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THE CONCEPT OF BIOLOGICAL POTENCY AS APPLIED TO CLOSELY RELATED ANTIBIOTICS

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In the wide field of microbial growth and its inhibition, concerted discussion about biological assay for potency is usually left to workers dealing with the therapeutic applications of an antibiotic, when it becomes necessary to specify the substance with the precision required by governmental regulation and by national pharmacopoeias. Before this stage, the worker manages with his own methods of assay, based sometimes on his personal ideas of what potency means; and the transition to generally agreed measures and methods of measurement may be difficult. In fact, agreement about biological potency is usually reached far too late in the development of an antibiotic. With each new substance there is a good case for the earliest possible attempt to codify and to specify it as an inhibitor of microbial growth.

The Concept of Biological Potency

First let us consider exactly what is meant by potency, and, in particular, how far the concept of potency, as it has been developed in the last 60 years of pharmacology and immunology, is applicable to the newer field of antibiotics. There is a good reason for dealing with such a familiar subject in an elementary fashion, because, although biological assay is in essentials quite simple, the variety and, indeed, the confusion of methods, and the minutiae of the actual practice of assay, often obscure the fundamental principles and so lead into error.

All statements about potency are comparative. For example, we can say that a botulinum toxin is one of the most potent of the bacterial toxins only when we know something about the activity of all bacterial toxins; and it follows that if we wish to make a numerical statement about potency, that statement must in the first place be in the form of a ratio. Thus, preparation X is ten times as potent as preparation Y. This potency ratio is more conveniently expressed as a single figure, and in practice it is usual

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to fix the potency of one preparation of the substance at unity, and measure the potency of other preparations as a multiple or fraction of the unit potency.

Biological potency is by definition a measure of activity in some biological system. The biological system may be a whole animal, such as a cat or guinea-pig; part of an animal, such as a preparation of blood-cells; or a culture of bacteria. Whatever it is, our first idea of potency is in terms of action on a biological system. This is only natural, but from the point of view of the logical assayist it is unfortunate, because most of the troubles in assay arise from the conception of potency as the amount of a substance that has a certain effect on a certain biological system. For example, the

TABLE I. CURRENT METHODS OF MEASURING BIOLOGICAL POTENCY

Reference point	Standard	Unit of potency
A chemically defined substance	Chemical and physical specification (or a *chemical* standard)	Weight
Active material, chemically undefined	Stable preparation	Agreed weight of a unique preparation
A biological system	A "myth"	No valid unit

potency of digitalis extract used to be defined as that dose which killed a cat during slow intravenous injection, and was measured in " cat-units ". The value, as a rigorous measure of potency, of the statement "one cat-unit of digitalis " can be readily seen, however, when it is paraphrased, as a thoroughly objective physiologist might do it, thus : " There is a certain range of lethal doses of this preparation of digitalis which, on the average, happened this week to kill the twenty miscellaneous cats that my cat-dealer managed to collect for me in the districts near my laboratory ". This is an extreme example, because in many assays great care is taken to select biological material that is as uniform as possible. Superficially considered, the microbiologist is fortunately placed for obtaining a homogeneous population of test-objects. He may start with a strain of *Staphylococcus aureus* casually acquired in routine clinical practice, but this is soon tamed and freeze-dried into a semblance of uniformity. In achieving this, however, he has by no means eliminated all gross variability from his test strain, because the biological system he finally uses in assay is not a given strain of microbe, but a complex population of the microbes growing in conditions where they are susceptible to a multitude of environmental changes. It is true that a group of workers thoroughly familiar with their strains, media, and methods of assay, can make reproducible measures of potency in terms of their biological system. But a great deal of the art of such an assay could not be put down on paper in a completely unambiguous fashion. Some of it

would prove to be indescribable and, as a result, the measure of potency would to that extent be a measure private to that group of workers, and therefore lacking general validity. It would be a measure only of a certain range of effective doses in selected and partly indefinable circumstances. Valid biological assay in fact aims at eliminating as far as possible any bias, controllable or otherwise, in the biological system by comparing test and standard preparations simultaneously on the same system, and measuring, in effect, the inherent variability of the system by designing the assay so as to yield a proper estimate of error.

Measures of Potency

There are three current methods of measuring, and attempting to express, the biological potency ratio (table I).

First, the pharmacologist's ideal, in which the activity of a preparation is compared with that of a defined chemical substance. There need be no agreed standard specimen of the chemical. A specification which, like a monograph in a pharmacopoeia, defines the biologically active substance in chemical terms, is a valid " standard " wherever chemistry and physics are competently practised. It may be convenient to maintain an authentic preparation of the substance for use in assay—we have, for example, reached this stage with chloramphenicol, whose potency is sufficiently defined by the weight of the drug—but the authentic preparation is a *chemical*, not a biological standard.

