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CULTURED CELLS FROM RENAL CORTEX OF HIBERNATORS AND NONHIBERNATORS

REGULATION OF CELL K^+ AT LOW TEMPERATURE

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SUMMARY

Cells were grown as primary monolayer cultures from kidney cortex of guinea pigs (nonhibernators), hamsters and ground squirrels (both hibernating species). When plates of cells were placed at 5 °C, cells of guinea pigs lost 37 % of their K^+ in 2 h and those of the hibernator lost about 10 %.

Uptake of ^{42}K into the cells exhibited a simple, single exponential time course at both temperatures. Unidirectional efflux of K^+ was equal to K^+ influx in all cultures at 37 °C and, within limits of error, in hibernator cells at 5 °C. Efflux was 3- to 5-fold greater than influx in guinea pig cells at 5 °C.

After 2 h in the cold the ouabain-sensitive K^+ influx remaining (7–15 % of that at 37 °C) was about the same in the cells of the 3 species. Cells from active hamsters and from hibernating ground squirrels, however, exhibited significantly greater pump activity after 45 min in the cold (19 and 14 %, respectively). The stimulation of K^+ influx by increasing $[K^+]_o$ did not show an increase in $K_m{}^+$ at 5 °C in cells of guinea pigs and ground squirrels. Lowering $[K^+]_c$ and/or raising $[Na^+]_c$ by treatment in low- and high-K^+ media caused only slight stimulation of K^+ influx, except in cells of ground squirrels at 5 °C in which the stimulation was at least 11-times greater than at 37 °C or in cells of guinea pigs at either temperature.

This altered kinetic response of K^+ transport to cytoplasmic ion stimulation with cooling accounted for about one-third of the improved regulation of K^+ at 5 °C in ground squirrel cells; the other two-thirds was attributable to a greater decrease in K^+ leak with cooling. The inhibition of active transport by cold in all 3 species was much less severe than that previously seen in any ($Na^+ + K^+$)-ATPase of mammalian cells.

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INTRODUCTION

The purpose of using primary cultures of renal cells to investigate K^+ retention and K^+ fluxes at low temperature is 3-fold.

Ever since the first demonstrations that nerves and hearts of hibernators continued to function at a low temperature even when excised from the organism, it has been recognized that a logical extension was to see if this capacity is uniquely retained by cells of hibernators after a longer sojourn, and several cell generations, in vitro [1]. Until now this question has been examined only by using the beating of cultured cardiac cells as a criterion of continued function and with this technique the expectation has failed to be confirmed [1–3]. K^+ retention at low temperature, which has been shown to be superior in a variety of tissues of hibernators prepared in various ways, would provide a more quantifiable criterion [4–6].

The second purpose, assuming persistence of adaptation in culture, is to use the cultured cells as a model for studying the effect of cold on active cation transport and passive permeability. With respect to the cold adaptation of hibernators, it has already been shown in red blood cells that greater retention of K^+ at low temperature depends upon less sensitivity of the Na^+/K^+ pump and greater sensitivity of passive permeability to cooling [5]. While there is some evidence that a similar statement could be made for kidney cells [4], the unsuitability of kidney slices for isotopic flux measurements has not permitted a detailed analysis of these issues. The features of kidney slices that complicate the interpretation of fluxes are complexity of structure and cell heterogeneity, and cultured cells may offer some relief from these limitations.

This possibility has a significance beyond the restricted problem of adaptation in hibernators, because the effect of cooling on the physical state of the membrane and on the correlated activity of membrane-bound enzymes in eukaryote cells has become an issue of wide interest [7–11]. Curiously, such studies have neglected the the effect of cold on Na^+/K^+ transport even while its inhibition of $(Na^+ + K^+)$-ATPase has been a central issue [10, 12, 13]. In this field the cells of hibernators could offer a useful contrast or control.

Although erythrocytes would perhaps be more appropriate for a deeper dissection of the effects of cold on kinetic features of the transport mechanism (e.g., partial fluxes), kidney cells would not only allow comparison in a synthetically active type of cell, but might also permit exploration of longer term cellular control and alteration of membrane activities in response to environmental challenge.

A final reason for using primary cultures is to contribute to the establishment of this technique as a way of studying transport properties of renal cells in isolation.

METHODS AND MATERIALS

Preparations of cultures

As donors of kidneys for preparing cultures, individuals were used from 2 species which hibernate, Syrian hamsters (*Mesocricetus auratus*) and 13-lined ground squirrels (*Citellus tridecemlineatus*), and from 1 species which does not, guinea pig. Both active and hibernating ground squirrels and hamsters were used. The active individuals were used at any time of the year directly out of the animal room. The hibernating individuals were allowed to cycle into and out of a bout of hibernation

at least twice before they were used, and they were killed while in a bout with a cheek pouch temperature of, at most, 7 °C.

The animal was killed with an overdose of Diabutal and the kidneys removed as rapidly as possible and placed in culture medium. The capsules were removed as aseptically as possible and the kidneys placed in a culture dish with fresh medium. While the kidney was held by the hilus with sterile forceps, strips of cortex were cut away with fine scissors. Care was taken not to include any medulla. The cortical strips were washed 4 times in sterile culture medium.

To disaggregate the cells in the cortical strips, the strips were first placed in a sterile 5 ml syringe and expelled into a second such syringe with an 18 gauge needle attached to it. The tissue mass was then expelled into a bottle containing 0.25 % trypsin in culture medium (hog trypsin, Nutritional Biochemicals) and incubated at 37 °C for about 40 min. The cells were then centrifuged at about 450 × *g* for 10 min and then added to 100 ml culture medium and stirred with a sterile magnetic stirrer. 2 ml of this uniform suspension were then pipetted into 35 mm Falcon plastic tissue culture dishes. After 24 h the medium was changed. This change of medium washed away clumped material not adhering to the dish.

Incubation conditions and growth properties

The culture medium was Dulbecco's modified Eagle's Medium with 100 ml of calf serum (Grand Island Biological Co.), 50 000 units of penicillin and 200 mg of streptomycin added per liter.

The cells were cultured for 3 days in an incubator at 37 °C in an atmosphere of air and 5 % CO_2 and at a pH of 7.4.

Although the cell population consisted of a mixture of types, in general the

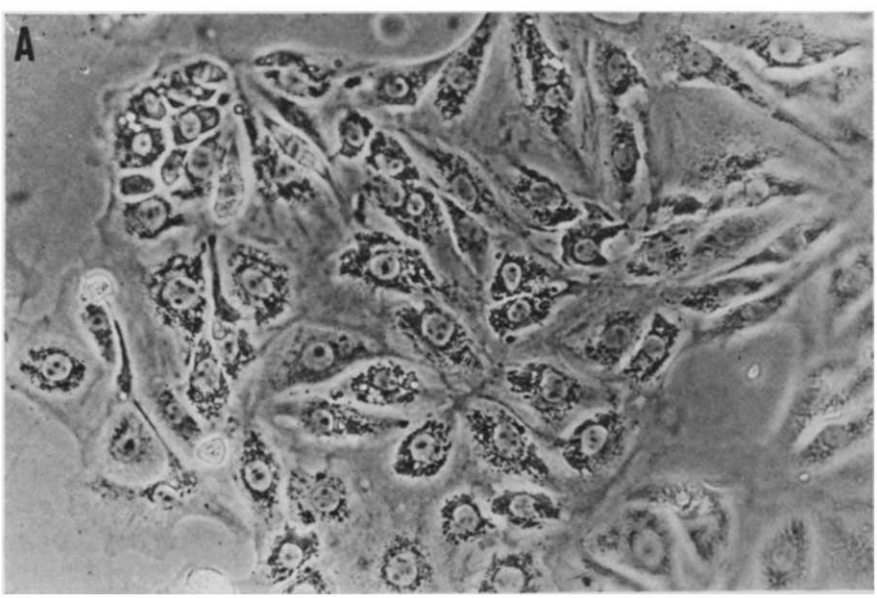

Fig. 1A. For legend see opposite page.

