ELECTRICAL PROPERTIES OF NORMAL AND TRANSFORMED MAMMALIAN CELLS

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ABSTRACT The transmembrane potential difference, *Em*, and DC membrane resistance were measured in 3T3 and polyoma virus-transformed 3T3 cells. *Em* was a function of cell density and was −12 and −25 mV for the normal and transformed cells, respectively. The external concentrations of K^+, Na^+, and Cl^- were varied in order to study the nature of the differences between the two cell types. The relative permeability of ions was calculated to be: P_{Na}/P_K, 1.0; P_{Cl}/P_K, 1.88; P_{Na}/P_{Cl}, 0.53 for 3T3 cells, and 0.27, 1.75, and 0.15 for the transformed cells. In contrast to the normal cells, P_{Na}/P_K varied as a function of the external K^+ concentration for the transformed cells. It was emphasized that the manipulation of variables directly affecting the electrical properties of cells also involves the indirect manipulation of a network of interconnected physiological determinants.

INTRODUCTION

Plasma membrane alterations seem ubiquitous following neoplastic transformation of mammalian cells. Change in membrane composition, architecture, and function (Stoker, 1971; Nicolson, 1976) can be measured by a variety of biological (Abercrombie and Heaysman, 1954; MacPherson and Montagnier, 1964), immunological (Habel, 1961; Prehn, 1971), biochemical (Buck et al., 1970; Hakomori and Murakami 1968), and biophysical techniques (Ambrose et al., 1956; Sherbert, 1978). The use of in vitro grown cells affords the opportunity to investigate membrane aberrations associated with parameters of cellular growth regulation. Of considerable interest is the role of the ionic distribution across the membrane in influencing regulatory pathways of cell growth control.

In general, the electrical properties of nonexcitable cells differ from those measured for excitable tissue such as nerve and muscle. The transmembrane potential difference, *Em*, is much less electronegative (−10 to −60 mV) in cultured mouse fibroblasts (Lamb and MacKinnon, 1971; MacDonald et al., 1972), human HeLa cells (Borle and Loveday, 1968), and leukemic cells (Schaefer, 1972) than that observed for classical measurements of nerve (−70 to −90 mV) (Hodgkin and Horowicz, 1959). The *Em* seems determined by two variables, the relative permeability of the membrane to diffusable ions and the concentration (activity) gradient of these ions across the membrane. When considered together with ion transport systems (such as Na^+-K^+ stimulated ATPase) and observations that other properties of transformed cells differ from their normal counterparts, it seems reasonable that the ionic environment of the cell may influence cellular physiological systems. Although the role of the major cations Ca^{++}, K^+, and Na^+ in growth regulation remains elusive, recent work has demonstrated abberations in the permeability, *P*, of potassium in transformed cells (Spaggiare et al., 1976; Adam et al., 1979). Early studies by Cone (1969; 1971) suggested that the *Em* and *P* for cultured cells varied both as a consequence of neoplastic transformation and as a function of cell density in culture (Cone and Tongier, 1973), a condition known to impose growth regulatory events. Manipulation and measurement of the concentration and permeabilities of Na^+ and K^+ in several cellular systems (Pollack and Fisher, 1976; Booustra, 1981; Stambrook et al., 1975) strengthens the notion that at least a qualitative description relating ion concentration, membrane permeability, *P*, *Em*, mitosis, and cell growth and differentiation can be formulated.

However, there has been no reported study of the electrical parameters of a transformed cell model using a systematic variation in the external ion concentrations. Such variations allow insight into the nature of the electrical properties. This report describes alterations of electrical properties of 3T3 mouse fibroblasts following transformation with polyoma virus (Py3T3).

