SOME EFFECTS OF 1-METHYL-4-PHENYL ETHYL ISONIPECOTATE (DEMEROL) AND 6-DIMETHYLAMINO-4,4,-DIPHENYL-3-HEPTANONE (AMIDONE[1]) UPON THE METABOLISM OF RAT BRAIN TISSUE IN VITRO[2]

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The similar *in vivo* effects of morphine, demerol, and amidone prompted the following studies on rat brain metabolism to correlate, if possible, *in vivo* activity with *in vitro* effects on mechanisms of biological oxidation. These compounds possess entirely different chemical structures but pharmacological studies indicate that demerol (1) and amidone (2) *in vivo* are morphine-like in action.

Quastel and others (3, 4, 5) have investigated the actions of anesthetics and hypnotics on brain metabolism and have been able to show that parallels exist between *in vivo* and *in vitro* activity. For example, the ability of a series of barbiturates to inhibit the oxygen uptake of brain tissue respiring in glucose-Ringers solution is roughly proportional to their hypnotic potency (3). On the basis of metabolic studies on brain tissue preparations, Michaelis and Quastel (6) proposed that narcotics act by inhibiting the oxidation by brain of carbohydrate. According to Grieg (7) the inhibition is due to the binding of reduced flavoprotein with cytochrome b or other intermediate, thus interrupting the "main line of biological oxidation".

Morphine apparently exerts a different action upon metabolic processes. We have substantiated the results of Seevers and Shideman who showed that 0.12% morphine has no effect on the oxygen uptake of rat cerebral tissue respiring in glucose-Ringers. Presumably morphine does not affect the mechanisms of biological oxidation in a manner similar to other narcotics which have been studied.

The following experiments suggest that amidone and demerol likewise do not conform to Quastel's theory of narcosis although they are capable of exerting profound effects on brain tissue metabolism.

Methods. Oxygen consumption and anaerobic glycolysis were measured by the direct method of Warburg (8, 9) at 37.2° C. Adult albino rats of the Slonaker-Wistar strain were sacrificed without previous preparation or medication. The brains were removed and placed in a cold box (10) as quickly as possible. Cerebral cortex slices were cut with a razor and template (11). Cerebral cellular homogenates were prepared in a Potter homogenizer (12). The tissues were suspended in a modified Krebs-Ringer solution containing 0.2% glucose or other substrate. In order to obtain a medium corresponding more nearly

[1] Supplied through the courtesy of Dr. K. K. Chen, Lilly Research Laboratories as "Dolophine" or 10820.

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to the composition of interstitial fluid, the amounts of potassium and calcium specified in Krebs' formula (9) were reduced from 5.1 to 4.0 and 5.4 to 3.2 millequivalents per liter respectively. Phosphate buffer was used for oxygen uptake measurements; bicarbonate buffer for anerobic glycolysis studies. The gas phase was oxygen for slices, air for homogenates and a 95% nitrogen 5% carbon dioxide mixture for anaerobic glycolysis studies. The last traces of oxygen were removed from the anerobic glycolysis vessels by means of a small piece of yellow phosphorus placed in the center well. Carbon dioxide was absorbed from the aerobic vessels by 0.1 ml. of 10% potassium hydroxide in the center well. The drugs (as hydrochlorides) were made up in Ringer's solution and added to the tissue from the sidearm after a control period of 60 or 90 minutes, each vessel thus serving as its own control. In addition, controls were run for the entire experimental period.

Effects on Glucose Oxidation. *Morphine.* Seevers and Shideman (13) found that 0.12% (0.0016M) morphine sulfate did not alter the respiration of brain slices in glucose-Ringers. Our measurements (figure 1) indicate that even higher concentrations are without effect. 0.005 and 0.01 M morphine hydrochloride failed to depress the oxygen uptake of brain slices. If uniform distribution were to be assumed, these concentrations would correspond to *in vivo* doses of 1.9 and 3.8 grams per kilogram, respectively.

