Passive Potassium Transport in LK Sheep Red Cells

Effects of Anti-L Antibody and Intracellular Potassium

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ABSTRACT   The passive K influx in low K (LK) red blood cells of sheep saturates with increasing external K concentration, indicating that this mode of transport is mediated by membrane-associated sites. The passive K influx, $^iM_K^i$, is inhibited by external Na. Isoimmune anti-L serum, known to stimulate active K transport in LK sheep red cells, inhibits $^iM_K^i$ about twofold. $^iM_K^i$ is affected by changes in intracellular K concentration, $[K]_i$, in a complex fashion: increasing $[K]_i$ from near zero stimulates $^iM_K^i$, while further increases in $[K]_i$, above 3 mmol/liter cells, inhibit $^iM_K^i$. The passive K influx is not mediated by K-K exchange diffusion. The effects of anti-L antibody and $[K]_i$ on passive cation transport are specific for K: neither factor affects passive Na transport. The common characteristics of passive and active K influx suggest that $^iM_K^i$ is mediated by inactive Na-K pump sites, and that the inability to translocate Na characterizes the inactive pumps. Anti-L antibody stimulates the K pump in reticulocytes of LK sheep. However, anti-L has no effect on $^iM_K^i$ in these cells, apparently because reticulocytes do not have the inactive pump sites which, in mature LK cells, are a consequence of the process of maturation of circulating LK cells. The results also indicate that anti-L alters the maximum velocity of both active and passive K fluxes by converting pumps sites from a form mediating passive K influx to an actively transporting form.

INTRODUCTION

This report is concerned with the effects of isoimmune anti-L serum and intracellular potassium on passive potassium transport in low K (LK) sheep red blood cells. The results are considered in terms of the relationship between the mechanisms for active and passive transport of K in these cells.

Sheep are dimorphic with respect to the ability of their red cells to pump Na and K. Red cells from HK sheep, with high K and low Na concentrations, have higher pump fluxes than LK cells (high Na and low K concentrations) (Kerr, 1937; Evans, 1954; Tosteson and Hoffman, 1960). This genetically determined dimorphism (Evans and King, 1955; Evans et al., 1956) is due in part to a difference in number of active transport sites per cell (Dunham and Hoffman, 1971a). The two cell types also differ in kinetic properties of the pump sites

(Hoffman and Tosteson, 1971); in particular, intracellular K is a much more effective inhibitor of the pump in LK cells than in HK cells (Glynn and Ellory, 1972). The inhibition is apparently by competition with Na for translocation loci of the intracellular aspect of the pump sites (Ellory et al., 1972; see also Hoffman, 1966; Hoffman and Tosteson, 1971; Knight and Welt, 1974).

At very low concentrations of intracellular K, $[K]_i$, raising $[K]_i$ stimulates the pump in HK cells (and also in LK cells pretreated with anti-L antibody; Dunham and Blostein, 1976). Only inhibition of the pump with increasing $[K]_i$ is observed in untreated LK cells. Stimulation of the pump by K_i had previously been reported in red cells of goats (Ellory et al., 1972; Sachs et al., 1974b) and humans (Garay and Garrahan, 1973). There are other types of interactions of K_i with the Na-K pump in red cells: one for one exchange of K (Glynn et al., 1970; Sachs et al., 1974b) and complete reversal of the pump (Lew et al., 1970), but these modes of operation of the pump have not been reported for sheep red cells.

In addition to lower pump fluxes, LK cells also have higher passive fluxes of K and Na than do HK cells (Tosteson and Hoffman, 1960). Passive K influx in LK sheep cells approaches saturation as the external K concentration, $[K]_o$, is increased (Dunham and Hoffman, 1971a), indicating that passive K transport in these cells is not entirely by simple passive diffusion. The much lower passive influx in HK cells showed no sign of saturation as a function of $[K]_o$. (Passive transport is used here to mean the flux measured in cells treated with ouabain.)

The M-L blood group antigen system in sheep is associated with the HK-LK dimorphism in that HK cells have only M antigen and the L antigen is found only on LK cells (Rasmusen and Hall, 1966). Anti-L antiserum, raised in HK sheep immunized with LK cells, stimulates active K transport in LK cells fourfold or more (Ellory and Tucker, 1969). Passive K transport is reduced by some preparations of anti-L serum (Ellory et al., 1972; Dunham, 1975, 1976) but not by others (Lauf et al., 1971; Lauf, 1974). Recently it was shown that anti-L serum can contain two populations of antibodies with different effects on K transport in LK sheep cells (Dunham, 1976). One antibody, anti-L_p, stimulates the pump. The primary action of the other antibody, anti-L_1, is reduction of passive K transport, though it also stimulates the pump. The immunology of the M-L dimorphism has recently been reviewed (Lauf, 1975).

