Biochemical Pharmacology, Vol. 32, No. 8, pp. 1433–1440, 1983.
Printed in Great Britain.

0006-2952/83/081433-08 $03.00/0

5′-DEOXYADENOSINE METABOLISM IN VARIOUS MAMMALIAN CELL LINES

PETER G. W. PLAGEMANN* and ROBERT M. WOHLHUETER
Department of Microbiology, Medical School, University of Minnesota, Minneapolis, MN 55455, U.S.A.

(*Received* 28 *June* 1982; *accepted* 9 *November* 1982)

Abstract—5′-Deoxyadenosine (5′-dAdo) was rapidly cleaved to adenine by cell-free, dialyzed extracts of Chinese hamster ovary (CHO), Novikoff rat hepatoma and HeLa cells in a phosphate-dependent reaction, but not by extracts from L929, L1210 and P388 cells. Radioactivity from [5′-^{3}H]5′-dAdo was incorporated into the acid-soluble pool (uptake) by whole CHO, Novikoff and HeLa cells almost as rapidly as from labeled adenosine or adenine (all at 5 μM extracellular concentration). Radioactivity in the acid-soluble pool was mainly associated with a component identified as 5-deoxyribose-1-phosphate. Compared to ribose-1-phosphate, 5-deoxyribose-1-phosphate was metabolically highly stable. A second labeled component, however, was formed slowly and accumulated mainly in the medium. Its formation was greatly stimulated by hypoxanthine and, under conditions where their deamination was not blocked, by adenosine and 2′- and 3′-deoxyadenosine. The second product was 5′-deoxyinosine synthesized from hypoxanthine and 5-deoxyribose-1-phosphate by purine nucleoside phosphorylase. Cleavage of 5′-dAdo by whole cells was dependent on the continuous removal of the product adenine, since uptake was greatly reduced in cells deficient in adenine phosphoribosyl transferase and 50 μM adenine strongly inhibited 5′-dAdo cleavage. The results are consistent with the view that 5′-dAdo is a substrate for 5′-methylthioadenosine phosphorylase and that its use as a non-metabolizable substrate for the nucleoside transport measurements is limited to cells lacking this enzyme.

5′-Deoxyadenosine (5′-dAdo) has been found not to be significantly phosphorylated, deaminated or metabolized in any other way by L1210 murine leukemia cells and has, therefore, been considered an ideal model substrate for studies of nucleoside transport in mammalian cells [1, 2]. In the meantime, however, it has been reported that 5′-dAdo is an efficient substrate for 5′-methylthioadenosine (MTA) phosphorylase of Sarcoma 180 cells [3] and plant cells [4]. We have found that 5′-dAdo is rapidly phosphorolyzed by a number of other, but not all, mammalian cell lines we have tested. The availability of [5′-^{3}H]5′-dAdo made it possible to study the fate of the 5-deoxyribose moiety in cells, and the effect of natural nucleosides, purine bases, and of deficiencies in adenosine kinase, adenine phosphoribosyl transferase (APRT) and hypoxanthine phosphoribosyl transferase (HPRT) on the intracellular metabolism of 5′-dAdo.

MATERIALS AND METHODS

The various cell lines (Table 1) were propagated in suspension culture as described previously [8, 9] and were demonstrated to be free of mycoplasma contamination by uridine/uracil incorporation [10] and cultural methods. Cells were harvested by centrifugation from late exponential phase cultures and suspended to (5–20) × 10^6 cells/ml of basal medium 42B (BM42B, Ref. 11). The suspensions were supplemented with [5′-^{3}H]5′-dAdo (Moravek Biochemicals, Brea, CA), unlabeled 5′-dAdo (supplied by Dr. H. Hogenkamp), and other unlabeled nucleosides or purine bases as indicated in appropriate experiments. The suspensions were incubated at 25°, and at various times the cells from duplicate 0.5-ml samples were collected by centrifugation through an oil mixture [12], and the cell pellets were analyzed for radioactivity [12]. Radioactivity per pellet was corrected for substrate trapped in extracellular space, as estimated by the use of [carboxyl-^{14}C]inulin and normalized to intracellular H_2O volume [12]. The extracellular H_2O space in the cell pellets generally did not exceed 12% of the intracellular H_2O space.

Uptake and metabolism of 5′-dAdo by intact cells was monitored by fractionating intracellular and extracellular radiolabeled components of the cells. Replicate samples of suspensions were collected by centrifugation and suspended in a boiling solution of 10 mM EDTA, pH 7.5. This suspension and the cell-free medium were heated in a boiling water bath for 1 min and then clarified by centrifugation. The supernatant fluids were analyzed by ascending chromatography on 3MM Whatman paper with a solvent composed of 6 vol. of butanol and 1 vol. of H_2O (solvent 30) as described previously [11].

