was also able to diminish the action of malachite green and that of its active derivatives (Table 1).

TABLE 1

Dilution of the bacteriostatic substances:	10^{-5}	10^{-6}	10^{-7}
Malachite green alone	–	–	++
Malachite green + leucobase diluted 10^{-4}	–	++	++
Carbinol bases alone	–	–	++
Carbinol bases + leucobase diluted 10^{-4}	+	++	++
Malachite green bisulphite alone	–	–	++
Malachite green bisulphite + leucobase diluted 10^{-4}	–	++	++

– no growth; + weak growth; ++ regular growth.

These assays were carried out with a strain of scarlatinous streptococcus (Dochez) in peptone-glucose broth and in Goodman medium.

Such antagonism may be caused (a) by neutralizing the active compound directly through some chemical or physicochemical process (formation of new compounds or complexes, adsorption, etc.); (b) by opposite influences upon the milieu; (c) by some biochemical mechanism, as by inhibition of entering the toxic compound into the cell through competition or by opposite influences upon metabolistic processes, etc.

We are now investigating these possibilities and can already state that malachite green and its leucobase do not change oppositely the redox potential of the milieu (*cf.* Ingraham[2]).

E. FISCHER
O. HOFFMANN
E. PRADO

INSTITUTO BACTERIOLÓGICO DE CHILE,
SANTIAGO DE CHILE

SCIENTIFIC APPARATUS AND LABORATORY METHODS

RELATIVE POTENCY AS APPLIED TO THE ASSAY OF PENICILLIN

A SIMPLE estimation of the relative potency of penicillin, insulin[1] and other drugs can be made under conditions where the biological indicator gives a graded linear response when plotted against the logarithm of the dose. An unknown, U, is compared with a standard, S, at two concentration levels such that the dilution ratio $U_2 : U_1 = S_2 : S_1$. The two doses of the unknown are selected so that they will have the same potency as those of the standard, in so far as this can be determined in advance. The four doses are applied at random to sets of four quite similar biological units. The sets in turn are repeated until the potency of the unknown has been determined with the desired precision.

The data are given in Table 1 for a cylinder-plate assay of penicillin which meets these requirements. Four glass cylinders were placed on the inoculated agar of each petri dish. Two were filled with different doses of an unknown preparation (U_1, U_2) and two with corresponding doses of a standard (S_1, S_2). The diameter in millimeters of the cleared area about each cylinder on the following day is shown for each plate or set in the left side of the table.

In this experiment the calculations are shortened through the adoption of a ratio between concentrations such that $U_2 = 3.16\ U_1$ and $S_2 = 3.16\ S_1$, giving a log-ratio of $I = 0.500$. This modification was first proposed by Dr. Lloyd C. Miller, to whom I am indebted for the data in Table 1, and it has been used successfully for penicillin assays at the Winthrop Chemical Company, Inc.[2] Both U_2 and S_2 are prepared initially to contain 2.0 U/cc of penicillin, the unknown in the present case having an assumed potency of 400 U/mg. The weaker dilutions are obtained by adding 2.31 cc of S_2 and U_2 to vessels containing 5.00 cc of buffer solution.

The first step in the analysis is to compute four differences for each plate, as shown in the second part of Table 1. The successive differences are those of the unknown minus the standard at the high (D_1) and at the low (D_2) dosage levels and of the high minus the low dose for the unknown (D_3) and for standard (D_4). These initial differences are then used to obtain the basic computing units (y). The values for $y_1 = D_1 + D_2$ total the effect of the differences between the two preparations, while those for $y_2 = D_3 + D_4$ total the effect of the differences between the two dosage levels. The differences $y_3 = D_1 - D_2 = D_3 - D_4$

TABLE 1

Plate No.	Diameter in mm for				$D_1 = U_2 - S_2$	$D_2 = U_1 - S_1$	$D_3 = U_2 - U_1$	$D_4 = S_2 - S_1$	$y_1 = D_1 + D_2$	$y_2 = D_3 + D_4$	$y_3 = D_1 - D_2 = D_3 - D_4$
	U_2	U_1	S_2	S_1							
1	25.8	20.8	25.6	20.4	.2	.4	5.0	5.2	.6	10.2	–.2
2	25.8	21.0	25.2	20.4	.6	.6	4.8	4.8	1.2	9.6	0
3	25.4	20.4	24.8	20.0	.6	.4	5.0	4.8	1.0	9.8	.2
4	25.8	20.8	25.2	20.4	.6	.4	5.0	4.8	1.0	9.8	.2
									3.8 = T_1	39.4 = T_2	.2 = T_3

[1] C. I. Bliss and H. P. Marks, *Quart. Jour. Pharmacy and Pharmacol.*, 12: 182, 1939.

[2] Ingraham, *Jour. of Bact.*, 26, 573, 1933.

[2] L. C. Miller, J. H. Bailey and W. F. Warner (in preparation).

test whether the dosage-response curves for standard and unknown are parallel. Computing y_3 in two ways checks the correctness of the original differences. The computing units are then summed for each column to obtain T_1, T_2 and T_3, respectively.

