Extracellular ATP (1 mM) inhibited the growth of Friend virus-infected murine erythroleukemia cells (MEL cells) but had no effect on dimethyl sulfoxide-induced differentiation. ATP (1 mM) also caused changes in the permeability of MEL cells to ions. There was an increased influx of $^{45}\text{Ca}^{2+}$ from a basal level of 5 pmol/min to 18 pmol/min/10^6 cells to achieve a 2-fold increase in steady-state $\text{Ca}^{2+}$ as measured at isotopic equilibration. $\text{Ca}^{2+}$ influx was blocked by diisothiocyanostilbene disulfonate (DIDS), an inhibitor of anion transport. ATP also stimulated $\text{Cl}^{-}$ uptake, and this flux was inhibited by DIDS. The ratio of ATP stimulated $\text{Cl}^{-}$ to $\text{Ca}^{2+}$ uptake was 1.6:1. $\text{K}^{+}$ and $\text{Na}^{+}$ influx were also stimulated by ATP, but phosphate uptake was inhibited; the $\text{Na}^{+}$ influx dissipated the $\text{Na}^{+}$ gradient and thus inhibited nutrient uptake. ATP-stimulated $\text{K}^{+}$ influx was ouabain inhibitable; however, the total cellular $\text{K}^{+}$ decreased due to an ATP-stimulated ouabain-resistant $\text{K}^{+}$ efflux. $\text{Na}^{+}$ influx and $\text{Ca}^{2+}$ influx occurred by separate independent routes, since $\text{Na}^{+}$ influx was not inhibited by DIDS. The effects observed were specific for ATP ($K_{u}$, MgATP = 0.7 mM) since AMP, GTP, adenosine, and the slowly hydrolyzable ATP analogue adenylylimidodiphosphate were without effect. The major ionic changes in the cell were a decrease in $\text{K}^{+}$ and increase in $\text{Na}^{+}$; cytoplasmic pH and free $\text{Ca}^{2+}$ did not change appreciably. These ATP-induced changes in ion flux are considered to be responsible for growth inhibition.

Extracellular ATP (1 mM) induced ion fluxes and inhibited growth of Friend Erythroleukemia Cells

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Changes in cation flux across the plasma membrane may play a pivotal role in the regulation of cellular growth and differentiation (1-4). Early changes in $\text{Na}^{+}$, $\text{H}^{+}$, $\text{Ca}^{2+}$, and $\text{K}^{+}$ fluxes across the plasma membrane have been demonstrated in quiescent fibroblasts stimulated to grow on addition of serum (3, 5, 6). However, the mechanism by which these changes influence growth and differentiation has not been characterized. Extracellular ATP has also been shown to affect cation fluxes in a variety of tumor cell lines. Ehrlich ascites cells (8), TA3 ascites cells (9), and monolayers of 3T6 cells (10) when exposed to ATP undergo a process of efflux of nucleoside pools of sugar phosphates and influx of several cations. ATP has also been reported to increase $\text{Na}^{+}$ and $\text{Ca}^{2+}$ influx in mast cells (11) and intestinal cells, although in the latter case the changes were of transitory nature (12). Rapaport et al. (13, 14) reported that extracellular ATP had a selective inhibitory growth effect on human transformed cells compared to normal cells.

The mechanism by which exogenous ATP induces permeability changes and inhibits cell growth is not known. However, in view of the known effects of extracellular ATP on various cell lines, we have studied its effect on MEL cell growth and differentiation. These cells are of particular interest since early changes in cation fluxes have been implicated as important signals for Me2SO-induced erythroid differentiation (7).

We report that ATP inhibits growth but has no effect on Me2SO-induced differentiation. ATP also brings about an increased and independent influx of $\text{Na}^{+}$ and $\text{Ca}^{2+}$ and an efflux of $\text{K}^{+}$. These effects are specific for ATP and are considered to be responsible for growth inhibition.

