Paralysis is regularly prevented by brewers' yeast, is cured by a water extract of yeast,[3] and has responded promptly (8–12 hours) to synthetic biotin[4] therapy in seven attacks in four dogs. The biotin was dissolved in physiological saline and administered subcutaneously. The therapeutic dose is approximately 100 gamma per kilo.

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PRELIMINARY NOTE ON THE INACTIVATION OF ANTIBIOTICS

DURING the course of investigations on antibiotic substances of plant origin[1] an antibiotic active against both Gram positive and negative organisms was isolated from *Allium sativum*. During the course of chemical studies of this antibiotic, the reaction with cysteine was investigated. It was found, as is the case with penicillin, that the antibiotic is rapidly inactivated by cysteine.

A number of other antibiotics of thallophyte and spermatophyte origin available in this laboratory were tested in the presence of cysteine. In every case, cysteine gave complete inactivation or marked diminution of antibiotic activity. Gram-positive antibiotc activity is more susceptible to cysteine inactivation than the Gram-negative activity.

The following antibiotics were inactivated: penicillin, citrinin, gliotoxin, clavacin (patulin or claviformin), pyocyanine; the active principles of *Allium sativum, Ranunculus acris* and *R. bulbosus, Erythronium americanum, Asarum reflexum,* Bassica species and *Arctium minus*. The antibiotic principles of *Allium sativum, Erythronium americanum, Asarum reflexum* and *Arctium minus* will be described in greater detail later.

The testing procedure was as follows: Water solutions of each of the antibiotics were divided into two portions. One portion was used as a control and to the other was added solid sodium biocarbonate adequate to maintain a pH of approximately 7 and cysteine hydrochloride. The solutions were allowed to stand for 30 to 60 minutes, then tested for antibiotic activity against *Staphylococcus aureus* and *Bacillus paratyphosus* A by the Oxford cup method.

This antagonistic effect of cysteine was similarly displayed by cysteine esters (methyl and ethyl), but not by S-methyl cysteine, methionine, alanine or serine. Other –SH compounds such as glutathione and thioglycollic acid had either no effect or a much weaker action.

This inactivation is especially unusual in the light of the widely different chemical types of antibiotics involved. The nature of the reaction of cysteine with some of the antibiotics is known; others are being investigated. In the known instances, cysteine reacts irreversibly with the antibiotics. However, this may not be true of all the antibiotics. Quantitative relationships of the antagonistic activity of cysteine and related compounds are being studied and will be reported later. It is suggested that possibly the fundamental mode of action of certain classes of antibiotics involves their ability to interfere with the normal function of sulfhydryl groups in bacterial metabolism. This has already been observed in some specific instances as by Fildes,[2] in his investigation of the mode of action of mercury as an antibacterial agent; by Eagle,[3] who observed that the anti-spirochetal action of arsphenamine could be counteracted by cysteine; and by Atkinson[4] in her work with penicidin.

That the sulfhydryl group is essential to cell proliferation has been demonstrated and discussed by Hammett.[5,6]

This note is published with the desire that other investigators having access to different antibiotics will test such substances for inactivation by cysteine.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

ENHANCED PRODUCTION OF PENICILLIN IN FLUID MEDIUM CONTAINING CELLOPHANE[1,2]

THE observation was made that young colonies of *Penicillium notatum* in fluid medium show a tendency to develop nearer the side walls of the vessel than

[3] Kindly supplied by Dr. C. N. Frey, of the Fleischmann Laboratories, Standard Brands, Inc., New York, N. Y.

[4] Kindly supplied by Dr. D. F. Robertson, of the Merck Company, Inc., Rahway, N. J.

[1] This work was begun before the appearance of the article by Osborn, *Brit. Jour. Exper. Path.*, 24: 227, 1943, and as a result, many of the plants tested have been duplicated.

[1] From the Laboratories of Bacteriology, The Mount Sinai Hospital, New York, N. Y.

[2] The author wishes to acknowledge thankfully the accurate and capable assistance of Miss Alice Fisher.

[2] Fildes, *Brit. Jour. Exper. Path.*, 21: 67, 1940.

[3] Eagle, *Jour. Pharmacol.*, 66: 436, 1939.

[4] Atkinson, Stanley, *Australian Jour. Exper. Biol. Med. Sci.*, (4)21: 249, 255, 1943.

[5] Hammett, Hammett, *Protoplasma*, 15: 59, 1932.

