REFERENCES

Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
Dawson, R. M. C. & Richter, D. (1950). *Amer. J. Physiol.* **160**, 203.
Dickens, F. (1936*a*). *Biochem. J.* **30**, 661.
Dickens, F. (1936*b*). *Biochem. J.* **30**, 1233.
Dixon, K. C. (1936). *Biochem. J.* **30**, 1479.
Elliott, K. A. C. & Henry, M. (1946). *J. biol. Chem.* **163**, 361.
Gore, M., Ibbott, F. & McIlwain, H. (1950). *Biochem. J.* **47**, 1.
Hawk, P. B., Oser, B. L. & Summerson, W. H. (1947). *Practical Physiological Chemistry.* London: Churchill.
Himwich, W. A. & Himwich, H. E. (1946). *J. Neurophysiol.* **9**, 133.
Kety, S. S., Woodford, R. B., Harmel, M. H., Freyhan, F. A., Appel, K. E. & Schmidt, C. F. (1948). *Amer. J. Psychiat.* **104**, 765.
Klein, J. R. & Olsen, N. S. (1947). *J. biol. Chem.* **167**, 747.
McIlwain, H. (1951). *Biochem. J.* **50**, 132.
McIlwain, H., Buchel, L. & Cheshire, J. D. (1951). *Biochem. J.* **48**, 12.
McIlwain, H. & Grinyer, I. (1950). *Biochem. J.* **46**, 5.
Meyerhof, O. & Geliazkowa, N. (1947). *Arch. Biochem.* **12**, 405.
Ochoa, S. (1941). *J. biol. Chem.* **141**, 245.
Schmidt, C. F., Kety, S. S. & Pennes, H. H. (1945). *Amer. J. Physiol.* **143**, 33.
Utter, M. F., Wood, H. G. & Reiner, J. R. (1945). *J. biol. Chem.* **161**, 197.
Warburg, O. (1930). *Metabolism of Tumours.* Tr. F. Dickens. London: Constable.

Adenosinetriphosphatase Activity of Brain

By MARION B. R. GORE
Biochemical Laboratories, Institute of Psychiatry (British Post-Graduate Medical Federation, University of London), Maudsley Hospital, S.E. 5

(*Received* 17 *February* 1951)

Phosphatases which act on adenosinetriphosphate (ATP) have a wide distribution in nature and are present in many mammalian tissues. DuBois & Potter (1943) described a method of assay of such an enzyme in various tissues including brain. The adenosinetriphosphatase (ATPase) of brain has been relatively little investigated, however, compared with that of muscle.

The important discovery that the contractile protein, myosin, is a highly active enzyme which splits inorganic phosphate from ATP, was made by Engelhardt & Ljubimowa (1939). The relationships between myosin, ATP and various ion concentrations in the contraction of the muscle fibre have been studied in detail mainly by Szent-Györgyi and his colleagues (Szent-Györgyi, 1947). The problem of how far the relationships existing in muscle represent a general principle which is used by other specialized tissues as a source of energy for activity is an interesting one. There is evidence that changes in functional activity are associated with changes in ATP, phosphocreatine and inorganic phosphate in brain (Stone, Webster & Gurdjian, 1945; Klein & Olsen, 1947). The enzymic hydrolysis of ATP has not, however, been shown to be a reaction essential in providing energy for the functional activity of brain.

The presence of an ATP-splitting enzyme in brain and the controlling effect which the concentration of this enzyme has on the rate on anaerobic glycolysis of glucose and fructose by brain homogenates, slices and extracts, has been described in a series of papers by Meyerhof and his colleagues (Meyerhof, 1947; Meyerhof & Geliazkowa, 1947; Meyerhof & Wilson, 1947, 1948, 1949). The importance of an adequate supply of ATP in the maintenance of glycolysis and the effect of the ATPase in brain homogenates and extracts in controlling this process has also been shown by Utter, Reiner & Wood (1945) and Utter (1950).

The present study was made to examine the properties of the ATP-splitting enzyme in brain tissue and to test the effect on it of compounds structurally related to ATP and various drugs which act on the central nervous system. The activity of brain tissue in splitting ATP has been compared with its inorganic pyrophosphatase (PPase) activity (Gordon, 1950).