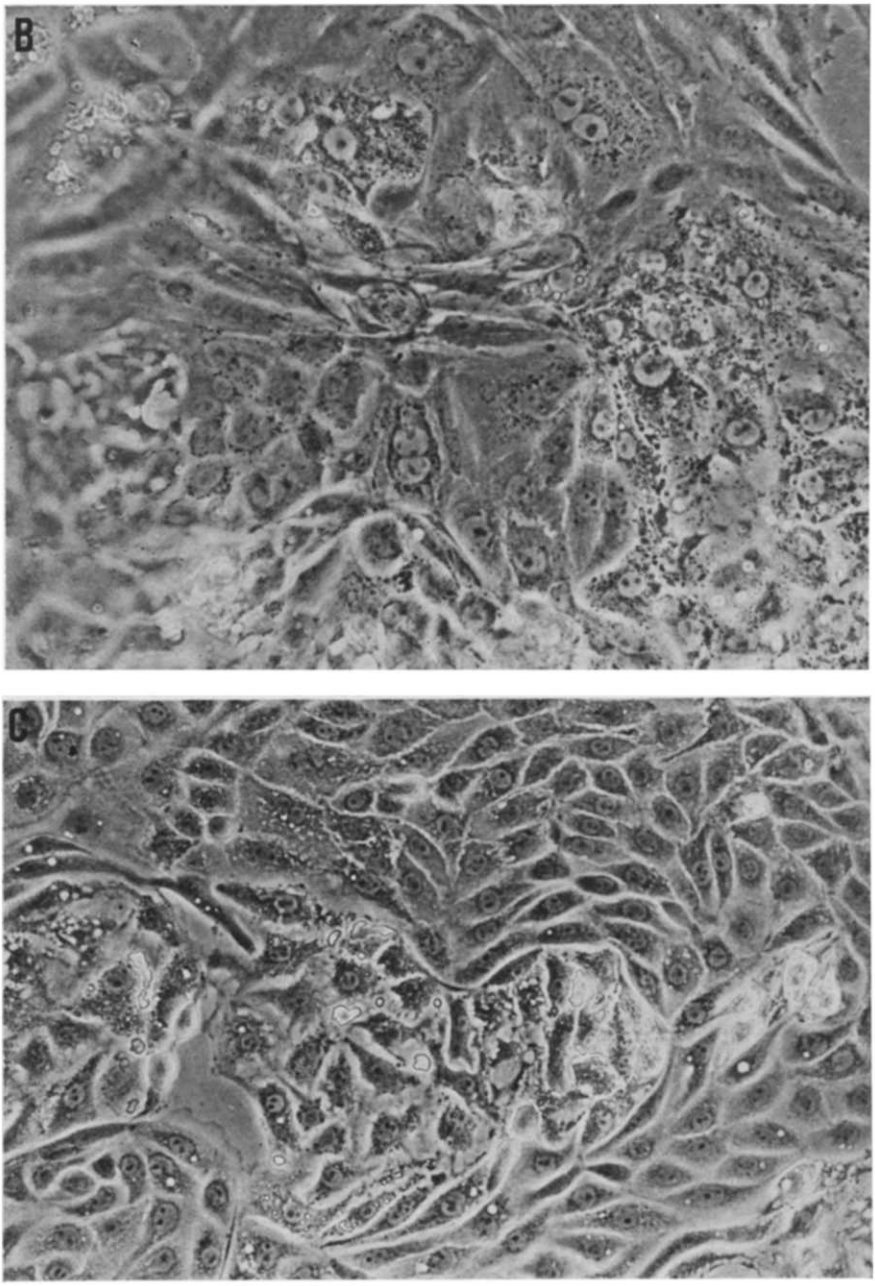

Fig. 1. Cells of kidney cortex on third day after explant. A. Guinea pig. B. Hamster C. Ground squirrel. Magnification approx. 2300 X.

cells from the 3 species were quite similar morphologically. They grew as monolayers with an epitheloid rather than a fibroblastic appearance (Fig. 1). They form small groups of cells, which as they enlarge coalesce to form large patches of cells (Fig. 1). In 3 days these patches had not yet coalesced to fill the plate, as they would have done if allowed to grow for a longer period.

Measurement of unidirectional fluxes and cell content of K^+ and Na^+

For determination of ^{42}K uptake at 5 °C the plates of cells were first transferred to a low temperature CO_2 incubator. About 45 min was required for the temperature of the medium to approach within 1 °C of that of the atmosphere (Fig. 2). At selected times after the plates had been placed in the cold (usually 45, 120 and 240 min), 0.2 ml isotonic saline solution containing ^{42}K (5 mM, about 1 μC) was injected into the culture medium and the plates were returned to the incubator. (The same procedure was used for plates at 37 °C, except, of course, no equilibration period was necessary). After an appropriate interval (for unidirectional fluxes, 10 min at 37 °C and 15 min at 5 °C) the plates were removed from the incubator, washed 3 times in ice-cold, K^+-free saline and drained. The cells were then extracted in 2 ml 10 % trichloroacetic acid. This extract was then placed in counting vials and their radioactivity measured by Cerenkov radiation in a Nuclear Chicago Mark II Liquid Scintillation Counter.

For measurement of efflux of K^+, cells were first equilibrated with the ^{42}K for 3 h before being transferred to the low temperature incubator. To determine efflux 1 pair of plates was washed 3 times in medium at the same temperature, the medium was replaced and the plates returned to the incubator. After the requisite period the plates were removed, washed 3 times in ice-cold, K^+-free medium, extracted and counted (providing "a_{cell_t}" in Eqn. 4). Another pair of plates was washed directly in ice-cold, K^+-free medium, providing a value of cell activity before efflux had occurred ("a_{cell_o}" in Eqn. 4).

Total cell K^+ was usually measured in the same cells as those in which isotopic labelling was measured by waiting for sufficient decay of radioactivity and diluting the sample in the counting vial, adding lithium as an internal standard and determining K^+ emission in a Baird flame photometer. When Na^+ was to be determined the final wash solution was made isotonic with sorbitol instead of with NaCl.

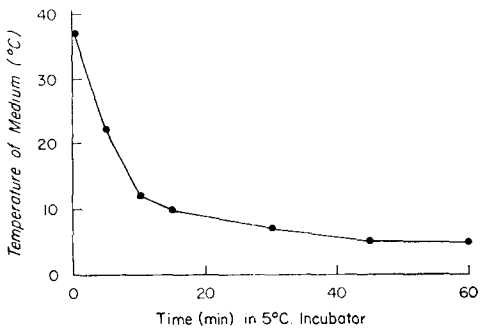

Fig. 2. Time course of temperature change in culture medium after transfer of culture plate to low temperature incubator set at 5 °C. Plate was 35 mm in diameter and contained 2 ml medium, the same conditions as used for plates with cells throughout.

Computation of fluxes

From the radioactivity accumulated during the measured time interval at 37 °C or 5 °C influx of K^+ was calculated using Eqn. 1 which applies to steady-state conditions:

$$M_i = \frac{d(^{42}K)/dt}{S_{med}} \tag{1}$$

Where: M_i = influx of K^+ (mol/min-plate); $d(^{42}K)/dt$ = change of cpm/min-plate; S_{med} = Specific activity of medium (cpm/mol K^+).

Since there was only a small (less than 3 %) change in cell K^+ content during the course of the exposure to isotope, this equation was also used to compute fluxes even in cases that were not strictly steady-state.

In some cases in this study, however, most notably in the experiments in which cell K^+ and Na^+ was varied, a state of rapidly changing cell K^+ concentration existed during the course of the measurement of ^{42}K uptake, and the use of Eqn. 1 was not appropriate. In such cases the following equation was used [14, 15].

$$^{i}M_K = \frac{Q \cdot dS_c/dt}{S_m - S_c} \tag{2}$$

where Q = quantity of K^+ in cells (μmol/plate); S_c = specific activity of cell and S_m = specific activity of medium (cpm/μmol); and dS_c/dt = rate of change of specific activity.

Rates of K^+ efflux were calculated using the following equations:

$$M_o = \frac{d(^{42}K)/dt}{S_{cell}} \tag{3}$$

$$d(^{42}K)/dt = a_{cell_o} - a_{cell_t} \tag{4}$$

Where: M_o = efflux of K^+ (μmol/min-plate); S_{cell} = specific activity of cells (cpm/μmol K^+) at $t = 0$; $d(^{42}K)/dt$ = change in radioactivity on plates (cpm/min); a_{cell_o} = activity of plates before efflux determination; a_{cell_t} = activity of plates after "t" min washout.

Rate coefficients (k_e) for efflux were calculated by assuming that the efflux of K^+ is described by first order kinetics and thus is proportional to cell K^+ concentration $(K^+)_{cell}$:

$$M_o = k_e(K^+)_{cell} \tag{5}$$

Ouabain treatment

In order to subdivide the total K^+ influx into ouabain-sensitive and -insensitive components, influxes were also determined on cells pretreated with ouabain. Cells of hamster, a species with very low sensitivity to ouabain, were preincubated with 3 mM ouabain for 45 min at 37 °C before measurement of fluxes at 37 °C or before transfer to the low temperature incubator. Cells of guinea pigs and ground squirrels, which have greater sensitivity, were preincubated for 10–15 min at 37 °C in 160 μM ouabain before any other treatment (i.e., change of medium, cooling, or introduction of isotope into medium.) When the experimental protocol called for changes of medium the subsequent media for ouabain-treated cells all contained ouabain.

Altering extracellular K^+

The $[K^+]_o$ (extracellular K^+) was altered by making experimental media with K^+ concentrations varying between 1.8 and 22 mM. Other constituents were 1.25 mM $CaCl_2$, 12.5 mM glucose, 44.4 mM $NaHCO_3$, 0.9 mM NaH_2PO_4 and calf serum (10 % v/v). The NaCl concentration varied between 80 and 100 mM, depending inversely upon the K^+ concentration. Since a small and potentially variable amount of K^+ was contained in the calf serum, the K^+ concentration of the final medium was verified by flame photometry.

