Electrical Stimulation *in vitro* of the Metabolism of Glucose by Mammalian Cerebral Cortex

By H. McILWAIN, G. ANGUIANO (British Council Bursar) AND J. D. CHESHIRE
*Biochemical Laboratories, Institute of Psychiatry (British Post-Graduate Medical Federation,
University of London), Maudsley Hospital, S.E. 5*

(Received 17 February 1951)

Glucose is the main substrate of the brain *in vivo* and is converted almost entirely to carbon dioxide, water and lactic acid. The rates of both the oxidation and the glycolysis increase on stimulating the brain *in situ* by electrical and also by some other means. Following our development of apparatus for electrical stimulation of portions of separated tissues (McIlwain, 1951), we have now examined the changes brought about by stimulation in the use of glucose by the cerebral cortex, measuring both the loss of this substance and the formation of its products.

EXPERIMENTAL

Media; procedure. Glycylglycine saline was that of McIlwain, Buchel & Cheshire (1951). Details given by McIlwain (1951) were followed, unless otherwise stated, with respect to phosphate and bicarbonate salines, manometric measurements, preparation of tissue, tissue-holding electrodes, and electrical stimulation. The experimental procedure described there was also followed, with the following additions. At the end of a manometric experiment the vessels were promptly removed from the thermostat and a portion (usually 0.5 or 1 ml.) pipetted into CuSO_4 for determination of lactic acid according to Barker & Summerson (1941). Another portion (usually 0.2 or 0.5 ml.) was taken for glucose according to Hagedorn & Jensen (see Hawk, Oser & Summerson, 1947).

Vessels and reagents in relation to lactic acid formation. Any failure in aeration increases lactic acid formation by brain tissues. Most experiments were carried out in vessels similar in size and shape to ordinary manometric apparatus, which is known to give adequate aeration. The virtues of the normal design were shown when, to alter electrical conditions, differently shaped vessels were used. In some of these, increased lactic acid was found in the absence of any stimulation. They were not further used and are not described. In all experiments recorded below, the stimulated and control tissues have been in vessels of closely similar shape and size.

In many experiments before and during the present studies, the formation of lactic acid by brain cortex slices has been found to be independent of moderate changes in the weight of tissue/unit volume of fluid. Commonly about 20 mg. fresh wt./ml. has been used (McIlwain & Grinyer, 1950). During some experiments, however, lactic acid formation per unit weight of tissue has been found to increase with increasing fluid/tissue ratio, being increased for example by 50% when the ratio was trebled. This would introduce errors in computing the acid formation from the CO_2 exchange in bicarbonate salines. The reason for this occasional behaviour was not definitely established, but was possibly related to the

quality of the distilled water employed. Water redistilled from all-glass apparatus, and whenever possible a uniform tissue/fluid ratio, have therefore been employed in the present experiments.

Tissue-holding electrodes and lactic acid formation. Previous studies showed a variety of electrode types to have little effect on the respiratory rates of slices fixed in them. Change in lactic acid formation has, however, been observed with some electrodes. An instance is given in Exp. 2, Table 1. Other increases, in gauze electrodes, have been 22 and 24% (electrodes *B* in phosphate and bicarbonate salines respectively). On the other hand, some wire electrodes led to lowered lactic acid formation. Thus electrodes *C*, in phosphate-glucose saline with vessels *A*, led to a fall of 30%. The relative instability of aerobic glycolysis is generally realized (Warburg, 1930).

The changes quoted above are opposed to, or are small in comparison with, those reported below to result from applied potentials. Nevertheless, it was necessary to eliminate this factor, and all observations on the effects of stimulation on lactic acid formation have therefore been based on comparisons between slices all of which have been held in the same type of tissue-holding electrodes, but of which some only were stimulated.

Effect of current on medium alone. We have noted (McIlwain, 1951) that under some conditions—avoided in these experiments—the passage of currents suitable for stimulating nervous tissues could result in acid formation from glucose. Although it did not seem likely that lactic acid would be formed, the method for lactic acid determination was not entirely specific, and it was therefore necessary to determine lactic acid in solutions through which currents corresponding to the experimental ones were passed, in the absence of tissue. These never showed any appreciable quantities of apparent lactic acid. Results in two media are quoted in Table 1, Exps. 1 and 7. Other experiments gave: electrodes *A* with glycylglycine glucose saline, <4% of that by the slices; electrodes *A*, in phosphate-glucose saline, <6% of that of the slice.

