ACTION OF ETHER AND ETHER ANESTHESIA ON BRAIN OXIDATIONS

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The influence of narcotic agents of different chemical types on the aerobic oxidative processes of central nervous system tissue has been investigated and a definite inhibitory effect found for the barbituric acid (1), morphine (2), urethane and other (3) chemically defined groups. The influence of inhalation anesthetics on the respiration of brain tissue has not been unequivocally determined partly because of the difficulties inherent in handling these volatile agents by the various manometric techniques available.

Quastel and Wheatley (4) have reported marked decreases in oxygen uptake of surviving brain tissue after *in vitro* treatment with relatively high concentrations of ether. Holmes and Bülow (5) failed to obtain any significant decrease in oxidation by treating brain tissue with concentrations of ether more nearly resembling those effective in the intact animal. Both studies were carried out with the direct method in which the Barcroft apparatus was used; small variations in the partial pressure of ether contained in the manometer vessels may modify the apparent oxygen uptake of the test tissue tremendously. Comparison of results with different volatile agents is as difficult as absolute estimation since the fat solubility of these different compounds varies widely and is an important source of error (6, 7).

In the present experiments the indirect method of Warburg (8, 9), based on the difference in solubility of carbon dioxide and oxygen in aqueous media, was used to investigate the effects of ether on oxidations of the central nervous system in two ways: (a) the effect of varying concentrations of ether on surviving brain tissue and (b) the oxygen consumption of brain tissue of rats anesthetized with ether for a prolonged period before the experiment. The single advantage of using the Warburg apparatus is that if the indirect method is used the tissue may be kept in a medium in equilibrium with an atmosphere of oxygen containing 5% carbon dioxide throughout the experiment. The sensitivity to variation in the partial pressure of ether caused either by liberation or removal of ether by the fatty brain tissue is as great as with direct manometric methods and again changes in pressure cannot be considered entirely due to changes in tension of oxygen or carbon dioxide.

Since the output of carbon dioxide is determined simultaneously with oxygen uptake, however, the disturbing effect of variations in the partial pressure of a foreign gas such as ether vapor may be

detected more conveniently than by direct methods, by examining the value obtained for apparent excess carbon dioxide produced if a respiratory quotient of 1.0 is assumed. This quantity, $Q_G^{O_2}$, expressed as mm.^{3} of excess CO_2 per mg. dry weight of tissue per hour, represents normally the amount of carbon dioxide liberated by aerobic glycolysis by the action of the resultant lactic acid on the bicarbonate media, and is generally about 2-3 mm.^{3} Variations caused by differences in the partial pressure of a foreign gas must be below this magnitude to escape observation.

Experimental

Effect of ether added to surviving brain tissue. A series of normal tissues obtained from brains of white rats were examined in the Warburg apparatus at 37° preliminary to the ether experiments. It was found that tissue slices gave less consistent results than finely minced whole brain, and that slightly higher and more consistent results were obtained using the special medium of Krebs and Henseleit (10) than with Warburg's solution; accordingly all experiments reported here were carried out on minced whole brain tissue and with a bicarbonate medium more closely resembling the electrolyte equilibrium in blood serum than does Warburg's solution. The stimulating effects of 0.2% glucose, succinic acid and lactic acid were noted with tissue allowed to autoxidize for from two to five hours.

Results obtained by adding ether to brain tissue were inconclusive because of the difficulty in establishing a permanent equilibrium between the gas, liquid, and emulsified fat phases present. Equilibration by means of passing a slow stream of oxygen containing 5% carbon dioxide and saturated with ether vapor at 30° was unsuccessful even when continued for as long as 60 minutes; values for the apparent $-Q_{O_2}$ (mm.^{3} of oxygen consumed per mg. dry weight of tissue per hour) obtained after this treatment in seven cases varied from 7.3 to 74.3, with an average of 39.9, while the pre-treatment oxygen consumption of the seven tissues varied from 9.1 to 11.95 mm.^{3} per hour for two to three hours before treatment. Better equilibration was obtained in experiments in which freshly excised minced brain tissue was allowed to soak in the manometer vessels, in media to which definite amounts of ether had been added, for one hour while gas saturated with ether vapor passed through the apparatus. Tissues so treated in media containing 150 mg.% of ether and equilibrated for an hour with gas saturated with ether vapor at 30° showed apparent complete inhibition of oxidation ($Q_{O_2} = -0.25$) while similar tissue treated in media containing 120 mg.% of ether and equilibrated for a like period with gas saturated at 20° showed but slight inhibition when compared with untreated tissue examined simultaneously. An examination of the $Q_G^{O_2}$, however, showed that in those experiments in which the tissues were treated with ether in the gas phase, the tissue was still absorbing ether rapidly even after prolonged periods of shaking in an attempt to reach an equilibrium; while in the ex-

