HAEMOLYTIC ANAEMIA IN CAPTIVE BLACK RHINOCEROSES: POTENTIAL STRATEGIES FOR PREVENTION AND THERAPY[a]

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In the dozen years since Miller and Boever first described a haemolytic syndrome in 17 captive black rhinoceroses (*Diceros bicornis*)[4], the number of affected animals has more than doubled, with an overall mortality rate approximating 75%. In some instances, sudden, massive haemolytic crises occurred in otherwise healthy appearing rhinoceroses following exposure to certain drugs or chemicals. In others, acute haemolytic episodes were observed as a complication of underlying disorders, such as infections, chronic mucocutaneous ulcerative disease and hypophosphataemia. Thus, both primary and secondary forms of the haemolytic syndrome appear to exist. Perhaps because of its sudden onset and often rapid progression to death, acute haemolysis has never been documented in the wild, but it has occurred both in zoo-born and wild-captured animals; and haemosiderosis in some may be interpreted as evidence of chronic or recurrent haemolysis[3].

Since this syndrome has become the leading cause of death within the captive population of *D. bicornis*, extensive investigations have been undertaken to determine its etiology, none of which has generated evidence to indict either haemoglobinopathies, membrane defects or autoimmune phenomena[1 2]. Studies fo the metabolic capacities of rhinoceros erythrocytes, however, have revealed a number of extraordinary differences compaired to other mammalian red cells[5 7 8 9 10 11]. Some of these, either alone or in combination, might be responsible for premature haemolysis, since they appear to reflect a pattern of impaired red cell capacity to neutralize ambient oxidants that are generated during many physiologic and most pathologic processes.

As evidence of increased susceptibility to oxidant-induced damage, erythrocytes of the black rhinoceros normally contain significant concentrations of sulfhaemoglobin and 10 - 15% Heinz bodies, and exhibit strongly positive ascorbate-cyanide and Heinz-body tests with marked glutathione instability. Additionally, rhinoceros red cells catabolize glucose and other sugars at very slow rates, particularly through the oxidant-neutralizing pentose phosphate pathway [hexose monophosphate (HMP) shunt], with an impaired capacity to divert glucose catabolism through the HMP shunt in response to suddent oxidant challenge[5 7 8 9 10].

These characteristics, coupled with a number of clinical similarities, led to an hypothesis that the haemolytic syndrome of black rhinoceroses represented a functional equivalent of the most common enzymopathy affecting humans, glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, even though G-6-PD activity in rhinoceros red cells assayed *in vitro* was quantitatively several times higher than in humans[6]. The basis for this hypothesis was the extraordinary dearth of high-energy phosphate in rhinoceros erythrocytes, their ATP concentration being only 2-5% of humans and other mammalian species. Since ATP is required to generate substrate for HMP shunt metabolism, its short supply might prove rate-limiting for acceleration of shunt activity that normally occurs in response to increases in ambient oxidants. Experimental evidence for this hypothesis is accumulating, and even though the concept remains unproven, it has provided a rationale for potential preventative and therapeutic strategies in the management of captive black rhinoceroses[6].

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An important salvage pathway in human erythrocytes, the incorporation of adenosine into the adenine nucleotide pool may be crucial to maintenance of the precariously low levels of ATP extant in rhinoceros red cells. *In vitro*, adenine nucleotide concentrations can be increased 10- to 20-fold, up to human levels and above, if rhinoceros erythrocytes are incubated with adenosine and glucose or other simple sugars, a process requiring inorganic phosphate. Since red cells of human subjects with hypo- or hyperphosphataemia, respectively, contain lower or higher than normal concentrations of ATP, alteration of plasma phosphate levels in rhinoceroses might permit a measure of control over red cell ATP *in vivo* and thereby alter their tendency to haemolyze.

This hypothesis has received prelimiary tests in two black rhinoceroses at the Dallas and Oklahoma City zoos with encouraging results. One female with severe chronic mucocutaneous ulcerative disease, anaemia (non-haemolytic), and hypophosphataemia, was maintained on high-phosphate dietary supplements for over three months. Periodic venous blood specimens showed variably fluctuating, progressive increases in total red cell adenine nucleotides, eventually achieving levels four- to fivefold higher than mean values for normal control animals.

Another adult female with mucocutaneous ulcerations and hypophosphataemia experienced a primary haemolytic episode with gross haemoglobinuria and rapid loss of two-thirds of her red cell mass. She was immobilized daily, then at longer intervals, to administer intraveneous infusions of phosphate which were associated with rapid variable elevations in red cell ATP, cessation of haemolysis, and gradual return of haemotocrit from a nadir of 16% to 45-48%.

These experiences substantiate conclusions drawn from *in vitro* experiments and illustrate the importance of avoiding or correcting hypophosphataemia to prevent haemolytic episodes consequent to inadequate red cell ATP. Since glycolysis is critical to ATP generation in mammalian erythrocytes, and phosphate is known to stimulate glycolysis, avoidance of other conditions which inhibit glycolysis, such as acidosis, also constitutes an important preventive measure.

Although the experience with one animal suggests that active haemolytic episodes might be interdicted by large infusions of intravenous phosphate, such a procedure carries obvious disadvantages and an unpredictable morbidity and mortality of its own. Emphasis therefore remains on prevention of haemolysis by avoidance of exposure to substances or conditions known to increase the potential for oxidant production[5,6,8,9,10]. These include several classes of drugs, such as sulphonamides, antimalarials, sulfones, nitrofurans, chloramphenicol, acetanilid, and possibly vitamin C and vitamin K analogues; as well as a number of chemical compounds, particularly those containing cyclic hydrocarbons, such as naphtalene and phenols, especially creosote, which may have direct hepatotoxic effects as well as potential capacity to initiate haemolysis. Given the haemolytic effects of certain plants, such as wild onion, oak and red maple leaves, in horses and other animals, the possibility of similar effects in rhinoceroses must be considered in design of captive and managed diets.

Preliminary data from current studies recently undertaken in collaboration with colleagues in the Department of Chemical Pathology at the University of Cape Town Medical School suggest that HMP shunt metabolism of black rhinoceros red cells may be stimulated significantly under appropriate conditions by an artificial electron carrier, such as methylene blue, or by ascorbid acid. Since both of these compounds can directly mediate peroxide production as well as glutathione oxidation, their potential therapeutic value will require careful and circumspect evaluation.

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