CCL. THE EFFECT OF NARCOTIC GASES ON BRAIN OXIDATIONS.

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IN a previous paper [Bülow and Holmes, 1932], published from the Pharmacological Laboratory, Cambridge, we dealt with the effect of narcotics on the oxygen uptake of the brain tissue. We were unable to observe any decrease in the respiration of brain tissue *in vitro* in the presence of ethylene, propylene or nitrous oxide, gases of known anaesthetic power. A recent paper of Quastel and Wheatley [1932] has, however, made it desirable to carry out a fresh investigation of the subject.

With fresh tissue, Quastel's experiments are in full agreement with our own, in that he observes no difference in the oxygen uptake of chopped brain tissue, incubated in presence of a mixture of nitrogen and oxygen, or of narcotic gas and oxygen in the same proportions, but he thinks that this agreement is misleading, the real inhibition of oxidation being hidden by the greater solubility of narcotic gases in the cell-lipoids. If he uses tissue which has been allowed to become greatly depleted of its oxidisable material by aeration for 3 hours, and then adds glucose, he obtains in the following 2 hours a distinct inhibitory effect of the narcotics, the cell-lipoids now being saturated with the gas.

On theoretical grounds alone we do not think that Quastel's criticism is valid. Brain tissue contains nearly 8 % lipoids. The solubility of acetylene in oil as determined by Meyer *et al.* [1920] is 1.8 cc. in 1 cc. solvent, so that 43 mm.³ of acetylene may be absorbed by 0.3 g. brain, and so disappear. In an experiment lasting 3 hours the inhibition which can be masked by the absorption of the narcotic gas is thus quite small, and indeed falls well within the limits of experimental error of the method. Apart from this, the manometers are evacuated and filled with the gas mixture three times. After immersion in the thermostat, 15 minutes are allowed for the attainment of temperature equilibrium, before the taps are closed. In all probability, therefore, the lipoids are already saturated with gas before readings are commenced. Experiment confirmed these conclusions. The presence of 0.03 cc. olive oil caused no effect on the gas pressure in the Barcroft vessels, filled with propylene, nor did any change appear during 3 hours' shaking.

It seems possible to explain Quastel's results on the following lines. The oxygen uptake of the cell may be due at first to systems not affected by narcotics, and it may be necessary for the tissue to suffer material damage if it is to show the effect of narcotics on certain specific processes. Quastel removes the material, the oxidation of which is not affected by narcotics, by incubating the brain for 3 hours. We endeavoured to obtain the same effect by washing the tissue several times with distilled water.

As the experiment shows, washing brain tissue three times with distilled water diminishes the velocity of the oxygen uptake as much as did the process

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 1. O_2 uptake of brain

alone $\bullet-\bullet$;
Na lactate $*-\bullet$;

washed with added glucose $\circ-\circ$;
washed with added Na succinate $\times-\times$.

Fig. 2. O_2 uptake of washed brain with added glucose in the presence of

ethylene 95% $\Delta-\Delta$;
acetylene 80% $\times-\times$;

propylene 80% $\bullet-\bullet$;
nitrogen 80%, 95% $\circ-\circ$.

Fig. 3. O_2 uptake of washed brain with added lactate in the presence of

ethylene 95% $\Delta-\Delta$;
acetylene 80% $\times-\times$;

propylene 80% $\bullet-\bullet$;
nitrogen 80%, 95% $*-\bullet$.

Fig. 4. O_2 uptake of washed brain and added succinate in the presence of

ethylene 95% $\Delta-\Delta$;
acetylene 80% $\times-\times$;

propylene 80% $\bullet-\bullet$;
nitrogen 80%, 95% $+--+$.

