Phosphates of Brain during *in vitro* Metabolism: Effects of Oxygen, Glucose, Glutamate, Glutamine, and Calcium and Potassium Salts

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It has been reported previously from this laboratory that labile phosphates can be resynthesized by portions of cerebral tissue suspended in suitable fluid media *in vitro* (McIlwain, Buchel & Cheshire, 1951). In the present paper the phosphates are further characterized and shown to include substances with the properties of adenine polyphosphates. Evidence was presented previously which suggested that concentrations, in the tissue, of the labile phosphates or of their breakdown products, conditioned the rates of overall processes such as respiration or glycolysis. To pursue this theme the effects on the phosphates of altering some constituents of the suspending media have been examined, choosing constituents which are known markedly to affect respiration and glycolysis. Oxygen, glucose, glutamate and glutamine (see Weil-Malherbe, 1938; Stern, Eggleston, Hems & Krebs, 1949), and calcium

Possible interference by phosphopyruvic acid in determination of phosphocreatine. Characterization of one of the labile phosphates resynthesized *in vitro* as creatine phosphate, depended previously on (a) its precipitation as Ca salt at pH 9 by 80% but not by 10% ethanol, (b) its determination as inorganic phosphate by Fiske & Subbarow's conditions (see Hawk, Oser & Summerson, 1947) but not by Lowry & Lopez's (1946) conditions, (c) the approximate equivalence between the quantity of creatine and of labile phosphate precipitated as described in (a). In these determinations, creatine was measured by Jaffe's reaction, which is not very specific and, in particular, is given also by pyruvic acid. Further, phosphopyruvic acid would be expected to be partly precipitated under the conditions (a) above. In interpreting the experiments with glutamic acid, it was particularly important to be aware of any possible interference from pyruvic acid, and therefore the maximum extent to which phosphopyruvic acid might contribute to the values obtained for creatine was estimated as follows.

Table 1. *Possible contribution by phosphopyruvate to phosphocreatine values*

(Guinea pig cerebral-cortex slices (1.48 g.) were distributed between three 100 ml. vessels each containing 15 ml. glycylglycine-glucose saline, and shaken in O_2 for 40 min. Determinations by titration with 0.005 M- $Na_2S_2O_3$ are described in the experimental section.)

	Ca-10% ethanol precipitate	Ca-80% ethanol precipitate
I_2 consumed (μ equiv./g. tissue)	4.71	9.83
Equivalent phosphopyruvic acid (μ moles/g. tissue)	0.79	1.64
Equivalent 'creatine' (μ moles/g. tissue)	0.07	0.15

and potassium salts (see Dickens & Greville, 1935; Canzanelli, Rogers & Rapport, 1942) have now been investigated in this way. The phosphates were found to be affected by all these substances, in a fashion which shows an interesting pattern of relationships to the concomitant changes in respiration and glycolysis.

EXPERIMENTAL

The salines were based on those described by McIlwain (1951a), and McIlwain *et al.* (1951). Any departures from these are described in the experiments concerned. Slices of cerebral cortex from guinea pigs were the main experimental material and were handled, and inorganic phosphates determined, according to McIlwain *et al.* (1951). Glutamate solutions were made by taking the L (+)-acid to pH 7.2 with NaOH.

Brain-cortex slices were prepared from two guinea pigs and caused to respire for 40 min. in glycylglycine-glucose saline. They were extracted with trichloroacetic acid and the Ca salts fractionated as indicated above. The precipitates were dissolved or suspended in water and the consumption of I_2 determined according to Lohmann & Meyerhof's (1934) method for phosphopyruvic acid. The I_2 may oxidize substances other than phosphopyruvic acid; however, a maximum figure for the phosphopyruvic acid could be given on the basis of the consumption of 6 equiv. I_2 by 1 mole of the acid (Table 1). The extent to which pyruvic acid reacted as creatine in the Jaffe determination was determined by finding the apparent creatine from 0.25, 1 and 5 μ moles of pyruvic acid, before and after the preliminary acid treatment involved in the Jaffe reaction. The treatment was not found to alter the apparent values, which were between 0.064 and 0.094 μ mole creatine/ μ mole pyruvate. The higher value has been used for calculating in Table 1 the possible interference of phosphopyruvate in the phosphocreatine

determinations. As a normal value for phosphocreatine in brain slices is $1.5 \mu\text{moles/g.}$, the interference could not be more than 10% and is probably less. There is thus no reason to doubt that the previous characterization of phosphocreatine was adequate.

