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The Effect of Anti-L on Ouabain Binding to Sheep Erythrocytes

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Summary. Binding of ^{3}H-ouabain was studied in high potassium (HK) and low potassium (LK) sheep red cells. In particular, we investigated the effect of anti-L, an antibody raised in HK sheep against L-positive LK sheep red cells, on ^{3}H-ouabain binding and its relation to K^+-pump flux inhibition in LK cells. HK cells were found to have about twice as many ^{3}H-ouabain binding sites and a higher association rate for ^{3}H-ouabain than homozygous LL-type LK cells. The number of ^{3}H-ouabain molecules bound to heterozygous LM-type LK cells is lower than that on LL cells, but the rate of ouabain binding is between that of HK and LL red cells. A close correlation was observed between the rates of ^{3}H-ouabain binding and fractional K^+-pump inhibition. Exposure of LM and LL cells to anti-L did not affect the number of ^{3}H-ouabain molecules bound at saturation, but increased the rates of glycoside binding and K^+-pump inhibition proportionately, so that for LK cells in the presence of anti-L, the rates of the two processes approximate those of HK cells. These data exclude the possibility that anti-L generates entirely new pump sites in LK sheep red cells, but suggest that the antibody increases the affinity of the existing $Na^+ - K^+$ pumps for the glycoside.

Sheep [10, 11, 24], goats [12], and cattle [9, 21] are known to possess red cells containing either high (HK) or low (LK) concentrations of intracellular potassium, $(K^+)_c$. These different $(K^+)_c$ steady states are reflected by the activities of the $Na^+ - K^+$ transport system: active $Na^+ - K^+$ transport and $Na^+ - K^+$ ATPase activity are several-fold higher in HK than in LK red cells [4, 35, 36]. In sheep these activity differences may be attributed in part to the number of $Na^+ - K^+$ pump sites as measured by ^{3}H-ouabain binding [5]. Kinetic studies, however, have shown that the lower $Na^+ - K^+$ pump activity in LK sheep red cells can be explained by the inhibitory action of $(K^+)_c$ on the intracellular aspect of the $Na^+ - K^+$ pump [20], and thus is not solely a function of a smaller number of pump sites.

Cross-immunization of LK sheep with HK sheep red cells induces iso-anti-M antibodies reacting only with M-positive HK (genotype MM) and heterozygous LK (genotype LM) red cells [33]. Immunization of HK

animals with LK cells produces iso-anti-L antibodies detecting the L-antigen, which is present only in LK red cells [32]. Anti-L has been shown to stimulate active $Na^+ - K^+$ transport dramatically in LK sheep and also goat red cells [6, 8, 30], suggesting that the L-antigen is an intimate part of the constituents comprising the $Na^+ - K^+$ pump in these cells. This effect of anti-L has been interpreted as due in part to a qualitative change of the $Na^+ - K^+$ pump: in sheep red cells anti-L decreases the inhibition by $(K^+)_c$ at the inner, sodium loading site of the pump [16, 30], and in goat LK red cells a similar effect of the antibody has been reported [34]. Thus, the L-antibody exerts its effect primarily at the inner surface of the membrane, though changes at the external K^+-loading site may also occur [26]. Furthermore, earlier findings of an increase in the number of ^{3}H-ouabain molecules bound per L-antibody-treated LK cell indicated that the antibody might also unmask occult pump sites [7, 30], but these reports omitted rate studies of ^{3}H-ouabain binding.

From the genetic and physiological point of view, it is important to know whether anti-L indeed unmasks new and potentially HK-type pumps in LK red cells; i.e., produces quantitative *and* qualitative changes. Accordingly, we have undertaken to compare the rate of ^{3}H-ouabain binding in its relation to the inhibition of K^+-pump influx in LK cells in the presence and absence of anti-L. The data indicate that, although higher than previously reported, the numbers of ^{3}H-ouabain binding sites per HK and LK sheep red cell are different, that the rate of ^{3}H-ouabain binding is faster in HK than in LK red cells, and that anti-L increases only the binding rate rather than the number of ^{3}H-ouabain molecules bound per cell. A preliminary report of these data has been presented elsewhere [22].

