THE INFLUENCE OF "FOLIC ACID" ON SPONTANEOUS BREAST CANCERS IN MICE[1]

IN a recent communication[2] we reported that a "folic acid concentrate" and a crystalline *L. casei* factor[3] ("folic acid") were found to be strong inhibitors of tumor growth.

In the following communication evidence for the therapeutic action of *L. casei* factor on spontaneous breast cancers in mice is presented. Further details will be given later.

Experimental: 149 mice from three different strains (Jackson Memorial Laboratory, strain A, Rockland strain and Bagg strain) bearing single spontaneous breast cancers were selected for the experiments. A definite diagnosis of malignancy was established by biopsy. The animals were kept on a normal diet (Rockland mouse pellets); 120 animals were divided into two groups (60 mice each) in such a way that they were matched as to strains and, as closely as possible, as to location and size of the tumors.

One of these groups of 60 mice received daily intravenous injections of 5 micrograms *L. casei* factor over a period of 4 to 6 weeks; the control group of 60 mice did not receive any injections. Another set of 29 mice were also treated with 5 micrograms of *L. casei* factor, but without "matched" controls. No toxic effect was observed in the treated animals.

Results are presented in Table 1.

TABLE 1

EFFECT OF L. CASEI FACTOR ON SPONTANEOUS BREAST CANCERS IN MICE

Number of mice	Dose injected	Number of healed mice	Number of living mice	Number of new tumors
60	5 micrograms	26	34	0
60	0	0	20	14
29	5 micrograms	12	15	1

It is evident from this table that intravenous injections of 5 micrograms of *L. casei* factor led to complete disappearance in 38 among 89 tumors. No tumor disappeared among the 60 controls. Fourteen controls developed new tumors, whereas one new tumor was observed among the treated mice. Among the 89 treated mice 49 are still living, including 33 healed animals.

The observation period extends from two to ten months for treated groups and controls. During this period, no local recurrences or new tumors were observed among the healed animals.

[1] This work was aided by grants of the International Cancer Research Foundation and the Lederle Laboratories, Inc.

[2] C. Leuchtenberger, R. Lewisohn, D. Laszlo and R. Leuchtenberger, *Proc. Soc. Exp. Biol. and Med.*, 55: 204, 1944.

[3] The *L. casei* factor was obtained through the courtesy of Dr. E. L. R. Stokstad and Dr. B. L. Hutchings, of the Lederle Laboratories, Inc.

Summary: Complete regressions of spontaneous breast cancers in mice were observed in 38 among 89 animals (43 per cent.). The treatment consisted of daily intravenous injections of 5 micrograms of *L. casei* factor ("folic acid"). The treated animals lived longer than the controls, especially the healed mice. The incidence of the development of new tumors was decreased among the treated mice as compared with the controls.

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PENICILLIN ASSAY[1]

IN SCIENCE for March 24, 1944, a method was given for determining the potency of penicillin.[2] The following is a further development and gives a simple statistical method of determining both the potency of antibiotic substances in terms of suitable standards and error of assay by use of a chart and a nomograph in conjunction with four figures obtained by certain additions and subtractions of the diameters of the zones of inhibition of the incubated plates.

The penicillin assay to which this statistical method applies directly involves four plates (Petri dishes) seeded with *Staph. aureus* or other appropriate organism. Four small glass cups are placed on each plate (metal cups or blotter discs may be used). The cups are then filled with penicillin so that each plate has two dilutions of the standard and two dilutions of the unknown made up so that one dilution contains .25 unit/ml and the other 1.00 unit/ml. The unknown is diluted according to its estimated potency. Thus the ratio of the two doses on both the standard and the unknown is 4 to 1 (or log of ratio of doses = log 4 = .602).

Both the formula for the potency as a per cent. of the standard and the formula for the ratio of the error of the potency to the potency have been graphed in relatively simple form for use in the laboratory[3] as shown in Figs. 1 and 2. To use the nomograph, simple additions and subtractions have to be made on the measurements of the diameters of the zones of inhibition to obtain V, W, R_v and R_w.

[1] The author wishes to gratefully acknowledge the encouragement and assistance of Dr. W. Edwards Deming, of the Bureau of the Budget, and also the drafting of the chart and nomograph by Pete James, of the Food and Drug Administration.

[2] "A Rapid Quantitative Method for the Determination of Penicillin," M. B. Sherwood, E. A. Falco and E. J. deBeer, SCIENCE, 99: 247.

[3] Enlarged graphs and the procedure employed by the Food and Drug Administration in their routine assay of penicillin may be obtained by writing to the Division of Bacteriology of the Food and Drug Administration. Mathematical derivation of the method will be published elsewhere.

An example may clarify the procedure. The data are given in Table 1.

