PHARMACOLOGICAL EXPERIMENTS ON INTACT PREPARATIONS

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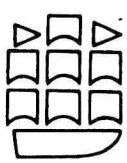

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CONTENTS

CHAPTER I

GENERAL METHODS

INTRODUCTION

This book is intended to be a companion to *Pharmacological Experiments on Isolated Preparations*. There are, however, big differences between the two types of experiment. The effects of a drug in a whole animal are complicated by the possibility that what is observed is the consequence of the drug acting at many sites at once. This is very important, for example, when considering the effects of drugs on the blood-pressure, where the change in pressure observed is the consequence of many actions, which may even oppose each other. The experiments are also complicated because the effect produced will depend partly on how the drug is administered, whether it reaches its sites of action immediately or only slowly over a long period. The action of the body on the drug is also important. The effects observed will depend upon the rate of excretion and metabolism of the drug and may be quite different from those observed with isolated preparations, where the drug is added in a known concentration at a known time to a small piece of tissue and washed out at a known time. The effects of suxamethonium and decamethonium on the isolated rat diaphragm preparation, for instance, are virtually indistinguishable but on an intact preparation, such as the cat tibialis (p. 37), the effects are quite different. Intact preparations have the great advantage that they indicate the likely clinical effects of a drug much better than isolated preparations but they have the disadvantage that it may be much more difficult to work out how the drug is acting. Clearly both types of experiment are necessary.

For practical reasons, however, experiments with intact preparations are much more difficult to organize for students. They usually take a lot of time. Many preparations involve lengthy dissection and the time available for testing drugs is limited. As the drugs cannot be 'washed out', the use of too big a dose may involve waiting, perhaps for as long as an hour, while the effects wear off. The experiments are more expensive than those with isolated preparations, require a greater manual skill, and it is often difficult to arrange for students to see what is going on satisfactorily. In Britain experiments on living animals come under Home Office control and must only be performed by people who have the necessary licence and certificates (p. 31). For all these reasons this book involves more description of the methods for setting up preparations than its companion and a smaller number of actual experiments with drugs. It is hoped, however, that these descriptions will be useful because the preparations can be used for a variety of experiments both for teaching and research.

Some experiments are included which involve human subjects. These do not come under the Cruelty to Animals Act, 1876, but the legal and ethical aspects of experiments with students are complex and the wisdom of attempting many experiments in man may be questioned. The examples chosen here, however, have been in use for many years and can confidently be regarded as safe.

The first chapter describes procedures such as anaesthesia and dissection, which are common to many of the preparations. After this, the book is divided into sections according to the effects studied; actions affecting the peripheral nervous system, actions affecting the central nervous system, actions on particular organs, and lastly, actions of the body on drugs.

For scientific reasons as well as for legal and moral ones, it is most important to see that laboratory animals are properly cared for. Good experimental results are unlikely to be obtained from animals in poor condition; for example, a sick animal may well die under anaesthesia. The same is true if an animal is frightened.

Any person who works with animals is strongly advised to consult the UFAW handbook, *Care and Management of Laboratory Animals*, 1967, Edinburgh: Livingstone. This gives detailed information about how animals should be housed and fed. It also includes a most useful account of how to handle animals, which should be read to supplement the brief account given below.

Handling Animals

In this book only five species are used, cats, rabbits, guinea-pigs, rats and mice. Wherever possible these should be handled frequently in the animal house. This applies especially to cats and, to a lesser extent, to rats. If a cat is used to being handled and is held by someone with whom it is familiar, it is possible to induce anaesthesia by injection of a barbiturate, or inhalation of halothane without causing any distress. The combination of a frightened animal and a frightened scientist may well cause the animal to die under the anaesthetic, particularly if it is potent.

For anaesthesia with a mask, cats are best held by two people. One grips the scruff of the neck firmly with one hand and the front legs with the other. The second person holds the back legs with one hand and the mask with the other (Fig. 1). If the animal is not frightened, it should not be necessary to put a cloth round the legs to prevent it scratching. A third person may be necessary if the mask is being sprayed with ethyl chloride. It is important that the animal holders should be experienced enough to restrain the animal firmly and not let go, without at the same time gripping it so strongly that they cause pain. Cats suffer extreme distress if they are placed in a box into which ether is blown and even with other volatile anaesthetics the use of a box makes it difficult to gauge the depth of anaesthesia.