Materials and Methods

Sheep Red Cells

Blood was drawn from healthy Dorset sheep by jugular venipuncture, with heparin as anti-coagulant, and was used on the day obtained. The cation and antigenic genotypes of all of the sheep had been previously determined by measuring intracellular cation content (Perkin Elmer atomic absorption spectrophotometer), and by hemolytic assays with anti-M and anti-L antibodies as described previously [28, 31].

Incubation Media

Most experiments utilized a "K-free" medium of composition (mM): 130 NaCl, 10 Tris-Cl (pH 7.4), 10 glucose, and 20 sucrose, 290 mOsm (Osmette osmometer, Precision Systems). The cells were washed 4 times in this medium. After resuspension in the same medium at 10% hematocrit, the extracellular potassium concentration, $(K^+)_o$,

was found to be less than 0.2 mM; after 5 hr of incubation at 37 °C, $(K^+)_o$ increased to about 0.4 mM. In experiments where the effect of $(K^+)_o$ on ^{3}H-ouabain binding was assessed, KCl was substituted for an isosmotic amount of sucrose.

^{3}H-Ouabain Specific Activity

^{3}H-ouabain was obtained from New England Nuclear Corp. (Cambridge, Mass.) in benzene/ethanol (1:9) solution (Lot #184–196); the specific activity given by the manufacturer was 13 Ci/mmole. The organic solvents were evaporated by a stream of filtered air and the ouabain taken up in 10 mM Tris-Cl buffer, pH 7.4. The concentration of ouabain in the sample was measured spectrophotometrically at 220 nm (Beckman ACTA II spectrophotometer). A molar extinction coefficient ($\varepsilon = 16{,}800$) was determined experimentally using cold ouabain (Sigma Chemical Co., St. Louis, Mo.) treated similarly to the ^{3}H-ouabain; i.e., evaporation of a standard benzene/ethanol solution and uptake in 10 mM Tris-Cl buffer. Assuming all of the ^{3}H activity resided in ouabain, the specific activity of the sample was determined to be 12 Ci/mmole. This number, corrected for decay, was used to calculate the data presented here.

^{3}H-Ouabain Binding

In order to facilitate rapid sampling and to be able to measure ^{3}H-ouabain binding and ^{42}K-influx on the same cell sample, the following modification of a technique described by Kepner and Tosteson [23] was used. Cells were incubated at 37 °C and 10% hematocrit in the final suspension. Timing was begun on addition of prewarmed solutions of ^{3}H-ouabain to give final glycoside concentrations of between 1 and 10×10^{-8} M. If ^{42}K-influx was not measured, 0.5 ml of suspension was transferred directly into polypropylene centrifuge tubes containing 9 ml ice cold $MgCl_2$ solution (120 mM, buffered with $MgCO_3$, pH 7.6) layered over 2 ml dibutyl phthlate. (Preliminary experiments indicated that free ^{3}H-ouabain did not partition into the phthlate phase.) The tubes were centrifuged at $12{,}000 \times g$ for 2 min in a Sorvall RC 28 centrifuge at 4 °C to pellet the cells beneath the organic phase; the $MgCl_2$ solution was removed and replaced. Then, the second aliquot of $MgCl_2$ solution was aspirated along with the phthlate, and the cells were lysed in 8 ml ice cold 10 mM Tris-Cl, pH 7.4. Subsequently, the membranes were pelleted at $27{,}000 \times g$ for 15 min at 4 °C. The supernatant was removed, and the optical density (OD) at 527 nm measured (Gilford 300N Microspectrophotometer) to determine the volume of cells in each sample. The membranes were washed 3 times with 3 ml of ice cold 10 mM Tris buffer by alternate vortexing and centrifugation. Control experiments demonstrated that up to six washes did not remove bound tritium from the membranes. After washing, the membranes were dissolved in 1 ml of 0.1 N NaOH by heating at 60 °C overnight. Dissolved membranes were transferred quantitatively to counting vials, washing the tubes 3 times with 5 ml aliquots of scintillation fluid (Aquasol, New England Nuclear).

Tritium was counted in a Beckman Liquid Scintillation Counter (Model L 250). Absolute counting efficiency was determined for each sample by means of internal and external standards and varied from 21 to 26% among samples. Vials were stored 2 days in the dark to reduce chemiluminescence. Background counts were less than 40 cpm and were, of course, subtracted from sample counts before calculations. An average sample vial contained 500 to 2000 cpm. The amount of tritium bound to cells incubated with 10^{-4} M cold ouabain in addition to ^{3}H-ouabain was less than 10 cpm above samples incubated without ^{3}H-ouabain, indicating very little binding of tritiated contaminants.

