ANESTHESIA. XLV: EFFECT OF ETHYL ETHER ON OXIDATIVE-PHOSPHORYLATION IN THE BRAIN

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For more than a century, various theories of narcosis have been proposed. None has proved to be completely satisfactory in explaining the phenomenon of anesthesia. Of special interest are the observations of Quastel and his associates (1, 2), who showed that anesthetics inhibit the oxygen consumption of brain tissues in vitro. That this effect is of primary importance in producing the state of anesthesia has been questioned by several investigators. It has been pointed out that only a few agents produce this effect in concentrations that produce narcosis in animals. It also has been pointed out that the depression in oxygen consumption may be only a result of the effect rather than a cause (3).

In reviewing the subject of narcosis, McElroy (4) came to the conclusion that many of the inhibitors which affect cellular activity do so by interfering with either the utilization or the formation of energy-rich phosphorylated intermediaries. The possibility of interference with utilization was investigated by Lu and Krantz (5). These workers found no significant influence on ATPase activity by various anesthetics and hypnotics in therapeutic concentrations. However, Brody and Bain (6) investigated the effects of anesthetics and hypnotics on the generation of high energy phosphate, with the result that a significant depression in phosphate was found to occur in vitro under the influence of barbiturates in concentrations which had only a slight effect on oxygen consumption. The uncoupling effect was qualitatively similar to that produced by 2,4-dinitrophenol (7). However, Levy and Featherstone (8) found that the gaseous anesthetics xenon and nitrous oxide have no effect on either oxygen or phosphate uptake by guinea pig mitochondrial preparations at anesthetic concentrations. In view of these findings concerning the action of volatile anesthetics, the authors considered it worthwhile to investigate the action of diethyl ether as an uncoupler of oxidative-phosphorylation.

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Methods

The mitochondrial homogenates were prepared from the whole brains of male white rats weighing 150 to 200 Gm. The rats were killed by a blow on the back of the neck, and decapitated. The brains were removed quickly and placed in 9 volumes of 0.25 molar sucrose maintained at zero in an ice bath. The brains were broken up in a glass tissue crusher, transferred to an Arthur H. Thomas model No. B-711 homogenizer, and reduced to a homogeneous suspension by hand. The suspension was then fractionated in a centrifuge at zero in 3 stages. The first centrifugation was carried out at 305×g for 5 minutes. The supernatant fluid was pipetted off and recentrifuged again under the same conditions. The supernatant fluid was then recentrifuged at 2310×g for 25 minutes. This last centrifugation brought down a small amount of thick white material which was the active fraction used in the experimental work. Although the active fraction was only a crude mitochondrial preparation, the centrifugation process removed a sufficient amount of interfering enzymic material to allow the preparation to carry out a significant degree of phosphorylation. The crude preparation was diluted 3 to 1 with cold 0.25 molar sucrose before adding to the Warburg vessels.

The hexokinase preparation employed by Cross et al. (9) was used as the high energy phosphate trapping system.

The respiration studies were done in the usual manner following the technique of Umbreit and Burris (10). The Warburg vessels were of standard 15 ml. capacity with a single side arm holding a gas exchange stem. Respiration was stopped by tipping in the side arm containing 0.2 ml. of 50 per cent trichloroacetic acid. Phosphate determinations were made according to the method of Sumner (11), and the P/O calculations were based on the extrapolation method of Cross et al. (9).

The method used in etherizing the Warburg vessels and the method used in estimating the ether concentration within the liquid phase of the vessels were those devised by Jowett and Quastel (2) and by Jowett (12).

Duplicate control vessels were run with each experiment in order that the experimental vessels could be compared with controls from the same mitochondrial preparation. If the values of the duplicate vessels were too far apart, neither value was used, or if the control vessels gave an average P/O ratio of less than 2.0, the experiments in most cases were discarded.

Results and Discussion

The effect of diethyl ether on the phosphate uptake of the rat brain mitochondrial preparation is shown in figure 1. The values corresponding to the points on the graph are given in table 1. Each of the
values represents the average of duplicate vessels. The concentration range goes well beyond that of the surgical anesthetic concentration. This generally is considered to be between 100 and 150 mg. per cent. It readily can be seen that the uncoupling effect within this range corresponds to about 20 per cent depression. Also it is apparent that the depression of phosphate uptake becomes more severe with increasing ether levels.

![Graph](image)

**Fig. 1.** Each flask contains: $2 \times 10^{-3}$ M tri-hydroxy methyl aminomethane buffer pH 7.4, $1 \times 10^{-2}$ M KPO$_4$ buffer pH 7.4, $2 \times 10^{-2}$ M glucose, $2 \times 10^{-4}$ M Na pyruvate, $8 \times 10^{-3}$ M MgCl$_2$, $2 \times 10^{-3}$ M K fumarate, $1.5 \times 10^{-3}$ M NaF, $5 \times 10^{-4}$ M ATP, 0.2 ml. of hexokinase, 1.0 ml. of mitochondrial preparation, 0.2 ml. of 2N KOH in center well, 0.2 ml. of 50 per cent TCA in side arm, total volume 3.0 ml. Temperature of bath 25°C. Readings taken every 5 minutes for 25 minutes.

The effect on oxygen uptake as such did not follow the same depression curve as did the phosphate uptake, but rather, it followed a random variation along the control level with some indication that a stimulation in oxygen uptake had occurred.

The possibility existed that the yeast hexokinase trapping system was being inhibited rather than the high energy phosphate generation by the mitochondria. This possibility was eliminated by assaying for the phosphorylating ability of the enzyme under various ether concentrations. Results showed no significant changes in this activity.

The significant degree of uncoupling found to occur at concentrations equivalent to surgical anesthesia may well indicate that the resultant decrease in high energy phosphate generation is one of the
primary causes of the state of anesthesia produced by diethyl ether. This would agree with the hypothesis proposed by McElroy (4) for the action of narcotics and extended by Brody and Bain (6) to explain the anesthetic action of certain barbituric acid derivatives. This does

<table>
<thead>
<tr>
<th>Concentration of Ethyl Ether</th>
<th>F/O Control Vessels</th>
<th>F/O Etherized Vessels</th>
<th>Per Cent Depression</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.006 Molar</td>
<td>2.25</td>
<td>2.06</td>
<td>8.5</td>
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<td>0.010</td>
<td>2.49</td>
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<td>40.5</td>
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<tr>
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<td>1.88</td>
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<td>34.0</td>
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</table>

not agree with the results found by Levy and Featherstone for the action of nitrous oxide and xenon. However, it may be that the concentration of gases used in their studies did not correspond to a plane of anesthesia in which a significant uncoupling effect would be apparent.

**SUMMARY**

The effect of diethyl ether on the oxidative-phosphorylation of rat brain mitochondrial preparations was studied. An uncoupling effect of approximately 20 per cent was found to occur at anesthetic concentrations.

It is proposed that these data further substantiate the hypothesis that one of the means by which anesthetics exert their action is by the uncoupling of oxidative-phosphorylation.

**REFERENCES**