Secondly, assay in terms of a biological standard. The material is ill-defined, but stable and active; and because it is ill-defined, only one preparation can serve as the master-standard, and as the unique repository of the unit of potency. That is to say, an impure substance can be made universally valid as the basis of a potency measure only by agreement that a given preparation shall be regarded as the standard. The international biological standards are in this category; and they hold in the biological field the same position as the standard metre and the standard gram hold in that of physical measurement (Miles [4]).

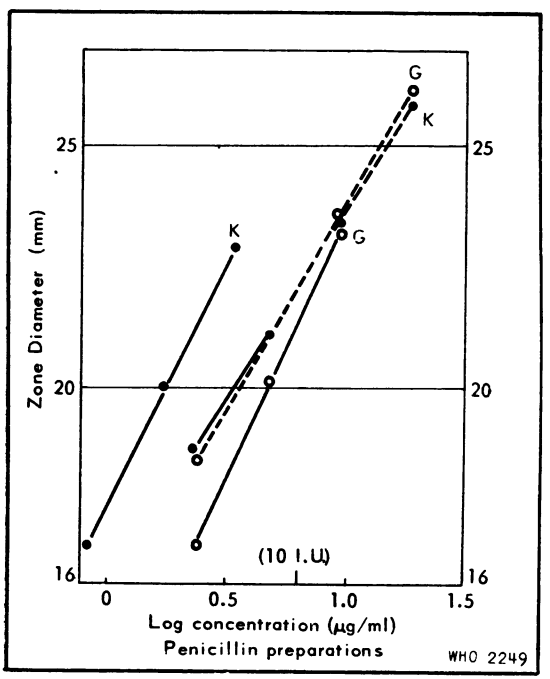
Thirdly, as already discussed above, there is direct assay in terms of the response of a biological system. Although, within a given laboratory, comparable estimates of potency can sometimes be made without reference to a standard preparation, neither a " standard " biological system nor any " unit " based on it can be fully defined for use elsewhere. In a rigorous analysis—and for standardization the utmost rigour is a sine qua non—this "unit" is not valid, and the standard biological system is a myth. In the absence of standardizable substances we sometimes have to admit myths into biological assay, but in these circumstances it would be well always to bear in mind that our indulgence in mythology is very much faute de mieux.

Requirements for Valid Biological Assay

Accepting the principle of measuring the potency ratio in terms of a fixed stable material standard, we must next consider the conditions in which the assay of a test preparation against the standard preparation will be valid.

(1) The biological system used is, in respect of the substance assayed, a random sample of the population of test-objects. It is easy to get random samples of microbial populations, but not so easy to eliminate bias in the

FIG. 1. DOSAGE-RESPONSES OF PENICILLINS G AND K, ASSAYED BY A MODIFIED CYLINDER-PLATE METHOD AGAINST STAPHYLOCOCCUS AUREUS AND BACILLUS SUBTILIS



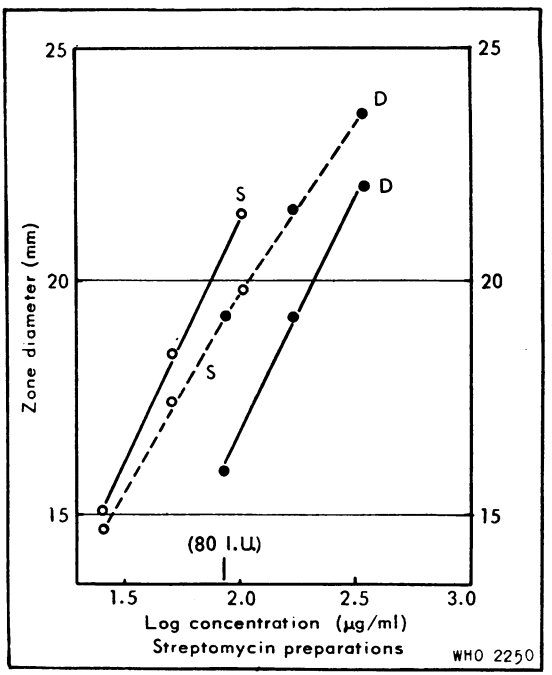
liquid culture or the plate used in the actual assay. In cylinder plate assay, for example, a small variation in the depth of the agar may greatly affect the diameter of the inhibition zone.

(2) The response of the biological system to the standard preparations and the test preparations is produced by the active principle it is desired to measure. The antibiotic expert is fortunate in that he is not, as yet, required to measure therapeutic potency; the assay measures antibacterial potency,

and its translation into anti-infective potency is left to the clinician. (This condition is more important with other substances; for example, in digitalis assay, the step from the poisoning of cat or pigeon muscle to curing heart-disease in man must first be established if the assay is to have any value.)

(3) The ratio of potencies obtained is independent of the biological system used. Theoretically, the same answer should be obtained whether an antibiotic is assayed with leptospirae, lactobacilli, or leuconostoc, provided that the various micro-organisms are susceptible to the active principle.

