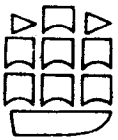
Introduction to Research in Medical Sciences

A. Cuschieri,
P.R. Baker,

Introduction to Research in Medical Sciences

A. Cuschieri, M.D., Ch.M., F.R.C.S. (Ed.), F.R.C.S. (Engl).
Professor and Head of Department of Surgery, University of Dundee

P.R. Baker, B.Sc., Ph.D.
Lecturer in Surgical Science, Department of Surgery, University of Dundee;
Formerly, Lecturer, Department of Surgery, University of Liverpool



CHURCHILL LIVINGSTONE
Edinburgh London and New York 1977

CHURCHILL LIVINGSTONE
Medical Division of Longman Group Limited

Distributed in the United States of America by Longman Inc., 19 West 44th Street, New York, N.Y. 10036 and by associated companies, branches and representatives throughout the world

First published 1977

ISBN 0 443 01466 3

Library of Congress Cataloging in Publication Data

Cuschieri, Alfred.
Introduction to research in medical sciences

Includes index.
1. Medical research. I. Baker, P. R. joint author. II. Title. [DNLM: 1. Medicine 2. Research. W20.5 C984i]
R850.C87 610'.7'2 76-22624

Printed in Great Britain
by Butler & Tanner Ltd, Frome and London

Preface

The need for this book became apparent to the authors from the number of postgraduate students inquiring about suitable introductory literature on medical research. While there are many excellent books and monographs relating to the subject, each covers in the main a certain aspect of research such as experimental design, statistical analysis, laboratory procedures, etc. To the authors' knowledge a broadly based introductory account dealing with the subject of research in medical sciences as a whole is not available, and this book has been written to fulfil this requirement. It is intended primarily for the young medical graduate who desires to spend a period of his postgraduate training in a research department undertaking investigative work, and is therefore designed to set the scene for his commitment. It should also be of interest to other graduates working in the biological and medical sciences.

The book deals mainly with the basic principles involved in the setting up, and execution of, research projects right through to the stage of data analysis and presentation of results. Although throughout the book strong emphasis is made on information of a practical nature, it is not intended as a laboratory manual. A guide to further reading is given at the end of each chapter and includes reference books which the authors have found to be particularly useful.

We have endeavoured to avoid controversial issues and to express the general consensus of opinion, although it is virtually impossible not to incorporate some personal viewpoints in a book of this nature. In that this book reflects the research experience gained over a number of years by a clinician and a scientist working in close co-operation, it is hoped that the account has not omitted important issues, although it cannot purport to cover all the aspects of what is essentially a very loosely defined subject.

Finally, it is hoped that this book will not only provide introductory information on research in medical sciences, but will also stimulate interest in the discipline amongst clinicians in general.

1976

A.C.
P.R.B.

Acknowledgements

The authors would like to express their sincere thanks to Mrs Marion Thomas and Miss Lynn Jones for undertaking the typing of the manuscript, and to Miss Maureen Crowley for her help in compiling the index. We are also indebted to Mr K. Sanderson, medical artist, for some assistance with the illustrations and to the Department of Photography at the University of Liverpool for the excellent prints used to illustrate the handling of laboratory animals.

Our thanks also go to the various technicians with whom we have been closely associated in our research endeavours, as they have in many indirect ways materially contributed to the preparation of this book.

Contents

1. The Nature and Principles of Experimental Work

The experienced research worker attains his proficiency in investigative work after a period of disciplined training that enables him to acquire the ability to recognize a problem and to analyse its various aspects so as to formulate a logical working hypothesis that might explain it. He then has to choose the most effective laboratory methods that are at his disposal in order to test this hypothesis. This training requires not only experience with the various laboratory techniques, but also a trained mind that can dissect a problem into its essential components in a logical sequence, and analyse the findings of investigative studies. Further requirements include scrupulous honesty, intellectual integrity, persistence and sheer hard work. The initial idea inevitably requires months of hard reckoning and hours of seemingly endless experimental work before flaws can be detected in the design of the protocol. It is evident from the above considerations that the best introduction to investigative work is achieved by a period of attachment to an experienced research team.

