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443

NATURAL VARIATION IN THE BLOOD PROTEINS OF WHITE AND BLACK RHINOS

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SUMMARY

Starch gel electrophoresis patterns of blood from 48 white rhinoceroses Ceratotherium simum simum Burchell, 1817 and 10 black rhinoceroses Diceros bicornis bicornis Linn., 1758 from the Hluhluwe and Umfolozi Game Reserves disclosed different haemoglobin, transferrin and albumin types. This technique detected presumptive genetic variation in the white rhinos but not in the black rhinos and might be used either on its own or combined with blood grouping to characterize different populations of one species.

INTRODUCTION

During a biological and veterinary survey of the rhino population in the Hluhluwe and Umfolozi Game Reserves, blood samples of white and black rhinos became available. Both species are of great interest from the phylogenetic point of view and many different theories exist on the evolution of Rhinocerotidae. being the most peculiar family of the order Perissodactyla. Since karyotype studies did not give a satisfactory answer to the evolution of Rhinocerotidae (Heinichen 1968) the present investigation was undertaken to characterize the two different species.

MATERIALS AND METHODS

All rhinos were immobilized in the wild state, with a mixture of Etorphine hydrochloride (Reckitt and Colman), Acetylpromazine (Boots), and the alkaloid Hyoscine hydrobromide, using a projectile syringe. Both males and females, most of them adult, were used for different aspects of biological and veterinary research. Blood was collected from the ear vein into dry bottles to obtain the serum samples, and into bottles containing the following anti-coagulant: 2.5 g.NaCL + 30.0 g.Na Citric. + 0.23 g. NA Cyanid, to 1000 ml. Agua dest., for the erythrocyte samples. These samples were packed in ice and sent in thermos flasks to Onderstepoort. The clotted samples were centrifuged to obtain the serum. The erythrocytes from anti-coagulant samples were washed three times with physiological saline; thereafter they were used either in different haemolytic and agglutination tests, or frozen before the electrophoretic separation of haemoglobin was performed, or both. The detailed description of the starch gel electrophoretic technique used for the separation of haemoglobins, transferrins and albumins is given by Osterhoff (1968), who also gave details regarding the haemolytic and agglutination tests.

RESULTS

Haemoglobin determinations could be performed on all 48 samples of white rhino blood and on all 10 samples of black rhino blood. Each species showed a typical migration pattern, the pattern of white rhino having only one band while the black rhino possesses two haemoglobin fractions similar to the haemoglobins in horses (Efremor & Braend 1965) and African buffalo (Osterhoff et al., 1969) No genetic variation could be established in either population (Fig. 1).

For transferrin investigations, all samples of both species could also be included. All black rhinos showed only one pattern while the white rhinos showed clear polymorphism in the transferrins. The different genetical variants were named AA, showing only one faster migrating band, AB, possessing a faster and slower band, and BB having only the slower migrating band (Fig. 2.)

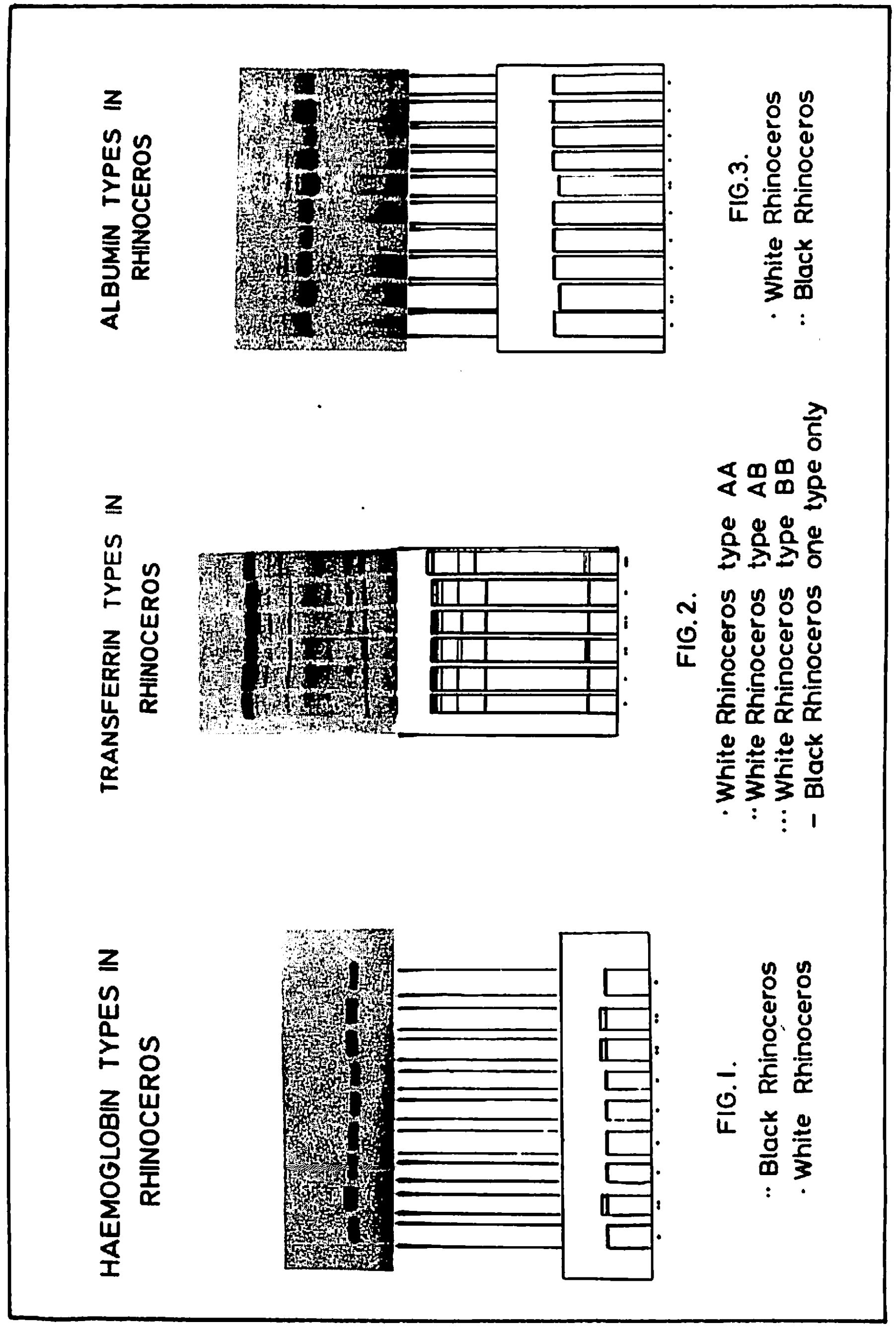
The frequency of the three types was as follows: 31 animals being of the AA, 12 of the AB and 5 of the BB type. The gene frequency calculation would give us the frequency of Tf^A as being .77 and of Tf^B as .23, but such a calculation is of little value in such a small sample of material. However, it is proved without any doubt that a clear genetic polymorphism exists in the white rhino population.

