The Toxic Effects of Oxygen on Brain Metabolism and on Tissue Enzymes

1. BRAIN METABOLISM

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Although the convulsive effects of exposure of animals and man to high oxygen pressures† have been known since Paul Bert (1878) first described the strychnine-like convulsions caused by breathing pure oxygen at about 4 atm. pressure, it is a remarkable fact that the effects of H.O.P. on the metabolism of the central nervous tissues have not been investigated hitherto. One would have expected this study to be stimulated by the close resemblance between oxygen convulsions and other types of convulsion which are definitely central in origin, a resemblance which is fully supported by the close similarity of the cortical electrical changes in both types of convulsion. Yet such work that has been already done is almost entirely confined to the effects of oxygen pressure on muscle (Bean & Bohr, 1938, 1940), investigations which have influenced these authors to ascribe rather a high importance to these peripheral changes in relation to the general effects produced. This, however, is hardly justifiable until similar studies have shown that the central nervous system is, in fact, less susceptible than muscle to oxygen. It may be said at the outset that it seems from our results that the reverse is true, and that brain cortex is one of the most sensitive tissues in the body to poisoning by high-pressure oxygen. This finding is more in accord with the views expressed by Behnke, Johnson, Popper & Motley (1935), who consider that the toxic effects of oxygen are referable mainly to a direct toxic action on the nervous system.

Whatever the physiological mechanism may be, a qualitative and quantitative examination of the poisoning of brain respiration by H.O.P. would seem to be a primary step towards its elucidation. There seems to be only one publication, that of A. L. Meyer (1927), relating to the effect of H.O.P. on

brain metabolism; this, however, records merely the diminished oxidation of guaiacum by hydrogen peroxide when these reagents are added to brain tissue, after the latter had been exposed to 4 atm. of oxygen for 4 hr. The reaction studied bears no clear relationship to the energy-yielding processes of brain, and the observations hardly warrant the author's conclusion that H.O.P. 'appears to exert a definite retarding influence on the metabolic activity of brain tissue'.

During the course of this investigation we have received a number of valuable personal communications from Stadie (1943) who has been investigating similar effects to those reported here.

As distinct from the effects of high oxygen pressures, those of oxygen at 1 atm. on brain metabolism have been the subject of two quite recent investigations. At the commencement of this work, in March 1943, Quastel (1943) reported to us some unpublished experiments made earlier by Mann & Quastel, and now released for publication (Mann & Quastel, 1946). They had found that the oxygen uptake of chopped or sliced rat brain in presence of glucose was better maintained in air than in oxygen at 1 atm. They also found that, whereas the oxidation of lactate and pyruvate was similarly poisoned by oxygen at 1 atm., that of succinate was not, when brain suspensions were used. The experiments to be described confirm the greater susceptibility of the glucose, lactate and pyruvate oxidation systems, especially in tissue slices. Quastel & Mann found that the toxic effect of oxygen on tissue oxidation could be duplicated by the addition of small quantities of hydrogen peroxide, and they tentatively suggested that the mechanism of oxygen poisoning might be a very slow formation of hydrogen peroxide, in amount so low that its rate of reaction with catalase might be too slow for its effective detoxication by this enzyme. They also suggested that damage to a flavoprotein constituent of the respiratory system of brain might be a factor in oxygen poisoning, but their experimental evidence was insufficient to prove either of these hypotheses. Nor have we been able to obtain any positive evidence for either of

* This work was completed and submitted as a Confidential Report (No. R.N.P. 44/146, U.P.S. 51) to the Royal Naval Personnel Research Committee of the Medical Research Council in March 1944. The experimental material of the present paper is the same, but the Discussion has been brought up-to-date.

† Abbreviated to H.O.P. This term is used to denote pressures of oxygen above one atmosphere.

these views; on the contrary, we advance a different hypothesis, but the nature of the two mechanisms suggested by Mann & Quastel makes their direct experimental proof extremely difficult. The evidence presented in this and the following paper (Dickens, 1946) is, on the whole, against the importance of either mechanism in oxygen poisoning of brain.*

Elliott & Libet (1942), who of course had no knowledge of this work of Mann & Quastel, published essentially similar findings with regard to the effect of oxygen at 1 atm. on the oxidation of glucose by finely homogenized brain and by slices of brain cortex. In their experiments with the former material in presence of glucose the rate of oxygen uptake in pure oxygen was equal to that in air during the first hour of the experiment, but it fell off slightly during the second hour, and 'in the third hour and fourth hour it was only about 55 and 35 %, respectively, of the rate in air'. With slices of cortex they found that, owing to the better penetration of oxygen into the slice, the respiration rate was initially higher in oxygen than in air, but in oxygen it fell much faster, until in the third and fourth hours it was only 80 and 40 % of that in air. They concluded: 'These observations of the slowly developing inhibitory effects of high oxygen tension on brain-tissue respiration may be related to the fact that prolonged oxygen breathing has adverse effects and that oxygen under increased pressure can produce convulsions in experimental animals and in man.'

It is important to note that Elliott & Libet used for the suspension of the brain tissue a saline medium which departed widely from the usual Ringer's solution, in that it contained potassium and sodium ions, but no calcium or magnesium. As will be shown below, the absence of these divalent cations was probably partly responsible for the very large effect of oxygen at 1 atm. pressure which Elliott & Libet observed; our own observations, made in Ringer's solution containing calcium ions, showed considerably less toxic effect of oxygen at 1 atm. Their experimental conditions were thus extremely favourable for revealing this otherwise rather feebly toxic action.

Since this investigation is confined almost entirely to brain tissue it is not proposed to review here work on other tissues, which is admirably summarized by Stadie, Riggs & Haugaard (1944). From their review the following apt quotation from Paul Bert (1878) may be extracted: 'One finds clearly

demonstrated the apparently paradoxical result that under the influence of very high oxygenation of the blood, the tissues oxidize less, organic combustions diminish in energy, production of carbon dioxide, excretion of urine, the destruction of sugar in the blood are diminished, and that as a result temperature falls.' As far as these conclusions can be applied to isolated brain tissue, it will be shown in this paper that they are amply confirmed.

EXPERIMENTAL

High-pressure methods

The dearth of publications on the toxic effect of H.O.P. on brain metabolism and upon tissue enzymes is undoubtedly due to technical difficulties in the measurement of tissue respiration and enzyme activity under pressure. One possible solution was indicated by the work of Libbrecht & Massart (1937), who enclosed Warburg manometers in a pressure tank only 1 m. high and 60 cm. in diameter, fitted with external controls. A much-improved version of this type of apparatus has been described fully by Stadie & Riggs (1944); the latter's description makes it evident that an efficient apparatus of this kind demands elaborate engineering construction.

In order to by-pass this difficulty, two ways will be described for the measurement of metabolic reactions during and after exposure to oxygen under pressure. In the first (A, below), the manometric apparatus used for measurement of tissue metabolism was included, together with the observer, in a large pressure chamber, and then the pressure inside the chamber was raised to the desired level. This method permits continuous readings of the metabolic exchange while under pressure, but is limited to pressures at which nitrogen intoxication of the observer is insufficient to prevent reliable readings. The long decompression time necessary to avoid 'bends' in the observer is also inconvenient at higher pressures, but the advantage of a continuous record offsets these drawbacks.

This method has one considerable advantage over that of Stadie & Riggs, in that any kind of manipulation of the manometers can be done as easily as in the laboratory, since the observer is inside the chamber. For example, it is easy to fill some manometers with air and others with oxygen while under pressure, and in fact controls in air were regularly run for comparison with specimens of the same material (e.g. brain slices) in oxygen in one and the same experiment. The oxygen may be displaced from any vessel at any stage of the experiment, and reagents or substrates may be tipped in from one or two side-bulbs attached to the manometer vessel. All these things would involve extreme elaboration if they were to be done in a sealed tank from the outside.

* Dr Quastel kindly informed us of these preliminary findings, and has discussed with us the whole question from time to time in the light of the experiments to be described. These discussions have been very helpful throughout. The paper of Mann & Quastel (1946) appears simultaneously with the present publication.

The second method (B, below) is very simple but does not permit continuous readings; it has enabled much work to be done while the large compression chamber was in use for other purposes.

A. *Manometric measurement of tissue metabolism and of enzyme activity under pressure, using the large compression chamber.* The compression chamber, of 9500 l. capacity, was that originally used by Boycott, Damant & Haldane (1908). A working bench was installed, and a compact type of Warburg manometric apparatus was constructed, capable of holding seven open-type Haldane-Barcroft-Warburg manometers in the usual shaking arrangement (cf. Warburg, 1930). The water-bath was electrically heated and its temperature was maintained at 37.5° by means of a hand-operated series-parallel switch; this simple expedient worked quite satisfactorily and avoided sparking, which might have been troublesome with the usual thermoregulator circuit.

The animal tissue or enzyme preparation was placed in the Warburg vessels outside the chamber; these were connected to their manometers, and the whole arrangement was passed through the manhole into the chamber, followed by the observers, who were then sealed in. At this stage the manometer vessels were shaking in the bath at 37.5° at the rate of 90 swings/min., which was maintained throughout. The upper taps of the manometers being open to the air of the chamber, the pressure was raised to the desired level, usually 3–4 atm. No change of level of the manometers occurred, since both limbs were open. One extra vessel, containing no tissue or enzyme, was always used as a thermobarometer to correct all the readings for any fluctuations in the temperature of the bath or the pressure within the chamber. The shaking rapidly caused the vessel contents to come into equilibrium with the rising pressure during compression. As soon as the chosen chamber pressure was attained, the upper limbs of those manometers which were to be filled with oxygen were connected to an oxygen cylinder and the gas was passed through the shaking vessels for 3 min., the tap-form stoppers of the vessels allowing the oxygen stream to escape during this process. The taps of all the vessels, including any that were to be left filled with air instead of oxygen for comparison purposes, were then closed, and the oxygen cylinder was disconnected.

Readings of the oxygen uptake were begun within a few minutes, and were continued at 20 min. intervals during the whole experimental time, which was usually 3½ hr., excluding the time required for decompression. In some cases readings were also taken at each stage of the decompression: since the taps had to be opened during decompression, the oxygen in the oxygen-filled vessels was largely replaced by air during decompression.

The accuracy was quite satisfactory, being only a little less than with the usual experiments at room pressure. Apart from the effects of nitrogen on the observers, which they confidently believe were very slight, the main additional error in the high-pressure work is caused by the difficulty of controlling exactly the pressure within the tank. The thermobarometer vessel is an exceedingly sensitive pressure indicator, since 1 atm. is equal to 10,000 mm. of the Brodie fluid with which the manometer is filled. The pressure ought not to vary by more than 1 or 2 mm. during each set of readings, i.e. by 1/10,000–1/5000 atm. With

practice, this ideal was nearly approached, by having one operator watching the thermobarometer and opening or closing a screwcock escape valve in such a way as to keep the reading of this manometer practically steady, while the other operator took the manometer readings. From time to time, a slight inflow of compressed air into the chamber had to be arranged similarly. After a few trial dives, a good degree of control was achieved. It should be emphasized that all these pressure changes were much too small to affect measurably the gauge pressure of the chamber, which of course was under the control of the operator outside the chamber throughout.

The reliability of this procedure was checked by several series of experiments in triplicate (Fig. 1), in which were followed the rates of oxygen consumption of three samples

Fig. 1. Respiration rate of brain tissue at 2.9 atm. in air or oxygen. Rat-brain cortex slices, suspended in phosphate-Ringer's solution with glucose present, at 37.5°. Triplicate estimations on same brain: — in oxygen. - - - - in air. [Abscissae: hr. of exposure. Ordinates: respiration rate, Q_{O_2} .]

of brain slices in air, and three more slices, from the brain of the same rat, in oxygen. The pressure of both gases was 2.9 atm. The agreement of the triplicates is about as good as is seen at room pressure, the fall of respiration in the three vessels with oxygen follows much the same course in all three, but the slight zigzag fluctuations are mainly due to this difficulty in pressure control. This error, however, leaves no doubt as to the reality of the poisoning effect with oxygen, and mean values give a high probability of satisfactory accuracy in the quantitative estimation of the poisoning by oxygen.

Warburg (1930, pp. 6–8) has shown that the vessel constants, used to convert the observed pressure changes to gas volumes, are independent of the initial external pressure. Warburg manometers may therefore be used at high or low pressures without change of the vessel constants.

B. *The use of the pressure-pot method for compression of tissue or enzyme preparations, followed by measurement of residual activity at ordinary pressure.* The apparatus used was designed expressly for this purpose by Dr E. H. J. Schuster. It consists of six brass tubes, each 3 × 12 cm.,

which screw into brass caps, the latter being provided with brass cocks and unions by means of which they are connected to a gas manifold. The latter is connected by a reinforced rubber tube ('acetylene-welding tubing') to a two-way brass cock and a pressure gauge. One limb of the two-way cock is connected to an oxygen cylinder by means of a fine adjustment valve, the other goes to a vacuum pump.

The brass tube assembly is shaken in the thermostat at 37.5° by a rocking device, which oscillates through an angle of about 40° on each side of the vertical, at a rate of 45 swings/min. Each of the brass tubes contains one glass tube, 2.5 × 10 cm., into which the enzyme preparation or the tissue in 2-3 ml. suspension fluid is placed. The oscillation ensures very good equilibration with gas, the surface of liquid in the tilted tubes being large. The whole arrangement is quickly evacuated, flushed out with oxygen, re-evacuated, refilled with oxygen, and when the pressure is correctly adjusted is placed in the bath and incubated under pressure for the desired period.

At intervals during the incubation the tubes may be removed after closing the brass cock attached to each pot. After removal from the tube, the tissue or enzyme is tested for its activity in the usual way at room pressure. When tissue slices were used they were usually transferred to fresh Ringer's solution in a Warburg manometer. Control experiments showed that the respiration of brain slices incubated in air or oxygen in this apparatus at 1 atm. was the same as that of control pieces simultaneously incubated in the same gas in Warburg vessels. This is a proof that the oxygenation and shaking were adequate, and in the actual experiments control slices in Warburg vessels could therefore be used for comparison with those incubated under pressure in the pressure pots.

