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BIOLOGICAL STANDARDISATION OF DRUGS

INTRODUCTION

VOLTAIRE defined 'therapeutics' as the pouring of drugs, of which one knows nothing, into a patient, of whom one knows less. Much water has flowed under the bridges since the above statement was made and fundamental advances have taken place in our knowledge of the chemistry and pharmacology of drugs as well as in the symptomatology and pathology of many disease processes in human beings. But the ancient sneer of Voltaire still expresses the general truth that there are *two variables* in therapeutics—the *patient* and the *drug*. As is well known, it is impossible to control two independent variables and, therefore, it is necessary to see which of the two we can standardise to our advantage. With regard to the *patient*, it is important to realise that no two individuals respond to any drug in an identical manner. Leaving aside certain abnormal cases of hypersensitiveness or idiosyncrasy so frequently met with in medical practice, considerable amount of evidence is available to show that there is a wide individual variation even in healthy individuals in the dose of a drug needed to produce an equal effect. If healthy patients do not react in an uniform manner, it is too much to expect that *diseased* human beings, on whom drugs are ordinarily intended to be employed, would respond uniformly. Such being the case, it is futile to attempt to standardise the patient. The only alternative, therefore, in the interest of the safety of patients and to ensure accuracy in therapeutics, is to standardise the other variable—*the drug*.

PHYSICAL, CHEMICAL AND PHARMACOGNOSTIC ASSAY

How can this standardisation be effected? The accurate use of drugs in therapeutics involves that the amount of the active principles given in each dose must be known or, at any rate, must not be subject to irregular variations. The majority of drugs that are used today are derived from vegetable, mineral and animal sources, and the estimation of the active ingredients in each of them can usually be done by the employment of well-known physical and chemical methods. In the case of vegetable remedies, a combination of botanical and chemical methods are often found adequate. Accepted methods for the standardisation of most of the drugs in common use are given in the recognised pharmacopœias, *e.g.*, the British Pharmacopœia, British Pharmaceutical Codex, the United States Pharmacopœia, the National Formulary, etc., and these methods can be easily employed by all laboratories engaged in the standardisation of drugs, medicinal, chemical, insecticides, cosmetics, etc.

BIOLOGICAL ASSAY

During the present century a group of potent substances, *e.g.*, hormones, vitamins, biological substances, etc., have been introduced into therapeutics whose active constituents are insufficiently known or when known, cannot be easily isolated quantitatively. Some of them are potentially dangerous and in many cases, for their safe and effective use, they must be administered by precise dosage. Since these cannot be assayed by chemical means, other methods depending upon their action on intact living animals or surviving isolated organs or tissues have to be adopted.

In the devising of these methods, advantage is taken of the fact that each of the substances exhibits *specific* biological properties. Thus, insulin administered to animals causes a reduction in the concentration of the blood sugar, old tuberculin produces a typical and specific

action when injected into tuberculous animals, administration of the vitamins to suitably-prepared animals will restore growth, cure scurvy, rickets and other diseased conditions, many of which can be experimentally produced in animals, and so on.

The principle underlying biological assay, as distinguished from chemical assay, is that a certain quantity of a drug will always produce the same degree of deflection from the normal in the same animal or in animals of the same species. This is not always absolutely true, for many conditions may alter the extent to which an animal reacts to a drug, and every precaution must be taken to keep all conditions identical in carrying out these tests. For example, the reaction varies inversely with the weight of the animals, and these must be taken as nearly identical as possible, and the dose must be calculated in terms of the weight of the animal. When great accuracy is required, the test must be done upon a series of animals sufficient to eliminate the variations and idiosyncrasies that cannot be controlled under ordinary circumstances.

The Importance and Scope of Biological Assay

The first official recognition of bio-assay was made in the ninth revision of the United States Pharmacopœia, 1916, by the inclusion of a physiological method of standardisation for cannabis and pituitary extracts. Since then, the method has gained steadily in importance and has been extended to a fairly large number of substances including biological products, such as antitoxic sera, vitamin and endocrine preparations, chemical transmitters, *e.g.*, acetylcholine, synthetic compcunds, *e.g.*, organic arsenicals and antimonials, chemotherapeutic remedies, *e.g.*, antimalarials, antibactericidals, etc., insecticides, disinfectants and other groups of drugs of the type of digitalis and similar glucosides, ergot, aconite, etc.

