2. Electrical pulses applied to cerebral cortex slices respiring in eserinized phosphate–glucose saline gave a progressive increase in the incubation medium of a substance acting as free acetylcholine, able to contract frog-rectus and leech muscle. Expressed as acetylcholine, the rate of increase was of the order 0·056 μg./g. fresh tissue/min.

3. In the absence of eserine, electrical pulses brought about a progressive decrease in the combined acetylcholine of cerebral cortex slices (of the order 0·047 μg./g. fresh tissue/min.), reversible on the cessation of applied pulses.

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Rapid Changes in Creatine Phosphate Level in Cerebral Cortex Slices

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Previous studies have shown that the application of both electrical pulses and agents such as potassium chloride and 2:4-dinitrophenol to slices of cerebral cortex results in an increase in oxygen uptake and aerobic lactic acid production and usually to a fall in the level of creatine phosphate in the slice (McIlwain & Gore, 1951; Gore & McIlwain, 1952). The changes in creatine phosphate have been measured at the end of a relatively long experimental period and give no indication of the rate at which the level is lowered, nor the rate at which creatine phosphate is resynthesized after a particular treatment. Further, it has not been possible to determine the effects of different substrates or agents such as metabolic inhibitors, convulsants or depressant drugs upon these two processes.

This paper describes a method which enables these changes to be measured during the passage of electrical pulses through slices of cerebral cortex. The results obtained have been compared with some similar changes brought about by potassium chloride.

MATERIALS AND METHODS

The saline medium contained NaCl 128 mM, KCl 6·3 mM, $CaCl_2$ 2·7 mM, $MgSO_4$ 1·28 mM, glucose 10·0 mM, and aminotrishydroxymethylmethane (Tris) 25 mM. The medium was at pH 7·4. The sample of Tris was recrystallized from water before use. The saline medium contained no detectable orthophosphate when estimated as described below. In experiments in which 1·3 mM-KH_2PO_4 was present in the medium, the requisite amount of KCl was omitted in order to maintain the K^+ concentration at 6·3 mM. In experiments in which the concentration of KCl was increased, the control saline used with slices was made isosmotic with an equal molarity of added NaCl.

Technique for measurement of rapid changes in phosphates in slices of cerebral cortex

Slices of cerebral cortex of guinea pigs or rats were prepared as described by McIlwain (1951*a*). Techniques previously used for determination of changes in phosphate levels when slices were subjected to different stimuli involved too long a manipulative period to be used in the present study. An apparatus was therefore devised which permitted rapid release and fixation of a slice in a protein precipitant, after an appropriate period of stimulation. Two types of holders are shown diagrammatically in Fig. 1. Slices weighing 100–140 mg., of the cerebral cortex of guinea pigs were incubated in 3·5 ml. of Tris–glucose saline contained in 25 ml. conical flasks under an atmosphere of O_2 for 30 min. The flasks were fitted with inlet and outlet tubes closed by Bunsen valves and were shaken during incubation. The time taken between the death of the animal and placing the flasks in the thermostat was usually about 20 min. After incubation, the flask contents were transferred to a dish containing about 4–5 ml. of warm saline and the slices

were floated into the holder. The holder and slice were then incubated in saline contained in a small constant-temperature bath, the saline being stirred with a vigorous stream of O_2. Incubation was for 5 min. after which electrical pulses were applied for the required period of time, and the slice immediately released into 3–5 ml. ice-cold 10% (w/v) trichloroacetic acid (TCA) contained in a small dish cooled in ice and water. For periods of 1–3 sec. it was necessary to remove the slice and holder from the incubation bath before applying pulses in order that release of the slice into TCA could be prompt. Since similar treatment of slices in the absence of pulses did not decrease the level of the creatine phosphate (Tables 2 and 3) it was considered that this treatment did not markedly affect the metabolism of the slice. (It has also been shown by McIlwain & Rodnight (1954) that slices moistened with saline containing glucose respire at a rate similar to that obtained if the slices were floating in a large volume of saline in manometric vessels.)

For measurement of the effect of KCl, the slice, after incubation for 30 min. in saline containing an additional quantity of NaCl equivalent to the amount of KCl whose effect was being studied, was placed directly into the high-KCl saline and was removed into TCA after an appropriate period. Slices were disintegrated in TCA, in a small glass tube with a fitting pestle and the fine suspensions were centrifuged at $-5°$, at 20000 *g*. This produced a clear supernatant which was used for the separation and determination of phosphates.

