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$^{86}Rb^+$ FLUXES IN CHINESE HAMSTER OVARY CELLS AS A FUNCTION OF MEMBRANE CHOLESTEROL CONTENT

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Summary

Steady-state fluxes of $^{86}Rb^+$ (as a tracer for K^+) were measured in Chinese hamster ovary cells (CHO-K1) and a mutant (CR1) defective in the regulation of cholesterol biosynthesis; the membrane cholesterol content of this mutant was varied by growing it on a range of cholesterol supplements to lipid-free medium (Sinensky, M. (1978) Proc. Natl. Acad. Sci. U.S. 75, 1247—1249).

Analogous to previous findings in ascites tumor cells, $^{86}Rb^+$ influx in the parent strain was differentiated into a ouabain-inhibitable 'pump' flux, furosemide-sensitive, chloride-dependent exchange diffusion, and a residual 'leak' flux.

On the basis of this flux characterization, $^{86}Rb^+$ pump and leak fluxes were measured in the mutant as a function of membrane cholesterol content. Pump and leak fluxes, when expressed per ml cell water, were independent of the cholesterol content of the mutant. Moreover, $^{86}Rb^+$ fluxes in the mutant were equal to those in the parent strain. Our data imply that the flux behavior of K^+ in the steady state is independent of the ordering of membrane lipid acyl chains.

Introduction

Recently one of us [1] has described the isolation and properties of a mutant (CR1) of the Chinese hamster ovary cell (CHO-K1) defective in the regulation of cholesterol biosynthesis. When the mutant was grown on delip-

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Abbreviation: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

idized tissue culture medium supplemented with different cholesterol concentrations, plasma membrane cholesterol and acyl chain order parameter could be caused to vary substantially [2]. The activity of the $(Na^+ + K^+)$-ATPase, measured in a membrane preparation under optimal conditions, decreased tenfold over this range (0.9 to 11 mol percent) of cholesterol content [3]. This is in line with results published for the maximal rate of $(Na^+ + K^+)$-ATPase in a reconstituted system [4,5], and fits into the concept [5] that the activities of membrane-bound enzymes may depend on membrane fluidity. However, data on the maximal rate of $(Na^+ + K^+)$-ATPase are not necessarily relevant for the living cell: in intact cells the $(Na^+ + K^+)$-pump generally functions at submaximal activity [6], maintaining an ionic steady state that is determined by both pump and leak processes.

Therefore, in the present study we set out to investigate how cholesterol content would influence the pump and leak fluxes of K^+ under steady-state conditions; as a tracer for K^+ we used $^{86}Rb^+$, which appears to be a satisfactory substitute in mammalian cells (e.g. [7]). In the first part of this paper we present evidence that, as was shown before for ascites tumor cells [8,9], the influx of $^{86}Rb^+$ in CHO cells could be accounted for by three components: an inwardly directed, ouabain-sensitive pump flux; one-to-one exchange diffusion that was suppressed by furosemide, or when chloride in the medium was replaced by nitrate; and a residual flux, presumably passive leak. Based on these data, conditions for the measurement of $^{86}Rb^+$ pump and leak fluxes in CHO cells were standardized. In the experiments described in the second part of this paper we used these conditions to determine the effect of membrane cholesterol level on those fluxes. It will be shown that, in contrast to the maximal rate of $(Na^+ + K^+)$-ATPase, steady-state pump and leak fluxes of $^{86}Rb^+$ were independent of membrane cholesterol content.

Methods and Materials

Media and cells

Cells were grown in 60 mm Petri dishes for 7 days in tissue culture medium F12 supplemented with 8% whole fetal calf serum, or with 8% delipidized serum supplemented with cholesterol as indicated. At the time of the experiments, cells had reached a titer between $2—5 \cdot 10^5$ cells/plate.

