LXXXIX. OXIDATIONS BY THE BRAIN.

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A FULL consideration of the work which has been carried out in relation to the causation and treatment of abnormal mental states shows that it is extremely desirable that researches be undertaken to discover how organic conditions, in psychotic states, differ from those existing in the normal. Much work has been carried out from a histological standpoint—particularly by Nissl, Alzheimer, Southard, Dunlop, Mott, Spielmeyer to quote only a few of the authors of many painstaking investigations. The results definitely suggest, in the very widespread disease of dementia praecox, organic changes occurring particularly in the nervous system. Mott [1920] states that the essential morbid change is one of nuclear decay most marked in the cortex and suggests that the histological findings are associated with deficient oxidation. Koch and Mann [1909] had already found, from analyses of the condition of sulphur in the brain, evidence of deficient oxidations. These results refer to dementia praecox. There is no question but that there occur marked histological changes in frankly organic mental diseases such as general paresis, which is of venereal origin, and treatment is on definitely organic lines, as in the clearing up or partial clearing up of general paresis by the action of malaria.

It is evident, however, that present histological methods of approach are most inadequate to show differences involving even considerable biochemical abnormalities. The fault lies in the technique. Histological methods involve the use of fixing and almost inevitably that of staining. Hardening a tissue in alcohol or treatment with the usual fixing agents produces changes in the tissue constituents which are greater than would ever be supposed to occur in the tissue when in the living condition. Dyestuffs are, in many instances, toxic to oxidising and even hydrolysing enzymes [Quastel and Wheatley, 1931, 2; Quastel, 1931]. It is not to be supposed that a fixed and stained section gives a true picture of the condition of a tissue in the living state. Morphological changes will be shown as well as the occurrence of foreign structures—but in an abnormal mental condition where possibly only relatively small differences from the normal are to be expected in the dynamics of the cell processes, the changes inherent in histological technique would obscure what changes may have been already present.

The alternative to the histological method of investigation is to examine the tissue along biochemical lines where every effort is made to preserve the tissue in the state in which it was present in the body and to determine by dynamic methods what differences exist between the tissues of abnormal mental states and those of the normal.

Work has been proceeding in this laboratory for the past two years on these lines—attention being focused on suitable methods to be used for comparative purposes. The lack of information on the biochemistry of the brain has made it imperative to study the tissues of normal animals before it has become profitable to make any study of the tissues of abnormal mental cases.

Histological work, as already mentioned, having pointed to the possibility of deficient oxidations in the cortex of certain psychotic cases, an endeavour has been made to study the oxidative processes of normal brain with a view to obtaining quantitative data which may be used subsequently for comparative purposes. The attempt has been made also to study these processes when brain is submitted to various environmental conditions, including those brought about by the presence of narcotics. This paper will be concerned with a description of normal oxidative processes in brain whilst a subsequent paper will indicate the influence of narcotics in general on these processes.

Technique.

Velocities of oxidation by brain tissue were determined in a Barcroft differential manometer, all experiments being carried out at 37°. The whole brain was used in the cases of small animals such as mice, rats, guinea-pigs, whilst the grey matter of cerebral cortex was used with larger animals. In all cases the *pia* was removed as carefully and completely as possible from the brain of the freshly killed animal. One of the most serious difficulties in working with human material is to obtain material less than 6 hours old. Animals were in most cases killed by bleeding, the brain being used as quickly as possible. Storage of the brain, when circumstances made this necessary, was always at 0°. The brain, after removal of the *pia*, was sliced with a scalpel and made into as homogeneous a mixture as possible. The homogeneity was shown by the identity of velocities of O_2 uptake by equal quantities of the mixed tissue. Many control experiments showed that this method of preparing tissue for examination gave the most consistent and reproducible results, the O_2 uptake of the tissue per unit quantity being the same whatever amount was taken. Experience showed that 0·5 g. was the most useful quantity to work with and in all experiments this amount has been used. Ashford and Holmes [1931] have shown that brain used in this way gives results in every way comparable with sliced tissue and our experience has been such as to confirm this and to indicate that chopped brain suffers no material damage, so far as its oxidative mechanisms are concerned, by the process of chopping.

The tissue was immersed in a phosphate buffer solution p_H 7·4 containing 0·6 % saline. Values of the rate of O_2 uptake of a normal guinea-pig brain showed no significant differences when examined in this way from those obtained with a tissue suspended in Ringer solution[1] [see also Ashford and Holmes, 1931]. Thus 0·5 g. of guinea-pig brain took up 871 mm.^{3} O_2 in a phosphate saline medium in 2 hours and 861 mm.^{3} O_2 in the same time in the Ringer medium. Phosphate buffer solution has been used in all except special cases to remove all possibility of appreciable p_H changes during the course of oxidation and to ensure the presence of an adequate supply of phosphate ions.

The procedure has usually been to place in the right-hand vessel of the manometer 1 cc. $M/5$ phosphate buffer p_H 7·4, 2 cc. saline (0·9 %) and 0·5 g. tissue, and the same with the exception of the tissue in the left-hand vessel. The apparatus is shaken at the temperature of the bath for 10 mins. to ensure temperature equilibrium, the taps are closed and the manometer levels are read every 15 mins. When substrates have been added to the tissue, the same volumes in the manometer vessels are preserved and care taken that no serious change in ionic concentration is effected by the addition of the substrate.

Autoxidation of brain.

A characteristic feature of fresh brain tissue is a high initial rate of oxygen uptake usually considerably greater than that of the same weight of muscle. (Leg muscle was usually taken.) A fresh guinea-pig brain takes up oxygen at an initial rate of about 200 mm.^{3} per g. of tissue per 15 min. and this rate is kept at very nearly a constant level for over 2 hours after which it begins to fall. Usually this rate has fallen to between two-thirds and half the initial value in 3 hours (see Figs. 1, 2, 4). There is very little difference between the rates of autoxidation of the brains of well-fed and starved animals. The total volume of oxygen taken up by 1 g. of brain tissue of the guinea-pig in 3 hours is approximately 2000 mm.^{3} Values of the oxygen uptake by the brain tissue of various animals are shown in Table I.

