IRON STORAGE SYNDROME IN RHINOCEROSES: Potential Role for Rhino Keepers in Prevention and Therapy

Donald E. Paglia, M.D., Emeritus Professor of Pathology & Laboratory Medicine, UCLA School of Medicine, and Principal Investigator, UCLA Hematology Research Laboratory, 10833 LeConte Ave., Los Angeles CA 90095-1732. <dpaglia@mcn.org>

Introduction

An inordinate number of unusual disorders affect rhinoceroses under captive conditions. Each species tends to develop characteristic problems, but none has been more dramatic than the multiple clinical syndromes that plague African black rhinoceroses in captivity. These are summarized in Table 1, and some will be discussed in greater detail here and by other speakers at this workshop. Notice how frequently descriptive titles are given to these conditions, implying (or, in the case of 'idiopathic', frankly admitting) that their causes are unknown, uncertain, or yet to be determined.

It has always seemed highly unlikely that so many disorders would occur in only one of the four rhino species unless there were some common contributing or causative factor. This presentation will focus on the excessive amounts of iron that accumulate under captive conditions in this species, as well as in Sumatran rhinos, and how iron overload might cause or contribute to the morbidity and mortality of the conditions listed in Table 1. Most importantly from the Rhino Keepers' point of view, potential preventative and therapeutic options will also be reviewed.

Investigations into the Nature of Hemolytic Anemia in Black Rhinos

Two decades ago, episodes of hemolytic anemia (the premature destruction of circulating red blood cells) emerged as one of the more important conditions affecting black rhinos in captivity (1). These 'hemolytic crises' were particularly dramatic because they often occurred suddenly in otherwise apparently healthy animals, they were associated with exposure to a variety of agents, and they were lethal in three out of four instances. Hemolytic episodes soon became recognized as the most common cause of death in captivity. The Hematology Research Laboratory at the UCLA School of Medicine was recruited at that time to help investigate this problem because our research teams had been responsible for the initial detection and characterization of over half of the red cell enzyme deficiency states known to cause hemolytic anemia in humans, in dogs such as the Basenji, and in other animal species.

Our initial studies revealed a number of extraordinary biochemical and enzymatic differences between rhinoceros red cells and those of other mammalian species (2,3). Some of these differences were so unusual and were of such magnitude that they would have been predicted to be incompatible with normal red cell function and viability if they were found in humans or other animals. Most of these related to the decreased capacity of rhino red cells to neutralize the oxidants that are invariably generated as byproducts of many physiological and pathological processes in oxygen-breathing animals (4-6).

Eventually, it became clear that reduced antioxidant capacity of red cells is a normal characteristic of all rhinoceroses, the blacks being more affected than other species, but it remains unproven whether such limitations are the principal defect in cases of sudden hemolysis (7). Nonetheless, it seemed reasonable to postulate that antioxidant capacity might also be diminished in other tissues besides red cells, making multiple organ systems susceptible to a wide variety of disorders (8). To test that hypothesis, we began to participate in necropsies, hoping to exchange our narrow focus on red cells for a more comprehensive view of the entire animal.

Serum Analyses and Necropsy Studies

Earlier necropsy reports of black rhinos dying throughout the U.S. frequently noted the presence of hemosiderosis, the abnormal accumulation of iron pigments (such as hemosiderin) in various tissues. Logically, this was often interpreted as a residual sign of previous hemolytic episodes, since breakdown of red blood cells releases the iron-containing pigment hemoglobin, which in turn is degraded and recycled by specialized macrophage cells throughout the body. From a pathologist's point of view, however, the huge amounts of iron pigments deposited and their pattern of distribution throughout various organs were incompatible with that interpretation and could more likely be explained by a fundamental disturbance in iron metabolism (9,10).

Figure 1 provides a typical example of the massive amounts of iron pigments that often accumulate in captive black compared to white rhinos. These sections of liver have been treated with Perl's Prussian blue stain, which induces an intense blue reaction with ferric iron compounds. In most cases, iron deposition in black rhino tissues, particularly liver, spleen, lymph nodes, bone marrow, endocrine glands, and intestines, is so severe that it doesn't even require a microscope to make the diagnosis. The intensity of the blue stain is readily apparent to the naked eye. A similar pattern of pathologic changes is characteristic of the dietary iron-storage disorder affecting subSaharan Africans, so-called Bantu siderosis (10).

The possibility that black rhinos might accumulate excessive iron stores in captivity was not an entirely new concept. On the basis of his comparative studies of serum iron compounds in black and white rhinos, Dr. Joseph Smith of the Kansas State School of Veterinary Medicine had previously suggested that captive black rhinos might be afflicted with an iron-storage disorder (11), but this was initially viewed by many as simply one more example of the multiple problems affecting this species in captivity.

Our studies of necropsy pathology in over fifty rhinos, however, have confirmed Dr. Smith's original hypothesis, unequivocally demonstrating the presence of pathological iron overloads in both the African black and Sumatran species (9,10,12,13).