Acid hydrolysis of phosphates from slices. This also required about 1.5 g. of slices, which were caused to respire, extracted, and fractionated by Ca-ethanol as indicated above. Each precipitate was then made up to 5 ml. in water, 5 ml. 2N-HCl added, and the mixture, in a stoppered tube, put into a bath at 100° . Specimens (2 ml.) were taken at 0, 7, 15 and 30 min. and their inorganic phosphate determined by Fiske & Subbarow's method (see Hawk *et al.* 1947).

RESULTS

Glucose and oxygen on phosphates

The resynthesis of phosphocreatine which took place in oxygenated salines containing glucose, did not take place in the absence of glucose or oxygen. Values are quoted in Table 2. The inorganic phos-

Table 2. *Glucose and oxygen on phosphates*

(Bicarbonate-glucose saline with Ca was used and the gases contained 5% (v/v) CO_2 . Experiments were at 37° for 40 min. Phosphates were determined after chemical separation.)

Conditions	Inorganic phosphate ($\mu\text{moles/g.}$)	Creatine phosphate ($\mu\text{moles/g.}$)
With glucose and O_2	3.82	1.33
No glucose; with O_2	5.02	0.31
With glucose and O_2	3.10	1.45
Glucose and N_2	3.96	0.31

phate in absence of glucose or oxygen was higher by an amount about equivalent to the fall in creatine phosphate. Respiration continued in the absence of glucose for much longer than the experiments of Table 2, but at a rate which was initially somewhat less than that in the presence of glucose, and which gradually fell further.

expected in the present preparations. These accordingly would be expected to contain adenine polyphosphates. Table 3 gives evidence indicating that this is the case. In examining the effects of different agents on the phosphates of slices as described in the following sections, our observations have at present been confined to the inorganic and creatine phosphates. This is because determination of, for example, the adenine polyphosphates by the present methods require more tissue, and also because the inorganic and creatine phosphates show the greatest susceptibility to applied agents *in vivo* (Stone, Webster & Gurdjian, 1945; Klein & Olsen, 1947).

Glutamate on phosphates

Determination after calcium separation. A major redistribution in the phosphates of brain-cortex slices took place when glutamate was added to media in which they were respiring. Table 4 (Exp. 1) shows the large fall in phosphocreatine which occurred when glutamic acid was added to media containing glucose. Glutamine had nearly the same effect. The fall took place even in the small quantity of phosphocreatine which remained in the slices after their metabolism in the absence of added substrate (Exp. 3). In all the experiments of Table 4 relatively little change in inorganic phosphate was associated with the addition of glutamic acid or glutamine.

When glutamate replaced glucose as substrate, changes in both inorganic phosphate and phosphocreatine were found. Table 5 shows this, together with the concomitant changes in respiration. Phosphocreatine fell markedly on replacing glucose by glutamate; inorganic phosphate and also respiration rose. Results derived from groups of experiments on two different occasions are given separately; no significant difference existed in values with glucose, but the variations between the two groups of experiments with glutamate as sub-

Table 3. *Acid hydrolysis of phosphate fractions from brain cortex*

(Experimental conditions as Table 1, using 1.4–1.8 g. of guinea pig brain-cortex slices. Hydrolysis with N-HCl at 100° .)

