Oscillator II (Fig. 1) is adjusted to produce an audible beat note suitable to the operator. The frequency of oscillator I is changed by the reactance tube modulator in accordance with the changes in heart potential, which are amplified by the low-frequency amplifier to a level necessary to actuate the reactance tube modulator.

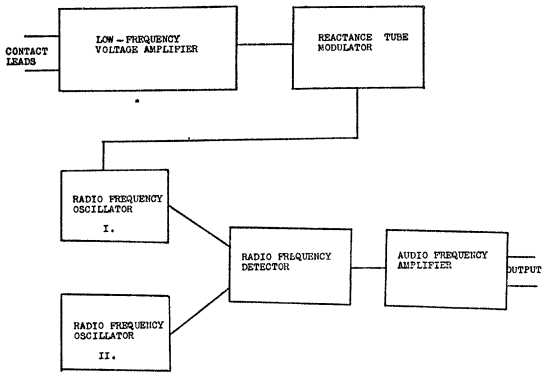

FIG. 1

The output of the detector is also amplified. Although a loud-speaker may be used, we have employed a button earphone so arranged (Fig. 2) that the sounds are conducted by rubber tubing into a standard stethoscope equipped with the conventional chestpiece. The auditory equivalents of the electrical potential changes may thus be cut into the normal stethoscopic sounds, which are not amplified or otherwise distorted. Although no attempt has been made to standardize on any given audio frequency, it is obvious that the heart can "play its piece" on any audible range of frequency—often with interesting effects.

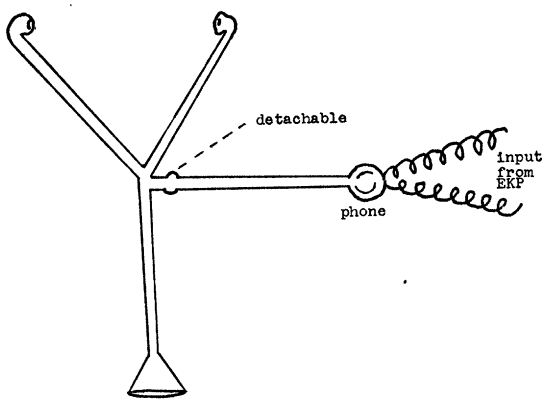

FIG. 2

Correlation of the electrical potential sounds with stethoscopic sounds in health and disease is now being attempted. Further correlation with the electrocardiogram will be tried by the use of a cathode-ray oscilloscope parallel with the electrocardiophone. Sound recordings of both the auditory equivalents of the electrical potential changes and the stethoscopic sounds will permit a more careful dissection of the interrelationship between the heart beat and the potential changes.

The technic should be distinguished from the simple amplification of the changes in heart potential *per se*: these changes are heard only as a clicking sound. For teaching purposes it is held undesirable to use electronic stethoscopic amplification since the resulting sounds depart in quality from those heard with the clinical stethoscope. Since the electrocardiophone utilizes a virgin medium, there is no such conflict here.

The technological details of the apparatus will be published elsewhere.

Simple Formulas for Calculating Percentage Potency in Three- and Four-Dose Assay Procedures

MARION B. SHERWOOD

The Wellcome Research Laboratories. Tuckahoe, New York

In many instances in which the log dose-response curves of unknown and standard substances are parallel straight lines simple formulas for the rapid calculation of the percentage potency of the unknown in terms of the standard are helpful. However, in order to make use of such formulas, it is essential that the assay be so designed that the unknown and standard materials are treated uniformly, *i.e.* the geometric relationship between the individual doses or concentrations of the unknown and standard must be identical, the same diluent must be employed for each, an equal number of dose or concentration levels of each reactant must be used, and the number of replicates per level of each dose must be uniform. Though the actual number of replicates is immaterial to the application of the formulas presented in this paper, it is recommended that, for accuracy's sake, three or four be employed. Moreover, since the formulas in their simplest expression give no indication of the error of an assay, it is suggested that the individual worker determine (a) the limits of accuracy within which the log dose-response is linear and (b) the precision of his proposed assay, so that he is assured of the applicability of these shortcuts.

