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Quantitative Aspects of Biological Assay

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A biological assay may be defined as any determination of toxicity or potency based upon the reaction of living matter. In a broader sense it covers research on new drugs but here we are concerned with the standardization of specific extracts, hormones, vitamins, sera, etc., where the concentration of active agent cannot be analyzed chemically. While the inherent precision of biological indicators is considerably less than that obtainable in analytical chemistry, this precision is measurable and potency can be estimated within any required limits of error.

Certain common factors underlying good assay technique may be listed in five categories.

(*a*) There should be substantial equivalence between the activity of the drug in a test animal and in man, so that samples producing the same reaction in the laboratory will have the same effect therapeutically. Since the biologically-standardized drugs often contain either impurities or several active principles in a complex mixture, two samples giving equal reactions in one species may give different reactions in another species. Assays in saline solution of crystalline standard insulin in comparison with the original impure standard gave an average potency some 19 per cent greater by the mouse method than by the rabbit, as shown in Table I. Similarly, Gold and Kwit (13)

Table I.—Assay of Crystalline Standard Insulin in Terms of the Original Impure Standard. Tests in Saline Solutions (14)

Laboratory	Units per mg. Rabbit Method	Units per mg. Mouse Method
Toronto		
(Hershey and Lacey)	21.3	24.8
(Scott)	....	24.0
England		
(A. B. Insulin Laboratories)	21.9	28.0
(Wellcome Laboratories)	....	27.0
Copenhagen	....	25.4
Indianapolis	21.9	....
Unweighted mean	21.7	25.8

have observed a three-fold difference in the therapeutic dose for man for preparations of digitoxin and of ouabain assayed as equipotent by the cat method. This factor deserves more careful study but is beyond the scope of the present report.

(*b*) The reaction should be sensitive to relatively small changes in dose and the characteristic curve relating these two terms should be determined experimentally. Assays are ill-contrived which depend upon an assumed direct proportionality between dosage and response, as in the U. S. P. XI parathyroid assay. The observed relation between the increase in the serum calcium

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of the dog and the log-dose of parathyroid extract has been plotted from the data of Lloyd C. Miller (15) in Fig. 1, after adjustment for differences between dogs (6). A comparison of the computed curve with that defined by the U. S. P. XI monograph on parathyroid extract illustrates the discrepancy that can arise in the case of a graded response. When based upon an all-or-none

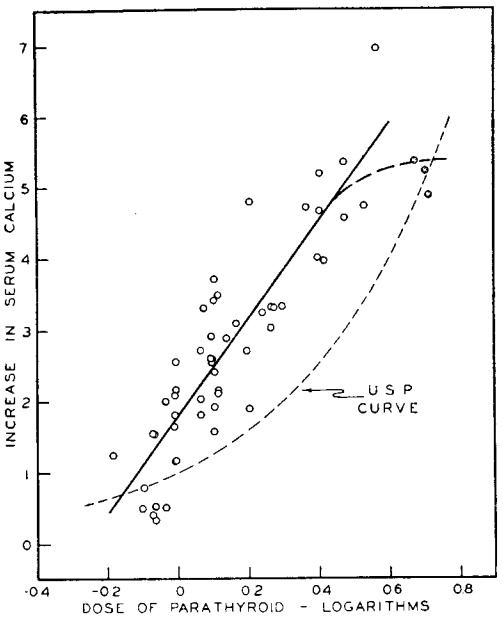

Fig. 1.—Dosage-Response Curve for Parathyroid Extract in Terms of the Increase in Serum Calcium of Dogs, Data of Lloyd C. Miller (6, 15), Showing the Discrepancy between the Observed Relation and That Assumed by the Pharmacopœial Monograph.

reaction, the characteristic curve relating dosage of drug and percentage effect is sigmoid and asymmetrical. A typical dosage-effect curve is that for the toxicity of ouabain to frogs as reported by Chapman and Morrell (8) and plotted in Fig. 2.

Normally the general form of the characteristic curve can be established with a single preparation of the drug. But the average or overall susceptibility of the test animals, which fixes the position of the curve, and to a lesser extent their sensitivity to a change in dose, determining the slope of the curve, tend to vary from one laboratory to another and from one time to another in the same laboratory. In the experiments behind Fig. 2, eight different "standard" curves were determined within a period of three weeks, each from 240 frogs in the same laboratory. Yet the individual curves differed significantly both in position as measured by the LD50 or its logarithm and in slope or b as computed from the transformed coördinates (Table II). Because of this

Table II.—Variations in "Standard" Dosage-Effect Curves for Ouabain. Each Curve Determined from 8 Groups of 30 Frogs by Over Night Technique, Computed in Terms of Log-Dose and Probit Mortality, Data of Chapman and Morrell (8)

