The scale of the method limits its accuracy to ± 5 % in a single determination, which is adequate for determinations of solubilities required for calculating manometer-vessel constants. Greater precision can be obtained by repeated determinations (see Table 1) and by increasing the dimensions of the apparatus. The special advantage of the present method compared with that of Davidson *et al.* (1952), lies in the fact that the initial pressure in the vessel is accurately measured, rather than estimated by extrapolation. In addition, temperature equilibration is ensured, as preliminary separation of the oil allows time for it to regain the temperature of the thermostat after cooling which occurs during evacuation.

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Electrical Pulses and the Metabolism of Cell-free Cerebral Preparations

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Metabolic response of sliced or chopped cerebral tissues to electrical pulses has shown several characteristics relating it to electrophysiological properties of the central nervous system (McIlwain, 1951*a*; 1954). Many of these properties can be interpreted as dependent on movements of ions to and from cells (see, for example, data collected by Eccles, 1953).

Certain changes in respiration and phosphorylation in cell-free preparations with applied electrical pulses (Abood, Gerard & Ochs, 1952) have been considered similar to those previously found in cell-containing systems. Responses in the two types of preparations have now been examined in parallel, and considerable differences found between them.

EXPERIMENTAL AND RESULTS

Particulate preparations

Glycolytic and respiratory responses to electrical pulses were less when cerebral tissues were very finely chopped and were doubtful or absent in 'homogenized' tissue, examined under conditions otherwise similar to those of the experiments with slices (Table 1*A*).

Media suitable for metabolism of sliced tissue are not optimum with cell-free preparations. In particular, in view of the connexion between changes in respiration, glycolysis, and phosphate derivatives in sliced cerebral tissue (McIlwain, 1952) it appeared desirable to examine preparations capable of oxidative phosphorylation. Two sets of conditions were chosen, using suspensions of the whole tissue, and a mitochondrial preparation.

Suspensions. In early experiments using electrodes of electrodeposited gold, partial but erratic inhibition of both respiration and phosphorylation was observed. Artifacts have previously been traced to the metal of the electrodes (McIlwain, 1951*a*) and molybdenum is free from many such effects (Ayres & McIlwain, 1953). Potential loss at molybdenum–saline interfaces is somewhat greater than at gold–or silver–saline interfaces, but full metabolic response in slices is readily obtained (Table 1*A*). Vessels with molybdenum electrodes were therefore used for examining effects of applied electrical pulses on suspensions under conditions in which phosphorylation was actively proceeding at some 200–300 μatoms P esterified/g. tissue/hr., with P/O ratios (atom/atom) of 1·2–1·8. Under these circumstances, pulses which with slices increased respiration twofold and glycolysis threefold, with suspensions increased oxygen uptake slightly (mean, 14 %) and phosphate esterification to nearly the same degree (10 %). The P/O ratio thus appeared to fall very slightly. This effect was extremely small in comparison with that which can be brought about by an agent such as 2:4-dinitrophenol (Table 1*B*) in the suspensions or by comparable pulses on the phosphates of sliced cerebral tissues (McIlwain & Gore, 1951).

It was concluded that the metabolic effects of electrical pulses on suspensions even during phosphorylation were of a different order from those brought about by pulses of the same characteristics on sliced tissues.

Mitochondrial preparations. The preparation made and handled as described by Brody & Bain (1952) was found to behave as indicated by those authors and to afford P/O ratios (atom/atom) approaching 2, while oxidizing pyruvate. Electrical pulses were without effect on respiration or phosphorylation. These experiments were, following the earlier description, carried out in media with 0·05 M potassium salts. Under these circumstances sliced cerebral tissues would already be respiring and glycolysing at their maximal rates and these would not be affected by applied pulses (Gore & McIlwain, 1952). Further experiments were therefore carried out with media progressively falling in content of potassium salts down to 0·2 mM. Electrical pulses applied to mitochondrial preparations in such media (0·2–5·0 mM-KCl) caused on the average a small change in phosphate esterification (+8%) and in oxygen uptake (+14%; mean value without pulses, $6{\cdot}31 \pm 0{\cdot}93$; average increase with pulses $0{\cdot}86 \pm 0{\cdot}55$ μmole O_2/mg. N/hr.; see the last six lines of Table 1*C*).