The number of ouabain molecules per cell was calculated from the relation below, modified after Dunham and Hoffman [5]:

$$B = \frac{\text{cpm}\left(\frac{100}{E}\right)N}{C \cdot V \cdot S \cdot \text{Ci}} \tag{1}$$

where

B = number of ouabain molecules bound per cell
cpm = counts per minute
E = counting efficiency
N = Avogadro's number
C = number of cells per ml (assuming MCV = 30 μ^3)
V = volume of cells in sample (ml)
S = specific activity of ^{3}H-ouabain, Ci/mole
Ci = Curie number, 2.22×10^{12} DPM/Ci.

Treatment of Cells with Antiserum

Anti-L antiserum (S-39) was obtained from Professor B. A. Rasmusen (College of Agriculture, Division of Animal Genetics, Univ. of Illinois, Urbana). Serum was dialyzed 36 hr at 4 °C against 100 volumes of the medium to be used in a given experiment. One volume of red cells at 10% hematocrit was preincubated at room temperature with approximately 2.5 volumes of the original serum for 30 min before the addition of ^{3}H-ouabain. Thus, the antibody concentration in the preincubation mixture was approximately 1/4 that in the original serum. Nonimmune HK serum (S-5), heated at 56 °C for 20 min, was used in control incubations.

^{42}K-Influx Measurements

When the influx of ^{42}K was measured, cells were incubated with ^{3}H-ouabain as described above. At timed intervals, aliquots of suspension were removed into 6 volumes of ice cold medium identical to the incubation medium, but lacking ^{3}H-ouabain and anti-L. Cells were centrifuged at 4 °C and washed 2 times in "K-free" medium and then quickly resuspended in sufficient "K-free" medium to give 10% hematocrit. If anti-L was being used, it was incorporated into this suspension and allowed 20 min at room temperature to rebind to the cells. Suspensions were warmed to 37 °C, and ^{42}K (specific activity about 50 mCi/mole; Cambridge Nuclear, Cambridge, Mass.) was added as the chloride to give a carrier concentration of approximately 10 mM $(K^+)_o$. After 1 hr, 0.5 ml samples were transferred into polypropylene centrifuge tubes containing $MgCl_2$ solution and dibutyl phthlate as described above. After centrifugation, washing and removal of the supernatants, cells were lysed in 10 mM Tris, pH 7.4, and ^{42}K was immediately counted in a well-type scintillation counter (Packard Autogamma). As in previous experiments [29], K^+-influx (^iM_K) was calculated according to Kepner and Tosteson [23]. Parallel flasks, containing either 10^{-4} M cold ouabain or no ouabain provided leak ($^iM_K^L$) or ouabain-uninhibited influxes, respectively. Active K^+-influx ($^iM_K^P$) is the difference between the total K^+-influx (^iM_K) and the ouabain-insensitive K^+-influx ($^iM_K^L$); per cent inhibition is:

$$\frac{{}^iM_K^P(\text{uninhibited}) - {}^iM_K^P(^3\text{H-ouabain})}{{}^iM_K^P(\text{uninhibited})} \times 100. \tag{2}$$

After ^{42}K counting, hemolysates were handled as described above for the determination of ^{3}H-ouabain binding. In this way active K^{+}-influx and ^{3}H-ouabain binding could be determined on the same sample of cells. Samples were kept on ice except for the one hour fluxing time and the 15 min necessary for counting ^{42}K. Preliminary experiments demonstrated that the loss of bound ouabain during this time period was insignificant.