There is little doubt that such a period of research training is beneficial in the making of a consultant clinician as it equips the young trainee with the ability to evaluate published work and instills in him a sense of purpose whereby methods of medical treatment are continually reassessed as beneficial to the patient or not.

Research can be divided into two broad types, fundamental or basic research and applied research, although there is a considerable overlap. The former involves the discovery of new phenomena while the latter is concerned with applying existing knowledge to new situations. Most medical research is of the second type where information unearthed by basic scientists (e.g. physicists, chemists, biologists) is applied to the diagnosis, treatment and prevention of human disease. Of course many medical research establishments also conduct basic research into the physiology and pathology of man. Generally speaking, however, the newcomer to medical research will be undertaking applied research.

It is often difficult to define with accuracy what instigates a particular research project. More often than not, however, a critical appraisal of the current knowledge on a particular subject prompts a certain question or questions that can only be answered after a carefully planned scientific study. Equally, a phenomenon may be observed which cannot be accounted for on the basis of the current knowledge, but a hypothesis can be formulated to explain it. In this event the

experiment is usually designed to test the prediction and therefore provide indirect evidence for or against the hypothesis. On other occasions research may be conducted to test another researcher's hypothesis and this may entail repeating his experiments. The need for this arises in two ways: either the original work was suggestive, but not conclusive, or the results obtained carry important practical connotations that affect treatment policies. The development of new and better methods of analysis may also make it necessary to repeat past investigations.

Experiments are therefore conducted principally to verify hypotheses, and the process of experimentation essentially involves the taking of certain observations (data) from a population specified by the hypothesis. The acceptance of a hypothesis entails the determination that an observed difference between two experimental groups is real and not the result of chance occurrence or intrinsic differences between the two groups. By the use of statistical analysis it is possible to make a decision whether to accept or reject the hypothesis for which the experiment was designed. Raw data are usually treated prior to statistical analysis and presented in some accepted format such as mean and variance.

Investigative work therefore follows certain logical steps which can be delineated as follows:

1. Original idea or problem
2. Critical review of literature
3. Formulation of a hypothesis
4. Statement of the problem
5. Experimental design
6. Experimental investigation
7. Accumulation of raw data
8. Treatment of raw data
9. Statistical analysis
10. Conclusions.

Review of literature

The main objective is a critical assessment of the information available on the subject matter in question. At the same time details are obtained of the best methods of investigation in current use and their reliability. A good review of the literature also helps to formulate the experimental plan or may suggest important alterations in the original design of the experimental protocol. A sensible start is to read the more comprehensive textbooks, annual reviews and monographs on the subject, bearing in mind that in an attempt at a clear exposition of the state of the knowledge these accounts may gloss over important controversial issues.

There are three main bibliographic tools available and all entail an

acquired ability to utilize the facilities of a medical library. Perhaps the most widely used is in the *Index Medicus* which is published monthly by the National Library of Medicine. Original articles and review papers are classified either under a subject or author index for the year in question.

The authors find the *Citation Index* very helpful. In this instance the search is started with a recent publication on the subject known to be pertinent. This is referred to as the starting reference. By reference to the appropriate section of *Citation Index*, a list is found of the current works which cite the starting reference. In the same index one can then look up works quoted on the list and obtain their full titles and complete bibliographic descriptions. Any of these articles could again be used as a starting reference to obtain another list of works that cite this starting reference, and so on.

Current Contents, published weekly, lists current tables of contents of most of the scientific and medical journals and is very useful as an up-to-date reference list because inclusion of published literature in such periodicals as the *Index Medicus* and the *Citation Index* lag behind publication of the scientific journals themselves.

The U.K. Medlars Information Retrieval Service provides a computer produced bibliography based on the *Index Medicus*. By contacting the Medlars Liaison Officer (Medical Library, University of Manchester) it is possible to obtain computer searches over recent and/or past years on the subject matter. In addition, 'update' searches can be obtained which will bring the reference list up-to-date. This system saves a lot of time that is required by the traditional hand search at a minimum of expense but it is only as complete as *Index Medicus* itself. Construction of the programme can also be difficult especially if a comprehensive search of several topics is required.

