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

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(*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

Table 1. *Testing for effects of pulses independent of tissue metabolism*

Experiments were at 37°; CO_2 when stated as present was at 5% (v/v). Pulses were alternating, of exponential voltage–time relationship, and obtained by charge and discharge of condensers collectively at 100/sec. In all experiments, the application of pulses caused a small volume change, of about 0·1 μl., which was reversed when pulses were switched off, and was previously shown (McIlwain, 1951) to be due to heating the vessels by a few hundredths of a degree by the pulses. Bicarbonate–glucose was used unless stated otherwise.

Electrode type (Ayres & McIlwain, 1953)	Metabolic conditions	Pulse: Peak potential (v)	Pulse: Time constant (msec.)	Result (progressive gas changes in μmoles/hr./vessel)
H, silver grid	N_2+CO_2	7–18	0·2–0·4	None*
	O_2+CO_2	7–15	0·3–0·4	None*
E, gold ring	N_2+CO_2	10–15	0·2–0·4	None*
	O_2+CO_2	10–18	0·2–0·4	None*
	3 mM Cysteine; O_2+CO_2	10	0·3	Gas absorption, 2
	—	15	0·4	Gas absorption, 4
	3 mM Cysteine, O_2; phosphate–glucose	15	0·4	Gas absorption, 8
	5% Yeast extract; N_2+CO_2	18	0·4	Gas evolution, 2
	0·8% Yeast extract, N_2+CO_2	18	0·4	Gas evolution, 0·4
	N_2+CO_2; yellow P	18	0·4	Gas evolution,* 4†
E, molybdenum ring	N_2+CO_2; yellow P	18	0·4	None*

* No apparent lactate when examined by the method of Barker & Summerson (1941).
† This change occurred also in the absence of glucose.

present experiments. However, under other conditions artifacts were found. Thus, if anaerobic conditions were maintained with yellow phosphorus in the centre well, application of pulses to electrodes of electrodeposited gold resulted for unexplained reasons in gas evolution from bicarbonate solutions in equilibrium with N_2+CO_2. This was not observed in vessels otherwise similar but with molybdenum electrodes; such electrodes were therefore employed in a few experiments in which strictly anaerobic conditions with yellow phosphorus were desired. Several added substances were without effect on gas-pressure changes under the conditions of the first four experiments of Table 1, but instances have been reported previously (Lewis & McIlwain, 1954), and others are included in Table 1, in which added substances cause such changes though the glucose of the salines does not. Effects of cysteine and a tissue extract are noteworthy.

RESULTS

Rate and stability of anaerobic glycolysis

Most of the present experiments concerned sliced guinea-pig cerebral cortex suspended in glucose–bicarbonate saline in equilibrium with N_2+CO_2. The extent of the formation of lactic acid, determined chemically in vessel contents at the end of experiments, and the extent of evolution of CO_2 from the bicarbonate, measured manometrically each 5 min., normally agreed (Table 2). Fig. 1 shows that the course of evolution of CO_2 is regular and reproducible until changed experimental conditions alter it.

Rates of glycolysis observed in different species and preparations are quoted in Table 3. Values with sliced cortex of rat and man are similar to those recorded by Dickens & Greville (1935), Weil-Malherbe (1938) and Elliott (1948), in their initial value and in their fall of some 20% in 90 min. Rates with guinea-pig tissues were similar. Table 3 shows that chopping the tissue to a suspension of particles 0·1–0·2 mg. in weight was not suitable for the present experiments as anaerobic glycolysis fell relatively rapidly, by 50–70% in 90 min.; fragments of 2–3 mg. in weight were therefore employed.

The cerebral cortex was not uniform in its level of anaerobic glycolysis (Tables 2 and 3). The outer slice, having the natural uncut outer surface of the hemisphere, yielded preparations of greatest glycolysis. Most of the remainder of the grey matter of the hemispheres was included in the next two slices cut, and these were 25% lower in glycolytic rate. With the agents studied, qualitative differences were not found between glycolysis in outer and inner slices. The difference in glycolytic rate may be compared with that in glycogen content (McIlwain & Tresize, 1956), and is not entirely explicable in terms of differences in swelling or dry weight of the tissues (see Experimental section).