Date, 1930	$LD50^{-8}$ per Gm. of Frog	Log-LD50	Slope b
Aug. 26	32.42 ±0.10	1.5108 ±0.0014	25.9 ±2.7
Aug. 28	28.51 ±0.39	1.4550 ±0.0060	15.3 ±2.1
Sept. 2	27.03 ±0.11	1.4319 ±0.0017	22.8 ±2.9
Sept. 4	30.54 ±0.10	1.4849 ±0.0014	24.9 ±2.5
Sept. 8	31.68 ±0.15	1.5008 ±0.0020	17.6 ±2.0
Sept. 10	32.03 ±0.12	1.5056 ±0.0017	22.2 ±2.8
Sept. 12	31.27 ±0.11	1.4951 ±0.0015	22.1 ±2.2
Sept. 16	31.21 ±0.10	1.4943 ±0.0014	27.8 ±2.9

variability, the curves on August 28th and on September 8th were omitted altogether and the remaining six curves adjusted for differences in LD50 in preparing Fig. 2. An erratically low mortality of 73 per cent was also omitted in computing the curve but it is included in the diagram. The essential relation between reaction and dose should be

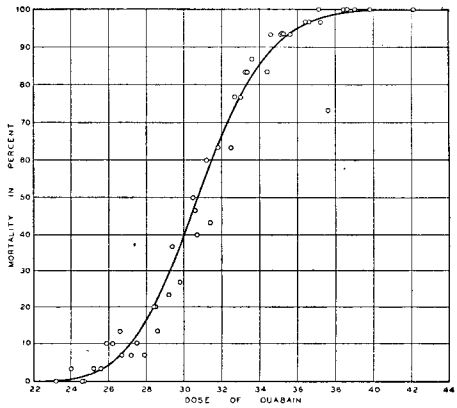

Fig. 2.—Dosage-Effect Curve for Ouabain in Frogs with Each Point Based upon a Separate Lot of 30 Frogs, Data of Chapman and Morrell (8). Six Different "Standard Curves" Have Been Adjusted to a Mean Level of Susceptibility to Facilitate Plotting Them in a Single Diagram.

determined, therefore, as an integral part of any self-contained assay. This is greatly facilitated where the reaction can be plotted as a straight line against the logarithm of the dose, so that an important objective in

analyzing the characteristic curve is to discover which criterion of effect best accomplishes this purpose.

(*c*) The unit of potency should be defined in milligrams of a stable reference standard of the drug and not in terms of biological effect. The concentration of each sample or unknown is adjusted so that it will produce quantitatively the same reaction as the standard when tested in parallel. This eliminates large, erratic differences caused by seasonal and secular change, by inherent and environmental differences between colonies of laboratory animals and by variations in manipulative detail. Susceptibility may change over such short intervals of time that the standard is included in each assay to correct differences similar to those shown in the first columns of Table II. There remain principally the unavoidable variations in sensitivity between the individuals of any given assay.

Although the concentration of an unknown can be adjusted by trial and error alone until it produces the same reaction as the standard, it is preferable to utilize rather than to ignore the underlying curve relating dosage and response. Determined as part of the assay, this curve enables the experimenter to convert an observed difference in the reactions to standard and to unknown to units of dosage and thence to relative potencies.

(*d*) Assays should be designed so that doses of the unknown and of the standard are administered under as nearly identical conditions as are practicable. By identifying unavoidable sources of variation and balancing them equally between the two samples of drug and the different concentrations of each, bias can be avoided and the experimental error reduced. The increase in precision with an effective design is illustrated by an experiment on the rabbit assay of insulin reported by Bliss and Marks (5). Here each of eight rabbits was given four different doses of insulin on four different days as determined by two Latin squares and the hypoglycæmic response measured from the mean percentage fall in blood sugar over a five-hour period. The different sources of variation have been segregated and appraised in Table III by the analysis of variance. By "quarantining" differences in susceptibility between individual rabbits and between days, they neither biassed the comparison between doses nor contributed to the estimate of experimental error. The variance ratios in the last column of Table III, computed by dividing the "mean squares" for each of the first three factors by that for experimental error, show that individual differences in susceptibility between rabbits and between days were many times larger than the experimental error, from which these terms had been removed, with a corresponding accentuation of the contrast between the dosage factor and the error.

Table III.—Partition of the Sources of Variation by the Analysis of Variance in an Experiment on the Rabbit Assay of Insulin, from Bliss and Marks (5)

Variation Due to	Degrees of Freedom	Sum of Squares	Mean Square or Variance	Variance Ratio (F)
Differences between rabbits	7	1702.20	243.17	8.28
Differences between days	3	403.72	134.57	4.58
Differences between doses	3	2330.58	776.86	26.45
Experimental error	18	528.68	29.371	1
Total	31	4965.18		

(*e*) Finally a biological assay should provide an objective measure of its experimental error as an integral part of the determination of potency. Even under well defined conditions assays vary in their inherent precision, which we measure in terms of the limits within which the true potency of the unknown may be expected to fall for any given odds. The Subcommittee on the Accuracy of Biological Assays for the British Pharmacopœia has determined the expected variation in the potency of certain drugs when the observed response is the same on both the standard and the unknown. In one official assay in 100 or at $P = 0.01$, the true potency is expected to exceed the limits given for the five selected assays in Table IV for an apparent potency of 100 per cent.