Analytical methods

Orthophosphate was determined in the extracts by the method of Berenblum & Chain (1938), as modified by Long (1943). Slightly larger volumes of solution were used than were described by Long but the procedure was essentially the same.

Inorganic phosphate, adenosine di- (ADP) and tri-phosphate (ATP) and creatine phosphate were separated from the extracts by the barium-fractionation procedure of LePage (1949). Generally the 5 ml. of TCA extract was adjusted to pH 8·4 with KOH (5 N and 0·1 N) and 1·0 ml. of 25% (w/v) barium acetate was added to precipitate the barium-insoluble salts. This procedure gave recoveries of $94 \pm 2{\cdot}8$% with quantities as low as 10 μg. of orthophosphate, in a total volume of 6–7 ml. This was the level of inorganic phosphate found in slices of cerebral cortex weighing 100–140 mg. wet wt. Inorganic phosphate was estimated directly in the barium-insoluble fraction and ATP was estimated as the difference between '10-min. phosphorus' and inorganic phosphorus. Creatine phosphate was determined in the barium-soluble fraction, after removal of excess of barium with sulphuric acid, by decomposition with ammonium molybdate for 30 min. at room temp. and determination of the orthophosphate formed (see Ennor & Stocken, 1948; Ernster, Zetterström & Lindberg, 1950). No attempt was made to precipitate barium creatine phosphate before analysis, since it has been adequately demonstrated (McIlwain, Buchel & Cheshire, 1951; McIlwain, 1952*a*) that creatine phosphate is the major labile phosphate present in the fraction, separated as above, from extracts of cerebral cortex slices which had respired in a glucose-containing saline.

Lactic acid. This was estimated by the method of Barker & Summerson (1941) in samples of the saline after the slices had respired for a definite time (Table 1).

Measurement of respiration of slices of cerebral cortex, in the presence and absence of applied electrical pulses, was made in the Warburg apparatus using the ring electrode vessels type *E* (McIlwain, 1951*b*). Electrical condenser pulses were supplied at 100/sec. from the apparatus of Ayres & McIlwain (1953) using a peak voltage of 18 v with a duration of 0·4–0·5 msec. The experimental conditions were similar to those described by Heald (1953).

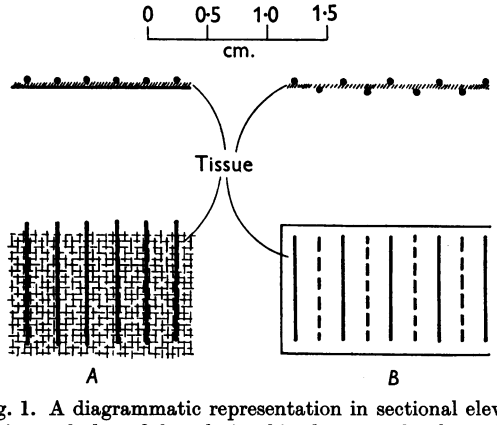

Fig. 1. A diagrammatic representation in sectional elevation and plan of the relationships between the electrodes and tissue slices. *A* represents a preliminary type of holder in which one electrode consisted of silver gauze and the other of silver wire. *B* represents a type of holder patterned after that described by Ayres & McIlwain (1953) (Fig. 2, diagrams *A–D*), in which both top and bottom electrodes were of silver wire.

RESULTS

Respiration and glycolysis in saline buffered with Tris

In Table 1 is shown the oxygen uptake and lactic acid production of slices of cerebral cortex, when metabolizing glucose in Tris–saline medium. On applying electrical pulses both respiration and glycolysis increased. The increase in lactic acid production was as great as that found in phosphate-buffered saline (McIlwain, 1951*b*), although the increase in oxygen uptake was somewhat less. Since ammonium ions at 10^{-3} M increase glycolysis in the absence of applied pulses, by some 220% (Weil-Malherbe, 1938; McIlwain & Gore, 1951), the values obtained in Table 1 indicated that ammonium salts were not present in the preparation of Tris used, in an amount sufficient to have any marked effect.

