PHARMACOLOGICAL EXPERIMENTS ON ISOLATED PREPARATIONS

By the staff of the
Department of Pharmacology
University of Edinburgh

Preface by
W. L. M. PERRY
O.B.E., D.Sc., M.D., F.R.C.P.E.

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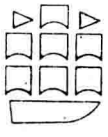

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PREFACE TO FIRST EDITION

The Department of Pharmacology in the University of Edinburgh is a very large one and provides teaching programmes for medical and dental students, for students of the Faculty of Science reading for degrees in chemistry and in biology, and for students proceeding to an honours degree in pharmacology. It has therefore been necessary to develop practical classes designed to meet the needs of each of these groups of students. We have thus accumulated, over the years, a wide experience of the sorts of experiments that are suitable for each purpose and that are reliable in the sense that they will work. This book has been written in the hope that our experience will be of value to others faced with similar problems of developing suitable practical courses.

Most practical courses include both experiments carried out on isolated tissues or organs and experiments using whole animals or man. These two kinds of experiment call for two kinds of apparatus, organization and skill. Furthermore experiments on whole animals must be arranged as demonstrations since, in the United Kingdom, they cannot be performed by students who are not normally licensed by the Home Office; and experiments in man always involve ethical considerations of one sort or another. The two kinds of experiment thus form quite distinct and separate groups each presenting its own particular problems. In this volume we deal only with experiments of the first kind, namely those using isolated preparations. All the experiments described can, therefore, be carried out by the students themselves. Included in the text, however, are three preliminary procedures, the sensitization of guinea-pigs to egg albumen (p. 75), the induction of oestrus in rats by stilboestrol treatment (p. 92), and the injection of heparin into rabbits (p. 108), all of which require for their performance in the United Kingdom a Home Office Licence. These procedures must therefore be carried out by a member of staff holding the necessary licence.

Practical classes serve different purposes for different types of student. In elementary classes they are illustrative, designed to show the student, for himself, and preferably in a dramatic way, what drugs will do. This information, otherwise, is available only at second hand from books or lectures; satisfactory films are seldom available. It is our practice in Edinburgh, when dealing with elementary classes, to set up all the experiments before the students arrive. All the preparations are therefore working satisfactorily at the start of the class. This does, of course, add to the burden upon both academic and technical staff, but the gain is very considerable. When students, however intelligent and interested they may be, are asked to set up experiments for themselves, they usually spend most of their available time struggling with unfamiliar techniques and often, even when they are careful, the preparations do not work well. It is much better that they should spend their limited time seeing exactly what drugs will do to the functioning preparation.

On the other hand more advanced students, in the final honours year, must learn to

comprehend the difficulties and limitations of techniques and must, to some extent, acquire practical skills. Their time is not so limited and it is our practice to require them to set up all their experiments for themselves. Furthermore when they carry out, as they do, some of the simpler techniques designed primarily for elementary classes, we will usually extend the scope of the exercises carried out with the preparation to include more sophisticated experimental designs or to enlarge the variety of drugs tested.

The choice of experiments for any particular course is essentially an exercise of judgement on the part of the staff concerned. We have indicated, for each experiment, the length of time required on average for its performance since time will often be a major factor in choice. Apart from this, it may be useful to point out that:

(i) a satisfactory general course for medical students can be built upon experiments 2, 7, 10, 12, 14, 20 and 29.
(ii) experiments 5, 6 and 15 are particularly suitable for chemistry students.
(iii) experiments 1, 9, 13, 17, 24 and 25 are more complex than the others and are mainly used for honours students.

In preparing this book we have drawn heavily upon Professor J. H. Burn's *Practical Pharmacology*, Blackwell, Oxford, 1952, which has for many years been the only, and a very valuable, guide to suitable experiments. Our experiments 4, 19, 20, 23, 28 and 29 are, indeed, adaptations of experiments described in that book.

I am deeply indebted to all the members of the staff of the department who have provided material for the book. Dr R. B. Barlow and Dr T. B. B. Crawford, who have the widest experience, have done the bulk of the writing. Mr L. J. McLeod has personally checked most of the experiments and himself designed experiments 8 and 31. We are all most grateful to the artists, Mr R. Callander, Mr I. Ramsden and Mr D. Lang and to the staff of Messrs E. & S. Livingstone, all of whom have helped us greatly.