The potency of the unknown is computed in logarithmic units. If the unknown is assumed to have the same potency as the standard, values such as those in the right hand and lower portion of Table 1 lead directly to the logarithm of relative potency, M′, by the formula

$$M' = \frac{IT_1}{T_2} = \frac{0.5 \times 3.8}{39.4} = 0.0482 \qquad (1)$$

where I is the dosage interval in logarithms and the numerical values are those obtained from Table 1. To express the results in Oxford units, the unitage assumed in carrying out the assay is used. The estimated log-potency (M) is given by the equation

$$M = \log(\text{assumed unitage or potency}) + M' = 2.6021 + 0.0482 = 2.6503. \qquad (2)$$

From the antilog of M the potency of the unknown has been assayed at 447.0 U/mg.

The above calculation differs from a similar proposal[3] in leading directly to an easy estimate of the standard error, s_M. While some factors in the determination of potency are not tested in a single assay—and these may be of first importance—the precision of a given technique is no greater than that indicated by the standard error. The latter depends upon several intermediate terms. The first of these, the slope of the dosage-response curve, is computed as

$$b = \frac{T_2}{2IN}, \qquad (3)$$

where N is the number of sets or plates in the assay. With $I = 0.500$, this may be simplified to

$$b = \frac{T_2}{N} = \frac{39.4}{4} = 9.85. \qquad (3a)$$

The standard deviation of a single response as computed from all relevant data is

$$s = \sqrt{\frac{S(y^2) - (T_1^2 + T_2^2 + T_3^2)/N}{12(N-1)}} = \sqrt{\frac{392.20 - 391.71}{36}} = 0.1167, \qquad (4)$$

where y refers to all individual values of y_1, y_2 and y_3 and $S(y^2)$ is the sum of their squares. The standard deviation in the response is divided by the slope to transform it from units of response (y) to units of log-dose (x), giving

$$\lambda = \frac{s}{b} = \frac{0.1167}{9.85} = 0.01185. \qquad (5)$$

The smaller the term λ the more sensitive is the assay for detecting differences in potency. The standard error of M or of M' is then equal to

$$s_M = \lambda \sqrt{\frac{1}{N}\left\{1 + \frac{T_1^2}{T_2^2}\right\}} = 0.01185 \sqrt{\frac{1.0093}{4}} = 0.0060. \qquad (6)$$

To realize the full precision of a given method, the assumed potency should not differ widely from the true value. Changes in technique which increase the inherent precision of an assay will reduce λ. With a standardized procedure, λ should be relatively stable from one assay to another.

Most tests of statistical significance are made in units of $M \pm s_M$. In original units an average standard error may be computed as

$$\text{standard error of potency} = 2.30 s_M \text{ (antilog M)}. \qquad (7)$$

In the present case the potency of the unknown would be reported as 447.0 ± 6.2 U/mg. This agreed well with a parallel assay of the same preparation, which gave a potency of 451.7 ± 5.6 U/mg.

The above procedure assumes that the dosage-response curves for standard and unknown are parallel. This assumption may be tested by the t-test for the significance of a difference where

$$t = \frac{T_3}{2s\sqrt{N}} = \frac{0.2}{2 \times 0.1167 \times 2} = 0.43 \qquad (8)$$

The observed value is referred to a suitable table[4] with 3(N-1) degrees of freedom and should not exceed the value for P = 0.05. In the present case no divergence from parallelism is indicated. The above design for an individual assay holds equally for those repeated frequently in the same laboratory, but in the latter case a modified calculation may be preferred.[5,6]

C. I. BLISS

CONNECTICUT AGRICULTURAL EXPERIMENT STATION AND YALE UNIVERSITY

[3] M. B. Sherwood, E. A. Falco and E. J. de Beer, SCIENCE, 99: 247, 1944.

[4] R. A. Fisher and F. Yates, "Statistical Tables for Biological, Agricultural and Medical Research," Oliver and Boyd, 1943.

[5] C. I. Bliss, *Jour. Amer. Statistical Asn.* (in press).

[6] C. I. Bliss and L. C. Miller (in preparation).

BOOKS RECEIVED

BROOKS, SUMNER CUSHING and M. MOLDENHAUER BROOKS. *The Permeability of Living Cells* (Protoplasma-Monographien Bd. 19). Pp. xviii + 395. J. W. Edwards, Ann Arbor, Mich. $5.00. 1944.

CANTAROW, ABRAHAM and MAX TRUMPER. *Lead Poisoning.* Pp. xiii + 264. Williams & Wilkins Co. $3.00. 1944.

CONRAD, VICTOR A. *Methods in Climatology.* Illustrated. Pp. xx + 228. Harvard University Press. $4.00. 1944.

GARRETT, S. D. *Root Disease Fungi.* Illustrated. Pp. 177. Chronica Botanica Company. $4.50. 1944.

MOVIUS, HALLAN L. *Early Man and Pleistocene Stratigraphy in Southern and Eastern Asia.* Illustrated. Pp. 125. 6 tables. Peabody Museum, Cambridge 38, Mass. $3.75. 1944.

NEVILLE, ERIC HAROLD. *Jacobian Elliptic Functions.* Illustrated. Pp. xiii + 331. Oxford University Press. 1943.

SELTZER, CARL C. *Racial Prehistory in the Southwest and the Hawikuh Zunis.* Pp. 37. 15 tables. Peabody Museum, Cambridge 38, Mass. $.75. 1944.

WILLETT, HURD C. *Descriptive Meteorology.* Illustrated. Pp. viii + 310. Academic Press, Inc. $4.00. 1944.