Volume 6

# EXPERIMENTS IN PHYSIOLOGY AND BIOCHEMISTRY

edited by G. A. KERKUT

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# Preface to Volume 1

The easiest way of learning how to perform an experiment is to watch someone else demonstrating it and then, using the same equipment, to try to copy the procedure. If one fails the first time one can watch again then repeat the process; any further failures can be corrected by being shown precisely what has gone wrong. For most experiments this is the best method of learning; however, it is often impossible to follow this procedure because the specific methods are not easily available in the laboratory where one is working.

Another system is to read through a series of instructions and attempt to follow them out. This is more difficult because the writer often infers practical experience not possessed by the reader. Furthermore, there may be minor practical details that are not immediately appreciated either by the reader or the writer. Nevertheless, this is the most commonly used method of learning how to conduct an experiment, and the work published in the scientific journals provides the necessary information and stimulus.

However, it is often difficult to follow experimental procedure from the published account in a scientific paper. This is frequently the fault of an editorial system which considers the "materials and methods" to be less important than the "results and conclusion", and most authors are persuaded to present these sections in a very condensed form.

The present volume is the first of a series in which it is hoped to supply sufficient practical details to enable the reader to follow and carry out the experiments for himself. The information is presented in detail, though possibly there may be too much detail for some people and not enough for others. Initially, only those experiments that could be performed in three hours were selected for the present volume. However, it was felt that there were also many experiments that would take longer in time but which could be broken down into smaller periods and so fit in with a rather more liberal practical programme. It is intended that at a later date the three-hour class type of experiments will be collected from this and subsequent volumes and published separately.

I should welcome suggestions from authors for future contributions. A detailed scheme for the arrangement of material is presented on p. xi. Such a lay-out is only tentative and can be modified according to the particular needs of individual experiments.

It is hoped that the body of practical information to be presented in this series will help to spread skill and experience from one Laboratory to another.

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G. A. KERKUT

*March* 1968

## Note on Vivisection

All experimentalists should note that most countries have rules and regulations concerning that performance of experiments on living animals.

In England, Scotland and Wales it is necessary that any experiments carried out on vertebrate animals should be performed in a Government licensed laboratory, by persons licensed to carry out the experiments, under the supervision and guidance of licensed persons. Failure to do this may bring about legal proceedings against the experimentalists.

The exact legal situation differs according to the country where the experiments are being carried out, but in all cases students are advised to ask their instructors for specific information.

Only animals that are lawfully acquired shall be used in this laboratory and their retention and use shall be in every case in strict compliance with state and local laws and regulations.

## Guiding Principles in the Care and Use of Animals

Approved by the Council of the American Physiological Society

Animals in the laboratory must receive every consideration for their bodily comfort; they must be kindly treated, properly fed and their surroundings kept in a sanitary condition.

Appropriate anesthetics must be used to eliminate sensibility to pain during operative procedures. Where recovery from anesthesia is necessary during the study, acceptable technic to minimize pain must be followed. Curarizing agents are not anesthetics. Where the study does not require recovery from anesthesia, the animal must be killed in a humane manner at the conclusion of the observations.

The postoperative care of animals shall be such as to minimize discomfort and pain and in any case shall be equivalent to accepted practices in schools of Veterinary Medicine.

When animals are used by students for their education or the advancement of science such work shall be under the direct supervision of an experienced teacher or investigator. The rules for the care of such animals must be the same as for animals used for research.

## Brand Names

Often in the experiment, a piece of equipment will be referred to by its trade, manufacturer's or supplier's name. It may be that you do not have this specific piece of *named* equipment in stock but that you have an equivalent or alternative make. In almost all cases their is nothing "magic" about the specific brand. It is mentioned because the author used it. When in doubt, it is advised that you carry out a trial experiment on your own equipment. This may be preferable to ordering the equipment BRAND X from your suppliers and finding when it is delivered some three months later that it is more expensive and worse than the model that you already have in the laboratory.

# Suggestions for Future Contributors

These volumes will provide full details of methods and specific experiments on the biochemistry and physiology of animals. It is intended that they will fill the gap that has been made by the restricted amount of space that journals provide to the "Materials and Methods" section of papers.

Where possible each account should provide very full experimental details so that:

(1) Research workers and advanced students will be able to perform the experiments with the minimum of difficulty.

(2) Technicians will know what equipment to set out and which chemical solutions will be required.

It will help if the material can be presented as a series of separate but linked experiments so that the reader will realize the precise task involved in each experiment. In some cases it may be necessary to give details as to how to construct a piece of equipment and how to test it. This would then be equivalent to an "experiment".

A *suggested* plan of the account is as follows though the authors can, where necessary, alter the layout to suit the particular case.

(1) Title of experiments.

(2) General principles that the experiments and methods will illustrate.

(3) Title of specific experiment.

(4) Apparatus required.

(5) Animals required.

(6) Chemical solutions required. Please give solutions in terms of g/ml instead of molarity of solutions.