RESULTS

Lactic acid formation

The formation of lactic acid in a variety of experimental media has been determined chemically at the end of different periods of metabolism. In addition, the promptness of glycolytic response to stimulation has been judged in bicarbonate salines, and the course of acid formation computed by applying Warburg's indirect method to obtain the CO_2 and O_2 exchange.

Table 1. *Lactic acid formation in tissue-holding electrodes, and on stimulation*

(All media contained glucose and Ca salts. Experiments were at 37° except no. 9, which was at 39°. With phosphate- and glycylglycine-buffered media, O₂ was used in the gas phase and NaOH paper in a centre well; with bicarbonate-buffered media, O₂-5% CO₂ was used in the gas phase. Electrodes were of silver. Results in Exps. 1*b* and 7*b* were calculated assuming a tissue weight equal to that of Exps. 1*a* and 7*a* respectively. Calculation of the percentage increase in lactic acid formation is described in the text. The increase in respiration, where quoted, was directly observed from 5 min. manometric readings. The voltage quoted in describing condenser pulses is that taken from a battery; the peak voltage when measured from the vessel leads by a cathode-ray tube was about 80% of that applied. The resistance of the vessel was such that the voltage of each pulse fell to one-third its original value in about 1 msec.)

Exp. no.	Metabolic arrangements			Electrical arrangements			Lactic acid produced			Respiration increase during stimulation (%) Mean, 63	
	Vessel type (see Fig. 3, McIlwain, 1951)	Medium*	Slices† fixed unless stated as free	Duration of experiment (min.)	Duration of stimulation (min.)	Stimulation† (V.)	Electrode type	Unstimulated whole exp. (μmol./g.)	Stimulated whole exp. (μmol./g.)		Increase during stimulation (%)
1 <i>a</i>	A	P	o	155	Three 21 min. periods	1.5, 3, 5 a.c.	A	76.5	104	87	—
1 <i>b</i>	A	P	None	155	Three 21 min. periods	1.5, 3, 5 a.c.	A	<6	<6	—	—
2 <i>a</i>	A	B	i	195	40-80; 126-166	2.5 a.c.	B	83	165	244	—
2 <i>b</i>	A	B	i	195	None	—	None	68	—	—	—
3 <i>a</i>	A ¹	G	i	40	5-40	2.9 a.c.	D	56	125	141	120
3 <i>b</i>	A ¹	G	o	40	5-20	2.9 a.c.	D	56	115	210	About 100
4 <i>a</i>	A ¹	P	i	221	48-91; 124-191	1.6, 2.5 a.c.	A	58	102	151	16, 43
4 <i>b</i>	A ¹	P	o	221	48-91; 124-191	1.6, 2.5 a.c.	E	43	56	60	9, 24
5 <i>a</i>	A ¹	P	o	220	54-94; 126-186	1.7, 2.5 a.c.	C	49	95	207	18, 53
5 <i>b</i>	A ¹	P	o, free	220	54-94; 126-186	1.7, 2.5 a.c.	C	—	46	0	—
6 <i>a</i>	A ¹	B	o	180	61-101; 141-180	2.3, 2.5 a.c.	D	56	80	80	—
6 <i>b</i>	A ¹	B	o, free	180	61-101; 141-180	2.3, 2.5 a.c.	D	—	59	5	—
7 <i>a</i>	B	B	i	215	Four 30 min. periods	1.2, 1.9, 2.9, 3.9 a.c.	B	102	185	151	—
7 <i>b</i>	B	B	None	215	Four 30 min. periods	1.2, 1.9, 2.9, 3.9 a.c.	B	<4	<4	—	—
8 <i>a</i>	A ¹	P	o	155	33-57; 81-106	6, 24 C	D	68	100	150	78, 100
8 <i>b</i>	B	P	i	155	57-81; 107-131	6, 24 C	B	92	111	67	—
	A ¹	P	i	130	66-98; 102-125	24 C	D	79	121	127	57

* P, phosphate; B, bicarbonate; G, glycylglycine.

† o, outer; i, inner.

‡ a.c., alternating current; C, condenser pulses.

