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Stimulation of Active Potassium Transport in LK Sheep Red Cells by Blood Group-L-Antiserum

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Summary. Anti-L serum prepared by immunization of a high-potassium-type (HK) (blood type MM) sheep with blood from a low-potassium-type (LK) (blood type ML) sheep contained an antibody which stimulated four- to sixfold K^+-pump influx in LK (LL) sheep red cells. In long-term *in vitro* incubation experiments, LK sheep red cells sensitized with anti-L showed a net increase in K^+ after two days of incubation at 37 °C, whereas HK-nonimmune (NI)-serum-treated control cells lost K^+. The antibody could be absorbed by LK (LL) sheep red cells but not by HK sheep red cells. Kinetic experiments showed that the concentration of external K^+($[K^+]_0$) required to produce half-maximum stimulation of the pump ($[Na^+]_0=0$, replaced by Mg^{++}) was the same (0.25 mM) in L-antiserum-treated or untreated LK cells. LK cells with different $[K^+]_i$ (Na^+ replacement) were prepared by the p-chloromercuribenzene sulfonate (PCMBS) method. At $[K^+]_0=5$ mM, pump influx decreased as $[K^+]_i$ increased from 1 to 70 mM in L-antiserum-treated LK cells, whereas LK cells treated with HK-NI-serum ceased to pump at $[K^+]_i=35$ mM. Exposure to anti-L serum produced an almost twofold increase in the number of pump sites of LK cells as measured by the binding of tritiated ouabain by LK sheep red cells. These findings indicate that the formation of a complex between the L-antigen and its antibody stimulates active transport in LK sheep red cells both by changing the kinetics of the pump and by increasing the number of pump sites.

Red blood cells of high-potassium-type (HK) and low-potassium-type (LK) sheep are different in both the active and passive transport of Na^+ and K^+ (Tosteson & Hoffman, 1960). Both the active transport of these ions and the Na^+-plus-K^+-stimulated and ouabain-sensitive ATPase (S-ATPase) are about four times more active in HK than in LK red cells (Tosteson, 1963). The two genetically distinct sheep red cell types differ also with respect to two antigens, the M- and L-antigens (Rasmusen & Hall, 1966; Ellory & Tucker, 1969; Rasmusen, 1969). The M-antigen is present on HK sheep red cells and the L-antigen on the red cells of homozygous LK sheep. The presence of both antigens in the heterozygote indicates a genetically closed

system. Although the inheritance of the M-antigen is associated with that of the HK character, it was recently shown that the reaction of anti-M antiserum with HK red cells does not affect the S-ATPase activity of these cells (Brewer, Eaton, Beck, Feitler & Shreffler, 1968), nor is the amount of antibody bound per HK red cell or membrane altered in the presence of ouabain, ATP or ions (Lauf & Tosteson, 1969). In contrast, the stimulation of active K^+ transport and S-ATPase in LK sheep red cells after exposure to L-antiserum reported by Ellory and Tucker (1969), and confirmed in this laboratory (Lauf, Rasmusen, Hoffman, Dunham, Cook, Parmelee & Tosteson, 1969; Tosteson, 1969), suggests that S-ATPase and L-antigen may be expressions of the same macromolecule(s) involved in the transport of cations across the red cell membrane.

This report describes in more detail the effect of anti-L serum on K^+ transport and, in particular, presents data on the binding of anti-L by LK cells, the kinetic properties of K-transport in anti-L-treated LK red cells, the net uptake of K^+ by LK cells cultured for prolonged times at 37 °C, and an estimation of the K^+-pump sites in LK cells in the presence and absence of the L-antibody.

