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Hemolytic anemia in black rhinoceroses has been noted with alarming frequency in recent years.[7] In several instances, death ensued, and an etiology of leptospirosis,[4] babesiosis,[2,6,9] and trypanosomiasis[6,9] has been proposed. In one instance, hemolytic anemia developed in a black rhinoceros being treated for tuberculosis.[5] In most, the etiology was unclear.

Clinical signs generally precede death by 2 hr to 2 days. Erythrocyte PCV may range from 4-15%, and erythrocyte and hemoglobin concentrations are correspondingly low. Hemosiderin may be deposited in the gut, lungs, and liver, and there may be evidence of centrilobular hepatic necrosis. Studies of erythrocyte osmotic fragility, hemoglobin electrophoresis, erythrocyte enzymes, glycolytic intermediates and several other measures have not distinguished between healthy and affected animals.[3,8]

Preliminary studies in the Michigan State University Comparative Animal Nutrition Laboratory established that plasma or serum alpha-tocopherol concentrations in 9 black rhinoceroses at 5 United States zoos were less than 1 ug/ml, and plasma alpha-tocopherol concentrations were immeasurable in 2 individuals that died in a hemolytic crisis. Since plasma tocopherol concentrations are related to dietary intake in other species, and since this vitamin is important as a free radical trapper in inhibiting phospholipid peroxidation and damage to cell membranes, it was suspected that these black rhinoceroses may have been deficient in vitamin E or their erythrocytes were unusually susceptible to peroxidation. Thus, this study was conducted to explore the stability of the black rhinoceros erythrocyte and to measure factors which may affect erythrocyte stability, such as plasma concentrations of alpha-tocopherol[1] and selenium.[11]

Heparinized blood samples were obtained from 10 black rhinoceroses in 7 zoos. Whole blood was shipped on ice (0°C) by air for overnight delivery to the Michigan State University Comparative Animal Nutrition Laboratory. Plasma was shipped on dry ice (-70°C) to the same destination. Within 24 hr of collection, samples of whole blood were exposed to a hydrogen peroxide hemolysis test, a time-dependent layering hemolysis test,[10] and an erythrocyte tonicity test.

Concentrations of hydrogen peroxide used included 0.01, 0.02, 0.04, 0.0625, 0.25, 1.25, and 2.5%. In contrast to studies of blood from swine, dogs, and humans, black rhinoceros erythrocytes were very resistant to peroxide-induced hemolysis. Instead of releasing hemoglobin into the supernate, the erythrocytes changed color and/or precipitated. At lower peroxide concentrations, the erythrocytes were a brick red, becoming progressively more brown with increasing peroxide concentrations. At no

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peroxide concentration was there the bright red color seen when the erythrocytes were hemolyzed in water.

The layering hemolysis test revealed little difference between animals.

Incubation of black rhinoceros erythrocytes with different concentrations of NaCl produced the results shown in Table 1. In three instances where an animal had been bled twice, about a year apart, some improvement in erythrocyte stability seemed apparent. During the year, attempts were made to improve dietary husbandry, but the details were not well documented. In a fourth instance where the same animal was bled twice, a month apart, duplication of the hemolysis values was excellent. Black rhinoceros no. 5 was bled during a hemolytic crisis, and the increased susceptibility of its erythrocytes to hypotonic NaCl solutions was readily apparent, particularly at NaCl concentrations of 0.5-0.8%. Based on differences between animals and assuming that these differences have predictive value for erythrocyte hemolysis in vivo, in vitro incubation of black rhinoceros erythrocytes at a NaCl concentration of 0.5% seems promising.

The analyses of plasma that were completed did not reveal a relationship between tocopherol or selenium concentrations and measures of erythrocyte stability. Alpha-tocopherol concentrations ranged from less than 0.1 to 0.4 ug/ml, and would be considered low in many other species. Plasma selenium concentrations ranged from 0.14-0.22 ug/ml and would be considered adequate.

While the stability in vitro of black rhinoceros erythrocytes to hypotonic NaCl solutions appears to differ between individuals, the etiology of in vivo hemolytic anemia remains unknown.

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Table 1. Hemolysis (%) of black rhino erythrocytes incubated in vitro with various concentrations of NaCl.

Rhino no.	Date	PCV (%)	NaCl (%) 0.8	0.7	0.6	0.5	0.4	0.3
1	5/87	42.8	0.5	2.5	11.4	66.8	91.7	--
2	5/87	31.8	0.6	3.0	12.6	71.9	91.8	--
2	4/88	39.9	0.6	1.8	6.3	34.8	93.8	99.0
3	5/87	39.4	0.6	1.6	7.4	42.3	92.4	--
3	6/88	36.7	0.6	0.5	2.5	16.7	77.0	97.6
4	5/87	29.0	1.2	2.3	7.9	50.6	94.0	--
5[a]	6/87	25.5	6.4	--	37.9	84.7	91.2	--
6	6/87	44.0	0.8	1.3	3.2	42.0	97.6	97.2
6	7/88	45.3	0.3	0.3	0.9	5.7	54.6	97.2
7	7/87	43.8	0.8	1.0	5.4	51.8	97.3	99.5
8	7/87	56.0	0.4	1.1	8.0	46.8	95.7	98.4
9	7/87	44.6	0.4	0.7	2.7	29.4	94.3	96.8
10	1/88	44.0	0.3	0.4	1.0	9.2	72.7	96.9
10	2/88	44.9	0.4	0.5	1.1	9.8	73.5	98.8

[a]Blood sample obtained during a hemolytic crisis.