SUMMARY: L1210 cells minitize growth after dilution with fresh medium during this time there occurs a transient increase in ornithine decarboxylase (ODC) activity. The addition of 20 to 25 nmol K⁺ or Na⁺ completely inhibits this induction of ODC activity with no effect on cell growth. These conditions also inhibit the increase of ODC activity in neocallstroma cells and in N1/2 cells which is induced by prostaglandin B₂ plus 3,4- dihydroxy-L-phenylalanine and by L5A fatal calf serum respectively. This inhibitory effect of low levels of cations on the induction of ODC activity in different cell lines suggests that the intracellular function of ODC, and of the products of the reaction it catalyzes (putrescine, spermidine and spermine), may be increasingly involved with changes in cation pools.

We have previously shown that the intracellular ornithine decarboxylase activity of L1210 cells can be affected by agents which disrupt the microtubule-microfilament cytoskeleton (1). This phenomenon was considered as a model system for reactions which link the perturbation of the cell membrane with intracellular changes in ODC activity.

ODC is the rate-limiting enzyme in polyamine synthesis and its intracellular activity in eukaryotic cells can be induced by increases in the rate of growth (2,3). The polyamines are implicated in RNA polymerase activity (4), tRNA methylation (5), polypeptide chain elongation (6) and other cellular functions. It is this breadth of association of these polyamines that makes it difficult to assess their exact role in cell metabolism.

In most of the reactions mentioned above, there occurs an overlap between the function of the polyamines and other cations; specifically potassium and magnesium (8). Alterations in the intracellular osmotic pressure (7), in the membrane potential (7) as well as changes in the transport of non-electrolytes (8) and in macromolecular synthesis (9,10) have been shown to be affected by
alterations of K+Na+ ratios in the interior and the exterior media of the cell. Furthermore it has been reported that lectin-stimulated lymphocytes (11) and Ehrlich ascites cells (12), virus transformed fibroblasts (13) and quiescent cells stimulated by serum (14) show increased K+ transport associated, in many cases, with changes in OCC activity (15,16). Studies in plant tissues have also emphasized that the plant polyamines and the physiological actions are

interchangeable (1,17). Because of these associations we have attempted to relate, in the present work, modifications of cation concentrations in the growth medium of cells grown in culture to fluctuations of intracellular OCC activity.

MATERIALS AND METHODS

The following compounds were purchased: [1-14C]-DL-ornithine, 30-50 mCi/mM, aqueous; [3H]methylamine, [3H]methylamine, and [3H]nicotinic from New England Nuclear, Boston, Mass.; horse serum, fetal calf serum, Fischer's medium and essential medium from Grand Island Biological, prostaglandin E1 (PGE1) from Dr. J. Fiske, Upjohn Co.; l-iodobilirubin-1-methylamine (IIMB) from Aldrich Chem. Co. All other chemicals were of reagent grade and were obtained from Fisher Scientific Co., Springfiel, N.J.

Cell Culture. L1210 cells were grown as previously described (18). The cells were grown to the late log phase (1.3 x 10^5 cells/ml) and then diluted with fresh Fischer's medium plus 10% horse serum to about 4 x 10^3 cells/ml for subsequent studies. Different amounts of cations were added to the fresh medium to observe their effects on the induction of OCC activity after cell dilution. 8-E cells were grown on 100 mm Falcon plastic plates in Eagle's minimal medium plus 1% fetal calf serum. At confluency, the plates were rinsed with saline, fresh medium minus serum was added and 24 hrs later OCC activity stimulated by the addition of fresh medium plus 15% fetal calf serum. The cells were harvested 2 hrs later and OCC activity assayed. Neuroblastoma cells were grown on 100 mm Falcon plastic plates in Dulbecco's modified Eagle's minimal medium with 10% fetal calf serum. At confluency, the plates were rinsed with Dulbecco's medium containing 25 mM HEPES pH 7.4 and no serum. HEPES and PBS (0.15 M in ethanol) were added to each plate containing the serum free medium at the final concentration of 2.5 mM and 10 mM respectively. The cells were harvested 6 hrs later and OCC activity assayed.

ASSAY OF OCC Activity. Ornithine decarboxylase was determined by measuring the release of CO2 from [1-14C]-DL-ornithine as previously described (1). The specific activity of the enzyme is expressed as molecules 14C02 released per mg protein per hour.