K^+ influx into kidney cells of guinea pigs and ground squirrels was measured in these media at 37 °C and 5 °C after the cells had been in the low temperature incubator in their growth medium for at least 2 h. The uptake of ^{42}K was initiated in the experimental medium with altered $[K^+]_o$ by removing the growth medium and replacing it with the experimental medium already containing the radioactive K^+ and prewarmed to 37 °C or precooled to 5 °C.

K^+ influx was expressed in terms of cell protein, measured directly on the plates used for influx after extraction by trichloroacetic acid by the method of Lowry et al. [16].

Altering intracellular K^+ and Na^+ concentrations

In order to alter $[K^+]_c$ and $[Na^+]_c$ (cellular or cytoplasmic K^+ and Na^+ concentrations), cells of kidneys of guinea pigs and ground squirrels were incubated for 10–45 min in experimental media with either low or high K^+ concentrations. The high K^+ medium contained 100 mM NaCl and 40 mM K^+ while the K^+-free medium contained 100 mM NaCl and 40 mM tetramethylammonium chloride. The other constituents were the same for both and were, 1.25 mM $CaCl_2$, 1.0 mM $MgSO_4$, 5.0 mM Na_2HPO_4, 0.85 mM NaH_2PO_4, 10 mM glucose.

Cells whose K^+ influx was to be determined at 5 °C were incubated for an additional hour in the experimental medium while they were in the low temperature incubator and cooling. The uptake of K^+ in 6 mM K^+ was initiated at 37 °C and 5 °C by replacing the experimental medium with prewarmed or precooled growth medium containing ^{42}K.

Expression of results

In this report fluxes are expressed in relative terms in one of two ways, either the ratio of a flux at 5 °C relative to the comparable flux at 37 °C (for example, ${}_5{}^iM_K{}^P/{}_{37}{}^iM_K{}^P$, ouabain-sensitive flux at 5 °C relative to ouabain-sensitive flux at 37 °C) or the influx under any condition as a fraction of the total K^+ influx at 37 °C (for example, ${}_5{}^iM_K{}^P/{}_{37}{}^iM_K$, ouabain-sensitive influx at 5 °C as relative to total influx at 37 °C). Either fraction is often stated as a per cent in the text. The cell K^+ concentrations shown in Fig. 3 and Table 2 were also computed as relative to that at 37 °C in untreated cells. In all cases these relative values were obtained by comparing the amount of K^+ or ^{42}K of sister plates from the same culture. Such treatment rests upon the assumption that the cell compartment of K^+ exhibits a small variation amongst the plates of a single culture. This was found to be true with respect to duplicate or triplicate samples in each experiment. In a trial determination of cell K^+ on 30 plates of a single culture from a guinea pig the standard deviation was 7 % of the mean value, and the greatest difference from the mean that could be generated by selection of extreme high or low values was 10 % for duplicates and 4 % for triplicates.

RESULTS

Retention of intracellular potassium

The simplest and most direct test of whether ion regulation is being maintained by cells at low temperature is to measure cell K^+ contents after a period in the cold. Primary cultures of cells of kidney cortex were exposed to 5 °C for periods up to 4 h, and samples were taken at several intervals for determination of K^+ content (Fig. 3).

After 45 min of cooling, by which time the temperature of the medium had approached to within 1 °C of the 5 °C atmosphere (Fig. 2), the cells of adult guinea pigs, hamsters and ground squirrels all had lost between 6 and 13 % of their original K^+, and the difference in loss between that of the guinea pig cells and that of the cells of the 2 hibernating species is not significant at this stage ($P > 0.05$). The cells of the guinea pig, however, continued to lose K^+ so that after 120 min in the low temperature incubator (75 min actually at 5 °C), they only contained 64 % of their original K^+. In contrast, the cells of hamsters and ground squirrels lost little K^+ in the cold over longer periods of incubation.

The K^+ content of cells of hamsters and ground squirrels at 120 min in cold was significantly greater than that of guinea pig. (In subsequent studies with cold exposure lasting for up to 2 days no further K^+ loss has been observed in cells from ground squirrels and hamsters).

The values for hamsters and ground squirrels in Fig. 3 combine the determinations from active and hibernating individuals. No difference in K^+ retention was seen between these 2 subgroups (Table I), although differences were observed in influx of K^+ (Tables II and IV, see below).

Since newborn mammals are frequently more cold resistant than adults and since studies with kidney slices of kidney cortex from young guinea pigs had been

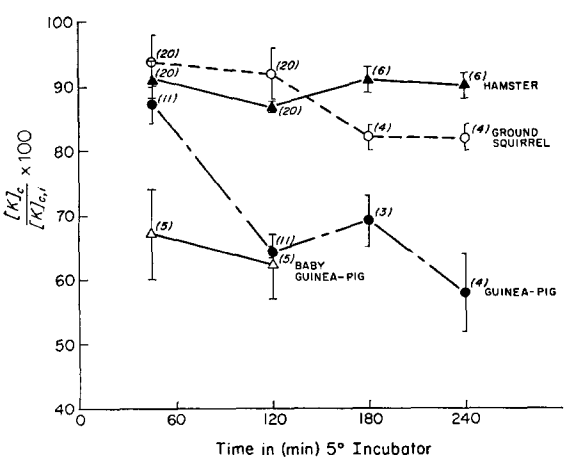

Fig. 3. Time course of change in content of K^+ of cells grown from kidney cortex following transfer of culture plates to low temperature incubator set at 5 °C. Ordinate: $[K^+]_c/(K^+]_{c,1}$ is the ratio of K^+ of cooled cells to that of the same number of cells on sister plates at 37 °C. Means (with S.E.) are based upon determinations in cultures made from separate individuals. Numbers of cases are indicated in parentheses.

TABLE I

RETENTION OF K^+ BY CELLS GROWN FROM KIDNEYS OF HIBERNATING AND ACTIVE HAMSTERS AND GROUND SQUIRRELS

Individual animals are same as shown as combined data in Fig. 3. Means, and numbers of cases (in parentheses) represent results from kidneys of separate animals. S.E. are shown for N greater than 5.

Species	Condition	Cell K^+ content (per cent of original)			
		45 min*	120 min	180 min	240 min
Hamster	Active	93±4 (11)	87±2 (11)	96 (3)	92 (4)
	Hibernating	88±5 (9)	88±6 (9)	87 (3)	86 (3)
Ground squirrel	Active	92±3 (10)	90±6 (10)	77 (2)	81 (2)
	Hibernating	98±6 (10)	93±2 (10)	87 (2)	83 (2)

* Time in 5 °C incubator

TABLE II

UNIDIRECTIONAL K^+ FLUXES IN PRIMARY CULTURES OF CELLS FROM KIDNEY CORTEX

Species	Condition of of donor	Temperature (°C)	Time in 5 °C incubator (min)	N	Influx	Efflux
Guinea pig	Active	37		10	1.00*	1.07±0.04
		~ 5	0**	8	0.22±0.06	0.51±0.07***
		~ 5	45	15,6	0.09±0.01	0.34±0.09***
		5	120	15,7	0.10±0.01	0.24±0.04***
Hamster	Active	37		4	1.00*	1.02±0.02
		~ 5	0	4	0.37±0.02	0.37±0.03
		~ 5	45	9,4	0.19±0.01	0.18±0.06
		5	120	9,4	0.15±0.03	0.13±0.03
	Hibernating	37		4	1.00*	0.92±0.04
		~ 5	0	4	0.39±0.06	0.36±0.03
		~ 5	45	9,4	0.14±0.03	0.22±0.06
		5	120	9,4	0.16±0.04	0.17±0.05
Ground squirrel	Active	37		10	1.00*	0.93±0.05
		~ 5	0	10	0.24±0.06	0.31±0.06
		~ 5	45	11,6	0.09±0.01	0.11±0.05
		5	120	11,6	0.07±0.01	0.10±0.04
	Hibernating	37		5	1.00*	0.98±0.01
		~ 5	0	3–5	0.32±0.03	0.55±0.06***
		~ 5	45	12,4	0.14±0.02	0.17±0.04
		5	120	12,4	0.12±0.02	0.09±0.04

* All fluxes are shown as relative to influx at 37 °C in same culture. Hence no S.E. is given for influx for 37 °C.

** Uptake of ^{42}K was measured for 5 min. In case of cells first put into low temperature incubator influx was measured for initial 5 min. Medium temperature is between 37 °C and about 12 °C at this time (Fig. 2).