METHODS

Cell Lines

Stocks of Swiss 3T3 and polyoma-virus transformed 3T3 (Py3T3) cells were kindly provided by Dr. M. Burger, Basel, Switzerland. The cells were grown in Falcon T-25 flasks at 37°C (Falcon Labware, Div. of Becton, Dickinson and Co., Oxnard, CA), equilibrated at 5% CO_2 in air using Dulbecco and Vogt's modification of Eagle's medium (DMEM), supplemented with 10% heat-inactivated fetal calf serum and antibiotics (Grand Island Biological Co., Grand Island, NY). Cells were seeded at low densities (10^3 cells cm^{-2}) and fresh DMEM was exchanged 2–3 times

weekly. Preliminary studies indicated that 3T3 cells became confluent in a monolayer (homogeneous cuboidal epithelial morphology, non-overlapping) at ~4–5 × 10^4 cells cm^{-2}. In contrast, the Py3T3 cells exhibited a spectrum of fibroblastic morphology with many large, multinucleated cells, and the cells tended to grow in large colonies and clusters, the center of which were many cell layers deep. Saturation density for this cell line was ~8–10 × 10^5 cells cm^{-2}, 20-fold higher than the 3T3. The 3T3 cells did not cause tumors in Swiss albino mice inoculated subcutaneously with 10^6 cells in contrast to 100% incidence of tumor growth in those animals injected with Py3T3 (D. Erwin and J. Killion, unpublished results).

Electrical Measurements

The *Em* and transmembrane resistance, *Rm*, of individual cells was measured with glass micropipette electrodes (Chowdhury, 1969) mounted on a micromanipulator (Narishige, Tokyo, Japan). All measurements were performed under conditions similar to other studies using cultured cells (Cone and Tongier, 1973; McDonald et al., 1972; Stambrook et al., 1975). The microelectrodes were filled with 1 M NaCl, had tip potentials (V_t) of <10 mV, and tip resistances (R_t) between 20 and 60 MΩ. All intracellular measurements were corrected by an estimated change in V_t and R_t due to the change in the ionic environment of the microelectrode tip upon cellular insertion. This estimation was made by changing the bathing solution for >35 microelectrodes from 140 mM NaCl, 10 mM KCl, to 140 mM KCl, 10 mM NaCl, and recording any changes in V_t and R_t. Correlation of ΔV_t and ΔR_t resulted in no corrections for R_t and all V_t corrections were < -2 mV. Measurements were performed at 35°C, unless otherwise indicated, and the potential difference between the reference electrode and the microelectrode was measured by an electrometer (M4A, W-P Instruments Inc., New Haven, CT) coupled via a calomel electrode and KCl salt bridge. The output was fed to a low-level DC amplifier that drove the final amplifier and was displayed on a strip chart recorder. R_t was displayed as a voltage drop when a square-wave pulse of current was passed between the electrodes using the bridge circuit of the electrometer. Fig. 1 shows a tracing of an actual intracellular *Em* and R_{t+m} measurement and serves to define the criterion of a successful measurement. The V_t was about +6 mV and R_t was ~30 MΩ prior to cell penetration. The tip was lowered next to individual cells (visualized at a magnification of 320), care being taken to avoid the nucleus. V_t became slightly more positive as the cell membrane was dimpled, and the immediate negative intracellular potential, *Em*, was about −35 mV. R_{t+m} was measured during the time of stable measurement, and V_t and R_t returned to their original values following removal of the electrode. Stable recordings could be made from a single cell for up to 1 h without removal of the electrode, and the same cell possessed the same *Em* and *Rm* when sampled more than once; however, repeated cell penetrations ultimately resulted in loss of cell integrity. Table I lists the components of the balanced salt solution (BSS) used to bathe the cells during penetration with the microelectrodes. The solution BSS-N refers to normal ionic concentrations associated with DMEM. Potassium concentration (BSS—K_{1-3}^+) was varied by equimolar replacement of NaCl (with Cl^- remaining constant, whereas Na^+ (BSS—Na_{1-4}^+) and Cl^- (BSS—Cl_{1-3}^+) were varied in a manner that held the concentration of the other major ions constant. Ca^{++} and Mg^{++} were constant at 1.8 and 0.8 mM, respectively. Glucose was present at 20 mM. Electrical measurements were made within several minutes of replacing the DMEM medium with the appropriate BSS solution (pH 7.2–7.4). This methodology allowed a systematic study of ion effects that could not have been performed in the presence of serum.