Table 1. *Electrical pulses and metabolism of cerebral preparations*

Preparations were from guinea pig tissues, between concentric electrodes, 10·5–11·5 mm. apart in vessels *E* (McIlwain, 1951*b*). To these were applied alternating condenser pulses of peak voltage 18–20 v and time-constant 0·4–0·5 msec., at 100/sec. Similar electrical pulses of peak potential 8–10 v gave lesser effects in all cases. Chopped tissue was prepared according to McIlwain & Buddle (1953) and suspensions to Case & McIlwain (1951). When examined histologically, the latter were found to contain very few intact cells; most nuclei were damaged but some small blood vessels were relatively intact. Mitochondrial preparations were the fraction II–R_3 of Brody & Bain (1952) from 'homogenization' of cerebral cortex in ice-cold 0·25 M sucrose. Yeast hexokinase was Slater's (1953) preparation, and cytochrome *c* and diphosphopyridine nucleotide were omitted since in agreement with Brody & Bain (1952) they were found to be without effect on respiration and phosphorylation. Adenylic acid was used in place of adenosine triphosphate and found satisfactory; fluoride was always included; other details followed Brody & Bain's description. The experiments of section *A* were in phosphate-buffered glucose saline; in *B* and *C* values from experiments with applied electrical pulses are in italics.

Preparation	Electrode	*A*. Response to electrical pulses (% of rate in their absence): Respiration	*A*. Response to electrical pulses (% of rate in their absence): Lactic acid formation
Slice, 0·35 mm. thick, particles of 2–60 mg.	Au	209±17 (5)*	288±51 (5)*
	Mo	203±17 (5)*	276±46 (5)*
Chopped tissue, particles of 10 μg.	Au	153, 150	176, 198
Suspension	Au	100, 112	90, 109
		B. Oxygen uptake (μmoles/g./hr.)	Phosphate esterified (μmoles/g./hr.)
Suspension 1	—	79, 73	272, 264
Suspension 1	Mo	*90, 88*	*282, 330*
Suspension 2	—	101, 92	292, 260
Suspension 2	Mo	*108, 128*	*312, 306*
Suspension 2 with 5×10^{-5} M 2:4-dinitrophenol	—	83, 90	14, 0
Suspension 3	—	74, 69	210, 202
Suspension 3	Mo	*82, 83*	*234, 196*
Suspension 3 with 5×10^{-5} M 2:4-dinitrophenol	—	60, 65	8, 0
		C. Oxygen uptake (μmoles/mg. N/hr.)	Phosphate esterified (μmoles/mg. N/hr.)
Mitochondrial; 37°; 50 mM-KCl	—	5·79±0·45 (5)*	20·6±2·0 (5)*
	Mo	*6·14±0·38* (5)*	*21·6±3·6* (5)*
Mitochondrial; 25°; 50 mM-KCl	—	2·24, 2·28	7·56, 9·56
	Mo	*2·24, 1·92*	*8·08, 7·96*
Mitochondrial; 37°; 5 mM-KCl	—	6·30, 6·78	24·2, 28·4
	Mo	*7·33, 6·90*	*27·4, 27·4*
Mitochondrial; 37°; 1 mM-KCl	—	7·34, 7·83	18·8, 21·8
	Mo	*9·08, 8·00*	*22·2, 21·4*
Mitochondrial; 37°; 0·2 mM-KCl	—	5·74, 5·10	22·8, 21·6
	Mo	*6·61, 6·34*	*25·4, 24·6*

* Standard deviation and number of experiments.

Control experiments; effects on individual enzymes

Because in preceding experiments, change in oxygen uptake with applied electrical pulses is not large and manometric measurement in such systems can be affected by many events other than tissue respiration, the following control observations were carried out. They are analogous to others reported previously (McIlwain, 1951*a*) in experiments with sliced tissues.

(1) Pulses were displayed with an oscilloscope throughout metabolic experiments to ensure that electrical energy was always being expended in the medium at the same rate. Any fall in rate could lead to contraction of the gas in the manometric vessel, and thus simulate oxygen absorption. This observation was all the more necessary because experiments with 'homogenates' were briefer than with slices and periods without pulses could not always be arranged before and after the period with pulses. The pulses were alternating and so lessened the possibility of electrolysis.

(2) Medium without tissue was exposed to pulses in the vessels used in respiratory experiments. No change took place. Certain electrode metals do however cause such changes (McIlwain, 1951*a*) and electrodeposited gold can also do so with substances more labile than normal media constituents. Thus oxidation of 10^{-4}M adrenaline in the phosphate saline is catalysed under the conditions of Table 1*A*, while oxidation of glucose and pyruvate is not. Cerebral preparations might produce from media constituents more labile substances and therefore after two experiments with suspensions, the reaction mixture was centrifuged and the supernatant exposed to pulses in an electrode vessel *E* (McIlwain, 1951*b*). The mean change with four such mixtures was small but could have contributed half of the 14% apparent increase in oxygen uptake observed. Pulses also might produce material affecting respiration. This is undoubtedly the case with some metals as electrodes but molybdenum, silver and electrodeposited gold were found free from such action under the present conditions. These experiments would not however control effects of substances transiently produced by tissue suspensions or by pulses.

