

4. The percentage of a dose of phenylacetic acid excreted in 24 hr. conjugated with glycine was 53, and with glucuronic acid 5. The corresponding values for phenylacetamide were 55 and 4, respectively.

5. No significant increase in the excretion of ethereal sulphate was observed after the adminis-

tration of any of the four compounds under investigation.

We wish to express our thanks to Mr T. M. Dauncey and Mr P. S. W. Wilkins for assisting with some of the analyses.

Part of the cost of this work was defrayed by a grant from the Lady Scott-Moncrieff Fund of the University of Birmingham.

#### REFERENCES

- Baumann, E. & Herter, E. (1877). *Hoppe-Seyl. Z.* 1, 244.  
 Folin, O. (1905-6). *J. biol. Chem.* 1, 131.  
 Gonnermann, M. (1902). *Pflug. Arch. ges. Physiol.* 89, 493.  
 Hanson, S. W. F., Mills, G. T. & Williams, R. T. (1944). *Biochem. J.* 38, 274.  
 Kanzaki, I. (1932). *J. Biochem., Tokyo*, 16, 105.  
 Nebelthau, E. (1895). *Arch. exp. Path. Pharmacol.* 36, 451.  
 Nencki, L. v. (1873). *Arch. exp. Path. Pharmacol.* 1, 420.  
 Peters, J. P. & Van Slyke, D. D. (1932). *Quant. Clin. Chem.* 2, 449. London: Baillière, Tindall and Cox.  
 Quick, A. J. (1926). *J. biol. Chem.* 67, 477.  
 Raiziss, G. W. & Dubin, H. (1915). *J. biol. Chem.* 20, 125.  
 Salkowski, E. (1877). *Hoppe-Seyl. Z.* 1, 1.  
 Tulane, V. J. & Lewis, H. B. (1933). *J. biol. Chem.* 103, 151.  
 Weichselbaum, T. E. & Probst, J. G. (1938-9). *J. lab. clin. Med.* 24, 636.

## Toxic Effects of Oxygen and of Hydrogen Peroxide on Brain Metabolism

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(Received 23 October 1945)

This paper describes the nature of the toxic action of oxygen upon the respiration of minced brain tissue, and gives an account of experiments undertaken to discover how oxygen at 1 atm. pressure inhibits brain respiration. These experiments are of a preliminary nature and the conclusions therefore tentative.\*

### RESULTS

#### *Rates of respiration of minced rat brain tissue in air or in oxygen*

The respiration of minced rat brain was examined in a Warburg manometric apparatus at 37° in an atmosphere of air or of oxygen. The tissue was minced with a scalpel or scissors, as quickly as possible after removal of the brain from the animal. It was well mixed with four times its weight of 0.9% (w/v) NaCl solution, and 1 ml. of the suspension was added to a sodium phosphate buffer (pH 7.4

0.02M)-Locke medium (NaCl 0.13M; KCl 0.004M; CaCl<sub>2</sub> 0.002M) in a Warburg manometric vessel. The rates of oxygen uptake were recorded either in the absence of any added substrate or in the presence of 0.027M-sodium lactate or 0.027M-sodium pyruvate or 0.05M-sodium succinate. When glucose was added, its final concentration was 0.01M.

In Table 1 are typical results showing the rates of respiration of the tissue under the varying conditions. In the absence of added substrate, the rate of respiration of the minced tissue diminishes rapidly with time, falling off more rapidly with oxygen than with air, and the rate of fall is greatest in the third hour. The respiration in the third hour is, with air, about 35% of that in the first hour, whilst, with oxygen, it is about 20% of that in the first hour. At the end of the third hour, the tissue is respiring about twice as rapidly in air as in oxygen.

\* The work was discontinued early in 1941 to enable the authors to undertake other scientific work, and was reported to the Medical Research Council in the same year in view of the practical importance of toxic effects of oxygen at high pressure. Publication of the results was not allowed at the time. Subsequent to the finding of the facts stated in this paper came the publication of Elliott & Libet (1942) whose results largely confirm those stated here, and the review on oxygen poisoning by Stadie, Riggs & Haugaard (1944). Our results form the starting point of the more exhaustive work of Dr F. Dickens whose papers follow this (Dickens, 1946a, b).