In the present work, this reduction of passive K transport by anti-L was characterized. In the course of these experiments it was shown that intracellular K affects passive K transport in LK cells in much the same manner that it affects active transport in HK cells: stimulation by low concentrations and moderate inhibition at higher concentrations of K_i. These and other results suggest that passive K transport in LK sheep cells is mediated by inactive pump sites, and that one of the actions of anti-L serum is a conversion of inactive pumps to an active form. A preliminary report of some of these results has been presented (Dunham, 1975).

MATERIALS AND METHODS

Blood was drawn into heparin by venipuncture from adult male Dorset sheep, and was used on the day it was drawn. The red cells were washed by centrifugation in an isotonic solution containing NaCl (150 mM), glucose (5 mM), Tris-Cl (10 mM) at pH 7.5.

Unidirectional fluxes of Na or K were measured with ^{42}K or ^{24}Na obtained as chlorides from International Chemical Corporation. Influxes and effluxes were measured and calculated as described by Sachs et al. (1974b) and Dunham and Hoffman (1971a), respectively. The passive influx of K and the passive efflux of Na were taken as the fluxes measured in cells pre-exposed to 10^{-3} M ouabain for 5 min at 37°C in K-free medium. During measurement of the fluxes, ouabain concentration was 10^{-4} M. Active, or pump fluxes were the ouabain-inhibited fluxes. Intracellular concentrations of Na and K and relative cell volumes were measured as described previously (Sachs and Welt, 1967; Sachs et al., 1974b). To measure fluxes in Na-free medium, choline Cl was used as a substitute for NaCl. Purified choline chloride was obtained from Hoffman-Taff, Inc., Springfield, Mo.

Intracellular concentrations of Na and K were altered by reversibly increasing cation permeability with nystatin after the method of Cass and Dalmark (1973) as modified by Dunham and Blostein (1976).

Reticulocyte-rich preparations were obtained as described previously (Dunham and Blostein, 1976). Briefly, erythropoiesis was stimulated by phlebotomy and reticulocytes were concentrated by gradient centrifugation.

Anti-L antiserum was raised in HK sheep by intramuscular injection of LK cells (Tucker and Ellory, 1970; Dunham, 1976). Packed LK cells were mixed 1:1 with Freund's complete adjuvant, and injections of 5 ml each were made into the four upper limbs of the animal. After 1 mo, booster injections were made of cells mixed with incomplete adjuvant. Before use the serum was heated to 56°C for 20 min to inactivate complement, and then was dialyzed at 4°C for 18 h against 50 vol 150 mM NaCl or choline Cl with 10 mM Tris-Cl, pH 7.5. Before a flux was measured, cells were incubated with antiserum at 5–10% hematocrit at 32°C for 30 min. Control aliquots of cells were incubated at the same time either in an isotonic NaCl solution or in heated, dialyzed serum from a nonimmunized HK sheep.

Affinity of pump sites for ouabain was investigated from the time course of the onset of inhibition of the pump during exposure to ouabain (Sachs et al., 1974a). Aliquots of cells were incubated for various times in an isotonic solution containing 10^{-6} M ouabain and 5 mM K, with Na the principal cation. Typically 8 ml of a 5% hematocrit suspension was used. Ouabain binding was stopped by rapid chilling of the suspensions in an ice bath. After washing, fluxes were measured in: (a) these cells; (b) cells not exposed to ouabain (giving the uninhibited flux); and (c) cells pre-exposed to 10^{-3} M ouabain (giving the maximally inhibited flux). The logarithm of pump flux remaining was plotted against time of exposure to ouabain. This curve was taken as the time course of ouabain binding. A straight line would indicate a single rate constant of binding and therefore homogeneous affinity of the pumps for ouabain, whereas a line of decreasing slope would indicate a heterogeneity of the affinity of the pumps for ouabain.

RESULTS

Table I shows pump and passive influxes of K in LK sheep cells, both untreated and pre-exposed to anti-L serum. Results are shown for cells from three LK sheep. Anti-L stimulated the pump as reported previously (Ellory and Tucker, 1969; Lauf et al., 1970) and also caused sizable reductions in the passive K influxes. This was a consistent finding in this study with preparations of anti-L serum from two immunized HK sheep.

Kinetics of Passive K Influx

As mentioned above, passive K influx in LK sheep cells saturates as $[K]_o$ is

increased (Dunham and Hoffman, 1971a). Fig. 1 shows the kinetics of passive K influxes, presented as double reciprocal plots, measured at various $[K]_o$'s in anti-L treated cells and in control cells. The results fit straight lines for both types of cells, consistent with saturation kinetics for passive K influx in anti-L treated as well as control cells. The extrapolations for the two types of cells to the abcissa were nearly the same, giving a $K_{1/2}$, the $[K]_o$ at half-maximal passive influx, of about 7 mM. This $K_{1/2}$ of passive influx can be compared with $K_{1/2}$'s for active K influx of 3 mM in Na-containing medium and 0.2 mM in Na-free medium (Hoffman and Tosteson, 1971). Thus, the mechanism mediating passive K influx has a lower apparent affinity for K than does the active pump mechanism.