Experimental procedure

For the $^{86}Rb^+$ flux measurements, the culture medium was aspirated and the dishes washed once with 1 ml HEPES-buffered saline (for composition, see below). Then the cells were incubated for 90 min in 1 ml saline to allow the ion content to stabilize.

For the influx measurements, $^{86}Rb^+$ (20 μl, 100 $\mu Ci \cdot ml^{-1}$) was added at zero time after the 90 min incubation. When furosemide- and/or ouabain-inhibited influx was measured, the medium was replaced by 1 ml of saline containing the indicated concentrations of the inhibitors 20 s prior to addition of the label. Samples of the medium (20 μl) were taken for determination of extracellular radioactivity. After the indicated influx times, the medium was aspirated; individual Petri dishes were picked up by forceps and washed

sequentially in three beakers containing ice-cold phosphate-buffered saline. Subsequently, 1 ml 0.1 N NaOH was added, and the dishes were shaken for 30 min at 37°C to digest the cells. From each dish, a 0.5 ml sample was taken for determination of protein content, and a 0.4 ml sample for determination of radioactivity.

For the efflux measurements, $^{86}Rb^+$ (20 μl, 100 μCi · ml^{-1}) was added at the beginning of the 90 min preincubation period. At the end of this period the medium was aspirated and the dishes washed as described above. At zero time, 1 ml unlabeled saline was added, containing, where indicated, 1.2 mM ouabain. After the indicated efflux times the medium was aspirated and the plates processed as described for the influx measurements.

For the determination of 3-*O*-[^{3}H]methyl glucose-accessible space (3-*O*-methyl glucose space), cells were incubated for 60 min in 1 ml NO_3^--saline in the presence of the isotope (50 μl, 100 μCi · ml^{-1}) to allow for equilibration. At the end of this period, 20-μl samples of the supernatant were taken for determination of radioactivity, and the dishes processed as described above for $^{86}Rb^+$ influx and efflux.

All experiments were performed at 37°C, and all samples were analyzed in duplicate. Protein was determined according to Lowry et al. [10]. For measurement of radioactivity, 0.4 ml 0.1 N NaOH was added to the 20 μl supernatant samples. Both these samples and the cell digests were subsequently mixed with 4 ml Budget Solve (RPI) and counted in a Beckman liquid scintillation counter at the appropriate setting for either $^{86}Rb^+$ or $^3H^+$.

Saline solutions and chemicals

Cl^--saline contained (mM): NaCl, 100; KCl, 5; HEPES anion, 50, neutralized with NaOH, 25; $CaCl_2$, 1; $MgSO_4$, 1; glucose, 20. Final pH was 7.5. In NO_3^--saline, NaCl and KCl were replaced by $NaNO_3$ and KNO_3, respectively, and 10 mM sodium phosphate was added. Ouabain was purchased from Sigma. Furosemide was kindly provided by Dr. H.R. Dettelbach from Hoechst. Radiochemicals were obtained from N.E.N.

Calculations

For the calculations it is useful to define a label distribution volume, Q:

$$Q = \frac{cpm_c/0.4}{cpm_s/0.02} \cdot \frac{1}{P/0.5} \text{ (ml} \cdot \text{g}^{-1}\text{)}$$

where cpm_c = radioactivity of 0.4 ml cell digest, cpm_s = radioactivity of 0.02 ml supernatant sample, and P = protein content of 0.5 ml cell sample (in g).

Q has the dimensions of a volume per g protein, and indicates how many ml of supernatant would have to be cleared of label in order to account for the radioactivity associated with 1 g of cell protein.

The absolute value of the K^+ influx per g protein (in μmol $K^+ \cdot g^{-1}$) is obtained by multiplying Q by the extracellular K^+ concentration (5 μmol · ml^{-1}). The 3-*O*-methyl glucose space is equivalent to the distribution volume Q for this compound; as will be argued in this paper, it represents the intracellular water space. The absolute value of the K^+ influx per ml cell water (in μmol K^+ ·

ml^{-1}) thus is obtained by dividing the influx per g protein by the 3-*O*-methyl glucose space.

