

THE INFLUENCE OF NITROUS OXIDE ON THE METABOLIC ACTIVITY OF BRAIN TISSUE * † ‡

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THE anaesthetic properties of nitrous oxide have been known for more than 150 years and the agent has been used in surgical anaesthesia for more than a century. Until about twenty years ago, however, when workers began to study the effect of the agent on the metabolic activity of isolated tissues, nothing was known about its mode of action.

Bülow and her associates (1) in 1932 observed that an 80 to 20 mixture of nitrous oxide and oxygen did not inhibit the respiration (oxygen consumption) of homogenized (finely minced) guinea-pig brain preparations. In the same year Quastel *et al.* (3) found that the mixture of gases did inhibit the respiration of guinea-pig "whole brain" (pieces) in a medium containing glucose. Bülow (2) was unable to confirm Quastel's observation and, more recently, other workers (4, 5), have supported Bülow's findings. The present writers have been able to confirm the observations of both Quastel and Bülow and their associates.

The scheme shown in figure 1 indicates the sequence of enzyme-catalyzed reactions involved in the respiration of living cells (7). This scheme indicates the pathway along which the hydrogen atoms or electrons, removed in the oxidation (dehydrogenation) of the various substrates (succinate, isocitrate, lactate, etc.), are transported finally to combine with activated oxygen to form water. The components shown within the dotted enclosure have not been isolated in pure form, but it is known that they are present in the cell in insoluble submicroscopic particles, the mitochondria. Of the various enzymes involved, the dehydrogenases, and their respective co-factors or "co-enzymes" such as diphospho- and triphospho-pyridine nucleotides (DPN⁺ and TPN⁺), flavoprotein (diaphorase and TPN⁺-cytochrome reductase) and the cytochromes, are necessary for the transfer of hydrogen along the metabolic pathway.

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Methylene blue is a synthetic dye which, when added to the respiratory system, accepts hydrogen atoms at the stages indicated in figure 1 and thus by-passes certain parts of the natural sequence (8, 9). Similarly, ferricyanide, when added to the tissue preparation, accepts hydrogen atoms and thus excludes an even greater part of the normal pathway. These two reagents have been used for many years for studying particular steps in the respiratory system and for determining where in the reaction sequence various drugs interfere with the normal metabolic processes.

METHODS

In these studies the tissue preparation consisted either of rat brain slices or the homogenized tissue. The animal was killed by instantaneous decapitation and the brain was rapidly removed. The

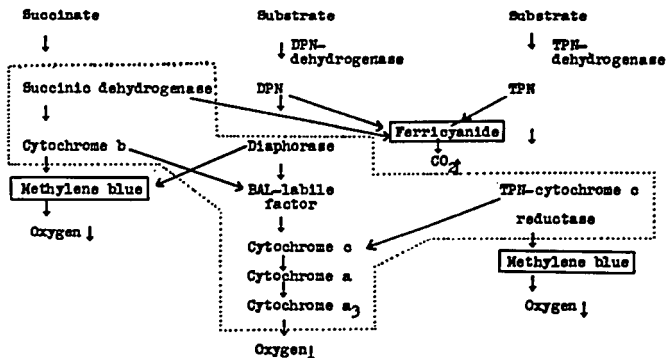


FIG. 1. Pathways of hydrogen transport in the cell.

slices (0.5 mm. in thickness) were cut with a Stadie-Riggs type of microtome in a humid chamber. Each slice was rapidly weighed and placed in a Warburg vessel containing a known volume of cold Krebs-Ringer medium (isotonic mixture of KCl, NaCl, KH₂PO₄, MgSO₄, and glucose). Nicotinamide (0.017 molar solution) was added to prevent the destruction of the endogenous DPN* by the enzyme DPN-ase of the tissue. In the preparation of the homogenates a weighed quantity of brain tissue was disintegrated in a Potter-Elvehjem type of glass homogenizer containing a calculated volume of Krebs-Ringer medium. In the calculation, the volume of 1 gram of tissue was assumed to be 1 ml., and the volume of medium was adjusted so that the final suspension would contain 100 mg. of tissue per ml.

The nitrous oxide-oxygen mixtures were prepared by displacement of a known volume of water in a 5 litre bottle. In view of the solubility of nitrous oxide in water (67 vol. per cent at 20 C.) The mixtures were freshly prepared for each experiment.

