TABLE 1

Rat No.	Original discrimination			After 30 weeks		
	Responses		Percentage	Responses		Percentage
	(In S^D)	(In S^Δ)	(In S^D)	(In S^D)	(In S^Δ)	(In S^D)
12	1,242	125	90.9	1,027	154	87.0
13	1,094	167	86.8	827	265	75.7
14	1,070	87	92.5	913	348	72.4
15	700	107	86.7	341	35	90.7
17	977	80	92.4	804	261	75.5
Sum	5,083	566	.....	3,912	1,063	.....

however, and the range of individual variation, this figure should be viewed as no more than a rough estimate of any population value. The important feature to note is that a substantial difference in rates is retained by all animals. Similarly, the total number of responses drops from a mean of 1,130 to one of 995, or about 11.3%.

Although some loss of discrimination and some decline in total responding is suggested by these figures, it is concluded that a well-established discrimination between two stimuli may largely be retained over a period of 30 weeks, under normal laboratory conditions; the breakdown of such a discrimination is not inevitable with the mere passage of time.

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Manuscript received June 18, 1951.

Effects of Indolebutyric Acid and Other Compounds on Virus Concentration in Plant Tissue Cultures

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In a previous paper by Kutsky and Rawlins (*1*), culture methods and a new method of analysis for tobacco mosaic virus in tobacco stem tissue cultures were described. It was reported that naphthalenacetic acid at 1.0 mg/l was effective in reducing the concentration of tobacco mosaic virus in tobacco stem tissue cultures. Further tests of compounds for effect on virus content are reported here.

The tissue culture technique and method of analysis were essentially the same as previously described (*1*). The characteristic nucleic acid absorption maximum at 260 mμ was used as a measure of virus concentration in processed extracts. By calculating the absorption ratio, T/C, of treated to control culture extracts one obtains an index of the effect of the added compound. For cultures where coloration was evident in the final fluid an added step—namely, precipitation of the virus in half-saturated ammonium sulfate and resuspension in water or $M/15$ phosphate buffer, pH 7, was inserted before the ultraviolet spectrophotometric analysis. Coloration, which occurred sporadically and interfered with the ultraviolet analysis, remained largely in the supernatant when the virus was precipitated with ammonium sulfate.

TABLE 1

EFFECT OF INDOLEBUTYRIC ACID ON CONCENTRATION OF TOBACCO MOSAIC VIRUS IN TOBACCO TISSUE CULTURES

Compound used	Concn (mg/l)	Terminal fresh wt of culture (mg)	Optical density/mg of tissue	T/C*	Av T/C
IBA	100	565	0.117	0.38	
Control	None	245	.306		
IBA	100	835	.144	.39	0.40
Control	None	315	.370		
IBA	100	590	.135	0.44	
Control	None	280	0.303		

* $\frac{T}{C} = \frac{\text{Optical density/mg treated tissue}}{\text{Optical density/mg control tissue}}$.

Twenty compounds were tested. The maximum concentration in each case is expressed as mg/l of solution brought to the pH of the medium (6.0) with 0.1 N sodium hydroxide: hydroxylamine, 1.0; sodium fluoride, 10; cobalt chloride ($CoCl_2 \cdot 6H_20$), 10; zinc chloride, 100; uranyl acetate, 100; ascorbic acid, 100; desoxyribonucleic acid, 2,000; ribonucleic acid, 1,000; colchicine, 10; *p*-hydroxyphenylglycine, 10; D-usnic acid, 0.1; 2,6,diaminopurine, 0.01; terramycin, 100; streptomycin, 1.0; subtilin, 100; caprylic acid, 10; phenylacetic acid, 10; phenylpropionic acid, 100; phenylvaleric acid, 100; indolebutyric acid, 100.

The first 19 substances were found to have no effect on the virus content at the concentrations listed. In most cases the concentration given is close to the maximum that the tissues can tolerate and still exhibit normal growth. Lower concentrations of all compounds were tried, but it was found that the maximal effect, if any, was produced at the highest concentrations that the tissue cultures would tolerate.