Materials and Methods

Antiserum Preparation

Isoimmune anti-L serum[1] was obtained after a subcutaneous injection of 1 ml of whole blood from a heterozygous LK donor (blood type ML) mixed with 1 ml of complete Freund's adjuvant into a homozygous HK recipient (blood type MM) sheep, followed four months later by two weekly intravenous injections of 10 ml of whole blood from the same donor. The antiserum was collected one week after the last injection and used in its unabsorbed or absorbed form. In the latter case, the antiserum was diluted 1:4 with saline and then absorbed with a variety of HK sheep bloods in order to remove M-system antibodies other than anti-L as well as antibodies belonging to yet different sheep blood group systems. The serum was heat-inactivated (56 °C, 30 min) prior to use. Isoimmune anti-M was prepared as described earlier (Rasmusen & Hall, 1966*b*). Non-immune sera (NI-sera) from HK or LK sheep were only heat-inactivated but not further preabsorbed in these studies. All sera were stored in small quantities at −20 °C and thawed prior to use.

Antibody Assay

Blood from healthy HK and LK sheep was freshly drawn and heparinized (10 USP units sodium heparin/ml). The red cells were washed in 150 mM NaCl, and 20% cell suspensions were made in a medium ("5 K medium") similar to that described by Ellory and Tucker (1969): 5 mM KCl, 140 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, and 10 mM Tris chloride, pH 7.6.

1 In this paper, the terms "group-L antiserum", "L-antiserum" and "anti-L serum" are synonymous.

To 0.25 ml of the 20% red cell suspension, 0.25 ml of the serum to be tested was added. The test tubes were incubated for 1 hr at 23, 32, and 37 °C, but for most of the experiments at 32 °C as given by Ellory and Tucker (1969). After incubation, the cells were diluted into 20 ml of the incubation medium. The samples were divided into 10-ml portions (hematocrit 0.25%) to which either 0.5 ml medium or 0.5 ml medium containing 10^{-2} M ouabain (final concentrations, 5×10^{-4} M) was added. After preincubation for 30 min at 37 °C, ^{42}K (75.5 mc/millimole; Cambridge Nuclear) was added to each test tube, and 5-ml samples were taken 30 and 150 min later. After separation of the supernatants, the cells were washed four times in a total of 32 ml of 120 mM $MgCl_2$ (pH 7.6) and lysed as previously described (Tosteson, Cook, Andreoli & Tieffenberg, 1967). Lysates were analyzed for hemoglobin, total K^+ and ^{42}K; supernatants were analyzed for total K^+ and ^{42}K. Hemoglobin concentration was estimated from measurements of the optical density of the lysates at 540 nm (Gilford 300 N Microspectrophotometer). Total K^+ and ^{42}K were measured by atomic flame photometry (Perkin-Elmer) and by solid crystal scintillation photometry (Packard Autogamma). From these measurements, K^+ influx (^iM_K) was computed as previously described (Tosteson *et al.*, 1967). ^iM_K in the presence of ouabain (10^{-4} M) was taken as a measure of leak influx ($^iM_K^L$), whereas the difference between ^iM_K and $^iM_K^L$ was assumed to be pump influx ($^iM_K^P$) (Tosteson & Hoffman, 1960).

Antibody Absorption

A 3-ml portion of washed packed red cells of either LK or HK type was mixed with 1 ml of anti-L serum of HK or LK-NI serum and incubated for 1 hr at 32 °C. After centrifugation at 10,000 rpm for 2 min, the supernatants were separated from the cells and geometrically diluted with incubation medium. Assay for remaining anti-L activity on the K^+-pump influx was done as described above.

Varying Cation Composition

LK sheep red cells were washed in 150 mM NaCl, and the internal cation composition was varied according to the method of Garrahan and Rega (1967) as modified by Hoffman and Tosteson (1969). The internal cation composition was adjusted to the desired values by suspending the cells in a medium containing the desired concentration of Na^+ and K^+ as the chloride salts as well as phosphate (5 mM, pH 7.5), sucrose (1.25%, 30 mM), $MgCl_2$ (2 mM) and 0.2 mM PCMBS (Sigma) for 16 to 24 hr at 4 °C. The cells were then spun down and "resealed" by incubation for 45 min at 37 °C in an identical medium except that it contained no PCMBS and did contain dithiothreitol (5 mM) and glucose (11 mM). The cells were finally washed four times in $MgCl_2$ as described in the antibody assay, and exposed to either anti-L serum or HK-NI serum; then K^+-pump influx was determined as described above.

