REFERENCES

1. L. B. MELLETT and L. A. WOODS, *Cancer Res.* **20**, 524 (1960).
2. D. S. KAUSHIK, M.Sc. thesis, Bradford University (1972).
3. W. C. J. ROSS, *J. chem. Soc.* 2257 (1950).
4. C. BENCKHUIJSEN, *Biochem. Pharmac.* **17**, 55 (1968).
5. *British Pharmacopoeia*, pp. 1242–1246. Pharmaceutical Press, London (1968).
6. C. G. BUTLER, D. S. KAUSHIK, J. MAXWELL and J. G. P. STELL, *J. Mond. Pharmac.* **4**, 359 (1967).
7. J. EPSTEIN, R. W. ROSENTHAL and R. J. ESS, *Analyt. Chem.* **27**, 1435 (1955).
8. R. TRUHAUT, E. DELACOUX, G. BRULE and C. BOHUON, *Clin. Chim. Acta.* **8**, 235 (1963).

Biochemical Pharmacology, Vol. 23, pp. 170–172. Pergamon Press, 1974. Printed in Great Britain.

Levels of nucleoside and nucleotide kinases in Rhesus monkey tissues

(*Received* 16 *April* 1973; *accepted* 6 *July* 1973)

THE NUCLEOSIDE and nucleotide kinases are involved in the phosphorylation of many analogues of chemotherapeutic interest. Consequently, the specificity of these enzymes with analogue substrates has been the subject of considerable investigation. However, there are no reported data which deal with the relative levels of these enzymes to which analogues would be exposed *in vivo*. Such information seemed an essential counterpart to the specificity data if a reasonable assessment of the quantitative significance of various routes of analogue metabolism was to be made. It was for this reason that this study was undertaken.

Two known mammalian nucleoside kinases act on purine nucleosides. One of these, adenosine kinase (EC 2.7.1.20), has been well characterized.[1,2] The other, only recently described,[3] acts on both deoxyguanosine and deoxyadenosine. Evidence has been reported which suggests that some mammalian cells very slowly phosphorylate inosine;[4,5] however, the independence of this activity from the above-mentioned nucleoside kinases has not been established. A previous study employed 6-methylthiopurine ribonucleoside as the substrate to determine the tissue levels of adenosine kinase.[6] In the present study, the natural substrate, adenosine, was used in the presence of the adenosine deaminase inhibitor, erythro-9-(2-hydroxy-3-nonyl)adenine. This compound, a generous gift of Dr. H. J. Schaeffer of these laboratories, is a potent inhibitor of adenosine deaminase from a number of different sources.* At the concentrations used in these experiments, it was found not to inhibit a purified preparation[1] of adenosine kinase, while completely inhibiting the deaminase. Further, complete inhibition of the deaminase in the tissue extracts was demonstrated by chromatography of reaction mixtures in a solvent[7] capable of separating adenosine and inosine.

In the presence of this inhibitor, adenosine kinase was detectable in all of the monkey tissues assayed (Table 1). Liver exhibited the highest activity. With the monkey tissue extracts, the levels of adenosine kinase were very much higher than those of the other purine nucleoside kinases assayed. Phosphorylation of deoxyguanosine was detectable only with kidney extracts while phosphorylation of inosine was not detectable with any extract (Table 1).

The two pyrimidine nucleoside kinases studied were uridine kinase (EC 2.7.1.48) and thymidine kinase (EC 2.7.1.21). There is yet another pyrimidine nucleoside kinase in mammalian tissues, deoxycytidine kinase, but its relationship to the activity toward deoxyguanosine is uncertain.[12–14] Uridine kinase was detectable in six of the 13 tissue extracts assayed, while thymidine kinase was detectable only in liver and brain extracts (Table 1). Again, the levels of both these enzymes were low as compared with those for adenosine kinase. Previous studies have shown that the bulk of these kinases are present in supernatant fractions of tissue homogenates.[15]

In mammals, at least three distinct enzymes catalyze the phosphorylation of purine nucleotides. Two of these enzymes are relatively specific for AMP as the phosphoryl acceptor but differ in their

*H. J. Schaeffer and C. F. Schwenden, personal communication.