MATERIALS AND METHODS

Effect of ATP on Cell Growth and Differentiation—Because of previous reports that extracellular ATP inhibits the growth of transformed cells, we investigated the effect of ATP on growth and differentiation of MEL cells. Fig. 1 shows that extracellular ATP inhibited the growth of MEL cells but had no effect on Me2SO-induced MEL cell differentiation.

Extracellular ATP (1 mM) Induces $\text{Ca}^{2+}$ Uptake—When MEL cells were exposed to 1 mM ATP at 25 °C $\text{Ca}^{2+}$ exchange into the cells was enhanced (Fig. 2a). The $\text{Ca}^{2+}$ uptake was linear for at least 10 min at 25 °C although a short lag was sometimes detected. The initial rate of $\text{Ca}^{2+}$ uptake was stimulated from a basal level of 5 pmol/min/10^6 to 18 pmol/min/10^6 cells in the presence of 1 mM ATP. The ATP-

1 The abbreviations used are: MEL cells, Friend virus-infected murine erythroleukemia cells; Me2SO, dimethyl sulfoxide; FCS, fetal calf serum; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; AMP-PNP, adenylylimidodiphosphate; AIB, $\alpha$-aminoisobutyric acid; DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonate; EGTA, ethylene glycol bis($\beta$-aminoethyl ether)-N,N',N''-tetraacetic acid; SITS, 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid.

2 Portions of this paper (including "Materials and Methods," Figs. 1 to 6, and Figs. 8-12) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-1026, cite the authors, and include a check or money order for $6.80 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

13717
induced Ca\(^{2+}\) uptake was most rapid when 145 mM choline chloride replaced NaCl in the external medium (Fig. 2b) (146 pmol/min/10^6 cells compared to the basal rate of 20 pmol/min/10^6 cells). In the presence of α-medium, external ATP caused an increase in total cellular Ca\(^{2+}\). ATP was added to MEL cells equilibrated with α-medium (1.8 mM Ca\(^{2+}\)) at 37 °C in the presence or absence of 1 mM ATP (Fig. 2c). Isotopic equilibration occurred after approximately 3 h. Twice as much 45Ca\(^{2+}\) was present in ATP-treated cells as in untreated cells.

ATP (1 mM) caused only a transient elevation of the cytoplasmic Ca\(^{2+}\) concentration in MEL cells. In the absence of ATP free Ca\(^{2+}\) was estimated to be 160 nM using Quin 2 fluorescence as an indicator. Addition of ATP caused a transient increase in Quin 2 fluorescence which peaked within 2 min and decayed back to the level in the control cells within 10 min (Fig. 3). At the peak, the free Ca\(^{2+}\) was estimated to be 240 nM. Readdition of ATP after 20 min had no effect on Quin 2 fluorescence (data not shown). Since under similar conditions ATP caused an increase in the Ca\(^{2+}\) influx rate which continued for several hours, these results suggest that uptake of Ca\(^{2+}\) by some cellular organelle (mitochondria or endoplasmic reticulum) is activated following ATP addition.

**ATP-induced Ca\(^{2+}\)** and Cl\(^{-}\) Uptake Are Inhibited by DIDS—It was found that ATP induced Ca\(^{2+}\) influx could be blocked by an anion transporter inhibitor DIDS (Fig. 4). This suggested that Ca\(^{2+}\) was cotransported with an anion resulting in an electroneutral flux. This possibility was substantiated by the finding that Cl\(^{-}\) transport was also stimulated by ATP (1 mM) and that this influx could also be inhibited by 200 μM DIDS (Fig. 5). The ratio of ATP-stimulated Cl\(^{-}\) uptake to ATP-stimulated Ca\(^{2+}\) uptake was 1.6:1 (Table I). This ratio indicates that one Cl\(^{-}\) is cotransported per Ca\(^{2+}\) since this Cl\(^{-}\) influx is electroneutral.