Formulas for calculating the potency are available when two similarly related doses of standard and unknown are utilized in assay procedures (*5*). The writer is not aware of any such simple formulas for the calculation of the activity in so-called three- or four-dose assay techniques. The desirability of employing more than two logarithmically related doses is apparent if one makes use of graphic representations of data. Obviously, a straight line is the only curve which can be drawn from the data available when only two doses each of standard and unknown are employed. If three or four doses are used, deviations in the linearity of the log dose-response curve become apparent. Moreover, the supplementary data obtained at additional dose levels with little extra work permits the determination of the individual regression lines or, in the case of the four-dose assay, the omission of data at the highest or lowest dose level of the unknown if such data lie outside the limits of linearity of the dose-response curve.

During the development of a thin filter paper disc-agar plate method for the assay of amylases in which the initial concentration of the standard and unknown enzyme extracts was 1 per cent and the three succeeding doses of each were decreased in a 1:5 ratio, a simple formula for calculating the percentage potency of so-called four-dose assays was developed. This formula, which has universal applicability to four-dose assays

under the restrictions given, may be expressed in its simplest form as follows:

$$\% \text{ Potency} = \text{Antilog}\left[2 \pm c + 5d\,\frac{(U_1 + U_2 + U_3 + U_4) - (S_1 + S_2 + S_3 + S_4)}{[3(U_4 + S_4) + (U_3 + S_3)] - [3(U_1 + S_1) + (U_2 + S_2)]}\right]^{1}.$$

In this formula, 2 = log 100, the factor for converting the ratio obtained to per cent potency; c = the positive expression of the log ratio between the corresponding doses, *i.e.* U_4 and S_4, of the unknown and standard. (In the formula, the sign of c is positive if the initial dose of the standard was greater, and, conversely, is negative if the initial dose of the standard was smaller, than that of the unknown); 5 = the constant for the four-dose assay; d = the logarithm of the successive dose intervals of both the standard and the unknown; and U_1, U_2, U_3, U_4, S_1, S_2, S_3, and S_4 = the sums of the individual responses of the unknown and standard, respectively, for each dose level, grading from the lowest to the highest.

In a similar manner, a formula was developed which is universally applicable to three-dose assays performed under the same restricted design. This formula in its broadest, but simplest, expression is as follows:

$$\% \text{ Potency of unknown} = \text{Antilog}\left[2 \pm c + \tfrac{4}{3}d\,\frac{(U_1 + U_2 + U_3) - (S_1 + S_2 + S_3)}{(U_3 + S_3) - (U_1 + S_1)}\right].$$

The terms in this equation have the same meaning as those in the four-dose assay, with the exception that the constant for the three-dose assay is $\frac{4}{3}$ instead of 5.

These formulas, under the restrictions of the test, have universal applicability to any assay in which the log dose-response curve has been shown to be a straight line and the curves of the unknown and standard are parallel. Graphs of the sums of the response at each dose level of both the standard and the unknown reactants give evidence of the parallism of their dose-response curves. If parallelism is not evident, the analysis of variance (*2*), factorial analysis (*3*), and the determination of lambda (*1*) may be necessary to interpret the significance of the assay. Any person familar with factorial analysis will recognize the numerators of the fractions given as the sum of the products dealing with the difference between samples, and the denominators as the sum of the products dealing with the slope of the dosage-response curve, *i.e.* S(xYp), which were given by Bliss and Marks in their factorial scheme for three- and four-dose assays (*3*). The application of the formulas presented here is, however, simpler than that of the potency formula given by these authors, *i.e.* $M = \frac{kID}{B}$. Moreover, both types of formulas give the same potency.[2]

These formulas have been applied in the Wellcome Laboratories to such widely divergent types of assay as agar diffusion methods for determining the potency of antibiotics or of amylases, turbidimetric procedures of assaying for a pneumococcus growth factor, and the mouse squeak test for the assay of analgesics. In the opinion of the author, a four-dose assay is preferable, since it permits the omission of the data from a single dose level (whether high or low), if the limit of linearity of the dose-response curve has been exceeded with the unknown. Hence, calculation of the potency on the basis of the three remaining dose levels may be made by applying the formula for the three-dose assay.

