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HIGH POTASSIUM AND LOW POTASSIUM ERYTHROCYTES IN CATTLE

BY P. CHRISTINAZ AND H. J. SCHATZMANN

From the Veterinary Pharmacology Institute, University of Bern, Switzerland

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SUMMARY

1. The K and Na concentrations in red blood cells (R.B.C.) of 251 animals of the Simmenthal breed and thirty-two animals of other cattle breeds were measured. $[Na]_{cells}+[K]_{cells}$ was 89·4 m-mole/l. cells. $[K]_{cells}$ varied between 7 and 70 and $[Na]_{cells}$ varied in the inverse sense between 15 and 87 m-mole/l. cells.

2. The frequency distribution of animals according to K content of R.B.C.S, which could best be fitted by two superimposed Gaussian curves, suggests that there are two distinct types of cells (high K (HK) cells and low K (LK) cells). Animals with HK cells were considerably less frequent than animals with LK cells.

3. Differences in breed, age or sex do not account for the difference in cation content of R.B.C.S.

4. Cold stored or PCMBS-treated HK cells show a more vigorous cation pump activity than equally treated LK cells.

5. At a Na concentration of 100 mM and a K concentration of 10 mM isolated haemoglobin-free membranes prepared from HK cells exhibit a higher activity of Na+K stimulated (ouabain inhibitable) ATPase activity per mg of protein than membranes from LK cells.

INTRODUCTION

Evans and co-workers (Evans & King, 1955; Evans, King, Cohen, Harris & Warren, 1956; Evans & Mounib, 1957; Evans, Harris & Warren, 1958; Evans, 1968) have shown that sheep of the same breed fall into two classes with respect to the K concentration within their red blood cells (R.B.C.S). The majority of individuals have low K (LK) cells and a minority has high K (HK) cells (Evans & Mounib, 1957), although the proportion of LK to HK animals may vary considerably between different breeds (Evans *et al.* 1958). The authors presented convincing evidence for the claim that the cell type is genetically determined and that the genetic

pattern can well be explained by the simple assumption of a single pair of alleles, the gene for LK being dominant over the gene for HK. Several investigations have elucidated the physiological basis for the difference in R.B.C. K (and Na) content (Sheppard, Martin & Beyl, 1951; Joyce & Weatherall, 1958; Tosteson & Hoffman, 1960; Tosteson, 1963, 1966, 1967, 1969). The salient feature seems to be a high number of Na – K pump sites per unit cell membrane surface in HK cells and a low number in LK cells (Dunham & Hoffman, 1969; see also Hoffman, 1969; Lauf, Rasmusen, Hoffman, Dunham, Cook, Parmelee & Tosteson, 1970). In addition, Tosteson & Hoffman (1960) found that the permeability of the membrane towards K and Na is different in the two cell types. The question as to whether there exist kinetic differences in the system for active cation transport in the two cell types is as yet under discussion (Hoffman & Tosteson, 1969; Tosteson, 1969; Whittington & Blostein, 1971).

In certain goat breeds a similar dimorphism with respect to alkali cation content of red cells was found (Evans & Phillipson, 1957).

In cattle the alkali cation content of R.B.C.S is less well known. A study on Ayrshire cattle suggested that only one type of animals with LK cells (with a mean value of 20 m-mole/l. cells and a range from 12 to 39 m-mole/l. cells) can be found (Evans & Phillipson, 1957), although the contention exists that a bimodal distribution is universally encountered in ruminants (Evans, 1968). We therefore decided to study the problem using Swiss cattle breeds. The survey included mainly animals of the Simmenthal breed but also a few Swiss brown (Braunvieh), Swiss black spotted (Freiburger Schwarzfleckvieh) and others. Since analysis of the results revealed no difference between breeds, the figures from all animals tested were pooled. An attempt was made to describe quantitatively the differences in passive permeability, Na – K pump activity and Na + K activated membrane ATPase by measuring net cation fluxes in the cold, net cation fluxes at 37° C and phosphate liberation from ATP by isolated membranes.

METHODS

Since it is known that LK sheep and cattle are born with high R.B.C. K-concentration which falls to a low steady level during the first weeks of life (Widdas, 1954; Wise, Caldwell, Parrish, Flipse & Hughes, 1947; Wright, Bradley, Nelson & Coghlan, 1958; Tosteson & Moulton 1959; Evans & Blunt, 1961; Blechner 1961; Tosteson, 1966) only animals above 4 months of age were used. In a first survey 283 animals were tested with respect to alkali cation content of R.B.C.S. Among these were 251 Simmenthal, eight Swiss Brown, seven Swiss black spotted and seventeen others, 193 bulls and ninety cows and heifers. Bulls were animals from the Centre d'insémination artificielle (Neuchâtel), cows and heifers were animals kept at different farms of the western part of the Swiss plateau. Climatic conditions and feeding habits were approximately similar for all animals tested. The sampling of blood took place

during the summer months. Blood was collected at the farm or in the slaughter-house into heparinized vials and brought to the laboratory in an ice cooled isolating box.

