. Beginners should be bullied into changing the magnification at frequent intervals otherwise they often tend to stay with one magnification throughout the anastomosis. A foot pedal control is invaluable for making these frequent changes although none of those on the market are ideal. They are operated most easily with bare feet and it is best to keep one foot on the pedal throughout the microsurgical phase of the operation.

As microsurgery entails long hours spent in the same position, the surgeon's posture is critical. The height of the stool should be adjusted so that the operator is seated comfortably right up against the table's edge and with a straight, not curved, spine. Fatigue sets in rapidly if the neck is craned or the back hunched to see through the eyepieces.

Adjusting the Microscope

1. Switch on the illumination and move the microscope stand and head until the ring of light is about 20cm from the near table edge.

2. Rotate the engraved dioptre scale on each eyepiece to the zero position, fold down or remove the rubber eyecups if you are wearing spectacles, and make sure the eyepieces are fully seated in their sockets.

3. Set the microscope at its lowest magnification either by the hand knob or by operating the footswitch on motorised zoom models.

4. Adjust the fine focus of the microscope according to the model so that it is in the centre of its travel. Most microscopes have 2' bench marks which are in line with each other at this zero position.

5. Draw a cross on a piece of paper laid flat on the worktop and put it in the centre of the field of view.

6. Move the microscope up and down until the cross is approximately in focus and tighten the clamp knob on the carrier arm (depending on model).

7. Adjust the distance between the two eyepieces (interpupillary distance) until the two images are superimposed on each other and fuse together. To make this rather critical adjustment, do not press your eyes up against the eyepieces but raise the head slightly so that your eyes are about a centimetre away from them.

8. Make sure the eyepieces are still pressed fully home into their socket and are properly seated.

9. With the microscope at its *highest* magnification, focus the cross exactly using the microscope's fine hand focus or zoom.

10. Now, *without deliberately altering the focus*, change the magnification to its lowest setting. Most microscopes are designed to be *parfocal* so, in theory, should stay in focus when moving down the magnification range but this in practice is not always the case, and minor adjustments may be necessary.

11. The separate focusing adjustments should now be made to suit the individual. If you wear spectacles with a correction of less than 3 dioptres it is usually more comfortable to remove them and adjust each eyepiece separately. Very near-sighted people, however, usually prefer to wear spectacles and keep the eyepiece adjustments at zero. Adjust each eyepiece setting to bring the cross into sharp focus, rotating the eyepieces in a clockwise direction: if you go past the position of sharpest focus, it is important to return fully anti-clockwise to the zero setting and start clockwise again.

12. Repeat steps 9, 10 and 11.

13. Make *final* focusing adjustments at the highest magnification.

14. Make a note of the dioptre setting for each eye.

15. Take microsurgical forceps in each hand and manipulate them in the field of view. Without removing your eyes from the eyepieces, change the focus and magnification repeatedly until each can be done easily. If the eyepieces have been adjusted correctly, the focus should remain sharp whatever the magnification.

ANIMAL PREPARATION AND ANAESTHETIC MANAGEMENT

General Hints

IN the United Kingdom, the Animals Scientific Procedures Act, 1986 expressly forbids the use of living animals for acquisition of microsurgical skills unless a project licence and personal licence are first obtained from the Home Office. Initially, some suturing skills can, and should be, mastered on synthetic materials or on freshly killed animals, fresh human placentae, or isolated tissues such as human peripheral nerve or fallopian tube pinned out on a dissecting board. Choice of these alternatives will depend on availability of material and the level of technical expertise present in the laboratory. Unfortunately, the value of these models is severely limited and basic training must inevitably be completed in animals.

As the animals have to be fully anaesthetized for long periods before they are killed on completion of the operation, it is imperative that they are healthy before starting. If rats are used, it is best to select animals weighing 250-300gms when their femoral arteries are about 1mm in external diameter and the rats are still young enough to be reasonably free of chronic respiratory disease and are not obese. Ordinary outbred (eg. Sprague-Dawley) rats are suitable. Any rats with obvious symptoms of respiratory distress such as wheezing or sneezing should be avoided. Similarly, where rabbits are to be used, they must be free of overt respiratory or intestinal disease and weigh between 2.5 and 3.0kg before they have become obese. The femoral arteries are about 1.5-2.0mm in external diameter in New Zealand White rabbits of 12 weeks of age; the sciatic nerve (2.0mm) is suitable for microneural dissections and anastomoses; and the oviducts are suitable for microsurgical reconstruction.

Anaesthetic Management

It is only possible here to provide a few simple guidelines. In general, it is better to use injectable agents for these long microsurgical procedures so avoiding the hazard of operator exposure to inhalational agents. Pentobarbitone sodium is commonly used in rats and rabbits but is a poor analgesic and has been replaced by safer and more effective agents. For long operations, it is essential to ensure good pulmonary ventilation, a constant oxygen supply, a source of heat to maintain body temperature, maintenance of fluid and electrolyte balance, and suction to aspirate the airway.

Rats: Combinations of sedative-analgesics such as fentanyl with tranquillisers such as fluanisone, diazepam or midazolam provide far safer anaesthesia than pentobarbitone. If these are not available, then it is generally safer to use relatively low doses of pentobarbitone concurrently with ketamine in preference to pentobarbitone alone. In some strains of rat, ketamine mixed with acepromazine is effective. As there are considerable strain differences to response to all these agents, it is only possible to make suggestions which you should try out first in the rats available to you.

Intramuscular (im) injections are made with a 10mm x 26 gauge needle into the belly of the hind limb muscles or over the shoulders. Intraperitoneal (ip) injections are made with the rat firmly held by an assistant: the rat should be on its back and slightly head down, and injection made just posterior and 2mm to one side of the umbilicus using a 16mm x 23 gauge needle (**Figure 16**).

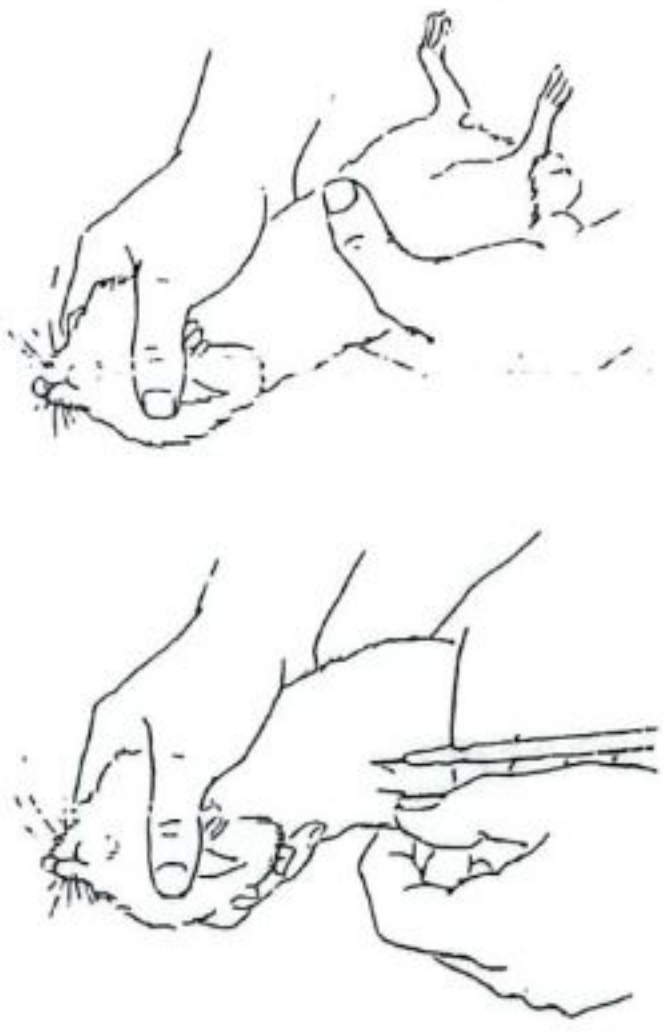


Figure 16. *Intraperitoneal injection of rat with anaesthetic agent.*

We use the following injectable agents in order of preference:

(i) Fentanyl-fluanisone (Hypnorm®) at 0.5ml/kg im given concurrently with diazepam (Valium 20®) at 2.5mg/kp ip produces surgical anaesthesia in 3-6min, and this lasts for 45-90 min. More fentanyl-fluanisone can be given at 0.2ml/kg to extend the effective period. It should be reversed with naloxone (0.3mg/kg) at the end of the operation.

(ii) Fentanyl-fluanisone-midazolam can be premixed (1 part Hypnorm®, 1 part Hypnovel®, and 2 parts water for injection) and injected by the ip route at 3.0ml/kg of the mixture to produce surgical anaesthesia in 5-10 min. This lasts for 75-150 min and can be further extended by injections of 2ml/kg every 60 min or so. It too can be reversed with naloxone (0.3mg/kg) at the end of the operation.

(iii) Ketamine at 60mg/kg im with pentobarbitone at 20mg/kg ip produces light surgical anaesthesia in 10-15 min and this lasts for 30-60 min.

(iv) Ketamine-acepromazine (premixed 15:1.0mg/kg ip) produces light surgical anaesthesia in 10-15 min and this lasts for 60-120 min.

Anaesthetic depth is assessed on the respiratory rate, and response to pinching the tail, interdigital web and ears. The latter is most sensitive in rats and loss of reflex activity is a good index of full surgical anaesthesia.

Cyanosis of mucous membranes, ears and interdigital webs must be regarded as indicating severe respiratory depression: in this event, artificial respiration and gentle insufflation with oxygen via an oral catheter after aspiration of any mucus from the airway (small bore plastic tubing attached to a 10cc syringe is adequate for aspiration) should be instituted immediately. If respiratory depression persists then doxapram at 1.5mg/kg should be administered ip.

Rabbits: The main problem encountered during long periods of anaesthesia in rabbits is respiratory depression especially if chronic upper respiratory tract infections are present. It is important therefore to supply oxygen to the face by mask or cone and preferable to ensure a patent airway by passing an endotracheal tube (3.5mm uncuffed paediatric tubes are ideal). Inhalational anaesthetics or injectable neuroleptanalgesics which can be readily reversed with specific antagonists are preferrable to injection with barbiturates.

Assessment of anaesthetic depth depends on the agent used. In general, withdrawal to toe pinch is lost as light surgical anaesthesia is reached. Effective anaesthesia is indicated by failure to react to ear pinch and when the photomotor and palpebral reflexes are lost. Loss of the corneal reflex indicates dangerously deep anaesthesia in rabbits. Rabbits are best restrained for injection on a non-slip surface by wrapping them securely in a towel. They will then almost invariably remain still. Intramuscular injections are made into the posterior thigh muscles or the muscles overlying the shoulder with a 16mm × 23 gauge needle. Intravenous (iv) injections are made into the lateral ear vein after the site has been carefully clipped, shaved and swabbed with alcohol. A 25mm × 23 gauge needle or better still a 21 gauge butterfly infusion set needle are inserted and taped in place.

In the absence of inhalational anaesthetic equipment, the safest injectable agents in rabbits are a combination of fentanyl-fluanisone (Hypnorm®) and diazepam (Valium 20®) given as separate injections. Fentanyl-fluanisone is injected im first at 1.0 - 3.0ml/kg of the commercial preparation and diazepam is injected iv at 1.0mg/kg. Surgical anaesthesia develops in 5-10 min and lasts for 25-45 min. This period can be extended by injecting fentanyl-fluanisone at 1.0ml/kg every 20-30 min as required.

Alternatively, xylazine (Rompun®) at 7mg/kg im followed 5 min later by ketamine (Vetalar®) at 20mg/kg im produces light surgical anaesthesia in 10-15 min. Further dosages of ketamine can be given at 15mg/kg im every 30-60 min as needed.

Having induced anaesthesia in this way it is now possible to maintain the animal for long periods on inhalational anaesthetic agents using very low concentrations (0.5 - 1.0%) of halothane, enflurane or methoxyflurane. The oxygen flow should be 2 litres/min supplied either to a Magill circuit and face mask or an Ayre's T-piece connected to a 3.5mm paediatric endotracheal tube.

Operating Site Preparation

Both in rats and rabbits it is far better to clip hair from the operating site with the finest grade of clipper head (Oster 40®) rather than attempt to shave it. Loose hair should then be removed with a small hand-held vacuum cleaner before the site is finally covered with a depilatory aerosol foam. This should be removed after 2-3 minutes. Finally, the site can be swabbed with alcohol. For the exercises described later, it is worth clipping a wide area including the lower abdomen, hind legs and scrotum.

Rats are then laid out in the supine position on the operating board with the hind legs taped close to the board. The fore limbs should not be taped out as this will make thoracic excursions difficult and increase the likelihood of respiratory failure. The head and neck should be slightly flexed and not stretched straight out as this will diminish airway patency. Finally, at this stage, the mouth and pharynx should be cleared by retracting the tongue and aspirating any mucous with small bore plastic tubing attached to suction or a 10cc syringe.

Rabbits too are laid in the supine position and the same rules apply. It is very important not to stretch the hind legs out in an extended position as it is easy to cause posterior hemiplegia. Similarly, it is important not to stretch the forelimbs out and down so that thoracic movements become laboured.

List of Useful Pharmacological Agents

Anticoagulant: Heparin sodium supplied at a concentration of 1000 iu/ml is useful both in the whole animal and diluted at 1000 iu in 1000ml of isotonic 0.9% saline for irrigating vessels and the immediate operative site. In rats, approximately 100 iu/100 gm body weight provides adequate anti-coagulation, whilst in rabbits approximately 300 iu/kg is sufficient.

Vasodilating agent: Procaine HCL (1% solution) or lidocaine 1% (Xylocaine®) are useful in rats and rabbits applied undiluted directly to the vessels and allowed at least 5 minutes to relieve spasm.

Agent for euthanasia: A strong solution (18%) of pentobarbitone sodium (Expiral®) is ideal for rats (2ml ip) and rabbits.(5ml iv).

Respiratory stimulant: Doxapram (Dopram® 20 mg/ml) is a direct stimulant of the central respiratory centre which is effective in rats at 1.5mg/kg ip and in rabbits at 1.0mg/kg iv to reverse respiratory depression.

Anaesthetic agents: Pentobarbitone (Sagatal® 60mg/ml) is not ideal for rats or rabbits. However, if no other agent is available it should be diluted 1:1 in isotonic saline and given initially at 40mg/kg ip to induce deep narcosis and thereafter further doses of 10mg/kg ip every one or two hours as needed.

Fentanyl-fluanisone (Hypnorm®) is a very useful drug combination in both rats (0.4ml/kg im) and rabbits (0.2ml/kg im) and rabbits (0.2ml/kg im) to provide deep sedation and excellent analgesia.

Diazepam (Valium 20® 5mg/ml) is useful in rats (2.5mg/kg ip) and rabbits (1.0mg/kg iv) to produce muscular relaxation and surgical anaesthesia after Hypnorm® has been injected im.

