IN THE LABORATORY

An Instrument for Plotting ED_{50} Curves

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Several methods for plotting ED_{50} curves have been described. De Beer (*2*) employed a permanent board with several instruments which enabled him to plot the curve and compute the results accurately and rapidly. Litchfield and Fertig (*3*) have described a rapid and accurate method for the calculation of the ED_{50} value, as have Miller and Tainter (*4*), who employed a special probit paper. Many investigators have used all of these methods, but thus far no instrument has been described which would allow a choice of the method to be used. The instrument described below has been used in our laboratories for over a year and has given accurate results with any of the above methods as well as with the longer Bliss (*1*) method.

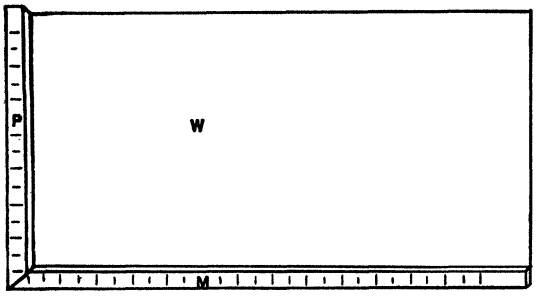

FIG. 1. Completed instruments. P = probit or per cent response scale; M = millimeter graph paper scale; W = Winthrop Probit Paper on plywood base.

The instrument (Fig. 1) was constructed from a piece of plywood 15 x 12 x $\frac{1}{4}$ inches, bordered by two pieces of molding 1 x $\frac{3}{8}$ x 15 inches and 12 inches respectively. To the plywood was glued a piece of Winthrop Probit Paper.[1] To the left-hand molding was attached the two scales cut from another piece of the same paper. While being glued, these scales were aligned so that part of the scale extended over the inner edge of the molding and fitted flush with the plywood. Attached to the lower molding was a piece of millimeter graph paper, which was divided into an arbitary scale allowing the plotting of log doses to 3 or more places, depending upon the accuracy desired. The entire board, including scales, was painted with a solution of cellulose nitrate in acetone to protect it against wear.

For plotting the ED_{50} of estrogenic response, where there is a definite logarithmic ratio between each dose, the scales on the plywood are used. The board is covered with a piece of tracing paper, and each dose response is marked off. The provisional and corrected curves relating log dose to probit or per cent response are obtained in the conventional manner, depending upon the method used. In plotting LD_{50} data, the scales on the plywood base of the board are covered with a piece of blank paper, the scales on the molding being used to obtain the dose-response curve. The log-dose values on the horizontal scale are chosen so that the greatest spread between doses will be obtained. This has a tendency to flatten the curve and make the results more accurate. The calculations for the provisional and corrected curves are done in the manner described by the method used.

[1] Obtained from the Winthrop Chemical Company, Rensselaer, New York.

References

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Conversion of Heart Potentials Into Auditory Equivalents

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For purposes of our research, it became desirable to have an instrument that would convert to auditory equivalents the changes in electrical potential occurring during the cardiac cycle. In consulting the literature, we found many instruments that involve the simple electronic amplification of the heart sounds (which may then be recorded graphically or phonographically) or the graphic or oscillographic recording of the changes in heart potential: amplifying stethoscopes, recording stethoscopes, stethographs, and electrocardiographs; but we were unable to find what we have termed an electrocardiophone or EKP: an instrument that, instead of expressing the heart potentials in graphic form, expresses them in auditory equivalents.

In developing a frequency modulation amplifier suitable for our purposes, preliminary difficulties were met in handling the extremely low frequencies without distortion. These difficulties have been largely overcome, although alternating-current fields and similar electrical conditions must be avoided.

The basic circuit of the electrocardiophone utilizes the fact that two radio frequencies will produce a beat frequency when fed into a suitable detector circuit. Since the beat frequency is equal to the difference between the frequency of oscillator I and oscillator II, it will be an audible frequency if the oscillators are within a few kilocycles of each other.

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.

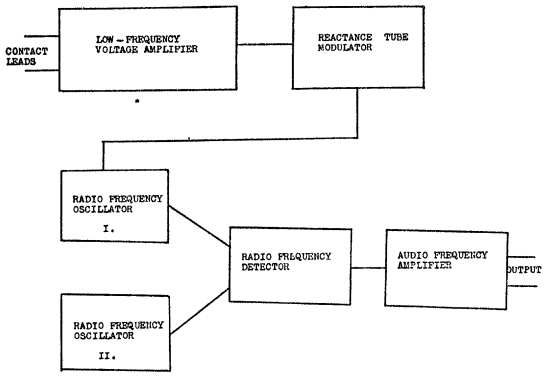

FIG. 1

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.

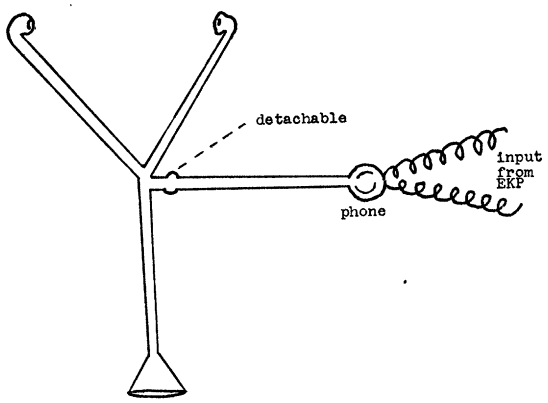

FIG. 2

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

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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