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# Mycoplasma Contamination Alters 2′-Deoxyadenosine Metabolism in Deoxycoformycin-Treated Mouse Leukemia Cells

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Deoxycoformycin-treated P388 and L1210 mouse leukemia cells salvage 2′-deoxyadenosine from the medium only inefficiently, because deoxyadenosine deamination is blocked and its phosphorylation is limited by feedback controls. Mycoplasma contamination at a level that had no significant effect on the growth of the cells increased the salvage of deoxyadenosine >10 fold over a 90 min period of incubation at 37°C, but in this case deoxyadenosine was mainly incorporated into ribonucleotides and RNA via adenine formed from deoxyadenosine by mycoplasma adenosine phosphorylase. Deoxyadenosine was an efficient substrate for this enzyme, in contrast to 2′,3′-dideoxyadenosine which was not phosphorolyzed. Mycoplasma infection was confirmed by the presence of uracil phosphoribosyltransferase activity and by culture isolation. The contaminant has been identified as *Mycoplasma orale*. Mycoplasma infection had no effect on the deamination and phosphorylation of deoxyadenosine and adenosine, on the salvage of hypoxanthine and adenine, or on the degradation of dAMP and dATP by the cells or on their acid and alkaline phosphatase activities.

**Key words: deoxyadenosine metabolism, adenosine phosphorylase**

Deoxyadenosine (dAdo) salvage by mammalian cells is generally quite rapid, but only because dAdo is rapidly deaminated in most mammalian cells and converted intracellularly mainly to ribonucleotides via deoxyinosine (dIno)→hypoxanthine (Hyp)→IMP [1]. When adenosine (Ado) deaminase is blocked genetically or chemically, increased amounts of dAdo become converted to dATP with resulting cytotoxicity [2]. However, dAdo salvage, which, under these conditions, can be considered a two step process comprised of carrier mediated transport across the plasma membrane followed by intracellular phosphorylation [3–5], is relatively inefficient in mammalian cells [2]. Salvage activity is low presumably due to feedback inhibition of deoxycytidine (dCyd) and Ado kinases, which have been implicated in the phosphorylation of dAdo [6–8]. However, previous studies have shown that the salvage of dAdo as well as its cytotoxicity

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in Ado deaminase-deficient or inhibited human lymphoma cells or mouse L1210 leukemia cells is stimulated rather than inhibited by high concentrations of dipyridamole [2,9,10], a potent inhibitor of nucleoside transport in these cells [2,5]. We also observed that dAdo salvage was much more rapid in another mouse leukemia cell line, P388, than in L1210 cells, and inhibited by dipyridamole [2]. In spite of this increased salvage, P388 cells were about 10 times more resistant to dAdo cytotoxicity than L1210 cells. We have now discovered that this high dAdo salvage activity was due to contamination with *Mycoplasma orale,* which, like all mycoplasmas, possesses Ado phosphorylase activity [11–15]. Our results show that this enzyme also catalyzes the phosphorolysis of dAdo and the resulting adenine (Ade) is rapidly salvaged by the P388 cells. This mycoplasma contamination had no significant effect on the growth rate of the P388 cells and was not readily detectable by the uridine (Urd)/uracil (Ura) incorporation method used for the detection of mycoplasma in cell cultures [16–18], even though the mycoplasma possessed considerable Ura phosphoribosyltransferase activity, another mycoplasma-specific enzyme [15,18–21].

## MATERIALS AND METHODS

### Cell Culture

P388 and L1210 mouse leukemia cells have been propagated in this laboratory for over 10 years [22]. The P388 line has been recently found to be contaminated with mycoplasma and is designated P388-M. A new line (designated P388 in this paper) has been recently established from a culture obtained from the American Type Culture Collection. All cells were propagated in suspension culture in Eagle's minimum essential medium (MEM) for suspension culture supplemented with non-essential amino acids, sera, and extra D-glucose as described previously [2]. The cells were enumerated in a Coulter counter.