The consumption of oxygen by the tissue or the evolution of carbon dioxide was measured with the conventional Warburg apparatus at 37.5 C. The solution of the substrate (succinate or lactate) was placed in the side-bulb of the vessel. When the oxygen uptake was to be measured, two sets of vessels with identical contents were used, with 20 per cent KOH filter paper placed in the center well to absorb the carbon dioxide evolved during the experimental period. One set

TABLE 1
THE EFFECT OF NITROUS OXIDE ON THE RESPIRATORY ACTIVITY OF SLICES OF RAT-BRAIN CORTEX

System	-Q _{O₂}						Q _{CO₂}		
	Without Methylene Blue			With Methylene Blue			With Ferricyanide		
	Air	80:20 N ₂ O:O ₂	% Inhib.	Air	80:20 N ₂ O:O ₂	% Inhib.	95:5 N ₂ :CO ₂	95:5 N ₂ O:CO ₂	% Inhib.
Control (no substrate)	7.8	3.7	53	8.4	2.2	67	5.6	4.5	20
Control + succinate	13.6	5.5	60	12.2	5.4	56	17.7	18.5	+4
Control + lactate	11.7	5.8	50	8.8	4.0	55	6.4	5.2	19
Control + iso-citrate	9.6	3.9	59	9.6	3.8	60	6.6	4.9	26

For Aerobic Experiments:

Main compartment: 1.8 ml. of cold Krebs-Ringer-phosphate-glucose.
Center well: 0.2 ml. 20% KOH and filter paper—30 mm. square.

For Anaerobic Experiments:

Main compartment: 1.8 ml. of cold Krebs-Ringer-bicarbonate-glucose.
Side bulb: 0.2 ml. 11% potassium ferricyanide (neutralized).

Temp.: 37.5 C. Gas Phase: As indicated in table. Total Volume: 2.2 ml.

Substrates: All substrates (0.2 ml.) were placed in the side bulb of the Warburg Vessel, which on addition to the main compartment produced a final concentration of 0.01M.

of vessels attached to their manometers was filled ("gassed") with an N₂O-O₂ mixture (80:20) while the other was filled with air (N₂/O₂:80:20).

The anaerobic metabolic activity of the tissue preparation was estimated by the volume of carbon dioxide liberated from bicarbonate buffer in the presence of ferricyanide and a gas mixture of nitrogen and carbon dioxide (95:5) according to the method of Quastel and Wheatley (6). The ferricyanide was placed in the side-bulb of the vessel.

In all the experiments the preparations were incubated for 10 minutes at 37.5 C. in the Warburg bath. The contents of the side-bulb of each vessel were then tipped into the tissue suspension in the main

compartment and a reading was taken on the manometer. The oxygen uptake or the carbon dioxide evolution was measured periodically thereafter over the period of an hour. The results were calculated as Q values. (The symbol " $-Q_{O_2}$ " designates μ l of O_2 taken up per mg. dry weight of tissue per hour. The positive quantity, " Q_{CO_2} ," signifies the μ l of CO_2 evolved per mg. dry weight of tissue per hour.)

RESULTS

Experiments with Brain Slices.—The respiratory activity of rat brain slices under aerobic conditions in the presence or absence of nitrous oxide is indicated by the data in table 1.

TABLE 2
THE EFFECT OF NITROUS OXIDE ON THE METABOLISM OF RAT-BRAIN HOMOGENATES

System	Microlitres O_2 Consumed per Flask per Hour						Microlitres CO_2 Evolved per Flask per Hour		
	Without Methylene Blue			With Methylene Blue			With Ferricyanide		
	Air	80:20 $N_2O:O_2$	% Inhib.	Air	80:20 $N_2O:O_2$	% Inhib.	95:5 $N_2:CO_2$	95:5 $N_2O:CO_2$	% Inhib.
Control (no substrate)	110	100	10	210	166	21	125	106	15
Control + succinate.	257	211	18	380	332	12	550	600	+9
Control + lactate	142	110	22	272	192	29	124	107	15
Control + iso-citrate	152	126	16	180	152	16	137	112	18

For Aerobic Experiments:

Main compartment: 1.0 ml. of brain homogenate.

Center well: 0.8 ml. of Krebs-Ringer-phosphate-glucose.

For Anaerobic Experiments:

Main compartment: 1.0 ml. of brain homogenate.

