

Antibody-Induced Alterations in the Kinetic Characteristics of the Na:K Pump in Goat Red Blood Cells

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SUMMARY The kinetic characteristics of the Na:K pump in high potassium (HK) and low potassium (LK) goat red cells were investigated after altering the intracellular cation concentrations. At low concentrations of intracellular K (K_e), increasing K_e at first stimulates the active K influx in HK cells, but at higher K_e the pump is inhibited. These results suggest that in HK cells K_e acts both at a stimulatory site at the inner aspect of the pump and by competition with intracellular Na (Na_e) at the Na translocation sites. In LK cells, K_e inhibits the active K influx and the sensitivity of LK cells to inhibition is much greater than the sensitivity of HK cells. Exposure of LK cells to an antibody (anti-L), raised in an HK sheep by injection of LK sheep cells, increased the active K influx at any given K_e . The effect of the antibody was greater at higher intracellular K concentrations, and in cells with very low concentrations of K the antibody had little effect on the pump rate. The failure of anti-L to stimulate the pump in low K_e LK cells was not due to failure of the antibody to bind to the cells. Anti-L combining at the outer surface of the cell reduces the affinity of the pump at the inner surface for K at the inhibitory sites. The maximal pump rate in LK cells at optimal Na and K concentrations is less than the maximal pump rate of HK cells under the same circumstances.

INTRODUCTION

Populations of sheep can be divided into a group of individuals with high (HK) and a group with low (LK) red blood cell potassium concentrations (Evans, 1954). This characteristic is determined by a single genetic locus with two alleles (Evans et al., 1956). The red cell K concentration is associated with the activity of the ouabain-sensitive cation transport system; the ouabain-sensitive K influx into HK sheep red cells is about seven times as great as that into LK sheep red cells (Tosteson and Hoffman, 1960; Dunham and Hoffman,

1971). Similar differences between HK and LK cells have been reported in the Na, K-ATPase activity (Tosteson, 1963) and in the number of ouabain binding sites, a measure of Na:K pump sites (Dunham and Hoffman, 1971).

Until recently it seemed that these findings could be explained simply by a greater number of pump sites in HK sheep cells than in LK cells. Several reports, however indicated that the situation is more complicated than this. Hoffman and Tosteson (1971) have studied the effect of altering the intracellular cation concentration on the activity of the ouabain-sensitive K influx. In both HK and LK cells the ouabain-sensitive K influx fell as K_e increased and Na_e decreased, but the LK cells were much more sensitive to this manipulation than the HK cells; in fact, at an Na_e/K_e ratio of 2/1 the ouabain-sensitive K influx in LK cells was almost zero. Since Na_e and K_e were varied reciprocally, it was not possible to decide with certainty if the effect was due to decreasing Na_e or increasing K_e , but from the shape of the curves it seemed more likely that the effect was an inhibition of the pump by K_e . The characteristics of the partial reactions of the Na, K-ATPase of membranes prepared from HK and LK sheep red cells also differ qualitatively. Although the Na-stimulated ATPase activity is 10 times greater in HK membranes than in LK membranes, the ADP-ATP exchange reaction is only 2.7 times greater in HK membranes (Whittington and Blostein, 1971). It therefore seems likely that, in addition to a lower absolute number of pumps in LK sheep cells, the pumps which are present are qualitatively different from those of HK cells.

Injecting red cells from an LK sheep into an HK animal raises an antibody (called anti-L) which, when allowed to react with LK cells, produces at least a threefold stimulation of the ouabain-sensitive K influx (Ellory and Tucker, 1969). By determining the characteristics of the anti-L-stimulated K influx, it should be possible to decide whether the stimulation produced by anti-L results from the uncovering of pump sites which are present but inoperative in the nontreated cell or from an alteration in the kinetic behavior of the functioning pumps (or both). When this type of experiment was carried out in LK sheep cells both effects seemed to occur, i.e., the number of ouabain binding sites was increased, and the sensitivity of the K influx to inhibition by internal K was decreased (Lauf et al., 1970), although this interpretation is somewhat uncertain since internal Na and K were varied simultaneously (Glynn and Ellory, 1972).

Populations of goats exhibit the same HK-LK dimorphism as do sheep (Evans and Phillipson, 1957) and LK goat red cells respond to anti-L raised in HK sheep with a 1.5-8-fold increase in ouabain-sensitive K influx (Ellory and Tucker, 1970 *a*). Using membranes from LK goat cells, anti-L has been shown to reduce the inhibition of the Na, K-ATPase produced by K; this inhibition presumably results from competition between Na and K for the Na sites at the inner surface of the membrane (Glynn and Ellory, 1972).

The present paper reports experiments designed to investigate in further detail the mechanism by which anti-L exerts its effect. The experiments were performed on red cells from goats rather than sheep. Goat red cells provide a more satisfactory experimental system for study since the ratio of the K pump to K leak is greater in LK goat cells than in LK sheep cells. A preliminary report of this work has been published (Ellory et al., 1972).

METHODS

Preparation of Antiserum

Anti-L was prepared by intramuscular injection in homozygous HK sheep of washed red cells from homozygous LK sheep mixed with Freund's complete adjuvant (Tucker and Ellory, 1970). Whole blood was obtained from the immunized sheep without anticoagulant. The serum was separated from the clot, heated at 56°C for 20 min to inactivate complement, and stored at -20°C. Before use, the serum was dialyzed for 36-48 h at 4°C against 10 vol of a solution which contained (mM): Na or choline 170, Mg 1, PO₄ 27, Cl 128, pH 7.4; the solution was changed three times. Before use adenosine 10 mM and glucose 5 mM were added to the dialyzed serum.

Preparation of Cells

Blood was obtained by jugular venipuncture from one of three Nubian goats, two LK and one HK, using heparin as anticoagulant. The cells were separated from the plasma and washed three times (by centrifugation at 4°C) in about 10 vol of a 107 mM MgCl₂ solution.

Alteration of Intracellular Cation Concentrations

This was accomplished by a modification of the method of Garrahan and Rega (1967). The method involves increasing the permeability of the cells to cations using *p*-chloromercuribenzenesulfonic acid (PCMBS), allowing the cells to equilibrate with solutions of the desired composition and then reversing the effect of PCMBS with dithiothreitol (DTT). Washed cells were suspended at 5% hematocrit in a solution which contained (mM): PCMBS 0.1, Mg 1, Cl 147, PO₄ 3.4, glucose 5, and varying concentrations of Na, K, and choline to make a total of 152 mM, pH 7.4. In order to maintain constant cell volume, sucrose in concentrations varying from 0 (all choline solutions) to 85 mM (choline-free solutions) was added. The amount of sucrose necessary to maintain constant cell volume was found by trial and error. The suspension was incubated with shaking at 4°C for 84 h; the cells were separated from the solutions and resuspended in fresh solutions at 24, 48, 60, and 72 h after the incubation began. After 84 h the cells were separated from the solutions and resuspended in solutions identical to the PCMBS solutions except that PCMBS was omitted, PO₄ was 27 mM and Cl 120 mM, and dithiothreitol 5 mM and adenosine 10 mM were included (with Na and K in the PCMBS solution replaced with choline in the dithiothreitol solution when K_e or Na_e was kept very low). The suspensions were incubated for 1 h at 37°C to reverse the permeability change and the cells were then removed from the solutions and washed three times using 107 mM MgCl₂ solution. Using this

technique it was possible to obtain cells with Na_c less than 5 mmol/liter RBC and K_c less than 1 mmol/liter RBC, and of normal volume (estimated by measuring the ratio of the hemoglobin concentration of a red cell suspension to the hematocrit of the same suspension).

Exposure to Anti-L Antiserum

Cells, either fresh or after alteration of intracellular cation concentrations, were suspended at 5% hematocrit either in dialyzed antiserum (antibody-treated cells) or in a solution of cation composition identical to that against which the antiserum was dialyzed, and containing adenosine 10 mM and glucose 5 mM (control cells). It has been shown that exposure of LK cells to serum from an HK nonimmunized animal does not alter the activity of the Na:K pump so it was unnecessary to include serum with the cells not exposed to anti-L. The suspensions were incubated for 30 min at 32°C and the cells were then removed from the solution and washed three times with 107 mM MgCl_2 solution.