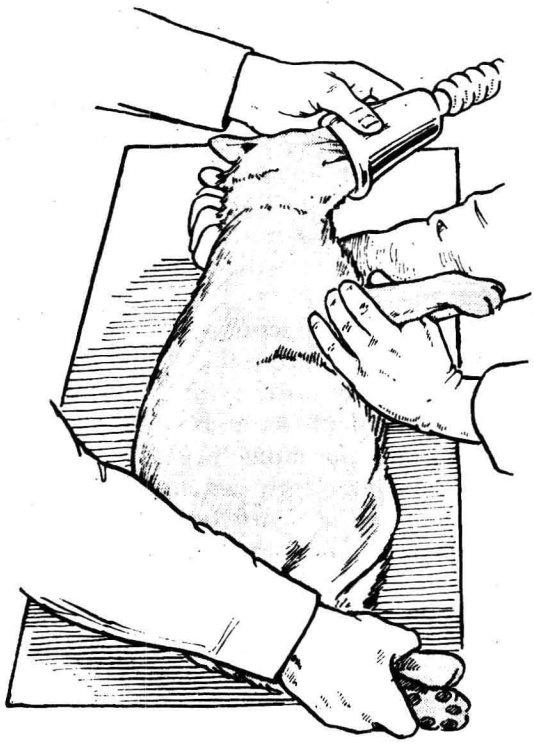

FIG. 1. Holding a cat for anaesthesia.

Rabbits can be held like cats for anaesthesia with a mask. For injections, also, two people are needed, one of whom holds the animal into his side while restraining the front legs, while the second person makes the injection into the marginal vein of the ear (Fig. 2). Rabbits will struggle if placed on a slippery surface but will remain quiet if their feet are on a rough surface and their back is hard up against something. If the rabbit is placed in a box which restrains movement without undue pressure then only one person is needed to make the injection.

Cats and rabbits can be lifted by the scruff of the neck and supported by a hand or arm below. It may be wise to wear gloves but this should be unnecessary if the animals are really tame, in which case it may be easier to lift them with two hands, rather than to use one hand for holding the scruff of the neck. With guinea-pigs, which are much lighter, one hand is usually adequate for lifting them. They appear particularly to like to nestle into corners and can be conveniently carried in the crook of an arm or in the pocket of a laboratory coat. Rats and mice may be lifted by their tails without alarming them though they should be put on a flat surface as soon as possible. If this allows their feet any grip, they will then pull away from where they are held and the scruff of the neck can then be grasped in the other hand. They can thus be held securely while injections are made. Mice can even be held single-handed with the forefinger and thumb holding the scruff of the neck and the little finger holding the tail or legs; the other hand is then free to make an injection (Fig. 3).

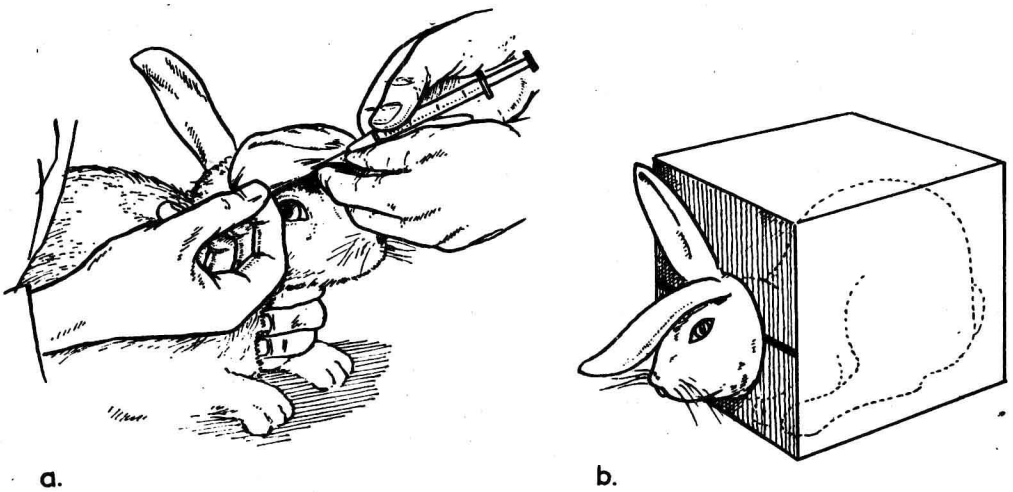

FIG. 2. Holding a rabbit; *a*, with an assistant; *b*, rabbit held in a box.