Increase on stimulation. It was easy to show large increases in lactic acid formation associated with electrical stimulation. Results are quoted in Table 1.

In most of these experiments stimulation was not applied for the whole experimental period. The effect of stimulation has therefore been calculated by assuming that in the experimental vessel the additional lactic acid was all formed during the period of stimulation. This gives results implying that lactic acid formation has increased to between 1.5 and 3.5 times its original rate during the various types of stimulation applied.

Kinetic experiments are described below. Here it may be noted that lactic acid formation by brain-tissue slices in general, independently of stimulation, is commonly not uniform, but greater in the first 20 min. or so of an experiment; and also that this calculation assumes a fairly prompt cessation of the increased rate of lactic acid formation. Even apart from the results of such calculations, however, values show a doubling of lactic acid to result from stimulation in some experiments. This occurred with inner and outer cortex slices, and in a variety of media and electrode arrangements which are listed in Table 1. It occurred as a result of the stimuli which had been found effective in causing increase in respiration, with sine-wave a.c. or with condenser pulses. Some forty experiments of this sort have been carried out, and in no case has a result opposed to those of Table 1 been encountered.

Effect of stimulation without tissue being fixed in electrode. It was feasible that the current involved in stimulation might convert a normal metabolic product of the slice into lactic acid. To examine this possibility lactic acid was determined at the end of experiments in which slices had been metabolizing (a) free, and (b) attached to electrodes; similar potentials were applied to each electrode and similar currents were found to flow in the vessels. Exps. 5 and 6 of Table 1 show that greatly increased lactic acid formation resulted only when the slices were attached to the electrodes. A close relationship to the stimulating electrode is thus necessary for increased glycolysis, as for increased respiration (McIlwain, 1951).

Manometric observations on the course of acid formation in bicarbonate-containing solutions. These observations have been used to show the promptness and course of the metabolic response to stimulation, and also to give information concerning the products. A relatively simple type of experiment suffices to give information on the first points, as is shown in Fig. 1. This gives the gas exchange caused by stimulated and unstimulated cortex slices in a bicarbonate-containing medium in equilibrium with O_2 -5% CO_2 . The medium and slices were in vessels with no absorbing agent. Stimulation (vessel C, Fig. 1) is seen to have caused a very prompt altera-

tion in the time course of the change in pressure. The alteration did not occur when the tissue was not attached to electrodes at which the potential change was occurring (curve B). The imposed alteration ceased promptly when stimulation was stopped. From experiments such as that of Fig. 1 it can be concluded that the metabolic response to stimulation is maximal within 2 min. of applying the potential gradient and ceases within about the same time. The changes may actually be more prompt and the value stated indicates the conclusions which can be drawn from experiments with ordinary manometric techniques.

Fig. 1. Change in pressure on stimulating cortex in bicarbonate-glucose saline. Vessels A^1 with electrodes F ; a.c. at 50 cyc./sec. applied as indicated. Gas phase: 5% CO_2/O_2 ; 37° ; saline about one-tenth of volume of vessel. Line A, slice without electrode; B, slice floating freely in vessel with electrode to which stimuli were applied; C, slice attached to electrode to which stimuli were applied. In order partly to eliminate the effect of different tissue weights and flask volumes, the results have been calculated to apparent $\mu\text{mol. } CO_2/g.$, using the K_{CO_2} of the vessels.

The pressure changes in such an experiment are fairly certainly the net result of O_2 uptake, CO_2 formation, and acid formation from glucose. The O_2 uptake and CO_2 formation, with brain cortex of respiratory quotient about unity (Dickens, 1936a), and in vessels and media of the present characteristics, would have as their net effect a gas absorption as the reaction product is the more soluble gas. This explains the fall in pressure in the latter part of the experiment in the absence of stimulation. Increase in pressure we have interpreted as due to acid formation. This has happened at the beginning of the experiment with all slices. Glycolysis in brain cortex slices is known to be active initially and then to fall (for examples and references see McIlwain & Grinyer, 1950). The best explanation of the increase in pressure during stimulation appears to be that it also reflects a higher rate of acid formation. The known

increase in respiration on stimulation would cause greater gas absorption unless the respiratory quotient exceeded unity which is unlikely with glucose as substrate. The increase in gas evolution on stimulation in experiments of this sort has indeed been found to be of the same order of magnitude as the increased lactic acid on stimulation, determined chemically at the end of the experiment. Thus: increased pressure from a slice in a vessel *A*, with electrodes *C*, 63 $\mu\text{mol. CO}_2/\text{g.}$; additional lactic acid, 74 $\mu\text{mol./g.}$ In a vessel *B*, electrodes *B*: additional CO_2 , 78 $\mu\text{mol./g.}$ and lactic acid, 97 $\mu\text{mol./g.}$ The discrepancy is of the order to be expected from the fact that changes in O_2 uptake and CO_2 formation on stimulation have been ignored.

