Purine Ribonucleotide Biosynthesis, Interconversion and Catabolism in Mouse Brain *in vitro*

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The relative rates of the synthetic, interconversion and catabolic reactions of purine metabolism in chopped mouse cerebrum were studied. The rates of incorporation of [^{14}C]adenine and [^{14}C]hypoxanthine into purine ribonucleotides were much less than the potential activities of adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase, and the rates of incorporation were stimulated by the addition of guanosine to the incubation mixture. The availability of ribose phosphates may be a limiting factor for the formation of ribonucleotides from purine bases. The rate of incorporation of [^{14}C]adenosine into purine ribonucleotides was at least seven- to eight-fold higher than that of adenine. The radioactivity in adenine ribonucleotides synthesized from adenine and hypoxanthine was about 100- and ten-fold respectively higher than that in the radioactive guanine ribonucleotides. The conversion of inosinate into guanine ribonucleotides was probably limited by the amount of inosinate available, and the conversion of adenine ribonucleotides into guanine ribonucleotides was probably limited by the activity of adenylylase deaminase. The rate of catabolism of [^{14}C]adenosine was low in comparison with its rate of utilization for ribonucleotide synthesis. A fraction of the [^{14}C]hypoxanthine was catabolized to xanthine and urate. [^{14}C]Guanine was completely converted into xanthine, mostly by the guanine deaminase that was released during incubation of chopped mouse cerebrum.

Information about many of the basic features of purine ribonucleotide metabolism and its regulation in brain is rather scarce. Evidence that purine ribonucleotides can be synthesized from preformed purines and from non-purine precursors in brain *in vivo* has come from observations that [^{14}C]glycine (Henderson & LePage, 1959), [^{14}C]adenine (Bennett *et al.*, 1960) and [^{14}C]guanine (Adams, 1965) are incorporated into brain nucleic acids after injection of these compounds into mice. In more detailed studies of purine metabolism in rat brain *in vivo*, Held & Wells (1969) measured the specific radioactivity of adenine and guanine nucleotides after intracerebral injections of [^{14}C]glycine, [^3H]adenine and [^3H]inosine. Azaserine markedly inhibited synthesis *de novo* of purine nucleotides soon after injection into the cerebral cortex of rats (Held *et al.*, 1969).

In brain slices [^{14}C]formate is incorporated into nucleic acids (Mannell & Rossiter, 1955), [^{14}C]glycine into phosphoribosylformylglycine amide (Howard *et al.*, 1970) and [^{14}C]adenine and [^{14}C]adenosine into adenine nucleotides (Forn & Krishna, 1971; Sharma & Singh, 1970; Shimizu & Daly, 1970; Shimizu *et al.*, 1969, 1970). Santos *et al.* (1968) measured the activities of a number of enzymes of

purine metabolism in extracts of rat cerebral cortex, and also determined the ability of cortical slices to take up a variety of purine ribonucleotide precursors and convert these into nucleotides and nucleic acids. Although it is clear that brain utilizes purine bases and non-purine precursors for purine ribonucleotide synthesis, little is known about the operation of the pathways of purine ribonucleotide metabolism as an integrated and regulated whole. We now report studies of the relative rates of the synthetic, interconversion and catabolic reactions of purine metabolism in mouse cerebrum *in vitro*, and of factors that regulate them.

Materials and Methods

Chemicals

[8- ^{14}C]Adenine (52.6 mCi/mmol), [8- ^{14}C]hypoxanthine (50 mCi/mmol), [8- ^{14}C]guanine (40 mCi/mmol), [8- ^{14}C]adenosine (47.2 mCi/mmol), [8- ^{14}C]inosine (32 mCi/mmol) and [1,2- ^{14}C]glycine (104 mCi/mmol) were obtained from Schwarz Bio-Research (Orangeburg, N.Y., U.S.A.). The purity of these radioactive compounds was established by the use of the t.l.c. system described below. Adenine and hypoxanthine contained no contaminants, adenosine contained 3% of inosine+hypoxanthine, inosine

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contained 6% of hypoxanthine, guanine contained 2.4% of xanthine, and glycine contained 7% of materials that chromatographed with the nucleotides. Non-radioactive purine bases, ribonucleosides and ribonucleotides were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). *N*-Hydroxyadenosine, 4'-thioadenosine, 6-methoxypurine ribonucleoside and 9-(2'-hydroxy-3'-hydroxymethyltetrahydrofuryl)-adenine were obtained from the Cancer Chemotherapy National Service Center (Bethesda, Md., U.S.A.).

