Morphology of Rhinoceros Platelets

L. DU PLESSIS,[1]* A.J. BOTHA,[2] AND K. STEVENS[1]
[1]*Department of Haematology, Institute for Pathology, University of Pretoria, Pretoria 0001, South Africa*
[2]*Unit for Electron Microscopy, University of Pretoria, Pretoria 0001, South Africa*

ABSTRACT Basic hematological information about rhinoceros species is limited. In this study, we have determined the platelet counts and described the platelet morphology of free-living white (*Ceratotherium simum*) and black (*Diceros bicornis*) rhinoceros using light and electron microscopy. The platelet counts of the two species were similar, but there were significant differences in platelet size as well as morphology between the species. The presence of large, nondiscoid platelet forms, resembling proplatelet or stress platelet forms, were observed in both, although not as often in the white rhinoceros as in the black rhinoceros. J. Morphol. 239:245–253, 1999. © 1999 Wiley-Liss, Inc.

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For a number of reasons, little information regarding the blood picture of the rhinoceros in the wild is available. Most hematological reports on the black rhinoceros, *Diceros bicornis,* refer mainly to hemolytic anemia, the major cause of death among captive black rhinoceroses (Chaplin et al., '86; Paul et al., '88; Jain, '93). Interest in their hemoglobin has revealed a β-globin chain hemoglobin polymorphism which does not appear to be involved in the hemolytic anemia (Fairbanks and Miller, '90). Reports on the white rhinoceros, *Ceratotherium simum,* concern mainly the chemical parameters of blood (Van Heerden et al., '85). However, with the increasing interest in wildlife, be it conservation or game farming, the need for basic hematological information has been emphasized. This study provides baseline information on the platelet count and morphology of both white and black rhinoceros species living in the Kruger National Park, South Africa.

MATERIALS AND METHODS

Blood was collected from six white rhinoceroses (*Ceratotherium simum*) and seven black rhinoceroses (*Diceros bicornis*) in the Kruger National Park. All the animals were captured for translocation. The animals were darted from a helicopter and immobilized with a cocktail consisting of 4 mg M99 (etorphine hydrochloride), 50 mg Stresnil (azaperone), and 7000 U hyalase.

Blood sampling

The blood was collected approximately 30 min after the animals were darted. Blood was drawn from each animal into commercially available vacutainer tubes. The first tube of blood collected from each animal contained EDTA as anticoagulant and was used for the full blood count and blood smears. The platelet counts were done as part of the full blood counts on an electronic blood cell counter (Coulter Counter Model T890, Coulter Electronics; Miami, FL). Another two tubes of blood, containing acid citrate dextrose (ACD) as the anticoagulant, were drawn from each animal and used for the ultrastructural studies.

Microscopy

Blood smears were stained with Wright's stain (Williams, '90) and examined by light microscopy for general cell morphology. Periodic acid–Schiff (PAS) procedures (Catovsky, '91) were also carried out on blood smears to test for the presence of glycogen in the platelets.

Blood samples for electron-microscopic studies were processed as soon as possible after the blood was drawn. The black rhinoceros samples were processed 30–45 min after blood sampling, but, due to logistic prob-

*Correspondence to: L. Du Plessis, Department of Haematology, Institute for Pathology, University of Pretoria, PO Box 2034, Pretoria 0001, South Africa. E-mail: ldupless@medic.up.ac.za

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lems in the Park, the white rhinoceros samples were processed 4 h after collection. Platelets were fixed in buffered glutaraldehyde and processed as described previously by Du Plessis et al. ('96) for both scanning (SEM) and transmission electron microscopy (TEM). The samples for SEM were coated with gold in a sputter coater (3 times 60 sec) and viewed in a JEOL 5800 (JEOL Electron Optics Instrumentation; Tokyo, Japan) scanning electron microscope. Thin sections of the platelets were stained with uranyl acetate and lead citrate (Reynolds, '63) and viewed in a Philips 301 (Philips Electron Optical Division; Eindhoven, The Netherlands) transmission electron microscope.

