

**Acetylcholinesterase and Glucose-6-Phosphate Dehydrogenase  
Activities in Erythrocytes of Fetal, Newborn and Adult Sheep.\*  
(31959)**

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The erythrocytes of newborn animals and newborn infants differ in many respects from the cells of adult individuals. The most studied differences concern the hemoglobin content and pattern and several metabolic and enzymatic properties. Although fetal erythrocytes have been extensively studied in regard to their hemoglobin characteristics, very little is known about their enzymatic properties. Our investigation concerns the measurement of the activities of two erythrocyte enzymes in fetal, newborn and adult sheep. The enzymes selected have been extensively investigated in humans and were chosen so as to represent the two compartments into which the mature mammalian erythrocyte can be delineated. Glucose-6-phosphate dehydrogenase (G6PD) is associated with the cytoplasm(1), while acetylcholinesterase (ACHE) is an integral part of the erythrocyte membrane(2). In addition, we have compared the activities of these two enzymes in erythrocytes separated by density into older and younger cell fractions.

*Materials and methods.* Blood from newborn and adult sheep was taken by venipuncture, and blood from sheep fetuses was obtained by fetal intra-arterial catheterization. These specimens were made available through the generosity of Dr. Arthur M. Silverstein, Wilmer Institute of Ophthalmology, Johns Hopkins University School of Medicine. The blood was collected in sequestrene and the red cells were washed 3 times with 20 volumes of ice-cold 0.15 M NaCl solution by centrifugation at 1,500 rpm for 5 minutes. The supernatant liquid and buffy coat were carefully removed after each centrifugation and an approximately 50% cell suspension in saline was

prepared after the last washing. An aliquot of this suspension was diluted 1:20 in ice-cold distilled water and the resultant lysate was assayed in duplicate for G6PD activity according to the method of Zinkham(3). For determination of ACHE activity, using the previously described colorimetric technique (4), replicate 1:500 dilutions of the erythrocyte suspension in 0.1 M phosphate buffer, pH 8.0 were made. The activities of both enzymes were related to hemoglobin concentration, the latter measured at 540  $m\mu$  as cyanmethemoglobin.

Sheep erythrocytes were segregated into fractions of older cells and of younger cells by the microgravimetric procedure described earlier(5) to which certain improvements have been made. The supernatant from thrice washed erythrocytes was suctioned off and the packed cells were mixed by repeated inversions of the tube. A 0.2 ml aliquot was adjusted with saline to a hematocrit of approximately 50% and set aside for determinations of the "original" enzyme activities. Six microhematocrit capillary tubes were filled with the thoroughly mixed cell suspension and one end of each tube sealed with vinyl plastic putty. The capillary tubes were centrifuged for 5 minutes at 12,500 rpm in a microhematocrit centrifuge. After measuring the total length of the centrifuged red cell columns, that part of the capillary tube containing the supernatant liquid was broken off and discarded. The top 10% and the bottom 10% of the erythrocyte columns were gently broken off at incision points made with a glass scorer and placed into properly labeled tubes containing 0.15 ml of 0.15 M NaCl solution. A homogenous suspension of the segregated red cell fractions was achieved by mixing the contents of the tubes with a Vortex Jr. mixer. This mechanical stress did

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TABLE I. ACHE and G6PD Activities of Ovine Erythrocytes.

	ACHE activity*			G6PD activity†		
	No.	Range	Mean $\pm$ S.D.	No.	Range	Mean $\pm$ S.D.
Fetal erythrocytes	13	35-53	44 $\pm$ 7	11	3.8-6.8	5.1 $\pm$ .5
Newborn "	6	36-48	41 $\pm$ 4	2	4.7;5.0	
Adult "	7	34-43	37 $\pm$ 4	7	1.0-2.0	1.6 $\pm$ .4

\* Acetylcholinesterase activity:  $\Delta$  OD/mg hemoglobin/min.

† Glucose-6-phosphate dehydrogenase activity:  $\mu$ moles TPN reduced/g hemoglobin/min.

not cause lysis of the red cells. Enzyme activities were measured in the "original" sample and in both cell fractions. The adequacy of this technique for the separation of erythrocytes into older and younger cell fractions was estimated by reticulocyte concentration(5).