Though a comparatively new method of standardisation, the utility and reliability of bio-assay have been established beyond doubt. The recent isolation of various hormones in chemically pure form and the newer ideas regarding the chemical transmission of nerve impulse are some of the fruits of biological assay. Drugs such as insulin or liver extract could not have been brought into clinical use unless it was possible to ensure a uniform product through biological standardisation. This method is also indicated if the chemical assay does not give a true value of the activity of the drugs, even when the active principles are chemically known. Thus, chemical assay may fail to separate optical isomers, such as *l*- and *d*-adrenaline, which differ greatly in their physiological activity. The efficiency of a new remedy may be judged with the action of a known sample by means of biological assay It has also been found valuable for the detection of small quantities of potent poisons in the body organs, blood or urine. Furthermore, biological assay is needed to supplement chemical assay in order to observe undesirable toxic effect or to gauge efficient therapeutic activity. An example of this is to be seen in the case of the arsphenamine group of drugs. This can be obtained as pure synthetic compounds of known chemical composition but minute differences in their toxicological behaviour have been found to exist from batch to batch. The animal body can detect such differences which are too fine to be identified by chemical or physical tests. On the whole, the great sensitivity of biological assay is an advantage over chemical assay. Thus, we can easily estimate acetylcholine or adrenaline in a dilution of one in one hundred millions or more. At present we do not know of any chemical method to detect or estimate such extraordinarily small quantities.

In spite of its advantages in certain directions, the biological assay methods must be regarded in most cases as merely a 'stop-gap' which allows the strength of drugs to be controlled before the chemical methods have been developed. Biological tests for some vitamins and hormones have already been replaced by physical and chemical methods, and this process will doubtless continue. Thus the preparations of ergot used to be assayed by the B.P. and is still being assayed by the U.S.P., by biological method. The chemistry of the active constituents of ergot are now better known and it has been found possible to standardise it by means of a colorimetric method. In the case of thyroid preparations also, the biological method has been largely replaced by the chemical method which determines the total iodine content of the glands. However, when biological methods and chemical methods for the assay of a pharmacologically active substance disagree so widely that the disagreement cannot be due to the error of the tests (as is sometimes encountered in hormone research) the biological method is, by definition, right and the chemical method must be assumed to be wrong.

Biological Standards and Their 'Units'

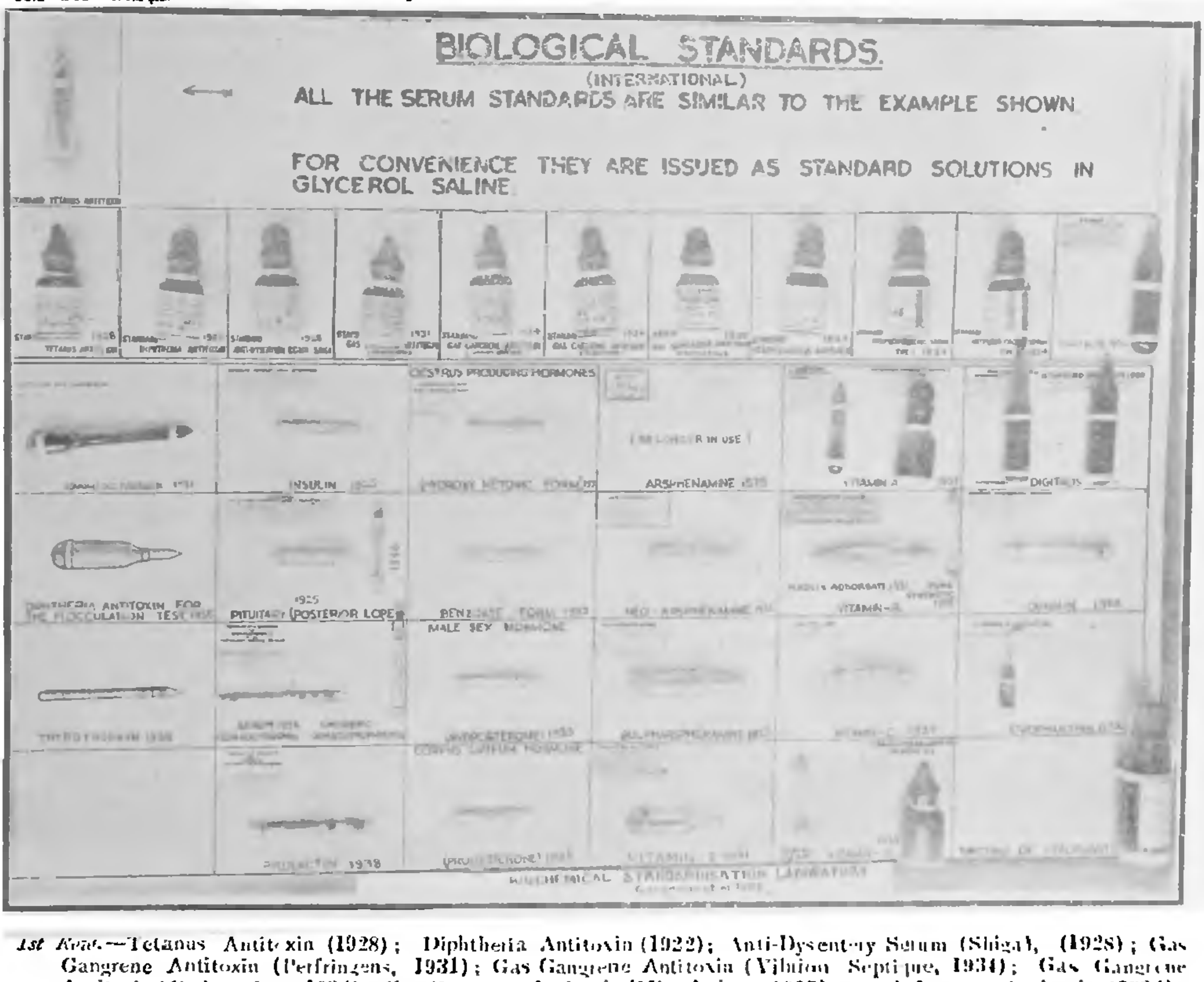
In biological assay, the object is to compare the effects of a preparation with those of a *standard* and whenever possible this standard should be a single, stable and pure chemical substance. For certain vitamins and hormones, for instance, it has already been found possible to take the active substances in chemically pure form as the standard, and to define this by its physical and chemical constants. In the case of adrenaline preparations, for example, there is no prescribed standard. It can be assayed against pure and *l*-adrenaline which is readily obtainable. But this is not always possible and biological standards have often to be employed in cases when a standard in a pure chemical form is not available. Where biological standards are used, the reason is that preparations issued for therapeutic use do not usually contain the active substances in its pure form and are frequently mixtures; so long as these conditions prevail, any method of assay other than the biological will accordingly be unpracticable.