Determination of Na and K concentration in R.B.C.*s.* The cells were washed three times at room temperature with about a fivefold volume of isotonic $MgCl_2$ solution (119 mM), which brought both Na and K concentration in packed cells to a minimum steady level. The white cells were discarded during the washing. After the last packing of cells in 5 or 3 ml. polyethylene tubes at 5000 rev/min the trapped intercellular fluid volume amounted to 5·4%, as judged by the haematocrit value measured in 1·1–1·2 mm bore glass tubes at 10000 rev/min for 10 min or longer. A correction was made for this in calculating intracellular Na and K concentration, whereas no correction was applied for the remaining intercellular fluid known to be present even under conditions of haematocrit determination. The water content of cells was determined by drying a weighed specimen of packed cells to constant weight in an oven at 100° C and over phosphorus pentoxide. The result was 68·4 ± 0·96 g/100 g cells. The specific gravity of packed cells was determined by weighing 25 ml. in a volumetric flask or by immersing a glass ball hanging from an analytical balance into a beaker filled with packed cells. The value found was 1·084. From these values the water content of cells was found to be 72·5 g/100 ml. This value was used to convert concentrations per ml. cells to concentrations per ml. cell water.

Estimation of active Na-K transport. (1) Whole blood was defibrinated by stirring with a wooden rod or prevented from clotting with heparin and stored for 1 week at 2° C in order to deplete the cells of K and load them with Na. Subsequently 11 mM glucose was added and the blood was incubated at 37° C for 6–8 hr with and without ouabain 10^{-4} g/ml. Na and K was determined in samples taken at different time intervals as described above or in the cells of a known amount of blood using the haematocrit to calculate cellular concentration. In some experiments Na and K were measured in the protein-free supernatant after haemolysis in water and deproteinization with an equal volume of 10% trichloroacetic acid. From the change in concentration net Na and K flux sensitive to ouabain was calculated by referring changes to the original volume.

(2) Following the procedure described by Garrahan & Rega (1967) cells washed in isotonic NaCl solution buffered with 20 mM Tris-Cl to pH 7·4 were exposed to 0·1 mM parachloromercuribenzene sulphonic acid (PCMBS) in a twentyfold volume of (mM) 150 NaCl, 15 Tris-Cl, 1 $MgCl_2$ (pH 7·2) solution during 20 hr in the cold. After this treatment the cells were washed once with and incubated for 1 hr at 37° C in the same solution containing in addition 11 mM glucose and 2 mM cysteine but no PCMBS. Subsequently the cells were washed three times with (mM) 140 choline-Cl, 15 Tris-Cl (pH 7·2) solution. The cells exchanged Na for K during the treatment but, unlike in human cells run as a control, equilibrium was not reached, the HK cells retaining more K than the LK cells. The transport experiment was carried out with these cells suspended in a tenfold volume of the following solution: (mM) 140 choline-Cl, 20 KCl, 1 $MgCl_2$, 2·5 K_2PO_4, 11 glucose, 2 cysteine (pH 7·3). Again, net ouabain sensitive fluxes were measured as described. Na movement was downhill, whereas K movement was against a very small or no gradient.

Since the active fluxes are exceedingly low in cattle cells, consistent results were obtained in most cases for K only. In one experiment following cold storage comparatively large fluxes were obtained, which allowed calculation of the electrochemical work done per hour by the Na–K pump according to

$$W = RT\left[{}^{p}\Phi^{net}_{Na} \ln\frac{[Na]_o}{[Na]_i} + {}^{p}\Phi^{net}_{K} \ln\frac{[K]_i}{[K]_o} + ({}^{p}\Phi^{net}_{Na} - {}^{p}\Phi^{net}_{K}) \ln\frac{[Cl]_o}{[Cl]_i}\right],$$

where ${}^{p}\Phi^{net}$ is the pump flux in mole/hr, $RT = 618$ and W is the work per hr in

kcal. The assumption was made that the chloride distribution (or the membrane potential) is equal in the two types of cells and similar to that in human red cells. The difference between the chloride distribution in human (Cl_o/Cl_i = 1·4), cattle (1·28) (Whittam, 1964) and sheep (1·46) (Tosteson & Hoffman, 1960) R.B.C.S is not large and the contribution of the electrical term is insignificant. The concentrations were the mean concentrations calculated from initial concentration in cell water, change in cellular concentration and initial values in the serum.

Measurement of the Na+K stimulated membrane ATPase activity. Haemoglobin-free isolated membranes made permeable by freezing and thawing were prepared according to the method of Garrahan, Pouchan & Rega (1969) as described previously (Schatzmann & Rossi, 1971). The assay medium was composed as follows: (mM) 100 Na, 10 K, 60 Tris, 5 Mg, 1 Tris-EGTA, 2·5 Na_2ATP, 180 Cl (pH 7·38). The sample size was 2·5 ml. and its protein content 25–28 μg/ml. Temperature was 38° C and incubation time 1, 2 and 3 hr. Na+K ATPase was taken to be identical with the difference between ouabain free samples and samples with ouabain 10^{-4} g/ml.

