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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

* British Council Scholar.

acid about 10% in excess of that required for interaction with the phosphocreatine. The mixture was then incubated in a thermostatically controlled water bath at 37°, and 2 ml. samples were pipetted out at suitable intervals (usually 20 min. and 1 hr.) into 2 ml. 10% (w/v) trichloroacetic acid at 0°. The reaction mixture was kept cool in ice during intervals of pipetting. Blanks were run with each series of experiments, omitting the enzyme preparation.

Determination of phosphocreatine. This was done according to the method of Stone (1943) with slight modifications. The reaction mixture after addition to trichloroacetic acid was allowed to stand for 15 min., centrifuged while being kept surrounded with ice and a 3 ml. sample of the supernatant removed for analysis. To this was added a drop of 0.1%

Extracts. In later experiments it was found advantageous to use the supernatant obtained on centrifuging the homogenate. In these experiments the homogenate was prepared using 2 ml. 0.9% NaCl/g. brain tissue, the homogenate was allowed to stand at 0° for 5 min. and then centrifuged in the cold. After pouring off the supernatant, the residue was suspended in the same volume of 0.9% NaCl as before and again homogenized. The centrifuging was repeated and the residue was homogenized a third time, using the same volume of 0.9% NaCl. The supernatants were combined and diluted to give an extract equivalent to 100 mg. of brain tissue/ml. This extract contained 42.4% of the N originally present in the tissue. All extracts mentioned in the tables were made in this way unless otherwise stated.

Table 1. *Splitting of phosphocreatine by brain homogenates*

(Total vol. 7 ml.; 0.033 M-glycylglycine at pH 7.)

Substrates added	Final dilution of homogenate (mg. tissue/ml.)	Inorganic phosphate (μg. P/ml. reaction mixture)			Phosphocreatine (μg. P/ml. reaction mixture)		
		0 min.	20 min.	1 hr.	0 min.	20 min.	1 hr.
None	28.6	13.3	14.7	21.3	0.7	0	0
Phosphocreatine	0	21.3	20.7	22.7	89.3	91.7	91.3
Phosphocreatine	28.6	45.0	90.0	96.0	89.3	46.0	34.3
None	9.5	1.0	0.33	1.33	0	0	0
Phosphocreatine	9.5	38.0	31.0	33.7	84.7	89.3	91.7
Phosphocreatine + adenylic acid	9.5	27.3	58.3	83.7	90.3	22.3	0
	2.4	13.7	24.7	41.0	98.3	83.3	41.3
	1.2	12.7	15.3	25.0	98.3	90.0	70.0

phenolphthalein and just enough finely ground $\text{Ca}(\text{OH})_2$ to turn it a pale pink. The $\text{Ca}(\text{OH})_2$ was rubbed in the tube with a glass rod, so that no undissolved particles remained, and the glass rod was washed down with a few drops of water. Ethanol which had been adjusted to a faintly alkaline reaction with NaOH was then added to a concentration of 10% (v/v), the solution was well mixed by swirling between the palms and allowed to stand in ice for 15 min. After centrifuging and decanting the supernatant, the precipitate was washed with 1 ml. of a solution of 5% calcium trichloroacetate in 10% (v/v) ethanol, prepared by carrying out a blank precipitation. The washings were added to the centrifugate, the volume made up to 10 ml. after addition of a drop of 5N-HCl and the phosphocreatine in a 2 ml. portion was determined by the method of Fiske & Subbarow as described by Hawk, Oser & Summerson (1947). The final volume for colour development was 10 ml. and an interval of 40 min. was allowed after addition of the reagents. The readings were taken in a Spekker absorptiometer.

Preparation of brain homogenates and extracts

Guinea pig brain was used in most of the experiments, though rat brain was found to be equally active; mention is made of the fact when rat brain has been used in any particular experiment. The guinea pig or rat was stunned by a blow on the neck, decapitated and the brain rapidly removed.

Homogenates. The brain was weighed quickly and transferred to a glass test-tube homogenizer containing a known volume of 0.9% NaCl. It was then homogenized and after this stage the brain preparation was kept throughout at 0° and dilutions were carried out with ice-cold 0.9% NaCl.