Inhibition of anaerobic glycolysis

By electrical pulses. Applied electrical pulses under a variety of circumstances decreased glycolysis of both inner and outer slices (Table 4). Electrodes of gold, silver, and molybdenum were of comparable action, and under the conditions used no artifacts were induced by pulses in the absence of tissue. Agreement between manometric and chemical measures of glycolysis in individual

experiments was usually closer (see Table 2) than the standard deviations of Table 4 might imply; the values averaged in Table 4 are from experiments with different animals on different days, and were not always alike in the time at which pulses were applied.

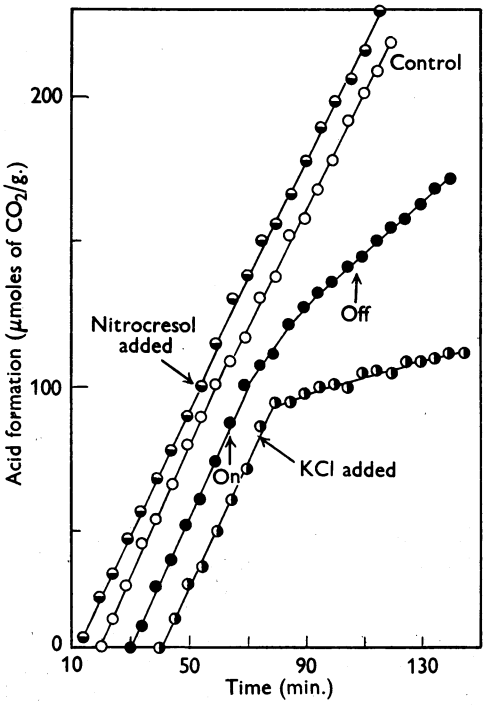

Fig. 1. Effect of pulses, 3:5-dinitro-*o*-cresol and potassium chloride on anaerobic glycolysis. Sliced guinea-pig cerebral cortex was in silver-grid electrodes *H* in bicarbonate–glucose saline. Condenser pulses of 15 v peak potential and 0·3 msec. time constant were applied at 100/sec. for the time indicated between the arrows 'on' and 'off'. The nitrocresol in the same saline was added from a side arm at the time indicated by the arrow to give a 5×10^{-5} M solution; KCl was added similarly to give a 33 mM solution. All ordinates of some curves are displaced for clarity.

That the effect of pulses was directly on the tissue was further shown by applying them to grid electrodes in salines containing tissue slices, but with the slices floating outside the electrodes. Glycolysis was then unaffected, while that of tissues within electrodes in the same experiment was greatly decreased as shown in the last line of Table 4; mean rates of CO_2 evolution were: tissue in grid: initially, 125; during pulses, 100–60; after, 44; tissue outside grid: initially, 131; during pulses, 113–105; after, 100. This latter fall occurred also if no pulses were applied (Table 3).

Table 2. *Acid formation and lactate formation compared*

Experiments were of 90–120 min. Guinea-pig tissues were sliced and cut to fragments of mean wt. 2–3 mg. These (total wt. 60–70 mg.) were floated in 3·5 ml. of bicarbonate–saline in vessels *E* (Ayres & McIlwain, 1953) to which pulses (15–18 v peak potential; time constant, 0·4 msec.) were applied as indicated. Lactic acid was determined according to Barker & Summerson (1941) and CO_2 from manometric observations. The CO_2 was measured directly for all except the first 10 and the last 1–5 min. of each experiment. During these periods it was assumed to be formed at the same rate as immediately after and before, and the total was then read by extrapolation from graphs such as Fig. 1. Observations on the same horizontal line refer to tissues from the same animal made in the same experiment.

	Vessels without pulses		Vessels with pulses	
Slice	Lactic acid (μmoles/g.)	CO_2 (μmoles/g.)	Lactic acid (μmoles/g.)	CO_2 (μmoles/g.)
Outer	273	255	137	128
	264	281	209	202
	269	258	263	239
	264	283	—	—
	302	281	—	—
Inner	171	178	145	129
	197	195	193	191
	227	225	162	155
	220	192	144	129
	183	185	140	127
	153	162	—	—

Table 3. *Rates of anaerobic glycolysis*

Rates were determined graphically from the course of CO_2 evolution from glucose–bicarbonate salines plotted as in Fig. 1. Fragments weighing 2–3 mg. were obtained by cutting slices 0·35 mm. thick, weighing them, and cutting with scissors. Chopped tissue included material from all depths in the cortex (mean wt. of fragments 0·1–0·2 mg.). Results are mean values of two or three experiments except when their number is stated in parentheses preceded by the S.E.M.