For Na and K determinations red cells or solutions were diluted with water such that the final concentrations were below 1 mM. These dilutions were measured on an EEL flame photometer run with coal gas from the main and air. Protein was measured according to Lowry, Rosebrough, Farr & Randall (1951), inorganic phosphate was determined according to Berenblum & Chain (1938). The isotonic $MgCl_2$-solution was checked by titration with EDTA using eriochrome black as indicator.

All chemicals were analytical grade from Merck if not mentioned otherwise. Parachlormercuribenzene sulphonic acid (PCMBS) was purchased from Sigma Chemicals, glucose was Analar grade of B.D.H., Na-ethylenediamintetraacetic acid (EDTA) and eriochrome black were obtained from Siegfried (Switzerland), 2-2′ethylene-dioxy-bis-ethylimino-di(acetic acid) (EGTA) was from Fluka (Switzerland), ouabain was the DAB product of Merck and heparin was 'Liquemin' Roche.

RESULTS

Frequency distribution of animals according to the alkali cation content of R.B.C.s

In Fig. 1*A* the cellular Na and K concentrations are plotted against each other. Each point represents one animal. The calculated regression line intersects with the co-ordinates at 89·4 m-mole/l. cells indicating the average total cation content which corresponds to 123 mM in the cell water after correction for cell solids and extracellular fluid.

The sum of the concentrations of Na and K in the cell water is considerably less than that in plasma. Bernstein (1954) already observed that in OX R.B.C.S this discrepancy is larger than in a number of other mammalian R.B.C.S examined.

Fig. 1*B* shows the frequency distribution for Na and K separately. Both distributions are markedly skew. The values for K vary between 7 and 70 m-mole/l. cells and those for Na between 15 and 87 m-mole/l. cells. The K distribution shows a large maximum at 20 m-mole/l. and a smaller peak at 50 m-mole/l., which is not matched by a clear-cut elevation

at the low values on the Na histogram. However, this might be due to blurring of the Na distribution caused by the fact that Na is more difficult to measure accurately.

It is clear that the K data cannot be fitted by a Gaussian distribution (for the deviation from the corresponding normal curve, estimated by the χ^2 test, a $P \ll 0{\cdot}001$ is obtained). Therefore the question had to be resolved whether we deal with a homogeneous population with a skew distribution or whether the data reflect the presence of two (or more) superimposed normal distributions in the population. The arguments that seem to make the second assumption more probable are as follows.

(1) The cellular K content is a continuous magnitude with defined theoretical limits (the lower limit is given by the Donnan distribution, the upper limit by the extreme case where passive permeability is negligible compared with pump fluxes for Na and K, leading to the K concentration approaching the total cation content). In general such values tend to distribute according to a Gaussian curve in sufficiently large samples.

(2) In Fig. 2*A* the population was divided at 36·25 m-mole/l. into two groups and the corresponding normal curves are shown (dashed lines). The compound curve resulting from the two normal curves (continuous line) fits the experimental data quite well in all but one class (marked with an asterisk). Omitting this class gives a $P \cong 0{\cdot}2$ for the deviation of the histogram from the curve in a χ^2 test.

(3) Similarly, a plot of the integral of the compound curve from Fig. 2*A* on a linear probability chart (Fig. 2*B*) fits the data reasonably well (continuous line), whereas a smooth positively skew curve fitted to the data by eye, under the condition that it has the same integral, matches the experimental points poorly at high K concentrations (dashed line). The appearance of two straight portions in the probability plot joined by an S-shaped part is indicative of two normal distributions being at the basis of the distribution found.

In the two groups obtained by the arbitrary division at 36·25 m-mole/l. the number of animals from a particular breed, of animals below 2 years of age or of either sex was not statistically different from the number expected from the whole population. It seems safe to conclude, therefore, that the tail of the distribution is not due to breed, age or sex.

Na-K transport across the cell membrane

(*a*) *Active transport.* R.B.C.s from three typically HK animals (mean K concentration 60 m-mole/l. cells) were compared with R.B.C.s from three LK animals (mean K concentration 22·3 m-mole/l. cells). Cold storage or treatment with PCMBS was used to increase Na and lower K concentration before the experiment. Both types of cells showed extrusion of Na

and uptake of K against the electrochemical gradient (Fig. 3*A*). In both types the active transport was completely blocked by ouabain (Fig. 3*A*). Table 1 shows the ouabain inhibitable net K movements measured during 3 and 6 hr. When measurements extended over two successive 3 hr periods the two results were averaged, because no deviation from linearity was