**Ouabain-sensitive and ouabain-resistant =Rb\(^{+}\) uptake were linear for more than 10 min** (Fig. 6). The ouabain-sensitive =Rb\(^{+}\) uptake was stimulated from a basal level of 0.46 nmol/min/10^6 cells to 0.70 nmol/min/10^6 cells in the presence of ATP, suggesting stimulation of Na\(^{+}\)/K\(^{+}\)-ATPase. No significant change (<10%) could be detected in ouabain-resistant =Rb\(^{+}\) uptake indicating that ATP is not merely acting by opening nonspecific channels.

**Effects of ATP on Na\(^{+}\) Uptake—Na\(^{+}\) was allowed to reach isotopic equilibrium in MEL cells suspended in α-medium at 25 °C (Fig. 7). ATP (1 mM) was then added, and a net increase in cellular Na\(^{+}\) was measured with time. ATP stimulated Na\(^{+}\) influx which then reached a new steady-state value after 20 min. This ATP-induced Na\(^{+}\) influx could be further enhanced by addition of EGTA (3 mM) to the media, indicating that ATP-induced Na\(^{+}\) permeability is not dependent on influx of extracellular Ca\(^{2+}\).

In view of the effects of DIDS on Ca\(^{2+}\) uptake by ATP, the Na\(^{+}\) uptake induced by ATP was also studied in the presence of DIDS (Fig. 8). 200 μM DIDS without ATP caused a small but significant increase in cellular Na\(^{+}\). The mechanism of this increase is not clear. After a 40-min incubation in ATP plus DIDS, the cellular Na\(^{+}\) was higher than in the presence of ATP alone or DIDS alone. Error in the data prevented an accurate comparison of initial rates of ATP-stimulated Na\(^{+}\) uptake in the presence and absence of DIDS. Since 200 μM DIDS completely blocked the ATP stimulation of Ca\(^{2+}\) uptake but did not prevent ATP from elevating the cellular Na\(^{+}\), these two effects must occur on separate transport systems. These results also indicate that a Cl\(^{-}\) influx does not accompany the ATP-stimulated Na\(^{+}\) influx since DIDS inhibits ATP-stimulated Cl\(^{-}\) influx (Fig. 5).

**ATP-induced Na\(^{+}\) Uptake Is Not Affected by Amiloride or Furosemide—Na\(^{+}\) was allowed to reach isotopic equilibrium in MEL cells suspended in α-medium at 25 °C. The cells were then preincubated with amiloride (1 mM) or furosemide (100 μM) for 10 min, and then ATP (1 mM) was added and a net increase in cellular Na\(^{+}\) was measured with time. Amiloride, an inhibitor of Na\(^{+}/H^+\) exchange had no effect on ATP-induced Na\(^{+}\) influx (data not presented). This finding was substantiated by the lack of effect of ATP on intracellular pH of MEL cells as measured by 5,6-carboxyfluorescein diacetate (data not shown). Furosemide (100 μM), an inhibitor of the Na\(^{+}/K^+\)/Cl\(^{-}\) transport system, also had no effect on ATP-induced Na\(^{+}\) uptake (Fig. 9).

**Enhanced Na\(^{+}\) Efflux Is Enhanced by ATP**—The effect of ATP on

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**Table I**

Initial rates of ion influx

<table>
<thead>
<tr>
<th>Ion</th>
<th>Initial rate (nmol/min/10^6 cells)</th>
<th>α-medium</th>
<th>ATP (1 mM)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(^{2+})</td>
<td>0.896</td>
<td>0.944</td>
<td>0.581</td>
<td>0.24</td>
</tr>
<tr>
<td>Cl(^{-})</td>
<td>0.384</td>
<td>1.392</td>
<td>0.601</td>
<td>0.192</td>
</tr>
<tr>
<td>Na(^{+})</td>
<td>0.46</td>
<td>2.190</td>
<td>2.190</td>
<td>0.314</td>
</tr>
<tr>
<td>Rb(^{+})</td>
<td>0.46</td>
<td>2.190</td>
<td>2.190</td>
<td>0.314</td>
</tr>
</tbody>
</table>

*Estimated by initial rate of isotope exchange into cells.