TABLE 1

PENICILLIN PLATE-ASSAY[4]

Plate No.	s_L .25 u/mg mm	s_H 1.0 u/mg mm	u_L estimated .25 u/mg mm	u_H estimated 1.0 u/mg mm	v or $(u_L + u_H) - (s_L + s_H)$	w or $(s_H + u_H) - (s_L + u_L)$
1	16.0	22.5	15.0	20.0	−3.5	11.5
2	16.2	22.5	14.5	19.5	−4.7	11.3
3	16.0	22.5	15.0	22.0	−1.5	13.5
4	15.0	22.0	14.0	21.0	−2.0	14.0
Sum	63.2	89.5	58.5	82.5	−11.7=V	50.3=W
Range	...	...	...	...	3.2=R_v	2.7=R_w

[4] Data from the laboratories of the Division of Bacteriology, Food and Drug Administration.

The columns headed s_L, s_H, u_L, and u_H (diameters of zones of inhibition on low and high doses of standard and unknown) are totalled and the values of v and w are calculated. The values of V and W are checked by separately adding the columns headed v and w. R_v is the range of the v column. R_w is the range of the w column.

The graph shown in Fig. 1 is entered with V = − 11.7

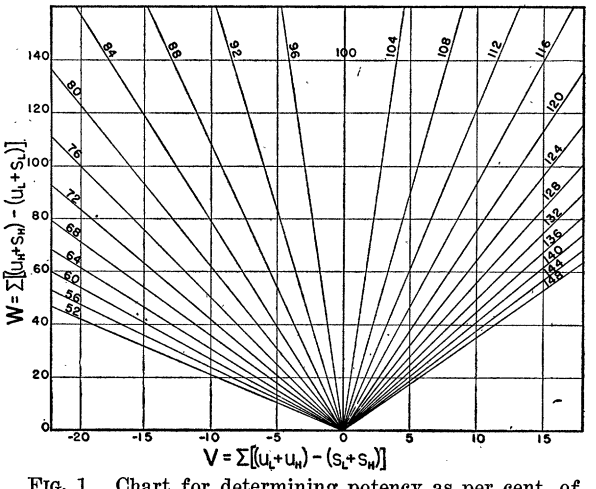

FIG. 1. Chart for determining potency as per cent. of a standard from the formula:

$$\text{Potency} = \text{antilog}\left(2 + \frac{.602V}{W}\right)$$

V and W are calculated from diameters of zones of inhibition. The chart is entered with coordinates V and W and the potency is read off the radial lines. The ratio of doses is 4: 1.

and W = 50.3 and the potency is found to be 72 per cent. of the standard.

To calculate the error of the assay, the inside scales on the right-hand side of the nomograph shown in Fig. 2 are entered with V = 11.7 and W = 50.3 (only absolute values of V, W, R_v and R_w are used in the calculation of the error of the assay). By means of a straightedge connecting first the values of V and W, marking the point of intersection of the straightedge and the diagonal line and then connecting this marked point and R_w, read the value of Q (.65). T is equal to $Q^2 + R^2_v = 10.66$. The left-hand side of Fig. 2 gives the value of the ratio of the error of the assay to the potency to be .087 by connecting the value of W (or 50.3) with the value of T (or 10.66). The error of the assay equals $.087 \times 72 = 6.3$.

However, if $11.4R_w$ is greater than W the slope of the assay does not differ significantly from zero by Student's *t* test. This is an indication of faulty procedure, and when the fault is located, the assay should be repeated. If the potency lies outside the limits given in Fig. 1, the assay should be repeated using a higher or lower dilution. In practice one may also wish to test the parallelism of the lines, however, lack of parallelism influences the size of the error of the assay.

The charts as given apply directly only to assays in which the ratio of the high dose to low dose equals 4 to 1. The potency chart can be used for any number of plates and should be remade for ratios of doses other than 4: 1, although the following formula gives an approximation within one per cent.:

$$\text{Corrected Potency minus } 100 = (\text{Potency from chart minus } 100)\frac{\text{log ratio of doses}}{.602}.$$

The nomograph for the error of the assay applies directly only if 4 plates are used. However, it can be used for any number of plates and any ratio of doses by multiplying the resultant ratio of the error of assay to the potency by the appropriate figure given in Table 2.

TABLE 2

Number of Plates N	Ratio of doses 4 : 1	Ratio of doses 3.16 : 1	Ratio of doses 2 : 1
2	1.2901	1.0715	.6451
3	1.0534	.8749	.5267
4	1.0000	.8306	.5000
5	.9896	.8219	.4949
6	.9949	.8263	.4974
7	1.0071	.8364	.5035
8	1.0226	.8493	.5113

The error of the assay calculated here or in any penicillin assay estimates only how closely one assayist can check himself on any given set of dilutions of unknown and standard. It does not include any errors of weighing or errors due to variations in materials or subdivisions of a lot of penicillin. Since very minute amounts of materials are used in this type of penicillin plate-assay, appreciable errors in weighing are likely to occur. An assay could be designed to include these errors by using two or more weighings and stock solutions of standard and unknown on each assay. Any statistical formula using the data from one series of, say, 4 plates from the same weighing and dilution can not estimate errors other than those accounting for some measurable

