

HAEMOGLOBIN AND ERYTHROCYTE POTASSIUM TYPES IN SHEEP AND THEIR INFLUENCE ON OXYGEN DISSOCIATION AND HAEMOGLOBIN DENATURATION

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Summary

The influence of haemoglobin type and erythrocyte potassium concentration on oxygen dissociation curves and rate of denaturation of haemoglobin by alkali have been studied in Southdown sheep.

A significant difference has been found between haemoglobin types in respect to their oxygen dissociation curves and rate of alkali denaturation.

A significant difference was found between the oxygen dissociation curves of high potassium (*HK*) and low potassium (*LK*) sheep. No significant difference was found between *HK* and *LK* sheep in respect to the rate of denaturation of their haemoglobin by alkali.

An association has been found, within a potassium group, between erythrocyte potassium concentration, oxygen dissociation curves, and rate of denaturation of haemoglobin by alkali. The possible significance of the results is discussed.

I. INTRODUCTION

The existence in British breeds of sheep of two haemoglobin types, based on their movement under paper electrophoresis, has been demonstrated. These haemoglobin types are genetically determined in a simple Mendelian manner, neither being dominant (Evans *et al.* 1956).

Van der Helm, van Vliet, and Huisman (1957) found two haemoglobin types in Dutch sheep and also investigated some of the properties of the two haemoglobins, including resistance to denaturation by alkali. They found the electrophoretically fast-moving haemoglobin, called Hb-A by Evans *et al.* and Hb-II by themselves, was more resistant to denaturation by alkali than the electrophoretically slower-moving haemoglobin, called Hb-B by Evans *et al.* and Hb-I by van der Helm, van Vliet, and Huisman. Huisman, van Vliet, and Sebens (1958) showed that Hb-II had an oxygen dissociation curve to the left of Hb-I.

Jonxis (1949) noted a relationship between the oxygen affinity of adult and foetal haemoglobins and their resistance to alkaline denaturation in four different species. He concluded that the higher the resistance of a haemoglobin to denaturation by alkali the less its affinity for oxygen. This proposed relationship is the opposite of that which can be obtained from the data of Huisman and co-workers.

Evans (1954) has shown that sheep may also be classified into at least two groups on the basis of their erythrocyte potassium and sodium concentrations, high potassium (*HK*) animals having an erythrocyte potassium concentration ($[K_e^+]$) of 70–95 m-equiv/l, and low potassium (*LK*) animals 8–26 m-equiv/l.

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Kennedy and Valtis (1954) and Henderson (1928) have shown that in certain anaemias haemoglobin has a lessened affinity for oxygen, and Williams *et al.* (1937) have demonstrated an increase in erythrocyte potassium in anaemia.

Because potassium is involved in the reactions of haemoglobin within the erythrocyte it was thought possible that *HK* sheep may have different oxygen dissociation curves from those of *LK* sheep and also that the relatively small but consistent differences in $[K_e^+]$ between sheep, which have been demonstrated in both the *HK* and *LK* type of cell (Evans 1957), might also be associated with differences in oxygen dissociation curves.

The experiments reported here were designed to examine these possibilities and to relate oxygen affinity, $[K_e^+]$, and rate of alkali denaturation in a flock of Southdown sheep selected on the basis of their haemoglobin type and $[K_e^+]$.

II. MATERIALS AND METHODS

(a) Sheep

Two-year-old Southdown ewes were used. They were fed 800 g lucerne chaff per day in pens, and blood samples were taken by jugular vein puncture before the morning feed at 9.00 a.m.

The sheep were divided into four groups on the basis of their haemoglobin and potassium type as follows:

- | | |
|----------------------|----------------------|
| (1) <i>HK</i> , Hb-A | (3) <i>HK</i> , Hb-B |
| (2) <i>LK</i> , Hb-A | (4) <i>LK</i> , Hb-B |

(b) Methods

Oxygen dissociation curves and haemoglobin denaturation rates (with the exception of those in Table 3 which were in triplicate) were determined in duplicate on single blood samples. Haemoglobin concentration, haematocrit, and potassium determinations were carried out in triplicate.