RESULTS

Occurrence of creatine phosphokinase in brain. Brain homogenates at a final dilution of 1 g. tissue/35 ml. were found to split phosphocreatine, but this reaction stopped when the dilution of the homogenate was increased to 1 g./105 ml. (Table 1). However, at the latter dilution, the addition of adenosine-5-phosphoric acid brought about the splitting of phosphocreatine at an even faster rate than with higher concentrations of homogenate in the absence of adenylic acid. Thus the splitting of phosphocreatine takes place largely according to the reaction of Lohmann (1934) and not by a simple phosphatase type of enzyme.

Solubility of brain phosphokinase and its distribution in the homogenate. Table 2 shows that the phosphokinase activity of brain could be practically completely extracted by either 0.9% sodium chloride or water. All the activity did not pass into solution on a single extraction with either sodium chloride solution or water, but after the second extraction there was only a negligible amount of activity remaining in the solid residue. The homogenate was allowed to stand in the cold for 5 min. after each extraction and vigorously shaken for 1 min. before centrifuging. The values for inorganic phosphate suggested that there was a comparatively greater ATPase activity in the residue, especially

Table 2. *Solubility of the enzyme in 0.9 % sodium chloride and in water*

(A: brain homogenized with 4 parts 0.9 % NaCl, centrifuged and residue stirred up (in homogenizer) with 4 parts of the saline and centrifuged, stirred up again with 4 parts saline as before and centrifuged, the three extracts and the final residue tested for activity separately at final dilutions corresponding to 5.7 mg. tissue/ml. B: extraction carried out with glass-distilled water in place of 0.9 % NaCl; same procedure as A, final dilution corresponding to 4.8 mg. tissue/ml., 3 mM-MgSO₄.)

	Inorganic phosphate (μ g. P/ml.)			Phosphocreatine (μ g. P/ml.)		
	0 min.	1 hr.	Increase	0 min.	1 hr.	Decrease
<i>A. Saline extraction</i>						
First extract	15.3	38.0	22.7	94.3	0	94.3
Second extract	18.7	27.3	8.6	92.3	74.0	18.3
Third extract	13.7	19.0	5.3	98.3	93.7	4.6
Residue	16.0	31.7	15.7	97.9	84.7	13.0
<i>B. Water extraction</i>						
First extract	24.0	61.3	37.3	88.7	0	88.7
Second extract	15.3	36.3	21.0	91.7	19.7	72.0
Third extract	14.3	21.0	6.7	95.7	93.7	2.0
Residue	20.7	24.7	4.0	96.0	92.0	4.0

when extraction is carried out with 0.9 % sodium chloride. More of the phosphokinase activity is extracted by sodium chloride solution in the first extraction than by distilled water.

Table 3. *Activating effect of magnesium ions*

(Dialysed (2 hr.) extract at dilution of 2 mg./ml.; 90.5 μ g. phosphocreatine P/ml.)

Concentration of MgSO ₄ in reaction mixture (mm)	Phosphocreatine split in 20 min. (μ g. P/ml.)	Phosphocreatine split in 1 hr. (μ g. P/ml.)
0	0.6	18.1
0.5	50.0	85.2
1	58.1	90.5
2	57.2	90.5
5	37.1	90.5

Activating effect of magnesium ions. Dialysis against 0.9 % sodium chloride for 6 hr. completely abolished the phosphokinase activity of a brain extract. The activity was reduced to 86 % after 2 hr. dialysis and to 34 % after 4 hr. dialysis. The

tests were carried out at a final dilution of 5 mg./ml. (calculated on the weight of brain tissue from which the extract was prepared). The activity of the dialysed extract was not restored by the addition of boiled extract to a final dilution of 10 mg./ml., but such an addition to the undialysed extract enhanced its activity considerably. The activating effect of boiled extracts was due to its content of magnesium ions. At dilutions as low as 2 mg./ml. the activity even of undialysed extracts was found to be considerably enhanced by the addition of magnesium ions. The optimal concentration of magnesium ions added as magnesium sulphate was found to be 1–2 mm (Table 3). The rate of reaction was perceptibly slower at higher concentrations of magnesium sulphate, as for instance 5 mm. Both calcium ions (as calcium chloride) and manganese ions (as manganous sulphate) restored the activity of dialysed extracts, but were not nearly as effective as magnesium ions (Table 4). Potassium ions added as potassium chloride were without any activating effect on the enzyme.