Resynthesis and maintenance of creatine phosphate

When freshly cut slices of cerebral cortex were incubated in Tris–glucose saline, creatine phosphate was resynthesized. In Table 2 (Expts. 1–3) are compared levels of creatine phosphate and inorganic phosphate both in slices which were fixed in TCA

Table 1. *Respiration and glycolysis of cerebral cortex slices in glucose–saline buffered with aminotrishydroxymethylmethane, with and without applied electrical pulses*

Each manometer vessel contained 3·5 ml. Tris–glucose saline and 70 mg. tissue slices and 0·2 ml. 5N-KOH in the centre well. Gas, O_2; temp., 37·5°. Alternating condenser pulses at 100/sec. Peak voltage: 18v. Duration of experiments 90 min. Pulses were applied during the 30th–60th min.

	O_2 consumption (μmoles/g. wet wt./hr.)			Lactic acid production (μmoles/g. wet wt. tissue)	
Animal	0–30 min.	30–60 min.	60–90 min.	With pulses	Without pulses
Rat	72	100	69	57	33
Rat	71	108	68	57·5	37
Guinea pig	54	102	55	—	—

Table 2. *Resynthesis of creatine phosphate in rat cerebral cortex slices*

Slices incubated for 30 min. in glucose–saline, buffered with aminotrishydroxymethylmethane in O_2 at 37·5°. Each value was obtained by pooling two slices of 70–80 mg. wet wt. The columns marked with an asterisk denote values obtained by transferring the slices into TCA immediately after cutting and weighing.

		Inorganic phosphate (μmoles/g. wet wt. tissue)		Creatine phosphate (μmoles/g. wet wt. tissue)	
Expt. no.	Treatment	Before* incubation	After incubation	Before* incubation	After incubation
1	Slices incubated in Tris–glucose saline for 30 min. under O_2	5·0	3·5	0·95	1·50
2		6·0	2·4	0·55	1·42
3		4·4	0·8	0·71	1·02
4	Slices incubated while held in holder *A* for 5 min., after incubation for 30 min. as above	7·6	2·4	0·95	1·40
5		7·2	1·7	0·60	1·37

Table 3. *Decrease of creatine phosphate on passage of electrical pulses through slices of cerebral cortex, in holder Type* A

Saline and pulses as in Table 1. Pulses were applied for 1 min. Slices were incubated in the holders at 37·5° in saline stirred by an oxygen stream for 5–10 min. before passing pulses. These slices had been previously incubated in small flasks as described in Methods.

	Inorganic phosphate (μmoles/g. wet wt. tissue)		Creatine phosphate (μmoles/g. wet wt. tissue)	
Expt. no.	With pulses	Without pulses	With pulses	Without pulses
1	1·70	1·80	0·66	1·20
2	2·70	2·12	0·78	1·80
3	1·78	1·60	0·68	1·20

without incubation and in slices which had been incubated in the medium containing glucose for 30 min. The levels of creatine phosphate finally reached were similar to those found in bicarbonate- and glycylglycine-buffered media (McIlwain, Buchel & Cheshire, 1951; McIlwain & Gore, 1951).

Transfer of the slices to a holder (Fig. 1), followed by further incubation of the slice in oxygenated saline, did not result in lowered levels of creatine phosphate (Table 2, Expts. 4 and 5). Since synthesis and maintenance of labile phosphates is dependent upon adequate oxygenation of the medium and hence of the tissue slice (McIlwain, 1952*a*), the results in Table 2 showed that this condition was fulfilled.

Application of electrical pulses for 1 min. to the slices when in the holders, decreased the level of creatine phosphate to that found in the slices before incubation (Table 3). From these experiments it was concluded that the saline and working conditions were adequate for the maintenance of the slices in a good metabolic condition and that the electrodes permitted response to applied electrical pulses.

Effect of electrical pulses

Role of breakdown of creatine phosphate. When slices were electrically stimulated for different periods of time, the levels of creatine phosphate and inorganic orthophosphate changed as shown in Fig. 2. This curve was obtained using a preliminary type holder *A* (Fig 1). Essentially similar results were obtained using holder *B*. The breakdown of creatine phosphate was complete in 5–6 sec. and the level was not further decreased by the passage of pulses for an additional 55 sec. The rate of breakdown of creatine phosphate in either holder *A* or *B* was 1200–1400 μmoles/g. wet tissue/hr. Concomitant with the decrease in creatine phosphate, inorganic phosphate increased. The small initial rise in the level of creatine phosphate during the first second, accompanied by a corresponding decrease in the level of inorganic phosphate was not statistically significant ($P > 0·05$).