Oxygen dissociation curves were prepared by equilibrating 3-ml samples of blood with different oxygen tensions and fixed tensions of carbon dioxide (40 mm Hg), in tonometers of 300-ml capacity which were rotated on a specially constructed water-bath at 20°C for 30 min. The percentage saturation was determined by estimating the oxygen content by the method described by Hawk, Oser, and Summerson (1954).

Haemoglobin denaturation rates were determined by the method of Jonxis and Visser (1956), and the haemoglobin concentration was measured as oxyhaemoglobin in a grey wedge photometer. The haemoglobin type was determined electrophoretically using Whatman No. 3MM paper in a horizontal bath containing Tris-borate buffer at pH 9.1. The bath was run at 225 V for 16 hr.

Potassium estimations of whole blood and plasma were carried out with a flame photometer using the method of King and Wootton (1956). Erythrocyte values were calculated from these data and the haematocrit. Haematocrit determinations were carried out by centrifuging samples in microhaematocrit tubes for 10 min at 12,000 *g*.

III. RESULTS

(a) *Haemoglobin Denaturation Rates*

Mean values of haemoglobin denaturation rates for sheep within the four groups are given in Table 1. Hb-A was significantly ($P < 0.001$) more resistant to denaturation by alkali than Hb-B.

TABLE 1

MEAN DENATURATION RATES OF THE TWO TYPES OF HAEMOGLOBIN FROM SHEEP ERYTHROCYTES WITH DIFFERENT POTASSIUM CONCENTRATIONS. Significant differences within haemoglobin types are indicated. Mean values for Hb-A and Hb-B types are significantly different at the 0.1% level

Group	Sheep No.	Erythrocyte Potassium Concn. (m-equiv/l)	Rate of Haemoglobin Denaturation†
HK, Hb-A	E55	89.6	0.08090
	E91	90.9	0.07085
	E85	92.3	0.07872
LK, Hb-A	E99	14.3	0.07436
	E53	17.8	0.07487
	E80	19.9	0.07426
HK, Hb-B	E63	78.4	0.12566
	E93	81.0	0.11678
	E79	84.9	0.10614
LK, Hb-B	E59	12.3	0.15579
	E78	15.0	0.12581
	E82	19.3	0.11856

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., not significant.

† Expressed as the tangents of the angles formed by the intersection with the abscissa of the plot of percentage haemoglobin denatured against time.

(b) *Oxygen Dissociation Curves*

The data for oxygen dissociation curves are summarized in Figure 1, which gives the mean dissociation curves for all groups. The dissociation curves for Hb-A were significantly ($P < 0.001$) to the left of those for Hb-B between 22.5 and 37.0 mm Hg oxygen tension. These basic differences were, however, modified by two other factors.

Firstly, a significant difference ($P < 0.01$) existed between the dissociation curves of blood from *HK* sheep and those from *LK* sheep between 22.5 and 37.0 mm Hg oxygen tension, irrespective of the haemoglobin type of the erythrocytes.

Bloods with *HK* erythrocytes have oxygen dissociation curves to the left of those from bloods with *LK* erythrocytes (Fig. 1). Because the dynamic nature of the affinity of haemoglobins for oxygen (Barker 1957) might have led to anomalous results, the percentage oxygen saturation of blood from each of the four groups of sheep was determined simultaneously at oxygen tensions of 22.5 and 37.0 mm Hg. The results (Table 2) confirm the significance of the data for the dissociation curves and show that the difference between *HK* and *LK* sheep with Hb-A is significant at

