Differences in Cattle Globins

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1. Comparisons have been made between electrophoretic mobilities of the cattle haemoglobins, HbA, HbB, HbC, HbD and HbF, at pH 8.9. The fastest was HbB, then came in decreasing order HbF, HbC, HbA and HbD. 2. Globins were prepared from the main fractions of the five haemoglobins by CM-cellulose chromatography and investigated by starch-gel electrophoresis in four different buffer systems. In three of these (pH 2.0 and 11.8) the globins appeared as two bands on stained starch gels. The slowest bands, the α -chains, showed the same rate of migration in all five globins. The faster bands, the non- α -chains, differed, that of HbF being the fastest and that from HbC the slowest. The other three were intermediate with, however, very small difference between the non- α -chains from HbA and HbD. 3. At pH 1.8 in an acetate-phosphate-hydrochloric acid-urea buffer three bands appeared in all five globins of which the two slowest were indistinguishable in rates of migration, whereas the rates of migration of the third and fastest bands differed. Explanations for the occurrence of three bands are discussed.

Polymorphism of cattle haemoglobin (Hb) was first observed by Cabannes & Serain (1955) in Algerian hill cattle. They found two electrophoretically different haemoglobin components and three phenotypes. The two haemoglobins explained by two co-dominant alleles were designated HbA and HbB (Bangham, 1957; Salisbury & Shreffler, 1957; Grimes, Duncan & Lassiter, 1957), the latter being the faster at alkaline pH. A third haemoglobin, HbC, was reported by Vella (1958) in *Bos indicus*. The existence of HbC was confirmed by Crockett, Koger & Chapman (1963), Naik & Sanghvi (1965) and Osterhoff & Van Heerden (1965) in cattle of Indian and African origin. Efremov & Braend (1965) found a fourth adult cattle haemoglobin, HbD, in African Muturu cattle. Naik & Sanghvi (1965) reported a fifth adult haemoglobin, Hb Khillari, in Indian cattle. A sixth haemoglobin, HbF, electrophoretically different from the other five, occurs only in the foetus and in the newborn animal.

The differences between the two most common adult cattle haemoglobins, HbA and HbB, has been found in electrophoretic properties of the globins (Grimes & Duncan, 1959), the total amino acid composition of the haemoglobin, however, being the same (Højgaard, Moustgaard & Möller, 1960; Efremov, 1963). Grimes & Duncan studied the purified globins (A and B) by moving-boundary electrophoresis and found that at pH 10.7 one of

the two polypeptide chains of each of the globins had an identical mobility whereas the mobilities of the other chains differed. Muller (1961), using starch-gel electrophoresis and 'fingerprinting', found the α -polypeptide chains of globins A and F to be identical but that the other chains differed.

The main purpose of the work described below was to compare globins of the cattle haemoglobins, HbA, HbB, HbC, HbD and HbF.

MATERIALS AND METHODS

Fresh heparin-treated blood was obtained from a Norwegian cow homozygous with respect to HbA. Heparin-treated foetal blood came from two 7-8-month-old foetuses. Blood representing the types HbB and HbC was obtained from homozygous South African cows. The samples were sent by air and the anticoagulant was ACD (38.0 g. of sodium citrate, 3.6 g. of citric acid and 37.0 g. of glucose/l.) containing 2% (v/v) of 10% formalin. Blood representing type HbB was also obtained from African cows (Nigeria) together with blood from homozygous cows of type HbD. These latter blood samples were treated with heparin and also with streptomycin, and sent by air.

Haemolysates were prepared by the method of Drabkin (1946) and converted into the cyanmethaemoglobin form.

The main fractions of the five haemoglobins, also called HbA₁, HbB₁, HbC₁, HbD₁ and HbF₁, were isolated by CM-cellulose chromatography as described by Huisman, Martis & Dozy (1958). The homogeneity of the purified main fractions was checked by starch-gel electrophoresis in tris-EDTA-boric acid buffer, pH 8.9 (Gahne, 1961; Braend & Efremov, 1965). Globins were prepared according to the method of Anson & Mirsky (1930) modified by the use of

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cyanmethaemoglobin and low temperature (-10° to -20°). The freeze-dried globins were stored at -20° .

The globins were subjected to starch-gel electrophoresis at a concentration of 5 mg./ml. Four different buffer systems were used. Buffer 1, pH 2.0, consisted of tris (0.1M) and citric acid (0.8M). For making the gel the buffer was diluted with 4 vol. of water. The voltage output of the power supply was 180 v and the running time 5 hr. Buffer 2, pH 11.8, consisted of glycine (0.05M), NaCl (0.05M) and NaOH (0.07N). For making the gel the buffer was diluted with 1 vol. of water. The voltage output was 100 v and the running time 6 hr. Buffer 3, discontinuous system, was made up as follows: in the vessel, NaOH (0.2N) and boric acid (0.15M), final pH 11.7; in the gel, tris (0.1M), EDTA (0.02M) and NaOH (0.12N), final pH 11.8. The voltage output was 80 v and the running time 8 hr. Buffer 4, pH 1.8, consisted of sodium acetate (0.065M), NaH_2PO_4 (0.02M) and urea (0.05M), adjusted to pH 1.8 with HCl (1N). For making the gel the buffer was diluted with 1 vol. of water. The voltage output was 60 v and the running time 8 hr.

