Role of excessive maternal iron in the pathogenesis of congenital leukoencephalomalacia in captive black rhinoceroses (*Diceros bicornis*)

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Objective—To investigate the possibility that excessive maternal iron (overload) may contribute to development of congenital leukoencephalomalacia in captive black rhinoceroses.

Sample Population—Tissue specimens and serum samples from 18 rhinoceroses in 2 kindreds harboring 4 (possibly 5) affected female calves.

Procedure—Fresh and archival sera and necropsy tissue specimens were evaluated to determine the nature and extent of iron overload in captive and wild black rhinoceroses as well as other rhinoceros species.

Results—Quantitative serum and tissue assays of iron and iron analytes, corroborated by histopathologic findings, indicated that these kindreds carried the greatest body burdens of iron yet found among captive black rhinoceroses. Fourteen of 18 rhinoceroses had the highest serum ferritin concentrations measured among 64 black rhinoceroses in captivity in the United States. Dams of affected calves had serum ferritin concentrations 2 orders of magnitude higher than clinically normal humans, equids, or free-ranging rhinoceroses. A neonatal serum sample from 1 affected female calf had a high ferritin concentration (approx 100-fold increase), but a male sibling of another affected female did not, suggesting a possible sex disparity in fetal response to maternal iron overload. Morphologic hallmarks of hemochromatosis were prominent in dams and grandams of affected calves.

Conclusions and Clinical Relevance—Excessive maternal iron may affect female fetuses more than males, possibly inducing leukoencephalomalacia by catalyzing production of highly toxic hydroxyl free radicals during crucial periods of in utero development. Reduction of maternal iron overload may decrease the probability of developing leukoencephalomalacia and some other disorders commonly affecting rhinoceroses in captivity. (*Am J Vet Res* 2001;62: 343–349)

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Four captive eastern black rhinoceroses (*Diceros bicornis michaeli*), varying in age from 2 to 29 months, have died as a consequence of an extensive CNS degenerative disorder, leukoencephalomalacia.[1,2] All were females born in captivity at 3 different zoologic institutions from wild- and captive-born dams and sires. Central nervous system degenerative changes primarily involved myelinated portions of the cerebral hemispheres, extending as low as the level of the midbrain while sparing the pons, medulla, and cerebellum. Resemblance to similar lesions in humans and domestic animals prompted a wide range of studies for possible infectious, toxicologic, or inherited metabolic causes, all without success.

Leukoencephalomalacia is but 1 of a number of disease syndromes that affect captive black rhinoceroses[3] to the apparent exclusion of others in the family Rhinocerotidae, and a common denominator in cause or pathogenesis has long been sought to explain this disparity.[4] Past necropsy reports on black rhinoceroses have commonly reported generalized hemosiderosis as an incidental finding.[5,6] This has usually been interpreted as evidence of previous hemolysis, because hemolytic anemia has been among the most common causes of captive deaths in this species.[7-9]

Our necropsy experience, however, indicates that hemosiderosis more likely represents the morphologic manifestation of a true iron-overload syndrome,[10] a possibility originally considered by Smith et al.[11] The extent and anatomic distribution of tissue iron deposition in most instances was far beyond those attributable to purely hemolytic origins, mimicking precisely the pathologic patterns of a dietary-based acquired form of human hemochromatosis highly prevalent among sub-Saharan African Bantu.[10] This alternative explanation is additionally supported by results of biochemical assays of iron analytes in captive and free-ranging rhinoceroses, suggesting that excess iron may be a common contributor to some of the diverse disorders affecting this species in captivity, including congenital leukoencephalomalacia and a possibly skewed sex ratio among newborns.[12] Therefore, the purpose of the study reported here was to investigate the possibility that maternal iron overload may contribute to development of congenital leukoencephalomalacia in captive black rhinoceroses.

Materials and Methods

Genealogies of 4 calves with confirmed leukoencephalomalacia were established from the Black Rhinoceros **Studbook** (**SB**; **Fig 1**) numbers. Archival materials, including clinical lab-

oratory and necropsy reports, were obtained from each zoologic institution, and histopathologic findings were assessed by reviewing available slides, studying additional formalin-fixed tissue specimens, and by special stains, including Perl's Prussian blue for ferric iron and von Kossa for calcium. Stored frozen sera were obtained from individual institutions and from the central specimen repository for the Black Rhinoceros Species Survival Plan maintained at St. Louis Zoological Park. Serum iron concentrations, iron binding capacities, and transferrin saturations were determined at the UCLA Hematology Research Laboratory, using a quantitative colorimetric technique.[a] Tissue minerals were assayed by inductively coupled plasma argon spectroscopy at Oregon State and Kansas State University Colleges of Veterinary Medicine.