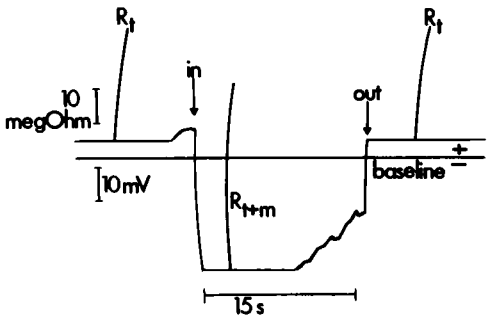

FIGURE 1 Tracing of a measured transmembrane potential difference. The baseline indicates 0 mV and the tip potential of the microelectrode was +5 mV (requiring a correction of −1 mV to intracellular reading). Electrode DC resistance is indicated by R_t (34 MΩ). Before cell penetration, there was a slight dimpling of the cell membrane, associated with a positive deflection, followed by penetration (arrow marked "in"), accompanied by an immediate deflection to about −30 mV, remaining stable for ~10 s, during which time a measurement of DC resistance across the electrode tip and membrane was made (R_{t+m}, 57MΩ). The electrode was withdrawn and the electrode tip returned to the original potential and resistance.

Careful consideration was given to the local cell density surrounding individual cells for the micropuncture studies. Most measurements were made upon cells that had few neighbors and were rarely touching another cell. Pilot studies indicated that except for extremely dense cell layers, the *Em* of cells did not significantly vary between regions of sparse and dense populations. The variation as a function of cell density in the flask is given in the Results section.

Conversion of *Rm* values to specific membrane resistivity (*Rms*) was done by estimating the surface area of attached 3T3 and Py3T3 cells. 3T3 cells were estimated at a disk of radius 20 μm and a height of 2 μm. The Py3T3 cells were approximated by two right cones, continuous at the base

TABLE I
COMPOSITION OF SOLUTION USED FOR MICROELECTRODE STUDIES

	Mannitol	NaCl	KCl	NaH_2PO_4	$NaHCO_3$	$N(CH_3)_4Cl$	NH_4HCO_3	Na_2SO_4
				mM				
BSS-N	—	109.5	5.4	1.0	44.0	—	—	—
BBS-K_1	—	95.5	20.7	1.0	44.0	—	—	—
BSS-K_2	—	64.0	52.5	1.0	44.0	—	—	—
BSS-K_3	—	23.0	92.5	1.0	44.0	—	—	—
BSS-Na_1	—	43.0	5.4	1.0	44.0	66.0	—	—
BSS-Na_2	—	—	5.4	1.0	44.0	109.0	—	—
BSS-Na_3	—	—	5.4	1.0	11.0	109.0	33.0	—
BSS-Na_4	—	—	5.4	1.0	—	109.0	44.0	—
BSS-Cl_1	30	43.0	5.4	1.0	44.0	—	—	33.0
BSS-Cl_2	43	22.0	5.4	1.0	44.0	—	—	43.0
BSS-Cl_3	54	6.0	5.4	1.0	44.0	—	—	50.0

with a radius of 15 μm and a height of 30 μm. The *Rms* is probably underestimated since no allowance was made for microfilopodia (Lamb and MacKinnon, 1971).

RESULTS

Consistent with observations on other cell lines (Adam et al., 1979; Cone and Tongier, 1973), the *Em* for Py3T3 cells was shown to be a function of cell density. The data of Fig. 2 compare the *Em* values for both cell types, and the results clearly demonstrate that at the cell density of 3–4 × 10^4 cells cm^{-2}, the transformed cells were more electronegative than the normal cells. The *Em* ranged from −22.7 ± 1.6 mV at sparse cell densities (<10^5 cells cm^{-2}) to −46.1 ± 2.4 mV at saturation density (~8–9 × 10^5 cells cm^{-2}). The membrane resistance, *Rm*, is shown in Fig. 3 as a function of cell density. The *Rm* changed significantly ($P < 0.05$) from 23.6 ± 1.6 MΩ at a cell density of 5 × 10^3 cells cm^{-2} to an average value of 34.8 ± 2.4 MΩ at the higher cell densities studied. The 3T3 cells had an *Rm* of 14.4 ± 1.3 MΩ. Hence, when compared on the basis of the constant cell density (2–4 × 10^5 cells cm^{-2}), the *Em* and *Rm* differed significantly between the normal and transformed cell ($P < 0.05$). When expressed as specific resistivity, the *Rms* was 1,100 Ω-cm^2 and 400 Ω-cm^2 for Py3T3 and 3T3 cells, respectively.

