Cation Metabolism in Relation to Cell Size in Synchronously Grown Tissue Culture Cell

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ABSTRACT In randomly grown tissue culture cells (mouse leukemic lymphoblast, L5178Y) the number, volume, and Na^+ and K^+ content increase as an exponential function with a doubling time of 11.3 hr. In synchronously grown cells the volume increase of the population and of single cells follows the same exponential function as in randomly grown cells. In contrast, the cation content fluctuates during a single cell cycle. About 1½ hr after the cell division burst (at the beginning of the *S* period), a net loss of K^+ occurs for a period of about 1 hr amounting to about 20% of the total K. Over the next 5 to 6 hr, the deficit in K^+ is eliminated. The Na^+ content shows a double fluctuation. It falls during the cell division burst, rises when the K^+ content decreases, falls again when K^+ content rises, and then increases again before the next cell division burst. The net fluxes of both Na^+ and K^+ are very small compared to the unidirectional fluxes (less than 5%), thus small changes in the balance of influx and efflux account for the changes in cation content during the growth cycle. Both unidirectional fluxes increase dramatically (by a factor of two) about 2 hr after the cell division burst, and then remain constant until after the next cell division. The pattern of electrolyte regulation during cell division does not follow a simple function such as cell number, cell surface, or cell volume, but must be related to specific internal events in the cell.

Electrolyte regulation in animal cells has been studied almost exclusively in nongrowing cells such as nerve (1), muscle (2), or erythrocytes (3–6), usually under steady-state conditions in which the efflux and influx of particular ions are equal and in which "uphill" and "downhill" movements are in balance. Such regulation in naked cells is related not only to the maintenance of ionic composition, but also to the regulation of size. Thus an increase or decrease in electrolyte content leads to swelling or shrinking, with the cells behaving at least approximately, as osmometers. The most rigorous analysis of the relation-

ship of electrolyte balance and cell size has been carried out in the red blood cell (7, 8). The predominant factors include the cellular content of Na^+ and K^+ and the colloidal osmotic component of the cellular proteins, the cation contents being determined by the balance of uphill and downhill movements (pumps and leaks).

In growing cells, the continuous increase in size presumably reflects a continuous increase in the cellular osmotic content. Because the alkali metal cations K^+ and Na^+ make up a large proportion of the cellular osmotic content, growth in volume presumably reflects in large part an accumulation of these cations (and accompanying anions). Growing cells must, therefore, be geared to continuous electrolyte accumulation rather than to maintenance of a steady state.

Tissue culture cells in suspension would seem to be an ideal biological material in which to study the relationship of electrolyte metabolism to the increase in size during growth. A few measurements of ion fluxes have been made (9, 10) and one set of observations has been reported concerning maintenance of cell size (11), but these represent the average behavior of a population of nongrowing cells in various stages of the cell division cycle. In the present study, techniques for achieving synchronization of cell divisions were applied to the problem. The particular cell chosen for study was L5178Y, a mouse leukemic lymphoblast (12), which can readily be grown in suspension, and in which the cell division cycle has been carefully determined (13). The changes in cell volume, cell number, K^+ and Na^+ content, and unidirectional fluxes of K^+ and Na^+ were estimated in both randomly growing and synchronized cultures.

METHODS

The cell line used in this study was a clone of mouse lymphoblast L5178Y cultured at 37°C in 15 ml screw cap tubes containing 5 ml of the well defined growth medium described by Fischer and Sartorelli (12). The K^+ and Na^+ concentrations of the medium are 5.4 and 136 mM, respectively. The cells were routinely resuspended in fresh media every 3rd day. Under these conditions the cells grow exponentially in suspension without shaking, with a reported generation time (T_G) of 11.8 hrs at 37°C. The life cycle under these specific conditions consists of 1.6 hr G_1, 8.1 hr S, 1.5 hr G_2, and 0.6 hr mitotic periods (13).

Partial synchronization of the cell population was obtained by two different methods: (*a*) cells were treated twice for 6 hr with 2×10^{-4} M thymine-2-deoxyriboside (TdR), with an intermission of 8 hr in TdR-free normal growth medium, and (*b*) cells were incubated in the presence of a mixture of 3.5×10^{-7} M methotrexate (kindly provided by Dr. J. M. Ruegsegger of the Lederle Laboratory) and 10^{-4} M hypoxanthine for 8 hr, then were transferred into the normal growth medium containing 2.5×10^{-6} M/liters of TdR, as suggested by G. A. Fischer. Both methods gave an average synchronization index (SI) of 0.65 (14). Other procedures for synchroniza-

tion were not so successful. Temperature shock at 4°C for 60 min sometimes resulted in a synchronization of 0.65 (SI) but the results were variable and changes in cell volume were observed. Attempts to synchronize by treatment with 5-fluorodeoxyuridine (FuDR) or ethylenediaminetetraacetic acid (EDTA) were successful.