		Rate of evolution of CO_2 (μmoles/g./hr.) during		
Species	Preparation	0–30 min.	30–60 min.	60–90 min.
Man	Sliced and cut	106	81	81
Guinea pig	First slices, cut	161±8·5 (14)	142±5·5 (9)	134±7 (9)
	Second slices, cut	121±6·0 (12)	105±6·6 (10)	99±6·3 (10)
	Third slices, cut	119±4·4 (11)	—	—
Guinea pig	Chopped	138	67	41
Rat	Chopped	122	66	45

Table 4. *Inhibition of glycolysis, using electrodes and pulses of various characteristics*

Inner slices or slice fragments of guinea-pig cerebral cortex (except*, in which chopped tissue was employed) were used in glucose–bicarbonate salines equilibrated with $N_2 + CO_2$ (95:5, v/v). Silver-grid electrodes *H* (Ayres & McIlwain, 1953) were used in vessels *A*; molybdenum and gold electrodes were in vessels *E*. When sufficient values are available the mean ±S.D. is quoted; others are averages of values differing by less than 15%. Anaerobiosis was maintained with yellow phosphorus except with gold electrodes, when the phosphorus was omitted. Alternating current is described by its frequency and virtual voltage.

Electrode type and material	Pulses	No. of expts.	Duration (min.) of expts.	Duration (min.) of pulses	Vessels without pulses: Lactic acid (μmoles/g.)	Vessels without pulses: CO_2 (μmoles/g.)	Vessels with pulses: Lactic acid (μmoles/g.)	Vessels with pulses: CO_2 (μmoles/g.)
Silver, grid	50 cyc./sec., 3v	6	70	60	110	121	58±13	57±7
Silver, grid	2000 cyc./sec., 3·5v	6	70	60	100	122	74±4	82±7
	Condenser pulses, time constant 0·4 msec. (v)							
Silver, grid	10	4	70	60	106	117	65±7	73±5
Gold, ring	15	5	95	30–35	200±21	195±16	153±13	142±17
Molybdenum, ring	18	3*	135	40–45	193	180	100	94
Molybdenum, ring	18	3	100	35	283	263	127	128
					Tissue outside electrodes, pulses applied			
Silver, grid	15	3	120	30	235	232	147	139

Table 5. *Glycolysis on applying pulses after different periods anaerobically*

Inner slices of guinea-pig cerebral cortex were suspended in vessels *E* and pulses of 18v, 0·4 msec. applied from gold electrodes; the lower rates of glycolysis were established within 10 min.

Time of commencing pulses (min. after placing at 37°)	Rate before pulses (μmoles of CO_2/g./hr.)	Lower steady rate established by pulses (μmoles of CO_2/g./hr.)
10	—	40
35	118	46
65	123	43

Pulses required appreciable time to inhibit glycolysis, and the inhibition after it was established was not lost when pulses were stopped. Fig. 1 shows some 20 min. to be needed to lower a rate of 132 μmoles/g./hr. to 54 μmoles/g./hr. by pulses of a given intensity. Continuing the pulses for a further 20 min. did not further lower glycolysis, though other agents or pulses of greater intensity could do so. Thus the slightly greater effect of the pulses of Table 5 occurred within 10 min.

Pulses of given characteristics were of similar effect when applied at different periods during an experiment (Table 5).

Comparison with other agents. Pulses increase aerobic glycolysis of cerebral tissues and also their respiration, effects which are paralleled by several agents, including nitrophenols, phenazine salts and potassium salts (Dickens & Greville, 1935; Dickens, 1936; Weil-Malherbe, 1938; McIlwain, Anguiano & Cheshire, 1951; McIlwain, 1952). Some similarities and differences are seen in the actions of these substances under anaerobic conditions. The effect of KCl parallels that of applied pulses (Fig. 1); like the pulses, its action has been shown to be irreversible in the sense that it is not restored to normal when the tissue is transferred to salines with ordinary levels of potassium salts (Dixon, 1949). 3:5-Dinitro-*o*-cresol, on the other hand, has no effect anaerobically in concentrations fully adequate for a large increase in aerobic glycolysis (Figs. 1 and 2). The two effects of potassium chloride, aerobically and anaerobically, though opposite in sense are brought about by similar concentrations of potassium salts.

