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# Endogenous Cyclic AMP Does Not Modulate Transport of Hexoses, Nucleosides, or Nucleobases in Chinese Hamster Ovary Cells

**Robert M. Wohlhueter, Peter G.W. Plagemann, and J.R. Sheppard**

*Department of Microbiology (R.M.W., P.G.W.P.) and Genetics and Cell Biology (J.R.S.) and Dight Institute for Human Genetics (J.R.S.) University of Minnesota, Minneapolis, Minnesota 55455*

In a previous study we have demonstrated that neither extracellular nor intracellular cyclic adenosine monophosphate (AMP) levels directly affect the uptake of nucleosides, nucleobases, or hexoses by various types of cultured mammalian cells. Uptake of these nutrients into cells, however, involves two processes operating in tandem: facilitated transport across the membrane and intracellular phosphorylation; and uptake rates generally reflect the rates of substrate phosphorylation rather than of transport. In the present study we have examined the question of whether substrate transport per se is regulated by intracellular cyclic AMP. Initially various cell lines, grown both in suspension and monolayer culture, were screened for their cyclic AMP response to prostaglandin $E_1$, isoproterenol, and inhibitors of cyclic AMP phosphodiesterase. Prostaglandin $E_1$ treatment of Chinese hamster ovary cells was selected as the system giving the largest and most consistent (50-fold to 100-fold) elevation of cyclic AMP. Rapid kinetic techniques were used to measure the transport of 3-O-methylglucose, thymidine, adenosine, hypoxanthine, and adenine in wild-type cells and in mutant sublines incapable of phosphorylating these substrates. In no case was an increase in intracellular cyclic AMP accompanied by a significant change in the rate of transport of these substrates, although prostaglandin $E_1$ slightly inhibited the transport of various substrates.

Key words: cyclic AMP; transport of nucleosides, nucleobases, and hexoses; Chinese hamster ovary cells.

Results of numerous studies have led to the view that hexose, nucleoside, and nucleobase transport in mammalian cells might be regulated in some manner by cyclic adenosine monophosphate (AMP). For example, the rapid increase in uridine uptake observed in density-inhibited or serum-starved, cultured mammalian cells consequent to mitogen stimulation was found to correlate with a decrease in intracellular cyclic AMP levels [1–7], and stimulation of cyclic AMP production by treatment of the cells with prostaglandin $E_1$ ($PGE_1$), theophylline, or dibutyryl cyclic AMP prevented the

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increase in uridine uptake caused by mitogens [1, 3]. Hexose uptake is also stimulated, although somewhat more slowly than uridine uptake, in mitogen-stimulated untransformed cultured cells [8] and lymphocytes [9, 10], and the stimulation in lymphocytes is counteracted by increases in intracellular cyclic AMP concentration [10]. Furthermore, the insulin-stimulated glucose uptake by fat cells is accompanied by a decrease in intracellular cyclic AMP [11, 12]. There exists also an inverse correlation between changes in hexose uptake capacity and intracellular cyclic AMP levels when cells become transformed to tumor cells [8, 13, 14].

In some studies incubation with high, exogenous concentrations of cyclic AMP or dibutyryl cyclic AMP were found to cause decreases in rates of uridine and thymidine uptake in Chinese hamster ovary (CHO) cells [15], Balb 3T3 [3], and neoplastic mast cells [16], and of hypoxanthine uptake in Chinese hamster lung cells [17]; and treatment of L cells with R020-1274, an inhibitor of cyclic AMP phosphodiesterase, lowered the uridine uptake rate concomitant with an increase in cyclic AMP concentration [18]. But in other studies, such treatments increased uptake rates of thymidine and uridine in CV-1 monkey cells [19] and mouse L cells [18], respectively, and of both substrates in human liver cells [20].

Results of experiments measuring hexose uptake have been equally contradictory; incubation of untransformed and murine sarcoma virus-transformed Balb 3T3 cells [13, 21] and human glioma cells [22] with dibutyryl cyclic AMP plus theophylline has been reported to result in an increase of glucose uptake, whereas a similar treatment of polyoma virus-transformed 3T3 cells caused an inhibition of deoxyglucose uptake [23]. Incubation of 3T3 cells with adenosine also resulted in a decrease in 3-O-methylglucose uptake, and indirect evidence suggested that such effect might be related to an increase in intracellular cyclic AMP [24].