First-order rate constants for $^{86}Rb^+$ efflux were obtained by linear regression from semilogarithmic plots of cellular $^{86}Rb^+$ content against time.

Results

Characterization of $^{86}Rb^+$ fluxes in CHO-K1 cells, and standardization of flux measurements

As mentioned in the Introduction, in ascites cells $^{86}Rb^+$ flux could be accounted for by three components: a ouabain-sensitive pump flux, an exchange diffusion flux that was inhibited by furosemide or by replacement of Cl^- in the medium by NO_3^-, and a passive leak flux [8,9]. The following experiments were performed to establish whether $^{86}Rb^+$ in CHO-K1 cells exhibited a similar flux pattern.

Fig. 1 shows the equilibration of $^{86}Rb^+$, in both Cl^-- and in NO_3^--containing saline. The initial rate of uptake in Cl^--saline was approximately double that in NO_3^--saline, but after 90 min the curves tended to converge. Uptake over the first 30 min was linear in both salines, and extrapolated to a zero ordinate intercept.

The initial rate of $^{86}Rb^+$ influx in NO_3^--saline was determined as a function of ouabain concentration. At 0.2 mM ouabain, influx was suppressed by 80%;

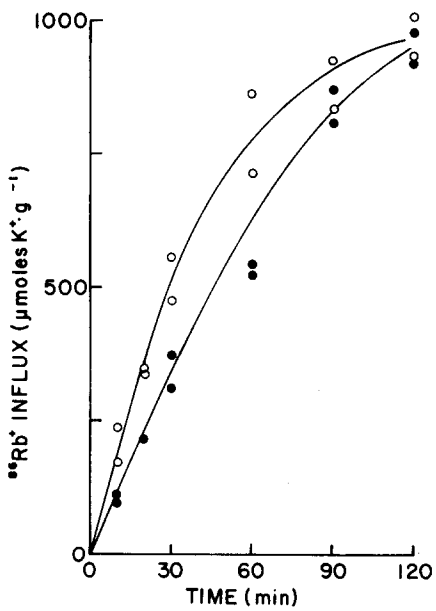

Fig. 1. $^{86}Rb^+$ influx in CHO-K1 cells in Cl^-- and NO_3^--saline. For this experiment and those shown in Fig. 2 and Table I, CHO-K1 cells were grown on F12 supplemented with 8% fetal calf serum. $^{86}Rb^+$ was added immediately after replacement of the culture medium by Cl^-- or NO_3^--saline, and influx determined as described in Methods. Data are expressed on a protein weight basis. o———o, Cl^--saline; •———•, NO_3^--saline.

TABLE I

EFFECT OF INHIBITORS AND NO_3^- ON $^{86}Rb^+$ FLUXES IN CHO-K1

Rate of influx is expressed on a protein weight basis. It was obtained from time points 10 and 20 min after addition of $^{86}Rb^+$; first-order rate constants for the efflux were calculated from duplicate determinations of cellular $^{86}Rb^+$ content at zero time and 40 min after replacing the medium by unlabelled saline. Numbers between brackets give the number of data points used for the calculation. Values are given plus or minus one standard deviation, except for duplicates, where values represent the average plus or minus half the difference.

	Rate of influx (μmol $K^+ \cdot g^{-1} \cdot min^{-1}$)		Efflux rate constant, k_e (min^{-1})	
Major anion:	Cl^-	NO_3^-	Cl^-	NO_3^-
Control	18.8 ± 1.8 (4)	11.8 ± 1.5 (4)	0.016 ± 0.001 (2)	0.009 ± 0.004 (2)
+ ouabain (1.2 mM)	9.1 ± 0.5 (3)	1.6 ± 0.3 (2)	0.014 ± 0.003 (2)	0.008 ± 0.004 (2)
+ ouabain (1.2 mM) and + furosemide (1.2 mM)	1.3 ± 0.3 (2)	1.5 ± 0.1 (2)	—	—

above 1 mM ouabain, uptake levelled off at approximately 10% of the control value (data not shown).