Long-Term Incubation Experiments

A complete description of the technique for incubation is presented elsewhere (Tosteson, 1969; Kepner & Tosteson, 1970). Blood was drawn from LK sheep under sterile precautions. The cells were washed in sterile saline and subsequently incubated in anti-L or HK-NI serum in volume proportions as described above. Without further washing, the cells were then transferred to culture bottles to make a 10^{-3} v/v suspension. The culture flasks were incubated at 37 °C for 7 days. The cell suspensions were stirred intermittently. Samples of the cultured cell suspensions were taken sterilely at various time intervals and analyzed for cation composition and/or K^+-pump influx as described above.

Determination of Bound Tritiated-Ouabain

Labelling and determination of bound ^{3}H-ouabain (T-ouabain) to red cells was carried out essentially as described by Hoffman and Ingram (1968) for human red cells as modified and applied to sheep red cells (Dunham & Hoffman, 1969). T-ouabain was obtained from New England Nuclear. The specific activity of 3.7 c/millimole was given by the manufacturer (Lot 184-125). Spectrophotometric analysis, however, indicated that the concentration of ouabain is only 1/4 of the stated amount. The molar extinction coefficient 1.54×10^4 mole was determined at the absorption peak 220 nm, using unlabelled ouabain-octahydrate (Sigma). Therefore, the specific activity is fourfold greater than that given by the manufacturer, provided that all ^{3}H activity resides solely in the ouabain. Calculations, therefore, were based on 14.8 c/millimole as specific activity of T-ouabain.

For each experiment, three conditions (in duplicates) were arranged for both anti-L- and HK-NI-serum-treated LK cells. Equal volumes of anti-L or HK-NI serum were added to one flask (flask I) containing 0.25 ml and to two flasks (flasks II & III) containing 3 ml of a 20% LK cell suspension (made in "K-free" medium: 145 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose and 10 mM Tris chloride, pH 7.6). All flasks were incubated for 1 hr at 32 °C and subsequently diluted 10-fold with the above K-free medium which also contained 25 mM CsCl shown to reduce nonspecific binding of ouabain (Dunham & Hoffman, 1969). T-ouabain was then added to flasks II and III to give a final concentration of 10^{-7} M, and nonradioactive ouabain was added to flask III (final concentration 10^{-4} M). Flask I (no ouabain) served for the control in the subsequent ^{42}K-flux measurements, and flask III containing the ouabain of low specific activity was used for determination of bound tritiated contaminants and estimation of the ouabain-insensitive K^+ influx. Uptake of T-ouabain was determined on the cells of flask II containing only 10^{-7} M T-ouabain. All flasks were now further incubated for 1 hr at 37 °C in a water bath shaker (Metabolyte, New Brunswick Scientific). Subsequently, flasks II and III were divided into 25- and 35-ml portions and washed twice with an equal volume of 5 K medium containing either 1/100 diluted anti-L or HK-NI serum plus 0.1 g% albumin. The cells from the 25-ml samples were directly used for T-ouabain counting (*see* below), whereas cells from the 35-ml samples were resuspended in the same volume of which 5 ml (equivalent to 0.25 ml of a 20% cell suspension in flask I) was used for flux measurement. Another 25 ml of the 35-ml sample was incubated simultaneously for the period of 2.5 hr of the flux experiment in order to estimate the loss of T-ouabain from the cells during this incubation period. The T-ouabain bound to the cells was extracted for liquid scintillation counting by suspending the washed packed red cells in about 0.75-ml volume and delivering 0.5 ml of it directly into a counting vial containing 15 ml Bray's solution. The number of red cells added to each counting vial was determined by measuring the amount of hemoglobin in 0.1 ml of the remaining 0.25-ml cell suspension. Bray's solution consists of 0.2 g POPOP, 4.0 g PPO, 100 ml absolute methanol, 20 ml ethylene glycol, 60 g recrystallized naphthalene and dioxane up to 1 liter. Immediately after addition of the cells to the counting vials, the samples were vigorously mixed with a vortex mixer to provide even mixing of the cells. The cells were then packed by centrifuging the counting vials for 15 min at 2,500 rpm. There was very little lysis of the cells in Bray's solution. Since the presence of the cells increased the background of the counts presumably due to fluorescence caused by hemoglobin, background samples using unlabelled cells were always prepared. The background was further minimized by storing the cells in the cold for several days before counting.

