THE HAEMOGLOBINS OF A SHEEP–GOAT HYBRID FOETUS AND THOSE OF THE PARENT SPECIES

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[*Manuscript received April* 10, 1967]

Summary

Electrophoretic examination of the haemoglobins of a sheep–goat hybrid foetus indicated the presence of haemoglobins found in the foetuses of the parent species only. No interaction products were found.

In vitro "hybridization" experiments indicate that the difference in electrophoretic mobility between sheep A, sheep B, goat A, goat F, and sheep F haemoglobins is due to a difference in the non-α-portion of the haemoglobin molecules. The absence of electrophoretically detectable haemoglobin interaction products in the hybrid foetus is explained by the finding that the α-polypeptides of the parent species are electrophoretically indistinguishable.

I. Introduction

The literature concerning human haemoglobins has recently been reviewed by Ingram (1963) and Huehns and Shooter (1965) and multiple haemoglobins in animals have been discussed by Gratzer and Allison (1960). In man, where the structure of the haemoglobins has been studied in most detail, the haemoglobin A (Hb-A) molecule consists of two identical α-polypeptide chains (α_2^A) and two identical β-polypeptide chains (β_2^A). The structure of the Hb-A molecule may therefore be represented by the notation $\alpha_2^A\beta_2^A$. The amino acid sequences of the α- and β-chains are determined by genes at two independent loci and abnormal haemoglobins may be of two types, α-chain variants and β-chain variants. The haemoglobin in foetal red cells, Hb-F, has the structure $\alpha_2^A\gamma_2^F$, where the γ-polypeptide is determined by genes at a third independent locus. All other mammalian haemoglobins which have been studied have the same basic structure of two α- and two β-polypeptide chains.

Although the abnormal haemoglobins in man are not generally found in such frequencies that they constitute a polymorphism (with the exception of haemoglobins S, C, and E in some areas), cattle, sheep, and goats are polymorphic for electrophoretic haemoglobin variants. Two variants Hb-A and Hb-B, occur commonly in adult sheep (Cabannes and Serain 1955; Harris and Warren 1955) and the haemoglobin of foetal red cells, Hb-F, is electrophoretically distinguishable from each of these. By *in vitro* mixing of sheep haemoglobin ($\alpha_2^{ov}\beta_2^{ov}$) with canine

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haemoglobin ($\alpha_2^{can}\beta_2^{can}$) at pH 4·7, only recombination products of the type $\alpha_2^{can}\beta_2^{ov}$ and $\alpha_2^{ov}\beta_2^{can}$ can be produced and it has been shown that the difference in electrophoretic mobility between sheep Hb-A, Hb-B, and Hb-F is due to a difference in the electrophoretic mobility of the non-α-portion of the molecule (Beale *et al.* 1966). Amino acid analysis of sheep haemoglobins has shown that the β-chains of Hb-A and Hb-B differ at seven amino acid residue positions (Boyer *et al.* 1966) so that one haemoglobin is not the result of a single amino acid substitution in the other as in the case of Hb-A and Hb-S in man.

Two adult haemoglobin phenotypes and a foetal haemoglobin have been described in the goat (Harris and Warren 1955). Khanolkar *et al.* (1963) also described these phenotypes and attributed them to two alleles at a locus controlling haemoglobin synthesis although no family data were published. The two phenotypes were thought to represent the A homozygote and the AB heterozygote. No goat with type B haemoglobin has been described in the literature.

The purpose of this paper is to describe the electrophoretic behaviour of haemoglobins from sheep and goat foetuses and from the foetus of a sheep–goat hybrid and of *in vitro* mixtures of haemoglobins from these foetuses and the parent species. Canine haemoglobin was also used in the latter experiments.

II. Materials and Methods

Blood was obtained from the umbilical vessels of a single sheep–goat hybrid foetus (aged 55 days) which was obtained by Caesarean section from a female goat (No. 51) during the course of the hybridization experiments described elsewhere (Alexander, Williams, and Bailey 1967).