The nature of these differences was studied by the following experiments. The data shown in Table II indicate that the temperature dependence of *Em* and *Rm* for the two cell lines were also different, the Py3T3 cells being sensitive to the temperature change from 35°C to 23°C, whereas the *Em* and *Rm* of the 3T3 cells did not significantly change. The *Em* and *Rm* of 3T3 cells were resistant to changes of external potassium, K_o^+, as indicated by the data of Table III. No significant change in *Em* or *Rm* was observed through a 10-fold increase in K_o^+ from 5.4 mM to 53 mM, until K_o was ~100 mM and the *Em* and *Rm* decreased ($P < 0.05$). Changes in the *Em* and *Rm* were also observed upon varying the concentrations of external Cl_o^- and Na_o^+, although the cells were relatively insensitive to these changes until the ion concentration was

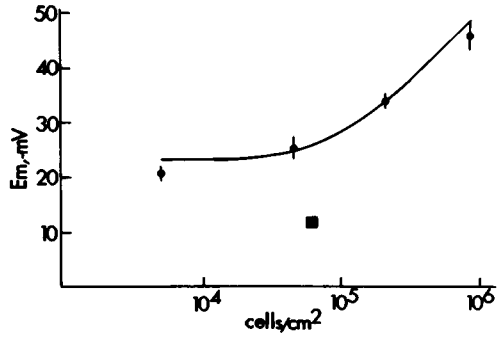

FIGURE 2 The transmembrane potential, *Em*, of 3T3 and Py3T3 cells as a function of cell density, cells-cm^{-2}. ●—●, Py3T3 cells, bars indicate mean ± se (minimum of 30 measurements per point). ■, 3T3 cells at single cell density of 4 × 10^4 cells − cm^2.

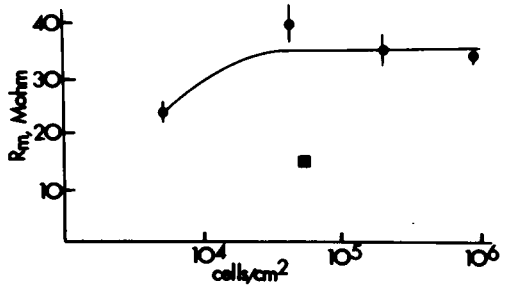

FIGURE 3 The DC membrane resistance of 3T3 and Py3T3 cells as a function of cell density, cells-cm^{-2}. When the value of the measurement did not significantly differ between two values of cell density, the graph was drawn through the average. ●—●, Py3T3 cells, each point is mean ± se of at least 30 measurements. ■, 3T3 cells at single density of 4 × 10^4 cells-cm^{-2}.

different by a factor of 4–5. In contrast to this insensitivity, the Py3T3 cells were very sensitive to alterations of the external ionic environment (Table IV). Significant depolarization of the *Em* was observed for nearly all of the measurements when K_o^- was raised or Cl_o^- and Na_o^+ was lowered.

DISCUSSION

The analysis of the data has been held until this section, because the method of analysis involves some assumptions and interpretations. The properties of 3T3 and Py3T3 cell systems summarized in Table V represent a classical model of in vitro transformation, where the normal cells do not grow in the syngeneic host and the cell line displays density-dependent inhibition of growth and is composed of a homogeneous-appearing cell population of self-limiting growth potential. On the other hand, the Py3T3 cells exhibited the morphological and growth changes associated with transformation (Nicolson, 1976), and injections of these cells resulted in a tumor. Both cell lines grew with an average doubling time of ~25 h, and when

