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Incorporation of Radioactive Phosphate into Lipids and Pentosenucleic Acid of Cat Brain Slices. The Effect of Inorganic Ions

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It is well known that an increase in the concentration of potassium ions, or a decrease in the concentration of calcium ions, causes an increase in the oxygen consumption and aerobic glycolysis of brain slices. There is also a decrease in the anaerobic glycolysis. More recently, McIlwain (1952a) and Gore & McIlwain (1952) have shown that if the concentration of K^+ in the medium is increased or the concentration of Ca^{2+} is decreased, respiring slices of guinea pig brain are unable to maintain their concentration of phosphocreatine and other labile phosphorus compounds.

Since it has been shown previously that the incorporation of inorganic phosphorus labelled with ^{32}P into the pentosenucleic acid (DeLuca, Rossiter & Strickland, 1953), phospholipid (Strickland, 1954), and other protein-bound phosphorus compounds (Findlay, Strickland & Rossiter, 1954) of respiring brain slices is dependent upon oxidative phosphorylation mechanisms within the slice, it seemed of interest to determine the effect of increasing the

concentration of K^+ or decreasing the concentration of Ca^{2+} on the incorporation of inorganic ^{32}P into these phosphorus-containing compounds. Since the addition of NH_4^+ , L-glutamate, or L-glutamine, also causes a decrease in the concentration of phosphocreatine in respiring brain slices (McIlwain, 1951, 1952a; Gore & McIlwain, 1952), the effect of these substances was also investigated.

A preliminary account of some of these experiments has appeared (Rossiter & Findlay, 1954).

METHODS

Slices of cat brain were incubated in Warburg flasks containing Krebs-Ringer bicarbonate, glucose and $[^{32}P]$ -phosphate. The vessels were filled with a mixture of 95% O_2 and 5% CO_2 and incubated in a bath at 37.2° for 4 hr., with shaking to ensure proper oxygenation. When inorganic ions were added or omitted, the medium was kept at the same tonicity by adding or omitting the requisite quantity of NaCl. Inorganic constituents were added as the sodium salt. Details of the preparation of tissue and incubation have been described by DeLuca *et al.* (1953).

After the period of incubation, the tissue was homogenized and the acid-soluble P, containing the greater part of the radioactivity, was removed as described by DeLuca *et al.* (1953). The lipid P was extracted and purified as described by Strickland (1952, 1954). The nucleic acids were extracted from the protein residue by the method of Hammarsten (1947) and the PNA nucleotides were further purified by two magnesium treatments. For brain slices the mixed nucleotides so obtained have a specific activity within the range given by the individual nucleotides separated chromatographically (DeLuca *et al.* 1953). The protein residue contains the residue organic P (ROP) and the 'phosphoprotein' (PP). These fractions were separated as described by Findlay *et al.* (1954). The ROP represents the fraction referred to as 'inositide P' by Logan, Mannell & Rossiter (1952). The fraction is analogous to the 'concomitant P' described by Davidson & Smellie (1952) for liver tissue. Middleton (to be published) has shown that this fraction, like the concomitant P of liver tissue, is comprised of a number of metabolically active P-containing compounds. The incorporation of ^{32}P into the PP fraction of brain was studied *in vivo* by Strickland (1952). More recently, experiments *in vitro* have been reported by Engelhardt & Lissovskaja (1953).

The specific activity of the PP was determined by the method of Ernster, Zetterström & Lindberg (1950), as were the specific activities of the purified lipid P, PNA nucleotides and ROP, after ashing with perchloric acid. In this method both the concentration and the radioactivity of the inorganic P are determined on the same sample. The optical density of the blue colour complex was read with a Beckman Model B Spectrophotometer at 730 μm . and the radioactivity due to the ^{32}P in the sample was determined on the same coloured solution using an M-6 liquid counter (20th Century Electronics, London, S.E. 27). The counting rate was corrected for background and decay and the specific activities were calculated on the basis of standard count of 8.1×10^5 impulses/min./ml. incubating medium.

