

should be saved and stored at -75 degrees C for future reference.

Possible future avenues of research identified at the meeting include: (I) repetition of many of the previous tests on additional black rhinoceroses and also on white and Indian rhinoceroses, (ii) further evaluation of the immunological status of these animals in addition to the continued use of the Coombs reagent, (iii) further evaluation of the stability of the black rhinoceros RBC and its hemoglobin, (iv) evaluation of the iron metabolism of this species and attempts to identify a possible chronic stage of the anemia process, and (v) an overall evaluation of the nutritional status of this species in captivity. One emphasis of the latter study should be the determination of vitamin E and selenium levels in both captive and wild populations. The importance of a multi-faceted diagnostic approach was emphasized in a species in which so little is known. In man, with a much broader data base available, the cause of less than 50% of nonspherocytic hemolytic anemia is identified.

Since the syndrome has not been reported in white and Indian rhinoceroses, results from these species may help to establish a comparative data base for the black rhinoceros. A blood collection protocol for diagnostic and genetic studies in black, white and Indian rhinoceroses has been distributed to North American and European institutions holding these species (copies are available on request from the senior author).

Finding the specific etiology for the hemolytic crisis so frequent in the captive black rhinoceros population rests on further research in the areas enumerated above and perhaps others yet to be identified. The authors welcome suggestions of additional tests and approaches to this perplexing problem in the successful maintenance of this species in captivity.

Authors' notes

- (i) Collecting large volumes of blood from the black rhinoceros can be difficult if the ear vein is used as the primary venipuncture site. Animals at St. Louis have been routinely bled from a large vein that passes over the medial carpus and ante-brachium. Though it is not always visible under the thick skin, a tourniquet applied proximally on the leg allows it to be palpated and cannulated. Up to one litre of blood has been collected rapidly from this site.
- (ii) Since the Cincinnati meeting, an additional three adult (14.16 and 24 years of age) black rhinoceroses have died of hemolytic anemia in North America. The deaths occurred from November 2 to December 17, 1986. Preliminary laboratory data from these cases parallels that from previous hemolytic events. Two of the cases were tested with the autoimmune reagents described in this paper, and both were negative. Further tests are pending. No common factors could be identified to link the cases.

HAEMATOLOGICAL STUDIES OF BLACK RHINOS IN ZIMBABWE

Summary of presentation by Raoul du Toit (IUCN African Elephant and Rhino Specialist Group),

co-authored by Beverley Paul (University of Zimbabwe)

Various haematological studies were carried out with blood samples from 31 black rhinos that were translocated from the Zambezi Valley, Zimbabwe, in mid-1986.

In a field laboratory, within 3 hours of the collection of each

sample, the following procedures were carried out: haematocrit, white blood count, red blood count measurement of haemoglobin, plasma protein, erythrocyte sedimentation rate, and osmotic fragility; preparation of slides for differential cell counts, reticulocyte counts and parasite screening. Additional blood samples from each animal were transported to Harare on wet ice, where standard blood analyses were performed on a Coulter counter (within at most 48 hours, and generally within 24 hours, of collection) in Harare, additional tests were carried out to investigate haemoglobin stability: isopropanol precipitation, heat test acidified glycerol lysis time test, and staining for Heinz bodies with methyl violet. Human blood specimens stored for similar periods were used as controls. Haemoglobin electrophoresis was performed on cellulose acetate. Glucose-6-phosphate-dehydrogenase was assayed using a commercial kit (Sigma), which had been supplied by St. Louis Zoo.

The findings of these investigations are to be published (*Journal of Zoology*, in press). Consistent results were obtained from the standard haematological tests, and measurements of haemoglobin, haematocrit, and cell count conform closely with those obtained by veterinarians at Whipanade Park, using blood from a few captive black rhinos. Thus it is felt that these data constitute reliable baseline information on the haematology of the species. Reticulocytes, not generally seen in rhino blood smeared occurred in some of the samples. The osmotic fragility of the red cells was somewhat greater than that of human red cells with 50% lysis occurring at a salt concentration of about 4.9 g/l. A significant observation was that all samples showed rapid precipitation of haemoglobin when incubated with isopropanol. Heinz bodies could be demonstrated by methyl violet staining in up to 10% of fresh red cells. Very high levels of G-6-P-D activity were found in the red cells.

These results, indicating an inherent tendency towards collapse of haemoglobin under oxidant stress, are obviously highly relevant to the problem of intravascular haemolysis. It seems unlikely that there is any single agent responsible for triggering haemolysis episodes; these are probably the end result of a variety of oxidant stresses.

There are indications that some die-offs of black rhinos in the wild could be related to haemolytic anaemia (e.g. about 30 rhinos died in Tsavo National Park in 1960-61, due to what was tentatively described as "nutritional anaemia"). With wild animals, it would be worth investigating if parasitaemia aggravated by inadequate nutrition, capture stress and other debilitating factors, is associated with haemolytic anaemia—abnormal haemoglobin and red cell enzyme systems may have developed in rhino as an evolutionary response to parasitaemia (as with sickle cell anaemia and possibly G-6-P D deficiency in humans), but under extra physiological stresses the balance could tip towards excessive haemolysis. The Zambezi rhinos, from which blood samples were taken, were translocated to another reserve in Zimbabwe, where at least 20% of them died some weeks after translocation. In the pathological examinations that were carried out on a couple of sick and dead rhinos in this group, an unidentified piroplasm parasite was found in blood smears to a greater extent than in blood smears taken at the time that the animals were first captured, and large amounts of haemosiderin were found in spleen and liver tissue. This indicates a possibility of the mortality being due to stress-induced parasitaemia and a degree of haemolytic anaemia (although it has also been suggested that the deaths were due to the use of the drug ivermectin, for controlling skin and gut parasites). Further

investigation of rhino blood parasites and haematology is intended in the hope of clarifying these health problems before more animals are lost in translocation operations, which will become an increasingly important part of rhino conservation in Africa.