When glucose is present as substrate, the same phenomenon is seen (Exps. 3 and 4, Table 1), the rate of fall of respiration in the third hour with oxygen present being much greater than with air present. The percentage fall in the rate of respiration in one experiment was 33 with air, and 75 with oxygen (Table 1). The brain tissue may be respiring in air at the end of the third hour at even double the rate of that in oxygen, though the initial rates of respiration may be higher in oxygen than in air.

The same phenomenon is exhibited by brain in the presence of sodium lactate (Exps. 5 and 6,

Table 1. Comparison of rates of fall of respiration of minced rat brain tissue in air and in O<sub>2</sub>

(About 0.2 g. of minced rat brain in 3 ml. 0.02M-sodium phosphate (pH 7.4)-Locke media with or without substrate. Gas: air or O<sub>2</sub>. Temp. 37°.)

Exp.	Substrate	Atmosphere	Oxygen consumed (μl.)			% fall of respiration in third hour $= \frac{A-C}{A} \times 100$
			First hour (A)	Second hour (B)	Third hour (C)	
1	None	Air	137.8	103.2	44.5	67
		Oxygen	128.2	69.3	18.0	86
2	None	Air	165.7	100.9	62.8	62
		Oxygen	156.6	75.5	36.8	76
3	Glucose (0.01M)	Air	225.3	190.1	150.9	33
		Oxygen	255.4	180.1	102.4	60
4	Glucose (0.01M)	Air	171.0	160.0	114.6	33
		Oxygen	179.3	131.3	44.6	75
5	Na lactate (0.027M)	Air	262.1	232.2	215.3	18
		Oxygen	245.5	213.0	143.3	42
6	Na lactate (0.027M)	Air	237.4	211.3	172.9	27
		Oxygen	243.9	157.3	83.9	66
7	Na lactate (0.027M)	Air	263.1	217.5	191.4	27
		Oxygen	274.3	189.0	119.0	57
8	Na pyruvate (0.027M)	Air	242.4	197.8	163.4	33
		Oxygen	238.1	136.3	83.4	65
9	Na pyruvate (0.027M)	Air	298.4	260.9	217.9	23
		Oxygen	280.2	194.2	162.5	42
10	Na succinate (0.05M)	Air	300.1	272.5	214.8	28
		Oxygen	328.6	285.1	211.3	35
11	Na succinate (0.05M)	Air	269.0	198.8	166.2	38
		Oxygen	267.7	189.3	156.8	41

Table 1) and in the presence of sodium pyruvate (Exps. 7 and 8, Table 1).

When the brain tissue respire in the presence of sodium succinate, however (Exps. 10 and 11, Table 1), the rate of fall of respiration in air is, within experimental error, the same as that in oxygen. The few experiments which have been carried out with succinate, under the given conditions, show that oxygen does not exert quantitatively the same toxic effect on the system concerned with the oxidation of succinate as on that of glucose, lactate or pyruvate.

#### *Anaerobic metabolism of brain tissue after exposure to air or to oxygen*

*Ferricyanide reduction.* Since the respiration of brain in presence of sodium lactate falls off more rapidly in oxygen than in air, experiments were undertaken to discover whether a gradual inactivation of lactic dehydrogenase would account for the difference.

The method adopted was that of Quastel & Wheatley (1938),\* in which the dehydrogenase activity of tissues is examined manometrically. It depends upon the measure-

ment of the rate of output of CO<sub>2</sub> when the dehydrogenase, in presence of its substrate, reacts anaerobically with sodium ferricyanide in a bicarbonate medium. Reduction of ferricyanide to ferrocyanide is accompanied by acid production which brings about liberation of CO<sub>2</sub> from the bicarbonate present.

When lactic dehydrogenase activity is examined by this technique it is necessary that HCN is present to fix the pyruvate, which would otherwise inhibit the system. In view of the findings of Mann & Quastel (1941), cozymase and nicotinamide must be present. Cozymase is broken down by the brain nucleotidases and would be absent from brain after incubation for 3 hr. at 37°, and must be added to complete the lactic dehydrogenase system. Nicotinamide must also be added to inhibit, specifically, the breakdown of the added cozymase by the tissue.