Tissue iron content of some specimens was also assayed at the Cellular and Molecular Pathology Laboratory at Kansas State University College of Veterinary Medicine along with serum ferritin and haptoglobin concentrations. Serum ferritin concentrations were determined by an ELISA test originally developed by Smith et al[13] for equids but modified on the basis of ferritin standards derived from black rhinoceros liver.[11] Haptoglobin was measured by the method of Makimura and Suzuki.[14]

Results

Iron analytes—Genealogies of the 2 kindreds were studied (Fig 1). Variables associated with sample collection and the results of serum assays were recorded. With only 4 necropsy-confirmed instances of leukoencephalomalacia, it could be entirely fortuitous that all affected calves were female, but the genealogic patterns suggest that females may be at greater risk for development of excess storage iron and its adverse effects. The dams of all 4 affected calves had maximum serum iron concentrations (range, 331 to 486 µg/dl) that were nearly double the mean (± SD) for 20 unrelated captive adult females (232 ± 84 µg/dl) with almost complete saturation of plasma transferrin (range 91 to 95%; **Table 1**). Additionally, the dams' maximum serum ferritin concentrations (range, 10,360 to 31,090 ng/ml) were approximately 5- to 10-times greater than the mean (± SD) for this same comparison population of captive adult females (2,864 ± 2,638 ng/ml). The latter, in turn, were 20 times higher than the mean (± SD) of 6 black rhinoceroses free-ranging in the wild (133 ± 68 ng/ml).

Of the samples assayed from 18 family members, 14 had serum ferritin concentrations that were higher than all but 4 of 46 other black rhinoceroses in captivity in the United States. Adult males and females in these kindreds typically had 20 to 50 times higher serum ferritin concentrations than their sex-matched comparison populations, with serum iron concentrations averaging 463 µg/dl (SD, 195 µg/dl; range, 154 to 764 µg/dl) and mean transferrin saturation of 89% (SD, 12 %; range, 54 to 99%).

Stored sera were available from only 2 of the 4 affected female calves. One (SB-360) was a single serum sample obtained after death, so its validity was uncertain. Three serum samples, however, were obtained from another affected calf (SB-460) between birth and 3 months of age. In that affected calf, values within reference range for serum iron concentration (108 µg/dl) and transferrin saturation (27%) were measured on the day of birth but were accompanied by a high serum ferritin concentration (14,590 ng/ml). This suggested that the serum ferritin concentration may have been a fetal response to maternal iron overload. Three weeks after birth, serum ferritin concentrations in this calf had decreased to 176 ng/ml, with concomitant increases in serum iron concentration (751 µg/dl) and transferrin saturation (91%). At 12 weeks of age, all 3 serum measurements were elevated (ie, 634 ng of ferritin/ml, 358 µg of iron/dl, 76% transferrin saturation) similar to those we have found in other captive-born male and female calves of comparable age. When this calf died at 16 months of age, serum sam-

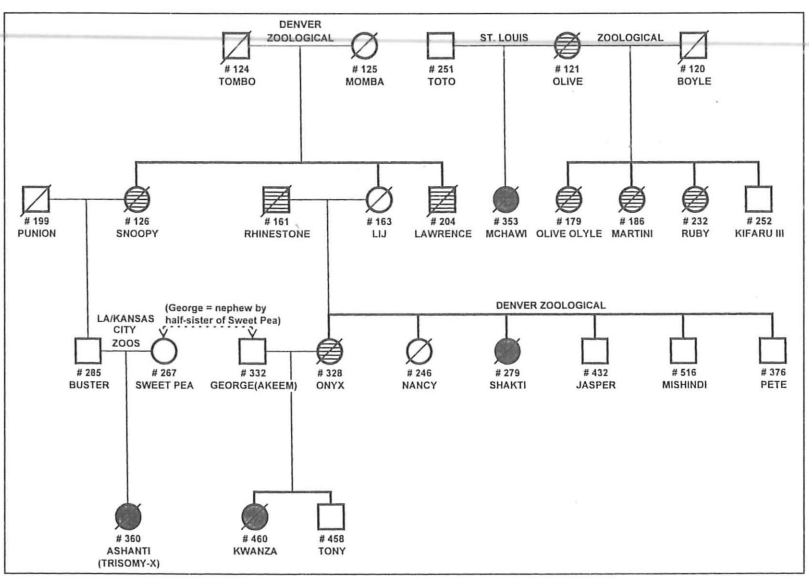

Figure 1—Genealogies of 2 kindreds with 4 calves (solid symbols) affected by fatal leukoencephalomalacia and a fifth (#246) suspected but unconfirmed affected calf. Black Rhinoceros Studbook numbers immediately below each symbol identifies individual rhinoceroses. Circles and squares, indicate females and males respectively. Diagonal lines signify deceased members. Horizontal cross-hatching identifies those with documented episodes of primary hemolytic anemia.

