# Haemoglobin, Transferrin and Albumin Types in *Equidae* (Horses, Mules, Donkeys and Zebras)

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## INTRODUCTION

Blood protein polymorphism provides the immunogeneticist with interesting tools for the search into possible relationships between different species and other problems of origin and domestication. In equidae we are in the fortunate position to have at hand three species of one genus and at the same time a hybrid between two of these species. Several investigations of protein polymorphism (Bangham and Lehmann, 1958; Podliachouk et al, 1965; Kaminski, 1965), enzyme differences (Kaminski and Cajos, 1964), blood groups (Podliachouk, 1965; Podliachouk et al, 1965) and immunological differences (Podliachouk et al, 1965) have been performed on horses, donkeys and their hybrids, but so far no zebras were included in any of these studies.
The present paper should be regarded as a preliminary study which will be followed by an investigation on a greater zebra as well as donkey and mule material with more family data included in the latter.

## METHODS AND MATERIAL

The material of 234 horses, 40 donkeys and 52 mules formed a mixed group of animals which are either bred at or purchased for the Veterinary Research Institute, Onderstepoort. No clear classification of breeds can be given with regard to the horses; the group consisted mainly of crossbred riding horses with relative few family data available. These horses also provided the female basis of the hybrids. A classification of the African donkey into breeds is almost impossible and no further description is necessary.

The zebras belonged to the species *Equus burchelli antiquorum*; most of the 38 samples included in the present study originated from animals in the Etosha Game Reserve, South West Africa. Several samples were collected in the Kruger National Park and other game reserves.

The determination of haemoglobins was performed by using the method of starch gel electrophoresis as described by Buschmann (1963) including the new technique of the preparation of starch as originated by Kristjansson (1963).

For transferrins the method of starch gel electrophoresis as described by Kristjansson and Hickman (1965) was applied in toto, the only difference being the double slicing of the gel to facilitate check readings of phenotypes.

Albumins were typed according to the method described by Braend and Efremov (1965) but also here, the preparation of starch was done according to techniques described by Kristjansson (1963).

## RESULTS

Fig. 1 shows the variation in haemoglobin obtained in the genus equidae.

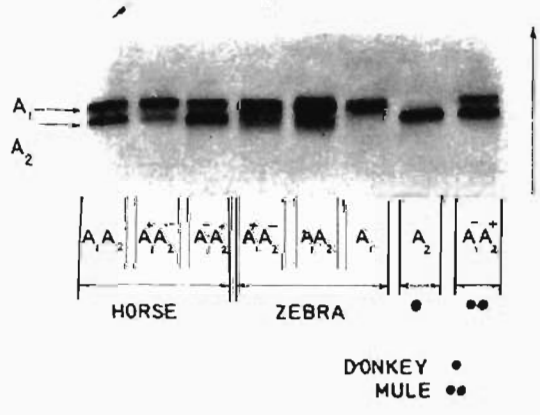


Figure 1. Haemoglobin types in equidae.

Three haemoglobin phenotypes could be established in horses, two of them being described before (Braend and Efremov, 1965; Schmid, 1965) and another phenotype which consisted also of two components but with a major band migrating slower than a secondary faster moving band.

Using the nomenclature introduced by Braend and Efremov (1965) and adding a plus or minus sign to indicate either heavy stained or very faint bands, the results can be described as follows. From 224 horse samples investigated 218 (97.5 per cent) re-

presented the normal $A_1A_2$-type, 5 animals (2.2 per cent) belonged to the $A_1^+A_2^-$-type and only one animal (0.3 per cent) to the $A_1^-A_2^+$-type.

The results of mules revealed that all 52 samples investigated represented the $A_1^-A_2^+$-type with proportional staining ability of both bands almost identical to the one horse sample mentioned. Samples of 38 donkeys showed only one band which could be classified as $A_2$-type, the band migrating slightly faster than the $A_2$-band of the horse.

In zebras three phenotypes could be established which are according to staining ability and migration rate the same as the horse haemoglobins. Of 30 zebras samples available for haemoglobin determination 19 (63.3 per cent) showed the normal $A_1A_2$-horse type, 10 (33.3 per cent) the $A_1^+A_2^-$-horse type and the remaining sample (4.3 per cent) only one component comparable to the $A_1$-horse type.

Results of transferrin determinations are presented in Table 1.