[6] Hammett, Chapman, *Growth*, 2: 223, 297, 1938.

towards the center of the surface of the fluid. The purpose of this investigation was to determine whether the introduction of some supporting material into the fluid medium could enhance the growth of the Penicillium and thereby increase the production of penicillin. In order to avoid a possible loss of penicillin on the solid surface, Cellophane No. 600 permitting rapid diffusion of the substance was selected for the work.[3] Cellophane in the form of an open bowl was inserted into "500 cc" Erlenmeyer flasks and filled with the fluid medium. The diameter, height and number of side folds of the bowls were varied in order to obtain different ratios of the surface of Cellophane to the total volume of the medium. The medium manufactured by Eimer and Amend for cultivation of *Penicillium notatum* was employed. The strains of the mold were maintained by serial transplants on agar medium. The titers of penicillin recorded indicate the highest dilution of the test material giving complete inhibition of growth of a standard suspension of stain H of *Staphylococcus aureus*.[4,5]

In the first group of experiments the submerged penicillin producer, strain NRRL 832 was used for inoculation. In the flask with Cellophane, on the 3rd day following inoculation there appeared numerous isolated colonies on the side folds and upper borders of the bowl. On the 6th day solid sheets of the mold showing yellow green pigment covered the upper third of the bag extending over the entire surface of the fluid. In the control flask containing the same amount of medium, growth was poor for a period of 5 to 6 days due to the unfavorable $\frac{\text{surface area}}{\text{total volume}}$ ratio (*i.e.*, 0.0775).[6] Although on the 9th day a solid pellicle formed, the growth remained considerably scantier than in the experimental flask for an additional period of 8 days. As may be seen from Fig. 1, the production of penicillin began in the flask with Cellophane on the 5th day following inoculation, namely, 3 days earlier than in the control. On the 8th day, when penicillin made its appearance in the control, the concentration of the drug in the experimental flask was already 30 times greater. During the following days the concentration rapidly increased in the control, remaining, however, markedly lower than in the flask with Cellophane.

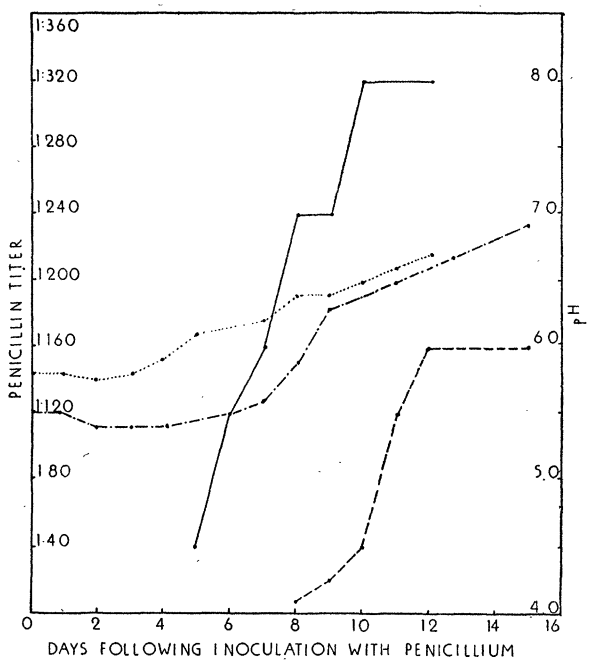

FIG. 1. *Inoculum:* suspension of spores in H_2O from a 4-day-old culture of strain NRRL 832, submerged penicillin producer. ———: daily determinations of penicillin in fluid culture containing Cellophane. *Medium:* 400 cc $\frac{\text{surface area}}{\text{total volume}}$ ratio 0.0775. — — —: daily determinations in a fluid culture without Cellophane. *Medium:* 400 cc $\frac{\text{surface area}}{\text{total volume}}$ ratio approximately the same, as above (the amount of Cellophane used above produces negligible displacement). . . . . : daily pH changes in Cellophone containing culture. — . —: daily pH changes in culture without Cellophane.

The remaining studies were carried out in order to determine the advantages of this method under conditions unfavorable for production of penicillin.

In the following experiment the submerged penicillin producer was used again. The inoculum was obtained, however, from a nine-day-old agar culture showing a white cottony growth. A culture incubated longer than 3 to 4 days and of the above appearance may be a weak penicillin producer.[6] As may be seen from Fig. 2, in the flask without Cellophane, penicillin 1:10 appeared on the 9th day and disappeared altogether on the 11th day following inoculation. In contrast, in the Cellophane containing flask penicillin appeared on the 6th day and reached the titer 1:80

[3] Cellophane was previously shown to be capable of increasing production of toxic substances of certain microorganisms, *i.e.*, *Staphylococcus* (L. Birch-Hirschfeld, *Z. Immunitätsforsch.*, 81: 260, 1933–34; Douglas McLean, *Jour. Path. and Bact.*, 44: 47, 1937), *E. typhosa, E. coli* and meningococcus, S. A. Morrell and Gregory Shwartzman, *Jour. Exp. Med.*, 67: 13, 1938.

[4] The author is thankful to Dr. Robert D. Coghill, of the U. S. Department of Agriculture, for supplying strains NRRL 832 and NRRL 1249.B21, and to Dr. Jackson W. Foster, of the Merck Research Laboratories, for the strain H of *Staphylococcus aureus*.