Exp.	Ca-10% ethanol ppt. Increased inorganic PO_4 ($\mu\text{moles/g.}$) after hydrolysis for			Ca-80% ethanol ppt. Increased inorganic PO_4 ($\mu\text{moles/g.}$) after hydrolysis for		
	7 min.	15 min.	30 min.	7 min.	15 min.	30 min.
1	1.34	1.94	2.0	0.36	0.46	1.04
2	1.92	—	2.55	—	—	—
3	1.47	—	2.53	—	—	—

The equilibrium which exists between adenine polyphosphates and creatine phosphate in muscle probably exists also in the brain *in vivo* (Klein & Olsen, 1947). Its existence in homogenates of cerebral tissues has been demonstrated in these laboratories (Narayanaswami, 1952) and would be

strate were real. The most likely point for such difference to arise was in the preparation or extraction of the tissue. The effect of changing substrate is, however, far beyond any such difference.

The values of Tables 4 and 5 were obtained after chemical separation of creatine and inorganic

phosphates. The inorganic phosphate was normally measured as the phosphate insoluble in calcium-10% ethanol by Fiske & Subbarow's method, but the same values were obtained by that of Lowry & Lopez (Table 6). Determination of the phosphate of the calcium-80% ethanol precipitate, by the same methods, showed the separation to have been good. Moreover, added glutamic acid did not inter-

glutamic acid corresponding to 13, 20 and 45 μ moles/g. respectively were therefore added to the extracts, and the usual separations and determinations of inorganic and creatine phosphates were performed. The findings were independent of added glutamic acid. A similar experiment, not recorded in the tables, showed them to be independent also of added glutamine.

Table 4. *Glutamate and glutamine on phosphates*

(All experiments were aerobic in bicarbonate saline with Ca salts, at 37° for 75 min. Glutamate was the L(+) acid taken to pH 7.2 by NaOH; glutamines A and B refer to two specimens for one of which we are indebted to Mr D. E. Hughes of Sheffield University. Phosphates were determined after chemical separation.)

Exp.	Substrate	Inorganic phosphate (μ moles/g.)	Creatine phosphate (μ moles/g.)
1	Glucose	3.27	1.35
	Glucose and glutamate (0.02M)	3.24	0.11
	Glucose and glutamate (0.004M)	3.15	0.36
2	Glucose	4.17	1.36
	Glucose and glutamine A (0.02M)	4.07	0.40
	Glucose and glutamine B (0.02M)	3.90	0.42
3	None	5.17	0.33
	Glutamate (0.02M) only	5.31	0.06
	Glutamate (0.004M) only	5.16	0.07

Table 5. *Effects on phosphates of replacing glucose by glutamates*

(Experiments were aerobic and phosphates determined after chemical separation. The group of six experiments was carried out some 6 months later than the group of 11 or 12.)

No. of experiments	Type of saline; substrate	Respiration (μ moles/g./hr.)		Inorganic phosphate (μ moles/g.)		Creatine phosphate (μ moles/g.)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
12	Glycylglycine; glucose (0.013M)	70.5	5.5	3.40	0.35	1.20	0.17
6	Glycylglycine; glucose (0.013M)	68	6	3.38	0.31	1.12	0.11
11	Glycylglycine; glutamate (0.02M)	79	4.4	5.22	0.22	0.32	0.07
6	Glycylglycine; glutamate (0.02M)	78	7	6.35	0.35	0.16	0.035

Table 6. *Separation of phosphates in trichloroacetic acid extracts of cerebral cortex*

(In Exp. 1, cortex slices had first respired for 40 min. in glycylglycine-glucose saline. In Exp. 2, cortex from the cerebral hemispheres was used 2 min. after death of the animal, and creatine phosphate added to the trichloroacetic acid extract.)

Exp.	Material extracted	Substance determined and method	Ca-10% ethanol ppt. (μ moles/g.)	Ca-80% ethanol ppt. (μ moles/g.)
1	Slices	Phosphate; Lowry-Lopez	3.72	0
	Slices	Phosphate; Fiske-Subbarow	3.85	1.99
2a	Cortex	Phosphate; Fiske-Subbarow	7.70	—
	Cortex + 1 mg./ml. glutamic acid	Phosphate: Fiske-Subbarow	7.94	—
	Cortex + 3 mg./ml. glutamic acid	Phosphate: Fiske-Subbarow	7.84	—
2b	Cortex	Creatine; Jaffe	—	1.23
	Cortex + 1 mg./ml. glutamic acid	Creatine; Jaffe	—	1.29
	Cortex + 3 mg./ml. glutamic acid	Creatine; Jaffe	—	1.19

fere with the separation or determinations (Exp. 2, Table 6). The arrangement in this case was as follows. Stern *et al.* (1949) showed slices initially containing about 18 μ moles/g. of glutamic acid, to increase this amount to up to 44 μ moles/g. during respiration in the presence of the acid. Amounts of