The cell number and the size distributions of the populations were determined by Coulter particle counter Model A (100 μ aperture) and Model B (70 μ aperture, with the calibration against erythrocytes whose average volume was determined to be $90\,\mu^3$ by hematocrit corrected for inulin space and cell count). It was necessary to resuspend the cells in isotonic NaCl just prior to the measurements made with these instruments.

The cell volumes were measured by three different methods: (*a*) the mean volume and modal volume were calculated from the data of Coulter particle counter Model B size distribution curves; (*b*) the packed volume of a known amount of cell suspension containing 1 to 2×10^7 total cells was determined after centrifugation at $1.20o \times g$ for 10 min in a horizontal rotor using thrombocytocrit tubes (van Allen, from A. H. Thomas, Philadelphia, Pa., graduated in 0.0005 ml) with snugly fitting Teflon plugs inserted in the open end of the capillary. With a magnifying glass, readings can be made to ± 0.1 microliter; (*c*) a single cell from the exponentially growing population was measured directly under the microscope using an eyepiece micrometer (disc, scale in 0.05 mm/division) calibrated by a stage micrometer. Because the cells are spherical their volume was easily calculated from the measured diameter. A specially designed microculture slide (Bausch and Lomb Optical Co., Rochester, N. Y.) was used which gives, when a cover glass (Thomas micro cover glass No. 1, 30×35) is mounted with 50% vaseline in paraffin seal, a culture space of about 0.5 ml capacity with a gap of 0.2 mm in height above the central platform of 18 mm square. About 0.15 ml of the cell suspension can be cultured for at least three generations with normal growth rate and cell shape. The platform was ruled into $\frac{1}{16}$ square mm to identify the position of each cell. The cells do not move out of position during the observation period.

After reading the cell volume in the thrombocytocrit, the packed cell mass in the capillary was quantitatively transferred into a small, dry, washed test tube by inserting a silver rod. The pellets in the tubes were dried overnight at 110°C for dry weight determinations (accuracy ± 0.1 mg). The extracellular space of the packed cell volume was estimated by measuring its inulin space (15). The average value (eight determinations) was 10.4% (± 0.9%). The water content in per cent of the net cell volume was 78%, assuming that the cell density is one. The relative water content did not change appreciably (less than 7%) during the cell division cycle.

To determine the cellular content of K^+, the packed cell mass was washed for 2 min with two times its suspension volume of ice cold isotonic NaCl solution. In order to evaluate possible errors due to losses of K^+, control experiments were performed with washing for 2, 3, and 4 min and the tests were made at various times in the cell cycle using synchronously grown cells. The K^+ loss was less than 0.5% of the cellular content per minute. Thus the maximum loss in the experiments was less than 1%. To determine the cellular content of Na^+, the cells were washed twice with 10 times the suspension volume of ice cold isotonic LiCl solution. The washed pellets in Vycor tubes were dried overnight at 110°C and were liquefied by heating over a gentle microburner flame with 0.10 ml of redistilled concentrated nitric acid, carefully avoid-charring. The residue was taken up in 5 ml of warm deionized water and the nitric

acid was driven off by gentle boiling with dropwise addition of formic acid (16). K^+ and Na^+ were determined on the Beckman DU flame photometer and calculated as mM/liter of cell water.

To measure K^+ influx, cells from actively growing cultures were resuspended in a prewarmed medium containing 8×10^{-2} μc/ml of $K^{42}Cl$ (Oak Ridge National Laboratory, Oak Ridge, Tenn.). At different times aliquots of known cell content were taken, spun down, and carefully washed with twice the suspension volume of ice cold NaCl isotonic solution. The washed cells were then counted for isotope. To measure K^+ efflux, the cells were first allowed to equilibrate with ^{42}K for 3 hr by adding

