The Glucose, Glycogen and Aerobic Glycolysis of Isolated Cerebral Tissues

By H. McILWAIN and MARGARET A. TRESIZE
Department of Biochemistry, Institute of Psychiatry (British Postgraduate Medical Federation, University of London), Maudsley Hospital, S.E. 5

(*Received* 12 *July* 1955)

The rapidity of response of many chemical processes in the brain to its excitation *in vivo* is impressive, and for adequate characterization requires study in a simpler system than the whole animal. The present paper concerns aspects of cerebral carbohydrate metabolism which have been studied in isolated tissues. Changes in the first few seconds of their excitation (McIlwain, 1951) have proved to differ qualitatively and quantitatively from the more sustained changes studied by ordinary methods over periods of minutes or hours.

Glycolysis when the brain is excited *in vivo* can reach 350 μmoles of lactate/g./hr., which is also reached in fortified homogenates, though in isolated cell-containing systems with various stimulating agents the most rapid sustained rate of glycolysis is one-third or less of this value (see McIlwain, 1955). By the use of apparatus permitting rapid handling of the isolated tissue (Heald & McIlwain, 1956) a brief burst of rapid glycolysis has now been observed *in vitro*. Associated studies have concerned glucose and glycogen as potential sources of the lactate, and have involved examining the distribution of glucose and lactate between tissue and fluid during *in vitro* experiments.

EXPERIMENTAL

Tissues

Most experiments employed adult guinea pigs weighing 350–500 g., of either sex and fed on a mixed diet. The cerebral cortex was prepared as slices 0·35 mm. thick and weighing about 120 mg., as described by McIlwain (1951). Three slices were cut successively from first one and then the other hemisphere, and the slices were handled throughout in the order in which they were cut. Their glycogen content varied with this order, as is described below. After cutting, slices were floated to the experimental saline, drained from excess of it, weighed on a torsion balance and placed in separate experimental vessels.

Incubation. Salines, based on those of Krebs, are described by Rodnight & McIlwain (1954). Respiration and acid formation in some experiments were measured in orthodox manometric apparatus, and the tissue was subsequently removed promptly for analysis by picking it from the vessel with a mounted, bent wire.

The majority of experiments, which involved exposing the tissues to changed experimental conditions for brief periods, were carried out in the apparatus of Heald & McIlwain (1956). Here the tissues (control and experimental) were held between grids of silver wire, in 5 ml. of the experimental saline in a thermostat at 37·5°. Incubation usually began 15 min. after the death of the animal. In some experiments alternating electrical condenser pulses were applied to the silver grids from the generator of Ayres & McIlwain (1953).

Transference of tissues after incubation. The slice was lifted in its holder from the fluid, leaving the tip of the holder touching for 2 sec. the side of the beaker which had contained it. The slice was then released to a fixing or other fluid, this transfer requiring only about 0·25 sec. During the period of 2 sec. it was considered that metabolic conditions in the tissue were not altered by the partial draining, for many metabolic characteristics of such tissue remained unchanged after much longer periods of incubation with minimal fluid (Rodnight & McIlwain, 1954). If the terminal period of an experiment included the application of pulses, this was continued during the draining.

The fluid transferred with slices of 120 mg. drained in this way was found by weighing to be 0·26 $\pm$ 0·06 g. (S.D.; 6 expts.). When the concentration of substance being determined was less in the fluid than in the tissue it was found satisfactory to calculate from this value the amount of substance transferred. In other cases the tissue was briefly rinsed, as described below. The quick-transfer apparatus was arranged to contain, in one or more of its 30 ml. beakers, 15 ml. of the medium used in incubation but which lacked the substance being determined. This medium was kept oxygenated and the slice was released from its holder to this medium, picked from it with a mounted, bent wire and put in the fixing agent. Rinsing and transfer then occupied about 3 sec., and pulses could not be maintained during this time.

Administration of general depressants and iodoacetate. For some of the experiments of Table 2, tissues were taken from guinea pigs which had been treated in one of the following ways. Pentobarbitone was injected intraperitoneally as a solution (Abbott Laboratories) of 60 mg./ml. in aqueous ethanol and propylene glycol, normally at 1 ml./kg. Reflexes were lost after about 10 min. The brain was removed after 20 min. A mixture of $N_2O + O_2$ (80:20, v/v) was delivered from a dentist's apparatus to a funnel over a guinea pig; after about 60–90 sec., when the animal was lying and breathing heavily and steadily, the tissue was removed. A *cyclo*propane–O_2 mixture was adjusted to produce similar effects, and administered in the same way. For as long as possible during their preparation, the tissues in N_2O and *cyclo*propane experiments were kept in the corresponding gas mixture, which was also bubbled through the salines in which the tissue was handled.