Results

Time Course of ^{3}H-Ouabain Binding to MM Type (HK), and LL and LM Type (LK) Sheep Red Cells

Fig. 1 shows the results of a typical series of experiments following the binding of ^{3}H-ouabain to cells with the time of incubation. At this ouabain concentration (2×10^{-8} M) binding proceeds relatively rapidly for the first hour, and increases more slowly for the remaining 4 hr. At higher concentrations of glycoside, binding saturates more rapidly, and the prolonged phase of binding is much less pronounced, as will be shown below. Table 1 presents the data for the eight sheep studied so far. The data on the cells of sheep HK 100 are derived from six separate experiments. Although the conditions (i.e. ^{3}H-ouabain concentration and time of incubation) are not identical for these experiments, the number given represents the number of glycoside molecules per cell at saturation, and the standard deviation permits an estimate of the reproducibility of the method. (The standard deviations of triplicate samples in a given experiment were generally smaller ($\leqq \pm 5\%$)

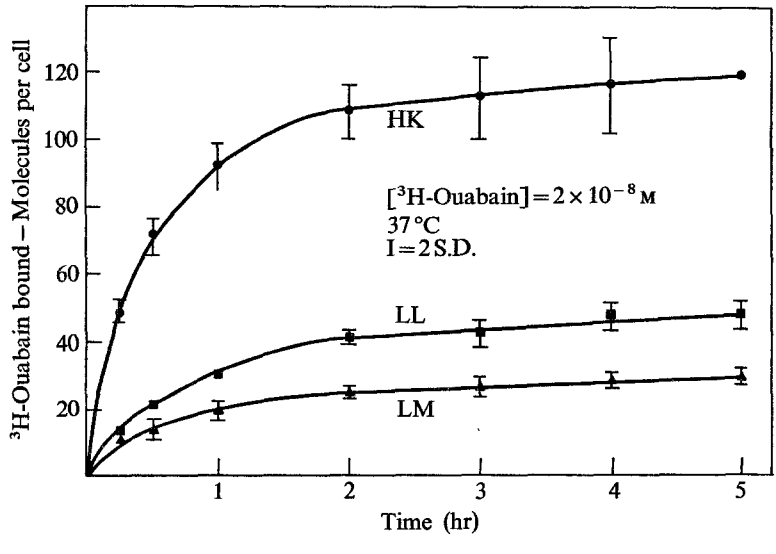

Fig. 1. Time course of ^{3}H-ouabain binding to LL, LM, and HK(MM) sheep red blood cells in "K-free" medium, as described in text

Table 1. Saturation levels of ouabain binding to sheep red cells

Sheep	$(K^+)_c$ $\left(\frac{\text{mmoles}}{\text{liter cells}}\right)$	Exp.	Ouabain molecules per cell
HK 100	84.8	n=6	113±9[a]
HK 101	83.7	D−23	135
LL 110	15.4	D−3	48
		D−5	52
LL 116	17.5	D−11	50
LL 115	21.8	D−15	75
		D−22	78
LM 114	16.4	D−4	30
		D−12	29
LM 112	15.2	D−7	30
LM 117	20.8	D−16	43
		D−24	32

[a] Mean ± SD.

than the deviation given in the Table.) The data for the LL animals seem to indicate considerable variation among the population; further testing of the entire flock is necessary to explore this possibility. It is, however, clear that LM red cells have generally lower (approximately 60%) maximal ^{3}H-ouabain binding sites than LL type cells.

Effect of K^+ on ^{3}H-Ouabain Binding

Fig. 2 represents an experiment designed to test the effect of K^+ on ouabain binding to sheep cells. In agreement with others [1, 13, 15, 17], we found that K^+ drastically reduces the rate of glycoside binding. Although binding was slower in the presence of 1.4 mM $(K^+)_o$, ouabain continued to associate with the cells as the incubation progressed. This suggests that steady-state levels of binding had not been reached, even after 5 hr of incubation. Although active K^+-influx was not measured in this experiment, other data indicate that in the presence of 10 mM K^+, 38 molecules of ^{3}H-ouabain bound per HK cell correspond to approximately 40% K^+-pump inhibition.

Effect of Anti-L on ^{3}H-Ouabain Binding

The effect of anti-L on ouabain binding to LK sheep cells is demonstrated in Fig. 3. Without the antiserum, and at low ouabain concentrations (2.0×10^{-8} M), binding is a slow process. At these concentrations, binding seems