Radiochemical purity of the original [5′-^{3}H]5′-dAdo was tested by chromatography with solvent 30 and high performance liquid chromatography (HPLC) on a Whatman Reverse Phase Partisil ODS-3, 10 μm, 46 × 250 mm column, eluted isocratically with a solution composed of 10 mM $(NH_4)_3PO_4$, 10 mM NH_4Cl and 6% (v/v) methanol, pH 4.8. About 25% of the radioactivity was associ-

* Address all correspondence to: Peter G. W. Plagemann, Department of Microbiology, Medical School, University of Minnesota, Mayo Memorial Bldg., Box 196, 420 Delaware St. S.E., Minneapolis, MN 55455, U.S.A.

Table 1. Cell lines and their enzyme-deficient variants used in present study

Cell line	Subline*	Phenotype†		
		HPRT	APRT	AK
Novikoff rat hepatoma	Wild type (N1S1-67)	+	+	+
	1-22 (TUB^r)	−	+	−
	1-23 (TUB^{r}DAP^r)	−	±	−
Chinese hamster ovary (CHO)	Wild type	+	+	+
	AR7‡	+	+	−
	TUB^r	+	+	−
	DAP12§	+	−	+
	DAP12TUB^r	+	−	−
	AR7DAP^r	+	−	−
HeLa	Wild type	+	+	+
L929 mouse	Wild type	+	+	+
P388 mouse leukemia	Wild type	+	+	+
L1210 mouse leukemia	Wild type	+	+	+

* TUB^r and DAP^r variants of Novikoff cells were selected from a HPRT-deficient subline (1-9-1, Ref. 5) by growth in suspension culture with increasing concentrations of tubercidin (TUB) and 2,6-diaminopurine (DAP) to maximum concentrations of 10 and 500 μM respectively. Resistant variants of CHO cells were selected in the same manner, except that the cells were grown in monolayer culture.

† + = wild type phenotype; − = <2% and ± = 5–20% of the activity of wild type cells as determined by appropriate assays of cell-free extracts. AK, adenosine kinase.

‡ An adenosine-resistant variant, supplied by Dr. G. F. Whitmore [6].

§ A DAP^r variant supplied by Dr. M. W. Taylor [7].

ated with a compound distinct from 5′-dAdo. The radiolabeled contaminant was not taken up by the cells, but all experiments reported here were conducted with [5′-^{3}H]5′-dAdo purified (≥ 98%) by HPLC, because the contaminant interfered with the chromatographic quantitation of the metabolic products of 5′-dAdo.

Chemical degradation of labeled 5′-dAdo was used to help identify metabolic products. For acid hydrolysis, [5′-^{3}H]5′-dAdo was mixed with unlabeled 5′-dAdo and HCl to final concentrations of 9 mM (7.4 μCi/ml) and 0.066 N respectively. Conversion of 5′-dAdo to adenine was monitored by HPLC and was complete after 20 min of incubation at 37°. (Incubation with 0.6 N HCl at 100° resulted in extensive degradation of the sugar moiety.) For deamination, [5′-^{3}H]5′-dAdo was mixed with unlabeled 5′-dAdo, glacial acetic acid and $NaNO_2$ to final concentrations of 2.5 mM (1 μCi/ml), 1.8 N and 1 M respectively [13]. Deamination was monitored by a decrease of absorbance at 258 nm and was complete after 100 min of incubation at 23°.

Cell lysates were prepared by sonicating a suspension of about 1×10^8 cells/ml of a buffer containing 10 mM NaCl, 1.5 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM triethanolamine and 10 mM Tris–HCl, pH 7.4 [11]. The lysate was centrifuged at 10^5 *g* for 30 min. The supernatant fraction was dialyzed overnight against a solution of 12 mM triethanolamine, 0.6 mM $MgCl_2$ and 0.06 mM EDTA (pH 7.5) and then assayed for 5′-dAdo phosphorolysis at 37°. Enzymatic conversion of 5′-dAdo was assayed by HPLC and by the spectrophotometric method of Saverese *et al.* [3]. The rate of adenine (Ade) production as monitored by HPLC was ten times faster than the rate of dihydroxyadenine production as measured in the xanthine oxidase-coupled assay. The discrepancy is probably to be accounted for by the inhibitory effect of the steady-state adenine concentrations (~40 μM) which are obtained in the coupled reaction. Activities reported here are those determined in the HPLC assay. For assay by HPLC, reaction mixtures of 200 μl contained 50 mM triethanolamine, pH 7.4, 10 mM NaH_2PO_4 (adjusted to pH 7.4), 5 mM 5′-dAdo and cell sap from about 5×10^7 cells. Aliquots of 50 μl were removed immediately after starting the reaction with 5′-dAdo, and 5′-dAdo and Ade were separated and quantitated by HPLC as described above. Parallel reactions were run without phosphate; in no case was any conversion of 5′-dAdo evident in the absence of phosphate.