of incubation in Quastel's experiments. The addition of glucose increases it distinctly (a solution that contains 0.025 % glucose gives nearly as great an effect as a solution of 0.25 %). The oxidation due to lactate is as great as that due to glucose, the effect of succinate being much more powerful (Fig. 1). These results, too, are in full agreement with Quastel's. But if the Barcroft vessels are now filled up with narcotic gas mixtures instead of air, and the oxygen uptake is compared with that which occurs in the presence of nitrogen-oxygen mixtures of the same oxygen content, there is no inhibition to be seen (Figs. 2, 3 and 4). This confirms our previous experiments and is in contradiction to Quastel's. The narcotics do not interfere with the oxidation either of glucose or of sodium lactate or sodium succinate. The last result, which is in agreement with Quastel's, seems to us of some general importance, and is difficult to bring into line with Keilin's studies [1925]. These indicate that the oxygen transference in the cell is inhibited by HCN, the activation of hydrogen by narcotics. The succinic acid dehydrase system is a typical dehydrase system, which has been much studied, and we should therefore expect it to be inhibited by these gases. As the experiments show, there is no such inhibition. On the contrary, in the experiments with ethylene the narcotic seems to have rather a stimulating effect. The uptake in a mixture of 95 % ethylene + 5 % oxygen is as large as that in 80 % acetylene or propylene and 20 % oxygen, and distinctly greater than that in 95 % nitrogen and 5 % oxygen.

EXPERIMENTAL.

0.3 g. of chopped mouse brain (the brains of 3 animals were usually mixed together) was suspended in about 10 cc. distilled water and centrifuged. The supernatant fluid was rejected. The process was repeated three times. The tissue was then placed in the right-hand vessels of Barcroft differential manometers which contained 3 cc. of Ringer solution, buffered with phosphate (4 parts of Ringer solution: 1 part of 0.2 M KH_2PO_4 solution) and brought to p_H 7.4. The left-hand vessels were filled in the same way, but without tissue. The filling with the gas was done by evacuating three times, and allowing the required mixture to run in. The CO_2 formed during the experiment was absorbed by 5 % KOH. All the gases were prepared in the laboratory, ethylene and propylene by treatment of ethyl and propyl alcohols with hot, water-free phosphoric acid, and acetylene from calcium carbide, the gas being washed with acidified $CuNO_3$ solution, dilute chromic acid, a mixture of $Ca(OCl)_2$ and CaO , and $NaOH$. The compositions of the gas mixtures employed were the same as in our previous experiments [1932], as they had been tested and proved to be narcotic for the mouse. (Quastel uses some lower concentrations.) The experiments were done at 36–38°, the apparatus being shaken for 15 minutes before closing the taps, and the readings taken at 10 minute intervals. The values are worked out for normal temperature and pressure. Each experiment was repeated at least four times, often more frequently. The results agreed very well.

As these experiments showed that with washed brain and added substrate narcotics caused no decrease in the oxygen uptake, we looked for another explanation of Quastel's results. An irreversible disturbance of the brain cells, due to a prolonged action of the narcotic, seemed to be a possibility.

To test this hypothesis we repeated Quastel's experiments, using his own method. Fresh brain tissue was shaken in Ringer solution, without adding substrate, and in the presence of narcotics. Controls contained a mixture of nitrogen and oxygen of the same oxygen content, and the same quantity of tissue. After 3 hours, when the rate of oxygen uptake had fallen distinctly, glucose was added and the apparatus refilled with the same gas mixture. In the following 2 hours we could confirm Quastel's observations. The oxygen uptake in this second period was decreased in the presence of acetylene and oxygen, compared with that in nitrogen and oxygen. But this effect was only given by acetylene. Ethylene and propylene did not act in the same way. With these gases the

Table I.