There are several methods available for filing information obtained from the literature search. The simplest consists of 3in × 5in filing cards kept in a file box and arranged alphabetically by the first author. On each card is written the full title of the paper with author/s name, journal, volume, year and pages. On the other side of the card are written the key words pertaining to the article in question (some journals provide the key words), whether the article is of a review nature or not and any other comments as to its usefulness.

Having reviewed the literature it is important to write down a critical account of the present state of knowledge on the subject as this is extremely important in formulating the experimental protocol. Incidentally, many heads of department put great emphasis on a written review of the literature by a candidate intent on embarking on a research project, and very often use it as a yardstick in assessing the suitability of the candidate.

The literature search, if done properly, will indicate to the investigator the key references on the subject, and reprints or photostats of these

should be obtained and filed in the laboratory for easy reference.

Hypothesis

The most important feature about a hypothesis is that it is a trial idea or suggestion which should not be accepted without adequate tests. Formulation of a hypothesis should, where possible, be based on accepted and proven knowledge thereby suggesting a logical sequence to what is already established as fact. Moreover, it must be specific rather than a vague general statement, and it must lend itself to a possible solution with the appropriate experimental techniques. Some would further add that the question it raises must not have been previously answered. One of the commonest types of hypothesis is the null hypothesis, meaning that there is no difference between the two groups other than that which can be attributed to chance. If statistical analysis reveals a significant difference between these two, the null hypothesis is usually rejected, and a new hypothesis would need to be formulated to explain the discrepancy.

Statement of the problem

The problem must be analysed and expressed in its simplest form. It may sometimes be feasible to dissect the problem into parts which are more easily answered separately. Furthermore, it is necessary to approach the issue in stages, starting with the simplest possible version.

Experimental design

There are several well established experimental designs. In the simplest type of layout, *the completely randomized design*, the treatments under study are allocated to all the units (e.g. animals) used in the study entirely at random. Other designs such as the Randomized blocks, Latin squares, Cross-over design, Graeco-Latin square etc., involve the construction of groups of units and utilize restrictive randomization. It is beyond the scope of this book to deal with the many and varied experimental designs and there are many excellent books on the subject. In any event, it is always advisable to obtain expert advice on this as the type of design will vary with the nature of the experiment.

Nevertheless, certain fundamental principles such as the use of controls, choice of samples, randomization, etc. apply to all research endeavours and must be strictly adhered to if results are to be meaningful.

Variables. These are defined as essential conditions which when set at a certain value ensure the occurrence of a given event. Thus, for example, in the boiling of water in a container the essential variables include the temperature of the flame, atmospheric pressure, the heat conductivity of the container and the purity of the water. In the design of an experiment careful consideration must be given to the nature of the

variables which prior information indicates as being the controlling ones. The investigator has to decide which variables can or should be controlled in the experiment. There are, of course, in any scientific study variables that cannot be controlled and whose exact effect on the results cannot be ascertained. The process of randomization will ensure an equitable distribution of these unknown variables between the experimental and control groups.

In experimental work, and this includes clinical trials, the principle often adopted is the method of difference. This applies to two sets of circumstances or groups that are alike in every aspect except one which is under investigation. In the event that a significant difference is obtained between the two comparable groups, this can be attributed in part or in whole to this singular difference between the two groups. This principle implies the use of controls with which 'the test group is compared, requires a careful choice of sample and necessitates randomization if we are to compare like with like.

