similar to grey in its resynthesis of phosphocreatine *in vitro* when provided with oxygen and glucose; glutamate again was much less effective. Thus with white matter also, circumstances supporting phosphocreatine and respiratory response to pulses are correlated.

That cerebral white matter shows much higher respiratory and glycolytic rates than sciatic nerve is presumably related to the quantity of connective tissue in the peripheral nerve. Relationships between the metabolic activities of grey and of white matter in the brain, as exhibited in the present work or in that of Lowry *et al.* (1954), can largely be explained by regarding white matter as similar to grey, apart from 'dilution' by metabolically less-active lipids of the myelin. Scope remains, however, for many differences in the level of individual enzymes in grey and white matter (see, for example, Naidoo & Pratt, 1951, 1952); data relevant to their allocation to different cell types are discussed by Brierley & McIlwain (1957).

SUMMARY

1. Subcortical white matter of the guinea pig, rabbit and man was capable of respiration and glycolysis in ordinary glucose salines at one-half to two-thirds of the rates found in the cerebral cortex. Under these conditions the guinea-pig subcortical white matter resynthesized phosphocreatine to a level of about 0·9 μmole/g.

2. In maintaining respiration glucose was replaceable by pyruvate; with succinate and glutamate (but not with acetate and citrate) respiratory rates were greater than in the absence of added substrate, but decreased with time. Glutamate did not, however, support resynthesis of phosphocreatine.

3. Respiration, glycolysis and phosphocreatine formation maintained by glucose responded to application of electrical pulses to the tissue. Pyruvate also permitted response but other substrates examined were ineffective in this respect.

4. Certain of these characteristics were examined also in tissues from the medulla, thalamus, midbrain and sciatic nerve.

We are greatly indebted to the Board of Governors of the Bethlem Royal Hospital and the Maudsley Hospital for allocation of research funds in support of these studies; and to Mr Murray Falconer, Guy's–Maudsley Neurosurgical Unit, for specimens of human cerebral tissues.

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The Composition of Isolated Cerebral Tissue: Purines

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(*Received* 4 *January* 1957)

In previous studies (McIlwain, Thomas & Bell, 1956; Thomas, 1956) it was shown that the composition of isolated cerebral tissues differed appreciably from that *in vivo*, but that the composition *in vitro* could be in part restored by specific additions to fluids in which the tissue was incubated. An attempt has now been made to maintain higher levels of nucleotides in cerebral slices respiring in media fortified with precursors of nucleotides.

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EXPERIMENTAL

Tissues

Rapid fixation of brain. The procedure adopted was that described by Kratzing & Narayanaswami (1953), liquid nitrogen being used. About 500 mg. of frozen powdered brain was weighed in a tube containing 10 ml. of 10% (w/v) trichloroacetic acid and homogenized.

Preparation of tissue and medium for metabolism. Six slices each of about 100 mg. wet wt. were cut from the cerebral cortex of a guinea pig. The slices were suspended in glucose–phosphate medium made according to Rodnight &

McIlwain (1954) and respiration was measured manometrically.

Extraction of tissue. On completion of metabolic experiments slices were rinsed quickly in saline; two slices were taken together and homogenized in 3 ml. of 10% (w/v) trichloroacetic acid at −5°. After being centrifuged at −5° the supernatant (*A*) was decanted and kept cold.

Separation and estimation of nucleotides, nucleosides and free purines

Initial separation was based on the method of Kerr & Seraidarian (1945*a*). This gave three final fractions, each of which was chromatographed on paper, and after elution the individual purines were estimated by measurement at their absorption peaks.

Removal of nucleotides. The pH of the supernatant (*A*) was adjusted to 8·0 with 40% (w/v) NaOH, made slightly acid with a drop of 10% acetic acid and treated with 0·12 ml. of 8% (w/v) uranyl acetate. The nucleotide–uranium precipitate was separated by centrifuging, washed with 1 ml. of diluted acidified uranium reagent and the washings were combined with the supernatant (*B*).