### Mycoplasma Assays

Several biochemical methods were used to assay cell cultures for mycoplasmas. One assay was based on the Urd/Ura incorporation method [16,17] (see Table I). Alternatively, cultures were directly analyzed for Ado phosphorylase or Urd phosphoribosyltransferase activities. The cells from 10 ml of stationary phase cultures (2–3 $\times$ $10^6$ cells/ml) were collected by centrifugation at 500*g* for 2 min and suspended in 0.2 ml of a lysing buffer (10 mM Tris-HCl, pH 7.4, 2 mM dithiothreitol, and 25 $\mu$M deoxycoformycin). The supernatant was centrifuged at 10,000*g* for 30 min and the pellet was also suspended in 0.2 ml of lysing buffer. Or 10 ml of cell culture was directly centrifuged at 10,000*g* for 30 min and the pellet suspended in lysing buffer. For the assay of Ado phosphorylase activity, 100 $\mu$l of lysate was mixed with 100 $\mu$l of 10 $\mu$M [$^3$H]dAdo in 100 mM phosphate buffer, pH 7.4. Where indicated, 10 $\mu$M [$^3$H]ddAdo was substituted for [$^3$H]dAdo. At various times of incubation at 37°C, 40 $\mu$l of reaction suspension was diluted with 160 $\mu$l of ice-cold $H_2O$ and the mixture immediately spotted onto 3MM Whatman chromatographic paper. The paper was chromatographed as described previously [23] with a solvent composed of 39 ml butanol, 22 ml ethyl acetate, 22 ml ammonium hydroxide, and 17 ml methanol (solvent 40), which separates dAdo, Ado, Ade, Hyp/inosine (Ino), and nucleotides. The lysates were assayed for Ura phosphoribosyltransferase activity [20,24] in the same manner, except that the reaction mixture

**TABLE I. Comparison of the Incorporation of 1 μM [$^3$H]uridine and [$^3$H]uracil (~400 cpm/pmol) by P388 (L1210) and P388-M Cells From Late Exponential Suspension Cultures at 37°C Under Different Experimental Conditions***

| Expt. No. | Cells | Cells/ml ($\times 10^6$) | Medium | Incub. period (h) | Substrate incorporation (pmol/$10^6$ cells) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Uridine | Uracil | Urd/Ura |
| 1 | P388 | 22 | A | 0.5 | 36 | 1.0 | 36 |
| | P388-M | 18 | A | 0.5 | 28 | 1.3 | 22 |
| | P388-M | 18 | A† | 0.5 | 38 | 1.4 | 27 |
| 2 | P388 | 10 | A | 1 | 17 | 0.13 | 131 |
| | P388-M | 8 | A | 1 | 21 | 1.5 | 14 |
| 3 | L1210 | 1.8 | B | 1 | 27 | 1.0 | 27 |
| | P388 | 1.3 | B | 1 | 25 | 1.2 | 21 |
| | P388-M | 1.3 | B | 1 | 10 | 2.6 | 3.8 |
| | P388-M | 1.4 | B | 1 | 7 | 2.2 | 3.1 |
| 4 | P388 | 1.5 | C | 24 | 36 | 11 | 3.3 |
| | P388-M | 1.1 | C | 24 | 96 | 34 | 2.8 |
| | P388-M | 1.1 | C | 24 | 91 | 75 | 1.2 |

*In Expt. 1 the cells were collected by centrifugation and suspended to the indicated cell density in serum free medium (A), and after 30 min of incubation, samples of the cells were collected by centrifugation through oil and analyzed for total $^3$H associated with the cells. A† indicates that the cells had been grown for 1 day before the assay in antibiotic-free medium. Expt. 2 was conducted in the same manner, except that after 1 h of incubation samples of the suspensions were analyzed for $^3$H in acid-insoluble material. In Expt. 3, growing cultures in growth medium (B) were supplemented with [$^3$H]Urd or [$^3$H]uracil and samples of the suspensions were analyzed for $^3$H in acid-insoluble material after 1 h of incubation. In Expt. 4 cells were collected by centrifugation and suspended to the indicated densities in RPMI containing 2% (v/v) fetal bovine serum (C). The suspensions were incubated for 24 h and samples thereof analyzed for $^3$H in acid-insoluble material. The cell densities did not increase significantly during the incubation period. All values are averages of duplicate samples. In Expt. 1, 3, and 4 duplicate independent cultures of P388-M were analyzed.

contained 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 50 mM KCl, 100 mM Tris-HCl (pH 7.5), 10 mM phosphoribosyl pyrophosphate, and 50 μM [$^3$H]Ura; the reactions were stopped by the addition of trichloroacetic acid to 0.2 N; and Ura and UMP were then separated by paper chromatography with a solvent composed of 30 ml 1 M ammonium acetate (pH 5) and 70 ml 95% ethanol (solvent 28).

The mycoplasmas present in P388-M cultures have been isolated and identified by immunological methods as *M. orale* by Dr. G. Kenny (Department of Pathobiology, University of Washington).