RESULTS

Potassium, calcium and ammonium. Table 1 shows that an increase in the concentration of K^+ from the usual 5.9 mM to 124 mM greatly depressed the incorporation of ^{32}P into the lipid P. In other experiments this effect was shown with concentrations of K^+ as low as 29 mM (see Table 9). The omission of Ca^{2+} from the medium caused a smaller reduction in the incorporation. The addition of NH_4^+ to give a final concentration of 29 mM caused a decrease of the same order as 124 mM K^+ . A further increase in the concentration of NH_4^+ to 118 mM caused a further decrease in the incorporation.

The effect of changing the concentrations of K^+ , Ca^{2+} or NH_4^+ on the incorporation of ^{32}P into the PNA was similar to that observed for the lipid P (Table 2). An increase in the concentration of K^+ , the addition of NH_4^+ , or the omission of Ca^{2+} , caused a decrease in the incorporation.

Findlay *et al.* (1954) showed that for brain slices respiring in a medium containing [^{32}P]phosphate and glucose, considerable quantities of the ^{32}P were incorporated into the ROP and PP fractions. Table 3 shows the result of an experiment in which the specific activities of these fractions were determined. It can be seen that the specific activities of both the ROP and the PP were considerably greater than the values reported in Tables 1 and 2 for the lipid P and PNA. An increase in the concentration of K^+ , or the addition of NH_4^+ to the medium, had a similar inhibitory effect on the incorporation of ^{32}P into the ROP and the PP fractions.

Table 1. *Effect of changing the concentration of K^+ , Ca^{2+} or NH_4^+ on the specific activity of the lipid P of slices of cat brain*

Slices incubated for 4 hr. at 37.2° in medium containing glucose (0.011 M) and [^{32}P]phosphate. Counts/min./ μg . P.

K^+ (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Expt. 1	Expt. 2	Expt. 3	Expt. 4
5.9	3.6	0	15.9	20.4	13.4	12.7
124	3.6	0	7.1	5.5	7.2	7.7
5.9	0	0	11.5	15.2	12.4	12.5
5.9	3.6	29	5.2	4.9	5.1	5.8
5.9	3.6	118	2.6	2.0	4.5	5.7

Table 2. *Effect of changing the concentration of K^+ , Ca^{2+} or NH_4^+ on the specific activity of the PNA of slices of cat brain*

Slices incubated for 4 hr. at 37.2° in medium containing glucose (0.011 M) and [^{32}P]phosphate. Counts/min./ μg . P.

K^+ (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Expt. 1	Expt. 2	Expt. 3	Expt. 4
5.9	3.6	0	27.2	26.6	21.7	25.3
124	3.6	0	7.8	10.3	16.6	—
5.9	0	0	19.4	—	15.3	21.6
5.9	3.6	29	8.8	11.8	15.1	14.4
5.9	3.6	118	1.9	1.9	—	4.0

Table 3. *Effect of changing the concentration of K⁺ or NH₄⁺ on the specific activity of the ROP and PP fractions of slices of cat brain*Slices incubated for 4 hr. at 37.2° in medium containing glucose (0.011 M) and [³²P]phosphate. Counts/min./μg. P.

K ⁺ (mM)	Ca ²⁺ (mM)	NH ₄ ⁺ (mM)	Residue organic P (ROP)	'Phosphoprotein' (PP)
5.9	3.6	0	432	1390
124	3.6	0	95	680
5.9	3.6	29	107	570
5.9	3.6	118	25	210

Table 4. *Effect of the addition of L-glutamate on the specific activity of the lipid P, PNA, ROP and PP of slices of cat brain*Slices incubated for 4 hr. at 37.2° in Krebs-Ringer bicarbonate containing [³²P]phosphate. Counts/min./μg. P. The solution of glutamate was prepared by titration of L-glutamic acid with NaOH to pH 7.4.