Typical results of an experiment (Table 2) make clear that exposure of brain tissue to oxygen brings about no apparent diminution of lactic dehydrogenase activity. This enzyme system, lactate-lactic dehydrogenase-cozymase-ferricyanide, has the same activity whether the tissue is exposed for 3 hr. to air or to oxygen at 1 atm. pressure. The inhibitive effect of oxygen on brain respiration in presence of lactate must clearly be operative on a factor other than lactic dehydrogenase itself.

*Methylene-blue reduction.* Minced brain tissue in presence of either glucose or lactate in a phosphate-Locke medium was exposed for 3 hr. to air or oxygen,

\* The reader is referred to this paper for full experimental details.

Table 2. *Lactic dehydrogenase activities of brain tissue after exposure to air or O<sub>2</sub>*

(0.2 g. of minced rat brain was incubated for 3 hr. at 37° in phosphate-lactate medium in either air or O<sub>2</sub>. Tissue suspension was then centrifuged and washed with 3 ml. Locke medium. Washed tissue was resuspended in 0.025M-bicarbonate-Locke medium with addition of 1 mg. cozymase, 0.3 ml. of 10% nicotinamide solution and 0.05M-NaCN. Ferricyanide-bicarbonate was placed in side tube. To one pair of tissues originally exposed to either air or O<sub>2</sub> no additional substrate was added; to another pair sodium lactate was added. Gas: 95% N<sub>2</sub> + 5% CO<sub>2</sub>. Temp. 37°.)

Atmosphere in which preliminary incubation of tissue in phosphate-lactate medium was carried out	Substrate added in subsequent part of experiment when ferricyanide was added under anaerobic conditions	CO <sub>2</sub> produced in 30 min. (μl.)	
		Exp. 1	Exp. 2
Air	None	41.5	54.5
Oxygen	None	45.0	49.1
Air	Lactate	162.9	148.3
Oxygen	Lactate	186.4	163.6

and the suspension was then transferred to a Thunberg tube. The tube was evacuated and the rate of methylene-blue reduction was recorded. The reduction time with brain tissue previously exposed to oxygen was always greater than that obtained with tissue previously exposed to air (Table 3).

Table 3. *Rates of methylene-blue reductions by brain tissue after exposure to air or O<sub>2</sub>*

(0.2 g. of minced rat brain was incubated in the Warburg manometric apparatus in phosphate-Locke medium with or without substrates for 3 hr. at 37°. Tissue suspension then transferred to Thunberg vacuum tubes and 1 ml. methylene-blue solution (1/10,000) added to each. Times of reduction of the methylene blue *in vacuo* at 37° were measured.)

Substrate with which brain was incubated in first 3 hr.	Atmosphere in which the brain was initially incubated	Subsequent reduction time of methylene blue (min.)
None	Air	51
None	Oxygen	86
Glucose (0.01 M)	Air	19
Glucose (0.01 M)	Oxygen	74
Lactate (0.027 M)	Air	6
Lactate (0.027 M)	Oxygen	26

The experiment was repeated with the modification that the tissue suspension after exposure to air or oxygen was centrifuged and washed with saline before transference to a Thunberg vacuum tube containing sodium lactate solution and methylene blue. After evacuation, substantially similar results were obtained, i.e. times of reduction of the dye with brain tissue previously exposed to oxygen were greater than those with brain tissue previously

exposed to air. Thus in one instance the reduction time with oxygen-exposed brain was 19 min. and that with air-exposed brain was 10.5 min.

These results with methylene-blue reduction suggest that the site of the toxic effect of oxygen lies in that part of the respiratory chain intermediate between cozymase and methylene blue. The possibility that the flavoprotein, diaphorase, was the factor involved was made unlikely by the fact that the difference between the reduction times of methylene blue disappeared when to the tissue was added a mixture of HCN, cozymase and nicotinamide (Table 4). It is evident that diaphorase is not affected by exposure to oxygen.

Table 4. *Rates of methylene-blue reduction by brain tissue, in presence of cozymase and nicotinamide, after exposure to air or O<sub>2</sub>*

(0.2 g. of minced rat brain was incubated in a Warburg apparatus in phosphate-Locke medium with or without substrate for 3 hr. in either air or O<sub>2</sub>. Tissue was centrifuged, washed with saline, and resuspended in the Warburg flask medium containing 0.025M-NaHCO<sub>3</sub>-Locke solution, 1 mg. cozymase, 0.3% nicotinamide and 0.05M-NaCN (made neutral with HCl). The medium contained either 0.027M-sodium lactate or no substrate. 0.2 ml. methylene-blue solution (1/1000) was placed in side tube of flask. After anaerobic conditions were established by gassing with 95% N<sub>2</sub> + 5% CO<sub>2</sub>, with yellow P in centre tube of flask, methylene blue was tipped into the brain suspension.)