We very much hope that this collection of experiments will be of real value to teachers of pharmacology. We also hope that they will adapt, modify and extend them so that practical classes in pharmacology may be a really useful and stimulating experience for students. We would particularly welcome comments, criticisms, and suggestions from any reader who shares this aim.

Edinburgh, 1968 W. L. M. PERRY

SECOND EDITION

This book has been revised and extended. We wish to thank John Bedwani, Ph.D. for help with the details of the chick oesophagus and guinea-pig vas deferens preparations and also particularly to thank Fiona Franks, B.Sc., for extensive checking of the text and of many of the experiments.

In deference to comments from reviewers we have abandoned any attempt to distinguish between chemical names and approved names.

Edinburgh 1970 PHARMACOLOGY DEPARTMENT STAFF

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CHAPTER ONE

MATERIALS

The experiments described in this book all involve the actions of drugs on pieces of tissue which have been taken from a freshly-killed animal and are kept alive in a suitable salt solution.

Physiological salt solutions: These must be carefully prepared with pure materials, e.g. of 'Analar' grade, and glass-distilled, or deionized, water. It is important to select the particular solution in which a tissue survives longest. The composition of a number of solutions and the preparations for which they are most suitable is shown in Appendix I. It is also important to aerate the solution with the appropriate gas mixture. For example, the continued aeration by pure oxygen of a solution containing a bicarbonate buffer will cause it to lose carbon dioxide and become alkaline.

Instead of weighing out the solid salts every time a solution is required, it may be more convenient to prepare concentrated stock solutions of some, or all, of the constituents, and to take appropriate volumes of these. By whatever method the solutions are prepared, the errors involved in making them should not exceed 1 per cent. For preparing 10 litres of Tyrode's solution, for example, the sodium chloride, glucose and sodium bicarbonate can conveniently be weighed out on any balance sensitive to 100 mg.; there is no need to use an analytical balance weighing to 0·1 mg. The other salts are usually added from stock solutions in volumes of 10 ml. or more, which can be measured with a 10 ml. graduated pipette. Because calcium chloride and magnesium chloride are very hygroscopic, it is best to add them from stock solutions whose composition has been checked by titration, e.g. against silver nitrate, with potassium chromate as indicator. Alternatively a ready-standardized solution (M/1) of calcium chloride can be obtained commercially and an equivalent amount of magnesium sulphate, 7 H_2O, which is not hygroscopic, can be used in place of magnesium chloride. The exchange of sulphate for chloride does not appear to matter; the proportional reduction in chloride is only small because most of the chloride is added as sodium chloride, and the amount of sulphate added is harmless. Instructions for preparing the salt solutions used in this book are given in Table I. It is best to add the calcium salt last, after all the other salts and most of the water, to avoid the risk of precipitating poorly soluble calcium salts.

Physiological salt solutions can be kept for about 24 hours, but they are good media

TABLE I

Preparation of physiological salt solutions

	Quantities for 10 litres of:				
	Frog-Ringer*	Krebs	Tyrode	Ringer-Locke	De Jalon
NaCl	65 g.	69 g.	80 g.	90 g.	90 g.
KCl 10 per cent	14 ml.	35 ml.	20 ml.	42 ml.	42 ml.
$MgSO_4$, $7H_2O$ 10 per cent	—	29 ml.	26 ml.	—	—
NaH_2PO_4, $2H_2O$ 5 per cent	1·3 ml.	—	13 ml.	—	—
KH_2PO_4 10 per cent	—	16 ml.	—	—	—
Glucose	(20 g.)	20 g.	10 g.	10 g.	5 g.
$NaHCO_3$	4 g.	21 g.	10 g.	5 g.	5 g.
$CaCl_2$ (Molar)	10·8 ml.	25·2 ml.	18 ml.	10·8 ml.	2·7 ml.
Aerating gas	air	$O_2 + 5\%$ CO_2	O_2 or air	pure O_2	$O_2 + 5\%$ CO_2