Determination of K Influx

Aliquots of 0.1 ml of packed cells were placed in each of a series of tubes and suspended in 0.1 ml of a solution which usually contained (mM): Na or choline 170, Mg 1, PO_4 27, Cl 128, adenosine 10, and glucose 5, pH 7.4. When required, ouabain 10^{-3} M was included. The tubes were incubated at 37°C, and the flux was started by adding to each tube 1 ml of a solution identical to that in which the cells were suspended except that part of the Na was replaced with K containing ^{42}K . When the concentration of K was varied, Na was kept constant and total cation concentration was maintained with choline. After incubating with shaking for either 0.5 or 1 h at 37°C, the flux was stopped by removing the tubes from the water bath and adding to each 5 ml of ice-cold 107 mM MgCl_2 solution. The cells were separated from the solution and washed twice in 5 ml of 107 mM MgCl_2 solution. After the last wash the cells were hemolyzed in distilled water and aliquots taken for counting and for determination of Na, K, and hemoglobin concentration. Aliquots of the solution containing ^{42}KCl were taken for counting and for determination of K concentration. Sometime during the course of the measurement, a suspension of the original cells was made and the hematocrit and hemoglobin concentration determined; from the ratio of the hematocrit to the hemoglobin concentration of the suspension and from the hemoglobin concentration of the samples counted for radioactivity the volume of cells counted and Na_c and K_c could be calculated (Sachs and Welt, 1967). A sample of the cells was also taken prior to the beginning of the influx measurement for determination of Na_c and K_c . The K influx was calculated according to the equation:

$${}^iM_k = K_c^* \times \frac{K_c}{K_s^*},$$

where iM_k is the K influx (millimoles/liter RBC, h), K_c^* is the radioactivity present in the cells (counts per minute per milliliter cells), K_c the K concentration of the incubating solution (millimoles per liter) and K_s^* the radioactivity in the incubating

solution (counts per minute per milliliter). Active K influx (${}^iM_K^P$) is calculated as the difference between the K influx measured in the absence of ouabain and that measured under identical conditions but in solutions containing 10^{-4} M ouabain.

Determination of K Outflux

The cells were prelabeled by including ${}^{42}\text{KCl}$ in the PCMB solution for the last 3 h of incubation at 0°C . After resealing with DTT solution and washing, the cells were incubated, with shaking, at 37°C in phosphate buffered solutions of composition similar to that of the solutions in which K influx was measured. The hematocrit was about 1%. Samples were taken 5 min after the incubation began and either 1 or 1.5 h later. The cells were separated from the suspension and an aliquot of the supernatant counted; in addition, an aliquot of the whole suspension was also counted. From these determinations the rate constant for K outflux ${}^o k_K$ (h^{-1}) was calculated as previously described (Sachs and Welt, 1967). ${}^o M_K$, the K outflux (millimoles per liter RBC, h) was calculated by multiplying ${}^o k_K$ by the intracellular K concentration determined from an aliquot of the cells used in the outflux measurement.

Na, K-ATPase Assay

Red cell ghosts were prepared by hemolyzing packed washed red cells in 20 vol of an ice-cold solution of 10^{-3} M Na_2EDTA brought to pH 7.0 at 0°C with Tris. The ghosts were separated by centrifugation at 27,000 *g*, frozen and thawed, and then washed until white in an ice-cold solution which contained (mM): NaCl 15.3, Tris Cl 1.7, Na_2EDTA 0.1, pH 7.5 at 0°C . The ghosts were concentrated and stored in this solution at -20°C . The ghosts after thawing were exposed to anti-L by the same method used for exposure of intact cells: 1 vol of ghosts were exposed to 20 vol of antiserum at 32°C for 30 min. After exposure, the ghosts were washed three times in the incubation medium which contained (mM): NaCl 140, KCl 10, Tris Cl 10, MgCl_2 1.25, pH 7.5 at 37°C . The membranes were then suspended in the same medium, incubated at 37°C , and the reaction was started by adding ATP (final concentration 0.1 mM) containing $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$. After a 30-min incubation the reaction was stopped by adding 2 vol of cold 6% perchloric acid. The ghosts were removed by centrifugation and the concentration of ${}^{32}\text{P}_i$ in the supernatant determined after removal of unhydrolyzed ATP by adsorption with charcoal (Heinz and Hoffman, 1965). Protein concentration of the ghost suspension was determined by the method of Lowry et al. (1951). Na, K-ATPase activity was taken as the difference between measurements made in ouabain-free solutions and those made in solutions containing 10^{-4} M ouabain.

ATP and Lactate Assay

ATP and lactate were estimated by enzymatic reactions which result in the stoichiometric oxidation of NADH or reduction of NAD; NADH concentration was measured fluorometrically (Lowry et al., 1964) or spectrophotometrically. 4 vol of a red cell suspension were added to 1 vol of ice-cold 60% perchloric acid solution with constant mixing. The mixture was allowed to stand for 15 min at 0°C and then remixed and centrifuged at 4°C . The clear supernatant was neutralized with KOH, allowed to

stand for 1 h at 0°C, and centrifuged. The supernatant was used for the determination of the concentration of ATP and lactate. ATP was measured with glyceraldehyde phosphate dehydrogenase and phosphoglycerate kinase, and lactate was determined with lactic dehydrogenase (Bergmeyer, 1963).

RESULTS

Fluxes in Fresh Goat Red Cells

Typical values of the K influx in red cells from the three animals used in this study are given in Table I. These measurements were made on fresh cells, i.e., within 5 h of drawing the blood and without altering K_e and Na_e . K_e is

TABLE I
TOTAL K INFLUX (iM_K), LEAK (OUABAIN-INSENSITIVE) K INFLUX (iM_K^L), AND PUMP (OUABAIN-SENSITIVE) K INFLUX (iM_K^P) IN FRESH GOAT CELLS BEFORE AND AFTER EXPOSURE TO THE ANTI-L ANTIBODY (ANTI-L) (K_e WAS 10 mM)

The measurements were made within 5 h of obtaining the blood, and the flux measurements were made in a solution composed of (mM): KCl 10, Tris Cl 10, NaCl 150, MgCl₂ 2.0, CaCl₂ 1.0, glucose 5, pH 7.5 at 37°C. The values are typical for cells from the animals used in this study. In order to minimize variations, all the experiments were performed using cells from two LK and one HK goat.

Goat	K_e	Na_e	Control			Anti-L		
			iM_K	iM_K^L	iM_K^P	iM_K	iM_K^L	iM_K^P
	mmol/liter RBC		mmol/liter RBC, h			mmol/liter RBC, h		
LK-1	35	54	0.412	0.147	0.266	1.165	0.153	1.012
LK-2	31	55	0.399	0.174	0.225	0.731	0.188	0.543
HK	73	10	0.769	0.148	0.621	0.773	0.142	0.641

about half as great in the LK cells as in the HK cells, and Na_e is more than five times greater in the LK cells. The lower K_e of the LK cells is associated with a K pump influx which is about a third as great as that of the HK cells. Exposure to anti-L produces a pronounced stimulation of the K pump influx in the LK cells, but does not affect the K pump influx in the HK cells. In addition, the K leak influx is about the same in HK and LK cells and is unaffected by anti-L.

ATP Concentrations and Lactate Production of Cells with Altered Cation Content

The concentration of ATP in goat red cells is normally less than 0.2 mmol/liter RBC, and the cells contain no detectable 2, 3 diphosphoglycerate (Harkness et al., 1970). It became evident during the course of these studies that cells subjected to alteration of intracellular cation concentrations by treatment with PCMBS were somewhat erratic in their maintenance of ATP concentration and in their production of lactate if glucose was the sole source of energy. However, incubation of PCMBS-treated cells in solutions

containing 27 mM phosphate and 10 mM adenosine in addition to 5 mM glucose resulted in maintenance of ATP at high levels and in adequate production of lactate. Table II gives the results of an experiment in which ATP concentration and lactate production were estimated in HK cells with low and high intracellular K concentrations and in control and anti-L-treated LK cells with low intracellular K concentration. The resealing solution and the incubation solution both contained 27 mM phosphate, 10 mM adenosine, and 5 mM glucose; in addition, the incubation solution contained 10 mM K. In each case the cells produced adequate amounts of lactate and, more important, ATP concentrations were high at the start

TABLE II
ATP CONTENT AND LACTATE PRODUCTION OF GOAT RED BLOOD
CELLS TREATED WITH PCMBS

Cell cation contents were altered by PCMBS treatment as described in the Methods section. After resealing and, where appropriate, treating the cells with anti-L or a phosphate-buffered saline solution, the cells were incubated at 37°C in a solution of the same composition as that used in measuring K influx; K_o was 9.6 mM. Samples were taken for the determination of ATP and lactate at zero time and 1 h later.