FIG. 2. DOSAGE-RESPONSES OF STREPTOMYCIN (S) AND DIHYDROSTREPTOMYCIN (D), ASSAYED BY A MODIFIED CYLINDER-PLATE METHOD AGAINST TWO DIFFERENT STRAINS OF MICROBES BELONGING TO THE BACILLUS SUBTILIS GROUP



——— *Bacillus subtilis* (I.C.I. strain)
- - - - - - - - *Bacillus subtilis* (Marburg strain)

(4) The active principle in the standard preparation is homogeneous; and the standard preparation contains no impurities having either a specific activity resembling that of the active principle, or substances which in any way modify the behaviour of the active principle. Thus a penicillin standard should contain only one kind of penicillin, and should not contain, for example, notatin, or any other antimicrobial substance.

(5) The active substance assayed in the test preparation is the same as the active substance in the standard preparation.

These conditions can be derived by an examination of the fundamental assumption of valid biological assay. One of the more recent discussions of these assumptions is that of Jerne & Wood,[3] who list three essential assumptions :

(*a*) The differences between responses in the several dose-groups of an assay are wholly caused either by differences in dosage or by random sampling.

(*b*) The expected response, U, is a determinable function of the dose, z, so that

$$U = F(z)$$

where U is a single-valued function of z, over the range of doses to be used.

$$U_s = F_s (z_s)$$

for the standard preparation, S, and

$$U_t = F_t (z_t)$$

for the test preparation, T.

Then for any selected value of U within this range, doses z_s and z_t can be found that give U; and the potency of the test preparation is the ratio : $R = z_s/z_t$. As it stands, the validity of this measure of potency is limited, because it does not necessarily hold when another value of U is chosen and equipotent doses of S and T are determined. A further restriction is needed.

(*c*) The response to both the standard and the test preparation is due solely to the same active principle, unmodified by any other substance that may be present in the preparations. If the ratio z_t/z_s is to be valid over the whole range of doses, it must be independent of U, the conditions of assay, and the species or kind of test-object used.

When this hypothesis of similarity of the active principle in standard and test preparations can be assumed, then in the two relations

$$U_s = F_s (z_s)$$

and

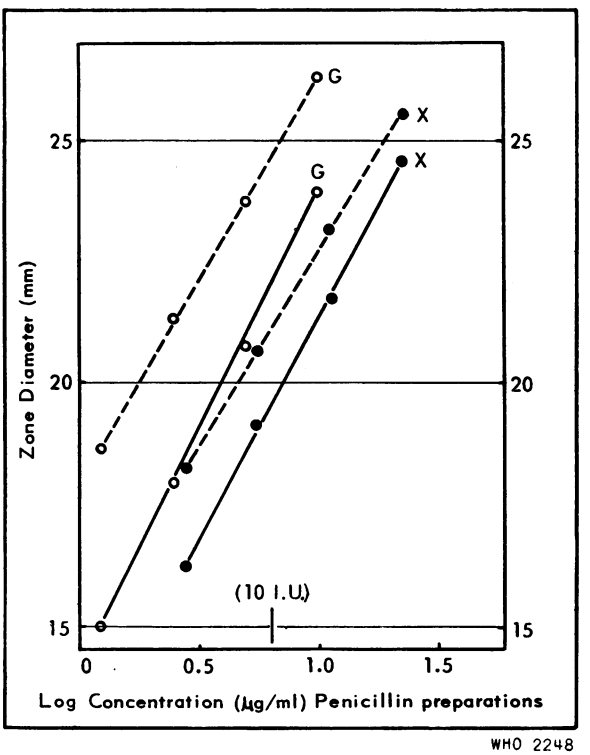
$$U_t = F_t (z_t)$$

the functions F_s ( ) and F_t ( ) are the same.

In the practice of assay, the dose z and the response U are usually transformed into quantities that have a linear relationship. The response is transformed in various ways, and is plotted against the logarithm of the dose. When the dosage-response lines to standard and test preparation are both linear and parallel (e.g., fig. 1, 2, and 3) the potency of one in terms of the other is estimated from the horizontal distance between the lines, which is the logarithm of the potency ratio. In cases of this kind, the potency is independent of the dose, and the particular assay is fully valid. But if the dosage-response lines are not parallel, as in fig. 5, this

distance is dependent on the level of response, and the result of the assay is said to have a restricted validity. The validity is, in fact, much more restricted than this statistical reasoning suggests because, formally speaking, there are no means whereby the result of such an assay can be confirmed. Because, by specifying a response level at which the standard and test preparations are to be compared, a " standard " biological system is automatically

FIG. 3. DOSAGE-RESPONSES OF PENICILLINS G AND X, ASSAYED BY A MODIFIED CYLINDER-PLATE METHOD AGAINST A "SMOOTH" AND A "ROUGH" COLONY VARIANT OF THE SAME STRAIN OF BACILLUS SUBTILIS



implied in which the selected doses would, on repetition of the assay, yield the same potency ratio; and the standardized biological system is a myth.