Although the autoxidation of brain tissue is always of a high order, the actual value is not constant, under our experimental conditions, for a particular animal species and it is impossible to use this value, therefore, as an index to the condition of a tissue. The order of variability is shown in the following figures which give the O_2 uptake in 90 min. by 0·5 g. fresh rabbit brain: 584, 431, 599, 685, 678 mm.^{3}

It was felt however that the extra oxygen uptakes due to the addition to the brain of various substrates or metabolites might prove to be constant and offer useful indices for judging the normality or abnormality of a tissue.

Glucose and sodium lactate (at concentrations of $M/30$) were first tested but these proved to be quite unsuitable under the given experimental conditions. The extra oxygen uptakes by these substances after 90 or 120 min.

[1] Ringer solution: NaCl 0·9 %, KCl 0·025 %, $CaCl_2$ 0·03 %, $NaHCO_3$ 0·015 %.

Table I.

The oxygen uptakes recorded in this table, which are representative of a large number of experiments, are mm.^{3} obtained after 90 min. at 37°, 0·5 g. brain tissue being used. Mixtures of several brains were made with the very small animals. Grey matter of the cerebral cortex was used in the cases of the cat, sheep and man.

Animal	Autoxidation	O_2 uptake in presence of succinate (0·05 *M*)	O_2 uptake in presence of *p*-phenylenediamine (21 mg.)
Mouse*	696	1300	2212
	480	1450	1850
Rat	788	1455	1635
	680	1680	1428
Guinea-pig	517	1438	1562
	645	1188	1480
Rabbit	599	1392	1560
	685	1320	1525
Cat	422	1040	1560
Fowl	766	1367	1598
	680	1480	1342
Pigeon*	844	2260	2310
Man	444	710	1402
Dog	390	680	1325
Sheep	540	960	—

* In these cases 0·25 g. brain was used and the figures are calculated for 0·5 g.

were small and irregular, and it was sometimes observed that the addition of glucose inhibited the initial rate of autoxidation of brain. It seems most likely that the irregularities were due to variations in the content of lactic acid initially in the brain. Ashford and Holmes [1931] have shown that the extra oxygen uptake due to lactate by brain in the presence of air does not become marked until the autoxidation has fallen away from its initial linear rate.

As is well known, a number of substrates will act as hydrogen donators in the presence of brain and these, on addition to tissue, will bring about an extra uptake of oxygen. Chief amongst these is succinate, which is oxidised freely and which is converted quantitatively into *l*-malate [Quastel and Wheatley, 1931, 1]. Now it has been found in studies on the connection between cell structure and cell activity [Penrose and Quastel, 1930] that the mobilisation of active hydrogen by the cell depends greatly on its integrity. Using bacteria it was found that disintegration of the cell structure resulted in a great diminution in the oxygen uptake induced by added hydrogen donators. The oxidation due to succinate was reduced over 90 %. Hence it was to be anticipated that any profound change in cell structure would be reflected in a lowered oxygen uptake by the brain in presence of hydrogen donators.

As sodium succinate is powerfully activated by normal brain tissue, it was chosen as a suitable hydrogen donator for the study of brain on these lines.

Another substance upon which attention was turned was *p*-phenylenediamine. Keilin [1929] has made it appear very probable that the *p*-phenylenediamine oxidase plays an essential part in the normal respiratory activity of

the cell and that the oxidase is identical with Warburg's "respiratory ferment." It has been shown [Penrose and Quastel, 1930] that the activity of the oxidase is independent of the integrity of cell structure, and that narcotics do not affect its action in the way that dehydrogenase activity is affected (Keilin). The oxidase, indeed, appears to belong to that category of substances to which cytochrome is related. The rate of oxidation of *p*-phenylenediamine depends on a tissue oxidase quite distinct from that responsible for the activation of the usual hydrogen donators and hence should give another index for judging the normality or abnormality of a particular tissue. Brain tissue oxidises *p*-phenylenediamine with great rapidity [*cf.* Holmes, 1930], grey matter being approximately four times as active as white.

Values of the oxygen uptakes found with the brain tissue of various animals in the presence of sodium succinate and neutralised *p*-phenylenediamine are recorded in Table I. These are typical of a large number of results.

It is noteworthy that the smallest animals give the largest velocities of oxidation of *p*-phenylenediamine. Mouse brain is much more effective than that of guinea-pig or rabbit, whilst the grey matter of cat, dog or man does not oxidise the diamine at a greater rate than does the whole brain of rabbit or guinea-pig. There appears to be an inverse relationship between the size of the animal (or its brain) and the ability of the brain to oxidise *p*-phenylenediamine. Such a relationship was also noticed by Vernon [1911, 1912] when studying the intensity of the indophenol reaction of various tissues. This relationship does not appear to extend to muscle. The muscle of mice, for instance, oxidises *p*-phenylenediamine at a slower rate than that of the guinea-pig. As a general rule the muscle is less active than the brain of the same animal in oxidising *p*-phenylenediamine. For instance whereas 0·5 g. rabbit brain took up 1560 mm.^{3} O_2 in 90 min. in presence of 21 mg. of *p*-phenylenediamine, 0·5 g. rabbit leg muscle took up 990 mm.^{3} O_2 under the same conditions. This diminished activity of muscle compared with brain is shown also in the oxidation of succinate. The extra O_2 uptake due to succinate (0·05 *M*) by 0·5 g. mouse brain was 604 mm.^{3}; the extra O_2 uptake under similar circumstances by 0·5 g. mouse leg muscle was 328 mm.^{3}

Of all tissues studied human grey matter showed the least activity towards succinate. Figures obtained with human material are open to the criticism that the brain is several hours old before examination can be made. It may be shown however that succinate oxidation by brain suffers no diminution on storage for 24 hours—whilst that of muscle diminishes considerably. Table II records values found for the autoxidation and for the oxidation of succinate with fresh rabbit brain and muscle and for oxidations with the same tissues 24 hours old (stored at 0°). The low oxygen uptakes found consistently with human material in presence of succinate appear to be characteristic of human tissue and are not due to the lapse of time between death and examination of the brain.