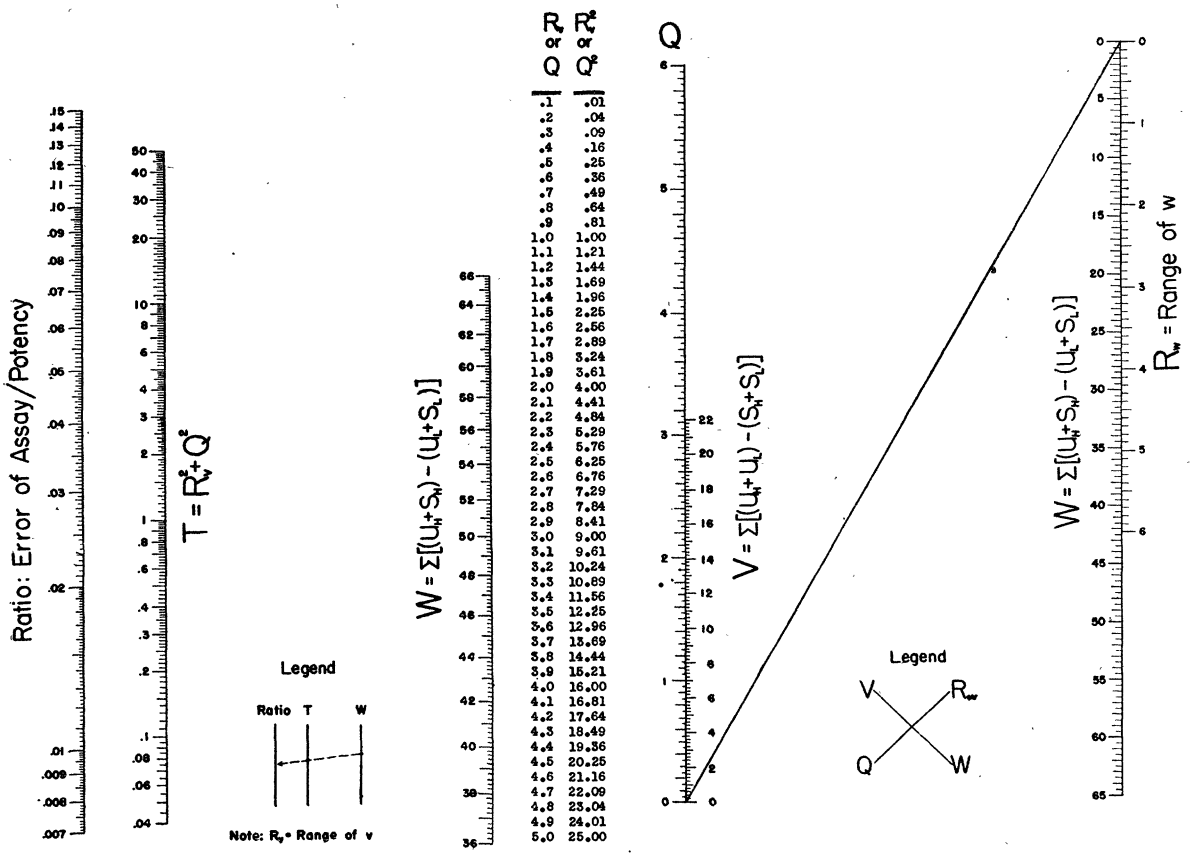

FIG. 2. Penicillin assay nomographs for estimating the error of the assay from the formula

$$\frac{\text{Error of Assay}}{\text{Potency}} = \frac{k}{W}\sqrt{R^2_v + \frac{R^2_w V^2}{W^2}}$$

V,W,R_v, and R_w are calculated from the diameters of the zones of inhibition on the two dose, four plate, penicillin assay in which the ratio of doses is 4: 1. A more complete table of squares such as Barlow's "Tables" should be substituted whenever possible for the brief table of squares given between the nomographs. Here k = 1.35, this being ln 4 times the square root of the number of plates all divided by the average number of standard deviations in the range.

variation within the assay. One can not state on the basis of this calculated error of assay that the lot of penicillin was, say, 650 ± 20 units/mg unless the method was so standardized and the results so kept in "statistical control" that any assayists at various laboratories can check each others' results as closely as one assayist can check himself.

The charts and methods can be used in similarly-designed assays of other drugs, *e.g.*, in the Vitamin A assay involving two-dose comparisons of standard and unknown, with four male rats from each litter.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MANOMETRIC APPARATUS FOR RESPIRATORY STUDIES OF SMALL ANIMALS

THE equipment described below was designed to aid in an investigation of chronic cyanide poisoning in the rat. It is presented because the method has certain advantages not found in other techniques and is adaptable to use in a variety of biological research problems.

The apparatus is essentially a constant volume manometer with a small pump to continuously circulate the gas (Fig. 1). It is simple and inexpensive and except for the air pump may be easily assembled from ordinary laboratory materials. The experimental animal is placed on a screen in a museum jar of about six liters capacity with the groundglass edge sealed with petrolatum to a piece of plate glass. A hole in the top plate contains a rubber stopper through