## Salvage of Radiolabeled dAdo and Purines

Cells harvested from mid to late exponential phase cultures (1.5 to 2.5 $\times 10^6$ cells/ml) were suspended to 1 to 4 $\times 10^7$ cells/ml of basal medium 42B (BM42B; [23]) containing, where indicated, 20–25 μM 2-deoxycoformycin. After 5 min of incubation at 37°C, the suspensions were supplemented with radiolabeled substrate as indicated in appropriate experiments. The suspensions were incubated at 37°C and at specified times the cells from 0.5 ml samples of suspension were separated from the medium by centrifugation through an oil mixture as described previously [2,25] and analyzed for radioactivity. Where indicated, replicate samples of cell suspension were analyzed for radioactivity in acid-insoluble material or for radioactivity in RNA and DNA as described previously [1]. For an analysis of other metabolic products, additional samples of cells were collected by centrifugation through oil and immediately extracted with 60%

(v/v) ethanol at −20°C [26,27]. The ethanol extracts were analyzed by ascending paper chromatography with solvent 28 which separates dATP, dAMP, and dAdo (plus Ade). For ascertaining the effectiveness of the deoxycoformycin treatment, the cell-free medium of cultures incubated with radiolabeled dAdo was removed immediately upon centrifugation of the cell suspension samples, and acidified and chromatographed with solvent 40 or a solvent composed of 79 ml saturated ammonium sulfate, 19 ml 50 mM phosphate buffer (pH 6), and 2 ml isopropanol (solvent 9; see ref. 1 and 28).

## Metabolism of dAdo and dAdo Phosphates in Cell Free Extracts

Cells suspended to 1 to 4 $\times 10^8$ cells/ml in a hypotonic buffer solution were disrupted by sonication as described previously [22] and the sonicate was clarified of cell debris by centrifugation at 10,000*g* for 30 min. The supernatant was assayed for various activities as follows.

For measuring dAdo deamination, clarified cell extracts were incubated at 37°C with 25 $\mu$M [$^3$H]dAdo in a solution composed of 50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 10 mM mercaptoethanol, and 50 mM KCl. Samples of the reaction mixture were heated at 100°C for 1 min and clarified by centrifugation and the supernatant was chromatographed with solvents 9 and 40 for the quantitation of $^3$H in dAdo, dIno, and Hyp.

For measuring the phosphorylation of dAdo, clarified cell extracts were preincubated with 50 $\mu$M deoxycoformycin and then incubated with 200 $\mu$M [$^3$H]dAdo in a solution composed of 100 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 4 mM dithiothreitol, 10 mM ATP, 10 mM NaF, 10 mM phosphoenolpyruvate, and 100 units pyruvate kinase/ml. At the specified times of incubation at 37°C, samples of the reaction mixture were diluted 5-fold with ice-cold water and duplicate samples thereof were spotted immediately onto DE81 Whatman filter paper discs. The filter discs were air dried, washed 3 times for 5 min in water, dried at 60°C, and analyzed for radioactivity. The procedure is modified from that described by Sarap and Fridland [8]. Its validity was ascertained by chromatographic analysis of the diluted reaction mixtures with solvent 28.

For measuring the degradation of dAMP and dATP the cells were disrupted in a hypotonic buffer either by sonication or by means of a Dounce homogenizer as described previously [29] and the cell lysates were centrifuged at 10,000*g* for 30 min. The supernatant fraction was removed and the pelleted material was suspended in buffer solution. The degradation of dAMP and dATP was measured in reaction mixtures composed of 100 mM Tris-HCl (pH 7.4), 150 mM KCl, 8 mM $MgCl_2$, 10 mM mercaptoethanol, 10 mM NaF, 1 mM[$^3$H]dATP or [$^3$H]dAMP, and the indicated concentrations of cell fraction. At specified times of incubation at 37°C, samples of the reaction mixture were diluted 5-fold with ice-cold water and duplicate aliquots of the dilution were immediately spotted onto two sheets of 3MM Whatman paper. The papers were developed with solvent 28 for the quantitation of radioactivity in dATP, dAMP, and dAdo (plus other nucleosides and nucleobases) and with solvent 9 or 40 for the quantitation of radioactivity in dIMP, dIno, Ade, and Hyp. For extracting the pools of [$^3$H]dAdo labeled cells or for stopping in vitro reactions involving dAdo and its nucleotides we avoided acids because of their acid lability. When dissolved in 0.5N trichloroacetic acid, [2-8-$^3$H]dATP and [2-8-$^3$H]dAMP at concentrations of 200 $\mu$M became completely degraded in 2 h at 0°C mainly to Ade, whereas [$^3$H]ATP and [$^3$H]AMP were completely stable under these conditions.