Midazolam (Hypnovel®) is a water soluble benzodiazepine which can be mixed with Hypnorm® (1 part Hypnovel®, 1 part Hypnorm® and 2 parts water for injection) to administer as one objection in rats at 3.0ml/kg ip to yield surgical anaesthesia.

Naloxone (Narcan®) is a specific opiate antagonist which reverses both respiratory/CNS depression and the analgesia (0.3mg/kg im).

Ketamine (Vetalar® 100mg/ml) is fairly useful in rats (60mg/kg im) and rabbits (20mg/kg im with atropine 1.0mg/kg) if given with other agents which act synergistically with it.

Xylazine (Rompun®) is useful in rabbits (7mg/kg im) if given with ketamine as it produces excellent muscular relaxation and reasonable surgical conditions.

Halothane (Fluothane®) is a useful inhalational agent which can be given to rats or rabbits at very low concentrations (0.5-1.0%) after initial anaesthesia has been induced with injectable agents.

PRELIMINARY SUTURING EXERCISES

Handling Microsurgical Instruments

BEFORE moving on to exercises in animals, the beginner should spend the first morning on preliminary exercises on a rubber practice card and on either 5mm diameter thin-walled silastic rubber tubing or, preferably, isolated rabbit aorta pinned out on a dissecting board. The first suturing exercises will be easier if 8/0 monofilament polyamide is used rather than 9/0 or 10/0. To restate basic points which have already been made:

(i) Adjust the microscope until you are certain your eyes do not ache.

(ii) Make sure your stool is adjusted to a comfortable height.

(ii) Arrange your instruments on a draped tray in a set pattern so that they are easily reached without stretching.

It is now time to learn the position of the hands. Microsurgical instruments are handled like a pen so the hands should be in the writing position, that is to say resting on the hypothenar eminences and slightly supinated, knuckles outward. The elbows, wrists and ulnar border of the hands should be supported on the table top, if necessary raised on folded towels to adjust their height so that the instruments point into the operative field without torque in the wrists. Movements within the microsurgical operating area should be restricted mainly to the fingertips.

Practice rolling a needle-holder in the right hand (if you are right handed) and holding microsurgical forceps in the left, bringing the tips of the instruments to within 2mm of each other in the same plane (i.e. the same distance away from the microscope objective).

Now practice picking up these instruments from the tray and bringing them into the microscope field observing these rules:

(i) Never move instruments at the same time as you move your eyes from the microscopic to macroscopic field and vice-versa.

(ii) Never move abruptly but at a slow rhythmic pace.

(iii) First move the instruments from the tray under direct vision into the general operative area.

(iv) Then move them into the magnified area whilst looking through the microscope at low (4x) magnification.

The reasons must be obvious. Not only are the microsurgical instruments delicate but they are sharp and can easily damage blood vessels and tissues in the patient as well as gloves and skin of any unfortunate assistant. With practice, these movements will become automatic, and instruments can be picked up and replaced without looking up from the microscope.

Rubber Practice Card

Handling needles and sutures is best learnt using a relatively large needle (130 micron) and suture (8/0 nylon) and a rubber practice card pinned in position under the microscope.

(i) Place the 8/0 suture in the 'park' provided in the magnified field (4x magnification).

(ii) Then grasp the needle just behind its midpoint so that when it is removed from its park it sits at right angles to the long axis of the needle holder and the tip points horizontally, not downward or up. With a flat-bodied needle, it should be stable in this position.

(iii) Now practice changing direction with the needle-holder by flexion or extension of the wrist and observe the needle point under the microscope as you roll the needle-holder in your fingers.

(iv) Practice placing the needle on the rubber and picking it up again (i.e. *not* using the park). Grasp the thread with the forceps about 1.5cm from the needle and raise it until the needle tip is just touching the rubber surface. The needle can then be easily grasped by the needle-holder *always* with the convex curvature of the jaws facing downward (Figure 17). Always put the needle down within the illuminated field.

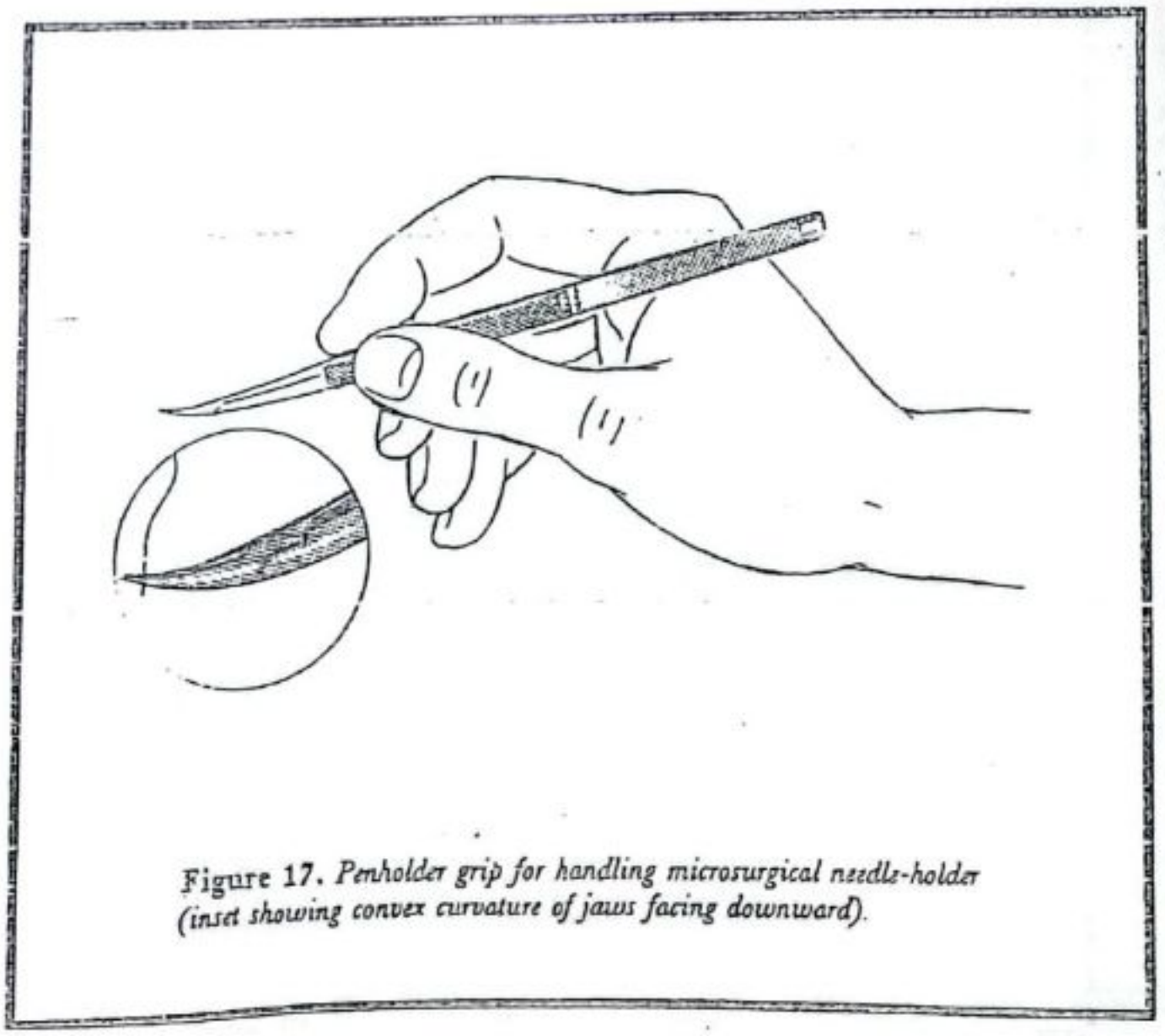


Figure 17. *Penholder grip for handling microsurgical needle-holder (inset showing convex curvature of jaws facing downward).*

(v) Now repeat (iv) but at a higher magnification (say up to 10x) and establish for yourself the most comfortable magnification for this procedure.

For the following knot-tying exercises, make a diagonal (top left to bottom right) incision in the rubber about 1cm long using scissors. Imagine that the rubber is a delicate living tissue lined with intima which can be easily damaged and learn the same rigid rules now which you will need later in your patients:

(i) Never grasp the thickness of the 'tissue' between the jaws of microsurgical forceps but evert it by placing the tips under the 'tissue', and allow the tips to spread about 1mm apart (magnification 10x).

(ii) Pass the needle through the 'tissue' at right angles to the slightly everted surface and at about a full tissue thickness from the edge using the dilator forceps as counterpressure (**Figure 18**).

(iii) With the microsurgical forceps now on the upper surface of the left side of the incision again as counter-pressure, pass the needle through the left side at right angles to the wall and at the same distance from the edge (**Figure 19**). The suture should be passed from right to left at 90° to the incision.

(iv) Pick up the tip of the needle and pull it through following the curvature of the needle and not attempting to pull it through in one straight movement, otherwise you will make large holes in tissues when you start on real vessels.

The knot tying procedure should now be practiced with the microscope at low magnification (6x magnification).

(i) The thread should be pulled through with the microsurgical forceps again at 90° to the incision and guided by the jaws of the needle-holders (**Figure 20**) until only 3mm of thread is left.

(ii) Place the needle on the periphery of the field and, with the microsurgical forceps, pick up the thread on the left of the incision about 1cm from the suture-holes (**Figure 21**).

(iii) Form a *double* loop around the tips of the needle-holder (**Figure 22**) by winding the thread clockwise around the jaws which are kept stationary close to the incision (i.e. working in the same plane just above the tissue).

(iv) Pick up the short end with the needle-holder tips (**Figure 23**) and pull with gentle tension whilst the double loops are allowed to fall from the jaws of the needle-holder thence over the short end of thread.

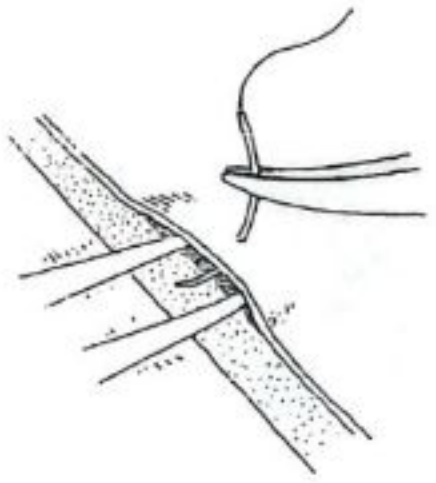


Figure 18.

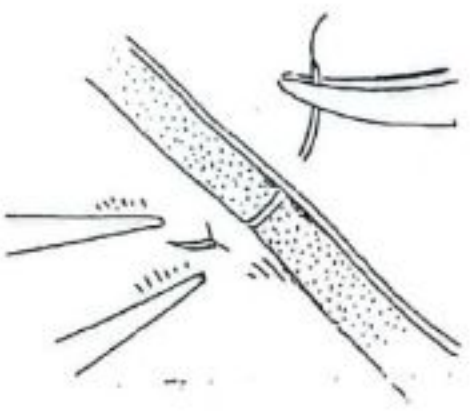


Figure 19.

Figure 20.

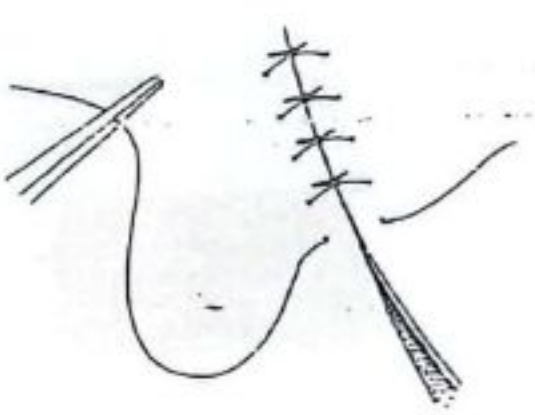


Figure 21.

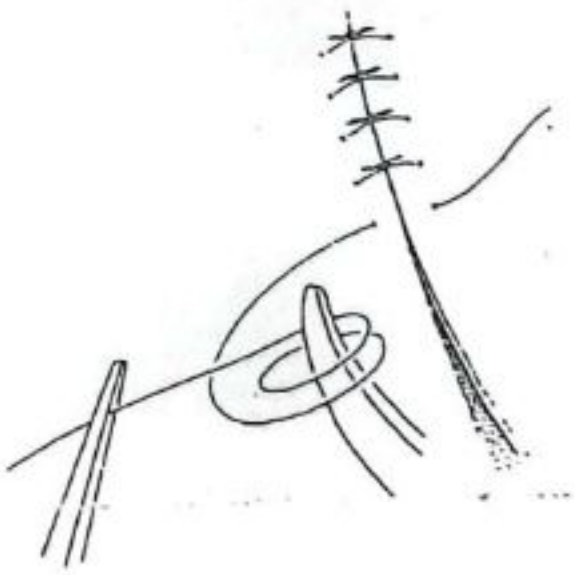


Figure 22.

Figure 23.

(v) The half-knot should now be placed accurately and then tightened carefully by tension on each length of thread until the edges of the incision come together without inversion, eversion or overlapping. Once the first half-knot is completed at the correct tension the second half of the manoeuvre should not overtighten it.

(vi) Place the needle-holder tips over the middle of the knot, pass a single loop of thread over it in the reverse direction, pick up the short thread again and pull both threads in opposite directions in a straight line, and at right angles to the incision.

(vii) For additional security, form another half-knot on top of and in the reverse direction to the previous half-knot.

(viii) Trim the short end of thread first, then grasp the long thread still attached to its needle and cut that close to the knot. By pulling on the thread, you will pull the needle into view ready to make the next stitch.

You have now completed a true "surgeons knot" which in practice you will only need for stay sutures but which are necessary in this case to hold the rubber material together. Later, you will find that in most situations you only need to make a 'square' or 'reef' knot in which a single loop is used on each half-knot. If the knots have been made correctly, the cut ends should lie at 90° to the incision and will not project downward into the incision nor interfere with the next stitch.

(ix) Repeat this with knots at 0.5mm intervals.

(x) Turn the card over to assess the suture lines.

(xi) Repeat this exercise with incisions at different angles e.g. diagonal upper left to right lower and so on.

Get into the habit at this early stage of releasing tissues and sutures whenever you have to emerge mentally or physically from the microscopic field. Otherwise, you will surely tear fragile tissues later on when operating on patients if your concentration is broken and you have to look up from the microscope.

Rabbit Aorta

For this exercise, an isolated 4cm length of rabbit aorta is pinned out on the cork board and cut in half. Again 8/0 monofilament nylon should be used to anastomose the two halves together. This is a good stage to learn the triangulation technique:

(i) Place two stay sutures (surgeon's knots) at 120° apart (Figure 24) one at 2 o'clock and the other at 10 o'clock and leave a long thread to each so that these can be held out.