EXPERIMENTAL

Enzyme preparations. A guinea pig was killed by a blow on the neck. The whole brain was excised rapidly, weighed and homogenized in 10 vol. of ice-cold distilled water, 0·154 M-NaCl solution, 0·5 M-KCl containing 0·03 M-$NaHCO_3$ and 0·2 M-Na_2CO_3, or Krebs-Ringer bicarbonate (Umbreit, Burris & Stauffer, 1949) from which $CaCl_2$ and KH_2PO_4 had been omitted. The homogenate was stored at 0° under toluene at this dilution. For measurement of enzyme activity further dilution of the homogenate to 1 g. tissue in

100 vol. of fluid was made. The tissue extracts obtained as supernatant solution on centrifuging the 1:10 homogenate and the tissue residue resuspended in an equal volume of homogenizing fluid have also been tested for ATPase and PPase activity.

For the experiments in which the effect of various compounds on brain ATPase was being measured, a preparation which contained ATPase relatively free from PPase was desired. This was prepared by homogenizing whole brain in 10 vol. of Krebs-Ringer solution. The tissue was extracted by stirring the homogenate mechanically for 30 min. at 0°. The mixture was then centrifuged, the supernatant removed and replaced with an equal volume of distilled water. After 10 min. stirring, the mixture was again centrifuged and the supernatant replaced with distilled water. Extraction was made for 30 min. This extract contained approximately 50% of the ATPase and 10% of the PPase activity of the initial homogenate.

Measurement of enzyme activity

Adenosinetriphosphatase. BaATP supplied by Boots Pure Drug Co. Ltd. was used to prepare the substrate for the test of ATPase activity. An analysis of the phosphorus content of the BaATP specimen used gave the figures: total P, 11·1%; labile P (10 min. hydrolysis in 1 N-HCl), 6·7%; inorganic P, 1·3%. A weighed sample of the commercial preparation was washed twice with small volumes of ice-cold 0·1 M-acetate buffer (pH 4·0) as suggested by Kornberg (1950) to remove inorganic phosphate present and then dissolved in the minimum amount of 0·1 N-HCl. The washing treatment reduced the percentage of phosphorus present as inorganic phosphate to 0·3% by weight. The Na salt was obtained by passing the acid solution through a column of Na-charged Zeo-Karb 216. The pH of the solution was brought to 7·4 with 0·1 N-NaOH and the molar concentration adjusted to 0·02. The ATP solution (0·5 ml.) was incubated in a stoppered tube with 1 ml. 0·1 M-glycylglycine buffer (pH 7·4) in the presence of $MgSO_4$ and KCl, each at a final concentration of $7·7 \times 10^{-3}$ M. The enzyme preparation (0·5 ml.) containing 0·5–2 mg. tissue, dry wt., was then added, the mixture well shaken, a 0·5 ml. sample removed quickly for estimation of phosphate present and the tube returned to the water bath at 37°. The initial and all subsequent samples were added to ice-cold trichloroacetic acid (final concn. 5%). Inorganic phosphate concentration was determined on the trichloroacetic acid filtrate by the method of Lowry & Lopez (1946). Results were calculated as mol. P liberated/hr./mg. tissue, dry wt.

Inorganic pyrophosphatase. The inorganic pyrophosphatase activity of brain preparations (Gordon, 1950) was estimated under the same conditions of pH, buffer and ion activation as the ATPase. $Na_4P_2O_7.10H_2O$ (A.R. quality), free from orthophosphate, was used as substrate at a final concentration of $2·5 \times 10^{-3}$ M.

RESULTS

Factors influencing activity of the ATPase

The effects of time of incubation, substrate concentration, enzyme concentration, pH, cation concentration and various buffers, have been measured and the results are shown in Figs. 1–4. The following results were obtained.

Time of incubation. The rate of decomposition of ATP by guinea pig-brain homogenate at pH 7·4 in the presence of $7·7 \times 10^{-3}$ M Mg^{++} and K^{++} was linear over a period of 20 min. (Fig. 1*a*). After 20 min. the activity slowly fell, possibly due to inactivation of the enzyme. Preheating the enzyme for 30 min. at 37° reduced its activity by 12%. On the basis of these findings samples taken after 15 min. incubation were used in measurement of enzyme activity.