Table I summarizes the effects of different incubation conditions on the rates of $^{86}Rb^+$ influx and -efflux. As in Fig. 1, influx in the absence of ouabain was approximately two times faster in Cl^--saline than in NO_3^--saline. In Cl^--saline, approximately 50% of $^{86}Rb^+$ influx was suppressed by 1.2 mM ouabain. In contrast, as mentioned above, this concentration of ouabain reduced influx in NO_3^--saline by nearly 90%. Furosemide reduced the rate of influx in Cl^--saline in the presence of ouabain to that found in NO_3^--saline; furosemide had no further effect in NO_3^--saline. The rate of efflux of $^{86}Rb^+$ was higher in Cl^--saline than in NO_3^--saline. However, neither in Cl^--saline nor in NO_3^--saline was the efflux rate significantly affected by ouabain. We have not determined the intracellular K^+ concentration in our cells; but it can be estimated from the distribution of $^{86}Rb^+$ after 90 min (which should be close to steady state) as approximately 1 mmol $K^+ \cdot g^{-1}$ (Fig. 1). From Table I it can then be calculated that, when Cl^- in the medium was replaced by NO_3^-, both in the absence and presence of ouabain influx and efflux were suppressed by approximately the same amount (7 μmol $K^+ \cdot g^{-1} \cdot min^{-1}$).

From the results shown in Fig. 1 and Table I it can be inferred that, as in ascites cells [8], the movement of $^{86}Rb^+$ in CHO cells is not restricted to a ouabain-inhibitable pump and passive leak fluxes: an additional, furosemide-inhibitable component is found in Cl^--saline. This component, analogous to that in ascites cells, presumably represents one-to-one K^+ exchange diffusion.

It might be mentioned here that efflux measurements generally gave rather scattered results, possibly because the cells were traumatized by the washing procedure. Protein contents per 0.5 ml cell sample (see Methods) varied widely between 0.02 and 0.2 mg, with the lower values found predominantly in the efflux experiments. It is possible that the cells started to detach from the Petri dishes during the flux measurements, especially after the washing procedure in ice-cold phosphate-buffered saline.

Determination of intracellular water

3-*O*-Methyl glucose is passively distributed in human diploid fibroblasts, and has been used as a marker for the water space in those cells [11]. We measured the uptake of 3-*O*-[^{3}H]methyl glucose as a function of time in CHO-K1 cells. When the data points were fitted by a first-order exponential curve, regression analysis showed the distribution at 60 min (the incubation time subsequently used) to be within 20% of the equilibrium value (Fig. 2). The fact that the uptake curves in Fig. 1 extrapolated to a zero-ordinate intercept indicates that the washing procedure was quite effective in removing the extracellular medium. Thus it seems justified to identify the 3-*O*-methyl glucose space with the intracellular water space.

$^{86}Rb^+$ pump- and leak fluxes as a function of membrane cholesterol content

CR1 cells were grown in medium supplemented with delipidized serum and cholesterol ranging from 0 to 10 $\mu g \cdot ml^{-1}$. As a result, plasma membrane cholesterol content increased from 0.9 to 10.8 mol percent, and the order parameter (calculated from the ESR spectrum of the spin probe 5-nitroxy stearic acid) from 0.663 to 0.697 [2]. Table II summarizes those data, and the values for $^{86}Rb^+$ fluxes as a function of cholesterol content. On the basis of the data presented above, we performed the flux measurements in NO_3^--saline in