(ii) Put each stay under tension and trap the thread between the head of a mapping pin and the cork board thus keeping the upper wall of the divided aorta under lateral tension.

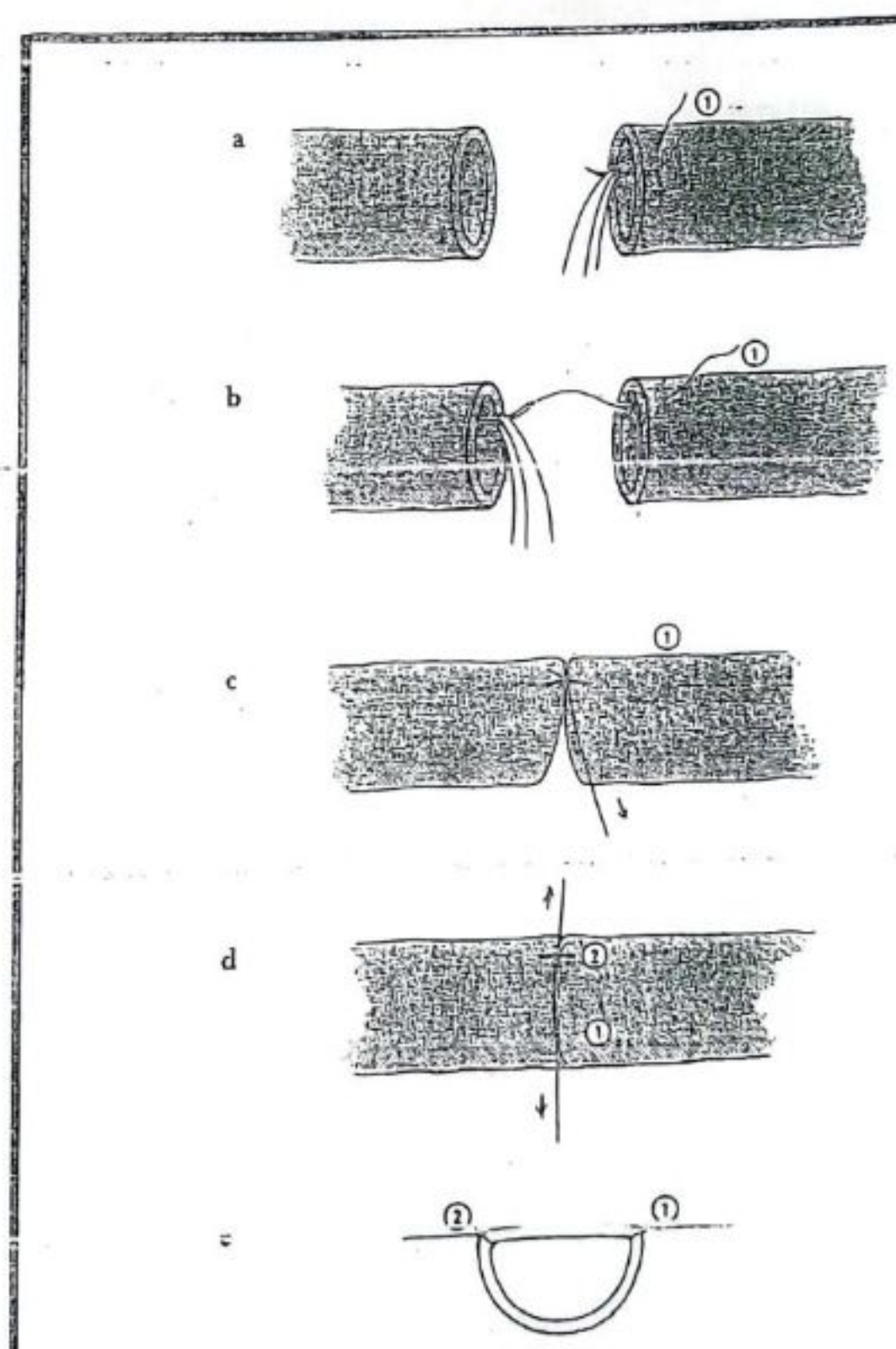


Figure 24. Stages of end-to-end anastomosis by triangulation technique. Placing first stay suture (1) a-c. *Plan view to show both stay sutures (1) and (2)* d. *Cross section* e *shows 'triangulation' effect of putting stays under tension.*

BASIC VASCULAR EXERCISES

Factors Affecting Success of Microvascular Anastomoses

FAILURE of patency in small blood vessels after anastomosis can be caused; by faults in microsurgical technique; by alterations in the laminar flow of blood; by a tendency to coagulation and thrombosis after operation due to release of coagulation factors; by spasm of the vessel musculature; or by a combination of each or any of these. Poor surgical technique is undoubtedly the most important and is the most susceptible to improvement.

Microsurgical faults: The most common technical mistake is to suture the front and back wall together thus constricting the lumen disastrously. Less obvious are tiny tags of adventitial tissue carried into the lumen on the needle point but sufficient to form a nidus for platelet aggregation in these small-bore tubes. The former problem is best avoided by using the triangulation technique described below, keeping the walls widely separated whilst sutures are placed, by repeated irrigation of the divided ends of the vessels and by making a careful inspection of the lumen before tying the final sutures. In the case of arteries, it is important to remove the loose adventitial tissue so that it is well clear of the suture line and less likely to be dragged into the anastomosis.

Gentle handling of the vessels is also of prime importance. The intima is particularly susceptible to damage so not only must the divided ends of vessels *never* be grasped within the jaws of microsurgical forceps but it is even dangerous to pick up and grip the whole vessel with these instruments. Instead, it should be manipulated by lifting gently from underneath with the sides of the forcep's jaws or by grasping it by its adventitia. Some workers suggest that even gentle mechanical dilation of the divided vessels is potentially damaging to intima and rely on pharmacological agents instead: however we have used special vessel dilators both to dilate the wall and to act as counterpressors with no obvious decline in patency rates so we believe they are valuable if used with tender care. Traumatic placement of sutures with tearing of needle holes and multiple punctures especially if the initial anastomosis has to be revised is far more likely to trigger platelet aggregation. Other potential causes of trauma are clumsy tests for patency, accidental cauterisation of the vessel wall whilst coagulating small adjacent vessels and application of excessive clamp pressure.

The divided edges of the vessels to be joined must abut each other for the full thickness of the wall (i.e. adventitia, media and intima). Blood components will be exposed to collagen and provoke thrombus formation whenever there are gaps in the endothelial lining, when insufficient sutures have been placed and when the vessel edge has been inverted and projects into the lumen.

Incorrect tension on each individual suture is also likely to cause problems. If they are too loose, a loop of nylon will project into the lumen to form a focus for thrombus formation, whereas if they are tied too tightly, the needle holes may tear and the vessel wall may be bunched up and undergo necrosis. Similarly if too big a bite of wall is taken, the edges of the vessel may be everted, inverted or constricted. The correct bite and tension is indicated by a small suture loop whose diameter is roughly equal to the wall thickness, and which is visible from the inside of the vessel.

If the whole anastomosis is put under longitudinal tension either because the natural elasticity of a divided artery has allowed too big a gap to form or because in the clinical situation, the vessels have been traumatized and need resection, then it is preferable to bridge the gap with an interpositional vein graft. Too much tension which cannot be reduced within a double clamp approximator will cause stenosis of the vessel and tearing of the suture holes. Again these will predispose to thrombosis.

Finally, kinking or longitudinal torsion will again alter laminar flow and predispose to loss of patency. There is really no excuse for this in laboratory work but it can be a problem clinically when positioning free flaps or replants.

Alterations in blood flow: Eddy currents and other forms of turbulent flow resulting from bends, kinks or gross distortion at the anastomotic site are likely to propagate thrombi downstream. Turbulent flow can be observed most easily in an end-to-side anastomosis but these cause surprisingly few problems so long as the suture line is not under tension and the incoming vessel is set at an angle between 60° to 45° with the direction of flow in the recipient vessel.

More important in any tissue transfer is poor flow or even complete stasis, particularly at the venous anastomosis. The latter will be at double risk if the arterial flow is inadequate because of partial patency and hence flow through the microcirculation in the tissue is sluggish. Most surgeons like to check the patency of the artery before proceeding to the venous anastomosis. Similarly, if a clamp is released then applied again, it is important to remove any static blood by vigorous irrigation with heparinised saline before it starts to coagulate.

Release of coagulation factors: Any tissue or organ which has been compromised by hypoxia or ischaemia will build up toxic metabolites, including lactic acid, hydrogen ions, oxygen-derived free radicals and high concentrations of potassium. These, together with thrombogenic factors such as bradykinins, serotonin and sensitised platelets, will be washed out of the tissue through the venous anastomosis once the clamps are released and further increase the risk of thrombogenesis, particularly if the flow is sluggish. Poor outflow is therefore the greatest risk in flaps and replants and has led to many attempts to improve results by local or systemic administration of inhibitors or platelet aggregation such as aspirin, persantin, ticlopidine and prostacyclin (PGI_2) as well as attaching leeches to the tissue. Their true worth awaits really critical evaluation but at least they do not appear to worsen the outcome whereas anticoagulants such as heparin and

dicumarol are probably contraindicated.

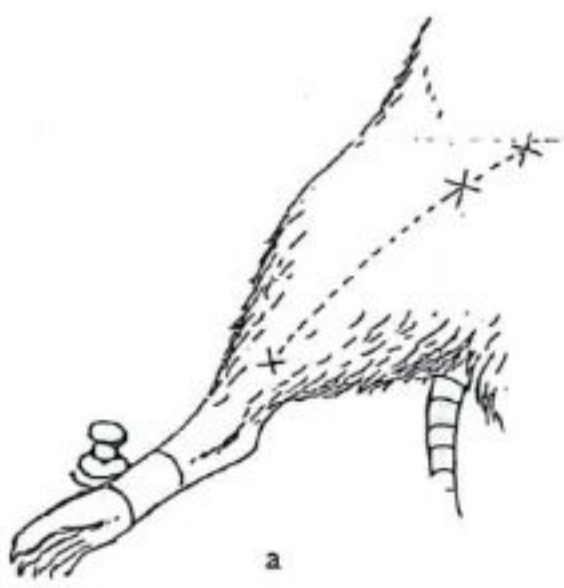
Spasm of the vessel musculature: Spasm is almost inevitable at some stage in a microvascular procedure and may be provoked locally: by surgical manipulation; by exposure at the operative site to an unusually low ambient temperature; by allowing the operative site to become dry; allowing blood or blood products in the operative field; and by abnormal pH and release of toxic factors from locally injured tissues. It may also be provoked systemically via the sympathetic system by release of catecholamines. Prevention consists of careful dissection and constant irrigation of the immediate viscinity of the anastomosis. Treatment is best achieved by drops of 1% procaine locally and allowing 10-15 minutes for the spasm to be relieved. It is important to relieve spasms *before* attempting an anastomosis, otherwise it is impossible to space sutures evenly and the suture line will leak once it is relaxed.

Dissection and Preparation of Rat Femoral Vessels

Exposure: Place the anaesthetised rat on its back and tape out the hind legs in a slightly extended position but do not extend the forelimbs or tape them down. The femoral triangle is bounded proximally by the inguinal ligament. The femoral vessels lie deep to a thick inguinal fat pad and are covered by a thin sheet of fascia. The first stage of the dissection is to make a longitudinal incision from the inguinal ligament to just above the knee (**Figure 27a**). The fat pad should be picked up and incised at the distal end and raised by blunt dissection until the femoral artery (0.8-1mm external diameter) and vein (2-2.5mm external diameter) are revealed together with the nerve entering the leg through the femoral canal. The fat pad should be retracted laterally to the left at the same time hooking a retractor over the abdominal musculature and inguinal ligament and retracting this medially (**Figure 27b**).

The full length of artery and vein can be visualised to the distal point of the femoral triangle. Here the femoral vessels give off the superficial epigastric artery and vein which ascend and supply the skin and subcutaneous tissue of the groin and posterior two-thirds of the abdomen. Halfway between these vessels and inguinal ligament, both the femoral artery and vein give off the profunda femoris vessels which dive deep into the underlying muscle; these are important in this dissection as they have to be ligated and divided to leave sufficient length of femoral vessel for the double approximating clamp to be placed and turned over easily.

Having thus exposed the whole of the femoral triangle, the next stage in the dissection is to separate the loose



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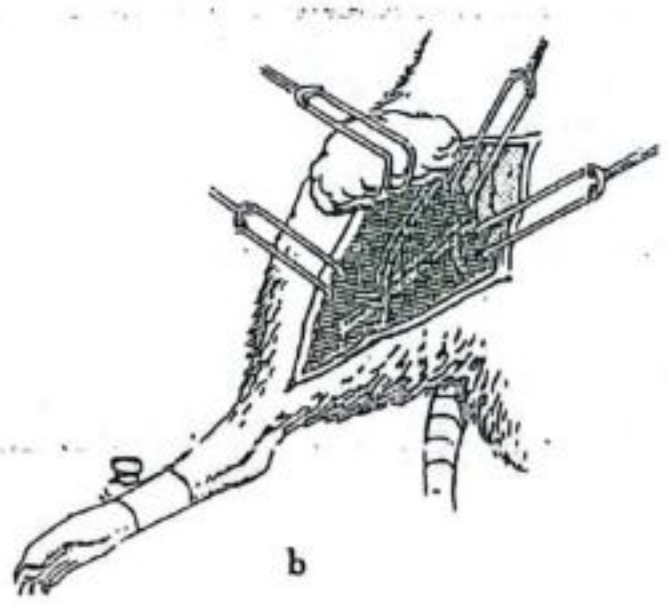


b

Figure 27. *Dissection of rat femoral triangle* a *skin incision* b *femoral artery and vein and retraction of fat pad.*

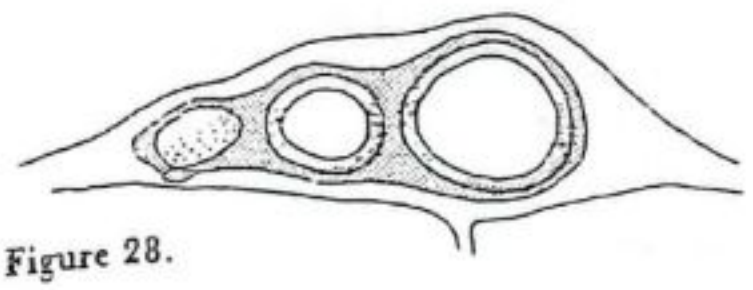


Figure 28.

