Metabolic Functions of Myo-inositol

VIII. ROLE OF INOSITOL IN Na⁺-K⁺ TRANSPORT AND IN Na⁺- AND K⁺-ACTIVATED ADENOSINE TRIPHOSPHATASE OF KB CELLS*

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SUMMARY

The effects of myo-inositol (inositol) deprivation on the Na⁺ and K⁺ transport system of KB cells and on the Na⁺- and K⁺-activated adenosine triphosphatase of cell-free extracts were investigated.

Kinetic studies under presteady state conditions showed that inositol deprivation causes a decrease of the initial rates of K⁺ influx and Na⁺ efflux. Both the $V_{\text{max}}$ of K⁺ influx and the $K_m$ for K⁺ are decreased.

The rate of ATP hydrolysis catalyzed by the membrane-bound, Na⁺- and K⁺-activated adenosine triphosphatase is greatly diminished in inositol-deficient cells. Both the $V_{\text{max}}$ and the $K_m$ values for K⁺ and Na⁺ are decreased. These changes are quantitatively identical with those observed in the case of Na⁺ and K⁺ fluxes across the plasma membrane of intact cells.

Inositol-deficient cells are unable to concentrate K⁺ to the same extent as normal cells, but the steady state concentration of intracellular Na⁺ remains within normal range. The lower intracellular concentration of K⁺ in inositol-deficient cells is not responsible for the impaired transport of $\alpha$-aminoisobutyric acid exhibited by these cells.

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The KB cells used in these studies (certified line No. 17 of the American Type Culture Collection) were obtained from Dr. H. Eagle in 1959 and have been maintained in monolayer and suspension cultures since then.

1 The abbreviations used are: inositol, myo-inositol; AIB, $\alpha$-aminoisobutyric acid; Na⁺-K⁺ pump, the Na⁺- and K⁺-activated, ouabain-inhibitable, membrane-bound ATPase.

2 The Krebs-Ringer-bicarbonate buffer contained the following components, in mM concentrations: KCl, 4.74; CaCl₂, 2.53; KH₂PO₄, 1.19; MgSO₄, 1.19; NaCl, 118.5; NaHCO₃, 24.9; and glucose, 2.0. The solution, equilibrated with a mixture of 95% O₂-5% CO₂, had a pH of 7.4.

Studies on the role of myo-inositol in the maintenance of normal function of the plasma membrane of KB cells¹ have shown that inositol² deprivation leads to an early impairment of the transport of various amino acids (1, 2). In the previous paper in this series (2), which dealt with the kinetics of $\alpha$-aminoisobutyric acid transport under presteady state and steady state conditions, it was shown that inositol deprivation in KB cells causes a decrease of the $V_{\text{max}}$ of AIB influx and efflux, without affecting the respective $K_m$ values. It was of interest, therefore, to determine whether or not functions of the plasma membrane other than amino acid transport are also affected by inositol deficiency. One such function is the translocation of Na⁺ and K⁺, thought to be mediated by the membrane-bound, Na⁺- and K⁺-activated, ouabain-inhibitable ATPase (Na⁺-K⁺ pump). The studies presented in this paper deal with the effects of inositol deprivation on the kinetics of Na⁺ and K⁺ fluxes in KB cells and on the kinetic parameters of the Na⁺-K⁺ pump in cell-free preparations.

EXPERIMENTAL PROCEDURE

Methods

Culture Conditions—The methods and media for culturing KB cells were described in an earlier publication (3). The cell density was maintained between 2 × 10⁶ and 5 × 10⁶ cells per ml. Unless otherwise stated, the deficient cells were grown for 3 days without inositol prior to being used in these studies. They were 90% viable, as compared to 98% for normal cells.