Comparison of effect of pulses under aerobic and anaerobic conditions

The two effects are brought about by pulses of similar intensities. Fig. 2 shows that, with condenser pulses of 100/sec. and time constant 0·3 msec., a peak voltage of 10v raises aerobic glycolysis to about half its anaerobic level. At this intensity, anaerobic glycolysis is depressed by about 40%. At 18v, both effects are intensified. Aerobically and anaerobically, the actions of sine-wave alternating current are both greater at 50 cyc./sec. than at 1000 cyc./sec.

It has also been found that when the glycolytic response of a tissue is damaged anaerobically, subsequent aerobic glycolysis is affected. This

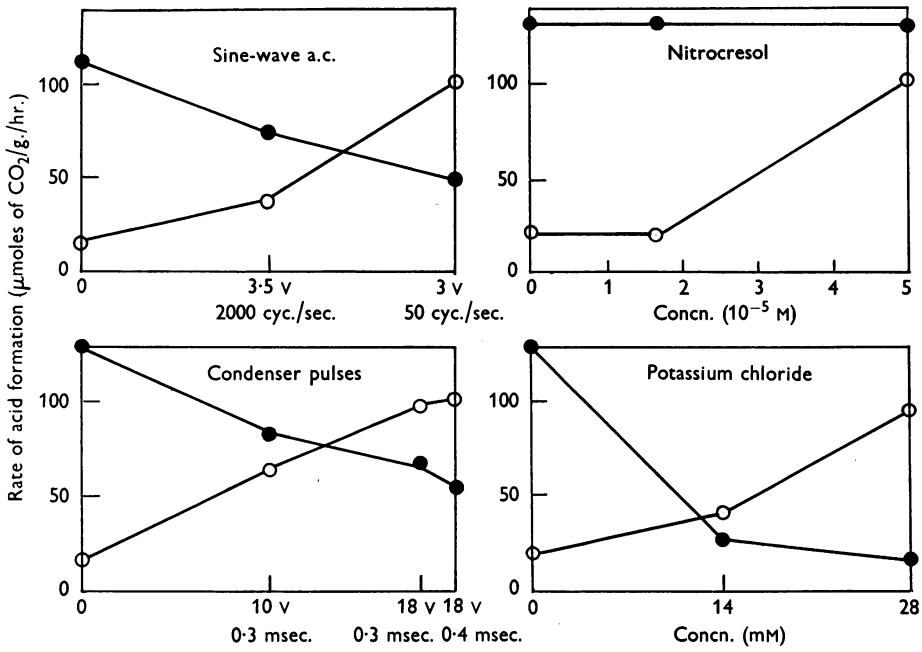

Fig. 2. Comparison of aerobic and anaerobic effects of pulses, KCl, and the dinitrocresol. Conditions for the anaerobic experiments (●) were as described in Fig. 1, except when otherwise indicated; aerobic experiments (○) employed the same tissue and apparatus, but were carried out in glucose–phosphate saline.