Iodoacetic acid (as a solution of the Na salt at pH 7·3 containing 100 mg. of the acid/ml.) was administered intraperitoneally at 4 ml./kg. Abnormal behaviour began at 8–10 min. after injection, and animals were stunned at 12–14 min. when convulsions appeared to be beginning.

Determination of glycogen and lactate

When in previous work glycogen and lactate have both been estimated in cerebral tissues, separate samples have been taken for the two determinations (e.g. Kerr, Hampel & Ghantus, 1937; Klein & Olsen, 1947; Gurdjian, Webster & Stone, 1949). As the present investigations concerned the concomitant change or lack of change in the two substances in small portions of tissue, a method was devised for determining them both in the same sample. Ethanol was chosen for the initial fixing of the tissue as it appeared to penetrate it quickly and leave it in a suitable physical form, still easily releasable from the holders, which were not damaged by the ethanol.

Method. The tissue was usually a slice weighing 120 mg., and six such specimens were handled successively at brief intervals. Each, in a quick-release holder, was allowed to drain for 2 sec. and released into 3 ml. of absolute ethanol in a 30 ml. squat beaker. After 3 min. it was washed from the beaker with the ethanol to a 15 ml. test tube and broken with a glass rod. The tube was centrifuged 10 min. later, and the ethanolic extract was returned to the same beaker; the residue in the tube was rubbed in a further 2 ml. of ethanol with the same rod, and after centrifuging the second extract was added to the first.

Glycogen was determined in the residue in the tube, which was well drained from ethanol on each occasion. Ethanolic KOH (1 ml. from a mixture of 2 vol. of ethanol and 1 vol. of 60%, w/v, aqueous KOH) was added and the estimation completed according to LeBaron (1955).

The beaker containing the ethanol extracts was placed with others from the same experiment over $CaCl_2$ in a desiccator which was evacuated and left at 3° for 6 hr. or more. The residue was then rubbed up with 5 ml. of 2% (w/v) $CuSO_4, 5H_2O$ and lactic acid determined according to Barker & Summerson (1941).

Control experiments. Barker & Summerson (1941) observed that ethanol did not interfere in the lactate determination. We found that two specimens of absolute ethanol gave no more than 0·2 μmole of apparent lactate/ml. when added as such to standard lactate solutions, and had no effect after evaporation in the manner described above, which also did not lead to loss of lactate. The extraction removed 98% of added lactate and was not facilitated by acidifying.

Tissue glucose

Tissue fixed in 95% or absolute ethanol was found to yield only part of its glucose to the ethanol. In the instances in which glucose was determined, separate slices were used. These were released to 15 ml. of glucose-free oxygenated medium at 37·5°, and transferred with a wire to 1 ml. of 0·1 N-$Ba(OH)_2$ in a 10 ml. tapered centrifuge tube, and immediately ground with a glass pestle shaped to fit the tube. Zinc sulphate soln. (1 ml., adjusted to leave the solution just pink to phenolphthalein) was added and the determination completed according to Nelson (1944).

RESULTS

Lactic acid distribution and formation

Content in normal tissue and fluid. The fluids in which the slices were immersed contained initially no lactic acid. The tissue immediately after death of the animals contained about 9 μmoles/g., but the greater part of this was lost to the fluids used in preparing the tissue. The lactate content of the slices of tissue after incubation was found to be relatively uniform (Table 1), independent of the order in which the slices were cut or of the depth in the cerebral cortex which the tissue had formerly occupied. Thus after 1 hr. at 37° the mean lactate concentrations in the second and third slices cut from a hemisphere were 103 and 88% of that in the first; in the second hemisphere, that of the second and third slices combined was 96% of that in the first. None of these values differed significantly from 100%. On the other hand, experiments carried out some time apart with different animals and different batches of media gave significantly different results. Contributing to the value of 1·51 ± 0·46 μmoles/g. of Table 1 were values for successive slices from the hemisphere of one animal, of 1·24, 1·42, 1·25, and from another, of 1·62, 1·91, 1·94.

Table 1. *Lactic acid of cerebral tissues after incubation in salines of different glucose levels*

Slices of cerebral cortex weighed 100–120 mg. Some, giving the data at zero time, were without contact with fluid. Others were in quick-transfer electrodes in 5 ml. of oxygenated, phosphate-buffered glucose–salines, and were fixed in ethanol after the periods stated. S.D and number of observations are given in one case; other results are averages of duplicates which differed by less than 15%.