### Nucleoside Triphosphate Content of Cells

The acid-soluble pools were extracted from 1–2 $\times 10^7$ cells harvested from late exponential phase cultures with trichloroacetic and perchloric acids and quantitated by high-performance liquid chromatography (HPLC) on a Whatman Reverse Phase Partisil ODS-3, 10 $\mu$m column (4.6 $\times$ 250 mm), and eluted isocratically for 5 min with a solution composed of 10 mM $KH_2PO_4$ (pH 3.5) followed by a linear gradient up to 800 mM $KH_2PO_4$ (pH 4.3) over a time interval of 40 min. The intracellular ribonucleotide concentrations were calculated on the basis of an intracellular water space of 10 $\mu$l/$10^7$ cells.

### Materials

[2,8-$^3$H]dAdo, [2,8-$^3$H]2′,3′-dideoxyadenosine (ddAdo), [2-8-$^3$H]dAMP, [2-8-$^3$H]dATP, [$^3$H-5]Ura, [$^3$H-5]Urd, [8-$^{14}$C]Hyp, and [8-$^{14}$C]Ade were purchased from Moravek Biochemicals, Brea, CA. Dipyridamole (Persantin) and deoxycoformycin (Pentostatin) were gifts from Geigy Pharmaceuticals, Yonkers, NY, and Parke-Davis and Co., Morris Plains, NJ, respectively.

## RESULTS

Figure 1 compares the salvage of dAdo by P388 cells that have been found to be contaminated with mycoplasma (P388-M), by P388 cells that have been recently obtained from the American Type Culture Collection, as well as by L1210 cells, which we have studied previously [2]. In the absence of deoxycoformycin (control), [$^3$H]dAdo salvage was about the same in all three cell lines (Fig. 1A–C) and radioactivity was mainly incorporated into ribonucleotide triphosphates (mainly ATP) and into RNA (data not shown), reflecting the rapid deamination of dAdo in these cells [1,2,22]. Treatment of the cells with 20 $\mu$M deoxycoformycin, which completely inhibited dAdo deamination (data not shown; [22]), had no significant effect on dAdo salvage in P388-M cells (Fig. 1A), whereas it drastically reduced dAdo salvage in P388 and L1210 cells (Fig. 1B,C). Deoxycoformycin-treated P388-M cells also incorporated [$^3$H]dAdo mainly into RNA rather than DNA, whereas incorporation in deoxycoformycin-treated P388 and L1210 cells was mainly into dATP and DNA (data not shown). As shown previously [2], treatment of L1210 cells with the nucleoside transport inhibitor dipyridamole greatly stimulated dAdo salvage in L1210 cells (see Fig. 1C) and its incorporation into dATP and DNA (data not shown), whereas it inhibited the salvage of dAdo by the P388-M cells (see Fig. 1A). Just as in L1210 cells, dAdo salvage by P388 cells was greatly stimulated by the dipyridamole treatment (Fig. 1B).

Combined, the results indicate that in deoxycoformycin-treated P388 cells that are free of mycoplasma, just as in L1210 cells, dAdo is mainly converted to dATP, which presumably mediates dAdo cytotoxicity. The greater resistance of deoxycoformycin-treated P388-M cells to the cytotoxic effects of dAdo [2] seems to correlate with the conversion of dAdo to ribonucleotides. Since the pathway dAdo→dIno→Hyp→IMP was blocked by treatment with deoxycoformycin, the conversion of dAdo to ribonucleotides in P388-M cultures most likely occurred via Ade. However, no free Ade was detectable either in the culture fluid of P388-M cells incubated with [$^3$H]dAdo or in ethanol extracts of these cells. On the other hand, a conversion of dAdo to ribonucleotides via Ade was supported by the finding that the salvage of 20 $\mu$M [$^3$H]dAdo by P388-M