Phosphates by differential stability. An earlier paper (McIlwain *et al.* 1951) described the determination of inorganic and creatine phosphates in brain extracts by the different specificities of Lowry & Lopez's (1946) and Fiske & Subbarow's methods. Of these, Lowry & Lopez's determined only in-

organic phosphate, and Fiske & Subbarow's determined inorganic and labile phosphates such as creatine phosphate. Table 6, Exp. 1, shows in an individual extract how this combination of determinations would operate. Table 7 gives values for labile and inorganic phosphates in the extracts, which show that the labile phosphate so determined corresponds to the creatine phosphate by calcium-ethanol fractionation when the method is applied to extracts from slices which had respired with glucose as substrate. However, this was not the case when the method was applied to slices which had respired in solutions containing glutamates. The values for labile phosphates were higher than those for phosphocreatine after calcium separation. This is shown clearly in Table 7 which summarizes some forty

interfere with the calcium separation. It appears likely that a labile phosphate other than creatine phosphate and the adenine phosphates accumulates in cerebral tissues respiring with glutamate.

Calcium and potassium salts on phosphates

The media normally employed in metabolic work with separated tissues contain calcium salts in concentrations approximating to those of blood plasma. The respiration of certain tissues, including cerebral cortex, increases when this calcium is omitted (Dickens & Greville, 1935; Canzanelli *et al.* 1942; Krebs, 1950). In the present experiments the effects of calcium salts have been examined under the three conditions quoted in Table 8, and consistent effects found. In the glucose-containing

Table 7. *Phosphates by Ca separation and by differential stability*

(Metabolism was in O_2 at 37° in the salines listed (referred to in experimental section) for 75 min. In bicarbonate salines the values given are means of several experiments, the number of which is given in parenthesis.)

Substrate; type of saline	Inorganic phosphate (μ moles/g.)		Labile phosphate (μ moles/g.)	
	By Ca separation	By stability	By Ca separation	By stability
Glucose; glycylglycine	3.40, 3.38	3.43, 3.06	1.20, 1.12	1.32, 1.4
Glutamate; glycylglycine	5.22, 6.35	6.7, 6.1	0.32, 0.16	0.78, 1.4
Glucose; bicarbonate	3.47 (6)	3.69 (8)	1.42 (6)	1.42 (8)
Glutamate; bicarbonate	5.3 (2)	6.8 (9)	0.07 (2)	1.57 (9)

Table 8. *Calcium and potassium salts on respiration, glycolysis and phosphate of cerebral-cortex slices*

(Experiments were with guinea pig cerebral-cortex slices. Numbers 1 to 3 were in one or two flasks of about 100 ml., each containing approx. 15 ml. saline and about 300 mg. tissue. Number 4 was in flasks of about 60 ml., with 8 ml. saline and about 100 mg. of tissues. Flasks were placed in a thermostat at 37° about 30 min. after death of the animal, and the manometric readings on which respiration rates were based, taken at 5 min. intervals. Exps. 1-3 ran for 40 min. and the rates were constant during this period, after which each flask in turn was sampled. Exp. 4 continued for 120 min.)