*** Difference between influx and efflux is highly significant statistically ($P < 0.01$). In all other cases difference is not significant ($P > 0.05$).

found to retain K^+ better during cold storage than those of adult [17], it was of interest to examine the K^+ loss in cells cultured from (whole) kidneys of several newborn (i.e., less than 1 day old) guinea pigs that we fortuitously acquired. As may be seen in Fig. 3, the cells of the newborn guinea pigs not only had the same reduction in

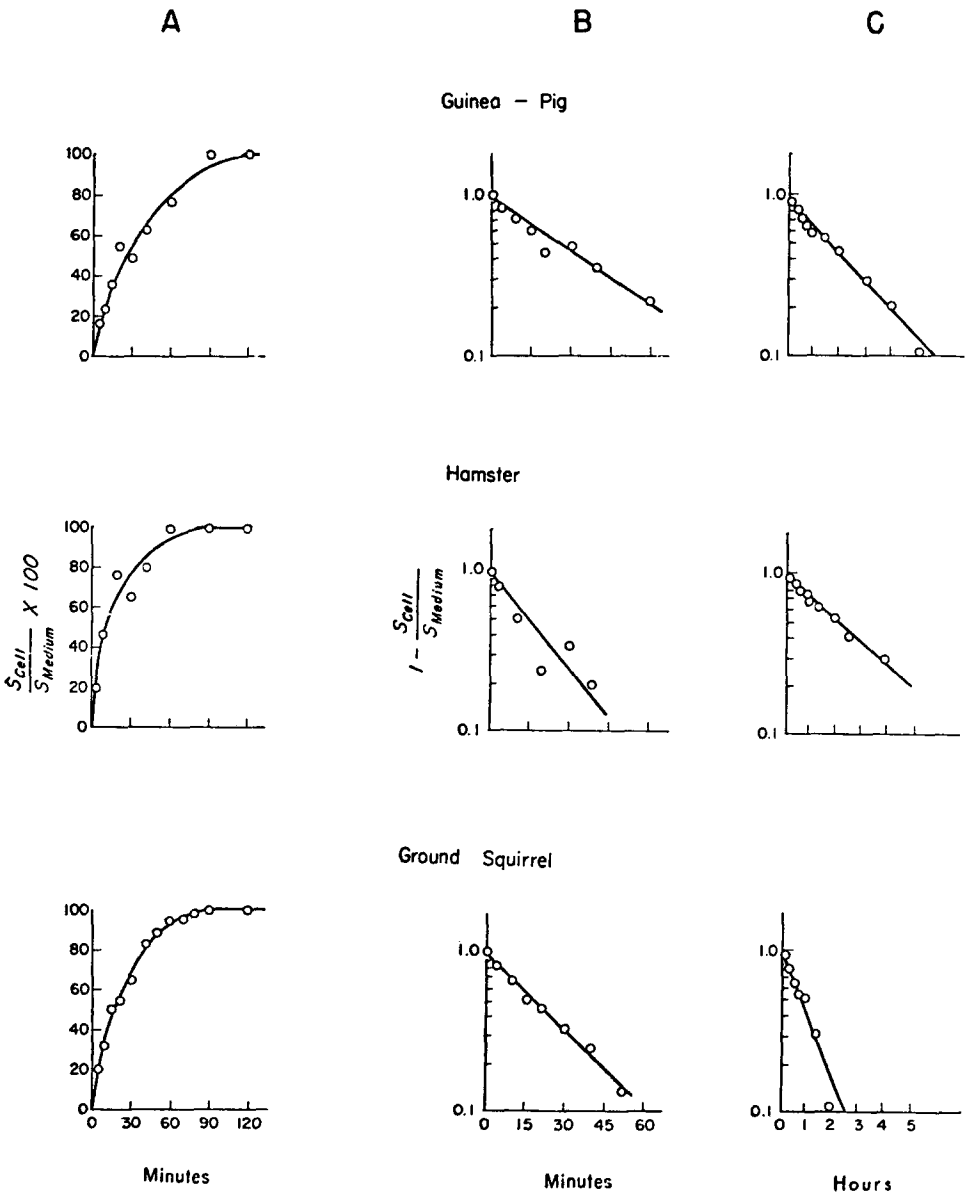

Fig. 4. A. Time course of ^{42}K uptake by kidney cells in culture at 37 °C. Points represent mean of duplicate samples of a single culture (single animal) of each species. B. Time course of equilibration of ^{42}K at 37 °C. For definition of S_{cell}, S_{med} see Methods. Points are computed from data in A (37 °C). Line shown is a linear regression line. C. Time course of equilibration of ^{42}K at 5 °C.

K^+ after 2 h in the low temperature incubator as those of adult guinea pigs, but even in the initial 45-min period of cooling lost 33 % of their K^+, more than that of any other group.

Unidirectional fluxes of K^+ in cultured kidney cells

The preceding results indicated a greater capacity of cells of the hibernating species to retain K^+ at low temperature, and in order to relate this difference to properties of the membrane it was necessary to measure fluxes of isotopic K^+.

When ^{42}K was introduced into the medium of kidney cells of the 3 species, the label in the cells reached the same specific activity as that in the medium within 90 min at 37 °C (Fig. 4a). Thus, the K^+ in the cells appeared to be totally exchangeable with that in the medium. When the uptake of K^+ was plotted as log relative specific activity, $(1 - a_t/a_\infty)$, as a function of time, the points were describable by a single straight line (although those for hamster were rather more scattered than the others). In accordance with a simple two compartment model [18] these results would indicate that the cells at 37 °C were behaving as a single homogeneous compartment. This was also true at 5 °C (Fig. 4c). Amongst the 3 species the half times for exchange varied between 15 and 30 min at 37 °C and between 90 and 180 min at 5 °C.

Curves for the time course of K^+ efflux at 37 °C in cells of guinea pigs and ground squirrels are shown in Fig. 5. From Fig. 4a and Fig. 5 it may be seen that for both efflux and influx it was feasible to measure an initial, nearly linear component even at 37 °C, when exchanges were most rapid, and thereby obtain a measure of unidirectional flux.

Effect of cooling on influx and efflux

Influxes and effluxes were measured at 37 °C and after several periods of cooling at 5 °C in cultures of kidney cells from hamsters and ground squirrels, both hibernating and active, and from guinea pigs.

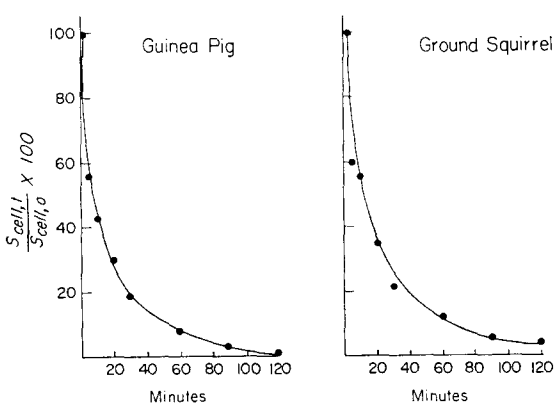

Fig. 5. Time course of loss of ^{42}K from cultured kidney cells at 37 °C. Cells were preloaded for 3 h and then washed 3 times with warm medium and placed in medium without isotopic label. Plates from the same culture were terminated after various periods of release by washing with ice-cold medium. Points represent means of duplicates from a single culture for each species.

In Table II, these results are expressed as a fraction of the influx at 37 °C. In all cases the influx and efflux were nearly equal at 37 °C, in accordance with expectation from the fact that the cells were in a steady-state with respect to K^+ concentration. At 5 °C in the cells from hamsters and ground squirrels the efflux was not significantly different from influx in any case ($P > 0.05$), except that of cells from hibernating ground squirrels during their initial period of cooling.

In contrast, the efflux of K^+ from cells of guinea pigs was significantly greater than influx ($P < 0.01$) at all times at 5 °C even during the first 10 min in the low temperature incubator, as the medium was undergoing its initial cooling from 37 °C to about 10 °C (Fig. 2).

The comparison of reductions in the separate fluxes is also of interest. Thus, at 2 h, by which time the temperature had been stabilized at 5 °C for over 1 h, the influx in guinea pig cells was 10 % of that at 37 °C, which is midway between that of

TABLE III

APPARENT FIRST ORDER RATE COEFFICIENT OF K^+ EFFLUX (k_e) FROM CELLS IN PRIMARY CULTURES GROWN FROM KIDNEY CORTEX

Means ± S.E. are shown. Numbers of cases given in parentheses.

Species	Condition of donor	Time in 5 °C incubator (min)	A	B	B/A
			Rate coefficient at 37 °C ($10^{-3} \cdot min^{-1}$)	Rate coefficient of cooled cells ($10^{-3} \cdot min^{-1}$)	
Guinea pig	Active (3–6)		33±7		
		0		12	0.40±0.02
		45		11	0.35±0.09
		120		11	0.33±0.04
Hamster	Active (4)		52±2		
		0		17±3	0.33±0.02
		45		12±3	0.24±0.06
		120		10±3	0.17±0.02*
	Hibernating (3–4)		52±2		
		0		17±3	0.34±0.05
		45		8±3	0.20±0.07
		120		8±3	0.15±0.03*
Ground squirrel	Active (4–5)		42±2		
		0		20±2	0.50±0.09
		45		6±3	0.09±0.03*
		120		6±2	0.10±0.04*
	Hibernating (3–5)		34±2		
		0		19±1	0.58±0.07
		45		7±1	0.21±0.03*
		120		4±1	0.14±0.02*

* Significantly different from corresponding value for guinea pig ($P < 0.01$).

active hamsters (15 %) and of hibernating ground squirrels (7 %) and not significantly different ($P > 0.05$) from that of either. (The influx into cells of awake hamsters and hibernating ground squirrels at 45 min, however, was significantly greater than that into guinea pig cells at that time.) On the other hand, the effluxes from guinea pig cells at 45 min and 2 h in the cold were 2- to 3-times as high as most of the efflux values from the cells of the two hibernating species.