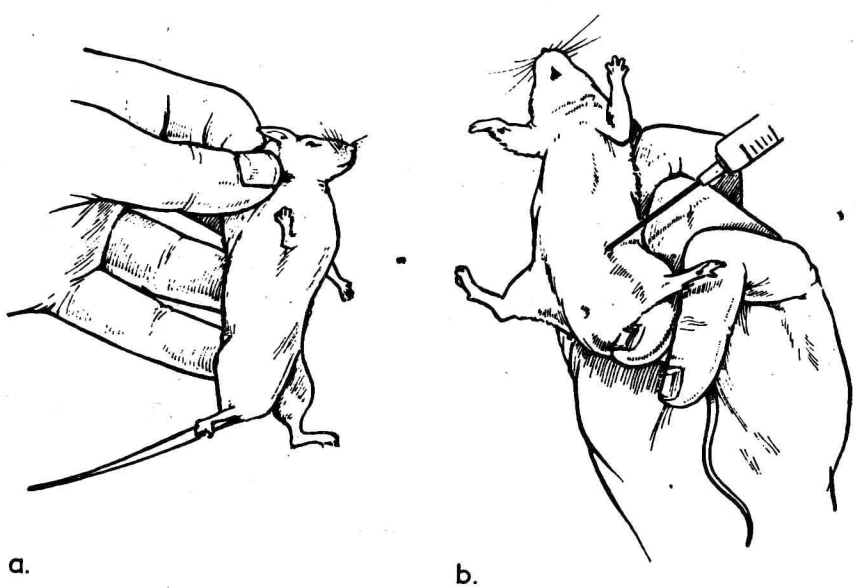

FIG. 3. Holding mice; *a*, for lifting; *b*, for an intraperitoneal injection.

Instruments

The choice of instruments depends very much on personal taste but the following instruments are likely to be needed and most of them are illustrated in Figure 4.

Large scissors are needed for general coarse surgery, e.g. for cutting the skin of cats and rabbits and for cutting lint and string. Blunt-ended scissors are convenient for blunt dissection: operating Mayo scissors with a 7½ inch straight conical blade are suitable.

Medium-sized scissors with either straight or curved blades are useful for cutting the skin and soft tissues of small animals and for cutting coarser sutures (1/0 and 3/0). Blunt-ended scissors are convenient for blunt dissection. 5 inch Mayo scissors, straight and curved or flat are suitable.

Medium fine scissors with either straight or curved blades are reserved for cutting blood vessels in cats and rabbits and fine sutures (5/0). Kilner's 4¾ inch straight scissors with an extra fine point, and Kilner's 4¾ inch curved or flat scissors are suitable.

Fine scissors should only be used for cutting fine blood vessels, e.g. the rat carotid artery and femoral artery or the cat lingual and dorsalis pedis arteries. The Iris, spring action, Birmingham pattern scissors are recommended. Some fine scissors have the blades set at an angle to the handles; this allows the points to be kept up, which is an advantage when cutting connective tissue around vessels, and for cutting fine blood vessels or the dura mater. Brudenall Carter's spring action capsular scissors are suitable.

Large forceps are needed for general work: 6 inch broad, fluted dissecting forceps are suitable. Some people find *large toothed forceps* are convenient for ensuring a firm grip of skin or large masses of tissue but they may tear. Treves dissecting forceps, 6 inch, 2 × 3 teeth are suitable.

Medium-sized curved forceps are convenient for blunt dissection or for passing ligatures under the trachea or large blood vessels of cats or rabbits. Dental packing forceps with curved points are suitable.

Medium-sized fine toothed forceps are ideal for ensuring a firm grip of smaller pieces of connective tissue or muscle and for holding the cut end of the trachea open. Iris forceps, straight fluted grip, fine tooth 1:2 are suitable.

Medium-sized fine curved forceps are used for delicate blunt dissection, e.g. beneath the trachea of the rat or around blood vessels in most animals, and for passing ligatures. Iris forceps, fluted grip, fully curved, are suitable.

Medium-sized fine straight forceps are needed for delicate blunt dissection. 3½ inch Ophthalmic dissecting forceps are suitable.

Fine pointed forceps are needed for picking up fine sutures, holding open or dilating cut blood vessels, and for stripping connective tissue from small blood vessels and nerves. Watchmakers forceps, 'Dumont' No. 4, are suitable.

Large haemostatic forceps, with straight or curved points, are required for clamping large blood vessels in the cat, or tissue masses containing bleeding vessels. 6 inch Spencer-Wells artery forceps, with either straight or curved points, are suitable. They should have box joints to ensure a firm grip.

