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RELATIONSHIP BETWEEN INTRACELLULAR K^+ CONCENTRATIONS AND K^+ FLUXES IN GROWING AND CONTACT-INHIBITED CELLS

B. DUCOURET-PRIGENT*, L. LELIEVRE, A. PARAF**,*** and A. KEPES

Institut de Biologie Moléculaire du C.N.R.S., Université Paris VII, 2, Place Jussieu, 75005 Paris (France)

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SUMMARY

The K^+ content and the K^+ flux were measured in the cell lines ME_2 and MF_2 isolated from plasmocytoma MOPC 173. Both cell lines were shown to have the same K^+ content and the same K^+ steady state flux per unit of surface area.

In ME_2 cells, no modification of the exchange movement was observed during contact inhibition. However, contact-inhibited cells exhibited an increased resistance to depletion, characterized by a lower K^+ net movement.

The $(Na^+ + K^+)$-ATPase measured in homogenates is poorly correlated to in vivo cation fluxes both because of the enhancement due, presumably, to the drop of K^+ concentration on the cytoplasmic face of the membrane and because of losses during preparation which can be conspicuous, especially in contact-inhibited cells.

The K^+ net flux is considerably increased when the intracellular K^+ level is reduced after preincubation of the cells in a K^+-free medium. Thus, internal K^+ seems to regulate the K^+ influx.

INTRODUCTION

It has been shown that the uptake of several solutes, aminoacids, nucleic acid bases, phosphate, etc. is strongly reduced when the density of cells in a culture reaches a certain threshold, especially when cells reach confluency (contact inhibition) [1, 2].

We have studied two cell lines derived from plasmocytoma MOPC 173, one of which, ME_2, exhibits contact inhibition and one, MF_2, which does not. It was found in this laboratory that the $(Na^+ + K^+)$-ATPase activity detected in the isolated membranes of the former sharply decreased at confluency, whereas the corresponding activity in the latter was not modified [3, 4]. In this paper we investigate the K^+ fluxes in the two cell lines before and after confluency in order to attain a new insight into the mechanism of contact inhibition.

* Present address: Laboratoire de Neurobiologie Moléculaire Faculté des Sciences, 35000 Rennes, France.

** On leave from I.N.R.A., Laboratoire de Virologie et d'Immunologie 78000 Thiverval-Grignon, France.

*** To whom requests for reprints should be sent.

MATERIALS AND METHODS

Cell lines

ME_2 and MF_2 cells, derived from MOPC 173, have been described previously [5]. Their relevant characteristic properties are summarized in Table I and typical growth curves are presented in Fig. 1. Cells were pregrown on roller flasks or Roux bottles so that a homogeneous population could be distributed into a number of Falcon flasks of 25 or 75 cm^2 surface area.

TABLE I

The diameter of trypsinized cells was measured on cell photographs with a micrometric reference. The given values are the mean of the measurement of a large number of cells (> 100).

Cell line	Morphology	Growth pattern	Trans-plantation	Protein per cell (pg)	Diameter, d, (μm)	Cell surface, d^2 (μm^2)	Cell volume, $d^3/6$ (μm^3)
MF_2	Fibroblastic	not contact-inhibited	yes	330	15 ± 1.5	700 ± 140	1770 ± 550
ME_2	Epithelioid	contact-inhibited	no	830	19 ± 2	1140 ± 240	3650 ± 1100

Culture medium

The culture medium contained Earle's medium, lactalbumine hydrolysate, yeast extract and Tris · HCl, pH 7.4, with 2 % calf serum added for ME_2 culture and 10 % for MF_2 [5]. The K^+ concentration was 7 mM and the medium was changed every three days for both cell lines.

Flux measurements for the ME_2 cell line were carried out on the 4th day of culture (density lower than 40 000 cells/cm^2) for non confluent cells, and on the 7th or 10th day (density higher than 80 000 cells/cm^2) for confluent populations. In MF_2 cells, the flux measurements were performed on the 4th day of culture, i.e. at confluency. At a later stage, the cells have a tendency to be washed out into the medium. In all cases the culture medium was renewed the day before the flux determination in order to avoid medium exhaustion. For the ME_2 cells it has been reported [6] that the medium change is followed by an increased thymidine incorporation with a maximum at the 12th hour, and that contact inhibition is recovered after 24 h.