TABLE I

THE EFFECT OF ANTI-L SERUM ON ACTIVE, $^iM_K^P$, AND PASSIVE, ^iM_K, K INFLUXES IN RED CELLS FROM THREE LK SHEEP

Sheep no.	$^iM_K^P$	^iM_K
1		
control	0.15	0.67
anti-L	0.75	0.18
2		
control	0.16	0.61
anti-L	0.24	0.22
3		
control	0.10	0.45
anti-L	0.18	0.26

Cells were pretreated with anti-L serum as described in the text. The same preparation of antiserum was used with all three aliquots of cells. Control cells were pretreated with isotonic saline. Fluxes (given as mmol/liter cells × h), are means of two determinations, measured in a medium with 10 mM K and with Na as the principal cation. Similar results were obtained in at least three other experiments on cells from each sheep. On cells from sheep no. 1, similar results were also obtained with antiserum from a different immunized HK sheep.

The above results show that anti-L reduces the maximal passive influx but not the apparent affinity for K of the system mediating passive influx. This effect of anti-L on passive K influx was reproducible qualitatively, but the $K_{1/2}$'s for both control and anti-L treated cells were sometimes two- to threefold higher. The reason for this variability is not known.

Inhibition by External Na

External Na is an inhibitor of active K influx in sheep red cells (Hoffman and Tosteson, 1971). Na may act as a competitive inhibitor (Sachs et al., 1975) and in a more complex manner as well (Cavieres and Ellory, 1975; Hobbs and Dunham, 1976). Fig. 2 shows the effect of Na-free medium compared to Na-medium on passive K influx in LK sheep cells. The flux was measured in untreated LK cells at various $[K]_o$'s in media with either Na or choline as the principal cation. In the
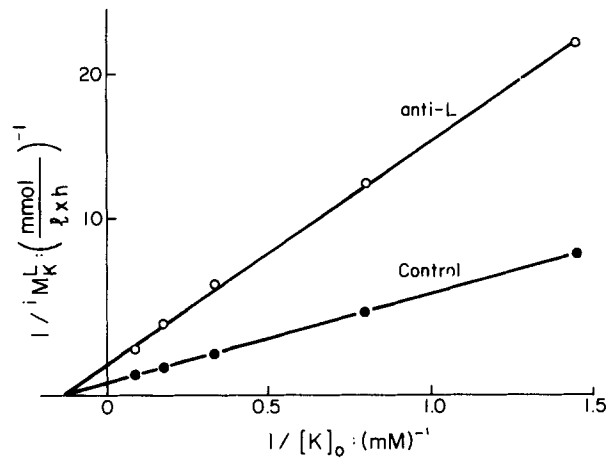
FIGURE 1.   Effect of anti-L serum on passive K influx in LK sheep red cells with varying external K concentration, $[K]_o$. Results are presented as double reciprocal plots. Pre-exposure to anti-L and measurement of fluxes were as described in the text. Fluxes are in millimoles/(liter cells × hour) plotted as reciprocals. Points represent means of two determinations. The lines were fitted by eye. Pump influxes measured at the same time at 10 mM $[K]_o$ were (mmol/liter × h): 0.09, control cells; 0.24, anti-L cells. In four other similar experiments the results were comparable in that the extrapolations to the abscissa were the same for control and anti-L cells. However in two of the experiments the extrapolation differed by a factor of 2–3.
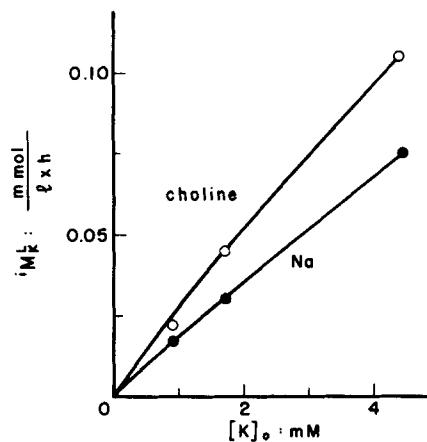

FIGURE 2.   Passive K influx in LK sheep red cells with and without external Na. $[K]_i$ = 28 mmol/liter cells after nystatin treatment. Ouabain-insensitive K influxes, $^iM_K^L$, were measured in media with 1, 2, or 4 mM K and with either Na or choline as the principal cation (146–149 mM). The points represent means of two determinations; the curves were fitted by eye. The same results were obtained in four other experiments, both with cells pretreated with nystatin ($[K]_i$ = 0.5–30 mmol/liter cells) and with fresh cells ($[K]_i$ = 6–8 mmol/liter cells).