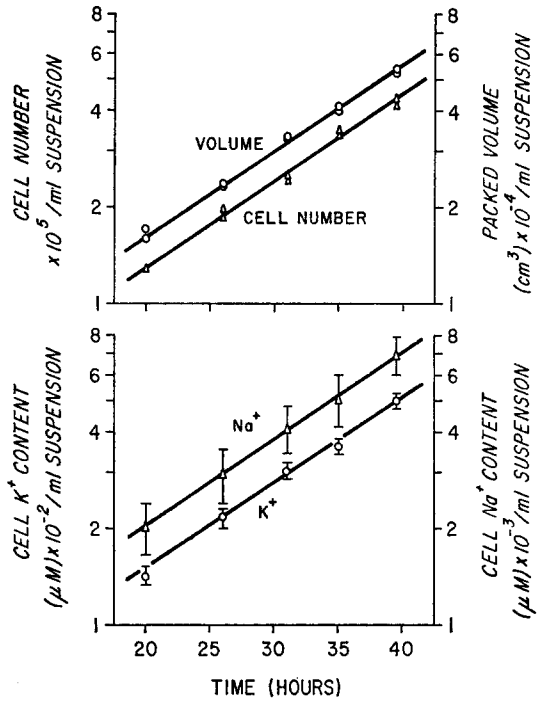

FIGURE 1. The increase in cell number, total packed volume (before inulin space correction), and total Na^+ and K^+ content in randomly growing cells. Bars indicate standard errors.

4×10^{-2} μc/ml of $K^{42}Cl$ to an actively growing cell suspension, then were washed twice with 10 times the suspension volume of ice cold saline, then resuspended in the prewarmed media. At given times aliquots of known cell content were taken, centrifuged, and the packed cell mass prepared for isotopic assay without further washing. Na^+ fluxes were measured the same way as K^+ fluxes except that 0.5 μc/ml of $Na^{22}Cl$ (Oak Ridge National Laboratory, Oak Ridge, Tenn.) was used and the cells were quickly washed twice with ten times the normal suspension volume of ice cold isotonic LiCl with thorough drainage of the supernatant fluid each time. Supernatants from the last washing and from the experimental suspensions were also counted. In every case the trapped extracellular solution contributed negligible errors to the radioactivity of the cells. Both the complete culture medium and a "defective medium" (the complete culture medium minus horse serum and glutamic acid) were used for flux measurements. The cells suspended in the defective medium did not change in

number or cytocrit or shape for at least 4 hr. Each assay sample contained not less than 16 μg of packed cell mass. Radioactivities of both ^{42}K and ^{22}Na were counted in a well-type scintillation counter with the ^{42}K measurements corrected for radioactive decay.

RESULTS

The initial experiments were carried out on randomly dividing cells. The increase in the numbers of cells followed a simple exponential function represented by a straight line on a semilogarithmic plot (second line from top in Fig. 1). The slope is equal to $0.693/2.3 \times T_G$, when the value of T_G, the generation time, is 11.3 hrs. A line of the same slope fits the data well for total

TABLE I

VOLUME AND K^+ AND Na^+ CONCENTRATIONS OF THE AVERAGE CELL IN A RANDOMLY DIVIDING POPULATION

Volume, μ^3	1112.4 ± 9.2 (98)*	1290.6 ± 15.6 (18)‡
K^+ (*mM/liter cell* H_2O)	136.4 ± 8.3 (26)	
Na^+ (*mM/liter cell* H_2O)	18.8 ± 3.8 (26)	

The data are the mean of the numbers of independent measurements indicated in parentheses with their standard errors of the mean.

* Volume obtained by cytocrit method after correction for inulin space.

‡ Volume from the size distribution curve and cell count from a Coulter particle counter, Model B.

cellular volume, total cellular content of K^+, and total cellular content of Na^+ (Fig. 1). It can be concluded, therefore, that cell number, cell volume, K^+ content, and Na^+ content all increase with the same exponential function that can be expressed

$$\frac{1}{Sc}\frac{dSc}{dt} = \frac{1}{V}\frac{dV}{dt} = \frac{1}{N}\frac{dN}{dt} \equiv \alpha \qquad (1)$$

where Sc is the cellular cation content, V the cellular volume, and N the number of cells. The average for α from seven independent estimates was 0.061 hr^{-1} ± 5 % (SE). From equation (1) it is obvious that

$$\frac{dSc/N}{dt} = \frac{dV/N}{dt} = 0 \qquad (2)$$

and that Sc/N and V/N, the average cellular cation content and average cellular volume, are in a steady state, independent of time. The average values for randomly growing cells are given in Table I. The cell volume estimated by the Coulter counter (1290 μ^3) is about 15 % larger than that estimated by the cytocrit method (1112 μ^3), perhaps due to the swelling of the cells in the serum-free medium (isotonic NaCl) that must be used in the Coulter counter

(17). The ratio of cellular Na^+ to K^+ is about 1 to 7.5, compared to that in the medium of 25.7 to 1, or a total discrimination in favor of K^+ of 192 to 1.