(3) In contrast with previous experiments on slices, those with suspensions and particulate preparations expose to a much greater degree enzyme

Table 2. *Electrical pulses and creatine phosphokinase*

Reactions were followed in a mixture containing in 3 ml., creatine phosphate (1·5 mM), adenylic acid (1·8 mM), glycylglycine (0·05M at pH 7) and extracts prepared from suspensions of guinea pig cerebral cortex, as described by Narayanaswami (1952). After and during incubation, 0·5 ml. samples were precipitated with trichloroacetic acid and creatine phosphate-P determined, as described by Narayanaswami (1952). Reactions were at 37° in vessels *G* (Ayres & McIlwain, 1953) with concentric electrodes of the metals described. Alternating condenser pulses, peak potential 18v and time-constant 0·5 msec., were applied as indicated.

Cerebral suspension (mg./ml.)	Electrode metal	Exposure to pulses: Before adding substrate (min.)	Exposure to pulses: During reaction	Phosphocreatine (μg. P/ml. reaction mixture): Initial	Loss at: 20 min.	Loss at: 40 min.
1	Au	0	–	84	12	40
1	Au	0	+	84	3	3
2	Au	0	–	84	51	75
2	Au	0	+	84	3	6
2·5	Au	0	–	106	56*	87†
2·5	Au	5	–	106	16*	22†
2·5	Au	33	–	106	15*	26†
2·5	Mo	5	–	106	48*	84†
2·5	Mo	33	–	106	30*	32†
2·0	Ag	0	–	88	33	67
2·0	Ag	0	+	88	0	0
2·0	Ag	40	–	88	0	0
2·0	Ag	40	+	88	0	0
2·5	Ag	2	–	83	0	8
2·5	Ag	5	–	83	0	0
2·5	Mo	0	–	103	22	72
2·5	Mo	2	–	103	25	78
2·5	Mo	20	–	103	14	52

* Loss at 30 min. † Loss at 60 min.

systems and intracellular elements directly to electrodes. Earlier observations of effects of electrical currents and pulses of various types afford examples of inhibition and acceleration recognized as probably secondary to electrolysis or to metals from electrodes (see Duclaux, 1899; Oppenheimer, 1910). Effects of applied pulses were therefore examined on two enzyme systems relevant to the control of respiratory level and phosphate transfer. Table 2 shows that creatine phosphokinase was markedly inhibited by even brief exposures to pulses employed in preceding experiments, from gold or silver electrodes. Inhibition with molybdenum electrodes was much less, but still observable (Table 2). This contrasts markedly with properties of these metals as electrodes with slices, when each afforded the same considerable increase in respiration and glycolysis (Table 1).

Liberation of inorganic phosphate from adenosine triphosphate by cerebral 'homogenates' under Gore's (1951) conditions was unaffected by pulses described in Table 2, from molybdenum electrodes.

DISCUSSION AND SUMMARY

It is to be expected that some changes immediately linking applied electrical pulses and reactions in cell-containing systems, will be reproduced in cell-free systems; and also that mitochondria may exhibit certain analogous phenomena depending on permeability barriers which nevertheless need not be the basis for reaction of cell-containing tissues. Present experiments however show pulses adequate or even a little excessive for maximal stimulation of respiration in cell-containing cerebral tissues to cause with suitable electrodes little or no change in oxygen uptake in presence of cerebral suspensions and mitochondrial preparations. Concomitant phosphorylation showed similarly little change. The changes observed in some cases may be secondary to deleterious effects induced by the pulses in sensitive intracellular systems, and of these an example has been encountered in creatine phosphokinase. Inhibition of the phosphokinase was greatly dependent on the metals used for electrodes. Comparable dependence on the electrode material was not found in working with cell-containing preparations of cerebral tissues.

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Applied Electrical Pulses and the Ammonia and Acetylcholine of Isolated Cerebral Cortex Slices

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Acetylcholine changes in the brain *in vivo* seem to be associated with its electrical activity (see Feldberg, 1945*a*). In particular it has been demonstrated that *in vivo* electrical stimulation diminishes considerably the total extractable acetylcholine of brain; further that the acetylcholine returns close to the normal level before convulsions start; during their progress the level is again lowered (Richter & Crossland, 1949). Electrical stimulation, or the administration of picrotoxin, have also been found to increase the free ammonia of the brain (Richter & Dawson, 1948) and this increase occurs before the development of convulsions.

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