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Effects of Electrical Pulses on the Distribution of Radioactive Phosphate in Cerebral Tissues

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It is now well established that in nervous tissue, as in muscle, the application of fluctuating electrical potentials results in marked changes in the levels, both of creatine phosphate and of tissue inorganic phosphate (see McIlwain, 1952, for review). Thus in both tissues the level of creatine phosphate falls while that of inorganic phosphate rises. Information regarding the nature of this change in cerebral tissues has, however, been lacking, and the questions of whether the increased inorganic phosphate does indeed arise from creatine phosphate, and of the route through which the phosphate of creatine phosphate passes during breakdown, do not appear to have been investigated. The problem assumes a greater significance when it is realized that the tissues containing creatine phosphate are those which, owing to the suddenness of events taking place, require a large and immediate source of energy.

In muscle the energy used appears largely in the form of mechanical work, but in cerebral tissues the energy requirements appear to be for the maintenance or re-establishment of a steady state in the continuous ionic changes associated with the

manifestation of electrical activity detectable, for example, by the electroencephalograph. Initially, therefore, it might not seem unreasonable to suppose that the pathway of creatine phosphate metabolism in brain may differ in some respects from that in muscle.

During an investigation of the rapid changes in slices of cerebral cortex of guinea pigs brought about by electrical pulses (Heald, 1954) it was found that the rate of breakdown of creatine phosphate appeared to be higher than that required to provide the 'energy-rich' phosphate involved in the metabolism of glucose. From this it was suggested that the 'energy-rich' phosphate released in response to pulses was not used solely in the phosphorylation of glucose. In an attempt to study this problem further, use has been made of radioactive phosphorus and the results obtained are described in this paper.

MATERIALS AND METHODS

Adenosine triphosphate, creatine phosphate and glucose 1-phosphate. These were samples described previously (Heald, 1956).

Radioactive orthophosphate. This was obtained as carrier-free orthophosphoric acid in dilute HCl, from the Radiochemical Centre, Amersham, Bucks. It was diluted to a convenient volume and stored in the refrigerator. Immediately before use a sample was heated with N-HCl for 20 min. at 100° to convert any pyrophosphate into orthophosphate, and cooled, and neutralized with an equal quantity of N-NaOH (cf. Ennor & Rosenberg, 1954). It was diluted with water to a concentration of $10 \mu\text{Ci/ml.}$ Samples of such solutions when tested by paper ionophoresis with sodium orthophosphate as carrier yielded no area of activity other than the orthophosphate area.

Tissues and salines. Guinea-pig cerebral slices were cut as described by McIlwain (1951) and were incubated in glucose salines buffered with aminotrihydroxymethylmethane (Heald, 1954). Usually the medium containing no added phosphate was used.

Technique of manipulation. Slices were floated into tissue holders placed in a beaker containing the saline and incubated in a thermostat (Heald, McIlwain & Nelligan, 1954). After 30 min. at 37.5° in O_2 , 0.2 ml. of a carrier-free solution of radioactive phosphate was added rapidly from a tuberculin syringe, and incubation was continued for the required period. The amount of NaCl added with the radioactive phosphate did not produce a change in concentration in the medium of more than 3%, and frequently the quantity was sufficient to produce a change of only 1%. The amount of phosphorus added was up to $0.2 \mu\text{g.}$ After a suitable period of incubation the slices were either transferred to 3 ml. of ice-cold 10% (w/v) trichloroacetic acid or else were rapidly transferred to 20 ml. of fresh saline contained in a small constant-temperature bath at 37.5° and stirred by a stream of O_2 . After 10 sec. incubation the holder and slice were transferred to a fresh 5 ml. of saline in a second beaker and re-incubated under O_2 for different periods as required. Slices were then either removed without further treatment and fixed in trichloroacetic acid or else were subjected to electrical impulses before placing in the trichloroacetic acid. Tissues were then disintegrated and the acid-soluble phosphates analysed as described by Heald (1956).

Radioactivity was measured on liquid samples in M6H counters (20th Century Electronics Ltd.), using an Ecko N 529 scaler unit. All counts were corrected for background and decay. Ionophoresis records were examined with a Panax GM Monitor, Model 50 (Panax Equipment Ltd., 173 London Road, Mitcham, Surrey).