Image analysis

Morphometrical analyses were done on TEM micrographs of the platelets taken at ×7500 magnification. At least 100 sections of different platelets were measured for each species. Only platelets in which the microtubules could be seen as distinct circles at both ends of the platelet sections were used for the measurements (Du Plessis et al., '97). The different measured parameters included area (square micrometers), external perimeter/circumference (micrometers), minimum and maximum projections (micrometers), width (micrometers), and the aspect ratio. The width is defined as the particle projection perpendicular to the maximum projection and may vary considerably from the minimum projection, while the aspect ratio is defined as the maximum projection divided by the width. The closer this value approaches the value of one, the more spherical the image. The work was done on a Tracor Northern Image Analysis System (Noran Instruments; Madison, WI, USA). The average number of organelles present in a section was also determined.

Statistical analysis of the parameters assessed was performed using a computer package (Statgraphics, version 5.0) as well as descriptive statistics for each parameter (i.e., mean and standard deviation). One-way analysis of variance, as well as multiple range analysis with a 95% Bonferroni correction, was carried out to determine possible differences between the two species.

RESULTS

Platelet counts

The platelet counts of the white rhinoceros varied from 370–513 × 10^9/L (mean 444.6 × 10^9/L, s.d. 51.8) and that of the black rhinoceros from 221–532 × 10^9/L (mean 377.2 × 10^9/L, s.d. 100.9). The figures for the black rhinoceros included that of a cow which calved 3 weeks after capture and had the highest platelet count of all the animals in this group (532 × 10^9/L). The platelet counts of the other black rhinoceroses varied from 221–426 × 10^9/L, with an average of 351 × 10^9/L.

Light microscopy

The platelets of the white rhinoceros generally appeared as round cells (Fig. 1a), while the black rhinoceros displayed platelets of different shapes and sizes (Fig. 1b). The ma-

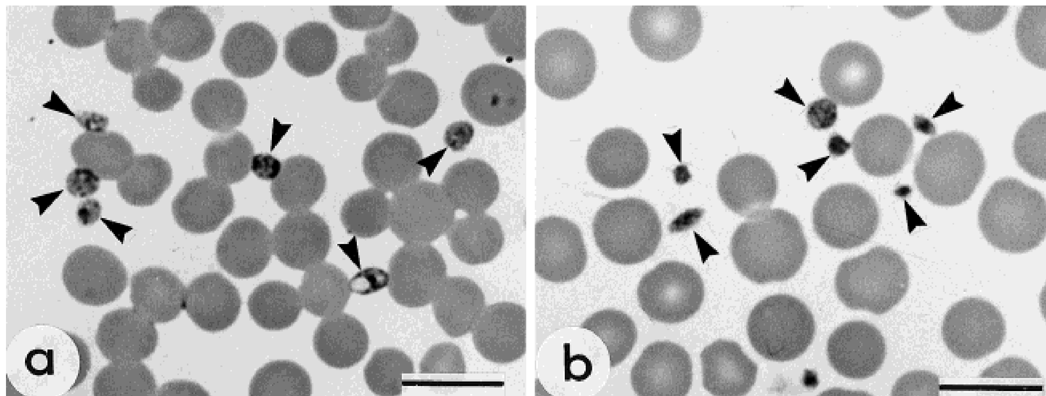

Fig. 1. Light micrographs of (**a**) white and (**b**) black rhinoceros blood smears. Arrowheads indicate platelets. Note the vacuole in the bottom right white rhinoceros platelet in panel a, typical of platelets exposed to EDTA after some time has elapsed. Bar = 10 µm.

jority of black rhinoceros platelets were small, oval-shaped cells, but occasionally large, round cells were observed. With Wright's stain, the cytoplasm in both species stained a light pink color with purple-staining granules visible. Few signs of activation (pseudopodia) or spreading of the cytoplasm on the glass slides were observed, although an occasional vacuole was noted in the white rhinoceros platelets (Fig. 1a). Both species stained positive for glycogen with the PAS technique.

Electron microscopy

Scanning electron microscopy

The white rhinoceros platelets were mainly discoid on SEM, but some were round and appeared flattened in side view (Fig. 2a). Black rhinoceros platelets appeared primarily oval-shaped; however, some marked variations were noticeable (Fig. 3a–c). Platelets varied from the typical oval discs to elongated cells, often extending completely across the microscopic field. These large