Controls. The use of a control is to nullify the effect of variables which may be operating in an unknown or uncontrollable way during the experiment and to eliminate chance occurrence. The essential requisite of controls is that they must be similar to the experimental group (same population), except for the change in the variable that is being investigated. Thus if an experiment is designed to study the effect of a particular operation, the control animals must not only be of the same species, but also have to be subjected to the same anaesthesia and a 'mock' laparotomy in order to be comparable with the test group—the *'sham operated' animal.* In biological research, the control animals must be matched with the experimental group so that they are as nearly alike in all pertinent features as possible, i.e. species, sex, age, etc. One cannot, of course, prove that the control series is exactly alike to the experimental series but important differences can certainly be excluded by the use of random sampling. In any event, there are statistical tests which determine whether the two groups could be regarded as being random samples of the population. It is possible to study a single experimental group where each unit (animal) acts as its own control. Thus the effect of a particular operation can be assessed by comparing preoperative with postoperative data in a sample of that particular population.

Choice of sample. A collection of individuals which form part of a class is called a sample of that class and since it is manifestly impossible to study whole populations, samples of populations (human or animal) are used in research. It is of course necessary that the samples selected must not be correlated with the attribute under study. On the other hand the sample selected will be dependent on the nature of the experiment.

Indeed in biological research careful consideration must be given to the selection and use of a particular animal species which whilst not introducing any bias to the results, is most suitable for the study in question (Ch. 3). In biological studies involving effect of drugs, vaccines,

etc., random sampling is often used as it is the safest way of selecting a sample that is truly representative of its class.

Randomization. The process of randomization is extremely important in biological research as animals of the same species show a variability in both health and disease. By randomization we ensure that the distribution of unknown variables is equal between the two groups (test and control) rendering them truly comparable. It also ensures that there is an equal chance for any of the experimental units (or animals) to receive any one of the treatments that are to be tested. This process of random allocation can be carried out by simple means such as the tossing of a coin or die, but the best and most convenient way is to use a table of random numbers. If after randomization, one group is found to vary from the other to a degree that would be unlikely to be due to chance alone (as judged by the appropriate test of statistical significance) one can then safely accept that the variable under study has produced a definite effect and refute the possibility that the result is due to the action of unknown variables.

Replication. When the class under study cannot be precisely defined and is subject to wide individual variation, a common problem in biological research, repetitive identical experiments (replications) are necessary in order to define a difference. The number of experiments needed for a given case depends on the magnitude of the expected differences, the uniformity of the material under study, and the precision of the methods used for obtaining the data. In this respect again the advice of a statistician should be obtained at the outset.

Experimental investigation

When the experiment has been planned, the next step consists of setting it up and thereafter obtaining the relevant data. Some general suggestions are pertinent at this stage but it must be stressed that the ability to execute an experiment efficiently is gained by experience and involves a period of training.

The methodology involved must be shown to be reproducible and this often entails the use of pilot studies on such matters as recovery rate, variability and standard error of any one particular analytical procedure. It is important that proficiency in the use of apparatus be gained before the study begins. Furthermore, the investigator must understand the theory on which the apparatus was designed. Very often calibration of instruments is necessary and this must be left to no one but the investigator himself. This holds as well for calibrations provided by the manufacturers particularly after the apparatus has been used by others (Chs. 4 and 6). Careful labelling of experimental and control material is of course essential right at the outset as otherwise endless confusion would inevitably result. Not infrequently after a few experiments it becomes obvious to the investigator that the original protocol has to be altered for a variety of reasons. In this respect an important maxim is

not to introduce more than one alteration at any one time. It is of course essential that data should be entered directly into a notebook at the time of the experiment. It is intolerable to rely on memory or use scraps of paper for primary recording because of the inevitability of both error and loss. The use of 'dry runs' is desirable when the investigator is not familiar with a particular experimental model to iron out problems before a crucial experiment is performed. Sometimes pilot studies are required to test the experimental protocol and/or to quantitate the expected difference and therefore indicate the number of replications required.

Accumulation of raw data

The information obtained when an investigative work is completed is referred to as the raw data. They may be of one or other type: qualitative or quantitative. *Qualitative, discreet* or *enumeration data* apply to items of a population that cannot be measured but which can be classified into antagonistic groups, e.g. number dead/number unaffected by therapy, etc. *Quantitative* or *continuous data* have a range of values which can be measured accurately, e.g. weight, serum concentration of a particular solute such as urea etc.