Similar HPLC analysis confirmed that 5′-dAdo is not acted upon by purine nucleoside phosphorylase or adenosine deaminase purified from bovine spleen (Sigma Chemical Co., St. Louis, MO).

RESULTS AND DISCUSSION

We have investigated the uptake of [5′-^{3}H]5′-dAdo by various mammalian cell lines and mutants thereof that are deficient in adenosine kinase, HPRT or APRT or a combination of these activities. The cell lines and mutants, and their properties, are summarized in Table 1, and typical time courses of [5′-^{3}H]5′-dAdo uptake are illustrated in Fig. 1. Wild type Novikoff, CHO and HeLa cells rapidly accumulated radioactivity to high levels; after 20 min of incubation, between 50 and 80% of the input radioactivity was cell-associated. A deficiency of Novikoff or CHO cells in adenosine kinase or HPRT had little or no effect on the ability of the cells to accumulate radioactivity from [5′-^{3}H]5′-dAdo, but a deficiency in APRT greatly reduced uptake (Fig. 1A–C). Similar results were obtained with adenosine kinase-deficient and double mutants of CHO cells of different origin (see Table 1). The extent of

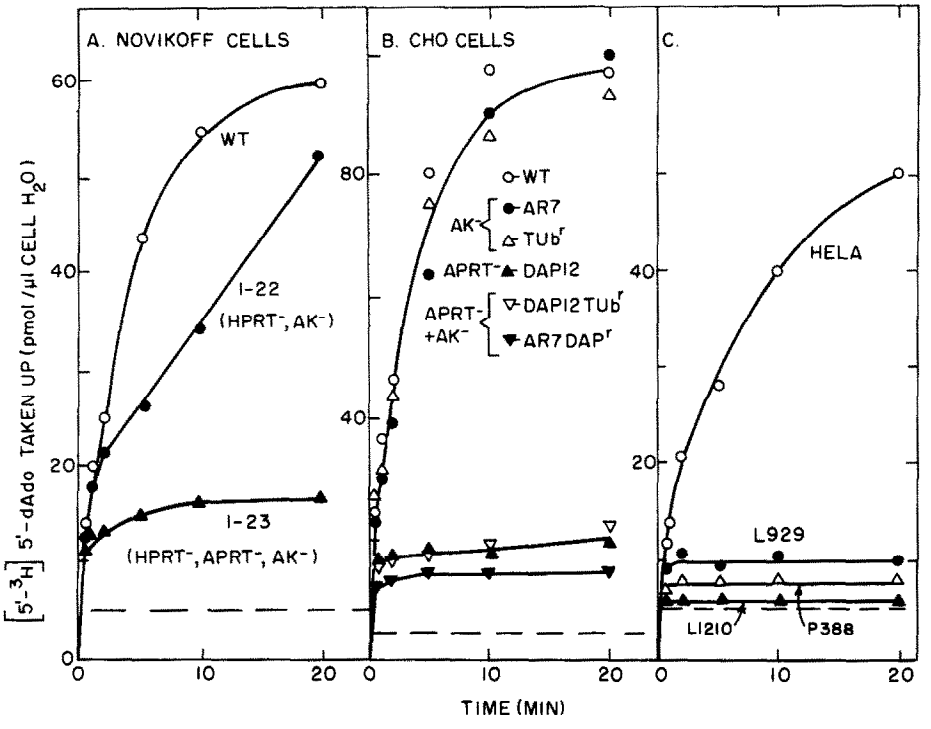

Fig. 1. Uptake of radioactivity from [5′-^{3}H]5′-dAdo by various cell lines and their enzyme-deficient variants. Suspensions of (0.2 to 2) × 10^7 cells/ml of basal medium were supplemented with 5 μM [5′-^{3}H]5′-dAdo (60–180 cpm/pmole). After various times of incubation at 25°, the cells from 0.5 ml of suspension were collected by centrifugation through oil, and the cell pellets were analyzed for radioactivity. All points represent averages of duplicate samples and have been corrected for radioactivity trapped in the extracellular space of cell pellets. The broken line indicates the extracellular concentration of 5-dAdo at zero time. After 20 min of incubation, replicate samples of cells were also collected by centrifugation and extracted in a boiling solution of EDTA, and the extracts and the cell-free medium were analyzed chromatographically (see Fig. 3).

accumulation of radioactivity within cells was also low in wild type L929, P388 and L1210 cells (Fig. 1C); radioactivity accumulated rapidly in these cells (within 1 min) to a level slightly above that in the medium (indicated by the broken line), but then remained constant.

