ERYTHROCYTE GLUTATHIONE POLYMORPHISM IN SHEEP

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Abstract

Reduced glutathione (GSH) levels of erythrocytes were determined in five sheep breeds. The Merino breed had a higher incidence of low GSH animals than the other breeds tested. High GSH type animals of the Border Leicester breed had a significantly lower mean GSH level than did higher GSH animals of the other breeds.

GSH levels were found to be associated with electrolyte levels, haemoglobin type, fleece weight, and body weight in Merino sheep.

I. Introduction

Since the discovery of "glutathione instability" of human red cells in drug-induced haemolytic anaemia (Beutler 1957) interest has been growing in the function of reduced glutathione (GSH) in the red blood cell. It has been shown that GSH is involved in protecting haemoglobin against irreversible oxidation denaturation, guarding membrane lipids against peroxidation, and shielding essential enzymes against inactivation (Jaffe 1970). Relationships between erythrocyte GSH levels and some production traits in animals (e.g. growth rate, body weight, fleece weight, milk yield) have been studied by various workers (Kidwell *et al.* 1958; Slepcov 1961; Charkey, Hougham, and Kano 1965; Mabon 1969; Owens, Siegel, and Van Kray 1970).

Smith and Osburn (1967) reported that three sheep in a flock of 104 had erythrocyte GSH levels which were less than 20% of the mean for the remaining animals. Tucker and Kilgour (1970) have since shown that the level of GSH in the red cells of sheep is controlled by a single pair of autosomal alleles, giving rise to two GSH types; high GSH (GSH^H) sheep have GSH levels above 55 mg/100 ml red blood cells, while animals with values below this level are classified as low GSH (GSH^h). The GSH^H allele is dominant. These authors have also shown an association between potassium concentration and GSH level in the red cell. The present paper describes the results of an experiment designed to measure erythrocyte GSH levels in sheep of different breeds, potassium types, and haemoglobin types. The effect of GSH type and concentration on fleece weight and body weight is also noted.

II. Experimental

Adult ewes of the Border Leicester, Corriedale, Dorset Horn, Poll Dorset, and Merino breeds were used. Merinos of known age, haemoglobin, and potassium types were obtained from the flock maintained by this Department. Ewes of other breeds were made available by the CSIRO Division of Animal Physiology, Pastoral Laboratory, Armidale, N.S.W. About 5 ml blood was obtained by jugular venipuncture and collected into EDTA vials. GSH levels in whole blood were estimated using an automated method (Roberts and Agar 1971). Erythrocyte

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GSH levels were then calculated using the haematocrit values. The automated GSH method of Roberts and Agar (1971) gives results that are approximately 20% higher than those obtained using the manual method of Beutler, Duron, and Kelly (1963). Because of this the classification of sheep into high and low GSH groups is not the same as that described by Tucker and Kilgour (1970). In the present paper sheep with GSH values below 65 mg/100 ml red blood cells are classified as GSH^h, while those with values of 65 mg/100 ml red blood cells or more are classified as GSH^H.

III. Results

(a) *Breed Differences in Erythrocyte GSH Levels*

Table 1 shows breed means for erythrocyte GSH levels. GSH^H animals were predominant in all the breeds analysed. No GSH^h animals were present in the Dorset Horn, Poll Dorset, and Border Leicester breeds and only one in the Corriedale breed.

Table 1

GSH levels in the erythrocytes of different breeds of sheep
GSH values given as milligrams per 100 ml red blood cells

Breed	GSH^H		GSH^h	
	No. of animals	Mean ± S.E.M.	No. of animals	Mean ± S.E.M.
Border Leicester	24	88·3±1·95	—	—
Corriedale	23	112·4±2·93	1	33·4
Dorset Horn	24	108·9±1·57	—	—
Poll Dorset	24	109·9±1·46	—	—
Merino	176	108·3±1·56	51	42·8±1·60

In contrast about 22% of the Merinos were GSH^h type. In the GSH^H animals, the Border Leicesters had a much lower mean erythrocyte GSH level than did any other breed.