Assay of Antibiotic Potency

Some of these points may be illustrated in the field of antibiotic assay. Plate assays of antibiotics are admirably suited for statistical analysis of this kind because the diameter of the zone of inhibition, over a wide dose-

TABLE II. COMPARISON OF FIRST INTERNATIONAL STANDARD PENICILLIN WITH PROVISIONAL SECOND STANDARD

Source of variation	Sum of squares	Degrees of freedom	Mean square	F	P
Between rows	28.7500	7	4.1071	1.07	> 0.20
Between columns . .	21.2500	7	3.0357	—	—
Between treatments . .	24731.3750	3	8243.7917	2150.07	< 0.001
Between substances.	1.5625	1	1.5625	—	—
Linearity	24727.5625	1	24727.5625	6449.21	< 0.001
Non-parallelism . . .	2.2500	1	2.2500	—	—
Residual	176.3750	46	3.8342 (error)	—	—
Total . .	24957.7500	63	—	—	—

Analysis of variance of the data from a single agar-plate *Bacillus subtilis* assay with 8 × 8 = 64 treatments

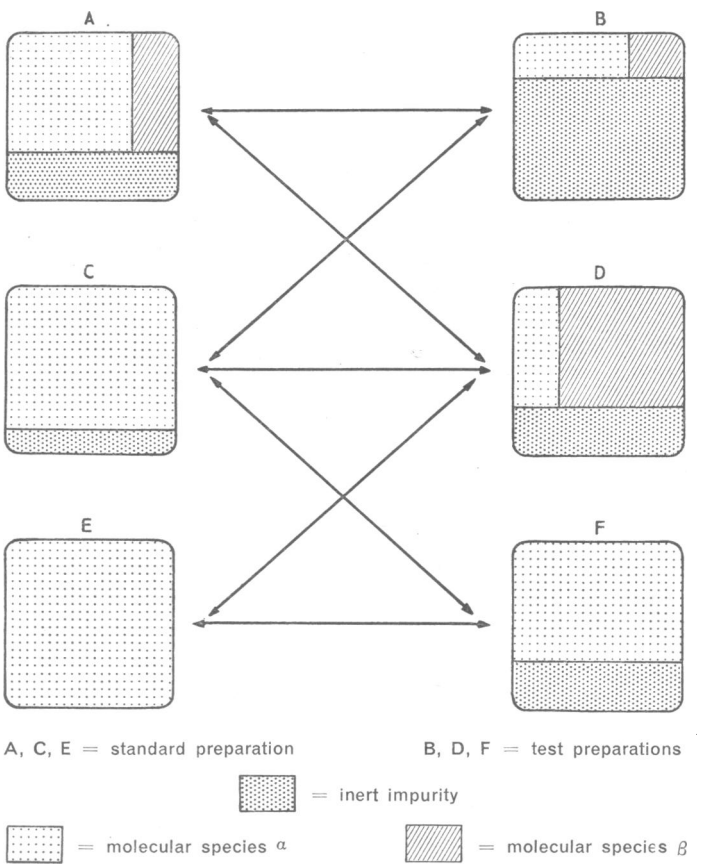
range, is linear with respect to dosage, and very accurate estimates of potency can be obtained. My colleagues in the Department of Biological Standards, Dr. J. H. Humphrey and Mr. J. Lightbown, to whom I am indebted for these illustrations, use a large, square agar-plate upon which eight rows of eight hollow (" fish-spine ") beads,[a] charged with antibiotic solution, can be arranged. The doses of standard and test preparations may be randomized in an eight-fold square. The analysis of variance of a single plate assay of this kind (table II), in which two almost pure specimens of benzyl penicillin were compared, shows that the variance due to position on the assay plate is small. Moreover, when the " between treatments " variance is broken down, that due to linearity is large and that due to deviation from parallelism is small. In this case, the " between substances " mean square is also small, because the two penicillins were almost equipotent.

In an assay like this, where the standard and test preparations are substantially pure, the conditions for valid assay summarized above are almost certainly fulfilled. In the assay of antibiotics in general, however, we have no right to assume they will be fulfilled, because either of the preparations may be impure. Suppose that the first usable crude preparation of an antibiotic is made into a standard (fig. 4 A) and, although we do not know it, it contains, besides inert impurities, two distinct molecular species (α and β) of the antibiotic—as distinct, say, as streptomycin and mannosidostreptomycin. Suppose also that the crude preparations assayed against this first standard contain only these two members of this particular family

[a] The idea of substituting, for the porcelain cylinder, these porcelain beads, which are used in the electrical industry for threading on to live-wires to form a flexible insulation, we owe to Dr. G. A. Stewart and Dr. R. H. Thorpe of Messrs. Burroughs Wellcome, London.

of antibiotics (fig. 4 B). If all the test preparations were like B, and contained the same proportions of α and β, the hypothesis of similarity holds, and assays in terms of A will be valid. But the likelihood of preparations like D and F and of even more complex mixtures makes any confident assumption of the hypothesis of similarity impossible. Both standard and test preparations are in fact likely to be heterogeneous.

FIG. 4. IMPLICATIONS OF ASSAY OF HETEROGENEOUS MATERIAL



With the next advance in purification it may be possible to produce a standard consisting of one molecular species, either pure or with some inert matter (C), but it may still be necessary to use it in the assay of heterogeneous preparations such as B or D.