This extraction procedure in Bray's solution removed virtually all of the T-ouabain from the cells as reported elsewhere (Dunham & Hoffman, 1969). Counting efficiency which was usually around 20% was determined in each experiment using internal and

external standards. Internal standards were made with 0.3, 0.5 and 0.7 ml of unlabelled cells in Bray's solution to which a known amount of ^{3}H-toluene was added. Absolute counting efficiency was plotted against the channels ratios for the external standards from which absolute counting efficiency of each test sample could be read. By knowing the number of cells counted, the counts per minute and specific activity of the bound ouabain, the number of bound T-ouabain molecules per cell could be estimated.

Results

K^+-Pump Influx of LK Sheep Red Cells after Reaction with Isoimmune Anti-L and Anti-M Antisera or with HK- or LK-NI Serum

Table 1 presents the K^+-influx of red cells from two different LK and one HK sheep subsequent to exposure to anti-L, anti-M, HK-NI and LK-NI sera. It is evident that the K^+-pump influx increased up to 5.5-fold in all LK cells exposed to anti-L, as compared to the controls (HK-NI). By contrast, $^iM_K^L$ in LK cells was unaffected by the unabsorbed anti-L serum used. Anti-M serum or HK-NI serum did not alter K^+-pump influx of LK cells, and anti-L or anti-M serum and HK or LK-NI serum had no effect on the K^+-pump influx of HK red cells. It was found that the stimulation of the K^+ pump was greatest when the exposure to anti-L serum was carried out at 32 °C, confirming the observation by Ellory and Tucker (1969). Fig. 1 shows the K^+-pump influx of L-antiserum-treated LK cells as a function of the relative concentration of L-antiserum. A concentration of anti-L of less than 1/20 of that present in the undiluted immune serum was sufficient to double $^iM_K^P$.

Table 1. *The effect of anti-L and anti-M on K^+ influx in LK and HK sheep red cells*

Test cells (blood group type)	Serum	K^+ influx [mM/(L cells) × hr]	
		Leak	Pump
LK 188 (LL)	Anti-L	0.076	0.61
	HK-NI	0.082	0.14
LK 2582 (LL)	Anti-L	0.180	0.36
	Anti-M	0.221	0.16
	HK-NI	0.218	0.10
HK S (MM)	Anti-L	0.026	0.35
	Anti-M	0.027	0.36
	HK-NI	0.034	0.38
	LK-NI	0.039	0.37

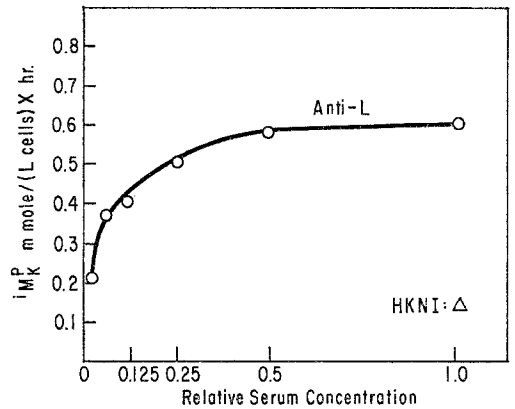

Fig. 1. Stimulation of K^+-pump influx in LK 188 red cells at various geometric dilutions of L-antiserum