Albumin types were also investigated and here again all animals of the black rhino group possessed only one slower migrating band while all members of the white rhino group possessed one faster migrating band (Fig. 3).

In the photograph of the starch block in Fig. 3 one is able actually to see another variation in the bands migrating in front of the albumin bands, the prealbumins. These bands are unfortunately very faint, but it seems that the white rhinos also show polymorphic variants in these fast migrating protein fractions. Genetically determined variations in these prealbumins have been described in horses (Gahne, 1966), pigs (Kristjansson, 1963) and sheep (Efremor Vaskov, 1968).

Esterase was the only enzyme investigated in 23 samples of white rhinos and 10 samples of black rhinos. Here too, however, a variation was found in the white rhinos which was not as clear as in the proteins mentioned above. All black rhinos again possessed only one type.

Agglutination tests using the sera of 10 white and 4 black rhinos and the erythrocytes of the same animals gave only negative results. In the haemolytic test, the white rhino cells reacted only with black rhino serum, when absorbed rabbit serum was used as complement. For the haemolytic tests, twelve sheep reagents and sixteen horse reagents were used. Only the erythrocytes of all white rhinos were lysed, while the red cells of all black rhinos showed a negative reaction. Erythrocytes of 22



white rhinos and 8 black rhinos were used in these tests and all reacted in the same way – all white rhinos positive with all reagents, and all black rhinos negative with all reagents.

DISCUSSION

While in the white rhino polymorphic variants could be established in the transferrins, prealbumins and esterases, the black rhinos showed no genetic variation whatsoever in any of the proteins investigated. With a material of ten black rhinos it would possibly be unwise to make final statements, but it seems that the genetic variation in the white rhino is certainly greater than in the black rhino. In work performed in cattle by the senior author (Osterhoff, 1968) one generally accepts that a breed with a greater variation is still in a developing stage and breeding and selection methods have changed the original variability very little. With higher selection intensity and inbreeding part of the original variation is usually lost.

Discussing the results obtained in the same light one would say that the white rhino still possesses a great amount of genetic variability, while the black rhino present in smaller numbers, could possibly be more inbred and could have lost some of the variability. Thenius (1966) says that animals with very high chromosomal numbers have undergone little adaptive change. The white rhino possesses 82 and the black rhino 84 chromosomes. Thus the argument that the decrease in chromosome number leads to a lessening of the scope of genetic variation certainly does not hold in the case of the rhinoceros.

It would seem that the problem cannot be solved through this very limited study and further work will be required to obtain a clear picture. However, there is no doubt that the new approach of investigating the natural variation in the different blood proteins is a very useful one.