Medium-sized haemostatic forceps, with straight or curved points, are useful for clamping small blood vessels in the cat or small tissue masses in the cat or the rat. 5 inch Halstead's 'Mosquito' straight and curved artery forceps are suitable.

Both large and medium artery forceps have ratchets so the pressure between the ends can be adjusted.

Small haemostatic forceps, with straight or curved points (*'Bulldogs'*) are needed for the temporary occlusion of blood vessels. Dieffenbach's artery forceps (straight or curved) are suitable. The 3 cm. size is used for cats and rabbits, the 2 cm. size for rats.

Needle-holding forceps are required for ensuring a firm grip on curved suture needles. Mathieu's spring catch, screw joint, 8 inch forceps are suitable: Spencer-Wells are not.

A fine blunt seeker is very useful for holding open cut blood vessels to aid the insertion of a cannula.

A large scalpel is useful for cutting skin quickly and cleanly. The Swann Morton No. 4 handle is suitable, used with a No. 20 disposable blade.

A medium-sized scalpel is useful for cutting small areas of skin and cutting open the trachea. The Swann Morton No. 3 handle is suitable used with disposable blades Nos. 10 and 11.

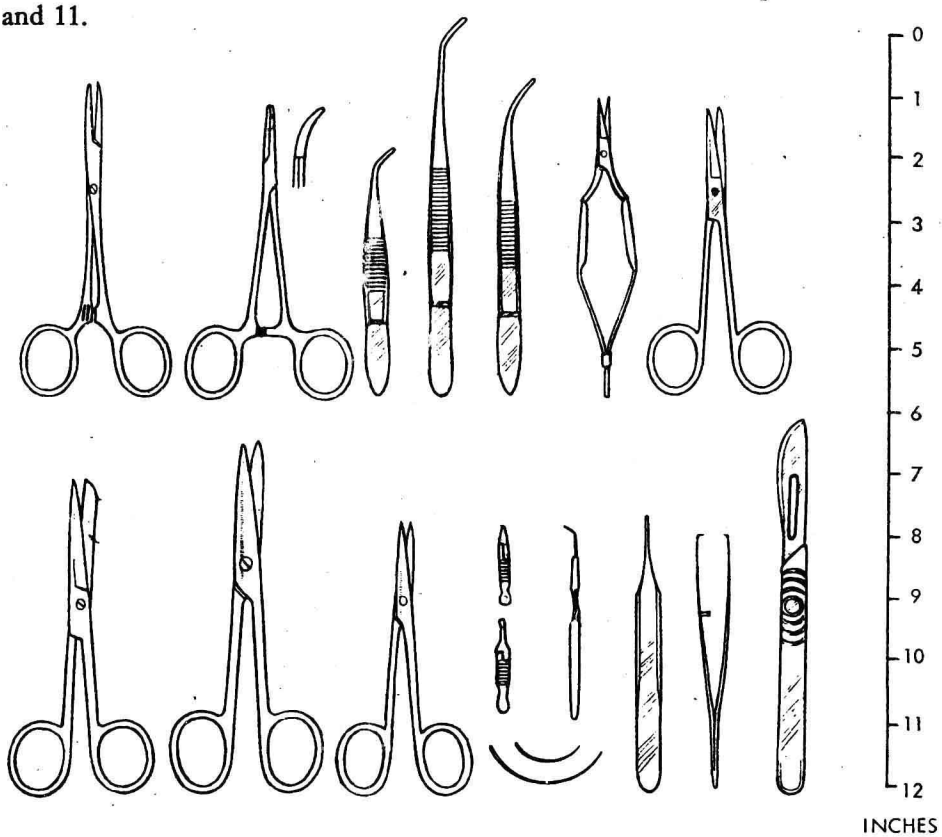

FIG. 4. Instruments most commonly used for dissection.

Cutting needles are necessary for stitching a skin. The smaller No. 3 needles are used for fine work in small animals. Hagedorn, ½ circle needle size, Nos. 3 and 10, and Hagedorn, straight needle size, Nos. 3 and 10, are suitable. Proper *suture* materials are needed for tying off blood vessels. Braided silk sutures of various colours are available on reels. 1/0 is coarse and 5/0 is very fine. Sizes 1/0, 4/0 and 5/0 are particularly useful.

A bone scraper is useful for cleaning the outer surface of bone. Faraboeuf's bone rougine, straight, 6½ inch is suitable.

Dissection

Most of the experiments in this book last for hours only and not for days and the animal is killed at the finish. Aseptic techniques and sophisticated surgery are not required but it is still a good principle to avoid unnecessary exposure of deep tissue or damage to it since this will cause the preparation to deteriorate.