(7) Experimental details. These should be very full, in numbered paragraphs, with diagrams where this will help show specific equipment, dissections technique, manipulative methods, etc. The authors should not assume too much "know-how" on the part of the reader. The reader may be an expert, but in a slightly different field and these experiments are to help him extend his technique.

(8) Sample results. These should be edited labelled traces, titration readings, tables, graphs, etc., together with full calculation of the result. The worker should see from these records exactly the sort of result that he should be able to obtain for himself.

(9) Trouble shooting. Notes about what can go wrong with the experiment. What to check first if the experiment is unsuccessful.

(10) Further ideas about experiments that can be carried out with this equipment.

(11) Bibliography. Further reading with notes as to the significance of the selected references. Full titles to papers and books should be given together with first and last page references.

There is no strict limitation as to number of words or figures, though authors are asked to be as concise as is concomitant with clarity.

# Contents

*Exp. in Physiol. and Biochem.* (1973). 6, 1–32.

# 1 | The Rat Blood Pressure Preparation

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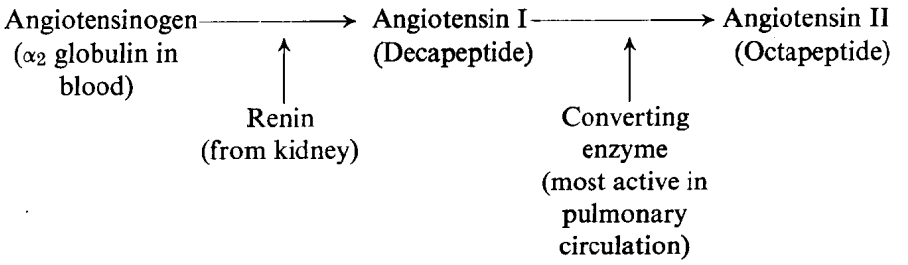
## Introduction

The rat blood pressure preparation is a very useful and sensitive bioassay system for many drugs which have an effect on the cardiovascular system. It can be used either qualitatively to investigate the mode of action of a drug, or quantitatively to assay samples of unknown concentration against standard samples of the drug.

The drug under investigation is given intravenously to an anaesthetized rat via a cannula in the femoral or jugular vein, and systemic arterial blood pressure is recorded using another cannula in the carotid artery. The drug is given intravenously rather than intra-arterially for two reasons. First, it is easier to inject against the low resistance in the low pressure venous circulation, and any error will not cause such a large loss of blood as can occur from the high pressure arterial side of the circulation. Secondly, a drug given intravenously will be carried directly to the heart where it is mixed with other blood before being pumped all round the body of the animal. Thus, intravenous injection leads to a more even distribution of the drug than the initially localized distribution of an intra-arterial injection.

In Section A below, general details are given on how to obtain a blood pressure recording from a rat. Some examples are given of interesting drugs which can be studied using this preparation.

In Section B, more specific details are given for the estimation of angiotensin II. This is the most potent naturally occurring pressor substance known. It is formed in the blood stream following the release of the hormone renin from the kidney. The series of reactions involved are summarized below.




The rate of release of renin from the juxtaglomerular cells of the kidney is normally the rate limiting step leading to the formation of angiotensin II which is the only physiologically active component of the series of reactions shown above. Angiotensin II is intimately involved in the maintenance of sodium balance in the body. This is effected firstly via a direct action on sodium reabsorption in the kidney tubule, and secondly by stimulation of the secretion of the sodium retaining hormone aldosterone from the adrenal cortex. The renin-angiotensin system may also be involved in the auto-regulation of renal blood flow. The physiological role of the systemic pressor effect is a little difficult to evaluate, but it is important under some pathological conditions, leading to certain forms of high blood pressure, hypertension.

## A. The Rat Blood Pressure Preparation

*Apparatus*

Dissecting instruments (must be free from formalin).

Fine and coarse scissors.
Very fine spring scissors are advantageous.
Fine and coarse forceps.
Fine curved end forceps.

Syringes: 1 ml with needles and one 5 ml.
Heart clip (bull-dog clamp)—see Fig. 4.
Cotton.

Plastic cannulation tubing—either polythene or nylon is suitable.
Sizes: 2·0–2·8 mm diam. (ext.) for trachea and 0·6–1·0 mm diam. (ext.) for blood vessels.

Glassware:

3 × 100 ml beakers.
2 × 10 ml (graduated) pipettes.
2 × 1 ml (graduated) pipettes.

Rough balance for weighing rats.
Rectal thermometer.
Bench lamp (also used to keep the animal warm).
Small retort stand with boss and clamp.
Narrow bore (max. 2 mm diam.) rubber pressure tubing.
Pen recorder (see Appendix).
Blood pressure transducer.
Three plastic three-way taps with same size fittings as syringes used.
Small mammal respirator pump. This is not essential but may well be very useful.

*Animals*

*Rats.* These should be at least 180 g weight. For most experiments animals of either sex are suitable. If a nephrectomy is to be performed on the animal (see Section B), then male rats are best as the ovary and uterus of females are in close proximity to the kidney.