Brain tissue

Adult Ha/ICR Swiss male mice were killed by cervical dislocation and decapitated. The brains were removed and rinsed briefly in 0.154M-NaCl. Slices (2mm thick) were prepared from cerebrum. In most cases two slices were then chopped in two directions by a McIlwain tissue chopper (Brinkmann Instruments Ltd., Toronto, Ont., Canada) so that prisms 0.3mm×0.3mm×2mm were obtained (McIlwain & Buddle, 1953). Cerebral slices (0.5mm thick) were also prepared with the McIlwain tissue chopper.

Incubation

Portions (50–100mg) of cerebral slices or chopped slices were each transferred to a 10ml Erlenmeyer flask containing 1ml of Krebs–Ringer medium (110mM-NaCl, 4.9mM-KCl, 1.2mM-MgSO₄, 25mM-sodium phosphate buffer, pH7.4, and 5.5mM-glucose), or Fischer's medium (Fischer & Sartorelli, 1964) containing 25mM-sodium phosphate buffer, pH7.4, and no bicarbonate. Tissues were dispersed by vigorous shaking and incubated in a shaking water bath for 30min at 37°C with 100% O₂ in the gas phase. The medium was then removed by aspiration and the tissues were washed twice with 5ml of pre-warmed medium and then resuspended in the same medium. Radioactive precursors were then added so that the final volume was 1ml.

To terminate the incubations, the entire contents of the flasks were each transferred to a tube containing 0.05ml of ice-cold 4.2M-HClO₄, homogenized and chilled, and the extracts were each immediately neutralized with 0.05ml of 4.42M-KOH. When chopped tissues were used, 0.1ml portions were removed at various intervals and each was denatured with 5μl of 4.2M-HClO₄ and transferred to a tube containing 5μl of 4.42M-KOH. In some experiments 0.1ml portions of the tissue suspensions were incubated (with 100% O₂ in the gas phase) with radioactive precursors in plastic tubes (1.0cm×7.5cm) instead of Erlenmeyer flasks. At the end of the incubation 5μl of 4.2M-HClO₄ was added to each tube and the contents were homogenized and neutralized as before. Portions (0.1ml) of tissue suspensions were transferred by an Eppendorf pipette (Brinkmann

Instruments, New York, N.Y., U.S.A.) with the tip cut off to provide a wider bore. On ten consecutive samples the standard deviation of the mean dry weight of tissue was ±14%. Results presented are those obtained with chopped tissues unless otherwise specified.

Some enzymes involved in purine metabolism were released into both types of incubation media during incubation of chopped cerebrum. Thus after 30min of incubation the medium contained purine nucleoside phosphorylase (purine nucleoside-orthophosphate ribosyltransferase, EC 2.4.2.1) and guanine deaminase (guanine aminohydrolase, EC 3.5.4.3) activities. By washing the tissues twice with 5ml of Fischer's medium, 96% of these activities could be removed. However, during further incubation in fresh medium purine nucleoside phosphorylase, guanine deaminase and adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) activities were released into the medium, so that after 45min the medium alone could convert 45% of added inosine (initial concentration 16μM) into hypoxanthine, 96% of added guanine (initial concentration 100μM) into xanthine, and 14% of added adenosine (initial concentration 10.6μM) into inosine+hypoxanthine, within 30min. Under these conditions (medium alone from 45min preincubation), however, hypoxanthine was not converted into inosine, xanthine was not oxidized, and the purine bases were not converted into nucleotides.