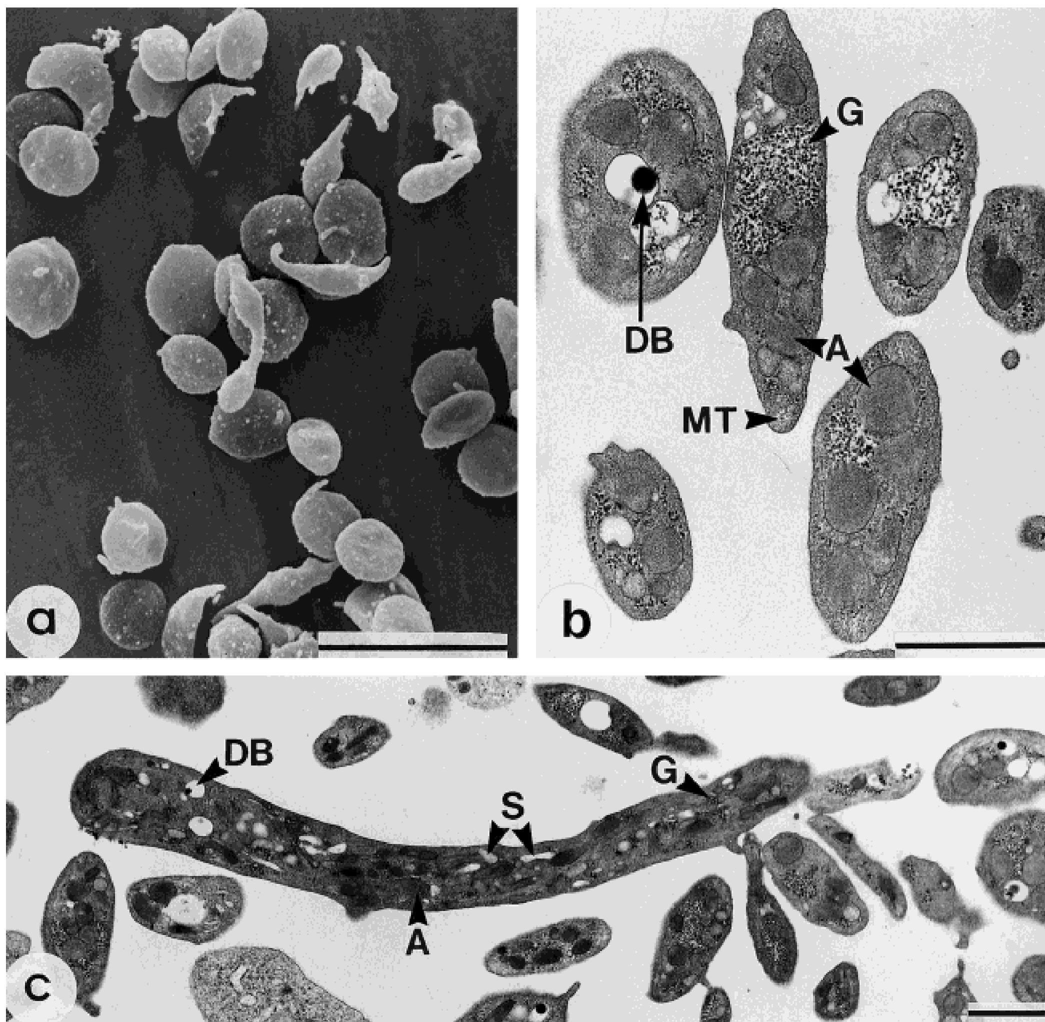

Fig. 2. Ultrastructure of white rhinoceros *Ceratotherium simum* platelets. **a:** Platelets displaying typical shapes of the platelets as seen with the scanning electron microscope. Bar = 5 μm. **b,c:** Cross-sections of platelets. Note the presence of the surface connecting canalicular system in the large platelet in panel c. A, α granules; DB, dense bodies; G, glycogen; MT, microtubules; S, surface connecting canalicular system. Bars = 1 μm.