K^+ flux measurement

For the measurement of K^+ influx during the steady state, 0.5 ml of a solution of ^{42}K (Commissariat à l'Energie Atomique, France) was added to 4 ml of the culture medium in 25 cm^2 falcon flask to give a final radioactivity of 3 μCi/ml. The incubation was carried out at 37 °C. Ouabain, when present, was added at the same time as ^{42}K.

At selected times, bottles were sampled in the following way. The incubation medium was discarded and the cells were washed three times with 5 ml 0.16 M NaCl at 4 °C (total washing time 30 s). After removal of the third wash, 1 ml of a 0.2 % sodium dodecyl sulfate solution was added. This caused immediate lysis. The cell lysate was collected and the culture flasks were washed 3 times with a total of 4 ml of water.

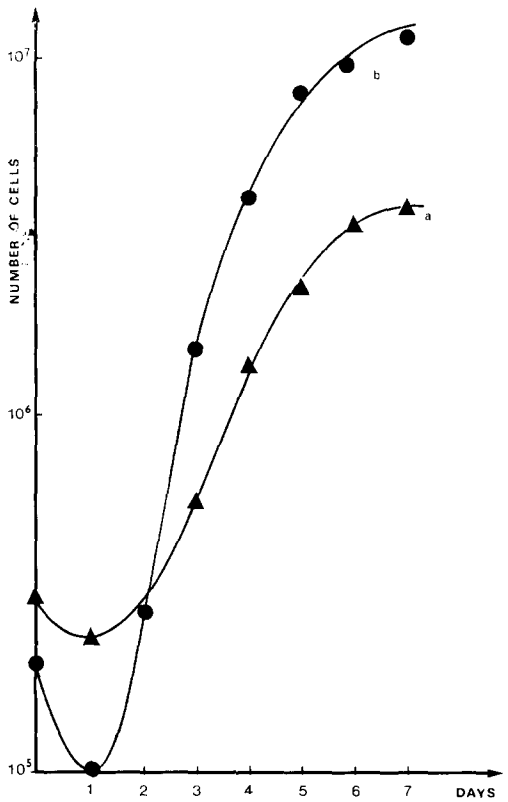

Fig. 1. Growth curves of ME_2 (a) and MF_2 (b) lines. Abscissa, days after plating; ordinate, number of cells per flask. ME_2 cells were plated at a density of $3 \cdot 10^5$ cells per flask, and MF_2 at $2 \cdot 10^5$ cells per flask.

0.5 ml of the cell lysate was mixed with 10 ml of Instagel (Packard) and the radioactivity was counted in an "Intertechnique" scintillation spectrometer.

The K^+ concentration was measured with a flame spectrophotometer (Jouan). Protein was estimated by the method of Lowry et al. [7].

For the efflux measurements, the ME_2 cells grown in 75 cm^2 Falcon flasks were incubated with 30 ml of medium containing ^{42}K (3 μCi/ml) at 37 °C for 4 h. After this equilibration, the culture was washed 3 times with 0.16 M NaCl at 37 °C and 25 or 50 ml of prewarmed medium was added per bottle to growing or stationary cells respectively. Aliquots of 0.5 ml were taken from the supernatant at close intervals and radioactivity was counted. The efflux evaluation presented the technical advantage of making it possible to conduct all the experiments on the same cell population. K^+ efflux was not measured in the MF_2 cells because they showed a tendency to detach from the flasks and pass into suspension, especially at confluency.

For the K^+ depletion, the cells were incubated 12 h at 4 °C in K_0 medium or 4 h at 37 °C in K_0 medium supplemented with 1 % of serum (K^+, 8.10^{-5} M). K_0 medium contained Earle's medium with KCl replaced by NaCl, and Tris · HCl buffer, pH 7.4.

RESULTS

Intracellular concentration of potassium. This was measured by flame photometry on cells of both cell lines at different stages of balanced growth. Intracellular potassium was 0.77 ± 0.09 μmol/mg cell protein in ME_2 up to a cell density of $2.3 \cdot 10^6$ cells per flask, i.e. $9.2 \cdot 10^4$ cells/cm^2, and therefore the steady state level of potassium does not seem to be subject to any obvious regulation linked to the state of confluency. The other cell line, MF_2, contained 0.80 ± 0.10 μmol K^+/mg protein, also independent from cell density up to the 4th day of culture. This value does not differ significantly from that of line ME_2.