TABLE II
EFFECT OF TEMPERATURE UPON THE TRANSMEMBRANE POTENTIAL DIFFERENCE AND DC MEMBRANE RESISTANCE OF Py3T3 AND 3T3 CELLS

Cell line	*T*	E_m*	*N*¶	*Rm*‡	*N*	Q_{10}§
	°C	−*mV*		MΩ		
	35	12.3 ± 0.9	44	14.4 ± 1.3	41	—
3T3	—	—		—	—	1.13 ± 0.22
	23	10.8 ± 0.7	33	19.9 ± 2.0	21	—
	35	35.2 ± 1.6	52	34.0 ± 2.8	42	—
PY3T3	—	—		—	—	2.62 ± 0.58
	23	13.4 ± 1.0	41	8.9 ± 0.4	34	—

*Transmembrane potential, BSS-N solution.
‡DC membrane resistance.
§Computed ratio TMP_{35}/TMP_{23} at 95% confidence limits.
¶Number of measurements.

TABLE III

EFFECT OF EXTERNAL ION CONCENTRATION ON THE TRANSMEMBRANE POTENTIAL DIFFERENCE AND DC MEMBRANE RESISTANCE OF 3T3 CELLS

Solution	I**	Em‡	N¶	Rm§	N
	mM	*−mV*		*MΩ*	
BSS-N‡‡	—	12.3 ± 0.9	44	14.4 ± 1.3	41
BSS-K_1	21	10.3 ± 0.6	33	13.4 ± 1.8	21
BSS-K_2	53	11.2 ± 0.6	32	14.0 ± 2.2	24
BSS-K_3	93	6.3 ± 0.3*	30	8.6 ± 0.7*	22
BSS-Cl_1	53	10.9 ± 0.9	43	14.2 ± 3.6	36
BSS-Cl_2	32	5.9 ± 0.4*	40	11.0 ± 1.6	37
BSS-Cl_3	17	7.4 ± 0.5*	37	8.5 ± 0.9*	33
BSS-Na_1	87	8.0 ± 0.6*	33	10.5 ± 1.4*	23
BSS-Na_2	44	11.8 ± 0.9	28	12.4 ± 1.1	20
BSS-Na_3	11	7.7 ± 0.6*	24	7.3 ± 0.6*	21
BSS-Na_4	1	5.3 ± 0.5*	31	5.1 ± 0.6*	24

*Significance ($p < 0.05$).
‡Transmembrane potential, cell density 5×10^4 cells/cm^2.
§DC membrane resistance.
¶Number of measurements.
**Concentration of the indicated ions.
‡‡Standard ion concentrations: Na^+ = 154.5 mM, K^+ = 5.4 mM, Cl^- = 114.9 mM.

TABLE IV

EFFECT OF EXTERNAL ION CONCENTRATION ON THE TRANSMEMBRANE POTENTIAL DIFFERENCE AND DC MEMBRANE RESISTANCE OF Py3T3 CELLS

Solution	I**	Em‡	N	Rm§	N
	mM	*−mV*		*MΩ*	
BSS-N‡‡		35.2 ± 1.6	52	34.0 ± 2.8	42
BSS-K_1	21	14.6 ± 0.9*	45	9.6 ± 0.7*	37
BSS-K_2	53	7.8 ± 0.8*	30	8.7 ± 1.4*	21
BSS-K_3	93	7.2 ± 0.7*	31	7.0 ± 0.6*	23
BSS-Cl_1	53	26.9 ± 2.1*	40	22.5 ± 2.2*	25
BSS-Cl_2	32	28.3 ± 1.8*	42	19.3 ± 1.7*	36
BSS-Cl_3	17	32.5 ± 2.7	30	32.8 ± 3.4	17
BSS-Na_1	87	29.5 ± 1.4*	47	18.3 ± 2.0*	28
BSS-Na_2	44	25.1 ± 2.0*	27	16.7 ± 1.6*	21
BSS-Na_3	11	29.5 ± 1.8*	30	21.0 ± 1.9*	15
BSS-Na_4	1	16.1 ± 0.8*	59	10.0 ± 1.8*	40