The Na^+ and K^+ content of the cells is determined by the influx and efflux of each cation. In the randomly growing population, with an exponentially increasing cation content, it is obvious that the influx always exceeds the efflux. The flux equations in common use for ions are derived for nongrowing cells in a steady-state system, and are therefore not directly applicable in the present case. A simple modification of the equations can, however, be applied. Assuming that the growing cells and the suspending medium are a closed, well mixed two compartment system, and that there is no isotopic effect, the movement of electrolytes across the cell membrane can be described in the following equation:

$$\frac{dp_c^* S_c}{dt} = \phi_i p_m^* - \phi_e p_c^* \tag{3}$$

where p^*, S, and ϕ are the specific activity (CPM/mM), amount of the electrolyte (mM), and flux (mM/min) respectively, and subscripts c, m, i, and e denote cell, medium, influx, and efflux respectively. Since there is a net flux which is characterized by equation (1),

$$\frac{dS_c}{dt} = \phi_i - \phi_e = \alpha S_c \tag{4}$$

And since the tracers are conserved in the system,

$$P_c^* S_c + p_m^* S_m = p_{c,\infty}^* (S_c + S_m) \tag{5}$$

where the subscript ∞ denotes the time of equilibration. Using equations (4) and (5), and assuming that $(S_c + S_m)/S_m$ and ϕ_i/S_c are constant, equation (3) was integrated and solved for the cellular specific activities to get the following equation,

$$\ln \frac{p_{c,\infty}^* - p_c^*}{p_{c,\infty}^* - p_{c,0}^*} = -\lambda t \tag{6}$$

where the subscript 0 denotes time zero and

$$\lambda \equiv \frac{S_c + S_m}{S_m} \frac{\phi_i}{S_c} = k_i + k_e + \alpha \tag{7}$$

and k_i, k_e are the first order rate constant defined as $k_i = \phi_i/S_m$ and $k_e = \phi_e/S_c$. λ can be obtained from the linear portion of the initial slope of the semi-log plot of equation (6), then the values of ϕ_i/S_c, k_i, and k_e can be calculated from equation (7). The value of S_c under the conditions of the experiment is

less than 0.013 of that of S_m, and changes only 0.061 (α) of its value per hour so that $\frac{S_c + S_m}{S_m}$ can be considered to be constant during the 2 hr experiment with the error less than 0.2 %.

A typical experiment in which the fluxes of labeled Na^+ and K^+ by the

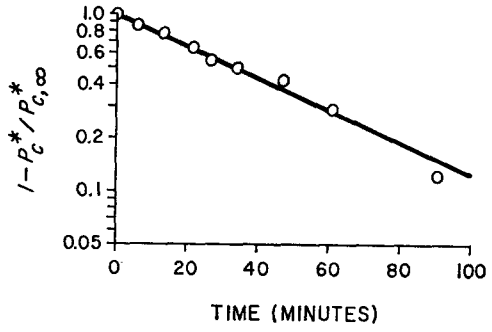

FIGURE 2. K^+ influx into a randomly dividing cell population.

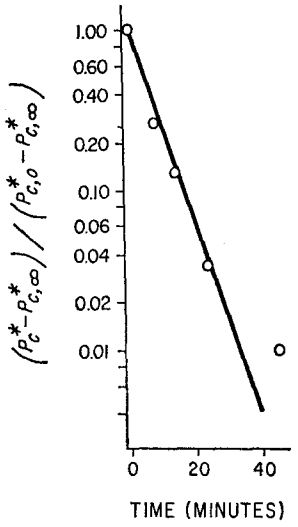

FIGURE 3. Na^+ efflux from a randomly dividing cell population.

cells were determined is given in Figs. 2 and 3, plotted according to equation (6). In each case, the data can be fitted by a straight line over at least a tenfold range. From the slopes of Figs. 2 and 3, the values of $\frac{\phi_i}{S_c}$ (min^{-1}) are 0.020 for K^+ and 0.14 for Na^+. These values are quite reproducible, ranging from 0.020 to 0.024 for K^+ (five estimates) and 0.13 to 0.14 for Na^+ (three estimates), despite the fact that the numbers of cells in the different populations differed by a factor of five. Thus ϕ_i/S_c is indeed time-invariant during the exponential growth.