Table 4. *Activating effect of calcium, manganese and potassium ions*

(Dialysed (2 hr.) extracts at final dilution of 2 mg./ml.)

Activator added	Concentration of activator (mm)	Phosphocreatine (μ g. P/ml.)		
		Present initially	Split in 20 min.	Split in 1 hr.
None	—	101.7	0.8	20.3
Magnesium sulphate	1	101.7	74.7	100.0
Potassium chloride	10	101.7	4.6	24.4
Potassium chloride	1	101.7	0	19.7
Calcium chloride	5	90.5	15.7	90.5
Calcium chloride	2	90.5	25.5	84.5
Calcium chloride	1	90.5	21.1	—
Manganous sulphate	1	97.0	36.7	93.9
Manganous sulphate	0.2	97.0	37.9	77.5

pH optimum of the forward reaction. The change: phosphocreatine + adenylic acid $\rightarrow$ creatine + ADP was optimally catalysed at a pH of 7, although decreasing the pH to 5.9 altered the reaction rate very little (Table 5). The rate of reaction decreased more steeply with increasing pH and was practically abolished at pH 8.9. Below pH 5.9 the splitting of phosphocreatine that took place was not dependent on the enzyme at all, the rate of splitting being almost the same in the absence of the enzyme.

Table 5. pH Optimum of brain phosphokinase

(Dilution of extract, 2 mg./ml.; 5 mM-MgSO₄; 92 μ g. phosphocreatine P/ml. originally present.)

pH	Buffer	Phosphocreatine split (μ g. P/ml.)		Phosphocreatine split in absence of enzyme (μ g. P/ml.) 1 hr.
		20 min.	1 hr.	
4.1	0.1 M-acetate, acetic acid	45	75	73.2
5.0	0.1 M-acetate, acetic acid	14	36.3	31.9
5.9	0.1 M-acetate, acetic acid	55	89.3	6.9
7.0	0.1 M-glycylglycine	60	89.3	0
8.2	0.1 M-glycine-sodium hydroxide	14.7	51.3	0.7
8.9	0.1 M-glycine-sodium hydroxide	6.3	6.0	0.3

Adenosinediphosphate as phosphate acceptor. When ADP was used as the phosphate acceptor in place of adenylic acid, the reaction was not so fast as with adenylic acid and was still incomplete at the end of 1 hr. (Table 6). The pH optimum was, however, within the same range. The evidence for the functioning of ADP as an alternative phosphate acceptor is, however, incomplete, since the possibility remains that an enzyme similar to myokinase might produce adenylic acid from the added ADP.

Table 6. Enzyme activity with ADP as phosphate acceptor

(Buffers as in Table 5; 5 mM-MgSO₄; 2.83 mM-ADP; dilution of extract 2 mg./ml.; 82.7 μ g. phosphocreatine P/ml.)

pH	Phosphocreatine split (μ g. P/ml.)		Phosphocreatine split in absence of enzyme (μ g. P/ml.) 1 hr.
	20 min.	1 hr.	
4.1	38.7	62.7	65.1
5.0	17.7	43.4	28.3
5.9	40.4	51.4	6.1
7.0	47.0	56.4	0
8.2	22.7	38.7	0.6
8.9	6.0	12.4	0.3

Stability of the enzyme. The activity of the enzyme did not decrease markedly on storage in the cold for periods of up to 8 days. The enzyme stored for 8 days split added phosphocreatine completely in 1 hr. at the usual concentration, though in the first 20 min. it split only 64 % of the phosphocreatine as compared to 85 % by the fresh preparation.

Synthesis of phosphocreatine by brain phosphokinase

The creatine phosphokinase of muscle is known to catalyse the synthesis of phosphocreatine from creatine and ATP (Lehmann, 1935; Banga, 1943). With preparations from brain it was found that a much higher concentration of the enzyme (20 mg. tissue/ml.) was required for synthetic activity than for the splitting of phosphocreatine (2 mg. tissue/ml.). It is probable that the presence of ATPase

in the brain extract limits the speed and extent of phosphocreatine synthesis, and this might explain the lesser synthetic activity at higher concentrations of brain extract. The pH range favouring this reaction (Table 7) in the presence of enzyme preparations from brain, seems to be the same as for muscle phosphokinase (Banga, 1943). The reaction proceeds at a measurable rate only above pH 8.2. An equilibrium is reached in about 40 min. and thereafter no increase in phosphocreatine can be detected. The reaction appears to be slower than with comparable concentrations of an extract from muscle.