TABLE 1. LEVELS OF NUCLEOSIDE* AND NUCLEOTIDE KINASES EXTRACTED FROM RHESUS MONKEY TISSUES†

Tissue	Substrate (nmoles phosphorylated/min/g of wet tissue extracted)‡						Protein extracted/g of wet tissue (mg)
	Adenosine	Deoxyguanosine	Uridine	Deoxythymidine	Guanosine-5′-monophosphate	Uridine-5′-monophosphate	
Liver	1200	< 10	< 10	51	170	310	100
Spleen	810	< 10	13	< 10	260	220	80
Lymph nodes	530	< 10	< 10	< 10	170	190	47
Kidney	500	16	21	< 10	600	220	100
Testis	360	< 10	10	< 10	190	100	48
Lung	270	< 10	< 10	< 10	150	130	60
Small intestine	220	< 10	19	< 10	180	60	74
Erythrocytes	200	< 10	24	< 10	43	59	320§
Brain	79	< 10	18	24	140	96	27
Heart	66	< 10	< 10	< 10	80	78	28
Skeletal muscle	63	< 10	< 10	< 10	32	120	80
Bone marrow	53	< 10	< 10	< 10	31	13	15§
Skin	38	< 10	< 10	< 10	30	11	12

* Activity with inosine and deoxythymidine-5′-monophosphate was not detectable (< 10) in extracts from every tissue listed.

† A 2-yr-old Rhesus monkey (*Macaca mulatta*) was anesthetized with ether and exsanguinated by cardiac puncture. Excised tissues were homogenized at 4° in twice their weight of 10 mM potassium phosphate-0·1 mM EDTA, pH 6·8, in either a Sorvall Omnimixer or a Potter homogenizer and centrifuged at 85,000 *g* for 30 min. A 2-ml aliquot of each supernatant fluid was applied to a G-10 Sephadex column (2 × 18 cm) which had been equilibrated with the eluting buffer, 50 mM Tris-HCl-4 mM dithiothreitol, pH 8·2, at 4°. Void volume fractions were combined. Protein concentrations of both the supernatan fluids and the combined fractions were determined by a spectrophotometric method[8] so the dilution which resulted from passage through the Sephadex column could be calculated. Assay mixtures (0·4 ml) contained $MgCl_2$ at 10 mM, Tris–HCl (pH 7·5 at 37°) at 50 mM, [8-^{14}C]purine nucleoside or [2-^{14}C]pyrimidine nucleoside at 1 mM or [^{14}C]5′-mononucleotide at 0·1 mM (from Schwarz/Mann, Orangeburg, N.Y.; nucleosides were diluted to 1–2 Ci/mole and nucleotides to 4–5 Ci/mole), Na_2ATP at 10 mM, dithiothreitol at 1 mM, and an appropriate dilution of the sephadexed extract. When adenosine was the substrate, the adenosine deaminase inhibitor, erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride, was also added to a final concentration of 0·072 mM. After 15 min at 37° the reactions were terminated by immersion in a boiling water bath for 90 sec. Control values were obtained by treating the extracts at 100° for 5 min before addition to the reaction mixtures. Products from nucleosides were separated from substrates by chromatography on Whatman No. 3 MM paper as previously described.[9,10] In no case was the phosphorolysis of either purine or pyrimidine nucleosides sufficient to significantly interfere with the kinase reaction. Isobutyric acid–conc. NH_4OH–water (57:4:39) was used as the solvent to separate the reaction products with GMP as substrate, and tert-amyl alcohol–formic acid–water (3:2:1) was used with UMP or dTMP as substrate. Each chromatogram was first scanned for radioactivity. These spots were then cut out and counted by liquid scintillation.[11] In all cases where nucleotide phosphorylation was detectable, the rate of dephosphorylation was much slower.

‡ Since comparisons of the catalytic potential of tissues rather than the extracts were desired, the enzyme levels were expressed as units extracted/g of tissue. It should be noted, however, that the comparisons based on data expressed in this manner are somewhat different from those based on the specific activities of tissue extracts (units of enzyme activity/mg of extracted protein). This was a consequence of the large differences in the amounts of soluble protein extractable from various tissues. The specific activity values, although not stated, can be calculated from the protein concentrations listed.

§ Milligrams of protein extracted/g of packed cells.

specificities toward phosphoryl donors.[16–18] With the third, guanylate kinase (EC 2.7.4.8), GMP and dGMP but not AMP are phosphoryl acceptors.[19,20] All of the tissue extracts studied had detectable levels of kinase activity toward GMP (Table 1). The level in kidney was more than twice that of any other tissue. The levels of activity toward AMP appeared relatively high with all extracts tested; however, attempts at quantitation were complicated by the presence of AMP deaminase.

At least two distinct pyrimidine nucleotide kinases are present in mammalian tissues. Evidence has been reported which suggests that UMP, CMP and dCMP are all efficiently phosphorylated by one enzyme which is referred to as deoxycytidylate kinase[21] (EC 2.7.4.5). This enzyme does not phosphorylate dTMP, which is phosphorylated by a distinct enzyme, thymidylate kinase (EC 2.7.4.9). Phosphorylation of UMP was catalyzed by all of the tissue extracts studied (Table 1) at rates comparable to those of GMP. Thymidylate phosphorylation was not detectably catalyzed by any of the tissue extracts. This is consistent with studies which show that levels of thymidylate kinase are low in adult tissues.[22,23]

The predominance of adenosine kinase among the nucleoside kinases was striking but perhaps not unexpected when the predominance of the adenine nucleotides *in vivo* is considered. In light of these high levels of adenosine kinase, the possibility must be considered that even relatively poor analogue substrates of this enzyme might be appreciably phosphorylated *in vivo*.