Uptake of radioactivity from [5′-^{3}H]5′-dAdo by CHO-AR7 cells was strongly inhibited by unlabeled 5′-dAdo, adenine, hypoxanthine, uridine (Fig. 2A), 2′-dAdo and adenosine (Fig. 2B). Cordycepin (3′-dAdo) had about the same effect as 2′-dAdo and adenosine (data not shown), and the inhibition by all three nucleosides was largely prevented by the presence of the adenosine deaminase inhibitor 2′-deoxycoformycin (dCF; Fig. 2B). dCF alone had no significant effect on 5′-dAdo uptake (cf. Fig. 2A and 2B). Essentially identical effects as those shown in Fig. 2 were observed for wild type CHO and Novikoff cells (data not shown).

The results of chromatographic fractionation of the extracellular and intracellular radiolabeled compounds may be summarized as follows. In control AR7 cells, most of the intracellular radioactivity was associated with a compound that failed to migrate in solvent 30 (Fig. 3A) and was adsorbed to polyethyleneimine-impregnated cellulose (data not shown). These properties are typical for phosphorylated compounds and, in agreement with this conclusion, treatment of this radiolabeled component with alkaline phosphatase altered its behavior in both tests. We conclude that it represented 5-deoxyribose-1-phosphate. This conclusion was substantiated by the finding that after alkaline phosphatase treatment practically all of the radioactivity comigrated in solvent 30 with the radiolabeled product of gentle acid hydrolysis of [5′-^{3}H]5′-dAdo (Fig. 4A and B), presumably 5-deoxyribose.

Most of the extracellular radioactivity remaining after 20 min of incubation was associated with unaltered 5′-dAdo (Fig. 3B), but some radiolabeled 5-deoxyribose-1-phosphate also seemed to be present in addition to a labeled component recovered in fractions 6–8, which comigrated with the product of chemical deamination of [5′-^{3}H]5′-dAdo and thus presumably was 5′-deoxyinosine (Fig. 4C). In agreement with these conclusions, adenosine, 2′-dAdo and hypoxanthine (and 3′-dAdo) all strongly inhibited the intracellular accumulation of total radioactivity (Fig. 2), and of labeled 5-deoxyribose-1-phosphate (Fig. 3), while gently stimulating the intracellular production of the compound migrating in fractions 6–8 and its accumulation in the medium (shown for 2′-dAdo in Fig. 3E and F). We have calculated the intracellular and extra-

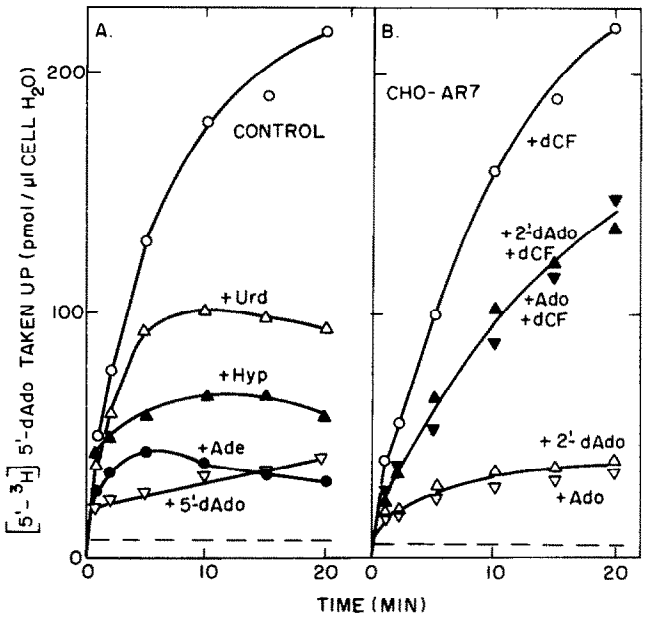

Fig. 2. Effects of various nucleosides and purine bases on the uptake of radioactivity from [5′-^{3}H]5′-dAdo by CHO-AR7 cells. Uptake was measured as in Fig. 1, except that, where indicated, samples of cell suspensions were supplemented with 50 μM 5′-dAdo, 2′-dAdo, adenosine (Ado), adenine (Ade), hypoxanthine (Hyp), uridine (Urd) and dCF along with radiolabeled substrate.