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444

A CHECK LIST OF THE BLOOD PARASITES RECORDED FROM THE LARGER WILD MAMMALS IN ZULULAND

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Species of Mammal	Parasite	Area Recorded	Date (Year)	Recorder	Reference
Spotted Hyaena *Crocuta crocuta*	Apparently *Trypanosoma brucei*	Zululand	1895	Bruce	1
Black Rhinoceros *Diceros bicornis*	Small piroplasms (Small *Babesia* or *Theileria* sp.)	Hluhluwe Game Reserve	1968	Bigalke & Keep	7
Square-lipped Rhinoceros *Ceratotherium simum*	Small piroplasms (Small *Babesia* or *Theileria* sp.)	Umfolozi Game Reserve	1967	Neitz, Bigalke and Keep	7
	Large *Babesia* sp.	Umfolozi Game Reserve	1968	Bigalke and Keep	7
Burchell's zebra *Equus burchelli*	*Trypanosoma* species	Zululand	1921	Mitchell	1
	Trypanosome congolense	Hlabisa Area –	1929	Neitz	1
	Small piroplasms (Similar to *B. equi*)	Umfolozi River	1929	Neitz	1
Warthog *Phacochoerus aethiopicus*	*Trypanosoma vivax*	Zululand	1921	Curson	1
	Small piroplasms	Umfolozi River	1929	Neitz	2
	Microfilaria	Lower Umfolozi	1929	Neitz	1
	Babesia trautmanni	Umfolozi Game Res.	1968	Neitz	3
Giraffe *Giraffa camelopardalis*	*Cytauxzoon* species	Ubizane Game Ranch Zululand	1967	McCully and Keep	8
Natal Red Duiker *Cephalophus natalensis*	Small piroplasms (*Theileria* type)	St. Lucia, Zululand	1968	Bigalke and Keep	7

Species of Mammal	Parasite	Area Recorded	Date (Year)	Recorder	Reference
Grey Duiker *Sylvicapra grimmia*	*Trypanosoma vivax*	Zululand	1921	Carson	1
	Small piroplasms (*Theileria mutans* type)	Umfolozi River	1929	Neitz	1
Steenbuck *Raphicerus campestris*	*Trypanosoma brucei*	Zululand	1903	Bruce	1
	Small piroplasms (*Theileria mutans* type)	Hlabisa District, Zululand	1929	Neitz	1
Common Reedbuck *Redunca arundinum*	Apparently *Trypanosoma brucei*	Zululand	1903	Bruce	1
	Small piroplasms	Hlabisa District & Umfolozi River	1929	Neitz	1
Mountain Reedbuck *Redunca fulvorufula*	Small piroplasms	Zululand Hlabisa District –	1929	Neitz	1
Waterbuck *Kobus ellipsiprymnus*	Small piroplasms (*Theileria mutans* type)	Hlabisa and Empangeni District and Umfolozi River	1929	Neitz	1
	Microfilaria	Empangeni district Zululand	1929	Neitz	2
Impala *Aepyceros melampus*	*Trypanosoma* species	Zululand	1921	Mitchell	1
	Piroplasms (*Theileria* type)	Ndumu Game Reserve	1967	Bigalke and Keep	7
	Borrelia theileri	Umfolozi Game Reserve	1968	Neitz	3
Blue Wildebeest *Connochaetes taurinus*	Apparently *Trypanosoma brucei*	Zululand	1895	Bruce	1
	Small piroplasms (*Theileria* species)	Hlabisa District & Umfolozi River	1929	Neitz	1
	Borrelia theileri	Umfolozi Game Res.	1968	Neitz	3
Bushbuck *Tragelaphus scriptus*	Apparently *Trypanosoma brucei*	Zululand	1895	Bruce	1
	Trypanosoma species	Zululand	1921	Mitchell	1
	Trypanosoma congolense	Empangeni District	1929	Neitz	1
	Trypanosoma vivax	Umfolozi River	1929	Neitz	1
	Small piroplasms (*Th. tragelaphi*)	Empangeni and Hlabisa Districts and Umfolozi River	1929	Neitz	1

	Koch bodies in spleen	Hlabisa District	1929	Neitz	1
	Babesia species	Hluhluwe Game Reserve	1968	Bigalke & Keep	7
Nyala Tragelaphus angasi	Small piroplasms (Theileria type)	Ndumu Game Reserve	1968	Bigalke & Keep	7
	Trypanosoma ingens – like	Ndumu Game Reserve	1968	Bigalke & Keep	7
Kudu Tragelaphus strepsiceros	Apparently Trypanosoma brucei	Zululand	1895 & 1903	Bruce	1
	Trypanosoma species	Zululand	1921	Mitchell	1
	Trypanosoma congolense	Hlabisa District – Zululand	1929	Neitz	1
	Trypanosoma vivax	Empangeni District & Lower Umfolozi	1929	Neitz	2
	Small piroplasms (Resemble Theileria mutans)	Umfolozi River	1929	Neitz	1
Buffalo Syncerus caffer	Apparently Trypanosoma brucei	Zululand	1895	Bruce	1
	Theileria mutans (Gonderia mutans)		1940		
		Zululand	1956	Neitz	4
	Theileria lawrencei (Gonderia lawrencei)	Zululand	1955	Neitz, Cantami and Kluge	5
			1955	Neitz	6
			1956	Neitz	4
Eland Taurotragus oryx	Small piroplasms of Cytauxzoon taurotragi	* Drakensberg Mountains, Natal	1968	Bigalke & Keep	7

* Not situated in Zululand, but within the Natal Parks Board Reserves.

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