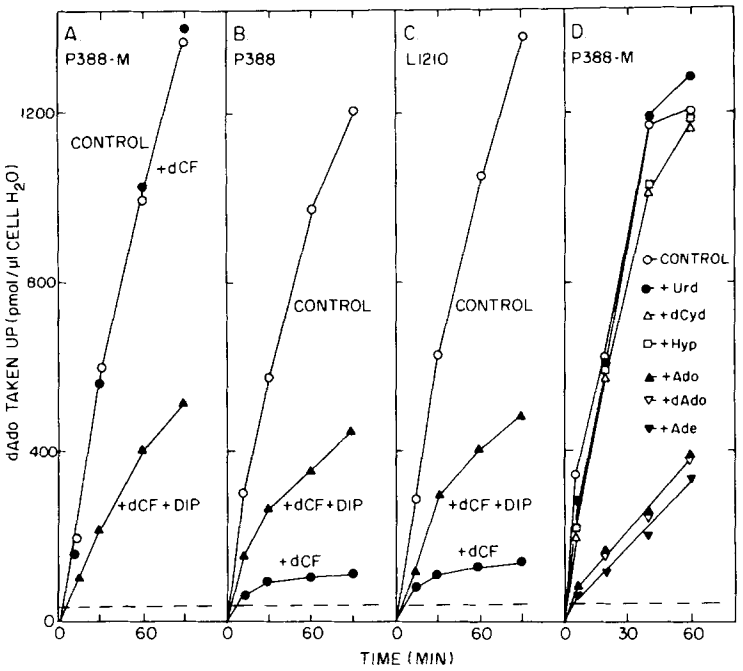


Fig. 1. dAdo uptake by P388-M (**A**), P388 (**B**), and L1210 (**C**) cells in the presence and absence of deoxycoformycin and dipyridamole and effects of various nucleosides and nucleobases on dAdo uptake by deoxycoformycin-treated P388-M cells (**D**). The cells were harvested from late exponential cultures and suspended to about $1 \times 10^7$ cells/ml (A–C) or $4 \times 10^7$ cells/ml (D) in BM42B containing where indicated 10 $\mu$M deoxycoformycin (dCF). After about 5 min of incubation at 37°C, samples of each suspension were supplemented with 20 $\mu$M [2,8-$^3$H]dAdo (50 cpm/pmol) and, where indicated, with 10 $\mu$M dipyridamole (DIP; A–C) or 100 $\mu$M of the indicated nucleosides or nucleobases (D). The uptake of $^3$H was monitored over a 90 or 60 min period at 37°C as described under Materials and Methods. All values are averages of duplicate samples of cell suspension. The broken lines indicate the intracellular concentration of dAdo-equivalents equal to that in the medium at 0 time.

cells was strongly inhibited by 100 $\mu$M unlabeled Ade as well as by 100 $\mu$M unlabeled Ado and dAdo (Fig. 1D), whereas the presence of 100 $\mu$M Hyp, Urd, or dCyd had no significant effect (Fig. 1D). In contrast, the salvage of [$^3$H]dAdo by P388 and L1210 cells that had not been treated with deoxycoformycin is inhibited by unlabeled Hyp, but only little by Ade [27]. The difference between P388 (and L1210 cells) and P388-M cells in dAdo salvage was unrelated to their ability to salvage Hyp or Ade. The rate of uptake of 50 $\mu$M [$^{14}$C]Hyp or [$^{14}$C]Ade was about the same in all three cell lines (50–70 pmol/$\mu$l cell water · sec at 37°C). These relatively high purine salvage activities explain the lack of accumulation of detectable levels of free Ade in cultures of P388-M cells during incubation with [$^3$H]dAdo. Ade was probably salvaged by the cells as rapidly as it was formed.

In other properties too the P388-M cells were indistinguishable from P388 (and L1210) cells. For example, their growth properties were comparable and their activities in transporting [2], deaminating, and phosphorylating dAdo and Ado (Fig. 2 and data now shown), in phosphorolyzing dIno (Fig. 2A), and in degrading dAMP and dATP (Fig. 3) were about the same. dAdo phosphorylation in deoxycoformycin-treated cell

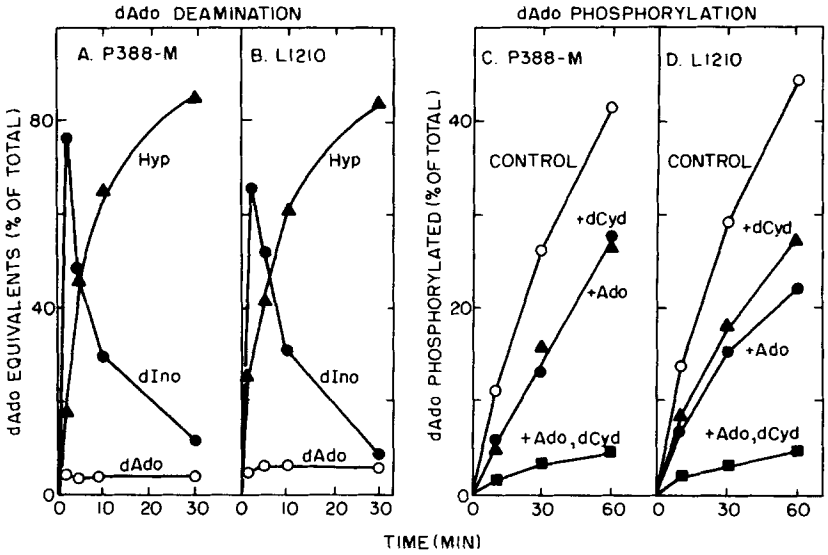


Fig. 2. Deamination (**A** and **B**) and phosphorylation (**C** and **D**) of dAdo by cell free extracts of P388-M and L1210 cells. The cells were disrupted by sonication and the cell debris was removed by centrifugation at 10,000*g* for 30 min. Samples of the supernatant were assayed for the deamination of 25 $\mu$M [$^3$H]dAdo or phosphorylation of 200 $\mu$M [$^3$H]dAdo as described under Materials and Methods. The reactions contained about 8 mg protein of cell extract/ml. Where indicated in C and D, the reactions were supplemented with 2.5 mM Ado, 2.5 mM dCyd, or both.