[5] C. M. McKee, G. Rake and A. E. O. Menzel, *Jour. Immun., Virus Res., and Exp. Chemoth.*, 48: 259, 1944.

[6] I. W. Foster, H. B. Woodruff and L. E. McDaniel, *Jour. Bact.*, 46: 421, 1943.

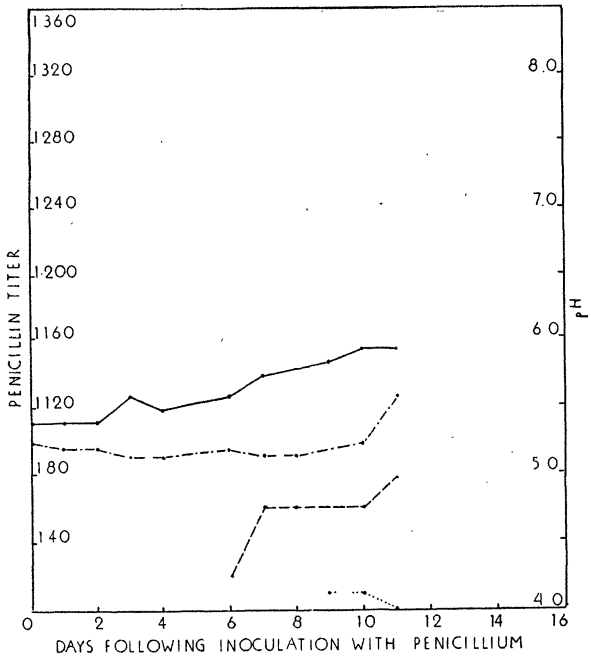

FIG. 2. *Inoculum:* suspension of spores in H_2O from a 9-day-old agar culture with white cottony growth of strain NRRL 832, submerged penicillin producer. — — —: daily determinations of penicillin in a fluid culture containing Cellophane. *Medium:* 400 cc $\frac{\text{surface ratio}}{\text{total volume}}$ ratio 0.0775. . . . . : daily determinations of penicillin in a fluid culture without Cellophane. *Amount of medium:* the same; $\frac{\text{surface area}}{\text{total volume}}$ ratio: approximately the same as above. ———: daily pH changes in the Cellophane containing culture. — . —: daily pH changes in culture without Cellophane.

on the 11th day. It may be also noted that the control flask yielded on the 6th day a moderate growth which became fairly abundant during the following 2 days. The experimental flask showed numerous colonies on the 3rd day and a heavy growth on the 5th day.

In the experiment described below strain NRRL 1249.B21, the surface penicillin producer was used. The flasks each contained 475 cc of the medium. The $\frac{\text{surface area}}{\text{total volume}}$ ratio of 0.0315 was extremely unfavorable for production of penicillin. Observations were made for 19 days. In the flask without Cellophane the highest penicillin titer obtained was 1:10. In the flask with Cellophane penicillin appeared on the 12th day. The highest titer 1:120 was reached on the 15th day, remaining approximately the same for the following 4 days. The growth was decidedly better in the experimental than in the control flask.

The changes in the H-ion concentration within the first 15 days of cultivation are given in Figs. 1 and 2. During this period cultures with Cellophane maintained consistently a somewhat higher pH level than the controls. A more significant effect of the Cellophane upon the pH was observed at later stages of growth not recorded in the figures. Thus, in control flasks having the favorable $\frac{\text{surface area}}{\text{total volume}}$ ratio 0.313 the pH usually rose sharply from 6.6–6.8 on the 14th day to 7.8–8.2 on the 19th day. Consistently during the same period the pH did not exceed 6.8 in the flasks with Cellophane.

SUMMARY

Enhanced production of penicillin is made possible by addition of Cellophane to fluid media. With Cellophane bags of suitable surface, the growth of the submerged and surface penicillin producing strains of *Penicillium notatum* is significantly faster and more abundant; penicillin makes its appearance earlier and reaches higher concentration in larger total volumes than in control cultures without Cellophane.[7] Thus the gain with the method described is both in the rate of production as well as in the total yield of penicillin. The enhancement also occurs under conditions unfavorable for development of penicillin, namely, (a) with degenerated cultures of the mold; and (b) when the surface penicillin producing strain is grown in cultures with an unfavorably small $\frac{\text{surface area}}{\text{total volume}}$ ratio (0.0315). There is also noted a markedly stabilizing effect of Cellophane upon the H-ion concentration of abundantly growing cultures during active production of penicillin. The stabilization is of significance, since the sharp rise in pH usually occurring in Penicillium cultures tends to destroy rapidly the penicillin.

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[7] In subsequent experiments in which large numbers of Cellophane strips instead of the bags were used, there was obtained markedly greater and faster production of penicillin than described in this report.

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