STATISTICAL METHOD IN BIOLOGICAL ASSAY

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PREFACE

In this book, I have attempted to provide a comprehensive account of designs and statistical analyses for biological assays, both as a textbook for the student of statistics and as a work of reference for the practitioner of bioassay. Simple introductions to the subject are to be found in C. W. Emmens's *Principles of Biological Assay*, and in special chapters of P. György's *Vitamin Methods* and J. H. Burn's *Biological Standardization*. My aim, however, has been to develop the theory and methods as far as seems practicable at this time, and to show the unity of the various aspects of the subject. Although considerations of length have compelled me to assume in my readers a reasonable familiarity with the basic statistical techniques used in scientific research (§ 1.5), I have given detailed explanation and illustration of all matters peculiar to the statistics of assays.

Many experimenters who are not themselves statisticians can, usefully and without undue difficulty, attain sufficient knowledge of statistical techniques to aid their own work. Nevertheless, I make no apology for emphasizing in this book the rôle of the professional statistician rather than that of a particular group of experimental scientists. The proper function of the statistician in scientific research and technology is no longer merely that of analysing and summarizing large bodies of numerical data: he must also be prepared to advise on the plan and economy of each investigation, in the light of its operational efficiency for a particular purpose. The efficiency attainable will depend upon the nature and reliability of existing information. The statistician must therefore be a major contributor to the answering of the question: " In the present state of knowledge about the nature and behaviour of certain materials and subjects of experimentation (or of non-experimental observation), how should the next investigation be planned in order to obtain the most trustworthy information on specified points from limited resources of materials, subjects, or time ? " He must later analyse the data from the investigation, report on the information they give for the immediate purpose, and be prepared to integrate this information with that previously existing, as a prelude to the

planning of further investigations on the same or related topics. The cycle of design, analysis, report, and integration recurs frequently in the work of the statistician, and the intimate relationship between its parts is particularly well exemplified in biological assay. A familiarity with the details of assay design and analysis is to-day necessary to many statisticians working as consultants in scientific research; in addition, a study of the manner in which the parts of this cycle interlock may be enlightening even to those whose chief interests have no connexion with bioassay.

I cannot adequately express my gratitude to Dr Eric C. Wood, who has for seven years stimulated my interest in biological assay. His persistent refusal to be satisfied by any incomplete answer to a question has compelled me to examine carefully the statistical techniques needed for assays, an inquiry which naturally disclosed gaps in the theory and practice of a branch of statistics that had scarcely been regarded as a unified whole. I have tried to make my own contribution to the filling of these gaps, but my part has mostly been to develop and express more fully the ideas of the pioneers of the subject—Bliss, Burn, Fieller, Gaddum, Hartley, Irwin, Trevan, to name only a few. Many of my own ideas, especially in connexion with slope ratio assays, originated in discussion with Dr Wood; he has given much thought to the logical basis, validity, and interpretation of assays; he has advised me on details of biological, chemical, and experimental theory and practice with which I am unfamiliar; finally, he has given most generously of his time in reading and criticizing the typescript of this book, and has suggested many improvements in matter and presentation.

In 1947, Professor J. H. Burn asked me to write a chapter on statistical methods for his *Biological Standardization*; in 1949, Professor Gertrude M. Cox invited me to lecture on biological assay in the Summer Session in Statistics at the University of North Carolina. I am grateful to Professor Burn and to Professor Cox for having caused me to put my thoughts on bioassay into logical order, for out of the work then done this book has grown.

My understanding of bioassay has gained immeasurably from conversations and correspondence with many other friends. I want to thank especially Dr Joseph Berkson, whose ideas on quantal responses have greatly influenced the argument of this book (though possibly not always in the way he would wish), and Dr. N. K. Jerne,

who, with Dr Wood, first formulated the ideas in Chapter 15. I am also glad to acknowledge my gratitude to Professor P. A. P. Moran, to my father, and to my wife, for reading the typescript of this book and commenting on style and content; to Miss M. Callow, for a final checking of almost all the calculations and for drawing the diagrams; to Mr A. L. Bacharach, for the many occasions on which he has drawn my attention to interesting problems in analysis and for permission to publish the data in Tables 12.1 and 14.2; to Dr W. F. J. Cuthbertson, for permission to publish the data in Table 16.1; to Professor R. A. Fisher, Dr F. Yates, and Messrs Oliver and Boyd, Ltd, for permission to publish full or abridged versions of Tables III, V, V_1, IV, and XII from their *Statistical Tables for Biological, Agricultural and Medical Research* as Appendix Tables I, II, III, IV, and XI; and to the Cambridge University Press for permission to publish Tables III and IV from my *Probit Analysis* as Appendix Tables VI and VII.

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SCHEMES OF STUDY

The arrangement of this book has been planned to give a comprehensive account of the subject for a student of statistical science who is already fairly familiar with less specialized techniques of analysis and with the principles of experimental design. The reader well grounded in the methods of statistical science should encounter little difficulty in a systematic study of the whole book, except, perhaps, in a few sections (such as §§ 2.7, 3.13, 14.3, 17.5, 19.3). Nevertheless, at his first trial he would be wise not to read each chapter fully, but rather should select the more important sections.