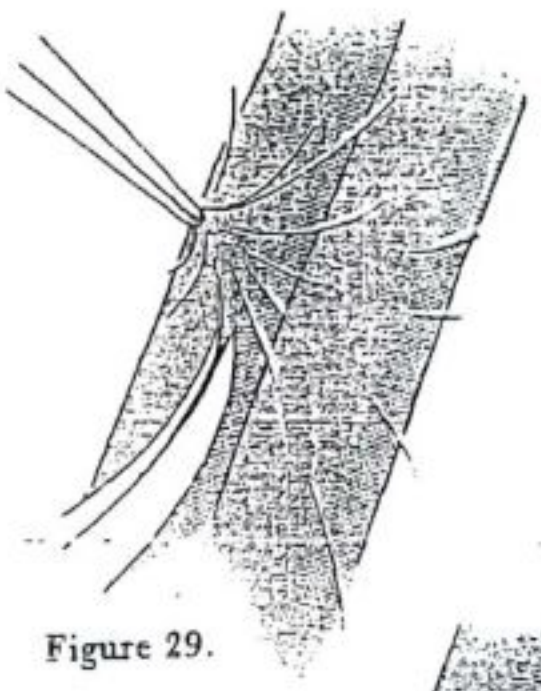


Figure 29.

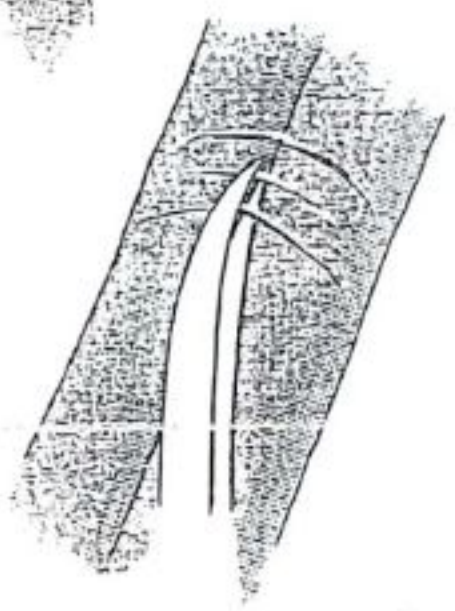


Figure 30.

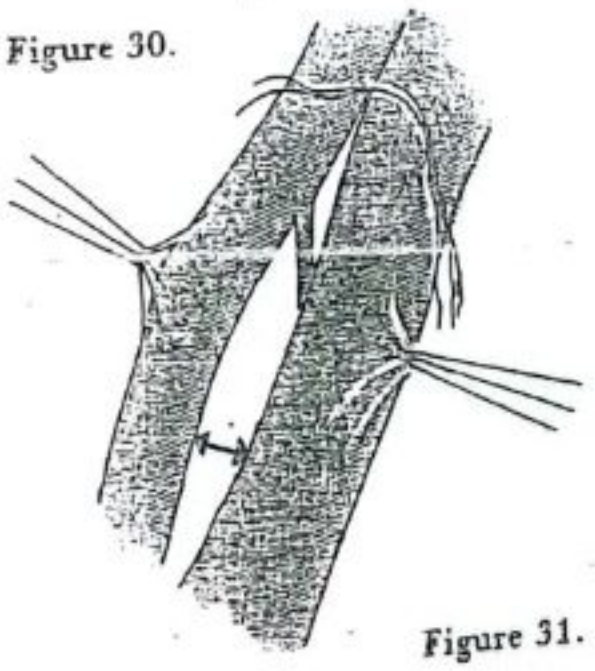


Figure 31.

overlying fascia and then prepare the artery and vein. First, under low magnification (4x) pick up the dense femoral sheath which surrounds the neurovascular bundle (**Figure 28**) with No. 5 microsurgical forceps gripping the sheath well away from the vessels. Nick the sheath at its distal end with the blades of the dissecting scissors parallel with the surface and slide one blade into the nick and thence away from you along the sheath (**Figure 29**). The sheath is thus opened longitudinally for the full length of the femoral triangle (**Figure 30**). The artery can now be freed from the septum by gentle lateral and longitudinal teasing using microsurgical forceps in each hand (**Figure 31**), preferably the No. D-5a vessel dilating type which have rounded atramatic points. We do not recommend the technique of holding the vessels with a pair of microsurgical forceps whilst cutting the adventitial tissue with scissors as this is more likely to result in tissue trauma or inadvertant stripping of the adventitia. Remember that the nutrient vasa vasorum run through the adventitia so this should be left as intact as possible: the vessels should be picked up only by the loosely attached adventitial tissue or raised from underneath with the sides of the closed forceps tips.

Time spent at this stage of preparation will be handsomely rewarded when the anastomosis has to be performed. It is essential to clear both artery and vein for their full length within the femoral triangle. However, it is also true that the vessels should be handled as little as is consistent with adequate exposure. Longitudinal stress must be avoided to minimise the risk of constriction and spasm. Having freed the artery for its full length, it is time to ligate and divide the profunda femoris artery. Using No. D-5a forceps, pass a 7/0 suture around the branch and tie off close to the femoral artery. Leave the ends of the suture long so that these can be pulled to one side. Gentle traction on the profunda femoris artery allows access for the second ligature to be placed before the vessel is divided between ligatures (**Figure 32a, 32b**).

Alternatively, it is possible to slide No. D-5a forceps under the profunda femoris artery, raise it gently clear of the parent vessels and then cauterise it.

The vein must be dissected with even more care. Scissors should definitely not be used and the surrounding tissues should be teased *away* from the vein with No. D-5a forceps. The veins are best handled by the small pieces of fat adherent to their wall. Once again the profunda vessels should be ligated and divided. Finally, the whole field is irrigated with physiological saline, any blood clots remaining are removed with cellulose microsurgery swabs and a piece of background material is slipped over the vein and under the artery so that the two vessels are temporarily isolated from each other (Figure 33a).

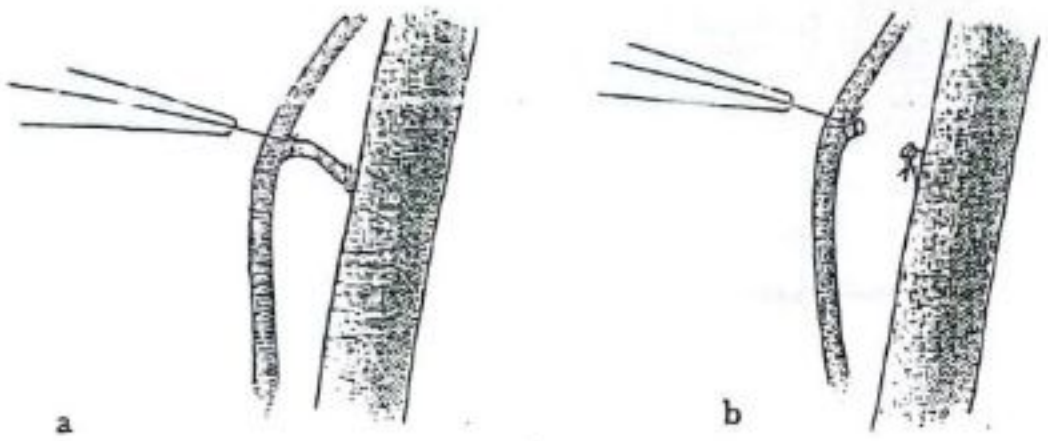


Figure 32.

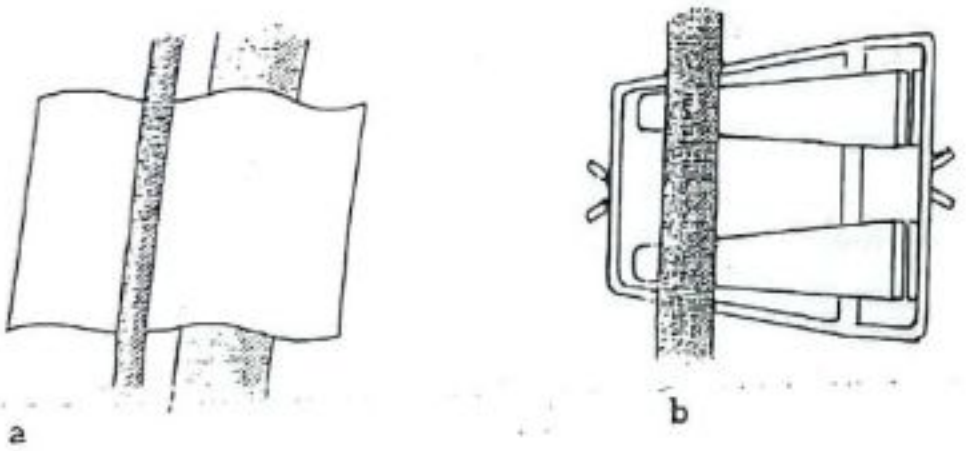


Figure 33.

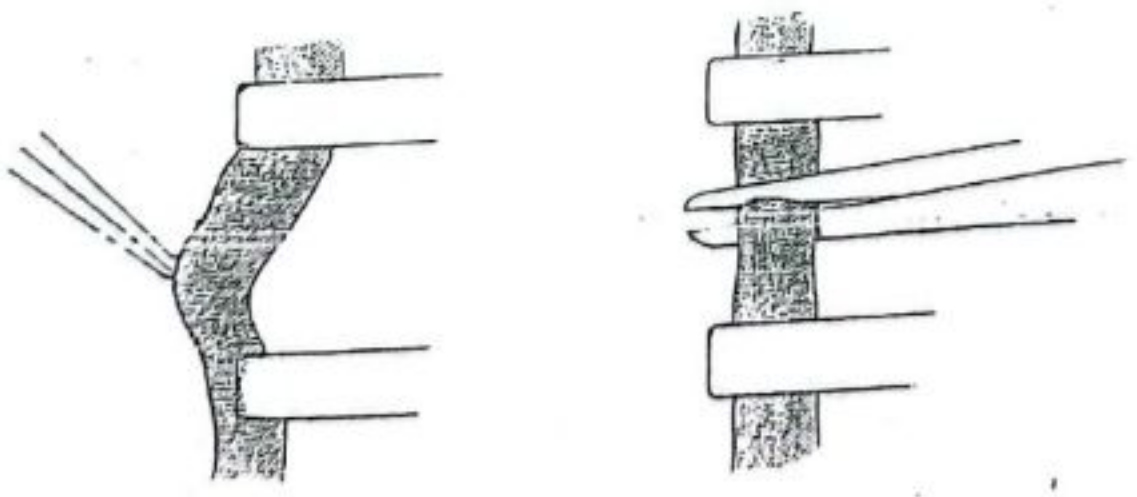


Figure 34.

Figure 35.

End-to-end Arterial Anastomosis

Applying the vessel approximating clamps: The routine described here is for the Acland vessel approximating clamp (ACC-1) but this can be adapted to other types of double clamp on a slide bar. The main point of having the two clips on a bar is to allow them to slide toward each other and thus ensure that the divided ends of the vessel are brought close together and are under no tension whatsoever whilst being anastomosed. Having rinsed the ACC-1 in heparinised saline, place it in the operating field viewed under low magnification and slide the two clips as far apart on the bar as possible. Then slide the whole frame over the background material and under the artery until just the clip tips project slightly beyond the vessel with the artery lying in a straight line over the two clips (Figure 33b). With the clamp appliers, open first the proximal clip, grasp the artery by its adventitia and pull it longitudinally into the open clip before allowing it to close. Now open the distal clip and again pull the artery longitudinally toward the centre of the frame, slide it into the clip and allow this to close. This manoeuvre ensures a relaxed length of artery between each clip. Do not twist the vessel and ensure that it is held at the *tip* of the clip otherwise, when divided, it will either leak or slip out of the wedge grip altogether (Figure 34).

Dividing the artery: Now, with the scissors at a right angle to the vessel wall, the artery should be transected halfway between the two clips with one decisive but controlled movement to achieve a clean cut through the full thickness of the vessel (Figure 35). The divided ends will retract immediately.

Irrigate the vessel with heparinised saline using a 30-gauge Rycroft air cannula attached to a 1ml syringe; this generates enough pressure to dislodge all but the most adherent clots from the lumen (Figure 36). Only if these refuse to budge should the vessel ends be folded back over the clips and stroked gently to remove any remaining clots (Figure 37). The clips are now slid gently toward each other until the retracted vessel ends are close together. If ample arterial length has been included in the clips initially whilst these are fully separated, then they will only need to be pushed together a little, thus leaving plenty of room to do the anastomosis without them getting in the way when the needle is being passed.

Figure 36.

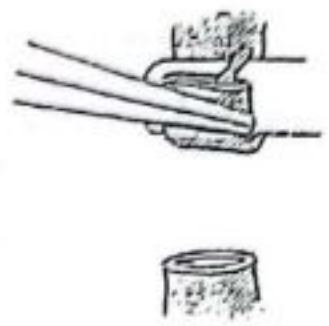


Figure 37.

Final preparation of the artery: The femoral triangle should now be thoroughly irrigated until it is full of fluid and the divided ends of vessel are floating freely. Only then is it possible to see the loose adventitial tissue properly. It is important to remove this for 1-2mm from the anastomosis for the reasons already stated above. The simplest method of doing this is to grasp the tissue with No. D-5a forceps, pull it gently over the vessel-end until a cone or sleeve is formed and then amputate it cleanly in one snip at the level of the underlying stump (Figure 38). The remaining tissue will retract back, leaving the vessel with a clean end (Figure 39).

The anastomosis: Interrupted sutures of 10/0 monofilament polyamide or monofilament polypropylene are used in all these exercises on rat femoral vessels. Some surgeons prefer a continuous suture pattern, particularly on veins, but in our experience it is never possible to appose the vessel walls so accurately or with equal tension around the anastomosis. The simplest technique (though not always possible in the clinical situation) is to join these vessels end-to-end using the so-called triangulation method. Two stay sutures are placed 120° apart so that when they are placed under tension the front wall of the anastomosis is stretched laterally and the back wall falls away from the front; it is then much less likely to be picked up inadvertently by the needle when completing the suturing of the front wall.