RESULTS AND DISCUSSION

1. Effect of cations on OCC activity of neuroblastoma and 8-E cells.

The basal OCC activity of confluent cultures of 8-E and neuroblastoma

cells is very low, however the enzyme activity can be stimulated approximately

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Table 1. Effect of cations on the induction of GCC activity of neuroblastoma cells and R-35 cells

<table>
<thead>
<tr>
<th>Growth medium* MgCl₂ mm</th>
<th>Induction medium* MgCl₂ mm</th>
<th>Cellular GCC activity µmoles CO₂/mg/hr</th>
<th>Inhibition Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroblastoma cells</td>
<td></td>
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<td></td>
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<tr>
<td>0</td>
<td>0</td>
<td>3.2</td>
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</tr>
<tr>
<td>0</td>
<td>10</td>
<td>1.6</td>
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</tr>
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</tr>
<tr>
<td>20</td>
<td>20</td>
<td>1.61</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>3.65</td>
<td>stimulation (14%)</td>
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R-35 cells

<table>
<thead>
<tr>
<th></th>
<th>MgCl₂ mm</th>
<th>Cellular GCC activity µmoles CO₂/mg/hr</th>
<th>Inhibition Per Cent</th>
</tr>
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<tr>
<td>20</td>
<td>0</td>
<td>0.43</td>
<td>stimulation (104)</td>
</tr>
</tbody>
</table>

* The composition of growth medium and induction medium were described in Materials and Methods.

200-fold within 2 hrs by the addition of medium plus serum, to serum-depleted R-35 cells or within 4 hrs by the addition of PGE₃ and DMSO to neuroblastoma cells. The addition of 10-20 mM MgCl₂ to the inducing media inhibits completely the rise in GCC activity in R-35 cells; the rise in GCC activity in neuroblastoma cells is inhibited by more than 50% (Table 1). In addition, this table shows that regardless of whether the cells were grown in the presence or absence of added Mg²⁺, the presence of added NaCl the induction of GCC. The cells are diluted in the absence of added NaCl.
1) In the present study, the presence of Mg^{++} in the induction medium prevents the induction of 5OC. The converse also obtains, because when stationary cells are diluted in the absence of Mg^{++}, 5OC activity is always induced.

2) Inhibition of the induction of 5OC activity in L1210 cells by cations.

Dilution of L1210 cells at late log phase or at stationary phase into fresh medium results in a stimulation of 5OC activity reaching a maximum approximately 2-4 hrs after dilution. Fig. 1 shows that if the fresh medium, at the time of dilution also contains 15 mM MgCl₂, the increase of 5OC activity is completely inhibited with no undue effects on the subsequent growth of the cells. A similar result is obtained if the dilution is done in medium with 15 mM NaCl or with 15 mM KCl (these concentrations of Na⁺, K⁺ and Mg²⁺ have little effect on 5OC activity when added to an in vitro assay). This involvement of the cations on the regulation of the induction of 5OC activity is
Fig. 2. Effect of 15 mM MgCl₂ on the induction of ODC activity of L1210 cells. L1210 cells were grown in normal medium to the late log phase and were diluted with fresh Fischer’s medium plus 19% horse serum in the presence (••) or in the absence (○) of added 15 mM MgCl₂. ••: L1210 cells were grown in Fischer’s medium with added 15 mM MgCl₂ for 2 generations and were then diluted into the normal fresh Fischer’s medium.

FURTHER EMPIRICALLY BY THE CONVERSE EXPERIMENT. FIG. 2 SHOWS THAT WHEN CELLS WHICH HAVE BEEN GROWN IN THE PRESENCE OF 20 mM MgCl₂ UP TO THE STATIONARY PHASE ARE DILUTED INTO NORMAL FRESH MEDIUM (i.e., TO normal MgCl₂), THEIR ACTIVITY ON AN ALMOST TWO-FOLD GREATER INCREASE IN ODC ACTIVITY THAN THE INCREASE IN ODC ACTIVITY USUALLY OBTAINED. THIS INDICATES THAT INCREASES IN ODC ACTIVITY A LOWERING OF Mg²⁺ CONCENTRATION INHIBITS THE INDUCTION OF ODC ACTIVITY AND FURTHER INCREASES THE INDUCTION OF ODC ACTIVITY.

3. THE ADAPTATION OF L1210 CELLS TO A HIGH Mg²⁺ MEDIUM.

L1210 cells grown in the presence of 20 mM MgCl₂ over several generations adapt slowly to this higher Mg²⁺ again show a normal induction of ODC activity with high Mg²⁺ media (Fig. 5). ODC activity in L1210 cells in Fig. 3 (A, B) refers to the 1st day of growth in the absence of MgCl₂; these results correspond to the 1st day of growth in the absence of MgCl₂. ODC activity which is almost equal to the normal induction of ODC activity + Mg²⁺ concentration; furthermore, in
Fig. 3. Adaptation of L1210 cells in the presence of 30 mM MgCl₂. L1210 cells grown in Fischer's medium and were diluted every 24 hrs (roughly 2 generations). a, L1210 cells at 1.2 x 10⁵ cells/ml were diluted in the absence (- - -) or in the presence (---) of 20 mM MgCl₂ to 3 x 10⁵ cells/ml. b, control cells were diluted with normal medium; the Mg-treated cells were diluted with 20 mM Mg²⁺ containing medium. c, control cells were diluted with normal medium and Mg-treated cells were diluted with 20 mM Mg²⁺ containing medium.

