

CONTRIBUTIONS TO THE STUDY OF BLOOD CONSTITUENTS IN DOMESTIC ANIMALS IN SOUTH AFRICA. 3. NORMAL VALUES FOR THE ACTIVITY OF GLYCERALDEHYDE-PHOSPHATE DEHYDROGENASE AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN THE ERYTHROCYTES OF SHEEP

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INTRODUCTION

One of the most prominent phenomena in both geeldikkop (tribulosis ovis) and enzootic icterus is chronic low grade intravascular haemolysis. In exacerbations of enzootic icterus this may assume the proportions of an acute or explosive haemolytic crisis, which is often associated with a variable degree of methaemoglobinocythaemia (Brown, Le Roux & Tustin, 1960; Brown, 1963, 1964). Wagner (1964) has demonstrated substantial differences in erythrocyte fragility and in the red cell methaemoglobin-reductase systems between sheep raised in the areas where these diseases are enzootic and those raised elsewhere. The nature of the methaemoglobin-reductase system in general has been discussed in a previous paper (Brown, 1963). At that time very little was known of it with regard to ovine erythrocytes. It is now believed that reduction of methaemoglobin in the red blood cell of the sheep proceeds through medium of hydrogen atoms removed from glyceraldehyde-phosphate during glycolysis via the direct Embden-Meyerhof pathway (Brown, 1964). Some of the results from which this conclusion was drawn are set out in the present paper together with the "normal" values of activity of ovine erythrocyte glyceraldehyde-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase. These had to be established before studies of this nature could be pursued.

MATERIALS AND METHODS

The animals used for this study, their nutrition and management were the same as reported previously (Wagner, 1964), viz. (a) *Group 1*: fully grown Merino sheep drawn from the pool of animals available for research work at this Institute. Approximately 75 per cent of these animals were purchased in areas where geeldikkop and enzootic icterus are enzootic, i.e. the Karoo and notably the Laingsburg and Beaufort West districts. The remainder were obtained from farms on the Transvaal Highveld. (b) *Group 2*: fully grown Merinos and Dorpers bred and raised at the experimental farm of the University of Pretoria, situated in a grass pasture area in which geeldikkop and enzootic icterus have never been observed.

The method of statistical evaluation of the data collected was as described previously (Wagner, 1964).

BLOOD CONSTITUENTS IN DOMESTIC ANIMALS IN SOUTH AFRICA

Glyceraldehyde-phosphate dehydrogenase (GAP-D) was assayed as outlined in the Sigma Technical Bulletin No. 10 (1961, Sigma Chemical Co., St. Louis, Mo.) and glucose-6-phosphate dehydrogenase (G-6-P-D.) by the method of Glock & McLean (1953).

A Unicam SP.500 spectrophotometer was used for both assays; pure substrates and enzyme preparations used for the standardization of the methods used were obtained from the Sigma Chemical Co. (St. Louis, Mo.) and potassium oxalate was used throughout as the anticoagulant in the collection of blood samples.

RESULTS

No significant difference between the two groups of sheep as regards the activity of erythrocyte GAP-D could be detected. The values obtained from the animals in Group 1 are therefore representative. The cumulative relative frequency curve and histogram constructed from these results are presented as Fig. 1 and 2 respectively and the conclusions drawn from these curves are indicated in Table 1.