TABLE 1. Distribution of transferrin types in equidae

| Horses (230) | | Donkeys (35) | | Mules (50) | | Zebras (38) | |
|---|---|---|---|---|---|---|---|
| Type | No. | Type | No. | Type | No. | Type | No. |
| DD | 25 | $D_dD_d$ | 19 | $D_dD$ | 8 | $D_zG$ | 2 |
| FF | 54 | $D_dH$ | 2 | $D_dF$ | 12 | GG | 2 |
| HH | 5 | $D_dI$ | 1 | $D_dH$ | 2 | GH | 3 |
| OO | 2 | $D_dM$ | 2 | $D_dO$ | 2 | GI | 2 |
| RR | 2 | $D_dO$ | 2 | $D_dR$ | 1 | GJ | 3 |
| DF | 72 | $D_dR$ | 2 | DI | 1 | GM | 1 |
| DH | 11 | MM | 1 | DJ | 1 | GO | 1 |
| DO | 9 | OO | 1 | DM | 6 | HH | 2 |
| DR | 5 | HR | 2 | FI | 3 | HJ | 2 |
| FH | 16 | IO | 3 | FJ | 1 | HO | 1 |
| FO | 13 | | | FM | 9 | HR | 2 |
| FR | 10 | | | FR | 1 | II | 2 |
| HR | 3 | | | HI | 1 | IJ | 4 |
| OR | 3 | | | HM | 1 | IM | 2 |
| | | | | MO | 1 | JJ | 2 |
| | | | | | | JM | 1 |
| | | | | | | MO | 1 |
| | | | | | | OO | 1 |
| | | | | | | RR | 1 |

A great variation in transferrin types was found and an enlargement of the nomenclature used in horse transferrins was appropriate. Each new type consisted also of two bands and the major band of the two was named according to its migration speed in agreement with the existing nomenclature (Braend and Stormont, 1964). A new type having a double band in the region of the major D-band in horses appeared rather frequently and it was decided to name the allele responsible for these two bands $Tf^Dd$, because it appeared in homozygous form only in donkeys and in heterozygous form only in mules.

In zebras two new combinations of bands were additionally found, apparently one allele ($Tf^Dz$) being responsible for two bands migrating faster than the Dd-bands and one ($Tf^G$) being responsible for two bands with the major band migrating between the F- and H-bands of horses.

Examples of migration rates of the new types are given in Fig. 2 and Fig. 3.

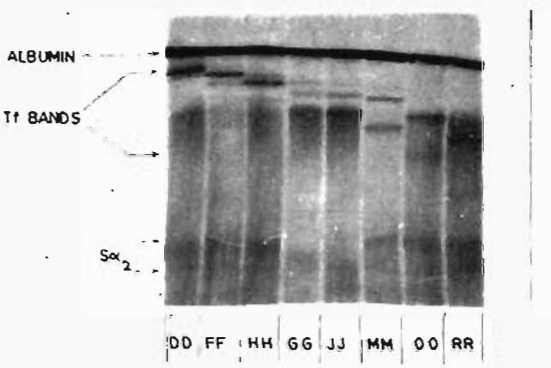


Figure 2. New homozygous transferrin types

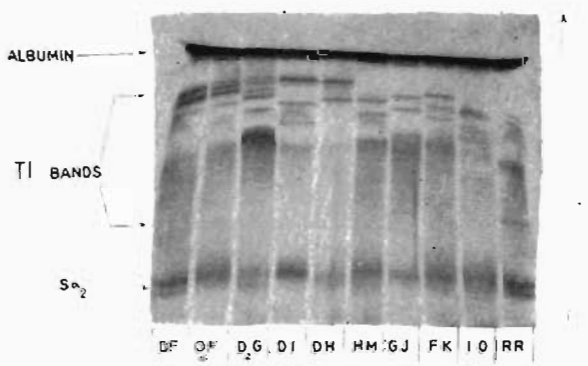


Figure 3. New heterozygous transferrin types

The frequencies of the different alleles are combined for all equidae investigated and shown diagramatically in Fig. 4.

Albumin typing allowed clear and simple separation into three bands which were designated A and B (acc. to Braend, 1964) and C, making provision for six albumin phenotypes. A photograph showing these phenotypes is presented in Fig. 5, the distri-

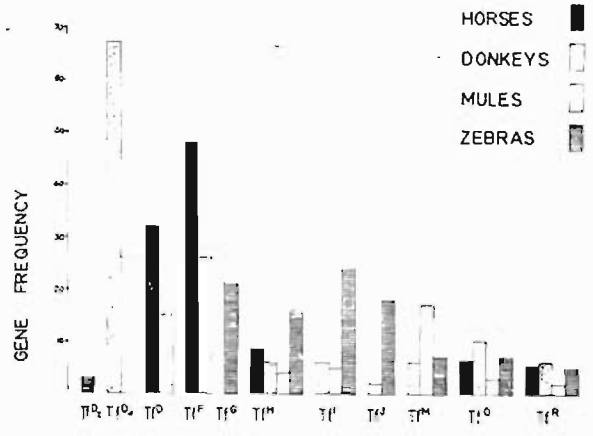


Figure 4. Frequency of transferrin alleles in equidae

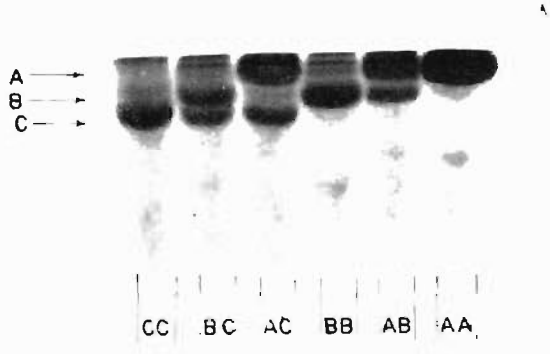


Figure 5. Albium types in equidae

bution of different phenotypes in the four groups of animals is shown in Table 2.