Measurement of K⁺ Influx and Na⁺ Efflux—KB cells harvested from their logarithmic phase of growth contained 24 to 28 mM Na⁺ and 90 to 100 mM K⁺. These steady state concentrations were also obtained when the cells were incubated in Krebs-Ringer-bicarbonate buffer, pH 7.4, at 37°C. In order to study the kinetics of net fluxes of K⁺ and Na⁺, as opposed to exchange fluxes, it was necessary to use cells in which the intracellular concentrations of these ions were removed from their steady state levels and then to measure the initial rates of K⁺ influx and Na⁺ efflux before the cells re-established steady state concentrations. In principle, this was achieved by first incubating the cells in Krebs-Ringer-bicarbonate buffer at 4°C. During this time, the cells lost K⁺ and gained Na⁺. In the intracellular concentrations of K⁺ and Na⁺ depended on the duration of the cold incubation and on the concentrations of these ions in the suspending medium. Following the cold incubation, the cell suspension was transferred to a 37°C bath in order to initiate K⁺ influx and Na⁺ efflux. The standard procedure adopted was as follows. The cells were harvested from their logarithmic phase of growth by centrifugation and were washed once with 200 vol.
umes of Krebs-Ringer-bicarbonate buffer. They were then suspended in this buffer (4 × 10^6 cells per ml), and 1 ml of cell suspension was added to centrifuge tubes which had been gassed with a mixture of 95% O₂-5% CO₂. The tubes were stoppered and incubated at 5° for 90 min with gentle shaking. At the end of this period, duplicate tubes were processed for determination of the intracellular concentrations of K⁺ and Na⁺ (zero time values); the remaining tubes were placed in a 37° bath to initiate K⁺ influx and Na⁺ efflux. At various intervals thereafter, the incubations were terminated, and the cells were analyzed for their K⁺ and Na⁺ content as described below. The difference between these values and the corresponding zero time levels gave the net K⁺ and Na⁺ fluxes. For initial rates, the incubation time at 37° was 5 min because the fluxes were constant and maximal during the first 10 min for normal cells and during the first 5 min in the case of inositol-deficient cells. In those experiments in which the K⁺ and Na⁺ concentrations of the Krebs-Ringer-bicarbonate medium were varied, appropriate amounts of choline-Cl, pH 7.4, were added, equivalent to the omitted amounts of these cations. These variations are indicated in each pertinent experiment.

**Determination of Intracellular Na⁺ and K⁺**—Cation fluxes were terminated by immersing the incubated cell suspensions in an ice bath and adding to them 10 ml of ice-cold 0.15 m choline-Cl, pH 7.4. Immediately, the samples were centrifuged at 1500 × g for 30 sec at 0°, and the cell pellets were rapidly suspended in 11 ml of ice-cold choline-Cl and centrifuged again. The supernant fluids were decanted, and the tubes were allowed to drain for 30 min at 5°. The inner walls of the tubes were wiped with clean tissue paper, and the pellets were suspended in 1 ml of deionized water. In preliminary experiments, it was established that washing the cells with choline-Cl as described above did not cause any measurable loss of intracellular Na⁺ or K⁺.

The extraction of Na⁺ and K⁺ from the cells was done by freezing and thawing the cell suspensions four times, followed by dilution with 3 ml of deionized water and incubation at room temperature for about 18 hours. The samples were then chilled in ice and centrifuged at 1500 × g for 30 min. The resulting clear supernatant fluids were analyzed for Na⁺ and K⁺ by flame photometry with the use of a Beckman model DU flame spectrophotometer. Na⁺ was measured at 589 nm and K⁺ at 768 nm. The emission signals (luminosities) were recorded on a linear recorder, and the cation concentrations were calculated from standard curves constructed with known concentrations of Na⁺ and K⁺. The reproducibility of the measurements was better than 98%, and duplicate cell incubations varied by less than 7%.

In those experiments in which the Na⁺ and K⁺ concentrations of the extracellular medium were measured, the incubations at 37° were terminated by chilling the cell suspensions to 0° for 45 sec without the addition of choline-Cl. After centrifugation, the clear supernatant fluids were analyzed for Na⁺ and K⁺ by flame photometry.

The procedure described above for extracting the intracellular Na⁺ and K⁺ was compared with two other methods, namely, extraction with 2% (v/v) nitric acid at room temperature for 18 hours and extraction with 10% (v/v) acetic acid at 100° for 1 hour. All three methods gave identical results.