	Gas mixture $N_2 : O_2 = 4 : 1$	Gas mixture $CH \equiv CH : O_2 = 4 : 1$	Inhibi- tion %
O_2 uptake in mm. ³ of 1 g. tissue alone in the 4th and 5th hours	304	262	14
Extra O_2 in presence of glucose (0.025 %)	561	125	78
	Gas O_2 previously $N_2 : O_2$	Gas O_2 previously $CH \equiv CH : O_2$	
O_2 uptake in mm. ³ of 1 g. tissue alone in the 6th and 7th hours	388	278	28
Extra O_2 in presence of glucose (0.025 %)	602	247	59
	Gas mixture $N_2 : O_2 = 1 : 1$	Gas mixture $CH \equiv CH : O_2 = 1 : 1$	
O_2 uptake in mm. ³ of 1 g. tissue alone in the 4th and 5th hours	297	280	—
Extra O_2 in presence of glucose (0.025 %)	398	217	45
	Gas O_2 previously $N_2 : O_2$	Gas O_2 previously $CH \equiv CH : O_2$	
O_2 uptake in mm. ³ of 1 g. tissue alone in the 6th and 7th hours	235	108	54
Extra O_2 in presence of glucose (0.025 %)	318	142	55
	Gas mixture $N_2 : O_2 = 4 : 1$	Gas mixture $CH_3CH_2 = CH_2 : O_2 = 4 : 1$	
O_2 uptake in mm. ³ of 1 g. tissue alone in the 4th and 5th hours	255	390	—
Extra O_2 in presence of glucose (0.025 %)	820	790	—
	Gas O_2 previously $N_2 : O_2$	Gas O_2 previously $CH_3CH_2 = CH_2 : O_2$	
O_2 uptake in mm. ³ of 1 g. tissue alone in the 6th and 7th hours	170	187	—
Extra O_2 in presence of glucose (0.025 %)	720	618	14
	Gas mixture $N_2 : O_2 = 19 : 1$	Gas mixture $CH_2 = CH_2 : O_2 = 19 : 1$	
O_2 uptake in mm. ³ of 1 g. tissue alone in the 4th and 5th hours	—	197	—
Extra O_2 in presence of glucose (0.025 %)	643	563	12
	Gas O_2 previously $N_2 : O_2$	Gas O_2 previously $CH_2 = CH_2 : O_2$	
O_2 uptake in mm. ³ of 1 g. tissue alone in the 6th and 7th hours	—	115	—
Extra O_2 in presence of glucose (0.025 %)	553	435	21

oxygen uptake was the same as that of the controls (Table I). The experiments were carried on for another 2 hours, after adding substrate once more and filling up the apparatus now with pure oxygen instead of the gas mixtures used before. The results were as expected. We observed a decreased oxygen uptake in the vessels which had previously been filled with acetylene, a normal one in those which had contained ethylene or propylene in the previous 2 hours (Table I). This indicates that the inhibitory effect, if it occurs, is an irreversible one. Acetylene is known to be a cell poison, and these irreversible effects may be ascribed to its toxic action on the cells during the prolonged period during which they are exposed to it.

It was found, in further experiments, that after adding as substrate sodium

Table II.

	Gas mixture $N_2 : O_2 = 4 : 1$	Gas mixture $CH \equiv CH : O_2 = 4 : 1$	Inhibi- tion %
O_2 uptake in mm. ³ of 1 g. tissue alone in the 4th and 5th hours	321	342	—
Extra O_2 in presence of 0.05M sodium succinate	1969	1680	15
O_2 uptake in mm. ³ of 1 g. tissue alone in the 6th and 7th hours	Gas O_2 previously $N_2 : O_2$ 205	Gas O_2 previously $CH \equiv CH : O_2$ 140	30
Extra O_2 in presence of 0.05M sodium succinate	1855	1610	13
O_2 uptake in mm. ³ of 1 g. tissue alone in the 4th and 5th hours	Gas mixture $N_2 : O_2 = 1 : 1$ 320	Gas mixture $CH \equiv CH : O_2 = 1 : 1$ 280	12
Extra O_2 in presence of 0.05M sodium succinate	1820	1990	—
O_2 uptake in mm. ³ of 1 g. tissue alone in the 6th and 7th hours	Gas O_2 previously $N_2 : O_2$ 144	Gas O_2 previously $CH \equiv CH : O_2$ 123	14
Extra O_2 in presence of 0.05M sodium succinate	1740	1840	—
O_2 uptake in mm. ³ of 1 g. tissue alone in the 4th and 5th hours	Gas mixture $N_2 : O_2 = 4 : 1$ 332	Gas mixture $CH_3CH_2 = CH_2 : O_2 = 4 : 1$ 310	—
Extra O_2 in presence of 0.05M sodium succinate	1950	1820	—
O_2 uptake in mm. ³ of 1 g. tissue alone in the 6th and 7th hours	Gas O_2 previously $N_2 : O_2$ 203	Gas O_2 previously $CH_3CH_2 = CH_2 : O_2$ 175	15
Extra O_2 in presence of 0.05M sodium succinate	2180	2035	—
O_2 uptake in mm. ³ of 1 g. tissue alone in the 4th and 5th hours	Gas mixture $N_2 : O_2 = 19 : 1$ 420	Gas mixture $CH_3CH_2 = CH_2 : O_2 = 19 : 1$ 335	21
Extra O_2 in presence of 0.05M sodium succinate	630	2015	-230
O_2 uptake in mm. ³ of 1 g. tissue alone in the 6th and 7th hours	Gas O_2 previously $N_2 : O_2$ 300	Gas O_2 previously $CH_3CH_2 = CH_2 : O_2$ 195	35
Extra O_2 in presence of 0.05 M sodium succinate	2730	2055	25
O_2 uptake in mm. ³ of 1 g. tissue alone in the 4th and 5th hours	Gas mixture $N_2 : O_2 = 19 : 1$ 420	Gas mixture $CH_2 = CH_2 : O_2 = 19 : 1$ 335	21
Extra O_2 in presence of 0.05M sodium succinate	460	1465	-220
O_2 uptake in mm. ³ of 1 g. tissue alone in the 6th and 7th hours	Gas O_2 previously $N_2 : O_2$ 300	Gas O_2 previously $CH_2 = CH_2 : O_2$ 195	35
Extra O_2 in presence of 0.05M sodium succinate	2930	2000	32