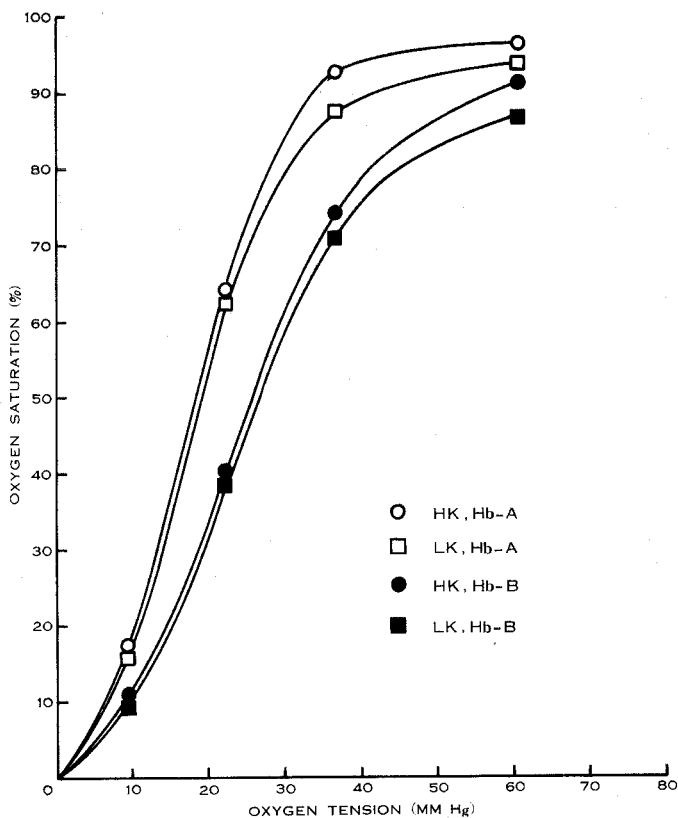


Fig. 1.—Influence of haemoglobin and potassium type on the oxygen dissociation curves of blood from the four groups of sheep.

the 1% level and between those with Hb-B at the 5% level. Table 1 shows, however, that there was no significant difference between the haemoglobin from *HK* or *LK* sheep within either of the haemoglobin types on the basis of its resistance to denaturation by alkali.

Secondly, the differences in $[K_e^+]$ which occur within the two major potassium groups appeared to be associated with differences in both the oxygen dissociation curve and the rate of alkaline denaturation of the haemoglobin. The data for oxygen dissociation curves indicate that as $[K_e^+]$ rises within a potassium group the dissociation curve shifts to the right (Fig. 2). Although the differences are small they tend to be confirmed by the variation in denaturation rate. A high $[K_e^+]$ within

either the *HK* or *LK* group was associated with greater resistance to denaturation by alkali in those animals with Hb-B (Table 1), although not in the results for Hb-A in the main experiment (Table 1). *LK*, Hb-A blood was critically re-examined in another experiment and significant differences were found (Table 3).

IV. DISCUSSION

The results show that the two haemoglobin types, Hb-A and Hb-B, found by Evans *et al.* (1956) in British breeds of sheep are the same with respect to their oxygen dissociation curves and alkali denaturation rates as the two haemoglobins, Hb-II and Hb-I, discovered by van der Helm, van Vliet, and Huisman (1957) and Huisman, van Vliet, and Sebens (1958) in Dutch Texel sheep. This supports the supposition of Huisman and co-workers that the haemoglobin types found in British and Dutch sheep are the same.

TABLE 2
EFFECT OF HAEMOGLOBIN AND POTASSIUM TYPE ON THE
OXYGEN UPTAKE OF SHEEP BLOOD

Group	Oxygen Saturation of Haemoglobin (%)	
	Oxygen Tension 22.5 mm Hg	Oxygen Tension 370 mm Hg
HK, Hb-A	63.1	93.6
LK, Hb-A	59.3	90.6
HK, Hb-B	38.0	73.8
LK, Hb-B	36.9	71.1

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

The results, however, conflict with the conclusion drawn by Jonxis (1949) for the relationship between oxygen affinity and resistance to alkaline denaturation in foetal and adult blood. It is probable, therefore, that the factors causing differences in oxygen affinity and alkaline denaturation between adult and foetal haemoglobins are different from those which give rise to the differences between the two adult sheep haemoglobins.

Van der Helm, van Vliet, and Huisman (1957) have shown differences in the amino acid composition and the solubilities of the two haemoglobins in buffered solutions, as well as the differences in mobility under electrophoresis and resistance to alkali. It appears that the difference in the oxygen dissociation curve between the two haemoglobin types is due to intrinsic differences in the haemoglobin molecule.