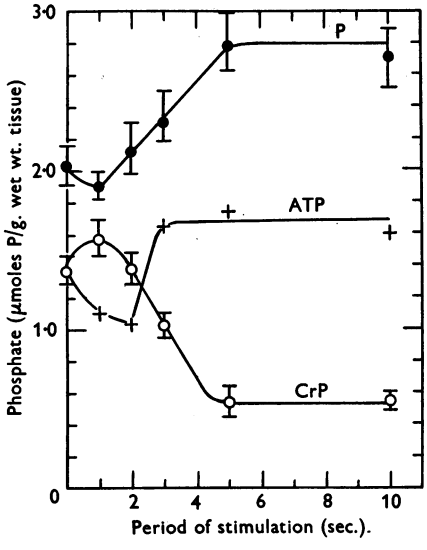

Fig. 2. Changes in creatine phosphate (CrP), inorganic phosphate (P) and adenosine triphosphate (ATP), in slices of guinea pig cerebral cortex during the passage of electrical pulses. Saline and conditions as in Methods. The horizontal lines above and below the experimental points in this and the following figures represent the limits of the S.E.M. of at least six determinations.

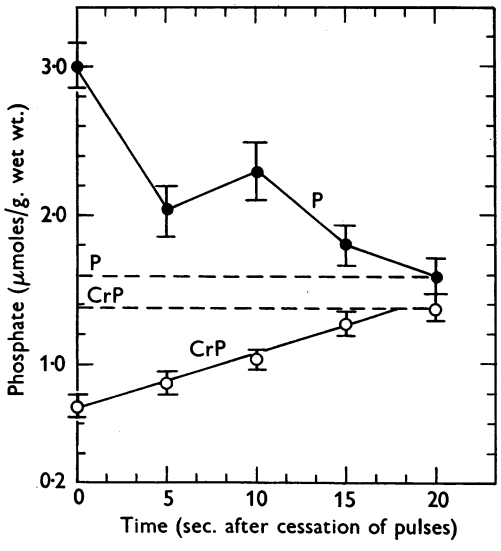

Fig. 3. The resynthesis of creatine phosphate (CrP) in slices of guinea pig cerebral cortex after the passage of impulses for 7 sec. Saline and conditions as in Methods. The dotted lines represent the levels of inorganic phosphate (P) and creatine phosphate in the slice before the passage of pulses.

Changes in adenosine triphosphate levels. In brain, as in muscle, the breakdown of creatine phosphate can be mediated by an active creatine phosphokinase (Narayanaswami, 1952). This system requires the presence of ADP, or adenylic acid, as phosphate acceptor. Previous studies had shown that in slices of cerebral cortex under good metabolic conditions, the adenosine polyphosphates were almost solely in the form of ATP with little or no ADP present (Kratzing & Narayanaswami, 1953). Since from these results it seemed that a breakdown of ATP to ADP might precede the breakdown of creatine phosphate, it was considered that a change in the level of ATP in the slices, as a result of this conversion, might be detectable during the first few seconds after passing pulses. Measurements of the acid-labile phosphate in the barium-insoluble fraction during the first 5–6 sec. of the passage of electrical pulses (Fig. 2) suggested that a decrease in ATP level did indeed precede a fall in the level of creatine phosphate.

Rate of resynthesis of creatine phosphate. Advantage was taken of the ease with which slices could be rapidly fixed after a given treatment to determine the rate of resynthesis of creatine phosphate after the passage of electrical pulses for short and long periods of time. In Fig. 3 are shown the changes in creatine phosphate and inorganic phosphate when pulses were passed through the slices for 6–7 sec. and the slices were then allowed to remain in the oxygenated saline for differing periods of time. Resynthesis of creatine phosphate was complete in about 20 sec. and was accompanied by a loss of inorganic phosphate from the slice. The rate of resynthesis was 150 μmoles/g.