The values recorded in Table I though fairly descriptive of the activities

Table II.

	O_2 uptake in mm.^{3} in 1 hr.	O_2 uptake in mm.^{3} in presence of succinate in 1 hr.	Extra O_2 uptake in mm.^{3} in 1 hr.
Brain	539	1150	611
,, 24 hrs. old	410	1135	725
	(2 hrs.)	(2 hrs.)	(2 hrs.)
Muscle	124	870	746
,, 24 hrs. old	45	502	457

of the brain tissue of various animals fail on the whole to give the information which it was the object of the investigation to discover. Reliable and consistent values were required which could be used as indices of the normal condition of brain tissue. The values found, though hovering near a mean value were not sufficiently consistent for the purposes required. It became clear that the method would have to be abandoned unless a modification of the experimental technique was adopted which would lead to consistent and reproducible results.

The value which was found to be most subject to variation was the rate of autoxidation, pointing to the presence of variable amounts of lactic acid and other substances in the brain of freshly killed animals. The question which arose was whether the extra oxygen uptake due to the addition of a metabolite such as succinate would be independent of the rate of autoxidation. If succinate, of which the brain initially contains but a trace, is oxidised independently of other material in the tissue, the extra O_2 uptake due to this substance would be a constant descriptive of the tissue under investigation. It has been found, however, that the extra oxygen uptake due to succinate varies inversely with the rate of autoxidation and it is now certain that the rate of oxidation of succinate is greatly dependent on the presence of other oxidisable material in the brain. This is shown as follows.

Oxidation of succinate.

0·5 g. of chopped guinea-pig brain was placed in each of the right-hand vessels of three Barcroft manometers. In the first was placed 1 cc. $M/5$ phosphate buffer p_H 7·4, 2 cc. saline and 0·5 cc. of 0·4 M sodium succinate, in the second was placed the same with the exception of the succinate and in the third was placed the same with 0·5 cc. of 0·4 M NaCl replacing the succinate. After attainment of temperature equilibrium the taps of the apparatus were closed and the rates of oxygen uptake in the three manometers read. Two hours later the contents of the first vessel were examined polarimetrically, the volume being made up to 5 cc. and 1 cc. glacial acetic acid and 10 cc. 14·2 % ammonium molybdate solution being added. This was filtered and the filtrate examined in a 1 dm. tube using the mercury green line. [For details of this method of estimation of *l*-malic acid, see Quastel, 1931.] One hour later—*i.e.* after autoxidation had proceeded in the remaining Barcroft manometers

for 3 hours—0·5 cc. of 0·4 *M* sodium succinate solution was added to the second vessel, the rate of oxygen uptake having dropped to about 2/3 its initial value. The rates of oxidation in the two vessels were measured for a further 2 hours, after which the contents of the two vessels were examined polarimetrically by the method described. The following were the figures obtained:

First vessel (succinate added initially)	
Oxygen uptake in 2 hrs.	1690 mm.^{3}
Rotation found	+0·44°
Second vessel (succinate added after 3 hrs. autoxidation)	
Oxygen uptake in the second period of 2 hrs. ...	1667 mm.^{3}
Rotation found	+0·68°
Third vessel (no succinate added at any time)	
Oxygen uptake in first period of 2 hrs.	745 mm.^{3}
Oxygen uptake in second period of 2 hrs.	250 mm.^{3}
Rotation found	0·00°
Extra O_2 uptake due to succinate in first vessel ...	945 mm.^{3}
Extra O_2 uptake due to succinate in second vessel ...	1417 mm.^{3}

It is clear from the figures quoted that the extra oxygen uptake due to addition of succinate varies inversely with the content of oxidisable material in the brain and that the *l*-malic acid formed (as shown by the rotation) varies in the same way. Now it is known that fumarate is transformed into *l*-malate in the presence of brain with great rapidity [see Quastel, 1931], and hence the rotations shown represent equilibrium values between the fumarate and *l*-malate. It is easy to show that the uptake of 1000 mm.^{3} of O_2 at 37° in the oxidation of succinate corresponds to the formation of *l*-malate whose rotation, measured in the manner described above, would be +0·48°. Hence the rotation corresponding to the extra O_2 uptake of 945 mm.^{3} would be +0·45° and that corresponding to an uptake of 1417 mm.^{3} would be +0·67°. The figures found were +0·44° and +0·68° respectively, showing that the extra oxygen uptakes are almost exactly equivalent to the *l*-malic acid formed. Thus the extra O_2 uptake is entirely concerned with the oxidation of succinate and it is evident that the oxidation of succinate by the brain is diminished by the presence of other oxidisable material in the brain.

It was decided in view of this result to modify the original experimental technique by allowing the brain tissue to autoxidise for 3 hours, *i.e.* till the rate of O_2 uptake had fallen to between 2/3 and 1/2 of its initial uptake, before adding substrates or metabolites. With this modified technique constant and reliable results have been obtained.

Oxidation of the sugars.

The addition of glucose at a concentration of 0·025 % to a brain tissue which has been greatly depleted of its autoxidisable materials results in a quick recovery in the rate of oxidation to a level nearly that of the initial rate (Curve A, Fig. 1). This new level remains constant for a lengthy period. The

rate of oxygen uptake after addition of glucose is directly proportional to the amount of tissue present. This is shown by the following figures:

Amount of tissue to which glucose has been added g.	O_2 uptake in 2 hrs. mm.^{3}	O_2 uptake. / Amount of tissue mm.^{3}
0·5	868	1736
0·3	568	1866
0·2	366	1830
0·1	166	1660

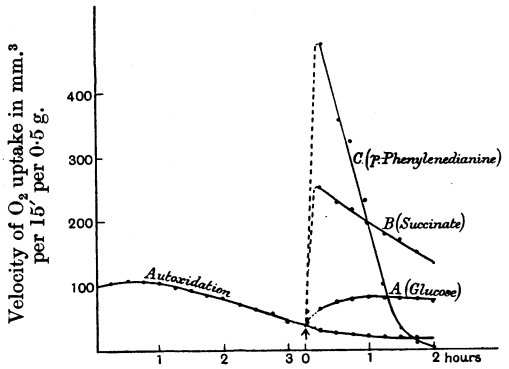


Fig. 1. Effects of addition of glucose (0·025 %) sodium succinate (0·05 *M*) and *p*-phenylenediamine (21 mg.) on rate of O_2 uptake of guinea-pig brain.