*Estimated by final rate of isotope exchange into cells.

Net Na\(^{+}\) uptake was measured after reaching isotopic equilibrium (see Fig. 7).

Ouabain-sensitive =Rb\(^{+}\)-K\(^{+}\) exchange rate (see Fig. 6).

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**Fig. 7. Effect of ATP (1 mM) on 22Na\(^{+}\) influx into MEL cells.** Cells were suspended at 5 × 10^6 cells/ml in α-medium and incubated with 22NaCl (6 μCi/ml) at 25 °C. After isotopic equilibrium had been attained (30 min), ATP (1 mM) with and without EGTA (3 mM) was added. Samples were removed at intervals and processed as described under "Materials and Methods." C, control; O, ATP; and A, ATP + EGTA.
MEL cells which had been initially preloaded with $^{86}$Rb$^+$ in α-medium at 25 °C for 4 h is shown in Fig. 10. Addition of ATP caused a rapid efflux of $^{86}$Rb$^+$ from MEL cells, even though at the same time there was an ATP-induced influx of $^{86}$Rb$^+$ (Fig. 6). The slight reduction of ATP-induced $^{86}$Rb$^+$ efflux seen in the presence of DIDS may have been due to greater influx of Na$^+$ (Fig. 8) and thus an enhanced Na$^+$-K$^+$-ATPase activity counteracting $^{86}$Rb$^+$ efflux. Assuming $^{86}$Rb$^+$ acts as a tracer for K$^+$ and assuming a K$^+$ concentration of 200 nmol/10$^6$ cells (17), then the K$^+$ content decreases by approximately 40 nmol/10$^6$ cells within 30 min. This decrease more than offsets the increase in cellular Na$^+$ during this time (10 nmol/10$^6$ cells; Fig. 7).

ATP Hydrolysis and Effect of AMP, AMP-PNP, Adenosine and GTP on Ca$^+$ Uptake—Addition of 1 mM ATP (1 µCi/ml) to MEL cells suspended in α-medium at 25 °C resulted in hydrolysis of ATP with time (Fig. 11). There was very little hydrolysis of ATP in the absence of cells. The initial rate of ATP hydrolysis was approximately 1 nmol/min/10$^6$ cells. The percentage of hydrolysis of ATP was 30% by 5 h in the presence of 5 x 10$^6$ cells/ml. The effect of AMP, adenosine, GTP, and the slowly hydrolyzable ATP analogue AMP-PNP was also studied by examining Ca$^{2+}$ uptake in Na$^+$-Ringer’s buffer. All the above compounds were without effect on Ca$^{2+}$ uptake suggesting that ATP hydrolysis may be important (data not presented).

Effect of ATP on P$_v$ Uptake—Ca$^{2+}$ uptake by mitochondria is known to be stimulated by P, and thus we looked to see if ATP enhanced P$_v$ uptake by MEL cells. In fact, it was found that ATP inhibited 3P$_v$ uptake by MEL cells (Table 1), once again suggesting that ATP affects specific transport systems.

\[ \text{[ATP] Dependence of Ca}^{2+} \text{Uptake} \]

Fig. 12 shows that the rate of Ca$^{2+}$ uptake induced by ATP is dependent on ATP concentration. The $K_m$ for MgATP was approximately 0.7 mM.