Many regular practitioners of biological assay, however, would be reluctant to regard themselves as statisticians, primarily because they lack formal mathematical training. Some understanding of the statistical principles and techniques on which bioassay depends is necessary to them; I am convinced that they can attain a good working knowledge without burdening themselves unduly with mathematical theory. Although this book has not been written for them, judicious choice of sections for study may enable them to gain something useful from it.

In order to guide those who do not propose to read the whole book consecutively, I have prepared suggestions for a first reading by four different categories of readers. These are :—

(A) *The professional statistician desiring a short course in biological assay.* Unless he is already experienced in applications of statistical methods to biological experimentation, he ought first to read one or two of the general accounts of bioassay mentioned in § 1.2, in order to appreciate better the background. This course of study might form part of the programme of instruction for a graduate in mathematics who is beginning to specialize in statistical science.

(B) *The non-mathematical user of biological assays.* He will find much of this book beyond him unless he knows something of the normal, t, χ^2, and variance ratio distributions,

significance tests, the analysis of variance, and the elements of experimental design. He ought first to study these in one of the textbooks mentioned in § 1.5; he may find his understanding of the present book further helped by reading either Emmens (1948) or Chapter III of Burn *et al.* (1950).

(C) *The reader, whether statistician, biologist, or chemist, who requires a general survey of the function of statistical science in biological assay.* The sections suggested for him are those which outline the main features of methods and give simple numerical examples, without much emphasis on details of argument or calculation.

(D) *The reader whose interest lies almost entirely in assays based on quantal responses.* Though he will wish to give most of his attention to the theory and practice of probit analysis and related techniques, he ought at least to read enough of sections from chapters other than 17–21 to enable him to relate these topics to the general theory of bioassay.

The table that follows lists the sections recommended to readers in each of these classes. In general, I would advise that they be read in the order of the book, but in some respects alterations could reasonably be made; for example, all or part of Chapter 15 could be read immediately after Chapter 8, or Chapters 7 and 8 could be deferred until any later stage that seemed convenient. The reader in class B may find that §§ 4.13–4.18 are more easily understood after §§ 5.1 and 5.3. Of course, the sections listed are not necessarily completely self-contained: when the reader encounters references to other sections, he should pursue such of these as seem important to his interest.

Chapter	Sections to be read			
	A	B	C	D
1	All	All	1–5, 9	All
2	1–5, 9–11, 14, 15	1–5, 9–11, 14, 15	1–3, 9, 11, 14, 15	1–5, 9–11, 14, 15
3	1–6, 9–12, 14–16	1–5, 9, 11, 14–16	1–5, 14–16	All
4	1–18, 21, 22	1–13, 15–18, 21, 22	1–12, 21, 22	1–12, 21, 22
5	1–5	1, 3–10	1, 9, 10	—
6	All	1–8, 10, 11	1–8, 11	1–8, 11
7	1–10	1–6, 9, 10, 13	1–6	—
8	1–4, 6	1, 2, 4, 6	1, 4, 6	—
9	1–6, 8, 9	1–4, 8	1–4, 8	—
10	1–4	1–3, 7	1, 2, 4, 7	—
11	1, 2	1, 2	1, 6	—
12	1–4, 6	1, 6	1, 6	—
13	1	1, 4	1, 4	—
14	1, 4	4	—	—
15	All	1–5	All	1–5
16	—	—	—	—
17	1–8, 10, 11, 15	1–4	1–4	All
18	1–3, 6	1, 2, 6	1, 2	All
19	1–3, 5, 8	1, 2, 5, 7	1, 2, 5, 7, 8	All
20	1–3, 5, 6, 12	1, 2, 12	1, 2, 12	All
21	5	—	—	All
22	All	1–3	1, 2, 4	All

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 The purpose of biological assay

Biological assays are methods for the estimation of the nature, constitution, or potency of a material (or of a process) by means of the reaction that follows its application to living matter. For example, a substance might be identified by means of a characteristic reaction produced in a particular species of organism. Qualitative assays of this type, however, do not present any statistical problems. Quantitative assays, with which this book is concerned, are similar to methods of physical measurement or of quantitative chemical analysis in that their function is to provide numerical assessment of some property of the material to be assayed ; an essential part in this assessment is played by measurement of growth or other changes in animals, plants, animal tissue, micro-organisms, or some other form of living matter. An assay is thus a form of biological experiment, but the interest lies in comparing the potencies of treatments on an agreed scale instead of in comparing the magnitudes of effects of different treatments. The experimental technique may be the same as is used in a purely comparative experiment, but the difference in purpose will affect the optimal experimental design and the statistical analysis. An investigation into the effects of different samples of insulin on the blood sugar of rabbits is not necessarily a biological assay ; it becomes one if the experimenter's interest lies not simply in the changes in blood sugar but in their use for the estimation of the potencies of the samples on a scale of standard units of insulin. Again, a field trial of the responses of potatoes to various phosphatic fertilizers would not generally be regarded as an assay ; nevertheless, if the yields of potatoes are to be used in assessing the potency of a natural rock phosphate relative to a standard superphosphate, and perhaps even in estimating the availability of phosphorus in the rock phosphate, the experiment is an assay within the terms of the description here given.