	Expt. 1		Expt. 2		Expt. 3			
	Lipid P	ROP	Lipid P	ROP	Lipid P	PNA	ROP	PP
Glucose (0.011 M)	6.4	66	11.8	150	22.7	26.0	305	1360
Glutamate (0.01 M)	3.0	37	6.6	59	9.4	—	167	490
Glucose (0.011 M) and glutamate (0.01 M)	3.9	33	6.8	72	14.4	20.1	156	730
No substrate	—	—	—	—	7.7	—	103	670

Table 5. *Effect of the addition of L-glutamine on the specific activity of the lipid P, PNA, ROP and PP of slices of cat brain*Slices incubated for 4 hr. at 37.2° in Krebs-Ringer bicarbonate containing [³²P]phosphate. Counts/min./μg. P.

	Expt. 1				Expt. 2			
	Lipid P	PNA	ROP	PP	Lipid P	PNA	ROP	PP
Glucose (0.011 M)	29.5	42.4	440	1260	12.1	26.1	162	540
Glucose (0.011 M) and glutamine (0.01 M)	17.9	30.6	366	1040	8.7	16.3	93	400

Table 6. *Effect of adding glucose either before or after the slices on the specific activity of the lipid P and PNA of slices of cat brain*Slices incubated for 4 hr. at 37.2° in Krebs-Ringer bicarbonate containing [³²P]phosphate. Counts/min./μg. P.

	Expt. 1		Expt. 2	
	Lipid P	PNA	Lipid P	PNA
No substrate	15.0	23.5	3.3	16.5
Glucose (0.011 M) added before slices	21.3	31.4	11.3	27.5
Slices added before glucose (0.011 M)	16.9	27.0	6.7	20.8

Glutamate and glutamine. Findlay, Rossiter & Strickland (1953) showed that L-glutamate failed to support the incorporation of ³²P into the PNA of cat brain slices, and Strickland (1954) reported a similar finding for lipid P. Since McIlwain (1951, 1952*a*) showed that in the presence of L-glutamate or L-glutamine, respiring slices of guinea pig brain failed to maintain their concentration of phosphocreatine, even in the presence of glucose, the effect of glutamate and glutamine on the ³²P incorporations was studied further.

Table 4 shows that if the glucose of the incubating medium was replaced by L-glutamate, the incorporation of ³²P into the lipid P, PNA, ROP and PP was greatly decreased. Expt. 3 shows that in the

presence of glutamate, the incorporation did not differ greatly from that found in the absence of added substrate. The table also shows that the incorporation was very little greater if glucose was added together with the glutamate. The presence of the glutamate inhibited the extra incorporation usually brought about by the addition of glucose.

From Table 5 it can be seen that the addition of L-glutamine had an inhibitory effect on each fraction similar to that produced by L-glutamic acid.

During the experiments with glutamate it was found that, if the slices of brain tissue were allowed to remain at room temperature for a short period in the absence of glucose, the incorporation of ³²P into both the lipid P and the PNA was considerably

reduced. Table 6 shows the results of two experiments in which the glucose was added to the medium either before the slices, as was customary, or after the addition of the slices, but before the vessels were filled with $\text{O}_2\text{-CO}_2$ gas mixture. It can be seen that for both lipid P and PNA the slices that were exposed to the glucose-free medium for some minutes did not take up the ^{32}P as well as slices not so exposed.

These results recall the earlier report of Dickens & Greville (1933) that a short period of anaerobiosis in the absence of glucose caused a great reduction in the anaerobic glycolysis of brain slices. Subsequently, Macfarlane & Weil-Malherbe (1941) reported that a similar period of anaerobiosis without added substrate caused a reduction in the concentration of easily hydrolysable P, probably mostly adenosine triphosphate (ATP).