Table IV.—Average Limits of Error (P = 0.99) of Selected Biological Assays in the British Pharmacopœia (7); the Response Assumed Equal on Unknown and on Standard

Vitamin A (5 weeks' growth)	37–272 per cent
Vitamin C (lesions of scurvy)	82–139 per cent
Vitamin D (line test)	49–215 per cent
Staphylococcus antitoxin (intravenous injection in mice)	92–108 per cent
Antipneumococcus serum, Type I (injection of mixtures of serum and culture)	57–176 per cent

It is evident that official assays varied widely in their precision. These average limits of error were based upon equal reactions to standard and to unknown and frequently would be larger due to a difference in the reaction. Since individual assays or the general level of precision in a given laboratory may differ considerably from the average limits shown in the table, an integral part of each assay should be a determination of its experimental error. Hence the data for computing this error should be obtained routinely in a good determination of relative potency.

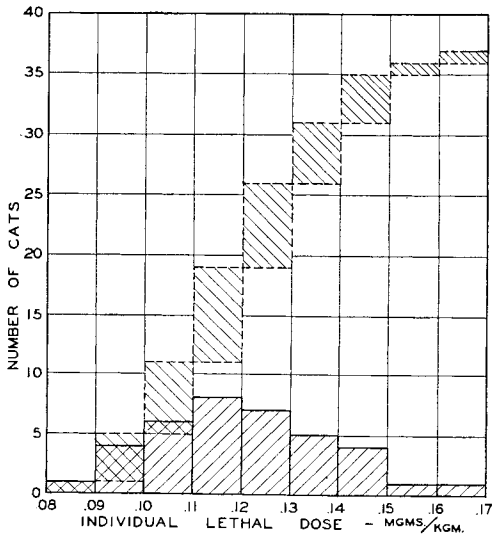

Fig. 3.—Frequency Distribution of the Individual Toxic Doses of Ouabain in 37 Cats, Data of Chen, Chen and Anderson (9), Plotted Directly at the Base of the Diagram and above It as a Cumulative Curve.

The application of concrete statistical procedure to problems of biological assay depends in part upon the nature of the reaction used to measure drug potency. Assays may be classified under three main headings: (1) those based upon an all-or-none reaction, (2) those based upon reaction time and (3) those depending upon a graded response.

(1) Many assays depend upon an all-or-none or qualitative reaction and an unknown is judged to have the same potency as the standard when equal percentages of test animals react characteristically to corresponding dosages of the two samples. The dosage-effect curve relating the percentage of positive reactions to the dosage of drug is typically an asymmetrical sigmoid curve, such as that shown in Fig. 2. The animals in an experiment differ in their susceptibility to the drug and the curve owes its shape to this variation. Because the variation conforms to the well-known normal distribution within the limits of experimental error, the dosage-effect curve can be converted easily to a straight line which greatly facilitates its analysis.

The statistical nature of the dosage-effect curve and its transformation can be understood most readily in terms of the cat method for assaying cardiac glucosides, where the just-toxic dose is measured directly for each individual by slow intravenous infusion. Because of individual differences in susceptibility, the required amount of drug varies from one cat to another even after adjustment for differences in body size. A typical series is supplied by the just-toxic dose of ouabain for 37 cats as determined by Chen, Chen and Anderson (9). These have been arranged in an increasing order, grouped in nine equal dosage intervals and plotted along the base of Fig. 3, the largest number of cats occurring at an intermediate dose with the numbers diminishing at larger and smaller doses. The resulting bell-shaped curve is the familiar one descriptive of biological variation. Each block along the base of Fig. 3 was then moved vertically upward until its lower edge coincided with the upper edge of the next preceding block, transforming the curve to the cumulative sigmoid form with the number of cats dying at all doses up to and including any given amount of ouabain plotted against the dose. This curve has been replotted in Fig. 4 with the ordinate changed to percentages and is

statistically identical with Fig. 2, which describes the reaction of frogs to the same drug. The difference between them is one of experimental technique, the several points

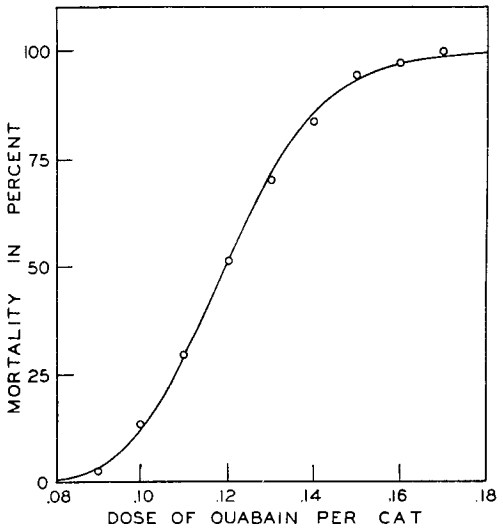

Fig. 4.—Dosage-Effect Curve for Ouabain in Cats, Computed from the Data in Fig. 3.

in Fig. 4 representing different readings on a single series of 37 cats, while each of those in Fig. 2 is the percentage reaction of a separate group of 30 frogs to a single dose of ouabain administered uniformly to each frog in the group.