Type	Cells		ATP \pm SEM		Lactate \pm SEM
	Na_e	K_e	$t = 0$	$t = 1$ h	
	mmol/liter RBC		mmol/liter RBC		mmol/liter RBC, h
HK	39.9	0.3	0.83 \pm 0.03	0.99 \pm 0.06	3.23 \pm 0.03
HK	42.0	33.0	1.05 \pm 0.01	1.48 \pm 0.02	3.34 \pm 0.02
LK control	45.4	0.7	0.99 \pm 0.05	1.26 \pm 0.03	2.30 \pm 0.02
LK anti-L	41.8	0.6	0.92 \pm 0.02	1.16 \pm 0.08	2.05 \pm 0.06

and rose over the course of the incubation. It seems likely, therefore, that ATP is not rate limiting for the Na-K pump when cells are prepared and incubated in solutions with high concentrations of phosphate and adenosine as will be the case in the experiments considered below.

Effect of Altering Intracellular Na and K on the Active K Influx of HK Cells

The affinity of the pump for intracellular Na and K was investigated by two types of experiment; in one, K_e was held constant and Na_e varied, and in the other Na_e was held constant and K_e varied. In both cases, the total intracellular cation content was held constant with choline. At normal cell volume the total cation content of goat red cells is 80–90 mM RBC. In cells with high K_e , therefore, it was not possible to obtain concentrations of Na_e as high as could be obtained in the low K_e cells.

Fig. 1 presents the results of an experiment in which K_e was held constant in HK cells. It is apparent that at low K_e the increase in the active K influx as Na_e increases is rapid, but in cells with a higher concentration of K the

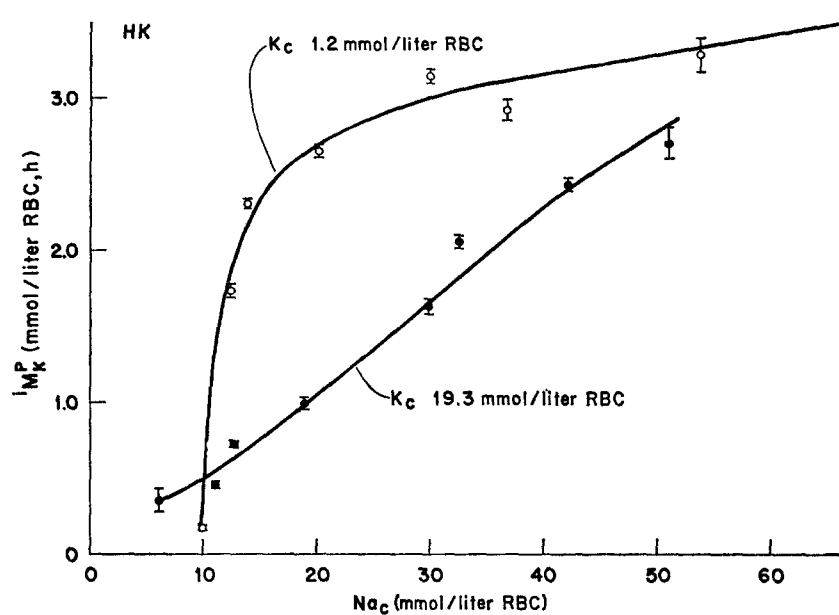


FIGURE 1. $I_{M_K}^P$ (ouabain-sensitive or pump influx of K) vs. Na_c (intracellular Na concentration) in HK goat red cells. K_o during the determination of the flux was 2.6 mM. In this and in succeeding figures (except Figs. 6 and 8) each point is the mean of four determinations \pm SEM. This and subsequent curves were drawn by eye unless otherwise indicated.

active K influx increases much less rapidly with Na_c ; at Na_c 19 mmol/liter RBC, equal to the K_c , the pump rate is only about a third of its maximal value. These findings are similar to those of Hoffman and Tosteson (1971) for sheep red cells, but it should be kept in mind that in the present experiments K_c was held constant as Na_c was varied. In many of these experiments it was not possible to reduce Na_c below 10 mmol/liter RBC when K_c was held at a relatively low concentration. However, Na_c was low enough to eliminate almost completely the ouabain-sensitive K influx.

Fig. 2 presents the results of a representative experiment in which the active K influx in HK cells was measured at constant Na_c . With low Na_c the ouabain-sensitive K influx falls as K_c increases, but becomes constant at 1 mmol/liter RBC, h at high K_c . At high Na_c , the active K influx at first increases as intracellular K increases, reaches a peak at a K_c of 8 mmol/liter RBC, and then decreases as K_c continues to increase.

Taken together, these experiments can be partly explained if intracellular K competes with Na at the pump site at the inner surface of the membrane at which Na stimulates pump activity (Hoffman, 1962; Hoffman and Tosteson, 1971). If Michaelis-Menten kinetics are followed, and if there is a single site

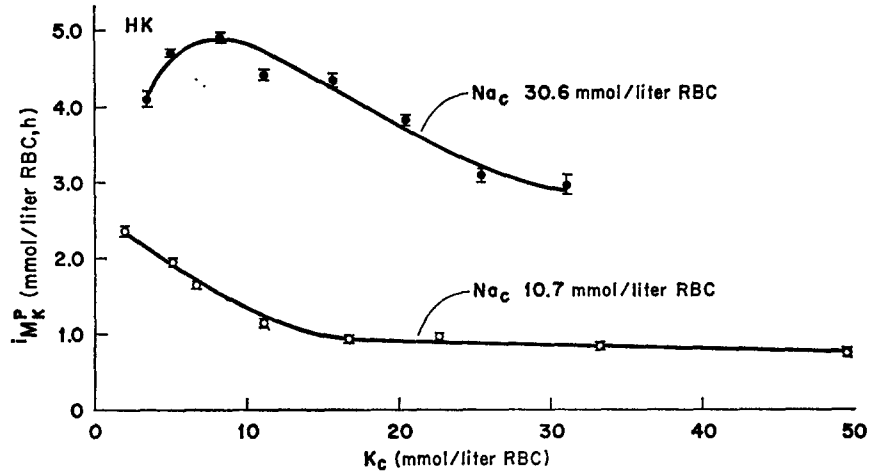


FIGURE 2. iM_K^P vs. K_c (intracellular K concentration) in HK goat red cells. K_o was 9.2 mM.

at which competition between Na_c and K_c occurs, the relation between the active K influx and intracellular Na and K should be described by

$$iM_K^P = \frac{(iM_K^P)_{\max}}{1 + \frac{K_{Na}}{[Na]_c} \left(1 + \frac{[K]_c}{K_K}\right)}, \quad (1)$$

where $(iM_K^P)_{\max}$ is the maximal active K influx at any given $[K]_o$, K_{Na} is the apparent Michaelis constant for Na_c and K_K the inhibitor constant for K_c . The equation predicts that increasing $[K]_c$ will increase K_{Na} as is seen in Fig. 1. If $[Na]_c$ is held constant and $[K]_c$ increases, the equation predicts that the active K influx will fall monotonically with increasing $[K]_c$ and approach zero as a limit. The results plotted in Fig. 2 deviate from these predictions in two respects. At high Na_c (top curve) the active K influx at first increases with increasing K_c . Such an effect cannot be due to K_c acting as a competitive inhibitor and must arise from some other cause; this will be further discussed below. At low Na_c (bottom curve), the active K influx falls with increasing K_c but does not approach zero as a limit. Such a deviation might be explained by at least two mechanisms. If there is a significant ouabain-sensitive K-K exchange (Glynn et al., 1970), it is possible that the ouabain-sensitive K influx at high K_c is in large part accounted for by the influx component of the exchange; evidence will be presented below that a ouabain-sensitive K outflux can be demonstrated in these cells which may reflect the existence of a K-K exchange. An alternative explanation for the failure of the ouabain-sensitive K influx to approach zero as K_c increases is that the pumps in these cells are not homogeneous as far as their relative affinities for intracellular Na and K

are concerned. If some of the pumps have a much lower affinity for K_e than the rest, it would be expected that a plot of the active K influx vs. K_e will not approach zero within the range of concentrations used.