Precipitation of free purines. At this stage it was found convenient to remove the uranium from the supernatant (*B*). This was done by adding 40% (w/v) NaOH to give a pH of 8·0, followed by a drop of 10% acetic acid. The uranium precipitate was centrifuged off, resuspended in 1 ml. of N-H_2SO_4 and reprecipitated by making alkaline, the washings being added to the supernatant. Free purines were separated from the nucleosides in the supernatant by adding 0·25 ml. of N-H_2SO_4 to give a pH of 2·0, followed by 0·2 ml. of M-$AgNO_3$. After standing in the cold for 30 min. the purine–silver complex was removed by centrifuging, washed twice with water and the first washings were combined with the supernatant (*C*).

Removal of nucleosides. By adjusting the supernatant (*C*) to pH 8·0 with alkali it was possible to precipitate the nucleoside–silver complex. After centrifuging, the precipitate was washed twice with water.

Extraction of purines from purine–silver complex. The complex was suspended in 3 ml. of N-HCl and heated in a boiling-water bath for 1 hr. After cooling and centrifuging the clear solution was decanted. The residue was re-extracted with 1 ml. of N-HCl and the washings were added to the supernatant.

Extraction of purines from nucleotide–uranium precipitate. This was achieved by boiling in 3 ml. of N-H_2SO_4 for 1 hr. After cooling, the uranium was removed by making alkaline as described above. Before applying chromatography it was necessary to purify the purines by reprecipitating with acid $AgNO_3$ as described above. The purine–silver complex was disrupted by boiling in N-HCl.

Extraction of purines from nucleoside–silver precipitate. After boiling in 3 ml. of N-H_2SO_4 for 1 hr. and cooling, the free purines were removed by acid $AgNO_3$ and extracted in boiling N-HCl.

Chromatographic separation and estimation of purines. The three final fractions containing free purines in N-HCl, derived from the nucleotide, nucleoside and free purine extracts of brain, were evaporated to dryness. The residues were taken up in small volumes of HCl, applied as spots to a sheet of Whatman no. 1 filter paper and run by the descending method in an aqueous solution of propan-2-ol and HCl made up according to the instructions of Wyatt (1951). For good resolution of a mixture of adenine, guanine and hypoxanthine it was necessary to run the solvent for 2–3 days. After the chromatograms had been dried the position of the purines was located under an ultraviolet lamp. The disks of paper were cut out and eluted with 4 ml. of N-HCl in a test tube. Suitable blanks for paper were obtained by cutting out spots of equal size from a free 'lane' immediately adjoining that carrying the purine. After standing overnight, the eluates were read at the absorption peaks on a Uvispek spectrophotometer. In N-HCl pure specimens of adenine, hypoxanthine and guanine absorbed maximally at wavelengths of 262, 250 and 245 mμ respectively. Standards were run and solutions of pure substances submitted to the above procedure gave recoveries of 96–100%.

RESULTS

Rapid fixation of guinea-pig brain gave a value of 2·95 μmoles of adenine nucleotides/g. of tissue (Table 1), a figure which agreed with that of Kratzing & Narayanaswami (1953), who used a different method of assay. The presence of guanine and hypoxanthine nucleotides was suggested by Kerr (1942) and in later work definitely established by Kerr & Seraidarian (1945*b*). The present study, with chromatographic methods, gave greater opportunity of detecting a variety of compounds, but the only nucleoside detected was adenosine at 0·3 μmole/g. of tissue. Of the free purines only hypoxanthine was found at 0·1 μmole/g. of tissue.

Table 1. *Results from guinea-pig brain handled in the frozen state throughout and from slices fixed immediately after cutting from guinea-pig cerebral cortex*

Results are expressed in μmole/g. of tissue ± standard deviations, with number of observations from different animals in parenthesis. Animals from which the frozen brains were obtained had been dropped intact into liquid nitrogen.

Preparation of tissue	Nucleotides (μmoles/g. of tissue)			Nucleosides (μmole/g. of tissue)			Free purine (μmole/g. of tissue)		
	Adenine	Hypoxanthine	Guanine	Adenine	Hypoxanthine	Guanine	Adenine	Hypoxanthine	Guanine
Frozen intact brain	2·95 ± 0·12 (3)	0·1 ± 0·01 (3)	0·65 ± 0·11 (3)	0·3 ± 0·05 (3)	0	0	0	0·1 ± 0·01 (3)	0
Slices fixed after cutting	1·64 ± 0·1 (6)	0·1 ± 0·05 (6)	0·38 ± 0·05 (6)	0·75 ± 0·2 (6)	0·20 ± 0·05 (6)	0	0	0·2 ± 0·01 (6)	0

The time taken before fixation of slices cut from cerebral cortex caused a marked reduction in the concentrations of nucleotides (Table 1). This loss was accompanied by an increase in adenosine, inosine and free hypoxanthine.