Merino sheep and Saanen goat foetuses were obtained from the Metropolitan and Export Abattoirs Board, Gepps Cross, South Australia. These were approximately the same age as the hybrid foetus, age being estimated by a crown–rump measurement (Cloete 1939).

Haemoglobin was prepared by lysis of washed erythrocytes and was examined without further purification.

Haemoglobins were "hybridized" *in vitro* using an adaptation of the method described by Itano and Singer (1958) and Itano and Robinson (1959) as follows: 2 ml of haemoglobin solution of each species, representing an eight times dilution of red cells, were mixed together; 2 ml of this mixture were dialysed against 0·2M sodium acetate buffer, pH 4·7, and the remaining 2 ml (constituting a control) were dialysed against distilled water. Dialysis was continued for approximately 17 hr when the sacs were transferred to a solution of gel buffer at pH 8·6 for 8 hr. After centrifugation at 1000 *g* for 15 min the supernatant was subjected to electrophoresis.

Starch gel electrophoresis was carried out using the Tris–EDTA–borate buffer system attributed to O. Smithies by Huehns and Shooter (1965). Haemoglobin was routinely detected by staining with dianisidine, except when photographs were required when amido black was used.

III. Results

The relative mobilities of sheep, goat, and canine haemoglobins are shown in Figure 1.

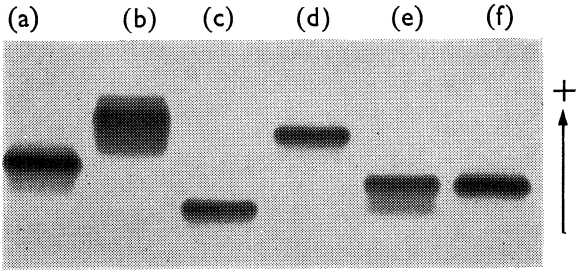

Fig. 1.—Starch gel electrophoretogram of sheep, goat, and canine haemoglobins: (*a*) sheep Hb-F; (*b*) goat Hb-F; (*c*) canine Hb; (*d*) sheep Hb-A; (*e*) sheep Hb-B; (*f*) goat Hb-A. Note minor components in (*a*), (*b*), and (*e*).

Figure 2 shows the results of electrophoresis of various foetal haemoglobins and *in vitro* foetal–canine "hybridizations". It is apparent that the haemoglobin of the sheep–goat hybrid has both sheep Hb-F and goat Hb-F [Figs. 2(*c*), 2(*e*)]