**DISCUSSION**

It has been shown in this study that extracellular ATP can inhibit the growth of MEL cells but has no effect of MeSO$_4$-induced differentiation. Furthermore, extracellular ATP can elicit changes in the membrane flux of Na$^+$, Ca$^{2+}$, and $^{86}$Rb$^+$ in MEL cells. The ATP-induced changes in ion flux are such that both the Ca$^{2+}$ and Na$^+$ levels in the cell increase approximately 2-fold (Figs. 2C and 7). Na$^+$ and Ca$^{2+}$ appeared to enter by separate independent routes, since Ca$^{2+}$ uptake could be selectively inhibited by DIDS with no inhibitory effect on Na$^+$ uptake. The consequence of the rise in Na$^+$ level was a loss of the electrochemical sodium gradient as reflected by the loss of Na$^+$-dependent amino acid uptake. ATP also caused a net decrease in cellular K$^+$ due to an ATP-stimulated ouabain-resistant K$^+$ efflux. The effects observed were specific for ATP ($K_m$ for ATP = 0.7 mM) since AMP, GTP, adenosine, and the slowly hydrolyzable ATP analogue AMP-PNP were without effect.

The inhibition of growth of MEL cells by ATP may be a consequence of the decrease in intracellular K$^+$. It is well documented that levels of intracellular ions, K$^+$ in particular, can influence the rate of growth through changes in the initiation of translation of mRNAs (26). The associated decrease in amino acid uptake may also slow down growth of MEL cells. It is also important to note that altering Na$^+$ and K$^+$ levels had no effect on differentiation. Although ATP caused a transient rise in cytoplasmic Ca$^{2+}$ it had no long-term effect on free Ca$^{2+}$ or cytoplasmic pH. This is consistent with the earlier report (7) that suggested that a prolonged rise in free Ca$^{2+}$ was essential for MEL cell differentiation. It seems that the bulk of the Ca$^{2+}$ uptake in MEL cells may be sequestered by mitochondria and endoplasmic reticulum as indicated by the Quin 2 experiment where only a transient rise in free Ca$^{2+}$ was seen even though there was a 2-fold increase in total Ca$^{2+}$. It should also be pointed out that although it is difficult to completely exclude the possibility that ATP-stimulated $^{46}$Ca$^{2+}$ uptake is occurring in mitochondria from lysed cells, the Quin 2 experiment indicated that some of the Ca$^{2+}$ uptake is into intact cells.

A similar increase in Na$^+$ and Ca$^{2+}$ influx has been observed for mast cells and Ehrlich ascites tumor cells incubated with ATP (8, 11, 19, 20). However, the changes in Na$^+$ and Ca$^{2+}$ ion flux induced by ATP in MEL cells were not of a transitory nature. This is different from the findings of Kimmich and Randles (12) with intestinal cells where transient changes in Na$^+$ and Ca$^{2+}$ were seen. Also, in the intestinal cells ATP-induced Na$^+$ influx could be blocked with SITS, an anion blocker like DIDS. With MEL cells, Na$^+$ influx could not be blocked with DIDS, nor with amiloride or furosemide. However, DIDS did block both Ca$^{2+}$ and Cl$^-$ influx, suggesting that these ions are cotransported. ATP was also found to cause cellular K$^+$ efflux, a finding similar to that reported by several other workers (10, 19).

The increase in steady-state levels of Na$^+$ and Ca$^{2+}$ was not due to an increase in cell volume by uptake of bulk medium from the environment since no change in cell water was observed using the $^3$H$_2$O/$^1$C$\text{glucose}$ method (data not presented). The decrease in P$_v$ uptake (Table 1) would also rule out this possibility. In addition, calculations based on data in Table 1 indicate that Na$^+$ efflux through the Na$^+$K$^+$-ATPase is 0.69 nmol/min/10$^6$ cells before ATP addition which increases by a further 0.36 nmol/min/10$^6$ cells on addition of ATP assuming 3Na$^+$:2K$^+$ exchanging via Na$^+$K$^+$-ATPase. The initial increase in Na$^+$ uptake following ATP addition was 0.58 nmol/min/10$^6$ cells. This value is somewhat greater than the ATP-stimulated efflux through the Na$^+$K$^+$-ATPase (0.36 nmol/min/10$^6$ cells). Thus, when cytoplasmic Na$^+$ reaches a steady state at the elevated level (Fig. 7), Na$^+$ efflux through a non-(Na$^+$K$^+$)-ATPase transport system must also increase.