Table 7. Effect of pH and enzyme concentration on synthesis of phosphocreatine

(2.8 mM-ATP; 5.5 mM-creatine; 5 mM-MgSO₄; 0.1 M-glycine-sodium hydroxide buffer.)

pH	Dilution of enzyme extract (mg. tissue/ml.)	Phosphocreatine formed (μ g. P/ml.)	
		20 min.	1 hr.
7.0	20	0	0
8.2	20	3.3	0
8.9	20	18.7	14.7
8.9	40	11.7	5.0
8.9	10	8.3	10.3

The effect of structural analogues, drugs of central action and some inhibitory agents

Various drugs known to act on the central nervous system were tested for their effect on the phosphokinase activity of brain extract (Table 8). These included the convulsants caffeine, leptazol, picrotoxin and strychnine, the anticonvulsants

Table 8. *Effect of various substances on enzyme activity*(Enzyme extract at dilution of 2 mg./ml.; 1.0 mm-MgSO₄.)

Exp.	Inhibitor added	Concentration of inhibitor (mm)	Phosphocreatine split (μg. P/ml.)		Inhibition based on 20 min. values (%)
			20 min.	1 hr.	
A	None	—	55.3	61.0	—
	Caffeine	10	56.6	61.0	Nil
	Leptazol	10	56.9	61.0	Nil
	Phenobarbitone	10	53.8	61.0	Nil
	Dial (5:5-diallylbarbituric acid)	10	57.8	61.0	Nil
B	None	—	33.3	82.3	—
	Adenine	2	39.1	82.3	Nil
	Adenosine	10	36.0	82.3	Nil
	Picrotoxin	2	35.7	82.3	Nil
	Mesantoin (3-methyl-5:5-phenylethylhydantoin)	2	30.3	82.3	Nil
	Morphine	1	39.9	82.3	Nil
	Atropine	1	40.8	82.3	Nil
C	None	—	53.1	81.7	—
	Strychnine	1	52.5	81.7	Nil
	Barbitone	10	49.3	81.7	Nil
	Tridione (3:5:5-trimethyl-2:4-oxazolidinedione)	10	50.6	81.7	Nil
	Iodoacetate	1	0	0	100
	Iodoacetate	0.5	—	3.4	—
	Iodoacetate	0.2	24.2	43.4	57
D	None	—	52.4	73.5	—
	Sodium fluoride	50	54.6	53.1	Nil*
	Sodium fluoride	10	37.7	60.0	28
	Sodium fluoride	1	42.7	73.5	18
	2:4-Dinitrophenol	1	43.3	68.5	17
	(+)-Tubocurarine	1	51.8	71.5	Nil

* Fluoride at this concentration was found to interfere with the estimation of phosphocreatine.

phenobarbitone, tridione (3:5:5-trimethyl-2:4-oxazolidinedione) and mesantoin (3-methyl-5:5-phenylethylhydantoin), and the narcotics barbitone, dial (5:5-diallylbarbituric acid) and morphine. None of

effect on the reverse reaction of phosphocreatine synthesis, since this takes place at a different pH and some of these substances might be expected to behave differently with an altered state of ionic equilibrium (Table 9). However, no marked reduction of synthetic activity could be detected with any of the substances examined.

Table 9. *Effect of various substances on phosphocreatine synthesis*

(Dilution of extract, 20 mg./ml.; 0.1 M-carbonate-bicarbonate buffer at pH 8.77; 5.6 mm-creatine; 3.2 mm-ATP.)

Added substance	Concentration (mm)	Phosphocreatine formed (μg. P/ml.)	
		20 min.	40 min.
None	—	10.3	13.1
2:4-Dinitrophenol	0.1	10.0	13.4
Dial (5:5-diallylbarbituric acid)	10	6.7	13.0
Phenobarbitone	10	6.3	11.9
Leptazol	10	8.3	10.8
Caffeine	10	6.9	9.9
Tridione (3:5:5-trimethyl-2:4-oxazolidinedione)	10	10.1	11.7

these drugs, even when present at the high concentrations indicated, had any significant effect on the splitting of phosphocreatine by brain extracts. A few of these drugs were also studied for their

Adenine and adenosine, which are structurally related to the substrates, were also found to be without any effect on enzyme activity. Of the miscellaneous substances tested, namely fluoride, iodoacetate, atropine, 2:4-dinitrophenol and (+)-tubocurarine, only iodoacetate inhibited enzyme activity significantly, 100 % at 1 mm and 57 % at 0.2 mm, while the inhibition caused by fluoride (28 % at 10 mm) was not as high as the activating effect of magnesium ions might lead one to expect.