Response of the Ouabain-Sensitive K Influx of LK Cells to Alterations in Na_e and K_e and the Effect of Anti-L

The results of an experiment in which the active K influx was measured in LK cells at constant low K_e and varying Na_e are shown in Fig. 3; the upper

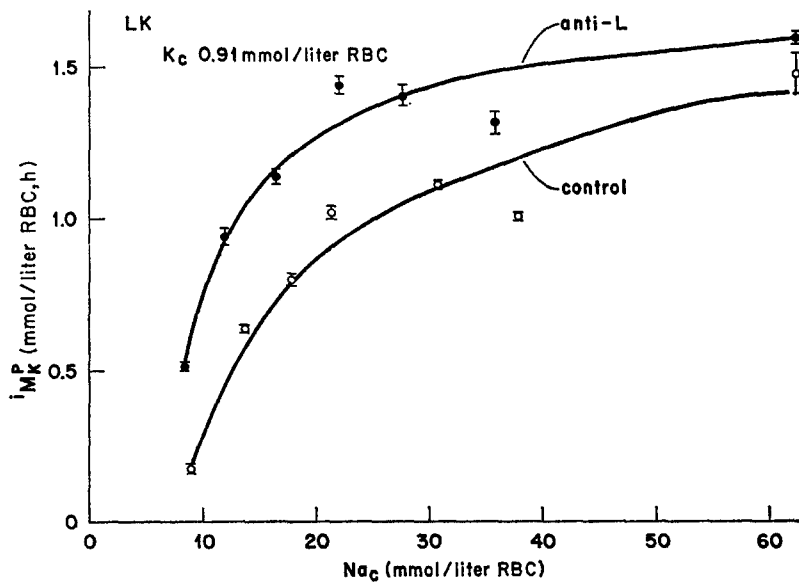


FIGURE 3. $i_{M_K}^P$ vs. Na_e in LK goat red cells. The two curves were obtained from experiments on the same batch of cells; cells used for the upper curve were exposed to anti-L. K_e during the determination was 2.5 mM.

curve is for cells exposed to anti-L and the lower curve is for control cells. The curve for the control cells is similar to the comparable curve obtained with HK cells (upper curve of Fig. 1) except that the absolute values of the fluxes are much lower and the curve for the LK cells is shifted to the right. Exposure of the cells to anti-L shifts the curve to the left and, except for the lower absolute values of the fluxes, the curve for the anti-L-treated cells is about superimposable on the comparable curve for HK cells (upper curve of Fig. 1). It appears from Fig. 3 that even at very low intracellular K concentrations anti-L increases the apparent affinity of the pump for Na_e . It is not clear, however, that this is a direct effect of the antibody on K_{Na} (Eq. 1); it will be shown below that LK cells are so sensitive to inhibition by K_e at low

Na_e that the antibody may be accomplishing its effect on the apparent affinity for Na_e by decreasing the affinity for K_e (i.e., by increasing K_K) even though K_e is very low.

Fig. 4 is a plot of the active K influx in LK cells, both control and anti-L-treated, as a function of Na_e in cells with a higher K_e than in Fig. 3, 18.6 mmol/liter RBC. In control cells the active K influx begins to increase rapidly only at concentrations of Na_e higher than about 40 mmol/liter RBC. Anti-L causes a greater active K influx at each Na_e , but the active K influx for the

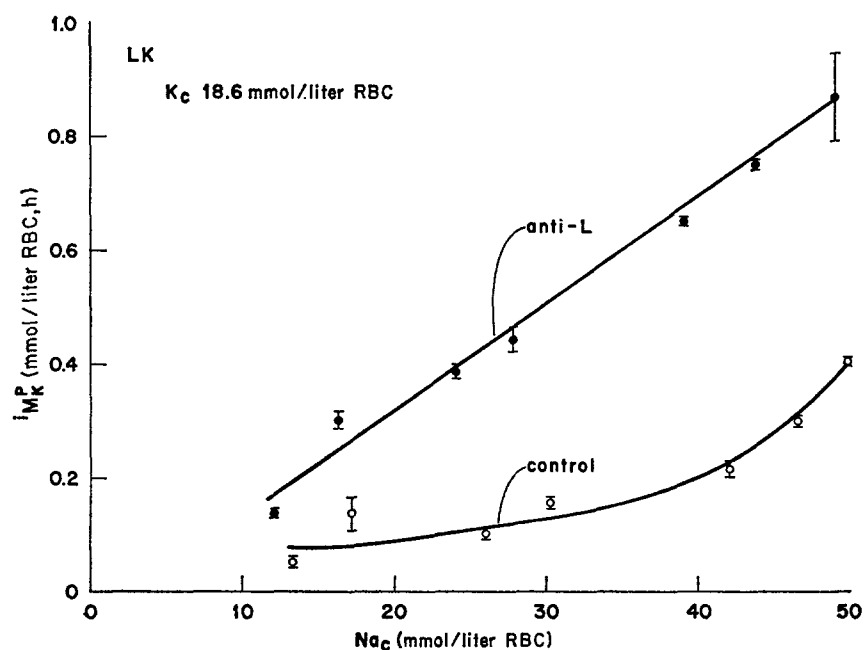


FIGURE 4. iM_K^P vs. Na_e in LK goat red cells. The upper curve was obtained from cells exposed to anti-L. K_e was 2.0 mM.

anti-L-treated LK cells does not reach that obtained from HK cells under similar circumstances (Fig. 1, lower curve). Anti-L therefore increases the apparent affinity of the pump for Na_e in high K_e LK cells, but not to the level found in HK cells of similar K content.

The relation between the ouabain-sensitive K influx in LK cells and the intracellular K concentration at a constant low Na_e is shown in Fig. 5. In both control and anti-L-treated cells the K influx decreases as K_e increases, but, as with HK cells (Fig. 2), does not approach zero as a limit at very high K_e . Fig. 6 is a plot of the reciprocal of the active K influx against the intracellular K concentration from the data of Fig. 5. If the effect of K_e is solely

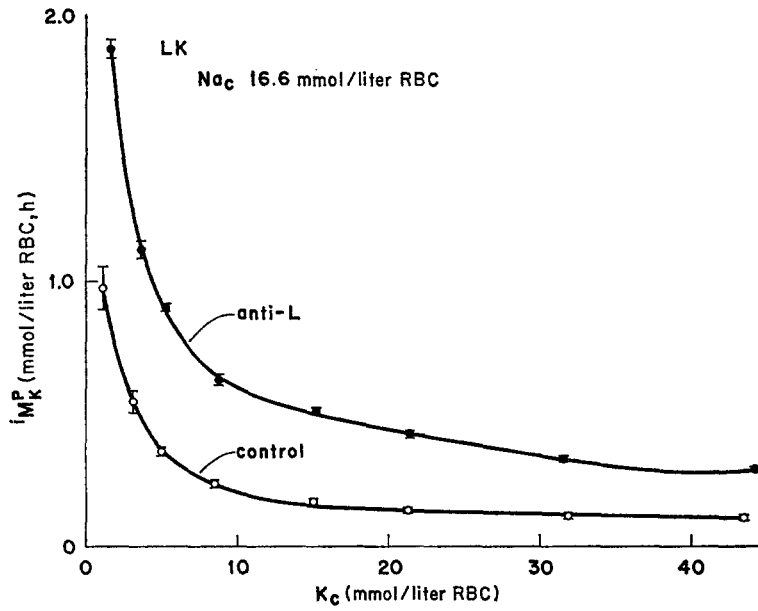


FIGURE 5. iM_K^P vs. K_c in LK goat red cells. The upper curve is for cells exposed to anti-L. K_o was 9.2 mM.