Chromatography

The neutralized tissue supernatant-fluid extracts were applied in 10μl portions to Baker–Flex polyethyleneimine–cellulose thin layers on Mylar sheets (Canadian Laboratory Supplies, Edmonton, Alberta, Canada) for separation of purine ribonucleotides, or to Eastman–Kodak unsubstituted cellulose thin layers on Mylar sheets (Fisher Scientific, Edmonton, Alberta, Canada) for separation of purine bases and ribonucleosides (Crabtree & Henderson, 1971). For some studies purine ribonucleotides, xanthine, glycine, inosine and hypoxanthine were separated on polyethyleneimine–cellulose thin-layer sheets by development in 70% (v/v) propan-1-ol. The respective *R_F* values were 0, 0.15, 0.26, 0.56 and 0.66.

The results represent the radioactivity (c.p.m.) in each metabolite, as determined by the method of Crabtree & Henderson (1971), rather than the total amount of each metabolite. Glucose was determined by the glucose oxidase method of Huggett & Nixon (1957). ATP was determined by the fluorimetric method of Greengard (1965).

Results

Purine ribonucleotide synthesis

Purine ribonucleotides may be synthesized *de novo*, via adenine phosphoribosyltransferase (EC 2.4.2.7)

and hypoxanthine/guanine phosphoribosyltransferase (EC 2.4.2.8), and via adenosine kinase (EC 2.7.1.20) and inosine kinase. The rates of these processes in chopped brain tissue were measured, and factors that influence them were studied.

Synthesis *de novo*. [^{14}C]Glycine was used to measure the rate of purine biosynthesis *de novo*, and maximal rates of nucleotide formation (7 pmol/45 min per mg) were attained with 50 μM -glycine. The rates of incorporation into total purine nucleotides were similar in both Krebs–Ringer phosphate medium and Fischer's medium, indicating that the amino acids in the latter (especially glutamine) had no effect on purine synthesis *de novo*. Indeed, addition of

0.2–0.7 mM-glutamine to Krebs–Ringer phosphate solution did not stimulate nucleotide synthesis.

Adenine phosphoribosyltransferase and hypoxanthine/guanine phosphoribosyltransferase. The lowest concentrations of adenine and hypoxanthine required to give maximum rates of total purine nucleotide synthesis were 10 and 18.5 μM respectively, as shown in Fig. 1(a). Fig. 1(b) shows the time-course of nucleotide synthesis from high concentrations of these two purine bases.

The possibility was considered that nucleotide synthesis from [^{14}C]adenine and [^{14}C]hypoxanthine was limited by the availability of 5-phosphoribosyl 1-pyrophosphate; this substrate may be synthesized

Fig. 1. Purine nucleotide synthesis from adenine and hypoxanthine in chopped mouse cerebrum

(a) Chopped cerebrum (about 70 mg/ml of Fischer's medium), which had been preincubated and washed, was divided into 0.1 ml portions. Each portion was incubated at 37°C with shaking in an atmosphere of 100% O_2 in the presence of various concentrations of [^{14}C]adenine (o) or [^{14}C]hypoxanthine (●) for 30 min and 45 min respectively. (b) Chopped cerebrum (about 70 mg/ml of medium), which had been preincubated and washed, was incubated at 37°C in an atmosphere of 100% O_2 in Fischer's medium containing 25 mM-phosphate with 100 μM -[^{14}C]adenine (o) or 46 μM -[^{14}C]hypoxanthine (●). At various times portions were removed. Radioactivity in total acid-soluble purine nucleotides was counted, and the results are expressed as pmol/mg wet wt. of tissue, according to the method of Crabtree & Henderson (1971). Each point represents an average of four analyses in two experiments. The average variation of individual values from the mean was less than 10%.

Fig. 2. Effect of guanosine on the synthesis of purine nucleotides in chopped mouse cerebrum

Chopped cerebrum was incubated, as described in Fig. 1, in Fischer's medium containing 25 mM-phosphate with (a) 40 μM-[¹⁴C]adenine and (b) 97 μM-[¹⁴C]hypoxanthine in the presence (●) or in the absence (○) of 0.5 mM-guanosine. At various times portions were removed for counting of radioactivity in total purine nucleotides. Each point represents an average of four analyses in two experiments. The average variation of individual values from the mean was less than 10%.