Assuming that the volume of plated cells was the same as that of the trypsin-treated cells (Table I), the intracellular K^+ concentration would be 175 ± 25 mM for ME_2 cells and 149 ± 25 mM for MF_2 cells. A dramatic decrease of K^+ content was observed upon addition of 1 mM ouabain as shown in Fig. 2. In the presence of this inhibitor, the K^+ level fell to 0.25 μmol/mg protein with a half-time of approximately 30 min. In spite of this drop, more than 90% of the cells retained their viability according to the test with Trypan blue.

It was hoped that a better measure of the cation pump activity could be achieved by the study of unidirectional fluxes of K^+ at the steady state.

Rate of K^+ exchange at the steady state. Inward flux was measured by adding $^{42}K^+$ to the growth medium of a large number of culture flasks in the same stage of growth (at the 4th or 10th day) and by sampling duplicate flasks at each predetermined incubation time. As shown in Fig. 3, the $^{42}K^+$ uptake in ME_2 cells exhibited an exponential increase corresponding to an influx of 0.55 μmol K^+/mg protein per h. The

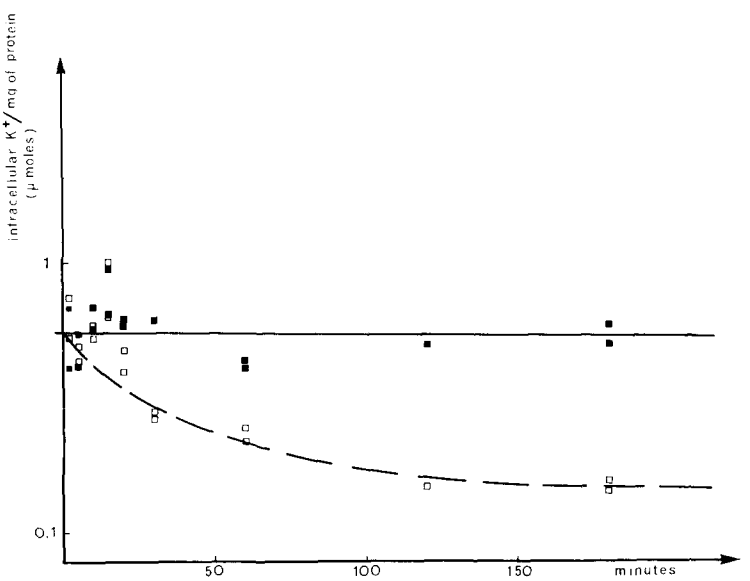

Fig. 2. Intracellular K^+ in ME_2 during incubation with ^{42}K. (■), control; (□), with 10^{-3} M ouabain. Abscissa, min incubation with $^{42}K^+$; ordinate, μmol K^+/mg protein.

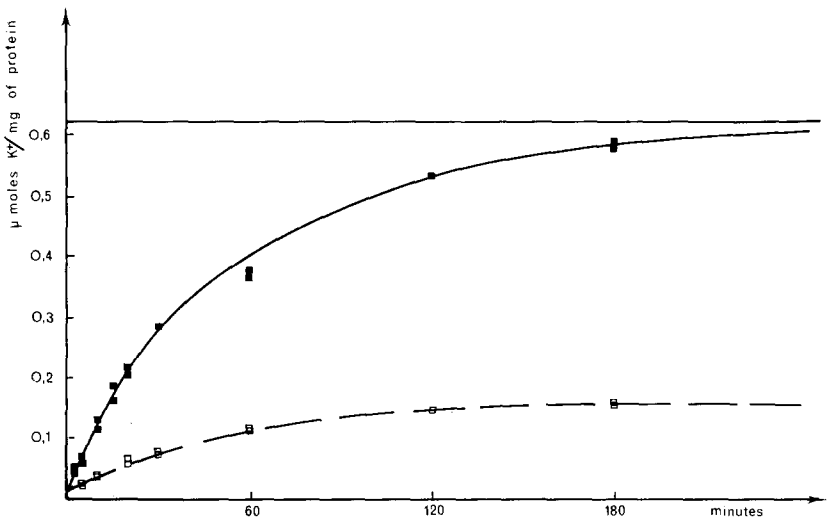

Fig. 3. Uptake of $^{42}K^+$ by ME_2 cell lines incubated in the culture medium (K^+ = 7 mM). (■), control; (□), with 10^{-3} M ouabain. Abscissa, min incubation with $^{42}K^+$; ordinate, uptake of K^+ in µmol K^+/mg protein. Each point is made in duplicate from two separate cell plates. The specific activity of the incubation medium at zero time is $9 \cdot 10^{11}$ cpm/mol K^+.