Opening the skin. After clipping or shaving the hair from an area, the skin is either cut open with a sharp scalpel or a longitudinal wedge of skin is cut away with medium or large scissors. The edges are now retracted to expose the underlying tissues and bleeding vessels are cauterized or temporarily clamped with haemostatic forceps. Surgical diathermy may be used instead of a scalpel or scissors for cutting the skin. It is less likely to cause bleeding and may well be essential if large amounts of anticoagulants are to be used.

Blunt dissection is used as much as possible. This involves tearing or pushing through connective tissue along the lines of cleavage. Blunt dissection is either done with the fingers, in large animals, or by inserting closed blunt-ended scissors or forceps and opening them. This method is quick and has the advantage that very small blood vessels seal more easily when their ends are torn than when they are cut. Larger or medium-sized blood vessels should be tied and cut or cauterized and cut to prevent troublesome bleeding later in the experiment, particularly if the blood pressure is made to rise or when an anticoagulant has been given to the animal.

Bleeding. During dissection it sometimes happens that a fairly large blood vessel is accidentally torn or cut and there is a sudden flow of blood. This should be stopped immediately by applying pressure with a swab and finger and thumb. If this is not enough the vessel is identified, clamped off with haemostatic forceps and tied. To tie off a vessel that is already cut, check that the thread is placed between the cut end of the vessel, secured by the clamp and the tissue. It is often convenient to pass the tie loosely round the clamp, pull it down to the tip and slide it off onto the vessel. It is always better, however, to anticipate trouble and to tie off even quite small vessels before cutting or tearing them. To tie off a vessel before cutting it, two threads (5/0 for small vessels, 3/0 for larger ones) are passed under it and then tied at least 2 mm. apart. The ends of these threads are held taut so that the vessel is slightly stretched. The vessel is cut and the ends of the threads then trimmed. If an electric cautery or diathermy is available it is convenient to coagulate small vessels instead of tying them and this saves considerable time. There are two main types of cautery tip, one of which can be used for cutting and the other, which is very small, for sealing small vessels.

Injections

For any injection the needle must be sharp and of suitable size (Table I). Even if

TABLE I

		I.V.	I.P.	S.C.	I.M.	Oral
Mouse	Needle size	27 G ½ in.	25 G ⅝ in.	25 G ⅝ in.	25 G ⅝ in.	18 G 2 in.
	Max. dose volume	0·4 ml.	1ml.	0·4 ml	0·4 ml.	1 ml.
Rat	Needle size	—	25 G 1 in.	25 G 1 in.	25 G 1 in.	—
	Max. dose volume	—	2 ml.	1 ml.	0·4 ml.	—
Cat	Needle size	—	21 G 1½ in.	25 G 1 in.	25 G 1 in.	—
	Max. dose volume	—	5 ml.	2 ml.	2 ml.	—
Rabbit	Needle size	25 G 1 in.	21 G 1½ in.	25 G 1 in.	25 G 1 in.	no. 9 catheter
	Max. dose volume	10 ml.	5 ml.	2 ml.	2 ml.	5–10 ml.
Guinea-pig	Needle size	—	25 G 1 in.	25 G 1 in.	25 G 1 in.	—
	Max. dose volume	—	2–4 ml.	1 ml.	0·5 ml.	—

it need not be sterile, there is no justification for re-using it if it is at all blunt, because disposable needles are not expensive. Disposable syringes, too, are cheap and much more robust than glass ones. Though it is just possible that some drugs may dissolve slightly in the plastic, this does not usually necessitate using glass ones. In many experiments sterility does not matter, and syringes, as opposed to needles, may be re-used many times, especially if they are being used always for the same drug solution. In these circumstances an appreciable saving in expense is made by re-using 'disposable' plastic syringes. These can even be used for different drugs provided they are well washed. A few drugs are reputedly particularly difficult to wash out and it is probably wisest to use a fresh syringe, for example, with secondary or tertiary bases, but with quaternary salts the useful life of a syringe can be very long, being limited only by the tendency for the graduation to become worn away. This can be reduced by placing transparent tape over

the markings. The main disadvantage of using plastic syringes is the difficulty of dislodging air bubbles trapped on the sides. It may be necessary to tap the syringe quite sharply several times to remove them. Some syringes are so designed that small bubbles are not likely to enter the needle but it is always important to check that no air is injected. At present all plastic syringes have Luer fittings and this pattern is becoming standard. It may be difficult to obtain very fine needles, e.g. for the injection into the tail of the mouse, for which a 27 gauge needle is required, with Luer fittings. Needles with Record fittings and glass Record syringes will then be required.