The normal distribution or curve of error with which these curves are to be identified is shown in Fig. 5 in the more familiar, bell-shaped form. The "dosages" along the base are in terms of standard deviations, 0 representing the mean (M). In order to avoid negative values, it is convenient to add 5 to each standard deviation, the resulting unit being known as a "probit" (1). Of the characteristics of this well-known curve, the one of immediate interest is the relation between the probit and the proportion (p) of the total area under the curve ($p + q$) lying to the left of a perpendicular (such as xz) erected at any point (x) along the base. The proportionate area, which may be identified with the proportion or percentage killed, has been plotted against a theoretical dosage or probit in Fig. 6 and again we have a sigmoid curve paralleling those in Figs. 2 and 4. Disregarding the known dosage of ouabain, each observed percentage (or proportion) in Fig. 2 or 4 can be spotted on the theoretical curve in Fig. 6 and the corresponding probit or "thoretical dosage" read from the base. When the probit is plotted against the actual dose of ouabain in

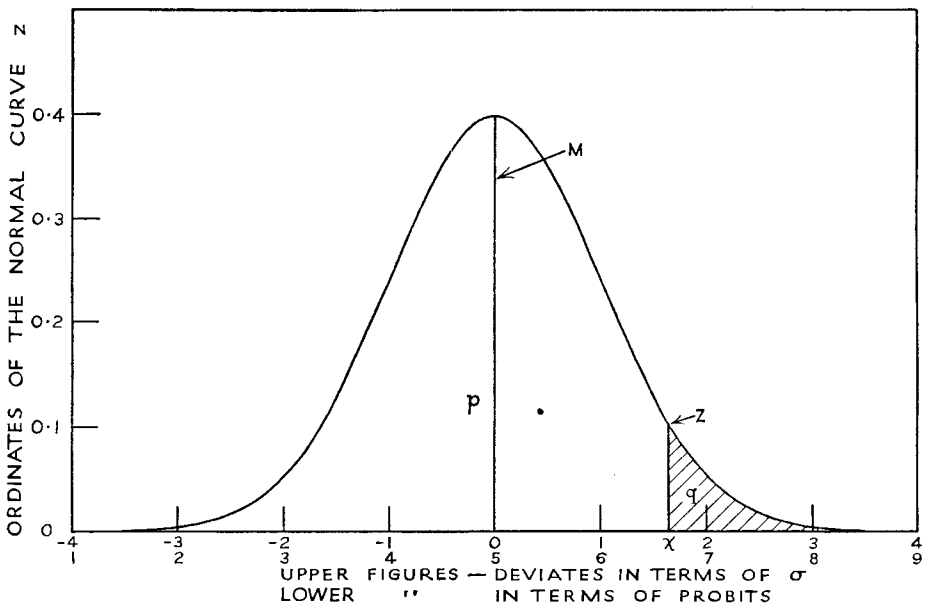

Fig. 5.—The Theoretical Normal Curve of Error, Believed to Underlie the Variation in the Susceptibility to Drugs, after Bliss (2). The Abscissa Corresponds to the Log-Dose and the Proportion of the Total Area to the Left of Any Given Vertical, Such as xz, to the Percentage Effect.

logarithmic units, the resulting diagram of inferred dose against the observed dose defines a straight line (Figs. 7 and 8). Gaddum (12) has given the curve in Fig. 6 in a form suitable for interpolating the normal equivalent deviation or "N. E. D." from the

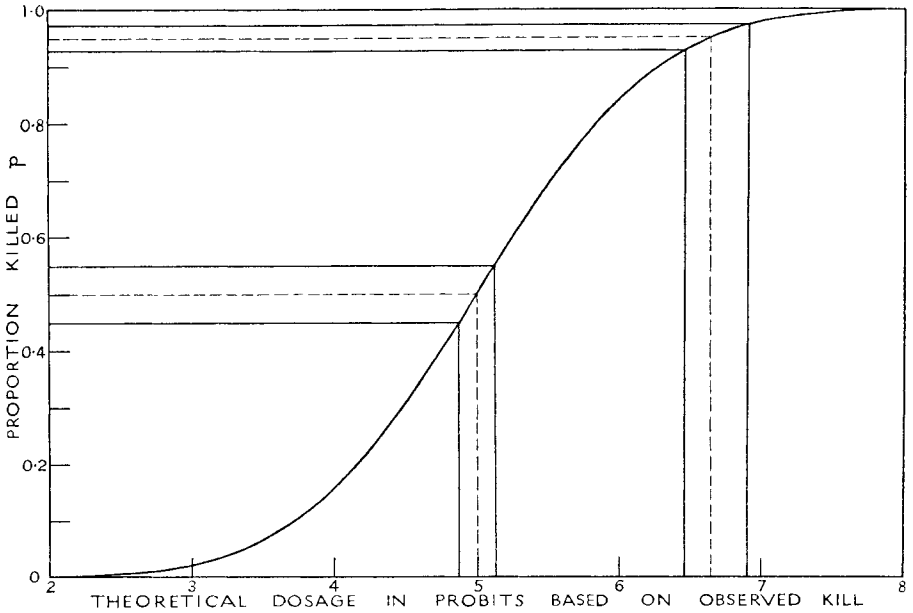

Fig. 6.—The Theoretical Normal Distribution of Fig. 5 Plotted in the Cumulative Form, from Which the Observed Percentage Reaction on the Ordinate Can Be Transformed to the Theoretical Dosage or Probit Along the Abscissa.