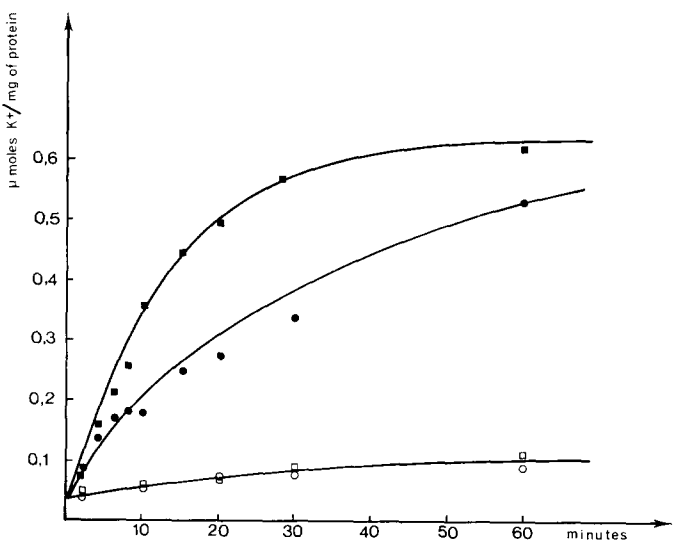

Fig. 4. Uptake of $^{42}K^+$ in ME_2 cells K^+-depleted by preincubation of 4 h in a K^+ medium with 1 % of serum (final $[K^+] = 8 \cdot 10^{-5}$ M) at 37 °C. Incubation with $^{42}K^+$ for measurement of the influx is made in the culture medium. The specific activity of this medium is $33 \cdot 10^{11}$ cpm/mol K^+. Cells in growth phase: (■), control; (□), with 10^{-3} M ouabain; cells in contact inhibition: (●), control; (○), with 10^{-3} M ouabain.

same results were found in growing and in contact-inhibited cells.

It was found that maximal inhibition required a 10^{-3}M concentration of ouabain. At this concentration the residual K^+ influx was 0.10 μmol/mg protein per h. This same value was found whatever the growth stage of the culture. This corresponds to an inhibition of 82 %. Possibly the residual flux is due partly or totally to free diffusion.

Rate of K^+ influx in K^+-depleted cells. K^+ depletion experiments have shown that cells incubated in a medium containing no K^+ at low temperature take a rounded shape, and that this change is likely to suppress cell-to-cell contact in a previously confluent culture. In order to keep the cells closer to physiological conditions and morphologically as similar as possible to a normal culture during K^+ depletion, 4 h incubation at 37 °C was used in a medium containing no added K^+ except that contained in 1 % serum ($8 \cdot 10^{-5}$ M). The intracellular K^+ dropped from 0.80 μmol/mg protein to 0.30 in the growing cells and to 0.62 in the contact-inhibited cells. When cells depleted of their intracellular K^+ as a result of this incubation were covered again with normal culture medium containing $^{42}K^+$, they exhibited initial rates of K^+ net uptake of 1.70 μmol/mg protein for the non-confluent culture and 0.84 μmol/mg protein for the confluent culture, which was less efficiently depleted. In both instances the ouabain-resistant influx was the same, i.e. 0.10 μmol/mg protein (Fig. 4).

Rate of K^+ efflux. At the steady state, we observed the same K^+ efflux, of 0.65 μmol/mg protein, in growing as in contact-inhibited cells, with an exponential exchange (Fig. 5). This value is slightly above the influx value, but the extra washings done before the efflux measurement could account for this difference. As for the influx, the half-time of exchange was about 45 min. However, a comparison between growing and contact-inhibited ME_2 cells incubated in K^+-depleted medium ($8 \cdot 10^{-5}$ M) showed a reproducible difference in the overall time course of release (Fig. 6):

