the latter type of protocol, *N*-demethylation of morphine was unchanged in 9000 *g* liver supernatant from rats maintained at 1 ATA when incubated at 21 ATA [18].

In the present study we have failed to demonstrate any significant effect of high atmospheric pressure on the metabolism of drugs *in vitro*. The decreased effect of some drugs under high pressure cannot be explained by a general increase in drug metabolism at the cellular level. The impact of other pharmacokinetic factors needs to be clarified in order to answer the question of how pressure affects drug actions.

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Substrate activites for adenosine kinase and adenosine deaminase in relation to the cytotoxicities of some O^6-alkyl derivatives of 8-azainosine and 8-azaguanosine

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Ribonucleosides of O^6-alkyl-8-azahypoxanthines and 6-(alkylthio)-8-azapurines at low concentrations are toxic to cultured H. Ep. # 2 cells [1]. It was anticipated that these 8-aza derivatives would have properties similar to those of the corresponding purine nucleosides and, therefore, that they would be substrates for adenosine kinase (AK) (EC 2.7.1.20), and that lines lacking this enzyme would be resistant to their action [2]. However, two lines of H. Ep. # 2 cells lacking AK activity and highly resistant (200 to 1000-fold) to adenosine analogs, such as *S*-methyl-6-thiopurine ribonucleoside, showed relatively low degrees of resistance (5 to 20-fold) to O^6-methyl- and O^6-ethyl-8-azainosine [1]. Inhibition of the cells deficient in AK indicated either that the unphosphorylated nucleosides had activity or that there was a pathway other than direct phosphorylation for their conversion to cytotoxic nucleotides. Since O^6-methylinosine is a substrate for adenosine deaminase (ADA) (EC 3.5.4.4) [3, 4], it appeared that the action of ADA might be responsible for the activity of O^6-alkyl-8-azainosines in the kinase-deficient cells. The product of this reaction would be 8-azainosine, which is cytotoxic as a result of its conversion to 8-aza-IMP either by direct phosphorylation or by an alternative pathway not involving AK [5] (Fig. 1). Another possible metabolic fate (not shown in Fig. 1) for O^6-alkyl-8-azainosines is their conversion directly to O^6-alkyl-8-azahypoxanthines by the action of purine nucleoside phosphorylase. These bases were not available for study, but they would be expected to be non-toxic because the closely related thio compound, *S*-methyl-6-thio-8-azapurine, was not toxic to H. Ep. # 2 cells at a concentration of 300 μM [1].

To obtain evidence on the metabolism of these nucleosides, we have examined the activities of selected compounds as substrates for AK and ADA. We have reported earlier the qualitative observation that some of these compounds were substrates for these enzymes [6, 7], but it was desirable to obtain quantitative data, particularly to determine if the rate of removal of alkoxy groups by ADA was sufficient to have metabolic significance.

The study with AK was performed with preparations purified 135- or 263-fold from cultured H. Ep. # 2 cells. The reaction was measured by determining the amount of labeled monophosphate formed when the candidate nucleoside was incubated with the enzyme in phosphate buffer (pH 7.0)

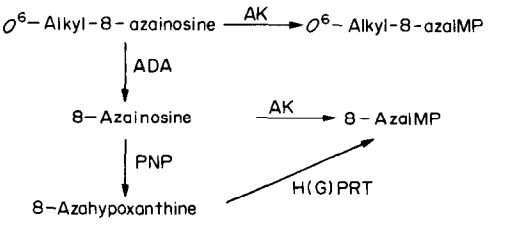

Fig. 1. Pathways for metabolism of O^6-alkyl derivatives of 8-azainosine. Abbreviations: AK, adenosine kinase; ADA, adenosine deaminase; PNP, purine nucleoside phosphorylase; and H(G)PRT, hypoxanthine (guanine) phosphoribosyltransferase.

containing [γ-^{32}P]ATP and $MgCl_2$. The assay and the procedure for purification of the kinase were the same as reported earlier [8], except that dithioerythritol was added, at a concentration of 5 mM, to the enzyme eluted from the Sephadex G-100 column and was present at this concentration through the remaining steps of the purification. Assays for activities as substrates for ADA were performed spectrophotometrically with an enzyme preparation from calf intestine (Sigma Chemical Co., St. Louis, MO). The assays were accomplished by measuring the changes in absorption that occurred at wavelengths at which the differences in absorption between substrate and product were the greatest. The wavelengths used, and the differences in molecular extinction coefficients (ΔE) between substrate and product at this wavelength, were: O^6-methly-8-azainosine, 250 nm, ΔE, 4,000; O^6-ethyl-8-azainosine, 250 nm, ΔE, 3,800; O^6-methyl-8-azaguanosine, 260 nm, ΔE, 9,700; O^6-methylinosine, 270 nm, ΔE, 3,400; and *S*-methyl-6-thio-8-azainosine, 305 nm, ΔE, 13,900. For determination of the kinetic constants for both enzymes by Lineweaver–Burk analysis, fifteen concentrations of each nucleoside were used.