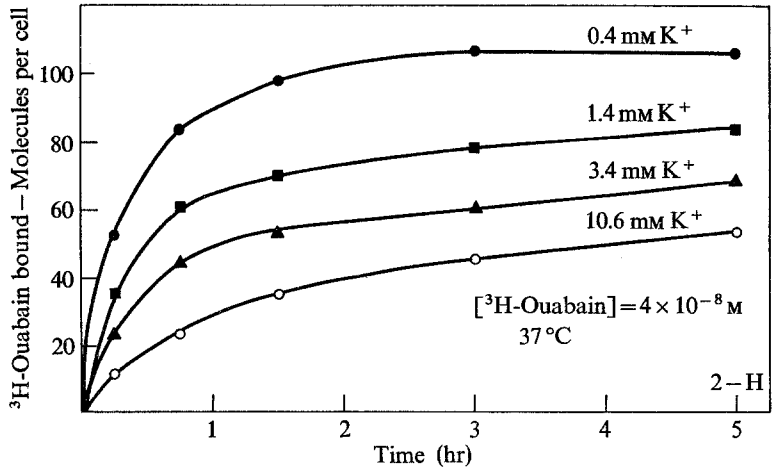

Fig. 2. Effect of K^+ on ^{3}H-ouabain binding to HK(MM) sheep red cells. Media as described in Materials and Methods. $(K^+)_o$ given was measured at the end of the incubation period (5 hr)

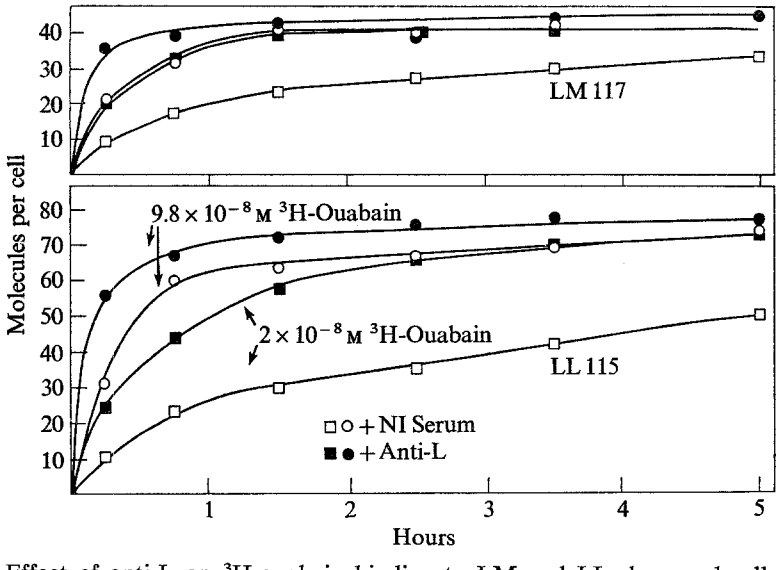

Fig. 3. Effect of anti-L on ^{3}H-ouabain binding to LM and LL sheep red cells. Open symbols (○, □) represent incubation with ^{3}H-ouabain in the presence of nonimmune serum; closed symbols (●, ■) in the presence of anti-L. Squares (□, ■) represent ^{3}H-ouabain concentration of 2.0×10^{-8} M; circles (○, ●) 9.8×10^{-8} M; "K-free" medium

to be incomplete even after 5 hr, since in another experiment with LL 115, the binding of 45 molecules of ^{3}H-ouabain per cell correlated with approximately 50% inhibition of active K^+-influx (*cf.* Fig. 4). However, at 9.8×10^{-8} M ^{3}H-ouabain, the rapid initial phase of binding is more prominent,

and receptor sites are virtually saturated at 2 hr, after which there is very little additional association. It is readily apparent from the graphs that anti-L increases the rate of ^{3}H-ouabain binding to both types of LK cells. It is equally clear from these data that the number of glycoside receptors on both types of cells is unaffected by the antibody.

Correlation of K^+-Influx with ^{3}H-Ouabain Binding

In order to validate the assumption that ^{3}H-ouabain binding represents the association of the glycoside with the K^+ pump, experiments were undertaken to correlate binding with inhibition of active K^+-influx. Fig. 4 represents the results of such experiments with LL and LM cells, with and without anti-L. In each panel, percent K^+-pump inhibition and percent of maximal ouabain binding are plotted on the same axis versus time of incubation with ^{3}H-ouabain. The correlation between inhibition and binding is maintained through the time period; noteworthy is the fact that K^+-pump inhibition proceeds slowly after $1^1/_2$ hr, parallel to the slow increase in the amount of ^{3}H-ouabain bound during this time.

It is clear from the data in Fig. 4 that anti-L does not change the relationship between binding of glycoside and inhibition of active K^+-influx.