Conditions of exposure in the first 3 hr.	Substrate for methylene-blue reduction	Reduction time (min.)
No substrate. Air	No substrate	30
No substrate. Oxygen	No substrate	30
Sodium lactate. Air (0.027 M)	Sodium lactate (0.027 M)	8
Sodium lactate. Oxygen (0.027 M)	Sodium lactate (0.027 M)	7

*Pyruvic oxidase.* The difference between the reduction times of methylene blue (Table 3) might be due to the higher accumulation of pyruvate in the brain tissue exposed to oxygen. It is well known that if pyruvate produced by lactate oxidation is not removed either by adequate respiratory activity or by a fixative such as cyanide, its accumulation causes inhibition of the lactic dehydrogenase. However, if the differences between the rates of methylene-blue reduction recorded in Table 3 were due to different rates of pyruvate accumulation, simple washing of the tissue should cause the subsequent rates of reduction in presence of lactate to be identical. This is not the case, and the possibility that pyruvate accumulation accounts for the longer reduction time with brain exposed to oxygen may be discarded.

When brain tissue is allowed to respire in a pyruvate-phosphate-Locke medium in air or in

oxygen for 3 hr. at 37° and the tissue is then centrifuged and washed, the tissue exposed to oxygen gives in presence of pyruvate (0.027M) and methylene blue a much longer reduction time than that exposed to air (under the experimental conditions stated in Table 3). For example, in one experiment, the reduction time of 0.2 ml. methylene blue (1/1000) was 30 min. for tissue exposed to oxygen and 7.5 min. for tissue exposed to air. It is apparent, therefore, that the toxic effect of oxygen may be located in the enzymic mechanisms responsible for the oxidation of pyruvate. At least two possibilities present themselves: (a) the enzyme pyruvic oxidase is poisoned by high tensions of oxygen, a likely possibility if the enzyme is a thiol protein; (b) pyruvic oxidase activity involves a flavoprotein sensitive to oxygen.

Table 5. *Xanthine oxidase activities of liver after exposure to air or O<sub>2</sub> or N<sub>2</sub>*

(Rat liver was finely suspended, after chopping, in twice its weight of saline. 1 ml. of the suspension after dialysis was added to a Warburg manometer vessel containing sodium phosphate buffer, pH 7.4, whose final concentration was 0.03M. Hypoxanthine was made up in a 0.05M solution in 0.05N-NaOH. 0.2 ml. of this solution was placed in a side tube of the manometer vessel and tipped in a main vessel after thermal equilibrium. Where hypoxanthine was used, the phosphate buffer in the main vessel was pH 7.1; this became pH 7.4 after tipping in the hypoxanthine (cf. Ball, 1939). Experiments were carried out in air and in oxygen at 37°.)

Exp.	Substrate	Oxygen uptake (μl.) in		
		First hour	Second hour	Third hour
1	None. Air	23.3	29.6	9.0
	None. Oxygen	22.6	28.2	6.8
	Hypoxanthine (0.003M). Air	115.2	99.3	28.2
	Hypoxanthine (0.003M). Oxygen	104.2	48.3	11.2
2	Liver suspension alone	55.8	—	—
	Liver suspension plus 0.003M-hypoxanthine	195.6	—	—
	Liver suspension previously incubated for 1 hr. in oxygen	25.8	—	—
	Liver suspension (previously incubated for 1 hr. in oxygen) plus hypoxanthine	86.3	—	—
	Liver suspension previously incubated for 1 hr. in nitrogen	32.8	—	—
	Liver suspension (previously incubated for 1 hr. in nitrogen) plus hypoxanthine	152.3	—	—

#### *Susceptibility of flavoproteins to oxygen poisoning*

At least one flavoprotein, diaphorase, seems unaffected by oxygen under our experimental conditions. Xanthine oxidase—presumably a flavoprotein—can be inactivated by traces of H<sub>2</sub>O<sub>2</sub> (c. 10<sup>-6</sup>M) produced during the oxidation of the substrate by its enzyme (Bernheim & Dixon 1928; Dixon 1925). It is easy to show that xanthine oxidase of liver—in presence of hypoxanthine—is inhibited by oxygen to a much greater extent than by air. Moreover, the enzyme preparation is inhibited by oxygen even when no substrate is added, though it is likely that traces of substrate are still present in the enzyme preparation (Table 5).