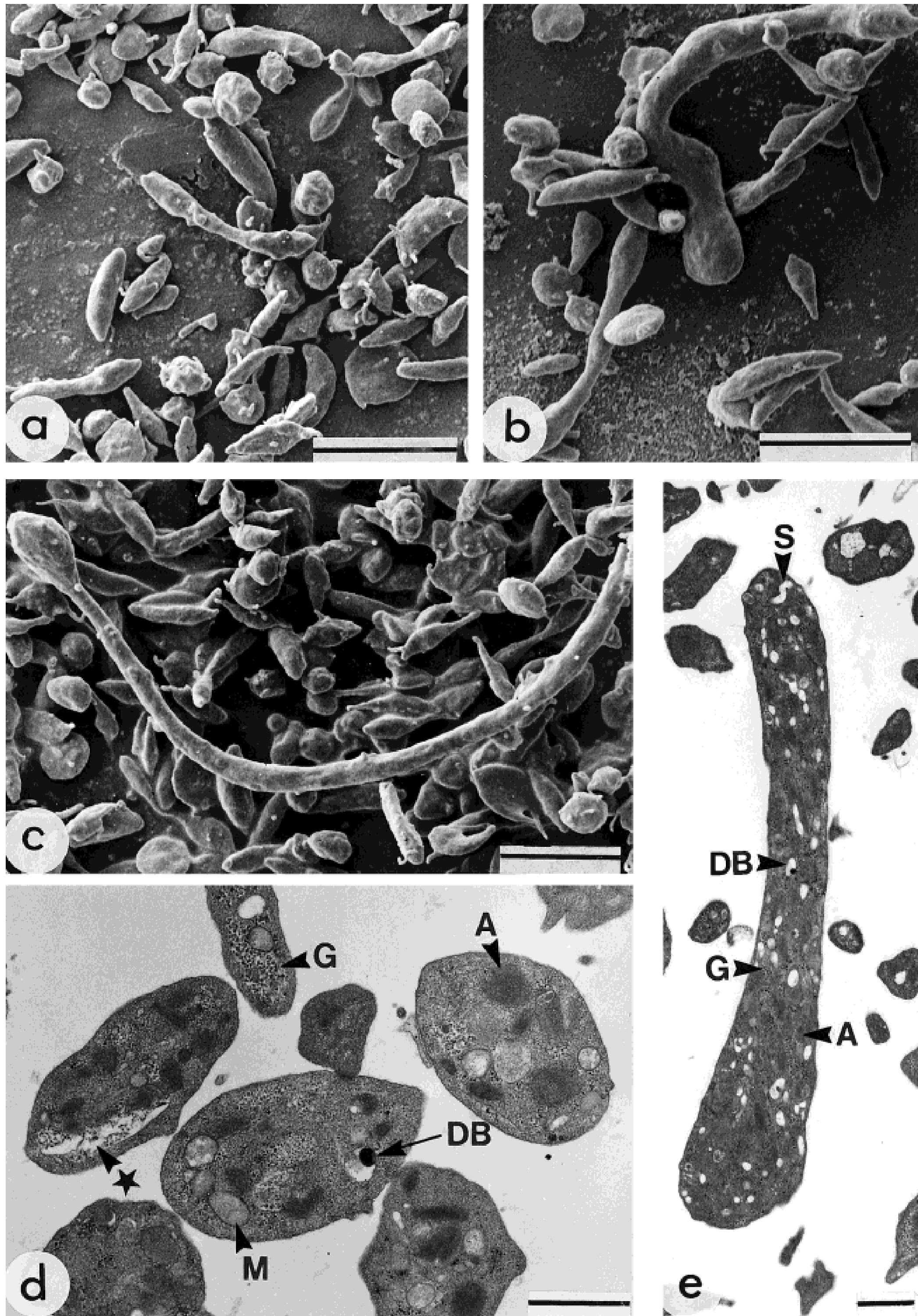

Fig. 3. Electron micrographs of black rhinoceros *Diceros bicornis* platelets. Note the unusual-shaped platelets seen on the SEM micrographs (**a–c**). **d,e:** Display cross-sections of the platelets as seen on TEM. Note the paucity in the cytoplasm (*) in the glycogen region in panel d. Panel e displays a section through one of the larger platelets as seen on the SEM. A, α granules; DB, dense bodies; G, glycogen; M, mitochondria; S, surface connecting canalicular system. a–c: Bars = 5 μm. d,e: Bars = 1 μm.

platelets often had a dumbbell appearance, while the oval-shaped cells appeared lentiform in side view and as flat discs from the top. A few elongated platelets were also noted in the SEM preparations of the white rhinoceros, although not nearly as many as in the case of the black rhinoceros. Pseudopodia were rarely seen in either species.

Transmission electron microscopy

In both species, the different shapes noted with SEM were not as evident with TEM, in which the platelets generally, depending on the plane of section, appeared round or oval-shaped (Figs. 2b,c, 3d,e) with few pseudopodia. In some microscopic fields, platelets of variable size and form were found, possibly representing the large structures seen on SEM. It is not surprising that these large cells were only rarely seen, as the chance of a section through the longitudinal plane of such large platelets is small.