L1210 cells adapt slowly to this higher Mg²⁺ level until they reach the stage where they again show a normal induction of G6C activity even when diluted at stationary phase with high Mg²⁺ media (Fig. 3). The figure shows that the induction of G6C activity in L1210 cells in Fischer's medium is inhibited by 20 mM Mg²⁺ (Fig. 3a); this result corresponds to that obtained with K⁺ (Fig. 1). However, if the L1210 cells are grown in a high Mg²⁺ medium for 2 generations and then diluted at stationary phase with a high Mg²⁺ medium, a low but definite induction of G6C activity is observed (Fig. 3b). In addition, if the L1210 cells have been grown for more than 4 generations in a high Mg²⁺ medium, then dilution at stationary phase with a high Mg²⁺ medium results in an induction of G6C activity which is almost equivalent to that of the control (Fig. 3c). These results emphasize that the cell adapts to the new environment with a normal induction of G6C activity in the presence of what was an inhibitory Mg²⁺ concentration; furthermore it appears that the phenomena we are observing...
are central to the function of the cell, but that the particular conditions that are required to elicit them will vary depending upon the prior conditioning of the cell.

4. The effect of cations on macromolecular synthesis in L2120 cells.

Although the three cations inhibit the induction of ODC activity at 10-20 mM, they have no adverse effect on cell growth. We also found that they do not inhibit the incorporation of thymidine into DNA and leucine into protein. However, a small percentage (less than 10%) stimulation of uridine incorporation in the presence of exogenous cations is consistently observed (data not shown). The results clearly indicate that KCl and NaCl2 as well as NaCl, when added in concentrations which have no apparent effect on the growth rate of the cells or on macromolecular synthesis, can abolish the characteristic normal induction of ODC which occurs after the withdrawal of growth in L2120 cells and after the stimulation of growth of neuroblastoma and n-B cells.

This study pertains strictly to the inhibition of the induction of ODC activity by cations and should be differentiated from the inhibition of the basal intracellular activity of ODC. Changes in the latter have been shown by Murao et al. (19) to occur at high changes of osmolarity of the surrounding medium for instance, an increase by 50 mM NaCl inhibits the basal ODC activity by less than 50%. This effect should be contrasted with the almost complete inhibition of induction of ODC activity noted in the present experiments with 15 mM NaCl.

The inhibition of the induction of ODC activity does not appear to be due to osmotic changes but due to cation specific effects. The osmolarity of Placzer's medium is approximately 325 mM KCl (20). Adding 10 to 20 mM KCl increases the osmolarity by approximately 7% to 15% and causes a complete inhibition of the induction of ODC activity. However, we find that an increase of the osmolarity by the addition of 20 to 40 mM sucrose, inhibits the induction of ODC activity only by 30% to 50%. Consequently, the change in osmolarity is not sufficient in itself to have a profound effect on the inhibition of the induction of ODC activity. Furthermore, the marginal inhibition of ODC activity that we have observed, when the osmolarity of the medium, may well be a reflection of those cations which are the precursors of ODC.

In addition, experiments with chloride and the sulfates ions show some changes seen to be cation activity in n-B cells is less sensitive. In contrast, the induction of ODC is sensitive to changes in Na+ and K+.

Our current working hypothesis is that ODC activity in response to restimulation of induction of ODC activity does not result in a cellular response, but rather a linkage between the microtubule-microfilament system.

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observed, when the osmolarity of the medium is changed by the addition of sucrose, may well be a reflection of changes in intracellular concentrations of those cations which are the proximal effectors of the inhibition of the induction of OOC.

In additional experiments we find that the same effect is produced by the chloride and the sulfonium ions of a given cation. In contrast, we find that some changes seem to be cation specific. For instance, the induction of OOC activity in B-35 cells is less sensitive to changes in Na⁺ than to changes in K⁺; in contrast, the induction of OOC activity in L-1210 cells is equally sensitive to changes in Na⁺ and K⁺.

Our current working hypothesis is that the inhibition of the induction of OOC activity in response to metabolic perturbations (1) and the inhibition of the induction of OOC activity observed with small changes of cation pools may be causally related; it is possible that fluctuations of cation pools may act as a linkage between the microtubule-microfilament cytoskeleton and OOC.

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