A more precise assessment of CO_2 output (displacement and formation) and O_2 uptake has been made by Warburg's indirect method. There are certain difficulties in applying this method to the present experiments. Those relating to the different volumes of solution in the two vessels were obviated, as noted above. More important was the difficulty of ensuring equal stimulation in the two vessels which is necessary for joint calculation of the gas exchange. Potentially, the variables are very large. In practice we have found that most cortex slices cut first, second or third and trimmed so that about two-thirds of their area remains, and fitted as uniformly as possible to electrodes made as similarly as possible, give fairly similar results. In doing this stimuli have been used which approached those giving maximal response. Table 3 gives results from two experiments and the chemically determined lactic acid in the pairs of vessels are seen to be in reasonable agreement.

The course of the gas exchange in one experiment is quoted in Table 2. Stimulation more than doubled the O_2 absorbed and the CO_2 evolved. The stimulated rates of both processes were uniform over the hour of observation. If it is supposed that the respiratory quotient has remained unity, the CO_2

from sources other than respiration can be calculated and compared with the lactic acid determined chemically. This is the most likely source of such extra CO_2 . The comparison is made in the last two columns of Table 3. These show approximate agreement, which is taken as indicating that the above assumptions are not greatly in error; the experiments as a whole are limited by the difficulties described in the preceding paragraph.

Table 2. Increased CO_2 and O_2 exchange on stimulation

(Cortex slices of about 60 mg. were used in two pairs of vessels of which one pair was stimulated as described in Table 3, after a period of 16 min. at 37° . An additional vessel was included in the experiment and taken from the bath at this time; it gave the lactic acid formed before the period during which manometric readings were taken. The corrected pressure changes *H* were observed in vessels ($\mu\text{molar constants } K_{\text{O}_2}, K_{\text{CO}_2}$) containing 7 ml. saline, and those *h* in vessels ($\mu\text{molar constants } k_{\text{CO}_2}, k_{\text{O}_2}$) containing 3 ml. The total volume of the vessels was about 12 ml., and it will be seen that these volumes gave adequate differences in the net gas exchange in the vessels. (First pair: k_{CO_2} , 0.0512; k_{O_2} , 0.0441; K_{CO_2} , 0.0409; K_{O_2} , 0.0238. Second pair: 0.0573, 0.0499, 0.0518, 0.0347, respectively.)

Time (min. after putting to thermostat)	<i>H</i> (mm.)	<i>h</i> (mm.)	O_2 ($\mu\text{mol./g.}$)	CO_2 ($\mu\text{mol./g.}$)
Stimulated vessels				
15	0	0	0	0
30	1	27	- 36	62.5
45	- 6	50	- 76	126
60	- 18	68	- 114	186
75	- 26	83	- 145	232
Unstimulated vessels				
15	0	0	0	0
30	3	4	- 4	12
45	1	8	- 23	40
60	2	15	- 42	76
75	1	21	- 64	113

Table 3. Stimulation in bicarbonate-glucose solutions

(Experiments used inner slices of about 60 mg. in vessels *B*, with electrodes *B*. Stimulation was by sine-wave a.c. at the voltage quoted; experimental arrangement and calculation of gaseous exchange was as described in Table 2. The two values for lactic acid refer to vessels containing large and small volumes of saline respectively.)