The 10/0 sutures are handled in the same way as the 8/0 in the exercise described earlier (p. 21). Again the needle is grasped just behind its mid-point in the needle-holder and withdrawn from the needle 'park' in the packet. Place the needle in the operative field (4 to 6x magnification), grasp the nylon about 6mm from its needle with forceps and lift it so that the needle point is just resting on the tissue. Pick up the needle by grasping it with the tips of the needle-holder just behind the needle mid-point (Figure 40). It should now lie comfortably at right angles to the jaw of the holders but if it needs adjustment, the grip

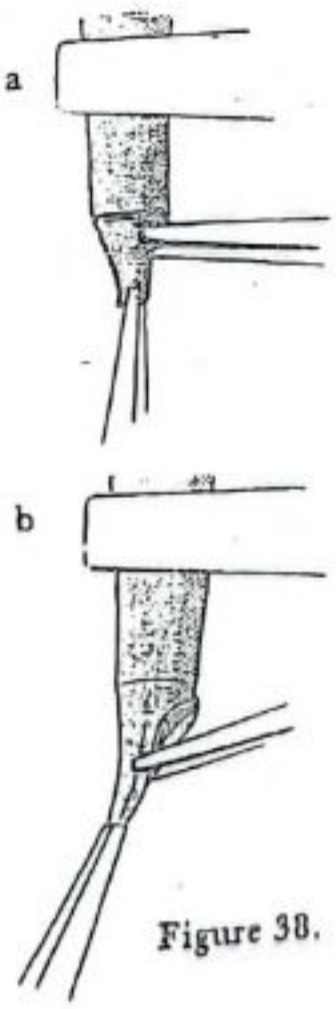
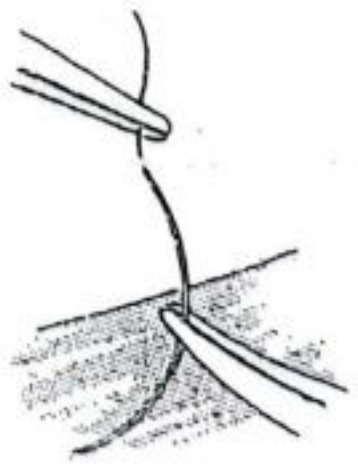


Figure 38.

Figure 39.



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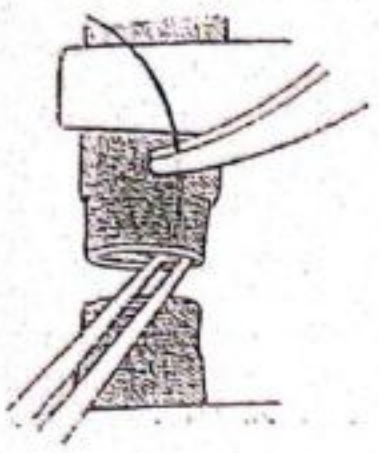

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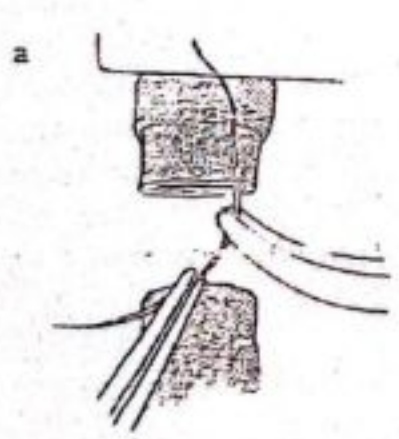

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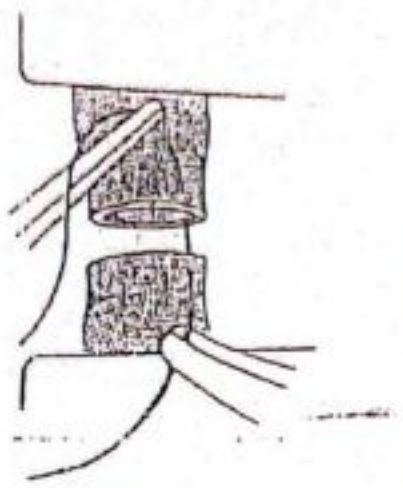
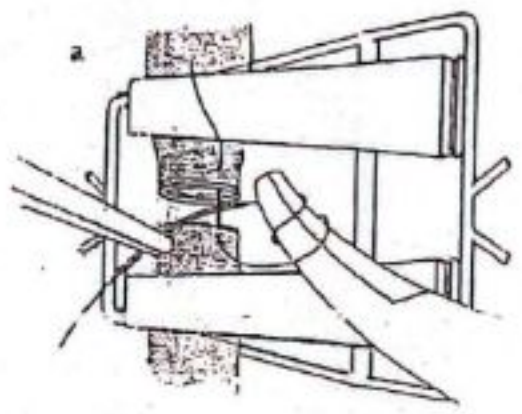
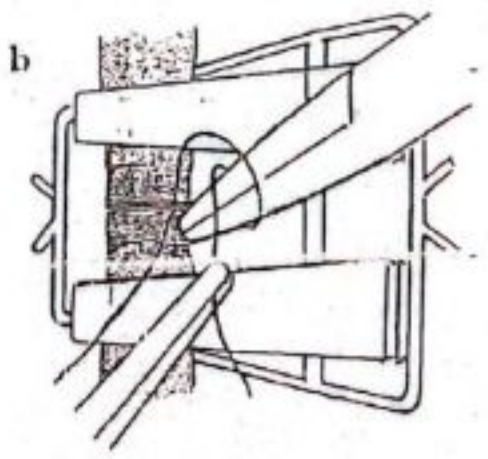


Figure 42.





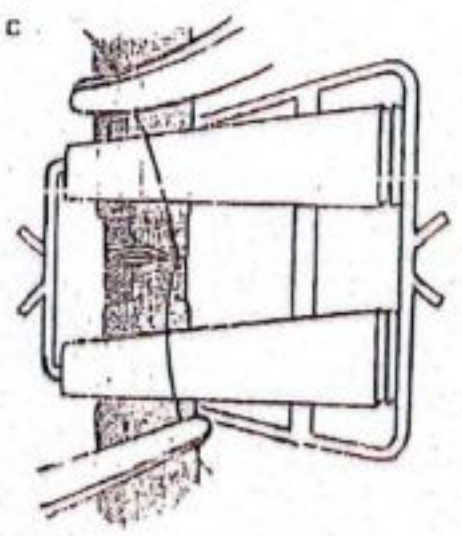


Figure 43.

should be slightly relaxed and the needle should be brushed gently backwards over the backing material.

The first stay suture is now placed at 2 o'clock around the circumference. Using the mid range magnification place the closed tips of vessel-dilating (No. D-5a) forceps just inside the lumen of the proximal vessel and slightly open them. Then place the needle tip overhead and slide it back to a point of entry about the thickness of the vessel wall from the edge. Pass the needle perpendicularly through the vessel wall so that it emerges between the forcep's tips acting as counterpressor (**Figure 41a**) and follows the curve of the needle until over two thirds of its length is through (**Figure 41b**). Transfer the needle-holder to the lumen, grasp the needle about its mid-point and pull it through clear of the wall together with about 4mm of suture. The adventitia of the distal vessel is now grasped with the forceps and is used as a counterpressor whilst the needle is passed perpendicularly from the vessel lumen outward (**Figure 42a, 42b**). The holder grip is again transferred to the front segement of the needle and the suture is slowly pulled through in one continuous movement, using the closed forceps to guide the suture through the proximal entry point in a straight line and so prevent it tearing and enlarging the hole toward the edge of the vessel (**Figure 42c**). Leave about 3-4mm of suture projecting from the entry point. Alter the magnification to about 6x and lay the needle down near the edge of the visual field where it can again be picked up easily. Now grasp the long arm of suture with the forceps about 1cm from the exit point and form a double loop around the tip of the needle-holders by winding the suture clockwise whilst keeping the holder tips stationary close to the vessel edges (**Figure 43a**): this avoids the risk of pulling the whole suture through inadvertently so that the entire procedure has to be started again. The short arm of the suture is now picked up in the holder jaws and pulled from top to bottom at the same time as the long arm still gripped by the forceps is carried over from bottom to top. The

double throw must lie flat against the tissue at right angles to the suture line, and just bringing the vessel edges together until they abut each other. To form the second part of the surgeon's knot, place the needle-holder tip over the middle of the existing half-knot (**Figure 43b**), pass a single loop of suture over it in the reverse direction, pick up the short thread again and then pull both threads in opposite directions in a straight line and at right angles to the vessel edges (**Figure 43c**). For additional security, place a third tie as a forehand loop. Now trim only the short end of the suture. Again using forceps and needle-holder, secure the stay suture in the cleat in a figure-of-eight placing the stay under gentle traction (**Figure 44**). Now place the second stay on the opposite side of the front wall 120° apart and secure it to the other cleat with sufficient traction to stretch the anastomosis line laterally (**Figure 45**). The back wall should now fall away from the front. Remember, however, that the more lateral tension you use the closer the vessel ends will need to be approximated if longitudinal tension is to be avoided.

The front wall is now sutured using square or reef knots which must lie flat against the anastomosis line. Under no circumstances must 'granny' knots be made as the ends can easily project through the suture line and into the lumen. First, place one suture (the holding suture) equidistant between the two stay sutures (**Figure 46, 3**) and leave one end long so that it can be grasped with forceps to raise and stabilise the vessel walls whilst intermediate sutures are placed. Now turn the clamp over *before* tying this suture taking great care that the cleats do not snag or puncture the adjacent vein, and make sure that you have not picked up the back wall. If all is well, return the clamp back again and complete the reef knot. The next suture is placed near the first stay suture, and thereafter they are placed at intervals approximately one needle diameter apart working toward the middle. Then the other quadrant of the front wall is completed in the same way (**Figure 47**). The long uncut end of the middle suture has been used to hold the anastomosis for each of these sutures thus avoiding all handling of the media or intima, and this technique is simpler than picking up the adventitia with forceps. Each suture must penetrate the full thickness of the vessel wall and the bite should be about the same as the vessel wall thickness. For these intermediate sutures it is easier to pass the needle through both sides of the anastomosis in one movement without releasing the holder.

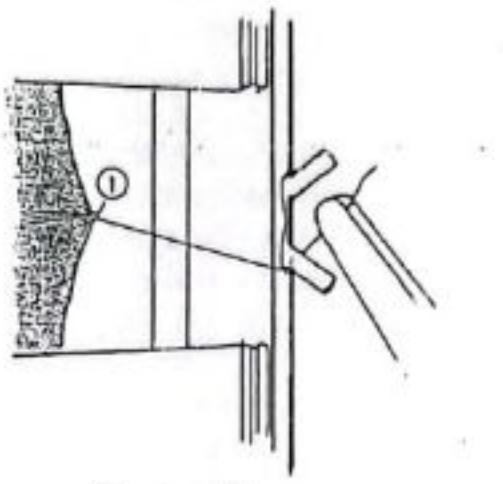


Figure 44

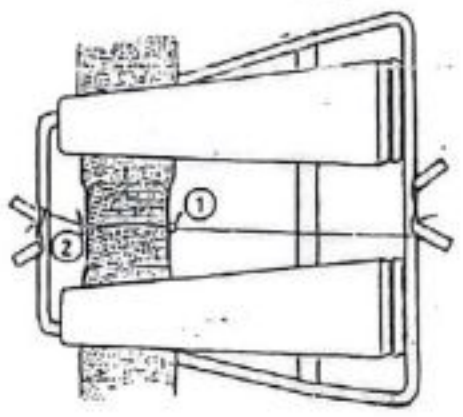


Figure 45

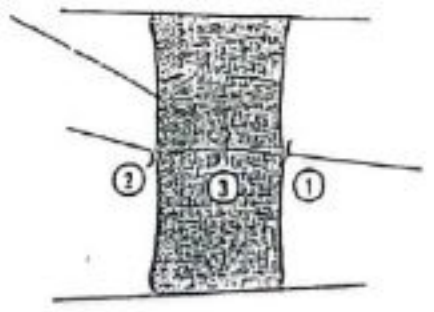


Figure 46

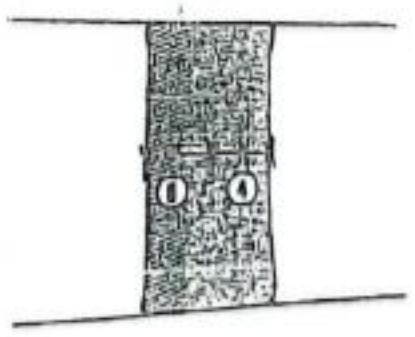


Figure 47

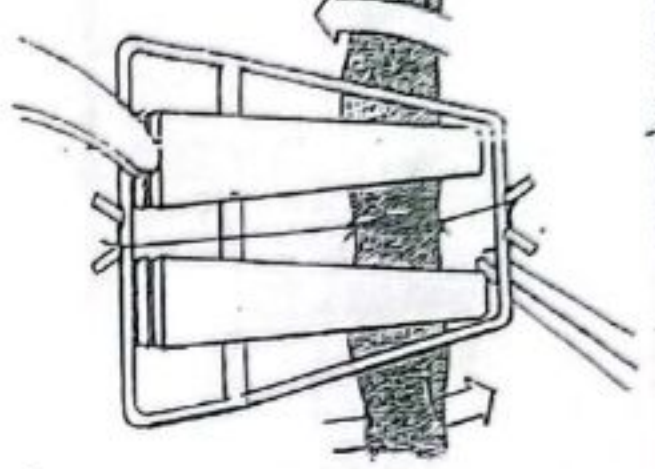


Figure 48

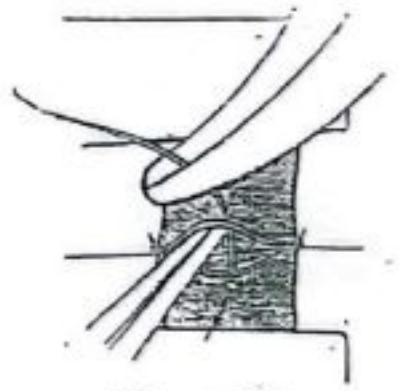


Figure 49

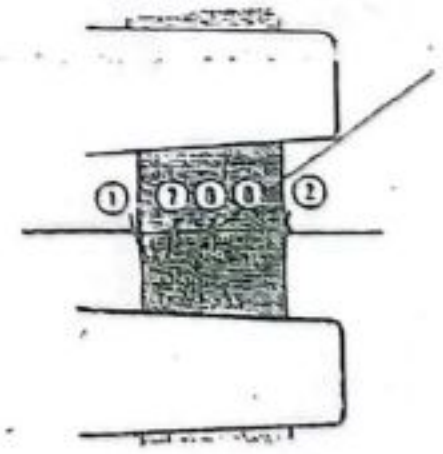


Figure 50

Figure 51

Having completed the front (or anterior) wall, the approximating clamp is rotated 180° with the artery in place so that the back wall is revealed (Figure 48). However, before lifting the clamp, it is essential to irrigate the field and ensure that the metal is not adherent to surrounding tissues. The clamp frame is raised vertically with the needle-holders, and No. D-5a forceps are used to press down on the background material at the same time as they grasp the opposite side of the frame and slide it over the irrigated (hence slippery) plastic. Before starting to suture the back wall, check again that the lumenal side of the front suture line looks good, ensuring particularly that there are no obvious loops of nylon projecting to indicate knots tied too loosely, and then irrigate the lumen with heparinised saline. Finally, because in rotating the clamp the artery has been twisted and subjected to longitudinal tension, it is important to nudge each clamp a little closer together to ensure that the suture line is not under tension.