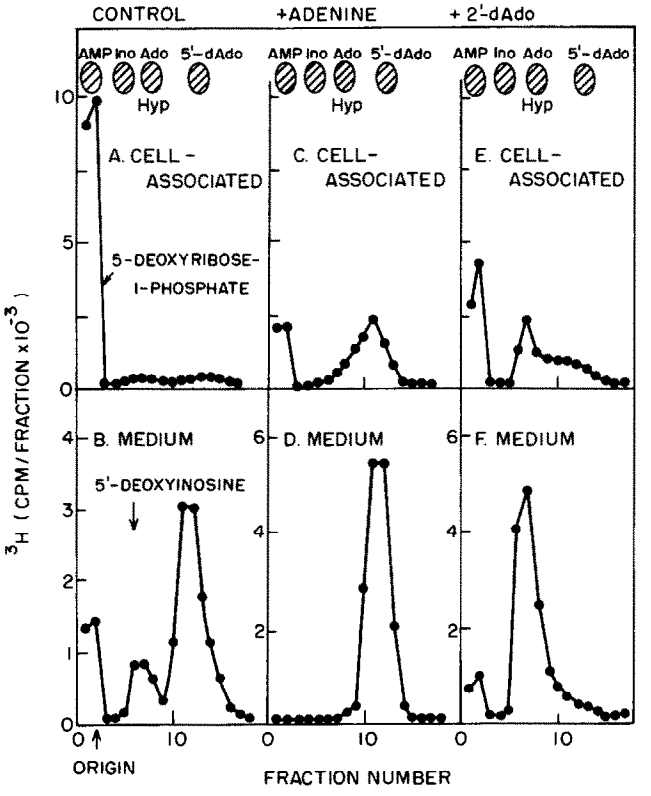

Fig. 3. Chromatographic separation of radiolabeled compounds from cultures of CHO-AR7 cells after a 20-min incubation with 5 μM [5′-^{3}H]5′-dAdo. The samples were from the experiment illustrated in Fig. 2 and show chromatographic profiles for radioactivity associated with the cells and the extracellular medium of control cells (A and B), and suspensions supplemented with 50 μM adenine (C and D) or 2′-dAdo (E and F). The positions of appropriate unlabeled markers are indicated on top of each set of profiles.

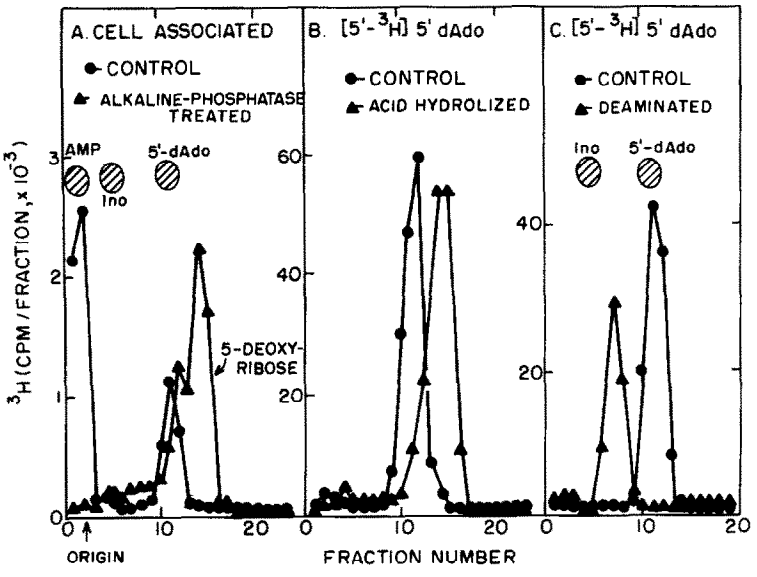

Fig. 4. Chromatographic separation of cell-associated radioactivity before and after incubation with alkaline phosphatase (A), of [5′-^{3}H]5′-dAdo and of products formed from it by acid hydrolysis (B), and chemical deamination (C). An EDTA extract was prepared from CHO-AR7 cells after 20 min of incubation with 5 μM [5′-^{3}H]5′-dAdo and a sample thereof was incubated with 2 units of alkaline phosphatase (Sigma Chemical Co.) per ml at 37° for 10 min. Acid hydrolysis and chemical deamination of [5′-^{3}H]5′-dAdo were conducted as described in Materials and Methods. All materials were chromatographed with solvent 30.

cellular concentrations of labeled components (expressed per μl cell H_2O) and these are summarized in Table 2. Inhibition of adenosine deaminase by dCF prevented these effects of adenosine and 2′-dAdo (Table 2) and of 3′-dAdo (data not shown), as well as the ability of them to diminish the intracellular accumulation of radioactivity from 5′-dAdo shown in Fig. 2. Thus, deamination of adenosine, 2′-dAdo and 3′-dAdo seemed to be prerequisite to their observed effects. The presence of adenine almost completely inhibited the metabolism of 5′-dAdo (Fig. 3C and D and Table 2). Uridine greatly inhibited the accumulation of 5-deoxyribose-1-phosphate from 5′-dAdo, but slightly increased the formation of 5′-deoxyinosine (Table 2). Time courses of the formation of the various intracellular and extracellular products of 5′-dAdo metabolism in control CHO-AR7 cells and in the presence of 2′-dAdo are illustrated in Fig. 5.