The results showed that there was no essential difference in the toxic effects of exposure to oxygen observed by this method and by method A in the large compression chamber. In neither case was there any indication of a restoration of respiration on returning a slice of brain from oxygen to air. Nor was there any evidence that the drastic and sudden pressure changes involved in method B—evacuation, compression, decompression—has any deleterious effect *per se* on brain-cortex respiration. Hence, the results of either method may be accepted with confidence as giving a true measure of the extent of oxygen poisoning under the conditions used.

METABOLISM OF BRAIN TISSUE DURING AND AFTER EXPOSURE TO OXYGEN

General manometric methods for measurement of brain metabolism

Preparation of the tissue. Brain tissue of albino rats was used in all experiments except where other tissues of the rat were used for comparison. The animals were killed by a blow on the neck, and the tissue was immediately prepared.

Brain-cortex slices of thickness less than 0.5 mm. were cut freehand with a razor moistened with Ringer's solution. Before transferring to the vessels for the experiment, they were lightly blotted on filter paper and weighed on a torsion

balance calibrated to 0.2 mg. Control experiments showed that the dry weight of tissue prepared in this way was equal to one-sixth of the wet weight. This method, originally adopted because of the probable loss of weight of the slices during prolonged shaking and consequent errors in the final dry weight determined at the end of the experiment in the conventional manner, was so satisfactory that it has now been adopted for all experiments. The slices were almost entirely free from white matter.

Chopped whole brain was used in some experiments; it was prepared by dividing the tissue with scissors in Ringer's solution until the pieces were fine enough to be sucked into a pipette and the suspension (200 mg. of fresh brain in 1 ml.) was measured into the vessels.

Homogenized brain (whole or cortex) was prepared by the all-glass homogenizer as described by Potter & Elvehjem (1936). Since calcium ions are inhibitory to tissue respiration when the tissue is so finely divided, M/30 phosphate buffer, pH 7.4, without added salts was used for suspension. The homogenization proceeded with ice cooling. The suspension usually contained 200 mg. of fresh tissue/2 ml. This preparation is also very useful for the study of succinoxidase in brain; it could be centrifuged and the resuspended tissue was still highly active after washing in this way. Interfering substances could thus be removed very conveniently (see Dickens, 1946).

Preparation of Ringer's solution. The measurement of tissue respiration is very much simpler in phosphate-containing medium exposed to oxygen or air than in bicarbonate-carbon dioxide buffered media. In view of the complications involved in the use of carbon dioxide at high pressures, especially the shift of pH of the medium for a given carbon dioxide percentage in the gas mixture, it was decided to use the simpler method, though it is recognized that solutions containing bicarbonate have many advantages as a more physiological medium. Nevertheless, the ability to absorb the respiratory carbon dioxide with alkali, and thus to obtain direct readings of tissue respiration on the scale of the manometer, and the constancy of pH of the vessel contents, regardless of the gas pressure, strongly recommend the use of phosphate buffered solutions for the purposes of the present experiments.

Two types of Ringer's solution have been used; they had the composition shown in Table 1.

Table 1. *Composition of saline media*

	I. Phosphate saline of Krebs (1933) (mg./100 ml.)	II. Simple phosphate-Ringer (cf. Dickens & Simer, 1930) (mg./100 ml.)	Mammalian serum (cf. Krebs & Henseleit, 1932) (average mg./100 ml.)
Sodium	324	350	320
Potassium	17	10	22
Calcium	10	7	10
Magnesium	2.6	0	2.5
Manganese*	0	0	0.004-0.025
Chloride	438	470	370
PO ₄	144	155	10
SO ₄	10	0	11
HCO ₃	0	0	54 vol. %
CO ₂ (at 38°)	0	0	2.5 vol. %
pH	7.4	7.4	7.4

* Reiman & Minot (1920): value for whole human blood.

Unless otherwise stated, solution II was used in all experiments. Solution I, which was used in our preliminary experiments with brain slices, differs mainly in its higher content of potassium and calcium, and in its magnesium content, magnesium being absent from solution II. Not until this work was in a fairly advanced stage did it become clear that the presence of magnesium and the higher calcium content may have a profound effect on the sensitiveness of the suspended tissue to oxygen. The protective action of physiological concentrations of these salts probably explains why, in our first experiments at 1 atm., we were unable to duplicate the results of Mann & Quastel for tissue slices; in these early experiments the period was also too short for the slight poisoning effect of oxygen at 1 atm. to be clearly demonstrable. After changing over to the second medium we had no difficulty in confirming Mann & Quastel's observations at 1 atm. This protective action of magnesium, and probably also to a less extent of calcium, will be discussed again after the experiments have been described.

Metabolites. Unless otherwise stated, glucose was the metabolite added to the Ringer's solution. It was used as 10 % solution, added to give a concentration of 0.2 %. When pyruvate was used, it was prepared freshly from the redistilled acid by neutralization of the diluted solution with sodium hydroxide. All additions of this kind were neutralized and were usually added as 0.2M-solutions of the sodium salts (e.g. sodium pyruvate, sodium lactate, etc.) to give the desired final concentration in the medium.

Measurement of glycolysis. The glycolysis was measured in only a few experiments, and these were made in the bicarbonate-saline medium of Warburg (1930), which resembles phosphate medium II (see above) but contains

bicarbonate instead of phosphate: glucose content 0.2 %. The methods used for this have been fully described (Warburg, 1930).

RESULTS

(1) *The effect of oxygen on the respiration of brain slices*

(a) *Earlier experiments at 1 atm. in Krebs's phosphate-saline with glucose.* These preliminary experiments (Table 2) are mostly of too short duration to be of much value in connexion with oxygen toxicity, but they indicate the reproducibility of *Q*-values in successive periods of the experiment. The maximum error is 9 %. In addition, Exp. V, Table 2, shows that respiration at 1 atm. of oxygen can continue for a 5 hr. period without any greater fall than respiration in air. Since we have never seen this behaviour in Ringer's solution without magnesium (solution II, p. 148), we concluded that the presence of this salt, the higher calcium content, and also perhaps the closer resemblance in composition of Krebs's saline to mammalian serum (cf. column 4, Table 1), were responsible for the constancy of respiratory rate in these experiments. Supporting evidence for this view will be given later. That the thickness of the slice is not a factor protecting it against oxygen in these experiments is shown by the data of Table 3. The third column of this table shows that with these very thin slices—about as thin as it is possible

Table 2. *Preliminary experiments: relative respiration rate in air and in pure oxygen at 1 atm.*

(Results are expressed as percentage of mean respiration throughout the whole experimental period for each slice.)

I. Oxygen															
30 min. period	Exp. no. I		II		III	IV		V		VI				Range	Mean %
No. 1	99	99	98	102	103	98	100	98	96	93	109	98	94	93-109	99.5
2	101	99	104	104	99	102	100	101	109	100	97	92	102	92-104	99.5
3	101	102	98	96	99	—	—	102	96	104	105	107	102	96-107	99
4	—	—	100	98	99	—	—	95	96	104	91	102	102	91-104	98.5
5	—	—	—	—	—	—	—	95	90	—	—	—	—	90, 95	93
6	—	—	—	—	—	—	—	104	109	—	—	—	—	104, 109	106.5
7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	107	98	—	—	—	—	98, 107	102.5
9)	—	—	—	—	—	—	—	99	108	—	—	—	—	99, 108	103.5
10)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

II. Air															
30 min. period	Exp. no. I		II		III	IV	V		VI		Range	Mean %			
No. 1	(88)*	98	95	95	90	(74)*	112	100	98	90	96	101	90-112	93	
2	99	98	100	101	99	107	94	100	104	99	98	96	94-107	99.5	
3	102	102	105	98	107	92	98	—	108	92	103	106	92-108	99	
4	—	—	—	104	103	101	98	—	102	94	101	96	94-104	100	
5	—	—	—	—	—	—	—	—	96	100	—	—	96, 100	98	
6	—	—	—	—	—	—	—	—	109	108	—	—	108, 109	108.5	
7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	98	108	—	—	98, 108	103	
9)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10)	—	—	—	—	—	—	—	—	89	108	—	—	89, 108	98.5	

Table 3. *Preliminary experiments: respiration rates of thin brain slices in pure oxygen, and of control slices in air at 1 atm.*

Rat no.	Thickness of slice (mm.)	O ₂ pressure at deepest layer (calc.) (atm.)	Respiration rate (Q_{O_2}) for 30 min. period							
			No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 8	Nos. 9, 10
			I. In pure oxygen (external oxygen pressure 0.93 atm.)							
IV	0.23	0.62	12.9	13.5	—	—	—	—	—	—
V	0.23	0.62	11.7	13.3	11.7	11.7	10.9	13.3	12.0	13.2
VI	0.26*	0.54	15.8	14.0	15.3	13.1	—	—	—	—
Above as percentage of mean respiration rates										
IV	—	—	98	102	—	—	—	—	—	—
V	—	—	96	109	96	96	96	109	98	108
VI	—	—	109	97	105	91	—	—	—	—
II. In air (external oxygen pressure 0.19 atm.)										
IV	0.31	0.00	9.5	9.5	—	—	—	—	—	—
V	0.17	0.02	12.9	14.3	13.2	13.5	14.4	15.6	15.6	15.5
VI	0.34	0.00	8.5	8.7	9.2	9.0	—	—	—	—
VI	0.30	0.00	10.0	9.5	10.5	9.5	—	—	—	—
Above as percentage of mean respiration rates										
IV	—	—	100	100	—	—	—	—	—	—
V	—	—	90	99	92	94	100	108	108	108

* Ringer solution without magnesium.

Table 4. *Respiration rate of rat-brain-cortex slices in oxygen or air at 1 atm.*

(Phosphate-Ringer's solution with 0.2 % glucose. Temp. 37.5°. Slices from brain of same rat compared in oxygen or in air.)

Rat no.	Diet* (see p. 162)	Respiration rate in oxygen at 1 atm. after (% of initial Q_{O_2})					Respiration rate in air at 1 atm. after (% of initial Q_{O_2})				
		2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.
1	No yeast, 10 days	92	93	78	74	71	97	95	87	84	81
		90	88	80	81	76	97	90	75	66	52
3	" 17 "	83	63	62	44	—	102	82	72	61	—
							88	70	62	67	—
5	" 23 "	92	86	76	66	57	95	88	82	79	80
		93	81	74	67	60	100	86	85	82	80
7	" 30 "	91	82	78	64	56	95	88	83	83	80
		99	90	85	74	68	109	104	107	104	105
9	" 37 "	91	81	73	62	53	96	90	88	80	75
		93	82	81	74	70	98	95	92	84	79
2	Yeast-fed, 10 "	95	78	64	55	45	90	85	78	69	67
		85	76	64	65	55	98	94	92	82	80
4	" 17 "	80	76	70	65	—	87	85	74	67	—
		83	76	67	60	—	92	96	92	97	—
6	" 23 "	94	80	72	67	67	98	88	88	85	87
							94	86	84	75	72
8	" 30 "	87	78	68	62	60	97	81	80	72	68
		87	77	66	60	56	94	89	85	77	76
10	" 37 "	91	81	73	62	53	110	105	95	74	69
		93	82	81	74	70	106	97	90	75	76
11	Ordinary mixed diet	102	92	90	82	—	98	98	96	83	—
12	"	97	90	80	77	69	108	97	80	80	68
13	"	104	77	71	69	—	93	65	54	51	—
14	"	92	87	75	64	—	96	101	99	94	—
	Mean of 'no yeast'	91.5	82	76	67	64	98	89	87	79	79
	Mean of yeast-fed	88.5	78	70	63	58	96.5	90.5	86	77	74
	Mean of mixed diet	99	86.5	79	73	(69)	99	90	82	83	(68)
	Average of all in oxygen at 1 atm.	92	82	76	66	59	Average of all in air at 1 atm.				
							98	90	85	78	76

* Since the effect of varying the diet is so slight as to be insignificant without a much larger series of observations, all the results are considered together in this table. The dietary experiments are described in detail in the text.

to cut them by any existing technique—there is in the experiments in pure oxygen a very high tension of oxygen even at the deepest layer of the brain slice (0.54–0.62 atm.). This calculation is based on the data of Warburg (1930, pp. 77–81), but the value of the diffusion coefficient for brain tissue is one determined from the experimentally observed value of the 'limiting thickness' (Warburg, 1930) of brain cortex (Dickens, unpublished observations). It will be noted how thin must brain slices be cut for adequate oxygen diffusion within the slice when air is used in the gas space of the vessels (Table 3, II, col. 3); for complete penetration to the centre of the slice the thickness must not exceed about 0.17 mm. Since it is only occasionally, even after much practice, that slices so thin as this can be cut, it will be noticed that the Q_{O_2} of most experiments in air is distinctly lower than that in oxygen, at least during the earlier readings before oxygen poisoning has become large. This is evidently the result of inadequate oxygenation of the interior of the slice when air is used. Consequently in many of the experiments in air the slice must have been subjected throughout to some degree of anoxia in its deepest parts. This, of course, is due to the absence of capillary circulation in the isolated tissue.

(b) *Long-term experiments in the respiration of brain-cortex slices in air or oxygen at 1 atm. Tissue suspended in simple phosphate-Ringer's solution containing glucose.* For these experiments saline II (Table 1) was used, containing no magnesium. All experiments were run for at least 5 hr. and sixteen for 6 hr.

These results (Table 4) confirm the findings of Quastel & Mann that oxygen at 1 atm. pressure has a toxic effect as compared with air at the same pressure. At 1 atm., however, the toxic effect is almost within the limits of error for the first 2 hr., and becomes clearly evident only in the fifth and sixth hours of exposure.