Again place the first (holding) suture equidistant (Figure 49) between the two stay sutures and, after tying a square knot, leave one end uncut so that this can be grasped in forceps to pull the back wall away from the front and stabilise it whilst the needle is passed for the intermediate sutures (Figure 50). It is also used to put slight lateral tension and to aid in even placement of the sutures — otherwise it is easy to place those nearest the stay sutures too far apart with disastrous consequences later when blood flow is resumed. Now, complete the anastomosis as before, suturing each quadrant separately and working from the stays to the centre. Remember throughout to use high magnification to place the sutures and low magnification whilst tying the square knots. Depending on the size of vessel we use 8 to 10 sutures for the complete anastomosis (Figure 50).

After thoroughly irrigating the field, the clamp is now rotated back to its original position and the artery is checked to ensure that it is not stuck to the metal frame. Divide the stay sutures close to the knots. The distal clip is opened first and No. D-5a forceps are used to slide the vessel out of the clip. Blood should immediately fill the vessel back to the proximal clip having crossed the suture line. Oozing of blood must be expected for a few minutes but profuse bleeding must be considered a surgical failure and the suture line should be inspected under high magnification. Once the anastomosis appears good, open the proximal clip, and remove the approximator and place it in heparinised saline. Observe the anastomosis under low magnification, apply gentle pressure over the suture line with microsurgical swabs and wait 2-3 minutes for the suture holes to become plugged and all bleeding to stop. Re-examine the suture line and if bleeding starts again re-apply the swab; only if blood is obviously pumping out in pulsatile fashion should it be necessary to insert another suture.

The description above assumes not only easy access but also vessels of comparable diameter. In some clinical circumstances it is not possible to use a double approximating clamp in which case single clamps have to be used and the stay sutures have to be attached to Kee clamps heavy enough to hold them out under tension. It may also be impossible to use the triangulation technique because the vessels cannot be turned over easily in which case it may be necessary to suture the back wall first with one suture then gradually work round until the anastomosis is completed (Figure 51).

There are various ways of coping with discrepancy in vessel size. The first solution is to do an end-to-side anastomosis but if this is not possible, there are three other techniques available.

(i) If the discrepancy is relatively minor then it is possible to dilate the smaller vessel by gently inserting dilating forceps and spreading their tips but of course there is then the risk of damaging the endothelial intima.

(ii) If the difference is greater, then the end of the smaller vessel can be transected on a 45° diagonal thus providing a large diameter edge to anastomose (Figure 52a, 52b).

(iii) Alternatively, the end of the small vessel can be incised longitudinally from the end to create a 'fishtail' which can then be sutured to the larger vessel (Figure 53a-53e).

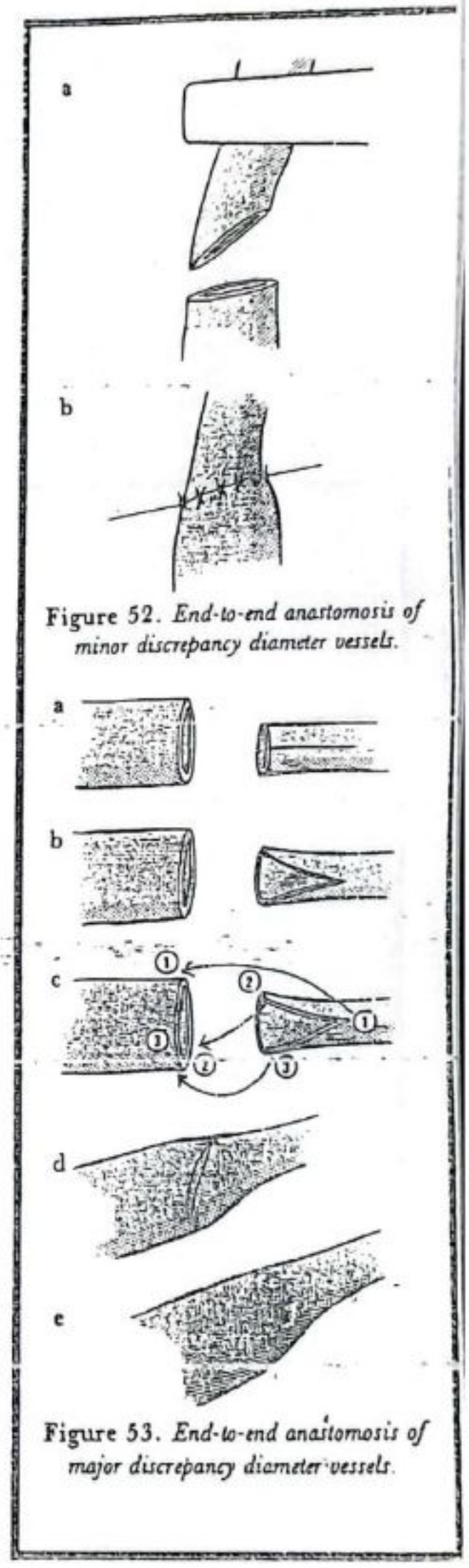


Figure 52. *End-to-end anastomosis of minor discrepancy diameter vessels.*

Figure 53. *End-to-end anastomosis of major discrepancy diameter vessels.*

In each of these methods, it is difficult to perform the simple triangulation technique so great care must be taken not to suture the front to back walls by mistake. It is also even more important to ensure that the vessels are under absolutely no tension, otherwise the smaller vessel is likely to tear. Similarly, you must ensure that the sutures are placed at the same relative position around the circumference of the small and large vessels, otherwise the final sutures will be impossible to match up.

Assessment of vessel patency: Even under high magnification it is difficult to check that the vessel is patent by simple observation because longitudinal pulsation can be transmitted distal to the anastomosis. However, if arterial tributaries (eg. the superficial epigastric artery) are pulsating, then the femoral artery must be patent. A more reliable test is to place a pair of closed forceps under the artery distal to the anastomosis, gently raise the vessel until the blood flow is almost occluded and then slowly lower it again (**Figure 54**). If the vessel is patent, you should observe alternating collapse and filling with the pulsatile flow and a 'flicker' of blood over the partially occluded position. These are the only permissible tests in clinical work or where the animal is to survive after operation. Where the exercise is completed and the animal is to be killed, the distal segment can either simply be transected and blood flow observed or the 'milking' test can be performed. The latter is very reliable but too traumatic to recommend in survival surgery. The vessel is occluded just distal to the anastomosis with a pair of forceps (**Figure 55a**) before the vessel is 'milked' for 3-4mm distally with another pair of forceps which are then used to occlude the vessel. There is thus an empty length of vessel lying between two occluding forceps and if the anostomosis is patent, this should rapidly refill as soon as the proximal pair of forceps are released (**Figure 55a, 55b**). If the anastomosis is not patent, it is sometimes possible to open it by manipulation of the vessels with forceps or by stroking a microsurgical swab over it. However, it is usually better to accept defeat, reapply the approximating clamps, excise 0.5mm on each side of the suture line, and start again.

End-to-end Venous Anastomosis

The veins are very thin walled, lack a substantial muscularis and are easily torn. They are best handled by the small pieces of fat adherent to them.

The profunda femoris vein should be divided between ties and not simply cauterised otherwise they frequently continue bleeding afterwards. Once freed for its whole length from the inguinal ligament to the superficial epigastric vein, background material should be slipped *under* the vein and over the artery to isolate the two vessels from each other. Now slide each clip as far apart as possible on the track and position the ACC-1 approximating clamp under the vein. Slide the vein into the distal end first, gently pulling

Figure 54.

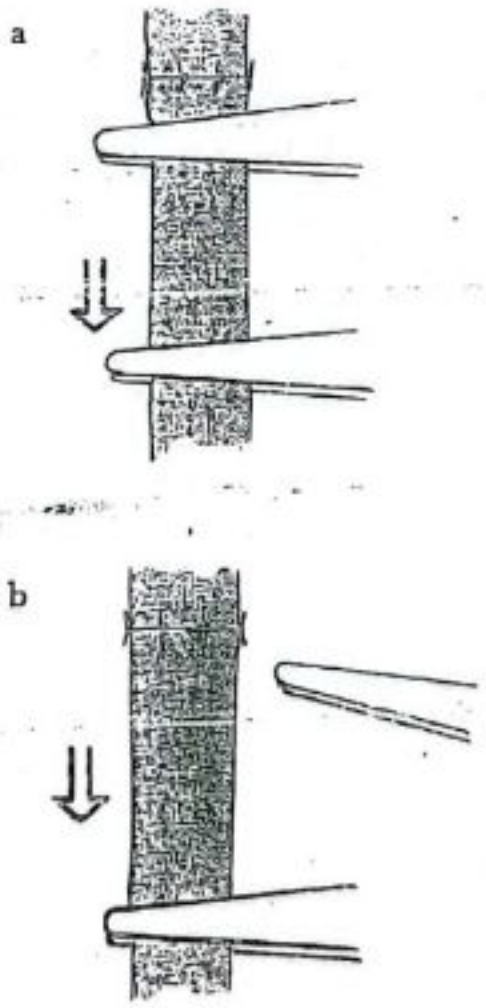


Figure 55.

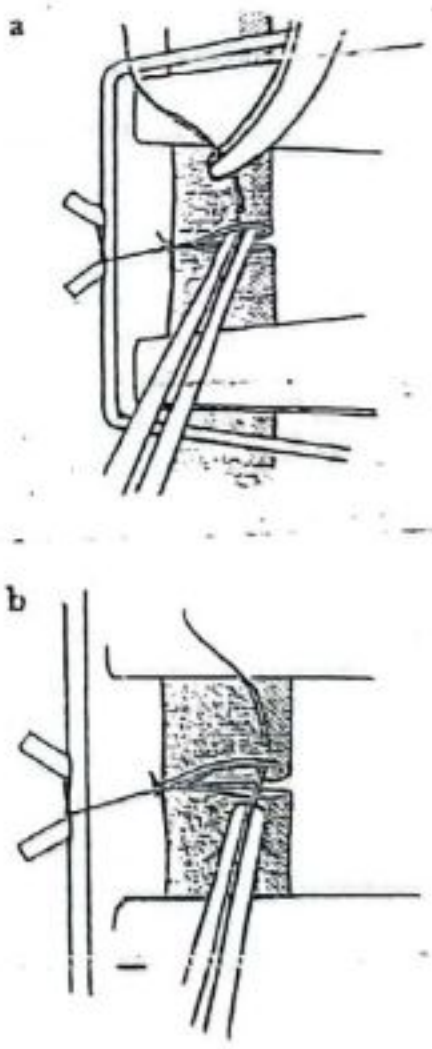


Figure 56.

longitudinally as much vein as possible into the framework of the ACC-1. Repeat this with the proximal clip. In each case, make sure the vein is as close to the tips as possible otherwise the full width of vessel may not be occluded and the divided ends will leak. Now clean off any loose tags of adventitial tissue and adherent fat but do not attempt to strip a sleeve of adventitia as in the artery or you will end up with no vessel wall to join. Transect the vein with one clean movement of the scissors, irrigate with heparinised saline and fold back each segment of vein over its clamp to display the lumen and orientate the vessel walls. Then slide the two clamps toward each other to approximate the vessel ends yet leaving sufficient working space between them to perform the anastomosis in comfort. Because veins are so thin-walled and friable, they have an even more pronounced tendency for the front and back walls to adhere together so accurate placement of the two stay sutures 120° apart is more important (**Figure 56a, 56b**). We like to submerge the veins in heparinised saline and place the 10/0 stay sutures at high magnification (16x) but dry the vessel ends whilst the knots are being tied. The stays are attached to the cleats as before with just enough traction to ensure that the front wall is under sufficient lateral tension to hold the two edges together. Now place a holding suture in the front wall equidistant between the stay sutures but, before tying, rotate the clamps and examine the lumen of the vein to ensure that the needle has not picked up the back wall (**Figure 57a**). Then form a reef knot leaving one end long enough to grip with forceps and pull to the left side whilst other sutures (**Figure 57b**) are placed at about two needle diameters apart, starting nearest to the first stay suture, and working in toward the holding suture (**Figure 57c**). Then place similar sutures tied as reef knots in the other segment, having pulled the holding suture to the right. We believe it is worth rotating the clamps and checking every suture before proceeding to the next to ensure that the back wall has not been picked up. Once the front wall is completed, rotate the approximator through 180°, nudge the clamps together a fraction and thoroughly irrigate the field with heparinised saline. The lumenal surface of the anterior wall is now finally inspected to ensure that it is correctly apposed. The back wall anastomosis is performed slightly differently in that a holding suture is placed equidistant between the stay sutures as before leaving one end long for traction, but each segment is further subdivided with sutures which are tied but left with one end long. The spaces between are then filled with sutures starting from the st sutures and working in and these are tied as reef knots. Between 8 and 10 sutures will be sufficient for these vein The anastomosis is completed by rotating the approximator back to its original position, cutting the stay sutures and releasing the clamps (proximal first, distal next) and then applying a wet swab until oozing of blood stops. It is worth instilling a few drops of 1% procaine around the femoral vessels at this stage to ensure full dilation and minimise the risk of stasis in the vein.

As discussed earlier the risk of thrombosis at the venous anastomosis far higher than on the arterial side partly because of the greater technical difficulty and risk of prolonged clamping, but also because of slower flow or even stasis for a time after the clamps have been opened.

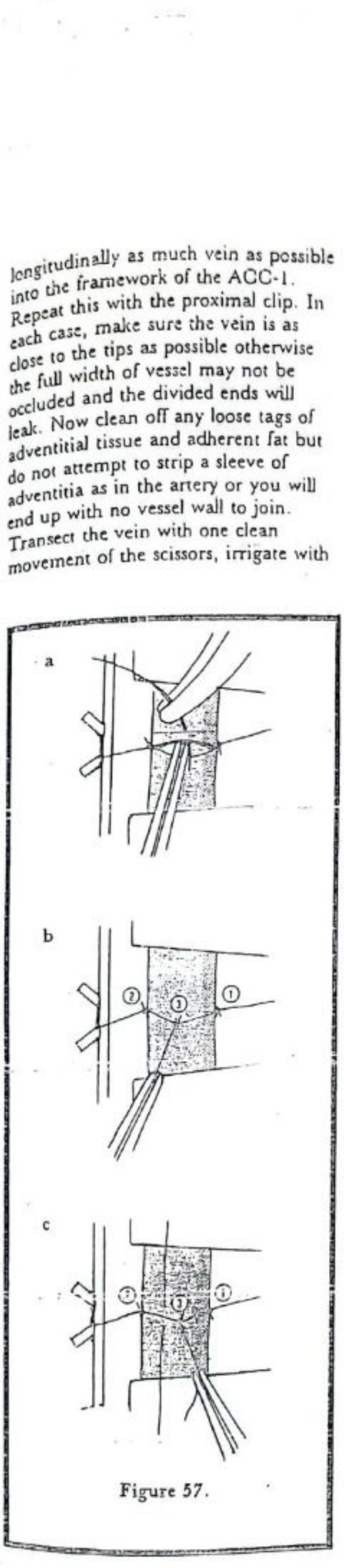


Figure 57.