both from glucose via the pentose phosphate pathway and from ribonucleosides via phosphorolysis and pyrophosphorylation. If the synthesis of 5-phosphoribosyl 1-pyrophosphate from glucose were limiting for the synthesis of nucleotides from purine bases in cerebral slices, then addition of nucleosides or stimulation of glucose conversion into pentose phosphates might result in increased synthesis of purine ribonucleotides. Accordingly the effect of 0.5 mM-guanosine on the rates of incorporation of radioactive adenine and hypoxanthine into nucleotides was examined (Fig. 2). The rates of incorporation were stimulated in both cases. Cytidine, uridine and xanthosine (all at 1 mM) were without effect. Attempts to stimulate nucleotide synthesis by addition of Methylene Blue (0.01–0.1 mM) or phenazine methosulphate (0.05 mM) to stimulate the oxidative pathway of pentose phosphate synthesis from glucose (O'Neill *et al.*, 1965) were unsuccessful.

Less than 0.2 pmol of radioactive nucleotide/mg of tissue was detected when [8-¹⁴C]guanine (50 μM) was supplied to chopped cerebrum. The activity of guanine phosphoribosyltransferase assayed by the

method of Murray *et al.* (1969), in a crude extract of cerebrum, was 0.25 nmol of GMP formed/min per mg of protein or 16 pmol/min per mg of tissue. The inability of guanine to be incorporated into nucleotides is probably due to rapid deamination of the substrate both intracellularly and extracellularly (see below).

A variety of purine analogues and derivatives have been shown to inhibit the hypoxanthine phosphoribosyltransferase of human erythrocytes (Krenitsky *et al.*, 1969; Lau & Henderson, 1972a), and the conversion of hypoxanthine into nucleotides in intact adenocarcinoma 755 cells (Hill, 1970) and in Ehrlich ascites-tumour cells (Lau & Henderson, 1972b). Several of the inhibitors used in the latter study were also tested in the brain system in an attempt to identify compounds that might be of use in future studies of the function of this enzyme in brain. When chopped cerebrum was incubated for 60 min in Fischer's medium containing 25 mM-phosphate and 46 μM-[¹⁴C]hypoxanthine, *N*-hydroxyadenosine inhibited nucleotide formation by 81%, 4'-thioadenosine by 53%, 6-methoxypurine ribonucleoside by 78% and 9-(2'-hydroxy-3'-hydroxymethyl)tetrahydro-

Fig. 3. Purine nucleotide synthesis from adenosine in chopped mouse cerebrum

Chopped cerebrum was incubated, as described in Fig. 1, in Fischer's medium containing 25 mM-phosphate with 4.2 μM - $[^{14}\text{C}]$ adenosine (○) or 21.2 μM - $[^{14}\text{C}]$ adenosine (●). At various times portions were removed for counting of radioactivity in total purine nucleotides. Each point represents an average of six analyses in three experiments. The average variation of individual values from the mean was less than 8%.

furyl)adenine by 69%; inhibitor concentration was 1 mM.

Adenosine kinase and inosine kinase. The rate of incorporation of $[^{14}\text{C}]$ adenosine into total purine nucleotides in chopped cerebrum is shown in Fig. 3. This substrate is utilized for nucleotide synthesis at a rate seven- to eight-fold higher than is adenine.

Measurement of the possible incorporation of inosine into nucleotides via inosine kinase was complicated by extracellular purine nucleoside phosphorylase activity in the incubation medium, as mentioned above. Thus any nucleotide formation detected could have been derived from hypoxanthine that was formed from inosine during incubation. This problem could be alleviated by addition of non-radioactive guanosine to the medium. In the presence of 0.5 mM-guanosine the formation of hypoxanthine was completely inhibited when chopped cerebrum was

incubated with inosine. Phosphorolysis of guanosine was facilitated by the continued removal (by deamination) of guanine. Under these conditions only 0.7 pmol of nucleotides/mg of tissue was formed during the 60 min of incubation in the presence of 27.2 μM - $[^{14}\text{C}]$ inosine and 0.5 mM-guanosine. This amount could be accounted for by the small amount of hypoxanthine present as an impurity in the radioactive inosine. It seems likely that inosine kinase activity was absent from mouse cerebrum, although there is still the possibility that the guanosine present might have competitively inhibited this reaction.