Table 1—Maximum serum iron analyte values in dams of calves with leukoencephalomalacia relative to means (±SD) of comparison populations

Animal	No. tested	Adult or juvenile	Mean age (y)	Sex	Ferritin (ng/ml)	Iron (μg/dl)	Transferrin saturation
Black rhinoceros (*Diceros bicornis*)	4	Dams*	17.5	F	18,900 ± 7,880	387 ± 59	93 ± 2
	20	Adult*	9.3	F	2,864 ± 2,638	232 ± 84	72 ± 21
	12	Adult*	12.0	M	1,542 ± 509	212 ± 33	63 ± 18
	7	Juvenile*	2.2	F	687 ± 271	252 ± 22	61 ± 23
	7	Juvenile*	2.6	M	1,186 ± 874	250 ± 60	66 ± 23
	6	Adult†	NA	Both	133 ± 68	101 ± 19	28 ± 6
White rhinoceros (*Ceratotherium simum*)	7–8	Adult*	NA	Both	41 ± 36	133 ± 46	38 ± 15
Indian rhinoceros (*Rhinoceros unicornis*)	3–4	Adult*	NA	Both	96 ± 141	123 ± 55	32 ± 10
Sumatran rhinoceros (*Dicerorhinus sumatrensis*)	2–3	Adult*	NA	Both	798 ± 134	222 ± 65	91± 3
Horses (range)	NA	Adult	NA	Both	43–261	50–198	22–44
Human (range)	NA	Adult	NA	Both	40–160	65–165	20–50

*Captive in the United States. †Free-ranging in Zimbabwe.
F = Female. M = Male. NA = Not applicable.

ples obtained postmortem had lower values for all 3 analytes, comparable to those measured in a postmortem sample from another affected calf (SB-360). It remains unclear whether these were simply postmortem artifacts or reflections of agonal changes in serum ferritin and transferrin concentrations, both of which are acute-phase inflammatory reactant proteins.

Because virtually all specimens were archival, those available from dams of the 4 offspring that developed leukoencephalomalacia unfortunately did not coincide with their periods of pregnancy. For 1 calf (SB-328), however, the maternal specimen had been obtained 28 months before birth of calf SB-460, (or only about 1 year before gestation began), so the serum analyte values more likely reflected those existent during fetal development in utero. In that instance, maternal serum iron concentration was high at 374 μg/dl, a concentration commonly found among captive black rhinoceroses;[10,11] but transferrin was 95% saturated and serum ferritin concentration was 20,140 ng/ml, 150-times higher than the mean of 6 black rhinoceroses residing lifelong in natural habitats (Zimbabwe) and comparable in magnitude to the concentrations of approximately 16,000 to 20,000 ng/ml measured in dam SB-328 2.2 to 2.4 years earlier(Table 1).

Male calf SB-432 provided the only other day-of-birth values available from a sibling of a female with leukoencephalomalacia (SB-279), and the male calf had serum ferritin concentrations (116 ng/ml) that were within reference range despite maternal concentrations of approximately 30,000 ng/ml. Thus, 1 male offspring of a dam with severe hemochromatosis was born with serum ferritin concentrations within reference range, whereas an affected female had a high serum ferritin concentration at birth that decreased rapidly after birth then increased progressively with age similar to other captive calves of both sexes. This raised the possibility that male fetuses may be less vulnerable to the transplacental effects of maternal iron overloads.

Additionally, 1 (SB-360) of 2 affected female calves tested had a triple-X karyotype, whereas the other (SB-353) had the typical chromosome count of 84.[15] In the absence of appropriate samples, karyotypes of the other affected calves remain unknown. Serum haptoglobin concentrations were within reference range for most adults and in the 1 affected female tested, thereby mitigating against active hemolysis.

Among the comparison populations, it is further notable that the few Sumatran rhinoceroses available for testing also had high serum iron concentrations, ferritin concentrations, and transferrin saturations, but white and greater 1-horned rhinoceroses did not (Table 1).