End-to-side Anastomosis

In the rat, the most convenient and simplest model for end-to-side anastomosis is to attach the end of the femoral artery to the side of the femoral vein. Both vessels are prepared as before to gain maximum length and the profunda femoris vessels are divided between ligatures. Then slip the backing material under *both* vessels and instil a few drops of 1% procaine around them to prevent spasm. Now using a single Acland clamp, occlude the artery as near to the inguinal ligament as possible, ligate the distal end of the artery close up against the superficial epigastric origin and transect the vessel straight across close to the tie (**Figure 58**); some surgeons prefer to transect the artery obliquely but we find this creates more problems. Then remove adventitial tissue by the sleeve technique. The approximating clamp is next positioned under the femoral vein as close to the inguinal ligament as practicable; it is important to clamp the vein here so that the anastomosis can be performed proximal to the point where the femoral artery has been divided so that no tension will be placed on the vein wall. With each clip

as far apart as possible on the track, slide the vein into the proximal clip first and the distal clip next as this ensures a ballooned vein in which a venotomy can be created with less risk of damaging the opposite wall (Figure 59).

Slide the clips toward each other slightly to release any tension (Figure 59). Now bring the end of the prepared artery alongside the vein so that the artery lies in a gentle curve without tension and create a venotomy equal to the outside diameter of the artery (Figure 60). This is achieved under magnification by tenting up the vein wall, creating the smallest possible hole using Vanna's scissors, flushing out the vein with heparinised saline and then inserting the closed tips of No. D-5a forceps and opening them slowly lengthwise along the vein until the hole is stretched to the required size. Do not cut out a window of tissue in veins as this invariably results in a larger hole than required. Only in an end-to-side anastomosis of artery-to-artery is it necessary to remove any tissue, in which case the vessel wall is again tented up with No. D-5a forceps and either cut straight across with curved Vanna's scissors or incised in two directions with straight Vanna's scissors to remove a diamond shaped piece of vessel wall. Having created a venotomy of the correct size, it is important at this stage to examine it under high magnification to ensure that its edges are cut clean and that no periadventitial tissue is invading the lumen.

Range the end of the artery alongside the venotomy ready for suturing. For each suture, take normal sized bites out of both artery and vein, avoiding larger bites on the venous side which can cause constriction later and space the sutures as close together as you would in an artery-to-artery anastomosis. Place the first suture at the proximal end of the venotomy passing the needle from the outside of the vein to the inside thence from inside the artery to the outside. Pull the suture through very slowly until the edges of the vessels just meet and make a surgeon's knot but leave one end long so that it can be used as a traction

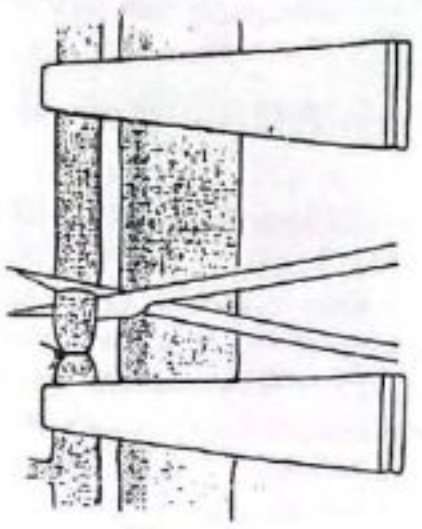


Figure 58.

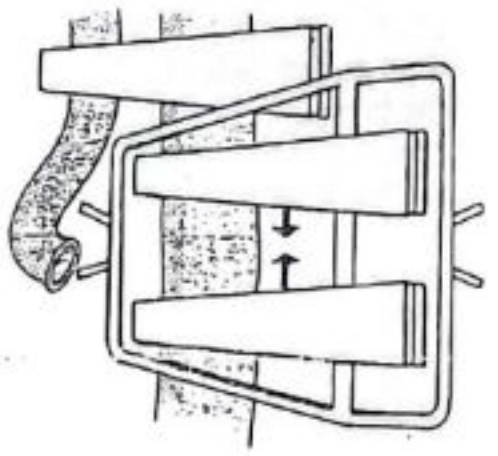


Figure 59.

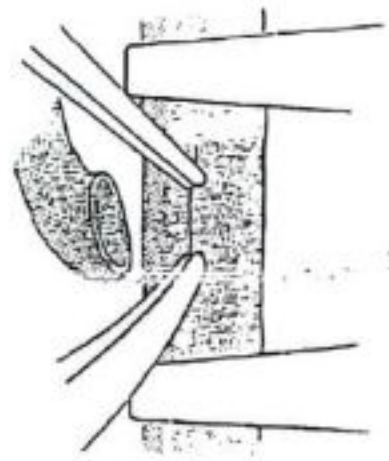


Figure 60.

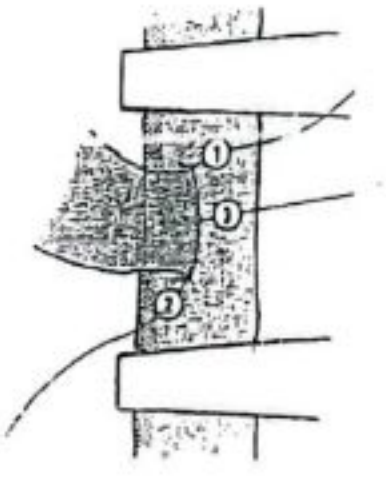


Figure 61.

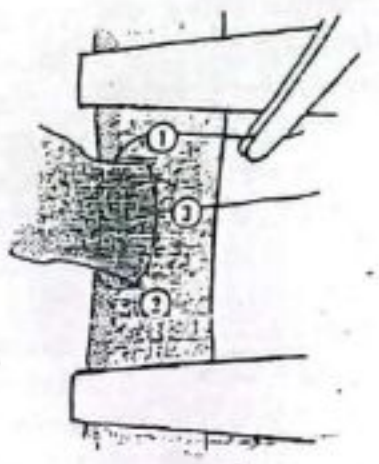


Figure 62.

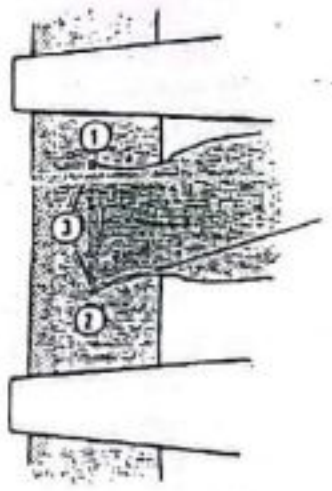


Figure 63.

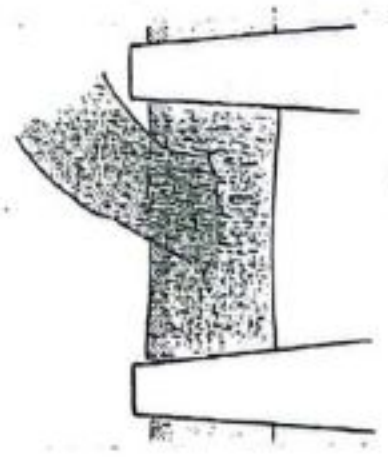


Figure 64.

stay. Now place the second suture at the opposite end of the venotomy, this time passing the needle outside of the artery to inside thence inside the vein to outside. Again carefully tighten the suture, make a surgeon's knot and leave one end long enough to fix to the furthest cleat on the approximator frame but do not cleat it out at this stage. Now place a third suture in the front wall half-way between the two stays, taking great care not to pick up the opposite walls of artery or vein (Figure 61). Keeping the vein filled with heparinised saline helps avoid this problem, but at all events it is worth checking the lumen before tying this suture. Make a reef knot leaving one end long (Figure 61). This is then used as a holding traction suture to pull the front wall away from the back whilst two other sutures are placed in the front wall, one in each segment between stay and holding suture. Again, these are carefully checked before knots are tied. Once these are completed all three knots are trimmed to the correct length.

To expose the other side, the long end of the second stay suture is passed under and around the back of the artery (Figure 62). It is used to retract the artery and expose the back walls for anastomosis and is stayed out to the cleat farthest from the anastomosis (Figure 63). After inspecting the lumenal side of the front wall anastomosis and again irrigating with heparinised saline, the back wall can be completed in identical fashion to the front (Figure 63). Now trim all long ends of knots and cut the stay sutures. Check that there are no obvious gaps when inspected at high magnification and place extra sutures if the gaps look dangerous (Figure 64). Remove the approximating clamps from the vein, distal clip first so that the vein fills, then the proximal clip, and finally remove the single arterial clamp. If the anastomosis is patent, bright red blood should be observed mixing turbulently with venous blood in the vein (Figure 64). If the anastomosis is oozing, put gentle pressure over it with a damp swab for a few minutes before re-examining it under magnification. Finally instil a few drops of 1% procaine around the vessels to prevent or reverse any constriction.

Interpositional Vein Graft

Because so much clinical microvascular surgery involves repair of avulsed and damaged vessels which have to be resected, and since we know that it is preferable to interpose a vein graft with two anastomoses rather than risk a single anastomosis subjected to longitudinal tension, the interpositional vein graft must be included in any microvascular training programme for clinicians. A segment of vein can be used to bridge a gap between the divided ends of either an artery or another vein. The suturing technique is essentially the same as that described for the end-to-end anastomosis earlier. The only real difficulty likely to be encountered is discrepancy in size which complicates the suturing itself and may lead to constriction or conversely ballooning with consequent rheological complications and added risk of thrombosis.

In the rat model, a segment of superficial epigastric vein is removed, reversed and sutured end-to-end between the divided end of the femoral artery. This vein when fully dilated is approximately the same diameter as the femoral artery. First prepare the femoral artery in the normal way and ligate and divide the profunda femoris artery, taking great care not to damage the delicate superficial epigastric vessels during the initial dissection. Instil 1% procaine around the femoral artery.

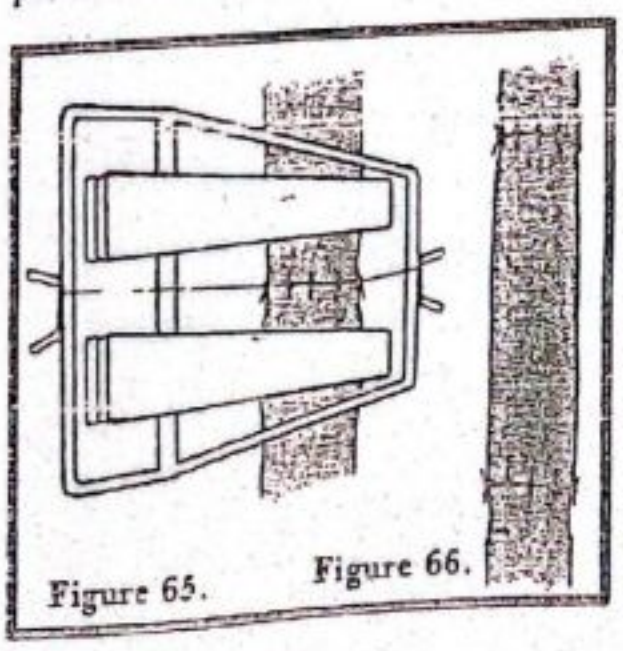


Figure 65. Figure 66.

Now dissect the superficial epigastric vein from its origin at the femoral vein to its bifurcation into the fat pad, releasing a length of some 1cm, and ligate it with 10/0 suture at each end. There is only one valve situated right at the junction with the femoral vein in this model, so there is strictly no need to reverse the vein, but it is as well to practice reversal ready for clinical work when it is important that valves open in the direction of blood flow. Hence, a marker (10/0 suture) should be placed in the vessel wall *toward* the distal end of the vein but not *at* the end as this will interfere with the anastomosis later on. Now transect the superficial epigastric vein at right angles proximally but leave the distal end attached whilst preparing the artery so that it does not get lost. Irrigate the superficial epigastric vein with heparinised saline using the 30 gauge Rycroft air cannula attached to a 1ml syringe. Next place a single clamp on the femoral artery as near to the inguinal ligament as possible and another distally close to the junction with the superficial epigastric vessels, and slide a piece of background material over the femoral vein and under the artery. Then place the approximating clamp midway between the two single clamps and divide the artery between the two clips which are positioned as far apart as possible. Natural retraction of the arterial segments is usually sufficient to create the correct space for the graft to fill comfortably without tension and it is unnecessary to excise any artery.

Remove adventitia from the cut ends of the artery and irrigate with heparinised saline. Now open the distal clip to release the distal segment of artery which will retract out of the way and will not bleed as the single clamp is still in place further down the line. Irrigate that part of the artery at this stage. The superficial epigastric vein is now divided at its distal end and the graft is laid alongside the approximator clamp with the marker stitch towards the proximal end of the femoral artery and is filled with heparinised saline to straighten it and correct any lengthwise twists. Open the distal clip of the approximator and slide in the vein graft with enough length protruding proximally to carry out the anastomosis comfortably. Now complete an end-to-end anastomosis with sutures spaced as closely as if it were an arterial end-to-end anastomosis, otherwise it will leak after high pressure blood flow is restored (Figure 65). Do not test the patency of this first anastomosis by releasing the proximal single clamp, otherwise the blood will clot by the time the second anastomosis has been completed. When the proximal anastomosis is complete, remove the approximating clamp and flush through with heparinised saline. Reposition the approximator so that the distal end of the vein graft can be secured in the proximal clip and the distal segment of artery can be held in the distal clip. The vein and artery to be joined must be relaxed and not twisted; Trim the length of the graft to about half of its original 1cm length otherwise it will be too long once it is filled with blood. Then complete the second (distal) anastomosis and remove the approximating clamp. Finally, remove the distal single clamp and allow time for blood to flow back across both anastomoses before removing the proximal single clamp. With a damp swab, apply gentle pressure for a few minutes before examining the two suture lines for leaks and for patency. Instil 1% procaine around the vessel. The graft should now pulse normally with the artery and should be approximately the same diameter although ballooning a little toward the middle (Figure 66).

PERIPHERAL NERVE REPAIR

General Notes

EACH trunk of a peripheral nerve comprises groups of nerve fibres gathered together into bundles termed fascicles. The nerve trunk is invested in loose epineurium, whilst each fascicle is surrounded by perineurium. It is really impossible to differentiate between sensory and motor fascicles as they are each made up of a mixture of sensory, motor and sympathetic nerve fibres.

It is possible to repair peripheral nerves by suturing together the epineurium, the perineurium or a combination of the two. Sutures (9/0 or 10/0) on cutting needles have been designed for this task. Whatever the method of anastomosis, the surgeon must seek close apposition of fascicles with blood vessels aligned and complete reduction of torque (i.e. longitudinal twist) and tension. Tension is *the* most common cause of failure as it inhibits the vascular supply to the nerve and increases the influx of fibrocytes.