The observed effects of extracellular cyclic AMP or dibutyryl cyclic AMP on substrate uptake rates, however, are probably secondary; they become apparent only after hours of incubation with cyclic nucleotides, whereas exposure of several lines of cultured mammalian cells to 1 mM dibutyryl cyclic AMP had no immediate effect on their uptake of uridine, thymidine, or deoxyglucose [25, 26]. Furthermore, we have previously shown that changes in intracellular cyclic AMP level induced in such cells by treatment with papaverine, $PGE_1$, or isoproterenol had no effect on the uptake of these substrates nor did it correlate with the inhibition of uridine, hypoxanthine, or deoxyglucose uptake caused by some of these substances [26].

One complicating factor in all the studies summarized above is that they were conducted with cells in which the substrates were rapidly metabolized so that substrate uptake rather than transport through the membrane was measured. Uptake here is defined as the intracellular accumulation of radioactivity derived from extracellular, radiolabeled substrate. Uptake thus reflects the tandem operation of facilitated transport and intracellular phosphorylation plus subsequent metabolic conversion [27, 28]. Recent studies have shown that long-term (1–10 min) rates of uptake of nucleosides [20–32], nucleobases [33], and deoxyglucose [34] by various cell lines reflect mainly the rate of phosphorylation of these substrates rather than the rate of transport of the unmodified substrate through the plasma membrane (for review see Wohlhueter and Plagemann [35]). Our previous results [26], therefore, did not unequivocally rule out an effect of cyclic AMP on the transport step per se. In the present study, we have examined this possibility by measuring the transport of nucleosides, nucleobases, and hexoses directly in cells incapable of metabolizing the substrates.

## MATERIALS AND METHODS

### Cell Culture

Sublines of CHO cells deficient in adenosine kinase ($AK^-$), thymidine kinase (T6y-4), and hypoxanthine guanine phosphoribosyltransferase (VT-29, $6TG^r$) were obtained from Dr. L. Siminovitch (University of Toronto) and a CHO line deficient in adenine phosphoribosyltransferase (DAP-12; Taylor et al [36]) from Dr. M. Taylor (Indiana University). These lines were all derived from proline auxotrophic clones of CHO cells (for convenience here designated "wild-type"). All lines of CHO cells, and lines of mouse lymphoma P388 cells, mouse L cells, and HeLa cells, were routinely propagated in suspension culture in Eagle's minimal essential medium for suspension culture supplemented with nonessential amino acids and 10% (v/v) heat-inactivated, fetal bovine serum as described previously [30, 31]. Cultures were incubated on a gyrotory shaker, except for cultures of more than 500 ml, which were incubated with magnetic stirring. Monolayer cultures in 25 $cm^3$ Falcon flasks were established from suspension cultures and incubated in a $CO_2$ incubator. The growth medium was the same as used for suspension cultures, except that its base was Eagle's minimal medium for monolayer culture. Cells were enumerated with a Coulter counter. Cultures were routinely assayed for mycoplasma contamination by measuring the relative reates of incorporation of 5 $\mu$M [$^3$H]uridine (40–80 cpm/pmole) and 5 $\mu$M [$^3$H]uracil (40–80 cpm/pmole) into acid-insoluble material over a 30–60 min time period, similar to the method described by Schneider et al [37]. The incorporation of uracil was consistently very low in all cell lines used in this study and the ratio of uridine to uracil incorporation was $> 500$, indicating absence of mycoplasma.