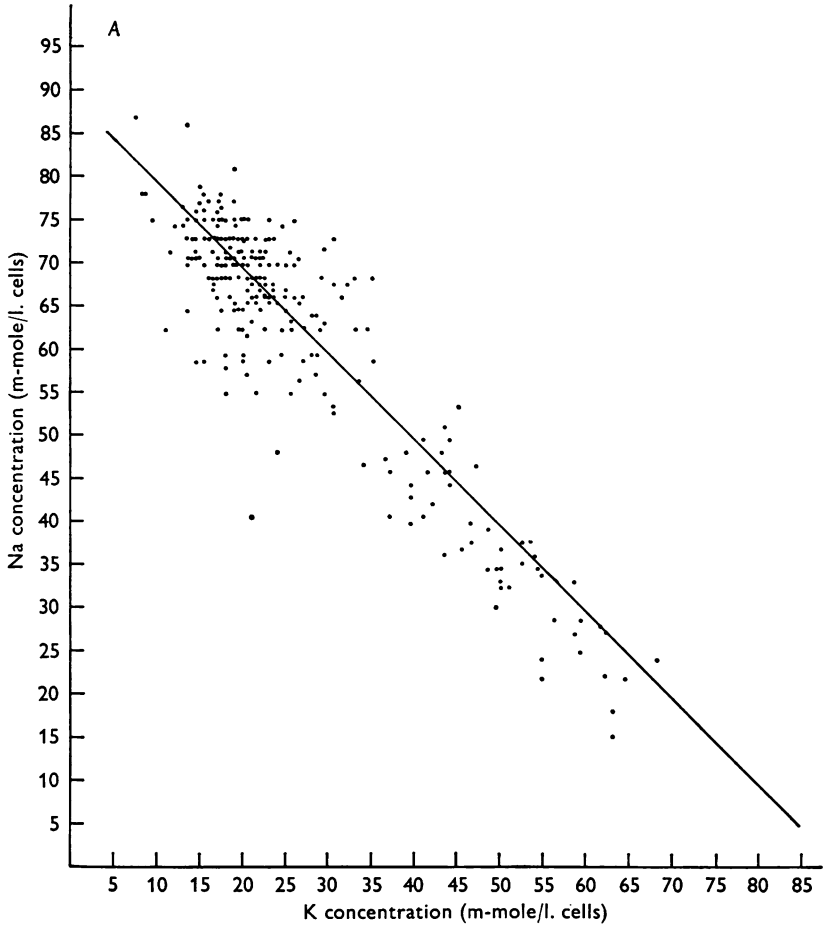

Fig. 1a. For legend see opposite page

detectable. It may be seen that HK cells transported K at a faster rate than LK cells in spite of the fact that the average gradient against which transport took place was larger in HK cells. Consistent results for Na transport were obtained only in one experiment in which the fluxes were particularly large. The result is given in Table 2 which also contains the work done by the pump. The work performed by HK cells was 2·5 times as large as that done by LK cells. When the regression line through all

the points of the experiment (six measurements) was calculated it appeared that the figure for Na flux in LK cells given in the Table probably overestimates the true value. The difference in work, therefore, is a lower estimate of the true value. The coupling ratio (number of Na ions transported per one K ion) calculated from the regression line for LK cells is 1·34.

(*b*) *Passive movements.* During the cold storage period of the experiment of Table 2 cellular Na and K concentrations were measured (Fig. 3 *B*). Net K efflux and Na influx were therefore known. The mean cation gradients were calculated from the initial concentration in cell water, the

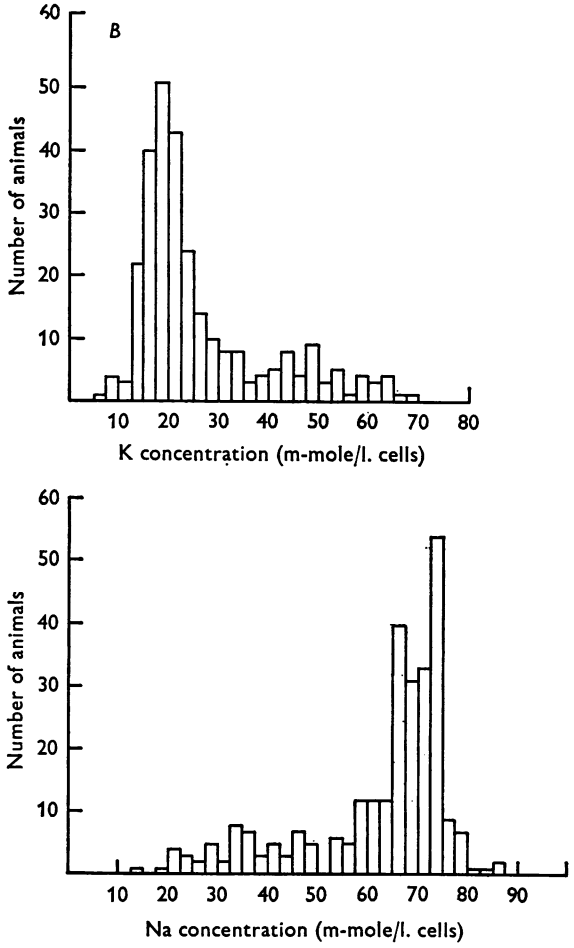

Fig. 1. *A*, plot of cellular Na concentration *vs.* cellular K concentration in red blood cells from 283 animals. Each point represents one animal. *B*, frequency distribution of cellular Na and K concentration in 283 animals.