Rat Sciatic Nerve

Exposure: After anaesthetising the rat and shaving the dorsal hind-quarter, the animal is laid on its right side (head to the operator's left) on the operating board and its left hind-leg is taped out in the extended position. With a scalpel, a 3cm long skin incision is made running parallel with the junction of the biceps femoris and the gluteus maximus muscles. Using bow scissors, the skin is bluntly dissected from the underlying muscles and is then retracted. Again with the bow scissors, separate but do not cut these two muscles and divide adherent tissue by blunt dissection, taking care not to rupture the superior gluteal or the medial femoral circumflex vessels. The sciatic nerve now lies to the right covered by the biceps femoris muscle and can be visualised once the gluteus maximus and biceps femoris muscles have been retracted with either a Jenson's retractor or paper clips attached to rubber bands. Using the microscope at low magnification, the nerve can be gently freed for a length of about 2cm from its pelvic origins to the site where it divides into three branches (the tibial, sural and peroneal nerves). Great care must be taken not to rupture the epineural sheath.

Epineural repair: This repair consists of 6 or more sutures joining the epineural connective tissue investing the nerve trunk. Background material should be placed under the nerve initially and the field should be irrigated. Next, at a convenient site, a plastic tongue depressor is slid under the nerve to provide a solid base over which to divide the nerve. Under low magnification, the nerve is sharply transected in one single movement preferably with a diamond or ruby scalpel. Nerves must not be cut with scissors which tend to compress then shear them in traumatic fashion. The divided nerves will retract a considerable distance so it may be necessary to move the rat's leg slightly to allow coaptation without tension. When the nerve ends are examined under high power you will usually find that the sciatic nerve of rats is divided into three fascicles. It is important to align the proximal and distal nerve segments so that the fascicles are matched in their correct rotation, using the angle of incision, the size and grouping of the fascicles and epineural vascular topography as visual guides to matching. Epineurium should not be stripped from the nerve segments as this is important to the vascular supply of nerve anastomoses. Neuroplasm will bulge or even exude from the nerve ends but do not attempt to cut it off since more will appear and be lost. Now grasp the epifascicular epineurium with No. D-5a microsurgical forceps working under 6 to 10x magnification and pass a 9/0 or 10/0 suture at the 12 o'clock position (**Figure 67**). Take 1mm bites out of the epineurium and pass the needle so that it lies parallel with the surface just beneath the epineurium, not into the perineurium, and taking care not to transfix any of the fascicles. The needle can be easily seen passing beneath the epineurium. This distal nerve stump should now be grasped with forceps and the needle passed across the anastomosis and through the epineurium from inside to out. The knot is tied as a surgeon's knot but one end is left long to use as a stabilising stay whilst placing the other sutures. Now place the second stay suture 120° from the first in the 4 o'clock position (**Figure 67**). It is important to place these stay sutures accurately otherwise the ends of the nerves will not be properly opposed. Tie this as a surgeon's knot, again leaving one end long, and taking care not to tie too tightly otherwise the nerve ends will again be misaligned.

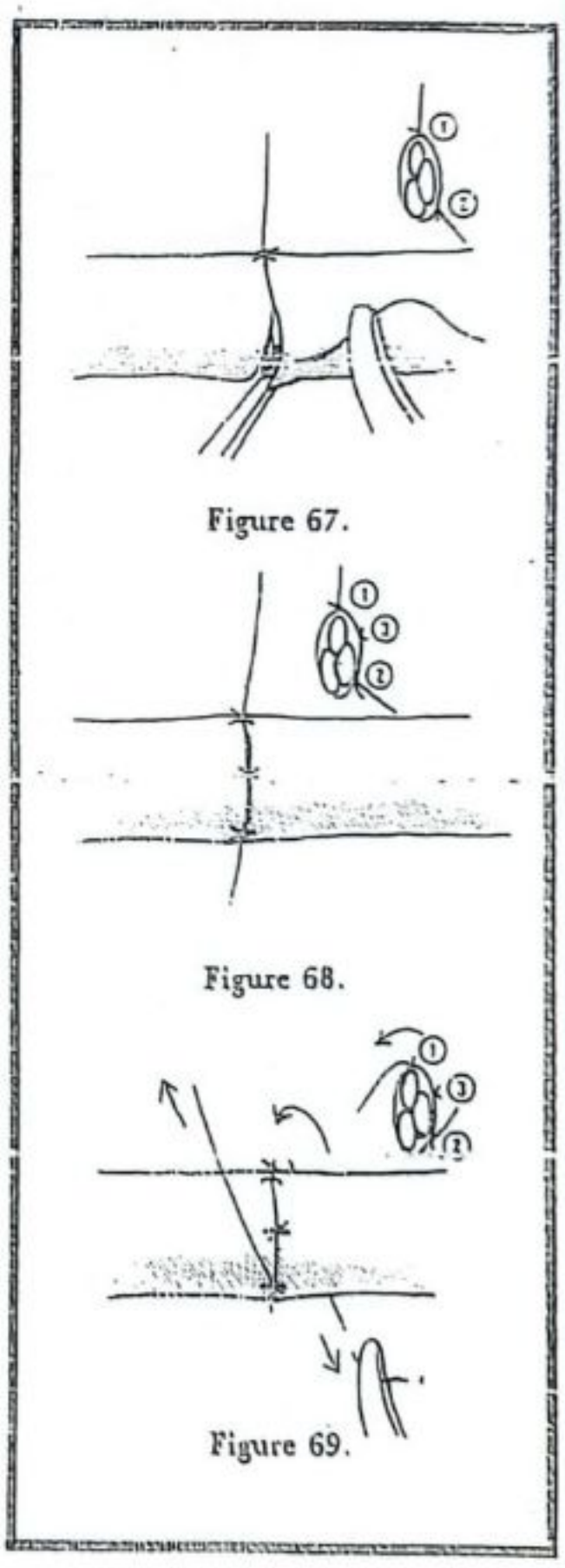


Figure 67.

Figure 68.

Figure 69.

Place a third suture midway between the two stays tying a simple square knot and cutting both ends short (Figure 68). The nerve is now rotated about its longitudinal axis using the stay sutures (Figure 69) and the fourth suture is placed at the original 6 o'clock position, the fifth suture at 8 o'clock and the sixth suture at the 10 o'clock position respectively (Figure 70). Each of these is tied as a square knot with the ends cut short, then the stays are cut and finally the nerve is allowed to rotate back to its normal position. Finally, irrigate the field and remove the background material. Throughout this exercise it is essential to anastomose nerve ends under minimal tension and remember that too many sutures can be as hazardous as too few; in the former case, constriction and scar tissue may result from a surfeit of foreign material whilst too few sutures allow neuroplasm to escape from the epineural sheath and neuromas can form.

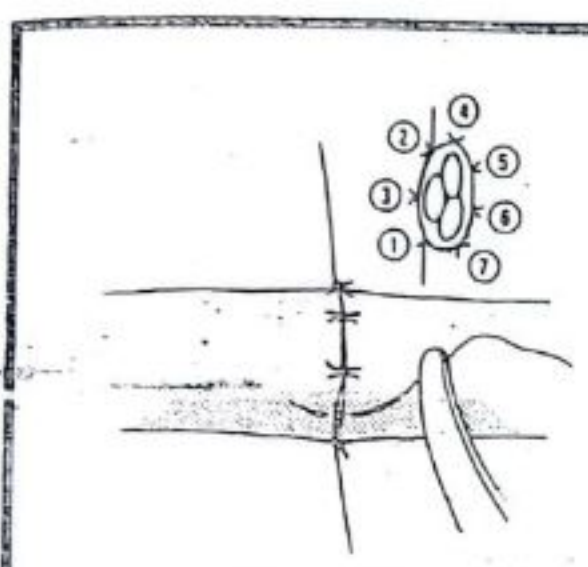


Figure 70.

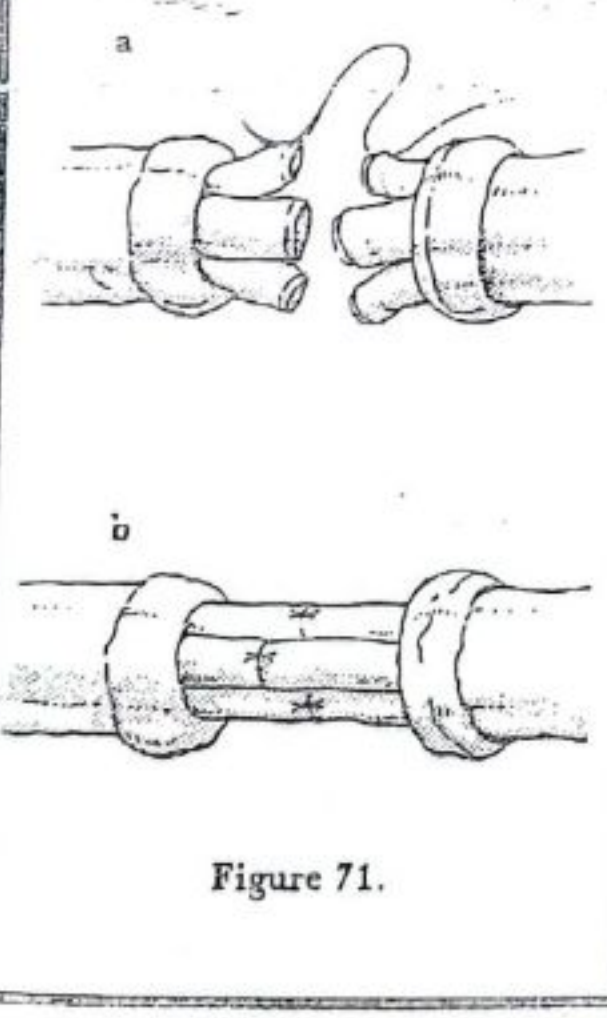


Figure 71.

Interfascicular repair: The sciatic nerve is exposed and dissected clear for sufficient length to allow transection 5inm proximal to its division into tibial, sural and peroneal nerves. Then using the microscope at 10x magnification, dissect and roll back the peripheral epineurium for a distance of about 1mm then separate each fascicle gently teasing apart the interfascicular epineurium. Now transect each fascicle. It is possible to place two sutures 180° apart in the two smaller fascicles passing the needle (9/0 or 10/0) through the perineuriun only (**Figure 71a, 71b**). In the larger fascicle it may be possible to place three sutures. Finally, the epifascicular epineurium is anastomosed with several sutures, the field is irrigated and the background material is removed.

Interpositional nerve grafts: To mimic the clinical situation in which interpositional nerve grafts are used to join damaged nerves without undue tension, the rat sciatic nerve is removed from one leg via the standard surgical approach and grafted into the opposite limb. It is possible to harvest between 1.0 and 1.2cm of donor nerve in which case excise only 0.5cm from the opposite sciatic nerve as the stumps will spring back and create a gap to be bridged of at least 1.0cm. The graft is then sutured in position using the standard epineural anastomoses described above.

TUBAL RECONSTRUCTION

General Notes

OVIDUCTS must only be joined where they are in healthy condition and any areas of stenosis, fibrous adhesion or necrosis must be excised first. The same basic rules for microsurgical reconstruction apply but in addition it is essential that the suture material does not intrude into the lumen otherwise ciliial function and transport of ova will be compromised.

Adult female rabbits of 2.5-3.0kg body weight provide a sultable model for developing and learning microsurgical skills relevant to clinical tubal reconstruction.

Anastomosis of Rabbit Oviducts

Exposure: After clipping and preparing the abdomen of the rabbit from xiphisternum to pubis, a midline incision about 10cm in length is made in the skin. A small incision through the peritoneal wall is made with the scalpel tip and this is then extended using round-ended scissors. The rabbit gut should be gently lifted out of the abdomen wrapped in damp swabs and then laid outside the animal taking care not to impede blood supply by twisting the vasculature. It is important to ensure a clear operative field by retraction of the abdominal wall and packing the cavity with damp swabs. It is also very important to irrigate the field constantly, to use damp swabs which will minimise abrasive trauma and to ensure efficient haemostasis by means of cautery, thus reducing the risk of adhesions and stenosis, particularly at the site of anastomosis.

Using the microscope at low magnification, locate the ovary, infundibulum, oviduct and uterine horn but disturb and mobilise as short a length of oviduct as necessary to carry out the anastomosis. Choose a site which is approximately mid-way between the fimbriated infundibulum and the isthmic-uterine junction where there are usually slightly fewer blood vessels. Gently grasp the oviduct with No. D-5a forceps and dissect it free of surrounding fat and adjacent blood vessels. Use bipolar coagulation where necessary but with restraint, remembering that the nutrient vessels are necessary for rapid healing at the anastomosis. Under no circumstances must the oviduct be crushed between forceps and care should be taken not to rupture the thin-walled and delicate blood vessels coursing longitudinally along the surface of the mesosalpinx. Clear a length of tube about 1cm long, ligating any larger longitudinal vessels before dividing the oviducts as they are very difficult to cauterise afterwards. After placing a strip of background material under the tube and irrigating the field thoroughly, place two single Winston modified Acland tubal clamps as far apart as possible to allow maximum space to carry out the anastomosis. Now divide the oviduct between clamps and meticulously cauterise any vessels bleeding in the muscular wall, taking care not to injure the exposed epithelium. Again irrigate the oviducts under 10x magnification and finally check that they are ready for suturing.

To carry out the anastomosis, use 10/0 monofilament polyamide on a cutting needle. In rabbits, the mesosalpinx and myosalpinx are virtually inseparable so these are encompassed in a single layer of sutures but the needle must on no account be passed into the endosalpinx. Place the first two sutures at 120° apart in the anterior wall taking bites of about 0.5mm on each side of the anastomosis and tie square knots with one end left long as a stay for manipulating and stabilising the tube. Now place the third suture midway between the first two. To expose the posterior wall, rotate the clamps through 180° and place the fourth suture halfway between the original stay sutures, leaving one end long as another stay, and then proceed with intermediate sutures working from the outer stays toward the middle just as in the vessel anastomoses. Finally, rotate the clamps back so that the oviduct lies in its normal orientation, irrigate the area and remove the clamps. The tear in the mesosalpinx is now repaired with interrupted sutures. To test for patency, a 30-gauge Rycroft cannula is passed into the infundibulum, held in place manually and methylene blue dye is injected into the oviduct and its passage along the lumen observed under the microscope at low magnification. Before closing the abdomen, ensure that there is no blood oozing from any points in the reconstruction field, and thoroughly irrigate the area to avoid the risk of adhesions. Then repair the laparotomy incision using interrupted chromic gut (0) for the combined muscle and peritoneum sutures, and 2/0 braided silk interrupted horizontal mattress sutures to close the skin. Under no circumstances should continuous suture patterns be used in rabbits for these abdominal repairs.