Wild type and 1-22 Novikoff cells and wild type

Table 2. Concentrations of labeled 5′-dAdo, 5-deoxyribose-1-phosphate (5-dR-1-P) and 5′-deoxyinosine (5′-dIno) after 20 min of incubation of CHO-AR7 cells and wild type Novikoff cells with 5 μM [5′-^{3}H]5′-dAdo in the absence and presence of 50 μM concentrations of various nucleosides and nucleobases*

		Labeled compounds (pmoles/μl cell H_2O)						
		Cell-associated			Cell-free medium			Total products
Cells	Additions	5-dR-1-P	5′-dIno	5′-dAdo	5-dR-1-P	5′-dIno	5′-dAdo	(5-dR-1-P + 5′-dIno)
CHO-AR7	None	186	13	18	30	19	135	248
	2′-dAdo	22	15	4	14	203	112	264
	2′-dAdo + dCF	96	22	18	15	38	199	171
	Adenosine	20	13	4	28	204	120	265
	Adenosine + dCF	106	23	23	19	28	230	176
	Hypoxanthine	18	8	2	22	238	124	286
	Uridine	76	10	3	23	67	194	176
	Adenine	9	2	19	7	7	344	25
N1S1-67-WT	None	106	4	13	6	2	3	118
	Adenosine	70	7	7	7	48	3	132
	Adenosine + dCF	102	4	13	4	2	8	112
	Hypoxanthine	33	9	2	9	81	3	132

* Values for CHO-AR7 cells are from the experiment illustrated in Fig. 2. Those for wild type Novikoff cells (N1S1-67-WT) were obtained in a similar experiment. Intracellular and extracellular concentrations of labeled compounds are expressed as pmoles/μl cell H_2O to facilitate comparison and were calculated on the basis of the total amounts of radioactivity taken up by the cells and remaining in the medium and its distribution in the various chemical species as indicated by analysis with solvent 30 (see Fig. 3).

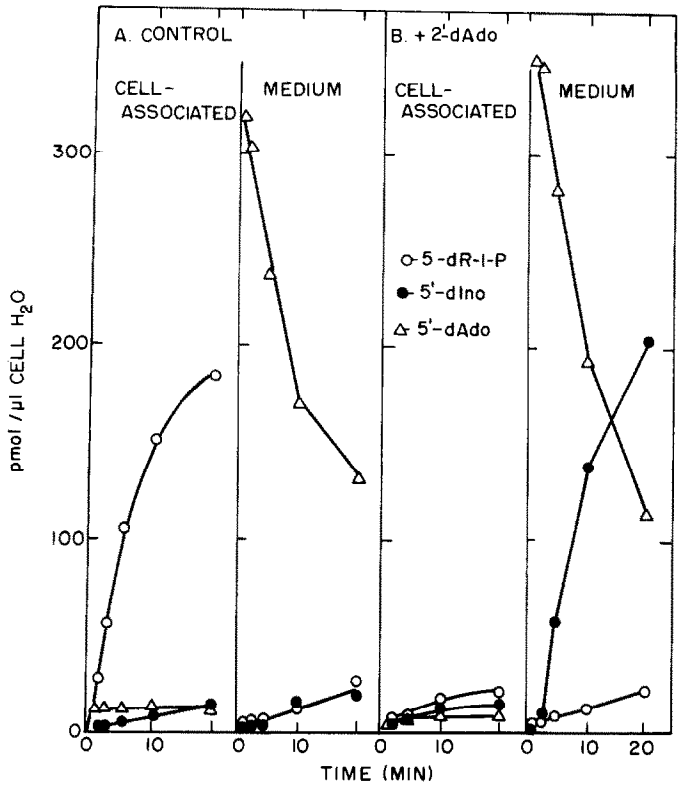

Fig. 5. Time courses of metabolism of 5'-dAdo by CHO-AR7 cells in the absence and presence of 2'-dAdo. The experiment was conducted as illustrated in Fig. 2. At the indicated times, the cells from additional samples were extracted with a boiling solution of EDTA, and samples of the extracts and the cell-free medium were chromatographed with solvent 30 (see Fig. 3). The concentrations of 5'-dAdo (△—△), 5'-deoxyinosine (5'-dIno, ●—●) and 5-deoxyribose-1-phosphate (5-dR-1-P, ○—○) were calculated on the basis of the total amounts of radioactivity taken up by the cells (see Fig. 2) and remaining in the medium and the chromatographic separations. To facilitate comparison, the concentrations in both the cells and the medium are expressed per µl of cell H_2O.

CHO cells behaved similarly to CHO-AR7 cells: radioactivity accumulated intracellularly mainly in the form of 5-deoxyribose-1-phosphate, and concurrent addition of adenine nucleosides stimulated the extracellular accumulation of 5'-deoxyinosine (Table 1 and data not shown).

APRT-deficient variants of these cells behaved quite differently: the intracellular accumulation of labeled 5-deoxyribose-1-phosphate was reduced 90–95% and little 5'-deoxyinosine was detectable in the culture fluid (data not shown). The extent of metabolism of 5'-dAdo observed in cultures of L1210, P388 and L929 cells resembled that in APRT-deficient CHO and Novikoff cells; after 20 min of incubation only 5–10% of the intracellular radioactivity was associated with 5-deoxyribose-1-phosphate and the amounts in the medium of 5'-deoxyinosine were insignificant.