Precisely similar results are obtained when a saturated benzaldehyde solution is substituted for the hypoxanthine solution (care is taken in experiments with benzaldehyde to omit potassium hydroxide from the inner cups of the manometer vessels) (Table 6).

Table 6. *Benzaldehyde oxidation by liver suspension after exposure to O<sub>2</sub> or N<sub>2</sub>*

(Conditions as in Table 5, but benzaldehyde (a saturated aqueous solution) substituted for hypoxanthine. 2.0 ml. liver suspension taken. 37°.)

Conditions of the experiment	O <sub>2</sub> (μl.) taken up in first hour
Suspension alone	56.5
Suspension alone plus 1 ml. of benzaldehyde solution	115.6
Suspension alone previously exposed to O <sub>2</sub> for 1 hr.	26.2
Suspension alone previously exposed to O <sub>2</sub> for 1 hr. plus 1 ml. of benzaldehyde solution	37.1
Suspension alone (previously exposed to N <sub>2</sub> for 1 hr.)	36.1
Suspension alone (previously exposed to N <sub>2</sub> for 1 hr.) plus 1 ml. of benzaldehyde solution	107.6

Another flavoprotein—*d*-amino-acid oxidase—shows greater sensitivity to oxygen than to air, but is protected from inactivation by the substrate, e.g. *dl*-alanine (Table 7).

The protection of *d*-amino-acid oxidase by its substrate from the toxic influence of oxygen distinguishes, to some extent, this enzyme from that involved in the pyruvate oxidizing system in brain. Here, as already stated, the respiratory system is gradually inactivated by oxygen even in the presence of the substrate, but clearly the experimental results are insufficient to decide whether pyruvate exerts any protective action or not.

The results which have been recorded make it obvious that flavoproteins may be inactivated by

Table 7. *d-Amino-acid oxidase activities of kidney extracts after exposure to air, O<sub>2</sub> or N<sub>2</sub>*

(*d*-Amino acid oxidase preparation was made by grinding rat kidneys with sand and ten times their weight of water. The suspension was centrifuged. 1 ml. of the supernatant fluid was used as the source of the enzyme. Rates of oxygen uptake were observed in the Warburg manometric apparatus, the manometer vessels containing the enzyme preparation, phosphate buffer (0.03M), pH 7.4 and *dl*-alanine (M/15) as substrate. Gas: air or oxygen. 37°.)

	O <sub>2</sub> uptake (μl.) in		
	First hour	Second hour	Third hour
Exp. 1			
<i>dl</i> -Alanine. Air	145.6	98.2	75.3
<i>dl</i> -Alanine. Oxygen	177.7	88.5	53.3
Exp. 2			
Enzyme preparation (previously exposed to nitrogen for 1 hr. at 37° in absence of <i>dl</i> -alanine) plus <i>dl</i> -alanine	133.1	—	—
Enzyme preparation (previously exposed to nitrogen for 1 hr. at 37° in presence of <i>dl</i> -alanine) plus <i>dl</i> -alanine	205.9	—	—
Enzyme preparation (previously exposed to oxygen for 1 hr. at 37° in absence of <i>dl</i> -alanine) plus <i>dl</i> -alanine	15.5	—	—
Enzyme preparation (previously exposed to oxygen for 1 hr. at 37° in presence of <i>dl</i> -alanine) plus <i>dl</i> -alanine	159.7	—	—

oxygen and conceivably pyruvic oxidase may lie in the class of oxygen-sensitive flavoproteins. It would be simpler, however, to interpret the toxic influence of oxygen as due to its inactivation of thiol groups, essential for the activity of the oxygen-sensitive enzymes. Barron (1936) has found that pyruvate oxidation of gonococci is sensitive to atmospheric oxygen and is inhibited by very low concentrations of quinone. The thiol-enzyme urease is inhibited by very low concentrations of quinones (Quastel, 1933) whose reactions with thiol compounds have been described by Snell & Weisberger (1939). Peters (1937) has already suggested the thiol nature of pyruvate oxidase, and the recent work of Barron & Singer (1945) confirms this.

