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The Effect of Experimental Anaemia on the Levels of Glutathione and Glycolytic Enzymes of the Erythrocytes of Normal and Glutathione-deficient Merino Sheep

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Abstract

The effects of experimental anaemia on the levels of reduced glutathione (GSH) and the activity of glycolytic enzymes in the erythrocytes of normal and GSH-deficient Merino sheep were investigated. There was a rise in red cell GSH levels in both groups of sheep; the magnitude of this response was, however, quite different. When expressed as a percentage of the initial value, the rise in GSH level was 18% in normal and 263% in GSH-deficient animals. There was also an increase in the activities of various enzymes following phlebotomy but this increase was similar in the two groups of sheep.

Introduction

The tripeptide glutathione is an essential cellular constituent and in its reduced form (GSH) is responsible for the maintenance of membrane sulphydryl (–SH) groups in the functional reduced form and also in the case of the red blood cell of the –SH groups of haemoglobin. Deficiencies of the enzymes that are required for GSH synthesis, or those associated with the pentose phosphate pathway, lead to increased susceptibility of haemoglobin to oxidative destruction. Several cases of such enzyme deficiencies resulting in haemolytic anaemia have been reported in the medical literature (Beutler 1971).

A genetic deficiency of GSH has been reported in the red blood cells of some sheep (Smith and Osburn 1967; Tucker and Kilgour 1970; Agar *et al.* 1972*b*). This deficiency has been attributed to the lowered level (approximately 55% of normal) of activity of γ-glutamyl-cysteine synthetase, one of the enzymes essential for *de novo* synthesis of glutathione from its precursor amino acids (Smith *et al.* 1973; Young *et al.* 1974). Despite lower levels of GSH, these sheep have normal haematological and biochemical parameters (Tucker and Ellory 1971; Agar *et al.* 1972*b*; Agar and Smith 1973; Smith 1973; Agar *et al.* 1975*b*).

As a further extension of these studies, the effect of experimental anaemia on the concentration of GSH and the activities of glycolytic enzymes has now been investigated in order to gain a better understanding of glucose metabolism in general and glutathione metabolism in particular of the red blood cells from normal and GSH-deficient sheep. The results are presented in this paper.

Materials and Methods

Eight adult Merino ewes, four normal and four GSH-deficient, were used. Approximately 5 ml blood was collected by jugular venepuncture into tubes containing EDTA as anticoagulant.

Normal values of various parameters were established over a period of several weeks. The animals were then made anaemic by the removal of about 600 ml blood on days 0, 1, 2 and 4.

Packed cell volume and haemoglobin concentration were determined by standard laboratory procedures. GSH levels in whole blood were measured using the 5,5-dithiobis(2-nitrobenzoic acid) method (Beutler *et al.* 1963). Red blood cell GSH values were then calculated using the haematocrit values and were expressed as milligram per 100 ml of red blood cells.

The methods used to measure the levels of the different enzymes have been recently described (Agar *et al.* 1975*a*). These methods provide optimal conditions for extraction of the enzymes from the red blood cells as well as for the assay of enzyme activities. The enzyme activities were calculated as μmoles per minute per gram haemoglobin and are referred to as enzyme units (e.u.).

Results

Normal Values

The mean level of GSH in the red blood cells of normal sheep (98·6 ± 11·3 mg/100 ml) was about four times greater than the mean values recorded in the GSH-deficient red blood cell (23·8 ± 4·80 mg/100 ml) (see Table 1).

Table 1. Levels of GSH and enzyme activities in the red blood cells of normal and GSH-deficient sheep

Each value is mean ± S.E.M.