(c) *The respiration of brain-cortex slices in air or oxygen under pressure: conditions (other than the higher pressures) the same as (b) above.* Table 5 contains measurements made in the large compression chamber at pressures of 2.9 and 3.86 atm. (or 62.5 and 94 ft. depth, respectively*) (see Method A). Table 6 contains similar observations for 3.38, 4.40 and 5.08 atm. (78.5, 112 and 135 ft. respectively), made in the 'pressure-pot' apparatus (see Method B). The average values are summarized here, the example chosen being the experiments in the compression chamber at 2.9 atm., the pressure for which the most complete data are available. Oxygen at this pressure is markedly toxic, much more so than at 1 atm. (cf. Table 4). The effect is still

a delayed one at this pressure, and during the first hour of exposure there is little difference between the fall of respiration in air and oxygen; the fall is hardly measurable in either gas until the second hour of exposure. The poisoning effect of pure oxygen becomes quite clearly evident during the first half of the second hour, and from that stage onwards it is progressive and continuous. After 180 min. the respiration has fallen to half its original

Fig. 2. Mean fall of respiration rate of brain tissue determined at various pressures of air or oxygen. Rat-brain-cortex slices. Phosphate-Ringer's solution with glucose. Temp. 37.5°. [Writing on curves: 2.9 atm. O₂ (10 exp.); 1 atm. O₂ (24 exp.); 0.2 atm. O₂ (air—26 exp.); air at 2.9 atm. (6 exp.). Abscissae: hr. Ordinates: fall of initial respiration rate (%).]

value in pure oxygen, while the tissue in air at the same pressure continues to respire at a perfectly steady rate. Fig. 2 shows clearly the average time course of the poisoning of brain respiration under the influence of air and oxygen at 1 and at 2.9 atm.

Does nitrogen protect brain respiration against oxygen? A remarkable feature of high-pressure air now comes to light. It will be seen from Fig. 2 (cf. Table 5) that there is no significant fall of brain respiration whatever during 3½ hr. exposure to 2.9 atm. of air. Now the partial pressure of oxygen in air at 2.9 atm. is 0.61 atm. Consequently, if air under pressure had the same effect on brain respiration as would the oxygen contained in it, i.e. if the dilution of the oxygen owing to the nitrogen (and rare gases) in air had nothing but a diluent effect, one might expect the toxicity of air at 2.9 atm. to lie between those of air at 1 atm. and oxygen at 1 atm.

In fact, as the table shows, air at 2.9 atm. is practically completely non-toxic in 3½ hr. exposure. During the same period the data of Table 4 show that brain slices under similar conditions were poisoned 21 % in oxygen at 1 atm., and 12.5 % in air at 1 atm. Thus, the *prima facie* conclusion would be that air under pressure is much less toxic than oxygen would be if it were breathed at a pressure equal to the partial pressure of oxygen in the compressed air. In other words, the nitrogen of the air has apparently exerted some protective action.

* On the basis of the convention that 1 extra atm. is equivalent to a depth of 33 ft. of sea water.

Exp. no.	Pressure atm.	Time of exposure (min.)										
		30	60	90	100	120	150	170	180	200	210	230
		Inhibition of respiration by oxygen (as percentage of initial rate)										
1	3.4	5	0	0	—	21	—	—	—	—	—	—
2	3.4	—	—	—	—	—	—	66	—	86	—	94
		—	—	—	—	—	—	60	—	86	—	92
3	3.4	—	—	—	—	—	67	—	—	—	—	—
		—	—	—	—	—	41	—	—	—	—	—
4	3.4	—	—	—	—	—	—	—	66	—	—	—
5	3.4	—	—	—	—	—	—	—	—	—	63	—
6	3.4	—	—	—	—	—	45	—	64	—	—	—
7	3.4	—	—	—	—	—	45	—	—	—	—	—
Mean %	3.4	(5)	0	0	—	21	50	63	65	86	(63)	93
8	4.4	—	—	45	—	63	—	—	—	—	—	—
9	4.4	—	—	—	—	78	—	—	—	—	—	—
10	4.4	—	—	—	60	—	—	—	—	—	—	—
11	4.4	0	—	—	—	74	—	(69)	—	—	—	—
12	4.4	—	—	—	—	—	—	86	—	—	—	—
Mean %	4.4	0	—	45	60	72	—	86	—	—	—	—
		Time of exposure (min.)										
		45	80	110	120							
13	5.0	18	—	61	—							
		30	—	54	—							
		24	—	53	—							
14	5.0	—	59	—	89							
Mean %	5.0	24	59	56	89							

This conclusion is, unfortunately, hardly justified by the present data. In the first place, the animals used were not the same in all experiments. Secondly, the diets were different in the two series, at 1 and at 2.9 atm., although our experiments have, admittedly, failed to demonstrate any definite effect of the different diets used. Thirdly, as has been already shown (Table 3), in air at 1 atm. the tissue slice is anoxic in its deeper layers, which may possibly account for part of the fall of respiration observed in air at 1 atm., a fall which does not occur in air at 2.9 atm. Hence it is not, perhaps, justifiable to conclude that the fall of respiration at 0.6 atm. of pure oxygen (the partial pressure of oxygen in air at 2.9 atm.) would lie about midway between the corresponding decreases of respiration in air and oxygen; for at 0.6 atm. of oxygen, a slice of 0.35 mm. thickness would be at the upper limit of permissible thickness for oxygen to penetrate fully to its deepest layer. Since this was about the thickness of good razor slices of brain, it may be that 0.6 atm. of oxygen actually provides the optimum concentration of oxygen for slices of this thickness. If this suggestion were to prove correct, the toxic effect of oxygen would be at a minimum when the anoxic condition was just avoided, but when there was no considerable excess of oxygen above the minimum requirements for complete oxygenation of those parts of the tissues most remote from the source of oxygen (i.e. the surface of the slice *in vitro*; the capillary *in vivo*).

The evidence of a protective effect of nitrogen against oxygen poisoning was not supported by the results of an experiment in which the toxic effects of 5 atm. of air were compared with those of 1 atm. of oxygen, i.e. the oxygen partial pressures were substantially the same, but in the experiment at 5 atm. there was a fourfold dilution with nitrogen. This experiment had perforce to be made in the 'pressure-pot' apparatus, owing to the high pressure needed. Table 7 shows that the respiration of brain

Table 7. *The effect of air at 5 atm. compared with that of pure oxygen at 1 atm. on the respiration rate of brain tissue*

(Material: slices of rat-brain cortex. Medium: Ringer's solution containing glucose and phosphate buffer, without magnesium. The respiration rates were all measured in pure oxygen, except that of the control portion which was in air.)

Time (hr.)	Measurement of respiration rates (Q_{O_2})				
	In O_2 at 1 atm. through-out	Exposed to air at 5 atm., then transferred to O_2 at 1 atm. for measurement. Slice no.			
		1	2	3	4
1	12.2	—	—	—	—
2	12.2	—	—	—	—
3	10.8	9.4	—	—	—
4	8.8	—	7.6	—	—
5	6.9	—	—	6.5	—
6	4.7	—	—	—	5.3
					through-out*
					7.5
					7.5
					7.4
					7.2
					7.2
					6.9

* The low values measured in air are due to inadequate diffusion owing to the low oxygen tension (cf. Table 3). Note, however, the constancy in 1 atm. air compared with the other two sets of readings.

slices is no better maintained in air at 5 atm. than in pure oxygen at 1 atm.

There is thus no clear positive evidence of any protective action of nitrogen against the toxic effect of oxygen on brain respiration.

(d) *Time course of the poisoning of brain respiration by oxygen at pressure.* The action of many drugs on tissues and particularly of drugs of which the action is weak but nevertheless evokes a response of the all-or-none type in the individual cells, may be represented by a sigmoid type of response curve (Clark, 1933). This type of curve may be regarded as the integration of the distribution curve of the individual sensitivities of the cell population exposed to the destructive action of the drug. If this curve is that of a 'normal' distribution, a symmetrical response curve will be obtained, of which the point of inflexion will correspond with the dosage (or time of exposure) which produces a 50 % poisoning. Such a curve will be a 'probability curve' and can be plotted from a probability table.

Fig. 3. Time course of poisoning of respiration of brain tissue at 2.9 atm. in pure oxygen, with control in air at same pressure. . = oxygen. x = air (each point represents one observation). - - - - Mean values (oxygen and air) observed. — Probability curve calculated for 50 % poisoning at 3 hr. (see text). Rat-brain-cortex slices in phosphate Ringer's solution with glucose at 37.5°. [Abscissae: hr. of exposure. Ordinates: poisoning of brain respiration (%).]

The time-action curve of exposure of brain tissue to H.O.P. is of this general type (Fig. 3). In this figure the solid curve plotted is based on a probability table such as that published by Bliss (1935). The points are those experimentally determined, and the position of the solid curve corresponding to 50 % poisoning is arranged to coincide approximately with the mean time actually observed. This curve adequately represents the mean poisoning effect for each time of exposure to oxygen at the pressure used (2.9 atm.), and this is more accurately proved by plotting the average readings for each period of the experiment on a scale of probabilities

as ordinates, against a linear time scale as abscissae, when the points are found to lie very close to a straight line (Fig. 4).

Fig. 4. Poisoning of brain respiration by oxygen, on scale of probability units: pressure 2.9 atm. oxygen. $\circ$ — $\circ$ = mean of ten observations for each period. Other data as in Fig. 3. [Abscissae: hr. of exposure. Ordinates: poisoning (%), on probability scale.]

(e) *The irreversibility of oxygen poisoning of brain respiration; and the effect of convulsions caused by H.O.P. on the subsequent oxygen uptake of rat's brain.* No reversal of the oxygen poisoning of brain-tissue respiration has ever been observed when the tissue was transferred to air instead of oxygen. This applies whether decompression to atmospheric pressure was carried out or not. Continuous readings taken in the compression chamber at each stage of decompression gave no indication of reversal; all that happened was that further fall of respiration was sometimes averted when air replaced the oxygen.

Nevertheless, the *in vivo* effects of oxygen are reversible, inasmuch as the acute symptoms of oxygen poisoning clear up rapidly on transferring the subject to air. On this point, the hypothesis that the symptoms may be due to injury to only a few brain cells does not appear to offer a very satisfying explanation. It would be possible to argue that a degree of poisoning which is so slight as to injure perhaps only a fraction of 1 % of the cortical cells may be reversible, even though the later stages of poisoning when measurable damage to tissue respiration has occurred are not reversible. A possible alternative explanation will be advanced in the Discussion.

Measurement of the respiration of cerebral cortical tissue of a rat which had just undergone violent convulsions in 4 atm. of oxygen, and was passed out from the large compression chamber through the air-lock and killed without delay, gave the results shown in Table 8.

These figures are of similar order to those obtained with normal rat brain, but of course only a gross poisoning would be detectable, except perhaps by a vast number of experiments.

Table 8. *Respiration rate (Q_{O_2}) of slices of cortex of a convulsed rat*

Period (min.)	Q_{O_2} (quadruplicate estimations)			
	9.2	9.3	10.7	—
0-20	8.8	9.4	10.5	10.0
20-40	9.5	10.0	11.5	11.8
40-60	—	—	10.5	9.8
60-80	—	—	10.8	10.3
120-140	—	—	11.8	10.5
140-160	—	—	—	—

(f) *The relationship of the poisoning of brain respiration to the oxygen pressure.* By combining all the results obtained at different values of H.O.P. (Tables 5, 6), some conclusions may be made as to the comparative sensitiveness of brain respiration at various pressures.

Our observations cover only the range 0.2 (air) to 5.08 atm. oxygen pressure. The effects of air and oxygen at 1 atm. are so small that the experiments have to be very prolonged to produce poisoning effects sufficiently outside the limits of error for any reasonably accurate quantitative calculations to be based on them. In very long experiments the 'wear and tear' factors become noticeable, and may complicate the interpretation. For pressures above 1 atm., Table 9 illustrates the relationship between the times necessary to give definite percentage poisoning and the prevailing oxygen pressures: for the calculation of the mean times for 25, 50 and 75 % poisoning the time-course established for 2.9 atm. of oxygen (probability curve) was again plotted against the actual readings obtained for the various pressures, using a probability scale of percentage poisoning, and drawing through the observed points the best straight line to fit the observations (cf. Fig. 4). The results, which are to be regarded only as approximations, are given in Table 9.

These figures suggest that there is an inverse proportionality between the time of exposure necessary for a given percentage of poisoning and the pressure of oxygen to which the brain tissue is subjected. On the whole, the proportionality is more exact if the excess pressures above 1 atm. ($= P - 1$, in Table 7) are taken, instead of the total pressure of oxygen. It cannot be claimed, however, that the determinations of percentage poisoning at any pressure (other than 2.9 atm., for which an extensive series of estimations was made) were of sufficient accuracy to enable one to choose between the two sets of figures, given as the products of ($P \times t$) and ($P - 1$) $\times t$, respectively, in Table 7. The second of these formulae would require no poisoning at 1 atm. of oxygen, whereas in fact some poisoning has been observed at this pressure. Without a large number of comparative determinations it is not justifiable to press this analysis further.

Table 9. *Relationship between oxygen pressure and time required for a given poisoning effect on brain-tissue respiration*

Depth (ft.)*	Oxygen pressure (atm.) (P)†	Average times (t min.) of exposure for poisoning			Product (t × P) for poisoning			Product (P - 1) t for poisoning		
		25 %	50 %	75 %	25 %	50 %	75 %	25 %	50 %	75 %
0	1	(246)	?	?	(229)	?	?	—	—	—
62.5	2.9	117	184	230	340	530	668	222	350	437
78.5	3.38	88	150	195	298	508	660	210	356	464
112	4.40	70	100	130	308	440	572	238	340	442
135	5.08	56	80	105	285	406	535	228	327	429
		Mean			308	471	609	224	343	443
		Max. diff. from mean			±32	65	63	14	16	21

* 33 ft. sea water = 1 extra atm.

† In the moist gas.