Pathologic findings—Necropsy materials from 8 of 18 members of both kindreds were examined, including 3 of the 4 affected calves, 2 of their dams, 1 sire, and 2 grandparents. Widespread iron deposition was observed in all 5 adults. Organ distribution was similar but more extensive in degree to that which we have now observed in necropsies of a number of unrelated captive black rhinoceroses.[10] Spleen, liver, bone marrow, and lungs were the organs most consistently involved, with less common (but nonetheless prominent) iron deposition in intestines, lymph nodes, adrenals, thyroid, pancreas, heart, female (but not male) reproductive organs, and other tissue specimens. Most of the iron pigments were deposited within macrophages in these organs, but parenchymal cells were also commonly and extensively involved. Hepatic portal macrophages and sinusoidal Kupffer cells were heavily engorged with hemosiderin, and course and fine iron-containing granules were abundant in the cytoplasm of hepatocytes, most prominently in periportal regions of the lobules (**Fig 2**). In more severely involved rhinoceroses such as dams SB-163 and SB-328 and grandam SB-125, there was evidence of hepatocellular degeneration, regenerative nodularity, and bile duct proliferation, and in 1 rhinoceros (SB-125), a low-grade hepatocellular carcinoma was found in a distinctly cirrhotic liver.

Extensive deposits of iron pigments were also observed in splenic and bone marrow macrophages. These were sometimes so densely packed in the marrow that hematopoietic elements were sufficiently excluded to account for anemia or pancytopenia. Macrophages in

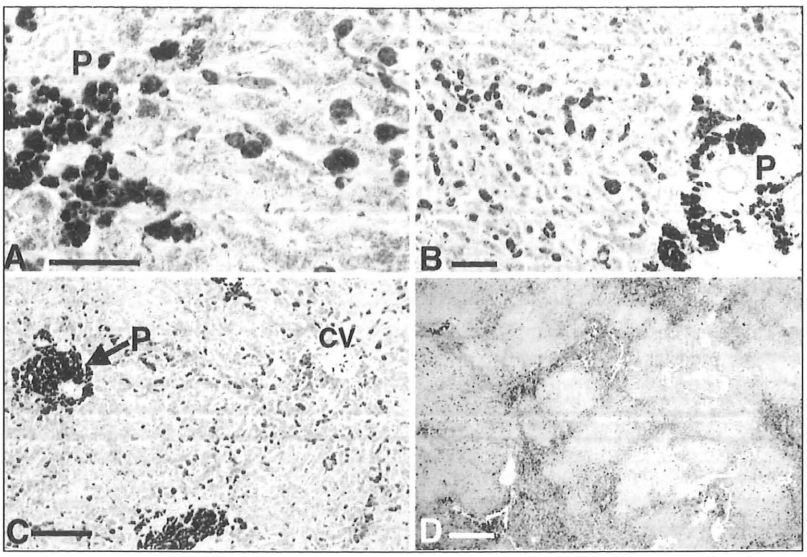

Figure 2—Photomicrographs of liver sections from black rhinoceroses demonstrating patterns of maternal and grandam hepatic iron deposition after the use of Perl's Prussian blue stain for ferric iron. A—Dense iron containing aggregates in portal (P) macrophages and Kupffer cells of dam SB-328. Hepatocytes are heavily laden with course and fine granules of iron-containing material and have focal degenerative changes. Bar = 100 μm. B—Panlobular parenchymal and reticuloendothelial hemosiderosis with bile duct proliferation in hepatic portal (P) areas of dam SB-163. Bar = 100 μm. C—Intense portal (P) iron deposition and early fibrosis with panlobular hemosiderosis of dam SB-328. CV = Central vein. Bar = 250 μm. D—Bridging fibrosis and regenerative nodularity in liver from grandam SB-125. Central pallor in regenerative nodules results from lower iron content of younger hepatocytes relative to older, heavily laden peripheral cells. Bar = 1 mm.

the pulmonary alveolar interstitium and in hepatic and lymph node sinusoids also appeared hyperplastic and laden with inordinate amounts of iron-containing fine and course granular or globular material. One of the most distinctive lesions involved dense iron deposits in abundant macrophages of the lamina propria and submucosa of the intestine, extending as far as the colon, with the highest concentrations occurring proximally, especially at the tips of duodenal villi.