In further studies most attention was paid to nucleotides. When slices were incubated in oxygenated medium containing glucose, adenine and guanine nucleotides were maintained at levels of 0·89 and 0·17 μmole/g. of wet tissue respectively (Table 2). The value for adenine nucleotides was a little higher than that obtained by Kratzing & Narayanaswami (1953), a medium buffered with glycylglycine and an enzymic method of estimation being used. The difference between the *in vivo* and the *in vitro* values represents a considerable loss of nucleotides, and therefore attempts were made to increase their synthesis in slices by adding nucleotide precursors to the suspending medium at concentrations very much greater than the physiological ones (Table 2). In rabbit's blood no nucleotides were detected and the only free purine present was adenine, at 0·01 mM (A. McCoubrey, private communication). Of the materials used, the free purines adenine and hypoxanthine, at 1 mM concentrations, although found to be assimilated by slices from the medium to the extent of 0·4 μmole/g. of wet tissue, had little effect on the adenine nucleotide concentration, adenine producing a slight increase. Slices were also able to take up 0·4 μmole of guanine/g. of wet tissue from the medium, but the level of guanine nucleotide was hardly affected. With added nucleosides greater success was obtained. Adenosine made available to tissue at 1 mM concentration caused a raising of adenine nucleotide from 0·89 to 1·27 μmoles/g. of wet tissue. Inosine, although not so efficient as adenosine, maintained a level of 1·13 μmoles/g. wet wt. Slices were able also to increase their content of guanine nucleotide from 0·17 to 0·21 μmole/g. wet wt. when guanosine was present in the medium. About 0·4 μmole of these nucleosides/g. of wet tissue was incorporated into slices from the medium, illustrating a greater availability for resynthesis of nucleotides from added nucleosides.

A previous study (Thomas, 1956) has shown that slices can take up creatine from media when incubated under good metabolic conditions. This assimilation of creatine was associated with a marked accumulation of resynthesized energy-rich phosphate in the form of phosphocreatine. Under the same conditions added creatine alone had no effect upon the resynthesis of nucleotides (Table 2), but if it was present with adenosine and guanosine there was an increased resynthesis of nucleotides (Table 3). After 3 hr. incubation figures of 1·56 and 0·27 μmoles/g. of wet tissue were obtained for adenine and guanine nucleotides respectively.

Table 2. *Levels of adenine and guanine nucleotides in slices incubated in supplemented saline*

Slices were incubated at 37·5° for 100 min. in 3·5 ml. of oxygenated medium in manometric vessels with NaOH and paper in the centre well. The additional materials quoted were present in medium to which slices were added. Results are expressed in μmoles/g. of wet tissue, followed by standard deviations and the number of observations from different animals in parenthesis. With no additions to medium no nucleosides were detected in slices but hypoxanthine was at 0·1. The added purines and nucleosides were all assimilated by slices from medium at a concentration of about 0·4 μmole/g. of wet tissue.

Additions to medium (mM)	Nucleotides (μmoles/g. of wet tissue)	
	Adenine	Guanine
None	0·89±0·03 (6)	0·17±0·02 (6)
Adenine (0·85)	1·01±0·02 (3)	0·15±0·01 (3)
Hypoxanthine (0·8)	0·86±0·02 (3)	0·14±0·02 (3)
Guanine (0·75)	0·87±0·03 (3)	0·19±0·01 (3)
Adenosine (1)	1·27±0·01 (4)	0·18±0·01 (4)
Inosine (1)	1·13±0·02 (3)	0·18±0·01 (3)
Guanosine (0·75)	0·88±0·03 (4)	0·21±0·01 (4)
Creatine (1)	0·85±0·03 (3)	0·16±0·02 (3)

Table 3. *Effect of time of incubation on content of adenine and guanine nucleotides of slices in contact with a mixture of adenosine, guanosine and creatine*

Slices were incubated in oxygenated medium at 37·5° in vessels with NaOH and paper in the centre well, and removed at different times. A mixture of adenosine, guanosine and creatine each at a final concn. of 1 mM was added to the medium. Control experiments without additions were run for the same periods of time. Results are given in μmole/g. of wet tissue followed by the number of observations on different animals in parentheses. When two values are quoted they refer to different animals.