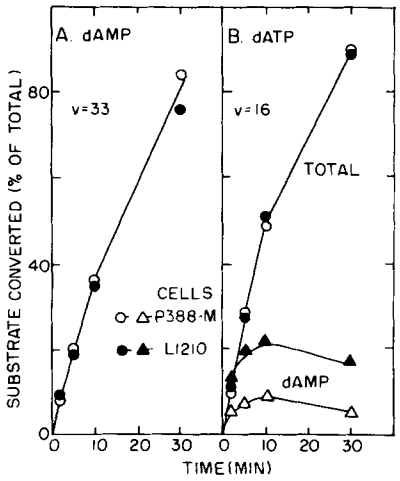


Fig. 3. Degradation of dAMP (**A**) and dATP (**B**) by cell free membrane fractions from P388-M and L1210 cells. The cells were disrupted with a Dounce homogenizer. The lysates were centrifuged at 10,000*g* for 30 min and the pelleted material was suspended in buffer solution. This fraction was assayed for the degradation of [$^3$H]dAMP and [$^3$H]dATP as described under Materials and Methods (protein concentration = 1 mg/ml in A and 3 mg/ml in B). The velocities of hydrolysis of dAMP and dATP (v) were estimated from the initial linear portions of the progress curves and are expressed as nmol/mg protein min.

free extracts was about equally inhibited by Ado and dCyd (Fig. 2B), which is consistent with the view that both Ado kinase and dCyd kinase are involved in the phosphorylation of dAdo [6,7]. Both dATP and dAMP nucleotidase activities were almost exclusively recovered in the particulate membrane fraction when the cells were disrupted by homogenization with a Dounce homogenizer, and the lysate was centrifuged at 10,000*g* for 30 min (data not shown). The main end product of both reactions was dAdo. Hyp was formed only slowly, which indicates that the membranes of the cells were largely devoid of Ado deaminase activity. On the other hand, when dAMP or dATP were incubated with whole cell lysates the predominant end product was Hyp (data not shown). In neither case were significant amounts of dIMP formed. Other experiments showed that the alkaline and acid phosphatase activities of P388-M and L1210 cell extracts were also about the same (about 40 and 3 nmol/mg protein · min, respectively, at 37°C) as were as their contents of ATP, GTP, UTP, and CTP (about 2.5, 0.6, 0.6 and 0.2 mM, respectively).

The only apparent difference between P388-M and P388 (and L1210) cells with respect to growth in culture and dAdo metabolism was the conversion of dAdo to ribonucleotides presumably via Ade. In mammalian cells, Ado is converted to Ade, though rather inefficiently, by 5′-methylthioadenosine phosphorylase [30,31], and 2′-dAdo most likely is also a substrate for this enzyme. However, this activity is lacking in P388 and L1210 cells [30,32] and no other enzyme that converts Ado or dAdo to Ade has been detected in mammalian cells. The lack of 5′-methylthioadenosine phosphorylase has been confirmed in our present strains of these cells. We have prepared radiolabeled 5′-methylthio[$^3$H]Ado from [$^3$H]5′-adenosylmethionine according to established procedures [33] and have shown that it is not metabolized by either P388-M or L1210 cells (data not shown). The alternative, therefore, was contamination of the P388-M cells by mycoplasma, which is known to possess Ado phosphorylase activity [11–15].

In a previous study [2] we failed to detect mycoplasma in P388-M cultures by the Urd/Ura incorporation method [16]. This method is based on the finding that mycoplasma, but not mammalian cells, possesses an Ura phosphoribosyltransferase which promotes efficient salvage of Ura in contaminated cell cultures via direct phosphoribosylation of Ura to UMP. In contrast, mammalian cells salvage Ura poorly, via Ura→Urd→UMP, even when they possess Urd phosphorylase activity, because of limiting intracellular levels of ribose-1-phosphate and unfavorable kinetic properties of mammalian Urd phosphorylase for salvage of Ura under physiological conditions [24]. Furthermore, mycoplasma expresses high Urd phosphorylase activity [34,35], which results in rapid degradation of [$^3$H]Urd and depression of Urd salvage by contaminated mammalian cell cultures [24]. Thus mycoplasma infection of cell cultures is generally associated with drastic decreases in Urd incorporation and increases in Ura incorporation which result in diagnostic decreases in the Urd/Ura incorporation ratio [16,17]. We have reexamined [$^3$H]Urd and [$^3$H]Ura incorporation by P388-M and P388 (L1210) cells under various experimental conditions (Table I). The incorporation of Urd was 10–30 times higher than Ura incorporation in all three types of cells. Urd incorporation was only slightly reduced in P388-M cell cultures and Ura incorporation only slightly increased when compared to P388 (L1210) cell cultures, whether the cultures were incubated with the substrates for 0.5, 1, or 24 h or at different cell densities or with