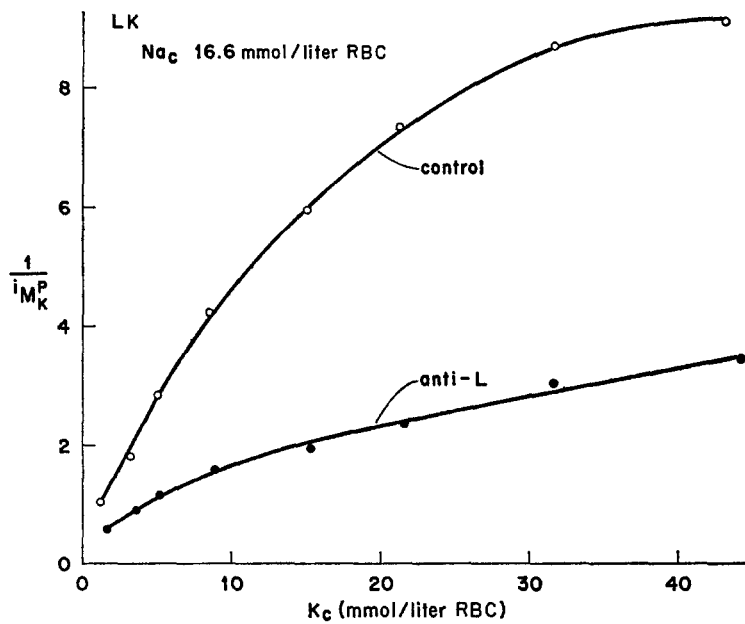


FIGURE 6. $1/iM_K^P$ vs. K_c in LK goat red cells. The data are from the experiment plotted in Fig. 5.

due to competitive inhibition of the activation of the pump by Na_e , then, from Eq. 1:

$$\frac{1}{iM_K^P} = \frac{1}{(iM_K^P)_{\max}} + \frac{K_{\text{Na}}}{(iM_K^P)_{\max} [\text{Na}]_e} \left(1 + \frac{[\text{K}]_e}{K_K} \right), \quad (2)$$

and the reciprocal of the active K influx should increase linearly with K_e if Na_e is constant. The deviation from linearity in Fig. 6 reflects the failure of the active K influx to approach zero at high K_e seen in Fig. 5; obviously, $1/iM_K^P$ is too low at high K_e because iM_K^P is too high.

Fig. 7 is a plot of the active K influx in LK cells with a higher fixed Na_e than in Fig. 5. Fig. 8 is a plot of the reciprocal of the active K influx vs. K_e from the data of Fig. 7. Straight lines of Fig. 8 were obtained by the method of least squares (the point for the lowest K_e was excluded); the theoretical curves obtained fit the data fairly well both when plotted directly (Fig. 7) and as the reciprocal of the flux (Fig. 8) if in each case the point for the lowest K_e is ignored. The deviation of the reciprocal plot from linearity seen when the measurement is made with low Na_e cells (Fig. 6) is not apparent when cells with higher Na_e are used.

If the curves of Figs. 6 and 8 are extrapolated to zero K_e , the intercept at the ordinate in each case is about the same for the experiment with anti-L-

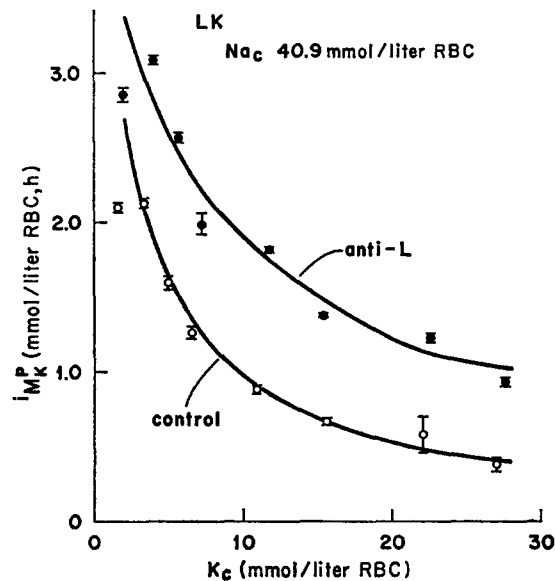


FIGURE 7. iM_K^P vs. K_e in LK goat red cells with high Na_e . The upper curve is for cells exposed to anti-L. The upper curve is a plot of the function (obtained from Fig. 8) $iM_K^P = 4.17/[1 + (K_e/8.3)]$; the lower curve is $iM_K^P = 4.78/[1 + (K_e/2.56)]$. K_e was 9.4 mM.

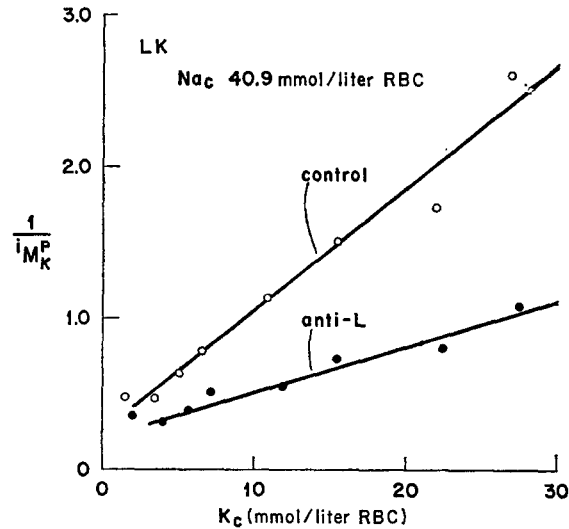


FIGURE 8. $1/iM_K^P$ vs. K_e ; the data are from the experiment plotted in Fig. 7. The lines were fitted to the points (excluding in each case the point at the lowest K_e) by the method of least squares. The line for the experiment using anti-L-treated cells is $1/iM_K^P = 0.209 + 0.0289 K_e$ and that for the control cells is $1/iM_K^P = 0.209 + 0.0816 K_e$.

treated cells as for that with control cells. From Eq. 2 it is seen that at zero K_e :

$$\frac{1}{iM_K^P} = \frac{1}{(iM_K^P)_{\max}} \left(1 + \frac{K_{Na}}{Na_e} \right).$$

If K_{Na} is not much affected by anti-L (Fig. 3), and if $[Na]_e$ is much greater than K_{Na} , as is the case in the experiments of Fig. 8, then the intercept at zero $[K]_e$ should be proportional to $(iM_K^P)_{\max}$, the maximal K influx as a function of K_e and Na_e . Since the intercept is the same for the curves from the anti-L treated cells and from the control cells, it is suggested that anti-L exerts its effect only by altering the relative affinities of the pump for Na_e and K_e without increasing the maximal pump activity. However, the conditions under which these measurements were made were such that it is not clear from the direct plots of Fig. 5 and 7 that anti-L did not increase the active K influx in cells with very low K_e . Therefore an experiment was performed in which the average K_e was kept lower by using a lower K_o and by making the measurement of K influx over a half-hour period; Na_e was kept as high as possible in order to reduce the effect of whatever K_e was present. The results of the experiment are in Fig. 9. At the very lowest K_e , the effect of anti-L on the cells of both LK animals is very small but increases as K_e increases. Even though anti-L has very little effect in the absence of K_e , nevertheless LK cells with very low K_e have an active K influx markedly lower than that present in HK cells of similar cation content. Since only three animals were used in

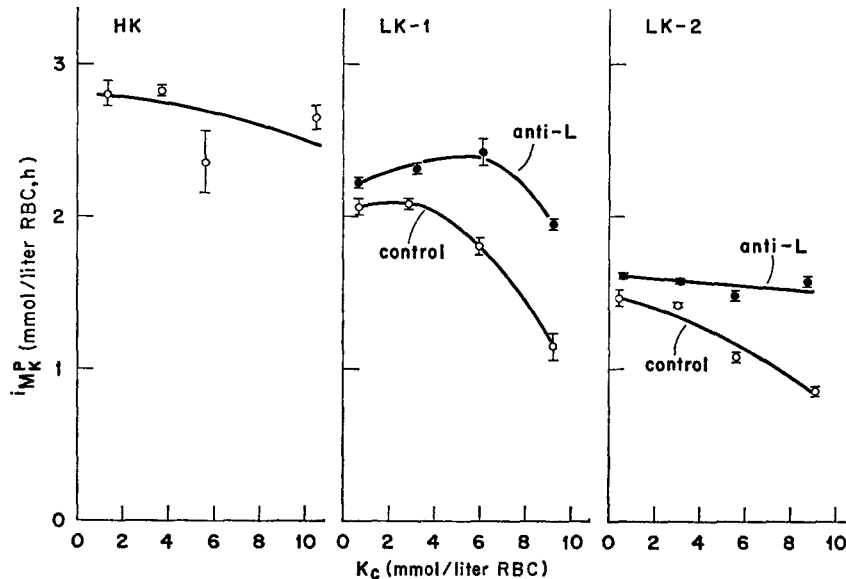


FIGURE 9. iM_K^p vs. K_e in HK cells and control and anti-L-treated LK cells from two animals. The average Na_e during the course of the experiment was 63.2 mmol/liter RBC for the HK cells, 64.2 for the LK-1 cells, and 60.2 for the LK-2 cells. K_o was 2.7 mM.

these studies, it is possible that the differences between the pump rates of the LK cells and that of the HK cells at very low K_e can be attributed to individual variations in pump activity not associated with the HK-LK dimorphism. Ellory and Tucker (1970a) have reported values of active K uptake of cells from four HK goat (species unspecified) and of anti-L-treated cells from nine LK goats; the range for the HK cells was 0.542–0.862 mmol/liter RBC, h and for the anti-L-treated LK cells 0.643–1.341 mmol/liter RBC, h. On the other hand, it is possible that the pumps of LK cells, in addition to being more sensitive to competitive inhibition by K_e , differ in some other way from those of HK cells or that there are fewer pumps per LK cell. If this is so, the second difference is not altered by treatment with anti-L.