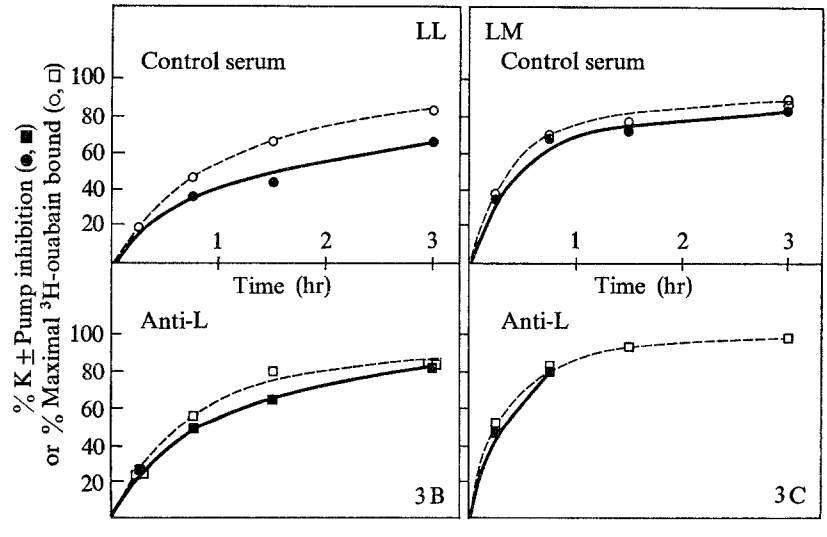

Fig. 4. Time course of K^+-pump inhibition and ^{3}H-ouabain binding to LL and LM sheep red cells in the presence or absence of anti-L. Open symbols (○, □) represent the percent of maximal ^{3}H-ouabain binding, and closed symbols (●, ■) represent percent inhibition of active K^+-influx. The upper panels show experiments in the presence of nonimmune serum; lower panels illustrate data for anti-L incubations. ^{3}H-ouabain concentration was 4×10^{-8} M in "K-free" medium

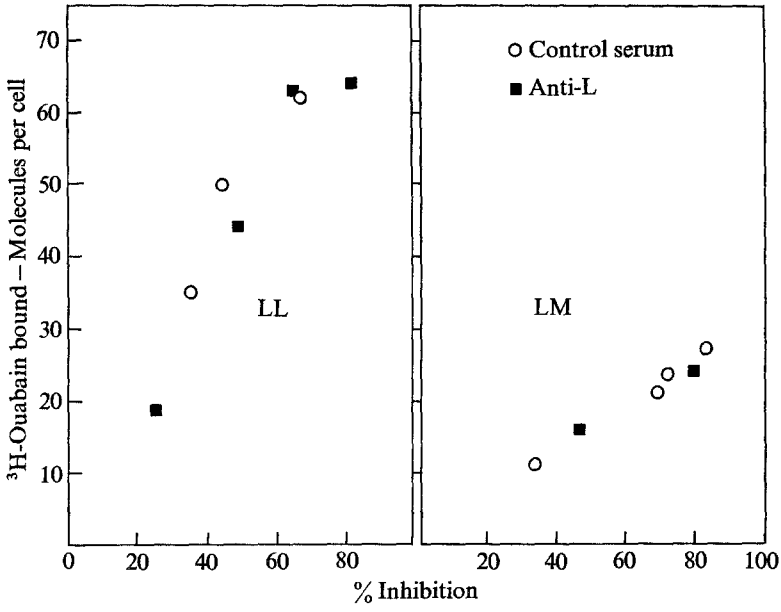

Fig. 5. Percent K^+-pump inhibition versus ^{3}H-ouabain binding to LL and LM sheep red cells. Open circles (○) represent preincubation with nonimmune serum; closed squares (■) with anti-L. ^{3}H-ouabain concentration was 4×10^{-8} M in "K-free" medium

This is also demonstrated in Fig. 5, in which the number of ouabain molecules bound per cell is plotted versus the % inhibition of active K^+ influx obtained. In addition, these data strengthen the conclusion that the number of K^+ pump sites does not increase with the addition of the antibody; such a change would cause the data points for cells treated with antibody to be shifted upwards on the ordinate of Fig. 5.

