The observation that ether in sub-lethal concentrations had no effect on oxygen uptake confirms the results of Jowett and Quastel. With regard to the hypothesis that ether acts as an anesthetic agent because of an inhibitory influence on oxidations in the central nervous system, the evidence from the rat, guinea pig and cat is considered strongly negative.

With even the lowest concentrations of ether there was a small but significant increase in lactic acid output. This appeared to be a unitary phenomenon, for, as the concentration of ether was raised, the extra lactic acid did not change appreciably until a definite inhibition of oxygen uptake occurred. The increase in lactic acid output by low concentrations of ether was small compared with that produced by cation imbalance[8,9] and by various dyes.[10] Since it appears with concentrations found in blood during light as well as deep anesthesia, the effect may be of interest in theoretical considerations of the mechanism of action of ether in anesthesia.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

LARGE-SCALE PRODUCTION OF PENICILLIN[1]

LARGE-SCALE production of penicillin is hampered by the necessity of: (1) providing a surface area exposed to the air of approximately 500 sq cm per liter of culture medium and of (2) growing the organism, *Penicillium notatum*, for 5 to 8 days under such conditions. Consequently, the production of large quantities of penicillin requires the use of a large number of culture vessels over a period of time. The idea occurred to the author that the fungus might grow well and produce penicillin continuously in a constant flow of medium trickling over a column of wood shavings in a setup similar to that commonly employed in the "quick" or "generator" process for the production of vinegar from wine or cider by the acetic acid bacteria. In the acetic acid generators a relatively large surface area per unit of volume is exposed to freely circulating air and medium with the result that the acid is produced rapidly and in quantity. The following experiments were designed on a laboratory scale to determine the feasibility of producing penicillin under conditions similar to those prevailing in vinegar generators.

The experimental apparatus consists of a glass tube 4 feet long and 2 inches in diameter containing a 3-foot column of wood shavings supported on a 4-inch layer of larger pieces of wood. In the original apparatus a stream of air was circulated upward through the column, but as the shavings were rather fine, the organism tended in time to block the upward passage of air as well as the downward flow of medium. It was found, however, that this can be prevented by the use of large shavings and a looser packing of the column. A downward flow of air also proved satisfactory.

In the present arrangement, the top of the tube is closed with a two-holed rubber stopper carrying: (1) a glass tube for the entrance of air filtered through a column of sterilized cotton and (2) a thick-walled capillary tube drawn out to a fine point for the delivery of the culture medium. From a large flask supported above the column, the fresh medium flows through a siphon connected to the capillary tube, which delivers it drop by drop on top of the column of wood shavings. The rate of flow is controlled by the diameter of the capillary tip and also by a pinchcock on the rubber tubing connecting the capillary tube to the reservoir of medium. The bottom of the glass column is closed with a one-holed rubber stopper fitted with a glass exit tube which allows the escape of air as well as of medium. This exit tube is connected by means of a piece of rubber tubing to a collecting flask equipped with a two-holed rubber stopper through which passes: (1) a tube for the liquid and air to enter the flask and (2) an air exit tube connected to the suction line. Medium, rubber tubing and collecting flasks are sterilized in the autoclave and fitted aseptically to the appropriate tubes of the culture apparatus. The latter, packed with wood shavings and with stoppers in place, is sterilized by allowing steam to flow through it for two hours.

Three culture columns as described above are now in operation. One of these columns consists of two such tubes in series and is used to determine the effect of doubling the length of the culture column. All studies were carried out in a 26° C. constant temperature room.

After the columns are sterilized and the medium reservoir and collecting vessels attached thereto, the shavings are well wetted with culture medium and

[1] Aided in part by the Fluid Research Fund, Stanford University School of Medicine.

[8] C. A. Ashford and K. C. Dixon, *Biochem. Jour.*, 29: 157, 1935.

[9] F. Dickens and G. D. Greville, *Biochem. Jour.*, 29: 1468, 1935.

[10] F. Dickens, *Biochem. Jour.*, 30: 1233, 1936.

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inoculated with a spore suspension of *P. notatum*.[2] Czapek-Dox medium (Florey et al.[3]) containing 4 per cent. glucose and 0.1 per cent. Difco yeast extract was employed. A continuous flow of the medium is started 24 hours after the inoculation and growth of the fungus usually becomes well established by the 5th day. In one of the columns the medium was allowed to collect and was drained off daily instead of continuously. In this case there was a marked inhibition of growth, even with vigorous aeration. This column was, therefore, converted after several days to continuous flow arrangement. Ordinary surface cultures of *P. notatum* in the same medium served as controls for comparative purposes on the amount of penicillin produced.