Na-free medium, the influxes were about 25% higher than in Na medium. Therefore, external Na appears to be an inhibitor of passive K influx.

K:K Exchange

Experiments were undertaken to determine if anti-L reduces passive K influx by reducing a ouabain-insensitive K:K exchange of the sort observed in goat red cells (Dunham and Bleier, 1973; cf. Sachs et al., 1974b). The cells were first loaded with ^{42}K. Washed aliquots of the cells were exposed to anti-L and then washed again. K efflux was measured in control and anti-L treated cells in four kinds of media, with 20 mM K or K-free, and these two media each with 10^{-3} M ouabain. Na was the principal cation. The results of such an experiment, presented in Table II, show first that K efflux was reduced nearly twofold by

TABLE II

EFFECT OF ANTI-L ON K EFFLUX IN LK SHEEP RED CELLS WITH AND WITHOUT EXTERNAL POTASSIUM AND OUABAIN

	K efflux (mmol/liter cells × h)			
	Control		anti-L	
	K-free	20 mM K	K-free	20 mM K
Control	1.38	1.23	0.70	0.78
Ouabain	1.41	1.28	0.72	0.73

Cells were loaded with ^{42}K and treated with anti-L as described in the text. Fluxes were measured in either K-free medium or medium with 20 mM K, and in each of these media with or without ouabain (control). For measuring the efflux in ouabain-treated cells, the cells had been pre-exposed to 10^{-4} M ouabain at 37°C for 10 min in K-free medium. The principal cation in the media was Na (NaCl + KCl = 150 mM). The fluxes are means of determinations on two flasks of cell suspensions. The flux for each flask was in turn the mean calculated from samples taken at three consecutive 30-min intervals. Intracellular K concentration was 5.5 mmol/liter cells. Similar results were obtained in two other experiments. Pump and leak influxes of K were measured simultaneously on aliquots of the same cells. Anti-L treatment caused a 1.85-fold increase in the pump and a 1.74-fold decrease in the passive influx.

anti-L, as expected from the results on passive K influx. Second, ouabain has no effect on K efflux in either cell type in the presence or absence of K_o. Finally, removal of K_0 had no effect on K efflux in anti-L treated cells. There is a suggestion of a small increase in K efflux upon removal of K_0 in control cells, but K:K exchange would be manifested by a reduced K efflux in K-free medium, not an increase. Therefore, there is no K:K exchange, either ouabain-sensitive or ouabain-insensitive, in LK sheep cells, and the effect of anti-L cannot be considered in the context of such a mechanism.

Anti-L on Na Efflux

To determine the specificity of the action of anti-L on cation transport, the effects of anti-L on Na effluxes were measured. Table III shows effluxes of Na into choline-medium for control and anti-L treated cells. Anti-L stimulated active Na efflux fourfold, but there was no effect of the antiserum on passive Na

efflux. Therefore the effect of anti-L on passive cation efflux is specific for K, though active transport of both K and Na are stimulated.

Passive K Influx and $[K]_i$

The foregoing results are consistent with mediation of passive K transport by a mechanism which has some properties in common with the Na-K pump. Since K_i interacts with the pump, the effects of varying $[K]_i$ on passive influx in LK cells were determined; the results are shown in Fig. 3. Fig. 3a shows the results of one representative experiment on untreated and anti-L-treated cells. It appeared that, at low concentrations, increasing $[K]_i$ stimulated passive K influx, while with a further increase in $[K]_i$, the influx was inhibited. To substantiate that these effects are real, summaries of a number of experiments are presented in Fig. 3b and c. Fig. 3b shows the results of 10 experiments with the fluxes

TABLE III

EFFECT OF ANTI-L SERUM ON NA EFFLUXES IN LK SHEEP
RED CELLS

| | Na efflux (mmol/liter cells × h) | | |
	Total	$^oM_{Na}^i$	$^oM_{Na}^p$
Control	1.14±0.022	0.96±0.019	0.19
Anti-L treated	1.72±0.031	0.96±0.008	0.76

The cells were loaded with ^{24}Na and treated with anti-L as described in the text. Effluxes were measured in media containing (mM): choline Cl, 130; KCl, 20; NaCl, 1.0; glucose, 5; Tris-Cl, 10; pH 7.5. The passive efflux, $^oM_{Na}^i$, was that measured from cells exposed, before the flux was measured, to 10^{-4} M ouabain for 10 min at 37°C in the choline medium without K. The pump efflux, $^oM_{Na}^p$, is the difference between total efflux and $^oM_{Na}^i$. The intracellular concentrations were (mmol/liter cells): $[K]_i = 11.6$ and $[Na]_i = 82.4$. Each value is the mean of fluxes determined on four flasks of cell suspensions. The flux for each flask was in turn a mean calculated from samples taken at three consecutive 45-min intervals. The errors are SEM for $n = 4$.