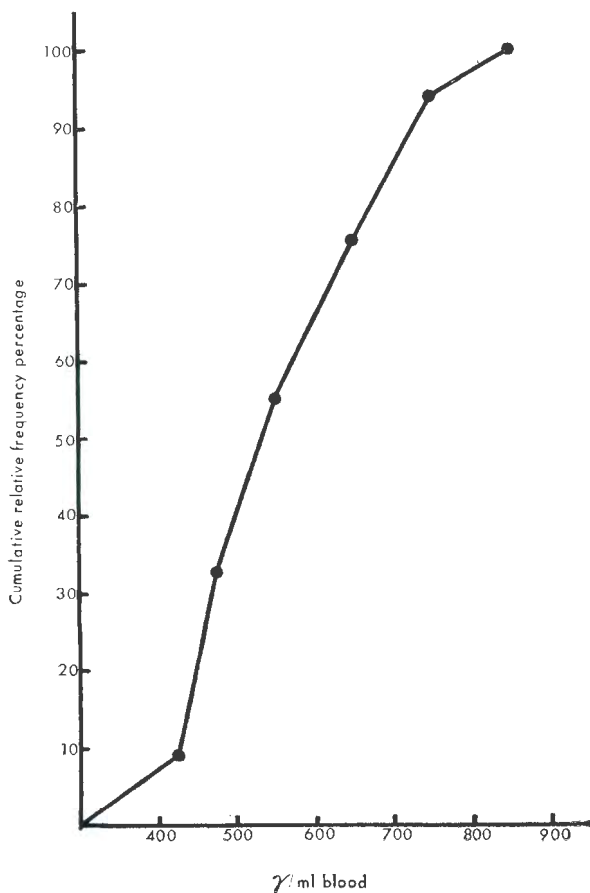


FIG. 1.—Cumulative relative frequency curve for Glyceraldehyde-3-phosphate dehydrogenase

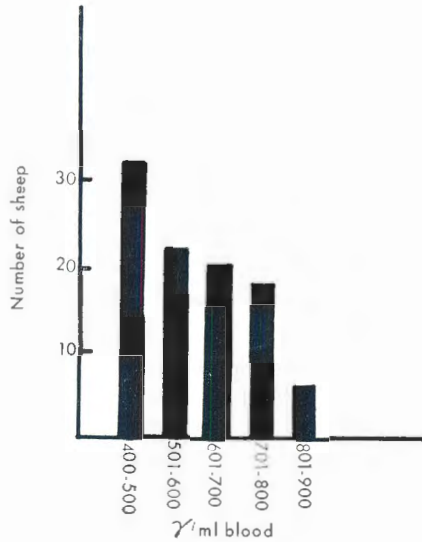


FIG. 2.—Glyceraldehyde-3-phosphate dehydrogenase

TABLE 1.—Ranges found for glyceraldehydephosphate dehydrogenase (GAP-D) in ovine erythrocytes

Figure shown by Median (50%)	Ranges		
	80%	10% lower	10% upper
535 (n = 98).....	425-730	400-424	731-900

NOTE.—The figures in the table refer to micrograms per ml of blood

Using the method of Glock & McLean (1953) for the assay of G-6-P-D, we were unable to detect any activity of this enzyme in ovine erythrocytes. The methods of Kornberg & Horecker (1957) and Bernstein (1962) were then applied in order to confirm these results. Once again no G-6-P-D activity could be found. As further confirmation washed erythrocytes from a number of different species of animals were tested for G-6-P-D activity by the methods of Kornberg & Horecker (1957). The results are presented in Fig. 3. This assay method depends on the rate of reduction of nicotine-adenine-dinucleotide phosphate (NADP) by the enzyme in the presence of glucose-6-phosphate. The figure depicts the decrease in NADP concentration plotted against time in seconds. The negligible activity of G-6-P-D in the erythrocytes of sheep and goats is obvious when compared with that in the red cells of other species.