Exp.	Type of saline; substrate	K^+ (mm)	Ca^{++} (mm)	Respiration (μ moles/g./hr.)	Inorganic phosphate (μ moles/g.)	Creatine phosphate (μ moles/g.)	Lactic acid (μ moles/ g./exp.)
1	Glycylglycine; glucose	6.7	0	107.4	4.4	1.03	28
	Glycylglycine; glucose	6.7	2.85	66.4	—	1.49	20
2	Bicarbonate; glucose	6.2	0	—	3.56	0.54	—
	Bicarbonate; glucose	6.2	0.71	—	3.51	0.68	—
	Bicarbonate; glucose	6.2	2.85	—	3.24	1.22	—
3	Glycylglycine; glutamate	6.7	0	86.6	7.28	<0.15	—
	Glycylglycine; glutamate	6.7	0.71	72.2	6.85	<0.15	—
	Glycylglycine; glutamate	6.7	2.85	62.0	5.60	<0.15	—
4	Glycylglycine; glucose	6.7	2.85	68	4.1	0.93	46
	Glycylglycine; glucose	106.7	2.85	103	5.1	0.45	161

experiments carried out at different times in the course of about 18 months in four different media. Both in bicarbonate- and in glycylglycine-glucose salines, the values for inorganic and creatine phosphates show reasonable agreement when measured by the two methods. With glutamate as substrate, all values tended to be higher when determined by differential stability; they were also less regular. As explained above, glutamate or glutamine do not

media, omission of Ca^{++} or lowering of Ca^{++} concentration led to lower creatine phosphate and possibly to higher inorganic phosphate concentrations. In the glutamate medium, the creatine phosphate was already so low that a further change was not detected, but the increase in inorganic phosphate was clear.

When the concentration of potassium salts in balanced salt mixtures is increased, respiration and

glycolysis increase (Ashford & Dixon, 1935; Dickens & Greville, 1935; Canzanelli *et al.* 1942. Here again (Table 8) these actions are seen to be associated with lowered creatine phosphate.

DISCUSSION

In the present study, substances have been encountered which bring about the following group of changes in brain-cortex slices: decreased content of creatine phosphate, increased inorganic phosphate, increased respiration and increased glycolysis. This group of changes has long been recognized to follow increased activity in muscle, and more recently similar phenomena have been shown in the brain *in vivo*. The technique used in the present series of studies with sections of tissue *in vitro* has, however, enabled an interesting variety of agents to be studied under uniform conditions. The agents now observed to cause these changes have been high K^+ concentration, low Ca^{++} concentration, glutamates and glutamine. Previously (McIlwain & Gore, 1951) 2:4-dinitrophenol and electrical stimulation were shown to cause similar changes.

This group of changes is a specific one which differs from those caused for example by narcotics under the same conditions (Buchel & McIlwain, 1950); many other substances including some convulsants and anticonvulsants (Anguiano & McIlwain, 1951, and unpublished) have been found to be relatively inert, or again, to behave differently. In attempting to understand the control of carbohydrate metabolism in nervous tissues, it is therefore important to seek a basis for the group of changes caused by potassium and calcium salts, glutamate, glutamine, dinitrophenol and electrical stimulation. The following suggestions are made in this connexion:

(1) Increased glucose usage might be the first common factor in the action of these substances, its phosphorylation being at the expense of the energy-rich phosphates, and the increase in inorganic phosphate coming from further metabolism of the intermediary hexose phosphates. Normally, however, increased glucose catabolism leads to esterification of inorganic phosphate and not to its formation.

(2) Activation or inhibition of tissue phosphatases, phosphokinases or comparable systems might be the primary effect. Thus calcium and potassium salts have opposing effects on adenosinetriphosphatase of brain (Gore, 1951). Evidence against this view in the case of 2:4-dinitrophenol was given by Case & McIlwain (1951). It has been investigated in the case of K^+ by Lipsett & Crescitelli (1950).

(3) Processes best described in terms of the functional activity of the tissue, might be primarily involved. This appears the most suitable explanation

in the case of electrical stimulation (McIlwain & Gore, 1951; McIlwain, 1951*a*). Here the chemical events *in vitro* closely parallel those following stimulation *in vivo*, when clear evidence is seen of increased activity in the central nervous system. The increased respiration and glycolysis then appeared to follow from increased concentrations of inorganic phosphate and phosphate acceptors resulting from utilization of energy-rich phosphates. At this point, the mode of action of the dinitrophenol and of electrical stimulation became similar (McIlwain & Gore, 1951). With respect to Ca^{++} and K^+ salts, glutamate and glutamine, it is significant that these are all normal constituents of nervous tissues or their environment. Although it is not necessary that a common mechanism should exist for the effects of all these agents, the following reasons can be seen for their action paralleling that of electrical stimulation.