**Assay of Na⁺- and K⁺-Activated ATPase**—This enzyme was assayed by the method of Post and Sen (4) by means of a particular fraction which was isolated from cell-free extracts as follows. The harvested cells were washed once with 50 volumes of ice-cold choline-Cl, pH 7.4, and were suspended in a buffer of glycglycine and imidazole (30 mM each), pH 7.4, containing 0.75 mM EDTA. The cell suspension (10^6 cells per ml) was sonically disrupted at 0° for 5 sec in a Branson sonifier (model W-185-C) at an output setting of 5. Under these conditions, there was 100% cell breakage. After centrifugation at 47,000 × g for 20 min, the resulting pellet was resuspended in 30 volumes of the same buffer and recentrifuged. The washed pellet was suspended in the same buffer at 2 to 3 mg of protein per ml and was used as the enzyme source. In preliminary experiments, it was established that all the enzymatic activity of the sonically disrupted sample was recovered in the washed particulate fraction prepared from normal or inositol-deficient cells. The assay mixtures (1.5 ml) were incubated at 37° for 30 min, and the reaction was terminated by chilling the samples to 0°, followed by centrifugation at 1500 × g for 10 min. The clear supernatant fluids were analyzed for inorganic phosphate by the Fiske and SubbaRow method (5). Appropriate incubation mixtures from which either the ATP or the enzyme was omitted served as controls. The rate of ATP hydrolysis catalysed by the Na⁺-K⁺ pump was derived by subtracting the rate of ATP hydrolysis obtained in the presence of ouabain (0.13 mM) from that obtained in the absence of ouabain. Alternatively, the activity of the Na⁺-K⁺ pump was derived by subtracting the rate of ATP hydrolysis in the absence of Na⁺ and K⁺ from that in the presence of these cations. Both methods gave identical results. Under the assay conditions described, the rate of ATP hydrolysis catalysed by the Na⁺-K⁺ pump was proportional to the amount of enzyme preparation added, provided that the total amount of ATP hydrolyzed did not exceed 30% of the initial ATP level. It should also be noted that the particulate fraction used as enzyme source was free of any ATPase, the activity of which, in the presence of Mg²⁺, could be further stimulated by the separate addition of either Na⁺ or K⁺.

**14C-AIB Influx**—Initial influx rates of 14C-AIB were determined as described previously (1). The incubation medium was Krebs-Ringer-bicarbonate, pH 7.4, and the 14C-AIB concentration was 0.2 mM.

**Analytical Methods**—The procedures used for determining the cell number, the cell water, the cell viability, and the intracellular 14C-AIB were described previously (6).

**Materials**

1-14C-AIB was purchased from Calbiochem, ouabain (g-strophanthin) and Tris-ATP from Sigma. All other materials were of reagent grade.

**Results**

**Kinetics of K⁺ Influx and Na⁺ Efflux at Presteady State Conditions**—Normal and inositol-deficient cells were incubated at 37° in Krebs-Ringer-bicarbonate buffer, pH 7.4, following a preliminary incubation at 5°, the standard procedure described under "Methods." The time course of K⁺ influx and Na⁺ efflux is shown in Fig. 1. It can be seen that deficient cells exhibit decreased rates of K⁺ influx and Na⁺ efflux and are unable to concentrate K⁺ to the same extent as normal cells. The steady state concentration of intracellular K⁺ is 9.05 mM in normal and 55 mM in deficient cells. With respect to Na⁺, its steady state concentration is about 28 mM for both types of cells.

The kinetics of net fluxes of these cations is shown in Fig. 2. The net fluxes, derived from the data of Fig. 1, represent the
FIG. 1. Time course of K⁺ influx and Na⁺ efflux. Normal and inositol-deficient cells were suspended in Krebs-Ringer-bicarbonate buffer at a density of $4 \times 10^6$ cells per ml and were incubated at $37^\circ$ for 90 min. They were then transferred to a $37^\circ$ water bath, and the cation fluxes were measured as a function of time, as described under "Methods."

FIG. 2. Kinetics of net fluxes of K⁺ and Na⁺. The fluxes were derived from the data of Fig. 1 by subtracting the intracellular concentrations of K⁺ and Na⁺ at various times of incubation from the respective concentrations at zero time of incubation. -- - , normal cells; - - - , inositol-deficient cells.

differences between the zero time value and the subsequent values. It is seen that the initial rate (amoles per min per ml of cell water) of Na⁺ efflux is 8.7 in normal and 3.15 in deficient cells. Similarly, the initial rate of K⁺ influx is lower in deficient cells, being 3.3 for normal and 2.7 for deficient cells. This difference becomes more pronounced after the first 5 min of incubation when the rate of K⁺ influx in deficient cells drops to a value of 0.72, in contrast to normal cells which maintain a high and constant rate for 15 to 20 min.