Of special interest was the comparison of the enzyme levels of the brain to those of other tissues. It was previously shown that levels of hypoxanthine-guanine phosphoribosyltransferase (H-G PRTase) are unusually high in primate brain as compared with other tissues.[24,25] The data presented in Table 1 show that other enzymes involved in nucleic acid precursor metabolism are not elevated in brain. This suggests that the over-all purine and pyrimidine metabolism of primate brain is not unusually rapid. Therefore, the elevated H-G PRTase levels in primate brain and the neurological disorders associated with a severe deficiency of this enzyme in humans[26] suggest a special dependence on hypoxanthine and/or guanine salvage in this tissue. It is not yet known if this apparent dependence on salvage is accompanied by an insufficiency of *de novo* purine nucleotide biosynthesis in primate brain.

The Wellcome Research Laboratories,
Burroughs Wellcome Co.,
Research Triangle Park, N.C. 27709, U.S.A.

THOMAS A. KRENITSKY
RICHARD L. MILLER
JAMES A. FYFE

REFERENCES

1. B. LINDBERG, H. KLENOW and K. HANSEN, *J. biol. Chem.* **242,** 350 (1967).
2. H. P. SCHNEBLI, D. L. HILL and L. L. BENNETT, JR., *J. biol. Chem.* **242,** 1997 (1967).
3. V. KRYGIER and R. L. MOMPARLER, *J. biol. Chem.* **246,** 2745 (1971).
4. K. J. PIERRE and G. A. LEPAGE, *Proc. Soc. exp. Biol. Med.* **127,** 432 (1968).
5. M. R. PAYNE, J. DANCIS, P. H. BERMAN and M. E. BALIS, *Expl Cell Res.* **59,** 489 (1970).
6. D. H. W. HO, J. K. LUCE and E. FREI, III, *Biochem. Pharmac.* **17,** 1025 (1968).
7. S. H. LOVE and C. N. REMY, *J. Bact.* **91,** 1037 (1966).
8. J. B. MURPHY and M. W. KIES, *Biochim. biophys. Acta* **45,** 382 (1960).
9. T. A. KRENITSKY, S. M. NEIL and R. L. MILLER, *J. biol. Chem.* **245,** 2605 (1970).
10. T. A. KRENITSKY, *J. biol. Chem.* **243,** 2871 (1968).
11. T. A. KRENITSKY, R. PAPAIOANNOU and G. B. ELION, *J. biol. Chem.* **244,** 1263 (1969).
12. R. L. MOMPARLER and G. A. FISCHER, *J. biol. Chem.* **243,** 4298 (1968).
13. J. P. DURHAM and D. H. IVES, *J. biol. Chem.* **254,** 2276 (1970).
14. D. H. IVES and J. P. DURHAM, *J. biol. Chem.* **245,** 2285 (1970).
15. J. P. DURHAM and D. H. IVES, *Expl Cell Res.* **70,** 97 (1972).
16. M. CHIGA and G. W. E. PLAUT, *J. biol. Chem.* **235,** 3260 (1960).
17. L. A. HEPPEL, J. L. STROMINGER and E. S. MAXWELL, *Biochim. biophys. Acta* **32,** 422 (1959).
18. L. NODA, in *The Enzymes* (Eds. P. D. BOYER, H. LARDY and K. MYRACK), 2nd edn, Vol. 6, p. 139. Academic Press, New York (1962).
19. R. P. MIECH and R. E. PARKS, JR., *J. biol. Chem.* **240,** 351 (1965).
20. H. SHIMONO and Y. SUGINO, *Eur. J. Biochem.* **19,** 256 (1971).
21. Y. SUGINO, H. TERAOKA and H. SHIMONO, *J. biol. Chem.* **241,** 961 (1966).
22. H. H. HIATT and T. B. BOJARSKI, *Biochem. biophys. Res. Commun.* **2,** 35 (1960).
23. H. H. HIATT and T. B. BOJARSKI, *Cold Spring Harb. Symp. Quant. Biol.* **26,** 367 (1961).
24. F. M. ROSENBLOOM, W. N. KELLEY, J. MILLER, J. F. HENDERSON and J. E. SEEGMILLER, *J. Am. med. Ass.* **202,** 175 (1967).
25. T. A. KRENITSKY, *Biochim. biophys. Acta* **179,** 506 (1969).
26. J. E. SEEGMILLER, F. M. ROSENBLOOM and W. N. KELLEY, *Science, N.Y.* **155,** 1682 (1967).