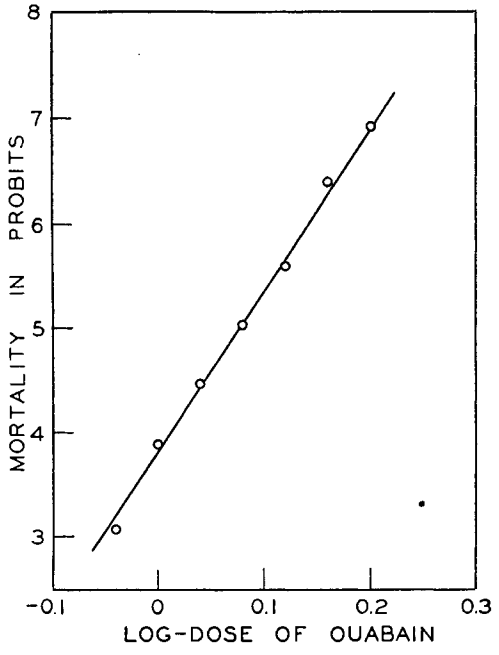

Fig. 7.—The Dosage-Effect Curve for Ouabain in Cats Replotted from Fig. 4 with Transformed Coördinates, the Log-LD50 Being the Mean of the Log-Doses for the Individual Cats and the Slope the Reciprocal of the Standard Deviation.

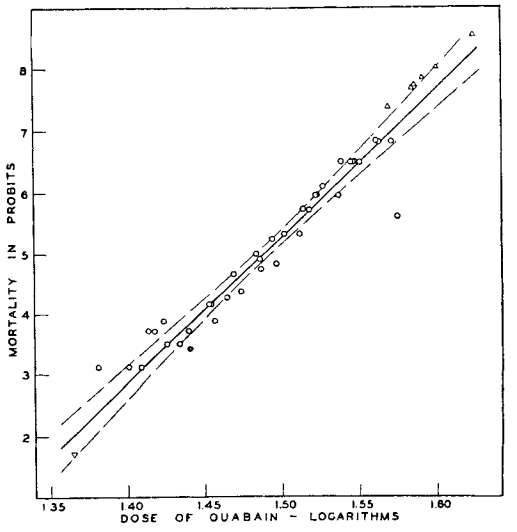

Fig. 8.—The Dosage-Effect Curve for Ouabain in Frogs Replotted from Fig. 2 with Transformed Coördinates. The True Value Lies within the Curved Lines within Odds of 99 in 100, the Individual Curves Being Corrected for Overall Variations in Susceptibility and Two out of Eight Series Omitted due to a Significantly Flatter Slope. The Isolated Record at 5.6 Probits Was Omitted from the Final Computation.

percentage effect, but it is more convenient to use specially ruled logarithmic-probability paper or a table of probits (2, 11). The more general indirect method of thus measuring the distribution of susceptibilities from the percentage reaction of separate lots of animals is that referred to as the dosage-mortality or the dosage-effect curve.

The transformation to a straight line has provided a rational interpretation of the dosage-effect curve and has shown that to obtain uniform increases in effect with most drugs the dosage must be increased not by equal increments but by a constant proportion. When in linear form, these curves can be computed so as to estimate the dosage producing any required percentage effect and the limits within which it has been determined for any given odds. The dosage-effect curve in Fig. 8, for example, as determined from six of the eight series, would not be expected to vary in slope beyond the curved lines of the diagram more than once in 100 times if redetermined from a similar number of equivalent frogs. The fact that in 2 of the 8 series the frogs were not equivalent emphasizes the desirability of so conducting assays that the slope can be checked.

By administering two or more doses of both standard and unknown, a curve can be determined separately for each and their inherent validity tested objectively. If the unknown sample produces in fact the same biological effect as the reference standard, these two straight lines should be substantially parallel, a single combined slope will fit both series of observations adequately and the estimate of potency holds at all dosage levels. Prigge's data (16) on the assay of a diphtheria aluminium antitoxin from the percentage survival of guinea pigs may be taken as an example. These have been plotted in Fig. 9 in terms of probits and logarithms. With 23 to 25 animals on each dose, the observations for both standard and unknown could be fitted adequately by parallel lines as tested by $\chi^2 = 3.86$ with 4 degrees of freedom for the assay as a whole. Then on the log-dosage scale the horizontal distance between the two parallel lines, designated by the symbol M, measures the relative potency of the unknown in terms of the reference standard. The standard error of M, s_M, is readily determinable and after adjustment for the assumed unitage of the unknown, both M and s_M can be transformed to the percentage or proportionate potency with a table of logarithms. In the example of Fig. 9, computation gave $M = 1.507 \pm 0.083$ or the aluminium antitoxin was assayed as 32.12 ± 6.15 as potent as the standard.