normalized to 1.0 at the highest $[K]_i$ in each experiment (about 30 mmol/liter cells). The results are presented in this fashion to emphasize the inhibitory effect obtained upon raising $[K]_i$ from about 3 mmol/liter. To emphasize the stimulation at low $[K]_i$, the results of four additional experiments are shown in Fig. 3c with the fluxes normalized to the lowest $[K]_i$ in each experiment. These effects of $[K]_i$ on passive K influx are comparable to the effects of varying $[K]_i$ on the pump flux in HK sheep cells (Dunham and Blostein, 1976) and in goat cells (Ellory et al., 1972; Sachs et al., 1974b).

As with the specificity of the action of anti-L on passive cation transport, the specificity of action of $[K]_i$ on passive cation influx was investigated. Na influx was measured at two external Na concentrations in cells with various $[K]_i$'s. As shown in Table IV, Na influx did not vary with $[K]_i$, and the effect of $[K]_i$ on passive cation transport, like the effect of anti-L, is specific for K transport.
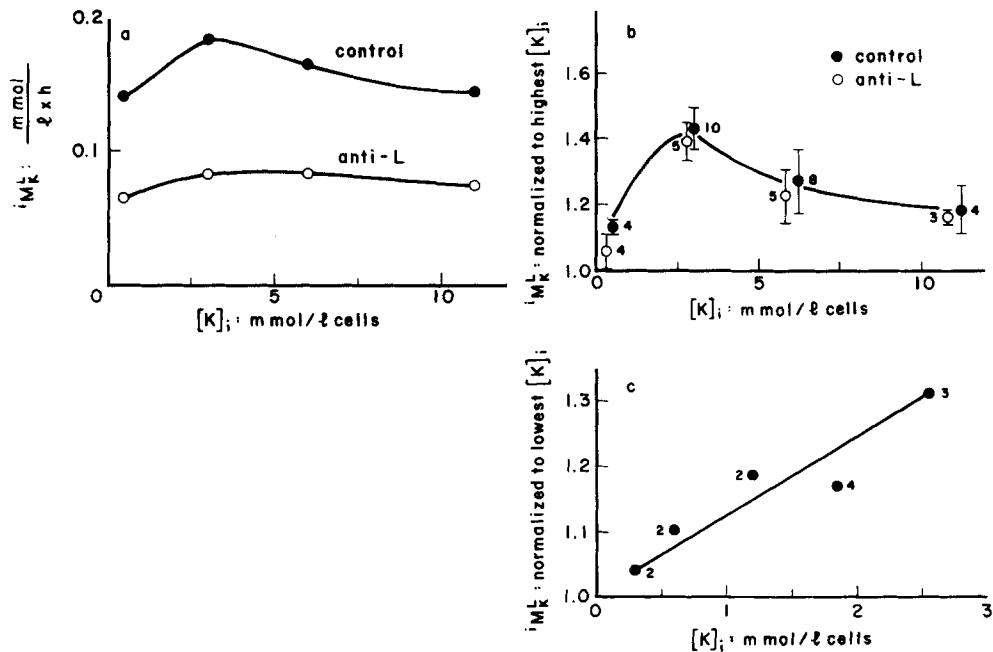
FIGURE 3. Effect of varying intracellular K concentration, $[K]_i$, on passive K influx, $^iM_K^L$, in LK sheep red cells. $[K]_i$ was varied with nystatin. Influxes were measured in media with approximately 4 mM K and with Na as the principal cation. (a) Results of one representative experiment on control cells, i.e. without pretreatment, and on cells pretreated with anti-L. The points represent means of two determinations. (b) Results of 10 experiments with the fluxes normalized to 1.0 at the highest $[K]_i$ in each experiment (28–31 mmol/liter cells). Results are shown for both control and anti-L treated cells. Numbers next to symbols are numbers of determinations for each mean. Errors are SEM. (c) Results of four experiments with the fluxes normalized to 1.0 at the lowest $[K]_i$ in each experiment (0.2–0.4 mmol/liter cells). (b) and (c) contain no values in common.

TABLE IV

EFFECT OF VARYING INTRACELLULAR K ON INFLUX OF NA ($^iM_{Na}^L$) IN LK SHEEP RED CELLS

$[K]_i$	$^iM_{Na}^L$ (mmol/liter cells × h)	
	[Na]$_o$	
	150 mM	*15 mM*
12	3.8	1.03
6	3.7	1.04
3	3.9	1.10
0.4	3.9	1.06

Methods for varying intracellular K concentration, $[K]_i$, and for measuring Na influx are described in the text. The media contained no K. External Na concentration, [Na]$_o$, was either 150 mM or 15 mM (+ 135 mM choline Cl). The fluxes are means of 2 determinations. The same results were obtained in 2 other experiments.