The influx of Na^+ and K^+ was also estimated under nongrowing conditions, in a steady state with regard to cations, by suspending the cells in a defective medium. The efflux was the same as the influx within experimental error. Furthermore, the flux data were indistinguishable from those of Figs. 2 and 3 for growing cells. This finding is not surprising in view of the fact that the net flux in growing cells is so small compared to the influx (0.001 compared to 0.020 min^{-1} for K^+, and an even greater difference for Na^+). It is, in fact, within the experimental error of the measurement of influx. Consequently, in equation (7) the value of α representing the net flux is so small that it is not

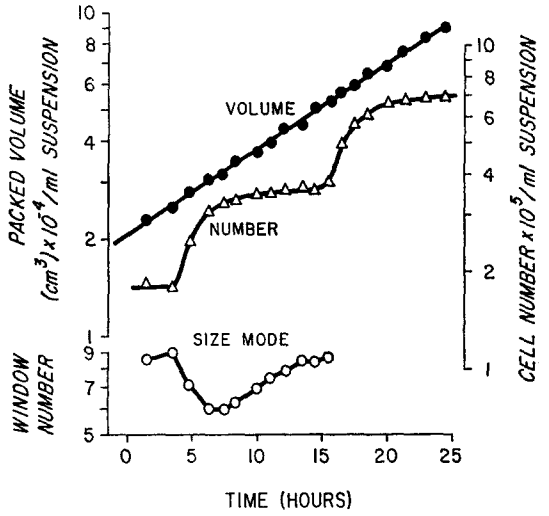

FIGURE 4. Changes in cell count, total packed volume, and size mode in synchronously dividing population. Synchronization was induced by TdR double treatment with transfer into the normal growth media at time $t = 0$. The size mode is given in terms of the window number of the Coulter particle counter Model B. The window number is roughly proportional to cell volume.

an important factor in estimating unidirectional fluxes. The accumulation of K^+ by the growing cells results, therefore, from the condition that the influx of the cation is about 5 % larger than the efflux, and the accumulation of Na^+ from the condition that the influx is about 1 % larger than the efflux. The large differences between the unidirectional fluxes and the rate of growth are also obvious from the exchange times. Half of the K^+ exchanges in 33 min and half of the Na^+ in 5 min, whereas the cell cycle and its consequent doubling of the K^+ and Na^+ content take 11.5 hr.

The values for cell size, Na^+ and K^+ contents, and fluxes of Figs. 1–3 represent an average value for the cells in the population, and the constancy of the values for the "average" cell in the population is a reflection of true exponential growth. The average values do not, however, give any indication concern-

ing changes in electrolyte balance and size of individual cells as they pass through the cycle. For this reason a series of experiments were carried out on populations of cells partially synchronized in their cell division cycle.

In a partially synchronized population of cells, the total volume as measured by the cytocrit, increased as an exponential function (Fig. 4). The slope of the line is $0.693/2.3 \times T_g$, the same as that found for the volume change in randomly growing cells (Fig. 1). Yet the increase in numbers of cells indicates that cell divisions occurred almost entirely in a 2½ to 3 hr period, with few or no divisions for a 6 to 7 hr period. The modal size determined by the size-frequency distribution (Coulter counter) fell sharply during the period when

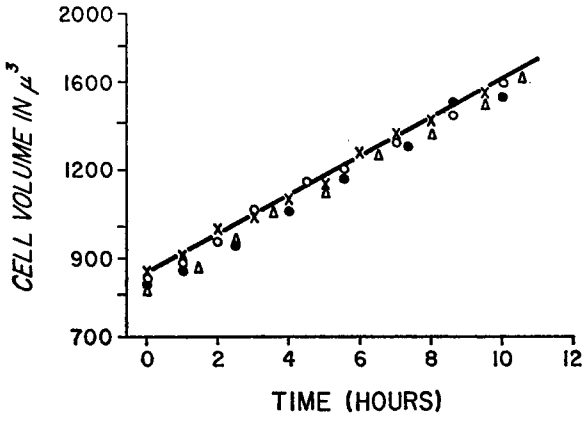

FIGURE 5. Volume increase of single cells during a single division cycle. Each point represents a mean of measurements of four single cells growing in a specially designed microculture slide. Time on the abscissa is normalized for each cell starting immediately after each division and measurements were continued until the next division occurred.

cell numbers increased, then increased during the period when cell numbers were constant. From these observations, it can be concluded that each individual cell must increase in volume in the same exponential fashion as the total population, and that after division each daughter cell does the same.