### Transport Studies

Cells were harvested from exponential phase suspension cultures by centrifugation at 400 g for 2 min and suspended to about $(1-3) \times 10^7$ cells per milliliter in basal medium 42 (BM42; Plagemann [38]) or glucose-free BM42B, as indicated in the appropriate experiments. The suspensions were supplemented where indicated with 60 or 120 $\mu$M $PGE_1$, incubated at 37° for 5 min, equilibrated at 25°, and then assayed for substrate transport. Substrate influx was measured against an intracellular concentration presumed to be effectively zero at zero time (the zero-trans protocol [27, 39]) using a rapid mixing/sampling technique which has been described in detail previously [27, 31]. Briefly, samples of 448 $\mu$l of suspension were mixed with 61 $\mu$l of a solution of labeled substrate at short intervals by means of a hand-operated dual syringe apparatus. The mixtures emerging from the mixing chamber were dispensed into 12 tubes mounted in an Eppendorf microcentrifuge. The tubes contained 100 $\mu$l of an oil mixture (final density = 1.034 g/ml). After the last sample had been mixed, the centrifuge was started, and within an estimated 2 sec, the cells had entered the oil phase, thus terminating transport. For sampling times in excess of 2 min, cell suspension and substrate solution were mixed in the same proportion provided by the mixing apparatus, and 509-$\mu$l samples were removed at appropriate times and centrifuged through oil. The culture fluid and oil were removed and the cell pellets were analyzed for radioactivity.

Total water space and extracellular water space in cell pellets obtained by centrifuging cells through the oil phase were determined in parallel runs in which substrate was replaced by [$^{14}$C]carboxylinulin in $^3H_2O$ [27]. All values for cell-associated radioactivity were corrected for radioactivity in the extracellular space of cell pellets,

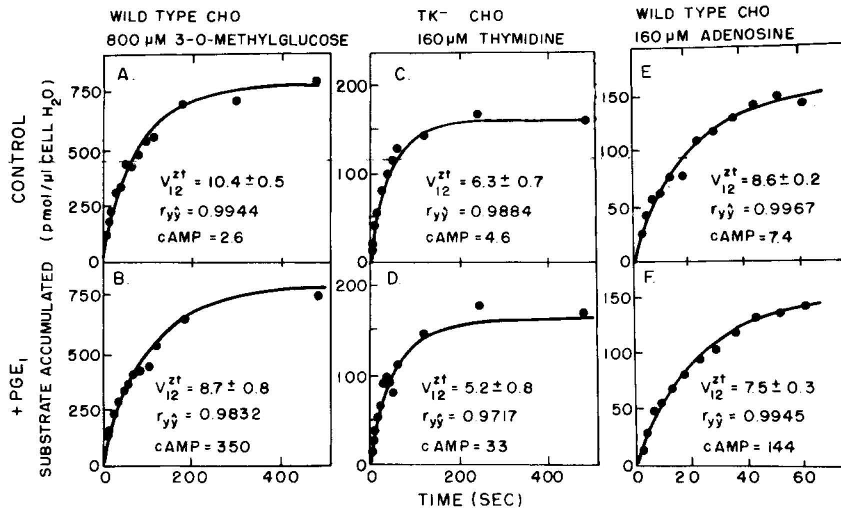


Fig. 1. Effect of $PGE_1$ pretreatment on the transport of 3-O-methylglucose (A, B), thymidine (C, D), and adenosine (E, F). Cells were harvested from exponential phase cultures of wild-type CHO (A, B, E, F) or thymidine kinase-deficient ($TK^-$) CHO (C, D), and suspended to about $1.3 \times 10^7$ cells per milliliter in BM42B (C–F) or glucose-free BM42B (A, B). One portion of each suspension was supplemented with 120 μM $PGE_1$, and the treated (B, D, F) and untreated (A, C, E) suspensions were incubated at 37° for 5 min and then thermally equilibrated at 25°. In E and F both untreated and $PGE_1$-treated suspensions were also supplemented with 10 μM 2-deoxycoformycin during preincubation. Aliquots of the suspension were extracted for assay of intracellular cyclic AMP as described under Materials and Methods. Other aliquots of the suspension were assayed at 25° for transport of the appropriate substrates by the rapid kinetic technique; the substrate concentrations were 800 μM 3-O-methyl-[$^3$H] D-glucose (190) cpm/μl); 160 μM [$^3$H] thymidine (720 cpm/μl), or 240 μM [$^3$H] adenosine (860 cpm/μl). Radioactivity per cell pellet was corrected for radioactivity trapped in extracellular water space and converted to pmoles/μl cell water ($S_{2,t}$) on the basis of the experimentally determined intracellular water space. The intracellular and extracellular water spaces in A and B, in C and D, and in E and F were 22.2 and 3.3, 8.4 and 3.2, and 10.9 and 5.3 μl/cell pellet, respectively. Equation 1 was fit to the time courses of substrate accumulation whereby all R parameters were constrained to equal each other and K was fixed at 5, 0.2, and 0.2 mM for 3-O-methylglucose, thymidine, and adenosine, respectively. The correlation coefficient ($r_{y,\hat{y}}$) is indicated for each fit. $v_{12}^{zt}$ values were calculated from K and the computed R parameters according to Equation 2.

and converted to pmoles per microliter cell water on the basis of the experimentally determined intracellular water space (Total water space – Inulin space/Cell pellet).