Absorption Studies

Table 2 shows the results of absorption experiments. Absorption of anti-L with LK 188 cells reduced the effect of the antiserum on the K^+-pump

Table 2. *Effect of anti-L on K^+ influx of LK cells after absorption with LK and HK cells*

Experiment	Treatment	Relative serum concentration tested	K^+ influx [mM/(L cells) × hr]	
			Pump	Leak
93a	Absorption with LK 188 cells	0.5	0.45	0.101
		0.25	0.36	0.145
		0.125	0.34	0.193
	Unabsorbed	0.5	0.51	0.064
		0.25	0.48	0.072
		0.125	0.45	0.112
	HK-NI control	0.5	0.12	0.109
93b	Absorption with LK 188 cells	0.5	0.44	0.113
		0.25	0.38	0.145
		0.125	0.25	0.195
	Unabsorbed	0.5	0.53	0.071
		0.25	0.52	0.072
		0.125	0.47	0.109
	HK-NI control	0.5	0.153	0.136
95	Absorbed with LK 188 cells	0.125	0.34	0.322
	Absorbed with HK S cells	0.125	0.53	0.269
	Unabsorbed	0.125	0.50	0.316
	HK-NI control	0.125	0.16	0.147

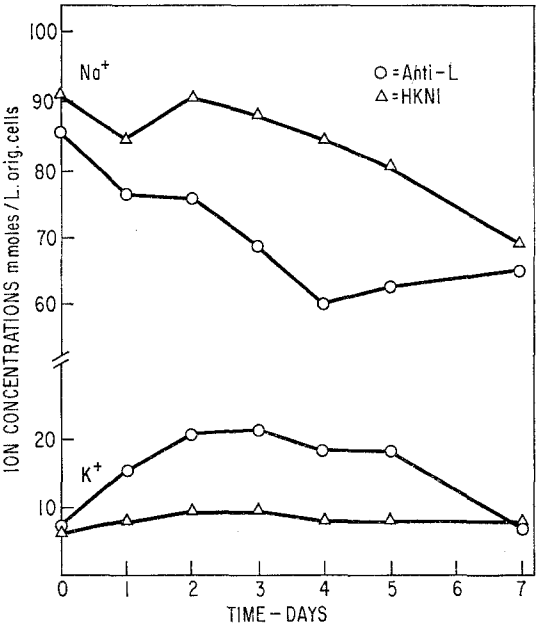

Fig. 2. Cation changes in anti-L-treated LK red cells incubated *in vitro*. Net accumulation of $(K^+)_i$ occurred until the third day only in cells treated with L-antiserum

flux of the test cells (LK 188), particularly when the absorbed serum was tested at a dilution of 1:8. Experiment 95 shows that absorption of anti-L with HK cells did not decrease the activity of the anti-L serum on the K^+-pump flux in LK cells. It should, however, be noted that in this experiment the leak flux appeared to be enhanced when LK cells were incubated with either absorbed or unabsorbed L-antiserum. Further evidence that anti-L was in fact absorbed by LK cells was obtained by measuring the K^+-pump flux in the cells used to absorb the antibody in experiment 93. The K^+-pump flux was found to be increased two- to threefold compared to the usual four- to fivefold. Since these cells had been washed in approximately 40 volumes of the incubation medium, loss of antibody from these cells during washing may have occurred.