The induction of ion flux observed in MEL cells seems to be very specific for ATP, since AMP, GTP, adenosine, and the nonhydrolyzable analogue of ATP, AMP-PNP, were without effect. Also, the permeability changes observed are dependent on ATP concentration with $K_m$ of 0.7 mM for MgATP (Fig. 12). This specificity and concentration dependence for ATP has also been noted by other workers in both the tumor cell line and mast cells.

Orthovovanadate (100 µM), an inhibitor of cation transporting ATPases, had no effect on the ATP-induced ion changes (data not presented), a finding similar to that reported by Dicker et al. (10) where 10 µM vanadate had no effect on the influx of nucleotide pools from 3T3 cells induced by extracellular ATP.

Extracellular ATP, it seems, can also influence the excitatory properties of rat dorsal root neurones. Jahn and Jessell (21) found that depolarization of dorsal horn neurones could be induced by ATP through an increase in Na$^+$ conductance. However, in their system analogues of ATP such as AMP-PNP and β,γ-methylene ATP were also effective, indicating that ATP hydrolysis was not essential for the activation of Na$^+$ influx. It would appear that the specificity and the reactions elicited by extracellular ATP depend very much on the type of cell investigated.

It is unclear what the physiological role may be for the changes in membrane permeability induced by extracellular
ATP. It is conceivable that ATP may be a circulating hormone. A possible transmitter action of ATP in the central and peripheral nervous systems has been suggested (22, 23), and there is evidence of ATP being released into the circulation following peripheral nerve stimulation (24). Also, release of histamine from mast cells, serotonin from platelets, and adrenaline from chromaffin granules is associated with large amounts of ATP being released into the circulation (25). Thus, following a pertinent stimulation, the translocated ATP could impinge on the membrane of adjacent cells, effecting an increase in permeability and, therefore, spreading the initial signal. The presence of ecto-ATPases and ecto-5'-nucleotidase would serve to limit or terminate the phase of increased permeability.

In conclusion, we have shown an effect of extracellular ATP on membrane permeability of MEL cells to specific ions. An increase in Na+ and Ca2+ influx occurred by separate routes, the latter being DIDS inhibitable. Also, there was a decrease in total cellular K+ which may have caused the observed inhibition of MEL cell growth.

Acknowledgments—We thank workers in Lewis Cantley's laboratory for useful discussion and general support during this work. We also thank Maria Aveni for technical assistance in maintaining the cells.

REFERENCES
18. Deleted in proof
**Ion Fluxes in Friend Cells**

**Figure 8.** Effect of furosemide (100 μM) on 3H influx into Friend cells.

Cells were pretreated with furosemide (100 μM) for 10 min and then 3H ATP was added. Samples were removed at intervals as described under 'Materials and Methods'. Control (o); control + furosemide (Δ); ATP (★); ATP + furosemide (■).

**Figure 9.** Time course of ATP hydrolysis by Friend cells.

Cells were treated with 100 μM ATP for 10 min and then ATP (0) and ATP + furosemide (■).

**Figure 10.** Effect of ATP (1 μM) and 8-BrcAMP (200 μM) on 3H influx from NR.

Cells were pretreated with ATP (1 μM) for 10 min and then 3H ATP was added. Samples were removed at intervals as described under 'Materials and Methods'. Control (o); control + ATP (Δ); ATP (★); ATP + 8-BrcAMP (■).

**Figure 11.** Effect of ATP on 3H uptake by Friend cells.

Cells were treated with ATP for 1 min at 37°C. ATP (0) and ATP + 8-BrcAMP (■).

**Figure 12.** Effect of ATP on 3H uptake by Friend cells.

Cells were treated with ATP for 1 min at 37°C. ATP (0) and ATP + 8-BrcAMP (■).