To evaluate transport rates an integrated rate equation (developed for zero-trans influx to conform to the simple carrier model of Eilam and Stein [39]) was fit by the method of least squares to time courses of accumulation of substrate to transmembrane equilibrium (for details see Refs. 28, 31):

$$S_{2,t} = S_1 \left[ 1 - \exp - \frac{t + (R_{21} + R_{ee}S_1/K)S_{2,t}}{KR_{00} + R_{12}S_1 + R_{21}S_1 + S_1{}^2R_{ee}/K} \right] \quad (1)$$

where $S_{2,t}$ = concentration of substrate inside the cell at time t ($S_{2,0} = 0$); $S_1$ = exogenous substrate concentration (and is taken as a constant); K = substrate carrier dissociation constant; and the R terms are resistant factors proportional to the time of a round of the

carrier as defined by Eilam and Stein [39] and equal to the reciprocals of the corresponding maximum velocities. Equation 1 was fit to individual time courses of intracellular substrate accumulation at a single substrate concentration (see Fig. 1) with all R constants held equal (corresponding to a completely symmetrical carrier) and with K fixed (at 5 mM for 3-O-methylglucose, 200 $\mu$M for thymidine, 200 $\mu$M for adenosine, 1.5 mM for hypoxanthine, and 2 mM for adenine). These values of K were estimated in previous studies with CHO cells (summarized in Ref. 28 and unpublished observations).

Justification for the assumption of complete carrier symmetry is available as yet only for nucleoside transport in Novikoff rat hepatoma cells [31], but the reported zero-trans transport data for other cell lines, including CHO cells, support the view that carriers for nucleoside and nucleobase transport in cultured cells in general are symmetrical [28], ie, that transport is indifferent to direction and to whether the carrier is loaded or empty.

Initial velocities of zero-trans entry ($v_{12}^{zt}$) were calculated from K and the estimated R constant ($R_{12} = R_{ee} = R_{oo}$) according to the original zero-trans differential equation of Eilam and Stein (Eq. 18, Ref. 39) at $S_2 = 0$:

$$v_{12}^{zt} = \frac{KS_1}{K^2 R_{OO} + KR_{12} S_1} \qquad (2)$$

### Cyclic AMP Analyses

Samples of (2–7) × $10^7$ cells were collected by centrifugation, washed twice in phosphate buffered saline and extracted with 0.5–2 ml of a solution of cold (0°) 5% (w/v) trichloroacetic acid. The acid extracts were assayed for cyclic AMP by a modification of Gilman's method as described previously [14, 26] and for protein by the method of Lowry [40].

### Chemicals

Radiochemicals were purchased as follows: 3-O-[$^3$H-methyl] methyl-D-glucose from ICN (Irvine, California); [$^3$H-methyl] thymidine and [G-$^3$H] hypoxanthine from Amersham/Searle (Arlington Heights, Illinois); [8-$^3$H] adenosine from Schwarz/Mann (Orangeburg, New York); and [$^{14}$C-carboxyl] carboxylinulin and $^3H_2O$ from New England Nuclear (Boston). Chemicals were obtained as follows: papaverine from Eastman Organic Chemicals (Rochester, New York) and isoproterenol and 1-methyl-3-isobutylxanthine from Sigma (St. Louis). $PGE_1$ was a gift from Dr. J. Pike, Upjohn Co. Other chemicals were reagent grade from standard suppliers.