Initial glucose of fluid (mM)	Time of incubation (min.)	Lactic acid: In fluid (μmoles/ml. of fluid)	Lactic acid: In fluid (μmoles/g. of tissue)	Lactic acid: In tissue (μmoles/g. of tissue)
(No fluid)	0	—	—	8·62
10	30	0·42	20·2	1·36
10	60	0·62	23·7	1·51 ± 0·46 (15)
10	120	0·60	31·4	0·82
2·5	60	0·61	22·8	1·22
1·0	60	0·24	10·9	0·97

The concentration of lactate after incubation was in all cases higher in the tissue than in the surrounding fluids (Table 1). Thus under the conditions most frequently employed, in a fluid with 10 mM glucose for 1 hr., lactate in the tissue was at about 2·5 times its level in the fluid. This difference was greater earlier in the experiment, when the rate of formation of lactate was greater, and fell further after incubation for 2 hr. The rates of formation in these experiments were about 20–30 μmoles of lactate/g./hr. and are typical of tissue prepared and handled in this way (McIlwain & Buddle, 1953; Rodnight & McIlwain, 1954). The total lactate formed after incubation for 1 hr. fell only a little when the initial glucose of the fluid was lowered from 10 to 2·5 mM, but fell to less than half with 1 mM glucose, as noted under other experimental conditions (McIlwain, 1953). In the present experiments the volume of fluid was about 40 times that of the tissue, so that, in spite of the distribution described, the fluid contained 90–97 % of the total lactate formed.

Electrical pulses and lactate formation. The pulses sharply increased the tissue lactate. Fig. 1 shows this in tissues after incubation for 1 hr., in response to pulses of a potency just greater than previously found necessary for maximum metabolic effect. In 7 sec. the tissue lactate had increased by over 50 %. By contrast, changes in the lactate of the fluid during the whole 80 sec. of such experiments were small.

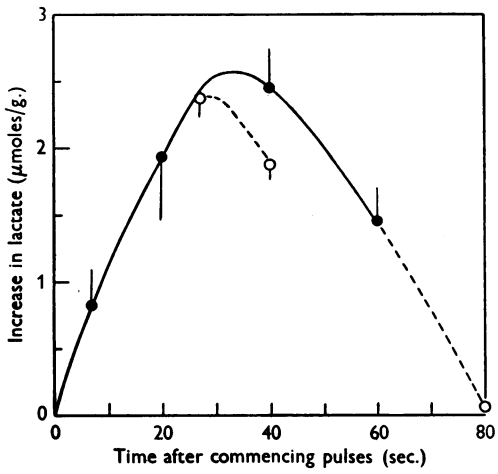

Fig. 1. Changes in the lactic acid of guinea-pig cerebral cortex after applying electrical pulses for the periods indicated. Prior to application of pulses all tissues had respired in phosphate salines with 10 mM glucose for 1 hr. Condenser pulses were alternating, of peak potential 10 v, time-constant 0·8 msec., and at 100/sec. Continuous lines and filled-in points (●) give the change with pulses applied to the moment of fixation; open points (○) and broken lines refer to experiments in which the pulses, applied until the preceding filled-in point, were followed by a period without pulses before the tissue was fixed. Vertical lines extend from the points for a distance corresponding to the S.D., described more fully in the text, and derived from six tissue specimens.

Each experimental point of Fig. 1 gives the mean difference between two groups of slices from the same one or two experimental animals. The filled circles give the differences between slices to which no pulses have been applied and others with pulses for the periods indicated. The standard deviations quoted in Fig. 1 refer to these differences, all of which are highly significant (giving t values corresponding to levels of significance of less than 0·01). The open circles (Fig. 1) give differences between slices allowed to metabolize for a few seconds after pulses ceased, and others, which after pulses for the same duration in the same experiments were fixed immediately pulses ceased. Again the mean differences were significant at the 0·01 level except that, 20 sec. after applying pulses for 20 sec., the lactate of the tissue was not appreciably different from its value when the pulses ceased.

The initial rate of lactate formation was remarkably high, being 420 μmoles/g./hr. This, however, lasted for only about 20 sec., after which, even if pulses are continued, lactic acid at first increased more slowly and then declined. The slower increase may simply reflect loss from tissue to solution. After 60 sec. application of pulses fluids averaged 22·7 μmoles of lactate/g. of tissue, while without pulses values were 21·2. The difference of 1·5 μmoles/g. is nearly equal to that of Fig. 1 between the maximal lactate of the tissue and its content at 60 sec., but had a P value of 0·1. It is naturally more difficult to measure changes of a few μmoles of lactate in the fluid which contains much more than does the tissue. Decline in lactate on continuing the application of pulses from 20 or 40 to 60 sec. appears, however, to reflect a decrease in its formation.

When pulses were stopped before lactate formation was maximal, the tissue lactate continued to rise for several seconds. When pulses were stopped at a time when lactate was already falling, the fall also continued (Fig. 1). There is thus an interesting inertia in the metabolic response to the pulses. On the other hand, the commencement of the change was prompt, the rate during the first 7 sec. being higher than in subsequent periods.