It was found in agreement with Loebel's results [1925] that the addition of fructose and mannose results in an increased rate of oxygen uptake. Glycogen and galactose have very little effect on the restoration of the rate of O_2 uptake whilst mannitol and gluconic acid are inert under the given experimental conditions. The pentoses, arabinose and xylose, were also investigated and found to be inert. The results are shown in Table III.

Table III.

O_2 uptake in mm.^{3} by 0·5 g. brain tissue (guinea-pig) largely depleted of autoxidisable material by autoxidation for 3 hours prior to addition of substrates. The sugars were all added in saline solution.

Time (min.)	30	60	90	120
Saline	96	170	225	269
Glucose (0·06 %)	165	396	647	893
Fructose (0·06 %)	200	400	601	784
Mannose (0·025 %)	160	332	516	662
Galactose (0·06 %)	148	250	319	375
Mannitol (0·06 %)	95	176	245	299
Arabinose (0·06 %)	74	135	186	232
Xylose (0·06 %)	84	157	228	289
Glycogen (0·06 %)	99	201	292	375
Sodium gluconate (0·025 %)	82	148	205	260
Sodium succinate (0·05 *M*)	493	927	1316	1620
Sodium fumarate (0·0125 *M*)	99	183	254	302
Sodium lactate (0·125 *M*)	176	401	628	848
Sodium lactate (0·0025 *M*)	139	265	385	490
Sodium pyruvate (0·0125 *M*)	192	395	601	802
Sodium glutamate (0·05 *M*)	144	291	436	559
Serum (0·5 cc.)	160	321	472	630

Oxidation of lactic and pyruvic acids.

As is now known from the work of Loebel [1925] and Ashford and Holmes [1931], the addition of sodium lactate and sodium pyruvate to the brain results in a prolongation of the steady rate of oxygen uptake. The addition of lactate at so low a concentration as 0·0025 *M* to brain tissue greatly depleted of oxidisable material results in a restoration of the linear uptake of O_2 by the brain. This is shown in Fig. 2, which gives the results of an experiment carried on for 9 hours. The rate of autoxidation had fallen to 2/3 the initial level in 3 hours, when 0·9 mg. lactic acid (as sodium lactate) was added to the tissue. It will be seen that the rate of oxidation in presence of the lactate falls off slowly in marked contrast to that in the presence of succinate where

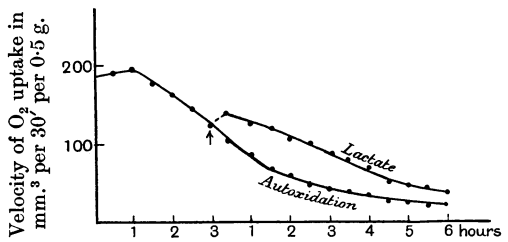


Fig. 2. Effect of addition of 0·9 mg. lactic acid on rate of oxidation of guinea-pig brain.

the rate falls off quickly (compare Curve B, Fig. 1, the concentration of succinate initially being 0·05 *M*). The volume of extra oxygen consumed, after oxidation had proceeded in presence of the added lactate (0·9 mg.) for 6 hours was 444 mm.³ and oxidation was still taking place at a fairly high rate. The theoretical oxygen uptake for the complete oxidation of this amount of lactate is 768 mm.³ at 37°. There seems little doubt that the theoretical oxygen uptake would have taken place had it been possible to carry out the experiment for a sufficiently long time.

This point is of some importance in connection with the statement of Ashford and Holmes [1931] that less oxygen is taken up by brain tissue than can be accounted for by the disappearance of lactate. It seems clear from a study of the rate of oxidation that even if lactic acid is converted by the brain into some substance which is not estimated as lactic acid it is eventually entirely oxidised. Reference will be made to this later.

It has been suggested [Toenissen and Brinkman, 1930] that the path of oxidation of lactic acid lies through pyruvic acid, αδ-diketoadipic acid and thence through succinic and fumaric acids. Now experiment shows that fumaric acid is exceedingly slowly oxidised by the brain (see Table III), though *l*-malic acid is formed from it with great rapidity. The very slow rate of oxidation of fumaric acid should bring about an accumulation of this substance (and, therefore of *l*-malic acid) among the products of oxidation of lactic acid—for both lactic and pyruvic acids at small concentrations are

oxidised at a higher rate than relatively large concentrations of fumaric acid. Ashford and Holmes [1931] could find no evidence of accumulation of a ketonic acid. Polarimetric examination of the products of oxidation of lactic acid in the presence of brain showed no trace of the formation of *l*-malic acid and it must be concluded that the evidence does not support the hypothesis that lactic acid (or the major part of it) is oxidised through succinic acid.

The addition of lactate or pyruvate to brain tissue which has been partially depleted of its autoxidisable material restores the rate of oxidation to a level equal to or a little higher than that brought about by the addition of an equivalent quantity of glucose. This result is in agreement with the hypothesis, for which Holmes has produced evidence, that glucose must be converted into lactic acid for its oxidation to occur in presence of the brain. The conclusion, too, is supported by the fact that the addition of glucose to brain tissue consuming lactate does not increase the rate of oxidation. This is shown by the following figures:

	mm.^{3}
O_2 uptake in 2 hrs. by 0·5 g. brain (guinea-pig) partially depleted of oxidisable material by autoxidation previously for 3 hrs.	236
O_2 uptake in 2 hrs. in the presence of lactate (0·025 *M*) by 0·5 g. of the same tissue	756
O_2 uptake in 2 hrs. in the presence of glucose (0·0125 *M*)...	589
O_2 uptake in 2 hrs. in the presence of glucose (0·0125 *M*) and lactate (0·025 *M*)	628

It has been shown by Loebel [1925] and it is indicated in the values recorded in Table III that fructose is oxidised by brain tissue at a rate approaching that at which glucose is attacked. Yet according to Loebel fructose gives rise to little or no lactic acid in presence of brain. The conclusion would be that fructose is oxidised on lines different from those of glucose oxidation.