The most frequently used methods of injection are subcutaneous, intraperitoneal, intramuscular, intravenous and oral. For a subcutaneous injection the skin is lifted up and the needle pushed firmly through it. The point of the needle should be quite free to move between the skin and the muscle. If the needle is of a suitable length or the syringe is held with the hand below it (Fig. 5) there is no possibility of inserting the needle too far into the animal. The position of the hand can then be altered so that the thumb presses the plunger. Either side of the back of an animal is most suitable for a subcutaneous injection. If there is no one available to hold the animal, however, it may be necessary to make the injection higher up towards the head because one hand will be needed to hold the scruff of the neck of the animal. In this way it is possible for one person to inject mice, rats or guinea-pigs and it is sometimes possible to inject docile rabbits or cats without assistance.

For an intraperitoneal injection the procedure is similar to that for a subcutaneous one, but it is the skin of the abdomen which must be held taut. The animal should be gripped by the scruff of the neck and by its tail or hind limbs, though with rats it may be better to place the hand right round the shoulders and hold the forelegs crossed firmly over the chest (Fig. 6). With rabbits it is advisable to hold the animal close against one's body (Fig. 7). The needle is pushed firmly through the abdomen in a region where it is not likely to penetrate the liver, kidney, spleen or bladder and the plunger pressed.

For an intramuscular injection the procedure is similar to the subcutaneous injection except that the site must be a muscular part of the body, usually in the region of the thigh or buttock. With this injection the tip of the needle should not be free but should have penetrated the muscle.

For an intravenous injection the procedure varies from one species to another. In the experiments in this book, intravenous injections in cats, guinea-pigs and rats are made under anaesthesia. In conscious rabbits, however, injections can be made into the marginal vein of the ear. The rabbit is held suitably (p. 4) and the fur is removed from the vein at the top outside edge of the ear either by plucking or with clippers. The vein is made to distend by occluding the flow at the base of the ear with the thumb and forefinger. It is advisable to shine a lamp on the ear (not directly on the face of the rabbit). This serves to warm the ear as well as to illuminate it. It may also be necessary to rub the edge of the ear gently. A needle can easily be inserted into the vein when it is distended, the thumb and forefinger released and the plunger of the syringe pressed in (Fig. 8). As a general principle the plunger of the syringe should be withdrawn to see if blood appears, though this should not be done with the mouse tail vein injection.

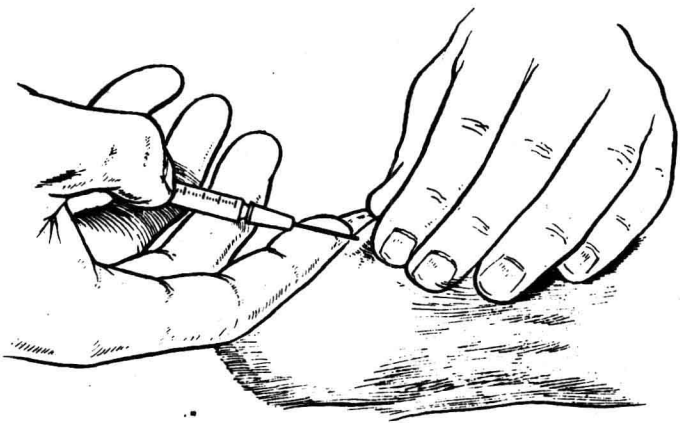

FIG. 5. A subcutaneous injection. The skin is lifted up and the needle inserted at an angle of about 45°.

FIG. 6. Holding a rat.

FIG. 7. Holding a rabbit for an intraperitoneal injection.

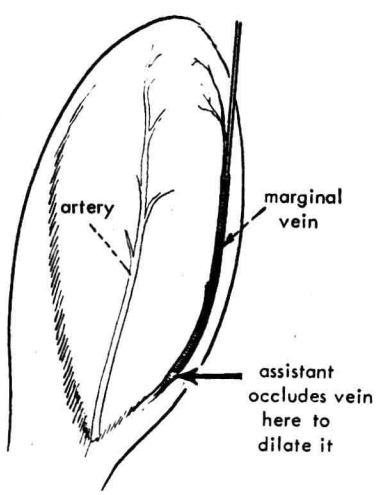

FIG. 8. Injection into marginal vein of rabbit's ear.