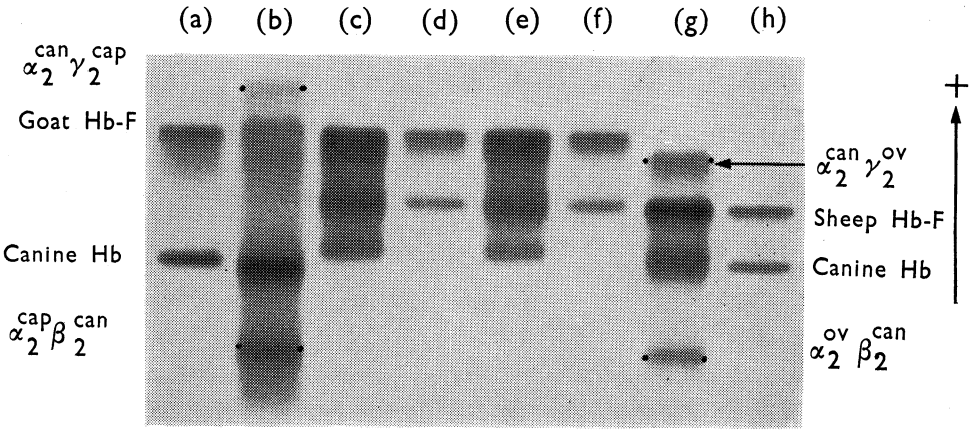

Fig. 2.—Formation of hybrid haemoglobins between canine haemoglobin and sheep and goat foetal haemoglobins. Interaction products are marked with two dots. (*a*) Canine Hb and goat Hb-F, control mixture; (*b*) canine Hb and goat Hb-F dissociated and recombined; (*c*) and (*e*) haemoglobins of a sheep–goat hybrid foetus; (*d*) and (*f*) goat and sheep Hb-F dissociated and recombined — note absence of any recombination products; (*g*) sheep Hb-F and canine Hb dissociated and recombined; (*h*) sheep Hb-F and canine Hb, control mixture.

but there is an additional major component migrating behind sheep Hb-F. There is also a faint band in this region (which did not reproduce in the photograph) with a mixture of dissociated (pH 4·7) and recombined sheep Hb-F and goat Hb-F [Figs.

2(*d*), 2(*f*); cf. Fig. 1(*a*)]. *In vitro* hybridization of foetal sheep and goat haemoglobins with canine haemoglobin [Figs. 2(*b*), 2(*g*)] produced two interaction products, the slower interaction product having the same mobility in each case. The results of other workers (Beale *et al.* 1966) indicate that the fast interaction products have the composition $\alpha_2^{can}\gamma_2^{ov}$ and $\alpha_2^{can}\gamma_2^{cap}$ and the slow interaction products $\alpha_2^{ov}\beta_2^{can}$ and $\alpha_2^{cap}\beta_2^{can}$.

The results of *in vitro* hybridization experiments using adult haemoglobins are shown in Figure 3. The mobility of the slow interaction product formed with canine haemoglobin was the same for sheep Hb-A [Fig. 3(*b*)], sheep Hb-B [Fig. 3(*c*)],

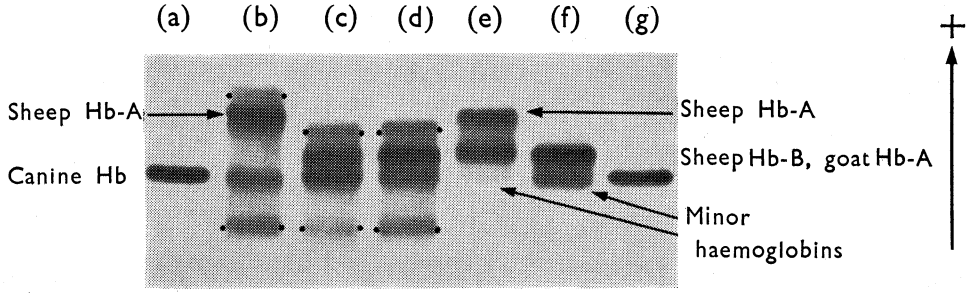

Fig. 3.—Formation of hybrid haemoglobins between canine and sheep and goat adult haemoglobins. All haemoglobin mixtures dissociated and recombined and interaction products are marked with two dots. (*a*) and (*g*) canine Hb; (*b*) canine Hb and sheep Hb-A; (*c*) canine Hb and sheep Hb-B; (*d*) canine Hb and goat Hb-A; (*e*) sheep Hb-A and goat Hb-A — note absence of recombination products; (*f*) sheep Hb-A and goat Hb-A — note absence of recombination products and trailing minor component. Presumed constitution of recombination products in (*b*), (*c*), (*d*) as follows:

	(*b*)	(*c*)	(*d*)
Fast	$\alpha_2^{can}\,\beta_2^{ovA}$	$\alpha_2^{can}\,\beta_2^{ovB}$	$\alpha_2^{can}\,\beta_2^{capA}$
Slow	$\alpha_2^{ovA}\,\beta_2^{can}$	$\alpha_2^{ovB}\,\beta_2^{can}$	$\alpha_2^{capA}\,\beta_2^{can}$

and goat Hb-A [Fig. 3(*d*)]; the fast interaction product formed with sheep Hb-B and goat Hb-A had the same mobility, but was slower than the fast interaction product formed with sheep Hb-A. No interaction products were formed when either of the sheep variants were hybridized *in vitro* with goat haemoglobin A [Figs. 3(*e*) and 3(*f*)]. The bands nearest to the origin in Figures 3(*e*) and 3(*f*) are the minor bands of the respective sheep haemoglobins.