FIG. 3. Lineweaver-Burk plots of the initial influx rate of K⁺ versus extracellular concentrations of K⁺, in normal (A) and inositol-deficient (B) cells. The cells were first incubated in Krebs-Ringer-bicarbonate buffer lacking K⁺ for 90 min at $5^\circ$ in order to deplete them of K⁺. They were then centrifuged and resuspended in fresh Krebs-Ringer-bicarbonate buffer containing various concentrations of KCl (final concentrations, 1, 2, 4, and 8 mM) and were incubated at $37^\circ$ for 5 min. At the end of this period, the intracellular concentrations of K⁺ were determined as described under "Methods." The influx velocities (V) represent net fluxes (amoles of K⁺ per ml of cell water) derived by subtracting the initial intracellular concentration of K⁺ (4.9 mM for normal and 2.5 mM for inositol-deficient cells) from the values obtained after incubation for 5 min.

Kinetic Parameters of Na⁺ and K⁺ Transport System—The results described above prompted further experiments designed to determine the effect of inositol deprivation on the $V_{max}$ and $K_m$ for K⁺ under presteady state conditions. The experimental procedure was as follows. Normal and inositol-deficient cells were harvested and washed once with 200 volumes of ice-cold 0.15 M choline-Cl, pH 7.4. They were resuspended in Krebs-Ringer-bicarbonate buffer lacking K⁺ ions and were incubated at $5^\circ$ for 90 min. The samples were then centrifuged, and the cells were resuspended in the same buffer mentioned above at a density of $4 \times 10^6$ cells per ml. This suspension (1 ml) was added to centrifuge tubes containing 20 μl of the appropriate concentration of KCl, and K⁺ influx was initiated by placing the tubes in a $37^\circ$
The assay mixture (1.5 ml) contained the following, in mM concentrations: glycylglycine, 20; imidazole, 20; ATP, 3; NaCl, 100; KCl, 20; MgCl₂, 5; EDTA, 0.53; and enzyme extract, 0.2 to 0.5 mg of protein. The final pH was 7.4. A similar incubation mixture contained, in addition, 0.13 mM ouabain. The mixtures were incubated at 37° for 30 min, and the amount of Pi released was measured colorimetrically. The rate of ATP hydrolysis catalyzed by the Na⁺-K⁺ pump was derived by subtracting the rate of ATP hydrolysis in the presence of ouabain from that obtained in its absence.

In other experiments, the influx rate was linear for at least 5 min, and duplicate incubations varied by less than 7%. The results of a typical experiment are shown in Fig. 3, in which influx velocities are plotted against the extracellular concentrations of K⁺, according to the method of Lineweaver and Burk. From these plots, the Vₘₐₓ and Kₘ values are derived. Normal cells have a Kₘ of 1.23 mM and a Vₘₐₓ of 16.1 (μmoles of K⁺ per ml of cell water), whereas deficient cells have values of 0.96 and 4.0, respectively. In other similar experiments, the Kₘ of deficient cells was as low as 48% of that of normal cells (see Table II). Thus, inositol deprivation decreases both the maximal velocity of K⁺ influx and the Kₘ for K⁺. It should also be noted that similar results were obtained when the extracellular Na⁺ was 2 mM instead of 140 mM.

Repeated attempts to determine the same kinetic parameters for Na⁺ influx were unsuccessful because of the difficulty experienced in loading the cells with Na⁺ at the desired low concentrations (4 to 30 mM) from which the Na⁺ fluxes emanate.

Kinetic Parameters of Na⁺-K⁺ Pump—Because Na⁺ and K⁺ transport across the plasma membrane is thought to be mediated by the membrane-bound, Na⁺- and K⁺-activated ATPase, one can infer that the observed effects of inositol deprivation on Na⁺ and K⁺ transport reflect changes in the activity and kinetic properties of this enzyme. Direct evidence that this is indeed the case was provided from measurements of the activity of this enzyme by use of a particulate fraction prepared from normal and inositol-deficient cells. The preparation of this fraction and the assay system were described under "Methods." Table I shows that the rate of ATP hydrolysis catalyzed by the Na⁺-K⁺ pump of inositol-deficient cells is about 40% of that of normal cells.