(2) *Comparison of the sensitivity of brain respiration to H.O.P. with that of other tissues*

The respiration of various rat tissues was measured (a) in oxygen at 1 atm., (b) similarly, after exposure to 4.4 atm. of oxygen for 3 hr. Brain cortex, liver, kidney, and lung tissue was cut into the usual thin slices. Spinal cord was opened out along the line of the dorsal spinal artery, and sliced laterally. Testicular tubules were teased out and rinsed in Ringer's solution.

Table 10. *Sensitivity of various rat tissues to H.O.P.*

(Medium: Phosphate-Ringer's solution with glucose. Tissue respiration measured in oxygen at 1 atm.)

Tissue ...	Brain cortex	Spinal cord	Liver	Testis	Kidney	Lung
Initial respiration ($\dot{Q}_{O_2}$)	8.8	2.6	9.4	8.0	18.1	4.4
Do. after 3 hr. O_2 at 1 atm.	8.3	1.5	6.7	3.8	14.1	3.8
Do. after 3 hr. O_2 at 4.4 atm.	1.25	0.25	1.5	2.4	9.3	2.9
As % of control at 1 atm.	15	17	22	63	66	76

Nervous tissue is the most sensitive to H.O.P. of the representative tissues tested (Table 10). Rat diaphragm was found by Stadie (1943, personal communication) to be more resistant than lung tissue to oxygen; hence muscle is one of the most resistant tissues. Liver tissue is quite sensitive, but the respiration of testis, kidney and whole-lung tissue is comparatively resistant to oxygen.

(3) *The effect of state of division of the tissue on sensitivity to oxygen*

The finer state of division in minces and homogenates of brain will presumably permit a better diffusion of oxygen to the metabolically active

centres, and thus increase the sensitivity of the preparation to any given pressure of oxygen.

The sensitivity to oxygen is in fact greater, the finer the subdivision of the tissue (Table 11). This is most readily seen from the estimated times of exposure necessary for the rate of respiration in oxygen to fall to 50 % of that in air, which are as follows:

State of brain tissue...	Slices (Tables 4, 5)	Chopped (Table 11)	Homogenized (Table 11)
Time for 50 % fall in oxygen:			
Pressure 1 atm. ...	About 7 hr.	4½ hr.	3½ hr.
Pressure 2.9 atm. ...	3 hr.	—	1 hr. 40 min.

The times are about halved for the homogenized tissue as compared with slices, but the fact that the respiration of chopped and homogenized tissue is steadily falling even in air makes accurate comparison difficult. It should also be remembered that the homogenized tissue was suspended in a different medium (see p. 148).

(4) *Effect of oxygen on the utilization of substrates other than glucose*

Hitherto, all experiments have been made in a glucose-containing medium. Other substances concerned in the carbohydrate metabolism of brain, of which the oxidation is poisoned by oxygen, include fructose, lactate and pyruvate. Table 12A, Exp. (i), shows that when brain slices are exposed to H.O.P. in presence of glucose, and their activity in oxidizing various substances is subsequently tested in the usual way, the slices can still oxidize succinate, and to a less and variable degree glutamate, but glucose, lactate and pyruvate oxidation is almost completely abolished under the same conditions of exposure to oxygen. When the brain slices are exposed to H.O.P. in presence of glucose, lactate or pyruvate (Table 12A, Exp. (ii)), the ability to oxidize glucose is lost also. The general conclusion is that all these stages of glucose oxidation are poisoned, with the exception of the succinate stage.

Table 11. *Toxic effect of oxygen on the respiration of chopped and homogenized brain*

(The following are the results of typical experiments. The medium contained glucose.)

(a) Chopped whole brain of rat

	Oxygen at 1 atm. hr. of exp.					Air at 1 atm. hr. of exp.				
	2	3	4	5	Initial	2	3	4	5	Initial
	% of initial Q_{O_2}				Q_{O_2}	% of initial Q_{O_2}				Q_{O_2}
Triplicates	70	43	24	14	11.7	76	59	38	33	8.9
	70	42	23	14	11.2	80	60	45	30	8.9
	70	43	22	10	11.4	76	58	42	30	8.7
Mean	70	43	23	11	11.4	77	59	42	31	8.8
Ratio: $\frac{\% \text{ in oxygen}}{\% \text{ in air}}$	0.91	0.73	0.55	0.35						

Respiration in oxygen = 50 % of that in air after c. 270 min.

(b) Whole brain homogenized

	Oxygen at 1 atm. hr. of exp.					Air at 1 atm. hr. of exp.				
	2	3	4	5	Initial	2	3	4	5	Initial
	% of initial Q_{O_2}				Q_{O_2}	% of initial Q_{O_2}				Q_{O_2}
Triplicates	68	40	22	13	9.3	85	63	45	35	7.5
	71	39	22	14	8.1	80	60	47	32	8.0
	72	42	20	14	8.2	78	61	46	35	7.9
Mean	70	40	21	14	8.5	81	61	46	34	7.8
Ratio: $\frac{\% \text{ in oxygen}}{\% \text{ in air}}$	0.86	0.66	0.46	0.41						

Respiration in oxygen = 50 % of that in air after c. 210 min.

Ditto under pressure (different brain tissue)

	Oxygen at 2.9 atm. hr. of exp.						Air at 2.9 atm. hr. of exp.					
	$\frac{1}{2}$ -1	1-1 $\frac{1}{2}$	1 $\frac{1}{2}$ -2	2-2 $\frac{1}{2}$	2 $\frac{1}{2}$ -3	Initial	$\frac{1}{2}$ -1	1-1 $\frac{1}{2}$	1 $\frac{1}{2}$ -2	2-2 $\frac{1}{2}$	2 $\frac{1}{2}$ -3	Initial
	% of initial Q_{O_2}					$Q_{O_2}^*$	% of initial Q_{O_2}					$Q_{O_2}^*$
Triplicates	80	44	28	21	13	5.4	82	69	65	63	52	5.7
	81	38	26	19	10	5.4	87	72	62	62	47	5.6
	86	44	(36)†	(32)†	(22)†	5.1	78	65	58	58	52	6.6
Mean	82	42	27	20	12	5.3	82	69	62	58	50	6.0
Ratio: $\frac{\% \text{ in oxygen}}{\% \text{ in air}}$	1.00	0.61	0.44	0.35	0.24							

* These experiments were done in the large compression chamber, and the initial Q_{O_2} has fallen somewhat owing to the inevitable delay during compression.† In this experiment the oxygen in the vessels was displaced by air after 1 $\frac{1}{2}$ hr. The bracketed figures show that the further fall of respiration was slowed to the same rate as in air, but no reversal of oxygen poisoning occurred.

That even the persistence of succinate oxidation after exposure is largely dependent on the presence of glucose during the incubation under H.O.P. is shown by Table 12A, Exp. (iii), in which the succinate oxidation fell to about one-third of its normal rate when glucose was omitted from the medium in which the tissue was exposed to H.O.P., while, when glucose was present, the succinic oxidation was mostly protected. Dr Quastel has pointed out that this observation may be related to the 'sparing' action of lactate (or glucose) on succinate oxidation (Quastel & Wheatley, 1932), whereby oxygen might be diverted from the succinate system to the other substrate.

As with glucose oxidation, that of the other substrates becomes more sensitive to oxygen the more finely divided the tissue (Table 12B, C). In homogenized brain tissue, even incubation for 2 hr. in oxygen at 1 atm. pressure causes the succinoxidase activity to fall to two-thirds of the value seen in another portion incubated simultaneously in nitrogen (Table 12C). With liver tissue the fall of succinoxidase activity in oxygen is even greater. To produce an approximately similar fall of oxidation of succinic acid by *slices* required about the same time of exposure to oxygen at 4.4 atm. (Table 12A, Exp. (iii), glucose absent). In the case of succinoxidase the intactness of the tissue

Table 12. *Effect of oxygen pressure on the oxidation of lactate, pyruvate, succinate and fructose*A. *Rat-brain cortex—slices*

- (i) Incubated under H.O.P. in glucose-containing medium and respiration measured after exposure, in presence of the substrates shown. Pressure during exposure, 3.4 atm. Time of exposure to oxygen, 2½ hr.

Substrate during measurement	Rate of oxygen uptake (Q_{O_2}) after exposure, during the period			
	0-½ hr.	½-1 hr.	1-1½ hr.	1½-2 hr.
Glucose, 0.2 %	3.5	3.4	2.9	—
Lactate, 0.01 M	3.8	4.0	2.8	—
Pyruvate, 0.01 M	2.7	2.7	1.8	—
l(+)-Glutamate, 0.01 M	5.7	4.4	3.3	—
Succinate, 0.01 M	8.5	6.5	5.3	—
None added	3.5	1.8	1.8	—

Controls to above; tissue not subjected to H.O.P.

Glucose	14.5	13.3	13.2	13.2
Lactate	9.3	9.2	9.3	9.3
Pyruvate	13.0	12.6	12.1	13.4
Glutamate	10.8	10.1	7.9	7.5
Succinate	11.7	8.9	6.6	6.5
None added	7.7	4.5	2.2	0.8

(In some experiments glutamate oxidation was more damaged by H.O.P. than in the above.)

- (ii) Incubated under H.O.P. in the presence of the substrate shown below. Respiration rate measured after exposure, upon the tissue suspended in glucose-containing medium. Pressure during exposure 4.4 atm. Time of exposure 100 min.

Substrate during exposure to H.O.P.	Rate of oxygen uptake (Q_{O_2}) after exposure, during the period			
	0-½ hr.	½-1 hr.	1-1½ hr.	1½-2 hr.
Glucose, 0.2 %	3.6	2.4	1.6	1.2
Lactate, 0.01 M	5.0	4.1	3.6	2.8
Pyruvate, 0.01 M	1.8	1.8	1.8	1.8

- (iii) Protection of succinate oxidation by the presence of glucose during exposure to H.O.P. Pressure during exposure 4.4 atm. Time of exposure, 130 min.

Substrate during measurement	Transferred after exposure to H.O.P. to Ringer's solution containing the substrates shown: respiration rate then measured (at 1 atm.)	
	Exposure to H.O.P. without glucose in medium	Exposure in presence of glucose, 0.2 %
	Q_{O_2}	Q_{O_2}
	Glucose	4.1
Succinate	3.4	9.3
None added	1.5	2.7

- (iv) Measurement of respiration of rat-brain-cortex slices while under H.O.P. (in the compression chamber): (a) glucose and fructose, (b) succinate, present as substrates.

(a) Pressure 2.9 atm. pure oxygen. Glucose and fructose oxidation

Substrate	Respiration rate (Q_{O_2}) during period (min.)							
	20-40	40-60	60-80	80-100	100-120	120-140	140-160	160-180 180-200
Glucose, 0.2 %	14.4	13.7	13.8	11.9	12.2	10.4	10.1	8.6 7.5
Fructose, 0.2 %	14.8	11.4	11.4	9.3	8.8	8.4	8.2	6.5 5.7

(In this experiment, fructose oxidation falls a little faster than glucose, but the difference is slight.)

Table 12 (*cont.*)

(b) Pressure 4.6 atm. of pure oxygen or of air. Succinate oxidation.

No glucose present; succinate concentration, 0.05 M

Respiration rate (Q_{O_2}) during period (min.)

	Measured in 4.6 atm. oxygen					Measured in 4.6 atm. air				
	0-20	20-40	40-60	60-80	80-100	0-20	20-40	40-60	60-80	80-100
Succinate added at once (0 min.)	10.9	9.3	7.2	5.7	5.7	10.5	11.7	8.1	8.1	7.2
Succinate added after 20 min. at pressure in O_2 or in air (duplicates)	9.3	6.8	6.0	5.7	—	10.2	12.0	6.9	7.0	—
	9.3	7.0	6.3	5.7	—	12.9	11.7	7.9	6.3	—

(The poisoning of oxidation of succinate by H.O.P. is shown, but it is much less than that of glucose, which at this pressure is 50 % in 100 min. compared with a sample in air at the same pressure.)

B. Rat brain (whole brain)—chopped. Oxidation of various substrates at 1 atm. in oxygen or air

Respiration rate as % of initial respiration; period (hr.)

Substrate	Oxygen at 1 atm.					Air at 1 atm.				
	Initial Q_{O_2}	2	3	4	5	Initial Q_{O_2}	2	3	4	5
Glucose, 0.025 M	9.6	74	50	28	14	7.3	74	53	35	17
Lactate, 0.025 M	11.8	67	42	24	13	9.3	66	45	34	24
Pyruvate, 0.025 M	9.7	97	51	25	15	8.4	56	26	19	17
None added	7.4	32	12	9	6	4.5	50	23	17	12

C. Rat-brain cortex—homogenized. Comparison of succinate oxidation after exposure of the homogenized brain to (a) oxygen at 1 atm., (b) nitrogen at 1 atm.

Temp. 37.5°; period of exposure 2 hr. in each case. Succinate oxidation rate was measured in oxygen at 1 atm. For comparison, homogenized liver of the rat was simultaneously studied.

Since cytochrome is washed out of the tissue on homogenizing, a preparation of cytochrome c, made from horse heart (Keilin & Hartree, 1937) was added. Final conc. of cytochrome, 4×10^{-8} mol./2 ml. (2 ml. liquid/vessel). Substrate: succinate, M/30.

Period (min.)	Oxygen uptake (μ l./40 mg. fresh tissue)			
	Brain cortex after 2 hr. in		Liver after 2 hr. in	
	Oxygen	Nitrogen	Oxygen	Nitrogen
0-30	76.5	118	108	281
0-60	150	227	205	499

Activity remaining after exposure to oxygen; as % of that in nitrogen:
66 % 39 %

apparently exerts an appreciable protection, and, in presence of glucose, this protection becomes almost complete.