different media or whether we measured the incorporation of the substrates into total cell material (Expt. 1) or into acid-insoluble material (Expt. 2–4). A substantial difference in the incorporation of Urd and Ura into acid-insoluble material was observed only in one experiment (Expt. 2); the Urd/Ura incorporation ratio was 10 times higher in P388-M than P388 cell cultures, but solely because of a higher incorporation of Ura by P388-M cell cultures. The ratios, however, were clearly much lower and the differences between P388-M and P388 cell cultures much smaller than reported by other investigators for mycoplasma-infected and uninfected cell cultures [16,17]. Nevertheless, the data in Table I suggest the presence of Ura phosphoribosyltransferase in P388-M cell cultures, and indeed considerable activity was detectable by an in vitro assay both in the culture fluid and associated with the cells (Fig. 4B). No such activity was found in the cultures of P388 and L1210 cells and other mammalian cell lines [24].

Furthermore, Ado phosphorylase activity was readily and consistently detectable in the culture fluid of P388-M cells (Fig. 4A) and lysates of pelleted P388-M cells, but not in equivalent preparations of cultures of P388 and L1210 cells (Fig. 4C). Under conditions where pelleted material from P388-M cultures almost completely converted dAdo to Ade, no significant formation of Ade was detectable in reactions with equivalent preparations of P388 cells (Fig. 4C). The finding that no radioactivity was recovered in Hyp (Fig. 4C) indicates that dAdo deamination was completely blocked by the deoxycoformycin present in the reaction mixture. Interestingly, dAdo was an efficient substrate for the mycoplasma Ado phosphorylase, whereas ddAdo was resistant to phosphorolysis (Fig. 4A).

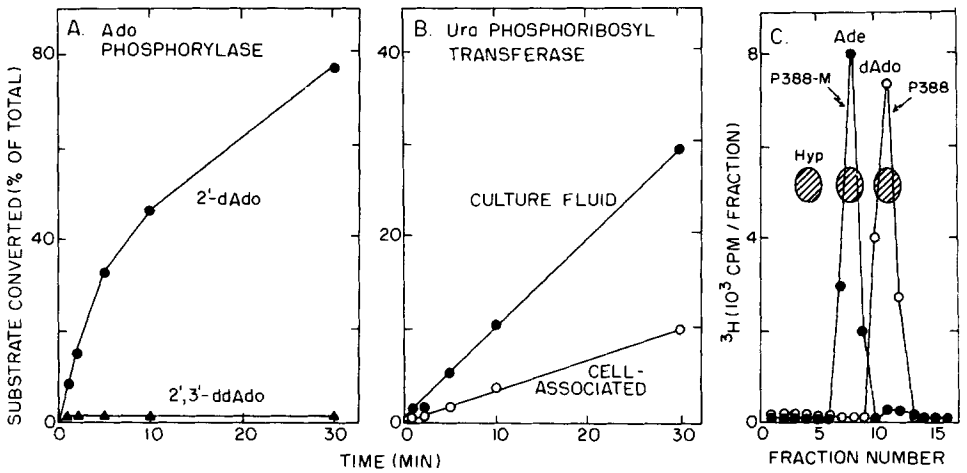


Fig. 4. Ado phosphorylase (**A**) and Ura phosphoribosyltransferase (**B**) activities in cultures of P388-M cells and chromatographic profiles of Ado phosphorylase reaction mixtures with lysates from P388 and P388-M cell cultures (**C**). Cell lysates and material harvested by centrifugation from the culture fluid were prepared and assayed at final concentrations of 3 and 1.25 mg protein/ml, respectively, as described under Materials and Methods. The culture medium preparation was assayed for the phosphorolysis of dAdo and ddAdo (A) and both fractions were assayed for Ura phosphoribosyltransferase activity (B). In C, 10 ml of late exponential phase cultures of P388 and P388-M cells were centrifuged at 10,000*g* for 30 min and the pelleted material was suspended in lysing buffer. Samples of the lysate were incubated with 5 $\mu$M [$^3$H]dAdo in phosphate buffer for 30 min and the reaction mixtures were then chromatographed with solvent 40.