Exp. no.	Duration (min.)		Respiration during stimulated period and corresponding times in controls		CO_2 formation		Excess of total CO_2 over total O_2 ($\mu\text{mol./g.}$)	Lactic acid during observational period, determined chemically ($\mu\text{mol./g.}$)
	Whole exp.	Observational period at voltages quoted	Rate ($\mu\text{mol./g./hr.}$)	Total uptake ($\mu\text{mol./g.}$)	Rate ($\mu\text{mol./g./hr.}$)	Total evolution ($\mu\text{mol./g.}$)		
1 <i>a</i>	76	—	76	64	126	113	49	47, 68
1 <i>b</i>	76	16–76, 2.8 V.	150	145	222	232	87	99, 96
2 <i>a</i>	62	—	68	43	122	66	23	21, 18
2 <i>b</i>	61	22–62, 3.0 V.	124	75	196	124	49	69, 62

Table 4. *Increased utilization of glucose on stimulation*

(Experiments were with guinea pig-brain cortex at 39° in phosphate-glucose saline with Ca. The initial concentration of glucose was, however, decreased to about $m/300$. Vessels *A*¹ and electrodes *D* were used with 5 or 8 ml. of the saline and 15–20 mg. tissue/ml. Condenser pulses were at 100/sec. and a.c. at 50 cyc./sec. Observation of respiration began at 7–10 min. after placing in a thermostat and no correction has been made for the unmeasured respiration.)

Exp. no.	Treatment	Duration (min.)		Glucose at end of experiment ($\mu\text{mol./vessel}$)	Glucose used ($\mu\text{mol./g.}$)	Lactic acid formed ($\mu\text{mol./g.}$)	Respiration ($\mu\text{mol./g.}$)	Glucose corresponding to lactic acid and respiration (see text) ($\mu\text{mol./g.}$)
		Metabolism	Stimulation					
1a	None; no slice	—	—	24.9	—	—	—	—
1b	Slice; no stimulation	180	—	17.5	64	—	216	—
1c	Slice; 3.2 V.	180	60	17.4	71	—	240	—
1d	Slice; 4 $\mu\text{F.}$, 24 V.	180	120	15.1	85.5	—	290	—
2a	None; no slice	—	—	26.2	—	—	—	—
2b	Slice; no stimulation	160	—	18.7	70	72	167	64
2c	Slice; 3.1 V. a.c.	160	150	15.6	97	141	177	100
2d	Slice; 4 $\mu\text{F.}$, 24 V.	160	150	13.3	115	131	252	108

Respiration

The increase in respiration previously found (McIlwain, 1951) has been observed during the present experiments. Instances are recorded in Tables 1–4. In several of these the effect of a given stimulus on lactic acid and respiration could be compared, and in almost all such cases the percentage increase in lactic acid formation was greater than that in respiration.

Glucose utilization

The increases in respiration and in lactic acid formation which have been found to follow stimulation, give expectation of finding increased glucose usage. That this occurs is shown in Table 4. It occurred in response to a.c. and to condenser pulses, and the additional usage was greater the longer the duration of stimulation. In these experiments the condenser pulses were the more effective means of stimulation as judged by respiration and glucose utilization.

We have also examined the balance of metabolism with glucose as substrate. *In vivo*, CO_2 and lactic acid account for almost all the glucose consumed (Himwich & Himwich, 1946). *In vitro*, the tissue slices respire in the absence of added substrate; they are not necessarily maintained in a steady state during an experiment, and substrates may be contributed by the slice. Dixon (1936), however, quotes a conclusion of Dr E. G. Holmes that lactic acid and CO_2 mainly account for the glucose disappearing during respiration of brain-cortex slices in its presence. This was the case during our experiments (Table 4). In assessing these experiments we have supposed all the O_2 consumed to be used in oxidizing glucose in the ratio of 6 O_2 :1 glucose; and all the lactic acid to be formed from glucose in the ratio

2 lactic:1 glucose. The glucose corresponding to the observed respiration and glycolysis could then be compared with that observed to be lost, as has been done in the last column. Approximate agreement was then found. In order to measure with reasonable accuracy the glucose disappearing, its total quantity was reduced to about $3.3 \times 10^{-3} M$. This might be expected to increase the tendency of the slices to oxidize autogenous substrates, but the results suggested that this did not occur to any large extent. During stimulation also, the increased quantities of glucose metabolized were largely accounted for by respiration and by the lactic acid formed.

DISCUSSION

The effects of fluctuating electrical potential gradients on the metabolism of portions of mammalian brain can now be compared *in vivo* and *in vitro* with respect to glycolysis as well as respiration. The stimuli in tissue sections under the newly devised conditions increased glucose usage and lactic acid formation. The glycolysis rose from initial values of 15–40 $\mu\text{mol./g./hr.}$ and reached about 100 $\mu\text{mol./g./hr.}$ The increase occurred promptly, being complete within 2 min., which represents the limit of measurement of the present methods; response may have been more prompt.