Synthesis of ADP and ATP. The relative rates of the synthesis of ATP, ADP and AMP from adenine, hypoxanthine and adenosine are shown in Fig. 4. The proportions of radioactive ATP, ADP and AMP were roughly constant over the period of incubation when the rates of nucleotide synthesis were linear.

The amounts of adenine and hypoxanthine incorporated into the acid-insoluble fraction of cerebral slices were only 3% of the amount of radioactivity found in acid-soluble nucleotides.

Interconversion of purine nucleotides

Fig. 5 shows that 90% of the total nucleotide radioactivity derived from $[^{14}\text{C}]$ hypoxanthine was found in adenine nucleotides, with the remaining 10% mainly in guanine nucleotides. The conversion of inosinate into guanylate was not stimulated when 1 mM-nicotinamide and 2 mM-glutamine were added to chopped cerebrum incubated in Krebs-Ringer phosphate medium. Experiments with $[^{14}\text{C}]$ adenine showed that under a variety of conditions only about 1% of the radioactive adenine nucleotides were converted into IMP.

Synthesis of cyclic AMP (adenosine 3':5-cyclic monophosphate)

Preliminary experiments were conducted on the effects of noradrenaline on $[^{14}\text{C}]$ adenine metabolism. In chopped cerebrum previously incubated with $[^{14}\text{C}]$ adenine for 60 min, according to the procedure of Shimizu and co-workers (Shimizu & Daly, 1970; Shimizu *et al.*, 1969, 1970) and of Forn & Krishna (1971), the addition of noradrenaline (0.1 mM final concentration) for 10 min led to a sixfold increase in the percentage of total nucleotide radioactivity in the form of cyclic AMP (from 0.5 to 3.1%). These results are similar to those reported by Forn & Krishna (1971). In the presence of 0.1 mM-noradrenaline the total amount of nucleotides synthesized from $[^{14}\text{C}]$ adenine during 60 min incubation was twice as great as in control samples (230 compared with 100 pmol/mg); the basis for the latter observation must be studied in more detail.

Fig. 4. Synthesis of individual adenine nucleotides from adenine, hypoxanthine and adenosine in chopped mouse cerebrum

Chopped cerebrum was incubated, as described in Fig. 1, in Fischer's medium containing 25 mM-phosphate with (a) $100\text{ }\mu\text{M}$ - $[^{14}\text{C}]$ adenine, (b) $46\text{ }\mu\text{M}$ - $[^{14}\text{C}]$ hypoxanthine and (c) $21.2\text{ }\mu\text{M}$ - $[^{14}\text{C}]$ adenosine. At various times portions were removed for counting of radioactivity in ATP ($\circ$), ADP ($\bullet$) and AMP ($\triangle$). Each point represents an average of six analyses in three experiments. The average variation of individual values from the mean was less than 6%.

Catabolism of purine ribonucleotides and related compounds

By analysis of the amounts of radioactivity in various purines and purine ribonucleosides, estimates could be made of the relative rates of different catabolic processes in chopped cerebrum.

Dephosphorylation of ribonucleotides. This process is very slight under these conditions. Thus only trace amounts of radioactive adenosine, inosine or hypoxanthine were found (and no xanthine or its derivatives) after 60 min of incubation with $[^{14}\text{C}]$ adenine. These compounds are the expected products of the dephosphorylation of adenylyate. As shown in Fig. 5, ATP started to break down when chopped cerebrum was incubated for longer than 60 min, and the loss in radioactivity in ATP was accounted for almost entirely by increased radioactivity in ADP and AMP. Under such adverse conditions the amount of radio-

activity found in adenosine, hypoxanthine and inosine was about 2% of the total adenine nucleotide radioactivity.

Likewise no detectable radioactivity was observed in xanthosine, guanine or guanosine when $[^{14}\text{C}]$ -hypoxanthine was precursor, suggesting that the dephosphorylation of XMP and GMP was nil.

Adenosine deaminase. Relative to the rate of its utilization for nucleotide synthesis (Fig. 4) adenosine was catabolized only slowly in chopped cerebrum. The total amount of inosine and hypoxanthine formed from adenosine at various times is shown in Table 1. These values have been corrected for the amount of inosine originally present in the adenosine. The rate of deamination of adenosine is clearly dependent on the initial adenosine concentration.