For amphibian tissue use frog-Ringer solution.
For mammalian or avian skeletal muscle use Krebs' solution.
For intestine use Tyrode's solution.
For heart muscle use Ringer-Locke solution.
For the rat uterus preparation use De Jalon's solution.
Tyrode's solution is sometimes aerated with $O_2 + 5\%$ CO_2.
The frog-Ringer solution listed above contains more bicarbonate, phosphate and glucose than originally described. The glucose can be omitted, unless the preparation is working hard or for a long period. A frog-Ringer solution for electrophysiological studies is described on page 124.

for the growth of micro-organisms and must be chilled. If it is wished to prepare them more than 24 hours in advance of the time when they will be used, they can be stored in the refrigerator for longer periods, provided that the glucose and calcium salts are omitted and only added when the solution is required for use.

Treatment of tissues: The preparations must not be pulled about at any time. Any attempt to wash out the contents of a piece of gut, for instance, by blowing them out quickly with a pipette will almost certainly damage the gut beyond recovery. Particular care must be taken with nervous tissue. Damage is especially liable to occur when the preparation is being mounted in the physiological salt solution. If it is tied to a lever which has not been properly balanced it may be suddenly grossly overloaded and ruined. It can also be damaged during washing. If the bathing fluid is removed, the preparation loses buoyancy and will sag. This disturbance in itself may cause responses to be irregular for many seconds, or even minutes. If the preparation has a nerve connected to an electrode, e.g. the rat diaphragm preparation (p. 33), and the nerve is not long enough, it may be badly stretched during washing. It is best with many preparations to wash by overflow, i.e. by upward displacement instead of by draining downwards, though if the tissue is robust enough to tolerate washing by draining and refilling, this is often the more convenient method.

Tissues are very sensitive to metals, especially copper and mercury, but including iron. Pins for impaling tissues should preferably be made of platinum or tungsten. Tissues can also be mounted by tying them to a glass or Perspex hook. Both rubber and plastic tubing contain toxic material, added as fillers or antioxidants. This can usually be got rid of by boiling the tubing and then washing it in running water for 24 hours. Red rubber, however, is usually toxic even after this treatment and should be avoided, though it may be used to take the outflow away from a preparation to the sink.

Apparatus must be washed through with distilled water immediately after use to discourage the growth of micro-organisms, and the glass parts should be cleaned in chromic acid if the apparatus is to be stored for some time. Chromic acid can be prepared by taking a saturated solution of sodium dichromate and adding sufficient concentrated sulphuric acid to redissolve the CrO_3 which is formed. The sodium salt is used rather than the potassium salt because it is more soluble and the cleaning fluid must be made up in a beaker made of heat-resistant glass. Glassware should not be pickled in chromic acid for more than about 12 hours and must afterwards be washed thoroughly to remove traces of cnromium.

Control of temperature: Amphibian tissue will survive for long periods at room temperature and this may be steady enough for regular effects to be obtained without using a thermostat. Most of the experiments described, however, involve mammalian tissue and are made at body temperature. The preparations may actually last longer at lower temperatures, e.g. 30° C, but their sensitivity to drugs is often markedly altered by changes in temperature, even as small as 1° C. It is therefore essential to use a thermostat. Equipment sold for keeping tropical fish in aquaria is often capable of keeping the temperature steady within 0·5° C and is adequate for most purposes. More elegant, but more expensive equipment can be bought from laboratory suppliers.

Figure 1 shows a typical arrangement, which can be used for nearly all the experiments with mammalian tissues described in this book. The outer bath is made of Perspex and the inner bath, in which the preparation is mounted, is made of glass. The coils connected to this organ bath are necessary to allow the physiological salt solution to warm up, so that there is no temperature change when the tissue is washed. They should have a volume greater than that of the organ bath and preferably more than twice this.

It is also important not to add volumes of cold drug solution direct to the tissue, which are big enough to cause a fall in temperature. If the organ bath has a volume of 10 ml., it is necessary to avoid giving doses of more than 1·0 ml. of a drug solution whose temperature is 15° C because the glass of the organ bath is a poor conductor of heat. Drug solutions should, obviously, not be used immediately they have been taken from a refrigerator.