K Influx in Reticulocytes

Reticulocytes from LK sheep differ from mature LK cells in having a cation composition like that of HK cells, a pump flux much higher than in mature cells, and a much higher passive flux (Lee et al., 1966; Tucker and Ellory, 1971; Dunham and Blostein, 1976), and the maturation of LK cells is characterized by large reductions in both active and passive transport of K. Though the pumps on LK reticulocytes differ kinetically from pumps on mature cells, anti-L stimulates active K influx in reticulocytes (Dunham and Blostein, 1976). Accordingly it was of interest to determine if passive transport in LK cells is inhibited by anti-L before the inactivation of the bulk of the pump sites which occurs during maturation. Table V shows both active and passive K influxes in immature and

TABLE V

THE EFFECT OF ANTI-L SERUM ON K INFLUXES IN RETICULOCYTES FROM AN LK SHEEP

	$^iM_K^p$	$^iM_K^l$	$[K]_i$
	mmol/liter cells h		*mmol/liter cells*
Reticulocytes			
control	3.04	2.82	4.46
anti-L	4.04	2.97	3.10
Mature cells			
control	0.14	0.27	3.44
anti-L	0.47	0.20	4.06

Active, $^iM_K^p$, and passive, $^iM_K^l$, fluxes were measured. Reticulocyte-rich preparations (55% reticulocytes) were obtained from the top fraction after density gradient centrifugation as described in the text. Results are also presented for mature cells (bottom fraction) obtained at the same time from the same sheep. Intracellular K concentrations, $[K]_i$'s, were made the same in the young and mature cells by using nystatin. Cells were pretreated either with isotonic saline (control cells) or with antiserum as described in the text. Fluxes were measured in a medium with 4 mM K and with Na as the principal cation. The same results were obtained in one other experiment.

mature LK cells, both control and anti-L treated. The $[K]_i$'s in the reticulocytes and mature cells were made the same with nystatin. Both active and passive K fluxes were much higher in reticulocytes. In the mature cells, anti-L stimulated the pump more than threefold and reduced the leak somewhat. In reticulocytes the pump was stimulated to a lesser relative extent than in mature cells, but the absolute stimulation was nearly 10-fold greater. Finally, the passive K influx in reticulocytes was unaffected by anti-L. In this respect the mechanisms for passive K transport differ in mature and immature LK cells, and inhibition of passive transport by anti-L is possible only after inactivation of the pumps.

Anti-L on Active K Influx

To aid in interpreting the mechanism of action on anti-L on passive K transport, two kinds of experiments were performed on the effect of anti-L on active transport. In the first type of experiment, the effect of anti-L was determined as a function of $[K]_i$. In LK goat cells, the stimulation of the K pump by anti-L is

nearly abolished as $[K]_i$ is reduced below 1.0 mmol/liter of cells, indicating that the primary effect of anti-L in LK goat cells is not on the maximum velocity of the pumps, but is probably an alteration of the affinity of the pumps for K_i (Sachs et al., 1974 b). Fig. 4 shows active K influx in LK sheep cells with varying $[K]_i$. The relative magnitude of the effect of anti-L was greatest at higher $[K]_i$, up to 15-fold stimulation at $[K]_i$ of 20 mmol/liter. Nevertheless the stimulation in cells nearly K-free ($[K]_i$ = 0.4 mmol/liter cells) was more than twofold. Therefore, anti-L stimulates the maximal turnover rate of the pump in LK sheep cells, and the mechanism of action is not only through changes in affinity of the pump for intracellular K. Although stimulation of the pump with increasing $[K]_i$ was not seen in these experiments (cf. Dunham and Blostein, 1976), the slope of the
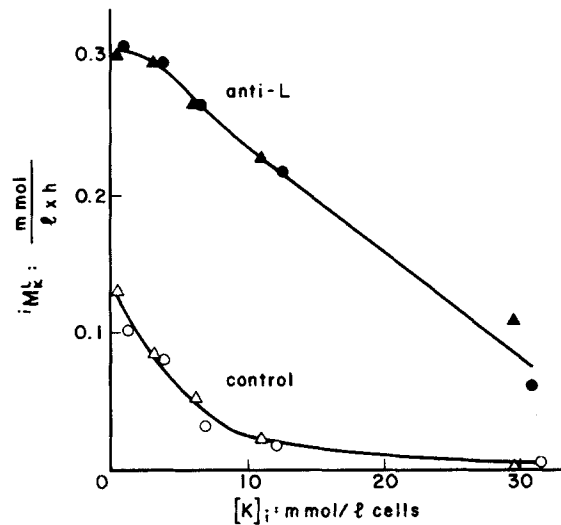

FIGURE 4.   Effect of anti-L serum on active K influx, $^iM_K^p$, in LK sheep red cells with intracellular K concentration, $[K]_i$, varied by use of nystatin. $[Na]_i$ was between 75 and 90 mmol/liter cells. Fluxes are given for control cells, i.e. without pretreatment, and for anti-L treated cells. Points represent means of two determinations. The same results were obtained in three other experiments.

curve is lowest at low $[K]_i$, a finding consistent with the stimulation seen in other studies.