## RESULTS AND DISCUSSION

Suspensions of cells have a great advantage over monolayer cultures in studies on the facilitated transport of nucleosides, nucleobases, and hexoses in that the density of cells in suspension can be manipulated in such manner [(2–5) × $10^7$ cells per milliliter] that the intracellular $H_2O$ space of the cells makes up at least 2% of the total water space of the suspension [27]. Thus, this proportion of total substrate added to the suspension will have accumulated intracellularly at equilibrium – a proportion that can be determined with reasonable accuracy. In contrast, in monolayer cultures at best only 0.2% of the total substrate can accumulate intracellularly in the absence of trapping

**TABLE I. Effect of Various Treatments on the Intracellular Levels of Cyclic AMP in Various Cell Lines Propagated in Suspension or Monolayers**

| | | Cyclic AMP (pmoles/mg protein) in | | | |
|---|---|---|---|---|---|
| Culture | Treatment | CHO | HeLa | P388 | L |
| Suspension[a] | None | 7.7 | 3.2 | 3.6 | 7.0[c] |
| | $PGE_1$ | 205. | 8.7 | 8.1 | ND[d] |
| | Papaverine | 9.4 | 4.7 | 4.1 | 8.0 |
| | Isoproterenol | 9.4 | 3.1 | 4.8 | ND |
| | MIX | 6.1 | ND | 4.3 | ND |
| | $PGE_1$ + MIX | 267. | ND | 8.0 | ND |
| Monolayer[b] | None | 27. | 16 | ND | 13 |
| | $PGE_1$ | 1,200. | 9 | ND | 40 |
| | Papaverine | 38. | 9 | ND | 23 |
| | Isoproterenol | 27. | 9 | ND | 17 |

[a]Cells were collected from exponential-phase cultures and suspended to $(0.5-3) \times 10^7$ cells per milliliter in serum-free BM42B containing, where indicated, 60 μM $PGE_1$, 200 μM papaverine, 100 μM isoproterenol, 0.5 mM 1-methyl-3-isobutylxanthine (MIX) or combinations thereof and incubated at 37° for 5 min. The cells from 4 ml of suspension were collected by centrifugation, washed twice in phosphate-buffered saline, and extracted with 0.5 ml cold 5% (w/v) trichloracetic acid. The acid extracts were analyzed for concentrations of cyclic AMP and protein as described in Materials and Methods.

[b]Cells were propagated in 25-$cm^3$ Falcon flasks and then analyzed as described above.

[c]Data from Sheppard and Plagemann [26].

[d]ND, not determined.

by phosphorylation. This low ratio of cell to total water hinders accurate estimates of intracellular substrate concentration. For these reasons it seemed preferable to use suspension cultures to assess the effect of endogenous cyclic AMP on substrate transport. Thus, the first prerequisite for these studies was to find cell lines in which cyclic AMP levels could be manipulated in some manner.

Previous studies have shown that the cyclic AMP levels of Novikoff rat hepatoma cells and mouse L cells propagated in suspension culture were unresponsive to treatment of the cells with $PGE_1$, isoproterenol, and various inhibitors of cyclic AMP phosphodiesterase [26]. We have now screened several other cell lines that can be grown in suspension culture for cyclic AMP response to such treatments (Table I). Treatment of CHO cells with $PGE_1$ was the only combination that elevated intracellular cyclic AMP levels by a factor of more than 2–3. In fact, in repeated experiments, the intracellular cyclic AMP level of wild-type CHO cells increased consistently between 20 and 100 times within 5 min of incubation with 60 or 120 μM $PGE_1$ (see also Table II). The elevated cyclic AMP levels persisted for at least 30 min in the presence of $PGE_1$ (data not shown). For comparative purposes we also determined the effect of the various treatments on intracellular cyclic AMP levels when the cells of the three lines that adhere to culture dishes were propagated in monolayer culture. In principle, the cell lines behaved the same whether propagated in suspension or monolayer culture, except that both the untreated and treated monolayer cultures possessed several times more cyclic AMP (expressed per milligram protein) than did the corresponding suspension cultures (Table I). A marked increase in cyclic AMP in response to $PGE_1$ has also been observed