But to have a *standard* preparation is not enough; its action must also be measurable and expressible in terms of a unit of activity. As has already been emphasised, it is not possible to determine the potency of any of these substances by simple observation of the effects

produced in an animal or a small group of animals. It is, however, possible to find out how much of a preparation, the potency of which is unknown, will produce the same biological effect as a definite quantity of another preparation the potency of which is known, provided the tests are carried out under strictly comparable conditions. The recognition of this fact has led to the definition of a biological *'unit'*. This indicates the degree of *specific biological activity contained in a certain weight of the standard.*

International Standards

The decision to have the question of biological standardisation internationally studied was taken by the League of Nations' Health Committee at its second session at Geneva in 1921. In 1924, the Health Committee decided to set up a Permanent Commission on Biological Standardisation consisting of experts from various countries. The Commission decided first, to establish *standard* preparations and second, to select *units* to express their potencies. By international agreement, a number of International Biological Standards have now been established and are available for use all over the world through 'national' distributing centres, so that a common notation and a common standard of reference are available to all research workers, assay laboratories and manufacturing institutions (see picture).



1st Row.—Tetanus Antitoxin (1928); Diphtheria Antitoxin (1922); Anti-Dysentery Serum (Shiga), (1928); Gas Gangrene Antitoxin (Perfringens, 1931); Gas Gangrene Antitoxin (Vibrion Septique, 1934); Gas Gangrene Antitoxin (Oedematiens, 1934); Gas Gangrene Antitoxin (Histolyticus, 1935); Staphylococcus Antitoxin (1934); Anti-Pneumococcus Serum (Type I, 1934); Anti-Pneumococcus Serum (Type II, (1934); Digitalis (1936).

2nd Row.—Old Tuberculin (1931); Insulin (1925); Oestrus producing Hormones (Hydroxy-ketonic form, 1932); Arsphenamine (1925); Vitamin A (β-Carotene, 1934); Digitalis (1925); Digitalis (Brit. Std.).

3rd Row.—Diphtheria Antitoxin for the Flocculation Test (1935); Pituitary (post lobe) (1925; 1940); Oestrus-producing Hormone Mono benzoate of Dihydroxy form (1937); Neoarsphenamine (1925); Vitamin B_1 (standard absorption product, 1931) (Pure synthetic, 1938); Ouabain (1928).

4th Row.—Thyrotrophin (1938); Gonadotrophins (Serum, 1938) (Chorionic, 1938), Male Hormone (Androsterone 1935); Sulpharsphenamine (1925); Vitamin C (*l*-Ascorbic acid) (1934); Strophanthin (Brit. Std.)

