ATP AND POTASSIUM CONTENT OF SHEEP ERYTHROCYTES

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Most mammalian erythrocytes are high in potassium content, low in sodium. Some mammals, such as the dog and cat, are exceptions to this general rule and have low potassium and high sodium levels in their red cells (Bernstein, 1954). A few species, including domestic sheep, are polymorphic for erythrocyte potassium: they fall into two general classes, some having high levels (HK) and others low (LK) (Kerr, 1937). It has been shown (Evans, et al., 1956) that this variation is inherited as a Mendelian character, with the LK gene displaying dominance over the HK gene. Thus, the LK phenotype includes animals heterozygous and homozygous for the LK gene.

The primary cause of the different electrolyte compositions of the two types of sheep red cells is not yet known with certainty. The potassium level in the red cell is maintained through the hydrolysis of

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adenosine triphosphate (ATP) by sodium-potassium dependent ATPase[1] (Post, *et al.*, 1960). Tosteson has studied this enzyme in the sheep erythrocyte, and his results indicate that the HK red cell has a four fold greater ATPase activity than the LK red cell (Tosteson, 1963). In his study, no distinction was made in the LK animals between red cells of sheep homozygous and heterozygous for the LK gene.

Although differences in ATPase activity may account for the differences in potassium content in the three sheep genotypes, variation in quantitative level of ATP, the other important factor in cation transport, may also be playing an important role. It is known that genetically determined variation in red cell ATP level exists in man (Brewer, 1967). Such variation, if it occurs in sheep erythrocytes, may interrelate with ATPase variation in determining potassium levels in the three sheep genotypes. A previous investigation, dealing only with the amounts of ATP bound to the erythrocyte stroma of sheep, had revealed no difference between HK and LK animals (Welt, *et al.*, 1963). The total ATP content of the sheep red cell has not previously been examined.

METHODS

Potassium measurements were made with a flame spectrophotometer, hemoglobin was measured spectrophotometrically as cyanmethemoglobin, and ATP levels of erythrocytes were assayed using a

[1] Sodium-potassium dependent ATPase is that ATPase activity requiring sodium and potassium for stimulation. This activity is completely inhibited by ouabain and is often referred to as sensitive ATPase.

previously published technique (Brewer and Powell, 1966). The sheep studied were of several breeds and crosses of breeds (Western Whiteface, Dorset, Tunis, Suffolk) maintained at Michigan State University. The potassium genotypes of the sheep were determined with the methods of Rasmusen and Hall (1966), through the concomitant measurement of the potassium content of the blood and typing for the M blood group of sheep. Rasmusen and Hall (1966) demonstrated that the HK gene is always associated with M positivity. Thus, animals homozygous or heterozygous for the HK gene are always M(+), but can be differentiated on the basis of potassium levels. Animals homozygous for the LK gene are all M(-).

RESULTS

The mean red cell ATP level of the HK sheep was about 31% higher (p = .001) than that of the LK animals, although there is overlap between the two groups (see Table 1). Among the LK sheep, the mean red cell ATP level of heterozygous animals did not differ significantly from that of animals homozygous for the LK gene (Table 1). The potassium levels of the three genotypes were significantly different from one another, including a higher mean level in the heterozygous animals than in those homozygous for the LK gene ($\underline{p}$ = .001). This substantiates, through another method of genotyping, the genealogically based work which reported significant differences between the two LK genotypes (Evans, *et al.*, 1956). Within the LK group as a whole, there was a positive correlation ($\underline{r}$ = +0.282; $\underline{p}$ =<0.02) between the levels of ATP and potassium within the red cells of individual animals (see Table 1).

TABLE 1:

COMPARISONS OF THE ATP AND K+ CONTENTS OF THE THREE POTASSIUM GENOTYPES OF SHEEP:

Genotype	Number	Mean ATP (μmoles/g Hb)	Mean Whole Blood K+ (mEg/g Hb)	Correlation Coefficient (K+ vs. ATP)	Significance of Correlation Coefficient
HK	10	2.31($\pm$.381)	0.266($\pm$.0252)	-----	-----
LK (hetero-zygous)	41	1.81($\pm$.222)	0.083($\pm$.0129)	+0.266	n.s.
LK (homozy-gous)	37	1.71($\pm$.360)	0.067($\pm$.0177)	+0.241	n.s.
LK (pooled: Hetero- + homozygous)	78	1.76($\pm$.288)	0.075($\pm$.0172)	+0.282	<0.02

Significance of differences of mean values ('*t*' test):

ATP

			t	*p*
LK (homozygous)	vs	HK (homozygous):	4.791	<0.001
LK (heterozygous)	vs	HK (homozygous):	5.573	<0.001
LK (homozygous)	vs	LK (heterozygous):	1.506	n.s.
LK (homozygous + heterozygous)	vs	HK (homozygous):	5.568	<0.001

K+

LK (homozygous)	vs	LK (heterozygous):	7.768	<0.001

DISCUSSION

According to the work of Tosteson (1963), the sensitive ATPase activity of the HK sheep red cell is four times that of the LK sheep red cell. His work suggests that the differences in electrolyte levels in the two types of sheep erythrocytes result from a difference in pumping rates. The ATP of the cell furnishes the energy for the electrolyte pump. In vitro incubation of erythrocytes of humans with hereditary spherocytosis has shown an increased rate of depletion of ATP (Robinson, et al., 1961; Mohler, 1965). This has been attributed to an increased rate of utilization of ATP for electrolyte pumping. One might expect, then, that differences in ATP levels in the two types of sheep erythrocytes would be in the direction of lower levels in the HK cell, since the higher pumping activity would result in a greater utilization of ATP. However, this is not the case. As shown here, the HK red cell has a mean level of ATP 31 percent greater than the LK red cell. These findings suggest that differences in sensitive ATPase activity as measured in erythrocyte membranes may not be the basic difference between the two types of sheep erythrocytes. The differences in sensitive ATPase activity, and in ATP levels, may both be secondary to some other genetically-determined variation. Our preliminary findings of dominance of decreased ATPase activity in the HK/LK heterozygote further support this view (Brewer et al.).

The data presented suggest that potassium levels may be influenced by the levels of ATP in the red cell. Even though the range of variation is not great, there is a significant positive correlation between ATP and potassium concentration in the LK sheep. It seems likely that the level of electrolytes in the sheep erythrocyte is influenced by both ATPase activity and content of ATP, and that the differences in these variables between the two types of cells may be a reflection of some unknown factor.

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