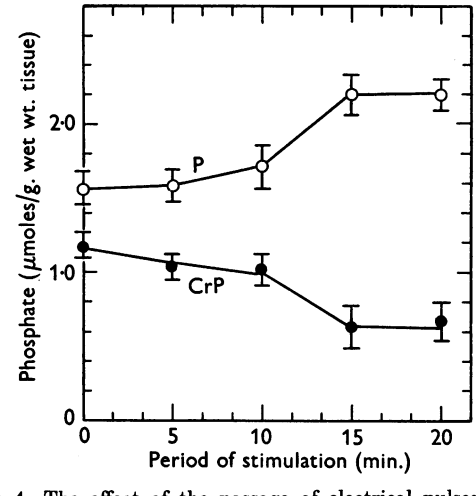

Fig. 4. The effect of the passage of electrical pulses for increasing periods of time on the ability of slices of cerebral cortex to resynthesize creatine phosphate within 20 sec. of switching off the pulses. Saline and conditions as in Methods. The ordinate represents the levels of creatine phosphate (CrP) and inorganic phosphate (P) found in the slices 20 sec. after switching off the pulses.

wet wt./hr. It was considered possible that after the passage of pulses for a long period, certain metabolites necessary for the rapid resynthesis of creatine phosphate might be depleted. Slices were treated in this way, for differing periods of time and allowed to recover for 20 sec. after cessation of pulses. The results are shown in Fig. 4, from which it is clear that continued passage of pulses decreased the ability of the slice to re-synthesize creatine phosphate.

In Fig. 5 is shown the resynthesis of creatine phosphate after passage of pulses for 20 min., both in a medium containing 1·3 mM-KH_2PO_4 and in the saline medium. The rate of resynthesis was much

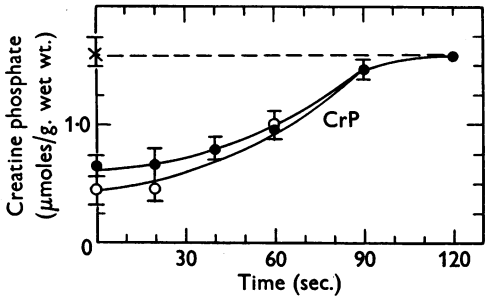

Fig. 5. The resynthesis of creatine phosphate (CrP) in slices of cerebral cortex after passing electrical pulses for 20 min. in two different salines. Saline 1, Tris–glucose saline; saline 2, Tris–glucose saline with 1·3 mM-KH_2PO_4. ○—○, Creatine phosphate resynthesis in saline 1; ●—●, creatine phosphate resynthesis in saline 2. The dotted line represents the level of creatine phosphate in slices before the passage of pulses.

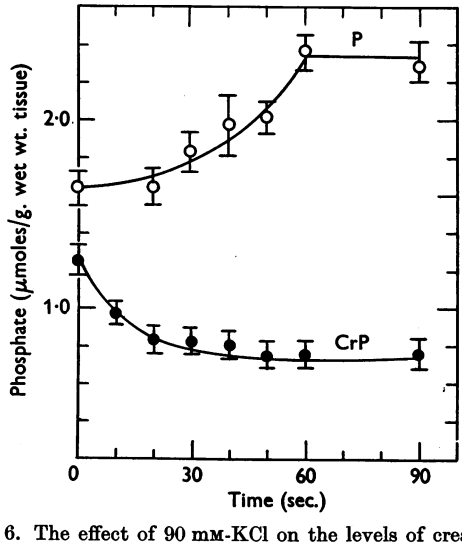

Fig. 6. The effect of 90 mM-KCl on the levels of creatine phosphate (CrP) and inorganic phosphate (P) in slices of cerebral cortex which had previously respired in Tris–glucose saline containing an additional 83·0 mM-NaCl.

slower than after applying pulses for a short period. The rate at the steepest portion, between the 80th and 90th sec., was 60 μmoles/g. wet wt./hr. Resynthesis was not affected by the presence of inorganic phosphate in the medium. The added phosphate made the values for inorganic phosphate in the slice too variable to be of use.

The effect of potassium chloride

Previous studies (McIlwain & Gore, 1951; McIlwain, 1952*a*) had shown that the increase in respiration and glycolysis when slices were treated with 0·1 M potassium chloride (Ashford & Dixon, 1935; Dickens & Greville, 1935) was accompanied by a decrease in creatine phosphate and an increase in inorganic phosphate. In this respect the effect was similar to that of electrical pulses. The similarity was greatest with 90 mM potassium chloride, at which level oxygen uptake was increased by 110 % of the control and lactic acid production by 70 %. When slices of cerebral cortex were incubated in a saline containing 90 mM potassium chloride for increasing periods of time the level of creatine phosphate decreased as shown in Fig. 6 falling most rapidly during the first 10 sec. at an initial rate of 120–130 μmoles/g. wet wt./hr. After 20 sec. the level of creatine phosphate had decreased by 80 % of the amount of the total decrease. In contrast, during this period, the level of inorganic phosphate had not markedly increased and did not begin to do so until after the 20th sec.