(5) *The effect of exposure to H.O.P. on the respiration, aerobic and anaerobic glycolysis of brain tissue*

When the respiration of brain tissue is poisoned by specific reagents such as cyanide, or entirely prevented by keeping the tissue anaerobic, the alternative energy source of brain—glycolysis—comes into operation. On the other hand, the so-called 'protoplasmic' or non-specific poisons destroy both types of metabolic activity. Oxygen behaves in this respect as a 'protoplasmic poison'. Slices of brain tissue were exposed to 4.4 atm. oxygen, in phosphate-Ringer's solution containing glucose, for

varying periods, and portions were transferred to bicarbonate-Ringer's solution in equilibrium at 1 atm. with gas mixtures of 95 % oxygen + 5 % carbon dioxide, or 95 % nitrogen + 5 % carbon dioxide. The aerobic and anaerobic glycolysis, and the respiration, were then measured in the usual way (Warburg, 1930).

As the respiration fell owing to oxygen poisoning, the aerobic glycolysis did not increase appreciably (with cyanide poisoning of respiration it rises almost exactly to the anaerobic level), and the anaerobic lactic acid formation was poisoned at about the same rate as the respiration (Table 13).

There is therefore a nearly parallel destruction of the enzymes necessary for both respiration and glycolysis. Since, in the above experiment, the tissue was transferred to atmospheric pressure for

Table 13. *Respiration and glycolysis of brain-cortex slices before and after exposure to H.O.P.*

(Oxygen pressure 4.4 atm. Exposure in phosphate-glucose-Ringer's solution. Measurement in bicarbonate-glucose-Ringer's solution.)

Time of exposure to H.O.P. (hr.)	...	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3
Respiration (Q_{O_2})		7.3	—	6.7	—	4.0	—	1.7
Aerobic glycolysis ($Q_G^{O_2}$)*		0.3	—	0.8	—	1.0	—	1.0
Anaerobic glycolysis ($Q_G^{N_2}$)		12.6	12.8	12.5	12.2	8.2	3.9	3.6

* R.Q. assumed equal to 1 throughout.

measurement of metabolic activity, this poisoning by oxygen is irreversible on transferring to normal pressure, both for respiration and glycolysis.

This result is important in consideration of the enzymes which are poisoned in brain tissue by oxygen.

(6) *The role of hydrogen peroxide in oxygen poisoning of brain*

Added hydrogen peroxide. Brain respiration is highly sensitive to traces of hydrogen peroxide. Table 14 shows the effect of the addition to respiring brain slices of two successive portions each of 0.2 ml. 1 : 9000 dilution of perhydrol, prepared in phosphate-Ringer's solution containing glucose. Each portion contained 0.12 mg. H_2O_2 , equivalent to

Table 14. *Effect of added hydrogen peroxide on rat-brain-slice respiration*

(Slices of rat-brain cortex suspended in phosphate-Ringer's solution containing glucose. Each manometric vessel had one slice, c. 12 mg. dry wt., suspended in 1.6 ml. The vessels had two side bulbs, from each of which at the stated interval H_2O_2 equivalent to 40 μ l. O_2 was added. CO_2 was absorbed by 0.2 ml. NaOH, as usual. A control vessel contained tissue without H_2O_2 . Gas space, oxygen at 1 atm. Temp. 37.5°.)

Period (min.)	Respiration rates (Q_{O_2})		
	No H_2O_2 added	H_2O_2 added as shown (duplicates)	
		9.9	11.1
0-20	8.1	(H ₂ O ₂ added at 20 and 50 min.)	
20-50	8.4	(Oxygen being evolved)	
50-80	7.0	(Oxygen being evolved)	
80-110	8.4	6.4	8.1
110-150	7.5	5.0	4.5
150-170	7.2	4.2	3.3
% of initial Q_{O_2} after 150 min.	89	42	30
Mean 36 %			

40 μ l. oxygen, and the total time of exposure to free peroxide lasted 60 min., during which time the peroxide was completely decomposed by the brain catalase. About 40 % of the brain respiration was poisoned by this treatment (Table 14).

This sensitivity to peroxide is no doubt associated with the low catalase content of brain tissue, and the consequently persistent effect. Fujita & Kodama (1931) found that the $Q_{catalase}$ of rabbit grey matter is 75, compared with 1700 in liver (of mouse). We obtained values of 53-73 for fresh grey matter of rat brain (Dickens, 1946). The catalase content of succinoxidase preparations made from pig heart in the usual way was so high that the addition of 0.1 ml. 1 % H_2O_2 had no measurable effect on the activity of this enzyme; the added peroxide, equiv. to 330 μ l. oxygen, was quantitatively decomposed in under 5 min. The Schales (1938) test for peroxide was quite negative shortly after the addition. On the other hand, the succinoxidase activity of homogenized rat-brain cortex was reduced to a very low level by the addition of minute traces of hydrogen peroxide (Table 15). The tissue shown in Table 15 was rather exceptionally sensitive, but poisoning of a similar order was regularly observed. Care was taken in these experiments to avoid as far as possible contamination with blood, which is rich in catalase.

Table 15. *Effect of traces of hydrogen peroxide on the succinoxidase activity of homogenized rat-brain cortex*

(Each vessel contained 40 mg. fresh wt. of cortex homogenized in 1 ml. M/30 phosphate buffer of pH 7.4. The vessels had two side bulbs, containing H_2O_2 and succinate, respectively. Water was added to the main part of the vessels to make the total volume 2 ml. Measurements in air at 37.5°, with NaOH in central well of vessel for absorption of carbon dioxide.)

Period (min.)	Control vessel:	Experimental vessels: bulbs con- tained H ₂ O ₂ equiv. to oxygen (μl.):		
	No H ₂ O ₂	3	33	333
0-10		Add H ₂ O ₂ to tissue at 0 min.; decomp. in 10 min.		
		Add succinate at 10 min.		
		Oxygen uptake (μl.) due to succinate		
0-15	50	18.5	1	3
15-60	117	41	4	9
% Poisoning of succinoxidase	64	97	93	

Is hydrogen peroxide formed in brain tissue exposed to oxygen? Amounts of H_2O_2 which poison the succinoxidase of brain (c. 5 μ g./ml. of suspension liquid; equiv. to 1.65 μ l. O_2 /ml.) are close to the lower limit of the sensitive Schales (1938) test. By working in complete darkness the chemilumi-

nescence due to the addition of 0.1 μg . H_2O_2 to brain tissue in presence of cyanide, or 1 μg . in absence of cyanide, was just detectable. On one occasion a positive reaction was observed in a blank experiment in which Ringer's solution alone was incubated in oxygen; it was attributed to autooxidation of a trace of the lanoline used for greasing the stoppers, but this could not be repeated. In spite of the very high sensitivity of this test, no positive reaction for peroxide was ever seen when brain tissue was present, with or without sodium azide or cyanide to inhibit catalase, which could be interpreted as due to peroxide formation by brain tissue. For these tests brain slices or homogenate were incubated in Ringer's solution exposed to pressures of oxygen from 1 to 4.4 atm. for 2 hr.

Lack of correlation between oxygen sensitivity and catalase content of various tissues. If the formation and accumulation of hydrogen peroxide were the primary cause of oxygen poisoning, one would expect those tissues with a low content of catalase to be highly sensitive to oxygen, and the converse. This is the case when brain and liver tissue are compared, as we have just seen, but the relationship breaks down completely when other tissues are studied. The tissues, arranged in their order of oxygen sensitivity (see Table 9) are:

brain > liver > testis > kidney > lung > muscle,

while the catalase content of these tissues, from various species, is, according to Fujita & Kodama (1931), in the ratio:

75 : 1700 : 180 : 500 : 470 : 70.

Thus brain tissue and muscle, which have respectively the highest and lowest sensitivity to oxygen, both have a very low catalase content, while liver, which is quite sensitive to oxygen, has the highest catalase content.

These facts make it difficult to believe that hydrogen peroxide could be *liberated* during the exposure of brain tissue to oxygen in amounts sufficient to explain the poisoning of brain respiration. There seems to be no very satisfactory method for eliminating the possibility that the hydrogen peroxide formed, if any, might have its destructive action localized to certain highly active centres spatially connected with the centres at which the peroxide itself originates, where it might be expected to undergo decomposition and thus escape detection.

The fact that succinoxidase of brain is so highly sensitive to peroxide, while glucose oxidation (in slices, at any rate) is more resistant, may be taken as evidence against the view that the oxygen poisoning of brain is due to formation of hydrogen peroxide; for the succinoxidase system of brain is more resistant to oxygen than is the glucose oxidation system.

(7) *Effect of oxygen poisoning of brain tissue of the addition of vitamins, coenzymes, narcotics, reducing substances, etc.*

An extensive series of experiments was made to see if oxygen poisoning could be prevented by the addition to respiring brain slices or homogenate of a wide variety of substances, coenzymes and vitamins, drugs for which beneficial effects in oxygen poisoning had been claimed, and a few other likely compounds. None of these attempts was successful, though transient prolongation of respiration was observed with some, and a few seemed to accelerate slightly the fall of brain respiration under the influence of H.O.P. (Table 16).

The effects included under the column headed 'Remarks' in Table 16 were in no case of such magnitude as to appear to warrant extended investigation. The lack of protective action of the narcotics and of bromide was disappointing in view of the marked prolongation of convulsion time observed by Marks (1944) with these substances in mice. Riboflavin and nicotinamide have been claimed by Fahr (1941) to protect guinea-pigs against convulsive effects of 4.5 atm. oxygen. Glutathione and cysteine have been stated to protect also (Massart, 1935; Fahr, 1941), but glutathione does not protect the respiration of brain tissue *in vitro* (Table 16). Marks (1944) could not obtain evidence of protective action *in vivo* with any of these substances. None of the members of the B group of vitamins tested protected brain respiration, in spite of their close connexion with carbohydrate metabolism; the effect of panthothenic acid, which was very slight, may well have been due to the calcium content of the preparation used. The effect of adenine, adenosine and cozymase in slightly prolonging the fall of brain-tissue respiration is curious but not large enough to be of much interest.

The most striking effect, one that was repeatedly observed, was the increased rate of fall of brain respiration in presence of even small traces (0.001M) of glutamic acid or glutamine. This was not due to excessive acid production by the tissue in presence of the amino-acids (cf. Weil-Malherbe, 1938), and the simultaneous presence of pyruvic acid did not remove the potentiation of oxygen toxicity by glutamate. It is possible that the effect is due to the very pronounced ability of glutamic acid to form complexes with heavy metals (Lehmann & Pollak, 1942). *In vivo* 10 mg. doses of glutamine given by injection to mice had no effect on the convulsion time (Marks, 1944).

The effect of diets rich or poor in vitamins of the B group on the resistance of the rats' brain tissue to oxygen. Since it was possible that only the prolonged administration of a substance, and its

Table 16. *Effect of various vitamins, coenzymes, drugs, etc., on the poisoning of brain-tissue respiration by oxygen*I. *Rat-brain cortex—slices.* Suspended in phosphate-Ringer's solution, with glucose (0.2 %) unless otherwise stated.

Substance added during exposure to oxygen	Conc. in Ringer's solution	Oxygen pressure (atm.)	Method*	Remarks: effect on brain poisoning by oxygen
Vitamins, coenzymes, etc.				
Aneurin	0.5 mg./ml.	2.9	A	No effect (i.e. same as control portion without addition)
Riboflavin	0.5 mg./ml.	2.9	A	No effect
Pyridoxine	2.5 µg./ml. 25 µg./ml.	2.9 2.9	A A	{ Increased rate of fall, larger with the larger dose; neither dose is toxic in air
Calcium d(+) -pantothenate	2.5 µg./ml. 5 and 20 µg./ml. 50 µg./ml.	2.9 3.4 2.9	A B A	
Nicotinamide	5 mg./ml.	2.9	A	Slight initial increase of respn. followed by usual fall
Nicotinic acid	0.5 mg./ml.	2.9	A	No effect
Ascorbic acid	0.5 mg./ml.	2.9	A	No effect
(Vitamin B complex	—	—	—	No effect: feeding experiment; Tables 4 and 17)
Adenylic acid (of muscle)	1.3 mg./ml.	2.9	A	This conc. slightly inhibits respn., but does not stop fall
Adenylic acid (of yeast)	1.3 mg./ml.	2.9	A	Same as muscle adenylic acid
Adenine	1 mg./ml.	2.9	A	A slightly delayed fall
Adenosine	1 mg./ml.	2.9	A	Initially increases resp. and delays its fall from about 140 to 180 min.
Cozymase	0.5 mg./ml.	2.9	A	Slight but delayed effect in arresting fall, after 2-3 hr. contact
Flavoprotein ('Old yellow enzyme')	5 mg./ml.	2.9	A	No effect
Metabolites				
Fructose (no glucose present)	0.2 %	2.9	A	Fall similar to that with glucose
Pyruvate	0.01 M	3.4	B	Fall slightly greater than without pyruvate
α-Ketoglutarate	0.01 M	4.8	B	Unlike glutamate and glutamine, does not affect the toxic action of O ₂
Glutamate	0.01-0.001 M	2.9 and 3.4	A and B	{ Accelerate the toxic effect of O ₂ ; the toxicity of O ₂ with both subs. is much increased with rising pressure
Glutamine	0.01-0.001 M	2.9 and 3.4	A and B	
Pyruvate + glutamate	0.01 M + 0.01 M	3.4	B	As toxic as glutamate alone
Narcotics, sedatives and miscellaneous				
Sodium bromide	0.05 %	3.4	B	No protection (2 exp.)
Luminal	0.05 %	3.4	B	No protection (2 exp.)
Chloretone	0.03 %	3.4	B	No protection
Ethanol	0.3 %†	3.4	B	Very slight protection
		4.8	B	No protection
Glutathione	0.5 mg./ml.	2.9	A	No effect
Various sulphhydryl compounds	0.5-5 mg./ml.	4.4	B	No protection
Ammonium chloride	0.19 mg./ml.	2.9	A	Increased rapidity of fall
(Metallic ions	—	—	—	see Table 18)
II. <i>Homogenized brain tissue</i>				
Nicotinamide	2.5 mg./ml.	1 atm. in laboratory as usual		{ No protection as compared with control in oxygen at 1 atm. The control in air at 1 atm. was slightly protected by cozymase; 1 exp. only
Adenylic acid (of muscle)	—			
Cozymase	—			

* Method A: Warburg manometers with observers in large compression chamber at pressure; Method B: Pressure pot apparatus. As described in text.