These patterns of hemosiderin deposition differed distinctly from those classically described for uncomplicated hemolytic disease[16] or hereditary hemochromatosis,[17,18] but they were virtually identical to those characteristic of dietary iron overload syndromes in lemurs[19] and in humans.[17,20,21] Accumulations of hemosiderin in small intestinal villi have been interpreted as strong evidence for enteric origin of excess iron.[22] Supporting this interpretation, we found no trace of intestinal iron deposition in an unrelated full-term stillborn female fetus with pronounced hemosiderosis in most other organs. Even though the duodenum typically absorbs iron, we observed heavy deposits in all segments of the intestine, including the colon in some rhinoceroses. This indicates that enhanced iron absorption may occur in these regions as well, raising questions about the relation between enteric microflora metabolism and its effects on bioavailability of iron.

In contrast to the adults, necropsy tissue specimens from 3 affected calves (SB-353, SB-460, SB-360) that died at 6, 16, and 29 months of age, respectively, revealed mild iron loads increasing in proportion to age and generally restricted to spleen, liver, and duodenal villus tips. This suggests that if any fetal iron excess existed in utero, it was mobilized in response to increasing demands for hemoglobin and myoglobin synthesis during postpartum growth. Isolated foci of iron-containing granules found in the CNS of 1 affected calf (SB-353) were not evident in regions with encephalomalacia, hemorrhage, or microabscess formation, therefore, appearing, to have no direct relation to the primary pathologic process.

Tissue specimen analyses—Frozen liver from 3 dams (SB-121, SB-163, SB-328) of calves with leukoencephalomalacia (SB-353, SB-279, SB-460, respectively) contained 5,500, 12,000, and 17,600 μg of iron/g of wet tissue. Tissue was not available from the fourth dam, but a grandam (SB-125) of affected calf SB-279 had an iron concentration of 14,000 μg/g.

These concentrations may best be compared with 11 (9 female) unrelated black rhinoceros adults ranging in age from 6 to 28 years that died in captivity in the United States with an average hepatic iron concentration of 3,830 μg/g. The wide range (176 to 11,000 μg/g) and SD (3,670 μg/g) reflect previous observations that iron overloads, whether measured directly by quantitative tissue analyses or indirectly by serum ferritin assays, increase progressively as a function of time in captivity.[10,11] The mean hepatic iron concentration in 7 juvenile (1 to 3.5 years in age) black rhinoceroses, for example, was only 416 μg/g (SD = 265 μg/g), and the reference range concentrations for horses were 100 to 300 μg/g.

Two free-ranging black rhinoceroses that were recently necropsied at reserves in Etosha, Namibia,[b] and Malilangwe, Zimbabwe,[c] were reported to have

hepatic iron concentrations of only 331 and 26 µg/g, respectively. In a third black rhinoceros, born and raised in a zoo-equivalent environment at Chipangali Wildlife Center in Zimbabwe until it died at 22 months of age, wide-spread hemosiderosis in necropsy tissue specimens was observed, and its hepatic iron was reported to be 1,765 µg/g,[c] further supporting the contribution of prolonged captive conditions to body iron loads, whether in the United States or Africa.

Discussion

Systemic or generalized hemosiderosis, the extensive deposition of iron pigments in multiple organs, has been commonly cited in necropsy reports of black rhinoceroses dying in captivity,[5,6] but in most instances it has been ascribed to previous hemolytic episodes, because hemolytic anemia has been a highly common condition in this population.[3,7-9]

In 1992, Kock et al[23] reported the lack of hemosiderosis in necropsy findings of free-ranging black rhinoceroses, and this was viewed as evidence that hemolytic disease did not affect rhinoceroses in their natural habitats. They also found that hemosiderin began to accumulate in organs beyond the spleen in translocated rhinoceroses that died after short intervals (approx 1 month) in confinement, and that the magnitude of these iron deposits as well as the number of organs affected increased progressively over the 2-year period of study.

Smith et al[11] observed a similar phenomenon as follows: in black rhinoceroses, serum ferritin concentrations (which indirectly reflect total body iron stores) increased progressively in proportion to age or time in captivity, and serum iron concentration and transferrin saturation were significantly increased, compared with captive white rhinoceroses. They speculated that this may be related to differences between natural and captive diets rather than a consequence of hemolysis, a possibility strongly supported by data reported here and previously.[10]

Taken together, these findings provide compelling evidence that captive conditions induce increased uptake of dietary iron resulting in clinically significant iron overloads. Such acquired hemochromatosis is most probably an effect of conversion from natural-browse based diets to manufactured pellets and domestic forages, which may contain comparable amounts of iron but lack many natural components (known and unknown) of native browse such as tannins and phytates that are capable of chelating iron into insoluble less bioavailable complexes. This hypothesis is supported by the observation that hemosiderosis and high iron analyte values commonly develop only in captive black and Sumatran species, both of which are obligate browsers, whereas rhinoceroses that are natural grazers (African white and Asian greater one-horned) do not have signs of iron overload when maintained in prolonged captivity.[10,11]