The latter comparisons do not, moreover, take into account the effect of the lowered cell K^+ content at 5 °C on efflux. A more meaningful comparison could, therefore, perhaps be made for the rate coefficients for K^+ efflux as computed by Eqn. 4 (see Methods). In Table III, it may be seen that this rate coefficient is about the same in ground squirrel and guinea pig cells at 37 °C, whereas at 5 °C it is about twice as great in guinea pig cells as in those of ground squirrels. The value of the rate coefficient at 5 °C as a fraction of that at 37 °C is 2- to 3-times as great in cells of guinea pigs as in those of ground squirrels. Cells of hamsters exhibited a greater rate coefficient at 37 °C than the other 2 species and about the same absolute value at 5 °C as those of guinea pigs, with the result that the relative reduction with 2 h of cooling was also about twice as great as that for guinea pigs.

Ouabain-sensitive and -insensitive K^+ influx

In order to determine the contribution of active K^+ uptake to the K^+ influx, plates of cells were treated with ouabain at 37 °C before cooling and exposure to ^{42}K. The K^+ influx at 5 °C remaining in the presence of ouabain varied between 11 % of ouabain-insensitive influx at 37 °C in cells from active ground squirrels and 25 % in cells from awake hamsters (Table IV). Again, the fraction in guinea pig cells was higher than that in cells of ground squirrels and lower than that of hamsters and not significantly different from either.

Ouabain-sensitive influx was determined at both experimental temperatures by subtracting the influx with ouabain from the total influx. In general, the relative values for ouabain-sensitive K^+ influx were parallel with those for total influx (Table

TABLE IV

OUABAIN-SENSITIVE K^+ INFLUX ($^iM_K^P$) AND OUABAIN-INSENSITIVE K^+ INFLUX ($^iM_K^L$) AT 5 °C (AS A FRACTION OF EACH AT 37 °C) IN CELLS IN PRIMARY CULTURES GROWN FROM KIDNEY CORTEX

Means ± S.E. are shown for given number of donors (N).

Species	Condition of donor	N	$_5{}^iM_K^P/_{37}{}^iM_K^P$		$_5{}^iM_K^L/_{37}{}^iM_K^L$	
			45 min*	120 min	45 min	120 min
Guinea pig	Active	9	0.07±0.02	0.07±0.02	0.14±0.02	0.14±0.02
Hamster	Active	5	0.16±0.02**	0.09±0.02	0.25±0.05	0.27±0.05
	Hibernating	9	0.11±0.02	0.07±0.01	0.19±0.03	0.17±0.02
Ground squirrel	Active	5	0.08±0.01	0.09±0.02	0.12±0.04	0.11±0.03
	Hibernating	9	0.14±0.01**	0.13±0.03	0.12±0.02	0.12±0.02

* Time in 5 °C incubator prior to measurement of K^+ influx.

** Significant difference from value for guinea pig ($P < 0.02$).

TABLE V

RATIO OF OUABAIN-SENSITIVE K^+ INFLUX TO TOTAL K^+ INFLUX AT 37 °C AND 5 °C IN CELLS GROWN IN PRIMARY CULTURES FROM KIDNEY CORTEX OF GUINEA PIGS, HAMSTERS AND GROUND SQUIRRELS

Species	Condition of donor (N)	Temperature (°C)	Time in 5 °C (min)	${}^{1}M_K{}^{P}/{}^{1}M_K$
Guinea pig	Active (9)	37	–	0.80±0.02
		5	45	0.63±0.07
		5	120	0.64±0.08
Hamster	Active (5)	37	–	0.89±0.08
		5	45	0.84±0.04
		5	120	0.77±0.03
	Hibernating (4)	37	–	0.91±0.07
		5	45	0.82±0.03
		5	120	0.80±0.07
Ground squirrel	Active (5)	37	–	0.78±0.03
		5	45	0.62±0.13
		5	120	0.75±0.04
	Hibernating (9)	37	–	0.69±0.03
		5	45	0.71±0.03
		5	120	0.70±0.07

IV, first two columns, compared with Table II), Thus, in cells of awake hamsters, there was a decline in active influx of between 45 min and 120 min in the cold from 16 to 9 % of the active influx at 37 °C, and the value at 45 min is significantly greater ($P < 0.01$) than that for guinea pig cells (7 %). Cells of hibernating ground squirrels also exhibited a ouabain-sensitive influx significantly greater than that of cells of guinea pigs at 45 min in the cold, but because of greater variation, not at 120 min.

The relationship of ouabain-sensitive to -insensitive K^+ influx at the two temperatures may be viewed in two ways. In Table V are given the ouabain-sensitive fluxes as a fraction of total influx at each temperature. Numerically, the average proportion of active influx declined slightly at 5 °C in both guinea pig and adult hamster, but not in ground squirrels. None of the differences, however, were significant, statistically ($P > 0.05$). Referring to Table IV on the other hand, it may be seen that for both guinea pigs and adult hamsters the fraction of ouabain-sensitive transport remaining at 5 °C is equal to, or less than, half the fraction of ouabain-insensitive flux remaining. These differences were significant statistically ($P < 0.01$). The reduction in ground squirrel cells, however, was parallel for the two components of influx.

Effect of $[K^+]_o$ *on* K^+ *influx*

The preceding results indicated a considerable reduction in K^+ influx with cooling in all groups of cells used, with some differences among the types of cells. In principle the overall reduction in ouabain-sensitive K^+ influx could be attributed either to change of rate of transport maximally stimulated by extracellular K^+ (V) or, at least in part, to change of the apparent affinity of the transport system for extracellular K^+ (K_m). In order to evaluate the contributions of these two possibilities in cells grown from kidneys of guinea pigs and ground squirrels K^+ influxes with and

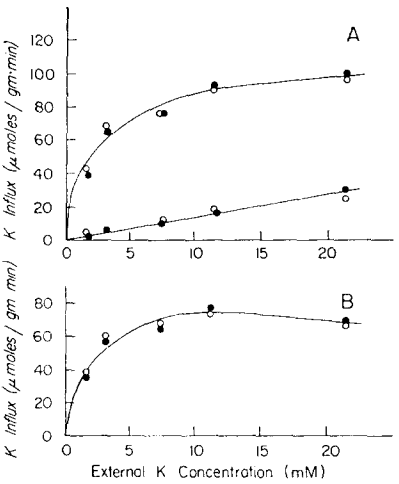

Fig. 6. Effect of extracellular K^+ concentration on K^+ influx at 37 °C in cells of guinea pig kidney cortex. A. Upper curve, total K^+ influx; lower curve, K^+ influx in the presence of ouabain. B. Ouabain-sensitive K^+ influx (difference of two curves in A.). Results from cultures grown from kidneys of two individuals are shown. Points represent mean of duplicate samples and fluxes are based upon mg protein content of the same plates of cells.

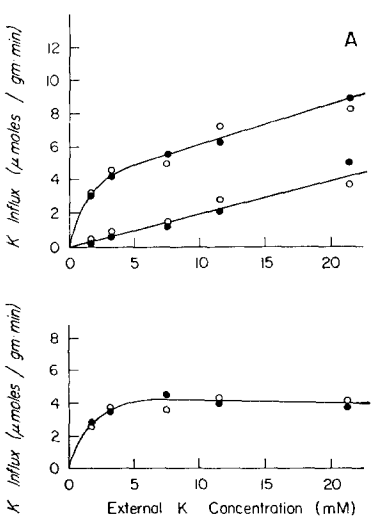

Fig. 7. Effect of extracellular K^+ concentration on K^+ influx at 5 °C in cells of guinea pig kidney cortex. A. Upper curve, total K^+ influx; lower curve, K^+ influx in the presence of ouabain. B. Ouabain-sensitive K^+ influx (difference between two curves in A). Other details as in Fig. 6.

without ouabain were measured with $[K^+]_o$ ranging from 1.8 to 22 mM. The results were similar for the 2 species and only the results for guinea pigs are shown (Figs. 6 and 7).

At 5 °C the total K^+ influx for both species resembles the behavior of human erythrocytes [19] in increasing $[K^+]_o$, a curvilinear portion at low $[K^+]_o$ and a linear component apparent at concentrations above 7 mM K^+. At 5 °C the influx of cells in ouabain rises strictly linearly with K^+ concentration. Subtracting the influx in ouabain from total influx yields a ouabain-sensitive component which shows a plateau at 7 mM K^+ and is half saturated at a concentration a little below 1.8 mM (near 1 mM) in cells of both species. Half saturation at 37 °C also occurs at a little below 1.8 mM (near 1 mM) for cells of both species, so that there is no apparent increase resulting from change of temperature in this property.