The cytoplasmic ground substance of both species had a granular appearance of moderate electron density. Mitochondria and dense bodies were scattered throughout the cytoplasm, and the marginal band of microtubules was present (Figs. 2b, 3d; see Table 1). Prominent glycogen granules and deposits were present. There was often a paucity of the cytoplasmic ground substance in these regions (Fig. 3d). The most noticeable difference between the organelles of the two species was the appearance of the α granules. In the white rhinoceros, these granules were easily identified as structures of moderate electron density surrounded by a single membrane (Fig. 2b). The granules, depending on the plane of section, displayed a variety of shapes and sizes. The α granules of the black rhinoceros were difficult to identify based upon their appearance alone. Granules varied in electron density, often with no clearly visible surrounding membrane (Fig. 3d). The differences in the α-granule morphology of the two rhinoceros species can also clearly be seen in Figures 2c and 3e. The characteristic surface connecting canalicular system of platelets was present but poorly developed in both species. In the few large platelets observed, the canalicular system was more obvious (Figs. 2c, 3e).

TABLE 1. Mean number of cytoplasmic organelles ± standard deviation present per platelet section[1]

Organelle	White rhinoceros	Black rhinoceros
α granules	4.00 ± 2.04	4.00 ± 1.92
Dense bodies	0.48 ± 0.68	1.19 ± 0.91
Mitochondria	0.73 ± 0.75	0.97 ± 0.76
Microtubules	15.58 ± 4.85	13.08 ± 4.37
% sections displaying glycogen	92.5 ± 26.5	94.5 ± 22.5

[1]Counts made on at least 100 platelet sections of each species.

TABLE 2. Mean dimensional values ± standard deviation of the platelet sections[1]

Parameter	White rhinoceros	Black rhinoceros
Area (μm^2)	1.180 ± 0.295	2.077 ± 0.510
External perimeter (μm)	4.829 ± 0.671	5.911 ± 0.774
Minimum projection (μm)	0.777 ± 0.144	1.218 ± 0.214
Maximum projection (μm)	2.077 ± 0.314	2.378 ± 0.377
Width (μm)	0.773 ± 0.145	1.225 ± 0.220
Aspect ratio	2.767 ± 0.622	2.002 ± 0.492

[1]Counts made on at least 100 platelet sections of each species.

Image analysis

The different measurements of the animals are given in Table 2. All these parameters displayed normal distribution curves, with the white rhinoceros sections smaller than the corresponding values of the black rhinoceros (Fig. 4). The only exception was the aspect ratio: the black rhinoceros had the smaller value of the two. However, the aspect ratio is an indication of the cell shape and not a direct measurement.

DISCUSSION

As with all hematological studies, the influence of any external factors—for example, the stress experienced during capture and the drugs used for the immobilization of the animals—should be considered when drawing any conclusions. Both the black and white rhinoceroses were subjected to the same degree of excitement, method of capture, drugs administered, and blood sampling methods. It is known that excitement and exercise can cause a temporary increase in platelet counts, the former because of platelet release by the spleen and the latter due to platelet release by the lungs (nonsplenic pool) (Jain, '93). The drugs used for the immobilization of the animals can also influence the blood pictures of animals. However, the influence of all these factors is eliminated after a period of approximately 30–40 min, when the platelet counts return to normal (Cross et al., 88). Since that was the minimum time elapsed before any of the blood samples were