† 'Over 90 % of persons with this concentration of alcohol in the blood are obviously drunk' (Goodman & Gilman, 1943).

incorporation into the actual tissue, might be effective, rats were fed on a synthetic diet with or without addition of B vitamins. The B group was selected for this test because of its known importance in carbohydrate metabolism.

The basal diet consisted of: casein, 20; rice starch, 75; salt mixture, 4; cod-liver oil, 1 part. The salt mixture contained NaCl, MgSO₄, Na₂HPO₄, KH₂PO₄, CaHPO₄, ferric citrate, and calcium lactate. Fifteen g. of basal diet/rat/day was made into a paste with water, and additional water was given *ad lib*. The yeast-fed group of rats received, in addition to the above diet, dried yeast to the amount of 5 % of the basal diet. The rats ate both diets completely up to the sixth day; afterwards the rats on basal diet left uneaten about half their ration each day. The yeast-fed rats ate all their diet throughout the experiment, which lasted 37 days.

The respiratory rate of slices of the brain cortex of these rats was measured after 10, 17, 23, 30 and 37 days feeding. One rat from each dietary group was killed on each of these days, and simultaneous measurements of respiration were made for each animal in air and in oxygen at 1 atm. The results have already been presented in Table 4. The rate of fall of respiration was if anything slightly greater in the yeast-fed animals, as compared with the non-yeast group, while on the usual mixed diet the fall was a little less than in either the yeast-fed or non-yeast groups. The differences, however, are probably not significant, and a much larger number of experiments would be needed to prove their reality or otherwise. On the whole, it may be concluded that feeding B-group vitamins does not exert any protective action; on the contrary, the brain tissue of yeast-fed rats is possibly slightly more susceptible. With the same material, one experiment was made at pressure. The rats had undergone 11 days' feeding, and the respiration of cortical tissue was measured in the large compression chamber in air and in oxygen at 2.9 atm. The results (Table 17) with and without yeast feeding are very closely alike, and they provide no evidence that the richness or lack of B vitamins has affected the susceptibility of brain respiration to H.O.P.

(8) *Protection of brain respiration against the toxic effect of oxygen by divalent cations (magnesium, manganese, cobalt)*

(a) *Magnesium ions.* A hint that the divalent metallic ions might be concerned in the stabilization of brain-tissue respiration against oxygen was provided by the earlier experiments at 1 atm. oxygen or air, in which, as already mentioned (p. 149), the presence of magnesium in the Ringer's solution then used probably accounted for our earlier failure to confirm Mann & Quastel's observations with tissue slices at 1 atm. Here a physiological concentration of magnesium was present (cf. Table 1), and this seems to have been sufficient to protect against the very slightly toxic effect of oxygen observed at this pressure in absence of magnesium (Table 4).

This interpretation is supported by the results of similar measurements made at higher pressures in presence and absence of magnesium. Reference to Table 5, Exps. I and II, will show that the mean fall of respiration in these two experiments (at 2.9 atm.) is significantly lower than in the other experiments of Table 4; these first two experiments were made in magnesium-containing Ringer's solution, while the others in Table 5 were in magnesium-free Ringer's solution (of composition as given in Table 1). There is, however, quite a large fall of respiration, even when a physiological amount of magnesium is present, at oxygen pressures of this order (2.9 atm.).

By increasing the magnesium content of the Ringer's solution above the normal physiological level, still more protection of brain respiration may be obtained (Table 18). The much smaller fall of respiration in the magnesium-containing media is clearly evident, especially in the first 2½–3 hr. of the first experiment. A curious feature, which has not been further studied, is the slow but considerable acceleration of brain respiration during the compression in air, when the medium contains abnormally high amounts of magnesium. This is denoted by the minus sign of the 'fall' of respiration,

Table 17. *Respiration of brain tissue of rats fed on diets with or without yeast supplement. Measured at 2.9 atm. oxygen or air*

(Feeding period 11 days. Diets as in text. Slices of cerebral cortex in phosphate-Ringer's solution with glucose at 37.5°.)

		Respiration rate (Q_{O_2}) as % of initial rate						
Period (min.) ...	Gas	40–60	60–80	80–100	100–120	120–140	140–160	160–180
No yeast added	Air	103	108	110	97	101	101	104
	O ₂	99	100	92	83	76	66	56
	(dupl.)	102	93	88	77	69	66	55
With yeast supplement	Air	103	109	107	99	103	106	106
	O ₂	98	98	95	92	77	67	46
	(dupl.)	98	94	91	93	76	71	63

Table 18. *Effect of the magnesium content of Ringer's solution on the oxygen poisoning of brain-tissue respiration*

(Slices of rat-brain cortex suspended in phosphate-Ringer's solution of composition I (Table 1), containing Na, K and Ca; compared with slices in similar medium but containing also Mg ions. Glucose 0.2 %.)

I. Experiments in large compression chamber; pressure 2.9 atm.

Hr. of exp. ...	Oxygen					Air						
	Initial Q_{O_2}	Fall of resp. as % of initial value				Initial Q_{O_2}	Fall of resp. as % of initial value					
		1	2	$2\frac{1}{2}$	3		$3\frac{1}{2}$	1	2	$2\frac{1}{2}$	3	$3\frac{1}{2}$
Mean of all previous results (Table 5)	13.9	3.5	18	28.5	47	66	12.0	-1	0	5	-1	-1
This exp.:												
In Ringer without Mg	13.1	4	14	27	47	65	14.5	-8	-7	-1	+1	+1
With $Mg=2.7 \times 10^{-3}M^*$	12.6	5	-10	-2	18	44	11.0	-16	-35	-27	-20	-24
With $Mg=10^{-2}M$	13.6	—	-5	8	33	45	11.0	-19	-45	-18	-22	-22

* This is approx. three times normal serum Mg content (other exp. about ten times).

II. Experiment in pressure-pot apparatus; pressure 4.4 atm.

(Exposure was for 100 min. in phosphate-Ringer's solution containing glucose, with or without the addition of sufficient $m/6$ $MgCl_2$ to bring the Mg content to about seven times the normal serum Mg. The tissue was then rinsed in ordinary phosphate-Ringer's solution, without Mg, and the respiration rate measured (glucose present).)

Conditions during exposure	Q_{O_2} after exposure
In absence of Mg	3.5
In presence of $7.7 \times 10^{-3} M$ Mg	6.5

in Table 18, and is probably to be considered, in part at any rate, as a slow removal of an initial slight inhibition of respiration by magnesium as the experiment proceeds. That the protective effect of magnesium is merely due to its narcotic action seems improbable in view of the fact that other narcotics (luminal, chlorotone, bromide, alcohol) do not protect (Table 16), and because manganese and cobalt are also protective (*vide infra*), in fact much more strongly so than is magnesium.

(b) *Cobalt ions*. At this stage of the investigation the work on phosphoglucomutase, detailed in Part II (Dickens, 1946), was in progress, and we were impressed by analogies between the behaviour of brain-tissue respiration towards oxygen and magnesium, and of this enzyme towards oxygen, which inhibits it, and towards magnesium ions, which accelerate its action. The fact that the action of phosphoglucomutase is also accelerated very strongly by other metals besides magnesium (cobalt, manganese), while it is inhibited by copper and zinc ions, even in low concentrations, led us to try the effect of these metals on oxygen poisoning, with the surprising result that traces of cobalt or manganese were found to give a definite, and in some instances complete, protection of brain-tissue respiration against the toxic effect of oxygen.

The results with cobalt are shown in Table 19. Almost complete protection has been observed with concentrations of cobalt salts from $5 \times 10^{-5} M$ upwards. Lower concentrations ($10^{-5} M$) are insuffi-

cient to give more than slight protection (see Exps. I-III, Table 19). In absence of glucose following the exposure to oxygen, the respiration of brain tissue, even when protected by cobalt during exposure to H.O.P., is very low. This shows that it is a true glucose oxidation that has been preserved in the brain tissue by the presence of cobalt during exposure to H.O.P. (Exp. III, Table 19). Oxidation of lactate and pyruvate is also protected against H.O.P. by cobalt (Exp. II, Table 19). However, cobalt is unable to restore an already poisoned brain respiration (Exp. III, Table 19).

There are some variations in the value of Q_{O_2} in Table 19, even when cobalt has been present during exposure to H.O.P. (cf. Exp. I, duplicates with $2 \times 10^{-4} M$ cobalt, for example). It should be remembered, however, that the brain tissue has been exposed to quite a long period of handling (over 100 min.) before these measurements began, and the values observed are, in fact, about as good as experience would lead one to expect. One noteworthy feature is the gradual rise of Q_{O_2} sometimes seen after transfer from cobalt-containing Ringer's solution to cobalt-free (e.g. Table 19, Exp. III, on transferring from $2 \times 10^{-4} M$ cobalt to Ringer's solution with glucose; period 0-30 and 30-60 min. after transfer). At 1 atm. of oxygen, cobalt does not affect brain respiration (glucose present) until the concentration rises to $10^{-3} M$, when it is very slowly toxic. The concentrations used in the above experiments were all below this level.

(c) *Manganese ions and comparison with some other metals*. Among the other metals tested, manganese was the only one with a protective action

Table 19. *Effect of traces of cobalt in protecting brain-cortex respiration against the toxic effect of oxygen*

A. Slices of rat-brain cortex suspended in phosphate-Ringer's solution containing *glucose, with or without cobalt*. After compression under oxygen, the slices were rinsed, and the Q_{O_2} measured, in cobalt-free Ringer's solution at 1 atm. The control sample was maintained throughout in air at 1 atm.

Oxygen pressure: 4.4 atm. for 100–105 min. Method B.

Exp. no.	Cobalt salt	Q_{O_2} measured after exposure in presence of following cobalt concentrations (M)						Air control (no cobalt)
		0	10^{-5}	5×10^{-5}	10^{-4}	2×10^{-4}	3×10^{-4}	
I	Acetate (dupl.)	3.6	—	—	7.5	11.3	—	—
		4.6	—	—	—	7.2	—	—
II	Acetate (dupl.)	2.0	—	9.1	6.4	8.6	9.4	8.3
		2.6	—	—	—	—	—	—
III	Acetate							
	0–30 min.	1.8	3.0	—	—	5.3	—	8.2
	30–60	1.9	2.9	—	—	7.0	—	6.1
	60–90	1.9	2.3	—	—	7.5	—	5.2
	Same brain: but after exposure to H.O.P. transferred into <i>glucose-free</i> Ringer for the following measurements of tissue respiration:							
	0–30	2.8	—	—	—	2.7	—	9.2
	30–60	1.9	—	—	—	2.0	—	5.0
	60–90	1.8	—	—	—	1.4	—	1.5
IV	Sulphate	3.1	—	—	—	7.7	—	—

B. Slices of rat-brain cortex in phosphate-Ringer's solution containing *lactate or pyruvate, with or without cobalt*. After compression under oxygen at 4.4 atm. for 100 min., the slices were rinsed, and the measurement of Q_{O_2} made, in cobalt-free Ringer's solution containing the same substrate as during the compression.

Substrate ...	Q_{O_2} after exposure to H.O.P.		
	Glucose (0.2 %)	Lactate (0.01 M)	Pyruvate (0.01 M)
Without added cobalt	3.5	5.0	1.8
Cobalt, 2×10^{-4} M, present during exposure to H.O.P.	—	9.2	8.2

C. Lack of restoration of oxygen poisoned respiration by subsequent addition of cobalt. (Slices of rat-brain cortex suspended in phosphate-Ringer's solution with glucose. Poisoned by 100 min. exposure to 4.4 atm. oxygen.)

$$Q_{O_2} \begin{cases} \text{Before adding Co} & = 2.1 \\ \text{After adding CoSO}_4, 2 \times 10^{-4} \text{ M} & = 1.5 \end{cases}$$

Another similar exp. at 5 atm. oxygen for 80 min. gave a similar result.

comparable with, or even surpassing, that of cobalt. The concentrations of manganese necessary were very similar to those of cobalt; 2×10^{-4} M-manganese gave nearly complete protection of brain respiration against the toxic effect of 4.4 atm. of oxygen for 100 min. The protection was very sustained; after transfer to manganese-free Ringer's solution at 1 atm., Q_{O_2} remained virtually constant for 2 hr. when this concentration of manganese was present during exposure of brain slices to H.O.P. (Table 20). Lower concentrations of manganese (5×10^{-5} M) gave quite a good degree of protection, but after transfer to fresh Ringer's solution the respiration rate was rather less constant than when the higher concentration of manganese was present during incubation under H.O.P.

Nickel salts and iron salts in 2×10^{-4} M-solution

gave a slight degree of protection (Table 20). Ferrous salts could not, of course, be present with these high oxygen pressures, and ferric sulphate was used for these experiments: it was largely precipitated as phosphate on adding to Ringer's solution, but evidently enough remained in solution to give some protection. These two metals exerted only a transient effect, and the respiration of brain tissue fell off fairly rapidly on transferring the tissue to ordinary Ringer's solution after H.O.P. Copper salts gave even less protection, and the fall of respiration after transfer was still greater. Zinc ions in the highest non-toxic concentration (2×10^{-4} M), as determined at 1 atm. of oxygen, gave no protection against 4.4 atm. H.O.P. for 100 min., and were perhaps slightly toxic under H.O.P. (cf. Table 20).

Table 20. *Effect of traces of other metals (Mn, Ni, Fe, Cu, Zn) on the stability of brain respiration towards H.O.P.*

(Conditions as in Table 19. Slices of rat-brain cortex in phosphate-Ringer's solution containing glucose were exposed to oxygen pressure in the presence of the concentration of metal salt named below. After the exposure, the slices were rinsed and transferred to ordinary Ringer's solution containing glucose, for measurement of the remaining respiratory activity.)