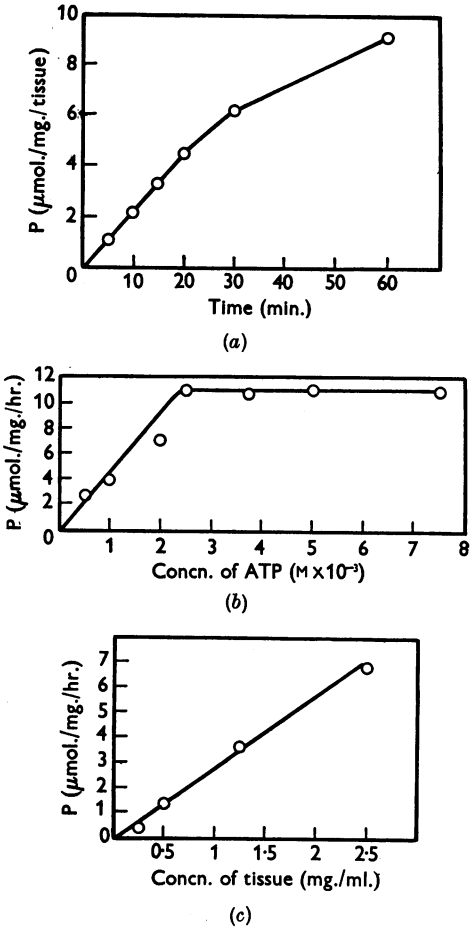

Fig. 1. Effect of (*a*) time of incubation, (*b*) substrate concentration, and (*c*) enzyme concentration on liberation of inorganic phosphate from ATP at 37°. Guinea pig-brain homogenate in 0·1 M-glycylglycine (pH 7·4) containing Mg^{++}, $7·7 \times 10^{-3}$ M, and K^{++}, $7·7 \times 10^{-3}$ M. In (*a*) and (*c*) ATP concentration is 5×10^{-3} M.

Substrate concentration. The activity of the enzyme was maximal with a substrate concentration of $2·5 \times 10^{-3}$ M (Fig. 1*b*). Increase in concentration of ATP to $7·5 \times 10^{-3}$ M produced no increase in the rate of liberation of inorganic phosphate. The

concentration of ATP used in the experiments in this study was 5×10^{-3}M.

Enzyme concentration. The amount of phosphate liberated from ATP was directly proportional to the weight of tissue present over the range 0·25–2·5 mg. (dry wt.)/ml. (Fig. 1c). Brain homogenates and extracts were diluted so that the concentration of tissue in the incubation mixture was in this range.

Buffers. The effect of several buffers on the rate of enzyme action was studied and the results are shown in Table 1. The rate of liberation of inorganic phosphate was greatest in 0·1M-glycylglycine buffer and this has been used in all experiments. The relatively high rate of activity in glycylglycine buffer may be due to some effect on inhibiting ions or on ion balance exerted by the glycylglycine. In their assay of ATPase activity in various tissues including brain DuBois & Potter (1943) used a diethylbarbiturate

Table 1. *Effect of buffer on ATPase of guinea pig brain*

(Guinea pig-brain homogenate incubated with 5×10^{-3}M-ATP and $7{\cdot}7 \times 10^{-3}$M Mg^{++} and K^+ at 37°.)

Buffer	pH	Rate (μmol. P/mg./hr.)	Percentage of highest rate
Glycylglycine, 0·1M	8·0	7·9	100
Bicarbonate, 0·072M (gassed with 5% CO_2)	8·0	6·0	76
Glycine, 0·1M	8·2	4·7	60
Diethylbarbiturate, 0·1M	8·0	2·5	32

buffer in which, according to Table 1, the enzyme reaction takes place at one-third of the rate of that in glycylglycine buffer.

Optimum pH. When the effect of pH on the enzyme activity was measured there appeared to be two maxima, one at pH 7·4 and the other at pH 8·2 (Fig. 2). This might be due to the presence of more

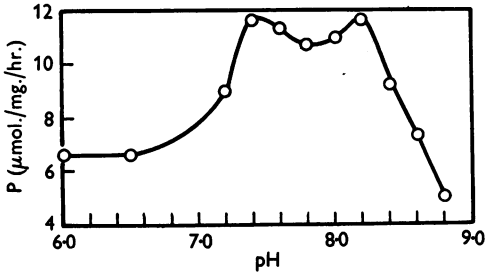

Fig. 2. Effect of pH of medium on ATPase activity of brain at 37°. Medium and ATP concentrations as Fig. 1.

than one enzyme in the brain homogenate acting on ATP or to the existence of a different pH optimum for the splitting of the first and second labile phosphate of ATP.