Glycogen

Normal cerebral glycogen of about 4–6 μmoles of hexose/g. of fresh tissue falls rapidly when the brain is removed and sectioned for metabolic experiments (Kerr, 1936; see Table 2). LeBaron (1955) showed that the glycogen could be restored to such sections during 4 hr. respiration in glucose salines. The present experiments commenced with attempts to

obtain this level of glycogen in a shorter time, so that the effects of agents upon it could be more readily studied.

Attempted preservation of 'native' glycogen. It was easy to preserve a higher level of glycogen in cerebral tissues by administering general depressants before removing the brain (Table 2). Pentobarbitone was more effective than nitrous oxide or *cyclo*propane; these tend to be lost from the animal while the brain is being removed and from the tissue during its preparation, though the tissue was as far as possible handled in their presence. Administration of iodoacetate also enabled a higher level of cerebral glycogen to be preserved in the separated tissue. In all these instances the glycogen of the cerebral cortex was found to be unevenly distributed, the outer part of the hemispheres, quoted first in Table 2, being appreciably richer than the inner parts. A similar trend in glycogen level of the tissue after incubation was also found, in accordance with LeBaron (1955).

Curiously, it was not found possible to preserve the glycogen of tissues from anaesthetized animals during subsequent *in vitro* metabolism. The tissue of column 2 of Table 2 had already been prepared for metabolic experiments by cutting it into sheets 0·35 mm. in thickness; yet when these were put into oxygenated glucose salines their glycogen fell in 10 min. to 15–50% of its previous value. At least 10 min. is required under these conditions for other tissue constituents to reach stable levels, and the tissue was not therefore examined at shorter intervals. After incubation for 30 and 90 min., the glycogen in tissue from anaesthetized animals had reached levels no higher than those of tissue from animals stunned without anaesthesia, when the initial glycogen levels were low. Even when the tissue was kept in the presence of a depressant by adding the drug to the saline, the glycogen proved unstable. As the presence of a depressant was undesirable in subsequent experiments, the volatile agents nitrous oxide and *cyclo*propane were chosen, and were removed from medium and tissue after a few minutes at 37° by changing from, for example, a *cyclo*propane–oxygen mixture to pure oxygen.

Resynthesis of glycogen. Attempts to accelerate the resynthesis of glycogen have been only partly successful. Provision of lactate as well as glucose, and of glucose at higher levels, was without effect, but citrate and glutamate decreased the synthesis. Glucose 1-phosphate (5 and 20 mM; we are indebted to Roche Products for this material)

Table 2. *Glycogen in guinea-pig cerebral slices before and after incubation in different salines*

In all cases the brain was removed as rapidly as possible and the cerebral hemispheres were prepared as though for a metabolic experiment. This was done by opening the hemisphere at the lateral ventricle and cutting three successive slices from it. The three glycogen values quoted refer to these slices in the order in which they were cut, the first being from the outer part of the hemisphere. Values in the second column refer to tissues fixed immediately after weighing, about 7 min. after death of the animal; and in the fourth column to tissues fixed within a sec. after removal from the incubation medium. Glycogen was determined according to LeBaron (1955) without preliminary fixation with ethanol. Incubation fluids: see text.

Before incubation		After 30 min. (or 10 min.*) at 37°	
Preparation of animal	Glycogen in successive slices (μmoles of hexose/g.)	Saline	Glycogen in successive slices (μmoles of hexose/g.)
Pentobarbitone	4·85, 3·33, 2·92	Phosphate	1·11, 0·65, 1·03
Pentobarbitone	3·80, 2·22, 1·01	Bicarbonate	1·17, 0·55, 0·60
Pentobarbitone	—	Serum–bicarbonate (1:3)	1·02, 0·54, 0·47
Pentobarbitone	—	Glutamate–bicarbonate	0·55, 0·31, 0·22
Nitrous oxide	2·23, 1·83, 1·43	$N_2O + O_2$–phosphate	— 0·31, 0·33
Nitrous oxide	—	$N_2O + O_2$–phosphate	1·12*, 0·31* —
*cyclo*Propane	3·33, 1·89, 1·69	*cyclo*Propane + O_2–phosphate	— 0·35, 0·33
*cyclo*Propane	—	*cyclo*Propane + O_2–phosphate	1·07,* 0·37* —
Iodoacetate	2·96, 1·22, 1·12	—	—
		After 90 min. at 37°	
Stunned	0·67, 0, 0·37	Phosphate	1·8, 1·26, 1·18
Stunned	0·03, 0·56, 0·59	Bicarbonate	2·16, 0·92, 1·47
Stunned	—	Citrated human plasma	0·44, 0·17, 0·24
Stunned	—	Guinea-pig serum	— 1·24, 1·62
Stunned	—	Liver extract–bicarbonate	1·65, 1·42 —
Stunned	—	Phenobarbitone (0·3 mM) phosphate	1·59, 1·47 —
Pentobarbitone	4·09, 1·82, 1·44	Phosphate	1·59, 1·24, 1·02
Pentobarbitone and exsanguination	2·41, 1·03, 0·99	—	—

yielded less glycogen and lactic acid than did glucose. Different fluid volumes and the addition of homologous sera, of liver extracts and slices, and of fluids in which slices of liver or brain had been incubated were not advantageous, but in some cases decreased the glycogen synthesized by the cerebral tissue.