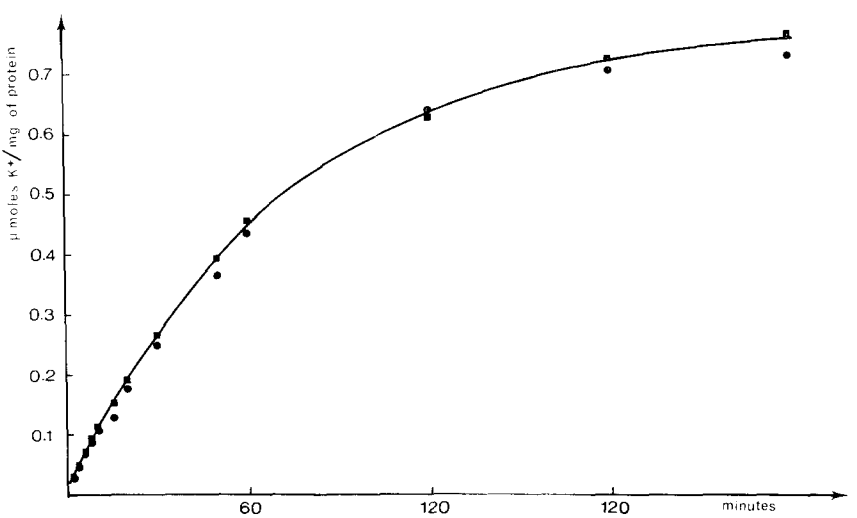

Fig. 5. $^{42}K^+$ release of preloaded ME_2 cells in the culture medium. The specific activity of the intracellular exchangeable K^+ is $8.7 \cdot 10^{11}$ cpm/mol K^+ at zero time. (■), cells in growth phase; (●), cells in contact inhibition.

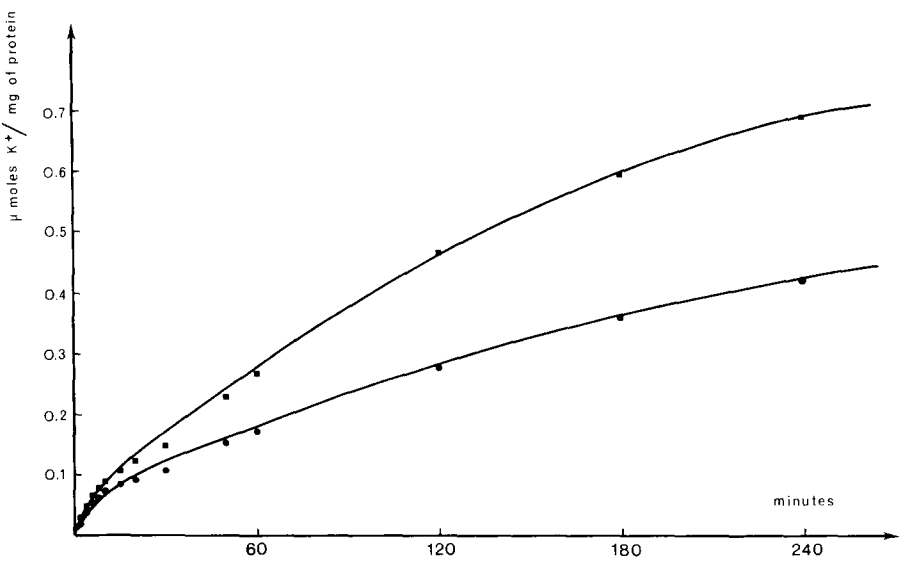

Fig. 6. $^{42}K^+$ release of preloaded ME_2 cells in a K_0 medium with 1 % of serum (Final $[K^+] = 8 \cdot 10^{-5}$ M). The specific activity of the intracellular exchangeable K^+ is $8.7 \cdot 10^{11}$ cpm/mol K^+ at zero time. (■), cells in growth phase; (●), cells in contact inhibition.

After 4 h of incubation, the former contain 30 % of the initial radioactivity while the latter still contain 70 %.

Rate of K^+ influx in MF_2 cells. In the presence of 10^{-3} M ouabain, the K^+ concentration decreased to a steady level of 0.25 μmol/mg protein after 1 h of incubation, i.e. 70 % of the intracellular K^+ came out of the cell as observed with ME_2 cells.

The influx at the steady state was 0.8 μmol/mg protein, and was reduced to 0.12 μmol/mg protein per h by 10^{-3}M ouabain. The ouabain-sensitive K^+ influx is 0.68 μmol/mg protein per h, which would require a $(Na^+ + K^+)$-ATPase activity in vivo of 0.34 μmol Pi/mg protein/h.