Indolebutyric acid, like naphthalenacetic acid, is

[1] It is a pleasure to acknowledge the generous aid and advice given by T. E. Rawlins. The 2,6,diaminopurine was kindly given by R. L. Thompson, Department of Microbiology, Indiana University Medical School, and was supplied to him by G. H. Hitchings, Wellcome Research Laboratories. Terramycin was kindly supplied by Peter Ark. Subtilin was obtained through the courtesy of H. Humfeld, of the USDA Western Regional Research Laboratory. The D-usnic acid was generously supplied by J. C. Lewis, of the USDA Western Regional Research Laboratory. The phenylvaleric acid was synthesized by James Cason, of the University of California Department of Chemistry.

known to be active as a synthetic plant growth hormone (*2*). In two separate series of experiments it was found to be effective in reducing the virus concentration of the tissue cultures. The results of one series at 100 mg/l (Table 1) indicate an average ratio of T/C of 0.40. It was noted that the callus produced in the presence of indolebutyric acid (IBA) at 100 mg/l had a cinnamon-buff (Ridgway) appearance similar to that produced by naphthalenacetic acid at 1.0 mg/l. IBA was not tried at higher concentrations than 100 mg/l, but it was found to be similarly effective at 10 mg/l. At both concentrations it was noted that the terminal weight of the treated cultures was significantly higher than that of the control cultures. This would establish the growth-promoting property of IBA in mosaic-infected tobacco tissue cultures.

It is interesting to note that in contrast to naphthalenacetic acid and IBA, phenylacetic acid at 10 and 1.0 mg/l does not change the amount of virus present, although it is known to possess growth-promoting properties (*2*).

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Manuscript received August 6, 1951.

A New Method for Detecting Penicillinase Production by Staphylococci

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Bondi, Spaulding, Smith, and Dietz (*1*) in 1947 described a method for rapid determination of susceptibility of infecting microorganisms to penicillin and other antibiotics. While using this method to study the incidence of penicillin-resistant staphylococcal infection in Farouk I University Hospital,[2] it has been found that it could also be used as a basis for a test to detect whether the strains studied produced penicillinase. Several other methods have been described for this purpose in recent years (*2–9*).

A blood agar plate that has already been dried in the incubator is uniformly streaked with a small loopful (using a loop 1 mm in diam) of a 24-hr broth culture of a known penicillin-sensitive strain of *Staphylococcus aureus*. With fine-pointed forceps sterilized in an alcohol flame, several sterilized filter paper disks (6.5 mm in diam) are individually dipped into a penicillin solution of 15 u/ml and then put on the streaked plate, at least 20 mm apart (after Bondi *et al.* [*1*]). One of the disks, which is given no further treatment, serves as a control, and its position is marked on the back of the plate. On each of the remaining disks a loopful of a 24-hr broth culture of one of the strains to be tested for penicillinase production is carefully placed, with appropriate identification on the back of the plate. In this way several strains are tested on one plate (usually 4 when using the ordinary 9-cm diam plates). A control disk is left in every plate used.

TABLE 1

Disk	Inhibition zone	Penicillinase production
Control	21 mm	
Strain A	Negative	Positive
Strain B	21 mm	Negative

The results are read after aerobic incubation of the plates at 37° C for 24 hr. Table 1 and Fig. 1 illustrate an experiment including one penicillinase-producing strain and one non-penicillinase-producing strain.

A zone of complete inhibition of growth of the streaked strain measuring over 20 mm in diameter is observed around the control disk. It is also present around the disk saturated with a culture of a non-penicillinase-producing strain. This is due to the inhibiting action of the penicillin, which is left intact in both conditions.

The disk saturated with a culture of a penicillinase-producing strain, on the other hand, is surrounded with very little or no inhibition. The streaked strain usually grows right to the disk, but often the growth is less marked in the immediate vicinity. The tested strain sometimes shows, beyond the rim of the disk, a concentric heavy growth which can easily be differentiated from the comparatively light streaked growth (Fig. 1). Presumably this is the result of destruction or inactivation of the penicillin by the enzyme produced by the tested strain.

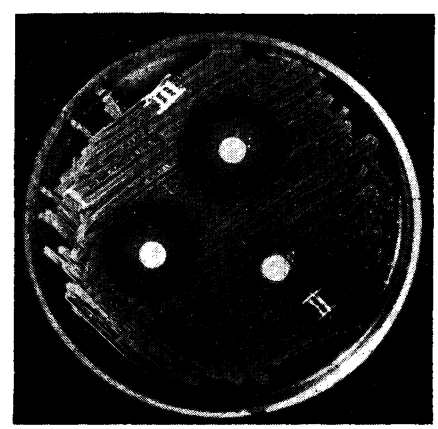

FIG. 1. I, growth inhibition around control disk; II, penicillinase positive test (no growth inhibition); III, penicillinase negative test (growth inhibition as control).

In some experiments, a second control was used by placing a loopful of sterile broth on one disk. But there was no difference between this and the previ-

[1] Thanks are due to M. M. Moussa, who prepared the photograph.

[2] This is the subject of another report.