Long-Term Incubation Experiments

Fig. 2 shows the results of long-term incubation experiments with LK sheep red cells in the presence and absence of anti-L serum. The cation content found in the cell samples from the culture each day indicates that a net uptake of K^+ and simultaneous decrease of the internal Na^+ occur in L-antiserum-treated LK cells until the third day. This change was not

Table 3. *In vitro incubation of anti-L-treated LK sheep red cells*

Sheep	Serum	K^+ influx [mM/(L cells) × hr]					
		Day 0		Day 2		Day 7	
		Leak	Pump	Leak	Pump	Leak	Pump
LK 188	Anti-L	0.06	0.44	0.01	0.45	0.12	0.08
	HK-NI	0.07	0.17	0.07	0.17	0.09	0.07
LK 183	Anti-L	0.21	0.27	0.18	0.23	0.09	0.13
	HK-NI	0.31	0.12	0.59	0.06	0.15	0.08

observed in control cells treated with HK-NI serum and incubated for the same length of time. Data on the K^+-pump influx and leak of these cultured red cells can be seen in Table 3. It is evident that anti-L continues to exert its stimulating effect on the K^+-pump influx of LK cells for the first few days when incubated at 37 °C. When cell samples were taken from the culture at times after the second day, ${}^iM_K^p$ decreased generally and the difference between L-antiserum-treated and HK-NI-treated LK cells disappeared. These findings are consistent with the results in Fig. 2 where the temporarily elevated $[K^+]_i$ fell to its original value at the seventh day of the experiment. Since the changes in cell K^+ and Na^+ content observed in these experiments could not occur simply from changes in the exchange diffusion or leakage properties of the membrane, these results demonstrate conclusively that anti-L treatment indeed stimulates active K^+ and Na^+ transport in LK sheep red cells. An explanation for the loss of anti-L effect after the third day of incubation will require further experiments.

Effect of Anti-L on Kinetic Characteristics of the K^+-Pump Flux in LK Sheep Red Cells

Fig. 3 shows the effect of $[K^+]_0$ on K^+-pump influx in LK sheep red cells exposed to anti-L and HK-NI serum. In this experiment, NaCl in the medium was replaced by an isoosmotic amount of $MgCl_2$. It can be seen that the maximum value of ${}^iM_K^p$ was more than five times higher in LK cells treated with anti-L than in LK cells incubated in HK-NI serum. In contrast, no change occurred in the apparent affinity of the pump sites for $[K^+]_0$ in L-antiserum-treated LK cells since $K_{1/2}$ (the $[K^+]_0$ required for half-maximal activation of ${}^iM_K^p$), determined from reciprocal plots, was close to 0.25 mM for both anti-L- and HK-NI-serum-treated LK sheep red cells. The $K_{1/2}$ value for HK red cells was found to be 0.6 mM (Hoffman & Tosteson, 1970). Therefore, it can be tentatively concluded that the pump

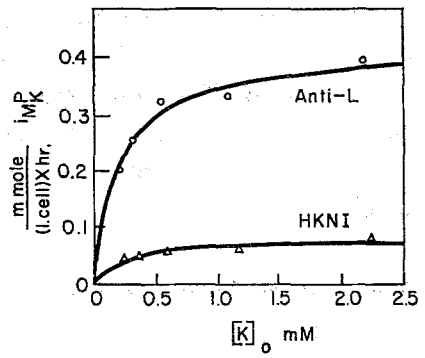

Fig. 3. K^+-pump influx in LK sheep red cells exposed to L-antiserum or HK-NI serum as a function of $[K^+]_0$; $[Na^+]_0 = 3$ mM, Mg^{++} replacement

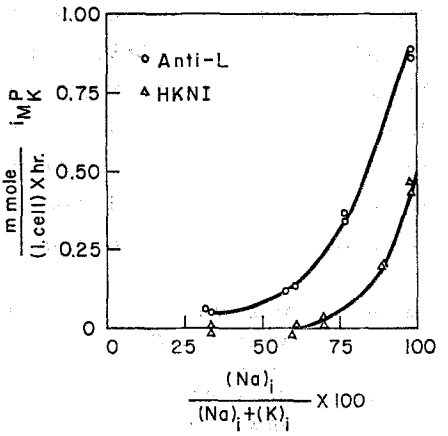

Fig. 4. K^+-pump flux in LK cells exposed to L-antiserum or HK-NI serum at various $[K^+]_i$ and $[Na^+]_i$. $[K^+]_0 = 5$ mM, $[Na^+]_0 = 130$ mM. The K^+-pump flux inhibition at 35 mM $[K^+]_i$ appears to be relieved when the cells were previously exposed to anti-L

sites of LK cells treated with anti-L did not change to pump sites of HK character.