Binding of Anti-L to LK Cells with Very Low K_e

The fact that anti-L had very little effect in the very low K_e cells might be due to a failure of the antibody to bind to such cells. In order to evaluate the possibility that anti-L does not bind unless K is present inside the cell, the binding of anti-L to permeable (frozen-thawed) ghosts was evaluated in solutions with and without K. The ghosts were exposed either to anti-L or to a control solution, washed, and the Na,K-ATPase activity was measured; the results are in Table III. Since anti-L pretreatment stimulates Na,K-ATPase activity whether or not K was present during the antibody treatment,

anti-L binding apparently does not require K_e . This conclusion was supported by a second kind of experiment using intact cells. In the experiment summarized in Table IV, cells with low K_e were exposed to anti-L or to a control

TABLE III

EFFECT OF K ON THE BINDING OF ANTI-L TO LK GHOSTS

Ghosts were exposed to anti-L which had been dialyzed against a Tris-buffered saline solution containing either K 0.02 mM or 1.1 mM; control ghosts were simultaneously exposed to a solution identical to that against which the antiserum had been dialyzed and containing the same K concentrations. The ghosts were then washed and incubated at 37°C in a solution containing (mM): NaCl 140, KCl 10, Tris Cl 10, MgCl₂ 1.25, pH 7.5 at 37°C, with and without ouabain 10⁻⁴M. The reaction was started by adding [γ^{32} P]-ATP at a final concentration of 100 μ M. The determinations were done in quadruplicate.

[K] _o in AL solutions	Ouabain-sensitive Na, K-ATPase		
	Control	Anti-L exposed	Δ
<i>mM</i>	<i>μmol P_i hydrolyzed/mg protein \times h \pm SEM</i>		
0.02	1.21 \pm 0.22	8.29 \pm 1.03	7.08 \pm 1.05
1.1	3.41 \pm 0.26	10.87 \pm 0.26	7.46 \pm 0.36

TABLE IV

EFFECT OF K_e ON THE BINDING OF ANTI-L TO INTACT LK RED CELLS

Intracellular cation concentration was altered as described in the Methods section. The cells were then exposed to anti-L (anti-L-treated) or to a solution of composition similar to that against which the serum was dialyzed (control). Half of each batch was then incubated in a phosphate buffered choline chloride solution at 37°C for 0.5 h; the other half was incubated in a phosphate buffered KCl solution. The cells were then washed three times in MgCl₂ solution and ouabain-sensitive $^3M_K^P$ determined in a phosphate buffered NaCl solution. K_e during the measurement of $^3M_K^P$ was 2.2 mM.

	After incubation in 160 mM choline		After incubation in 160 mM K	
	K_e	$^3M_K^P \pm SEM$	K_e	$^3M_K^P \pm SEM$
	<i>mmol/liter RBC</i>	<i>mmol/liter RBC, h</i>	<i>mmol/liter RBC</i>	<i>mmol/liter RBC, h</i>
Control	1.3	1.130 \pm 0.030	4.8	0.936 \pm 0.016
Anti-L-treated	1.0	1.139 \pm 0.021	4.2	1.156 \pm 0.022

solution of ionic composition similar to that of the solution containing the antibody. The cells were then washed, and half of each batch was incubated in a solution with a very high concentration of K in order to raise K_e ; the other half were incubated in a K-free solution. The cells were again washed, and the active K influx determined. If anti-L was bound to the low K_e cell, it

was expected that, even though there was no stimulation of the active K influx in the cells which remained low K_e , a stimulation would be seen in the cells in which K_e was raised. The expectation was fulfilled, and it seems clear that the ineffectiveness of anti-L in low K_e cells is not due to a failure of the antibody to bind.

Stimulation of Active K Influx in HK Cells by K_e

The stimulation of the ouabain-sensitive K influx in HK cells by increasing concentrations of K_e described above (Fig. 2) was unexpected; at the time we first noticed this effect (Ellory et al., 1972) we were not aware of any other circumstance in which intracellular K serves as an activator of the Na:K pump. But recently Garay and Garrahan (1973) reported that the maximal value of the ouabain-sensitive Na outflux from human red blood cells into solutions containing Na and K is stimulated as intracellular K increases; the effect was reported to be maximal at a K_e of about 30 mmol/liter RBC water.

To further characterize this phenomenon in HK goat cells, the active K influx was measured as a function of K_e at four different Na concentrations; the measurements were made at a relatively low K_e (Fig. 10) and at a higher K_e (Fig. 11). At the lowest Na_e , K_e acts solely as an inhibitor of the active K influx at both extracellular K concentrations. Activation of the K influx becomes apparent at higher intracellular Na concentrations. In the experiment performed at low K_e (Fig. 10), the stimulation by K_e even at the highest Na_e

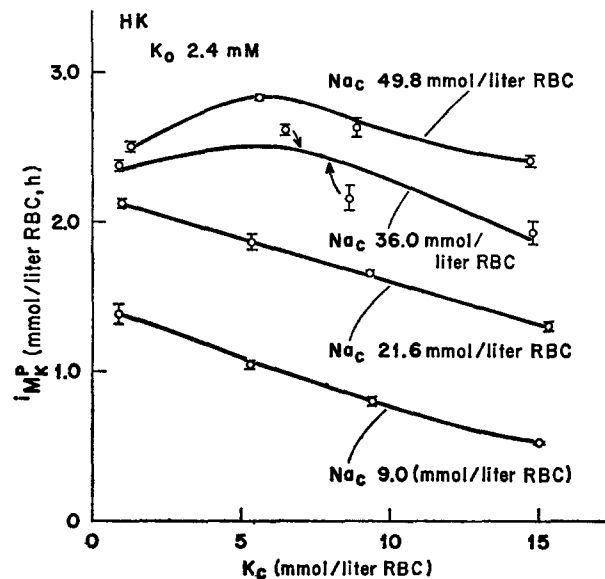


FIGURE 10. $i_{M_K}^P$ vs. K_e in HK cells at four intracellular Na concentrations. K_o was 2.4 mM.

was small and apparently reached a maximum at a relatively low K_e ; as K_e increased further, the active K influx was inhibited. However, in the experiment performed at high K_e (Fig. 11), K_e stimulated the K influx in the cells with the highest Na_c to a greater degree and the stimulation continued to higher intracellular K concentrations.

The effect of intracellular K thus appears to be biphasic. K_e competitively inhibits the activation of the pump by Na_c ; this effect is best seen at low intracellular Na concentrations. Under other circumstances, K_e seems to increase

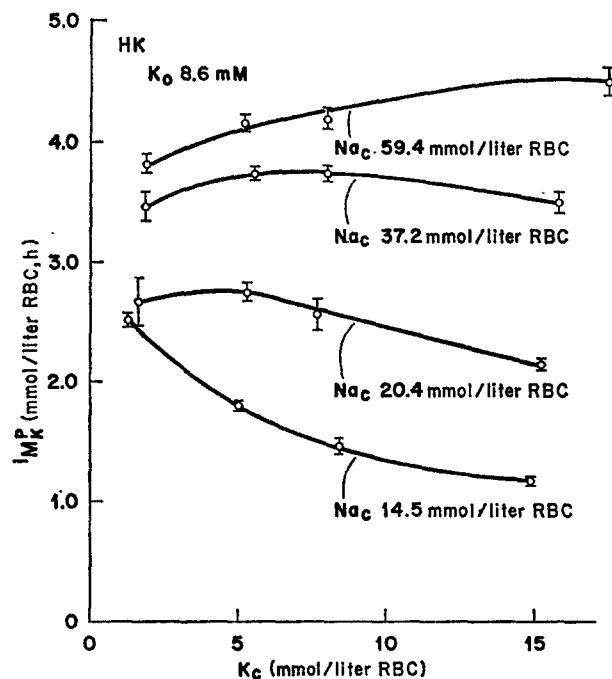


FIGURE 11. ${}^iM_K^P$ vs. K_c in HK cells at four intracellular Na concentrations. K_e was 8.6 mM.

the ouabain-sensitive K influx; this effect is best seen at high intracellular Na concentrations and was most apparent when the measurements were made at high K_e . The competitive inhibition appears straightforward, but the effect of K_e as a stimulator of the K influx is more ambiguous. While it is possible that K_e is increasing the activity of the Na:K pump as suggested by Garay and Garrahan (1973) for human red cells, an alternative mechanism might be operative. One such mechanism is a ouabain-sensitive K:K exchange. Such an exchange, which does exist in these cells (Ellory and Lew, 1969), would cause the ouabain-sensitive K influx to increase as intracellular K increases as a result of an increasing K:K exchange. This will be evaluated in the next section.