The rate of flow of medium has been varied from 400 to 1,000 ml daily and the sugar content from 1 to 4 per cent. with little influence on penicillin titer. After growth is well established, yeast extract does not appear to be essential for penicillin production, but a 4 per cent. concentration of sugar may be of some value inasmuch as all but a fraction of a mgm of sugar per ml is utilized during the flow of the medium through the growth column.

One column has been in satisfactory operation and free from bacterial contamination for 15 days. The rate of flow of the medium through this column has varied from 600 to 800 ml daily and the effluent has had a pH of 7.0–7.3. Serial dilutions in broth of the penicillin-containing effluent were inoculated with *Staphylococcus aureus*. Growth in the various dilutions following 20 hours incubation at 37° C. was compared with that of a control culture of *S. aureus* and with serial dilutions tests on ordinary surface cultures of *P. notatum*. Typical results are presented in Table 1.

TABLE 1

PENICILLIN TITER IN CONSTANT FLOW AND ORDINARY CULTURES OF PENICILLIUM NOTATUM

Fluid from	Age of culture in days	Dilutions				
		1–10	1–20	1–40	1–80	1–160
Constant flow apparatus	5	–	–	–	‡	+
	10	–	–	–	–	+
	15	–	–	–	‡	+
Ordinary culture, maximum titer	6	–	–	–	–	+

– no growth, ‡ slight growth, + full growth of *S. aureus*.

It is apparent that the penicillin titer of the effluent from the constant flow apparatus approaches that observed with the fluid from ordinary surface cultures of *P. notatum*. Only one strain of *P. notatum* and one medium has been thoroughly tested thus far. Higher titers might well be obtained with other strains of the fungus or in other media. In incomplete studies the substitution of corn steep liquor in place of yeast extract more than doubled the amounts of penicillin produced. The constant flow method appears to have the advantage that once growth of the organism is well established, penicillin is produced continuously and a large volume of penicillin-containing liquid can be obtained with a minimum of equipment in a short period of time.

Doubling the length of column by connecting two tubes in series appears to increase the penicillin concentration in the effluent to a slight extent. Some difficulty was experienced in obtaining satisfactory growth in the second column due to the rapid depletion of the culture medium. Therefore, the influence of an increased rate of flow of a more concentrated medium was tested, but bacterial contamination was encountered before entirely conclusive results could be obtained.

Further studies on factors influencing the production of penicillin in a flow of medium continuously trickling in a thin stream over the fungus growing on a column of shavings are in progress. The prevention of bacterial contamination appears to be the most difficult, but not insurmountable, obstacle to the production of penicillin on a large scale. The apparatus employed in this study may also prove satisfactory for the growth of other aerobic organisms producing antibiotic agents. The results of these preliminary studies suggest that a fairly high penicillin titer can be obtained under the conditions described and that penicillin may be produced rapidly and in large quantities in acetic acid generators.

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[2] The culture of *P. notatum*, from the original Fleming strain, was provided through the courtesy of the Cutter Laboratories, Berkeley, Calif.

[3] E. P. Abraham, E. Chain, C. M. Fletcher, H. D. Gardner, N. G. Wheatley, M. A. Jennings and H. W. Florey, *Lancet*, 241: 177–88, 1941.

BOOKS RECEIVED

Essays in Biology. In Honor of Herbert M. Evans. Illustrated. Pp. xxxi + 684. University of California Press. $10.00.

DUBLIN, LOUIS I. *A Family of Thirty Million.* Illustrated. Pp. xvi + 496. Metropolitan Life Insurance Company.

LUCK, JAMES MURRAY. *Annual Review of Biochemistry.* Illustrated. Pp. ix + 704. Annual Reviews, Inc. $5.00.

MURRAY, RAYMOND W. *Man's Unknown Ancestors.* Illustrated. Pp. xiii + 384. The Bruce Publishing Company. $3.50.

RAVEN, C. E. *Science, Religion, and the Future.* Pp. x + 125. The Macmillan Company. $2.00.

SMITH, H. T. U. *Aerial Photographs and Their Applications.* Illustrated. Pp. xiv + 372. D. Appleton-Century. $3.75.

WHITE, MARSH W. *Practical Physics.* Illustrated. Pp. x + 365. McGraw-Hill. $2.50.