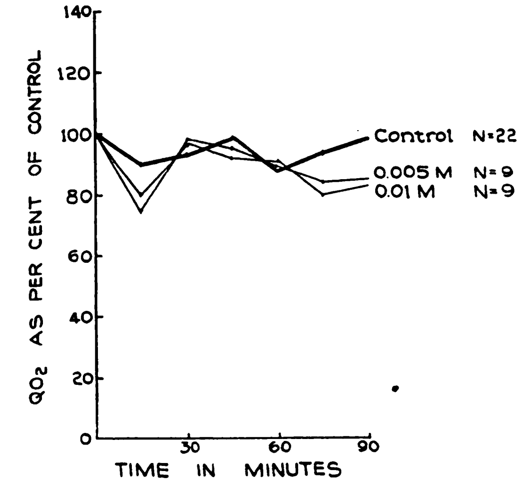
Demerol. The effect of a series of concentrations of demerol upon the oxygen uptake of rat brain cortex slices is illustrated in figure 1. Considering the control Q_{O_2}'s (wet weight) for each concentration as 100%, the rate of oxygen uptake during the 90 minute period following addition of the drug is expressed as per cent of the control Q_{O_2}. Unlike morphine, demerol inhibits brain slice respiration but only at concentrations which correspond to very high *in vivo* doses. The lowest concentration of demerol to show inhibition of oxygen uptake (0.005M) is equivalent to 1.9 grams per kilogram, assuming uniform distribution.

Amidone. The action of various concentrations of amidone on the respiration of rat brain cortex slices is shown in Figure 1. The ordinate is expressed in per cent as described for demerol. This substance stimulates brain slice respiration at concentrations between 0.00016 and 0.0006 M but is a powerful inhibitor at concentrations above 0.001M. Apparently amidone exerts a dual effect, best shown at a concentration of 0.0006 M, resulting in a stimulation of respiration followed by depression. Amidone is a much more powerful inhibitor of tissue respiration than is demerol and its ability to stimulate oxygen uptake is not shared by either morphine or demerol. However, its effects are produced by concentrations much higher than are necessary to produce analgesia *in vivo*, assuming uniform distribution. The concentration of amidone which stimulates oxygen uptake over the entire experimental period is equivalent to 55 milligrams per kilogram (0.00016 M) and 0.001 M is equivalent to 345 milligrams per kilogram.

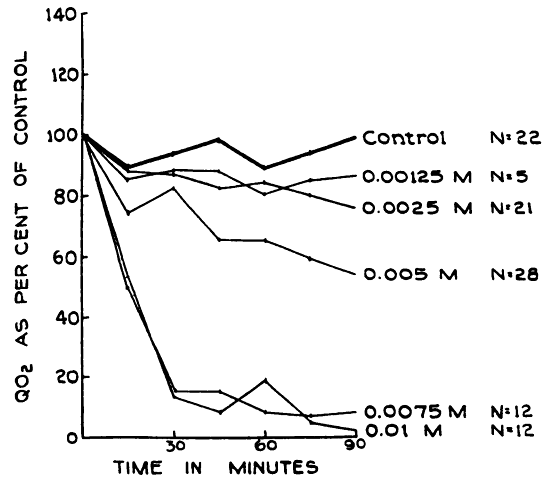
Site of Action. Demerol and amidone in concentrations capable of inhibiting the oxidation of glucose do not inhibit the oxidation of succinate. This would indicate that these agents do not depress the cytochrome-cytochrome oxidase system (7).

The inhibition of cerebral homogenate respiration produced by demerol and amidone is not reversed by 6×10^{-5} M methylene blue in contrast to the complete reversal by methylene blue of sodium pentobarbital inhibition of similar preparations. Since methylene blue can serve as a hydrogen carrier between dehydrogenases and oxygen (7), demerol and amidone apparently inhibit the dehydrogenases involved in carbohydrate oxidation.

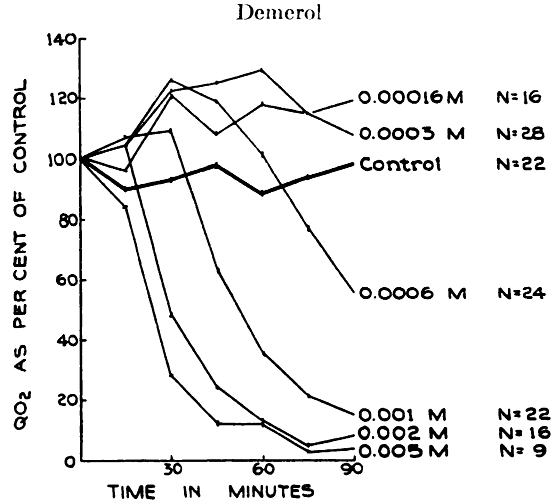
Anaerobic glycolysis of cerebral homogenate preparations is inhibited both by demerol and amidone as illustrated in table 1. The concentrations used were those which strongly inhibited brain slice oxygen uptake. Since these drugs decrease the anaerobic production of lactic acid, an action must occur before the separation of anaerobic and aerobic cabohydrate metabolism (14). This ability to inhibit anaerobic glycolysis is not shared by such narcotics as chloretone (6) or propazone (15). Because of the inhibition of anaerobic glycolysis it might be inferred that the principal action of demerol and amidone is to limit the supply of intermediary metabolites at a point previous to the separation of anaerobic and aerobic carbohydrate metabolism. However, this hypothesis is untenable because the oxidation of d-lactate and pyruvate is also inhibited by demerol and amidone as shown in table 2.



