hyperplastic epidermis is an important feature in this experimentally induced precancerous condition.

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SURVIVAL OF NORMAL CELLS IN PENICILLIN SOLUTIONS LETHAL TO MALIGNANT CELLS

STUDIES at the Wistar Institute of Anatomy and Biology, made possible through the kindness of Dr. M. R. Lewis and Dr. W. H. Lewis,[1] have revealed a selective lethal effect of penicillin upon rat and mouse sarcoma cells, of which a full account will be published later.

In roller tube cultures, untreated sarcoma cells, grown with normal fibroblasts derived from explants of muscle from the same strain of tumor host, grow fully as vigorously as the normal cells. However, upon addition of penicillin (Squibb's sodium salt of penicillin), the sarcoma cells were selectively damaged. With proper choice of the dosage level, it was found possible to kill all the sarcoma cells without damaging the normal fibroblasts. Higher dosage damaged the non-malignant cells, but the dose required was two to three times that required to produce an equivalent injury in malignant cells.

Damage was arbitrarily classified as incipient (granularity of 50 per cent or more of the cells, and a faintly withered appearance of the cell membrane), marked damage (rounding, coagulation or disintegration of the cells, short of 100 per cent), and lethal (no living cells visible). Table 1 shows the totals of

TABLE 1

NUMBERS OF EXPLANTS OF SARCOMA CELLS AND OF NORMAL FIBROBLASTS SHOWING DIFFERENT GRADES OF DAMAGE. COMBINED TOTALS OF ALL EXPERIMENTS.

	None	Incipient	Marked	Lethal	Totals
Normal	112	37	57	0	206
Sarcoma	0	29	156	78	263

explants classified according to damage shown. These results include four induced rat tumors and one induced mouse tumor. Another induced mouse tumor, not included in the data of Table 1, did not show a definite selective response.

In twenty-five experiments, the treated tumor cultures were implanted into rats of the corresponding 100 per cent-susceptible strain. All cultures graded as "lethal damage," and most of those graded as "marked damage" failed to produce tumors, whereas the untreated cultures produced tumors.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A RAPID, QUANTITATIVE METHOD FOR THE DETERMINATION OF PENICILLIN

THE following assay method affords a rapid, convenient and relatively accurate method of determining the potencies of antibiotic substances in terms of suitable standards. Since assays may be completed during the course of a working day, the method is particularly useful as a guide to the time of maximum production of penicillin, for the control of isolation procedures of various antibiotic agents, in the determination of the amount of deterioration and for related problems.

Earlier experiments[1] on the use of filter paper as a matrix to support mold growth led to the development of the present assay method in which the antibiotic substance to be assayed is placed on sterile, absorbent paper discs on the surface of inoculated nutrient agar. The function of the paper is to act as a reservoir from which the antibiotic substance diffuses into the agar where it inhibits the growth of the test organism. This results in a clear zone surrounding the disc. It was found that the diameter of this zone of inhibition is proportional to the amount of antibiotic substance present and, for practical purposes, may be considered a straight-line function of the log concentration.

The following example illustrates the simplicity of the assay. Nutrient agar,[2] approximately pH 7.0, was seeded with *B. subtilis* spore suspension[3] so as to contain approximately 2×10^5 spores per cc and 25 cc portions of this medium were pipetted into 90 mm petri dishes (depth of the agar approximately 4 mm). Four sterile, filter paper discs, diameter 15.3 mm,[4] were evenly spaced upon the agar. By means of a

[1] We are indebted to the Carnegie Institution of Washington Department of Embryology and to the International Cancer Research Foundation, as well as to the Wistar Institute of Anatomy and Biology, for aid in carrying out this work.

[1] M. B. Sherwood, *Jour. Bact.*, 43: *Proc.*, 779, 1942.

[2] G. L. A. Ruehle and C. M. Brewer, *U. S. Dept. of Agriculture Circ.* 198, 1929.

[3] J. W. Foster and H. B. Woodruff, *Jour. Biol. Chem.*, 148: 723, 1943.

[4] Discs were cut from E. and D. No. 615 filter paper with a sharpened cork borer.

4 mm, 24-gauge nichrome wire loop,[5] 2 loopfuls of the undiluted standard (in this case a *P. notatum*[6] filtrate of known potency) were placed upon one disc and, after flaming the loop, a second disc was treated with 2 loopfuls of 1:4 aqueous dilution of the standard filtrate. Similarly, 2 loopfuls of the undiluted unknown (the unfiltered infranatant solution from a growing culture) were placed on the third disc and 2 loopfuls of a 1:4 dilution of the unknown on the fourth disc. Plates were prepared in quadruplicate and incubated immediately after treatment. An incubation period of 5½ hours at 37° C resulted in growth sufficiently heavy to permit measurement of the clear zone of inhibition with a mm rule. The results are given in Table 1.