In fact, we do not reach the valid assay until we know without question that the standard is like E and test preparations are like F.

Consequences of Heterogeneity of Standard and Test Preparations

What are the practical consequences of ignoring, not only the state of the standards, but the purity of our statistical criteria ? First, the potency ratio may vary with the biological test system; and second, there may be a deviation of the dosage-response line from the expected. Thus, an expected linear response may be non-linear, or the two dosage-response lines may be different (that is, the slopes of the dosage-response lines may not be parallel).

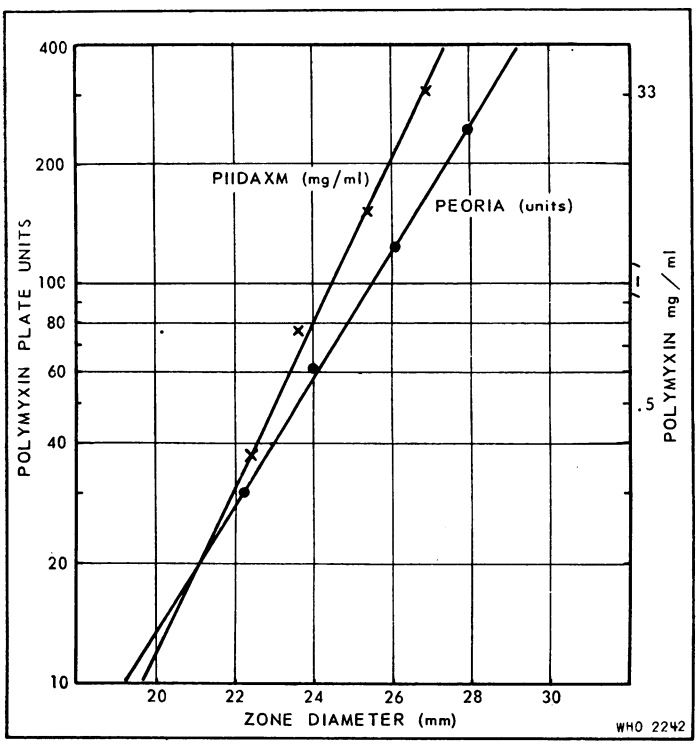
In the field of antibiotics the classic example of variation of potency with the biological system is, of course, that discovered by Schmidt, Ward & Coghill [6] with early penicillin preparations, when they changed from *Staph. aureus* to *Bacillus subtilis*—a discovery which led directly to the analysis of penicillin into a family of antibiotics. Fig. 1 and 2 illustrate the change in the potency ratio with change of biological system, the first for penicillins G and K with *Staph. aureus* and *B. subtilis*, and the second for streptomycin and dihydrostreptomycin when one strain of the *B. subtilis* group is substituted for another. Even a change within a test strain can alter potency ratios substantially. Thus in fig. 3, penicillins G and X are compared on a strain of *B. subtilis* and on a rough-colony derivative of that strain isolated from the edge of an inhibition zone in an assay plate. Such a result serves to underline the dangers of attempting to put into practice the concept of biological assay directly in terms of a "standardized" organism.

So much for difference in potency ratio due to heterogeneity. Within a family of antibiotics it is more difficult to find examples of differences in the slope of the dosage-response lines. Fig. 5, however, reproduced from Reese & Eisenberg's paper [5] on polymyxin assay, illustrated their reason, which proved to be well founded, for suspecting that polymyxin preparations contained more than one type of the antibiotic. Slopes do vary between widely different antibiotics, but not, as might be expected, by analogy with other types of substance. On Dr. Humphrey's and Mr. Lightbown's plates, aureomycin, terramycin, chloramphenicol, streptomycin, and dihydrostreptomycin, using either *Staph. aureus*, *Sarcina lutea*, or one of two strains of *B. subtilis*, gave slopes which were remarkably similar. (It is possible that plate assay masks dissimilarities that might be reflected by differences in slope for, in the plate, slope is probably determined mainly by the rate of diffusion of the antibiotics through a coarse agar gel, and not by peculiarities of the reaction of the different antibiotics with the test organism.)

The implications of heterogeneity in measurements of potency are worth stressing, because in the known antibiotics we already have many examples of a fundamental heterogeneity—not the accidental heterogeneity due to synergistic impurities, but the existence of several members of a family. This is true of the penicillins, the streptomycins, the bacitracins,

the polymyxins, the gramicidins, and others. Moreover, since we are dealing with the products of organisms whose striking characteristic, whether they are considered as genetic individuals or as populations, is an extreme readiness to adapt themselves in their synthetic as well as in their other reactions to changing environment, chemical or otherwise, we shall be wise if, with the new type of antibiotic, we assume that it is heterogeneous

FIG. 5. PLATE ASSAY OF TWO POLYMYXIN PREPARATIONS AGAINST BRUCELLA BRONCHISEPTICA*



The logarithm of the dose is plotted against zone diameter.