succinate, instead of glucose, the oxidation was not affected even by the presence of acetylene. (A distinct inhibition, which we once observed, is certainly to be explained by an experimental error.) In the ethylene-oxygen mixture, with its small content of oxygen, we again observed the same stimulating effect of the narcotic as was mentioned above. The oxygen uptake in a mixture which contained only 5 % oxygen was nearly as high as in a mixture with 20 %, and much higher than that in a mixture of 95 % nitrogen and 5 % oxygen. In the following period, when the vessels were filled with pure oxygen, the condition of affairs was reversed. The oxygen uptake in the vessel which previously contained O_2 and N_2 was much higher than in that which previously contained O_2 + narcotic. These results were confirmed with propylene in low concentrations (Table II).

Each experiment was carried out with 0.5 g. mouse brain. The vessels of the Barcroft apparatus contained 3 cc. of the Ringer solution mentioned above, but without addition of substrate. The filling with the gas mixture of the required composition was performed in the same way as in the previous experiments. The manometers were read every 10 minutes, the temperature being about 37°. After 3 hours, 0.3 cc. of a 0.25 % glucose solution (or 0.5M sodium succinate solution) was placed in both cups of two of the manometers, two other apparatus remaining without addition of substrate. The vessels were refilled in the same way as before and with the same gas mixtures. After 2 hours' further shaking the same quantity of glucose (or succinate) was added, the gas mixture was removed, and the manometers were filled with pure oxygen. The evacuation and running in of oxygen were repeated twice to remove the previous mixture as completely as possible. The readings were continued for a further 2 hours. The values given in the table are typical of a large number of results, the volumes of oxygen being corrected for normal temperature and pressure.

SUMMARY.

The suggestion of Quastel, that in incubation experiments the solubility of narcotic gases in lipoids may give rise to an apparent uptake of oxygen, is disproved.

Narcotic gases (ethylene, propylene, acetylene) have no inhibitory action on the oxygen uptake of brain tissue, depleted of its substrate by washing with distilled water, to which glucose, lactate or succinate has been added.

Experiments similar to those of Quastel in which, after depletion of initial substrate by preliminary shaking in Ringer solution with the appropriate gas mixture, the oxygen uptake is measured in the presence of ethylene or propylene, show no inhibitory effect of these gases. The inhibition caused by acetylene, which Quastel observed, was confirmed.

On carrying on the investigations for another two hours, and incubating now in pure oxygen, the inhibition continued in those vessels, which had previously been filled with acetylene, while the presence of ethylene and propylene in the two hours prior to these measurements caused no decrease in the subsequent oxygen uptake in pure oxygen. The inhibition described by Quastel, therefore, is an irreversible toxic effect, due to a too prolonged period of exposure to acetylene.

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