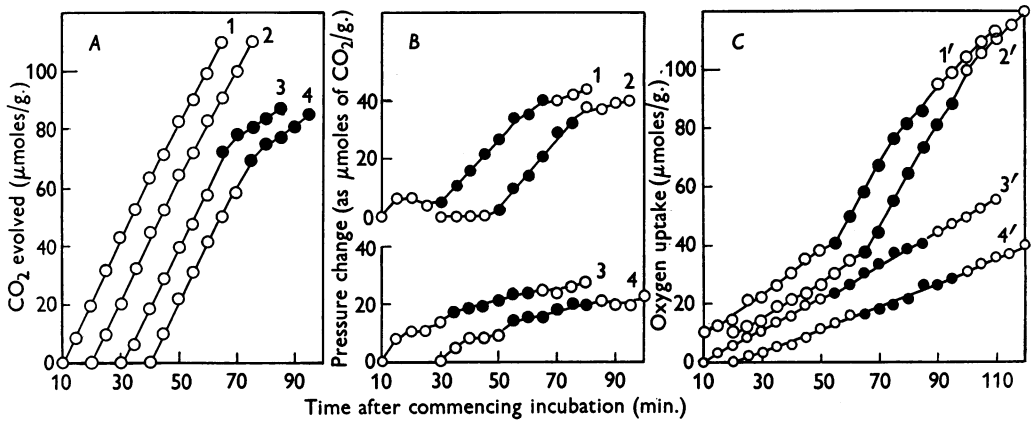

Fig. 3. Subsequent aerobic effects of pulses applied anaerobically. Part *A* was in vessels *E* with gold-ring electrodes; part *B* followed 10 min. after part *A*, the N_2+CO_2 of *A* being replaced by O_2+CO_2; conditions otherwise were the same. *C* gives the second, aerobic, part of an experiment in silver-grid electrodes *H* which commenced under the conditions of Expt. *A*, except that pulses were 15 v and applied for 20 min., commencing 10 min. after the vessels were put at 37°. ○, No pulses applied; ●, pulses applied, in *A* and *B* at 18 v peak potential, 0·4 msec. and in *C* at 15 v, 0·4 msec., in all cases exponential and at 100/sec. Media: *A* and *B*, bicarbonate–glucose saline; *C*, phosphate–glucose saline. For manipulations, see text; and for lactate determined chemically, see Table 6. All ordinates of some curves are displaced for clarity.

Table 6. *Subsequent aerobic effects of pulses applied anaerobically*

Two sets of experiments are described here and in Fig. 3. The first, *A* and *B* of Fig. 3 and 1–6 of the table, lasted anaerobically for 65 min., and aerobically for 80 min. The second, *C*, of Fig. 3 and 1′–6′ of the table, lasted 36 min. anaerobically, and 110 min. aerobically. The order in which the vessels were handled in the experiments was 1, 3, 6, 5, 4, 2. Pulses (see Fig. 3) were applied during the aerobic part of the experiments.

Vessel (see Fig. 3)	Pulses during anaerobic part	Vessel removed immediately (see Fig. 3)	Lactic acid in vessel when removed (μmoles/g. of tissue)	Change in lactic acid aerobically (μmoles/g.)
1	–	After *B*	196	+37
2	–	After *B*	210	+51
3	+	After *B*	140	– 2
4	+	After *B*	152	+10
5	–	Before *B*	159	—
6	+	Before *B*	142	—
1′	–	After *C*	70	+70
2′	–	After *C*	77	+77
3′	+	After *C*	28	+28
4′	+	After *C*	39	+39
5′	–	Before *C*	103	—
6′	+	Before *C*	89	—

action of condenser pulses is shown in Fig. 3 and Table 6. Tissues whose anaerobic glycolysis had been decreased by pulses were of normal glycolytic rate aerobically until pulses were applied, when they proved incapable of response. The mean value for lactate formed during the aerobic part of the experiment was 4 μmoles/g. (Table 6) in distinction to the 44 μmoles/g. from the tissues not treated electrically in the anaerobic part. This effect of pulses is in contrast to their action under aerobic conditions when glycolysis can be increased repeatedly (McIlwain *et al.* 1951).

Pulses applied anaerobically affect also the subsequent respiratory response of the tissue to pulses. The experiment of Fig. 3*C* was commenced in the same way as that of the preceding paragraph, except that pulses were applied to vessels 3′ and 4′ earlier, and the anaerobic part of the experiment lasted only 36 min. The vessels were then removed from the manometers, their bicarbonate–saline was removed, the vessels and tissue (held by the grid electrodes) were washed twice with 2 ml. of oxygenated phosphate–saline at room temperature, and NaOH and paper were placed in the centre wells; oxygen was passed to equilibrate, and the vessels were returned to 37°, when the observations of Fig. 3*C* were made. The low initial respiratory rates in this figure (30–45 μmoles of O_2/g./hr.) would be expected to follow from the anaerobiosis and change of media (compare McIlwain, 1953). However, pulses yielded rates of 99–110 μmoles/g./hr. in those tissues which had not received pulses anaerobically, while those which had showed no subsequent respiratory response. In this case, as fresh media had been provided for the aerobic part of the experiment, the lactic acid formed then could be observed directly. In Table 6, glycolysis in the tissue previously exposed anaerobically to pulses was at normal average rates of 15 and 21 μmoles/g./hr. In the tissue not so exposed the level was markedly higher, presumably owing to the tissue's continuing ability to respond to pulses. By the end of the experiment of Fig. 3*C*, the tissues not exposed to pulses anaerobically had returned to normal respiratory rates of 60 μmoles/g./hr.; those exposed remained lower.

