Mathematics of Biological Assay

IF in a biological assay the mean responses, y_{11}, y_{12}, of two groups of n animals to doses of a standard preparation the logarithms of which are x_{11}, x_{12}, are compared with the mean responses, y_{21}, y_{22}, to doses of the preparation under test the logarithms of which are x_{21}, x_{22}, it is often possible to express the relationship between dosage and response adequately by the equations :

$$Y_1 = a + bx, \quad Y_2 = a + b(x - M), \qquad (1)$$

in which M is a constant difference between log-doses producing equal responses. It is usual to choose pairs of doses having a fixed ratio, so that $(x_{12} - x_{11}) = (x_{22} - x_{21}) = d$. Writing

$$R = \tfrac{1}{2}(y_{22} - y_{21} + y_{12} - y_{11}),$$
$$S = \tfrac{1}{2}(y_{22} + y_{21} - y_{12} - y_{11}),$$
$$\text{and } T = \tfrac{1}{2}(y_{22} - y_{21} - y_{12} + y_{11}),$$

it may be shown that M is estimated by

$$M = \tfrac{1}{2}(x_{22} + x_{21} - x_{12} - x_{11}) - Sd/R, \qquad (2)$$

since R/d is the estimated increase in response per unit increase in log-dose[1]. In a recent communication[2], E. C. Wood has shown that, if a quadratic term is added to the response curves, still keeping the condition that there shall be a constant ratio between equally effective doses, so that

$$Y_1 = a + bx + cx^2, \quad Y_2 = a + b(x - M) + c(x - M)^2, \qquad (3)$$

equation (2) still estimates M.

It would be unfortunate if this interesting fact were allowed to conceal the limited applicability of the four-point assay. Equations (1) express the hypothesis that the responses are related to the log-doses by two parallel straight lines, and the data themselves provide a test of the adequacy of this hypothesis. T is a measure of the departure from parallelism of the straight lines connecting the responses to the pairs of doses ; if s is the residual standard error in an analysis of variance of the responses by the $4n$ animals (differences corresponding to doses and to litters or any other relevant classification having been eliminated), the standard error of T, as also of R and S, is $s/\sqrt{n}$, and the significance of T may then be judged by a t-test.

Equations (3) express the hypothesis that the responses are related to the log-doses by quadratic curves, still with the condition that the curves are identical save for a constant ratio of potencies ; the four parameters, a, b, c and M, may be determined so that equations (3) reproduce the experimental mean responses exactly, leaving no degrees of freedom for assessing the adequacy of the hypothesis. There may sometimes be strong *a priori* reasons for believing that the standard and test preparations have response curves of identical form, in spite of a difference in potency ; a quadratic equation such as (3) may then be a sufficiently good approximation, even when a linear is unsatisfactory, and the relative potency is estimated as the antilogarithm of the expression in equation (2). Without this belief, it is unjustifiable to assume the existence of a constant relative potency, and the four-point assay cannot give a valid result unless a low value of T indicates the adequacy of the hypothesis expressed by equations (1). In all cases of uncertainty it is desirable to test at least three doses of both preparations, so that more information on the response curve may be obtained. Without such precautions, a spurious simplicity may appear in the results of an assay, a single figure being said to represent the potency of a test preparation relative to a standard, when, in fact, the relative potency depends on the level of response at which a comparison is made.

The standard error of M, as given by equation (2), is[1]

$$s_M = \pm \frac{sd}{R^2}\sqrt{\frac{R^2 + S^2}{n}}.$$

For any chosen level of probability a value of t may be obtained corresponding to the number of degrees of freedom on which s is based ; provided that $g = t^2 s^2/nR^2$ is small, the fiducial limits to M are $M \pm ts_M$, but when g is large the precise formula

$$M - \frac{g}{1-g}\frac{Sd}{R} \pm \frac{tsd}{R^2(1-g)}\sqrt{\frac{R^2(1-g) + S^2}{n}}$$

must be used.

D. J. FINNEY.

Rothamsted Experimental Station,
Harpenden, Herts.
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[1] Bliss, C. I., and Marks, H. P., *Quart. J. Pharm. Pharmacol.*, **11**, 192 (1939).
[2] Wood, E. C., NATURE, **153**, 84 (1944).

Tautomerism of Cyanamide

THE constitution of cyanamide, since its first synthesis in 1851, has been the subject of a very large number of publications. Whereas the chemical properties of this substance point to the structures NH_2CN and NH : C : NH, its physical properties are held[1] to favour the former. The modern tendency is to regard cyanamide as a mixture of these isomers in tautomeric equilibrium[2], with the position of equilibrium considerably in favour of the cyanide structure.

The equilibrium $NH_2CN \rightleftharpoons NH : C : NH$ is a special case of amidine tautomerism, and the tautomeric behaviour of amidines has already been correlated[3] with their associated (hydrogen-bond) structure. Since many of the physical properties of cyanamide are consistent with a high degree of molecular association, it appeared of interest to examine the molecular condition of cyanamide and its N-substituted derivatives to see whether a similar correlation prevailed. A sufficient number of cyanamide derivatives has now been examined to provide overwhelming support for this suggestion, namely, that the tautomerism of cyanamide and its monosubstituted derivatives (RNHCN $\rightleftharpoons$ RN : C : NH) is due to their molecular association.

Molecular weight measurements have been made cryoscopically in benzene solution, or, in cases where the solubility was too low at the freezing point of benzene, in naphthalene. Cyanamide itself was not sufficiently soluble in either solvent to give reliable results, and was measured cryoscopically in nitrobenzene solution. In spite of the donor character of this solvent, and its consequent tendency to simplify the solute molecules, a 2 per cent solution of cyanamide in nitrobenzene showed an association factor of well over 2·0. Molecular weight determinations have been made on more than a dozen N-substituted cyanamides, which are found to fall into two distinct classes : those possessing an unsubstituted hydrogen atom (RNHCN) are markedly associated, their molecular weight rising rapidly with