*Significance ($p < 0.05$).
‡Transmembrane potential, cell density 2×10^5 cells/cm^2.
§DC membrane resistance.
¶Number of measurements.
**Concentration of the indicated ions in millimoles per liter.
‡‡Standard ion concentrations: Na^+ = 154.5 mM, K^+ = 5.4 mM, Cl^- = 114.9 mM.

considered as a function of cell density, the *Em* and *Rm* increase (increase of *Em* meaning more electronegative), support the reasoning of Cone (1971) concerning the correlation of high *Em* values with less proliferative cells. The present results are also consistent with the data of both Adam et al. (1979) and Cone and Tongier (1973) who observed density-dependent increases in *Em*, and the present study shows that the ion conductance also decreases as the cell density of the culture increases. The *Rm* increased rapidly at a cell density where very few cell-cell contacts were formed, suggesting the presence of diffusable factors that altered ion conductance.

As a starting point for data analysis, consider that the *Em* is related to the ion permeability, *P*, the ion concentration, *X* (i, inside and o, outside the cell, respectively), and further, that this relationship is dominated by the two ions K^+ and Na^+ (Cl^- being passively distributed) from the Goldman equation (Goldman, 1943), and as discussed by Tupper (1972)

$$E_m = \frac{RT}{F} \ln \left\{ \frac{P_K[K]_o + P_{Na}[Na]_o}{P_K[K]_i + P_{Na}[Na]_i} \right\}. \quad (1)$$

Where *R* and *T* have their usually thermodynamic meanings and *F* is the Faraday constant, we may write

$$\exp\left(\frac{EmF}{RT}\right) = \frac{[K]_o(1-\alpha) + \beta\alpha}{[K]_i}. \quad (2)$$

Where $\alpha \equiv P_{Na}/P_K$, $\beta \equiv [K]_o + [Na]_o$, and α Na $\ll [K]_i$, and Eq. 2 is linear with respect to $[K]_o$ and of slope $(1-\alpha)/[K]_i$ and of *y*-intercept $\alpha\beta/[K]_i$. If we plot exp (EmF/RT) as a function of $[K]_o$, we can solve for α and $[K]_i$. The data of Fig. 4 demonstrate the insensitivity of 3T3 cells to changes in $[K]_o$, and using Eq. 2, $\alpha = 1$ and $[K]_i$ = 237 mM. This result is in excellent agreement with the permeability data of Adam et al. (1979) (P_{Na}/P_K ~0.75 to 1.0 at 37°C, 1 mM Ca^{++}) for 3T3 and transformed 3T3 cells. Viewed from another point, in considering the two cell types at a given cell density (late logarithmic growth), the *Em* of 3T3 cells was less electronegative (−12 mV) than Py3T3 cells (−25 mV) and demonstrates that, in contrast to suggestion (Cone and Tongier, 1973), transformed cells do not necessarily possess lower *Em* values because of their proliferative capacity. Hence, this particular feature of Cone's discussion of the relationship between the *Em* and mitotic activity seems mistaken. Superimposed on all of these data is the consideration of *Em* as a function of the cell cycle itself, nicely demonstrated using neuroblastoma cells (Boustra et al., 1981).

TABLE V

SUMMARY OF THE GROWTH AND ELECTRICAL PROPERTIES OF NORMAL 3T3 CELLS AND TRANSFORMED PY3T3 CELLS

Parameter	3T3	Py3T3
Saturation density	4–5 × 10^4 cells cm^{-2}	3–10 × 10^5 cells cm^{-2}
Doubling time	29 h	23 h
Em (5 × 10^4 cells cm^{-2})	−12 mV	−25 mV
Rms	400 Ω-cm^2	1,100 Ω-cm^2
Temperature coefficient, *Em*	1.13	2.62
P_{Na}/P_K	1.0, constant	0.27, variable
P_{Cl}/P_{Na}	0.53	0.15
P_{Cl}/P_K	1.88	1.75