DISCUSSION

Potassium. Ashford & Dixon (1935) and Dickens & Greville (1935) found that an increase in the concentration of K^+ in the medium caused an increase in both respiration and aerobic glycolysis, and a decrease in the anaerobic glycolysis, of brain slices from the rabbit and rat, respectively. These findings were confirmed by a number of workers (Canzanelli, Rogers & Rapport, 1942; Lipsett & Crescitelli, 1950). McIlwain (1952a) and Gore & McIlwain (1952), in addition to confirming the increase in respiration and aerobic glycolysis, showed that in the presence of excess K^+ , brain slices were unable to maintain their concentration of phosphocreatine.

The lower concentration of labile P in slices respiring in a medium containing excess K^+ raises the obvious question of whether the K^+ might stimulate phosphatases responsible for the hydrolysis of labile phosphorus compounds. Gore (1951) found that the adenosinetriphosphatase of brain homogenates was stimulated by an increase in the concentration of K^+ . However, results obtained with homogenates of brain tissue are not directly comparable with those obtained with slices, for in both homogenates and extracts an increase in the concentration of K^+ or a decrease in the concentration of Na^+ increases, rather than decreases, anaerobic glycolysis (Racker & Krinsky, 1945; Utter, 1950; Muntz & Hurwitz, 1951a). Muntz & Hurwitz (1951a) and McIlwain (1952a) have summarized the evidence for the view that the effect of increasing the concentration of K^+ is not directly on the phosphatases that break down labile phosphorus compounds.

Lipsett & Crescitelli (1950) suggested that K^+ might increase the oxygen consumption of brain slices by stimulating the K^+ -sensitive ATP-phosphopyruvate transphosphorylase reaction. If this

were so, the addition of K^+ should increase, rather than decrease, the concentration of labile P and the incorporation of ^{32}P into lipid P and PNA. It is not immediately apparent why an increase in the concentration of K^+ in the medium should cause a depletion of labile P. McIlwain (1952a) suggested that in brain slices much of the metabolism is directed towards the maintenance of a high gradient of K^+ between the inside and the outside of the nerve cells. To maintain the same gradient in the presence of a high concentration of K^+ in the medium, a greatly increased expenditure of energy would be necessary. The respiring brain slice can concentrate K^+ , presumably by processes similar to those described by Turner, Eggleston & Krebs (1950). This reaction is energy-consuming and could conceivably decrease the quantity of labile P available for other reactions.

Calcium. Dickens & Greville (1935), Canzanelli *et al.* (1942) and Krebs (1950) showed that the oxygen consumption of brain slices was increased when Ca^{2+} was omitted from the medium. McIlwain (1952a) and Gore & McIlwain (1952) showed that the omission of Ca^{2+} , like an increase in the concentration of K^+ , caused an increase in both oxygen consumption and aerobic glycolysis and a decrease in the ability of the slices to maintain their level of phosphocreatine.

That these antagonistic effects of K^+ and Ca^{2+} on brain slices may have a counterpart *in vivo* is suggested by the observation of Dubner & Gerard (1939) that K^+ enhanced and Ca^{2+} diminished the spontaneous rhythm of the geniculate body of the cat, and by the finding of Libet & Gerard (1939) that K^+ and Ca^{2+} had opposite effects on the olfactory bulb of the isolated frog brain. Another link between the effect of inorganic ions on the metabolism of brain slices and physiological function is provided by the interesting report of Gore & McIlwain (1952) on the response of respiring brain slices to electric stimuli applied *in vitro*. These workers found that the addition of NH_4^+ , the omission of Ca^{2+} , or an increase in the concentration of K^+ , abolished the metabolic response to stimulation *in vitro*.