#### *Effects of hydrogen peroxide*

The addition of small quantities of hydrogen peroxide to minced brain tissue has effects on its respiration very similar to those brought about by its exposure to oxygen (Table 8). The toxic action of the hydrogen peroxide is greatly increased by

the presence of sodium azide which presumably inactivates the catalase present.

Table 8. *Effects of H<sub>2</sub>O<sub>2</sub> on brain respiration*

(About 0.2 g. of minced rat-brain tissue was added to phosphate-Locke media (to give a total volume of 3 ml.) in Warburg manometric vessels, as described under Table 1. To the brain suspension was added hydrogen peroxide, with or without 0.002M-sodium azide. The media contained either no added substrate, or sodium lactate (0.027M) or sodium succinate (0.05M). Rates of oxygen uptake in atmosphere of air only. 37°.)

Substances added to the tissue suspension	O <sub>2</sub> taken up in first hour (μl.)
Exp. 1	
Sodium lactate	290.7
Sodium lactate plus 0.002M-sodium azide	222.2
Sodium lactate plus 0.2 ml. of 0.02 vol. H <sub>2</sub> O <sub>2</sub> (≡ 4 μl. O <sub>2</sub> )	258.8
Sodium lactate plus 0.2 ml. of 0.02 vol. H <sub>2</sub> O <sub>2</sub> plus 0.002M-sodium azide	85.3
Exp. 2	
0.2 ml. of 0.02 vol. H <sub>2</sub> O <sub>2</sub>	155.8
0.2 ml. of 0.02 vol. H <sub>2</sub> O <sub>2</sub> plus sodium azide	38.4
0.2 ml. of 0.02 vol. H <sub>2</sub> O <sub>2</sub> plus sodium lactate	251.3
0.2 ml. of 0.02 vol. H <sub>2</sub> O <sub>2</sub> plus sodium lactate plus sodium azide	61.3
0.2 ml. of 0.02 vol. H <sub>2</sub> O <sub>2</sub> plus sodium succinate	362.1
0.2 ml. of 0.02 vol. H <sub>2</sub> O <sub>2</sub> plus sodium succinate plus sodium azide	263.1

Results indicate that respiration of brain tissue in presence of lactate is reduced after exposure to concentrations of hydrogen peroxide of the order of 0.0001M, the reduction of respiration being greatly enhanced by the simultaneous addition of 0.002M-sodium azide. The inhibitive effect of the hydrogen peroxide on the respiration of minced brain tissue in presence of sodium succinate and sodium azide is less than on that of the tissue in presence of sodium lactate and sodium azide.

The toxic effects of hydrogen peroxide may be seen in the subsequent rates of reduction of methylene blue in presence of sodium pyruvate by the brain tissue after exposure to small quantities of hydrogen peroxide in presence of sodium azide (Table 9). The experimental results show that hydrogen peroxide at a concentration of the order of 0.0001M, in the presence of sodium azide, greatly inhibits the pyruvate oxidizing system, and that the presence of lactate in the brain does not protect

its respiratory system from the toxic effects of hydrogen peroxide.

Although few, the experiments with hydrogen peroxide indicate a distinct possibility that the toxic action of oxygen on brain respiration may be due to the intermediate formation of hydrogen peroxide. The latter may operate in the cell at concentrations below the threshold for breakdown by catalase, or, conceivably, by coupled oxidation

in the presence of catalase (Keilin & Hartree, 1936; Pugh & Quastel, 1937).

Whether or not hydrogen peroxide formation is indispensable for the development of oxygen toxicity in brain respiratory systems, it is evident that there is much in common between the effects of oxygen and of hydrogen peroxide. This would be readily explained if the factor primarily involved is a thiol enzyme such as pyruvic oxidase, and if, therefore, the metabolic process most concerned in oxygen toxicity is that concerned with the breakdown of pyruvic acid in the brain. The admittedly few experiments which we have been able to carry out so far have led us to this conclusion.