(b) *GSH Types and Erythrocyte Electrolyte Levels*

Table 2 shows erythrocyte electrolyte levels in high potassium (HK) and low

Table 2

Erythrocyte potassium and sodium levels in Merino sheep of different GSH types

Sheep potassium character	GSH^H		GSH^h		Significance levels between GSH^H and GSH^h groups (*t*-test)
	No. of animals	Mean ± S.E.M.	No. of animals	Mean ± S.E.M.	
	Potassium level (m-equiv/l red blood cells)				
HK	64	80·5±1·12	12	78·7±3·07	n.s.
LK	105	19·7±0·56	30	18·1±1·05	n.s.
	Sodium level (m-equiv/l red blood cells)				
HK	65	21·3±0·74	12	19·0±2·14	n.s.
LK	105	88·0±0·91	30	92·1±1·59	$P < 0·05$
	Sodium + potassium level (m-equiv/l red blood cells)				
	167	105·4±0·61	42	106·7±1·58	n.s.

potassium (LK) sheep (Evans 1954) of high and low GSH types. There was a significant difference in erythrocyte sodium concentration between LK sheep of different GSH types.

(c) *GSH Types and Haematocrit, Body Weight, and Fleece Weight*

Comparisons were made between GSH^H and GSH^h animals in the Merino flock for the characters shown in Table 3. There were no significant differences in means for body weight or fleece weight between the two groups. Haematocrit was, however, significantly higher in the GSH^H group ($P < 0{\cdot}02$).

TABLE 3

HAEMATOCRIT, BODY WEIGHT, AND GREASY FLEECE WEIGHT IN GSH^H AND GSH^h MERINO SHEEP

Character	GSH^H		GSH^h		Significance levels between GSH^H and GSH^h groups (t-test)
	No. of animals	Mean ± S.E.M.	No. of animals	Mean ± S.E.M.	
Haematocrit (%)	177	33·3±0·25	50	31·8±0·55	$P < 0{\cdot}02$
Body weight (kg)	163	26·5±0·33	42	27·6±0·68	n.s.
Fleece weight (kg)	168	2·13±0·032	44	2·13±0·059	n.s.

(d) *Effect of Age, Potassium Type, and Haemoglobin Type on GSH Levels*

An analysis of variance was carried out to assess the effects of age, haemoglobin type, potassium type, and the interactions between these variables on GSH levels. Only GSH^H animals of Hb AB and Hb B types were used in this analysis because of the small numbers of GSH^h and Hb A animals. Because of unequal subclass numbers an approximate analysis was carried out on group means using as an error the within subclass mean squares. No significant differences due to age, potassium type, or haemoglobin type were found and it would appear that any main effects and interactions are masked by the very large error component. However, despite this very large variation within groups the group means plotted in Figure 1 show a significant difference

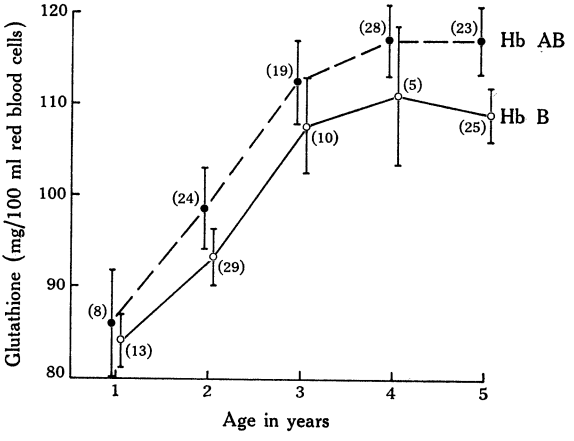

Fig. 1.—Mean erythrocyte GSH levels (±1 S.E.M.) within haemoglobin types and ages in Merino sheep. High GSH animals only; number of animals in parenthesis.

between haemoglobin types within ages, using the sign test, $P < 0{\cdot}03$. Age differences showed a significant linear trend which just failed to reach the 1·0% level in both the AB and B animals (Fig. 1).