5th Row.—Prolactin (1938); Corpus Luteum Hormone (Progesterone, 1935), Vitamin E (1941); Vitamin D (Irradiated ergosterol, 1931) (Calciferol, 1934); Tinct. of Strophanthus (Brit. Std.).

Methods of Biological Assay

While the Standards Commission recommended suitable methods, the methods remain open to progressive modification and improvement or discovery of a new method in accordance with experience and research in this field of study The methods may vary while the activity of the standards is immutable. It is important that the method of assay must be one which measures the important therapeutic principle. Several different methods can be used, provided they measure the same active principle. The result of assay should be the same whatever method is used, and in every case, a 'control' assay should be run with the 'standard', side by side with an unknown sample using the same technique. In general, the methods of biological assay may be divided into two classes:—

(A) *The "All or None" Reaction.*—The 'all or none' biological response indicates that a given reaction is either present or absent. Although perhaps, 'death' occurs more frequently than other end points, the method has been applied to many criteria, such as convulsions, systolic standstill of the heart, the presence of cornified cells in the vaginal epithelium, negative blood smears and survival in a therapeutic test. The biological assay based upon the toxicity test, the assay of œstrogenic hormones and vitamin B are familiar examples of this type of measurement.

(B) *The 'Graded Response' Reaction.*—Another large group of assays depend upon a graded dose-effect response, where the extent of the reaction varies with the dose. Examples are the hypoglycemic response of the rabbit to insulin, the height of contraction of guineapig uterine muscle to posterior pituitary extract, the level of serum calcium in the dog following treatment with parathyroid extract, and the growth of depleted mice under different dosages of vitamin A. Since each individual reaction is quantitative rather than qualitative, it contributes more information per animal than in the preceding type or assay. It is important, however, to choose a dose for the *standard* and for the *unknown* sample, which produces a *median* response. An essential requirement for assay is that equal active doses should produce equal effects. Further, a significant difference in dosage must give rise to an unmistakably different effect.

Sampling Error in Bio-Assays

The most serious difficulty in biological assay is that due to the variability of the 'test objects', *i.e.*, living animals and some of the most serious errors have arisen from a failure to appreciate its true dimension. This error is called the "sampling error". No physiological method has any value which does not eliminate or estimate the animal variations. Two kinds of variation have to be considered: the relatively persistent differences of sensitiveness between one individual and another, and the variations of sensitiveness seen in a *single individual* from day to day, or in a single isolated organ or tissue during the course of an experiment.

Individual variations can be eliminated when the same animal or organ can be used for successive or alternate tests. But even in this case the variations from one time to another are not eliminated. In fact only too often the animal may become sensitised.

When the assay is carried out on different groups of individuals, the difficulties are greater. It is a common habit to assume that, if the results obtained from three successive animals happen to agree, the right answer has been obtained, further results which do not happen to agree being discarded. Such a habit of discarding some unwelcome figures introduces an error in biological assay. If in ten estimations, for example, nine give reasonable agreement and the tenth is considerably higher, the mere fact that the tenth has occurred means that the true *mean* is a little higher than the figures which apparently agree. The operation of the 'law of averages' is important in biological assay.

Fortunately, the sampling error can be estimated by statistical computation. We can, by employing the well-established laws of mathematics, calculate whether the difference in the average potencies of two samples is merely due to variation in the animals selected or is actually 'significant' ('T' value) and is to be taken into account. Similarly, by applying the computation of 'Probability' ('P' value), we can find the best number of animals to employ in an experiment by which an accurate result can be obtained. However, the mechanism of the physiological action of drugs is of so complex a nature that numerous factors may introduce serious errors in the estimated results. It is easy to familiarize oneself with the technique of bio-assays but it is difficult to eliminate or judge the various errors likely to occur. No biological assay can be considered valid unless it takes into consideration all the principles enunciated above.

Bio-Assay of Drugs in India

With the advice of Sir Henry Dale, P.R.S., N.L., lately Director of the National Institute for Medical Research, London, Prof. R. N. Chopra first introduced biological methods for the standardisation of digitalis preparations in the laboratories of the School of Tropical Medicine, Calcutta. Meanwhile, biological standardisation of sera, vaccines, antitoxins, etc., was being carried out at the Central Research Institute, Kasauli, and at the Haffkine Institute in Bombay. Under the able guidance of Sir Robert McCarrison, the Nutrition Research Institute at Coonoor, South India, also adopted approved methods for bio-assay of vitamins and vitamin products.