The difference in the oxygen dissociation curves between *HK* and *LK* bloods is probably not due to the same factors as those which give rise to the differences in oxygen dissociation curves between Hb-A and Hb-B. No obvious difference

has been found between the haemoglobin from *HK* sheep and that from *LK* sheep within a haemoglobin type with respect to their electrophoretic properties or their resistance to denaturation by alkali. The difference in alkali denaturation between *LK*, Hb-B and *HK*, Hb-B in the present experiments is not significant (Table 1). It thus appears that the factor or factors giving rise to the differences in oxygen dissociation curves between potassium types are associated with the intact erythrocyte. Kennedy and Valtis (1954) attributed most of the change in the oxygen dissociation curve, which they observed in anaemias, to an alteration in erythrocyte

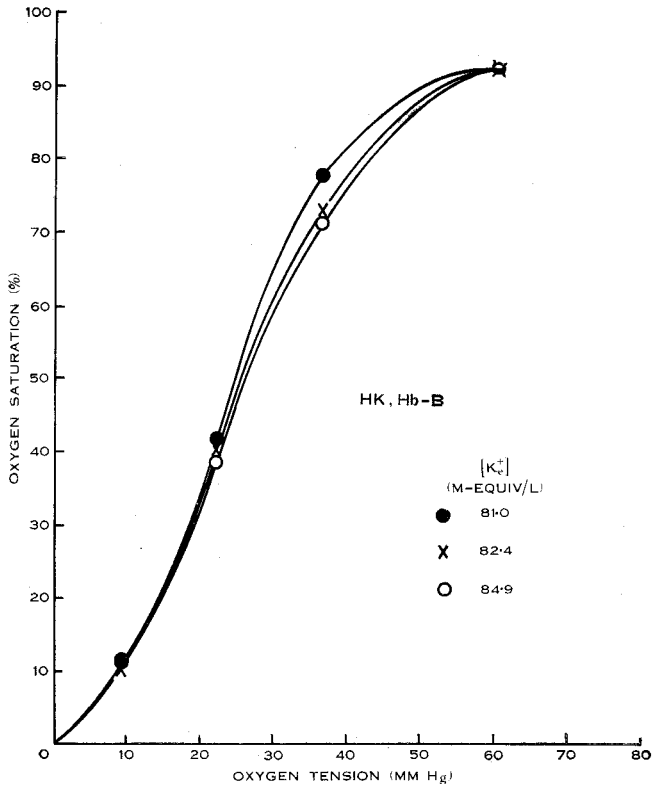


Fig. 2.—Differences in the oxygen dissociation curves of sheep blood associated with small but constant differences in $[K_e^+]$ within a *HK*, Hb-B type.

pH. It is possible therefore that differences in the dissociation curves of *HK* blood and *LK* blood could be due to differences in the internal pH of the two types of erythrocytes.

It has long been known, however, that the oxygen dissociation curves for foetal and adult human blood vary in their relationship to each other under certain conditions. When whole bloods are studied the foetal oxygen dissociation curve is slightly to the left of that of the mother (McCarthy 1943). On haemolysis, the foetal curve does not shift whereas the adult curve is shifted to the left to such an extent that the relationship between the two curves is reversed (McCarthy 1943; Allen,

Wyman, and Smith 1953). Allen, Wyman, and Smith dialysed similarly prepared haemoglobin solutions from foetal and maternal blood simultaneously against a common surrounding solution of restricted volume to equalize the concentrations of their diffusible components, and found that oxygen dissociation curves prepared from these solutions gave similar results. They suggested that the difference between their dialysed and non-dialysed solutions could be due to differences in the environment of the haemoglobin rather than to the intrinsic differences between foetal and maternal molecules. Various ions are, of course, known to affect the dissociation curve of haemoglobin solutions (Sidwell *et al.* 1938). Thus, the differences in the dissociation curves which are discussed in this paper could be due to variation in a specific ion effect on the haemoglobin caused by the different ionic environments within the two types of cell.

TABLE 3
MEAN DENATURATION RATES OF Hb-A FROM *LK* ERYTHROCYTES
WITH DIFFERENT POTASSIUM CONCENTRATIONS

Group	Sheep No.	Erythrocyte Potassium Concn. (m-equiv/l)	Rate of Haemoglobin Denaturation†
LK, Hb-A	E8	17.0	0.0855
	E23	18.0	0.0819
	E11	23.0	0.0742
	E20	24.0	0.0697

** $P < 0.01$; n.s., not significant.

† See footnote, Table 1.