In other experiments, the Vₘₐₓ and Kₘ values of K⁺ and Na⁺ were determined with enzyme preparations from normal and inositol-deficient cells. Fig. 4 shows Lineweaver-Burk plots of rates of ATP hydrolysis against K⁺ concentrations. From these data, the Vₘₐₓ (μmoles of P₁ released in 30 min by an amount of enzyme corresponding to 10⁶ cells) and Kₘ (mM) values were calculated to be 0.24 and 1.47, respectively, for normal cells. In the case of inositol-deficient cells, the Vₘₐₓ is 0.11 and the Kₘ is 0.68. Similar results were obtained for Na⁺, as shown in Fig. 5. In the case of normal cells, the Vₘₐₓ is 0.21 and the Kₘ is 22.2, whereas with deficient cells these values are 0.10 and 10.7, respectively. Thus, as in the case of Na⁺ and K⁺ transport in intact cells, inositol deprivation causes a decrease of the Vₘₐₓ and Kₘ (for K⁺ and Na⁺) values of the Na⁺-K⁺ pump. It should be pointed out that these Kₘ values represent apparent Kₘ values obtained under the stated experimental conditions, because it is known that the concentration of each of these ions for half-maximal activation of the Na⁺-K⁺ pump depends on the relative concentrations of these ions in the incubation medium (7-12).

Correlation of Changes of Na⁺-K⁺ Transport and of Na⁺-K⁺ Pump—In order to establish a quantitative correlation between the effects of inositol deprivation on the Na⁺-K⁺ transport system in intact cells and those on the Na⁺-K⁺ pump in cell-free preparations, experiments were carried out in which the kinetic parameters were measured under the same conditions. Table II shows the correlation between the changes in the kinetic parameters of the Na⁺-K⁺ pump and of Na⁺-K⁺ transport in intact cells. The results indicate that the changes in the kinetic parameters of the Na⁺-K⁺ pump and of Na⁺-K⁺ transport in intact cells are closely correlated.
sults presented in Fig. 1 have shown that inositol-deficient cells contain less K+ (55 mm) than normal cells (96 mm). The question, therefore, arose as to whether or not the impaired AIB transport of deficient cells (2) is the result of their lower K+ content. Experiments were carried out in which normal and inositol-deficient cells were incubated under the conditions described in Fig. 1, and, at various times during the period of Na+ and K+ fluxes (see Fig. 1), aliquots were withdrawn from the two flasks and were further incubated in the presence of 0.2 mM 4C-AIB for 1 min at 37°. After termination of the incubations, the cells were analyzed for their 4C-AIB content. The following results were obtained. The initial influx rate of AIB (molecules per 10^6 cells per min) in normal cells was 1.4 and was not significantly affected by variation of the intracellular concentrations of K+ (between 20 and 100 mm) and Na+ (between 28 and 140 mm). As expected, inositol-deficient cells exhibited a lower rate of AIB influx, equal to 0.68, which remained unchanged when the intracellular concentration of K+ varied between 24 and 50 mm and that of Na+ varied between 29 and 90 mm. Hence, the impairment of AIB transport of inositol-deficient cells cannot be ascribed to their lower K+ content.

**DISCUSSION**

The studies described in this paper constitute a continuation of the work initiated in this laboratory on the role of inositol in maintaining normal structure and function of the plasma membrane of mammalian cells. Earlier investigations have shown that inositol deprivation leads to severe impairment of the amino acid transport system of KB cells and to inability to concentrate various amino acids. A consequence of this defect was shown to be the loss of various biosynthetic functions which depend on an adequate availability of intracellular amino acids, such as, the synthesis de novo of nucleotides, nucleic acids, and proteins (1, 2).

The results presented in the present paper demonstrate that another important function of the plasma membrane, namely, the translocation of K+ and Na+, is also impaired by inositol deprivation. This defect manifests itself kinetically by a decrease of the Vmax of K+ influx and Na+ efflux and by a decrease of the Km for K+.