To confirm the above interpretation, the diameters of growing single cells were directly measured under the microscope using a specially designed microculture slide (see Methods for details). Since each cell is almost perfectly spherical in shape, its volume was easily estimated from the measured diameter. The results from 16 sets of such measurements are shown in Fig. 5. It is clear that each individual cell increases in volume in an exponential fashion. Furthermore, the slope of the line in Fig. 5 which fits the points reasonably well, is the same as that which describes the volume changes measured by the cytocrit method in synchronized (Fig. 4) and randomly growing populations (Fig. 1).

Knowing that the increase in volume in individual cells follows a simple exponential function, an average cell volume for a random population can be calculated[1] to be 1183 μ^3, which agrees reasonably well with the cytocrit and Coulter counter data of Table I, 1112 and 1291 μ^3, respectively.

Synchronously growing cells were sampled periodically for estimates of Na^+ and K^+ content. 12 experiments were carried out with a typical case presented in Fig. 6. The dashed line represents the increase in total cell volume, with a slope of α, as discussed in relation to Figs. 1, 4, and 5. In sharp contrast to the exponential increase in both Na^+ and K^+ content in randomly growing cells, a complex pattern is observed in synchronized cells. Although the total content of K^+ in the population of cells doubles in each generation, in the 2 to 3 hr immediately after the cell division burst, a net loss occurs, followed after 6 hr by a rapid gain for 2 hr at a rate about three times that of the volume increase, α. Thereafter the rate of net influx proceeds at about the same rate as the volume increase until the next cell division cycle. The dip in K^+ content was observed in each of the 12 experiments. In terms of the concentration of K^+ in cell water, it represents a 20 to 30 % decrease. In 8 of the 12 experiments a small dip in K^+ content was also observed about an hour before the division burst occurred. The significance of this finding is questionable.

The pattern for Na^+ was more complicated. The size and exact timing of the fluctuations were variable, but in every case two dips in Na^+ content were

[1] In the exponentially growing random population, the age distribution is

$$n = n_0 e^{-0.693\, t/T_G} \tag{8}$$

where n and n_0 are the numbers of cells at ages t and 0 respectively. Since the volume of a single cell increases as an exponential function of its age,

$$v = v_0 e^{0.693\, t/T_G} \tag{9}$$

where v and v_0 are the single cell volumes at ages t and 0 respectively. Then the total cell volume in the population V is

$$V = \int_0^{T_G} n\, v\, dt = \int_0^{T_G} n_0\, e^{-0.693t/T_G} v_0\, e^{0.693t/T_G}\, dt = n_0\, v_0\, T_G \tag{10}$$

Also, the total number of cells in the population N is

$$N = \int_0^{T_G} n_0\, e^{-0.693t/T_G}\, dt = \frac{n_0\, T_G}{1.386} \tag{11}$$

Then, the average cell volume of the population V_a is

$$V_a \equiv \frac{V}{N} = 1.386\, v_0 \tag{12}$$

seen in each cycle. At the time of division the Na^+ content falls. It rises again when the K^+ loss occurs, and falls a second time when net K^+ uptake is rapid. Because the K^+ content is seven times as high as that of Na^+ the sum of K^+ and Na^+ is almost parallel to the K^+ curve, but about 15 % higher. In the period following cell division the contribution of the alkali metal cations to the osmotic pressure of the cell is presumably reduced by some 20 to 30 %, and