Manometric measurement of phosphokinase activity

In an attempt to follow the time course of the phosphokinase reaction in greater detail than was practicable by the technique of colorimetric estimation of phosphocreatine remaining after varying periods of incubation, several experiments were carried out to test the feasibility of a manometric method of following this reaction. The reaction mixture in the main compartment of the manometric vessel contained, in a volume of 2.5 ml., 4–10 μmoles sodium phosphocreatine, 4.5–12 μmoles adenylic acid, and NaHCO₃ to give

a final concentration of 5.89 mM. The enzyme preparation with 0.03 M-MgSO₄ in 0.5 ml., was in the side arm. The gas phase was N₂ containing 5% (v/v) CO₂. The enzyme solution was tipped into the main compartment when the manometer readings were steady, and readings were taken at 5 min. intervals. At the end of 1 hr. the phosphocreatine remaining in the solution was estimated by the usual method. It was found that the reaction proceeded to completion only with relatively high concentrations of enzyme (10 mg. tissue/ml.); at these concentrations the ratio μ moles CO₂ absorbed to μ moles phosphocreatine split approximated to unity, as would be expected if the creatine liberated by the reaction absorbed 1 equivalent of CO₂. However, at lower concentrations of enzyme, the reaction did not proceed to completion and the ratio of CO₂ absorbed to phosphocreatine split decreased to nearly 0.5. Reactions other than that of the phosphokinase were found to be taking place with the higher tissue concentrations, as inorganic phosphate was formed. The method was not examined further.

DISCUSSION

It is evident from this study that phosphocreatine is not degraded in brain by a simple hydrolytic reaction, but that the energy-rich phosphate bond is transferred to a phosphate acceptor, adenylic acid or ADP, so that the stored energy in phosphocreatine largely remains available. In muscle, phosphocreatine acts as a reservoir of energy-rich phosphate bonds. The enzyme system described here might prove to be responsible for the first step in the utilization of phosphocreatine for the functional activity of the central nervous system.

Under optimum conditions of pH and concentration of magnesium ions the extract from 2 mg. brain can bring about the splitting of 2.5 μ moles of phosphocreatine in 20 min. This corresponds to 3750 μ moles/g./hr. Dawson & Richter (1950) found that electrical stimulation *in vivo* produced a 50% fall in the phosphocreatine content of the brain of rats in 1 sec. At the optimal rate we have found that brain phosphokinase could split 1.04 μ moles of phosphocreatine/g. tissue/sec. As rat brain contains 3.16 μ moles/g., this is of the order reported by Dawson & Richter. We have also observed a slowing of the reaction after the first 20 min. and it is possible that the rate of phosphocreatine splitting might be even greater during the first few seconds. Using the values given by Klein & Olsen (1947) for the glucose and lactic acid content of the brains of cats in their normal condition and after a 10 sec. convulsive period, McIlwain, Anguiano & Cheshire (1951) have estimated possible maximal rates for the metabolic utilization of glucose by brain. They arrived at a figure of -370 μ moles glucose/g./hr. The rate at which the phosphokinase of brain releases high-energy phosphate is thus much greater than is necessary to ensure the phosphorylation and utilization of glucose at the above rate. It is possible that in the intact cell the activity of the phosphokinase is

modified by histological and other factors, for instance the concentration of phosphate acceptors. This is an aspect of the enzyme system not dealt with in the present work and deferred for further study.

It is of interest to compare phosphokinase activity with the activities of other enzyme systems present in brain and concerned with the degradation or transfer of energy-rich phosphate groups. Gore (1951) has shown that under optimum conditions the ATPase activity of guinea pig brain is as high as 10.2-13.6 μ moles P/mg./hr. This is on the basis of dry weight of tissue and is equivalent to 1600-2176 μ moles P/g./hr. for wet tissue, and thus is of the same order of activity as observed for phosphokinase. The values reported for hexokinase activity, on the other hand, are very much lower. Meyerhof & Wilson (1948) found that on incubation with a 1:5.5 brain extract, the decrease in glucose was 1.2 μ moles/12 min. This leads to a value of 66 μ moles P transferred/g./hr. Thus phosphokinase in brain could form ATP at a considerably faster rate than would be required for the full activity of hexokinase.