Phosphorus. This was determined as orthophosphate by the method of Berenblum & Chain (1938) as modified by Long (1943). Since this method yields the phosphate as a complex in isobutanol the quantity of phosphate and the radioactivity can be measured in the same solution, thus permitting a precise estimate of specific activity (cf. Ernster, Zetterstrom & Lindberg, 1950; Ennor & Rosenberg, 1952).

Alternating condenser pulses. These were supplied at 100/sec. from the apparatus of Ayres & McIlwain (1953) with a peak voltage of 18 v and a duration of 0.4–0.5 msec.

Units. The specific activity is defined as the counts/min./ $\mu\text{g.}$ of phosphorus. The relative specific activity is defined as the specific activity of a phosphate in the slice, such as creatine phosphate, calculated on the assumption that the specific activity of the original medium was 2×10^4 . Thus in each experiment the actual specific activity of the incuba-

tion medium was determined and the quotient, 2×10^4 /specific activity of the medium, used to multiply the specific activity of any phosphate of the slice. In the majority of experiments the specific activity of the medium was between 1.5 and 1.9×10^4 .

RESULTS

Though the process of phosphate transport into the slice appears to be complex (Greenberg, 1952) it seemed reasonable to suppose that the amount of labelled phosphate entering would depend partly on the amount of unlabelled phosphate in the medium surrounding the tissue slice. This was found to be the case. Fig. 1 shows the specific activity found in creatine phosphate in slices, incubated either in a medium to which 6.3 mM phosphate had been added or in a medium initially free from phosphate. The specific activity was related to a solution count of 10^5 counts/min./ml., and it is seen that in the high-phosphate medium a period of some 18 min. was required for the activity of the creatine phosphate to reach a level attained in 3 min. by slices incubated in the 'phosphate-free' medium. Actually it was found that the saline initially free from phosphate had in fact accumulated orthophosphate during the preliminary incubation period. Though the quantities averaged $4.25 \mu\text{moles}$ of phosphate/g. wet wt. of tissue over a large number of experiments, there was some variation from experiment to experiment, even though the ratio, wt. of tissue:vol. of saline, was maintained nearly constant. For this reason it was considered desirable to calculate the relative specific activities of the individual phosphates on

Fig. 1. Rate of incorporation of radioactive phosphate into creatine phosphate in guinea-pig cerebral slices. ●, Medium with no additional phosphate; ○, medium containing 6.3 mM potassium phosphate. Salines and conditions as in Methods.

the basis of the specific activity of the incubation medium rather than on the basis of a standard count in solution (cf. Sacks & Culbreth, 1951). By maintaining the specific activities of the incubation media reasonably constant a comparison of different experiments by simple proportion could be made. As will be shown later, this does not apply to media whose specific activities differ widely. From the above experiments it was concluded that the use of a phosphate-free saline was desirable since the short incubation time needed to build up a high activity in creatine phosphate would minimize the chance of a more even distribution of labelled phosphate taking place throughout the tissue. This would permit subsequent changes in radioactive phosphate distribution to be more easily detected.

Removal of extraneous radioactive phosphate. Calculations of the absolute rate of renewal and turnover of phosphates within a slice are possible only if the intracellular concentration of radioactive phosphate is known (Zilversmit, Enteman & Fishler, 1943). To try to obtain such a value the majority of workers have estimated the contribution to tissue radioactive phosphorus made by the extracellular phosphorus. The techniques used have been critically examined by Ennor & Rosenberg (1954), who concluded that assessments of this type are open to certain errors which have not yet been overcome. Thus in some instances the values calculated for the extracellular contribution are greater than the total actually found (Sacks & Altschuler, 1942). Also, during procedures involving either perfusion (Kalckar, Dehlinger & Mehler, 1944) or, with slices, washing in fresh saline at 0° for periods of 30 or 60 min. (Furchgott & Shorr, 1943), phosphate exchange within the tissue proceeds at a measurable rate, rendering the final results difficult of interpretation in terms of events taking place at the supposed termination of the experiment. In view of this it was decided not to try to estimate extracellular space in the cerebral slices, but to use a washing technique of brief duration, although sufficient to yield reproducible results for total tissue radioactive orthophosphate.