normal values for Na concentration (154 mM) and K concentration (5 mM) in plasma (H. Gerber, personal communication), the net flux and the haematocrit value. By dividing the net flux by the electrochemical potential gradient a measure of membrane permeability (P_K, P_{Na}) is obtained

$$P_K = \frac{{}^{l}\Phi_K^{net}}{RT \ln \frac{[K]_i \cdot [Cl]_i}{[K]_o \cdot [Cl]_o}}, \qquad (1a)$$

$$P_{Na} = \frac{{}^{l}\Phi_{Na}^{net}}{RT \ln \frac{[Na]_o \cdot [Cl]_o}{[Na]_i \cdot [Cl]_i}}, \qquad (1b)$$

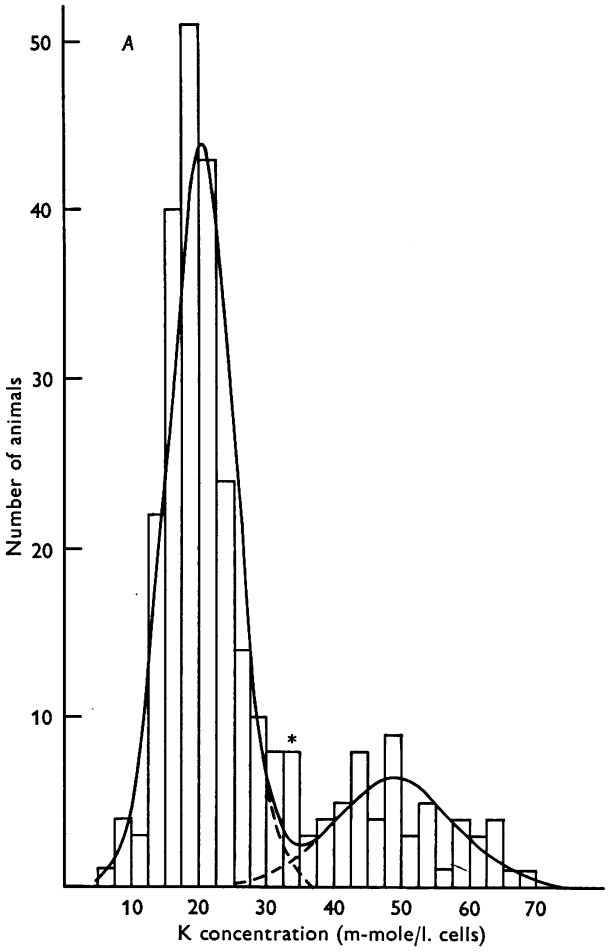

Fig. 2A. For legend see opposite page.

where $^{l}\Phi_{K}^{net}$ and $^{l}\Phi_{Na}^{net}$ are the net leak fluxes. P_{Na} and P_{K} correspond to the rate constants calculated by Tosteson & Hoffman (1960). $\alpha = P_{Na}/P_{K}$ gives the permeability of Na relative to that of K. Using for the chloride distribution $(Cl_i/Cl_o) = 0{\cdot}78$ (Whittam, 1964, p. 81) α was found to be 2·65 for LK cells and 1·52 for HK cells. This is the inverse of what Tosteson & Hoffman (1960) found for sheep cells, where LK cells are relatively more permeable to K, but in cattle the difference between HK and LK