Number of inorganic phosphate groups liberated and specificity. The number of inorganic phosphate groups split from ATP by brain ATPase was measured at pH 7·4 and 8·2. The result is shown in Fig. 3. There was very little difference in the rate of

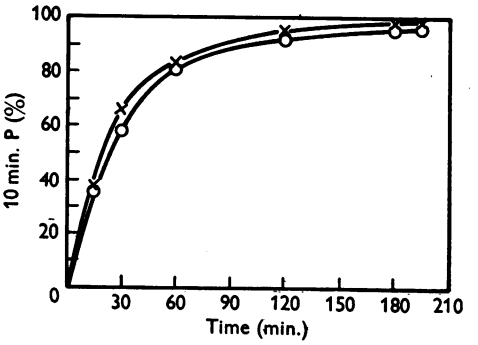

Fig. 3. Number of phosphate groups split from ATP by brain. Medium and ATP concentration as in Fig. 1. Labile phosphate was measured after 10 min. hydrolysis at 100° in 1N-HCl. ×—×, pH 8·2; ○—○, pH 7·4.

hydrolysis of ATP at different pH, and in both conditions two phosphate groups were split from the ATP molecule. One phosphate group was split from ADP when this was used as substrate at pH 7·4. The enzyme did not act on hexosediphosphate and β-glycerophosphate.

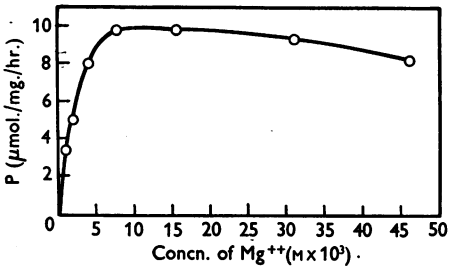

Fig. 4. Effect of Mg^{++} concentration on ATPase activity of brain at 37°. Medium and ATP concentration as in Fig. 1.

Effect of Mg^{++}, Ca^{++} *and* K^+. The activity of the enzyme was slight in the absence of Mg^{++} ions. Optimum activation was given by a final concentration of $7{\cdot}7 \times 10^{-3}$M-Mg^{++} in the incubation mixture (Fig. 4). Both in the absence of Mg^{++} ions and in the presence of the optimum Mg^{++} ion concentration, the addition of Ca^{++} to the medium in concentrations as low as 6×10^{-4}M produced inhibition of the enzyme (Table 2). In the presence of $7{\cdot}7 \times 10^{-3}$M-Mg^{++}, K^+ increased the rate of liberation of inorganic phosphate. The medium used for measuring enzyme activity contained Mg^{++} and K^+ in amounts which gave a final concentration of each of $7{\cdot}7 \times 10^{-3}$M.

These results differ from those of DuBois & Potter (1943), who found that the ATPase of ten different

Table 2. *Effect of* Mg^{++}, K^{+} *and* Ca^{++}, *on ATPase of guinea pig brain*

(Guinea pig-brain homogenate incubated in 0·1 M-glycylglycine with 5×10^{-3} M-ATP at 37°.)

Medium containing final concentration ($M \times 10^{-3}$)			Rate (μmol. P/mg./hr.)
Mg	K	Ca	
0	0	0	1·2
7·7	0	0	5·9
7·7	19	0	9·9
7·7	7·7	0	10·2
7·7	3	0	8·9
7·7	7·7	1·3	4·3
7·7	7·7	0	13·6
7·7	7·7	55	4·3
7·7	7·7	1·3	5·05
7·7	7·7	0·6	7·35
0	7·7	0	2·6
0	7·7	11	1·4
0	7·7	5·5	1·4
0	7·7	2·8	1·3

tissues, including brain, was activated by Ca^{++}. The ATPase of brain they report to be three times more active in the presence of 3×10^{-3} M-Ca^{++} as in its absence. They also found Mg^{++} in concentration above $2{\cdot}4 \times 10^{-3}$ M to inhibit liver ATPase. Other workers, however, support the findings of this study by reporting either Mg^{++} activation or Ca^{++} inhibition or both of ATPase in nervous tissue. (Greville & Lehmann, 1943; Feldberg & Mann, 1945; Epelbaum, Sheves & Kobylin, 1949; Binkley & Olson, 1950).

Effect of various substances on ATPase

Sodium fluoride is known to be a phosphatase inhibitor and has been found to inhibit brain ATPase. The effect of some other common enzyme inhibitors on ATPase has been examined. An attempt to find an inhibitor specific for ATPase has been made by examining the effect of substances structurally related to ATP on the enzyme. Torda & Wolff (1948) studied the effect of some centrally active substances and alkaloids on muscle ATPase. A similar examination on brain ATPase was clearly desirable and this has been made.