(e) Relationships between Erythrocyte GSH Levels and the Parameters Measured

Table 4 shows correlation coefficients obtained for the relationships between erythrocyte GSH levels within GSH types and the parameters measured in this study. Most of these relationships show only a low degree of correlation although some are highly significant. Because of the age variations in the animals, partial correlations for both fleece weight and body weight with GSH level, corrected for age, were calculated in the GSH^H group. These partial correlations, although still statistically significant, were of a very low degree (for fleece weight $r = 0{\cdot}2110$, $P < 0{\cdot}001$, for body weight $r = 0{\cdot}2352$, $P < 0{\cdot}001$). No obvious age effects were noted for haematocrit and sodium or potassium or both levels.

TABLE 4

CORRELATION COEFFICIENTS FOR THE RELATIONSHIPS BETWEEN ERYTHROCYTE GSH AND THE PARAMETERS LISTED, IN GSH^H AND GSH^h SHEEP

Character	GSH^H			GSH^h		
	n	r	Significance level	n	r	Significance level
Erythrocyte potassium						
HK sheep	64	0·6122	$P < 0{\cdot}001$	12	−0·6359	$P < 0{\cdot}05$
LK sheep	105	0·1156	n.s.	30	−0·2085	n.s.
Erythrocyte sodium						
HK sheep	64	−0·2130	n.s.	12	0·4217	n.s.
LK sheep	105	−0·1020	n.s.	30	0·0302	n.s.
Erythrocyte potassium + sodium	166	0·2451	$P < 0{\cdot}01$	42	−0·3362	$P < 0{\cdot}05$
Haematocrit	176	0·3480	$P < 0{\cdot}001$	51	0·1513	n.s.
Body weight	162	0·3898	$P < 0{\cdot}001$	42	0·0999	n.s.
Fleece weight	167	0·3208	$P < 0{\cdot}001$	44	0·0587	n.s.

IV. DISCUSSION

Tucker and Kilgour (1970) appear to be the first to report breed differences for erythrocyte GSH values in sheep. They have demonstrated differences not only in the distribution of the gene frequencies for GSH^H and GSH^h types but also in the mean GSH levels within each glutathione type. Other reports of GSH levels in sheep (Kidwell *et al.* 1958; Klinski and Dyatlova 1960; Wagner 1964; Sundukov 1967) have been expressed in terms of whole blood GSH concentrations and are not, therefore, directly comparable with the values presented here. Of the six breeds examined by Tucker and Kilgour, only the Finnish Landrace had an appreciable number of GSH^h animals. No obvious explanation presents itself for the observed differences in GSH^h gene frequency between breeds, or for the breed differences in mean GSH level within GSH type. The two breeds that have been shown to have a relatively high GSH^h gene frequency, Finnish Landrace and Merino appear to be quite dissimilar in origin and characteristics.

Sheep haemoglobins A and B differ from each other in their oxygen dissociation characteristics, Hb A having a greater affinity for oxygen than Hb B (Huisman, van Vliet, and Sebens 1958; Dawson and Evans 1962). Addition of GSH to human blood shifts the oxygen dissociation curve to the right, i.e. decreases the oxygen affinity of haemoglobin (Horejsi 1970). Our results indicate that in the GSH^H group Hb B animals tend to have a lower erythrocyte GSH value than do Hb AB animals (Fig. 1). These findings are contrary to the theoretical expectation. From the information available in the medical literature one would expect higher GSH levels in the red cells of Hb B sheep than in Hb A, although the relationship between GSH levels and the oxygen affinity of the red cell may be different in man and sheep. This relationship is presently being investigated by the authors.

Haemoglobin A sheep have higher haematocrit values than Hb B sheep (Mounib and Evans 1959). If GSH level is also modified by haemoglobin type (Fig. 1) it is not unreasonable to conceive a relationship between haematocrit and GSH levels. Table 3 shows that GSH^H animals have significantly higher haematocrit values ($P < 0{\cdot}02$). Also a positive correlation between erythrocyte GSH level and haematocrit ($r = 0{\cdot}3480$, $P < 0{\cdot}001$) was found in the GSH^H group (Table 4). This correlation was, however, not significant in the GSH^h group. Kidwell *et al.* (1958) working with sheep were unable to find any relationship between these characters. A negative relationship between hematocrit and erythrocyte GSH level has been reported in man (Cernoch and Malinska 1966).