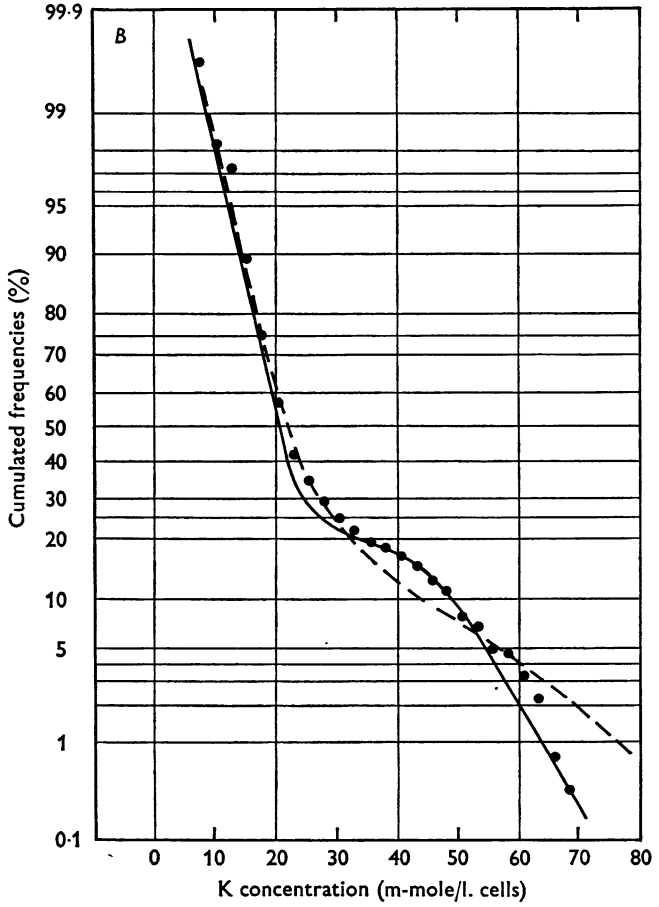

Fig. 2. *A*, frequency distribution of K concentration in red blood cells of 283 cattle with two normal curves giving a good statistical fit. The curves have the same integral as the experimental data. The tail of the distribution is not accounted for by age, sex or breed. *B*, the same data plotted on a linear probability chart (filled circles). The continuous line corresponds to the continuous curve in *A*, the dashed line corresponds to a skew distribution fitted to the histogram of *A* by eye, disregarding the hump at high K concentrations.

cells is less marked. Inserting the concentrations in plasma and in the cell water of fresh cells from the experiment (K_{LK} = 40 m-mole/l., Na_{LK} = 92; K_{HK} = 79, Na_{HK} = 28) into the equations and assuming that the rate constants for downhill movements at 37° C are equal to those at 2° C, (disregarding the factor 1·1 due to T in (1a) and (1b)) the leak fluxes

TABLE 1. Ouabain inhibitable K uptake into Na-loaded cattle R.B.C.S

Loading procedure	Expt. no.	Animal no.	Low K cells (LK) (m-mole/l. cells.hr)	Animal no.	High K cells (HK) (m-mole/l. cells.hr)
			A		*B*
PCMBS-cysteine	1	1	0·23	4	1·93
		2	−0·07	5	0·50
Cold storage	2	1	0·235	4	0·56
		2	0·185	5	0·365
Cold storage	3	3	0·65	6	1·68
Mean ± S.E. of mean			0·246 ± 0·116		1·007 ± 0·33
			P for $B-A$ against 0 = 0·05		

Numbers in Table are the average of two successive 3 hr periods, except for Expt. no. 3 with only one 3 hr period. Temp. 37° C, glucose added 11 mM, ouabain 10^{-4} g/ml. Medium for PCMBS-treated cells see text. Cold storage cells incubated in plasma (Expt. no. 2) or serum (Expt. no. 3). Average K content of fresh LK cells: 22·3 m-mole/l. cells, of fresh HK cells: 60 m-mole/l. cells.

TABLE 2. Cold stored cattle blood incubated at 37° C in serum with 11 mM glucose added (expt. of Fig. 3A). Ouabain inhibitable net Na and K movements, mean concentration gradient across the cell membrane and electrochemical work calculated

Type of cells	Net K flux (m-mole/l. cells.hr)	Net Na flux (m-mole/l. cells.hr)	Mean gradient $[K]_i/[K]_o$	Mean gradient $[Na]_o/[Na]_i$	Electrochemical work (kcal/l. cells.hr)
High K cells (HK)	1·68	1·74	3·29	2·13	1·80
Low K cells (LK)	0·65	1·32*	2·41	1·34	0·73

Single experiment. Whole blood was defibrinated and stored for 8 days at 2° C. The experiment lasted for 7 hr. Intracellular Na and K was measured in the cells of 1 ml. blood and the concentration per litre cells calculated from the haematocrit. The concentration change between 0·5 and 8 hr was measured in a control and a sample with ouabain 10^{-4} g/ml. The difference between these two samples was taken to calculate ouabain inhibited fluxes. A calculated regression line through all points (6) of the experiment showed that the Na flux for low K cells given in the Table probably over-estimates the true value.* Mean concentration in the cell water was calculated as indicated in the text. $[Cl]_i/[Cl]_o$ was taken as 0·72. Work was calculated as indicated in text. [K] in fresh HK cells was 57 m-mole/l. cells and in fresh LK cells 29 m-mole/l. cells.