Although the outer bath only contains tapwater, it will become contaminated with salt solution, especially because preparations are often washed by overflow (p. 3); it must be drained at the end of each experiment—switch off the heater first!

Recording devices: In most of the experiments the effect which has to be measured is a change in length or tension; in a few it is a change in volume, or a change in the rate of flow of fluid through the tissue.

The simplest and cheapest device for measuring a change in length is a suitable lever, examples of which are shown in Figure 2. The length of the lever is usually chosen so as to magnify the response between 5- and 7-fold and the load on the lever is usually between 0·5 g. and 1 g. The writing point on the tip of the lever makes a mark on the smoked paper attached to the drum of a kymograph by transparent adhesive tape and a permanent record of the experiment is obtained by varnishing the paper. If a plastic varnish such as 'Vinylac' is used, the tracing is dry in a few minutes. If the response of the preparation is sluggish it may be necessary to attach a vibrator to the upright on which the lever is mounted to prevent the writing-point sticking (p. 38) but this is quite unnecessary with preparations which give fairly rapid responses.

If the response is very rapid, e.g. the twitch of voluntary muscle or the contraction of heart muscle, a lightly sprung lever must be used. With other responses, e.g. the contracture of a piece of ileum, a simple lever is adequate. This is often fitted with a frontal writing point, so that the response appears as a straight line on the paper, instead of as a curved one. With such a lever, however, the relationship between the shortening of the tissue and the length of the line on the paper is not a fixed ratio, nor does the load stay constant. With an 'auxotonic' lever the load is deliberately planned to increase as the size of the contraction increases. For true isotonic recording, i.e. with a load which is constant whatever the size of the contraction, electrical devices are often superior, and they are the only really satisfactory way of recording isometrically, i.e. of recording increases in tension when the length of the tissue is kept constant. Their disadvantages are their expense and (often) their complexity, which may make it difficult for students to use them correctly. An isotonic lever can however easily be modified for electrical recording (Fig. 2*g*) in such a way that it provides a flexible and easily understood system which is satisfactory for experiments with smooth muscle.

In experiments in pharmacology, however, the imperfections of levers as recording devices may not be as serious as they are in physiology. The pharmacologist is largely

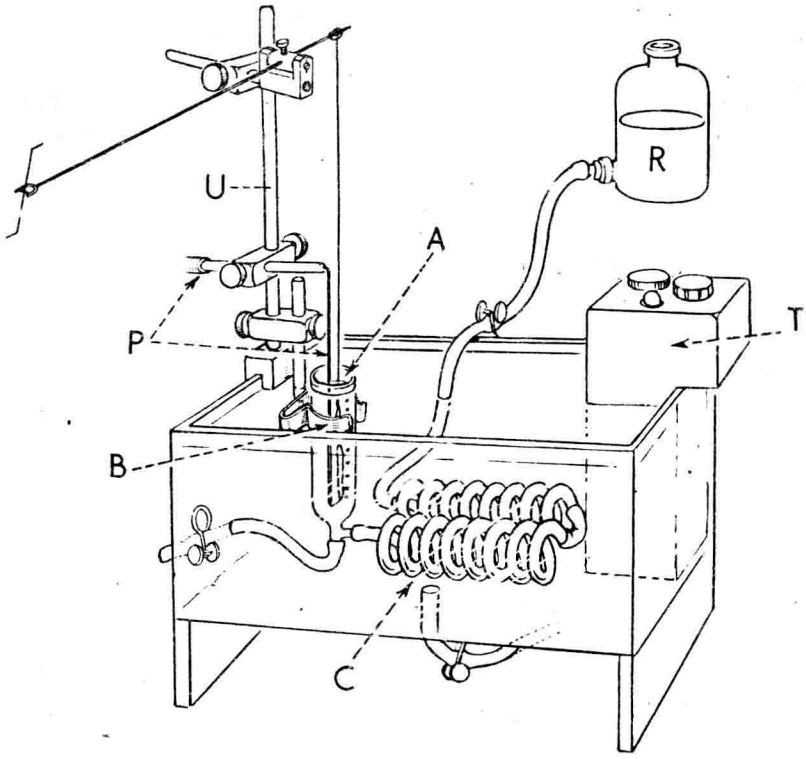
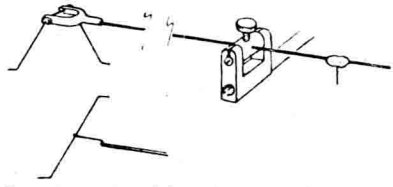