Ammonium. Weil-Malherbe (1938) reported that the addition of NH_4^+ to the medium containing brain slices, like an increase in the concentration of K^+ , caused an increase in the oxygen consumption and aerobic glycolysis, and a decrease in the anaerobic glycolysis. Gore & McIlwain (1952) confirmed these findings and showed that the addition of NH_4^+ , again like an increase in the concentration of K^+ , impaired the ability of respiring slices to maintain their level of phosphocreatine. Muntz & Hurwitz (1951a) showed that in brain extracts, as opposed to brain slices, the addition of NH_4^+ increased aerobic glycolysis, but had little effect on the breakdown of ATP. A number of

Table 7. *Effect of the addition of L-glutamine on the specific activity of the lipid P, PNA, ROP and PP of slices of cat brain incubated under different conditions*Slices incubated for 4 hr. at 37.2° in medium containing glucose (0.011 M) and [³²P]phosphate. Counts/min./μg. P.

	Lipid P	PNA	ROP	PP
Expt. 1				
No glutamine, no NH ₄ ⁺	29.5	42.4	440	1260
No glutamine, NH ₄ ⁺ (29 mM)	8.6	15.3	134	580
Glutamine (0.01 M), NH ₄ ⁺ (29 mM)	5.9	11.0	85	600
Expt. 2				
No glutamine, normal K ⁺	12.1	26.1	162	540
No glutamine, K ⁺ (29 mM)	8.2	15.3	109	440
Glutamine (0.01 M), K ⁺ (29 mM)	7.1	17.4	87	390

glycolytic enzymes that are known to be K⁺-sensitive, viz. hexokinase, phosphohexokinase and ATP-phosphopyruvate transphosphorylase, are sensitive to NH₄⁺ in the absence of K⁺ (Muntz & Hurwitz, 1951*b*).

Braganca, Faulkner & Quastel (1953), in their attempt to explain the inhibitory effect of NH₄⁺ on the aerobic formation of acetylcholine by brain slices, suggested that the high concentration of NH₄⁺ might increase the formation of glutamine from glutamic acid, a reaction demonstrated in brain slices by Krebs (1935*b*) and in cell-free systems by Elliott (1951). The reaction is energy-consuming and in cell-free systems it has been shown to require an adequate supply of ATP. Such a reaction could conceivably decrease the quantity of ATP available for synthetic reactions, such as the formation of acetylcholine and the incorporation of ³²P into lipid P, PNA and other P-containing fractions. However, in our experiments neither ATP nor excess glutamine was found to reverse the inhibitory effect of NH₄⁺. Table 7 shows the results of two experiments in which glutamine was added. In most instances the low specific activity brought about by the addition of NH₄⁺, or the increase in the concentration of K⁺, was decreased still further by the addition of glutamine, a result that is not surprising when the inhibitory effect of glutamine, demonstrated in Table 6, is taken into consideration.

The inhibitory effect of NH₄⁺ on ³²P incorporation is of some interest in relation to the finding that the injection of NH₄⁺ can produce convulsions in experimental animals (Sapirstein, 1943; Torda, 1953). It is also known that there is an increase in the concentration of NH₄⁺ in the brain after convulsions produced by the administration of convulsant drugs (Richter & Dawson, 1948; Torda, 1953), fluoroacetate (Benitez, Pscheidt & Stone, 1954), or after electrically induced convulsions (Richter & Dawson, 1948).

Glutamic acid and glutamine. Like the addition of NH₄⁺ or excess K⁺ to the medium, the addition of L-glutamate or L-glutamine causes an increase in aerobic glycolysis and a decrease in the anaerobic

glycolysis of brain slices (Weil-Malherbe, 1938). Glutamate and glutamine decreased the ability of guinea pig brain slices to maintain their level of phosphocreatine (McIlwain, 1951, 1952*a*). It is not surprising, therefore, that glutamate was found to inhibit the incorporation of ³²P into the P-containing compounds. The preliminary report of Engelhardt & Lissovskaia (1953) also demonstrated that glutamate inhibits the incorporation of ³²P into the PP of brain slices.