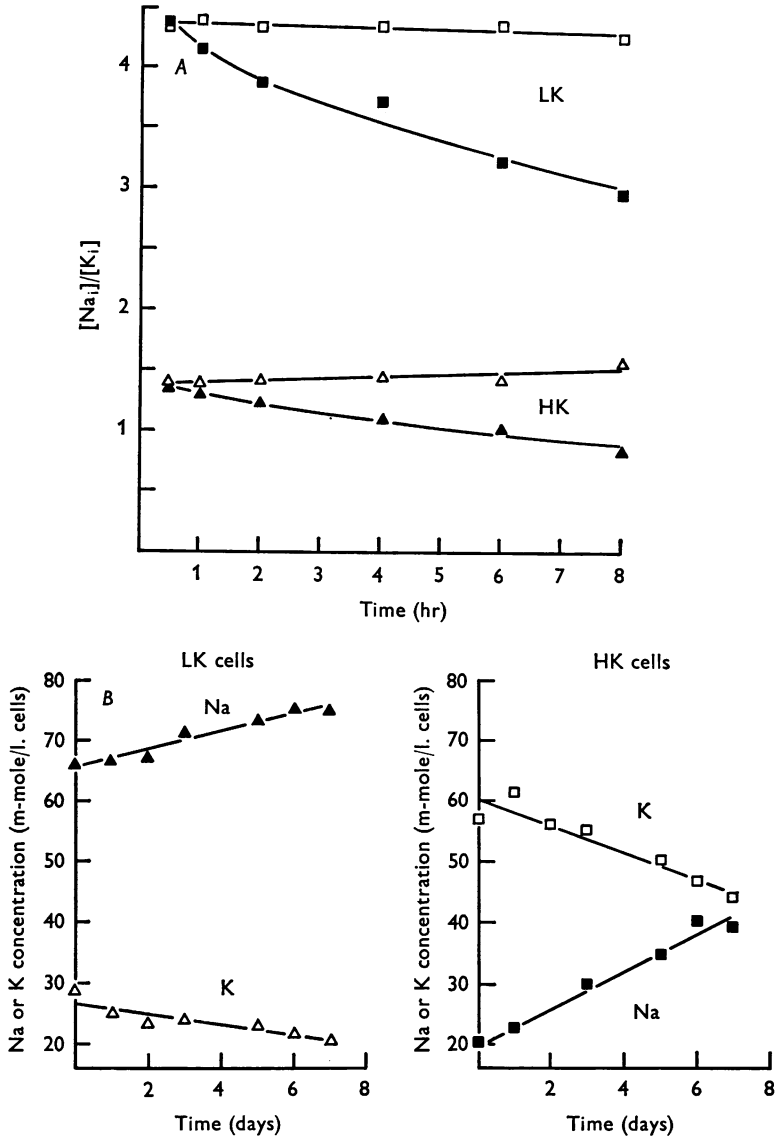

Fig. 3. *A*, whole blood stored for 8 days at 2° C. At 0 time 11 mM glucose was added and the blood was incubated at 37° C. Na and K content of cells was measured at intervals and the cellular concentration per unit original cell volume was calculated. Ordinate: ratio of these concentrations, abscissa: time. Above: LK cells, below: HK cells. ■, ▲, without ouabain; □, △, with ouabain 10^{-4} g/ml. Decrease of Na/K indicates activity of the Na – K pump. Same experiment as Table 2. *B*, changes in cellular Na and K concentration during the cold storage period, preceding experiment of A, ordinate: Na or K concentration in m-mole/l. cells, abscissa: time in days. Left: LK cells, right: HK cells. Experiment used to calculate the figures shown in Table 3.

under normal conditions (steady-state conditions) are obtained. The result is shown in Table 3. The assumption of equal leak fluxes at different temperatures is not beyond doubt, but alternative procedures (measuring leak fluxes at 37° C in the presence of ouabain, or in starved cells) are not utterly satisfactory either. From Table 3 it may be seen that Na flux and K flux are nearly equal to each other in both types of cells but that they are about 2·5 times as large in HK cells as in LK cells. Since pump fluxes under steady-state conditions in plasma are equal to leak fluxes it can be concluded that the coupling ratio of the pump (number of Na ions exchanged for one K ion) is not far from unity. For this conclusion it is only necessary to postulate that a change in temperature changes the rate constants for Na and K movements in both cell types by the same factor.

TABLE 3. Leak fluxes calculated for fresh cells in plasma (m-mole/l. cells.hr) at 2° C

	$\Phi_{Na}(a)$	${}^{1}\Phi_{K}(b)$	(a/b)
HK	3·86	3·02	1·28
LK	1·5	1·21	1·24

P_{Na} and P_K were calculated from net fluxes during cold storage of the experiment of Table 2, using eqns. (1*a*) and (1*b*). By inserting these and concentrations in cell water of the same cells in the fresh state and normal plasma values in (1*a*) and (1*b*) ${}^{1}\Phi_{Na}$ and ${}^{1}\Phi_{K}$ were obtained. For 37° C the figures must be multiplied by 1·1 according to eqns. (1*a*) and (1*b*). Temperature might also change properties of the membrane.

Na + K activated membrane ATPase

About equal amounts of packed cells from two LK animals and two HK animals respectively were pooled and haemoglobin free permeable membranes were prepared from them. Fig. 4 demonstrates that the initial activity that can be suppressed by ouabain 10^{-4} g/ml. in the presence of 100 mM-Na and 10 mM-K was about 3 times as large in HK cells as in LK cells. After 2 hr the rate of P_i liberation dropped markedly in the HK cell membranes. This is not due to exhaustion of ATP because by 2 hr only a very small fraction of the ATP added was hydrolysed. The decline of activity, therefore, reflects either a deterioration of the preparation after long incubation at 37° C or possibly a spontaneous resealing of the membrane vesicles originally made permeable towards ATP and cations by freezing and thawing.