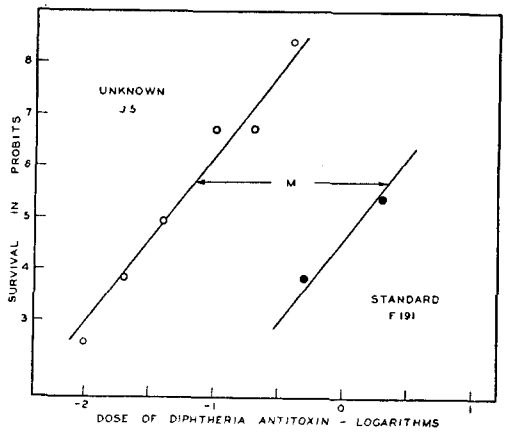

Fig. 9.—The Biological Assay of a Diphtheria Antitoxin from an All-or-None Response as Computed from Prigge's Data (16), the Horizontal Distance between the Parallel Lines (M) Being the Log-Ratio of Potencies.

(2) Occasionally the reaction time to a qualitative response varies sufficiently with the dose that it can be used as an assay criterion. In most all-or-none assays, results are scored when the reaction is substantially complete, so that a later listing would reverse comparatively few scores. Dosages are adjusted to fall within the range giving from 5 to 95 per cent response and the reaction time is noted primarily to insure that the ratings are not made before the drug has had a chance to exert its full effect or after secondary factors may have entered the picture. Although the reaction time may be relatively independent of individual susceptibility to a drug, in some cases these two aspects are linked so closely that the results from the larger doses can be scored much earlier than those from small doses. It then becomes practicable to work within a higher dosage range and to use reaction time as the index of effect.

When measuring drug action in terms of

the reaction time, the time to reach a constant end-point should be related to the dose. This does not occur when the percentage of individuals which have reacted is noted at some arbitrary interval after treatment, for both the level of effect and the speed of the reaction would contribute to such a percentage. Instead, periodic records should be continued throughout the experiment, so as to estimate for each dose the time at which one-half of the animals reacted. The estimation of this value from the original data is often facilitated by the use of logarithmic-probability paper or of equivalent techniques, which provide an unbiased estimate of the median time and of the standard deviation without distortion from individuals which react either erratically or not at all (3). The end-point, log-RT50, then represents equivalent levels of susceptibility at all dosages of both standard and unknown and may plot as a straight line against the log-dose. It should then be possible to obtain parallel lines for standard and unknown that are suitable for the estimation of relative potency in terms of M. As a criterion for drug assay, the quantitative aspects of reaction time have been explored less fully than those based upon the dosage-effect curve.

(3) Most other biological assays depend upon a graded or quantitative reaction and potency is estimated from the average of the individual responses at one or more dosages of drug. In contrast with an all-or-none reaction, a graded response frequently can be measured in several ways. The reaction to a larger dose may be both greater in intensity and longer in duration than that to a smaller dose, so that a dosage-response curve could be constructed from any one of several terms. Figure 10 shows the course of

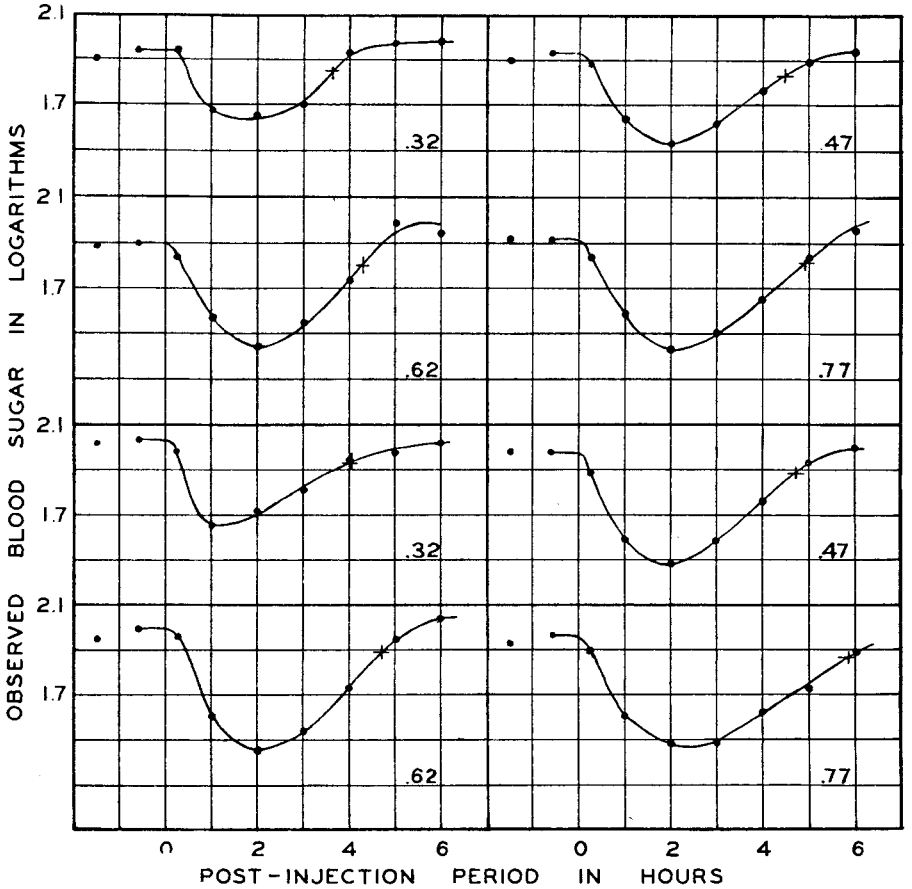

Fig. 10.—Typical Blood-Sugar Curves for an Individual Rabbit Following the Injection of Different Doses (in Logarithms) of Insulin, the Upper Four after One Day without Food and the Lower Four One Day Later, Data of Bliss and Marks (5).