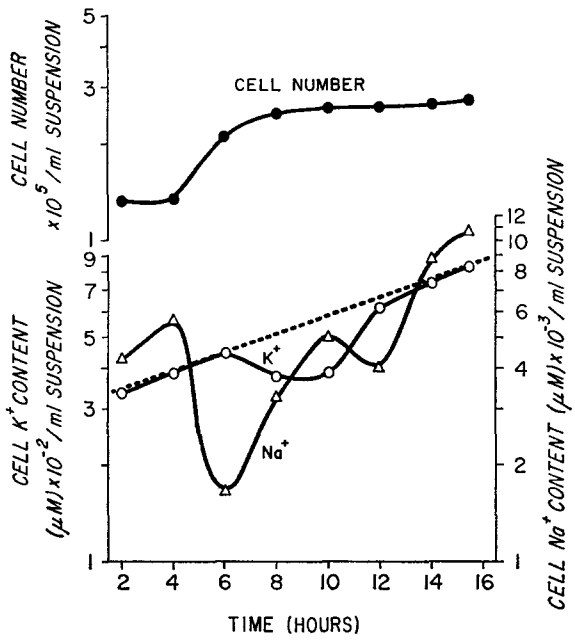

FIGURE 6. Changes in cell count and total cellular Na^+ and K^+ contents in a synchronously growing population. Synchronization was induced by methotrexate-hypoxanthine-TdR treatment. $t = 0$ on abscissa is the time the cells were transferred into TdR containing growth media. The dashed line represents total packed volume, and is arbitrarily positioned for comparative purposes, to give the slope for the increase in volume.

some other osmotic components (perhaps amino acids) compensate, because no corresponding dip is seen in cell volume.

The slope of the curve for K^+ content in Fig. 6 represents the net flux. It varies from -1.5α to 3α, where α is 0.001 min^{-1}, the slope of the net increase of K^+ in randomly growing cells (equation 1 and Fig. 1). In order to evaluate the factors involved in the changes in net flux, an attempt was made to measure the unidirectional flux of K^+ at different stages of the cell cycle. To avoid the complicated changes in net flux in the growth medium, the measurements were made under steady-state conditions by resuspending samples of synchronized cells at different stages into the "deficient" medium in which no further growth occurs and in which the K^+ content remains constant. At the

time of transfer a fixed amount of ^{42}K was added to the medium, and its uptake by the cells was measured at 5 to 7 min intervals. Total cellular ^{42}K activities were plotted against time, and the initial rate of uptake[2] was taken to represent the K influx of the population of cells at the time they were removed from the growth medium. The data are plotted on a relative, semilogarithmic scale in Fig. 7, taking the predivision flux and the predivision number of cells as 1.0. For about 2 hr after the cell division burst, the total influx, ϕ_i, of the population does not change. Thus no change in influx occurs during the cell division process itself. Each mother cell has the same total

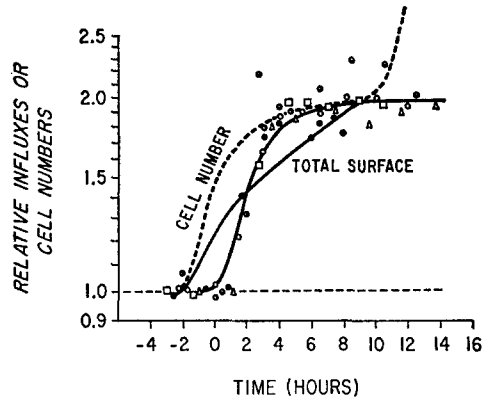

FIGURE 7. Relative changes in K^+ influx, cell counts, and total cell surface in a synchronously growing population. Six independent sets of measurements, each of which is represented by the different symbols, were normalized against the cell counts curve by setting the time of inflection of the cell counts curve as the reference point on the time scale. The line for the influx data was drawn by eye. For more detail, see text.

influx as its two daughter cells. But 2 hr after the cell division burst the total influx of the population increased rather dramatically to a new level. This increase is not parallel to the increase in volume which is linear on the semilog

[2] At constant S_c, and for the experiment in which the tracer was added initially only to the medium, equation 6 can be converted to the following equation:

$$P_c^* = P_{m,0}^* \left(\frac{S_c}{S_m + S_c}\right)(1 - e^{-\lambda t}) \tag{13}$$

where P^* is equal to p^*S, subscript 0 denotes time 0, and λ is as expressed in equation 7. If the exponential term in this equation is expanded in series and divided by t, as t approaches zero, we get

$$\lim_{t \to 0} \frac{P_c^*}{t} = p_{m,0}^* \, \phi_i \tag{14}$$

This expression gives the initial slope of the curve of equation 13 and ϕ_i can be calculated from this equation.

plot. Nor is it directly related to the increase in cell surface which can be calculated from the cell size and number distributions.[3] Rather, it is parallel to the change in numbers of cells, suggesting that at about 2 hr after cell division the rate of influx for each individual cell is increased markedly (almost double).

The time at which the increased influx in Fig. 7 occurs is the same time at which the net flux is negative and the cells are losing K^+ (Fig. 6). It must be concluded therefore that the efflux is transiently increased to an even greater extent than the influx, exceeding it for several hours. Thereafter, the efflux falls below the influx with the resulting net accumulation of K^+ in the later stages of the cell division cycle. The differences between the rates of influx and efflux are never very large because, as pointed out previously, the unidirectional fluxes are some 7 to 20 times as large as the net fluxes.

