and more or less equal distribution through all the cutaneous tissues. Details of these studies on different types of skin-penetrating vehicles, as well as further discussion of the histochemical method itself, will be published shortly.[4]

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## ETHER AND METABOLISM IN THE CEREBRAL CORTEX[1]

JOWETT and Quastel[2] have concluded that anesthetic concentrations of ether do not inhibit by more than the experimental error the respiration of the gray matter of the cerebral cortex of the rat or guinea pig. That another metabolic process might be affected by ether was suggested by an observation made on slices of cerebral cortex from cats under ether anesthesia.[3] Measured in phosphate medium, the lactic acid output was greater in slices from these animals than from controls. The present report is concerned with the effect of ether applied *in vitro* on the oxygen uptake and lactic acid output by cat cerebral cortex slices.

The procedure was as follows. First, the oxygen uptake in Ringer-phosphate-glucose medium in six vessels with oxygen in the gas phase, was observed for 45 minutes. Second, a quantity of medium with or without ethyl ether dissolved in concentrations up to 40 mg per cc was added to each vessel through the venting plug by means of a syringe fitted with a long needle, to make a final concentration of up to 9 mg per cc. Third, oxygen uptake was followed for from two to six hours more. The details of the manometric technique were as reported elsewhere[4] except that the shaker speed was 80 cycles per minute. Fourth, metabolism was stopped by tipping in HCl solution from the side arm, and the concentration of ether in the medium was estimated manometrically with a temperature change of 1.5° by the method of Jowett.[5] Fifth, the lactic acid that accumulated in each vessel was determined by the method of Barker and Summerson.[6] In the control vessels the lactic acid content was higher at the end of the experiment than at the end of the first 45 minutes.

Data for 15 cats and the standard errors of the means are given in Table 1, in four groups according to ether concentration. In the first group the concentration was such that it would not produce unconsciousness if present in the blood.[7] In the second group the ether concentration would produce full anesthesia. The higher concentrations would be lethal. Oxygen uptake is recorded as a percentage of the initial rate for each vessel corrected for any change in rate with time in the control vessel, to which an addition of medium without ether had been made. Lactic acid is reported as the difference between the amounts in the vessel containing ether and in the control vessel, divided by the number of hours of exposure to ether.

TABLE 1

EFFECT OF ETHER ON THE OXYGEN UPTAKE AND LACTIC ACID OUTPUT BY SLICES OF CEREBRAL CORTEX OF THE CAT IN RINGER-PHOSPHATE-GLUCOSE MEDIUM

| Ether concentration | | | Oxygen uptake | | | | | | Extra lactic acid output | |
|---|---|---|---|---|---|---|---|---|---|---|
| Range | Mean | | 1st hour | | 2nd hour | | 3rd hour | | | |
| mg/cc | mg/cc | M × 1000 | c.*/o. | Per cent. of control | c.*/o. | Per cent. of control | c.*/o. | Per cent. of control | c.*/o. | mg/g/hr |
| 0.025–0.4 | 0.2 | 2.7 | 6/14 | 97 ± 1.85 | 6/14 | 95 ± 2.09 | 3/3 | 90 ± 6.46 | 6/14 | 4 ± 0.93 |
| 0.7 –1.6 | 1.3 | 17.5 | 9/15 | 100 ± 1.34 | 9/15 | 101 ± 1.29 | 5/11 | 100 ± 2.00 | 9/15 | 3 ± 1.19 |
| 2.0 –5.3 | 3.7 | 50 | 7/14 | 103 ± 1.73 | 7/14 | 106 ± 1.89 | 5/8 | 111 ± 3.63 | 7/14 | 5 ± 0.62 |
| 6.7 –9.6 | 8.4 | 113 | 6/9 | 107 ± 6.1 | 6/9 | 86 ± 8.8 | 6/9 | 43 ± 6.9 | 6/9 | 14 ± 2.75 |

* Number of cats/number of vessels.

[4] MacKee, Herrmann, Baer and Sulzberger, *Jour. Clin. and Lab. Med.*, 1943 (in press).

[1] Aided by a grant from the Milton Fund of Harvard University to Henry K. Beecher.

[2] M. Jowett and J. H. Quastel, *Biochem. Jour.*, 31: 1101, 1937.

[3] H. K. Beecher and F. N. Craig, *Jour. Biol. Chem.*, 148: 383, 1943.

[4] F. N. Craig and H. K. Beecher, *Jour. Neurophysiol.*, 6: 135, 1943.

[5] M. Jowett, *Biochem. Jour.*, 31: 1097, 1937.

[6] S. B. Barker and W. H. Summerson, *Jour. Biol. Chem.*, 138: 535, 1941.

[7] H. W. Haggard, *Jour. Biol. Chem.*, 59: 783, 1924.

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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