Krebs [1931] has shown that the addition of iodoacetic acid to the brain prevents the oxidation of glucose, presumably by attacking the mechanism by which glycolysis is effected. It was thought possible that iodoacetic acid would not affect the oxidation of fructose if the production of lactic acid is not an important path of the breakdown of this sugar. The following experiments were therefore undertaken.

Action of iodoacetic acid.

Sodium iodoacetate solution was added to fresh brain tissue to give a final concentration of *M*/4000. Autoxidation was allowed to take place for 3 hours, after which the effects of the addition of glucose, lactate *etc.* were determined. The results are noted in Table IV.

It will be seen that the oxidations of glucose, fructose and mannose are greatly inhibited (over 90 %). The oxidations of lactate and pyruvate are also affected but not to the same extent.

Holmes [1930] has shown that glucose oxidation proceeds independently of a phosphoric acid ester stage. Iodoacetic acid inhibits, presumably, a direct glycolysis of glucose. It is conceivable that fructose oxidation proceeds through a phosphoric ester stage and that iodoacetic acid inhibits the forma-

Table IV.

O_2 uptakes by 0·5 g. guinea-pig brain tissue in the presence of various substrates after treatment with $M/4000$ iodoacetic acid and autoxidation for 3 hrs.

Time (min.)	30	60	90	120
Saline	41	77	105	126
Sodium lactate (0·0125 M)	104	213	317	408
Sodium pyruvate (0·0125 M)	105	211	316	410
Glucose (0·025 %)	55	100	132	171
Fructose (0·025 %)	58	104	140	168
Mannose (0·025 %)	61	105	138	168
Sodium glutamate (0·05 M)	87	176	255	324
Serum (0·5 cc.)	114	195	265	317
Sodium succinate (0·05 M)	498	922	1280	1578

tion or breakdown of the ester in the manner suggested by Lohmann [1931]. On the other hand hexosediphosphoric acid is only attacked very slowly by brain tissue and a sample of synthetic 6-hexosemonophosphoric acid[1] proved to be inert, as shown by the following figures.

	mm.^{3}
O_2 uptake by rat brain in 2 hrs.	244
O_2 uptake by rat brain in presence of sodium hexosediphosphate (0·25 %) ...	302
O_2 uptake by rat brain in presence of sodium 6-hexosemonophosphate (0·06 %)	240
O_2 uptake by rat brain in presence of glucose (0·06 %)...	583

The brain tissue was partially depleted of oxidisable material by prior oxidation for 3 hours.

The possibility remains that fructose gives rise to lactic acid at a very slow rate but sufficient to provide lactic acid in a concentration which would give the observed rate of oxidation. The question must remain in abeyance until more is known of the mechanism of fructose breakdown in presence of brain. In the meantime, the action of iodoacetic acid on the oxidation of glucose does not constitute, in view of its effect on fructose, evidence that glucose necessarily passes through lactic acid for its oxidation to take place. There is indeed an entire lack of evidence as yet to show how far iodoacetic acid affects the initial activation of carbohydrates by brain tissue, *i.e.* preliminary to subsequent metabolic changes. The same criticism applies to the action of sodium fluoride which inhibits glycolysis and glucose oxidation to a greater extent than lactate oxidation [Holmes, 1930; Ashford and Holmes, 1929]. It applies also to the action of oxalate which has a marked toxic action on the oxidation of glucose by brain; indeed oxalate at a concentration of 0·025 M is so toxic that it inhibits the oxidation of lactate.

Relative effects of phosphate buffer and Ringer solution.

It has been shown that lactic acid production from glucose proceeds independently of added phosphate ions and that it proceeds actively in a bicarbonate buffer [Ashford and Holmes, 1929]. Experimental results shown in Fig. 3 indicate that if brain tissue is depleted of much of its oxidisable material

[1] We are much indebted to Baeyer Products Ltd. for a gift of calcium hexosediphosphate and to Dr P. A. Levene for a sample of 6-hexosemonophosphate.

in the presence of Ringer solution the subsequent addition of glucose gives rise to a diminished extra oxygen uptake. The addition of phosphate buffer solution at the same time as the glucose brings about, however, the usual increased rate of oxygen uptake by the brain.

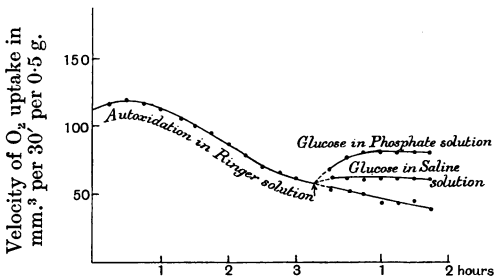


Fig. 3. Effects of addition of glucose (0·025 %) in phosphate buffer solution p_H 7·4 and in saline respectively on rate of O_2 uptake by guinea-pig brain suspended in Ringer solution.

It is observable in all experiments where glucose is added to brain tissue suspended in Ringer solution that acidity develops, a p_H of 6·8 being obtained. Lactate in phosphate buffer at this p_H is only very slowly oxidised, as the following results indicate: the brain tissue had been allowed to autoxidise in the usual way for 3 hours prior to addition of lactate.

	mm.^{3}
Total O_2 uptake by lactate (0·025 *M*) in presence of 0·5 g. brain tissue suspended in phosphate buffer at p_H 6·8	218
Ditto but with tissue suspended in phosphate buffer at p_H 7·4	450

It is probable therefore that the lack of oxidation of glucose in Ringer solution by brain is due to the development of a relatively high hydrogen ion concentration.