Morphine



Demerol



Amidone

FIG. 1. THE EFFECT OF MORPHINE, DEMEROL AND AMIDONE ON THE OXYGEN UPTAKE OF BRAIN SLICES

N = Number of Vessels

QO_2 = ml. of oxygen per gram of tissue per hour

On the basis of present evidence it seems most probably that demerol and amidone inhibit brain tissue oxygen uptake by interrupting the "main line of biological oxidation" at the dehydrogenase level rather than at the flavoprotein level as is postulated for anesthetics and hypnotics.

COMMENT. In contrast to morphine, demerol and amidone exert marked effects on the oxidation of glucose by brain tissue *in vitro*. A difference in action is apparent in that only amidone is capable of stimulating oxygen con-

TABLE 1

The effect of amidone and demerol on anaerobic glycolysis of cerebral homogenate preparations

	VESSELS	$Q_G^{N_2}$*	PERCENT INHIBITION
Control	34	0.41	—
Amidone 0.002 M	18	0.17	59
Demerol 0.01 M	11	0.00	100

* $Q_G^{N_2}$ (wet weight) 60 min. after addition of drug. Ml. of CO_2 per gram of tissue per hour.

TABLE 2

The effect of amidone and demerol upon the oxidation of lactate and pyruvate by cerebral cortex slices

		CONTROLS	AMIDONE 0.001M	DEMEROL 0.01M
Sodium Lactate 0.02 M	Q_{O_2}	2.48	0.64	0.25
	percent inhibition		74	90
Sodium Pyruvate 0.02 M	Q_{O_2}	2.32	0.47	0.14
	percent inhibition		80	94

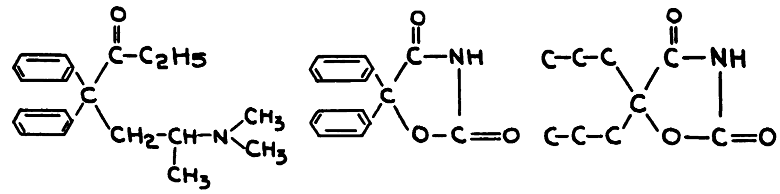
Each value represents the average wet weight Q_{O_2} of eight vessels 60 minutes after the addition of the drug.

sumption. If inhibitory action alone is considered, amidone is approximately ten times as potent as demerol. It is of interest that Scott and Chen (2) found the same ratio to hold in comparing the threshold analgetic dose for albino rats. However, the concentrations capable of inhibiting brain respiration *in vitro* were many times greater than might be expected after *in vivo* administration of the analgetic doses used (amidone 1 mg. per kg.; demerol 10 mg. per kg.).

No attempt will be made here to explain the stimulating action of amidone in low concentration on glucose oxidation. A similar action has been reported for the convulsant diphenyloxazolidinedione (16) in contrast to the purely inhibitory action reported for the central nervous system depressant propazone (di-n-propyl oxazolidinedione) (15). Comparison of structural formulas (figure 2) suggests that the stimulant effect is related to the presence of the benzene rings which are absent in propazone.

In spite of the varying effects of these drugs on oxygen uptake there is some similarity in their action upon the mechanisms of biological oxidation. Seevers and Shideman (13) found that morphine inhibits lactic, citric and glucose dehydrogenases, but not succinic and alcohol dehydrogenases. Our experiments show that demerol and amidone exert their inhibitory effects by action on dehydrogenases involved in glucose oxidation but do not interfere with the activity of succinic dehydrogenase.

Except for the findings on succinate oxidation there is no evidence that morphine, demerol and amidone exert their effects on oxidative processes in a manner similar to anesthetics and hypnotics. For this reason and because of the high concentrations required to produce *in vitro* effects it is felt that these analgetics do not conform to Quastel's theory of narcosis.



Amidone   Diphenyloxazolidinedione   Di-n-propyl oxazolidinedione

FIG. 2.

SUMMARY

The effects of demerol and amidone *in vitro* on the respiration and anaerobic glycolysis of brain tissue preparations have been studied by conventional manometric methods. Amidone is ten times as potent an inhibitor of glucose oxidation as demerol and stimulates oxygen uptake at low concentrations. The inhibitory action of these drugs is presumably due to an action on the dehydrogenases involved in glucose oxidation.

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