DISCUSSION

Examination of 251 animals of the Simmenthal breed and thirty-two of other breeds of cattle showed that the Na and K concentration in the R.B.C,S varies widely. The extreme values found for Na were 15 and 87 m-

mole/l. cells and for K, 7 and 70 m-mole/l. cells. The frequency distribution for cellular K has a large maximum at 20 m-mole/l. cells and a smaller peak at 50 m-mole/l. cells. There are several arguments in favour of the idea that the second maximum at high K concentrations is real and not simply due to statistical variation. The distribution can well be fitted by two superimposed normal distributions, suggesting that there are two

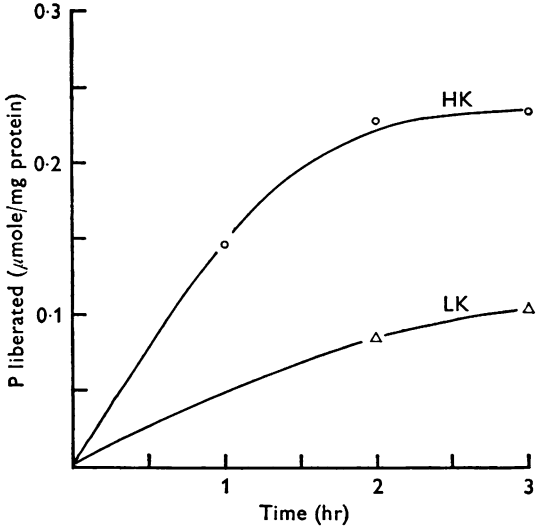

Fig. 4. Na + K stimulated ATPase activity of isolated membranes prepared from HK and LK cells. Temp. 37° C. Medium: (mM) Na 105, K 10, Tris 61, EGTA 1, Mg 5, ATP 2·5, Cl 180, pH 7·38. Ordinate: difference of inorganic phosphate liberated per mg protein between samples without and with ouabain 10^{-4} g/ml. Abscissa: time. ○ HK cells, △ LK cells.

distinct populations (Fig. 2*A*, *B*) of animals with HK and LK cells. The two groups do not contain different proportions of animals from one breed, one age group or of either sex. It seems warranted, therefore, to conclude that there exist two cell types within the same breed or age group and that the cation content of cells is not sex linked. Unlike in sheep the two groups of HK and LK animals overlap considerably. In analogy to the case of sheep R.B.C.S it seems probable that the alkali cation content of cells is determined genetically. However, we were unable to collect a sufficiently large number of animals among the offspring of parents with known cell type to prove the genetic basis or to describe how the genes segregate.

The HK cells undoubtedly display a higher rate of active alkali cation transport than LK cells *in vitro*, both in the absence and presence of an electrochemical gradient against which the transport operates (Tables

1 and 2). Accordingly, the electrochemical work done by the Na-K pump is higher in HK cells than in LK cells.

In order to obtain an estimate of the passive permeability of the cell membrane for Na and K the net K efflux and Na influx during cold storage were divided by the average electrochemical potential prevailing during the experimental period. A meaningful result can be expected from this procedure only if the following assumptions are correct: (1) the downhill movements obey the laws of diffusion, that is the two cations move independently from each other and the process operates far from saturation; (2) the ratio of passive Na flux to passive K flux at 37° C is nearly equal to that at 2° C or changes by the same factor in both cell types; (3) the model proposed by Tosteson & Hoffman (1960) is applicable, which postulates that movements through leak channels and pump channels do not influence each other. If these points are taken for granted the cold storage experiment leads to the following conclusions: (1) Na permeability appears to be higher than K permeability in both cell types but more so in LK cells; (2) the ratio of passive Na flux over passive K flux under steady-state conditions is equal in both cell types (Table 3); (3) it follows from (2) that the coupling ratio (Na ions transported per one K ion transported) of the Na-K pump is equal in both cell types. The results shown in Table 3 suggest that it is nearly 1:1. This conclusion is confirmed by the direct measurement of the net pump fluxes if the large error in the estimate of Na flux in LK cells is compensated by statistical treatment; (4) both leak and pump fluxes are larger in HK cells than in LK cells under steady-state conditions.

It may be concluded that the main reason for the different cation content in the two cell types is the higher pump activity in HK cells. In sheep cells it has been demonstrated by Dunham & Hoffman (1969) that labelling membranes with tritiated ouabain reveals a larger number of pump sites per unit membrane surface in HK cells. For cattle cells we have no similar information but a different density of pump sites might well be the reason for the different pumping activity in the two cell types in cattle, too.

The observed difference between HK and LK cells in Na + K stimulated, membrane bound ATPase activity (Fig. 4) is in accord with and confirms the assumption that one and probably the main cause for the different cation content is the more intensive active cation transport in HK cells compared with LK cells. It must be mentioned that ATPase was assayed at fixed Na and K concentration. It may be possible, therefore, that the rate of P_i liberation measured does not correspond to the maximum rate in the two cell types.

The possibility cannot be ruled out, however, that the differences in

relative cation permeability found contribute to the difference in cation content observed in the two cell types.

The work presented is part of the doctoral thesis submitted to the Veterinary Faculty by P. Christinaz. An account was given at the meeting of the Swiss physiologists.

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