Starch prepared from commercial Norwegian potato starch was used at a concentration of 13 g./100 ml. of buffer. The gels (horizontal) measured 22 cm. $\times$ 13 cm. $\times$ 0.6 cm. Staining was done with Amido Black.

RESULTS

Fig. 1 shows the relative mobilities at pH 8.9 of the main fractions of the five cattle haemoglobins. HbC₁ has a migration rate close to that of HbF₁. Components HbA₁ and HbD₁ also have migration rates very close to each other under our conditions of electrophoresis.

In Fig. 2 the electrophoretic behaviour of the globins in gel made from buffer 1 is shown. Two components usually appeared of which the slower moving is assumed to be the α -polypeptide chain (Muller, 1961) and the faster the non- α -chain. The slow bands of the respective five globins all have the same mobility, whereas the fast bands are different in most of the globins. The fastest is the non- α -chain (γ -polypeptide chain; Muller, 1961) of globin F and the slowest that of globin C. The non- α -chain of globin C stains considerably more weakly than the corresponding non- α -chains of the other four globins. The non- α -chain of globin B is intermediate in mobility between the non- α -chains of globins F and C, but it is clearly slower than those

of globins A and D. The latter two are not distinguishable in this photograph. However, repeated runs in buffers 2 and 3 indicated a small difference in migration rates for the non- α -chains of globins A and D also, that from globin D being slightly faster.

Fig. 3 shows the results obtained at pH 1.8 (buffer 4). Under these conditions the globins of all five types of haemoglobin appeared as three bands on stained starch gels. The slowest bands have the same rate of migration for all the globins. This is assumed to represent the α -polypeptide chain (see above). The middle bands of all five globins also appear with the same mobilities. The third bands, which are assumed to be non- α -chains, are clearly different in migration rates for globins F, B and C, where globin F has the fastest non- α -chain and globin C the slowest, that of globin B being intermediate. Also, under these conditions the non- α -chains of globins A and D appear indistinguishable from each other but different from the other three. Further, there are differences in the strength of the bands; thus the fastest band of

Fig. 2. Electrophoretic patterns of cattle globins in tris-citric acid buffer, pH 2.0.

Fig. 1. Relative mobilities of cattle haemoglobins in tris-EDTA-boric acid buffer, pH 8.9.

Fig. 3. Electrophoretic patterns of cattle globins in acetate-phosphate-urea-HCl buffer, pH 1.8.

globin C is markedly weaker than the corresponding bands of the other globins. For the middle bands differences in strength are also seen, that of globin F being the weakest. Finally, when haemoglobins from homozygous cattle were investigated in gel made from buffer 4 the three-band pattern again appeared.

DISCUSSION

It is known (see Wyman, 1948) that urea, acid or alkali may cause dissociation of haemoglobin into half-molecules of approximately equal molecular weight.

In our studies with the buffer systems where two main bands appeared on stained starch gels (buffers 1, 2 and 3) the results agree with those of Grimes & Duncan (1959) and Muller (1961), the slower band being the α -chain and the faster the non- α -chain. Grimes & Duncan (1959) also reported the finding of a third component of both cattle globins at pH 2.4. The mobilities of the respective three components were essentially the same for both globins. This agrees with our results for buffer 4, except that in our investigations one of the three bands of the two globins differed in migration rate. The appearance of three components at pH 1.8 in the presence of urea may have any of several explanations. The low pH may have caused chemical modification (with change in charge) of the non- α -chains. Another possible explanation would be the formation of polymer species large enough to be retarded by the gel. A third possibility would be splitting of polypeptide chains into sub-units. Reichmann & Colvin (1956), working with horse haemoglobin, found that at pH 3.7–4.0 the haemoglobin predominantly splits into half-molecules of different mobilities. As the pH decreased, however, an increasing proportion of both half-molecules dissociated into sub-units. A similar situation may exist for cattle haemoglobins. Muller (1961) reported that additional bands, except the single polypeptide chains, may appear, especially in old globins or in samples that have been treated rigorously. However, as we have obtained the same results with fresh haemoglobin we do not consider the last-mentioned conditions to be responsible for the appearance of three bands in our experiments.

In our investigations the α -polypeptide chains of the five cattle haemoglobins were indistinguishable from each other. That does not necessarily mean that they are identical. Differences may exist that cannot be detected by our present technique. This may mean that the α -chain is, in some respects, more important than the non- α -chains. That is in accordance with the existence of haemoglobin having the α -chain in both foetus and adult, whereas for the non- α -chains different chains are

found in foetus and adult in man and probably also in many domestic animals. This may have correlation with the viability of mutations. In human haemoglobin, for instance, several mutations in the α -chain are reported, although in total there seem to be more mutations in the β -chain (see Baglioni, 1963; Huisman, 1963). In cattle only few different haemoglobins have so far been found, and we do not know whether any of them may be described as abnormal. However, realizing the stronger natural selection in cattle than in man, we are not surprised to find the mutations in the non- α -chains only.

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