Treatment of raw data

Raw data are analysed and converted into meaningful values such as means, standard deviations, standard errors, etc. which apart from being a more convenient way of presenting one's data also allows easier comparison of experimental groups. The use of desk top calculators and computers are invaluable for this purpose. Not infrequently some form of transformation of the data is also necessary and this will be dealt with elsewhere (Ch. 10). In some cases particularly complex data may require treatment by large computers. Most research establishments have their own computer(s) or ready access to one, and personnel available for advice and help in composing programmes and interpretation.

Statistical analysis

In medical and biological research, the results obtained usually exhibit some scatter because of the effects of uncontrolled variables, and natural variability. This resulting scatter may be large enough to conceal the effects of the variables under study. It is the purpose of statistical analysis to provide ways and means of treating data so that the maximum information can be obtained with a known and predetermined risk of drawing a false conclusion, i.e. the probability that a difference obtained is due to chance. The principles of statistical analysis are outlined in Chapter 10. It is important at this stage, however, to stress that the appropriate test of statistical significance must be used and that the use of statistics does not render valid the results of a badly

designed or poorly conducted experiment. The advice of a statistician is essential in all but the simplest analysis and should be sought at the onset of the research project.

Conclusions

It is all too easy, once the data have been analysed statistically, to rush into making sweeping conclusions. There are many instances on record where this has been done, but where the conclusions made have not been borne out by later and possibly more extensive investigations. Before firm conclusions are made the researcher must take into account the many facets of his investigative work such as the number of observations, the accuracy and precision of his analytical methods, the effect of uncontrollable variables and the type of biological material used. In any event, statistical analysis only points to the probability that observed differences or similarities are or are not due to chance alone. It does not *prove* the hypothesis but only suggests the likelihood of it being correct or not. Finally, conclusions can only be made on the basis of the actual observations made by the investigator and not on inferences that these observations may imply.

FURTHER READING

Bright Wilson Jr., E. (1952) *An Introduction to Scientific Research.* New York: McGraw-Hill.

Cochran, W. G. & Cox, Gertude M. (1950) *Experimental design.* New York: John Wiley and Sons, Inc.

Fisher, R. A. (1966) *The Design of Experiments,* 8th edition. Edinburgh: Oliver and Boyd.

Hamilton, M. (1974) *Methodology of Clinical Research.* Edinburgh: Churchill Livingstone.

Kempthorne, O. (1952) *The Design and Analysis of Experiments* London: Chapman and Hall

2. Organization of Research

General considerations

In 1972 the Council for Scientific Policy, in a report on the future of the Research Council System in the United Kingdom, recognized three categories of scientific work: tactical, strategic and basic. Tactical research or science, which is financially supported by Departments of State and Industry, is directed to further their immediate executive and /or commercial functions. In this respect it has to be consumer-orientated. Strategic research is carried out on a broader scientific front but with due regard to national goals; it involves a variety of scientific disciplines working singly or in collaboration, and is needed as a foundation for tactical research. On the other hand, basic research has no specific application in view and is directed to ensure the advance of scientific knowledge and to maintain a body of suitably trained scientists in the many and varied disciplines.

It can be surmised from the above that research is performed by individuals who are employed in public and private industry, in Government Departments, in various Research Councils and Institutes, and in centres of higher education such as universities and polytechnical colleges.

Research into the medical sciences is becoming increasingly complex as its organization into multi- and interdisciplinary studies has become more frequent. Perhaps this has been the single most important innovation in medical scientific inquiry and it has resulted in clinicians working in closer association with scientists in other disciplines, such as physiology, biochemistry, chemistry, engineering, etc. Whilst it is not the aim of this chapter to deal with the organization of multidisciplinary studies, their importance is stressed as it seems highly probable that major medical advances will, in the future, emanate from those types of multidisciplinary endeavours. Indeed there is an increasing number of basic scientists working within clinical departments.