Enzyme inhibitors. The effect of various enzyme inhibitors on guinea pig-brain ATPase is given in Table 3. The table also shows the effect of the same concentration of inhibitor on PPase and this will be discussed below. The ATPase appears to be sensitive to low concentrations of substances known to react with thiol groups: copper sulphate, sodium iodoacetate and alloxan. Sodium fluoride is also an active inhibitor. Relatively high concentrations of sodium cyanide are necessary to produce inhibition and the enzyme was almost unaffected by the concentration of 2:4-dinitrophenol used.

Table 3. *Effect of enzyme inhibitors on ATPase and PPase of guinea pig brain*

(Second water extract of homogenized brain tissue in 0·1 M-glycylglycine, pH 7·4, with 5×10^{-3} M-ATP or $2{\cdot}5 \times 10^{-3}$ M-sodium pyrophosphate (PP) in presence of $7{\cdot}7 \times 10^{-3}$ M-Mg^{++} and K^{++} and test substance.)

Substance	Concentration ($M \times 10^{-3}$)	Inhibition (%)	
		ATPase	PPase
NaF	2·5	61	100
NaF	0·5	31	93
NaF	0·25	23	66
$CuSO_4$	0·1	32	87
$CuSO_4$	0·125	33	100
$CuSO_4$	0·01	− 8	4
NaCN	15	19	12
Sodium iodoacetate	2	29	50
Alloxan	1	17	23
Alloxan	10	50	82
2:4-Dinitrophenol	1·6	4	—
2:4-Dinitrophenol	0·3	7	—
2:4-Dinitrophenol	0·06	0	—

Substances structurally related to ATP and substances with action on central nervous system. The action of purine derivatives, barbiturate drugs and several alkaloids in the ATPase activity of brain has been assessed quantitatively. The results obtained with some of the substances tested are given in Table 4.

Table 4. *Effect of various substances on ATPase activity of guinea pig brain*

(5×10^{-3} M-ATP, $7{\cdot}7 \times 10^{-3}$ M-Mg^{++} and K^{+} and test substance in 1·5 ml. 0·1 M-glycylglycine, pH 7·4, at 37°. Second water extract of homogenized brain added, 0·5 ml.)

Substance	Concentration ($M \times 10^{-3}$)	Rate of reaction (as percentage of rate without added substance)
Adenine	5	77
Adenosine	20	84
Guanine	5	98
Xanthine	5	114
Caffeine	6·6	100
Caffeine	1·3	110
Glucose	20	88
Glucose	4	78
Glucose	0·8	89
Glutamate	20	90
Glutamate	4	93
Glutamate	0·8	96
Creatine	20	94
Aneurin	2	91
Aneurin	2	83
Adrenaline	2	83

Of the purine substances tested, adenine produced a slight inhibition of the enzyme at a relatively high concentration. The other purines appeared to have little effect. Meyerhof & Wilson (1949) report a small inhibition of the ATPase of brain homogenate by

octyl and decyl alcohols, which also have narcotic properties. None of the barbiturate drugs, phenobarbitone, soneryl (5-*n*-butyl-5-ethylbarbituric acid), dial (diallylbarbituric acid) or evipan (5-*cyclo*hexyl-1:5-dimethylbarbituric acid) produced any significant inhibition of ATPase in the concentrations 2×10^{-2}M, 4×10^{-3}M, $0{\cdot}8 \times 10^{-3}$M in the present investigations. Diethylbarbituric acid, used in 0·1M concentration as a buffer, did have considerable inhibitory effect (Table 1).

The alkaloids papaverine and narcotine in saturated solution, brucine at 2×10^{-3}M and colchicine at 10^{-3}M and $0{\cdot}4 \times 10^{-4}$M had very little inhibitory action on ATPase. Atropine at $0{\cdot}4 \times 10^{-3}$M produced an inhibition of 17 %.

Glucose and sodium glutamate and creatine were added to the reaction medium and produced only a slight inhibition of the enzyme. Meyerhof & Wilson (1949) state that the presence of glucose has an inhibitory effect on ATPase of complete brain homogenate. In the present experiments the rate of glycolysis was not measured, but was probably low due to removal of glycolytic enzymes in the Krebs-Ringer and first water extracts of the brain tissue.

The slight inhibition produced by aneurin and adrenaline are shown in Table 4.