**TABLE II. Effect of $PGE_1$ Pretreatment on Cyclic AMP Concentrations and Initial Transport Velocities for Various Substrates in Wild-Type CHO Cells and Cells of Various Enzyme-Deficient Sublines Thereof*.**

| | | | $v_{12}^{zt}$ (pmoles/μl cell $H_2O$·sec) | | [Cyclic AMP] (pmoles/mg protein) | |
|---|---|---|---|---|---|---|
| CHO cells | Substrate | μM | Control | $+PGE_1$ | Control | $+PGE_1$ |
| Wild-type | 3-O-methyl-glucose | 800 | 10.4 ± 0.5 | 8.7 ± 0.8 | 2.6 | 350 |
| | | 800 | 10.8 ± 1.0 | 9.6 ± 0.8 | 4.0 | 226 |
| | | 800 | 18.6 ± 1.1 | 12.8 ± 0.7 | 5.5 | 85 |
| | | 800 | 16.4 ± 3.2 | 13.4 ± 2.1 | 3.1 | 179 |
| $TK^-$ | Thymidine | 160 | 6.3 ± 0.7 | 5.2 ± 0.8 | 4.6 | 33 |
| $AK^-$ | Adenosine | 80 | 6.1 ± 0.9 | 4.6 ± 0.7 | 6.1 | 39 |
| | | 160 | 10.1 ± 0.6 | 8.3 ± 0.2 | 6.0 | 8.4 |
| Wild-type | Adenosine | 160 | 8.6 ± 0.2 | 7.5 ± 0.3 | 7.4 | 144 |
| $HGPRT^-$ | Hypoxanthine | 160 | 3.2 ± 0.2 | 2.2 ± 0.2 | ND[a] | ND |
| | | 320 | 4.7 ± 0.5 | 3.3 ± 0.3 | 4.7 | 5.4 |
| $APRT^-$ | Adenine | 240 | 2.9 ± 0.3 | 2.4 ± 0.3 | 4.6 | 4.3 |
| | | 240 | 5.3 ± 0.7 | 4.3 ± 0.6 | 4.6 | 5.9 |
| Wild-type | Adenine | 240 | 1.7 ± 0.2 | 1.2 ± 0.1 | 4.2 | 150 |
| | | 480 | 8.2 ± 1.6 | 6.8 ± 1.0 | 3.3 | 148 |

*Values are from the experiments illustrated in Figure 1 and from other experiments conducted in the same manner. $TK^-$, $AK^-$, $HGPRT^-$, and $APRT^-$ are CHO sublines deficient in thymidine kinase, adenosine kinase, hypoxanthine/guanine phosphoribosyltransferase, and adenine phosphoribosyltransferase, respectively.

[a]ND, not determined.

in monolayer cultures of CHO cells by O'Neill and Hsie [41], and the adenylate cyclase of various types of hamster cells has been shown to be greatly stimulated by prostaglandins [42]. $PGE_1$ also causes an increase in cyclic AMP in 3T3 cells [1, 3, 26, 43] and human WI38 cells [44], but these cells cannot be propagated in suspension culture. The adenylate cyclase of L929 is greatly stimulated by $PGE_1$ [42, 46], but our line of L cells is unresponsive whether propagated in monolayers or suspension. The responses of 3T6 cells and some transformed 3T3 cells to prostaglandins, on the other hand, are minimal [26, 42, 45].

Figure 1 illustrates typical time courses of accumulation of 3-O-methyl-D-glucose, thymidine, and adenosine to transmembrane equilibrium in untreated and $PGE_1$-treated CHO cells in suspension. Table II summarizes the initial transport velocities for these substrates and for adenine and hypoxanthine in untreated and treated cells as well as the intracellular cyclic AMP concentrations observed in these cells at the time transport was measured.

The transport of each substrate was measured in cells unable to metabolize that substrate. 3-O-methylglucose is not phosphorylated in mammalian cells [47], so that its transport could be measured in wild-type cells without complication of metabolism. Transport of thymidine, hypoxanthine, adenosine, and adenine was measured in sublines of CHO cells deficient in thymidine kinase, hypoxanthine/guanine phosphoribosyltransferase, adenosine kinase, and adenine phosphoribosyltransferase, respectively, in which sublines no significant phosphorylation of the substrates was detected during

**TABLE III. Direct Effect of $PGE_1$ on the Transport of Various Substrates in Wild-Type CHO Cells***

| | | $v_{12}^{zt}$ (pmoles/$\mu$l cell $H_2O \cdot$sec) | |
|---|---|---|---|
| Substrate | $\mu$M | Control | $+PGE_1$ |
| 3-O-Methylglucose | 800 | 9.2 ± 1.9 | 7.4 ± 1.5 |
| Adenosine | 160 | 7.2 ± 0.2 | 6.7 ± 0.4 |
| Thymidine | 320 | 10.8 ± 0.5 | 8.8 ± 0.4 |
| Hypoxanthine | 320 | 5.1 ± 0.4 | 3.3 ± 0.2 |
| Adenine | 320 | 2.3 ± 0.1 | 2.4 ± 0.2 |