*Results and discussion.* From Table I it can be seen that there were no significant differences in erythrocyte ACHE activity between adult animals, newborn lambs and sheep fetuses. ACHE activity was approximately 25% that of red cells from normal adult humans(4). Contrary to what is seen in man, erythrocyte ACHE activity of fetuses and newborn animals was not lower than in adult sheep. These observations indicate that the dissociation noted in humans at birth between ACHE activity and the activity of other erythrocyte enzymes was not universal. As has been noted for other species(2,4), sheep erythrocyte ACHE activity was exclusively associated with the cell membrane, since no activity could be detected in stroma-free lysates. No differences in  $K_m$  (ca.  $1 \times 10^{-4}$ ) were noted between the enzyme of human and of ovine origin.

A striking dissimilarity in G6PD activity between the erythrocytes from fetuses and adult animals was noted. Enzyme activity was approximately 3 times higher in fetal cells than in the erythrocytes from adult sheep (Table I). Adult animals showed an activity which was only 1/5 that of normal human adults(4). This relatively low G6PD activity could account for the controversy which existed on the presence of this enzyme in erythrocytes from adult sheep(6-9). As in humans, G6PD activity was higher in the erythrocytes from fetuses and newborn lambs than in the cells from adult animals, although the difference was greater than that seen in

man(4). The increased enzyme activity noted with fetal cells was not due to the higher mean corpuscular volume or to the lower mean corpuscular hemoglobin concentration which are characteristic of the blood of fetuses and newborn animals(10), but could be ascribed to the presence of reticulocytes and more young erythrocytes in the fetal blood.

When sheep erythrocytes were separated into lighter and heavier cell fractions, ACHE and G6PD activities were increased in the lighter fraction with higher reticulocyte content, and reduced in the heavier (older cell) fraction. Results of four typical separations are presented in Table II. These findings indicate that both fetal and adult sheep blood contain heterogenous populations of erythrocytes which can be resolved into discrete fractions of similar age, with the help of the technique described above. The basis for this method is the phenomenon that an increase in specific gravity is associated with the normal *in vivo* aging process of the red cell, which in turn is characterized by a gradual decline in the activities of several enzymes(5). As can be seen in Table II the pronounced difference in G6PD activity between fetal and adult cells persisted even in the fractions containing the older erythrocytes. This observation could be interpreted as an indication that the heavy fetal fraction had a relatively younger red cell population than the fraction containing the heavy adult erythrocytes. However, the possibility also exists that fetal and adult erythrocytes possess characteristically distinct enzymes which are inactivated *in vivo* at different rates.

While the precise physiological functions of erythrocyte ACHE remain unknown, the role of G6PD in the overall erythrocyte metabolism is well established. As the first enzyme of oxidative glycolysis, it generates reduced

TABLE II. Separation of Sheep Erythrocytes in Accordance with Their Density.

	Original			Light			Heavy		
	% reticulocytes	ACHE activity*	G6PD activity†	% reticulocytes	ACHE activity*	G6PD activity†	% reticulocytes	ACHE activity*	G6PD activity†
Ovine fetus 107 d. gestation	3.6	46	6.8	20.2	80	12.9	<.1	31	5.0
Ovine fetus 133 d. gestation	1.7	44	5.8	16.6	52	7.2	<.1	38	4.7
Newborn lamb 2 d. old	1.2	48	4.7	16.2	52	5.0	<.1	38	3.3
Adult sheep	<.1	36	1.8	.8	40	1.8	<.1	30	1.2

\* Acetylcholinesterase activity:  $\Delta$  OD/mg hemoglobin/min.

† Glucose-6-phosphate dehydrogenase activity:  $\mu$ moles TPN reduced/g hemoglobin/min.

TPN, which seems to be essential for the protection of the red cell. The relatively low levels of G6PD activity found in sheep erythrocytes are compatible with the observation that these cells do not reduce methemoglobin as efficiently as do normal human red cells (11). The finding of a 3-fold higher G6PD activity in fetuses and newborns than in adult sheep is probably not related to the high potassium levels seen in the erythrocytes of all newborn lambs(8), since no difference in the activity of this enzyme could be found between the red cells of adult animals with genetically-controlled high or low potassium concentration(8,12).

*Summary.* Acetylcholinesterase activity, essentially similar in fetal, newborn and adult sheep erythrocytes, was only 25% that of cells from adult humans. Erythrocyte glucose-6-phosphate dehydrogenase activity was 3 times higher in fetuses and newborn lambs than in adult sheep. Cells from adult animals had only  $\frac{1}{3}$  the activity seen in erythrocytes from normal adult humans. When sheep erythrocytes were separated into light and heavy cell fractions, the activities of both

enzymes were increased in the lighter fraction with higher reticulocyte content and decreased in the fraction containing the older cells.

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