Xanthine oxidase. Analysis of radioactivity in bases after incubation of chopped cerebrum with $[^{14}\text{C}]$ -

Fig. 5. Interconversion of purine nucleotides in chopped mouse cerebrum

Chopped cerebrum was incubated, as described in Fig. 1, in Fischer's medium containing 25 mM-phosphate with $46 \mu\text{M}$ - $[^{14}\text{C}]$ hypoxanthine. At various times portions were removed for counting of radioactivity in adenine nucleotides ($\circ$), guanine nucleotides ($\bullet$) and inosinate ($\square$). Each point represents an average of six analyses in three experiments. The average variation of individual values from the mean was less than 12%.

hypoxanthine showed that a considerable amount of xanthine and urate accumulated. The rates of formation of these compounds are shown in Fig. 6.

Guanine deaminase. It has been reported that the cerebellum of a variety of animals contained little or no guanine deaminase activity (Kumar *et al.*, 1967; Dawson, 1971), in contrast to a high activity of this enzyme in cerebrum. Table 2 shows the results of measurements of guanine deamination; in preparations of cerebrum no free guanine remained after 60 min incubation.

Inosine phosphorylase. Fig. 7 shows the rate of hypoxanthine formation from $[^{14}\text{C}]$ inosine, and of inosine formation from $[^{14}\text{C}]$ hypoxanthine. The

Fig. 6. Oxidation of hypoxanthine in chopped mouse cerebrum

Chopped cerebrum was incubated as described in Fig. 1, in Fischer's medium containing 25 mM-phosphate, with $46 \mu\text{M}$ - $[^{14}\text{C}]$ hypoxanthine. At various times portions were removed for counting of radioactivity in xanthine ($\circ$) and urate ($\bullet$). Each point represents an average of six analyses in three experiments. The average variation of individual values from the mean was less than 10%.

former process (which is about tenfold more rapid than the latter) takes place predominantly in the medium and is catalysed by enzyme that has leaked out of the chopped cerebrum. The high orthophosphate content of the medium also supports rapid phosphorolysis. The conversion of hypoxanthine into inosine apparently is intracellular and is limited at least in part by the availability of ribose 1-phosphate.

Discussion

These studies have demonstrated that chopped mouse cerebrum can utilize glycine for the synthesis of purine nucleotides *de novo*. Its rate of incorporation was about 30-fold higher than that observed in rat cerebral slices, as measured by the accumulation of phosphoribosylformylglycine amide (Howard *et al.*, 1970), and 12-fold higher than that observed in rat cerebral-cortical slices by Santos *et al.* (1968). The

Table 1. *Deamination of adenosine in chopped mouse cerebrum*

Chopped cerebrum was incubated in Fischer's medium containing 25 mM-phosphate, with 4.2 μ M- or 21.2 μ M- [14 C]adenosine, as described in Fig. 1. At various times portions were removed for counting of radioactivity in adenosine, inosine and hypoxanthine. Each value represents an average of four analyses in two experiments. The average variation of individual values from the mean was less than 10%.

Initial concentration of adenosine (μ M)	Incubation time (min)	Adenosine remaining (pmol/mg)	Hypoxanthine+inosine formed (pmol/mg)
4.2	0	85.4	0
	5	33.6	0
	10	18.6	5.9
	30	5.6	5.6
21.2	0	387	0
	5	278	0
	10	232	12.6
	30	117	66.4

Table 2. *Deamination of guanine in chopped mouse cerebrum and cerebellum*

Chopped cerebrum or cerebellum was incubated in Fischer's medium containing 25 mM-phosphate and [14 C]-guanine, as described in Fig. 1. At various times portions were removed for counting of radioactivity in xanthine and urate. Each value represents an average of four analyses in two experiments. The average variation of individual values from the mean was less than 10%.

System	Initial concentration of guanine (μ M)	Incubation time (min)	Xanthine+urate formed (pmol/mg)
Cerebrum	50	15	524
		60	578
Cerebellum	40	60	365

glycine concentrations used by these previous workers were 110 and 4.8 μ M respectively. In the latter case, at least, the concentration may not have been sufficient to promote the maximum rate of nucleotide synthesis. The similar rates observed when Krebs-Ringer phosphate medium and Fischer's medium were used indicates that supplementation with a variety of amino acids had no effect on the synthesis of nucleotides from glycine. The amount of [14 C]glycine incorporated into nucleotides represents a lower limit, since no consideration has been given to dilution of isotope by endogenous glycine. In rat brain glycine concentrations of 1.3–1.7 μ mol/g have been reported (Porcellati & Thompson, 1957).