In addition, we have measured the concentrations of iron compounds in blood sera and tissues from over one hundred captive and free-ranging rhinos of all four available species and found the same results. The rate of iron accumulation in affected rhino species was particularly alarming. Serum concentrations of ferritin, (an iron-protein complex that reliably reflects total-body iron stores), increased as much as tenfold above normal in as little as three years, and ferritin concentrations hundreds or thousands of times greater than normal were frequently found in long-term captive animals.

Taken together, these laboratory and necropsy findings indicate that rhinos usually have normal iron levels when they are newly born in captivity or living lifelong in their native habitats, but the African black and Sumatran species begin to accumulate iron rapidly and progressively under captive conditions, producing major pathological changes in multiple organs, particularly the liver. For those of you who have keeper responsibilities beyond rhinos, it is equally important to recognize that iron loading is a highly common and widely unappreciated problem that adversely affects many species of exotic wildlife when brought into captivity (14), including another perrisodactyl, tapirs (15).

Causes and Effects of Iron-Induced Cell Injury

Iron is an essential element for virtually all life forms, but its presence in excess is invariably harmful. Because of the marked toxicity of elemental iron, it is usually bound to various proteins *in vivo.* Free iron is an active catalyst for generation of highly destructive hydroxyl free radicals (16-18). These cause oxidative injury to cell and organelle membranes, to nucleoproteins, and to structural, functional and enzymatic proteins at multiple molecular sites. The clinical consequences of iron overload therefore depend on which organs are primarily affected and on the relative degrees of functional impairment produced by cellular damage.

An enormous body of experience with iron storage disorders in humans (19) and other animals has demonstrated that iron overburdens of the magnitude observed in captive rhinos can contribute to (if not directly cause) severe, life-threatening, multi-system disorders, some of which are clinically similar to those affecting these animals in captivity.

One of the most important effects of iron overload is an increased susceptibility to infections. Since microorganisms cannot proliferate and survive unless they obtain essential iron from their hosts, the virulence of invading microbes is greatly enhanced by the presence of freely available iron (20-22). Excess iron also impairs the ability of white blood cells and tissue macrophages to phagocytize and kill bacteria and other pathogens. These effects are very likely responsible for the high incidence of diverse and exotic infectious diseases known to affect African black and Sumatran rhinoceroses in captivity.

Additional evidence has also been accumulating that links iron overload to other disorders of captive black rhinos, for example, congenital leukoencephalomalacia (23), stress intolerance, and toxic hepatopathy. Although direct cause-and-effect relationships have not been unequivocally established, a number of plausible hypotheses have emerged linking iron overload and the diverse disorders affecting captive rhinos. Some of these are summarized in Table 2. Regardless of the validity of any of these hypothesized mechanisms, one axiom remains certain: Excess free iron is always biologically toxic and should be aggressively treated if not preventable.

Cause of Iron Accumulation in Captive Rhinos

Field studies have verified that black rhinos free-ranging in their native African habitats show no evidence of an iron storage problem, but soon after capture, they start to accumulate iron progressively (9,24). Since iron-storage syndromes in rhinos develop only in the two browser species, and not in the grazers, dietary factors are naturally suspect. Indeed, the only plausible iron source in these animals is via ingestion of food, soil, or other iron containing materials.

Mammals are not equipped with physiological mechanisms to excrete iron, so iron balance is regulated by the intestine which stops absorbing dietary iron when body stores are sufficient. African black and Sumatran rhinos, however, apparently did not evolve this feedback control capacity, so they continue to absorb iron from intestinal contents regardless of adequate, increased, or even massive body stores. These two rhino species apparently depend on alternative regulatory mechanisms for maintaining proper levels of iron. One likely possibility is their consumption of plants containing components capable of binding iron into insoluble, non-absorbable complexes. A huge number of such natural metal "chelators" are known to exist in browse, tannins being among the most prominent (25-27). A search for crucial chelators in native rhino browse has focused on tannins, but a number of other natural ingredients might be equally important to maintenance of normal iron balance.

Considerable effort has been devoted to defining the composition of natural rhino diets (28,29), but browser rhinos may forage on over 200 different botanical species, so it remains a practical impossibility to duplicate such complex diets for captive rhinos far removed from the native environments in which they evolved. Supplementation of captive diets with browse has not yet been sufficient to prevent or to significantly lower the rates of iron accumulation. Such supplements, however, are not self-selected by the rhinos from their native habitats, but instead are picked by humans dependent on seasonal availability.

The Rhino Keepers' Role in Prevention and Therapy of Iron Overloads

It seems unlikely that the problems of iron-storage disease will ever be effectively managed solely by dietary manipulation. Even if natural chelators could be identified and added to captive diets, they would be useful only as prevention against further uptake. Since *all* adult black and Sumatran rhinos so far studied have pathological

iron overloads proportional to their time in captivity, the excessive iron stores already present would not be reduced simply by preventing more uptake. Instead, these will persist unless removed by some form of active intervention. The operative term is 'active intervention', and this is the area in which rhino keepers could play a crucial role.