**TABLE II**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>System</th>
<th>Normal cells</th>
<th>Deficient cells</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmax</td>
<td>Na^+(^+)K^+ pump in vitro</td>
<td>0.23</td>
<td>0.12</td>
<td>52</td>
</tr>
<tr>
<td>Km(K+)</td>
<td></td>
<td>1.45</td>
<td>0.65</td>
<td>45</td>
</tr>
<tr>
<td>Km(Na+)</td>
<td></td>
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<td>10.40</td>
<td>47</td>
</tr>
<tr>
<td>Vmax</td>
<td>K^+ influx in intact cells</td>
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<td>0.19</td>
<td>50</td>
</tr>
<tr>
<td>Km(K+)</td>
<td></td>
<td>1.37</td>
<td>0.66</td>
<td>48</td>
</tr>
</tbody>
</table>

parameters of the Na^+\(^+\)K^+ transport system and of the Na^+\(^+\)K^+ pump were determined by means of the same populations of normal and inositol-deficient cells. The results of these experiments are summarized in Table II. It can be seen that inositol deprivation impairs the Na^+\(^+\)K^+ transport system in a manner which is quantitatively in good agreement with the impairment of the Na^+\(^+\)K^+ pump.

*Is Lower Steady State Concentration of Intracellular K+ in Deficient Cells Responsible for Impaired AIB Transport?—The results presented in Fig. 1 have shown that inositol-deficient cells contain less K+ (55 mm) than normal cells (96 mm). The question, therefore, arose as to whether or not the impaired AIB transport of deficient cells (2) is the result of their lower K+ content. Experiments were carried out in which normal and inositol-deficient cells were incubated under the conditions described in Fig. 1, and, at various times during the period of Na+ and K+ fluxes (see Fig. 1), aliquots were withdrawn from the two flasks and were further incubated in the presence of 0.2 mM 4C-AIB for 1 min at 37°. After termination of the incubations, the cells were analyzed for their 4C-AIB content. The following results were obtained. The initial influx rate of AIB (molecules per 10^6 cells per min) in normal cells was 1.4 and was not significantly affected by variation of the intracellular concentrations of K+ (between 20 and 100 mm) and Na+ (between 28 and 140 mm). As expected, inositol-deficient cells exhibited a lower rate of AIB influx, equal to 0.68, which remained unchanged when the intracellular concentration of K+ varied between 24 and 50 mm and that of Na+ varied between 29 and 90 mm. Hence, the impairment of AIB transport of inositol-deficient cells cannot be ascribed to their lower K+ content.

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The results presented in the present paper demonstrate that another important function of the plasma membrane, namely, the translocation of K+ and Na+, is also impaired by inositol deprivation. This defect manifests itself kinetically by a decrease of the Vmax of K+ influx and Na+ efflux and by a decrease of the Km for K+. Furthermore, inositol-deficient cells are unable to concentrate K+ to the same extent as normal cells. Because active transport of K+ and Na+ across the plasma membrane is thought to be mediated by the membrane-bound, ouabain-inhibitable, Na+- and K+-activated ATPase, it was important to establish whether the above changes have their counterpart in the activity and the kinetic parameters of this ATPase when studied in cell-free preparations. The results of these studies show that both the Vmax and the Km values (for K+ and Na+) of this enzyme are decreased in inositol-deficient cells, and that the magnitude and direction of these changes are identical to those obtained when transport of K+ and Na+ across the plasma membrane of intact cells is measured (Table II). These findings lend strong support to the overwhelming evidence existing in the literature (7) that this ATPase is indeed the carrier protein for Na+ and K+ (Na+-K+ pump). Evidence that most of the Na+-K+ pump of KB and L cells (mouse fibroblasts) is localized on the plasma membrane will be presented elsewhere.

The above results could be interpreted as indicating that the amount of the Na+-K+ pump per cell is decreased in inositol deficient cells, although the possibility that the results may reflect, instead, a partial inactivation of this carrier protein cannot...
be excluded. The finding that the $K_m$ values (for $K^+$ and $Na^+$) of the Na$^+$$-$$K^+$ pump and of $K^+$ influx in intact cells are lower in inositol-deficient cells (Table II) suggests that inositol deprivation results in conformational changes of the Na$^+$$-$$K^+$ pump or of the membrane environment where the pump is localized. It is worth noting that in the case of AIB transport, inositol deficiency does not change the $K_m$ values for AIB influx or efflux (2).