The much-needed fillip to the wider employment of standardization procedures in the field of drugs came through the establishment by the Government of India of the Bio-chemical Standardisation Laboratory under the direction of Sir R. N. Chopra. Though far from ideally equipped and not endowed with facilities commensurate with the intensity and importance *of the task it is required to tackle, this* Laboratory, during the last six years of its existence, has made significant contributions to this difficult field of work and by advice and guidance, has enabled the drug industry in India

to launch newer projects in the manufacture of glandular products and modern chemo-therapeutic remedies. Previous to the establishment of the Bio-chemical Standardisation Laboratory, opinion with regard to the physiological potency and therapeutic efficacy of products of this group could only be obtained from Britain, Germany or America. In the present state of India's progress in the field of drug manufacture, there is need for increased emphasis in the direction of biological standardization side by side with the development of synthetic and applied chemistry. There is need also for the development of 'Therapeutic Research Institutes' on the lines of the Nuffield Institute in Oxford where experimental medicine and clinical trial of promising drugs on human patients could be undertaken to supplement observations made in the biological standardization laboratories. Only by such organised efforts can India be made self-sufficient in the matter of her drug supply.

B. MUKERJI.

THE WAY AND SPIRIT OF SCIENCE*

BY GENERALISSIMO CHIANG KAI-SHEK

(Chairman of the Military Affairs Commission of the Republic of China and Supreme Commander of the Chinese Armies)

THE SCIENTIFIC APPROACH

PROCEED from the immediate to the distant, from the low to the high; attain the great through the small, the difficult through the simple. To accomplish great and important things, it is necessary to start from the nearest, simplest, and most minute matters, enlarging and expanding gradually in systematic order.

Competence in small things preceeds competence for big affairs; first know how to do commonplace things well; afterwards talk of doing work of special importance. Know how to solve small and easy problems; afterwards you may be successful in rare accomplishments. Never run after speculations and far-reaching conclusions, missing out immediate stages and hoping for lucky shots. Your work will only be superficial; short cuts will not lead to the desired aim, and nothing solid will be accomplished.

THE SCIENTIFIC SPIRIT

In order to investigate thoroughly all the phenomena of man and Nature, our attitude towards anything should be, not only to attempt understanding of what is not understood, and to make experiments where the previous experiments have not been satisfactory; but also to understand further what is commonly supposed to be already understood, and to improve further those experiments which have already been commonly considered satisfactory. The more knowledge grows, the more the lack of it is felt. The greater one's accomplishment, the more intensely the smallness of one's ability is realized. But it is through this very feeling of inadequacy and insignificance that knowledge and achievements can continually progress.

Never be satisfied with obtaining one result or even a small success. Whatever be the department of knowledge one is investigating or whatever kind of work one is to do, it is necessary before starting to consider properly the facts of the case, avoid all vagueness in defining the aim and be sure about the method to be employed. Once started, be determined to carry the project through, working unceasingly and meeting every obstacle with undaunted resource.

Many young men of to-day have no understanding of this principle. They will start to study a subject or work on a project, but when they meet the slightest obstacle or disappointment, instead of holding on doggedly and industriously, they will merely skip the difficulty and turn over to some other easier problem. This mental unsteadiness, chopping and changing time after time, will never bring any result.

There are also many who have as their main object in life the attainment of wealth or high official position, and if they find themselves engaged in work where these aims seem to have little or no hope of realization, they will discard it without any consideration, however important it may be, and creep into some new path. Such opportunists, consulting only their own selfish interests, have no responsibility to the country and the people.

THE SCIENTIFIC SYSTEM

Limitations. In doing anything we must first know the limitations of the question; in other words, one must be quite clear about one's object and aim. This is of prime necessity, for one can then concentrate time and energy upon possibilities within the frame of reference. Thus one can avoid all distractions from other sources; and one will not try to do several things at once. If we think of attacking one problem to-day, another to-morrow, and still another the day after to-morrow, finally nothing will be accomplished. In investigations and affairs it is essential to define the central idea. Only if this is done can we distinguish between the roots and branches of things, avoiding confusion between the first and the last. A clear idea of the order of things, which stands the test of practice, is required for the success of any research.

Management. All reasonable scientific organization must satisfy the following requirements: (a) a vertical definite dendritic order; (b) a horizontal intimate co-ordination. The vertical order embodies the relation between

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* Abridgment of an address by Generalissimo Chiang Kai-shek, given in 1942; translated by Huang Hsing-Tsung and Dr. Joseph Needham, F.R.S. Note that the word "Way" has a special significance in Chinese philosophy. The address is posted in all Chinese Government laboratories and workshops.