There is some evidence that L-glutamate is not an energy-yielding substrate for brain tissue (see Weil-Malherbe, 1950, 1952). This would explain the failure of glutamate to support ³²P incorporation, but it does not explain the inhibitory effect of glutamate when it is added in the presence of an adequate concentration of glucose. Possible explanations are:

(i) It is known that L-glutamate undergoes oxidative deamination in brain slices to form α-oxoglutarate and ammonia (Krebs, 1935*a*; Weil-Malherbe, 1936). The presence of NH₄⁺ has already been shown to inhibit ³²P incorporation. However, Krebs (1935*b*) and Weil-Malherbe (1936) have shown that, under the conditions attaining in brain slices, no ammonia accumulates because it reacts with more glutamate to form glutamine. As already seen, this is an energy-requiring reaction that could deplete the slices of their labile P.

(ii) Ternier *et al.* (1950) have shown that, in the presence of oxidizable substrate and L-glutamate, the outward diffusion of K⁺ from brain slices is reversed. Stern, Eggleston, Hems & Krebs (1949) showed that under these conditions glutamate is taken up by the slice. Both the K⁺ and the glutamate enter the brain cells against a concentration gradient. Such an energy-requiring process would be expected to deplete the tissue of labile P. If this hypothesis were true, the addition of glutamate should have an effect on the energy requirements of the slice similar to that produced by an increase in the concentration of K⁺. Evidence for this is the finding of Lipsett & Crescitelli (1950) that the stimulatory effect of K⁺ on the oxygen consumption

Table 8. *Concentration and specific activity of inorganic P of slices and medium before and after incubation under different conditions*Cat brain slices incubated for 4 hr. at 37.2° in medium containing glucose (0.011 M) and [^{32}P]phosphate.

	Medium		Slice	
	Concn. ($\mu\text{g. P/ml.}$)	Specific activity (counts/min./ $\mu\text{g. P}$)	Concn. ($\mu\text{g. P/g.}$)	Specific activity (counts/min./ $\mu\text{g. P}$)
Expt. 1				
Before incubation	104	6 620	241	466
After incubation				
No glutamine, normal K^+	113	4 960	336	3 040
Glutamine (0.01 M), normal K^+	116	4 880	301	2 700
No glutamine, K^+ (29 mM)	114	4 660	285	3 180
Expt. 2				
Before incubation	61	12 100	199	749
After incubation				
No NH_4^+	64	8 000	201	5 260
NH_4^+ (29 mM)	81	7 350	160	4 950

of brain slices was not observed when the medium contained both glucose and glutamate.

The ineffectiveness of glutamic acid as an energy-yielding substrate *in vivo* for experimental animals may be inferred from the report of Waelsch (1951) that the addition of glutamic acid to perfused cat brain caused a decrease in respiration. Maddock, Hawkins & Holmes (1939) found that glutamate, unlike glucose, was unable to abolish the changes in the electroencephalogram produced by the progressive hypoglycaemia of hepatectomized experimental animals. McIlwain (1951) showed that the addition of glutamate to respiring slices of guinea pig brain abolished the metabolic response to stimulation *in vitro*. However, the species studied is of some importance, for McIlwain (1953) reported that slices of human brain respiring with glutamate as substrate responded to *in vitro* stimulation. With the human tissue, glutamate did not abolish the usual response observed in the presence of glucose.

General. The possibility was considered that under the conditions where the ^{32}P incorporation was inhibited, non-labelled inorganic P might appear either in the slice or in the medium as a result of hydrolysis of P-containing compounds during the period of incubation. The effect of this would be to decrease the specific activity of the inorganic P of the medium and to increase the pool of inorganic P within the slice. McIlwain (1951; 1952a) and Gore & McIlwain (1952) reported that changes in the inorganic constituents of the medium, or the addition of L-glutamate, caused an increase in the concentration of inorganic P in the slice and McIlwain, Buchel & Cheshire (1951) showed that during the incubation of brain slices there was an increase in the concentration of inorganic P in the medium.