Comparison of ATPase and PPase activity

Early in this investigation the similarity of the conditions of optimum pH, ion activation and effective inhibitors of guinea pig-brain ATPase to the properties of brain inorganic pyrophosphatase described by Gordon (1950) was realized. When the PPase activity of a fresh guinea pig brain homogenized in water, sodium chloride or Krebs-Ringer was measured using the same medium as for ATPase measurement, but sodium pyrophosphate at a final concentration of $2{\cdot}5 \times 10^{-3}$M as substrate, it was found that ATPase and PPase activity of the same preparation were approximately equal. Three possible interpretations of this result were considered: (1) that the enzyme described by Gordon was also capable of acting on organic pyrophosphates; (2) that the ATP being used contained inorganic pyrophosphate as impurity (Bailey, 1949) in sufficient quantity to act as substrate for brain PPase; and (3) that there are two separate phosphatases present in brain, one of which reacts with ATP and the other with inorganic pyrophosphate. Several pieces of evidence indicate that brain contains both ATPase and PPase.

An inorganic pyrophosphate-free sample of ATP was prepared by the method described by Baddiley, Michelson & Todd (1949). Brain homogenates gave the same rate of liberation of phosphate with this preparation as with the non-purified sample.

An acetone-dried preparation of brain was made from guinea pig brain according to the instructions given by Gordon (1950). When the saline suspension of this material was tested for ATPase as well as PPase activity it was found that the ATPase activity was only a fraction (10–17 %) of the PPase activity. The fact that acetone drying destroys ATPase activity in brain tissue had previously been noted by Feldberg & Mann (1945) and Meyerhof & Wilson (1948).

Another difference between the enzymic breakdown of the two substrates was the degree of inhibition produced by certain concentrations of inhibitors, notably sodium fluoride and copper sulphate. This is shown in Table 3. It can be seen that concentrations of sodium fluoride, which produced 100 and 93 % inhibition of breakdown of inorganic pyrophosphate, affected ATPase by 61 and 31 %. Similar distinctions were seen at different concentrations of copper sulphate.

A further way in which ATPase and PPase activity was differentiated was in the distribution of the enzyme activity on these two substrates in the extracts and tissue residue of brain homogenates.

Table 5 shows the ATPase and PPase activity of guinea pig-brain homogenates prepared in water and different salt solutions and of the extracts and tissue residues obtained by centrifuging these homogenates. Guinea pig brain was homogenized in 10 vol. of ice-cold water, 0·154M-sodium chloride, 0·5M-potassium chloride containing 0·03M-sodium bicarbonate and 0·02M-sodium carbonate and Krebs-Ringer bi-

Table 5. *Comparison of ATPase and PPase activity of various homogenate fractions*

(Enzyme incubated in 0·1M-glycylglycine, pH 7·4, with 5×10^{-3}M-ATP or $2{\cdot}5 \times 10^{-3}$M-sodium pyrophosphate (PP) in presence of $7{\cdot}7 \times 10^{-3}$M-Mg^{++} and K^{++}.)

Guinea pig brain homogenized in	Homogenate Rate (μmol. P/mg./hr.) ATP	Homogenate Rate (μmol. P/mg./hr.) PP	Homogenate Ratio ATP/PP	Supernatant Rate (μmol. P/mg./hr.) ATP	Supernatant Rate (μmol. P/mg./hr.) PP	Supernatant Ratio ATP/PP	Residue Rate (μmol. P/mg./hr.) ATP	Residue Rate (μmol. P/mg./hr.) PP	Residue Ratio ATP/PP
Water	6·5	8·5	0·77	4·85	10·5	0·46	5·85	1·66	3·5
NaCl, 0·154M	7·4	5·3	1·4	3·1	10·5	0·29	8·7	2·06	4·2
Alkaline KCl, 0·5M	4·0	1·5	2·6	2·64	5·8	0·45	3·88	0	—
Krebs-Ringer bicarbonate	5·9	6·1	0·97	3·7	10·7	0·29	6·6	1·5	4·4
	4·0	4·15	0·97	2·4	7·1	0·34	7·0	1·6	4·35

carbonate solution in which there were no calcium salts or phosphate.

The rate at which the homogenates in water, sodium chloride and Krebs-Ringer split ATP and inorganic pyrophosphates was approximately equal. In the sodium chloride and Krebs-Ringer extracts of brain the PPase activity was three times as great as the ATPase activity; in water and potassium chloride the ratio ATPase/PPase was 0·45. In the tissue residues which were resuspended in homogenizing fluid the ATPase activity was approximately four times as great as the PPase activity.