DISCUSSION

The technique employed above has made possible the following of rapid changes in the levels of creatine phosphate and inorganic orthophosphate in slices of cerebral cortex when subjected to applied electrical pulses. While no attempt was made here to investigate these changes in detail, the results obtained permit a comparison of the rates of breakdown and resynthesis of creatine phosphate in intact slices, with rates of similar processes taking place either in brain *in vivo* or in brain preparations.

The effect of electrical pulses

The breakdown of creatine phosphate. On passing pulses, the level of creatine phosphate in the slices decreased at a rate of 1400 μmoles/g. wet wt./hr. This decrease did not occur immediately, but after a delay of 2–3 sec. This result differs from the *in vivo* results reported by Dawson & Richter (1950). These authors applied electrical pulses to the skulls of young rats for a period of 1 sec. or longer after which the animals were immediately dropped into liquid air. If this was done after 1 sec., brain creatine phosphate was decreased by 50 % (Dawson & Richter, 1950, Fig. 1), from which a rate of breakdown

of 4600 μmoles/g. wet wt./hr. can be calculated. However, since the time taken for the transfer of the animals was about 1 sec. and the time for freezing the cortex about 4 sec., it is possible that this value is too high. Breakdown of creatine phosphate *in vivo* may therefore follow a course similar to that shown in Fig. 2.

The rate of breakdown of creatine phosphate in slices is appreciably lower than the maximum rate of 3750 μmoles/g. wet wt./hr. found by Narayanaswami (1952) for a preparation of guinea pig brain creatine phosphokinase. The rate is also lower than that of the liberation of inorganic phosphate from ATP by brain preparations, which can proceed at 1600–2100 μmoles P/g. wet wt./hr. (Gore, 1951). However, creatine phosphate is broken down at a rate greater than that required for the phosphorylation of glucose. Thus, from the data of Klein & Olsen (1947), McIlwain, Anguiano & Cheshire (1951) calculated that the maximal rate of glucose uptake by cat brain stimulated *in vivo* with electrical pulses was 370 μmoles glucose/g. wet wt./hr. during the first few seconds. This accords well with the value of 390 μmoles glucose/g. wet wt./hr. observed as the maximal rate of hexokinase activity in rat brain 'homogenates' by Long (1951). If all the glucose is phosphorylated to hexose diphosphate the maximal requirement for 'energy-rich' phosphate is about 780 μmoles/g./hr. This value is considerably exceeded by the rate of breakdown of creatine phosphate and suggests that the 'energy-rich' phosphate released in response to pulses is not used solely in the phosphorylation of glucose.

Resynthesis of creatine phosphate. In contrast to the rate of breakdown, the rate of resynthesis of creatine phosphate after electrical stimulation had ceased was 150 μmoles/g. wet wt./hr. This compares with a value of 340 μmoles/g./hr. in rat brain *in vivo* calculated from the data of Dawson & Richter (1950). The period required for complete resynthesis was 17–20 sec. This result was obtained after passage of pulses for 7 sec. If pulses were passed for a longer period (Figs. 4, 5), creatine phosphate was no longer resynthesized within 20 sec. after switching off the pulses but required up to 90 sec. The maximal rate attained was 60 μmoles/g. wet wt./hr. This slower rate of resynthesis was not altered by the presence or absence of inorganic phosphate in the medium (Fig. 5). Failure to resynthesize creatine phosphate rapidly under these conditions suggests that factors, other than glucose, oxygen and inorganic phosphate are required.

It is considered, however, that such factors are not seriously depleted during the passage of pulses for a minimal period of 7 sec. This view is supported by the results in Fig. 4 which show that even after passing impulses for 5 min., the creatine phosphate level was almost completely restored within 20 sec. of cessation of pulses. The difference in time therefore between a 7 sec. application and that required to produce a measurable effect on the rate of creatine phosphate resynthesis is very large. It is possible then to compare the rate of resynthesis shown in Fig. 3 with the rate of breakdown (Fig. 2). These rates of 150 μmoles/g. wet wt./hr. and 1400 μmoles/g. wet wt./hr. for resynthesis and degradation, respectively, clearly show that lowering of the creatine phosphate level on passage of electrical pulses is due to an increased rate of breakdown and could not be solely due to a decreased rate of synthesis. Further, it seems unlikely that the increased breakdown is due to a suppression of oxidative phosphorylation by electrical pulses. Thus, assuming a P/O ratio of 3·0 (Ochoa & Stern, 1952) in the metabolism of glucose and knowing that the rate of uptake of oxygen by slices of guinea pig cerebral cortex under the above conditions is 55 μmoles/g. wet wt./hr. (Table 1) the total requirement for 'energy-rich' phosphate to maintain a steady state in the absence of pulses could not exceed 330 μmoles/g. wet wt./hr. Since this rate is a quarter of the rate of breakdown of creatine phosphate measured above, complete cessation of oxidative phosphorylation, if it occurred, could not account for the results obtained in Fig. 2.