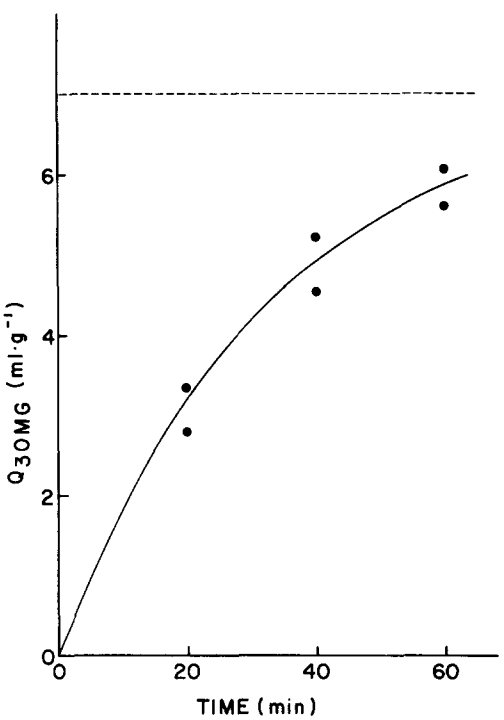

Fig. 2. Uptake of 3-*O*-[^{3}H]methyl glucose (30MG) in CHO-K1 cells. Uptake was determined and expressed as described in Methods. Data points are fitted by a first-order exponential curve of the form $Q_t = Q_\infty (1 - e^{-kt})$, in which Q_t and Q_∞ are the distribution spaces at times t and infinite, respectively. Q_∞ (dotted line) = 7.0 ml · g^{-1}; k = 0.030 min^{-1}; correlation coefficient r = 0.98).

TABLE II

$^{86}Rb^+$ FLUXES IN CR1 AS A FUNCTION OF MEMBRANE CHOLESTEROL CONTENT

The experiment was performed in NO_3^--saline, as described in Methods. Rate of influx is expressed either on a protein weight basis (g^{-1}) or on a cell water basis (ml^{-1}). It was determined from time points 10 and 20 min after addition of $^{86}Rb^+$. k_e was obtained by linear regression from a semilogarithmic plot of cellular $^{86}Rb^+$ content against time (time points: 20, 40, 60 min). Cell water was determined as 3-*O*-methyl glucose space after 60 min incubation. Ouabain concentration was 1.2 mM. Data are presented as in Table I.

Cholesterol suplement ($\mu g \cdot ml^{-1}$)	0	2.5	5	7.5	10
Membrane cholesterol content * (μmol cholesterol/ μmol PL)	0.009	0.047	0.073	0.091	0.108
Order parameter *	0.663	0.672	0.683	0.688	0.697
Cell H_2O ($ml \cdot g^{-1}$)	—	7.3 ± 0.3 (2)	7.8 ± 0.1 (2)	8.8 ± 0.2 (2)	7.2 ± 0.4 (2)
Rate of influx **					
Control (g^{-1})	12.0 ± 1.2 (4)	12.9 ± 0.9 (4)	11.2 ± 0.5 (4)	14.1 ± 1.2 (4)	11.9 ± 0.9 (4)
(ml^{-1})	—	1.75 ± 0.15	1.45 ± 0.05	1.60 ± 0.10	1.65 ± 0.15
+Ouabain (g^{-1})	0.90 ± 0.10 (2)	1.10 ± 0.10 (2)	1.25 ± 0.05 (2)	1.30 ± 0.05 (2)	1.10 ± 0.20 (2)
(ml^{-1})	—	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.16 ± 0.03
K^+ pump rate **					
(g^{-1})	11.1 ± 1.2	11.8 ± 0.9	10.0 ± 0.5	12.8 ± 1.2	10.8 ± 0.9
(ml^{-1})	—	1.60 ± 0.15	1.30 ± 0.05	1.45 ± 0.10	1.50 ± 0.15
k_e (min^{-1})	0.0087 ($r = 0.95$)	0.0093 ($r = 0.82$)	0.0098 ($r = 0.97$)	—	0.0087 ($r = 0.97$)

* See Ref. 2.