Oxidation of glutamic acid.

Glutamic acid (0·05 *M*) is oxidised at a comparatively slow rate by either fresh brain tissue or tissue which has been depleted of oxidisable material by previous oxidation for 3 hours. The following figures are typical for guinea-pig brain. (Glutamic acid was always neutralised with sodium hydroxide.)

	mm.^{3}
O_2 uptake of fresh brain (0·5 g.) in 1½ hours	770
O_2 uptake of fresh brain with glutamic acid (0·05 *M*) added initially (in 1½ hrs.)	942
O_2 uptake by brain in 1½ hours, after 3 hours prior autoxidation	200
O_2 uptake by brain in 1½ hours, with the glutamic acid added subsequent to the 3 hours autoxidation	388

Polarimetric examination of the products of oxidation of glutamic acid by brain tissue showed no indication of *l*-malic acid formation.

Iodoacetic acid (*M*/4000) had but little effect on the oxidation by brain of the amino-acid (see Table IV).

The action of serum.

It was interesting to observe the effects of the addition of serum to brain tissue which had been depleted of much of its oxidisable material. An immediate rise in the rate of oxidation took place. Values representative of the increased O_2 uptake due to serum are shown in Table III. The effect is of the same order as that of glucose or lactate and is due doubtless partly to the presence of these substances in the serum. A brain treated with iodoacetic acid showed an increased O_2 uptake in presence of serum (Table IV). Serum, as control experiments showed, does not interfere with the action of iodoacetic acid.

Oxidation of succinate and p-*phenylenediamine.*

Table V records typical values indicating the oxygen uptake by brain tissue of various animals in the presence of glucose, sodium succinate and *p*-phenylenediamine—the brain tissue having been allowed to take up oxygen

Table V.

O_2 uptake in mm.^{3} in 1 hour by 0·5 g. brain tissue, which had been allowed previously to take up oxygen for 3 hours, in the presence of glucose (0·025 %), sodium succinate (0·05 *M*) and neutralised *p*-phenylenediamine hydrochloride (21 mg.).

	Saline (autoxidation)	Glucose	Sodium succinate	*p*-Phenylene-diamine
Rabbit	162	229	790	1315
Mouse	154	345	1122	1534
Guinea-pig	154	287	870	1406
Bullock (grey matter)	179	250	790	1190

for 3 hours prior to the addition of substrates—and Curves A, B and C, Fig. 1, show the great differences in rates of oxygen uptake induced by them. The modified technique results in values being obtained which are remarkably uniform. The following figures show the extra O_2 uptake in $1\frac{1}{2}$ hours by 0·5 g. guinea-pig brain tissue in the presence of sodium succinate (0·05 *M*), using the new technique; 1066, 1066, 1055, 993, 989 mm.^{3} Considering that guinea-pigs from various sources were used and that the total experimental error involved in the determination of O_2 uptake is of the order of 5 %, the uniformity of the results speaks well for the utility of the method. Human material has not yet been investigated with the modified technique.

Table V shows the high oxidising activity of mouse brain towards glucose and succinate as compared with the brain of guinea-pig or rabbit. It is found that the initial rate of oxidation of glucose by mouse brain is more than double that found with guinea-pig or rabbit. The oxidation of succinate by mouse brain proceeds at a velocity 50 % greater than that by rabbit brain and the velocity of oxidation of *p*-phenylenediamine is also greater as was found in the preliminary experiments recorded in Table I. The value of the modification of the original technique is seen on comparing the results of Table I and Table V. In the early experiments owing to the high autoxidation of

brain it was impossible to obtain a fair estimate of the rate of attack of succinate—and experiments with glucose were out of the question. The new results confirm the previous conclusion that the rate of oxidation of various metabolites by brain tissue is inversely proportional to the size of the animal.

l-Malic acid production from succinate in the presence of lactate.

It has already been shown that the extra oxygen uptake and the *l*-malate production consequent on addition of succinate to a fresh brain are less than those obtained when succinate is added to a brain greatly depleted of its autoxidisable material. The question arises as to whether this is due to interference with succinate oxidation by some normal constituent of brain tissue. Since it is known that a part of the normal oxygen uptake of brain is due to lactate, experiments were devised to ascertain whether

(*a*) a mixture of succinate and lactate was oxidised at a rate higher than either of the two individually,

(*b*) *l*-malate production from succinate was affected by the presence of lactate.

Results are recorded in Fig. 4 which shows the effects of adding succinate, lactate and a mixture of succinate and lactate to brain tissue which had been

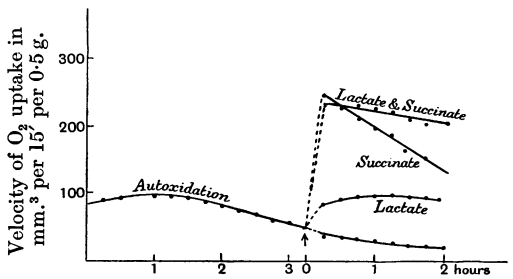


Fig. 4. Effects of addition of sodium lactate (0·0125 *M*), sodium succinate (0·05 *M*) and a mixture of these on rate of O_2 uptake of guinea-pig brain.

allowed to take up oxygen for 3 hours prior to addition of the substrates. It is clear that the velocity of O_2 uptake is not greater with the mixture of succinate and lactate than with succinate alone—but the high level in the rate of oxidation is retained for a longer period with the mixture than with the individual substrates. Polarimetric examination of the products of oxidation showed the following results:

Rotation found with lactate alone	0·00°
Rotation found with succinate alone	+0·66°
Rotation found with lactate + succinate	+0·48°

The oxidation of succinate is diminished by the presence of lactate, the amount of diminution being almost exactly equivalent to the amount of oxidation for which lactate alone is responsible. This is shown by the following figures which are typical of a number of experiments. They are the values

found after 2 hours' oxidation with 0·5 g. guinea-pig brain tissue which had been allowed to oxidise for 3 hours prior to addition of substrates. The sodium salts of lactic and succinic acids were used at concentrations of 0·0125 *M* and 0·05 *M* respectively.