Glycolysis in the brain *in situ* is also increased promptly by electrical stimulation. A quantitative comparison between the two conditions is more difficult to make. Our experiments have been arranged so that a normal steady state of metabolism has been replaced by a markedly higher steady state. In doing this we have caused the stimulating potentials to affect as much of the tissue as possible, and to cause something approaching the largest change possible in steady state of metabolism. Measure-

ments do not appear to have been made on the result of comparable electrical stimulation *in vivo*, probably because stimulation of a large part of the brain *in vivo* is not likely to lead to stable conditions. The stimulation has major effects on the rest of the organism. Thus, convulsions with large circulatory and respiratory changes take place in the whole animal, as well as vasomotor changes in the brain itself. Increased metabolism of the brain can, however, be detected by arterial-venous difference (cf. Schmidt, Kety & Pennes, 1945) followed by lowered rates in the post-convulsive phase (Kety, Woodford, Harmel, Freyhan, Appel & Schmidt, 1948).

A different technique has given some indications of maximal values for metabolic rates during very brief periods. This consists of analysis of the brain itself, fixed by rapid freezing in liquid air, at intervals of a few seconds during the convulsions which follow general stimulation of the central nervous system. Klein & Olsen's (1947) values for the glucose and lactic acid of the brains of cats in their normal condition and after a 10 sec. convulsive period, suggest changes at the rate of $-260 \mu\text{mol. glucose/g./hr.}$ and $+310 \mu\text{mol. lactic acid/g./hr.}$ In making this calculation we have supposed the change to be spread over a period of 23 sec., as Dawson & Richter (1950) showed large changes in related metabolites within a second of applying an electric stimulus, and in Klein & Olsen's (1947) experiments convulsions usually commenced 13 sec. after applying the stimulus. The initial rate may be more rapid than this; rates of change of intermediates measured by Dawson & Richter (1950) were much greater during the first 2 sec. than during the first 20 sec., but these concerned substances (inorganic phosphate, phosphocreatine, and hexose and adenosine phosphates) which can be both formed and used up by the brain. Lactic acid and glucose are not in this category, and the changes calculated on the basis of Klein & Olsen's figures are much higher than any found in the intermediates by Dawson & Richter. It is uncertain whether the changes calculated in the above fashion from Klein & Olsen's values represent the actual rates of reaction during the 23 sec. or whether they represent the increase in those rates (which is the authors' view), for entry of substances to the brain may be restricted. Maximal rates might thus be $-370 \mu\text{mol. glucose/g./hr.}$ and $+340 \mu\text{mol. lactic acid/g./hr.}$

These calculations give rates for glycolysis close to those which have been observed in fortified homogenates. These are between 300 and $450 \mu\text{mol./g./hr.}$ (Ochoa, 1941; Utter, Wood & Reiner, 1945; Elliott & Henry, 1946; Meyerhof & Geliazkova, 1947). Indeed the situation in Klein & Olsen's (1947) experiments can be regarded as close to the situation in a homogenate, in that the changes in Klein &

Olsen's work probably involved only intracellular substrates. Maximal rates obtained by either of these two methods are much above the values, of up to $100 \mu\text{mol./g./hr.}$, which we have now found to follow electrical stimulation of the slices.

This is, however, understandable for the following reasons: (1) The high rates *in vivo* last for some seconds only, and those in diluted homogenates are dependent on the addition of reserves of intermediary metabolites which are large in proportion to the tissue weight. (2) The slices *in vitro* must first assimilate the glucose they use; some dilution has taken place in them by their imbibition of fluid from the suspending medium, and some loss of reactants has occurred, e.g. of cozymase (Gore, Ibbott & McIlwain, 1950). Phosphocreatine also is only partly resynthesized. (3) The nutrient saline and conditions of electrical stimulation in our experiments may not be optimal.