the hypoglycæmic reaction of a typical rabbit to four doses of insulin after one and two days of fasting. The response could be measured from the maximum fall in blood sugar, from the time for the blood sugar to return to a given percentage of the initial value or from the average fall in blood sugar recorded periodically after treatment, and all of these are subject to differences in definition and in their relation to the initial level of blood sugar. Even after eliminating impractical criteria, several alternatives often remain from which one can be selected which will plot as a straight line against the log-dose over a wide range of dosages and have the largest ratio between the slope and the standard deviation about the computed line.

Most dosage-response curves flatten out toward a maximal reaction as the dose is increased or toward a minimum as the dose is reduced, but there is often a long central portion which can be considered as a straight line. In a study of the dosage-response curve for insulin by Bliss and Marks (5), for example, the mean percentage fall in blood sugar based upon five hourly readings was linear over a three- to four-fold change in dose (Fig. 11). In other cases a curvature has been observed at one or both ends and unless it can be rectified by a convenient mathematical transformation, only the central part of the complete curve is suitable for purposes of biological assay. This restriction in the range of response often increases the desirability of including three dosage levels of both standard and unknown in each assay, spaced equally on a logarithmic scale. In other cases, where curvature is exceptional and the use of three doses would prolong the assay undesirably, two doses of standard and two doses of unknown are indicated. In either case a relatively simple method of factorial analysis can be used for computing the log-ratio of the potency of the unknown to the standard (M), the experimental error of the determination (s_M) and a numerical test of the underlying assumption that the dosage-response curves for standard and unknown are equivalent to parallel, straight lines.

The method can be illustrated from a separate computation of the first day's reactions for 8 of the 12 rabbits used in Fig. 11. These data have already been analyzed initially in Table III and we will now examine the effect of dosage as a test assay, on the assumption

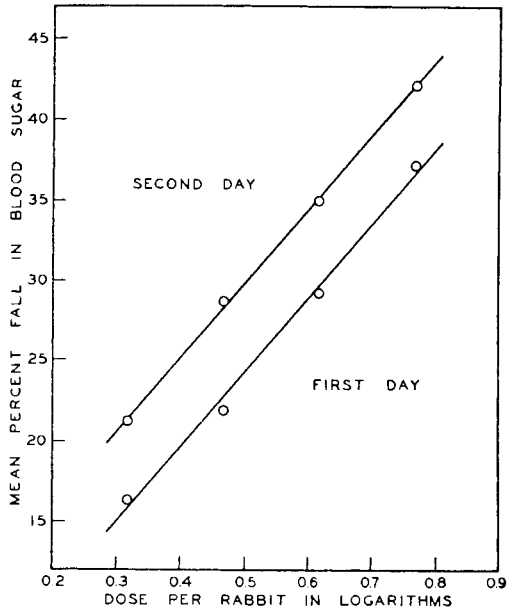

Fig. 11.—Dosage Response Curves Showing the Effect of Insulin upon the Blood Sugar of the Rabbit as Determined from 12 Rabbits, Each Tested Once with Every Dose on Both the First and Second Days of Starvation, from Bliss and Marks (5).

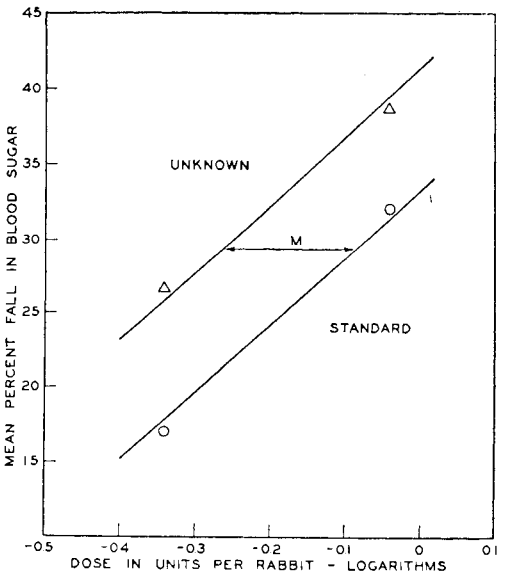

Fig. 12.—A Test Assay for Insulin Where the "Unknown" is a Known Dilution of the Standard, from Bliss and Marks (5).

that doses 1 and 3 represent the standard and doses 2 and 4 an unknown as plotted in Fig. 12. The estimated log-ratio of potencies was $M = 0.179 \pm 0.049$, which differed less than its standard error from the true value of $M = 0.1505$. Since the adjusted $[Y^2]$ measuring the difference in slope between the standard and the "unknown" of Fig. 12 fell within the experimental error, it verified the basic assumption of parallelism.