Research posts

Research in medical sciences is undertaken by medical and science graduates but not all of them will be intent on an academic career where a higher degree is considered to be essential. A frequent source of recruitment by university departments is from medical graduates wishing to embark on a career in hospital medicine because the attainment of a higher degree greatly improves the chances of

promotion. In university departments the organization of research is usually supervised by the head of the department in conjunction with senior members of his staff such as readers and senior lecturers. Within such an establishment, research personnel are graded, according to seniority and experience, into students, research assistants, research associates, fellows and temporary and full-time lecturers. Some of these research posts may carry in addition part-time clinical duties usually in the registrar or senior registrar grade. It is within this framework that the medical graduate intent on a period of investigative work for a higher degree is usually accommodated. Having decided on the particular discipline in which he is interested, his first priority is to contact the head of the department known to have that special interest. In fact the choice of any particular research establishment both within and outside university departments is one of the most important decisions that the young medical trainee has to make. In the end it must of necessity be a personal decision based on expert and carefully considered advice together with tactful enquiry and the availability of research posts. Perhaps the most important factor involved relates to the type of career the medical graduate has decided upon, so that he may ensure that his research will be relevant to the work of his known speciality.

Having chosen the department it is often desirable for an individual to formulate a research project before he approaches the head of the department. However, in some instances, he can be accommodated in ongoing research projects and this may be preferable if he has had no previous experience in investigative work. This must not, however, be interpreted as an easy substitute for original thinking, initiative and dedication which are essential attributes, without which there is little point in individuals attempting any investigative work. Joining an ongoing research project offers certain advantages to the inexperienced research worker as it allows for constant expert advice and guidance from the more senior members of the research team and ensures a period of training in methods of experimental work.

Financial support

At this stage, the financial considerations relating to salaries and other research expenditure need not concern the young medical graduate as usually the head of the department arranges for the necessary funds to be made available either from local sources or on application to one of the various grant-donating institutions. There are, however, in many countries a number of research scholarships that the aspiring candidate may apply for.

In most universities there is only a limited amount of money allocated to departments to support research so that reliance is heavily placed on financial support from outside sources. These may be local or regional such as hospital endowment funds, local industry, family foundations

and charitable organizations. Contributions from these local sources are perhaps the most useful method of financing new projects on a short term basis. Once promising results are obtained in this way, one can more confidently apply for supporting funds from larger grant-donating organizations. This requires formal application by established investigators to private foundations such as the Nuffield Foundation and the Wellcome Trust, national research organizations such as the Medical Research Council and the National Institute of Health, in addition to international bodies such as the World Health Organization. The nature of the proposed work usually suggests the appropriate institution to be approached for financial support. In any event, an application for a grant to any of these bodies must be detailed and presented in an orderly fashion. It must be realized that the success of the application is dependent on its scientific merits as judged by recognized experts in the field of study. Some institutions, such as the Medical Research Council, issue application forms with the relevant headings and instructions which must be strictly adhered to. Whether or not this is the case, any application for a grant must include a brief summary of the background of the proposed research work with the necessary key references, a detailed description of the proposed experimental protocol and the reasons for the request for financial support. In addition details of the techniques, analytical methods and animals to be used in the study must be stated. Finally the application must include an estimate of the expenditure involved on an annual basis. This will cover capital expenditure required for the purchase of particular items of equipment as well as recurrent expenditure relating to salaries (e.g. for technicians), animals (purchase and keep), operating theatre costs and other consumables such as glassware, reagents, etc. It is important that time and effort are spent to obtain as accurate an account as is possible of the estimated total expenditure. It is of course impossible to forecast the rise in the cost of living over a number of years and most grant-donating bodies make an allowance for this. A successful application carries with it a responsibility on the part of the investigator that the research be carried out as expeditiously as possible and annual progress reports on the results obtained and details of expenditure incurred have to be sent to the respective grant-awarding organization. Courtesy dictates that publications resulting from the research project must acknowledge the financial support by a particular organization.

Research departments

Most research establishments conform to a basic plan although, of course, important differences exist between various centres, largely depending on the discipline and the nature of the research work carried out. The more usual research establishment consists of a number of laboratories, study, consulting and seminar rooms, offices, library, storage rooms, workshops, and an animal house with its associated