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Effect of Imposed Serum Deprivation on Growth of the Mouse 3T3 Cell

DISSOCIATION FROM CHANGES IN POTASSIUM ION TRANSPORT AS MEASURED FROM [^{86}Rb]RUBIDIUM ION UPTAKE

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Decreased serum concentrations that substantially alter the growth of normal 3T3 cells alter neither the active and non-active components of unidirectional $^{86}Rb^+$ influx nor the intracellular K^+ content when compared with cells in exponential growth. Thus the changes in K^+ transport (measured with $^{86}Rb^+$ as an analogue for K^+ movements) that occur on density-dependent growth inhibition of the mouse 3T3 cell are not mimicked by serum deprivation of the cells before density inhibition.

It has been suggested that the cell membrane plays a primary role in growth control and that this role may be manifested through changes in membrane transport of macromolecular serum factors or low-molecular-weight substances such as essential amino acids or ions [for reviews, see Pardee (1964) and Holley (1972)]. In regard to ion transport, it has been observed that density-dependent growth inhibition of the mouse 3T3 cell and the chicken fibroblast cell is characterized by distinct changes in their K^+-transport properties (Spaggiare *et al.*, 1976; Brown, 1977; Weber, 1976) and activities of $(Na^+ + K^+)$-stimulated ATPase (EC 3.6.1.3) (Elligsen *et al.*, 1974; Kimelberg & Mayhew, 1975, 1976). Also, serum stimulation of quiescent 3T3 cells results in their initiation of a new growth cycle, and this is characterized by changes in K^+ transport and cellular K^+ content (Rozengurt & Heppel, 1975; Tupper *et al.*, 1977). On the basis of these observations regarding growth and K^+ transport, we have investigated the relationship between serum deprivation and K^+ transport in the mouse 3T3 and SV40-virus-transformed 3T3 cell. Concentrations of serum that distinctly alter the growth pattern of the cells do not alter their $^{86}Rb^+$ transport properties (an analogue for K^+ transport in these cells) or their intracellular K^+ concentrations.

Experimental

Balb/c 3T3 cells were obtained from the American Type Culture Collection at passage 86. The SV40-virus-transformed Balb/c 3T3 cells (passage 126) were generously given by Dr. George Poste, Roswell Park Memorial Institute, Roswell Park, NY, U.S.A. Stock lines were maintained in Dulbecco's modification of Eagle's medium plus 10% (w/v) calf serum as previously described (Tupper *et al.*, 1977; Tupper & Zorgniotti, 1977). Unidirectional K^+ influx was determined from the kinetics of uptake of $^{86}Rb^+$. The procedures are described in detail elsewhere (Spaggiare *et al.*, 1976). Briefly, cells were pulsed with a trace amount of $^{86}Rb^+$ for 10 min. Rb^+ has been shown to mimic the movement of K^+ under all the conditions used in these experiments (Spaggiare *et al.*, 1976). Over this time interval the uptake is linear with time, and the loss of K^+ in the presence of 0.5 mM-ouabain is less than 15% of the total intracellular K^+. Thus the uptake represents a unidirectional influx and the cells are in steady state or near steady state in the case of ouabain. At the termination of the pulse, the plates were washed four times with 200 mM-choline chloride/1 mM-Tris/HCl, pH 7.4. The cells were then lysed in 15 mM-LiCl, scraped from the plates and placed in liquid-scintillation-counting vials. The ^{86}Rb content was determined from the detection of Čerenkov radiation in a liquid-scintillation counter. The same samples were then analysed for K content with a flame photometer, with Li as internal standard. Portions were removed and used for protein measurement with the Biuret procedure. Parallel plates were washed in a manner identical with that for plates used for transport measurements, trypsin treated, and cell number and volume were determined with a Coulter cell counter. The specific radioactivity of the external medium (c.p.m. of ^{86}Rb/mol of K) was determined from portions of the incubation medium that contained a trace of ^{86}Rb. The unidirectional influx is expressed as mol of K^+/min per mg of protein) or mol of K^+/min per μl of cell volume). For the experiments reported here, the cells were plated at 1×10^5 cells/plate (4.8×10^3 cells/cm^2) in 5 ml of low-glucose Dulbecco's modified Eagle medium supplemented with 10% calf serum. They were in passages 18–23 in our hands. Under these plating conditions the 3T3 cells used had saturation densities of 6.6×10^4–7.9×10^4 cells/cm^2. This is noteworthy, since we have observed that the absolute unidirectional K^+ influx in the 3T3 cell is increased with increasing passage number and saturation density (Tupper, 1977).