Our study sought to investigate retrospectively the potential influence of iron excess on the development of congenital leukoencephalomalacia by examining archival materials from affected calves and their close relatives and comparing them to all available species of rhinoceroses, captive and those free-ranging lifelong in the wild. One shortcoming of these data was their dependence on stored sera and tissue specimens that were originally obtained at times often far removed from periods of pregnancy when iron overload would be capable of exerting toxic effects on the calves' in utero development. Nonetheless, maternal samples from 1 dam (SB-328) with serum ferritin concentrations in the 20,000 ng/ml range coincided closely (approx 1 year) with the onset of gestation, and her affected female calf (SB-460) had a high serum ferritin concentration on the day of birth (14,590 ng/ml). Because ferritins are multimeric macromolecules (approx 440 kd, depending on iron content), transplacental transfer of maternal ferritin seems unlikely. This calf's serum ferritin concentration decreased to 176 ng/ml by 3 weeks of age, indicating that the extremely high concentration in the first sample most likely reflected a fetal response to maternal iron overload. This was supported by necropsy findings of this and 2 other affected females that died between 6 and 29 months of age that revealed no more histologic evidence of generalized hemosiderosis than the progressive iron accumulation found in unrelated rhinoceroses as a function of age or time in captivity. One male sibling (SB-432), on the other hand, appeared to have serum ferritin concentrations within reference range at birth that then increased progressively with age. Born only 3 months before a sample from his mother had a serum ferritin concentration of 30,800 ng/ml, this male calf had a day-of-birth serum ferritin concentration of 116 ng/ml, suggesting that it may have been less affected by the transplacental influences of maternal iron excess.

Although it is widely accepted that serum ferritin concentrations provide the most accurate indirect measure of body burdens of iron,[24-26] it should be mentioned that direct correlations hold true only within a certain range and only in the absence of inflammation and other stressful conditions in which ferritin responds as an acute-phase inflammatory reactant.[27] Because ferritin protein has a turnover rate of only a few days,[28] its plasma concentration is limited by maximum synthetic rates, which in humans are sufficient to maintain plasma concentrations up to approximately 4,000 to 5,000 ng/ml. The high concentrations measured in these rhinoceroses, therefore, almost certainly include contributions from intracellular ferritin released by concurrent cellular destruction, providing biochemical corroboration of the tissue damage that was often apparent histologically. As serum ferritin concentrations extend into these higher ranges, quantitation also becomes less accurate, because sera must be serially diluted as much as 1,000-fold or more, possibly accounting for some of the variability among multiple assays for a given individual. Despite these uncertainties, histopathologic findings confirmed close correlations between serum ferritin assays and the extent of tissue iron deposition, lending credence to both.

By all methods of assessment (quantitative assays of iron analytes in sera and tissue specimens and histologic evaluation of total-body iron stores), these 2

kindreds included individuals with the most profound concentrations of iron overload yet found among any rhinoceros species. Systemic hemosiderosis, the morphologic hallmark of iron overload, was extreme in all 5 instances in which necropsy material from adults was available for review. Fourteen of the 18 highest (> 10,000 ng/ml) serum ferritin concentrations among 64 captive black rhinoceroses in the United States were found in immediate relatives of the 4 affected calves. Compared with reference range serum ferritin concentrations of approximately 100 to 200 ng/ml, the dams in all 4 instances had high concentrations, ranging from > 10,000 to > 30,000 ng/ml. Concentrations as high as 70,000 to > 350,000 ng/ml were encountered in some family members, fully 3 orders of magnitude higher than in clinically normal control animals.

It should be mentioned that mean serum ferritin concentrations in juvenile and adult captive black rhinoceroses were also increased 5 to 20 times above those observed in free-ranging rhinoceroses, emphasizing the relation between time in captivity and magnitude of iron overloads (Table 1).[10,11]

The appearance of congenital leukoencephalomalacia only in female calves may be fortuitous, but many other females in direct lineage also had remarkably high body burdens of iron, whereas males appeared to be less severely affected. In retrospect, a fifth calf (SB-246), also female, was suspected of possibly having had clinical signs of leukoencephalomalacia, but, unfortunately, that calf died accidentally without a comprehensive necropsy examination of the CNS. The discovery of a trisomy-X karyotype in 1 of 2 affected calves studied[15] further suggests an element of sex influence. Possibilities of coincidence were further diminished by examination of 3 male offspring of the same parents, which were born with neither biochemical nor histologic evidence of increased iron loads. If this apparent sex disparity is real, it may be 1 of the factors influencing a reported male predominance in surviving live births among captive black rhinoceroses.[12]

It should also be mentioned that a third of those included in the leukoencephalomalacia genealogies had histories of hemolytic episodes (Fig 1). Because erythrocytes of black rhinoceroses are known to be highly sensitive to oxidant-induced hemolysis,[29] this adds credence to a postulate formulated independently by Dennis[d] that hemosiderosis (ie, iron overload) may be the cause, rather than the consequence, of hemolytic disease in captive black rhinoceroses.