IV. Discussion

When a mixture of two haemoglobins is dissociated into subunits and recombined, two electrophoretically detectable interaction products are formed if all four polypeptide chains are electrophoretically distinguishable, e.g. α_2^{ov}, α_2^{can}, β_2^{ov}, β_2^{can}. If the mixture contains only three electrophoretically distinguishable polypeptides

then only one interaction product can be detected by electrophoresis, e.g. when canine haemoglobin (α_2^{can}, β_2^{can}) and haemoglobin Barts (γ_4^F) in man are "hybridized" the only new product detectable is $\alpha_2^{can}\gamma_2^F$. Thus, if the third major component of the sheep–goat hybrid haemoglobin [Figs. 2(*c*) and 2(*e*)] is an interaction product, one of the parent species must have a foetal haemoglobin with all four chains having the same mobility. This is not so since both sheep Hb-F and goat Hb-F form two interaction products with canine haemoglobin under the same conditions [Figs. 2(*b*) and 2(*g*)]. It must be concluded that the third major component of sheep–goat hybrid haemoglobin is not a new product formed during haemoglobin synthesis as a result of the recombination of polypeptides from the sheep and goat. Whether it is the result of an interaction between loci controlling haemoglobin synthesis or whether it is a haemoglobin breakdown product associated with the pathology of the dying foetus is unknown.

Examination of the recombination products after *in vitro* hybridization of goat and sheep haemoglobins with canine haemoglobin [Figs. 3(*b*), 3(*c*), 3(*d*)] reveals that goat Hb-A, goat Hb-F, sheep Hb-A, sheep Hb-B, and sheep Hb-F all give a slow interaction product which has the same mobility. This suggests that the α-chains of sheep and goat haemoglobins are indistinguishable electrophoretically and that the differences between goat Hb-A, goat Hb-F, sheep Hb-A, sheep Hb-B, and sheep Hb-F are due to the non-α-portion of the molecule.

It has been shown (Zuckerkandl and Schroeder 1961) that the α-chains of gorilla haemoglobin differ from the α-chains of human haemoglobin A by no more than two amino acid residues and that the β-chains differ by only one amino acid residue. Since none of these substitutions affect the net charge on the molecule, gorilla and human haemoglobins have the same electrophoretic mobility. The present study has shown that goat haemoglobins A and F and sheep haemoglobins A, B, and F have α-chains with the same mobility. However, by analogy with the comparison of human and gorilla haemoglobins, identical electrophoretic mobility does not necessarily indicate identical primary structure. Since the known haemoglobin variants in man which constitute genetic polymorphisms are β-chain variants (Hb-S, Hb-C, and Hb-E), and since both the cattle haemoglobin variants Hb-B, Hb-C, Hb-A, and Hb-D (Efremov and Braend 1965) and the sheep variants Hb-A and Hb-B (Beale *et al.* 1966) are β-chain variants, then one must conclude that there is strong selection against the survival of individuals with α-chain variation. As pointed out by Ingram (1963) this is probably due to the presence of α-chains in foetal ($\alpha_2\gamma_2$) and embryonic ($\alpha_2\epsilon_2$) haemoglobins. Since species as widely separated as the gorilla and man have few differences in primary haemoglobin structure, it is possible that the α-chains of sheep and goat haemoglobin have the same primary structure although conclusive evidence for and against this must await amino acid sequence studies.

V. Acknowledgments

We wish to thank the Metropolitan and Export Abattoirs Board for access to sheep and goat foetuses. During this work one of us (L.F.B.) held an Australian Dairy Produce Board Senior Postgraduate Studentship.

VI. References

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