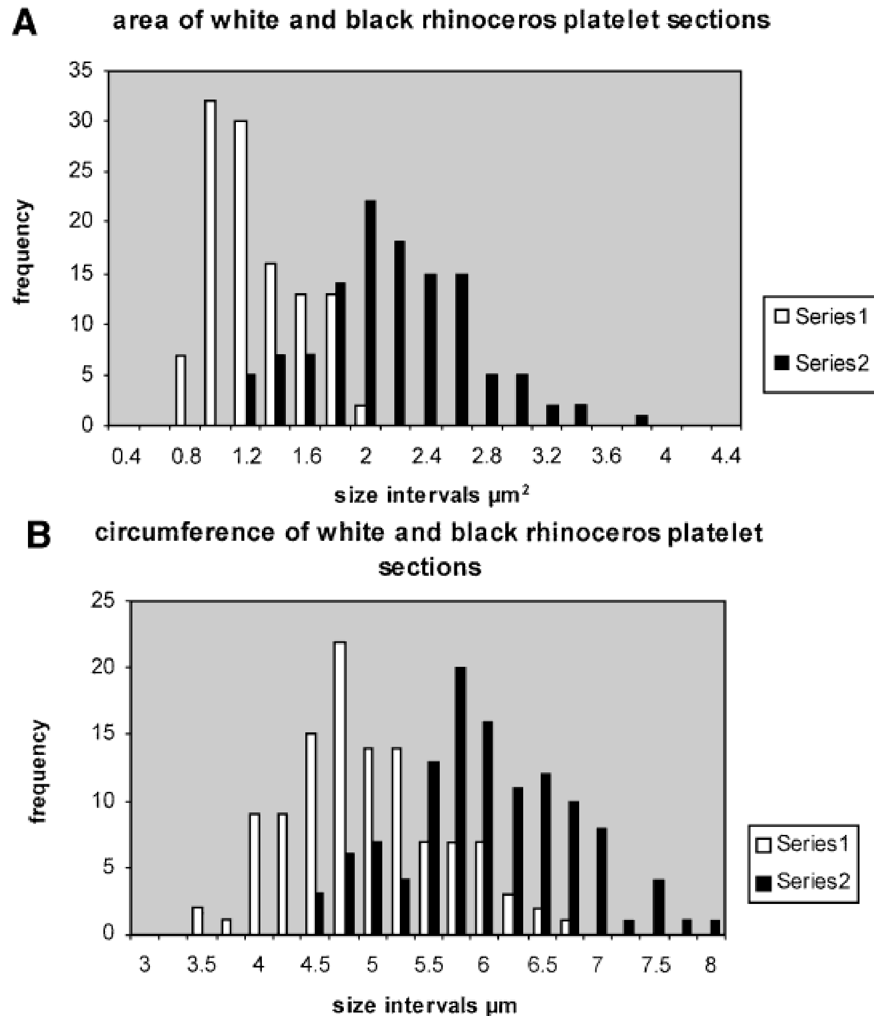

Fig. 4. Distribution curves of the different parameters measured to determine the platelet dimensions of the two rhinoceros species. Series 1 represents the white rhinoceros and Series 2 the black rhinoceros.

drawn, it is thought that these factors did not have major influences on the present results. The time elapsed between drawing the blood and processing the specimens for electron microscopy may have caused activation of the platelets. It is remarkable, therefore, that the platelets of the white rhinoceros showed only very few pseudopodia.

The platelet counts of the black rhinoceros revealed in this study (221–532 $\times$ 10^9/L) fall within the range given by Hawkey ('77) (154–634 $\times$ 10^9/L). It is known that the platelet counts in cows vary during the estrous cycle (Jain, '93) and that the platelet counts tend to fall during normal human pregnancies (Eastham and Slade, '92). However, the platelet count of the rhinoceros that calved 3 weeks after capture was considerably higher than that of her peers, which would suggest that the principle reported by Eastham and Slade ('92) for humans may not be applicable to the black rhinoceros. If the platelet count of the pregnant cow in this study is excluded, the range for the black rhinoceros will be 221–426 $\times$ 10^9/L, essentially within the 126–410 $\times$ 10^9/L (mean 223.5 and s.d. 67.7) range given by Paul et al. ('88), who used the same immobilizing drugs as in the