TABLE 1

DIAMETER OF ZONES OF INHIBITION IN MM

	Standard		Unknown	
	Diluted 1 to 4 or 25 per cent.	Undiluted or 100 per cent.	Diluted 1 to 4 or 25 per cent.	Undiluted or 100 per cent.
Plate I . . .	27.5	32.5	23.5	29.5
Plate II . .	27	31.5	23	28
Plate III . .	26.5	31	24	29
Plate IV . .	27	32	25	30
Sums . .	$108.0 = S_1$	$127.0 = S_2$	$95.5 = U_1$	$116.5 = U_2$

The potency expressed as percentage of the standard may be readily calculated with the aid of the following simple equation[7] in which 2 is the factor for converting to per cent., d is the log of the ratio of the greater dose to the smaller dose (here d = log 4 or 0.602) and the other terms are those indicated in the table.

$$\text{Potency} = \text{antilog}\left(2 + d\,\frac{(U_2+U_1)-(S_2+S_1)}{(U_2-U_1)+(S_2-S_1)}\right)$$

On substituting the data of Table 1, it was found that the unknown was 45 per cent. as potent as the standard.

$$\text{Potency} = \text{antilog}\left(2 + 0.602\,\left\{\frac{(116.5+95.5)-(127.0+108.0)}{(116.5-95.5)+(127.0-108.0)}\right\}\right)$$

Potency = antilog 1.6538 = 45%.

Using the method of factorial analysis described by Bliss,[8] it was found that the average standard error was 4.3 and that the slope of the line was 8.3.

Illustrating the reproducibility of results, two independent workers assayed two different preparations and found 11 per cent. and 12 per cent. potency for the weaker solution and 80 per cent. and 84 per cent. for the stronger.

In these antibacterial assays, the loop was found to give the same order of accuracy as micropipettes but to afford greater ease of manipulation and to eliminate much washing and sterilization.

Up to the present, experiments have been done with penicillin, clavacin and gliotoxin. *B. subtilis* was found to be a more convenient test organism than *Staph. aureus, E. typhosa,* or *D. pneumoniae* types I, II and III.

Since the response is affected by the temperature and duration of incubation, the volume of the dose, the size of disc, the depth of the agar and the number of *B. subtilis* organisms per plate, these factors must be kept relatively constant. A more complete study of the method, together with data on the above-mentioned variables, will be presented elsewhere.

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[5] Constructed as described in reference 2. The use of short, wide tubes led to more uniform loopfuls of solution.

[6] The authors are indebted to Dr. K. B. Raper, of the Northern Regional Research Laboratory, Peoria, Ill., for cultures of *P. notatum;* to Dr. S. A. Waksman, of the N. J. Agricultural Expt. Station, New Brunswick, N. J., for a culture of *A. clavatus;* and to Dr. J. W. Foster, of Merck and Company, Rahway, N. J., for cultures of *Staph. aureus* and *B. subtilis.*

[7] The equation is derived as follows: The average of the responses for the standard solutions, $\frac{S_2+S_1}{2N}$ (where N represents the number of responses per dose), is subtracted from the average of the responses for the unknown solutions $\frac{U_2+U_1}{2N}$ and the resulting difference is converted into a logarithm by dividing by b_c, the average slope of the two dose-response curves. The antilog of this logarithm is the potency of the unknown in terms of the standard and may be expressed on a percentage basis by multiplying by 100. It is more convenient, however, to carry out this multiplication while the potency ratio is still in logarithmic form by adding it to 2, the logarithm of 100. Combining $\frac{1}{2}\left(\frac{U_2-U_1}{Nd}+\frac{S_2-S_1}{Nd}\right)$, which is the formula for b_c, with the above steps results in the following equation:

$$\text{Potency} = \text{antilog}\left(2+\left[\frac{1}{\frac{1}{2}\left\{\frac{U_2-U_1}{Nd}+\frac{S_2-S_1}{Nd}\right\}}\right]\left[\frac{U_2-U_1}{2N}-\frac{S_2-S_1}{2N}\right]\right)$$

which reduces to the simple expression given in the text.

[8] C. I. Bliss and H. P. Marks, *Quart. Jour. Pharm. Pharmacol.*, 14: 182, 1939.

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BAILEY, C. H. *The Constituents of Wheat and Wheat Products.* Illustrated. Pp. 332. Reinhold Publishing Corporation. $6.50.

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