* From Reese & Eisenberg[4] (by permission of the editors of the *Annals of the New York Academy of Sciences*)

until it can be proved pure and homogeneous—that it is a family rather than an individual; and even when its individuality is established, we should always entertain the possibility that another member of the family may appear in the metabolic products of another organism.

It is not easy to resolve this difficulty of assay, even when the members of the family are defined and capable of isolation so as to provide separate standards, because, with only quantitative differences in specificity, it may be impossible, or at least impracticably laborious, to deduce the composition

of an unknown mixture from potency ratios determined with the various standards.

In the assays of G against K and G against X (fig. 1 and 3), the slopes were substantially parallel; this permits of a perfectly valid assay of these unlike substances, with the restriction, however, that a selected biological system is used. It is customary, in fact, to express the potency of K and X in units of G; these units, however, do not refer to a standard only, but to a standard used on a certain organism. We are thus immediately faced with the prime difficulty of defining the test organism with enough precision to ensure that it will behave in all hands as it did when the unitage was first established.

Early Establishment of Possibly Heterogeneous Standards

This brings us to the major contradiction in the practice of biological assay. It will be clear that we cannot assume the hypothesis of similarity (i.e., that all assays resemble the comparison of E and F in fig. 4) until a very late stage in the development of an antibiotic, when we have relatively pure preparations; and by this time biological assay, as with penicillin, is becoming superfluous. On the other hand, in the earlier stages of research when potency measures are important, we have no right, from the point of view of the rigorous biometrician, to depend on the estimates of potency we obtain. This contradiction can be resolved in practice by assuming from the start that the conditions for valid assay of potency are fulfilled and, during the research period of development, performing the assay so as to reveal statistical indications of heterogeneity. In other words, the search for evidence of formal invalidity should be used as a means of exploring the antibiotic.

The discovery and definition of heterogeneity, in the sense of there being more than one molecular species of an antibiotic, is an important stage in its development. This end is well served by the early establishment of a crude standard. There are many more reasons for adopting an early standard than the need to satisfy the rigorous biometrician. But potency is the raison d'être of antibiotic work, and a unit—even a temporary one—cannot be fixed too soon. The early performance of exact and statistically perfect assays against this standard, with meticulous scrutiny for small changes in potency ratios and for deviations from the expected dosage-response, will not, of course, yield the only, or even the first, indication of significant heterogeneity. The usual chemical manipulations, and powerful tools such as paper chromatography, as applied by Goodall & Levi,[1,2] and countercurrent extraction, are far more likely to discover heterogeneity. Nevertheless, heterogeneity so discovered cannot be satisfactorily defined, at least from the therapist's point of view, without accurate potency measures.

"Author's Preparations" as Standards

Although the international biological standards, as established and maintained by the World Health Organization, are in many ways models for these proposed early standards, their status is different. The international standard represents worldwide agreement about the suitability of the standard and about the weight of the standard preparation to which an international unit of potency is assigned. With a rapid exploration and development of the new antibiotic, crude early standards might require changing within a year or less; and although one of the benefits of the early standard is to establish a unit of potency that will have a permanent validity, yet with new knowledge—especially the recognition of more than one molecular species in the antibiotic—the unit might acquire a changed significance. Something more flexible in conception than the international standard is required, something that does not demand such extensive preliminary research and agreement. The WHO Expert Committee on Biological Standardization also establishes international reference preparations, with provisional units of potency, for biological substances that are not yet ripe—in the sense, for example, of widespread use in medicine or widespread interest in research—for promotion to full international standards. With these also, however, WHO, acting for its Member States, is responsible for specifying the standard preparation, and provisionally assigning unit potency to it. For the even more tentative and perhaps more temporary standard intended to facilitate the early work on an antibiotic, the Expert Committee on Biological Standardization has recommended setting up a collection of "author's preparations" of antibiotics.[7] The title is important, for it is intended to imply a definitive scientific publication about the antibiotic, designed not only to describe important facts about the substance, but to stimulate work by other scientists. After such a publication, it should be possible, without depriving the author of his rights to harvest the immediate scientific results of his work, to set aside a number of ampoules containing small samples of the material carrying the antibiotic activity and to assign a provisional unit of potency to a given weight of the material.

How the collection should be made is still a matter for debate. Workers might offer specimens of their antibiotic to WHO when they feel ready to do so; and WHO might ask for author's preparations, either on its own initiative or at the request of other workers in the field. Although the WHO expert committees on antibiotics and on biological standardization might offer suggestions about the standard and the provisional unit, the responsibility for specifying and, if necessary, modifying the standard would remain with the author, thereby ensuring the flexibility required in any attempt at valid standardization in a rapidly developing field.