Relationships between inorganic phosphate, creatine phosphate and oxygen uptake. Increase or decrease in the creatine phosphate level was accompanied by a decrease or increase in inorganic orthophosphate (Figs. 2–4). The close relationships in the variation in levels of these two components suggests that the orthophosphate measured was actually derived from the phosphorus of creatine phosphate, although the pathway by which this occurs is not clear.

So far, direct measurement of oxygen uptake by slices during the shorter periods studied has not been undertaken. Previous work (see McIlwain, 1952*b*) indicates that the increase in oxygen uptake of cerebral cortex slices in response to electrical pulses is prompt and ceases abruptly within 30 sec. or less after switching off the pulses. If extra oxygen consumption ceases immediately pulses are stopped, then from the rate of synthesis of creatine phosphate (Fig. 4) and the rate of oxygen uptake of unstimulated guinea pig cerebral cortex slices of 55 μmoles O_2/g. wet wt./hr. (Table 1), the ratio creatine phosphate formed/O_2 consumed (P/O ratios) is 1·35. If extra oxygen consumption continues at the higher rate of 102 μmoles/g. wet wt./hr., the P/O ratio becomes 0·75.

These values are of an order similar to those found for oxidative phosphorylation in brain, in more artificial systems. Thus, experiments with 'homogenates' of pigeon brain in which glucose was used as phosphate acceptor yielded P/O ratios of

1·7–2·0 for the oxidation of pyruvate (Ochoa, 1941) and 1·5–3·8 with rat-brain or guinea pig brain 'homogenates' (Eiler & McEwen, 1949; Case & McIlwain, 1951). It is to be emphasized, however, that in these instances comparison is limited since the structural integrity of the slices was destroyed and the addition of fluoride, as indicated by Case & McIlwain (1951), further altered the phosphorylative capacity of the systems.

The effect of potassium salts

Although the rate of decrease of creatine phosphate was initially rapid, the time required to reach the final level (Fig. 6) was some 10 times longer than was required for a similar effect to be produced by electrical pulses. In this respect the effect of potassium salt differs from that of electrical pulses. A more marked divergence is seen in the level of inorganic phosphate which, in the case of potassium stimulation (Fig. 6), did not begin to increase until 80% of the total breakdown of creatine phosphate had occurred, suggesting that the phosphate of creatine phosphate, ultimately appearing as inorganic phosphate, was present as an intermediate during the first 20–30 sec.

SUMMARY

1. Rapid changes in the creatine phosphate and inorganic phosphate levels in slices of cerebral cortex during the passage of electrical pulses were studied in a phosphate-free medium, buffered with aminotrishydroxymethylmethane.

2. On passage of pulses, creatine phosphate was broken down at a rate of 1400 μmoles/g. wet wt. tissue/hr., after an initial lag period of 2–3 sec. Breakdown was complete in 5 sec.

3. The breakdown was preceded by a slight fall in the level of ATP, which regained its original value when creatine phosphate breakdown commenced.

4. If the pulses were switched off within 7 sec. of application, creatine phosphate was resynthesized to the original level within 20 sec. The rate of resynthesis was 150 μmoles/g. wet wt. tissue/hr. and was almost linear with time.

5. After prolonged passage of pulses creatine phosphate was resynthesized only after 90 sec. This resynthesis took place more rapidly towards the end of this period than at the beginning.

6. Treatment of slices of cerebral cortex with 0·09M potassium chloride decreased the creatine phosphate level within 60 sec. to a level similar to that produced by electrical pulses in 5 sec.

7. The changes in level of inorganic phosphate in the slices, under the above conditions, mirrored the changes in creatine phosphate levels, with the exception of slices treated with excess of potassium chloride. In this case, the inorganic phosphate level did not rise until the creatine phosphate level had fallen by 80% of the total decrease.

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