Mycoplasmas have been isolated by culture methods from cultures of P388-M cells and identified by immunological methods as *Mycoplasma orale* (see Materials and Methods).

## DISCUSSION

Our results show that contamination with *M. orale* drastically alters the metabolism of dAdo by P388 cells (and of L1210 cells, data not shown) as a result of mycoplasmal Ado phosphorylase activity. Since practically all mycoplasmas have been found to possess this enzyme, this effect probably applies to infections by all mycoplasmas. In general, dAdo salvage by mammalian cells is very inefficient under conditions where dAdo deamination is blocked, probably because of feedback control of dAdo phosphorylation. Such control could explain why net uptake of 10 $\mu$M dAdo ceases after 5 to 10 min of incubation (Fig. 1B,C). Rapid turnover of dAdo phosphates does not seem to be involved [2]. Recent experiments have indicated that dipyridamole stimulates dAdo salvage by P388 and L1210 cells, because these cells express not only facilitated nucleoside transport, but also low levels of an $Na^+$-dependent, active transporter which is specific for Urd and purine nucleosides and is relatively resistant to inhibition by dipyridamole (Plagemann, submitted for publication). In the presence of dipyridamole these cells concentratively accumulate high levels of purine nucleosides, including dAdo, because dipyridamole inhibits the efflux via facilitated transport of the nucleoside actively transported into the cells. Increased intracellular accumulation of dAdo apparently drives its increased phosphorylation in deoxycoformycin-treated P388 and L1210 cells.

Mycoplasma infection also greatly stimulates the salvage of dAdo by P388 (and L1210) cells blocked in dAdo deamination (Fig. 1A), but in this case the stimulation reflects degradation of dAdo to Ade and salvage of the Ade by the mammalian cells. If Ade is released from dAdo by the action of extracellular mycoplasma, it is unclear why dipyridamole inhibits dAdo salvage by the mammalian cells since Ade transport in mammalian cells is not significantly inhibited by dipyridamole [5,36]. One possibility is that dAdo transport into mycoplasma is also sensitive to inhibition by dipyridamole. Regardless, it is clear that mycoplasma contamination can cause drastic changes in dAdo metabolism in cell cultures without affecting the growth of the mammalian cells or many other of their metabolic properties.

We consider the Ado phosphorylase assay superior to other biochemical assays for the detection of mycoplasma infections of animal cell cultures and use it routinely for this purpose. The enzyme has been reported to be present in all mycoplasmas [11,14], with the possible exception of one isolate *M. orale* [37], and it is present at relatively high activities. Furthermore, the assay is relatively simple and yields unambiguous results. In our routine assay, we centrifuge 10 ml of cell culture and lyse the pelleted material in a hypotonic buffer containing dithiothreitol and deoxycoformycin. A sample of lysate is incubated with 5 $\mu$M [$^3$H]dAdo in phosphate buffer for 30 min and then dAdo and Ade are separated by ascending paper chromatography. Fourteen samples can be chromatographed on a single sheet of paper. Under these assay conditions, lysates from infected cultures of at least 10 different cell lines have been found to consistently convert >90% of dAdo to Ade, whereas no significant degradation of dAdo is apparent with lysates from uninfected cultures (see Fig. 4C). The assay can be readily scaled down to the use of as little as 1 ml of cell culture.

The Urd/Ura incorporation method, on the other hand, yielded ambiguous results with cultures of P388-M cells that are infected with *M. orale* (Table I). The finding that the uptake of Urd was only little reduced in infected cultures suggests the presence of only low levels of Urd phosphorylase activity and this conclusion has been confirmed experimentally (data not shown). The uptake of Ura was higher in infected than uninfected cultures, but only relatively little, in spite of considerable Ura phosphoribosyltransferase activity in infected cultures. Cultures of HeLa cells infected with *M. orale* have previously been found to incorporate Ura only poorly compared to cultures infected with other mycoplasmas [18]. The reason for the low Ura incorporation by these infected cultures, in spite of the presence of Ura phosphoribosyltransferase, has not been resolved. It could result from an inefficient production of P-Rib-PP by *M. orale* or a high Km of the Ura phosphoribosyltransferase of this organism for P-Rib-PP. A precedent for the former suggestion is supplied by Novikoff rat hepatoma cells [38]. Stationary phase cultures of these cells fail to salvage Hyp, in spite of undiminished Hyp phosphoribosyltransferase activity, because of an inefficient generation of P-Rib-PP.

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