In Fig. 2 is shown the rate of removal of adherent radioactive phosphate when slices and holders, after incubation for 3 min. in the presence of labelled phosphorus, were rapidly transferred to 20 ml. of fresh phosphate-free oxygenated saline. It is seen that removal was virtually complete in 5 sec. Accordingly, a period of 10 sec. was chosen as a standard washing period before slices were subjected to further treatment. The constancy of the specific activity of phosphate within the tissue after washing was maintained within reasonable limits both from slice to slice and from one group of

Fig. 2. Rate of removal of radioactive phosphate adhering to cerebral slices and to the tissue-holder, when washed for different periods of time in warm oxygenated phosphate-free saline. The horizontal lines on either side of the experimental points indicate the s.e.m. of six determinations. Salines and conditions as in Methods.

slices to another. Thus in five experiments using different samples of radioactive phosphate the variation of the specific activities of each group varied from 7.8 to 11.2% of the average values. Since the amount of medium carried over into the washing saline by each slice and holder was found to be 0.42 ± 0.03 (6) ml., i.e. a variation of 7.1%, the above values for radioactive phosphorus in the slices were considered to be as good as could be expected. The procedure did not affect the metabolism of the slices as judged by the levels of creatine phosphate. Thus in three experiments the levels (with standard errors) were 1.38 ± 0.1 (4) 1.44 ± 0.19 (6) and 1.42 ± 0.05 (5), all of which are normal for tissues respiring in glucose salines (McIlwain, Buchel & Cheshire, 1951; McIlwain & Gore, 1951; Heald, 1954).

Time course of incorporation of radioactive phosphate in cerebral tissue. With the above information, an attempt was made to plot the time course of radioactive phosphate distribution in the soluble phosphates with a view to selecting a point most suitable for studying the effects of applied pulses. In Fig. 3 is shown the distribution of activity between the major acid-soluble phosphates determined in extracts of guinea-pig cerebral slices incubated for periods of up to 3 min. in the presence of radioactive orthophosphate, and washed for 10 sec. and again incubated in 5 ml. of fresh saline. Although the quantities incorporated appear to be considerable, this is due to the high activity of radioactive phosphorus in the incubation medium, for in fact not more than 1% of the labelled phosphate added was assimilated after 3 min. incubation.

During the initial period incorporation was most rapid into the γ -P of adenosine triphosphate, followed closely by creatine phosphate and more slowly by the hexose monophosphate group. On removal from the radioactive incubation medium and reincubation in fresh saline all the activities fell steadily. It is of interest that the curve for the γ -P of adenosine triphosphate crossed the curve for creatine phosphate below the peak of the latter. The possible significance of this will be discussed later. The relative specific activity of the tissue orthophosphate was much above that of any other component. This must in part be attributed to extracellular phosphate not removed by washing. The fall in this, and the other phosphates, can partly be accounted for by loss of labelled phosphate to the incubation medium. Thus measurement of orthophosphate in the second incubation medium revealed a small and variable but quite definite quantity, the values and standard errors being 0.29 ± 0.04 (9) μ mole/g. wet wt. of tissue after 2 min. further incubation, and 0.384 ± 0.185 (7) μ mole/g. wet wt. of tissue after 5 min. further incubation. The relative specific activities $\times 10^{-3}$ of this phosphate were 2.95 ± 0.95 (9) and 3.6 ± 1.0 (7) respectively, i.e. 14.7 ± 4.75 and $18.0 \pm 5.0\%$ of the

specific activity of the phosphate in the original incubation medium. This would appear to mean that of the orthophosphate appearing in the medium after the second incubation some 80–85% is derived from the tissue phosphates and does not arise from phosphate of the original medium adhering to the slices and being mechanically transferred. There was no general increase in the relative specific activities of the phosphates in the fraction with soluble barium salts not precipitated by ethanol. This fraction contained 1.3 μ moles of phosphate/g. wet wt. of tissue and reached a relative specific activity of 24.0 in the initial incubation period of 3 min. After the slice had been washed, and further incubated for 2, 5 and 10 min., the values were 21.0, 22.0 and 22.0 respectively. This fraction has been shown to contain some radioactive orthophosphate even after the ethanol precipitation (Heald, 1956), which in part accounts for the constancy of the relative specific activity. The fraction was not examined further.