Exp. no.		Oxygen pressure: 4.4 atm. for 100 min.					
I	Salt added ...	None	MnSO ₄	MnSO ₄	MnSO ₄	CuSO ₄	MgCl ₂
	Molarity of salt	0	2 × 10 ⁻⁴	5 × 10 ⁻⁵	10 ⁻⁵	2 × 10 ⁻⁴	7.7 × 10 ⁻⁵
			Respiration rates (Q _{O₂}) after exposure				
II	Salt added ...	None	8.0	6.3	6.8	4.5	6.5
	Molarity of salt	0	(well main- tained)	(falling)	(falling)	(falling rapidly)	(maintained)
			Protection by salt added				
II	Salt added ...	None	CoSO ₄	NiSO ₄	MnSO ₄	Fe ₂ (SO ₄) ₃	ZnSO ₄
	Molarity of salt	0	All 2.3 × 10 ⁻⁴				
			Respiration rate (Q _{O₂}) after above exposure				
		3.1	7.7	4.9	7.8	4.8	2.8
	Protection		+++	+	+++	+	-

DISCUSSION

These experiments have established the fact that the respiration of isolated cerebral cortical tissue is progressively and irreversibly poisoned by exposure to high oxygen pressures. Even at 1 atm. of oxygen, this effect slowly becomes evident when the respiration is compared with that in air. At pressures above 1 atm. the rate of poisoning steadily increases with rising oxygen pressure, and the time required to produce a given degree of poisoning is approximately inversely proportional to the oxygen pressure.

The time course at any given pressure of this poisoning is of a sigmoid type, and evidence has been presented that it conforms to a probability curve, with time as a linear abscissa and percentage poisoning as ordinate. Action curves of this type are familiar in pharmacology and their interpretation has been reviewed by Clark (1933) and Bliss (1935); the latter, especially, adopts the viewpoint that such curves are 'primarily descriptive of the variation in susceptibility of the individuals of a population' (Bliss, 1935). In the present instance, the assumption would be that the brain tissue is composed of cells whose individual susceptibility to oxygen is arranged according to a normal distribution curve; as the number of cells in even a small slice of tissue is large our experiments yield directly curves of this type. It is, however, worth noting that the interpretation of such curves is still controversial and that closely similar curves can be derived from different premises, e.g. autocatalytic and chain reactions and adsorption equations. But if the 'statistical' interpretation be adopted it would

imply that among the brain centres (according to Bliss, cells) a small proportion is very highly sensitive to oxygen.

Relationship of onset of symptoms to degree of oxygen poisoning in vitro. The importance of this interpretation of the measurements is that the symptoms of oxygen poisoning *in vivo* might be related to a stage of brain poisoning when the respiration of the brain as a whole would be only very slightly affected.

The time-pressure relationships for oxygen poisoning in divers have been investigated by several authors with varying results. Behnke *et al.* (1935) give as the 'comparatively safe' periods for a healthy man in pure oxygen, 4 hr. at 1 atm., 3 hr. at 2 atm., 2 hr. at 3 atm. and <45 min. at 4 atm. Under somewhat different conditions, Donald (1943), and Brown, Downman, MacIntosh & Williams (1944) observed toxic effects after much shorter exposures, and the former author obtained results conforming to one or other of the two equations:

$$t = \frac{1086}{d - 39.7} \quad \text{or} \quad \log t = 2.368 - 0.0152d,$$

where t min. is the mean time of onset of symptoms and d ft. the depth of dive when pure oxygen is breathed.* There is an obvious resemblance between these equations and the relationship described in the present work between the total oxygen pressure (P atm.) and the time (t min.) for a given

* The former of these equations was based on preliminary observations, and a more elaborate statistical analysis of Donald's figures was later made by Haldane & Spurway (1944).

percentage poisoning of brain respiration, which may be written

$$t = \frac{k}{P-a} \quad (\text{or } \log t = k' - \log (P-a)),$$

where k and k' are constants and a has a value between 0 and 1 (cf. p. 154).

In any attempt to compare such data one is confronted with the fact that the actual oxygen tensions at and within the tissue are unknown, both *in vivo* and *in vitro*. Neglecting this, and also the different species (man and rat), calculation shows that whilst the estimates of onset of toxic symptoms in oxygen at 3 atm. (= 66 ft. depth) vary from about 2 hr. (Behnke *et al.* 1935) to 41 or 23 min. (equations of Donald's observations, above), the percentage poisoning of the respiration of isolated brain tissue exposed to the same overall pressure of oxygen would be approximately (Fig. 4), after 2 hr. 20 %, and after 30 min. < 2 %. If the probable assumption is made that, for a similar external pressure of oxygen, the oxygen tension in the brain tissue *in vivo* is considerably less than that prevailing within the tissue *in vitro*, it is clear that only a low percentage poisoning of brain respiration could be expected to accompany the first clinical symptoms of oxygen poisoning. This statement would be invalidated if human brain tissue were much more sensitive to oxygen than rat brain, but as the convulsion times in man and rat are not widely different, this seems unlikely.

In spite of the low percentage poisoning of brain respiration which our measurements would lead us to expect as the outcome of periods of exposure to oxygen which clinically produce symptoms and signs of oxygen poisoning, there is a good deal of support for the view that these events are in fact related: (1) the pressure-time relationships for the two are closely similar; (2) nervous tissue is exceptionally sensitive to oxygen, both *in vivo* and *in vitro*; (3) oxygen inhibits particularly the oxidation of carbohydrate and its intermediates (glucose, fructose, lactate, pyruvate), which are known to be the main energy sources in brain metabolism.

A closely similar dilemma is encountered in attempts to explain the effects of narcotics on the basis of the inhibition of brain respiration which they produce *in vitro*. For example, a concentration of ethyl urethane which causes deep narcosis produces only 6 % inhibition of brain respiration; but, just as with oxygen poisoning, the inhibition produced by narcotics is strongest in nervous tissue, and there is evidence that the oxidation of carbohydrate is particularly affected (Quastel, 1939).

It will be assumed in what follows that these slight poisonings are causally related to the narcosis or oxygen toxicity which accompany them *in vivo*, although direct proof of this would be exceedingly

difficult to obtain. Two main lines of interpretation are available: (a) anatomical, (b) chemical. On the former it would be assumed that the poisoning of certain hypersensitive units was involved; these units could be cells performing specialized functions (cf. the views of Bliss (1935) already discussed), or else in Quastel's view (1939) 'specific areas or centres' of the brain. Quastel (1939) suggested that interference by narcotics with glucose oxidation at these centres caused, in effect, a local anoxaemia. The chemical explanation is fundamentally different, and is that adopted by Michaelis & Quastel (1941). It is assumed that narcotics or oxygen would interfere with the catalytic function of some component of brain tissue. In their later work on narcosis, Michaelis & Quastel (1941) consider that the narcotic interferes with the catalytic function of 'either a special flavoprotein or some component of the tissue respiratory system which plays an intermediate role between flavoprotein and cytochrome oxidase'. The chemical type of interpretation will be adopted in this discussion of oxygen poisoning, and it will be seen that there are strong arguments in its favour.

Relationship of oxygen poisoning to intermediary metabolism. The fact that several of the enzymes believed to be concerned in the carbohydrate metabolism of brain are known to be oxygen-sensitive (cf. Dickens, 1946) makes it attractive to consider the possibility that one such enzyme might be more affected than the others, and that oxygen poisoning might primarily be due to this. The relevant facts are: (1) oxidation of glucose, fructose, lactate and pyruvate is poisoned; (2) oxidation of succinate is more resistant, particularly in slices; (3) poisoning of the (predominantly carbohydrate) oxidation in brain tissue results in loss of ability to convert glucose to lactic acid (p. 158); (4) the poisoning is irreversible; (5) addition of a wide variety of coenzymes and related substances failed to prevent poisoning of brain respiration by oxygen; (6) narcotics also failed to protect; (7) addition of traces of magnesium, manganese or cobalt ions during the exposure to oxygen had a strongly protective action. Ability to oxidize glucose, lactate and pyruvate was thus protected (tested for cobalt), while manganese protected succinate oxidation against oxygen poisoning in homogenized brain. These metals were unable to restore an already poisoned tissue respiration.

The following are the resemblances and differences between oxygen poisoning and narcosis of brain tissue: narcotics poison the oxidation of glucose, lactate and pyruvate; that of succinate is more resistant. The lactic fermentation of brain tissue is only slightly poisoned by concentrations of narcotics which strongly inhibit respiration. The effect of narcotics is largely reversible (for lit. see Quastel,

1939). The effect of manganese and cobalt ions on narcotized brain has not been studied, but magnesium ions, which are themselves narcotic, inhibit brain respiration when they are present in excess; in physiological concentrations magnesium stabilizes brain respiration at a constant level (Dickens & Greville, 1935).^{*} While narcotics generally stabilize the tissue respiration at a low level (after a short preliminary period of adsorption), the toxic effect of oxygen, being irreversible, is continuous and progressive with the time of exposure.

It is suggested that the primary effect in oxygen poisoning of brain tissue may be the inhibition of pyruvic oxidase. The secondary effects of this would be the general poisoning of carbohydrate oxidation, since all known paths of carbohydrate oxidation converge at the stage of pyruvate. This would explain why oxygen-poisoned brain cannot oxidize glucose, fructose or lactate. The partial persistence of succinate oxidation after oxygen poisoning is probably explained by the partial survival of succinoxidase activity; probably R.Q. measurements would reveal that oxidation of succinate is incomplete in poisoned brain tissue. (Some of the results of Laser (1942) on variations of the R.Q. and/or aerobic acid production from succinate could also be interpreted in a similar way, although as only the 'apparent R.Q.' was measured these results are equivocal.) It is not so obvious why inhibition of pyruvic oxidase should destroy the ability of brain tissue to form lactic acid anaerobically (p. 158), even though the oxidase is not a component of the glycolytic system, but consideration shows that irreversible inactivation of the pyruvic oxidation system would be expected to lead to this result. Inactivation of this system would prevent the resynthesis of adenosine triphosphate, for Ochoa (1943) has shown that the oxidation of one-molecule of pyruvate is capable of yielding no less than fifteen high-energy phosphate bonds, a considerable pro-

portion of the total number formed in carbohydrate oxidation. Dickens & Greville (1933) observed the loss of glycolytic power of brain tissue after its exposure without substrate to anaerobic conditions even for a few minutes, and this effect was shown by Macfarlane & Weil-Malherbe (1941) to be due to loss of adenosine triphosphate; both effects were associated in time and were irreversible. Consequently, inactivation of the normal resynthesis of adenosine triphosphate resulting from poisoning of the pyruvic oxidase system is the probable explanation of the loss of ability of oxygen-poisoned brain tissue to form lactic acid anaerobically.

Powerful support for this view is provided by known properties of the enzyme system. Pyruvic oxidase of animal tissues, including brain, is a complex system in which the intermediate steps are not yet fully understood. The greater stability of the bacterial enzyme system have enabled Lipmann (1939*a, b*) to show that, in addition to the apoenzyme ('protein'), inorganic phosphate, diphosphoaneurin, a flavin, and (cf. Still, 1941) manganese, magnesium or cobalt are necessary for its activity. The primary reaction products are acetyl phosphate, the source of energy-rich phosphate bonds, and carbon dioxide. Barron (1936), Peters (1940) and Barron & Singer (1943, 1945) have brought forward evidence that this enzyme requires an intact —SH group to be present for activity. Pyruvic oxidase from gonococci is inactivated by exposure to gaseous oxygen (Barron, 1936), even at ice-box temperature. Animal pyruvic oxidase also requires magnesium or manganese (Ochoa, 1939; apparently cobalt was not tested).

These properties fit admirably those expected in the enzyme whose inactivation would account for oxygen poisoning, very strong support being provided by the inactivation observed with gaseous oxygen by Barron. This is reinforced by the fact that the same three metals that activate bacterial pyruvic oxidase (magnesium, manganese and cobalt) also protect brain-tissue respiration against oxygen (p. 162), other metals tested being far less effective. In view of the known —SH character of this enzyme and the known ability of these metals to form co-ordination compounds, there can be little doubt that the protective action of the metals against the toxic effects of oxygen is in some way associated with the preservation of essential —SH groups within the enzyme by these metals during the exposure of pyruvic oxidase to oxygen. If this is correct—and it should not be difficult to prove by direct experiment using bacterial pyruvic oxidase—the —SH group of pyruvic oxidase is almost certainly the seat of oxygen poisoning; although it would still remain to be shown that, in addition to pyruvic oxidase, other enzymes sensitive to oxygen and concerned in carbohydrate metabolism are not also inactivated: some of these other

^{*} The present work may be regarded as showing that oxygen is a 'stress factor', which reveals the need for trace metals (manganese, cobalt, in addition to magnesium) to stabilize fully the respiration of brain tissue. Hitherto the need for such substances had been shown in isolated enzyme systems. Brain tissue contains manganese in about 10^{-5} M. concentration (Lund, Shaw & Drinker, 1921), while human whole blood has one-half to one-tenth of this amount (Reiman & Minot, 1920). These are not far below the 'protective' concentrations required in magnesium-free Ringer's solution. It is suggested that for measurement of tissue respiration, which is usually done in 1 atm. O_2 to increase oxygen penetration, the Ringer's solution should contain approximately 10^{-5} M. manganese sulphate. A similar amount of cobalt salt might also be added. The improvement in Krebs & Henseleit's saline (Table 1) due to its content of magnesium has been demonstrated in this paper, and the above additions would probably reinforce this action (cf. Tables 18–20).

enzymes are considered in the following paper (Dickens, 1946).