SUMMARY

The biological potency of any substance is primarily measured in terms of a specific response of a biological system (animal, animal tissue, culture of microbe, etc.). When the substance can be precisely defined by chemical and physical means alone, a given weight always has the same biological potency, but when the substance is impure or not fully characterized, any statement of potency is universally valid only if it is in terms of a particular preparation of the same substance, because both the methods of production and the biological systems are variable. In these cases, potency by weight must be replaced by " unit " potency, defined as the activity of a given weight of a single stable preparation of the substance—i.e., a fixed, material standard whose status in biological standardization is similar to that of the standard metre and gram in physical mensuration. With such a standard, the potency of preparations to be tested is determined from the ratio of equipotent weights of standard and test preparations in the biological system employed, and expressed in arbitrary but unequivocal units.

The validity of a biological assay of potency in terms of a standard depends upon the following assumptions :

(1) that the biological test system represents a random sample of the test-object and that its response to the standard and test preparations is due to the active principle it is desired to measure ;

(2) that the ratio of potencies obtained in an assay is independent of the biological system used ;

(3) that the standard preparation contains neither impurities with an activity like that of the active principle, nor synergists, nor inhibitors ;

(4) that the biologically active constituent in the standard preparation is homogeneous ;

RÉSUMÉ

L'activité biologique d'une substance est mesurée dans son essence par la réponse spécifique du système biologique à laquelle elle est appliquée (animal, tissu animal, culture microbienne, etc.). Lorsque des moyens chimiques ou physiques suffisent à définir une substance, un poids donné de celle-ci a toujours la même activité ; mais, lorsque la substance est impure ou incomplètement caractérisée, l'indication d'activité n'est universellement valable que si elle est exprimée en fonction d'une préparation donnée de la même substance, car les méthodes de préparation et les systèmes biologiques comportent tous deux des variables. Dans ces cas-là, l'expression de l'activité en fonction du poids doit être remplacée par une « unité » d'activité, définie comme l'activité exercée par une quantité donnée d'une préparation stable de la substance, c'est-à-dire un étalon matériel déterminé, qui joue en standardisation biologique le rôle que joue le mètre-étalon ou le gramme-étalon dans les mesures physiques. Grâce à cet étalon, l'activité des préparations à éprouver est établie par comparaison de poids également actifs de la préparation d'une part et de l'étalon d'autre part, dans le système biologique employé; elle est exprimée en unités arbitraires, mais fixes.

La validité d'un essai d'activité biologique par rapport à un étalon repose sur les postulats suivants :

1) le système biologique représente un exemple pris au hasard des systèmes sur lesquels la préparation sera active ; sa réponse à l'étalon et à la préparation à essayer est due au principe actif qu'il s'agit de mesurer ;

2) le rapport entre les activités est indépendant du système biologique utilisé;

3) l'étalon ne contient aucune impureté ayant la même action que le principe actif, ni synergiques, ni inhibiteurs ;

4) le principe biologiquement actif de l'étalon est homogène ;

(5) that the active substance assayed is the same as the active substance in the standard.

In assaying the potency of antibiotics, especially during the early stages of their development, it cannot usually be affirmed that the preparations are free from synergists or are homogeneous. Synergists are usually removed during purification in making high-potency material, but heterogeneity of the active principle is an outstanding feature of many of the more fully developed antibiotics, even in the relatively pure state. Penicillin, streptomycin, bacitracin, and polymyxin, each is the name of a family of similar substances.

Heterogeneity must be particularly borne in mind in attempts to establish the criteria for the validity of assays of antibiotic potency. In making these attempts, however, the investigator is faced with a major contradiction—namely that biological assay is most necessary in the early stages of development of an antibiotic, when it is least possible to know whether the criteria are fulfilled. This difficulty is partly reflected in the late stage in its history at which a standard is set up for a given antibiotic. The contradiction is best resolved by making agreed standards at the earliest possible stage of research and assuming that they are in fact fulfilled, and by devising assays in such a way that analysis will yield the evidence for or against their validity.

The fully valid assay gives measurements that are independent of the biological system used. The heterogeneity of the penicillins was discovered because potency ratios between preparations changed when *Staphylococcus aureus* was replaced by *Bacillus subtilis*. The potency of penicillins, even in terms of a pure benzyl penicillin standard, consequently has a restricted meaning, being defined partly in terms of the biological system, which in a final analysis is indefinable.

When the assay is designed so that the measure of the biological response, or a

5) la substance active qui fait l'objet de l'essai est la même que la substance active de l'étalon.

Lors de l'évaluation de l'activité des antibiotiques, surtout aux premiers stades de développement, il est impossible d'affirmer que les préparations ne contiennent pas de synergiques et sont homogènes. Les synergiques sont généralement éliminés au cours de la purification des préparations très actives, mais l'hétérogénéité du principe actif est l'un des principaux caractères de plusieurs des antibiotiques les plus connus, même à l'état relativement pur. Pénicilline, streptomycine, bacitracine, polymyxine, sont chacune membre d'une famille de substances similaires.