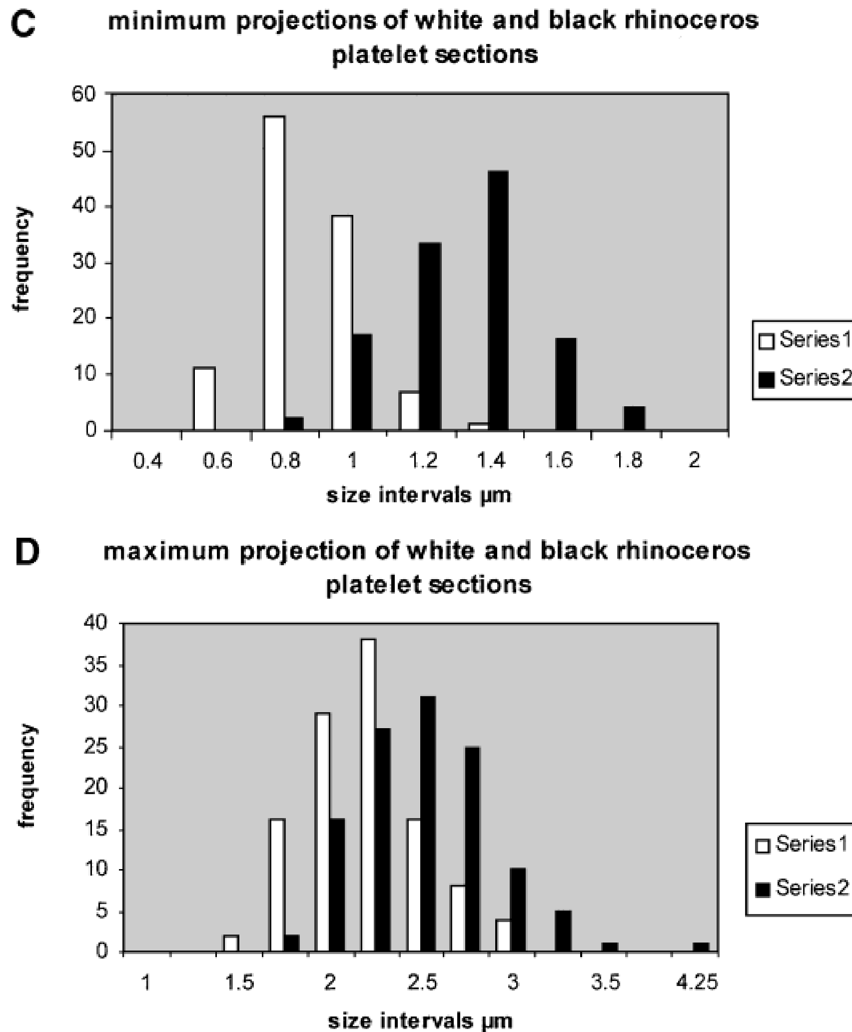

Fig. 4. (Continued)

present study. The few large platelets that were observed at the ultrastructural level were probably not counted as platelets by the cell counter and were too few to change the platelet counts significantly. Although the sample size was relatively small, the platelet counts of both the black and white rhinoceroses in this study displayed a normal distribution, with no statistically significant differences between the two species.

Mainly round to oval-shaped cells were seen by light microscopy. This is not surprising, as the blood was collected in EDTA, which is known to cause sphering of the platelets. There was no sign of the platelets spreading on the glass slides, a phenomenon often seen on blood smears, indicating activation of these cells (Ham et al., 79).

The typical morphological description of platelets is anuclear cells with granules scattered throughout the cytoplasm. Platelet size is notoriously variable. Under normal conditions, most platelet populations display a normal distribution curve with small as well as large platelets present (Scurfield and Radley, '81; Rφger et al., '92). However, Scurfield and Radley ('81) reported that there are certain conditions when larger platelets occur in the circulation (e.g., recovery from drug-induced thrombocytopenia and after

