PHARMACOPŒIAS AND FORMULARIES

THE PHARMACOLOGY OF THE BRITISH PHARMACOPOEIA, 1953

BY MILES WEATHERALL

THE changes in successive British Pharmacopœias reflect the changing judgments and fashions in the use of drugs, and it is interesting to observe how the eighth (1953) Pharmacopœia has developed from its predecessors. One section in particular shows a striking change, not so much in its content as in its approach; this is Appendix XV, the appendix which deals with biological standards and the biological standardisation of drugs. This appendix appeared first in the sixth B.P., in 1932, when it occupied 33 pages; it grew to 44 in 1948, and has now all but doubled its original size by occupying 60 pages.

Its expansion reflects partly the inclusion of new standards and methods of assay, but the most striking change is the introduction of 14 pages on statistical methods for the design and interpretation of assays. Previously, the method of analysis was left largely to the discretion of the assayist, and the recommended design of the assay sometimes provided data not readily amenable to the estimation of their error. In most instances, indeed, standard figures were quoted for the error of the assay when performed by the specified method. This declaration of the accuracy of an assay may be convenient for anyone who prefers to treat their results as naïvely as possible, but it is not really justifiable, as the introductory remarks in the 1953 version point out. Groups of animals vary not only in their mean sensitivity to drugs, but also in the extent to which they vary from one another, and the reliability of any particular assay depends on the variability of the particular group of animals used. So generally it is better to estimate the error of an assay from internal evidence and to calculate the limits of confidence of the result of the assay in this way than to use a standard figure obtained with different animals in other circumstances. Statistical methods in the new Pharmacopœia have now been revised and brought into forms which provide not only a satisfactory estimate of potency, but also an estimate of the error of potency and some checks on the validity of the assay.

The necessary designs and methods of analysis are applicable to various different assays, and so the statistical methods have been concentrated in a single prefatory section. Recipes are provided for estimation of the fiducial limits of error in the circumstances which are likely to arise in straightforward assays. The most complicated design considered is a twin cross-over test based on a (2 and 2) dose assay. The recipes achieve a nice balance between the more subtle refinements of statistical theory and simplification carried to a point at which gross inaccuracy is liable to occur. Model calculations are given which are straightforward to follow. Logarithmic transformation of measured responses is recommended for any that vary more than narrowly. Formal analyses of

variance have been avoided, and the examples are worked as far as is feasible in terms of individual deviations from the appropriate means. From the point of view of anyone unfamiliar with statistical methods this is probably an advantage, as it eliminates a certain amount of (to some people) incomprehensible algebra. What remains is probably still incomprehensible to these people, but much thought has evidently gone into introducing no more than the minimum of obscure sums.

It is interesting to observe that the probability which is now specified as "in practice equivalent to certainty" has been reduced from 0·99 to 0·95. This reduction in the former very rigid requirement has doubtless not been made without much consideration and presumably is intended to provide a more satisfactory balance between idealism and expediency.

The numbers of standard preparations listed to be used in biological assays has risen from 20 to 28. Two standards, vitamin A and tincture of strophanthus, have disappeared. Vitamin A has moved to an appendix of its own, as it is now standardised by its ultra-violet absorption spectrum, and tincture of strophanthus has been deleted from the Pharmacopœia. The 10 newcomers consist of aureomycin, dihydrostreptomycin, streptomycin, dimercaprol, tubocurarine chloride, globin zinc insulin, scarlet fever antitoxin, diphtheria antitoxin for flocculation test, and Anti-A and Anti-B blood-grouping sera. These new standards involve new recommended methods of standardisation, but most of the innovations closely follow patterns already laid down. The new antibiotics are assayable in much the same way as penicillin, and globin zinc insulin is assayed like insulin. The test for undue toxicity of dimercaprol specifies a (2×2) dose assay and an estimated toxicity not more than 110 per cent. of the standard: this contrasts with the analogous test for neoarsphenamine, which is more specifically designed, requires only 2 doses, and gives a self-evident answer. The reason for this difference in recommended procedure is not obvious. Only tubocurarine among the new standards involves the description of experimental methods which have not previously appeared in the Pharmacopœia, and both the rabbit head-drop and rat phrenic nerve diaphragm are suggested.

There are some changes in the proposed methods of assay of posterior pituitary extracts and of digitalis. The classical guinea-pig uterus has been displaced for oxytocic assays by the much more manageable rat uterus in a low-calcium low-dextrose medium, and the assay by depression of the chicken's blood pressure is described for the first time. Rather surprisingly, the pressor assay on rats, which is now widely used and is reliable and relatively quick, is not described: for this assay cats continue to hold their official position. The antidiuretic assay has been modified, so that the response of each rat is measured separately, and is measured as a function of the volume of urine passed at an appropriate time in the test, instead of the time to peak excretion of a group of rats being used. This is undoubtedly an improvement, as the amount of information wasted by the old method was very large.

The assay of digitalis on cats has been tidied up by requiring standards and unknowns to be run at the same time, instead of the sensitivity of the

laboratory supply of cats being checked from time to time with the standard preparation. The assay on guinea-pigs is described separately, and the determination of the lethal dose in anæsthetised pigeons has been added.

The main effect of all these changes is to provide a guide to the most convenient of the well established methods of assay, and to give simple standard procedures for designing and analysing the assays so that their reliability shall be calculable on evidence provided largely by the assays themselves. This is a substantial advance on the previous articles on the same topic and is a reminder that much is lost when variable data are treated with uncritical simplicity and the accuracy of assays is merely specified as good or poor, or by an unvarying figure.

(ABSTRACTS *continued from p. 476.*)

BACTERIOLOGY AND CLINICAL TESTS

Isoniazid-resistant Strains of Tubercle Bacilli, Development and Stability of. M. Barnett, S. R. M. Bushby and D. A. Mitchison. (*Lancet*, 1953, **264**, 314.) Strains of *Mycobacterium tuberculosis* resistant to isoniazid, whether produced *in vitro* or isolated from patients, were found to be unstable both in viability and in their degree of resistance, and two such strains and one resistant variant of H37RV were investigated in mice and guinea-pigs. The animals were infected by intravenous injection of a culture of the organism in a modified Dubos medium. Treatment was started immediately after infection, the isoniazid being given subcutaneously twice daily in doses of 10 mg./kg. of bodyweight, and was continued until the animal died or was killed at the end of the experiment. Previous reports that isoniazid is destroyed in Dubos medium at 37° C. were confirmed, but evidence that continuing growth in the medium was due in part to the development of resistance and not solely to destruction of the drug was provided by the fact that the resistance during the next passage through the medium was increased. Resistant variants of H37RV produced *in vitro* reverted to sensitivity if they had been in contact with the drug during only one subculture. If subcultured more than once in the presence of isoniazid, resistance was retained during several passages through drug-free medium. Viability of resistant variants was poor during the first 3 passages in the presence of isoniazid but thereafter it seemed normal. Repeated passages in the presence of isoniazid failed to produce a strain with an inhibitory end-point higher than 25 to 50 μg./ml. With 10 to 16 resistant strains isolated from patients, the resistance fell substantially during 3 subcultures in the absence of the drug. Mice and guinea-pigs infected with sensitive strains were completely protected by doses of 20 mg./kg. With mice infected by *in vitro* resistant strains, survival time was considerably increased. Guinea-pigs similarly infected were almost completely protected and a chemotherapeutic response to the drug by these animals is likely even when they are infected with a strain which will grow in the presence of 1 μg./ml. H. T. B.