The results of the enzyme assays are shown in Table 1. Both O^6-methyl- and O^6-ethyl-8-azainosine were substrates for the kinase, but their K_m values were higher and their V_{max} values lower than those for adenosine or O^6-methylinosine. O^6-Methyl-8-azaguanosine was not a substrate. As expected, *S*-methyl-6-thio-8-azainosine was a good substrate, with a V_{max} greater than that for adenosine. All five of the nucleosides assayed were substrates for ADA. O^6-methylinosine is a known substrate for the deaminase but was included here, along with adenosine, as a control substrate; our kinetic constants for this compound agree well with those reported by others [3, 4]. Substitution of an N-atom for C-8 of O^6-methylinosine increased the K_m 3-fold but increased the V_{max} even more (approximately 14-fold). O^6-ethyl-8-azainosine was a considerably poorer substrate than O^6-methyl-8-azainosine. O^6-methyl-8-azaguanosine was a moderately good substrate; its K_m was considerably less than that of O^6-methyl-8-azainosine and its V_{max} was about the same; it is not surprising that this compound was acted upon by ADA since O^6-methylguanosine is a known substrate [3, 4]. *S*-methyl-6-thio-8-azainosine had barely detectable activity as a substrate for ADA; the poor activity of this compound is in accord with observations of others on the inertness of *S*-methyl-6-thiopurine ribonucleoside to the action of ADA [9, 10].

The activities of the O^6-alkyl-8-azainosines and O^6-methyl-8-azaguanosine as substrates for ADA appeared to be great enough so that their ADA-catalyzed conversions to 8-azainosine or 8-azaguanosine could be a factor in their cytotoxicities. To obtain further evidence on this point, they were assayed for cytotoxicity to H. Ep. # 2 cells in the presence of 2′-deoxycoformycin [(R)-3-(2-deoxy-β-D-erythro-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol], a potent inhibitor of ADA [11, 12]. The results are shown in Table 2. The presence of 2′-deoxycoformycin was without effect on the cytotoxicity of O^6-ethyl-8-azainosine, partially prevented the cytotoxicity of O^6-methyl-8-azainosine, and completely prevented the cytotoxicity of O^6-methyl-8-azaguanosine.

The results with 2′-deoxycoformycin indicate that the action of ADA contributes to the cytotoxicity of O^6-methyl-8-azainosine but not to that of O^6-ethyl-8-azainosine. This conclusion is in accord with the fact that O^6-methyl-8-azainosine is a much better substrate for ADA (Table 1). However, the action of ADA apparently is not solely responsible for the cytotoxicity of O^6-methyl-8-azainosine, because (a) cells lacking AK activity had some degree of resistance to this compound [1]; and (b) 2′-deoxycoformycin only partially prevented its cytotoxicity (Table 2). Although the action of ADA does not appear to contribute to the cytotoxicity of O^6-ethyl-8-azainosine to cells that have both AK and ADA, it presumably is responsible for the low degree of resistance to this compound in cells lacking AK [1]. Observations with *S*-alkyl-6-thio-8-azainosines are also pertinent. Cells lacking AK activity were highly resistant to these compounds [1]; thus, direct phosphorylation appears to be the principal route for their activation. This conclusion is consistent with the results of Table 1 which show *S*-methyl-6-thio-8-azainosine to be a good substrate for the kinase and a very poor substrate for the deaminase.

With respect to mechanisms of action, it is noteworthy that the actions of AK and ADA on O^6-alkyl-8-azainosines lead to different cytotoxic nucleotides. The product of direct phosphorylation is an O^6-alkyl-8-aza-IMP, a type of nucleotide the mechanism of action of which has not been investigated, but which might be expected to be similar to that of the nucleotide of *S*-methyl-6-thiopurine. The product of the action of ADA is 8-azainosine, the toxicity of which probably results from its conversion to nucleotides of 8-azaguanine [14].