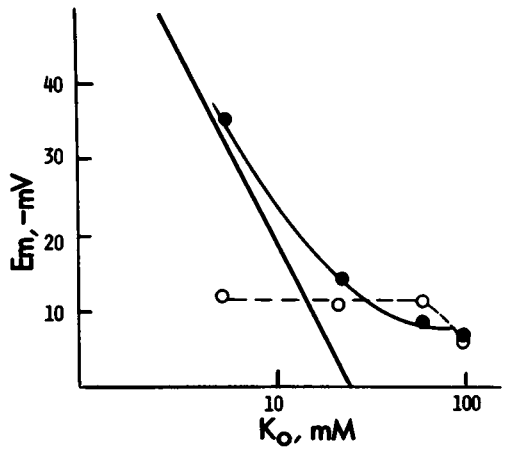

FIGURE 4 The transmembrane potential, *Em*, as a function of external K_o^+ concentration. ●—●, Py3T3 cells at cell density of 2×10^5 cells-cm^{-2}; O—O, 3T3 cells at cell density of 5×10^4 cells-cm^{-2}. Each point represents the mean of at least 30 measurements. Error bars (<1 mV) have been omitted for clarity. The solid line indicates a slope of −60mV per 10-fold change in K_o^+, (Nernst equation) for comparison with observed values.

The early rise in the *Rm* of the transformed cells was somewhat surprising in that it occurred during a portion of culture growth where there were only a few cell-cell contacts ($<5 \times 10^4$ cells cm^{-2}), suggesting that some metabolic situation may have made the cells "electrically aware" of the presence of other cells, in accordance with results discussed by Lowenstein (1972) and the ability of cells to exchange small-molecular weight material.

There is sufficient data on cultured cells to allow a mathematical analysis of the permeability properties of the 3T3 and Py3T3 cells. The intracellular potassium estimate of 237 mM is in close agreement with Cone's direct measurement of 205 mM (1973), recognizing that cultured cells have reported values of $[K]_i$ ranging from 150 to 206 mM (Lamb and MacKinnon, 1971; MacDonald et al., 1972; Borle and Loveday, 1968; Tupper, 1972; Sacks and MacDonald, 1972). Adam et al. (1979) showed that P_{Na} for 3T3 cells is the same order of magnitude as P_K (10^{-7} cm · s^{-1}) hence, compared with excitable tissue where P_{Na}/P_K is ~0.01 ($P_{Na} \sim 10^{-9}$ cm · s^{-1}) (Hodgkin and Horowicz, 1959), the ratio obtained from the present analysis can be explained by considering that the P_{Na} is greater than in the nonexcitable cell and approaches the permeability observed for K^+. Other work has measured P_{Na}/P_K from 0.33 to 1.0 (Lamb and MacKinnon, 1971; Borle and Loveday, 1968; Adam et al., 1979).

The analysis of P_{Na}/P_K for the transformed cells by the same method cannot be attempted with confidence, since the data of Fig. 4 show that the response of the *Em* for $[K]_o$ variation was not linear. One does notice that the change in *Em* as a function of $[K]_o$ tended to the Nernst line shown in the graph. The deviation observed can be approached by considering that the permeability ratio, P_{Na}/P_K, is a function of the K^+ and Na^+ distribution across the membrane.

We may estimate the value for P_{Na}/P_K by assuming that for a variation in the external concentration of potassium, P_{Na}/P_K will also vary, but in a manner such that the Goldman equation is obeyed. Hence, using $[K]_i = 205$ mM (Cone and Tongier, 1973) and $[K]_o = 5.3$ mM, then P_{Na}/P_K for the transformed cells was 0.27.

Stated another way, the *Em* observed for the Py3T3 cells at $[K]_o = 5.3$ mM can be explained by the Goldman relationship, if we assume a $P_{Na}/P_K = 0.27$, and at other values of $[K]_o$, the P_{Na}/P_K changes rapidly. The value of 0.27 is in good agreement with other reported values, such as 0.26 for neuroblastoma (Booustra et al., 1981), estimated at 0.5 for the data of Adam et al. (1979), and 0.33 for Chinese hamster ovary (CHO) cells (Stambrook et al., 1975). Summarizing this portion of the analysis, we find that the *Em* of the 3T3 and Py3T3 cells may be considered as a diffusion potential described by a modified Goldman equation, with the permeability ratio P_{Na}/P_K equal to unity in the normal cell, reduced at 0.27 in the transformed cell, and associated with ion distribution sensitivity in this latter cell type. Permeability changes associated with $[K]_o$ are not without precedence (Hodgkin and Horowicz, 1959; Claret et al., 1970; Nobel, 1956).