Fluxes in cells with altered $[K^+]_c$ *and* $[Na^+]_c$

The preceding results indicated that, with the exception of hibernating ground squirrels, cells deriving from individuals of hibernating species and cooled for 2 h at 5 °C did not have a higher ${}_5{}^iM_K/{}_{37}{}^iM_K$ (either total or ouabainsensitive) than cells from guinea pigs. Since the cells from awake hamsters and ground squirrels do retain K^+ better at 5 °C than those of guinea pigs, such a finding might tend to the conclusion that the difference in K^+ retention was entirely attributable to the slower leak at low temperature in the hibernator cells. On the other hand, in view of the fact that cell K^+ and Na^+ concentrations were rapidly changing at 5 °C in the guinea pig cells, but not in those of the hibernators, the ouabain-sensitive influxes in the two cases are not strictly comparable. Thus, work with erythrocytes has shown that not only is cellular Na^+ stimulatory to the Na^+/K^+ pump, but that cellular K^+ is inhibitory [20–22]. Assuming a similar relationship to have existed in the cultured kidney cells, the rate of ouabain-sensitive influx in guinea pig cells at 5 °C might have represented a maximum while that of, say, the ground squirrels might have been nearer a minimum.

In order to test this possibility, cells of kidneys from active ground squirrels and from guinea pigs were exposed as described in Methods to K^+-free medium or to medium with 40 mM K^+ with the intent of decreasing or increasing $[K^+]_c$, respectively.

Net ion movement

In general, treatment with K^+-free medium was more successful in decreasing $[K^+]_c$ than the treatment with high K^+ medium was in causing a rise. It was desirable to determine the rapidity with which cell ion contents reverted to normal at the two temperatures, and an example of single experiments with cells of both guinea pigs and ground squirrels, treated in K^+-free medium and returned to standard incubation medium (6 mM K^+) is shown in Fig. 8. In cells of both species the treatment lowered the $[K^+]_c$ and elevated the $[Na^+]_c$. Upon return to the 6 mM medium the values for cell K^+ and Na^+, expressed as a fraction of the unaltered concentrations, returned virtually to normal within half an hour at 37 °C in cells of both species and at 5 °C in the cells of ground squirrels. Guinea pig cells were unable to reaccumulate K^+ at 5 °C, although Na^+ extrusion did seem to occur. Since the recovery was so rapid in most instances, the flux determination had to be corrected for net K^+ movement, as described in Methods.

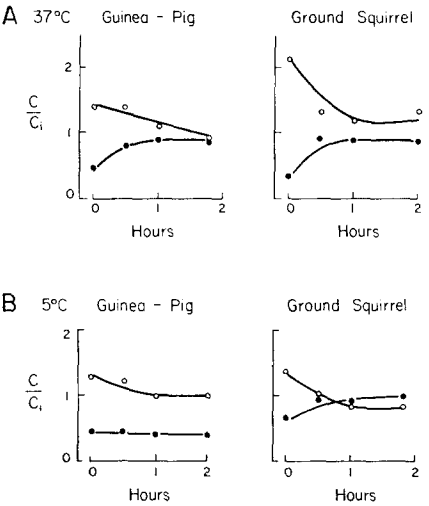

Fig. 8. Uptake of K^+ and extrusion of Na^+ in cultured kidney cells returned to growth medium (containing 10% calf serum) after exposure to K^+-free medium. A. 37 °C. Cells were exposed to K^+-free medium for 45 min. B. 5 °C. Cells were exposed to K^+-free medium for 15 min at 37 °C then for a further 45 min at 5 °C before they were returned to 5 °C. Open circles, cell Na^+; closed circles, cell K^+; values given at each time as a fraction of the initial before treatment. Points represent mean of paired samples from a single individual of each species.

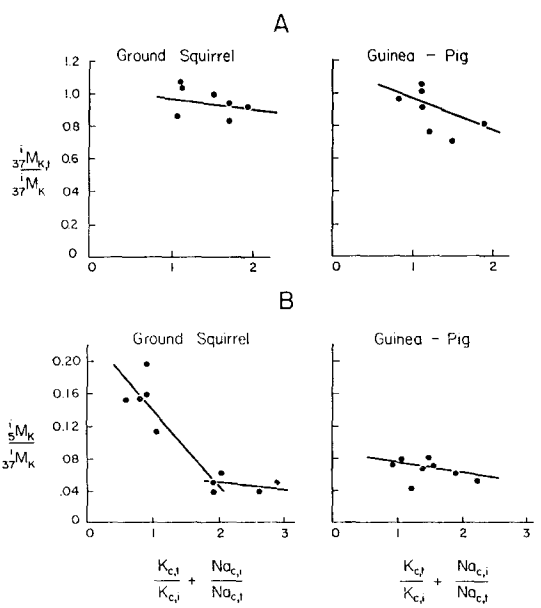

Fig. 9. Effect of intracellular K^+ and Na^+ on K^+ influx. Cells were exposed to high or low K^+ media as described in text and returned to growth medium for determination of ^{42}K uptake. A. Total K^+ influx at 37 °C (as a fraction of total influx in untreated cells). B. Total K^+ influx at 5 °C (as a fraction of total K^+ influx at 37 °C in untreated cells). Each point represents results from cultured cells from kidneys of a separate single animal. Curves shown are regression lines. (Two regression lines are shown for ground squirrels, for values below 2.1 and for values above 1.8.) ${}_5{}^1M_K$, total influx at 5 °C; ${}_{37}{}^1M_K$, total K^+ influx at 37 °C ("t" denotes "treated"); $[K^+]_{c,i}$, $[Na^+]_{c,i}$, cellular K^+ and Na^+ of untreated cells; $[K^+]_{c,t}$, $[Na^+]_{c,t}$, cellular K^+ and Na^+ of treated cells.

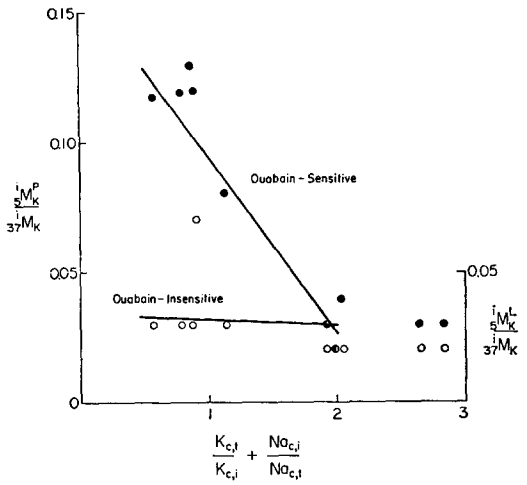

Fig. 10. Effect of $[K^+]_c$ and $[Na^+]_c$ on ouabain-sensitive and insensitive components of K^+ influx at 5 °C in cells of ground squirrels. Same experiments as shown in Fig. 9. Values for influx in presence of ouabain, ${}_5{}^iM_K{}^L/{}_{37}{}^iM_K$, (open circles) are subtracted from total influx (Fig. 9) to give ouabain-sensitive influx, ${}_5{}^iM_K{}^P/{}_{37}{}^iM_K$, (closed circles). Curves shown at values on the abscissa of less than 2 are regression lines. Slopes are −0.002 (ouabain-insensitive) and −0.0705 (ouabain-sensitive).

Unidirectional K^+ influx

In order to allow for changes in cell Na^+ as well as K^+, the dependency of influx of K^+ has been plotted in Figs. 9–11 as a function of the sum of the ratio of change of $[K^+]_c$ and $[Na^+]_c$. The term used is: $[K^+]_{c,t}/[K^+]_{c,i}+[Na^+]_{c,i}/[Na^+]_{c,t}$, where "t" indicates treated and "i" indicates initial, unaltered concentration. Assuming influences similar to those in erythrocytes, a decrease in either term should work in the direction of increasing active pump flux. (For convenience, this expression will hereafter be referred to as $[C]_c$.)

As seen in Fig. 9, total K^+ influx into cells of ground squirrels and guinea pigs showed only a slight rise with decreased $[C]_c$ at 37 °C and 5 °C except in cells of ground squirrels at 5 °C with values of $[C]_c$ of less than 2. Only in this case was the rise significantly different from zero, statistically (slope of −0.0885). That the increase in K^+ influx in ground squirrel cells at 5 °C was due to increase in ouabain-sensitive influx is shown in Fig. 10, in which the contributions of the ouabain-sensitive and -insensitive fluxes have been separated. Ouabain-insensitive influx did not change appreciably with $[C]_c$ (slope of −0.002, not significantly different from zero, $P > 0.05$) whereas the increase in ouabain-sensitive influx was highly significant (slope of −0.0705, $P < 0.02$).