The results with O^6-methyl-8-azaguanosine are different from those with the O^6-alkyl-8-azainosines. This compound was not a substrate for AK but was a moderately good substrate for ADA (Table 1). The fact that its cytotoxicity was completely prevented by deoxycoformycin (Table 2) indicates that the action of ADA is required for its biological activity and that it is not phosphorylated significantly to a toxic nucleotide by any kinase present in H. Ep. # 2 cells. The

Table 1. Kinetic constants for the action of adenosine deaminase and adenosine kinase on adenosine and some nucleosides of 6-alkoxy and 6-alkylthio-8-azapurines*

	Adenosine deaminase		Adenosine kinase	
Substrate	K_m (μM)	V_{max} (μmoles/min/mg)	K_m (μM)	V_{max}† (nmoles/min/mg)
Adenosine	29	435	1.8	222
O^6-methylinosine	50	2	91	285
O^6-methyl-8-azainosine	140	27	240	115
O^6-ethyl-8-azainosine	360	0.7	190	31
O^6-methyl-8-azaguanosine	22	20	‡	
S-methyl-6-thio-8-azainosine	1300	0.7	85	333

* For details of the assays see text.

† Two different preparations of kinase were used, one 135-fold and one 263-fold purified. The value of 222 nmoles/min/mg for adenosine is for the 263-fold purified preparation. The values obtained with the 135-fold purified preparation have been normalized to make them directly comparable to values for the 263-fold purified preparation.

‡ No phosphorylation of this substrate was detected under conditions of the standard assay.

Table 2. Effects of 2′-deoxycoformycin (DCF) on the cytotoxicity to H. Ep. # 2 cells of some O^6-alkyl-8-azainosines and O^6-methyl-8-azaguanosine*

Compounds and concentration (μM)	Colony formation: Per cent of control (range)
DCF, 3.8	97 (93–107)
O^6-methyl-8-azainosine, 0.18	24 (18–33)
O^6-methyl-8-azainosine, 0.18, + DCF, 3.8	68 (64–72)
O^6-methyl-8-azainosine, 0.35	4 (3–6)
O^6-methyl-8-azainosine, 0.35, + DCF, 3.8	17 (8–31)
O^6-ethyl-8-azainosine, 0.17	4 (3–5)
O^6-ethyl-8-azainosine, 0.17, + DCF, 3.8	5 (3–7)
O^6-methyl-8-azaguanosine, 3.4	24 (9–33)
O^6-methyl-8-azaguanosine, 3.4, + DCF, 3.8	92 (85–99)

* One hundred cells were placed in 4 oz prescription bottles containing control cultures with 10 ml of SRI-14 medium [13], and treated cultures with 10 ml of medium to which the candidate inhibitors had been added. After the cultures had been incubated at 37° for 7–10 days, the medium was decanted and the cells adhering to the glass were washed with phosphate-buffered NaCl solution (0.85%), fixed with Bouin's fixative, and stained with Giemsa stain. The microscopic colonies present were then counted. The cloning efficiency of control cultures was 40–70 per cent. Each value in the last column represents the average of three or more experiments run in triplicate. The concentration of DCF was that determined in previous experiments to be non-toxic and to give essentially complete inhibition of ADA.

product of the action of ADA is 8-azaguanosine, which would be cytotoxic as a result of its further metabolism, via 8-azaguanine, to nucleotides of 8-azaguanine [15]. The corresponding thio compound, *S*-methyl-6-thio-8-azaguanosine, has no toxicity to H. Ep. # 2 cells [7]; this fact is in accord with the absence of kinase activity for this type of compound and with the resistance of the 6-alkylthio group to the action of ADA. The ribonucleosides of O^6-alkyl-8-azahypoxanthines and O^6-methyl-8-azaguanine are rare examples of nucleosides that are converted by ADA to compounds that retain toxicity. To our knowledge, O^6-methyl-8-azaguanosine is the only example of a compound the biological activity of which is entirely dependent upon the action of ADA.

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Methacholine-induced attenuation of methylisobutylxanthine- and isoproterenol-elevated cyclic AMP levels in isolated rat atria

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Recent studies have shown that cholinergic agents, which have no significant effects on basal cyclic AMP (cAMP) levels, markedly lower cAMP elevations induced by: (a) epinephrine in rat uterus [1] and perfused rat heart [2]; (b) isoproterenol in rat ventricular slices [3], rat lung slices [4], bovine tracheal smooth muscle [5], guinea pig ileum [6] and human astrocytoma [7]; (c) norepinephrine in rat parotid gland [8]; (d) glucagon in rat ventricular slices [3] and per-