Incubation time (min.)	No addition to medium (μmoles/g. of wet tissue)		Addition to medium (μmoles/g. of wet tissue)	
	Adenine	Guanine	Adenine	Guanine
25	0·91, 0·90	0·18, 0·17	0·92±0·02 (3)	0·17±0·02 (3)
40	0·92, 0·91	0·18, 0·19	1·20±0·02 (3)	0·21±0·01 (3)
100	0·94, 0·93	0·18, 0·18	1·37±0·04 (3)	0·25±0·01 (3)
180	1·08, 1·06	0·19, 0·18	1·56±0·05 (3)	0·27±0·01 (3)

DISCUSSION

The rapid loss of energy-rich phosphates from the guinea-pig brain after death is not accompanied by a comparably large loss of total purines; some 80% of the original quantity remains in the tissue (see Kerr & Seraidarian, 1945*b*; McIlwain, 1955). However, during typical metabolic experiments with sliced tissue still further purine derivatives are lost, presumably to fluids in which the slices are incubated. As energy-rich phosphates are known to play an important part in cerebral tissues it is valuable to *in vitro* studies to attempt to maintain concentrations approaching those in the living animal. Incubation of cerebral slices in simple oxygenated glucose-containing medium permits appreciable resynthesis of phosphocreatine and adenosine triphosphate, but levels remain below those observed *in vivo* (McIlwain, 1951, 1955). With phosphocreatine, loss of the hydrolytic products to the medium largely accounts for this depletion in studies *in vitro*. When more creatine is made available to respiring slices there is increased resynthesis of phosphocreatine (Thomas, 1956). With nucleotides the picture has now proved to be similar. Although free purines themselves have little effect on resynthesis of nucleotides, when they are present in the form of adenosine and guanosine in the medium there is a higher content in the corresponding cerebral nucleotide. A combination of creatine, adenosine and guanosine added to medium produced the highest resynthesis of the energy-rich phosphates of creatine, adenosine and guanosine. The extreme rapidity of decomposition of these compounds is in direct contrast with their gradual formation by respiring slices. The same effect was obtained by Le Baron (1955) and McIlwain & Tresize (1956) in investigations on the resynthesis of glycogen, maximum glycogen being obtained after 3 hr. incubation.

These findings emphasize the presence and maintenance in cerebral tissues of simple guanine derivatives, which are attracting attention from other points of view (Sanadi, Gibson & Ayengar, 1954; Keller & Zamecnik, 1956).

SUMMARY

1. Levels of adenine, guanine and hypoxanthine nucleotides in the whole brains of guinea pigs immersed in liquid nitrogen were 2·95, 0·65 and 0·1 μmoles/g. of tissue respectively. Adenosine was at 0·3 and hypoxanthine at 0·1 μmole/g. of tissue.

2. Cortex slices fixed immediately gave 1·64, 0·38 and 0·1 μmoles/g. of tissue for adenine, guanine and hypoxanthine nucleotides respectively. Adenosine was at 0·75, inosine at 0·2 and hypoxanthine at 0·2 μmole/g. of tissue.

3. Slices incubated in oxygenated glucose-containing medium maintained adenine and guanine nucleotides at 0·89 and 0·17 μmole/g. of wet tissue respectively.

4. Additions of adenine, guanine, hypoxanthine and creatine at 1 mM to the medium had little effect on nucleotide content of incubated slices.

5. Slices respiring in medium containing 1 mM-adenosine raised adenine nucleotides to 1·27 and added guanosine increased guanine nucleotide to 0·21 μmole/g. of wet tissue.

6. In a mixture of adenosine, guanosine and creatine at 1 mM in medium, 1·56 and 0·27 μmoles of adenine and guanine nucleotides/g. of wet tissue were resynthesized in slices incubated for 3 hr.

I wish to thank Professor McIlwain for suggesting this investigation and for his continued encouragement and advice. My thanks are also due to Mr A. McCoubrey for helpful discussions on the method of estimation of purines.

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