In order to compare the response of ouabain-sensitive K^+ influx to altered cell K^+ and Na^+ at the two temperatures in cells of a single species, the influx was plotted in Fig. 11 as a fraction of the influx at $[C]_c$ of 2 at the respective temperature. When treated in this way it may be seen that the response is unaltered in cells of guinea pigs with temperature change and that the response in cells of ground squirrels at 37 °C is the same as in those of guinea pigs. When these normalized results from the 3 groups (i.e., guinea pigs and ground squirrels, 37 °C, and guinea pigs, 5 °C) are pooled and a single composite regression line computed, the resulting slope of −0.25 is

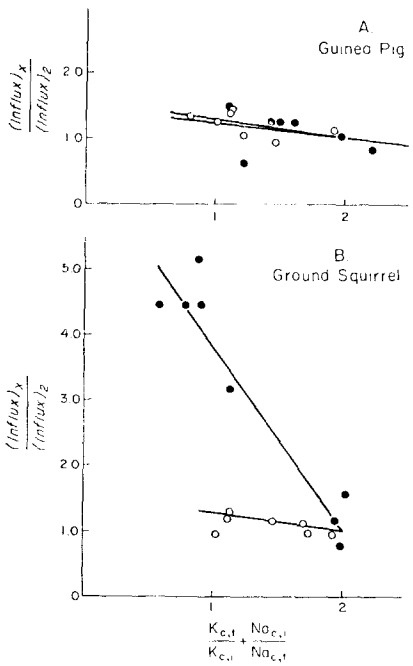

Fig. 11. Comparison of effect of cellular alkali cations on ouabain-sensitive K^+ transport at two temperatures, 37 °C (open circles), 5 °C (closed circles). A. Guinea pig. B. Ground squirrel. Ordinate: ouabain-sensitive influx at given value on the abscissa as a fraction of ouabain-sensitive influx at a value of 2 on the abscissa (i.e., unchanged $[Na^+]_c$, $[K^+]_c$). Same experiments as shown in Figs. 9 and 10 except that values for ground squirrels at values on the abscissa of greater than 2.1 are omitted. Slopes of the curves shown are: Guinea pig 37 °C, −0.28; Ground squirrel 37 °C, −0.19; Guinea pig 5 °C, −0.23; Ground squirrel 5 °C, −2.84.

significantly different from zero ($P = 0.035$). The most striking feature, however, is that the response of cells of ground squirrels to altered $[C]_c$ is much greater at 5 °C than at 37 °C. The slope of this response is −2.84, or 11-times that of the composite slope of the other 3 cases.

DISCUSSION

The results would seem to permit four conclusions:

1. Greater decrease of passive permeability with cooling in cultured kidney cells of hibernating species accounted for part of their greater retention of K^+ at 5 °C, as was previously found in freshly drawn erythrocytes.

2. Unlike erythrocytes, however, neither the total influx nor the ouabain-sensitive influx of K^+ was less sensitive to cold in the kidney cells of the hibernating species (exposed to cold for 2 h).

3. In kidney cells both of the hibernators and of the guinea pig the ouabain-sensitive K^+ influx was surprisingly high at 5 °C compared with expectation based on earlier and current studies with $(Na^+ + K^+)$-ATPase.

4. In the cells of ground squirrels a greater sensitivity of K^+ transport at 5 °C

to lowered cytoplasmic K^+ and elevated Na^+ contributed to greater K^+ retention in the cold.

These will be discussed in order.

Cold on passive permeability.

As described in Results, the reduction in passive leak of K^+ in guinea pig kidney cells between 37 °C and 5 °C is only a third to a half that in the cells of the hibernating species. This was revealed both in the measurement of K^+ efflux and in the computation of the rate coefficient of efflux which corrects for the effect of diminished K^+ concentration in the cell. Consideration of the rate coefficient also reveals the point, not apparent from the simple effluxes, that the decrease with cooling was progressive beyond 45 min in the cold in the cells from hibernators but not in those of guinea pigs. This would seem to imply either that the membranes of the hibernator cells do not fully respond to cooling instantly or that the main differences between guinea pig cells and those of the 2 species of hibernators lie at temperatures below 10 °C. (i.e., the temperature at the end of the "time 0" measurement, Fig. 2.) The finding of continued decrease in the rate coefficient of efflux in the cells of the hibernating ground squirrel between 45 min and 2 h in the cold adds credence to the possibility of a progressive change in membrane permeability, perhaps due to secondary factors.

Cold on active transport, hibernators vs. guinea pig

The failure to find a large and persistent difference in temperature sensitivity of the Na^+/K^+ pump between cells of hibernators and non-hibernators does not seem to agree with earlier results with kidney slices [4, 23]. In those earlier studies active transport was judged by the initial rate of net accumulation of K^+ in slices previously leached of K^+ (and loaded with Na^+) by brief anaerobic incubation. The difference in the 5/37 °C ratio of transport between hamsters (6 %) and guinea pigs (1 %) was striking. The apparent discrepancy need not be ascribed to changes resulting from growth in culture, since leached cultured cells showed the same sort of differences in net uptake of K^+ as previously seen in slices (Fig. 8). A more satisfactory explanation therefore, is the inadequacy of net uptake as the criterion for rate of transport in comparing two groups of cells with different passive permeability. Since what was being measured in the earlier kidney slice studies was basically rate of return to steady state, the disparity between initial rate of net uptake and unidirectional flux as measures of transport would have been greatest precisely in the case of the cells with the relatively greater leak (i.e., guinea pig cells). This interpretation clarifies the paradoxical finding in the slices of kidney cortex of guinea pig that while K^+ transport virtually disappeared, there was still an easily measured ouabain-sensitive, Na^+ dependent oxygen uptake amounting to nearly the same fraction of respiration as at 37 °C [23].

Cold on active transport, intact pump vs. ($Na^+ + K^+$)-ATPase

Willis and Li [24] reported in 1969 that cooling inhibited ($Na^+ + K^+$)-ATPase of renal cortex much more severely than it inhibited two correlates of transport in the intact cell, net K^+ uptake and ouabain-sensitive, Na^+-dependent respiration. Even though the enzyme preparation and the criteria for transport in that study were crude,

the conclusion seems to have been borne out by other studies of $(Na^+ + K^+)$-ATPase and by the flux determinations in the present investigation. Many reports have stressed the strong and discontinuous inhibition of the $(Na^+ + K^+)$-ATPase by cold and have generally attributed this to phase transition of the membrane lipids [10, 12, 13, 25–27]. In our hands the microsomal fraction of the kidney cortex has a 5/37 °C ratio of about 0.3 % in the kidney cortex of guinea pigs and about 1 % in that of hamsters and ground squirrels, much lower than that of 7–13 % seen for unidirectional, ouabain-sensitive K^+ influx in cultured kidney cells (Table IV). Of course, comparison of fluxes in cultured cortical kidney cells with an enzyme from the fresh kidney cortex is not ideal. Although we have not yet succeeded in making a satisfactory preparation of $(Na^+ + K^+)$-ATPase from the cultured cells, we have measured influxes in mouse 3T3 and L cells and find these also to have a low sensitivity (5/37 °C ratio of 10 %) compared with the high sensitivity of the $(Na^+ + K^+)$-ATPase of the same types of cells reported elsewhere [13, 27].

The severe inhibition of ouabain-sensitive K^+ influx at 5 °C previously reported in erythrocytes of guinea pigs would seem to be in accord with the "temperature breaks" commonly seen for $(Na^+ + K^+)$-ATPase. Ellory and Willis (unpublished), however, have recently found that even here the same kind of discrepancy exists between inhibition of transport and of enzyme in the same population of cells between 30 and 5 °C.

To find the source of this departure in behavior of these two otherwise highly linked aspects of transport, one might search either for alterations in the enzymatic activity caused by the procedures for isolation of the enzyme (disruption of the cell, selection by differential centrifugation, treatment with detergents, chelators, and chaotropic agents) or for compensatory responses of the intact cell to elevate pumping at the low temperature. In the latter category would be stimulation of the pump by altered cytoplasmic ion concentrations and uncovering of active pump sites at low temperature [28]. With regard to these last two possibilities the results shown in Fig. 11 would seem to rule out the first, kinetic stimulation, and our observations that inhibitorily specific ouabain binding does not increase at low temperature [29] probably rules out the second, increase in pump sites.

K^+ influx into cells with altered $[K^+]_c$, $[Na^+]_c$.

Two comparisons seem necessary in order to judge the significance of the large response of the ouabain-sensitive K^+ influx into cells of ground squirrel at low temperature. The first is to ask how the pump rate seen in the untreated cells at 5 °C (Table IV) compares with what would be expected from their altered alkali cation content at that temperature, based on the results in Fig. 9. The comparison is made difficult by the fact that in the cells in which $[K^+]_c/[K^+]_{c,i}$ was estimated (Fig. 3) we did not measure $[Na^+]_c$. However, in the cells of ground squirrels treated at 5 °C with low-K^+ medium, there was a strong inverse correlation between the two ions ($r = 0.67$, $P = 0.05$). If we apply the same relationship to the untreated cells, the expected pump rate can be computed on the basis of cell K^+ alone by

$$ {}_5{}^{i}M_K = a + d + b(1.68 - 1.51\ [K^+]_c/[K^+]_{c,i}), \tag{6} $$

where a is the ouabain-sensitive K^+ influx in cells with unchanged cation content, b is the slope of the regression curve in Fig. 9 taken as a positive number and d is the

ouabain-insensitive K^+ influx (about 0.03).