Il faut particulièrement tenir compte de l'hétérogénéité lorsque l'on tente d'établir les critères de validité des essais d'activité biologique. Le chercheur se heurte à un paradoxe : en effet, c'est aux premiers stades du développement d'un antibiotique que l'essai biologique est le plus nécessaire et c'est à ce moment qu'il est le plus difficile de se rendre compte si les conditions de validité sont remplies. On retrouve cette difficulté au dernier stade de l'histoire de l'antibiotique, lors de l'établissement de l'étalon. La meilleure façon d'y obvier est d'établir des normes dès les premiers stades des recherches, d'admettre qu'elles sont valables et d'organiser les essais de façon telle que l'analyse prouve leur validité ou leur non-validité.

L'essai dont la validité est parfaite donne des résultats indépendants du système biologique utilisé. L'hétérogénéité des pénicillines a été découverte lorsque l'on s'aperçut que les rapports d'activité entre les préparations changeaient si l'on remplaçait *Staphylococcus aureus* par *Bacillus subtilis*. L'expression de l'activité des pénicillines, même par rapport à un étalon de benzylpénicilline pure a, par conséquent, une valeur limitée, car elle est définie partiellement par rapport à un système qui, lui-même, en dernière analyse, n'est pas strictement définissable.

Lorsque l'essai est établi de façon telle que la mesure de la réponse biologique

mathematical transformation of the measure, is linear when plotted against the logarithm of the dose, a further test of validity is possible, namely parallelism of the dosage-response lines of standard and test preparation. Non-parallelism of the lines may be used as a second indicator of heterogeneity. Other indications of heterogeneity are available in slope-ratio assays.

With new antibiotics, the likelihood of heterogeneity makes it important that not only the general researches but also the routine methods of assay should be designed to discover it. In the light of experience with established antibiotics, the most likely indication in assays of heterogeneity is change of potency ratio with change of biological system. Differences in the slope of the dose-response in any one assay are relatively uncommon ; i.e., it appears that members of a family of antibiotics tend to produce the same dose-response slope. In so far as this is generally true, assays of the various members of a family in terms of one member (e.g., as currently practised with the penicillins) are likely to be formally valid—an important point in the setting-up of useful standards.

The establishment of a fixed material standard and a unit of biological potency early in the research on a newly discovered antibiotic is thus both practically and logically the best course to adopt, although it must be realized that the standard may have to be changed as the antibiotic is developed. The value of a standard is enhanced by international recognition : its early adoption may, however, mean the issue of a series of standards for the same substance, with a consequent risk of confusion. The difficulty can be solved by adopting the recommendation of the WHO Expert Committee on Biological Standardization, which proposes a hierarchy of stages for international standards : first, " author's preparations ", to be made internationally available after

— ou une transformation mathématique de cette mesure — est linéaire dans le diagramme log dose-réponse, une nouvelle épreuve de validité est possible, à savoir le parallélisme des courbes dose-réponse de l'étalon et de la préparation à essayer. L'absence de parallélisme de ces courbes peut être considérée comme un nouvel indice d'hétérogénéité. D'autres indices peuvent être cherchés dans les essais mettant en jeu le rapport entre les courbes.

Quand il s'agit d'antibiotiques nouveaux, l'hétérogénéité est si fortement probable que les recherches et les méthodes courantes d'essai elles-mêmes doivent être orientées de façon à la mettre en évidence. A la lumière des expériences faites avec des antibiotiques déjà bien connus, l'indice d'hétérogénéité le plus fréquent est la modification du taux d'activité lorsque l'on change de système biologique. Des différences dans les courbes dose-réponse d'un essai particulier quelconque sont plutôt rares ; c'est dire qu'apparemment les membres d'une même famille d'antibiotiques ont tendance à présenter la même courbe dose-réponse. Dans la mesure où cette observation peut être généralisée, les essais de divers membres d'une famille par rapport à l'un d'entre eux (méthode couramment appliquée aux pénicillines) ont des chances d'être valables, ce qui est important pour l'établissement d'étalons utiles.

Il est opportun, du point de vue tant pratique que logique, de faire choix d'un étalon matériel déterminé, dès les premiers stades des recherches d'un nouvel antibiotique. On se souviendra cependant qu'un changement d'étalon peut s'avérer nécessaire au cours des études. La valeur d'un étalon est rehaussée par le fait qu'il est reconnu internationalement. L'adoption d'un étalon, à un stade précoce des recherches déjà, peut impliquer, par la suite, le choix d'une série d'étalons successifs pour la même substance, source possible de confusion. La recommandation formulée par le Comité d'experts de l'OMS pour la Standardisation biologique propose une solution à ce problème. Elle préconise la hiérarchie suivante dans l'établissement des étalons : d'abord des

the publication of the discovery ; second, an " international reference preparation ", to facilitate later development and early therapeutic use of the antibiotic ; and finally, the definitive " international standard " for official pharmacopoeial specification of the antibiotic.

« préparations d'auteurs », mises à disposition sur le plan international, après la publication de la découverte ; ensuite une « préparation internationale de référence » qui faciliterait le développement ultérieur des recherches et permettrait d'employer l'antibiotique, sans retard, en thérapeutique ; enfin, l'« étalon international » définitif d'après lequel l'antibiotique serait officiellement caractérisé et décrit dans les pharmacopées.

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