Fig. 4 shows the effect of varying $[Na^+]_i$ and $[K^+]_i$ on K^+-pump influx of LK cells in the presence of anti-L or HK-NI serum. The most striking difference between these two curves is that inhibition of the K^+-pump occurs at a lower $[Na^+]_i$ (and a higher $[K^+]_i$) in cells treated with anti-L than in control cells. Normally, LK cells cease to pump at $[K^+]_i$ values of about 35 mM $([Na^+]_i/[Na^+]_i + [K^+]_i) \times 100 \cong 60]$. Under these circumstances, ${}^iM_K^P$ is still substantial in anti-L-treated cells. When the $[K^+]_i$ approaches that found in normal LK cells $([Na^+]_i/[Na^+]_i + [K^+]_i) \times 100 = 90]$, anti-L-exposed LK cells show a K^+-pump influx which is about four times greater than that

of LK cells exposed to HK-NI serum. Under this condition, HK red cells would have a pump activity which is about 10 to 20 times greater than that of normal LK cells (Hoffman & Tosteson, 1969). Thus, although the sensitivity of the K^+-pump influx to changes in internal cation composition was altered remarkably by anti-L, there were important differences between these cells and HK red cells.

Estimation of the Number of Pump Sites

Data on the uptake of T-ouabain simultaneously measured with the K^+-pump influx in LK cells exposed to anti-L or HK-NI serum are given in Table 4. The number of pump sites per cell was calculated assuming that one T-ouabain molecule blocks one pump site and that the inhibition of the pump is directly proportional to the number of ouabain molecules bound per cell (Dunham & Hoffman, 1969). Non-specific binding of T-ouabain was estimated from the amount of radioactivity remaining on the cells after addition of 5×10^{-4} M cold ouabain. Such nonspecific binding could be further reduced by performing the experiments in 25 mM CsCl which was added to the buffered medium used. The table shows the values of three different experiments on the same LK animal, indicating that the

Table 4. *Effect of L-antiserum on the number of T-ouabain molecules bound per LK sheep red cell*

Experiment	Serum	T-ouabain (M)/ preincubation	K^+-pump flux [mM/(L cells) × hr]	Inhibition (%)	Molecules T-ouabain/cell	Pump sites/cell
E 84	Anti-L	0	0.28			
		10^{-7}	0.12	57	70	122
	HK NI	0	0.11			
		10^{-7}	0.065	41	40	97
E 86	Anti-L	0	0.26			
		10^{-7}	0.076	71	45	63
	HK NI	0	0.001			
		10^{-7}	<0	71	21	30 [a]
E 88	Anti-L	0	0.38			
		10^{-7}	0.25	37	30	82
	HK NI	0	0.094			
		10^{-7}	0.059	37	10	27

[a] Since no K^+-pump flux was measured in this part of exp. 86, the degree of inhibition by 10^{-7} M T-ouabain could not be estimated. Therefore, the number of pump sites was computed on the basis of 71 % K^+-pump flux inhibition found in presence of anti-L.

number of pump sites is almost two times higher in the anti-L-sensitized cells than in the controls.