periments in which ether was present initially in both the liquid and gas phases, the tissue apparently liberated small amounts of ether possibly as a latent result of the small temperature rise occurring after passage of gas was stopped.

Effect of ether anesthesia on brain oxidations. Anesthesia was produced in individual rats by subjecting them to an atmosphere of oxygen containing 2.5 mM/l. of ether, in a closed 18 liter chamber, for one hour. At this concentration of ether vapor, induction is rapid and the animals remain in a state of deep surgical anesthesia throughout the period of exposure. Control rats were treated simultaneously with pure oxygen, although it was found that no difference in oxygen consumption of brain tissue resulted from so increasing the oxygen content of respired air for one hour. After 60 minutes of treatment the two animals were quickly killed by a sharp blow, the brain removed and rapidly minced and two 15-50 mg aliquots in each case rapidly weighed to the nearest 0.5 mg. The tissues were immediately placed in manometer vessels containing 3.0 and 8.0 cc. of Krebs and Henseleit's fluid at 37°. Each aliquot of tissue was carefully examined to insure fairly even dispersion in the fluid, since it was found in previous experiments that the presence of small aggregates of tissue tends to decrease the apparent Q_{O_2} by preventing adequate access to the dissolved oxygen. The entire process from the point of removal of the tissue from the rat through to the commencement of equilibration with 5% carbon dioxide required less time than was taken by a control group of similarly anesthetized rats to recover even to the point of light anesthesia.

Twenty-four animals were so treated in twelve individual experiments. Of these, tissues from seven anesthetized and seven oxygen-treated control rats were used for experiments in which glucose was added to the media to make a final concentration of 0.2%, single animals of each group being run concomitantly in pairs. Comparison of results so obtained showed that in six of the seven cases tissues from anesthetized rats showed substantially the same oxygen consumption per hour for two to three hours as was found for the control tissues run at the same time. In five cases the test tissues gave slightly higher

TABLE 1

Oxygen consumption of brain from untreated and ether anesthetized rats, in media containing 0.2% glucose

		Time in Minutes				
	Treatment	30	60	90	120	150
$-Q_{O_2}$	Controls	10.50	10.35	10.60	10.70	10.60
$-Q_{O_2}$	Ether	10.75	10.60	10.90	11.40	11.20

values than the controls, and in one case slightly less during the first hour. In the remaining experiment a marked apparent inhibition of respiration of the etherized tissue was present during the first hour,

but this could be accounted for by a considerable loss of ether from the fatty brain tissue caused by a slight rise in temperature as noted by a rise in the thermobarometer tube. Therefore experiments in which a correction of more than 5 mm. of Brodie's fluid was necessary as indicated by the thermobarometer manometer were discarded. If the temperature of the bath changes, the thermobarometer cannot be used as a complete control in experiments in which the other tubes hold fatty tissue containing even slight amounts of volatile agents, but it is obviously indispensable as a sensitive indicator as to whether or not such changes have occurred.

Results from a typical experiment in this group are given in Table 1. No inhibition of normal respiration is apparent under the conditions studied. The very close agreement of absolute values for respiration of control and test tissues is fortuitous.

TABLE 2

Oxygen consumption of surviving brain tissue from anesthetized rats during autoxidation and after addition of 0.2% glucose

Condition		Autoxidizing					After Glucose	
Time in Minutes		15	30	45	60	75	105	120
X_{O_2}....	Ether	2.15	3.30	4.00	4.50	4.55	2.15	4.90
X_{O_2}....	Control	2.40	4.25	6.05	7.70	9.45	2.25	4.65
$-Q_{O_2}$..	Ether	8.60	4.60	2.80	2.00	0.20	8.60	11.00
$-Q_{O_2}$..	Control	9.60	7.40	7.20	6.60	7.00	9.00	9.60

X_{O_2} = mm.^{3} O_2/mg. dry weight of tissue, absorbed in time interval above.
$-Q_{O_2}$ = mm^3 O_2/mg. dry weight of tissue absorbed per hour, calculated for each 15 min. period.

