Volume 4

# EXPERIMENTS IN PHYSIOLOGY AND BIOCHEMISTRY

Edited by G. A. KERKUT

ACADEMIC PRESS
London and New York

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# EXPERIMENTS IN PHYSIOLOGY AND BIOCHEMISTRY

Volume 4

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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.

DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY
UNIVERSITY OF SOUTHAMPTON
ENGLAND

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.

## Guiding Principles in the Care and Use of Animals

Approved by the Council of the American Physiological Society

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.

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 there is nothing "magic" about the specified 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.* (1971). 4, 1–33.

# 1 | Sterol Biosynthesis

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## A. The Biosynthesis of 3β-Sterols

### Animals and Apparatus

*Animals*

The details given refer to the snail *Arion rufus* but apply also to other gastropods like: Limax, Limnaea and Helix. In principle every species can be used for this experiment, but in several phyla of the animal kingdom the capacity of synthesizing 3β-sterols is absent (has been lost?).

The total fresh weight of the animals should be at least 100 g.

*Apparatus*

Three rectangular chromatography tanks for the ascending system on glass plates 20 × 20 cm. Each tank should be pre-equilibrated with, and used for one solvent only.

Homogenizer, electrically driven with cutting action and beaker of about 1 litre (Braun mixer).

Rotary evaporator (Büchi, Rotavapor R) with water jet aspirator.

Vacuum pump for evacuating desiccators.

Steam bath (Homef).

Fraction collector (L.K.B.).

Drying oven (Heraeus), attainable temperature at least 110°C.

Thin-layer coater (Motorized TLC coater, B.T.L.).

Balance, weighing milligrams to one figure (Mettler B5).

Balance, weighing milligrams to three figures (Mettler S6).

Liquid Scintillation Spectrometer (Packard Tri-Carb).

When available a small centrifuge (3000 rev/min) with centrifuge tubes.