Side bulb: 0.8 ml. of Krebs-Ringer-bicarbonate-glucose.

Temp.: 37.5 C. Gas Phase: As indicated in table. Total Volume: 2.2 ml.

Substrates: All substrates (0.2 ml.) were placed in the side bulb of the Warburg Vessel, which on addition to the main compartment produced a final concentration of 0.01M.

Taking the oxygen uptake in the presence of air as representing the "normal" respiratory activity of the tissue, it is evident that nitrous oxide caused a 50 per cent diminution in the oxygen consumption of the brain slices. The degree of inhibition was about the same with the succinoxidase, DPN-linked and the TPN-linked systems, respectively. Since the same degree of inhibition was obtained when methylene blue was added to these systems, it is evident that in all three of these systems the component inhibited must precede the cytochrome c in the reaction sequence. It may be inferred, therefore, that the cytochrome c — cytochrome oxidase system is not sensitive to nitrous oxide.

The anaerobic experiments with ferricyanide indicated that nitrous

oxide caused little or no interference with the anaerobic metabolic activity. It is clear that nitrous oxide did not interfere with the transfer of hydrogen by succinic dehydrogenase to ferricyanide. In the presence of methylene blue, however, the nitrous oxide produced a strong inhibition of the reaction, thus indicating that the sensitive component of the system is the cytochrome b.

With reference to the DPN-linked system, a high degree of inhibition of the oxygen consumption was produced when methylene blue was present as an electron acceptor. In the anaerobic system, on the other hand, in which the added ferricyanide can accept electrons from reduced DPN, only a slight degree of inhibition occurred. It may be inferred, therefore, that diaphorase—a flavoprotein—is one of the N_2O -sensitive components of the system. Since the addition of DPN

TABLE 3
THE EFFECT OF NITROUS OXIDE ON ANAEROBIC GLYCOLYSIS OF BRAIN

System	$N_2:CO_2$	$N_2O:CO_2$ (μ l CO_2 evolved)	% Inhibition
Control (contains ATP)	73	23	—
Control + glucose (10 mM)	160	45	72
Control + glucose-6-phosphate (20 mM)	230	60	74
Control + fructose-6-phosphate (20 mM)	220	56	75
Control + fructose-1, 6-diphosphate (20 mM)	142	38	73

Flask Contents:

Main compartment: 1.1 ml. of medium containing 0.007M NaCl;
0.08M $NaHCO_3$; 0.003M $MgSO_4$; 0.0035M KCl;
0.017M nicotinamide;
0.2 ml. DPN (4.5×10^{-4} M final concentration);
0.4 ml. rat brain extract (100 mg. tissue).

Side bulb: 0.2 ml. of phosphate buffer pH 7.4;
0.1 ml. 0.03M ATP;
0.2 ml. substrate.

did not decrease the degree of inhibition, it would appear that the slight interference observed with ferricyanide may be attributed to a slight sensitivity of the lactic dehydrogenase to nitrous oxide.

The similar results obtained with the TPN-linked system suggest that the flavoprotein (in this case TPN-cytochrome c reductase) is the sensitive component.

The results obtained with the brain slices thus indicate that nitrous oxide interferes with both the aerobic and anaerobic metabolic processes. Quastel *et al.* (3) in 1932 had observed that the gas inhibits the oxygen consumption of "whole brain." The relatively stronger inhibition observed in our experiments presumably may be explained by the more rapid diffusion of the gas into the tissue slices.

Experiments with Brain Homogenates.—The results obtained in

the aerobic and the anaerobic experiments with homogenates are presented in table 2.

The respiratory activity of the "control" preparations (i.e., without added substrate) under aerobic conditions was only very slightly diminished by the nitrous oxide. These results may be regarded as comparable with those obtained by Bülow (2) and other workers (4, 5) who observed no inhibition with nitrous oxide. In the presence of added substrates, such as succinate, lactate and isocitrate, on the other hand, a significant inhibition occurred.