	mm.³
O_2 uptake by tissue alone	231
O_2 uptake by tissue + lactate	756
O_2 uptake by tissue + succinate	1580
O_2 uptake by tissue + lactate + succinate	1752
Extra O_2 uptake due to lactate	525
Extra O_2 uptake due to succinate	1347
Extra O_2 uptake due to lactate + succinate ...	1521

Polarimetric examination:

Rotation found with succinate alone ... ... +0·66°

Rotation calculated for succinate + lactate $= (1521 - 525) \times \frac{0{\cdot}48°}{1000} = 0{\cdot}478°$

Rotation found for succinate and lactate ... ... +0·48°

The calculation of rotation is made on the basis that the rate of oxidation of succinate (in the mixture) is that of the mixture *minus* the rate of oxidation due to the lactate alone (this, it is assumed, being unaffected by the presence of succinate). The calculated and observed values of *l*-malate production in the mixture of succinate and lactate are identical within the limits of experimental error. Hence the oxidation of succinate by brain is "spared" by the presence of lactate to an extent equivalent to the amount of oxidation for which lactate alone is responsible.

This finding has an important bearing on Ashford and Holmes' investigation [1931] of the "Meyerhof quotient" in brain. These authors have found that more lactic acid disappears in the presence of brain than can be accounted for by the extra oxygen uptake. This could be explained by a lessened oxidation of proteins or lipoids in the presence of lactate, but Ashford and Holmes concluded that the sparing action was improbable because (unoxidised) breakdown products of protein or lipoid could not be found in a brain tissue consuming added lactate. The results given above, however, show definitely that lactate "spares" the oxidation of succinate and since it is unlikely that this sparing action is unique and that it only affects succinate there is every probability that the oxidation of normal constituents of the brain is spared by lactate. This is all the more significant when it is realised that the total oxygen uptake of brain is more than double that required to oxidise the entire quantity of lactic acid initially present.

Let us now consider the mechanism of this sparing action.

Mechanism of the sparing action of lactate on succinate oxidation.

Three suggestions offer themselves as explanations for this sparing action of lactate and these will be examined in turn.

A. Lactate in the presence of brain tissue and the succinate-fumarate system may not be directly oxidised but may reduce fumarate to succinate at a rate comparable with the formation of fumarate from succinate by oxida-

tion. Thus fumarate would not accumulate in the presence of lactate and a sparing action by the lactate would be evinced.

This suggestion can be experimentally tested. If lactate reduces fumarate at a speed at all comparable with succinate oxidation it is clear that the addition of lactate to fumarate in the presence of brain tissue will reduce the yield of *l*-malate by an amount proportional to the succinate formed. Accordingly 0·5 g. guinea-pig brain tissue was allowed to autoxidise in the usual way for 3 hours in the vessels of two Barcroft manometers. Sodium fumarate solution (0·025 *M*) was added to one vessel and to the other was added a mixture of sodium fumarate (0·025 *M*) and sodium lactate (0·025 *M*). The air in the manometer vessels was displaced by nitrogen and under these anaerobic conditions the tissues were allowed to remain for 2 hours. Polarimetric examinations of the contents of the two vessels were then made and it was found that the rotation (0·29°) was identical in both cases. No measurable reduction of fumarate by lactate had taken place and the possibility of lactate sparing by means of fumarate reduction must be dismissed.

B. Lactate may become adsorbed at the succinic acid oxidising enzyme, a competition between lactate and succinate taking place for the same oxidising catalyst. This suggestion was experimentally investigated in the following way.

Assuming that the sparing of succinate takes place by preferential adsorption of, or a competition by, lactate for the same oxidising enzyme, it is clear that if brain tissue can be so treated that lactate oxidation is inhibited whilst that of succinate is unimpaired, the addition of lactate to such a tissue should reduce the velocity of oxidation due to succinate. The amount of reduction would depend on the amount of adsorption of, or competition by, lactate for the succinate oxidising enzyme.

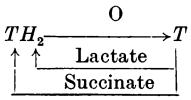
It is necessary at this juncture to anticipate certain results which will be published in full in a subsequent paper. They are to the effect that narcotics, in low concentrations, inhibit the oxidation by the brain of glucose, lactate or pyruvate and that they have no effect on the oxidation of succinate.

For the purposes of this experiment chloretone (0·12 %) was used. Brain (guinea-pig) tissue was allowed to autoxidise in the usual way in the presence of chloretone for 3 hours. After this interval the rates of O_2 uptake after addition of lactate (0·025 *M*), succinate (0·025 *M*), and a mixture of lactate (0·025 *M*) and succinate (0·025 *M*) were measured. The following were the results obtained (2 hours being the experimental period):

	mm.³
O_2 uptake by 0·5 g. tissue alone	236
O_2 uptake by tissue + lactate	298
O_2 uptake by tissue + succinate	1740
O_2 uptake by tissue + lactate + succinate	1750
Polarimetric examination:	
Rotation due to tissue + lactate	0·00°
Rotation due to tissue + succinate	+0·72°
Rotation due to tissue + lactate + succinate	+0·70°

It will be observed that with a chloretone-treated brain the presence of lactate does not appreciably affect the oxidation of succinate, either by reducing the oxygen uptake or the yield of *l*-malic acid. This result dismisses the possibility that lactate is adsorbed at or competes with succinate for the enyzme oxidising the latter.

C. The oxidation of both succinate and lactate may necessitate the intermediate action of a carrier, in the manner shown in the diagram. Assuming



this to be the case, the rate of oxidation of lactate or succinate or that of a mixture of the two will be determined by the rate of oxidation of the reduced form of the carrier TH_2 and by the rate of reduction of the oxidised form T. Lactate and succinate will compete for reduction of T, since only a limited quantity of this is available. Preferential attack of T by lactate may then simply depend on an easier access of T to the site of activation of the lactate molecule.