The metabolic inertness of 5'-deoxyadenosine in L1210, P388 and L929 cells is explained by their lack of 5'-dAdo phosphorylase activity. The activities of cell extracts from CHO, Novikoff and HeLa cells fell between 8 and 40 nmoles 5'-dAdo converted to adenine per mg cytosol protein per min at 37°, considerably higher than the activity reported [3] for S180 cells (but see technical note on assay above). The activity of CHO cells deficient in adenosine kinase (AR7) or in both adenosine kinase and APRT (DAP12TUB^r) was about the same as that of wild type cells. No activity was observed in the absence of phosphate. The activities of extracts from P388, L1210 and L929 cells were <5% of that of the other cell lines.

Variations in the uptake of 5'-dAdo in the other cell lines, all of which have 5'-dAdo phosphorylase activity, and the influences of other nucleosides on that uptake, may be rationalized in reference to the pathways depicted in Fig. 6. The phosphorolysis of [5'-^{3}H]5'-dAdo yielded unlabeled adenine and labeled 5-deoxyribose-1-phosphate, which mainly accumulated intracellularly, because the membrane was largely impermeable to negatively charged phosphorylated compounds. A small amount also accumulated in the medium with time of incubation (Fig. 5B), but whether as a result of lysis of a small proportion of the cells or of secretion cannot be decided on the basis of the present data. A continuing phosphorolysis of 5'-dAdo was dependent on the removal of the product adenine by phosphoribosylation. This is indicated by the limited phosphorolysis

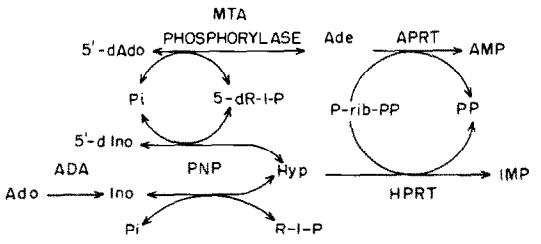

Fig. 6. Postulated pathways for the metabolism of 5′-dAdo in cells that possess MTA phosphorylase. Abbreviations: Ade, adenine; 5′-dIno, 5′-deoxyinosine; Ado, adenosine; Ino, inosine; Hyp, hypoxanthine; 5-dR-1-P, 5-deoxyribose-1-phosphate; R-1-P, ribose-1-phosphate; ADA, adenosine deaminase; and PNP, purine nucleoside phosphorylase.

of 5′-dAdo observed in cell variants that lack APRT and by the direct inhibition of phosphorolysis of 5 μM 5′-dAdo observed in the presence of 50 μM adenine.

The phosphorolytic cleavage of MTA in extracts from animal cells is also inhibited by adenine [14, 15], in a degree markedly dependent on the concentration of P_i [14]. This suggests that adenine exerts product inhibition of the MTA phosphorylase (K_m with respect to 5′-dAdo is 23 μM; cf. Ref. 3) as well as supporting the reverse reaction. The absence of APRT would result in much higher steady-state levels of adenine, and, consequently, greater inhibition of phosphorolysis of 5′-dAdo (and of MTA). Addition of 50 μM adenine exogenously would saturate APRT (K_m with respect to adenine about 1 μM; cf. Ref. 16) and thus have the same effect.

In contrast to the product inhibition by adenine, the accumulation of 5-deoxyribose-1-phosphate seems to have little effect on the phosphorolysis of 5′-dAdo. Although 5-deoxyribose-1-phosphate is readily degraded by alkaline phosphatase in cell-free extracts, we have observed little degradation to 5-deoxyribose in whole cells during 20 min of incubation. The usual metabolic route for ribose-1-phosphate, namely isomerization to ribose-5-phosphate, is precluded by absence of a 5-hydroxyl. 5-Deoxyribose-1-phosphate, however, seems to be an efficient substrate for purine nucleoside phosphorylase, giving rise to the formation of labeled 5′-deoxyinosine from [5′-^{3}H]5′-dAdo. Its formation is slow under normal conditions, probably because of the limited availability of hypoxanthine in cells, but is greatly stimulated by the addition of hypoxanthine or of adenine nucleosides which can give rise to hypoxanthine. The overall scheme is supported by our finding that the adenine nucleosides must be deaminated to exert their effects. Also, there seems to be a slight lag period in the extracellular accumulation of 5′-deoxyinosine suggesting a precursor–product relationship between 5-deoxyribose-1-phosphate and 5′-deoxyinosine. A direct deamination of 5′-dAdo to 5′-deoxyinosine is ruled out by a lack of effect of dCF on the metabolism of 5′-dAdo in CHO-AR7 cells (Fig. 2) and by studies with cell-free preparations or adenosine deaminase (Refs. 1 and 3, and the present paper). Similar evidence has been presented to indicate a sequential metabolism of 5′-isobutylthioadenosine by MTA phosphorylase and purine nucleoside phosphorylase to 5′-isobutylthioinosine [17]. Furthermore, 5′-deoxyinosine has been demonstrated to be an efficient substrate for purified human erythrocyte purine nucleoside phosphorylase [18]. All these results raise the likelihood that 5-methylthioribose-1-phosphate formed in the phosphorolysis of MTA may also be a cosubstrate for purine nucleoside phosphorylase resulting in the formation of 5′-methylthioinosine in the presence of hypoxanthine.