Table 9. *Effects of H<sub>2</sub>O<sub>2</sub> on methylene-blue reduction by minced brain in presence of pyruvate*

(About 0.2 g. minced rat-brain tissue was added to phosphate-Locke media (to give 3 ml.) in Warburg manometer vessels, as described under Table 1. To these media were added sodium lactate (0.027 M), sodium azide (0.002 M) or hydrogen peroxide or combinations of these. They were incubated in air for 1 hr. At the termination of this period, the tissue suspensions were centrifuged and washed and added to 3 ml. pyruvate (0.027 M)-Locke media containing 0.15 ml. of methylene-blue solution (1/1000). The rate of reduction of the dye under anaerobic conditions was recorded.)

Substances present in preliminary aerobic incubation of 1 hr. at 37°	Subsequent times of reduction of methylene blue, in presence of sodium pyruvate, under anaerobic conditions (min.)
Exp. 1	
Sodium lactate	2.75
Sodium lactate and sodium azide	3
Sodium lactate plus 0.2 ml. 0.02 vol. H <sub>2</sub> O <sub>2</sub>	3
Sodium lactate plus 0.2 ml. 0.02 vol. H <sub>2</sub> O <sub>2</sub> plus sodium azide	57
Exp. 2	
0.2 ml. 0.02 vol. H <sub>2</sub> O <sub>2</sub>	3.75
0.2 ml. 0.02 vol. H <sub>2</sub> O <sub>2</sub> plus sodium azide	39
Sodium lactate plus 0.2 ml. 0.02 vol. H <sub>2</sub> O <sub>2</sub>	3.5
Sodium lactate plus 0.2 ml. 0.02 vol. H <sub>2</sub> O <sub>2</sub> plus sodium azide	42

1. The respiration of minced brain tissue falls off to a greater extent in the presence of oxygen than in the presence of air, particularly when the respiration of brain takes place in the presence of glucose, sodium lactate or sodium pyruvate. The respiration of minced brain in presence of sodium succinate appears not to be so oxygen-sensitive.

2. The lactic dehydrogenase activity of brain is not affected by exposure of brain to oxygen.

3. Diaphorase of brain tissue is not inhibited by exposure to oxygen.

4. The evidence points to the pyruvate oxidizing system as constituting that part of the brain respiratory system most sensitive to oxygen.

5. *d*-Amino-acid oxidase is poisoned by exposure to oxygen, but the enzyme is protected by the presence of its substrate. Xanthine oxidase is poisoned by oxygen, probably through the intermediate formation of hydrogen peroxide.

6. It is suggested that pyruvate oxidase, as a thiol enzyme, is the factor which is poisoned by high tensions of oxygen. This may be accomplished either by oxygen itself, or by hydrogen peroxide which it is shown exerts similar toxic effects, especially in the presence of sodium azide, on brain respiration.

Our grateful thanks are due to the Medical Research Council and to the Rockefeller Foundation for grants which made the work possible.

## REFERENCES

- Ball, E. G. (1939). *J. biol. Chem.* **128**, 51.  
 Barron, E. S. G. (1936). *J. biol. Chem.* **113**, 695.  
 Barron, E. S. G. & Singer, T. P. (1945). *J. biol. Chem.* **157**, 221, 241.  
 Bernheim, F. & Dixon, M. (1928). *Biochem. J.* **22**, 113.  
 Dickens, F. (1946*a*). *Biochem. J.* **40**, 145.  
 Dickens, F. (1946*b*). *Biochem. J.* **40**, 171.  
 Dixon, M. (1925). *Biochem. J.* **19**, 507.  
 Elliott, K. A. C. & Libet, B. (1942). *J. biol. Chem.* **143**, 227.  
 Keilin, D. & Hartree, E. F. (1936). *Proc. Roy. Soc. B*, **119**, 141.  
 Mann, P. J. G. & Quastel, J. H. (1941). *Biochem. J.* **35**, 502.  
 Peters, R. A. (1937). *Perspectives in Biochemistry*, p. 41. Cambridge University Press.  
 Pugh, C. E. M. & Quastel, J. H. (1937). *Biochem. J.* **31**, 2317.  
 Quastel, J. H. (1933). *Biochem. J.* **27**, 1116.  
 Quastel, J. H. & Wheatley, A. H. M. (1938). *Biochem. J.* **32**, 936.  
 Snell, J. M. & Weisberger, A. (1939). *J. Amer. chem. Soc.* **61**, 450.  
 Stadie, W. C., Riggs, B. C. & Haugaard, N. (1944). *Amer. J. med. Sci.* **207**, 84.