** μmol $K^+ \cdot min^{-1}$.

TABLE III

A COMPARISON OF $^{86}Rb^+$ FLUXES IN CHO-K1 AND CR1

Both CHO-K1 and CR1 were grown on medium F12 supplemented with 8% delipidized fetal calf serum and 10 μg/ml cholesterol. The experiment was performed in NO_3^--saline, and cell H_2O and rates were determined as indicated in the legend to Table II. Data are presented as in Table I.

Cellular cholesterol content (mg/g cell protein)	13.5	40.0
Cell H_2O ($ml \cdot g^{-1}$)	6.9 ± 0.1 (2)	7.9 ± 0.4 (3)
Rate of influx		
Control		
($\mu mol\ K^+ \cdot g^{-1} \cdot min^{-1}$)	10.8 ± 0.2 (4)	12.7 ± 0.7 (4)
($\mu mol\ K^+ \cdot ml^{-1} \cdot min^{-1}$)	1.55 ± 0.05	1.60 ± 0.10
+Ouabain, 1.2 mM		
($\mu mol\ K^+ \cdot g^{-1} \cdot min^{-1}$)	0.95 ± 0.05 (2)	1.25 ± 0.05 (2)
($\mu mol\ K^+ \cdot ml^{-1} \cdot min^{-1}$)	0.14 ± 0.005	0.16 ± 0.01
K^+ pump rate		
($\mu mol\ K^+ \cdot g^{-1} \cdot min^{-1}$)	9.8 ± 0.2	11.4 ± 0.7
($\mu mol\ K^+ \cdot ml^{-1} \cdot min^{-1}$)	1.41 ± 0.05	1.44 ± 0.10

order to abolish the exchange component; we differentiated the remaining influx into a 'pump' and a 'leak' contribution by the action of 1.2 mM ouabain. When the results were expressed on a protein basis, both in the absence and presence of ouabain there were significant differences in influx rates (without apparent correlation to cholesterol content). For instance, mean values for 5 and 7.5 $\mu g \cdot ml^{-1}$ cholesterol were 25% apart. However, cellular water content, expressed on a protein basis, varied in a parallel way, so that values for $^{86}Rb^+$ influx when expressed per ml cell water were essentially identical for all cholesterol concentrations tested. Also the rate constants for $^{86}Rb^+$ efflux appeared to be independent of membrane cholesterol content.

Table III compares $^{86}Rb^+$ pump and leak fluxes in CHO-K1 cells (grown in medium F12 supplemented with 8% fetal calf serum) and in CR1 cells (grown on 10 $\mu g \cdot ml^{-1}$ cholesterol). Again, fluxes expressed on a protein basis were significantly different in the two cell types, but when expressed per ml cell water they were equal within the experimental error.

The results of Tables II and III can be summarized as follows: For all cultures, cell water ranged between 6.9 and 8.8 $\mu l \cdot mg^{-1}$ (without apparent correlation to cholesterol content). Expressed per ml cell water, the rate of K^+ influx was 1.60 ± 0.04 (S.D. of means) $\mu mol\ K^+ \cdot ml^{-1} \cdot min^{-1}$. This value could be differentiated into 'pump' and 'leak' fluxes of 1.45 ± 0.04 (S.D. of means) and 0.153 ± 0.003 (S.D. of means) $\mu mol\ K^+ \cdot ml^{-1} \cdot min^{-1}$, respectively.

Discussion

$^{86}Rb^+$ flux components in CHO cells

In this paper we set out to investigate the effect of membrane cholesterol content on the steady-state behavior of K^+ in CHO cells; as a tracer for K^+ we used $^{86}Rb^+$ [7]. The ionic steady state is determined by the interplay of pump and passive leak processes. However, in addition to pump and leak fluxes, plasma membranes from a variety of sources under certain conditions appear to

mediate exchange diffusion [9]. In the first part of this communication we present experiments indicating that CHO cells, like ascites cells [8], mediate a Cl^--dependent and furosemide-sensitive $^{86}Rb^+$ flux component. That this component, analogous to that in ascites cells, represents one-to-one exchange diffusion is supported by the following lines of evidence: (i) influx and efflux were suppressed by approximately the same amount when Cl^- in the medium was replaced by NO_3^- (text to Table I); (ii) the uptake curves for Cl^--saline and NO_3^--saline converge towards the same steady-state $^{86}Rb^+$ distribution (Fig. 1). This corroborates the assumption that NO_3^- suppressed a flux component which did not contribute to steady-state ionic balance.