Ten animals were similarly treated in five additional experiments in which glucose was not added to the media until after one to three hours of autoxidation. Here an extraordinary and quite unexpected shortening of the time of autoxidation of available carbohydrate was encountered. After addition of glucose to make a final concentration of 0.2%, the rate of oxygen consumption increased rapidly up to or even above the rate noted in the first fifteen minute period of autoxidation. An example of the usual result is given in Table 2, in comparison with an oxygen-treated control; the fall in rate of autoxidation of the ether-treated tissue was slightly slower than in other experiments in this group.

Deep ether anesthesia of even moderate duration brings about a marked hyperglycemia. High blood sugar concentrations have been associated with increased rate of brain oxidations in surviving tissue taken from hyperglycemic animals (2). It has been shown, however, that brain glycogen and total carbohydrate content fall during ether anesthesia (11) in rats and this effect outweighs the expected action of the increased blood sugar in enhancing the consumption of oxygen in the excised tissues.

An accurate relation between depth or duration of ether anesthesia and the resultant hyperglycemia is not handily found in the literature.

For the purposes of the experiments here reported it was desirable to know the time and degree of increase of blood sugar in rats anesthetized with 2.5 mM/1. of ether, for periods to one hour. Six fed rats comparable to those in respiration experiments were used for determination of these data. Blood was obtained at 10-minute intervals twice before beginning anesthesia and six times during anesthesia, by cardiac puncture. Sugar was determined by the Hagedorn-Jensen micromethod (12). Results are given in Table 3. It is apparent that the degree of ether hyperglycemia is related to some extent with the duration of the anesthesia as well as the depth of narcosis (13).

TABLE 3

Hyperglycemia of fed rats anesthetized with 2.5 mM/1. of ether for time periods to one hour; results expressed in mg. % glucose

Rat Number	Controls	Duration of Anesthesia, Min.						
	10	0	10	20	30	40	50	60
1 (female)	120*	124	186	203	235	262	252	—
2 (female)	134	120	150	185	149	156*	195	224
3 (female)	110	106	168	192	229	218	214	260
4 (male)	108*	117	174	233	243	249	242**	†
5 (male)	127	131	147	174	195	205	207	222
6 (male)	147	143	176	229	222	254	246	†
Ave. (female)	121	117	168	193	204	212	220	242
Ave. (male)	127	130	166	212	220	236	232	—
Control (female)	124	118	122	120	127	120	124	120
Control (male)	105	97	92*	103	106	101	94	103

*Determination on 0.05 cc. of blood.
**Blood aspirated from ventricle after death; glucose in extravasated blood in pericardium, 229 mg. %.
†Rat died.

Discussion

As has been emphasized by Warburg (14) and others (5), the cardinal problem to be clarified through information obtained by studying the action of depressant agents on brain oxidations is primarily not one of their qualitative action, but the quantitative relationship to tissue concentrations presumably present during *in vivo* action. In many cases other than in the *in vitro* action of ether here reported, the concentration necessary to inhibit respiration of surviving tissue is much greater than that effective in the intact animal in producing narcosis. If a relationship between narcosis and rate of brain oxidations is to be defended, two possible explanations of this anomaly may be suggested: either many narcotic agents act *in vivo* through selectively depressing certain anatomically segregated portions of the central nervous system through heightened concentration of the drug at these points or by an inherent greater susceptibility of these portions of nervous tissue to depressant action, or else the properties of brain tissue are profoundly changed by the manipulations necessary for *in vitro study*.. The apparently smaller susceptibility

in vitro, if the same mechanisms are involved in narcosis, would differ markedly from the drug responses obtained on excised tissues such as smooth muscle, in which the tremendously augmented response to doses far below those effective *in vivo* may indicate the loss of some protective system normally present in the intact animal, as suggested by Storm van Leeuwen (15). Possibly some clue could be obtained by comparing the actions within a homologous series such as the barbiturates on different fractions of the brain, since clinically it may be demonstrated that some difference in rate of depression of different functional levels occurs with these agents. A complicating factor in all work on surviving brain is that if we extrapolate Hill's results (16) on peripheral nervous tissue to central, it would appear that the energy requirements differ only slightly whether nervous tissue is functionally active or passive.