POPULATION AND VETERINARY STATUS OF BLACK RHINOS IN THE UNITED KINGDOM

*Summary of presentation by Richard Kock
(Zoological Society of London)*

Introduction

The black rhino population in the British isles numbers 12 at present: five wild-caught and seven captive-bred individuals. The latter derive from two genetic lines. One pair came direct from East Africa to the United Kingdom in 1950. The other genetic line is derived from a pair at Hannover which were wild-caught in 1955 and 1957, and two Whipsnade animals and one London animal which were also wild-caught. Fifteen animals in total were caught from the wild. Twenty-four individuals have been born in captivity since 1958. From 1969-1986, 24 deaths occurred including both captive-bred and wild-caught individuals. The major reason for this poor record includes a high mortality in both sexually immature and mature individuals. A relatively short reproductive period over the life span and a long calving interval are also problematic. Deaths in this species, when compared to the white rhinoceros in captivity, are premature.

Of the 24 deaths recorded, 21 died between October and May, there was one still-birth in July and two deaths between May and October, but both of these had been ill during the previous winter. In general the clinical syndromes recorded are associated with winter management, i.e. indoor housing, fluctuating climatic conditions, dry fodder nutrition and inactivity. There appears to be no sex or age susceptibility to illness.

Nine collections have exhibited the black rhino, including 5 currently.

From 1969-1986, 20 deaths occurred in collections as follows:

Chester (5), Marwell (2), Bristol (6), Dublin (3), Paignton (1), Manchester (2), Whipsnade (2), London (1) and Howletts (2). The most "successful" records are from London/Whipsnade and Howletts/Port Lympne. Only three post-natal deaths (two juveniles and one adult which was ill on arrival from Bristol) occurred in these collections. Five offspring from these two zoos are at present alive in Great Britain. The two animals at Howletts are in their sixteenth year of captivity and include a captive born animal. The animals at London/Whipsnade are over 20 years old. A major difference between these two collections and the rest is in feeding management, with more browse and green foods being provided during the summer due to a rural location.

Clinical histories 1969-1986

From the case records available only a few individuals died without clinical signs prior to death. The clinical signs have included nasal discharges; with muco-purulent material, serosanguinous fluid and frequently whole blood clots. Skin ulcers, diffuse and punctate in appearance and with a remarkably regular patterning over the skin surface, were common. A few cases presented with diarrhoea, laminitis or haemoglobinuria. During periods of illness animals were in general lethargic and on occasions inappetent. Many of the

animals have shown respiratory distress in the last two or three days of illness particularly where recumbency was evident.

Due to the difficulty of clinical examination without anaesthesia in this species clinicians rarely performed extensive diagnostics.

Haematology

The information available is primarily from the Zoological Society's collections (Table 13). It appears that the red cell numbers, haemoglobin concentrations, packed cell volumes and mean cell volumes are extremely variable in the individuals examined when compared with the white rhino. There appears to be no correlation between the time of year and the red cell/mean cell volume values or the presence of Heinz bodies. The Heinz body findings are unlikely to be significant as they are a common occurrence in white rhinos. High mean cell volumes have been recorded in several animals and this was suggested to be due to a vitamin B or folate deficiency. It may have been an indication of a response to red cell loss by haemolysis. In general, the black rhinoceros has lower red cell haemoglobin values, packed cell volumes and higher mean cell volumes than the white rhinoceros. The only comparative data to hand are from an individual case in another collection which showed dramatically lower red cell haemoglobin and packed cell volume values to those in the collection. It is worth noting here that none of the deaths in the Society's collections have

Table 13. Haematological data from African rhinos, obtained by the Zoological Society of London.

	Black rhino (n = 7)			White rhino (n = 16)		
	Lowest	Mean	Highest	Lowest	Mean	Highest
Red cell count (x 10 ¹² /l)	2.69	4.80	6.90	5.48	6.82	8.16
White cell count (x 10 ⁹ /l)	3.0	8.5	14.0	4.7	8.6	12.5
Haemoglobin (g/l)	9.76	14.70	19.64	13.94	17.03	20.13
Packed cell volume (%)	30.7	41.6	52.6	37.9	46.4	54.9
Mean cell volume (fl)	76.1	86.8	114.2	67.3	68.0	69.1
Mean cell Haemoglobin (pg)	28.5	30.6	36.3	24.7	25.0	25.4
Erythrocyte Sedimentation rate (mm/hr)	2.00	22.50	54.00	5.00	16.98	33.00
Platelets (x 10 ⁹ /l)	14.3	314.2	614.1	2.28	5.34	8.41
Reticulocytes						
Neutrophils (x 10 ⁹ /l)	0	0	0	0	0	0
Lymphocytes (x 10 ⁹ /l)	2.38	5.09	7.79	2.28	5.43	8.41
Monocytes (x 10 ⁹ /l)	0.00	0.24	0.95	0.00	0.32	0.83
Eosinophils (x 10 ⁹ /l)	0.00	0.22	0.72	0.16	0.41	1.00

been during a haemolytic crisis, as has occurred in other collections in this country and abroad.

Biochemistry

The biochemical parameters did not show any consistent abnormality except for very low plasma vitamin E levels of less than 0.1 mu/ml. Low values are seen frequently in white rhinos and elephants so this is difficult to interpret. Plasma vitamin A levels varied between 15-140 iu/litre. Very little