Effect of electrical pulses

The results obtained suggested that a suitable point at which applied pulses might show the greatest effect was 2 min. after removal of the tissue from the preliminary incubation medium. It had been shown (Heald, 1954) that the application of pulses to cerebral slices in a system similar to the one used here resulted in a rapid breakdown of creatine phosphate, which was complete within 5–7 sec. Accordingly, a period of 10 sec. was chosen as being sufficient to ensure that the rapid breakdown of creatine phosphate would be complete.

Slices from one cerebral hemisphere were incubated in the presence of radioactive phosphate, and washed and reincubated for 2 min. Pulses were then applied for 10 sec., the slice being fixed in ice-cold trichloroacetic acid during the 10th to 11th sec. Control slices from the other cerebral hemisphere were treated similarly but without applying pulses. The results of these experiments are shown in Table 1, from which it is apparent that pulses produced a marked change in the phosphates. Thus in tissue which had been electrically stimulated there was a decrease in the relative specific activity not only of the tissue orthophosphate but also of the γ -P of adenosine triphosphate, of creatine phosphate and of the hexose monophosphate group. The level of orthophosphate had risen and that of creatine phosphate had fallen, as compared with the control slices. There were also slight increases in levels of the hexose monophosphate group and adenylic acid. The probable nature of the fraction 'A' has been discussed previously (Heald, 1956), and although values for

Fig. 3. Time course of incorporation of radioactive phosphate into guinea-pig cerebral slices incubated in an aminotrihydroxymethylmethane-buffered saline containing glucose. Tissues were removed at the time indicated by the arrows A, washed for 10 sec. in saline and re-incubated in fresh saline. +, Orthophosphate; ●, adenosine triphosphate γ -P; ○, creatine phosphate; Δ, hexose monophosphates. The dotted line between the points at 3 and 5 min. indicates the possible change during this interval. The horizontal lines on either side of the experimental points represent the S.E.M. values. Several of these lines have been omitted for the sake of clarity.

relative specific activity are quoted here, the possibly composite nature of this fraction and the fact that it may contain variable quantities of radioactive orthophosphate which had not been precipitated as the water-insoluble barium salt makes comparisons of the two sets of values of little use.

In these experiments the sum of the counts found in the various fractions after deionization (Heald, 1956) was almost equal to the total counts found in the original extract, the recoveries and standard errors being $99.1 \pm 2.2\%$ (10) for stimulated tissues and $97.4 \pm 2.6\%$ (6) for unstimulated tissues. These values showed that little or no radioactive phosphate was being absorbed on the ion-exchange resins. Examination of the ionophoretograms with a Panax monitor did not reveal the presence of any active area other than those estimated and listed in Table 1.

The figures in Table 1 show clearly that there was a general decrease in the relative specific activity of

the phosphates in extracts of the tissues subjected to pulses. This suggested that radioactive phosphate was being transferred from acid-soluble to acid-insoluble phosphate, and accordingly determinations were made of the relative specific activity of the phosphates remaining in the tissue after trichloroacetic acid extraction. The results of these experiments are presented in Table 2, in which, in addition to relative specific activity of the residues, values are given for the relative counts found in the trichloroacetic acid extracts. It must be pointed out that these latter figures, although calculated to permit comparison between the extracts, have no precise significance as regards their magnitude. The relative specific activity of the residual tissue phosphate was in all cases greater in tissues subjected to pulses than in tissues not stimulated. Parallel with this there was a decrease in the relative total count found in the trichloroacetic acid extracts. The reasons for the variation between the individual experiments in the relative

Table 1. *Effect of electrical pulses on the distribution of radioactivity in the acid-soluble phosphates of guinea-pig cerebral tissues*

Conditions as described in Methods. Pulses: condenser pulses, 50 cyc./sec.; peak voltage: 18 v; duration: 0.4 msec.; period of application: 10 sec. Values given $\pm$ s.e.m. Figures in parentheses show the number of experiments.