During the course of the present experiments, however, the glycogen determined in the tissue after incubation for 1 hr. in glucose saline has been approximately doubled by apparently small changes in technique. The first lay in preparing the tissue rapidly. It was normally incubated 15 min. after death of the animal. That speed of preparation is important is shown by the finding (Table 3) that slices from the second hemisphere to be cut were consistently lower than the first in glycogen level after incubation. The second modification consisted of rapidly transferring the tissue to ethanol after incubation. When so determined, the glycogen of the tissue reached about half its original level after respiration for 1 hr. Glucose provided at 10 or 2·5 mM appeared to be in excess, for glycogen levels were then similar, reproducible, and higher than

Table 3. *Glycogen in cerebral cortex after* 1 *hr. at* 37°

Guinea pigs were stunned, and the brain was removed; any one of the hemispheres taken and three slices were cut successively from it, weighed, and placed in holders in saline of the glucose level stated. After 1 hr. at 37° slices were transferred to ethanol for determining glycogen and lactate. The second hemisphere was treated similarly as soon as slices had been cut from the first. Values with 2·5 and 1 mM glucose are for slices from first and second hemispheres. Glycogen levels are averages followed by their S.D. and number of observations.

Slice (order of cutting)	Glycogen (μmoles of hexose/g.)			
	In 10 mM glucose			
	Of 1st hemisphere	Difference, 1st – 2nd hemispheres	In 2·5 mM glucose	In 1 mM glucose
1st	2·35±0·16 (5)	0·14±0·06 (5)	2·22±0·17 (6)	1·94±0·16 (4)
2nd	2·08±0·26 (5)	0·12±0·08 (5)	2·00±0·56 (6)	1·57±0·1 (4)
3rd	1·80±0·13 (5)	0·18±0·08 (5)	1·67±0·15 (6)	1·46±0·08 (4)
All	2·05±0·9 (15)	0·15±0·08 (15)	1·95	1·65

Table 4. *Stability of the glycogen of guinea-pig cerebral cortex*

Each line gives results from six or twelve slices from one or two guinea pigs; half the slices in each experiment were incubated under the changed conditions described in the first column. Slices were prepared and incubated as described in Table 3 and were in oxygenated salines in 10 mM glucose except when stated otherwise. Pulses were applied after 1 hr. at 37° and recovery periods were the intervals between stopping pulses and fixing the tissue. The significance of the differences induced by changed conditions was assessed by the *t* test but is not quoted when the significance is obviously low. In the last two experiments N_2 was blown vigorously through the saline after the incubation; some seconds were involved in displacing the oxygen but the time was not determined. Insulin (British Drug Houses, A.B. 40) was at 0·5 unit/ml.

Changed conditions or applied agents*	Difference in glycogen content: slices of 1st – those of 2nd hemisphere (μmoles of hexose/g.)		
	Agent to 1st hemisphere	Agent to 2nd hemisphere	*P*
None (see Table 3)	0·15±0·08		
Pulses for 7 sec.	0·33	−0·02	>0·1
Pulses for 20 sec.	0·18	0·22	—
Pulses for 60 sec.	—	0·12	—
Pulses for 20 sec.; recovery, 7 sec.	—	0·05	—
Pulses for 20 sec.; recovery, 20 sec.	—	0·05	—
Pulses for 60 sec.; recovery, 20 sec.	—	0·07	—
Insulin	0·15	0·25	—
Pulses for 20 sec.; insulin	0·34	−0·06	<0·01
Pulses for 60 sec.; insulin	0·11	0·23	—
Pulses for 60 sec.; recovery 20 sec.; insulin	0·21	0·31	—
In mM glucose; pulses 20 sec.; recovery, 7 sec.	0·11	−0·17	>0·1
In mM glucose; pulses 60 sec.; recovery 7 sec.	−0·01	0·13	—
In 2·5 mM glucose; insulin	−0·04	0·11	—
In 2·5 mM glucose; pulses 20 sec.; recovery 7 sec.; insulin	0·09	0·36	—
Nitrogen for 1 min.	−0·12	0·17	0·05
Nitrogen for 5 min.	−0·59	0·63	<0·01

* Those named last in each line constitute the difference which is assessed in cols. 2–4.

with 1 mM glucose (Table 3). The resynthesis is thus sensitive to about the same range of glucose concentrations as is the formation of lactic acid.