Potential mechanisms by which iron overload may play a role in the pathogenesis of leukoencephalomalacia remain speculative, but the established generation of toxic hydroxyl free radicals by free iron in the presence of reactive oxygen species provides a logical pathway.[30-34] Lipid components of cells and organelles are particularly vulnerable to peroxidative damage, and contributions of excess iron to a number of neurodegenerative disorders such as hereditary ataxia (Friedreich's ataxia) are currently a focus of intensive investigation.[34]

Given the interdependence between copper and iron metabolism,[35] we have preliminary data that indicate that concurrent copper deficiency in some rhinoceroses may provide an intriguing alternative explanation, because copper is essential for proper myelination[36] as well as for mobilization of iron from storage sites in the reticuloendothelial system.[22,35,37]

Whether iron excess is causally related to development of leukoencephalomalacia in black rhinoceroses, the known deleterious effects of chronic iron overload in animals and humans have prompted consideration of potential preventative and therapeutic strategies. These include reduction of endogenous dietary iron or its bioavailability by dietary supplements such as tannins and other components known to bind iron into insoluble complexes. Therapeutic intervention by pharmacologic agents cuerrently available would be prohibitively expensive, but repetitive phlebotomies are likely to be at least effective, especiallyin the juvenile and younger adult populations with lesser burdens of iron.

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[a]Diagnostics Kit No. 565, Sigma Diagnostics, St Louis, Mo.

[b]Kinsell MJ, Zoo Pathology Program,Loyola University Medical Center, Maywood, Ill: Personal communication, March, 1999.

[c]Foggin CM, Wildelife Unit,Centeral University Research Laboratory,Harare,Zimbabwe: Personal communication, June, 1998.

[d]Dennis PM, Department of Wildlife and Zoological Medicine, University of Florida, College of Veterinary Medicine,Gainesille: Personal communication, November, 1997.

References

1. Miller RE, Cambre RC, de laHunta A, et al. Encephalomalacia in three black rhinoceroses *(Diceros bicornis). J Zoo Wildl Med* 1990;21:192–199.

2. Kenny DE, Cambre RC, Spraker TR, et al. Leukoencephalomalacia in a neonatal female black rhinoceros *(Diceros bicornis)*: report of a fourth case. *J Zoo Wildl Med* 1996;27:259–265.

3. Miller RE. Diseases of black rhinoceroses in captivity, in *Proceedings.* Symp Rhinos Game Ranch Anim 1994;180–185.

4. Paglia DE, Miller RE, Renner SW. Is impairment of oxidant neutralization the common denominator among diverse diseases of black rhinoceroses?, in *Proceedings.* Annu Conf Am Assoc Zoo Vet 1996;37–41.

5. Montali RJ, Citino SB. Pathological conditions in captive rhinoceroses. In: Ryder OA, ed. *Rhinoceros biology and conservation.* San Diego, Calif: Zoological Society 1993;346–349.

6. Lowenstine LJ, Munson L. Iron overload in the animal kingdom. In: Fowler ME, Miller RE, eds. *Zoo and wild animal medicine. Current therapy 4.* Philadelphia: WB Saunders Co, 1999;260–268.

7. Miller RE, Boever WJ. Fatal hemolytic anemia in the black rhinoceros: case report and a survey. *J Am Vet Med Assoc* 1982; 181:1228–1231.

8. Miller RE, Chaplin H Jr, Paglia DE, et al. Hemolytic anemia in the black rhinoceros—an update, in *Proceedings.* Annu Conf Am Assoc Zoo Vet 1986;7–8.

9. Miller RE, Chaplin H Jr, Paglia DE, et al. Hemolytic anemia in the black rhino. *Pachyderm* 1987;9:26–32.

10. Paglia DE, Dennis P. Role of chronic iron overload in multiple disorders of captive black rhinoceroses *(Diceros bicornis),* in *Proceedings.* Annu Conf Am Assoc Zoo Vet 1999;163–171.