DISCUSSION

The intracellular K^+ concentration was 175 ± 25 mM for the ME_2 line and 149 ±25 mM for the MF_2 line. Therefore the two phenotypes contain closely similar K^+ concentrations, consistent with the concentrations found in other mammalian cells as, for instance, 135 mM in S 180 cell line (Lubin) [8] or 200 mM in Girardi cells (Lamb and MacCall) [9].

The total influxes are 0.55 μmol/mg per h for ME_2 cells and 0.80 μmol/mg per h for MF_2 cells, and the influxes in the presence of ouabain are, respectively, 0.10 μmol/mg per h and 0.12 μmol/mg per h. So the ouabain-sensitive influx (which depends on the $(Na^+ + K^+)$-ATPase activity) is 0.45 and 0.68 μmol/mg per h, respectively. Assuming that the cell surface area during culture is about the same as in the trypsin-treated cell, the ouabain-sensitive influx can be calculated per unit area. For ME_2 cells, it is 32.8 nmol K^+/cm^2 per h and for MF_2 cells 32.2 nmol K^+/cm^2 per h. Comparisons between normal and transformed cells have already been made for the

active penetration of amino acids [10]. Foster and Pardee [1] have measured a two-fold increase in the uptake of α-amino isobutyric acid and of other amino acids by the the transformation of 3 T_3 cells by the polyoma virus. The apparent differences between normal and transformed cells might be less striking if the uptake were expressed per unit surface area.

Depletion experiments carried out on ME_2 cells revealed a consistent difference between exponentially growing cells and contact-inhibited cells. The former had lost more K^+ than the latter after 4h. Thus it appears that growing and contact-inhibited ME_2 cells are more different with respect to K^+ net movement than with respect to exchange. When the ME_2 cells were depleted in K^+, we observed an activation of the potassium pump. Thus, it seems that the lower the internal K^+ concentration, the higher the flux. These experiments suggested a regulation of the pump activity by the internal K^+ concentration. However, we cannot exclude a possible role of other permeant ions such as Na^+, Cl^- and H^+ [11]. Such a relationship between internal K^+ depletion and K^+ pump activation has been observed for red blood cells [12–14], heart muscle [15] and fat cell ghosts [16]. Knight and Welt [17] demonstrated that for human red blood cells intracellular Na^+, membrane potential and ATP concentration had no regulatory effect on the $(Na^+ + K^+)$-ATPase activity.

In the case where depletion attained 70 %, a fourfold activation of the transport activity was detected. Thus, if the flux measured in depleted cells is considered as reflecting the full activity of the transport system, the K^+ flux at the steady state uses only 25% of the transport capacity which has been observed by others [18, 19]. Our main goal was to obtain a new insight into the mechanism of contact inhibition by comparing in vivo and in vitro $(Na^+ + K^+)$-ATPase activity. Such a comparison has already been made in leukemic cells in culture [20]. It was reported that $(Na^+ + K^+)$-stimulated hydrolysis of one ATP molecule of the cells is related to the in vivo active transfer of 2.5 K^+ ions. This ratio was later found to be 2 [21].

In fact, we found the same active flux and the same intracellular K^+ concentration in both growing and contact-inhibited ME_2 cells. However, in previous papers [3, 4] we reported that homogenates from ME_2 contact-inhibited cells had a lower $(Na^+ + K^+)$-ATPase activity (0.1 μmol P_i/mg protein per h) than homogenates from ME_2 growing cells (1.8 μmol P_i/mg protein per h). The K^+ influx measurements reported here during steady state exchange can be accounted for by an $(Na^+ + K^+)$-ATPase activity of 0.22 μmol P /mg protein per h, at any state of culture confluence. We would like to emphasize that the ATPase activity measured in the cell lysate from contact-inhibited cells is insufficient, by a factor of 2, to account for the $^{42}K^+$ flux measured in vivo, but the ATPase activity found for growing cells is in an 8-fold excess. Thus the activity found in homogenates of ME_2 growing cells was in large excess over the requirement for the K^+ flux, whereas the homogenates of contact-inhibited cells contained not an excess but an insufficiency of the required activity. We conclude, therefore, that contact-inhibited cells lose ATPase activity in the preparation of homogenates, whereas growing cells do not. This might reflect a temporary fragility of membrane structure in the contact-inhibited state. The relatively high K^+ retention observed with contact-inhibited cells might be another aspect of such a structural modification.

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