Table 8 shows the results of two experiments in which the concentration and the specific activity of the inorganic P in both the medium and the slice

were determined before and after the 4 hr. incubation period. In each experiment there was a slight increase in the concentration of the inorganic P of the medium during the incubation period. The increase was slightly greater under conditions where the ^{32}P incorporation was inhibited. There was also an increase in the concentration of inorganic P in the slice during the incubation period. This increase tended to be less under the conditions where the ^{32}P incorporation was inhibited. These experiments are not directly comparable with those of McIlwain *et al.* (1951) and McIlwain (1952a), in that the incubation period was prolonged for 4 hr.

From Table 8 it can be seen that during the incubation there was, as to be expected, a decrease in the specific activity of the inorganic P of the medium and an increase in the specific activity of the inorganic P of the slice. The table also shows that under conditions where the ^{32}P incorporation was inhibited, there were only slight changes in the specific activity of the inorganic P of either the medium or the slice. This is well illustrated in Table 9, which shows the specific activities of the lipid P, PNA, ROP and PP for the two experiments reported in Table 8. Not only was the specific activity of each fraction decreased under conditions such as an increase in the concentration of K^+ , or the presence of glutamine or NH_4^+ , but the specific activity relative to that of the inorganic P of the slice was also decreased. These figures indicate that the inhibition of ^{32}P incorporation cannot be explained merely in terms of changes in the specific activity of inorganic P, either in the medium or in the slice.

The relative specific activity figures reported in Table 9 do not represent the turnover of the respective P-containing fractions, for part of the inorganic P of the slice is extracellular and part is intracellular. At present there is no satisfactory way of determining the specific activity of the intracellular portion of the inorganic P.

Table 9. *Specific activity and relative specific activity of lipid P, PNA, ROP and PP of slices of cat brain*Slices incubated for 4 hr. at 37.2° in medium containing glucose (0.011 M) and [³²P]phosphate.

	Specific activity (counts/min./μg. P)				Specific activity relative to specific activity of inorganic P of slice × 100			
	Lipid P	PNA	ROP	PP	Lipid P	PNA	ROP	PP
Expt. 1								
No glutamine, normal K ⁺	12.1	26.1	162	540	0.40	0.86	5.3	17.8
Glutamine (0.01 M), normal K ⁺	8.8	16.3	93	400	0.33	0.60	3.4	14.9
No glutamine, K ⁺ (29 mM)	8.2	15.3	109	440	0.26	0.48	3.4	13.8
Expt. 2								
No NH ₄ ⁺	27.3	—	—	—	0.52	—	—	—
NH ₄ ⁺ (29 mM)	8.2	—	—	—	0.17	—	—	—

In previous papers attention has been drawn to the similarity that exists between the conditions that depress the incorporation of ³²P into the lipid P and PNA of cat brain slices and the conditions, studied by McIlwain and his colleagues, that bring about a decrease in the concentration of creatine phosphate, ATP, and possibly other labile phosphate in respiring slices of guinea pig brain (see McIlwain *et al.* 1951; McIlwain, 1952*a, b*; Kratzing & Narayanaswami, 1953). These conditions include the absence of oxygen or glucose, replacing the glucose by L-glutamate or succinate, and the presence of a large range of metabolic inhibitors, including 2:4-dinitrophenol (Findlay *et al.* 1953; Strickland, 1954). To these may now be added the addition of L-glutamate or L-glutamine to the medium, the removal of Ca²⁺, the addition of NH₄⁺, and an increase in the concentration of K⁺.

SUMMARY

1. An increase in the concentration of K⁺, omission of Ca²⁺, or the addition of NH₄⁺ to the incubating medium caused a decrease in the incorporation of [³²P]phosphate into the lipid P, pentosenucleic acid (PNA), residue organic P (ROP) and 'phosphoprotein' P (PP) of slices of cat brain respiring in a Krebs-Ringer bicarbonate buffer containing glucose and inorganic phosphate labelled with ³²P.