TABLE 2. Distribution of albumin types in equidae

| Species (hybrid) | No. of animals | Albumin types | | | | | |
|---|---|---|---|---|---|---|---|
| | | AA | AB | BB | CC | AC | BC |
| Horse | 234 | 63 | 99 | 72 | 0 | 0 | 0 |
| Donkey | 40 | 0 | 0 | 0 | 40 | 0 | 0 |
| Mule | 52 | 0 | 0 | 0 | 0 | 21 | 31 |
| Zebra | 32 | 0 | 0 | 0 | 27 | 0 | 5 |

## DISCUSSION AND CONCLUSIONS

Different authors give different ratios of the two components $A_1$ and $A_2$ or horse haemoglobin (Studzinski, 1965; Braend and Efremov, 1965). Apparently these ratios may vary in the same animal; this was also revealed by retesting three samples which in previous tests showed only one band ($A_1$), but which in repeated tests showed an additional slow migrating faint band and had to be classified $A_1^+A_2^-$.

The horse possessing the $A_1^-A_2^+$-type was repeatedly bled and the correct typing confirmed. Chemically investigations should confirm the similarities of this type and the haemoglobin type found in all mules. Donkeys possess only the $A_2$-band, a configuration not yet seen in horses, zebras or mules. The zebra samples were also tested by Schmid and the results confirmed (Schmid and Osterhoff, 1966).

Although not all transferrin types listed in Table 1 could be controlled by family data or breeding tests, they can be considered as well established. In doubtful cases check runs with serum samples labelled with $Fe^{59}$ were performed to ensure correct identification. Furthermore, several horse serum samples were checked in the Norwegian laboratory and our correct typing was confirmed (Braend, 1966). The major bands of the I and J-types are very near the major H-band of horses, the naming being in agreement with the existing nomenclature. The inheritance of the phenotypic Dd-bands could be clarified in several donkeys and hybrid families. If the major band of the M-type found in donkeys, mules and zebras is in fact the same as the corresponding band in horses could however not be represented in our material. The alleles $Tf^R$, $Tf^O$ and $Tf^R$ were present in all species (hybrids) investigated.

The great variation in the frequency of transferrin alleles in zebra at the one hand and the concentration of the alleles $Tf^D$ and $Tf^F$ in horses at the other, which has been found in many studies (see also Gahne, 1966), could possibly give some indication with regard to the phylogeny of these species. It could be reasoned that along the long road of domestication and selection several of the transferrin genes originally present had been diminished or were lost completely.

The albumin typing gave clear results proving the observations of Stormont and Suzuki (1963) that the phenotypes A, AB and B were inherited as if controlled by a pair of codominat autosomal alleles. The extension of that genetic system to three alleles is appropriate. Frequency calculations revealed similar distributions of $Alb^A$ and $Alb^B$ in horses and mules appearing in the latter only in heterozygous form together with $Alb^C$. No difference between the phenotypes C in donkeys and zebras could be established be cause the above M-phenotype was not between these species. The zebra phenotype AC was not observed, but should be found in a extended material.

## ABSTRACT

Blood and serum samples of 234 horses, 40 donkeys, 52 mules and 38 zebras were collected and protein polymorphism was studied by means of starch gel electrophoresis.

Three haemoglobin phenotypes could be established in horses, one of them representing a new combination of a major band migrating slower than a secondary faint band. This type was also found in

all mules while all donkeys possessed only the slower migrating band. In zebras three types were found being similar to the phenotypes described earlier in horses.

Fourteen transferrin phenotypes were found in horses representing all known types except the phenotypes HO and all combinations of the H-type. New types were discovered in mules, donkeys and zebras which were named in accordance with the existing nomenclature for horse transferrins. The greatest diversity in transferrin types was found in zebras, followed by mules combining the transferrin types of donkeys and horses in heterozygous form. The gene frequencies of $Tf^D$ and $Tf^F$ in horses and $Tf^D$ in donkeys were prevailing.

Albumin studies revealed a clear separation into three phenotypic bands A, B and C, giving the following phenotypic combinations for the three species and the hybrids: horses AA, AB and BB; donkeys — only CC; mules — AC and BC; zebras — BC and CC with AC not yet found.

The usefulness of these studies in phylogenic investigations is indicated.

## SUMMARY

Protein polymorphism in horses, mules, donkeys and zebras was studied by means of starch gel electrophoresis.

Three haemoglobin phenotypes could be established in horses, one of them representing a new combination of a major band migrating slower than a secondary faint band. This type was also found in all mules, while all donkeys possessed only the slower migrating band. In zebras three types were established being similar to the phenotypes earlier described in horses.

Most of the known transferrin phenotypes could be established in horses. New types were discovered in mules, donkeys and zebras which were named in accordance with the existing nomenclature.

Albumin studies resulted in the identification of three bands and a clear species differentiation could be achieved.

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