Phosphate estimated	With pulses		Without pulses	
	Levels (μ moles/g. wet wt.)	Relative specific activity	Levels (μ moles/g. wet wt.)	Relative specific activity
Orthophosphate	1.92 ± 0.18 (7)	160 ± 6.3 (8)	1.12 ± 0.12 (4)	199.3 ± 9.2 (7)
Adenosine triphosphate γ -P	—	43.6 ± 6.4 (9)	—	110.6 ± 13.5 (8)
Creatine phosphate	0.55 ± 0.09 (8)	40.0 ± 5.9 (7)	1.15 ± 0.12 (6)	92.0 ± 10.0 (9)
Hexose monophosphate	0.64 ± 0.065 (9)	22.6 ± 4.4 (7)	0.52 ± 0.12 (6)	42.6 ± 6.2 (9)
Adenylic acid	0.76 ± 0.15 (8)	28.5 ± 8.5 (9)	0.48 ± 0.15 (5)	44.3 ± 9.0 (6)
Diphosphopyridine nucleotide	0.39 ± 0.14 (7)	4.9 ± 1.6 (6)	0.27 ± 0.038 (5)	5.7 ± 1.2 (6)
Fraction 'A' (Heald, 1956)	0.64 ± 0.10 (9)	70.0 ± 3.6 (8)	0.91 ± 0.09 (6)	139 ± 40 (5)

Table 2. *Effect of electrical pulses on the relative specific activities of the (tissue) residual phosphate, after extraction with trichloroacetic acid, and on the distribution of the relative counts in the extracts*

For methods see text. The relative counts/min. in the extracts are calculated as counts/min. of extract relative to a specific activity of 2×10^4 in the incubation medium. Data for Expts. 7-12 were obtained from experiments described in Table 1.

Expt.	Relative specific activity of residues			Relative counts in extracts		
	With pulses (1)	Without pulses (2)	Difference (1 - 2)	With pulses (3)	Without pulses (4)	Difference (3 - 4)
1	3.68	3.50	+0.18	—	—	—
2	3.85	2.78	+1.07	61 500	65 600	-4100
3*	9.1*	8.1*	+1.0	38 200	45 500	-7300
4	1.47	1.12	+0.35	23 800	25 500	-1700
5	1.75	1.08	+0.67	31 200	33 600	-2400
6	2.02	1.62	+0.40	32 600	38 400	-5800
7	—	—	—	15 200	19 200	-4000
8	—	—	—	24 800	23 000	+1800
9	—	—	—	16 600	19 700	-3100
10	—	—	—	31 800	33 400	-1600
12	—	—	—	28 200	36 200	-8000

* The tissues in this experiment were extracted once only with trichloroacetic acid.

specific activity of the residues is not clear. Possibly the efficiency of washing of the residues varies from experiment to experiment.

DISCUSSION

The most important of the results presented in this paper appear to be the changes taking place in the relative specific activities of both the acid-soluble and acid-insoluble phosphates on passing electrical pulses.

As stated in the introduction, the experiments were considered in the hope that they might indicate whether the increased orthophosphate found in the tissue after stimulation was derived from creatine phosphate. Calculations based on the data in Table 1 show that if the increase of $0.8 \mu\text{mole}$ for the phosphate/g. wet wt. of tissue was derived from the phosphate of creatine phosphate then the relative specific activity of the total orthophosphate would be 155.0. This value agrees almost exactly with the value of 160.0 found experimentally. However, a simple breakdown of creatine phosphate to creatine and orthophosphate should not result in any decrease in the relative specific activity of the residual creatine phosphate, whereas a decrease was found not only in this quantity but in the relative specific activity of the γ -P of adenosine triphosphate and in the hexose monophosphate group. Parallel with this there was a definite increase in the relative specific activity of the residual acid-insoluble phosphate.