Chopped cerebrum also can utilize purine bases for the synthesis of nucleotides. However, the concentrations of adenine and hypoxanthine required to promote maximum rates of utilization were very low. Presumably the other substrate for nucleotide formation, 5-phosphoribosyl 1-pyrophosphate may be a

limiting factor for the synthesis of nucleotides in chopped cerebrum. In brain most of the glucose is metabolized via the glycolytic pathway, because Hostetler & Landau (1967) found only 3–5% of the total glucose utilized to be metabolized via the pentose phosphate pathways in rat brain *in vivo*. Further, the oxidative pathway of the pentose phosphate shunt is limited by the capacity to regenerate NADP⁺ (O'Neill *et al.*, 1965), and any ribose phosphates formed can be channelled back into glycolysis via the non-oxidative pathway. The concentration in mouse brain of ribose 5-phosphate+sedoheptulose 7-phosphate is estimated to be 64 μ mol/g (Kauffman *et al.*, 1969). Therefore the availability of ribose phosphate may be a limiting factor for the formation of nucleotides from purine bases. The stimulation by guanosine of the formation of nucleotides that was observed in the present work is consistent with this idea.

Adenosine is utilized for the synthesis of nucleotides

Fig. 7. Inosine phosphorylase activity in chopped mouse cerebrum

Chopped cerebrum was incubated, as described in Fig. 1, in Fischer's medium containing 25mM-phosphate, with $46\mu\text{M}$ - $[^{14}\text{C}]$ hypoxanthine or $27\mu\text{M}$ - $[^{14}\text{C}]$ inosine. At various times portions were removed for counting of radioactivity in inosine (○) or hypoxanthine (●). Each point represents an average of six analyses in three experiments. The average variation of individual values from the mean was less than 10%.

at a rate seven- to eight-fold higher than that of adenine (Fig. 3). This difference may be even higher, since the concentration of adenosine used in these studies was not saturating. The rate of catabolism of adenosine is small in comparison with its rate of utilization for nucleotide synthesis (Table 1). Thus mouse cerebral adenosine kinase might play an important part in the reutilization of adenine compounds after the degradation of RNA in the brain.

The rate of conversion of adenine nucleotides into inosinate was very slow. Further, when ATP was broken down, only ADP and AMP accumulated (see Fig. 2a). Deuticke *et al.* (1966) and Matthias & Busch (1969) found that in the ischaemic brain of rat and rabbit adenine nucleotides were broken down via AMP, adenosine and inosine to hypoxanthine. Therefore the conversion of AMP into inosinate is likely to be the limiting step for the conversion of adenine nucleotides into guanine nucleotides.

Inosinate or xanthylate might accumulate if inosinate dehydrogenase (EC 1.2.1.14) or guanylate synthetase (EC 6.3.5.2.) respectively were rate limit-

ing for guanylate synthesis. However, the amounts of inosinate remained very low (Fig. 5) and no radioactivity was detectable in xanthylate. Since these nucleotides may also be dephosphorylated, the catabolites of inosinate and xanthylate must also be considered. Unfortunately, inosine and xanthine can also be formed directly from hypoxanthine, and these routes of metabolism cannot yet be distinguished from each other. It was suggested above that the availability of 5-phosphoribosyl 1-pyrophosphate may limit the formation of inosinate from hypoxanthine. Hence the concentrations of inosinate may never be high enough for significant rates of guanine nucleotide synthesis.

It has not yet been possible to study the conversion of guanine nucleotides into adenine nucleotides in mouse brain, because the high activity of guanine deaminase present in chopped cerebrum prevents the formation of a pool of labelled guanine nucleotides.