Potassium and calcium salts. The increased respiration and glycolysis which potassium salts induce are relatively specific to nervous tissues, and are not caused in testis, kidney, yolk sac and tumour tissues (Dickens & Greville, 1935), nor in chick embryo (Needham, Nowinski, Dixon & Cook, 1937). Movement of potassium salts is associated with the passage of nerve impulses, and one suggestion of how chemically derived energy might be applied in supporting the activity of the tissue is that it may perform osmotic work in maintaining the differential ion concentrations normal to the tissue. The K^+ concentration in the tissue is normally about 0.09M (see, for example, Hoagland & Stone, 1948) and in surrounding fluids about 0.005M. The external concentration of K^+ which affected phosphates, glycolysis and respiration was 0.03–0.1M. Increased intake of K^+ and also of water probably occur in nervous tissues in solutions high in K^+ (Fenn, 1940). If the activities of the tissues were directed to maintaining a ratio of 20:1 in K^+ between inside and outside its cells, the added K^+ would greatly increase the expenditure of energy necessary for such maintenance. This is undoubtedly an over-simplification of events, which also involve Na^+ and Ca^{++} . It gives an immediate analogy between the effects of high K^+ concentration and of electrical stimulation. It is deliberately presented as a contrast to the suggestions of Lipsett & Crescitelli (1950) without at present discussing the merits of the two views.

Antagonism between the effects of calcium and potassium salts is an extremely common physiological phenomenon (see Heilbrunn, 1947) and it is therefore not surprising that in the present experiments the effects of diminishing Ca^{++} concentration should be similar to those of increasing K^+ concentration. In particular the antagonism has been shown to hold with respect to metabolic character-

istics of mammalian brain (Dickens & Greville, 1935; Canzanelli *et al.* 1942) and to the electrical behaviour of separated portions of frog brain (Libet & Gerard, 1939). Considering specific ways in which lack of Ca^{++} may lead to increased utilization of energy-rich compounds, lack of Ca^{++} has been observed to lead to spontaneous discharge in nervous tissues (see Fenn, 1940; Monnier & Chevalier, 1942). The extent to which this can manifest itself in the present preparations is not known, but electrical activity occurs in isolated fragments of certain parts of the frog brain (Libet & Gerard, 1939). Further, calcium salts are observed to aid the 'sealing' of damaged cells, including those of nervous tissues (Heilbrunn, 1947). Damage to cells is inevitable in the present preparations, and by minimizing its effects calcium salts may reduce the amount of osmotic work which the cells are required to perform, and thus reduce also their respiration and glycolysis.

Glutamic acid. This is assimilated by brain slices against a concentration gradient (Stern *et al.* 1949) and presumably with the expenditure of energy, so that the increased respiration brought about by its addition might depend on its active assimilation. The acid was also found to have additional specific effects on the phosphates, which require further study, and which resulted in higher concentrations of inorganic phosphates than those found to follow any of the other agents listed above. It is possible that the labile phosphate accumulating under these conditions may be the γ -glutamyl phosphate suggested but not demonstrated by Elliott (1951) as an intermediate in glutamine synthesis in brain. Higher K^+ concentration is also found in the slices (Krebs & Eggleston, 1949; Krebs, Eggleston & Turner, 1951). Supplying the tissue with glutamic acid may therefore provide it with the means of carrying out more osmotic work and chemical synthesis. There is evidence that, anaerobically, glutamic acid limits glycolysis by depleting the tissue of adenine polyphosphates, for the glycolysis, so limited, is restored by added adenosinetriphosphate (Stern *et al.* 1949). When such depletion occurs aerobically, as in the present experiments, it can be understood on grounds referred to above, that glycolysis and respiration should be stimulated. This was observed by Weil-Malherbe (1938) and occurred during the present experiments.