From a pharmacological point of view, then, the case for a direct relationship of brain oxidative rates and narcosis is weak because: (a) concentrations of drugs active on the carbohydrate oxidation system *in vitro* are in many cases far beyond those producing narcosis in the intact animal; (b) satisfactory evidence of segregation of drug in the brain *in vivo* or of enhanced susceptibility of anatomically discrete centers to inhibition of glucose oxidation has been lacking; (c) active agents of types other than narcotics produce reversible changes in rate of oxidation in surviving tissue closely resembling (17) those brought about by narcotics; (d) oxidative processes in brain tissue have been shown to depend on chain reactions by means of the catatorulin effect (18) and through the difference in action of agents directly on oxidation systems in surviving brain and the action produced if the agents are allowed to incubate with the tissue for a period before addition of available carbohydrate: accordingly, at least as many different modes of action of drugs in inhibiting oxidations should be possible as there are steps in the reaction; (e) it appears difficult to estimate the occurrence of any large difference in energy consumption between actively functioning tissue and passive or even narcotized tissue which consumes considerable oxygen simply to maintain itself; (f) there is some question, at present unanswerable, as to whether the same mechanisms of oxidation function in surviving tissue as in the brain of the intact animal. As stated by Ashford and Dixon (19), "An even more disturbing possibility is that under artificial conditions a cell may actually develop a new and different mechanism. If this were so, it would throw into doubt much work done on excised tissues and extracts. At present it is simpler to postulate that there are different mechanisms . . . in oxygen and nitrogen . . .". (g) There is a constant difficulty inherent in studying isolated tissue—the absence of general humoral effects which cause or profoundly modify activity in the intact animal.

For biochemical information as to possible oxidative enzyme mechanisms present in brain tissue, the influence of potassium or phosphate or crystalline vitamin B on glycolysis in the brain, and the different types of substrates oxidized by nervous tissue, experiments on isolated

tissue serve very well. For data on the effect of narcotic agents on intact brain, their value is as yet questionable. Thus even the most complete of studies on the effects of narcotics on brain oxidations should not be taken to apply beyond the scope of the actual results found.

In the present work, the first part dealing with the direct action of ether on brain oxidations both when added to surviving tissue and when administered to the animal for a period before excising the tissue is a suitable target for all the above objections. The second part which deals with the time of autoxidizing the available carbohydrate store is subject to none of them, since it is agreed that the brain utilizes glucose both *in vivo* and *in vitro*.

Uchida (11) has examined the effect of chloroform, morphine, and urethane in addition to ether narcosis on both the glycogen and total carbohydrate content of rat brain. All these agents reduce both fractions of brain carbohydrate from 35-46% of the original value, and all have some action on the suprarenals. In work such as that of Gross and Pierce (2), therefore, an added complication is encountered. Peters *et alii* (18) have stated the main fault of using Quastel's technique for determination of the direct action of narcotics in inhibiting increased oxidation after the addition of glucose: "since the narcotic (or poison) is added during the preparative stage, the narcotic may well be influencing this preliminary stage, and no conclusions can really be drawn about the effect upon the oxidative stage." In addition to this, however, there is the further danger in work in which agents acting to reduce brain carbohydrate are given before excising the test tissue that depletion may have been so serious that a portion of the tissue dies before the period of autoxidation is over and the test of increased oxidation with increased glucose begun. In spite of the marked hyperglycemia with morphine and other narcotics, it is impossible to reconcile the high oxygen consumption during autoxidation of brains from non-tolerant rats receiving morphine (2) with the decrease in both glycogen and total carbohydrate reported by Uchida (11) unless the actions of morphine vary differently with respect to the magnitude of the dose.

Summary

The action of ether on processes of aerobic oxidation in surviving minced whole brain tissue of rats and the rate of oxygen consumption of surviving minced whole brain tissue taken from rats deeply anesthetized with ether were studied by the indirect method of Warburg. If ether produces any direct effect on brain oxidations when present in amounts effective in producing surgical anesthesia, this effect is either too slight or too rapidly and completely reversible to permit detection by the methods used. The effect of ether anesthesia in shortening the time of autoxidation, due to depletion of the available carbohydrate in surviving brain tissue, is demonstrated. Pharmacological implications of the action of chemical agents on brain oxidations *in vitro* are discussed.

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