It was of especial interest to determine the glycogen level of the tissue during the rapid changes in lactate recorded in Fig. 1. The glycogen proved remarkably stable under these conditions. Electrical pulses applied for different periods up to 1 min. caused no significant change in glycogen level (Table 4), though in this time lactate rapidly rose and fell by amounts nearly equivalent to the total glycogen of the tissue. In other experiments the tissue was fixed not immediately after pulses had been applied but after a brief interval of recovery after their passage. This might correspond more closely to situations in which large glycogen changes have been reported *in vivo* (Chance, 1951). However, *in vitro* no appreciable change was found either with excess (2·5 and 10 mM) or with a sub-optimum level (1 mM) of glucose. Insulin was included in several experiments (Table 4) with the intention of causing greater lability in the glycogen, but was without effect. An instance was found (Table 4) in which the presence of insulin during incubation for 1 hr. followed by pulses for 20 sec. significantly increased the glycogen of the tissue; the change was small.

Anoxia for periods of a minute or more caused loss of glycogen.

Glucose

As electrical pulses increase the lactate of cerebral tissues without appreciable effect on glycogen, glucose is the most likely source of the lactate. The hexose phosphates of the tissue appear unlikely as net source, though presumably they are intermediates, for their quantity is inadequate and they slightly increase rather than decrease in amount under conditions similar to those of the present experiments (Heald, 1956), and also on stimulation *in vivo* (Dawson & Richter, 1950).

The glucose content of sliced cerebral tissues as ordinarily prepared but handled in glucose-free salines was low, being about 0·3 μmole/g.; the initial 3 or 4 μmoles/g. in the brain *in vivo* presumably contributes largely to the lactate found in the tissue *post mortem* (p. 251). After respiration for 1 hr. in phosphate salines, the glucose of the tissue rose, but remained somewhat below the level of the saline. Thus with initially 10 mM glucose, which had fallen to 9·5–9·7 mM after 1 hr., the tissue glucose was 7·3 μmoles/g. The distribution of glucose *in vitro* (Table 5) was thus rather more in favour of the tissue than obtains *in vivo*, when tissue glucose is some 36 % of the plasma level (Klein & Olsen, 1947). This may be due to the presence of more extracellular fluid in the tissue examined *in vitro*. The distribution was notably different from that of lactate, which was at higher level in the tissue, presumably reflecting the utilization of glucose and formation of lactate in the tissue itself.

Table 5. *Glucose in slices of guinea-pig cerebral cortex*

Slices prepared and handled as described in Table 1 respired for 1 hr. in oxygenated phosphate salines of the glucose content noted. Some were then rapidly released in glucose-free saline and immediately transferred to 0·1 N-$Ba(OH)_2$. To others, pulses were applied as indicated, immediately before transfer. Mean values with S.D. and numbers of observations are quoted in some cases.

Initial glucose (mM)	Time pulses were applied (sec.)	Glucose (μmoles/g.)
10	0	7·31±0·35 (5)
10	20	6·44, 6·62
10	60	5·05, 6·14
2·5	0	1·30±0·09 (5)
2·5	20	0·35, 0·45
2·5	60	0·29, 0·21
1·0	0	0·19±0·02 (3)

Nevertheless, with each level of glucose examined, that found in the tissue was adequate to yield the additional lactate produced by pulses. That the glucose actually did fall as lactate rose is shown in Table 5. The decrease in 20 sec. was about 0·9 μmole/g., and only a further 0·2–0·5 μmole was lost in a further 40 sec. Thus the change, like that in lactate, ceases before glucose is exhausted.

DISCUSSION

The restraint normally imposed on metabolism in cerebral tissues is emphasized by the results shown in Fig. 2, which summarizes some of the present and earlier findings. Electrical pulses for a few seconds can induce lactate formation at 420 μmoles/g./hr., or over 20 times the resting rate, with corresponding loss of glucose. Formation of inorganic phosphate during the first 6 sec. of pulses is also at about 450 μmoles/g./hr. (Heald, 1954; its maximum rate during part of this time may reach 800 μmoles/g./hr.) but then ceases; the high rate of lactate formation continues for 20–30 sec. If a continuing tendency of the continuous pulses to produce phosphate is assumed, the stability of phosphate levels after 6 sec. could be accounted for by the expected esterification of 1 mole of phosphate/mole of lactate formed.

After 30 sec. with pulses, the tissue lactate falls markedly and that of the fluid rises correspondingly. The maintained rate of glycolysis is only one-fourth or one-fifth of the maximum rate. Nevertheless, inorganic phosphate does not rise at this point, although sufficient creatine and adenosine

phosphates remain in the tissue to yield 2 or 3 μmoles/g. of inorganic phosphate. Presumably either the effect of the pulses in this respect has become less, although they are still applied at the same intensity, or other phosphorylating processes have accelerated. Respiration is in fact known to increase with pulses to the extent of about 60 μmoles/g./hr., which is probably sufficient to esterify 360 μmoles of phosphate/g./hr., or approximately the deficit left when the level of glycolysis falls.