Discussion

^{3}H-ouabain binding has been employed by several investigators to estimate the number of $Na^+ - K^+$ pump sites on a variety of cell types [2, 5, 13, 18]. Our studies with sheep red cells were initiated in an attempt to elucidate the nature of the interaction by which anti-L stimulates active K^+ transport and the $Na^+ - K^+$ ATPase in LK sheep erythrocytes. In summarizing the results reported in this paper, four observations warrant emphasis: 1) the number of glycoside binding sites on HK cells is approximately double that on homozygous LK cells; 2) the heterozygous LM cells contain only 50–60% of the binding sites of the LL cells; 3) the rate of ouabain binding is fastest in HK cells, slowest in LL cells, and intermediate in the

heterozygote LM cells; 4) anti-L, while increasing the rate of ouabain binding to LK cells, does not alter the number of binding sites on each cell.

Dunham and Hoffman [5] have reported that the number of ouabain molecules necessary for 100% inhibition of the $Na^+ - K^+$ pump was 42 for HK and 7.6 for LK sheep red cells. Other workers, however, have found higher numbers of glycoside binding sites. Ellory and Tucker [7] reported 71 sites per HK cell and 37 for LK; Lauf *et al.* [30] found approximately 30 sites per LK cell. The reasons for these discrepancies are uncertain, but different technical approaches, different ^{3}H-ouabain specific activities, and the use of different sheep may be important factors. [1]

The problem of "nonspecific" binding of ouabain (that is, binding which is not molecularly associated with the K^+ pump) has plagued investigations attempting to quantitate the number of $Na^+ - K^+$ pump sites. Hoffman [18] has proposed that Cs^+ selectively prevents "nonspecific" ouabain binding, since the rate of inhibition of K^+-influx by ouabain was unchanged by incubation in the presence of Cs^+ (compared to incubation in Na^+ medium), while ^{3}H-ouabain binding was reduced. Other authors [3, 14] have reported that Cs^+ does reduce the binding of ouabain, but that the effect is a result of competition of the ion with glycoside for specific, that is, pump-associated sites.

However, in this report we have not made a rigorous attempt to separate specific from nonpump binding of ^{3}H-ouabain to LK cells for three reasons. First, glycoside binding saturates at low ouabain concentrations (Fig. 3); if the amount of ^{3}H-ouabain bound increases with time, the inhibition of active K^+-influx increases concomittantly (Fig. 4). Secondly, the plots of the number of ouabain molecules per LK cell versus percent K^+-pump inhibition (Fig. 5) are either straight (LM) or slightly convex (LL). These two facts indicate that the nonpump binding sites, if they exist, have the same or greater affinity for ouabain than the pump-associated sites. (Plots of ouabain bound versus percent K^+-pump inhibition for HK cells (not shown) are more complex, and can be interpreted as indicating some component of "nonspecific" binding, or heterogeneity of ouabain binding

1 There is a similar controversy about the number of glycoside receptor sites in human erythrocytes. Glynn [15] first estimated the number of sites to be 1200 or less per cell by following the disappearance of scillaren from the medium in which the cells were incubated. Hoffman and Ingram [19] first used ^{3}H-ouabain on human cells and reported 250 molecules per cells, a number later confirmed by Baker and Willis [2]. On the other hand, Gardner and Conlon [13] reported maximal binding of 1200 molecules per cell. Recently, Knauf, Proverbio and Hoffman [25] published data on the number of ouabain molecules bound to human red cell ghosts which was 2 to 4 times higher than their previous estimate, i.e. close to that reported by Gardner and Conlon [13].

or K^+ pump sites, or both. This subject will be dealt with in detail in a later publication.) Finally, our primary interest in this study was to describe the changes brought about in ouabain binding to LK cells by the interaction of the cells with anti-L, for which only a comparison of the number of sites before and after antibody treatment is necessary.

The experiments represented in Fig. 3 produced the somewhat surprising result that the maximal number of ouabain molecules which could be bound to LK cells was the same in the presence or absence of anti-L. This is contradictory to the previous reports from Ellory and Tucker [7] and from this laboratory [30], which had indicated an increase in the number of ouabain binding sites by the antibody. It is difficult to account for the discrepancy between our results and those of earlier studies, since ouabain binding in the earlier work was correlated with K^+-pump inhibition and the number of sites calculated by extrapolation to 100% inhibition [30]. However, we feel that the data presented in this report, examining the rates of glycoside binding and K^+-pump inhibition, provide a more complete picture of the ouabain/receptor interaction in these cells and the effect on this interaction of anti-L. In view of the data in Fig. 3 and the tight correlation of ^{3}H-ouabain binding with K^+-pump inhibition demonstrated in Figs. 4 and 5, we find the conclusion inescapable that anti-L does not change the number of ouabain binding sites on LK sheep red cells.