The possibility that the impaired AIB transport of inositol-deficient cells is the result of their lower content of $K^+$ (45 to 55 mM as compared with 90 to 100 mM of normal cells) can be excluded since the influx rate of AIB in normal cells is unaffected by variation of the intracellular concentration of $K^+$ between 20 and 100 mM. However, intracellular $K^+$ is essential for maintenance of the functional integrity of the AIB transport system of KB cells. The evidence for this conclusion and the role of the Na$^+$$-$$K^+$ pump in the active transport of AIB are presented in the following paper (15).

The discovery that one of the earliest manifestations of inositol deprivation in KB cells is the impairment of plasma membrane functions concerned with concentrative amino acid transport, translocation of $K^+$ and $Na^+$, and activity of the ouabain-inhibitable ATPase raises the question of whether or not these changes reflect a generalized poor condition of these cells in which almost any function would be depressed. That this is not the case was demonstrated in earlier studies in which evidence was presented showing that during the first 3 days of inositol deprivation, when the above functions are severely impaired, the deficient cells behave normally with respect to many other important functions. That evidence can be summarized as follows. (a) The rate of cell multiplication (14) and the protein and DNA content of deficient cells (3) remain at their normal values. (b) The rates of synthesis of acid-soluble purine nucleotides, nucleic acids, and proteins, as measured by the rate of incorporation of $^1$H-glycine or $^1$C-serine into these classes of compounds, are greater than those of normal cells (6). (c) Inositol-deficient cells maintain normal concentrations of acid-soluble nucleotides and RNA if glycine or serine are present in the growth medium (1 mM). The values for normal cells have been reported earlier (3). Omission of these amino acids from the growth medium leads to decreased rates of synthesis and lower concentrations of these compounds (3, 15). This decrease was shown to be the result of defective amino acid transport of inositol-deficient cells, as a result of which the cells are unable to maintain normal concentration gradients of glycine and serine across the plasma membrane. The leakage of these amino acids into the medium reduces their intracellular concentration below the level necessary for the normal rate of synthesis of nucleotides and RNA (8). It should be emphasized that supplementation of the medium with glycine or serine does not in any way prevent inositol-deficient cells from developing defective amino acid transport (6) and defective translocation of $K^+$ and $Na^+$ (the results are identical with those presented in Fig. 2 and Table I). (d) The levels of the following enzymatic activities of inositol-deficient cells are equal to or greater than those of normal cells: guanosine 5'phosphate and adenosine 5'-phosphate pyrophosphorylase, 5'-phosphoribosylpyrophosphamide, uridine 5'-diphosphopyrophosphatase, nucleotidase (acting on purine or pyrimidine nucleotides), inosine 5'-phosphate dehydrogenase, vanthosine 5'-phosphate aminase, nucleoside phosphorylase, guanase, and serine hydroxymethylase (6, 14, 15). (e) The percentage of viable cells (cells which do not take up trypan blue) remains above 90% as compared to 96 to 98% for normal cells (13). It is interesting that this test, when positive, indicates changes of the permeability properties of the plasma membrane without necessarily implying cell death, the latter meaning inability to divide. (f) The rate of oxygen uptake with or without added glucose, measured polarographically, is 8.0 ± 0.7 amoles per 10$^6$ cells per min for normal and inositol-deficient cells.

The biochemical profile of the inositol-deficient cells presented above and confirmed in innumerable experiments during the last 4 years clearly shows that during the first 3 days of inositol deficiency a number of complex and highly integrated cell functions remain normal even though transport functions of the plasma membrane are severely impaired. In the studies presented in this and the companion paper (13), it has been confirmed again that the rate of cell multiplication (cell doubling time of 32 ± 1 hours), the DNA content (20 ± 1 µg per 10$^6$ cells), and the protein content (0.3 ± 0.015 mg per 10$^6$ cells) of inositol-deficient cells are indistinguishable from those of normal cells.

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