Although the differences in the oxygen dissociation curve associated with *HK* and *LK* erythrocytes do not appear to be due to a difference in the haemoglobin molecule or its configuration, these factors could be involved in those variations in the curve which are associated with small but constant differences in $[K_e^+]$ (Fig. 2). Haurowitz, Hardin, and Dicks (1954) have shown that the configurational changes associated with de-oxygenation of bovine and rabbit haemoglobin leads to a decrease in the resistance to alkaline denaturation. Also variations in the resistance of haemoglobins to alkaline denaturation are usually associated with differences in their oxygen affinity (Jonxis 1949).

It is possible therefore that the results which relate $[K_e^+]$ and oxygen affinity within either of the potassium groups are due to an inherent individuality between sheep related to minor differences in the relationship of intracellular K^+ to the haemoglobin. But, in view of the significant differences in alkaline denaturation rates between haemoglobin from cells with different $[K_e^+]$ after K^+ - and Na^+ -free solutions had been obtained by dialysis (Dawson and Evans, unpublished data),

it seems equally reasonable to suggest that there are small differences in the haemoglobins which result in differences in intracellular $[K^+]$ as well as oxygen affinity and alkali denaturation rate.

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VI. REFERENCES

- ALLEN, D. W., WYMAN, J., and SMITH, G. A. (1953).—The oxygen equilibrium of foetal and adult human haemoglobin. *J. Biol. Chem.* **203**: 81–7.
- BARKER, J. N. (1957).—Role of haemoglobin affinity and concentration in determining hypoxia tolerance of mammals during infancy, hypoxia, hyperoxia and irradiation. *Amer. J. Physiol.* **189**: 281–9.
- EVANS, J. V. (1954).—Electrolyte concentrations in the red blood cells of British breeds of sheep. *Nature* **174**: 931.
- EVANS, J. V. (1957).—The stability of the potassium concentration in the erythrocytes of individual sheep compared with the variability between different sheep. *J. Physiol.* **136**: 41–59.
- EVANS, J. V., KING, J. W. B., COHEN, B. L., HARRIS, H., and WARREN, F. L. (1956).—Genetics of haemoglobin and blood potassium difference in sheep. *Nature* **178**: 849–50.
- HAUROWITZ, F., HARDIN, R. L., and DICKS, M. (1954).—Denaturation of haemoglobin by alkali. *J. Phys. Chem.* **58**: 103–5.
- HAWK, P. B., OSER, B. L., and SUMMERSON, W. H. (1954).—“Practical Physiological Chemistry.” 13th Ed. (McGraw-Hill Book Company Inc.: New York.)
- HELM, H. J. VAN DER, VLIET, G. VAN, and HUISMAN, T. H. J. (1957).—Investigations on two different haemoglobins of the sheep. *Arch. Biochem. Biophys.* **72**: 331–9.
- HENDERSON, L. J. (1928).—“Blood: A Study in General Physiology.” (Yale University Press: New Haven, U.S.A.)
- HUISMAN, T. H. J., VLIET, G. VAN, and SEBENS, T. (1958).—Sheep haemoglobins. *Nature* **182**: 171–2.
- JONXIS, J. H. P. (1949).—“Haemoglobin.” (Eds. F. J. W. Roughton and J. C. Kendrew.) (Butterworths Scientific Publications: London.)
- JONXIS, J. H. P., and VISSER, H. K. A. (1956).—Determination of low percentages of foetal haemoglobin in blood of normal children. *Amer. J. Dis. Child.* **92**: 588–91.
- KENNEDY, A. C., and VALTIS, D. J. (1954).—The oxygen dissociation curve in anaemia of various types. *J. Clin. Invest.* **33**: 1372–81.
- KING, E. J. and WOOTTON, I. D. P. (1956).—“Micro-analysis in Medical Biochemistry.” 3rd Ed. (J. and A. Churchill Ltd.: London.)
- MCCARTHY, E. F. (1943).—The oxygen affinity of human foetal and maternal haemoglobin. *J. Physiol.* **102**: 55–60.
- SIDWELL, A. E., JR. (1938).—The salt effect on the haemoglobin–oxygen equilibrium. *J. Biol. Chem.* **123**: 335–50.
- WILLIAMS, H. H., ERICKSON, BETTY, N., BERNSTEIN, S., HUMMEL, F. C., and MACY, I. C. (1937).—The lipid and mineral distribution of the serum and erythrocytes in pernicious anaemia. *J. Biol. Chem.* **118**: 599–618.