DISCUSSION

During the exponential growth of randomly dividing L5178Y lymphoblasts under the specified conditions of the experiments reported here, the parameters of the population such as total cell volume, K^+ and Na^+ content, follow a single exponential function. Thus for the average cell the volume, K^+ and Na^+ content, are time-invariant. From the data it can also be inferred that the unidirectional fluxes of K^+ and Na^+ are also time-invariant. It can be con-

[3] The total surface of a synchronously growing cell population can be attributed to two independent factors, individual cell growth and cell division. Relative change in the surface of an individual cell (a') can be expressed from the equation of cell volume growth (equation 10) by

$$a'/a_0' = e^{\frac{2}{3}0.693\, t/T_G} \tag{15}$$

where t, T_G, and subscript have the same meaning as was used in the text. Each division into two daughter cells without change in total volume results in the relative change of $2^{-\frac{1}{3}}$ in a single cell surface. Thus relative change in surface of the population due to cell division (a'') is

$$a''/a_0'' = \left(\frac{N}{N_0}\right)^{\frac{1}{3}} \tag{16}$$

Then the change in total surface of population A is the combination of equations (15) and (16)

$$A/A_0 = \left(\frac{N}{N_0}\right)^{\frac{1}{3}} e^{\frac{2}{3}0.693t/T_G} \tag{17}$$

The actual curve of the surface change in the semilog plot can be constructed easily by taking the log of equation (17)

$$\log\frac{A}{A_0} = \frac{1}{3}\log\frac{N}{N_0} + \frac{2}{3}\frac{0.693}{2.3}t/T_G \tag{17'}$$

and using the observed time course of N (cell number) within T_G.

cluded that the average cell is in a steady-state condition, with no external factors modifying its growth. Such behavior has been reported for L cells (17) but is not always fulfilled by other cultured mammalian cell lines such as monolayer cultures of HeLa cells (18). The uptake of labeled K^+ and Na^+ can be fitted by an equation for a closed, two compartment system (cells and medium), which differs from steady-state equations (19) only by the addition of α, the growth coefficient, to the exponential term. Thus the flux data are compatible with a single cellular compartment for the cations. Because the net flux, represented by α is less than 5 % of the unidirectional fluxes, the uptake of labeled K^+ or Na^+ by randomly growing cells cannot be distinguished experimentally from that by nongrowing cells suspended in a defective medium.

In synchronously grown cells both the net and unidirectional fluxes of K^+, and the net flux of Na^+ undergo remarkable changes during a single division cycle, changes that cannot be predicted from data obtained from the average cell in a randomly growing population. At the end of the G_1 period (1.6 hr) of the cell cycle, a net loss of K^+ occurs which lasts about an hour into the S period (8.1 hr). At about the same time a large and rather sudden increase in both influx and efflux occurs. In the latter two-thirds of the S period the net inward flux is relatively rapid and the deficit of K^+ is eliminated. The control of the net flux cannot be attributed specifically to changes in influx or efflux because on the average they differ by less than 5 % and a small shift in either flux can result in striking changes in K^+ content. The content of Na^+ in synchronized cells fluctuates even more than that of K^+. Technically, Na^+ fluxes are more difficult to determine. For this reason and because Na^+ contributes far less than K^+ to volume regulation, no systematic attempt was made to estimate unidirectional Na^+ fluxes. Preliminary observations indicated deviations from simple two-compartment behavior at certain stages of the cell division cycle.

Few data are available in the literature on Na^+ and K^+ fluxes of cultured mammalian cells. The present half-times of exchange for K^+ and Na^+ of randomly grown cells of 33 and 5 min are in reasonable agreement with values of 40 min for K^+ (9) and a fast component of 2 min (10) in Ehrlich ascites cells. The fluxes are considerably larger than those of nongrowing cells such as erythrocytes (35 hr for K^+ (reference 3) and 2.4 hr for Na^+ (reference 4)), and muscle (5.8 hr for K^+ and 42 min for Na^+) (2).

Although the cellular cations constitute a major part of the osmotic content of the cell and are presumably a major factor in determining cell size, the failure of the cation content to follow an exponential increase in individual cells indicates that other factors are involved in volume regulation. Presumably other osmotically active substances compensate for the cation deficit in the early part of the S period.

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