From the data presented in Fig. 3, it is apparent that anti-L increases the rate of ^{3}H-ouabain binding to LK cells. This is also demonstrated in Fig. 6, in which the percent of maximal ouabain binding is plotted versus time for HK cells and for the two types of LK cells in the presence and absence of antibody. This graph shows that the control LL cells bind ouabain more slowly than the other two cell types, and HK cells exhibit the fastest rate of binding; the heterozygote LM cells are intermediate in rate. The addition of antibody clearly changes the rate of glycoside binding in both types of LK cells, so that in both cases, the rate becomes nearly identical to that of HK cells.

In terms of the molecular mechanism of the anti-L effect, the findings reported here are consistent with the hypothesis that the antibody increases the cation turnover number of each site by affecting the cation affinity of the pump [30]. However, at the present time, we cannot exclude the possibility that ^{3}H-ouabain binds to active *and* inactive pump sites, and that anti-L transforms inactive into active pumps, and also changes the rate of ^{3}H-ouabain binding of *both* types of sites. It is important to note that LM-type LK red cells have an even smaller number of ^{3}H-ouabain molecules bound per cell, and that they bind glycoside faster than LL cells. The lower number

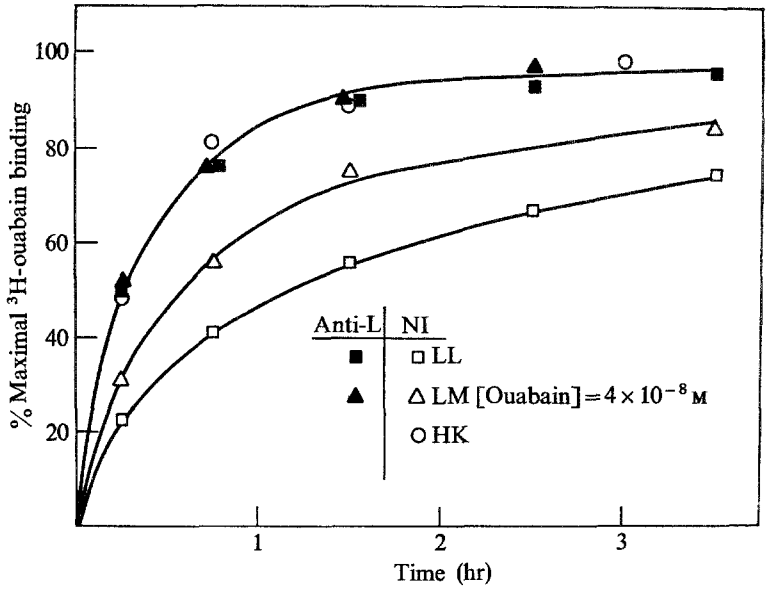

Fig. 6. Rate of ^{3}H-ouabain binding to LL, LM, and HK(MM) sheep red cells. Binding is expressed as percent of maximal binding. Open symbols (○, □, △) represent incubation in the presence of nonimmune serum (NI); filled symbols (■, ▲) in the presence of anti-L

of ^{3}H-ouabain binding sites in LM cells and our previous finding that K^+-pump activation by anti-L is less in LM cells than in the homozygote cells [26, 27] are of interest and require further studies.

Irrespective of these and other possibilities, it appears to be highly likely that the dominant Ka locus controls the LK-property by a smaller number of qualitatively different pumps. Anti-L may correct to some extent the qualitative, but not the quantitative differences between HK and LK cells.

Note Added in Revision: The results presented here are consistent with a recent report by Sachs *et al.* (J. R. Sachs, P. B. Dunham, D. L. Kropp, J. C. Ellory, J. F. Hoffman, 1974. *J. Gen. Physiol.* **64**:536), where it was shown that HK goat cells bound ouabain more rapidly than LK cells, and that anti-L (raised in sheep) increased the rate of ouabain binding of LK goat cells to that of HK goat cells.

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