ERYTHROCYTIC ATP DEFICIENCY AND ACATALASEMIA IN THE BLACK RHINOCEROS (Diceros bicornis) AND THEIR PATHOGENIC ROLES IN ACUTE EPISODIC HEMOLYSIS AND MUCOCUTANEOUS ULCERATIONS

Donald E. Paglia, M.D.
University of California Los Angeles, Los Angeles, CA, 90024-1732, USA

R. Eric Miller, D.V.M.*
St. Louis Zoological Park, St. Louis, Missouri 63110-1396, USA

Sudden episodes of severe hemolytic anemia are the leading cause of death among captive black rhinoceroses (accounting for 25 deaths in 33 affected individuals) and mild to severe oral and/or skin ulcerations also occur commonly in this species.[9] In regard to hemolytic anemia, studies to detect potential hemoglobinopathies,[3] autoimmune phenomena,[2] or exposures to specific toxins have been negative.[6] Although leptospirosis appeared to be involved in a high percentage of the cases,[4,7] it has not been identified in all hemolytic crises.[4,7] To date, studies of the mucocutaneous ulcerations have not determined a definitive etiology.[9]

Hemolytic syndromes in humans are commonly caused by enzyme defects which impair metabolic pathways required to neutralize oxidants in red blood cells (RBCs). The most common defect is hereditary deficiency of glucose-6-phosphatate dehydrogenase (G-6-PD), which predisposes affected individuals to hemolysis secondary to oxidant stress. Earlier studies at the UCLA Hematology Research Laboratory established that black rhinoceros erythrocytes possess RBC enzyme profiles which differ radically from all other known species. The black rhinoceros RBCs contain only 2%-5% of human RBC ATP levels and are relatively deficient in many RBC enzymes. Additionally, even though rhinoceros RBCs had abundant G-6-PD activity, they were found to be very susceptible to oxidants *in vitro*.

More recent studies indicate that low ATP concentrations in the rhinoceros erythrocyte may be rate-limiting in the anti-oxidant activity of the hexomonophosphate (HMP) shunt. Glutathione instability of rhinoceros erythrocytes has been corrected by incubation of the cells with adenosine and glucose which raised intracellular ATP levels to human equivalents.

In the small group of black rhinoceroses tested to date, adenosine kinase activity segregated into two groups, one with about half the activity of the other. This is consistent with a heterozygous deficiency that could increase susceptibility to oxidant-induced hemolysis, since this is the enzyme principally responsible for maintenance of ATP levels, already precariously low in rhinoceros RBCs. Glucose catabolism is essential for ATP salvage via the adenosine kinase pathway, thus it is crucial to avoid and correct conditions that inhibit glycolysis, such as acidosis or hypophosphatemia. Currently, this provides the metabolic rationale for aggressive correction of these two conditions which may occur as complications of other disorders.

In humans, there is strong evidence that G-6-PD deficiency and decreased RBC ATP concentrations may have evolved as a protection against malarial parasitism.[5] It seems reasonable to speculate that ATP deficiency may confer a similar protective advantage for rhinoceroses which are subjected to numerous hemic parasites in the wild.

An additional highly significant recent finding has been the marked deficiency of catalase activity (acatalasemia) in black rhinoceros RBCs. In some catalase deficient human populations, gangrenous oral ulcers (Takahara's disease) are a notable consequence of this deficiency.[11] In recent testing, one rhinoceros calf, exhibiting both leucoencephalomalacia and skin ulcers, was found to have only 2% of the catalase activity present in human erythrocytes. Since then, all additional black rhinoceroses tested, including several with ulcerative disease, have been acatalasemic, whereas, two white rhinoceroses (*Ceratotherium simum*) have not. In combination with impaired HMP shunt metabolism, acatalasemia may further decrease the ability of black rhinoceros cells to neutralize oxidant stresses and therefore may contribute to both mucocutaneous ulcerative disease and acute episodic hemolysis. Since white rhinoceroses nearly all the other metabolic characteristics of the blacks, but are not susceptible to hemolysis or ulcerative disease, it appears likely that catalase plays a pivotal role in the morbidity and mortality of these syndromes in the blacks.

Studies at UCLA to further outline the metabolism of the black rhinoceros RBC are continuing. In the interim, it seems prudent to regard all rhinoceroses as though they were clinically equivalent to G-6-PD deficient humans and to protect them from drugs and agents known to cause hemolysis in man. These include several classes of pharmaceutical compounds: antimalarials, sulfonimides, sulfones, nitrofurans, acetanilid, chloramphenicol and some vitamin K analogs, fava beans, and a number of chemical compounds (including wood preservatives, rodent control poisons or other pesticides, strong cleansers, particularly those containing naphthalene). In man, many other drugs have been associated with hemolysis, but their precise role is uncertain. These include aspirin, phenacetin, aminopyrine, acetaminophen, probenecid, vitamin C, dimercaprol, p-aminosalicylic acid and L-DOPA. Additionally, due to the induction of hemolysis in horses and other domestic species by consumption of certain oak and red maple leaves and wild onions and members of the *Brassica* (rape, kale) family,[1] these should be avoided as well.

In G-6-PD deficient patients, viral, bacterial, and rickettsial infections may also induce hemolysis. In black rhinoceroses approximately 50+% of the hemolytic cases studied had indications of infection with serovars of the spirochete bacterium *Leptospirosis interrogans*. Semi-annual vaccination of all black rhinoceroses with a killed bacterin (Leptoferm-5, Norden Pharmaceuticals, Lincoln, Nebraska 68521, USA) containing *L. interrogans* serovars *icterohaemorrhagiae*, *grippotyphosa*, *canicola*, *pomona* and *hardjo*) is recommended.[7]

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