Discussion

In confirmation of the results by Ellory and Tucker (1969), the data presented in this report show that the iso-immune anti-L antiserum prepared in HK sheep produced a dramatic increase in the K^+-pump influx in LK but not in HK sheep red cells. This effect could be demonstrated both by using a specifically absorbed anti-L serum (i.e., relatively devoid of antibodies not belonging to the anti-L type) and with unabsorbed antiserum containing such antibodies, indicating that non-L-specific antibodies produced during the isoimmunization do not interfere with the action of anti-L on K^+ transport in LK sheep red cells. Extensive studies were not undertaken, however, because of the unavailability of larger quantities of specifically absorbed anti-L reagent. It appears, therefore, that the experiments can be carried out with unabsorbed anti-L, thus dispensing with the several absorptions with a variety of sheep bloods usually necessary before one can detect a specific anti-red cell antibody by its lytic or agglutinating properties.

Anti-L exerts its effect on the K^+-pump influx only when used in relatively high concentrations. This finding parallels our observation that maximal immune lysis of LK cells by anti-L serum was achieved with an antiserum absorbed at 1:8 and diluted to 1:32 antiserum (*unpublished results*), placing the latter property in marked contrast to the potency of anti-M which produces complement-dependent lysis at much higher dilutions (Lauf & Tosteson, 1969). The significant reduction of the L-antiserum effect on active K^+ transport when diluted up to 1:32 would be compatible with a low affinity of the L-antibody for its antigen on the cell surface. This interpretation is supported by the fact that a large number of LK sheep red cells (more than 3×10^{10} cells/ml undiluted anti-L serum) are required to absorb an amount of antibody from the serum sufficient to reduce significantly its effect on the K^+-pump influx (Table 2). It is of interest that anti-L of another source also appears to be inactive with respect to the K^+-pump stimulation when dilutions higher than 1:16 are used (Ellory, 1969). Thus, immunization of homozygous MM (HK) sheep with heterozygous ML (LK) sheep blood to obtain the anti-L serum used in our experiment (Rasmusen, 1969) results in production of an antibody with at least some characteristics similar to that of Ellory and Tucker (1969). Comparative absorption studies with both anti-L sera presently available should clarify if there are differences between these antibodies and therefore several L-alleles. Studies presently underway in our laboratory indicate that the K^+-pump-

stimulating activity resides in the electrophoretically fast immunoglobulin fractions of the isoimmune serum used in the studies described in this paper.

The kinetic studies and T-ouabain binding experiments presented in this report are helpful toward finding an explanation for the unusual action of anti-L serum on active cation transport in LK sheep red cells. The kinetic studies of K^+ transport and T-ouabain binding experiments described in this report limit the range of tenable explanations for the unusual action of anti-L serum on active transport in LK sheep red cells. The results of the T-ouabain binding experiments are consistent with the conclusion that treatment with anti-L serum increases the number of active transport sites on LK sheep red cell membranes by a factor of about two. The data shown in Fig. 3 and Table 1 show that treatment with anti-L serum increases the maximum K^+ pumping rate four- to sixfold. It appears unlikely, therefore, that the effect of the L-antigen-L-antibody reaction on active K^+ transport in LK sheep red cells can be explained solely on the basis of an increase in the number of pump sites. Rather, it is necessary to suppose that the kinetic characteristics of at least some of the sites are altered as a result of the antigen-antibody reaction.

The effects of alterations in the $[K^+]_i$ and $[Na^+]_i$ on the rate of active K^+ transport shown in Fig. 4 are consistent with this conclusion. In particular, the action of anti-L treatment to produce a shift to the left in the curve relating ${}^iM_K^P$ to $[Na^+]_i$ suggests that the antigen-antibody reaction leads to the formation of new pump sites with kinetic characteristics similar to those observed in genetically MM (HK) rather than LL (LK) sheep red cells. However, the fact that anti-L treatment does not alter the apparent affinity of the pump for external K^+ (Fig. 3) is inconsistent with this view. Clearly, an adequate description of the molecular basis for the effect of anti-L on active K^+-Na^+ transport in LK sheep red cells must await further investigation.

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