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.

R. LEUCHTENBERGER
C. LEUCHTENBERGER
D. LASZLO
R. LEWISOHN

MOUNT SINAI HOSPITAL,
NEW YORK CITY

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.