Respiration rises within a minute or two; whether it has partly replaced glycolysis at 15 sec. and completely at 30 sec., as might be suggested by the decreasing rate of lactate accumulation seen in Fig. 2, is not known. In sympathetic ganglia, respiration increases on stimulation with a time constant of about 1 min. (Larrabee & Bronk, 1952). If in general this picture of a very prompt glycolytic response succeeded by a respiratory one is correct, reason can be seen for the tissue content of glycolytic and respiratory systems being such as to give approximately equal yields of labile phosphates in a given time. This equality could also be important anaerobically. Formation of lactate even by the brain *in vivo* is not, however, only an anaerobic process, for lactate comes from the brain in venous blood still 62% saturated with oxygen (Gibbs, Lennox, Nims & Gibbs, 1942): conceivably this is produced as in the experiments of Fig. 2 with increase of activity in a part of the brain. That the energy yield of glycolysis subserved some qualitatively different role from that of respiration was suggested earlier (McIlwain, 1953) on the basis of quite different evidence.

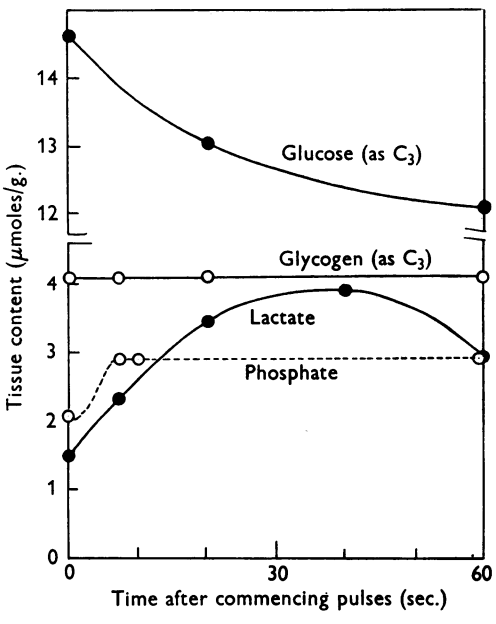

Fig. 2. Constituents of cerebral tissues after respiration for 1 hr. in oxygenated salines with 10 mM glucose (zero time in diagram) and their change with applied electrical pulses. Glucose and glycogen are expressed as three-carbon units. Phosphate levels are from Heald (1954).

SUMMARY

1. The glucose of cerebral tissues fell rapidly on excision, but was restored when the tissue respired in glucose saline; the level in the tissue remained below that of the saline.

2. The glycogen of the tissue, also rapidly lost on excision, was about half-restored after respiration for 1 hr. in glucose salines. General depressants *in vivo* preserved much of the glycogen from loss on excision, but loss nevertheless occurred on beginning respiration at 37°. Resynthesis was similar in 2·5 and in 10 mM glucose but less in 1 mM. After resynthesis, glycogen fell promptly when oxygen was excluded, but insulin and brief application of electrical pulses had little if any effect on its level.

3. The lactate of the tissue which had greatly increased *post mortem*, on respiration *in vitro* fell to levels akin to those *in vivo*, but remained greater than in a surrounding saline.

4. When electrical pulses were applied to the tissue, its glucose began within a few seconds to be converted into lactate. Conversion was at the rate of 400 μmoles of lactate/g./hr. for the first 20 or 30 sec., but greatly slowed after about 40 sec. of pulses. If pulses were stopped while the tissue lactate was still increasing, the increase continued for some seconds.

5. The maximal transitory rates of formation of lactate and of inorganic phosphate on applying pulses to the tissue were similar, and were also close to that of the formation of labile phosphates potentially associated with the observed increase in respiration. Lactate formation with continued pulses fell before glucose or phosphates were exhausted, and it is suggested that an initial momentary glycolytic response to excitation is largely replaced by a subsequent, sustained, increase in respiration.

REFERENCES

Ayres, P. J. W. & McIlwain, H. (1953). *Biochem. J.* **55**, 607.
Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
Chance, M. R. A. (1951). *Brit. J. Pharmacol.* **6**, 1.
Dawson, R. M. C. & Richter, D. (1950). *Amer. J. Physiol.* **160**, 203.
Gibbs, E. L., Lennox, W. G., Nims, L. F. & Gibbs, F. A. (1942). *J. biol. Chem.* **144**, 325.
Gurdjian, E. S., Webster, J. E. & Stone, W. E. (1949). *Amer. J. Physiol.* **156**, 149.