Some of the resemblances and differences between oxygen poisoning and narcosis now become capable of explanation. Pyruvic oxidase is strongly inhibited by chloretone (Michaelis & Quastel, 1941), and these authors assume that probably the flavo-protein component is that attacked by the narcotic. It is assumed that this action would be reversible, since narcosis is in general a reversible effect. In the hypothesis of oxygen poisoning just advanced, an entirely different group of the same enzyme complex, the —SH component, is considered to be that poisoned by oxygen, and in brain tissue this effect is irreversible, as would be expected, on removal of the high oxygen pressure. That it has not yet been possible to reverse it by reducing compounds such as cysteine and glutathione is not surprising in view of the great difficulty often encountered in reactivations of this kind (cf. Barron & Singer, 1945). The stable succinic dehydrogenase is reactivated, apparently rather slowly, by reduced glutathione (Hopkins & Morgan, 1938); but in cases where oxygen is the inactivating agent there may be a further complication due to formation of sulphoxides, etc. It seems not impossible that reactivation of an oxygen-inactivated enzyme in this way might occur more readily *in vivo*, where there are reserves of glutathione actually within the cell, and the whole architecture is designed to protect as far as possible the integrity of the components. Some such explanation might account for the reversibility of the signs of oxygen poisoning *in vivo*. The sensitivity to oxygen increases with the degree of disruption of the tissue, as has been shown (p. 155), and even in the tissue slice the conditions are far from physiological. It will be shown (Dickens, 1946) that oxygen poisoning is greatly influenced by trace substances, which, like the protective metallic ions described in this paper, might easily be lost to the tissue *in vitro* by diffusion into the suspension medium.

The fact that oxygen poisoning and narcosis act through the same enzyme complex, pyruvic oxidase, though at different centres, would explain the many similarities in these two inhibitions of brain metabolism. In this connexion it is of great interest that oxygen poisoning and narcosis are to some extent antagonistic *in vivo*. Thus breathing oxygen at pressure diminishes anaesthetic effect (Davidson, 1926; Bean, 1931), while Marks (1944) has shown that bromides and (cf. Hederer & Andre, 1940) barbiturates have a marked protective action in mice, preventing the onset of convulsions under oxygen pressure.

Such antagonisms are usually referred to competitions for the same enzyme, but if the suggestions made above are correct, this particular example is

unusual in that the two inhibitors—oxygen and the narcotic—are presumed to be adsorbed at different centres within the enzyme complex. Unfortunately, neither of these effects has been demonstrated *in vitro* upon brain metabolism, and we were unable to protect the respiration of isolated brain tissue by sodium bromide, luminal, chloretone, or ethanol (p. 160). Soskin & Taubenhaus (1943) showed that succinate, which remains oxidizable in narcotized brain tissue and also in oxygen-poisoned tissue, can act as an antidote to barbiturate poisoning, presumably because its oxidation maintained the energy supplies (and perhaps the resynthesis of energy-rich phosphate bonds?) in the narcotized tissue: Marks (1944), however, was not able to protect mice against oxygen poisoning by administration of succinate, and succinate is not protective *in vitro* (p. 157). Nor has the original observation been confirmed (Beyer & Latven, 1944; Lardy, Hansen & Phillips, 1944).

There are also curious anomalies in the effect of metal ions on oxygen poisoning *in vivo* as compared with that on isolated brain tissue. In animal tests, Marks (1944) found that subcutaneous injection into mice of salts of calcium, magnesium and cobalt in doses (expressed as free metal) of 10, 4 and 0.15 mg. respectively had a definite protective action against oxygen convulsions. This result agrees with the powerful effect of cobalt and the weaker effect of magnesium in protecting brain respiration *in vitro*; calcium was also sometimes slightly protective. But Marks also found that manganese salts—which are similarly active to cobalt in protecting brain respiration, and also, unlike cobalt, protect the succinoxidase system—did not protect mice against oxygen convulsions. The action of zinc salts is even more remarkable, for in the concentrations tested it did not protect brain respiring *in vitro* under high oxygen pressures, while Marks (1944) found that it significantly protected mice against oxygen convulsions in doses of 0.2 and 1.0 mg.*

* Our experiments suggest that it may be possible to use therapeutic effects of this kind as indicators of the enzymes concerned, and it would be highly desirable to see if other types of convulsion are affected by such metals. For example, epileptics are stated to have benefited from administration of magnesium salts (among many other substances), but cobalt and manganese do not appear to have received attention (for lit. see Penfield & Erickson, 1941). Zinc oxide has been used for this purpose (as Dr C. B. B. Downman pointed out to me), but is 'probably useless' (Sollman, 1942). Even though therapeutically disappointing, such experiments might perhaps indicate deficiencies of certain metallo- or —SH enzymes of the nervous system, or in toxic convulsions their poisoning.

Conversely, a study of enzyme deficiencies in diseases of cattle and sheep due to dietary deficiencies of cobalt and manganese might be of value, particularly if oxygen susceptibility could be made use of as a 'stress factor' (cf. footnote, p. 167).

In spite of these anomalies, the balance of evidence is that oxygen poisoning is connected with the inactivation of an —SH enzyme or enzymes, that the most likely single enzyme whose poisoning would account for most of the metabolic effects is a component of the pyruvic oxidase system, and that the enzyme which is inactivated by oxygen probably needs for its activity in addition to the —SH group one of the metals cobalt, manganese or magnesium. The possibility that other enzymes are simultaneously inactivated cannot be excluded, but as far as the metabolic changes are concerned it does not seem necessary that their poisoning need be assumed, except as a secondary consequence. It must be admitted that the discrepancy between Marks's (1944) observations on protection by metals *in vivo* and the present findings *in vitro* have not yet been satisfactorily accounted for, and unless it be assumed that certain of these metals behave differently in the living animal and in isolated tissue their explanation would seem to involve the inactivation of another enzyme system also. The complex nature of pyruvate oxidation in animal tissues may be found to account for some of the difficulties, and as the component reactions of this system are now rapidly being elucidated, it should be possible in the near future to investigate their individual susceptibilities to oxygen, their protection and activation by metals, and their inhibition by narcotics. In this way a clearer understanding of the mechanism and reversal of both oxygen poisoning and narcosis may reasonably be anticipated.

SUMMARY

1. A description is given of the methods evolved for the measurement of tissue metabolism and of enzyme activity during and after exposure to high oxygen pressures (i.e. above 1 atm.; abbreviated H.O.P.).

2. The respiration of brain tissue is slowly and irreversibly poisoned by exposure of the isolated tissue to H.O.P. Even at 1 atm. very slow poisoning by oxygen occurs, and above this pressure the rate of poisoning steadily increases. The time t of exposure necessary to produce a given percentage poisoning is approximately inversely proportional to the oxygen pressure P for the range $P = 1-5$ atm. Values are given illustrating the relationship.

3. The course of the oxygen poisoning of brain-tissue respiration proceeds according to a sigmoid curve when the time of exposure is plotted as a linear ordinate, and has the form of a probability curve. The interpretation of this time-action curve is fully discussed.

4. No evidence was obtained clearly showing that nitrogen of the air exerts a protective action as distinct from a diluent effect, at the pressures used.

5. No restoration of brain metabolism was observed when the oxygen-poisoned tissue was returned to normal pressure, nor could it be effected by any of the methods tried.

6. From all the data it is concluded that if the oxygen poisoning of brain respiration is, in fact, responsible for the convulsions produced by H.O.P., the clinical effects must occur when only a small percentage of brain respiration has been poisoned by oxygen. The analogy with some narcotics in this respect is emphasized.

7. The order of sensitivity to oxygen of the respiration of various rat tissues is as follows:

Brain cortex (finely divided) > Brain cortex (slices) > Spinal cord > Liver > Testis > Kidney > Lung > Muscle.

8. In addition to poisoning the oxidation of glucose, oxygen also poisons the ability of brain tissue to oxidize fructose, lactate and pyruvate. When the tissue is in the form of slices, and particularly if glucose is present during exposure to H.O.P., the ability to oxidize succinate is much less poisoned than that observed for the above substrates.

9. Under the influence of H.O.P. the ability of brain tissue to form lactic acid falls off at about the same rate as the ability to oxidize glucose, and the effect is likewise irreversible.

10. Brain respiration is poisoned by traces of hydrogen peroxide added to the tissue. Succinate oxidation is perhaps even more sensitive to hydrogen peroxide. The oxygen susceptibility of various tissues has no relationship to the amounts of catalase which they contain, whereas poisoning by hydrogen peroxide is much less in a catalase-rich tissue (liver) than in brain, which is very weak in catalase. No accumulation of hydrogen peroxide in brain tissue after its exposure to H.O.P. could be demonstrated by a sensitive test. It is concluded that evidence that H.O.P. poisoning of brain is due to accumulation of hydrogen peroxide is lacking.

11. Addition of the following had no great effect on the course of oxygen poisoning of brain: (a) vitamins B₁, B₂, B₆ and C, pantothenic acid, nicotinamide, nicotinic acid, adenylic acid (of muscle or yeast), adenine, adenosine, cozymase, flavoprotein; (b) fructose, pyruvate, α -ketoglutarate; (c) sodium bromide, luminal, chlorotone, ethanol; (d) glutathione and other —SH compounds, ammonium chloride.

12. Glutamic acid or glutamine (0.001M) increased the rate of fall of respiration of brain tissue under H.O.P.

13. Feeding rats on diets rich or poor in B vitamins had no significant effect.

14. The respiration of slices of brain cortex is protected against the toxic effect of H.O.P. by the

presence of salts of certain divalent metals. These metals, in the order of their efficiency, were: $Mn > Co > Mg > Ca$. The activities of manganese and cobalt were similarly high; calcium was much weaker than the other three. In addition, iron, nickel and occasionally copper were very slightly protective: zinc did not protect. The concentration of magnesium in normal serum is protective but higher concentrations are more so; with this metal protection is incomplete. Cobalt and manganese were highly active in $5 \times 10^{-5} M$ -solution and slightly higher concentrations often gave almost complete protection. Cobalt protected the oxidation of glucose, lactate and pyruvate by brain tissue; manganese protected succinate oxidation in homogenized brain. These metals are unable to restore an already poisoned respiration.

15. The general significance of these results is discussed. It is suggested that oxygen may poison primarily the pyruvate oxidase system, probably attacking the essential —SH group of the enzyme, the latter being assumed to be both activated and also protected against oxygen by the three metals magnesium, manganese and cobalt. Inhibition of pyruvic oxidase might be expected to interfere with the oxidation of glucose, fructose and lactate, and,

by causing a block in the regeneration of adenosine triphosphate, to inhibit the ability of oxygen-poisoned tissue to form lactic acid from glucose. It is not, however, excluded that other oxygen-sensitive enzymes may not be also poisoned.

Attention is drawn to the parallelism between the action of narcotics and of oxygen on cell metabolism, and also to some points of difference. Both interfere with pyruvate oxidation, but there is some reason to believe that they act upon different links in the enzyme complex.

This work was done during the period March 1943–March 1944 at the request of the Royal Naval Personnel Research Committee, Medical Research Council, at the National Institute for Medical Research, Hampstead. The experiments of Mann & Quastel (1946), which are published in an accompanying paper, were the starting point of this work and led directly to my being invited. I am indebted to Dr William C. Stadie for informing me in advance of publication of some of his work and granting permission for its citation here. I would like to thank the Medical Research Council for supporting this investigation and permitting this publication, and I wish to thank also my colleagues at Hampstead, especially Dr G. L. Brown, for many helpful discussions and for introducing me to life under high pressure. Mr A. W. Spratt accompanied me in the 'dives' and rendered admirable technical assistance.

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The Toxic Effects of Oxygen on Brain Metabolism and on Tissue Enzymes

2. TISSUE ENZYMES

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The evidence in Part 1 (Dickens, 1946) enables the following working hypothesis of the mode of action of oxygen on brain metabolism to be advanced with some confidence.

(1) Exposure to unphysiologically high tensions of oxygen causes irreversible damage to brain respiration and glycolysis.

(2) This damage is sustained by the enzymes themselves rather than by their coenzymes, as shown by the lack of general protection in presence of excess of the more important coenzymes.

(3) The following conditions tend to protect against oxygen poisoning: Presence of (a) glucose or (as shown in this paper) manganese ions, which protect succinoxidase in finely divided brain tissue; (b) ions of manganese, cobalt, or magnesium (and probably calcium), which protect the whole oxidative system in brain slices, with decreasing activity in the order of the above series.

(4) The brain enzymes poisoned by oxygen are mainly those concerned in carbohydrate metabolism. Oxidation of glucose, fructose, lactate and pyruvate is inhibited, while the succinoxidase system, at least in intact tissue (slices) is much more resistant. There is support for the view that the inactivation of an —SH component of the pyruvate oxidase system may be a primary stage in oxygen poisoning, the resulting interference with resynthesis of adenosine triphosphate could then lead to general breakdown of aerobic and anaerobic carbohydrate metabolism.

(5) It is, however, not excluded that, in addition to the pyruvate oxidase, another and earlier stage of carbohydrate metabolism might be inhibited, thus poisoning directly the oxidation of glucose and fructose.

It is hoped later to study the effect of oxygen on components of the pyruvic oxidase system. In the

present paper, some general evidence is collected on the mode of inactivation by oxygen of some other enzymes, particularly those which, like pyruvate oxidase, may play a part in brain metabolism. These experiments are of a preliminary and exploratory character, and serve mainly to define some conditions under which this type of inhibition may be expected to occur, and to investigate some examples of protection against oxygen poisoning in enzyme systems.

The general nature of the inactivation of enzymes by oxygen

A list has been compiled of enzymes known to be sensitive to oxygen, or capable of inactivation by comparatively mild oxidants, and therefore open to the suspicion of being oxygen-sensitive under the right conditions (Table 1). These observations were widely scattered, and probably others could be added: the only review found to bear on this subject, apart from the excellent one by Stadie, Riggs & Haugaard (1944) which appeared after this work was completed, is that of Hellerman (1937), which is limited to hydrolytic enzymes. As this author rightly remarks: 'In most enzyme studies, the effect of an ever present reagent, the oxygen of the air, has been generally neglected.'

There is another reason why such a list is inevitably incomplete, and that is the enormous effect on oxygen susceptibility of traces of contaminants, especially —SH compounds and metallic ions, but also other substances less well defined. The following