Evaluating this for $[K^+]_c/[K^+]_{c,i}$ of 0.90 (Table I) gives a value of 0.07 for influx at 5 °C (as a fraction of that at 37 °C), which agrees with the observed value of 0.07 (Table II). The ouabain-sensitive component of influx (0.05, see Table V), correspondingly, was nearly twice as great as the minimum value at $[C]_c = 2$ (0.03) and less than half of the highest rates achieved (0.12) with low $[K^+]_c$ high $[Na^+]_c$. The same comparisons cannot precisely be made for the guinea pig cells, but it is nevertheless apparent that with cooling alone their rate of influx was near maximum.

The other comparison of interest is to ask what influence the altered response in ground squirrel cells would have upon steady state K^+ concentration at the lowered temperature. If it is assumed that the rate coefficient for efflux is a constant over a wide range of $[K^+]_c$ (as was seen to be the case for guinea pig cells, Table III) one may then combine a form of Eqn. 2 with Eqn. 3 and solve for the steady state $[K^+]_c/[K^+]_{c,i}$ (Table VI).

When this is evaluated (Table VI) it may be seen that, as expected, if efflux were set equal to observed influx at 5 °C in ground squirrel cells, no change in $[K^+]_c$ would occur. When the actually observed efflux is used to compute k_e, a steady state

TABLE VI

COMPUTED STEADY-STATE VALUES OF $[K^+]_c/[K^+]_{c,i}$ IN CELLS OF KIDNEY CORTEX OF GROUND SQUIRRELS AT 5 °C WITH VARIOUS ASSUMED VALUES OF K^+ LEAK AND OF RESPONSIVENESS OF PUMP RATE TO CHANGES IN $[K^+]_c$ AND $[Na^+]_c$

Based upon:

$$\left[\frac{K_c^+}{K_{c,i}^+}\right]_{\text{steady state}} = \frac{a+d+1.68\,b}{k_e+1.51\,b} \tag{7}$$

Derived from Eqn. 6 and

$${}^{o}M_K/{}_{37}{}^{i}M_K = k_e([K^+]_c/[K^+]_{c,i}) \tag{8}$$

by solving for $[K^+]_c/[K^+]_{c,i}$ in the steady state when influx equals efflux.

Terms: G.S., ground squirrel; G.P., guinea pig; a, ouabain sensitive influx at $[C]_c = 2$ and 5 °C, (i.e., $a = 0.03$); d, ouabain insensitive influx at 5 °C ($d = 0.03$); b, "response" or slope or regression curve, Fig. 9, taken as a positive number, ${}^{o}M_K$ = efflux of K, and ${}_{37}{}^{i}M_K$ = influx at 37 °C.

	Description	b	k_e	$K_c^+/K_{c,i}^+$	Comments
1	G. S. response, G. S. leak	0.0705	0.0605	0.97–1.03	k_e based on assumption of equality of influx and efflux (Tables I, II)*
2	G.S. response, G.S. leak	0.0705	0.11	0.82	k_e value based on actually measured efflux (Table II)
3	G.P. response, G.S. leak	0.011	0.11	0.59	k_e same as 2
4	G.S. response, G.P. leak	0.0705	0.38	0.36	k_e is 0.24/0.64 (Table II, Fig. 3)
5	G.S. with G.P. response and G. P. leak	0.011	0.38	0.20	Not same as $K_c^+/K_{c,i}^+$ for G.P.

* In example 1 the value of 0.97 correponds to influx of 0.07 (Table I) and that of 1.03 to total influx of 0.06 (sum of $a = 0.03$ and $d = 0.03$, Fig. 10).

$[K^+]_c/[K^+]_{c,i}$ of 0.82 is predicted, which agrees with the value observed at 3 and 4 h in the cold (Fig. 3, Table I). If the ground squirrel cells had the guinea pig's response to change in $[C]_c$, then the steady-state level would have been 0.59, a value seen in guinea pig cells after 4 h in the cold. With the guinea pig's leak but the ground squirrel's response to changed $[C]_c$ the steady-state level would have been 0.36, and the minimum for ground squirrel cells with guinea pig pump and leak would have been 0.20 (i.e., 80 % loss of K^+). Thus, within the total scope of regulation from 20 to 82 % of original $[K^+]_c$, two-thirds may be attributed to the low leak at 5 °C and one-third to the increased responsiveness to lowered $[K^+]_c$, $[Na^+]_c$.

The value of 0.19 does not predict the minimum $[K^+]_c/[K^+]_{c,i}$ for guinea pig cells because their values of a, d and the relation of $[Na^+]_{c,i}/[Na^+]_{c,t}$ to $[K^+]_{c,t}/[K^+]_{c,i}$ would all be different. But there is no reason to suppose that they have achieved steady-state at 4 h. In other studies lasting up to 48 h one value of 0.23 has been observed (Foster and Willis, unpublished observations).

Kinetic alterations accounting for differences in transport rate with changed conditions or between similar cells are not common. To our knowledge the difference between HK and LK erythrocytes of sheep and goats and the reversal of the latter by anti-L antibody [30] is the only familiar and well described example. The occurrence in the present case raises the question of whether the change observed in the ground squirrel cells is due to an effect on all the pump sites or to a selection of pump sites, and whether in either case the change is a direct effect of cooling or is mediated through an accessory membrane component such as that suggested by the existence of anti-L antibody.

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REFERENCES

1 Lyman, C. P. and Blinks, D. C. (1959) J. Cell Comp. Physiol. 54, 53–64
2 Andrus, W. de W. (1969) Am. Zool. 9, 589
3 Lyman, C. P. and Jarrow, E. L. (1971) Exp. Cell Res. 68, 214–217
4 Willis, J. S. (1966) J. Gen. Physiol. 49, 1221–1239
5 Kimzey, S. L. and Willis, J. S. (1971) J. Gen. Physiol. 58, 634–649
6 Willis, J. S., Fang, L. S. T. and Foster, R. F. (1972) in Hibernation and Hypothermia: Perspectives and Challenges (South, F. E., ed.), pp. 123–147, Elsevier, New York
7 Raison, J. K. and Lyons, J. M. (1971) Proc. Natl. Acad. Sci. U.S. 68, 2092–2094
8 Lyons, J. M. (1972) Cryobiology 9, 341–350
9 Fox, C. F. (1972) in Membrane Molecular Biology (Fox, C. F. and Keith, A. D. eds.), pp. 345–359, Sinauer Associates, Inc., Stanford
10 Grisham, C. M. and Barnett, R. E. (1973) Biochemistry 12, 2635–2637
11 Barnett, R. E. and Palazzotto, J. (1974) Ann. N.Y. Acad. Sci. 242, 69–76
12 Charnock, J. S., Cook, D. A., Almeida, A. F. and To, R. (1973) Arch. Biochem. Biophys. 159, 393–399
13 Wisnieski, B. J., Parkes, J. G., Huang, Y. O. and Fox, C. J. (1974) Proc. Natl. Acad. Sci. 71, 4381–4385
14 Keynes, R. D. and Lewis, P. (1951) J. Physiol. 113, 73–98
15 Robertson, J. S. (1957) Physiol. Rev. 37, 133–154

16 Lowry, O. H., Rosebrough, N. T., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
17 Willis, J. S., Foster, R. F. and Behrends, C. L. (1975) Cryobiology 12, 255–265
18 Atkins, G. (1969) Multicompartment Models for Biological Systems, pp. 19–65, Methuen, London
19 Glynn, I. M. (1956) J. Physiol. 134, 278–310
20 Sachs, J. R. (1970) J. Gen. Physiol. 56, 322–341
21 Hoffman, P. G. and Tosteson, D. C. (1971) J. Gen. Physiol. 58, 438–466
22 Sachs, J. R., Ellory, J. C., Kropp, D. L., Dunham, P. B. and Hoffman, J. F. (1974) J. Gen. Physiol. 63, 389–414
23 Willis, J. S. (1968) Am. J. Physiol. 214, 923–928
24 Willis, J. S. and Li, N. M. (1968) Am. J. Physiol. 217, 321–326
25 Gruener, N. and Avi-Dor, Y. (1966) Biochem. J. 100, 762–767
26 Kimelberg, H. K. and Papahadjopoulos, D. (1972) Biochim. Biophys. Acta 282, 277–292
27 Kimelberg, H. K. and Mayhew, E. (1975) J. Biol. Chem. 250, 100–104
28 Grinstein, S. and Erlij, D. (1974) The Physiologist 17, 233
29 Willis, J. S., Zeidler, R. B., Hohla, D. H. and Foster, R. F. (1975) Cryobiology 12, 555
30 Glynn, I. M. and Ellory, J. C. (1972) in Roles of Membranes in Secretory Processes (Bolis, Liana, Keynes, R. D. and Wilbrant, W. eds.), pp. 224–237, Elsevier, New York