*Samples of a suspension of about $1.7 \times 10^7$ wild-type CHO cells per milliliter of BM42B (or of glucose-free BM42B for 3-O-methylglucose uptake measurements) were assayed for zero-trans accumulation of $^3$H-labeled substrates at 25° by the rapid kinetic technique described in Materials and Methods and illustrated in Figure 1. For [$^3$H]adenosine transport measurements, the cells were preincubated with 10 $\mu$M 2-deoxycoformycin at 37° for 5 min and then at 25° for 2 min. Where indicated, $PGE_1$ was added simultaneously with substrate to a final concentration of 120$\mu$M. Radioactivity per cell pellet was corrected for substrate trapped in extracellular space (3.9 $\mu$l/pellet). The intracellular space was 12.1 $\mu$l/cell pellet. Equation 1 was fitted to the time courses of substrate accumulation comprised of 12–15 time points encompassing 2.5 sec to 2 min (see Fig. 1) with all R constants held constant and K fixed at 5, 0.2, 0.2, 1.5, and 2.5 mM for 3-O-methylglucose, adenosine, thymidine, hypoxanthine, and adenine, respectively. The correlation coefficients for the fits were all $> 0.9$.

4 min of incubation with the indicated concentrations of substrate (data not shown). It is noteworthy that sublines lacking either adenine- or hypoxanthine/guanine-phosphoribosyltransferase failed to respond to $PGE_1$ treatment. As yet we do not know what significance is to be attached to this observation. The two sublines in question were obtained by us from two different laboratories; whether the histories of the parental lines might contribute to the mutants' unresponsiveness is unclear.

In any case adenosine and adenine transport was also measured in our strain of wild-type CHO cells, which do respond to $PGE_1$. We have demonstrated previously that the initial time courses of accumulation of adenosine, adenine, and other nucleosides and nucleobases are about the same in wild-type cells as in cells lacking the respective enzymes responsible for their phosphorylation, provided the substrate concentration greatly exceeds the concentration that saturates the phosphorylating enzyme [28, 35].

Together, the results of Figure 1 and Table II indicate that the 10-fold to 100-fold increase in intracellular cyclic AMP concentration after $PGE_1$ treatment had little effect on the initial zero-trans transport velocities for the various substrates examined. Although the transport velocities were, in general, marginally lower in $PGE_1$-treated than in untreated cells, there is good reason to believe that this difference is not mediated by cyclic AMP, but is attributable to a direct effect of $PGE_1$ on the transport of these substrates: 1) A similar, slight decrease in transport $v_{12}^{zt}$ was observed when $PGE_1$ was added simultaneously with substrate (Table III); 2) an inhibitory effect of $PGE_1$ was apparent in the hypoxanthine/guanine phosphoribosyltransferase-deficient cells, which do not respond to $PGE_1$ with elevated cyclic AMP levels; and 3) we have previously demonstrated that both $PGE_1$ and prostaglandin $F_{2\alpha}$ inhibit the uptake of nucleosides, hypoxanthine, deoxyglucose, and choline in Novikoff rat hepatoma cells, whose adenylate cyclase is also unresponsive to $PGE_1$ [48]. A recent report by Rozengurt et al [32] indicates that the involvement of cyclic AMP in uridine uptake by mitogen-

stimulated 3T3 cells is distal to the transport step. An observed decrease in cyclic AMP correlated with an increase in the rate at which uridine was phosphorylated, while the rate of transport remained unchanged.

We conclude that the zero-trans transport of various substrates is not regulated by endogenous cyclic AMP, but that $PGE_1$ at concentrations of 60–120 $\mu M$ causes a slight direct inhibition of the transport of these substrates. That extracellular cyclic AMP and its dibutyryl derivative similarly have no effect on the transport of these substrates can be deduced from the lack of effect of these cyclic nucleotides at a concentration of 1 mM on the uptake of these nucleosides, nucleobases, and hexoses [26].

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