Attempted prevention of anaerobic effects of pulses

Many mechanisms can be envisaged for the anaerobic depletion, and clues to its nature may be obtained by attempting to restore or antagonize the change induced by pulses. Restoration has not been achieved, but some substances have been found to antagonize the pulses.

No antagonizing effect was shown by fumaric acid, the substance most effective in restoring respiratory response to aerobically depleted tissue (McIlwain & Gore, 1953). Tissue extracts gave complex effects, for many themselves lowered anaerobic glycolysis, probably because they contained glutamic acid or glutamine (see Weil-Malherbe, 1938). Nevertheless, some antagonism to pulses was suggested, and a number of substances likely to be present in such extracts were examined. In view of the antagonism found by Wollenberger (1955) between protoveratrine and nicotinamide acting on anaerobic glycolysis, nicotinamide was tested and found active (Table 7; this result was first obtained by Miss J. L. Lewis). Nicotinic acid had little effect, but adenine also antagonized. A nucleic acid and ethylenediaminetetraacetic acid were ineffective. Further studies are in progress; some concerning anticonvulsant agents and sine-wave alternating currents have

Table 7. *Antagonism to anaerobic depletion by pulses*

Cut slices of cerebral cortex were suspended in gold-ring electrode vessels *E* some of which contained the added substances in glucose–bicarbonate salines with N_2+CO_2. Condenser pulses at 100/sec. and of peak potential 15 v, time constant 0·4 msec., were applied after 30–40 min. for 30–40 min., and the course of glycolysis was measured for about 100 min. in all. The decreases are computed from the initial and final rates.

Addition	Decrease in rate of anaerobic glycolysis by pulses (μmoles/g. of tissue/hr.) Without addition	With addition
Sodium fumarate, 20 mM	90	89
Ethylenediaminetetraacetic acid, 1 mM	109	103
Yeast nucleic acid, 10 mg./ml.	110	104
Adenine, 30 mM	118	48
Nicotinamide, 30 mM	92	42

already been reported (Greengard & McIlwain, 1955), and show that relatively low concentrations of given agents can be effective and can differ in their ability to antagonize pulses of different electrical characteristics.

DISCUSSION

A working hypothesis linking changes described now and previously (McIlwain & Gore, 1953) is as follows. Pulses aerobically in presence of glucose affect some responsive system in the tissue akin to that involved in its normal physiological activity. This brings into play the normal increase in respiration associated with increased activity and presumably coupled to processes which restore the tissue to its previous state of responsiveness. If oxygen or glucose is not available, such restoration does not occur. Several systems, themselves complex, are involved between the pulse and its response; they may be summarized as (McIlwain & Gore, 1953); (1) substrate-level changes; (2) phosphate changes; (3) ion transport; (4) membrane polarization. Certain of these or of their components have presumably failed under the different conditions examined.

Previously induced aerobic depletion was partial. The respiratory response specifically failed, leaving the glycolytic response; glycolysis in absence of pulses remained below the level to which pulses could raise it. This, and a restoration of respiratory response by malate or fumarate, suggested the defect to be in (1) above. The anaerobic depletion observed in the present experiments involves both responses. Although a way of restoring the changes which are induced by the pulses has not been discovered, agents antagonizing the changes have been found and may give more information on processes (3) or (4); the respiratory defect is distinct from that induced previously in not being restored by fumarate. Further details of system (2) have been obtained by Heald (1956). By such studies it is hoped to find more chemical detail of the linkage between applied pulse and metabolic response.

SUMMARY

1. Anaerobic glycolysis proceeded in outer slices from the cerebral cortex of guinea pigs at about 160 μmoles/g./hr. and in inner ones at about 130 μmoles/g./hr. Each rate fell about 20% after 90 min.; the fall was more rapid in more finely cut tissue, and comparable rates were observed in tissue from the rat and from man.

2. Electrical pulses of types which excite the brain *in vivo* or stimulate respiration and glycolysis aerobically *in vitro* decreased anaerobic glycolysis.

3. Decreased anaerobic glycolysis persisted after pulses were stopped, and the tissue so treated was found to have lost also its ability to respond aerobically to pulses, both in respiration and in glycolysis.

4. Several substances and preparations were without action on the glycolytic effect of pulses anaerobically, but the presence of adenine or of nicotinamide could partly prevent their effect.

I am greatly indebted to Mr A. McNeil and Miss M. A. Tresize for assistance during these experiments.

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