11. Smith JE, Chavey PS, Miller RE. Iron metabolism in captive black *(Diceros bicornis)* and white *(Ceratotherium simum)* rhinoceroses. *J Zoo Wildl Med* 1995;26:525–531.

12. Atkinson SJ, Dierenfeld ES, Foose TJ. Possible determinants of skewed natal sex ratios in captive black *(Diceros bicornis)* and Indian *(Rhinoceros unicornis)* rhinoceros in North America. Cumberland, Ohio: International Rhino Foundation,1997; 1–82.

13. Smith JE, Moore K, Cipriano JE, et al. Serum ferritin as a measure of stored iron in horses. *J Nutr* 1984;114:677–681.

14. Makimura S, Suzuki N. Quantitative determination of

bovine serum haptoglobin and its elevation in some inflammatory diseases. *Jpn J Vet Sci* 1982;44:15–21.

15. Houck ML, Ryder OA, Kumamoto AT, et al. Cytogenetics of the Rhinocerotidae. *Verh ber Erkrg Zootiere* 1995;37:25–32.

16. Muir R, Dunn JS. The retention of iron in the organs in haemolytic anaemia. *J Pathol* 1915;19:417–428.

17. Brink B, Disler P, Lynch S, et al. Patterns of iron storage in dietary iron overload and idiopathic hemochromatosis. *J Lab Clin Med* 1976;88:725–731.

18. Bothwell RH, MacPhail AP. Hereditary hemochromatosis: etiologic, pathologic, and clinical aspects. *Semin Hematol* 1998;35:55–71.

19. Spelman LH, Osborn KG, Anderson MP. Pathogenesis of hemosiderosis in lemurs: role of dietary iron, tannin, and ascorbic acid. *Zoo Biol* 1989;8:239–251.

20. Kinney TD, Hegsted DH, Finch CA. The influence of diet on iron absorption. I. The pathology of iron excess. *J Exp Med* 1949; 90:137–147.

21. Higginson J, Gerritsen T, Walker ARP. Siderosis in the Bantu of Southern Africa. *Am J Pathol* 1953;29:779–815.

22. Lee GR, Nacht S, Lukens JN, et al. Iron metabolism in copper-deficient swine. *J Clin Invest* 1968;47:2058–2069.

23. Kock N, Foggin C, Kock MD, et al. Hemosiderosis in the black rhinoceros (*Diceros bicornis*): a comparison of free-ranging and recently captured with translocated and captive animals. *J Zoo Wildl Med* 1992;23:230–234.

24. Cook JD, Lipschitz DA, Miles LEM, et al. Serum ferritin as a measure of iron stores in normal subjects. *Am J Clin Nutr* 1974;27: 681–687.

25. Lipschitz DA, Cook JD, Finch CA. A clinical evaluation of serum ferritin as an index of iron stores. *N Engl J Med* 1974;290: 1213–1216.

26. Jacobs A, Worwood M. Ferritin in serum. Clinical and biochemical implications. *N Engl J Med* 1975;292:951–956.

27. Finch CA, Huebers H. Perspectives in iron metabolism. *N Engl J Med* 1982;306:1520–1528.

28. Drysdale JW, Munro HN. Regulation of synthesis and turnover of ferritin in rat liver. *J Biol Chem* 1966;241:3630–3637.

29. Paglia DE, Miller RE. Erythrocytes of the black rhinoceros (*Diceros bicornis*): susceptibility to oxidant-induced haemolysis. *Int Zoo Yearb* 1993;32:20–27.

30. Slater TF. Free-radical mechanisms in tissue injury. *Biochem J* 1984;222:1–15.

31. Halliwell F, Gutteridge JMC. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. *Arch Biochem Biophys* 1986;246:501–514.

32. Saltman P. Oxidative stress: a radical view. *Semin Hematol* 1989;26:249–256.

33. Britton RS, Tavill AS, Bacon BR. Mechanisms of iron toxicity. In: Brock JH, Halliday JW, Pippard MJ, et al, eds. *Iron metabolism in health and disease.* Philadelphia: WB Saunders Co 1994;311–351.

34. McCord JM. Iron, free radicals, and oxidative injury. *Semin Hematol* 1998;35:5–12.

35. Owen CA. Effects of iron on copper metabolism and copper on iron metabolism in rats. *Am J Physiol* 1973;224:514–518.

36. Prohaska JR. Functions of trace elements in brain metabolism. *Physiol Rev* 1987;67:858–901.

37. Roeser HP, Lee GR, Nacht S, et al. The role of ceruloplasmin in iron metabolism. *J Clin Invest* 1970;49:2408–2417.