Anti-L on Rate of Ouabain Binding

In LK goat cells the affinity of the pumps for ouabain is heterogeneous, as indicated by a nonlinear time course of a logarithmic plot of ouabain binding (Sachs et al., 1974 a). The rate of ouabain binding was increased by anti-L treatment in the goat cells and is also increased in LK sheep cells (Joiner and Lauf, 1975). In neither study was there an indication of whether or not anti-L also reduced the heterogeneity of the affinity of the pumps for ouabain. The second type of experiment on stimulation of the pump showed that this is the case in LK sheep cells (Fig. 5). Aliquots of cells were first treated with anti-L, and

then the rate of ouabain binding was measured in control and anti-L treated cells as described above. The time course for ouabain binding to control cells indicates a heterogeneity of the affinity of the pumps for ouabain. Anti-L treatment increased the rate of ouabain binding, and also reduced the heterogeneity, in such a way that only about 3% of active transport remained when the line fitting the time course of binding departed from linearity. These results suggest that anti-L increases the rate of ouabain binding by increasing the ouabain affinity of the low affinity sites. The results do not relate to possible effects of anti-L on
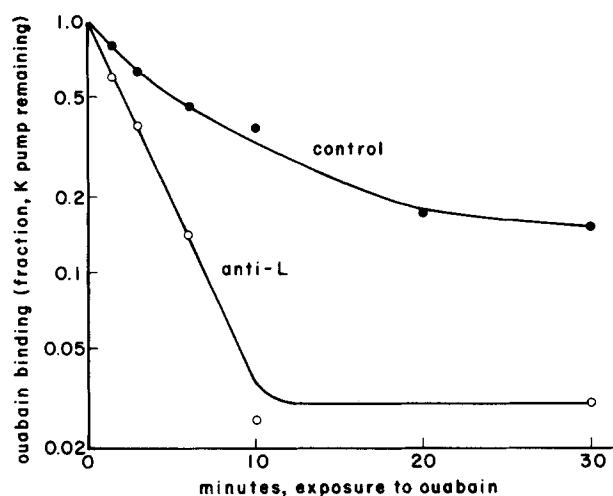

FIGURE 5. The effect of anti-L on rate of ouabain binding in LK sheep red cells. Rate of ouabain binding was determined, as described in the text, from the fraction of active K influx remaining (plotted on a logarithmic scale) after incubation for various times in media containing ouabain. The ouabain-incubation media contained 145 mM NaCl, 5 mM KCl, 5 mM glucose, 10 mM Tris-Cl (pH 7.5) and 10^{-6} M ouabain. The intracellular K concentration had been reduced with nystatin to (mmol/liter cells): 2.7, control cells; 3.3, anti-L cells. After the cells had been incubated with ouabain and washed by centrifugation, K influxes were measured in media with 4 mM K and with Na as the principal cation. The points represent means of two determinations. The results are representative of four other experiments.

maximum velocity of the pumps since they are expresed in terms of relative, not absolute fluxes.

DISCUSSION

The results of this study have bearing on the relationship between the mechanisms for passive and active K transport, on the action of anti-L antiserum on K transport, and on the processes of maturation of LK and HK red cells.

Passive K influx in LK sheep red cells is not by simple passive diffusion, but obeys saturation kinetics as a function of $[K]_o$ as shown in Fig. 1. There are two important similarities between active K influx in HK cells and passive K influx in LK cells. (a) The kinetics of the active and passive K influxes vary in the same

manner as a function of intracellular K concentration: both types of influx are stimulated and then inhibited as $[K]_i$ is increased from near zero. (b) Passive and active K influxes are both inhibited by external Na.

There is in addition an important difference between the active and passive modes, indicated by the effects of anti-L and K_i on K influx. Active K influx is coupled to Na efflux, but passive K influx is independent of Na transport. The evidence for this latter point is that both altering $[K]_i$ and treatment with anti-L affect passive transport of K but not the passive flux of Na (both of these procedures affect active transport of Na as well as of K). On the basis of these points of evidence, it is proposed that passive K influx is mediated by inactive pump sites, sites which have become inactivated during maturation of the immature LK cells.

The specificity of the passive transport sites for K is a provocative finding, but no molecular basis for this characteristic suggests itself.