	Normal sheep (n = 4)	GSH-deficient sheep (n = 4)
Reduced glutathione	98·60 ± 11·30	23·80 ± 4·80
Hexokinase	0·71 ± 0·08	0·69 ± 0·12
Glucosephosphate isomerase	20·70 ± 0·90	20·40 ± 1·10
6-Phosphofructokinase	0·35 ± 0·04	0·42 ± 0·04
Fructose-bisphosphate aldolase	1·65 ± 0·25	1·50 ± 0·12
Phosphoglycerate kinase	24·30 ± 2·80	24·70 ± 2·90
Pyruvate kinase	2·09 ± 0·38	2·37 ± 0·14
Lactate dehydrogenase	25·50 ± 1·00	27·50 ± 2·30
Glucose-6-phosphate dehydrogenase	0·94 ± 0·09	1·00 ± 0·11
Phosphogluconate dehydrogenase	0·42 ± 0·07	0·36 ± 0·06
Glutathione reductase (NAD(P)H)	1·85 ± 0·20	1·84 ± 0·21

The activities of various glycolytic enzymes in the red blood cells of normal and GSH-deficient sheep are also given in Table 1. No significant differences were observed in the enzyme levels in the two groups of sheep. These results are in agreement with the previous report of Agar and Smith (1973) on mixed breeds in America.

Effect of Experimental Anaemia on Packed Cell Volume, Haemoglobin Concentration and GSH

The packed cell volume of the four normal sheep decreased from a mean value of 36 to 14% by day 8; this drop was from 38 to 17% in the GSH-deficient sheep. During the same period haemoglobin concentration was decreased from 10 to 4 g/100 ml in normal and from 11 to 5 g/100 ml in GSH-deficient sheep (Fig. 1). The concentration of GSH in the red blood cells of all sheep increased after phlebotomy, reaching the highest value at day 10. When expressed as percentages of the initial values, these increases were 18% in normal sheep and 263% in GSH-deficient sheep. In both groups, GSH levels began to fall after day 10 and

continued for a period of about 30 days before returning to pre-experimental values (Fig. 1).

Effect of Experimental Anaemia on the Activities of Red Cell Enzymes

Glutathione reductase (NAD(P)H) (GR: EC 1.6.4.2)

There was a sharp rise in the activity of GR in both groups of sheep. Peak values were reached by day 10 and were maintained up to about day 35. Pre-experimental values were reached by day 60 (Fig. 2*a*).

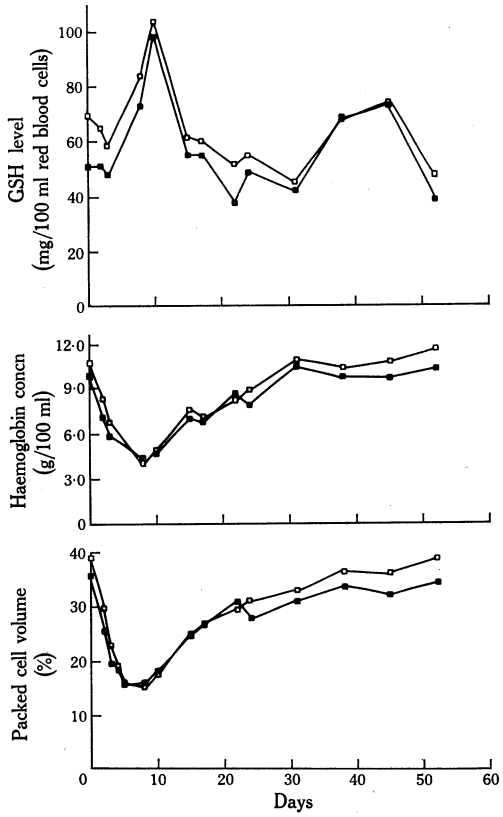

Fig. 1. Effect of experimental anaemia on packed cell volume, haemoglobin concentration and red blood cell GSH levels in normal (□) and GSH-deficient (■) sheep.

Pentose phosphate pathway enzymes

After a slight initial fall, there was a sharp increase in the enzyme activity of glucose-6-phosphate dehydrogenase (G6PD: EC 1.1.1.49) (Fig. 2*a*). The changes in activity of G6PD were similar in the two groups and followed a similar pattern to that of GR. The rise in the activity of phosphogluconate dehydrogenase (PGD: EC 1.1.1.44) was not consistent in either of the two groups and by day 35 levels were close to those observed before phlebotomy (Fig. 2*a*).