The results presented in Table 2 support the observation of Tucker and Kilgour (1970) that GSH^h animals have a lower mean erythrocyte potassium concentration than GSH^H animals in both HK and LK groups. However, our results are not statistically significant. It is possible that the association between erythrocyte GSH levels and electrolyte levels varies between breeds. From the results in Table 4 it appears that there may be fundamental differences between GSH types in the relationships between GSH and electrolyte levels. GSH is correlated positively with potassium and negatively with sodium in the high GSH animals, while in the low GSH group the sign of these correlations is reversed. This difference occurs in both HK and LK groups.

An increase in erythrocyte GSH with age has been reported in man (Bertolini, Palo, and Spinnler 1962; Goldschmidt 1970), rhesus monkey (Brown, Goodman, and Gavan 1970), and pigeons (Brown and Sharp 1970). The relationship between erythrocyte GSH and age in ruminants, however, has been the subject of conflicting reports. Reid, Ward, and Salisbury (1948) reported an increase in erythrocyte GSH with age in cattle. Kunkel, Stutts, and Shrode (1954) found no difference in GSH values between calves and adult cattle, while Gurtler, Stephan, and Kolb (1965), Podgorski and Majewski (1969), and Mabon (1969) have reported higher values in calves than in adults. In sheep, Kidwell *et al.* (1958) reported no difference between GSH levels of lambs and ewes. The results presented here (Fig. 1) suggest strongly that erythrocyte GSH values rise with increasing age up to 3 or 4 years. After this the value appears to remain constant, although older animals would need to be tested to confirm this. We have previously noted a rise in GSH level from birth to 90 days of age in the lamb (Agar and Roberts 1971).

Erythrocyte GSH levels have been shown to be associated with milk yield, growth rate and body size in cattle (Kidwell, Wade, and Hunter 1955; Slepkov 1961; Boriskenko 1961), body weight in chickens (Charkey, Hougham, and Kano 1965; Sabalina and Iogov 1967; Makrushin 1968; Owens, Siegel, and Van Kray 1970), and fleece weight in sheep (Saltykov 1956). It would appear from our results that both fleece weight and body weight are positively correlated with erythrocyte GSH levels in the sheep (Table 4). Although these correlations reach a high degree of statistical significance in the GSH^H group, even after correction for age, they appear to be too low to be used as predictors for growth. Taneja, Narayan, and Ghosh (1969) have shown a relationship between wool fibre diameter and the frequency of the LK gene and the actual level of potassium in the erythrocytes of LK animals. We have confirmed this relationship between erythrocyte potassium level and fleece weight and fleece quality (Evans *et al.*, unpublished observations). It is therefore possible that further investigations into the relationships of GSH and potassium in the red cell, body weight, and fleece weight may provide information of interest to livestock breeders.

A deficiency of GSH in the red cells of sheep is unaccompanied by any apparent haemolytic disorder (Smith and Osburn 1967). In the course of this and other studies we have observed sheep having GSH values ranging from less than 10 mg to 150 mg GSH/100 ml red blood cells; these animals were all apparently normal with regard to haemoglobin concentration, haematocrit, and erythrocyte and plasma electrolyte levels. It is difficult to believe that such great differences in red cell GSH level are not accompanied by other inherent differences of a compensatory nature in the biochemistry of the red cell. It is known that oxidized glutathione (GSSG) inhibits the activity of S-ATPase (Dick, Dick, and Tosteson 1969). The activity of S-ATPase is about four times greater in HK red cells than in LK cells (Tosteson 1963). The relationship of GSH to GSSG in the red cell and the possible effects of this relationship on S-ATPase is not yet clear and remains a key problem in the biochemistry of the erythrocyte. Studies of the level of GSSG in HK and LK sheep and GSH^H or GSH^h or both types of sheep and its possible role in active cation transport are in progress.

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