There is little doubt that 5′-dAdo is an efficient substrate for the nucleoside transporter of mammalian cells ([1]; P. G. W. Plagemann and R. M. Wohlhueter, unpublished results). The same most likely is true for 5′-dIno, which therefore is rapidly transported out of the cells upon its formation and accumulates in the medium until an intracellular–extracellular equilibrium is attained. Hypoxanthine, however, may have an additional adverse effect on 5′-dAdo phosphorolysis, namely by competing in its phosphoribosylation with adenine for cellular P-rib-PP. This effect, however, must be of minor importance, since even greater total amounts of 5′-dAdo became metabolized in the presence of hypoxanthine or adenine nucleosides than in their absence (Fig. 5 and Table 2).

One effect we have no explanation for at present is the strong inhibition of 5′-dAdo phosphorolysis by uridine. With regard to Novikoff cells, one could argue that uridine phosphorylase may function in the same manner as discussed already for purine nucleoside phosphorylase, but uridine is equally effective in inhibiting 5′-dAdo phosphorolysis in CHO cells, which lack uridine phosphorylase [19].

We have no direct evidence that the degradation of 5′-dAdo in Novikoff, CHO and HeLa cells is catalyzed by MTA phosphorylase, but this conclusion seems likely because L1210 and P388 cells which have been shown to lack MTA phosphorylase [3, 14] failed to degrade 5′-dAdo. We have also shown that the cleavage of 5′-dAdo in cell-free extracts of the various cell types is strictly dependent on the presence of phosphate and that cell-free extracts from P388, L1210 and L929 cells exhibit <5% of the activity observed in Novikoff and CHO cell extracts. But even in these cells some phosphorolysis of 5′-dAdo occurs. It is obvious, therefore, that caution is required in the use of 5′-dAdo as a nucleoside transport substrate.

Acknowledgements—The authors thank John Erbe and Wayne Brown for competent technical assistance and Colleen O'Neill for excellent secretarial help. This work was supported by USPHS Research Grant GM 24468.

REFERENCES

1. D. Kessel, *J. biol. Chem.* **253**, 400 (1978).
2. P. G. W. Plagemann and R. M. Wohlhueter, *Curr. Topics Membr. Transp.* **14**, 225 (1980).
3. T. M. Savarese, G. M. Crabtree and R. E. Parks, Jr., *Biochem. Pharmac.* **30**, 189 (1981).
4. A. B. Guranowski, P. K. Chiang and G. L. Cantoni, *Eur. J. Biochem.* **114**, 293 (1981).

5. J. A. Zylka and P. G. W. Plagemann, *J. biol. Chem.* **250**, 5756 (1975).
6. M. W. McBurney and G. F. Whitmore, *J. cell. Physiol.* **85**, 87 (1975).
7. F. R. Witney and M. W. Taylor, *Biochem. Genet.* **16**, 917 (1978).
8. G. A. Ward and P. G. W. Plagemann, *J. cell. Physiol.* **73**, 213 (1969).
9. P. G. W. Plagemann and R. D. Estensen, *J. cell. Physiol.* **104**, 105 (1980).
10. E. L. Schneider, E. J. Stanbridge and C. J. Epstein, *Expl Cell Res.* **84**, 311 (1974).
11. P. G. W. Plagemann, *J. cell. Physiol.* **77**, 213 (1971).
12. R. M. Wohlhueter, R. Marz, J. C. Graff and P. G. W. Plagemann, *Meth. Cell Biol.* **20**, 211 (1978).
13. N. O. Kaplan, *Meth. Enzym.* **3**, 873 (1957).
14. J. I. Toohey, *Biochem. biophys. Res. Commun.* **83**, 27 (1978).
15. A. J. Ferro, N. C. Wrobel and J. A. Nicolette, *Biochim. biophys. Acta* **570**, 65 (1979).
16. R. M. Wohlhueter and P. G. W. Plagemann, *Int. Rev. Cytol.* **64**, 171 (1981).
17. N. Kamati, E. H. Willis and D. A. Carson, *Biochem. biophys. Res. Commun.* **104**, 1335 (1982).
18. J. D. Stoeckler, C. Cambor and R. E. Parks, Jr., *Biochemistry* **19**, 102 (1981).
19. P. G. W. Plagemann, R. M. Wohlhueter and J. Erbe, *Biochim. biophys. Acta* **640**, 448 (1981).