Abbreviation used: ATPase, adenosine triphophatase.

Results and Discussion

The mouse 3T3 or SV40-virus-transformed 3T3 cells were plated in medium containing 10% serum. After 24h these cultures were exposed to fresh medium containing 10 or 4% serum (for 3T3 cells) and 10% or 0.5% serum (for SV40-virus-transformed 3T3 cells). The cells remained under these conditions for another 48h, at which time they were assayed for $^{86}Rb^+$ uptake and K^+ content. Before these experiments, growth curves were determined under similar conditions to establish the effect of these decreased serum contents on growth. Our results are in agreement with previous observations (e.g. Bartholomew *et al.*, 1976). Within 48h of serum stepdown the 3T3 cell is quiescent at a final saturation density proportional to the serum content of the medium. At this time the 3T3-cell cultures in 10% serum were in exponential growth and capable of at least another complete doubling. Exposure of the SV40-virus-transformed 3T3 cell to 0.5% serum resulted in an approximately halving of the cell number on the plates 48h after serum stepdown. It has been determined that this results from an elongation of all phases of the cell cycle in the transformed cell (Bartholomew *et al.*, 1976).

The components of unidirectional K^+ influx in the 3T3 cell fall into at least three categories (Spaggiare *et al.*, 1976): (*a*) an active ouabain-sensitive K^+ influx that contributes to net K^+ flux; (*b*) a furosemide-sensitive component of K^+ influx that does not contribute to net K^+ flux and fits the criteria of one-for-one K^+ exchange; and (*c*) an ouabain-insensitive furosemide-insensitive component that represents in whole or in part the passive diffusional component of K^+ influx. The magnitude of each of these components was evaluated by simultaneous addition of a concentrated ouabain or furosemide solution with the trace of ^{86}Rb to yield a final concentration of 0.5mM for the inhibitor in question; no medium change was involved. The results are given in Table 1. In 3T3 cells brought to quiescence by serum stepdown, the magnitudes of the total unidirectional $^{86}Rb^+$ influx and the fractions of total influx sensitive to ouabain and furosemide are equivalent to those of growing cells. Furthermore, the total K^+ content of the cells on a mol/litre-cell-volume or mol/mg-of-protein basis was unaltered and consistent with values obtained for numerous cell lines. The data are similar in the SV40-virus-transformed 3T3 cell (Table 1). A serum content in the medium of 0.5% slowed the proliferation of this cell. However, no concomitant change in membrane transport of $^{86}Rb^+$ or in intracellular K^+ concentration was observed. It is noteworthy that the absolute value for $^{86}Rb^+$ influx was greater in the SV40-virus-transformed 3T3 cells compared with that in the 3T3 cells in the present experiments. In our previous study (Spaggiare *et al.*, 1976) it was observed to be less in the transformed cell. Subsequent studies (Tupper, 1977) revealed that the absolute magnitude of total and ouabain-sensitive K^+ influx in the 3T3 cell increases with increasing passages of the cells, whereas it remains relatively constant in the transformed cell over a similar number of passages.