It is therefore significant that the increased rates in slices are comparable to the increases which can be induced in them by means other than electrical stimulation. They are greater than the effects of glutamate or absence of calcium salts, previously commented on (McIlwain, 1951), and approach those which can be caused by added substances such as 2:4-dinitrophenol, phenosafranin, and ethyl red (cf. Dickens, 1936b). A recent comparison from this laboratory showed that rates up to $125 \mu\text{mol. lactic acid/g./hr.}$ could be reached in this way (McIlwain & Grinyer, 1950). The electrically stimulated tissue thus appears to be respiring and glycolysing at close to its maximal rates. These are data to be taken into account in any judgement of what may be the counterpart in the brain *in situ* of such a stimulated condition. Judgement cannot, however, be made on the basis of biochemical data alone, and further investigations are in progress.

SUMMARY

1. The changing potential gradients which increase the respiration of slices of cerebral cortex, increase also its rate of utilization of added glucose and its rate of formation of lactic acid.

2. Respiration (the calculation assuming 6 mol. oxygen/mol. glucose) and lactic acid formation account to within 6% for the glucose lost, during stimulation as well as in its absence.

3. The formation of lactic acid, aerobically, can rise from 15 or $40 \mu\text{mol./g./hr.}$ to $100 \mu\text{mol./g./hr.}$ The change takes place as promptly as can be detected by the methods employed, i.e. within 2 min. It can be maintained for an hour or more.

4. Response to stimulation *in vitro* is thus in the same direction as in the brain *in situ*; it can be maintained much longer, but does not reach rates equal to those which appear to occur during the first few seconds after stimuli are applied *in vivo*.

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Adenosinetriphosphatase Activity of Brain

By MARION B. R. GORE

Biochemical Laboratories, Institute of Psychiatry (British Post-Graduate Medical Federation, University of London), Maudsley Hospital, S.E. 5

(Received 17 February 1951)

Phosphatases which act on adenosinetriphosphate (ATP) have a wide distribution in nature and are present in many mammalian tissues. DuBois & Potter (1943) described a method of assay of such an enzyme in various tissues including brain. The adenosinetriphosphatase (ATPase) of brain has been relatively little investigated, however, compared with that of muscle.

The important discovery that the contractile protein, myosin, is a highly active enzyme which splits inorganic phosphate from ATP, was made by Engelhardt & Ljubimowa (1939). The relationships between myosin, ATP and various ion concentrations in the contraction of the muscle fibre have been studied in detail mainly by Szent-Györgyi and his colleagues (Szent-Györgyi, 1947). The problem of how far the relationships existing in muscle represent a general principle which is used by other specialized tissues as a source of energy for activity is an interesting one. There is evidence that changes in functional activity are associated with changes in ATP, phosphocreatine and inorganic phosphate in brain (Stone, Webster & Gurdjian, 1945; Klein & Olsen, 1947). The enzymic hydrolysis of ATP has not, however, been shown to be a reaction essential in providing energy for the functional activity of brain.

The presence of an ATP-splitting enzyme in brain and the controlling effect which the concentration of

this enzyme has on the rate on anaerobic glycolysis of glucose and fructose by brain homogenates, slices and extracts, has been described in a series of papers by Meyerhof and his colleagues (Meyerhof, 1947; Meyerhof & Geliazkova, 1947; Meyerhof & Wilson, 1947, 1948, 1949). The importance of an adequate supply of ATP in the maintenance of glycolysis and the effect of the ATPase in brain homogenates and extracts in controlling this process has also been shown by Utter, Reiner & Wood (1945) and Utter (1950).

The present study was made to examine the properties of the ATP-splitting enzyme in brain tissue and to test the effect on it of compounds structurally related to ATP and various drugs which act on the central nervous system. The activity of brain tissue in splitting ATP has been compared with its inorganic pyrophosphatase (PPase) activity (Gordon, 1950).

EXPERIMENTAL

Enzyme preparations. A guinea pig was killed by a blow on the neck. The whole brain was excised rapidly, weighed and homogenized in 10 vol. of ice-cold distilled water, 0.154M-NaCl solution, 0.5M-KCl containing 0.03M-NaHCO₃ and 0.2M-Na₂CO₃, or Krebs-Ringer bicarbonate (Umbreit, Burris & Stauffer, 1949) from which CaCl₂ and KH₂PO₄ had been omitted. The homogenate was stored at 0° under toluene at this dilution. For measurement of enzyme activity further dilution of the homogenate to 1 g. tissue in