The two most obvious interpretations of these results appear to be either that (a) electrical pulses release unlabelled or weakly labelled phosphate from the insoluble phosphates, possibly as orthophosphate which by entering the intracellular phosphate pool becomes available for incorporation into adenosine triphosphate and thence to creatine phosphate and orthophosphates, or (b) pulses promote an exchange between a phosphate such as adenosine triphosphate and a weakly labelled or unlabelled organic phosphate more closely associated with the basic changes caused by electrical pulses (McIlwain & Gore, 1953) than adenosine triphosphate itself. These two interpretations are presented in Schemes A and B.

Scheme A is difficult to reconcile with the data. Thus although such change would result in a decrease of the relative specific activity of the intracellular orthophosphate there is no reason why the relative specific activity of adenosine triphosphate and of creatine phosphate, already below the relative specific activity of the tissue orthophosphate, should decrease still further. Neither does the scheme offer an explanation for a decrease in the level of creatine phosphate. While the possibility must remain that interactions of the various phosphates may take place in certain localized areas, such a hypothesis is difficult to test in the experimental system described, for pulses appear to be effective only in intact nervous tissue (Kratzing, 1951; McIlwain & Narayanaswami, 1954).

Scheme A

Scheme B

On the other hand, Scheme *B* permits a reasonable interpretation of the observed data. Thus the tissue organophosphate, labelled X-phosphate, can break down to yield orthophosphate, and can be rephosphorylated by adenosine triphosphate which is in turn rephosphorylated by creatine phosphate. During this process the residual acid-insoluble phosphates containing X-phosphate would increase in relative specific activities due to transfer of the adenosine triphosphate phosphorus. The net result of this process is a decrease in the level of creatine phosphate and an increase in the level of inorganic phosphate.

The rates at which such reactions must proceed to achieve the above results within 10 sec. are well within the rates found for two phosphate transferring systems known to occur in guinea-pig cerebral tissue. Thus the rate of transfer of the phosphate of creatine phosphate to adenosine diphosphate by cerebral creatine phosphokinase is 3600 μ moles of phosphate/g. wet wt./hr. (Narayananaswami, 1952), a rate sufficient to enable the total tissue creatine phosphate to be turned over some 6–7 times in 10 sec., the period during which impulses were applied. Similarly, the adenosine triphosphatase of cerebral tissue converts adenosine triphosphate into adenosine diphosphate and orthophosphate at a rate of 2000 μ moles/g. wet wt./hr. (Gore, 1951), sufficient to split cerebral adenosine triphosphate some 5–6 times in 10 sec. It does not seem unreasonable to suppose that the increased enzymic activity of cerebral tissues brought about by applied impulses, as shown by increased respiration and lactic acid production, should also include systems of the adenosine triphosphatase type.

The proposed existence of a phosphate such as X-phosphate is supported by other evidence. Thus with cat-cerebral slices in a glucose-saline medium, Findlay, Strickland & Rossiter (1954) have demonstrated a relatively rapid incorporation of externally added radioactive phosphate into a fraction thought to be phosphoprotein, which remains in the tissue residue after extraction with trichloroacetic acid. Further, it has been shown by Fleckenstein, Janke, Davies & Krebs (1954) that frog muscle contains an unknown phosphate which, on brief electrical stimulation for 1 sec. at 0°, releases orthophosphate before any change in the levels of creatine phosphate or adenosine triphosphate can be detected.

The changes in relative specific activity of creatine phosphate, adenosine triphosphate and orthophosphate as a result of electrical stimulation permit some observations to be made regarding the inhibition of oxidative phosphorylation supposed to be brought about by applied impulses. It has been stated (Abood, Gerard & Ochs, 1952; Abood,

Gerard & Tschirgi, 1952; Abood, 1954) that in systems of cerebral mitochondria supplemented with yeast hexokinase and glucose, the passage of electrical pulses from a variety of electrodes brings about a reduction in the amount of phosphorus esterified in relation to the increase in oxygen uptake. Such conclusions have been drawn on the basis of a decrease in the specific activities of the hexose monophosphate formed and of the orthophosphate in the system, though this latter was apparently heavily contaminated with up to 30 % of adenosine di- and tri-phosphates (Abood & Gerard, 1952). Certain of these effects are due to artifacts (McIlwain & Narayanaswami, 1954; Chappell & Greville, 1954), as agreed by Abood (1954). Others may be capable of interpretation by an exchange mechanism of the type outlined in Scheme *B*.