Heald, P. J. (1954). *Biochem. J.* **57**, 673.
Heald, P. J. (1956). *Biochem. J.* **63**, 242.
Heald, P. J. & McIlwain, H. (1956). *Biochem. J.* **63**, 231.
Kerr, S. E. (1936). *J. biol. Chem.* **116**, 1.
Kerr, S. E., Hampel, C. W. & Ghantus, M. (1937). *J. biol. Chem.* **119**, 405.
Klein, J. R. & Olsen, N. S. (1947). *J. biol. Chem.* **167**, 747.
Larrabee, M. G. & Bronk, D. W. (1952). *Cold Spr. Harb. Sym. quant. Biol.*, **17**, 245.
LeBaron, F. N. (1955). *Biochem. J.* **61**, 80.
McIlwain, H. (1951). *Biochem. J.* **49**, 382.
McIlwain, H. (1953). *Biochem. J.* **55**, 618.
McIlwain, H. (1955). *Biochemistry and the Central Nervous System*, p. 63. London: Churchill.
McIlwain, H. & Buddle, H. L. (1953). *Biochem. J.* **53**, 412.
Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
Rodnight, R. & McIlwain, H. (1954). *Biochem. J.* **57**, 649.

Anaerobic Glycolysis of Cerebral Tissues and a Second, Electrically-induced, Metabolic Defect

By H. McILWAIN
Department of Biochemistry, Institute of Psychiatry (British Postgraduate Medical Federation, University of London), Maudsley Hospital, S.E. 5

(*Received* 12 *July* 1955)

Electrical pulses increase aerobic metabolism by separated cerebral tissues in fashions similar to those resulting from application of comparable pulses to the brain *in situ*. Linkage between electrical stimulus and metabolic response is thus open to study in the isolated tissue and previous investigations suggested an outline of such linkage (McIlwain & Gore, 1953). With pulses, several reactions are accelerated to rates beyond those of restoring systems; creatine phosphate, a major reserve of metabolically available energy, falls, but ordinarily is probably being resynthesized continually as a result of glucose oxidation; respiration increases. With the intention of characterizing other components of linking systems, pulses were in earlier studies applied aerobically to tissues without added oxidizable substrate, and their effects then found not to be immediately reversible, though with added substances a partial return to normal was achieved. In the present experiments, pulses have been applied to tissues in the presence of glucose but absence of oxygen. A second metabolic defect has so been induced, and observations have been made on its nature and means of antagonizing it.

Under the anaerobic conditions chosen for the present study the main overall reaction brought about by the tissue is glycolysis yielding lactic acid. This is already known to be affected by agents germane to these investigations, and this knowledge has been extended in the present study.

EXPERIMENTAL

Tissues and fluids

Most tissues were from guinea pigs stunned by a blow on the neck, and were prepared as slices of defined thickness and position as described by McIlwain (1951). In some cases the slices were cut to fragments as follows. After weighing (60–70 mg.) they were placed in 0·5–0·8 ml. of the experimental saline and about ten cuts made through the suspended tissue with fine dissecting scissors, yielding about thirty fragments which were transferred to the experimental vessel with a 'shovel' of silver gauze mounted in a small handle. Chopped tissue was that described by McIlwain & Buddle (1953). Tissue from rats, or tissue obtained neurosurgically from man (I am greatly indebted to Mr Murray Falconer, Guy's–Maudsley Neurosurgical Unit, for the latter), was treated similarly.

The phosphate–saline was that of Rodnight & McIlwain (1954) usually with glucose at 10 mM. In the bicarbonate–saline $NaHCO_3$ (26 mM) replaced Na_2HPO_4; equilibration was with N_2+CO_2 (95:5, v/v) or O_2+CO_2 (95:5, v/v). The dry weight (105°) of tissues as used in the present studies was found to be $15\pm0{\cdot}2\%$ (8) (standard error and number of observations) of the 'wet weight' after draining on a glass surface at room temperature. Because of differences in glycolysis in the first slice cut ('outer') and the second and third cut from a hemisphere ('inner') these were examined separately and gave: outer, $15{\cdot}4\pm0{\cdot}3\%$ (4); inner, $14{\cdot}5\pm0{\cdot}3\%$ (4).

Manometric and electrical arrangements

Pulse generators and types of vessels were those previously described or referred to by Ayres & McIlwain (1953). Vessel and electrode arrangements were tested at frequent intervals throughout the experiments to ensure freedom from changes in gas pressure or in lactate, not dependent on tissue metabolism; such control experiments under aerobic conditions have been described at some length by McIlwain (1951) and by Narayanaswami & McIlwain (1954). The following additional information, more specifically related to anaerobic conditions, was obtained during the present experiments and is summarized in Table 1.

The majority of experiments of the present series were carried out under the conditions of the first four experiments of Table 1. As will be seen, they were free from any progressive change in gas pressure or in lactate which might simulate the metabolic events measured in the