The ATPase/PPase ratio could be further increased if the Krebs-Ringer homogenate was centrifuged and the tissue residue extracted twice with water. In the first water washing more PPase activity and very little ATPase activity was lost. The second water washing contained 30–50 % of the total ATPase activity of the homogenate and only 5–8 % of the total PPase activity. Although the potassium chloride homogenate seemed to show a relatively high ATPase/PPase activity, the ATPase rate was low and the enzyme appeared to be less stable than in the other salt solutions. Potassium chloride was therefore not used in making ATPase preparations.

In the water homogenates, one-fifth of the total ATPase activity of the complete homogenate was found in the extract; with sodium chloride and Krebs-Ringer homogenates only one-tenth of the ATPase activity was in the extract. This fractionation of brain tissue by extraction did not produce any great purification of the ATPase active fraction as can be seen in Table 5. Attempts to purify the enzyme by ammonium sulphate precipitation and acetone precipitation (Binkley & Olson, 1950) did not meet with success as, after dialysis, the activity of the isolated fraction was always extremely low.

DISCUSSION

It would seem from the present study, and from a number of independent reports, that there exists in mammalian brain an enzyme which liberates inorganic phosphate from ATP, and which is activated by Mg^{++} and inhibited by Ca^{++}. What role this enzyme plays in brain tissue is not fully understood. The function which Meyerhof has found it to fulfil, that of controlling anaerobic glycolysis of brain tissue by removing from the medium the energy-rich phosphates of ATP, seems a somewhat negative role for an active enzyme. No direct evidence has been obtained of what use is made of the energy released by the breakdown of ATP with the liberation of inorganic phosphate in brain.

The importance of the calcium-activated and magnesium-inhibited ATPase of muscle, myosin, in the functioning of that tissue overshadows all work on ATPases. There exists also, however, in muscle, a labile ATPase which is activated by Mg^{++} and inhibited by Ca^{++} (Keilley & Meyerhof, 1948) and whose optimum pH, 6·8, is much closer to physiological pH than that of myosin, 9·4. Keilley & Meyerhof go so far as to suggest that *in vivo* the magnesium-activated ATPase must be responsible for most of the dephosphorylation which takes place in muscle. It does not seem to be very helpful to attempt at this stage to interpret the effects of Ca^{++} and Mg^{++} on nervous tissue through effects on ATPase. Low concentrations of both Ca^{++} and Mg^{++} in the blood produce hyperexcitability of the nervous system. On the other hand, anaesthesia produced by high Mg^{++} concentration in the blood is combated by calcium salts.

Meyerhof & Wilson (1949) have drawn some distinctions between soluble ATPase (that found in an extract of brain homogenate) and absorbed ATPase (that associated with the tissue particles of a homogenate). These workers also note different proportions of soluble and absorbed ATPase in brain, tumours and chicken embryo. It has been noticeable that in the preparation of brain homogenates in different solutions a fraction of the total ATPase is always found in the extract. This is the fraction of brain ATPase which has been treated by acetone precipitations by Binkley & Olson (1950) and which has yielded material active on both ATP and inorganic pyrophosphate. There would appear to be ATPase fractions in brain and other tissue which differ not only in solubility but possibly also in other properties. The problem of the isolation of these fractions in a pure state remains to be solved.

SUMMARY

1. A description is given of some of the properties of a magnesium-activated enzyme in guinea pig brain which splits two phosphate groups from adenosinetriphosphate.

2. The enzyme is inhibited by calcium ions, thiol group reactants and sodium fluoride. It is not inhibited by some barbiturate drugs and alkaloids, and only slightly by some structurally related substances.

3. The action of brain homogenates on adenosinetriphosphate has been compared with its action on inorganic pyrophosphate. While the conditions of action on the two substrates is similar in some respects, there is evidence that two separate enzymes are acting on the different substrates.

I wish to acknowledge with gratitude the assistance of Mr F. A. Ibbott and Mr D. G. Redford in this investigation.