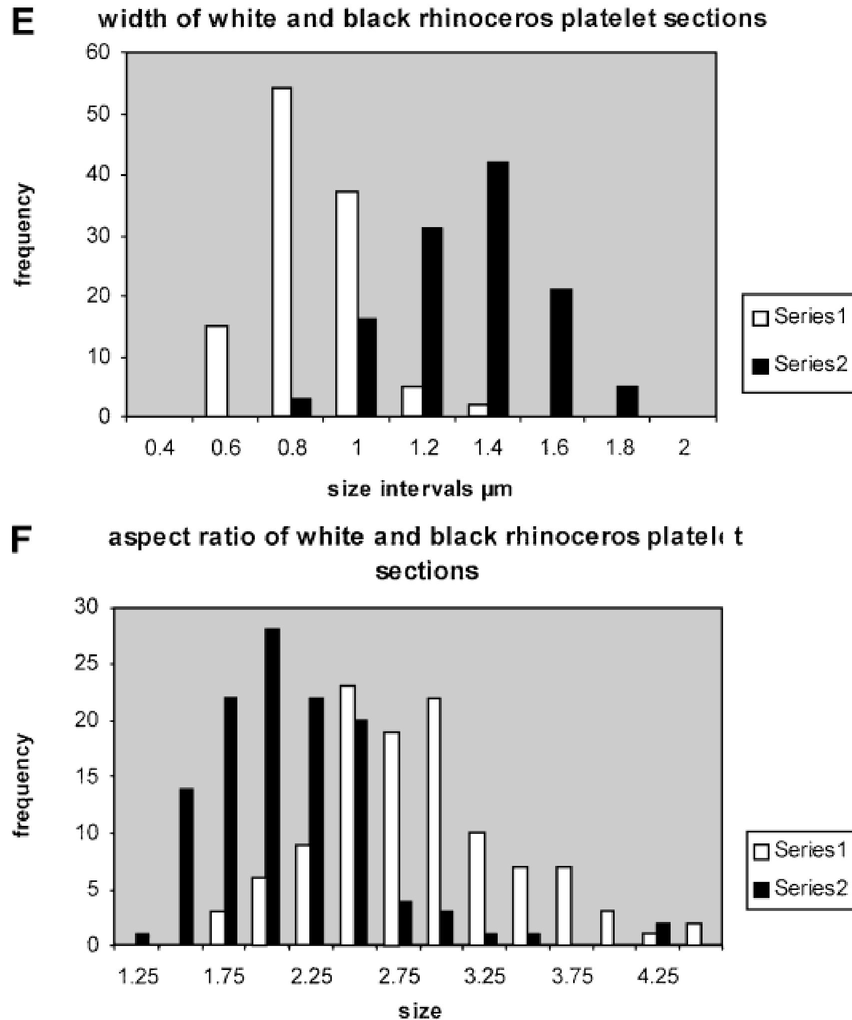

Fig. 4. (Continued)

acute hemorrhage). Frojmovic and Milton ('82) are of the opinion that an increase in large, circulating platelets may reflect increased platelet release from the splenic pool and bears no relation to the platelet production rate or megakaryocyte maturation. In addition, platelets produced during thrombocytopenia show an increase in the mean platelet volume but do not exceed the normal distribution curve (Jain, '93). According to the few platelet count values available for rhinoceros species (Hawkey, '77; Paul et al., '88), the animals in the present study displayed neither thrombocytosis nor thrombocytopenia to explain the presence of megathrombocytes. During periods of stress, however, platelet production is accelerated, releasing giant platelets called stress platelets by Tong et al. ('87) into the circulation. Such platelets should not be confused with proplatelets, the platelets formed by the fragmentation of megakaryocyte pseudopodia (Handagama et al., '87; Jain, '93). Whether the unusually large platelets seen in especially the black rhinoceros are in fact stress platelets is doubtful, for they occurred too soon after the period of stress that the animals undoubtedly experienced with the capture. The possibility that they are in fact proplatelets seems more likely. The antico-

agulant used when the blood is drawn plays a large part in the observation of proplatelets, as it is known that EDTA causes the proplatelets to sphere (Tong et al., '87; Hattori et al., '92). This explains why no proplatelets were observed on the blood smears. The sphering of the platelets over a period of time (4 h), even though the blood was drawn into ACD, possibly also explains why there were fewer proplatelets seen in the white rhinoceros samples.

The cytoplasmic organelles of the two rhinoceros species displayed the typical platelet ultrastructure and had a similar appearance except for the unusual appearance of the black rhinoceros α granules. The relevance of this finding is unclear. It cannot be attributed to fixation damage, as all the other organelles and membranes remained intact. It can therefore be concluded that the membrane of this particular organelle is variable.

For the comparative morphometric studies, a reliable marker was used to ensure that all the platelet sections measured were true cross-sections of the platelets (Du Plessis et al., '96). This marker, the microtubules, was used to determine whether cross-sections of platelets were true since a rotation through as little as 10% will blur these structures (De Wit et al., '87). The different parameters measured showed that the black rhinoceros platelets were significantly bigger than those of the white rhinoceros ($P < 0.001$).

In conclusion, it can be stated that, although the platelet counts of the species are similar, there were significant statistical differences in the morphology and size of the two rhinoceros species studied. This is surprising, as comparisons between other groups of closely related animals, such as cattle and the buffalo *Syncerus caffer*, showed no differences in platelet size and morphology (Du Plessis et al., '96). The reason for the morphological differences noted in the present study is not clear, but it appears that platelet morphology may vary in some closely related animals.

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