Ouabain-Sensitive K Outflux

It is important to have an estimate of the ouabain-sensitive K:K exchange in these cells both to evaluate the significance of the stimulation of the ouabain-sensitive K influx by K_e in HK cells and to find an explanation for the failure in both the HK and LK cells of the K influx to approach zero as K_e increases (Figs. 2, 5, and 7). In principle the best measurement to make is the ouabain-sensitive K influx in cells with very low Na_e (so that the Na:K exchange is inoperative) as a function of K_e . Under such circumstances, a ouabain-sensitive K influx which depends on intracellular K can be interpreted as a K:K exchange. While it is possible in Na-free human cells to demonstrate a ouabain-sensitive K influx which is dependent on intracellular K (Post and Sen, 1965; Sachs, 1972), such measurements were not possible in goat cells since we were unable to consistently reduce Na_e low enough to be sure that Na:K exchange was completely eliminated. The possibility of a ouabain-sensitive K:K exchange was therefore evaluated by measuring the ouabain-sensitive K outflux into solutions containing K. If it is assumed that the ouabain-sensitive K outflux into solutions containing Na and K represents a K:K exchange, and if the exchange is linked in a 1:1 ratio, then the outflux can be taken as equal to the ouabain-sensitive K influx occurring by the exchange pathway in the same solutions.

Table V contains the results of experiments in which the potassium outflux (oM_K) was measured in HK cells. In solutions containing Na and K, a

TABLE V
K OUTFLOW (oM_K) IN HK GOAT RED CELLS

Solution: Na_e 162 mM K_e 9.6 mM					
Na_e	K_e	Ouabain-sensitive $^oM_K \pm SEM$	Na_e	K_e	Ouabain-sensitive $^oM_K \pm SEM$
<i>mmol/liter RBC</i>		<i>mmol/liter RBC, h</i>	<i>mmol/liter RBC</i>		<i>mmol/liter RBC, h</i>
58.5	2.1	0.025 \pm 0.001	0.9	1.2	0.090 \pm 0.003
56.2	13.1	0.145 \pm 0.008	0.7	11.1	0.431 \pm 0.031
47.3	33.6	0.110 \pm 0.040	0.7	32.5	0.449 \pm 0.039
Solution: Na_e 0					
Na_e	K_e	$^oM_K \pm SEM$ K_e 9.6 mM	$^oM_K \pm SEM$ K_e 9.6 mM ouabain 10^{-4} M	$^oM_K \pm SEM$ K_e 0 ouabain 0	
<i>mmol/liter RBC</i>			<i>mmol/liter RBC, h</i>		
39.1	5.7	0.182 \pm 0.004	0.134 \pm 0.003	0.073 \pm 0.006	
37.6	27.4	0.858 \pm 0.022	0.633 \pm 0.022	0.370 \pm 0.011	
1.1	3.0	0.248 \pm 0.010	0.064 \pm 0.002	0.176 \pm 0.003	
1.0	23.8	1.516 \pm 0.048	0.507 \pm 0.026	0.694 \pm 0.036	

ouabain-sensitive K outflux can be demonstrated which appears to be saturated at a K_e of about 13 mmol/liter RBC. The maximum value of the K outflux in cells containing Na (0.145 mmol/liter RBC, h) is considerably smaller than the outflux from the low Na cells (0.449 mmol/liter RBC, h). If there is an equal ouabain-sensitive influx which exchanges for this outflux, then in going from zero K_e to a K_e of 13 mmol/liter RBC in high Na cells there should be an increase in the ouabain-sensitive K influx of about 0.15 mmol/liter RBC, h independent of the Na:K exchange. The actual increase as a result of increasing K_e in the ouabain-sensitive K influx of the cells with the highest Na_e (Fig. 11) was about 0.7 mmol/liter RBC, h. The mechanism of K:K exchange does not explain the stimulation by K_e of the ouabain-sensitive K influx.

The K:K exchange may, however, partly explain the failure of the ouabain-sensitive K influx in low Na HK cells to approach zero at high K_e (Fig. 2). The ouabain-sensitive K outflux in low Na HK cells (0.45 mmol/liter RBC, h) is almost as great as the ouabain-sensitive K influx in low Na HK cells at very high K_e (0.75 mmol/liter RBC, h).

The lower half of Table V gives the results of measurements of K outflux into Na-free solutions. If the ouabain-sensitive outflux is, indeed, a K:K exchange it should decrease if K is removed from the solution. Since it appears that in human red cells the pump can reverse in solutions free of K (Glynn et al., 1970) with a ouabain-sensitive K outflux exchanging for a ouabain-sensitive Na influx, the effect of the removal of K must be tested in Na-free solutions since reversal requires external Na. From the data of Table V it can be seen that in high Na_e cells removal of K has an even greater effect on the K outflux than the addition of ouabain; apparently there is a ouabain-insensitive K:K exchange in addition to the ouabain-sensitive one (Dunham and Bleier, 1973). In low Na_e cells removal of K reduces the K outflux, but in this case the effect is less than that of ouabain. In both cases removal of K reduced the K outflux supporting the assumption that the ouabain-sensitive K outflux reflects at least in part K:K exchange.

Measurements of the ouabain-sensitive K outflux were made in LK cells and the results are in Table VI. Although the results show a great deal of variability, the ouabain-sensitive K outflux seems to be greater in low Na cells than in the high and the fluxes are higher in the anti-L-treated cells than in the control. Qualitatively, this is what one would expect if the failure of the curves in Fig. 5 to approach zero as K_e increases is due to the operation of a K:K exchange. The deviation from a zero asymptote is greater for the cells treated with anti-L, and there is not much deviation in the high Na cells (the reciprocal plots of Fig. 8 are adequately described by straight lines). The quantitative comparison is not good enough to be certain that the alternate explanation can be ruled out, i.e., that some pumps have a very low affinity for K_e .

TABLE VI
K OUTFLOW ($^{\circ}M_K$) IN LK GOAT RED CELLS

Solution: Na _o 162 mM K _o 9.6 mM		Control	Anti-L-treated
Na _e	K _e	Ouabain-sensitive $^{\circ}M_K \pm \text{SEM}$	Ouabain-sensitive $^{\circ}M_K \pm \text{SEM}$
<i>mmol/liter RBC</i>		<i>mmol/liter RBC, h</i>	
42.1	3.0	0.002±0.019	0.053±0.028
42.3	13.5	0.015±0.051	0.074±0.022
1.2	3.2	0.057±0.008	0.089±0.013
1.1	11.8	0.026±0.051	0.297±0.057

Effect of Anti-L Exposure on the Affinity for K_o

Since treatment of LK cells with anti-L produces cells with greatly altered affinity for K at the inner surface of the membrane, it was of interest to determine whether the antibody also affected the affinity of the pump for extracellular K. Fig. 12 gives the results of an experiment in which the ouabain-