BLOOD CONSTITUENTS IN DOMESTIC ANIMALS IN SOUTH AFRICA

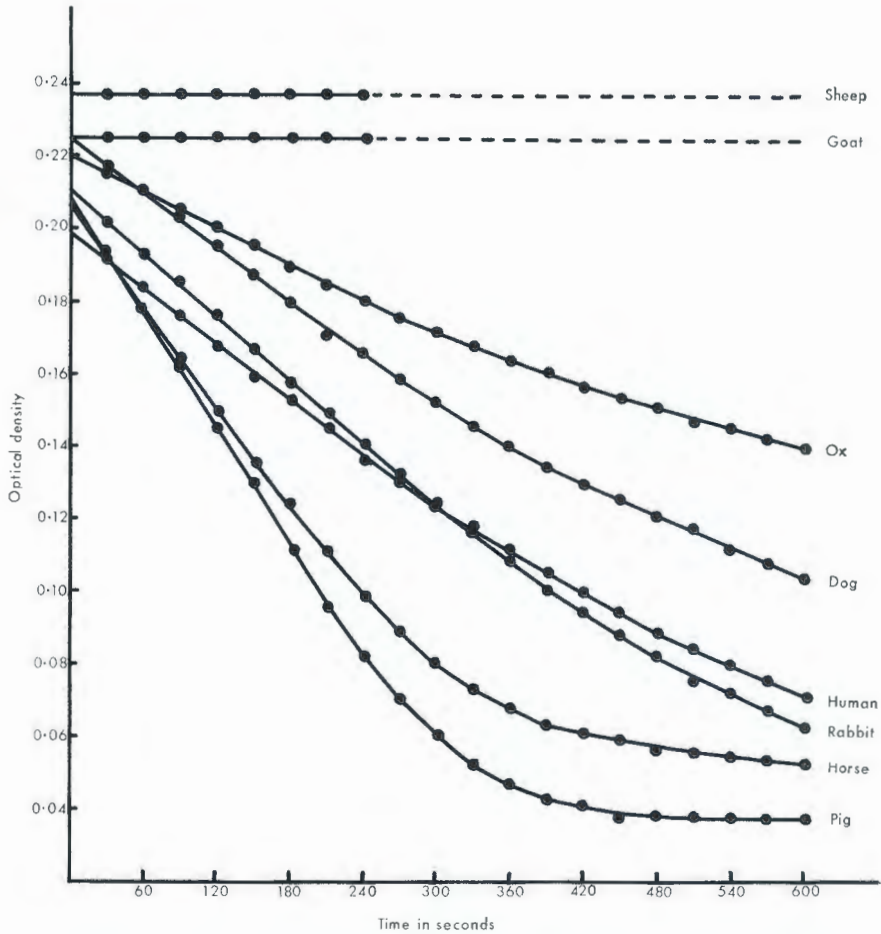


FIG. 3.—G-6-P-D assay on the erythrocytes of different species, showing rate of reduction of NADP (optical density changes of reaction medium plotted against reaction time in seconds)

DISCUSSION

Although we have not been able to demonstrate any significant differences in GAP-D activity between the red cells of sheep emanating from the Karoo and those from elsewhere, some important facts have been established. The 10 per cent lower, 80 per cent and 10 per cent upper limits for GAP-D activity in the erythrocytes of sheep maintained under field and experimental conditions have been shown to be 400–424, 425–730 and 731–900 micrograms per ml of blood respectively. The finding that G-6-P-D is absent from the red cell of the sheep and goat confirms the earlier studies of Carson (1960) and the contemporary work of Budtz-Olsen, Axten & Haigh (1963). It is highly probable that glycolysis in the ovine erythrocyte proceeds only via the direct Embden-Meyerhof pathway (Brown, 1964). The reaction rates

shown in Fig. 3 for G-6-P-D activity in the erythrocytes of different species are very interesting. Notable activity is seen in the red cells of the bovine, dog, horse, pig and rabbit as well as in the human erythrocyte on which a considerable amount of work has been done. The aerobic oxidation of glucose via the pentose monophosphate pathway may provide a convenient alternative glycolytic pathway in the domestic animals mentioned, should GAP-D activity fail for any reason. The ovine erythrocyte apparently, depending solely on GAP-D for glycolysis and methaemoglobin reduction, would be made more vulnerable in diseases such as enzootic icterus and geeldikkop. It is thus hardly remarkable that intravascular haemolysis, increased red cell fragility, and methaemoglobincythaemia feature prominently in the symptomatology of the acute episodes of these syndromes.

SUMMARY

Normal values have been established for the activity of glyceraldehyde-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase in the erythrocytes of sheep maintained under experimental and field conditions in South Africa. The 10 per cent lower, 80 per cent and 10 per cent upper limits have been established for the former enzyme as 400–424, 425–730 and 731–900 micrograms/ml of blood respectively. Negligible activity of the latter enzyme has been demonstrated in the ovine erythrocyte by three different methods. Glycolysis in the red blood cells of the sheep is believed to proceed mainly via the direct Embden-Meyerhof pathway. The importance of these findings with regard to the ovine disease syndromes, geeldikkop and enzootic icterus, is mentioned.

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