The sensitivity of the electrical parameters of both cell types to variations in the external chloride concentration suggests that we consider the Goldman equation (1943) further to estimate the ratio P_{Cl}/P_K.

For the 3T3 cells, $P_{Na}/P_K = 1.0$, we may write,

$$[K]_o + [Na]_o = \zeta[Cl]_i$$
$$[K]_i + [Na]_i = \zeta[Cl]_o \qquad (3)$$

where $\zeta \equiv P_{Cl}/P_K$ and solving these two equations yields $P_{Cl}/P_K = 1.88$ and $[Cl]_i = 83$ mM for the 3T3 cells. A similar calculation for the Py3T3 cells (using $P_{Na}/P_K = 0.27$) gives $P_{Cl}/P_K = 1.75$ and $[Cl]_i = 28$ mM. Now, the proper values for $[Cl]_i$ may be obtained by the Nernst equation, which is an independent check on the methodology above. Using $Em = -12.6$ mV and $[Cl]_o = 115$ mM for the 3T3 cells, Eqs. 3 and 4 yield $[Cl]_i = 80$ mM, in close agreement with the predicted value calculated above. For the Py3T3 cells, with a resting potential of −35.2 mV, we obtain $[Cl]_i = 30$ mM, comparing favorably with the 28 mM computed above. The computed values for P_{Cl}/P_K show that for the two cell lines, $P_{Cl} > P_K > P_{Na}$, consistent with other measurements in rat liver (Claret et al., 1970) and L cells (Lamb and MacKinnon, 1971). The ratio P_{Na}/P_{Cl} remains to be computed, and is 0.53 and 0.15 for the 3T3 and Py3T3 cells, respectively, compared with 0.54 obtained by Schaefer et al. (1972) for leukemic cells and 0.34 reported by Aull for ascitic tumor cells (1967).

The results presented here demonstrate that, in general, the electrical alterations observed in the transformed cells seem associated with membrane features that tend to lower ionic conductance, and with the observation that the temperature coefficient was a value consistent with enzy-

matic processes (>2), the result could be manifested as reduced ion mobility through the membrane; hence, a combination of ion redistribution and change in P_{Na}/P_K could easily give rise to the observed differences in *Em*. Alterations in both K^+ flux (Adam et al., 1979) and Na^+-K^+ ATPase activity (Yoshikawa-Fukada and Jojima, 1973) are well described in transformed cells, yet the pathways by which these phenomena are linked to the metabolic processes of growth regulation remain unknown. Permeability changes to a variety of metabolites are common with transformation (Foster and Pardee, 1969), and both increases and decreases in ion flux have been reported (Spaggiare et al., 1976; Adam et al., 1979).

Variations in the ratio P_{Na}/P_K might also affect regulatory events associated with cellular phenotype (such as saturation density, morphology, or growth rate). Large external concentrations of potassium are optimal for the growth of some cells (McDonald et al., 1972; Yoshikawa-Fukada and Jojima, 1973) and affects both the growth rate and morphology of other cultured lines (Stambrook et al., 1975).

The role of ions in cellular growth regulation including the aberrations associated with ion transport and changes in membrane composition and architecture seem common to transformed cells. As the *Em* is determined equally by the ion mobilities and the ion concentrations, which in turn are determined by membrane structure and enzymatic activity, it seems reasonable to picture the causal sequence of ion-mediated regulatory events to lie on a circle, along the circumference of which lie the equal effectors of metabolic activity, namely, permeability, *Em*, conductance, ion distribution, enzymes, protein synthesis, etc. The study of one variable becomes an indirect study of the other dependent (interconnected) variables.

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