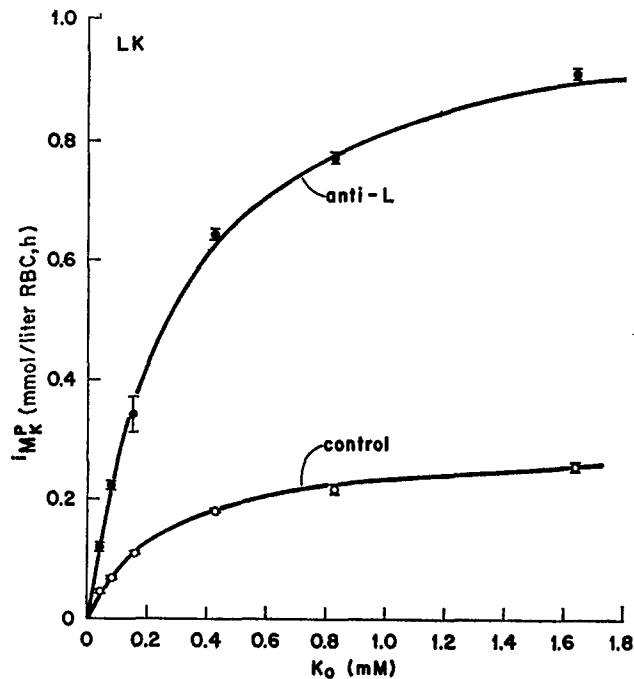


FIGURE 12. iM_K^p vs. K_o in LK goat red cells. The measurements were made using fresh cells in a solution which contained (mM): Choline Cl 150, MgCl₂ 2.0, CaCl₂ 1.0, Tris Cl 10, glucose 5, pH 7.4 at 37°C. KCl was replaced by Choline Cl. The curves (obtained from double reciprocal plots by the method of least squares) are: anti-L $iM_K^p = 1.06/[1 + (0.306/[K_o])]$, control $iM_K^p = 0.296/[1 + (0.264/[K_o])]$.

sensitive K influx was measured at varying concentrations of K_e using anti-L-treated and control LK cells. The maximal pump rate is much higher in the antibody-treated cells and the concentration of K at which the active K influx is half maximal (determined from double reciprocal plots) is somewhat greater in the anti-L-treated cells (0.31 mM) than in the control cells (0.26 mM). Since the kinetics of the Na:K pump in goat cells appear to be complex, it may be that the change in the apparent $K_{1/2}$ for K_e in the anti-L-treated cells if significant is in some way related to the change in the affinity for K at the inner membrane surface, or may be related to the maximal pump rate. It has been reported that anti-L does not alter the apparent $K_{1/2}$ for extracellular K in sheep cells (Ellory and Tucker, 1970 *b*; Lauf et al., 1970).

DISCUSSION

There were two main findings of this study: (*a*) In HK cells intracellular K not only acts as an inhibitor of the Na:K pump, but, at low levels of K_e , intracellular K stimulates the pump, and (*b*) the stimulation of the Na-K pump in LK cells by anti-L is largely or entirely through its reduction of the affinity of the intracellular aspect of the pump for K at the site on the pump where K acts as an inhibitor.

An unusual finding in these experiments was the biphasic effect of intracellular K on the pump rate of HK cells (Figs. 2, 11). As K_e rose from very low levels the ouabain-sensitive K influx at first increased and then decreased; it was shown that the stimulatory effect of intracellular K cannot be entirely explained as a manifestation of a K:K exchange. Intracellular K is therefore both a stimulator and an inhibitor of the pump in these cells, and it seems necessary to assume that K_e must interact with two different types of sites associated with each pump, one stimulatory and one inhibitory. A reasonable mechanism for the inhibitory effect is competitive inhibition by K_e of the interaction of Na_e with the Na pump sites; such competition between K_e and Na_e has been proposed to explain the inhibition of the pump by K_e in human cells (Hoffman, 1962) and in HK and LK sheep cells (Hoffman and Tosteson, 1971). The way in which K_e exerts its stimulatory effect is less obvious. One attractive possibility is to suppose that the stimulatory effect is exerted by combination of K_e with the K translocation sites. If the combination of intracellular Na with the pump occurs more rapidly if the K translocation sites are at the inside surface of the membrane and combined with K than if they are at the inside surface of the membrane and not combined with K, stimulation of the pump rate by intracellular K might be expected. Such a mechanism implies that the pump sites which combine with Na and those which combine with K are distinct. The mechanism also implies that when K_e is stimulatory the rate-limiting step in the operation of the pump is the step in which the pump interacts with Na_e . This may account for the observation that the stimulation by K_e is greater at high K_e than at low; at low K_e the

rate-limiting step might be the combination of the pump with K_e , while at higher K_e interaction with Na_e becomes rate limiting. Presumably the stimulation is better seen at high Na_e than at low because the affinity of the Na translocation site for K_e is so great that competitive inhibition between K_e and Na_e predominates at all but the highest Na_e . Stimulation of the pump by K_e is not clearly present in LK cells. However, if in these cells the sole effect of K_e is that of competitive inhibition of the combination of the pump with Na_e , it would be expected that the greatest rate of fall of pump rate with K_e would occur at the lowest K_e . Examination of Figs. 7 and 9 shows that this is not the case, so that perhaps K_e stimulation of the pump rate occurs in LK cells too but is masked by the much greater affinity for K_e of the Na sites of LK cells (even when treated with anti-L). As mentioned above, Garay and Garrahan (1973) have recently reported that K_e stimulated the pump rate in human cells.

The stimulation by anti-L antibody of the pump of LK goat cells seems to be due almost entirely to its alteration of the relative affinity of the pump for Na_e and K_e at the Na translocation site; the relative affinity for K_e is greatly reduced. It is not possible to be certain that anti-L does not increase the affinity of the pump for Na_e since anti-L reduced the apparent $K_{1/2}$ for Na_e even in LK cells with very low K_e (Fig. 3), perhaps due to the very high affinity of these cells for K_e . Nevertheless, the effect was small and it seems likely in view of all the evidence that the effect of anti-L is to reduce the affinity of the pump for K_e . There is very little effect of anti-L on the activity of the pump in LK cells when intracellular K is very low, and there is a small effect of questionable significance on the affinity for K_e . The failure of anti-L to stimulate the pump in low K_e cells is not due to a failure of the antibody to bind. It should be pointed out that it is not necessarily the case that all the pumps in the LK cells have the same relative affinity for Na_e and K_e ; it is possible that the pumps are heterogeneous and vary in their relative affinities for the intracellular cations. This action of anti-L is of particular interest since the effect of the antibody, which binds to the outside of the membrane, is manifest as a specific change in a functional characteristic at the inner aspect of the pump.

It has been reported that in LK sheep red cells the effect of anti-L is both to stimulate the maximal pump rate at very low K_e and to decrease the affinity of the pump for K_e (Lauf et al., 1970). Schmidt (1973) has fitted these data by a kinetic model which postulates that K_e competes with Na_e at the inner surface of the cell but that it requires two K ions to inhibit the pump; using this model it is necessary to postulate that anti-L both increases the maximal pump rate and lowers the affinity of the pump for K_e . On the other hand, Glynn and Ellory (1972) have reported that the same data can be fitted by a Michaelis-Menten model which assumes that K_e and Na_e compete at a single site; in this case it is necessary to assume only that anti-L decreases the

affinity of the pump for K_e and does not alter the maximal pump rate. In the experiments reported by Lauf et al., (1970) the lowest K_e obtained was 1.5% of the total cation content; assuming that the total cell cation content was 100 mmol/liter RBC, this means that K_e was 1.5 mmol/liter RBC. LK sheep cells seem to be even more sensitive to inhibition by K_e than goat cells and it seems likely that the conflict will not be resolved until the effect of anti-L is studied in LK sheep cells with K_e considerably lower than 1 mmol/liter RBC. Glynn and Ellory (1972) have reported that the effect of anti-L on the Na,K ATPase activity of LK goat membranes can be explained if it is supposed only that the antibody alters the relative affinity of the Na site for Na and K, which is consistent with the conclusions drawn from the present study.

Although the stimulation of the pump rate by anti-L in LK goat red cells occurs only in cells with intracellular K, Blostein et al. (1971) have reported that treatment of LK sheep red cell membranes with anti-L increases the Na-activated ATPase activity even in the absence of K.

The pump rate in LK cells in which K_e is very low or in anti-L-treated LK cells is less than the pump rate of HK cells when the measurements are made under similar circumstances. It was suggested above that since only one HK and two LK animals were used in these experiments, the differences may simply be due to individual variations among the animals independent of the HK-LK dimorphism, but such a fortuitous occurrence seems unlikely. Other possibilities for the lower maximal pump activity in LK cells might be suggested. It is possible that the absolute number of pumps in LK cells is less than that in HK cells as is the case for sheep cells. A second possibility is that the affinity of LK pumps for some substrate other than Na or K (e.g., Mg or ATP) is different than the affinity of HK pumps. We have investigated the effect of manipulation of intracellular Mg on the pump rate of HK and LK cells; the response of the two cell types seems to be the same. Finally, it is possible that the turnover rate of LK cells at optimal substrate concentrations is lower than that of HK cells. Whatever the cause of the difference in the maximal pump rate in HK and LK cells, it is not altered by treatment of the LK cells with anti-L.

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