REFERENCES

Baddiley, J., Michelson, A. M. & Todd, A. R. (1949). *J. chem. Soc.* p. 582.
Bailey, K. (1949). *Biochem. J.* **45**, 479.
Binkley, F. & Olson, C. K. (1950). *J. biol. Chem.* **186**, 725.
DuBois, K. P. & Potter, V. R. (1943). *J. biol. Chem.* **150**, 185.
Engelhardt, V. A. & Ljubimowa, M. N. (1939). *Nature, Lond.*, **144**, 669.
Epelbaum, S. E., Sheves, G. & Kobylin, A. A. (1949). *Biokhimiya*, **14**, 107. Cited in *Chem. Abstr.* (1949), **43**, 6263*d*.
Feldberg, W. & Mann, T. (1945). *J. Physiol.* **103**, 28*P*.
Gordon, J. J. (1950). *Biochem. J.* **46**, 96.
Greville, G. D. & Lehmann, H. (1943). *Nature, Lond.*, **152**, 81.
Keilley, W. W. & Meyerhof, O. (1948). *J. biol. Chem.* **176**, 591.
Klein, R. J. & Olsen, N. W. (1947). *J. biol. Chem.* **167**, 747.
Kornberg, A. (1950). *J. biol. Chem.* **182**, 779.
Lowry, O. H. & Lopez, J. A. (1946). *J. biol. Chem.* **162**, 421.
Meyerhof, O. (1947). *Arch. Biochem.* **13**, 485.
Meyerhof, O. & Geliazkowa, N. (1947). *Arch. Biochem.* **12**, 405.
Meyerhof, O. & Wilson, J. R. (1947). *Arch. Biochem.* **14**, 71.
Meyerhof, O. & Wilson, J. R. (1948). *Arch. Biochem.* **17**, 153.
Meyerhof, O. & Wilson, J. R. (1949). *Arch. Biochem.* **23**, 246.
Stone, W. E., Webster, J. E. & Gurdjian, E. S. (1945). *J. Neurophysiol.* **8**, 233.
Szent-Györgyi, A. (1947). *Muscular Contraction.* New York: Academic Press Inc.
Torda, C. & Wolff, H. G. (1948). *Amer. J. Physiol.* **152**, 86.
Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1949). *Manometric Techniques and Related Methods for the Study of Tissue Metabolism.* Minneapolis: Burgess.
Utter, M. F. (1950). *J. biol. Chem.* **185**, 499.
Utter, M. F., Reiner, J. M. & Wood, H. G. (1945). *J. exp. Med.* **82**, 217.

Actions of Electrical Stimulation and of 2:4-Dinitrophenol on the Phosphates in Sections of Mammalian Brain *in vitro*

By H. McILWAIN and MARION B. R. GORE
Biochemical Laboratories, Institute of Psychiatry (British Post-Graduate Medical Federation, University of London), Maudsley Hospital, London, S.E. 5

(*Received* 17 *February* 1951)

The expectation that sections of brain *in vitro* might respond to electrical stimulation was based on the finding (McIlwain, Buchel & Cheshire, 1951) that resynthesis of labile phosphates could be induced in such preparations. When metabolic response to stimulation was found (McIlwain, 1951 and McIlwain, Anguiano & Cheshire, 1951) our working hypothesis was that the increase in respiration and glycolysis was secondary to breakdown of labile phosphates. The labile phosphates had presumably been depleted in supporting additional activity in the slices; their breakdown products stimulate both respiration and glycolysis (for references see McIlwain, 1950). The possible nature of the additional activity is discussed below.

An immediate test of such hypotheses was to examine the concentrations of inorganic and creatine phosphates in slices of brain stimulated electrically. Very little data are available on concentrations of phosphate fractions in surviving tissue slices during *in vitro* metabolism, but the value of such data in understanding the overall metabolism of nervous tissue has been shown by McIlwain, Buchel & Cheshire (1951) and Buchel & McIlwain (1950). In particular, we were also investigating in this laboratory the effects of substances such as 2:4-dinitrophenol which disturb the phosphorylation normally associated with respiration. We have therefore, in the present paper, compared the effects of electrical stimulation and of 2:4-dinitrophenol on phosphates, respiration and glycolysis.

EXPERIMENTAL

Guinea pig-brain cortex was used, and prepared as described by McIlwain (1951), where details of the salines are also given. The electrode vessels employed were type A^1 of Fig. 3 of that paper, with the tissue-holding electrodes *D* of Fig. 2, made of silver. Six vessels were commonly used together, of which up to four were stimulated. Each vessel held a slice of 100–120 mg., and two or three of these were combined for determination of phosphates as described by McIlwain, Buchel & Cheshire (1951). In previous determinations of phosphate after *in vitro* metabolism, dependable values were obtained so long as the slices were homogenized in trichloroacetic acid within about 30 sec. of their removal from oxygenated nutrient salines. To do this in the present experiments, the following procedure was adopted.

At the end of an experiment one stimulated vessel, with its manometer, was removed from the bath while its stimulating current was still maintained, the outside of the vessel quickly wiped dry around its joint with the manometer, and the manometer removed. The current was switched off, and the tissue-holding electrodes removed from the lead-in wires