FIG. 1. *Typical apparatus for experiments with isolated tissues*

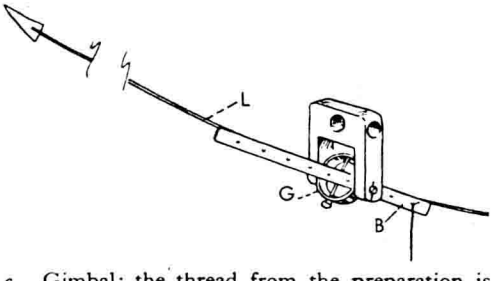
The preparation is placed in the glass organ-bath, A. One end is tied to the fixed pin, P, which may be hollow and serve as an aerator. The other end of the preparation is attached to a lever or force-displacement transducer, also mounted on the upright, U. The organ-bath, A, is held in place by a plastic-coated Terry clip, B, mounted on a brass rod. The circulator and thermostat, T, illustrated can be replaced for most experiments by apparatus sold commercially for aquaria, which is less expensive. The physiological salt solution in the organ-bath is changed at frequent intervals but the fresh solution from the reservoir, R, will have been standing in the coils, C, so the temperature of the preparation should not be altered when it is washed. If electrodes are required for stimulating the preparation, these can also be conveniently mounted on the upright, U.

FIG. 2. *Levers*

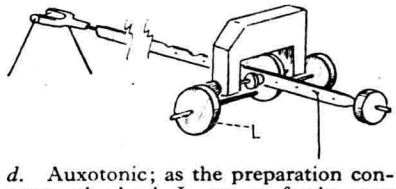
a. Sideways-writing; the simplest form of lever but it gives a curved line when the preparation contracts



b. Frontal writing; because the point is hinged and the lever writes frontally, it gives a straight line when the preparation contracts; the lower picture shows an alternative writing-point.

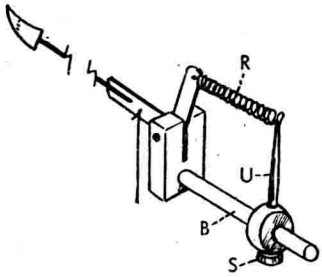


c. Gimbal; the thread from the preparation is attached to the bar B; the lever itself, L, is mounted in the gimbals, G, and the friction between the lever and the paper can thus be kept constant.

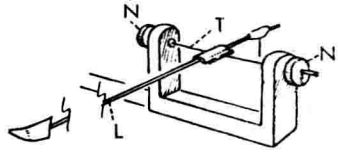


d. Auxotonic; as the preparation contracts, the load, L, moves further out from the fulcrum and so exerts a greater effect.