The pumps operating in the passive mode are less sensitive to inhibition of K_i than are the active pumps (compare curves for control cells in Figs. 3a and 4). If this is so, then increased effectiveness of K_i as an inhibitor is not associated with the process of inactivation. The most striking difference between active and inactive pumps lies in their interaction with Na_i: Na is translocated by active pumps but not by inactive ones. Thus, uncoupling of Na and K fluxes may be a primary event associated with inactivation. (It should be emphasized that, although passive Na fluxes are not mediated by inactive pump sites, a large fraction of passive Na transport is by a ouabain-insensitive Na-Na exchange [Tosteson and Hoffman, 1960].)

The stimulation of passive K influx by K_i resembles the stimulation of the active flux by K_i in HK cells and anti-L treated LK cells of both sheep and goats (Sachs et al., 1974b; Dunham and Blostein, 1976; the present report); K-K exchange cannot account for the stimulation (Table II). The concomitant stimulation and inhibition of K influxes by K_i suggest interaction of K with two classes of sites at the intracellular aspect of the pumps. These two classes of sites may be the "unloading" sites for K influx and the "loading" sites for Na efflux (with occupation by Na not resulting in translocation by inactive pumps). However, the data available do not allow the development of a model which takes into account all of the interactions of K_i with the pumps (cf. Garay and Garrahan, 1973; Sachs et al., 1974b).

The results of the present study are consistent with an earlier proposal that active and passive cation transport sites are interconvertible (Dunham and Hoffman, 1971a): pumps are inactivated during maturation of LK cells (Dunham and Hoffman, 1971b) and are activated by treatment with anti-L (Lauf et al., 1970). It was shown in Table V that there is insignificant inhibition by anti-L of passive K influx in immature LK cells (and substantial inhibition in mature cells). This result indicates the absence in the immature cells of sites (inactive pumps) which in mature LK cells mediate passive K influx and which are sensitive to anti-L. These inactive pumps arise during maturation by conversion of pumps from an active to an inactive mode. Total passive K flux also declines during maturation of LK cells, but this change is probably associated with K permeability unrelated to the Na-K pumps.

The results in Fig. 1 provide an indication of the nature of anti-L action on passive transport. The maximum velocity of passive K influx is reduced, but not the apparent affinity for K of the sites mediating the influx. This observation is consistent with anti-L simply reducing the number of sites mediating the passive influx.

It was recently shown that anti-L_1, one of the two populations of antibodies in anti-L serum, is responsible for the reduction in passive K transport (Dunham, 1976). At the same time anti-L_1 also increases the pump flux in LK sheep cells, as does anti-L_p, the other type of antibody. In one possible scheme of action of anti-L serum, anti-L_1 activates the inactive pump sites, sites which mediate only passive K transport before treatment. The sites' affinity for ouabain may increase as a consequence of the treatment with anti-L, perhaps from near zero (cf. Sachs et al., 1974a). Anti-L_p would have no effect on inactive pump sites, but would increase the pump flux at sites active before treatment by decreasing the inhibition of the pump by K_i. The results in Fig. 4 support these suggestions: the stimulation of the pump in cells essentially K-free represents stimulation of the maximum velocity of the pump. This would be an effect of the anti-L_1, an consequence of the conversion of sites from a passive to an active mode. (In a related finding, Blostein et al. [1971] demonstrated stimulation by anti-L of Na-ATPase activity in the absence of K in membranes of LK sheep cells.)

The change in the shape of the curve in Fig. 4 by anti-L treatment, presumably the effect of anti-L_p, corresponds to the effect seen previously with anti-L treatment of LK goat cells (Sachs et al., 1974b) and interpreted to indicate a reduced affinity for K_i at Na translocation sites.

Related observations on the affect of anti-L on LK goat cells are consistent with this scheme: (a) anti-L_1 has no effect on the pump in LK goat cells (Dunham, 1976); and (b) the primary effect of anti-L on the goat cells is on the relative affinities of the pumps for Na_i and K_i since the antiserum had little effect on cells with very low $(K)_i$ (Sachs et al., 1974b).

The results on affinity for ouabain (Fig. 5) are also consistent with the above scheme. Untreated LK cells have pumps with heterogeneous affinities for ouabain. The pumps with lower affinity may be partially inactivated sites with a higher relative affinity for K_i than for Na_i. The increased rate of ouabain binding at these sites in anti-L treated cells would be associated with their altered affinity for intracellular cations. The homogeneity of the pumps on anti-L treated cells in their affinity for ouabain is then a consequence of two effects of components of anti-L serum: activation of passively transporting sites, and alteration of pumping sites sensitive to $[K]_i$ and therefore partially inactivated before interaction with antiserum. The actions of anti-L_1 and anti-L_p differ in forms of the pump preferred, but may have in common an effect on the association of Na with the intracellular aspect of the pump mechanism.

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