The catabolites of hypoxanthine in chopped mouse cerebrum included xanthine and urate (Fig. 6), although on the basis of previous studies xanthine oxidase has been thought to be absent from crude extracts of brain from a number of animals (see Al-Khalidi & Chalglassian, 1965; Rappoport *et al.*, 1960; Rosenbloom *et al.*, 1967; Santos *et al.*, 1968).

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References

- Adams, D. H. (1965) *J. Neurochem.* **12**, 783
- Al-Khalidi, U. A. S. & Chalglassian, T. H. (1965) *Biochem. J.* **97**, 318
- Bennett, L. L., Jr., Simpson, L. & Skipper, H. E. (1960) *Biochim. Biophys. Acta* **42**, 237
- Crabtree, G. W. & Henderson, J. F. (1971) *Cancer Res.* **31**, 985
- Dawson, D. M. (1971) *Neurology* **21**, 621
- Deuticke, B., Gerlach, E. & Dierkesmann, R. (1966) *Pflügers Arch. Ges. Physiol. Menschen Tierl.* **292**, 239
- Fischer, G. A. & Sartorelli, A. C. (1964) *Methods Med. Res.* **10**, 247
- Forn, J. & Krishna, G. (1971) *Pharmacology* **5**, 193
- Greengard, P. (1965) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 551, Academic Press, New York
- Held, I. & Wells, W. (1969) *J. Neurochem.* **16**, 329
- Held, I., Wells, W. & Koenig, H. (1969) *J. Neurochem.* **16**, 537
- Henderson, J. F. & LePage, G. A. (1959) *J. Biol. Chem.* **234**, 2364
- Hill, D. L. (1970) *Biochem. Pharmacol.* **19**, 545
- Hostetler, K. Y. & Landau, B. (1967) *Biochemistry* **7**, 2961
- Howard, W. J., Kerson, L. A. & Appel, S. H. (1970) *J. Neurochem.* **17**, 121
- Huggett, A. St. G. & Nixon, D. A. (1957) *Biochem. J.* **66**, 12p

- Kauffman, F. C., Brown, J. G., Passonneau, J. V. & Lowry, O. H. (1969) *J. Biol. Chem.* **244**, 3647
- Krenitsky, T. A., Papaioannou, R. & Elion, G. B. (1969) *J. Biol. Chem.* **244**, 1263
- Kumar, S., Josan, V., Sanger, K. C. S., Tewari, K. K. & Krishnan, P. S. (1967) *Biochem. J.* **102**, 691
- Lau, K. F. & Henderson, J. F. (1972a) *Cancer Chemother. Rep.* in the press
- Lau, K. F. & Henderson, J. F. (1972b) *Cancer Chemother. Rep.* in the press
- Mannell, W. A. & Rossiter, R. J. (1955) *Biochem. J.* **61**, 418
- Matthias, R. & Busch, E. (1969) *Hoppe-Seyler's Z. Physiol. Chem.* **350**, 1410
- McIlwain, H. & Buddle, H. L. (1953) *Biochem. J.* **53**, 412
- Murray, A. W., Wong, P. C. L. & Friedrichs, B. (1969) *Biochem. J.* **112**, 741
- O'Neill, J. J., Simon, S. H. & Shreeve, W. W. (1965) *J. Neurochem.* **12**, 797
- Porcellati, G. & Thompson, R. H. S. (1957) *J. Neurochem.* **1**, 340
- Rappoport, D. A., Fritz, R. R., Allen, J. R. & Moraczewski, A. (1960) *J. Neurochem.* **6**, 21
- Rosenbloom, F. M., Kelley, W. N., Miller, J., Henderson, J. F. & Seegmiller, J. E. (1967) *J. Amer. Med. Ass.* **202**, 175
- Santos, J. N., Hempstead, K. W., Kopp, L. E. & Meich, R. P. (1968) *J. Neurochem.* **15**, 367
- Sarma, S. K. & Singh, U. N. (1970) *J. Neurochem.* **17**, 305
- Shimizu, H. & Daly, J. (1970) *Biochim. Biophys. Acta* **222**, 465
- Shimizu, H., Daly, J. & Creveling, C. R. (1969) *J. Neurochem.* **16**, 1609
- Shimizu, H., Creveling, C. R. & Daly, J. (1970) *Proc. Nat. Acad. Sci. U.S.* **65**, 1033