Certain other points deserve mention. In Fig. 3 the curves showing the relative specific activities of creatine phosphate and of the γ -P of adenosine triphosphate intersect at a point below the peak of the creatine phosphate curve. Such a result, interpreted along the lines developed by Zilversmit *et al.* (1943) for product-precursor relationships in phospholipid biosynthesis, would suggest that the γ -P of adenosine triphosphate is not the immediate precursor of creatine phosphate. The factors involved in relationships of this type have been re-examined by Hearon (1951), who concluded that while obedience to the simple product-precursor relationship implies that one compound is a precursor of the other, the reverse is not necessarily true. Other factors to be considered in interpreting the results must include the change in saline medium during the experiments and, in addition, the fact that the differences between the points plotted on the descending curves for adenosine triphosphate and creatine phosphate are not statistically significant. It is considered that the results in Fig. 3 are consistent with the view that the γ -P of adenosine triphosphate is the precursor of creatine phosphate (cf. Ennor & Rosenberg, 1954).

Table 1 shows that the level of the hexose monophosphate group changed little in response to pulses, though the relative specific activity decreased. The probably composite nature of this group has been discussed previously (Heald, 1956), and until detailed analysis has been performed changes in relative specific activity and their magnitude cannot adequately be assessed. The small increase in quantity in the stimulated tissues may be compared with the finding of Dawson & Richter (1950) that during the application of electrical pulses to the brains of rats *in vivo* cerebral hexose monophosphate increased. Apart from the difference between the *in vivo* and *in vitro*

systems, another difference lies in the method of analysis employed. Thus, while Dawson & Richter analysed the cerebral tissues by the method of Stone (1943), the fraction designated 'hexose-phosphate' consisted of all the phosphates other than creatine phosphate, which were not precipitated by calcium acetate or hydroxide from an alkaline solution containing 10% (w/v) ethanol. This fraction is known to contain many phosphates in addition to hexose monophosphates (Stone, 1943).

A final point concerns the use of the specific activity of orthophosphate in solution as a means of calculation of the relative specific activities of tissue phosphate. It is the practice of the majority of workers to arrange that in experiments *in vitro* the quantity of orthophosphate in the medium is greatly in excess of the quantity of phosphate in the tissue, thus ensuring that such changes as may occur are minimal. In the system used in this work additional orthophosphate was absent, and it was found desirable to maintain the specific activities of the solutions nearly constant. In such a system, if the period of incubation is short, a simple proportional relationship between the specific activity of the different media and an arbitrary standard specific activity can be used as a basis for calculation. If, however, the specific activities of the media vary widely this no longer holds. Thus in collaboration with Dr A. S. C. Ehrenberg, it was found in preliminary experiments that in media in which the specific activity varied from 5.68×10^3 to 3.62×10^4 the relationship between the specific activity of the medium and the specific activity of the creatine phosphate in the slice was given by

$$\sqrt{c} = (0.1m + 0.25)t + 2.00,$$

where c = specific activity of creatine phosphate, m = specific activity of medium $\times 10^{-3}$ and t = period of incubation in minutes.

Generally, the work described above shows that in cerebral tissues *in vitro* the rapid breakdown of creatine phosphate occurring in response to electrical pulses is accompanied by an equally rapid redistribution of the phosphates of the tissue, and the nature of the changes involved is being studied.

SUMMARY

1. A study has been made of the effects of electrical pulses applied for 10 sec. on the distribution of radioactivity amongst the acid-soluble phosphates of guinea-pig cerebral cortex.

2. In tissues exposed to pulses for 10 sec. the relative specific activity of inorganic phosphate decreased from 199.3 to 160, that of the γ -P of adenosine triphosphate from 110.6 to 43.6, and that of creatine phosphate from 92.0 to 40.0.

3. Accompanying the decrease in the relative specific activity of the acid-soluble phosphorus, the

residual insoluble phosphorus of the tissues exposed to pulses showed an increase as compared with the control tissues.

4. The results are discussed in the light of a proposed exchange mechanism operating between the acid-soluble and -insoluble phosphates.

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