It is possible to test this view by examining the action of lactate on the oxidation of succinate when this is present at low concentrations.

According to the view that the oxidation of a hydrogen donator by the cell depends upon the reduction of T to TH_2 and the subsequent oxidation of TH_2 to T, it is evident that the rate of oxidation of any donator will depend upon its rate of production of TH_2. A given concentration of a donator will bring about the production of a certain concentration of TH_2 which will determine the velocity of oxidation. Let this concentration, for a given concentration of lactate, be αc where c represents the total concentration of TH_2 available; similarly let the concentration for a certain quantity of succinate be βc. Then the velocity of oxidation due to the lactate will be $\kappa\alpha c$, that for the succinate will be $\kappa\beta c$, and that for the two together will be $\kappa\alpha c + \kappa\beta c$ (κ being a constant). Now keeping the concentration of lactate constant, let us increase the concentration of succinate. The limiting velocity of oxygen uptake by the cell is obviously κc; hence when the succinate concentration increases to the extent that $\beta = 1 - \alpha$, the limiting velocity of oxygen uptake of the cell has been achieved. A further increase in succinate concentration may be expected to involve a sharing of the total quantity of TH_2 between the succinate and lactate. By hypothesis, however, the availability of T is so much more favourable for lactate than for succinate, that such sharing will not take place, α remaining constant for the given concentration of lactate. Hence increase in succinate concentration cannot bring about a greater velocity of oxidation than $\kappa (1 - \alpha) c$, though in the absence of the lactate the velocity will become κc, owing to all the carrier being available.

Hence the sparing of oxidation of succinate by lactate will only take place

when the cell is working at its full capacity, the oxidation of lactate proceeding at a constant rate independent of the amount of succinate. With low concentrations of succinate, it is to be expected that much less "sparing" of oxidation by lactate will take place. Experiment confirms this:

	mm.^{3}
O_2 uptake in 2 hrs. by 0·5 g. guinea-pig brain tissue after previous oxidation for 3 hrs.	231
O_2 uptake by the tissue in presence of 0·0125 *M* lactate	756
O_2 uptake by this tissue in presence of 0·0125 *M* sodium succinate	638
O_2 uptake by this tissue in presence of a mixture of 0·0125 *M* lactate and 0·0125 *M* succinate	1102

Polarimetric examination showed that the *l*-malic acid production with succinate alone corresponded to a rotation of + 0·20° and that with the mixture of lactate and succinate, to a rotation of + 0·17°.

The fact that cytochrome [Holmes, 1930] is abundant in the grey matter of brain makes it possible that this substance is identical with the TH_2–T system.

Ashford and Holmes [1931] do not lean to the view that lactate has any appreciable sparing action on the oxidation of tissue components, their conclusion being based on the fact that unoxidised hydrolysis products of tissue or lipoid were not found in brain tissue in which lactate was being oxidised. This argument does not seem to be flawless, for it may be conceived that oxidation of a saturated (? lipoid) molecule may proceed normally to an unsaturated form without an entire break-up of the molecule preliminary to oxidation and this oxidation may be spared by lactate in much the same way as succinate oxidation is spared.

Such sparing action could not account for all Ashford and Holmes's results, for, as they point out, under certain circumstances more lactic acid disappears than can be accounted for by the total oxygen uptake. On the other hand it is observed that the total oxygen uptakes obtained in the presence of concentrations of lactate which are small enough to be comparable with those present initially in the brain, are sufficient to account for the entire oxidation of lactate. Only with relatively high concentrations of lactate does a considerable discrepancy arise and it seems probable that here a product is formed which escapes oxidation and which cannot be estimated as lactic acid. Similar experiments with pyruvate would probably prove to be instructive. In the case of bacteria, pyruvate is transformed into a substance which seems to regenerate pyruvate on subsequent treatment with alkali [Quastel and Wooldridge, 1929]. Whether this occurs with brain tissue is a matter for further investigation.

SUMMARY.

1. The rates of oxygen uptake by brain tissue of various animals and the effects upon these of the addition of a number of substrates have been studied. Of all tissues investigated human grey matter shows the least activity towards the oxidation of succinate.

2. A modification of the usual technique is described whereby brain tissue is allowed to become greatly depleted of its oxidisable material before substrates are added to it. Using this method constant and reproducible results are obtained for the rates of oxidation of glucose, sodium succinate, *p*-phenylenediamine *etc.* in the presence of brain tissue of various animals.

3. The rate of oxidation of added substrates to the brain varies inversely with the size of the animal. This generalisation does not apply to the oxidations of muscle.

4. Glucose, sodium lactate and sodium pyruvate at equivalent concentrations are oxidised at approximately the same rate by brain tissue. Glucose is not appreciably oxidised by brain in Ringer solution, this probably being due to development of a high hydrogen ion concentration which retards the oxidation of lactate. The addition of phosphate buffer to Ringer solution restores the rate of oxidation due to glucose. Lactate is probably completely oxidised by brain. The addition of serum increases the oxygen uptake due to brain.

5. Iodoacetic acid ($M/4000$) inhibits the oxidation by brain of glucose, fructose and mannose. This result is discussed in view of the statement that fructose does not give rise to lactic acid in presence of brain. Iodoacetic acid only partially inhibits the oxidation of lactate, pyruvate and glutamate. It has no effect on succinate oxidation.

6. The oxidation of succinate by brain tissue varies with its content of oxidisable material.

7. The presence of lactate "spares" the oxidation of succinate by brain. In a mixture of lactate and succinate, the oxidation of the latter (as measured by *l*-malate production) is inhibited by an amount exactly equivalent to the oxidation for which the lactate alone is responsible.

8. The mechanism of the sparing action due to lactate on succinate oxidation is discussed. It is shown that it cannot be due to reduction of fumarate by lactate, nor to competition by lactate with succinate for the enzyme oxidising the latter. It is concluded that the action is due to competition of lactate with succinate for the oxidised form of a carrier (? cytochrome). The significance of the sparing action of lactate is discussed.

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