2. The addition of L-glutamate or L-glutamine to the medium, either in the presence or the absence of glucose, caused a decrease in the incorporation of ³²P into the lipid P, ROP and PP.

3. The inhibition of ³²P incorporation was not the result of changes in the specific activity of inorganic P, either in the medium or in the slice.

4. The similarity between the conditions that adversely affect the incorporation of ³²P into the P-containing fractions of brain slices and those that cause a failure of brain slices to maintain their concentration of phosphocreatine is commented upon.

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Chemical Studies of Peripheral Nerve during Wallerian Degeneration

6. INCORPORATION OF RADIOACTIVE PHOSPHATE INTO PENTOSENUCLEIC ACID AND PHOSPHOLIPIN *IN VITRO*

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In previous papers from this laboratory the changes that occur in the concentration of lipids (Johnson, McNabb & Rossiter, 1949; Burt, McNabb & Rossiter, 1950), nucleic acids (Logan, Mannell & Rossiter, 1952b), and certain enzymes (Hollinger, Rossiter & Upmalis, 1952; Hollinger & Rossiter, 1952) have been described for peripheral nerves degenerating after nerve section or regenerating after nerve crush. After nerve section there is a decrease in the concentration of the principal lipid components of the myelin sheath of the nerve fibre (myelin lipids) and a decrease in the concentration of the residue organic phosphorus (ROP), referred to as 'inositide P' by Logan, Mannell & Rossiter (1952a). There is also a smaller decrease in the concentration of 'phosphoprotein' phosphorus (PP) and an increase in the concentration of both deoxypentose nucleic acid (DNA) and pentose nucleic acid (PNA), associated with the cellular proliferation and the infiltration of macrophages that accompany the degeneration. If the nerve subsequently regenerates (after crush instead of section), the concentrations of myelin lipids, ROP and PP gradually return to normal and the concentrations of DNA and PNA decrease.

Since it has been shown that inorganic phosphate labelled with ^{32}P is incorporated into both the phospholipin (Fries, Schachner & Chaikoff, 1942; Schachner, Fries & Chaikoff, 1942; Strickland, 1954) and the PNA (DeLuca, Rossiter & Strickland, 1953; Findlay, Rossiter & Strickland, 1953) of brain

slices respiring in a Krebs-Ringer bicarbonate medium containing glucose, it was decided to investigate the possible incorporation of ^{32}P into the lipids and PNA of normal, degenerating and regenerating peripheral nerve under similar conditions. In addition, observations were made on the incorporation of ^{32}P into the ROP and PP of normal, degenerating and regenerating nerve (see Strickland, 1952; Findlay, Strickland & Rossiter, 1954).

A preliminary report of some of these experiments has already appeared (Magee & Rossiter, 1954).

METHODS

The right sciatic nerve of a number of cats was either sectioned or crushed at the level of the greater trochanter of the femur. Details of the operations have been described previously (Johnson *et al.* 1949; Burt *et al.* 1950). No attempt was made to control the age, sex, or weight of the animals. After 8, 16, 32 or 96 days the animals were killed, and the segment of the nerve distal to the site of the operation was removed. At the same time a similar length of left sciatic nerve was removed to serve as a control. Each nerve was cleaned of adherent fatty and connective tissue, the epineurium was stripped off and the nerve was rapidly weighed. It was then cut into small lengths (about 1 mm.) and transferred to a Warburg flask containing Krebs-Ringer bicarbonate, glucose and [^{32}P]phosphate. The vessel was filled with a mixture of 95% O_2 and 5% CO_2 and incubated in a bath at 37.2° for 4 hr., with shaking to ensure proper oxygenation. Details of the incubation have been described by DeLuca *et al.* (1953).