Glycolytic enzymes

The level of hexokinase (HK: EC 2.7.1.1) began to rise immediately after the first day of phlebotomy and rose from a mean value of 0·71 to 1·81 e.u. in normal sheep

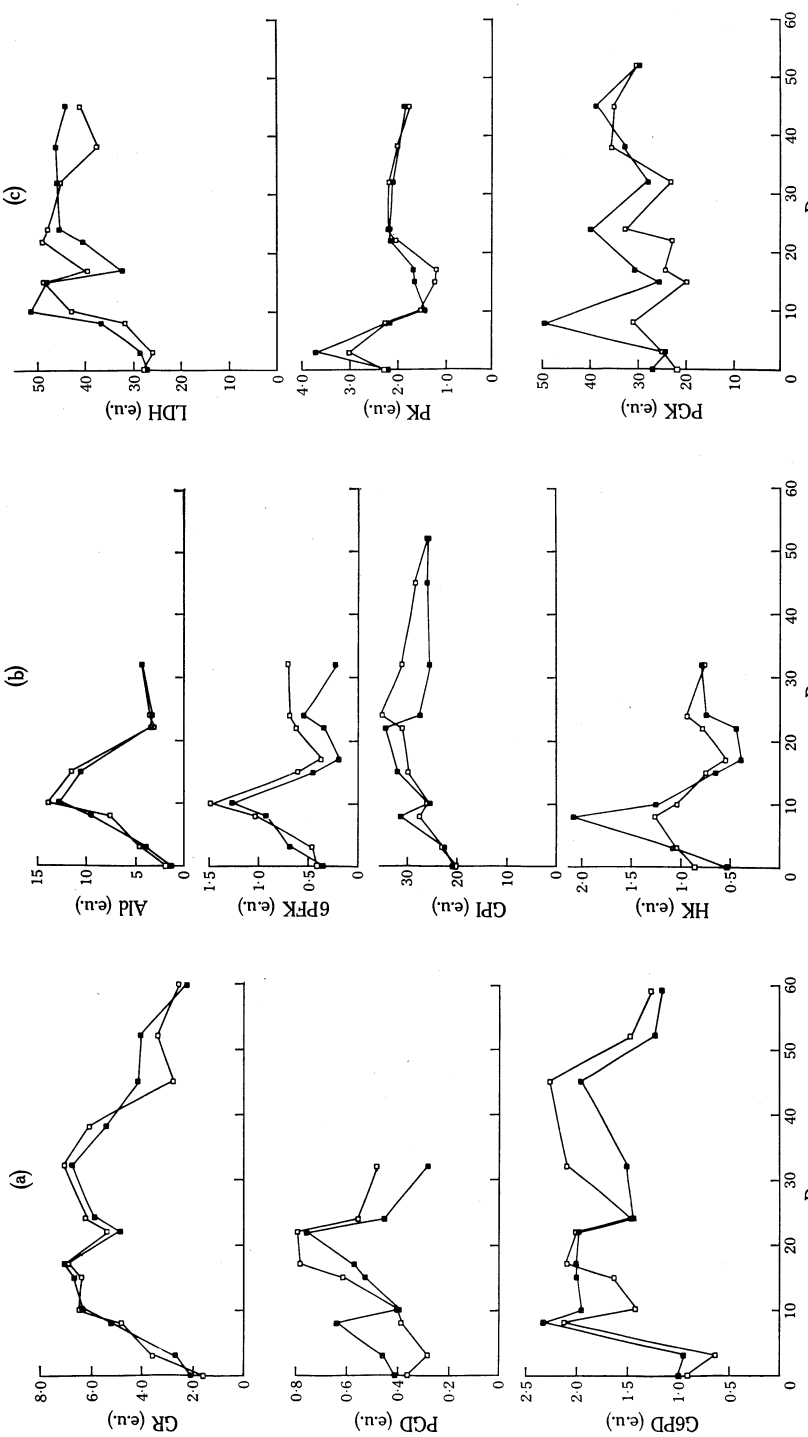

Fig. 2. Effect of experimental anaemia on the levels of (*a*) G6PD, PGD and GR, (*b*) HK, GPI, 6PFK and Ald, (*c*) PGK, PK and LDH activities in the red blood cells of normal (□) and GSH-deficient (■) sheep.