*Chemicals*

Tyrodes solution. This is a mammalian physiological saline which is used to make up all drug solutions and to flush drug doses into the animals. It is made up as shown below.

| *Chemical* | *Conc. in g/litre of water* |
|---|---|
| NaCl | 8·00 |
| KCl | 0·20 |
| $CaCl_2$ | 0·20 |
| $MgCl_2$ | 0·10 |
| $NaHCO_3$ | 1·00 |
| $NaH_2PO_4$ | 0·05 |

Anaesthetic. Sodium pentabarbitone (Veterinary Nembutal) (60 mg/ml) is recommended.
Heparin (anticoagulant).
Drugs to be tested on the blood pressure preparation. See Section A for suggested experiments.
Glycerol to lubricate the rectal thermometer.

### HANDLING AND ANAESTHETIZING THE RAT

You are well advised, if possible, to get someone with experience in handling rats to show you the best way to avoid getting bitten. A good method of picking up the rat is to grasp the animal by the tail as close as

possible to the body. Then, drawing the animal slowly backwards towards you on the bench place the other hand diagonally across the back of the animal (Fig. 1). Slide your thumb behind one front leg, under the thorax

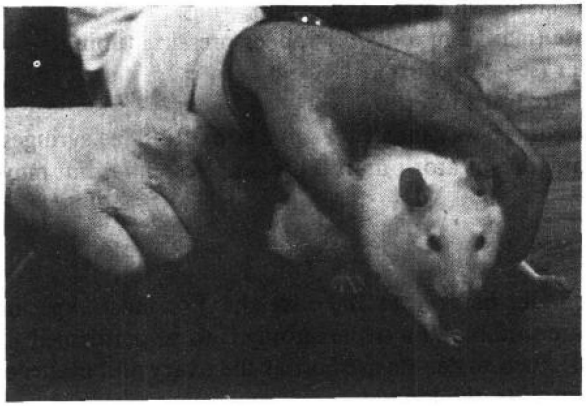


FIG. 1. A method of picking up a rat. Note position of the hands as the animal is drawn slowly along the bench towards the experimenter.

and across in front of the other leg onto the lower jaw. At the same time the fingers are wrapped round the thorax of the animal with the forefinger in front of the rat's other forelimb (Fig. 2.) Hold the animal firmly, but not too tightly or you may strangle it! This handling technique is most successful if the animal is picked up in one smooth movement, but this does require some practice.

The anaesthetic is given intraperitoneally, that is, into the abdominal body cavity. The best site for injection is on the midline of the abdomen, level with the anterior edge of the hind limbs (Fig. 2). This position gives a good chance

FIG. 2. A method of picking up a rat and administering anaesthetic intraperitoneally.

of avoiding injection of anaesthetic into the bladder or gut of the animal. The syringe needle should be jabbed in sharply, and when the tip is free to move in the peritoneal cavity the appropriate dose of anaesthetic injected. The actual dose given depends on the weight of the animal, and a dose of 1 ml/kg (60 mg/kg) Nembutal is used. The anaesthetic takes about 15–20 min to have maximum effect. The tests employed to gauge whether anaesthesia is sufficiently deep for the purposes of these experiments are as follows:

1. Absence of changes in the rate of respiration or in abdominal muscular tone on pinching the end of the tail.

2. Disappearance of the leg withdrawal reflex on pinching the toes.

3. Disappearance of the eye-blink reflex on touching the eye with a pair of forceps.

The third reflex is the last to disappear, and the animals are normally safe to work on if the first two groups of reflexes are completely suppressed.

If the initial dose of anaesthetic does not lead to a suitable depth of anaesthesia within 20 min, further injections of about 25% of the original dose should be given every 15–20 min until anaesthesia is satisfactory. During prolonged experiments booster doses of the anaesthetic (25% of initial dose) are given when required, as shown by the reappearance of one or more of the reflexes listed above. Normally this is about every $\frac{3}{4}$–$1\frac{1}{4}$ h.

## TRACHEOTOMIZING THE RAT

Frequently the nasal passages of an anaesthetized rat become congested with mucus. Insertion of a tube into the trachea (tracheotomy) will bypass this region and allow the animal to breath freely. For any non-recovery experiments with prolonged anaesthesia it is advisable to tracheotomize the rat before carrying out any other procedures.

With the anaesthetized rat lying on its back, make a transverse cut with coarse scissors across the neck region about half-way down the neck. This cut should be about 1 cm long. It reveals connective tissue and the salivary glands (Fig. 3). The connective tissue must be pulled away until a pair of longitudinal muscles overlying the trachea are revealed. Wherever possible it is best to tear connective tissue apart with forceps rather than cutting it. In this way if care is taken the tissue will give down the line of least resistance, i.e. through the connective tissue rather than through more complex structures such as large blood vessels. When clear, the muscle over the trachea can either be cut transversely and peeled back towards the thorax, or the muscles can be separated down the longitudinal axis, revealing the trachea.

Using the curved forceps, draw a piece of cotton under the trachea and tie a loose ligature round it. Prepare the tube for the trachea. A piece of tubing about 5 cm long and 2–2·8 mm diam. is suitable. Cut one end of this off at