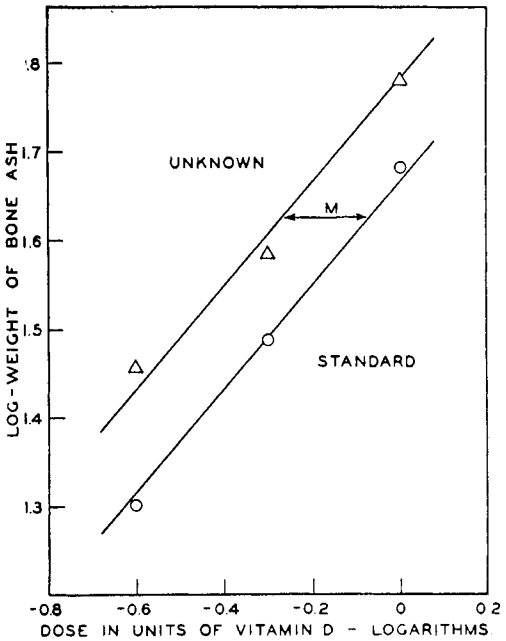

Fig. 13.—The Assay of the Vitamin D Content of a Sample of Cod Liver Oil from the Ash Content of the Bone, Data from Coward (10) as Re-analyzed by Bliss (4).

Bioassays include many unavoidable sources of variation known to the pharmacologist, such as differences between individuals, litters, sexes and dates of treatment. Sometimes these potential sources of error are balanced between the different doses of standard and unknown so that they are as comparable as possible, notably in the so-called cross-over test. This principle has been generalized and improved by means of restricted randomized designs coupled with computation by the analysis of variance, which has been illustrated in Table III. The newer technique not only segregates potential sources of variation, so that the comparisons of doses or of standard and unknown are unbiassed, but it eliminates them from the estimate of experimental error as well. These possibilities for increasing the precision of bioassays are only beginning to be explored.

Assay technique frequently adjusts each individual response for differences in the initial level from which the response is measured, for differences in body weight or for other concomitant factors. These adjustments are often arbitrary and differ from one worker to the next. Necessarily each assumes certain quantitative biological relations between the reaction and the initial or concomitant measure and if incorrect, the adjustment may even reduce the precision of the assay. For example, the use of the percentage ash in the femur of the rat to measure the potency of vitamin D implies a specific relation in the bone between its ash and organic content. This was sufficiently inaccurate in a case reported by Coward (10) and reëxamined by Bliss (4) that it increased the error of M to the same extent as if the number of test animals were halved in comparison with a computation of potency from the log-weight of ash alone without reference to the organic matter lost in ashing. The mean responses to the three doses of standard and of unknown have been plotted in Fig. 13 and in terms of the more accurate criterion $M = 0.199 \pm 0.023$ or upon adjustment for the assumed potency 197.7 ± 10.6 units of vitamin D per Gm. of cod liver oil as compared with 193.4 ± 15.0 units by the original criterion. If adjustment is needed, such concomitant variations can be corrected effectively by the analysis of covariance from the internal evidence of each assay. Covariance has the added advantage of allowing a more flexible assay procedure where several factors are involved as in the official assay for vitamin A.

SUMMARY

The potency of physiologically active agents which are too complex for chemical analysis is determined from the reaction of living matter by biological assay. Five factors characterize good assay procedure: (*a*) substantial equivalence between the activity of the drug in a laboratory test animal and in its therapeutic applications,

(*b*) high sensitivity to a change in dose, measured objectively by the curve relating-dosage and the function of response which over the widest range plots against it as a straight line, (*c*) the definition of potency in terms of a reference standard based upon the reaction to two or more doses of both standard and unknown in each assay, (*d*) the partition of potential variation equally between different dosages and between standard and unknown and (*e*) the determination of its experimental error as an integral part of each assay. Statistical procedures depend in part upon the nature of the response. Those based upon an all-or-none reaction show a sigmoid dosage-effect curve mirroring the individual variation in susceptibility. Since this follows the normal distribution, the curve can be rectified and used effectively for biological assay by transforming dosages to logarithms and percentage effect to probits or their equivalent. Similar procedures are of value when reaction time is used as an assay criterion, providing a biologically stable end-point for each dose. The dosage-response curve for graded reactions is often linear over a manageable dosage range. Especially in these cases the newer statistical designs and methods of analysis extend considerably the precision that can be attained from a given amount of biological material and time.

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Influence of Sex Life upon Resistance to Nostal and Pentobarbital*

By Harald G. O. Holck and Lewis D. Fink†

Agduhr and his collaborators have reported that sex life increases resistance in mice, rats and rabbits to such varied substances as methyl alcohol (1), ethyl alcohol (2), soluble barbital (3) and arsenic trioxide (4). Small, very gradually increasing daily doses were administered until the animals died. Mated female mice showed an increased resistance even though they failed to become pregnant (2). In general, mated female mice gained more in resistance than did the corresponding males (1, 2, 3). However, in the case of arsenic trioxide, mated male rats showed greater resistance. In all of these experiments the animals were divided into three groups: 1, two males together; 2, two females together; 3, male and female together.

EXPERIMENTAL

PART A. OBSERVATIONS WITH A SINGLE DOSE OF ISOPROPYL BROMALLYL BARBITURIC ACID (NOSTAL)

In this phase of the study 81 male and 120 female albino rats, bred from Wistar stock, were divided

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