and from 0·69 to 1·50 e.u. in GSH-deficient sheep by day 8. Values close to pre-experimental values were reached by day 35 (Fig. 2*b*). Glucosephosphate isomerase (GPI: EC 5.3.1.9) activity rose in both groups of sheep until about day 20, thereafter levels fell slowly. By day 50 levels were close to those observed before phlebotomy (Fig. 2*b*). The changes in the activities of 6-phosphofructokinase (6PFK: EC 2.7.1.11) and fructose-bisphosphate aldolase (Ald: EC 4.1.2.13) enzymes were similar in the two groups of sheep and were more or less identical to those observed for HK (Fig. 2*b*). The mean activity of phosphoglycerate kinase (PGK: EC 2.7.2.3) enzyme varied considerably throughout this experiment in both groups of sheep. No definite trends that could be attributed to anaemia were observed (Fig. 2*c*). After a sudden rise in the enzyme activity of pyruvate kinase (PK: EC 2.7.1.40) at day 3, the levels returned to their pre-anaemic values and remained at these levels throughout the remainder of the experiment (Fig. 2*c*). In both groups of sheep the activity of lactate dehydrogenase (LDH: EC 1.1.1.27) rose to a peak at about day 10 and remained elevated throughout the period of the experiment. Values obtained for the GSH-deficient sheep were consistently higher than those of the normal animals (Fig. 2*c*).

Discussion

In confirmation with the findings of Todd and Ross (1968) and Tucker and Kilgour (1973) the results obtained in the present experiment demonstrate that red blood cell GSH levels rise in sheep in response to anaemia. The magnitude of this response, however, appears to be quite different in normal and GSH-deficient sheep as shown in Fig. 1; the rise in GSH level was 263% in deficient and only 18% in normal sheep. These results suggest that the young cells in both normal and deficient sheep are similar with regard to GSH metabolism. With the advance in age of the cell, there is a greater decline in the GSH level in deficient red cells as opposed to the normal red cells. This situation in analogous to that of changes in potassium level in the red cells of high-potassium and low-potassium sheep during experimental anaemia. There is a 242% increase in the low-potassium sheep, but only 22% in the high-potassium type sheep (Blunt and Evans 1965).

The most interesting observation was that changes in the activities of red cell enzymes, in response to anaemia, were similar in the animals of the two groups (Fig. 2). This was in marked contrast to the changes observed in GSH levels discussed above. In general, it appeared that the enzyme activities were higher in the young than in the mature red cells in both groups of sheep. A rise in the activities of many red cell enzymes with increase in the age of the red cell has been shown in man (Yunis and Yasmineh 1969), rabbit (Rubinstein *et al.* 1956), cattle (Kaneko *et al.* 1969), and dog and horse (Smith and Agar, unpublished data). The species variation in the levels of enzyme activities are probably related to variability in the red cell life span and various other biochemical and physiological characteristics of the red blood cells.

It has already been reported that the haematocrit and haemoglobin concentration (Tucker and Ellory 1971; Agar *et al.* 1972*b*), red cell life span (Agar *et al.* 1975*b*), glycolysis (Agar and Smith 1973) and the concentration of glutathione precursors (Smith 1973) are similar in the red cells of normal and GSH-deficient sheep. Our results presented in Table 1 and Figs 1 and 2 provide further evidence that there are

probably no differences in the biochemistry and/or physiology of the red blood cells of sheep with different levels of GSH. Unlike the situation in man, where GSH deficiency is associated with non-spherocyte haemolytic anaemia, GSH deficiency in sheep seems to have no effect on the physiology of the red blood cell and, like potassium and haemoglobin type (Agar *et al.* 1972*a*), is yet one more example of a biochemical polymorphic character of red blood cells maintained by natural selection.

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