For the remainder of this paper we have assumed that in CHO cells, as in ascites cells [8,9], $^{86}Rb^+$ influx in NO_3^--saline represents the sum of a ouabain-inhibitable K^+ pump flux and a residual K^+ passive leak flux. This assumption needs some comments: (i) the ouabain-insensitive influx (as well as the presumably passive efflux) in NO_3^--saline may still contain other, as yet unidentified flux components; (ii) for the CHO system we have not rigorously established to which extent net K^+ fluxes are represented by unidirectional $^{86}Rb^+$ fluxes. Keeping this in mind, our characterization of $^{86}Rb^+$ fluxes in CHO cells allows us to draw some tentative conclusions concerning the energetics of the ionic steady state in those cells. In NO_3^--saline, ouabain had no significant effect on the rate of $^{86}Rb^+$ efflux (Table I). This indicates that the membrane potential was not significantly affected by blocking the $(Na^+ + K^+)$-pump. However, the efflux data were too scattered to exclude a small electrogenic pump contribution.

Furthermore, the membrane potential in CHO cells may be estimated from the Ussing flux ratio ([16]; in this case, the ratio of $^{86}Rb^+$ efflux and ouabain-resistant $^{86}Rb^+$ influx in NO_3^--saline):

$$V = \frac{RT}{F} \ln \frac{\text{efflux}/[K^+]_i}{\text{influx}/[K^+]_0}; \quad \text{and, since } (\text{efflux}/[K^+]_i) = k_e,$$

$$V = 26.6 \ln(0.009/0.031) = -33 \text{ mV}.$$

As mentioned, the internal K^+ concentration should be close to 1 mmol $\cdot g^{-1}$, or approximately 150 mM. These values for both the steady-state membrane potential and the intracellular K^+ concentration are comparable to those estimated for ascites cells [9]. The absolute magnitude of the $^{86}Rb^+$ pump and leak fluxes in CHO cells, expressed on a volume basis, was approximately twice that found in ascites cells under similar conditions ([8], Table II, assuming a cell water : dry weight ratio of 4). In view of the fact that the surface/volume ratio is probably larger in cells growing in a monolayer, it may be inferred that also the pump and leak activity per membrane area is quantitatively comparable for the two cell types.

Finally it might be pointed out that the driving forces for ion fluxes are directly related to ion concentrations rather than to the absolute amount of ions per cell. In comparing different preparations ion fluxes should therefore be related to cell water space rather than to cell protein content. The water-to-protein ratio in CHO-K1 and CR1 cells varied without apparent correlation to cholesterol content; this is probably due to the fact that protein content may

vary with the condition and state of growth of the cells. However, when expressed on a volume basis, K^+ pump and leak rates were equal for all cell cultures tested (Tables II and III).

Effect of membrane cholesterol on $^{86}Rb^+$ pump- and leak fluxes

The lack of effect of cholesterol content on the steady-state rate of K^+ pumping is in contrast to the finding [3] that the maximal turnover of the $(Na^+ + K^+)$-ATPase, measured in a membrane preparation, decreased with a factor 10 between the lowest and the highest cholesterol level tested. An explanation of this discrepancy should accomodate two additional facts: (i) in the steady state, the $(Na^+ + K^+)$-ATPase generally functions at submaximal activity [6], and (ii) cholesterol did not affect the steady-state K^+ leak fluxes (Tables II and III). Effectively, then, in the steady state the pump appeared to adjust its activity to the leak, or, more precisely: with increasing cholesterol content, the cells appeared to mobilize an increasing proportion of the decreasing total pump capacity in order to adjust to a fixed rate of leak. In view of the factor 10 mentioned above, we must then conclude that at the lowest cholesterol content tested maximally 10% of the total pump capacity was expressed in the steady state: this number should be compared to approximately 30% for red blood cells under standard conditions [6].