These observations lead one to expect that the substances studied here would change the response of cerebral tissues to electrical stimulation. This has been found to be the case with glutamic acid (McIlwain, 1951*b*) and ionic constituents (Gore & McIlwain, 1952).

SUMMARY

1. The content of certain phosphates has been investigated in slices of cerebral cortex which were metabolizing in balanced salines containing sodium, potassium, magnesium and calcium salts with chlorides, sulphates, phosphates and sometimes bicarbonates and glycylglycine.

2. When oxygen and glucose were added to the salines, such slices were found to contain creatine phosphate and adenine polyphosphates and their inorganic phosphate was about $3.5 \mu\text{moles/g.}$ fresh weight. In the absence of glucose or oxygen, the creatine phosphate fell and the inorganic phosphate rose.

3. The further addition of glutamic acid salts or of glutamine caused the phosphocreatine of the slices to fall, whether or not glucose was also present.

4. When glutamic acid salts replaced glucose as the added substrate, the inorganic phosphate of the slices became greater, and their phosphocreatine much less. A labile phosphate other than phosphocreatine and the adenine phosphates was found in the slices.

5. Lowering the concentration of calcium ion in the salines or increasing that of potassium ion, led to lowered phosphocreatine in the slices and sometimes to increased inorganic phosphates.

6. The various changes in salines described above, were associated with changes also in respiration and glycolysis in the slices. Reasons are given for considering these changes in many cases to be secondary to the changes in phosphates, and to represent a pattern of changes similar to that seen in the intact central nervous system in different states of activity.

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Creatine Phosphokinase in Mammalian Brain

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Different levels of activity in the central nervous system are associated with different contents of energy-rich phosphates and of inorganic phosphate in cerebral tissue (for references and a discussion see McIlwain, 1950). For this reason it was decided to study enzyme systems in cerebral tissue which bring about changes in these substances. Some properties of the adenosinetriphosphatases of brain have already been investigated in these laboratories (Gore, 1951). The present study deals with the systems which lead to the transfer of phosphate from phosphocreatine to adenylic acid or adenosinediphosphate. Lohmann (1934) showed that cold water extracts of minced muscle contain an enzyme which brings about the degradation of phosphocreatine. The phosphate group of phosphocreatine was transferred to adenylic acid resulting in the formation of 'adenylpyrophosphate' (adenosinetriphosphate). Lehmann (1935, 1936) found that this reaction was made up of two reversible bimolecular reactions namely,

Banga (1943) considered that two distinct enzymes were present in muscle, one catalysing the reaction between ATP and creatine and the other

catalysing the reaction between ADP and creatine. She obtained the first enzyme in a highly purified state.

The importance of the creatine phosphokinase system in maintaining the level of ATP in muscle has been recognized for some time, but its occurrence in brain does not seem to have been investigated. The present paper reports the presence of this enzyme system in brain and describes some of its properties. Effects on this enzyme system of a few substances structurally related to its substrates, and of some drugs which act on the central nervous system, have also been studied.

EXPERIMENTAL

Preparation of sodium phosphocreatine. This was carried out according to the method of Ennor & Stocken (1948). The product contained 8.05% phosphocreatine P and was practically free from inorganic phosphate.

Adenosinediphosphate. This was prepared as the Ba salt from commercial ATP by heating in the presence of MgCl₂ as described by Bielschowsky (1950). The adenylic acid used was a commercial preparation obtained from L. Light and Co. Ltd.

Determination of phosphokinase activity. The reaction mixture usually contained 1 ml. 0.015–0.016 M-sodium phosphocreatine, 1 ml. 0.018 M-adenylic acid adjusted to pH 7 with NaOH, 2.5 ml. of 0.1 M-glycylglycine buffer at pH 7, and 0.5 ml. enzyme preparation containing 0.01 M-MgSO₄. The solutions were mixed in that order and were kept cool in ice. This gave phosphocreatine at 3.0–3.2 mM, similar to its concentration in normal brain, and adenylic

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