We consider that the present findings bear consideration for the following reasons. Serum deprivation of exponentially growing populations of the 3T3

Table 1. *Effect of different serum concentrations on the components of $^{86}Rb^+$ transport, the intracellular K^+ content and cell proliferation in normal and SV40-virus-transformed 3T3 cells*

All cells were plated in Dulbecco's modified Eagle medium supplemented with 10%(w/v) calf serum. After 24h, fresh medium was added containing the indicated concentration of calf serum. After 48h the cells were assayed for $^{86}Rb^+$-transport properties and intracellular K^+ concentrations as described in the text. At this time, duplicate plates at each serum concentration were assayed for cell number to confirm the effect of serum deprivation on proliferation. Results are means ± S.E.M. for triplicate samples. The data are typical of three such experiments.

Cell type ...	3T3		SV40-virus-transformed 3T3	
Serum concentration (%) ...	10	4	10	0.5
No. of cells/plate	7.0×10^5	1.6×10^5	2.9×10^6	1.5×10^6
K^+ influx [nmol/min per μl, or nmol/min per mg (in square brackets)]				
Total	1.6±0.05 [11.9]	1.5±0.03 [12.2]	3.4±0.08 [18.7]	3.3±0.07 [20.4]
Ouabain-sensitive	0.8±0.03 [5.6]	0.8±0.06 [6.2]	2.1±0.12 [11.7]	2.0±0.09 [15.6]
Furosemide-sensitive	0.3±0.02 [2.3]	0.3±0.04 [2.8]	0.9±0.02 [5.0]	0.8±0.03 [6.7]
Intracellular K^+ content [mmol/litre, or μmol/mg of cell protein (in square brackets)]	178±11 [1.2]	161±14 [1.3]	150±8 [0.8]	156±11 [1.1]

cells does not result in an alteration of their $^{86}Rb^+$ transport or K^+ content over 48h, but it does alter their growth. In contrast, density-dependent growth inhibition of the 3T3 cell is characterized by 2–6-fold decreases in $(Na^+ + K^+)$-activated ATPase activity (Elligsen *et al.*, 1974) and 2–5-fold decreases in active $^{86}Rb^+$ uptake (Spaggiare *et al.*, 1976; Brown, 1977). Release from density-dependent growth inhibition mediated by serum stimulation results in 2–4-fold increases in $^{86}Rb^+$ influx and a 1.5-fold increase in K^+ content (Rozengurt & Heppel, 1975; Tupper *et al.*, 1977). Thus the present data indicate that the growth inhibition induced by serum deprivation of the 3T3 cell is not equivalent, as regards the nature of $^{86}Rb^+$ transport, to density-dependent growth inhibition exhibited by this cell at higher saturation densities. This suggests that the progression to quiescence in the normal cell may involve utilization of a serum factor or factors involved in the regulation of cation as well as other transport systems, and that this deprivation is not achieved by simple lowering of serum content in exponentially growing cells. These data also indicate that the enhanced degree of cell-to-cell contact associated with density-inhibited cells may be influential in the changes in K^+ transport observed under these conditions. These observations are analogous to differences in cyclic AMP concentrations observed in serum-deprived as against density-inhibited 3T3 cells. Oey *et al.* (1974) and Moens *et al.* (1975) failed to observe changes in intracellular cyclic AMP as 3T3 cells became confluent, but they did detect changes in serum-restricted cells, observations that suggest density-specific regulation and serum-specific regulation of cyclic AMP metabolism as well as K metabolism.

We also conclude that the differential sensitivity to serum deprivation of normal as against SV40-virus-transformed 3T3 cells does not appear to be directly related to their K^+-transport properties, since both cell types fail to exhibit significant changes in $^{86}Rb^+$ transport or K^+ content after serum stepdown, but exhibit distinct differences in growth response. These data also suggest that the two methods of obtaining quiescent cells (imposed serum deprivation or growth to saturation density at normal serum concentrations) do not result in cell populations with equivalent ion-transport properties. This may well extend to other transport systems and, conceivably, other cellular functions.

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