Under anaerobic conditions the degree of inhibition of metabolism with the homogenates was small and was comparable with that observed in the former experiments with slices. The circumstance that the percentage inhibition observed with the homogenates under anaerobic condition was about the same as under aerobic conditions in-

TABLE 4
RECOVERY FROM THE INHIBITORY EFFECT OF NITROUS OXIDE ON ANAEROBIC GLYCOLYSIS OF BRAIN TISSUE

	First 30 Minutes		Second 30 Minutes
	N ₂ :CO ₂	N ₂ O:CO ₂	N ₂ :CO ₂
Control + 10 mM glucose + 6 × 10 ⁻⁴ M ATP	350	—	—
Control + 10 mM glucose + 6 × 10 ⁻⁴ ATP	—	84	358*

* These flasks were regassed with 5% CO₂:95% N₂ and 6 × 10⁻⁴M ATP was added before any further measurements were taken.
Experimental conditions as in table 3.

dicates that some of the enzymes which operate under both conditions are about equally sensitive to the gas.

Influence of Nitrous Oxide on the Glycolytic Activity of Brain Tissue.—Brain tissue depends largely upon glycolysis for the energy required to drive its metabolic processes. In glycolysis, the breakdown of glycogen to lactic acid is accompanied by the liberation of energy which is captured through the synthesis of energy-rich phosphate esters, notably adenosine triphosphate (ATP). The glycolytic activity can be studied *in vitro* with the Warburg apparatus, by adding glucose, or certain phosphate esters of glucose, and ATP to extracts of brain tissue. The volume of carbon dioxide liberated from the bicarbonate buffer in the medium gives a measure of the quantity of lactic acid produced (10-12).

The influence of nitrous oxide on the glycolytic activity of extracts of rat brain is indicated in table 3.

Nitrous oxide causes a significant inhibition of the glycolytic ac-

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tivity. The degree of inhibition is the same with the phosphate ester of glucose as with glucose itself as the substrate.

The inhibitory effect is readily eliminated if the nitrous oxide is removed by gassing the system with a $N_2:CO_2$ (95:5) gas mixture and adding ATP. The recovery of the activity is indicated in table 4.

It is evident from the complete recovery that the inhibition of glycolysis by nitrous oxide causes no damage to the glycolytic system.

SUMMARY

A mixture of 80 per cent nitrous oxide and 20 per cent oxygen inhibits the oxygen consumption of rat-brain cortex slices. The inhibitory action of the gas is moderately enhanced when the tissue is incubated with various substrates.

The inhibitory action of nitrous oxide on the oxygen consumption of rat brain homogenates is negligible when this tissue is incubated in the presence of the gas mixture without added substrate. On the addition of any of several substrates the inhibition is slightly increased.

These results confirm the early finding of Quastel *et al.* that the gas mixture inhibits the oxygen consumption of guinea-pig "whole brain." Also, we have confirmed the results of Bülow and others that the gas mixture produces no inhibition on the respiration of brain homogenates incubated without added substrate.

Under anaerobic conditions, nitrous oxide has been found to exert only a slight inhibitory action on the metabolism of brain slices and homogenates.

In the hydrogen transport system, it has been found that cytochrome b of the succinoxidase system and the flavoproteins, diaphorase and TPN-cytochrome c-reductase, are sensitive to the gas.

The anaerobic glycolysis of brain tissue was significantly inhibited by nitrous oxide. It was found that the gas does not interfere with the enzymatic reactions that occur between the phosphorylation of glucose and the formation of fructose 1, 6-diphosphate. This inhibitory action of the gas is readily and completely reversed on replacing the nitrous oxide in the system with nitrogen, provided ATP is added.

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AFTERNOON: GENERAL SCIENTIFIC SESSION:

Effects of Atropine, Scopolamine and Meperidine Upon Man: A Method of Evaluating Hypnotic Drugs—Louis R. Orkin, M.D., Philip S. Bergman, M.D. and Norton Nathanson, M.D.

A Clinical Evaluation of Epidural Anesthesia in General Surgery—2000 Cases—Peere C. Lund, M.D., John Charles Cwik, M.D., and R. Magaziner, M.D.

Development and Testing of a New Respirator—Frederick H. Van Bergen, M.D., Joseph J. Buckley, M.D., Earl Arthur Schultz, M.D., and James H. Matthews, M.D.

A Study of the Mechanics of the Respiration Using the Pneumotachogram—Eva M. Kavan, M.D., and Francis J. Haddy, M.D.

The Use of a Steroid Anesthetic Agent—Frank J. Murphy, M.D., and Neri P. Gaudagni, M.D.

A Servosystem Using Succinylcholine for the Automatic Control of Degree of Neuromuscular Blockade—Edward Brazell, M.D., and John Abajian, M.D.

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