[1] The formula is derived in the following manner from the original Gaddum formula (*4*), *i.e.*

$$\text{log ratio of potencies} = \frac{\bar{y}_U - \bar{y}_S}{b} - (\bar{x}_U - \bar{x}_S),$$

where $\bar{y}_U$ = mean response of the unknown, $\bar{y}_s$ = mean response of the standard, b = combined slope of the dosage-response curves of the unknown and standard preparations, $\bar{x}_U$ = mean log dose of the unknown, and $\bar{x}_S$ = mean log dose of the standard. But the mean response of the unknown = $\frac{(U_1 + U_2 + U_3 + U_4)}{4N}$, where 4 = the number of doses, N = the number of replicates per dose, and U_1, U_2, U_3, and U_4 represent the sums of the replicates for each successive dose grading from the lowest to the highest; similarly, the mean response of the standard is $\frac{(S_1 + S_2 + S_3 + S_4)}{4N}$. Moreover, where X = the individual polynomial coefficients of the linear term and d = log dose interval, the slope of the unknown, *i.e.* $b = \frac{2S(xY_p)}{dNS(X^2)}$ becomes $\frac{2(3U_4 + U_3 - U_2 - 3U_1)}{dNS(X^2)}$ and that of the standard,

$$\frac{2(3S_4 + S_3 - S_2 - 3S_1)}{dNS(X^2)}.$$

Hence, the average slope of the unknown and the standard is

$$\tfrac{1}{2}\left[\frac{2(3U_4 + U_3 - U_2 - 3U_1 + 3S_4 + S_3 - S_2 - 3S_1)}{dNS(X^2)}\right].$$

But $X^2 = 20$ for a four-dose assay. Therefore, by substituting and combining terms, the average slope becomes

$$\frac{[3(U_4 + S_4) + (U_3 + S_3)] - [3(U_1 + S_1) + (U_2 + S_2)]}{20\,dN}.$$

By further substituting in the Gaddum formula, the following result is obtained: the ratio of potency of unknown to standard = $\frac{(U_1 + U_2 + U_3 + U_4) - (S_1 + S_2 + S_3 + S_4)}{4N} \cdot \frac{20\,dN}{[3(U_4 + S_4) + (U_3 + S_3)] - [3(U_1 + S_1) + (U_2 + S_2)]}$ $+(\bar{x}_S - \bar{x}_U)$; this reduces to the formula in the text, since $c = \bar{x}_S - \bar{x}_U$.

References

1. Bliss, C. I., and Cattell, McK. *Annu. Rev. Physiol.*, 1943, **5**, 479–539.
2. Bliss, C. I., and Marks, H. P. *Quart. J. Pharm. Pharmacol.*, 1939, **12**, 82–110.
3. Bliss, C. I., and Marks, H. P. *Quart. J. Pharm. Pharmacol.*, 1939, **12**, 182–205.
4. Gaddum, J. H. *Med. Res. Coun., Spec. Rep.*, 1933, Ser. 183.
5. Schild, H. O. *J. Physiol.*, 1942, **101**, 115–130; Sherwood, M. B., Falco, E. A., and de Beer, E. J. *Science*, 1944, **99**, 247–248; Knudsen, L. F. *Science*, 1945, **101**, 46–48.

[2] After this paper was in press, it was suggested to the author that, for convenience, the formula for the two-dose assay should be included. In order to make the previous formula analogous to the formulas herein presented the term c has been included, and the terms in its denominator have been rearranged. The revised formula for the potency of the unknown in a two-dose assay procedure is as follows:

$$\% \text{ Potency} = \text{Antilog } 2 \pm c + d\,\frac{(U_2 + U_1) - (S_2 + S_1)}{(U_2 + S_2) - (U_1 + S_1)}$$