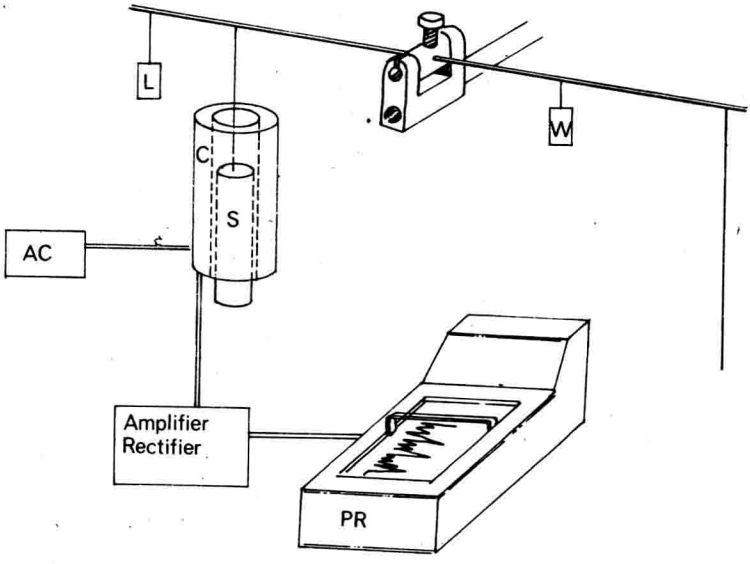
FIG. 2. *Levers*



e. Sprung; the thread from the preparation is attached on the same side of the fulcrum as the writing-point and acts in opposition to the return-spring, R, whose tension can be adjusted by unscrewing S and altering the position of the upright, U, on the bar, B.



f. Torsion; the lever, L, is mounted on a nylon thread, T, or fine metal strip, whose tension can be adjusted by the nuts, N.



g. Lever modified for electrical recording; as the muscle contracts the slug, S, is pulled into the differential transformer, C, and the increase in the induced current is amplified, rectified and moves the pen of the potentiometric recorder, PR. The weight, W, balances the weight of the slug, S, so the load on the preparation is the small weight, L. Note that the contractions are not magnified mechanically; the slug need move only small amounts, within the range in which its movement is directly related to the pen movement (check this!); the magnfication is achieved electrically. The system has appreciable inertia but functions well with relatively slow responses such as those of smooth muscle.

concerned with comparing concentrations of different drugs which produce comparable responses and it is not critical if the record does not absolutely indicate the response, because the responses to all the drugs should be affected to the same extent. The inertia of a lever, however, seriously limits its usefulness in experiments with very rapidly contracting tissues, such as isolated auricles. Although all the experiments in this book which involve effects on the length of a tissue are described for performance with levers, they can equally well be performed using electrical recording, if this is available (Fig. 3).

Some experiments involve changes in volume and these can be detected with a simple float recorder. Figure 4 shows such a recorder fitted to detect changes in the volume of the contents of a piece of gut. When peristalsis occurs, fluid is displaced back into the reservoir and, in its turn, the float will be displaced upwards by the air above the fluid. A decrease in the volume of the fluid inside the gut will therefore appear as an upward line and an increase as a downward line. Float recorders are often difficult to operate with small changes in volume because of the friction necessary if the writing point is to produce a trace on kymograph paper covered with soot. Sensitive pressure-transducers are available which are much more satisfactory.

Figure 5 shows how a float recorder may be used to detect changes in the rate of flow of fluid through a tissue.[1] The fluid is collected by a tube with a constricted end. When the rate increases, the head of fluid behind the constriction will rise and this will raise the float recorder attached to the air-lock. The apparatus is calibrated by observing the height of the line corresponding to a particular rate of flow, measured with a stop-watch and a measuring cylinder. This is done for a number of rates of flow and the height of the line on the record is plotted against the rate of flow.

When the rate of flow is slow this method is not satisfactory and it is necessary to use some kind of drop-counting apparatus. In some of these, the drop forms an electrical contact across two wires and the completion of the circuit activates a counting device. In others, the drop falls past a photo-electric cell and so activates the counter. With the Gaddum drop-timer, a lever rises upwards until a drop falls; a fast rate of flow is indicated by a series of short lines close together and a slow rate by a line rising far up the paper, possibly becoming level when the lever can rise no further. With a Thorpe drop-timer, the impulse provided by each drop causes the lever to rise one step, usually 1 mm.; it will continue to rise until, at the end of a fixed time interval selected from a time-clock, it returns to the starting line. The height of the trace therefore indicates the rate of flow.

Stimulators: In a number of experiments it is necessary to stimulate the preparation electrically. The shape and duration of the impulse is very important, particularly when voluntary nerve and muscle are involved. With the older type of stimulator, using an induction coil or a condenser-discharge device, it is impossible to obtain regular twitch responses at a slow rate from such preparations. The length and shape of the stimuli almost invariably cause repetitive firing and a short tetanic response, rather than a twitch. Stimulators which deliver rectangular-wave pulses of short duration are essential; these are available commercially and are not expensive.

[1] Stephenson (1948). J. Physiol. **107**, 162.