Convulsants and anticonvulsants are known to lead to changes in energy-rich phosphates of the brain *in vivo* (Stone, Webster & Gurdjian, 1945; Klein & Olsen, 1947). There are good reasons for thinking that these changes are secondary to changed functional activities in the central nervous system, but they could be achieved by a purely biochemical mechanism, such as a direct acceleration or inhibition of the phosphokinase system. No indication of such an effect was, however, found.

It is evident also that 2:4-dinitrophenol, which can markedly affect the phosphates in cerebral tissue (McIlwain & Gore, 1951), does not act on the creatine phosphokinase system. The relatively slight inhibition caused by fluoride ions at a concentration at which they might be expected to remove a large part of the magnesium ions from the system is interesting and can probably be explained by the strong affinity of the enzyme for binding magnesium ions. Iodoacetate, the most effective inhibitor found, inhibits respiration of brain slices at comparable concentrations when glucose is the substrate (Krebs, 1931) and it inhibits also a number of enzymes whose activity depends on thiol groups.

SUMMARY

1. Homogenates of mammalian brain have been found to split phosphocreatine in the presence of adenylic acid. The phosphokinase activity of brain homogenates can be recovered in the supernatant on centrifuging them.

2. Magnesium ions at 1-2 mM are necessary for full activity of the dialysed enzyme preparations; manganese and calcium ions are also effective as

activators though less so than magnesium. Potassium ions are inactive in this respect.

3. The pH optimum of the enzyme for the forward reaction, i.e. the splitting of phosphocreatine in the presence of adenylic acid lies in the range of 5.9–7; phosphocreatine is split optimally at the same pH range when adenosinediphosphate is used as the phosphate acceptor.

4. Preparations from brain also catalyse the

synthesis of phosphocreatine from creatine and adenosinetriphosphate above pH 8.2.

5. None of the drugs of central action which were examined had any significant effect on the activity of the enzyme. Iodoacetate inhibited it completely above a concentration of 0.5 mM and fluoride to a much less marked degree.

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Effects of Insulin and of Glycerol *in vitro* on the Incorporation of [carboxy-¹⁴C]acetate into the Fatty Acids of Lactating Mammary Gland Slices with Special Reference to Species Differences

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Udder slices from lactating ruminants (cow, ewe, goat) have been shown actively to utilize acetate as sole substrate with R.Q. > 1 (Folley & French, 1950), from which it was concluded that udder tissue of the ruminant can synthesize fat from acetate alone. On the other hand, slices of lactating mammary gland from non-ruminants (rat, rabbit) were inert to acetate, which, however, was utilized in the presence of glucose, the R.Q. then being of the same order (> 1) as in glucose alone. Moreover, addition of glucose to the medium increased the amount of acetate used by mammary gland slices from ruminants (sheep) and the R.Q. was increased. This was taken to indicate that glucose increases the utilization of acetate for fat synthesis under these conditions.

The effect of insulin *in vitro* on fat synthesis by mammary gland slices has also been studied by respiratory measurements. Addition of insulin to the incubation medium resulted in a marked in-

crease in the R.Q., $-Q_{O_2}$ and acetate uptake of rat mammary gland slices metabolizing mixtures of acetate and glucose, but had no effect on the respiration of the slices in acetate alone, from which it was concluded that in rat mammary tissue insulin enhances fat synthesis from acetate in the presence of glucose, but evokes no fatty acid synthesis in acetate alone (Balmain, French & Folley, 1950; Balmain & Folley, 1951). Sheep udder slices, on the other hand, were inert to insulin both in acetate alone and in acetate plus glucose (Balmain & Folley, 1951). The increase in the R.Q. of mammary gland slices from lactating rats in the presence of insulin has been confirmed by Hills & Stadie (1952).

Glycerol has been found to influence the metabolism of acetate plus glucose by rat mammary gland slices in a way similar to insulin in all respects save that the increase in R.Q. was less marked and, more important, glycerol did not increase $-Q_{\text{glucose}}$ while insulin did. This finding was interpreted as