One of the factors controlling pump mobilization may be the internal Na^+ concentration [6]: CR1 cultures grown on different cholesterol supplements may end up with different steady-state Na^+ content. Also, as shown for red blood cells [13], cholesterol could have a dual effect: while decreasing the maximal turnover of the $(Na^+ + K^+)$-pump, it might increase the apparent affinity of this enzyme for internal Na^+.

In line with our results, in red blood cells the maximal turnover of the $(Na^+ + K^+)$-ATPase was shown to decrease with cholesterol content [13], whereas the steady-state pump activity appears to be much less affected, or completely unaffected [14,15]. Also the maximal rate of $(Na^+ + K^+)$-ATPase in a reconstituted system was suppressed by cholesterol [4,5]. In contrast, Chen et al. [12] recently reported that the maximal rate of $(Na^+ + K^+)$-ATPase in a membrane preparation from L cells was independent of membrane sterol content, whereas the steady-state rates of K^+ pumping and leakage were both increased by sterol depletion. However, these results are most readily explained by assuming that the depletion procedure used (incubation with oxygenated sterols) increased K^+ permeability (and may have affected overall membrane integrity) without changing the direct environment of the $(Na^+ + K^+)$-ATPase; thus, data obtained by this method of sterol depletion may not be representative for the effects of membrane sterol content.

It should be stressed that the steady-state flux parameters derived here apply to the particular incubation conditions chosen: both membrane potential and K^+ fluxes are expected to be different during active growth [11,17], and in consecutive phases of the cell cycle [18]. However, our results are inconsistent with a model [12] in which membrane sterol content regulates the initiation and progress of the cell cycle by its effects on active K^+ transport.

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References

1 Sinensky, M. (1977) Biochem. Biophys. Res. Commun. 78, 863—867
2 Sinensky, M. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 1247—1249
3 Sinensky, M., Pinkerton, F., Sutherland, E. and Simon, F. (1979) Proc. Natl. Acad. Sci. U.S., in the press
4 Kimelberg, H.K. (1975) Biochim. Biophys. Acta 413, 143—156
5 Kimelberg, H.K. and Papahadjopoulos, D. (1974) J. Biol. Chem. 249, 1071—1080
6 Whittam, R. and Ager, M.E. (1965) Biochem. J. 97, 214—227
7 Mills, B. and Tupper, J.T. (1975) J. Membrane Biol. 20, 75—97
8 Bakker-Grunwald, T. (1978) Biochim. Biophys. Acta 513, 292—295
9 Tupper, J.T. (1975) Biochim. Biophys. Acta 394, 586—596
10 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
11 Villereal, M.L. and Cook, J.S. (1977) J. Supramol. Struct. 6, 179—189
12 Chen, H.W., Heiniger, H.-J. and Kandutsch, A.A. (1978) J. Biol. Chem. 253, 3180—3185
13 Claret, M., Garay, R. and Giraud, F. (1978) J. Physiol. 274, 247—263
14 Cooper, R.A., Arner, E.C., Wiley, J.S. and Shattil, S.J. (1975) J. Clin. Invest. 55, 115—126
15 Owen, J.S. and McIntyre, N. (1978) Biochim. Biophys. Acta 510, 168—176
16 Ussing, H.H. (1949) Acta Physiol. Scand. 19, 43—56
17 Villereal, M.L. and Cook, J.S. (1978) J. Biol. Chem. 253, 8257—8262
18 Mills, B. and Tupper, J.T. (1976) J. Cell. Physiol. 89, 123—132