As a group, rhino keepers are probably in the best position to contribute to enhancement of the health and welfare of captive black and Sumatran rhino populations by actively promoting reduction of iron overburdens. Therapy to reduce iron overloads in humans relies on pharmacologic chelators such as desferrioxamine (Desferal, Novartis Pharmaceuticals, NJ), which are prohibitively expensive, or on the ancient art of blood-letting, (an almost embarrassing alternative for a physician to be recommending to colleagues in this day and age!) Yet, each liter of blood contains about half a gram of hemoglobin iron. Significant amounts of iron, therefore, can be physically removed by phlebotomy, allowing the body to mobilize tissue deposits of iron to make new red blood cells in compensation.

Humans with iron overload are commonly treated by phlebotomy of as much as 500 ml of blood once or twice a week. A comparable amount for a black rhino would be fifteen times more, clearly impractical, but any volume removed would contribute to reduction of the total body iron burden. If animals were conditioned to tolerate frequent standing phlebotomies without sedation, significant amounts of iron could be extracted and discarded over a period of time without jeopardizing animal health. To the contrary, it is important to recognize that any reduction in body iron burdens would be beneficial and should reduce the probability of developing many of the disorders listed in Table 1, particularly their high susceptibility to infections.

Definitive guidelines for management of iron overload in humans have been established by the College of American Pathologists (30) and by the U.S. Centers for Disease Control and Prevention (CDC) (31). From these guidelines and our own experience, we have developed recommendations for a phlebotomy protocol (32) that could be applied to captive rhinos and tapirs (unpublished but available from the author).

Repetitive phlebotomy is already being attempted at some institutions, but it is time-consuming and requires dedicated keepers and veterinarians to train and bleed the animals. It also requires supportive institutional administrators who are willing to devote such resources to correct a problem that is generally not clinically apparent until end-stage disease has been induced in the liver, heart, or some other critical organ system. Since deleterious effects of iron overload are so insidious, an enormous educational effort will be required to bring this problem to widespread attention. Even greater efforts will be required of the rhino keeper and veterinary communities to actively intervene.

LITERATURE CITED

1. Miller RE. Hemolytic anemia in the black rhinoceros. In: Fowler ME (ed). *Zoo and Wild Animal Medicine.* WB Saunders, Philadelphia, pp. 455-458, 1993.

2. Paglia DE, Valentine WN, Miller RE, Nakatani M, Brockway RA. Acute

intravascular hemolysis in the black rhinoceros: Erythrocyte enzymes and metabolic intermediates. *Am. J. Vet. Res.* 47:1321-1325, 1986.

3. Paglia DE, Miller RE. Erythrocyte ATP deficiency and acatalasemia in the black rhinoceros *(Diceros bicornis)* and their pathogenetic roles in acute episodic hemolysis and mucocutaneous ulcerations. *Proc. Annu. Conf. Am. Assoc. Zoo Vet.*, pp. 217-219, 1992.

4. Paglia DE. Acute episodic hemolysis in the African black rhinoceros as an analogue of human glucose-6-phosphate dehydrogenase deficiency. *Am. J. Hematol.* 42:36-45, 1993.

5. Paglia DE, Miller RE. Erythrocytes of the black rhinoceros *(Diceros bicornis)*: susceptibility to oxidant-induced haemolysis. *Intl. Zoo Yearbk.* 32:20-27, 1993.

6. Paglia DE, Weber B, Baumgarten I, Harley EH. Radiometric assessment of hexose monophosphate shunt capacity in erythrocytes of rhinoceroses. *Am. J. Vet. Res.* 62:1113-1117, 2001.

7. Weber B, Paglia DE, Harley EH. Red cell metabolism in the black rhinoceros: Relevance to haemolytic disease. Abstracts of the International Elephant and Rhino Research Symposium, Vienna, Austria, p. 41, 2001.

8. Paglia DE, Miller RE, Renner SW. Is impairment of oxidant neutralization the common denominator among diverse diseases of black rhinoceroses? *Proc. Annu. Conf. Am. Assoc. Zoo Vet.*, pp. 37-41. 1996.

9. Paglia DE, Dennis P. Role of chronic iron overload in multiple disorders of captive black rhinoceroses *(Diceros bicornis). Proc. Annu. Conf. Am. Assoc. Zoo Vet.*, pp. 163-171, 1999.

10. Paglia DE. Captivity acquired hemochromatosis resembling Bantu siderosis in browser, but not grazer, rhinoceroses. *Blood* 96:484a, 2000.

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

12. Paglia DE, Radcliffe RW. Anthracycline cardiotoxicity in a black rhinoceros *(Diceros bicornis)*: Evidence for impaired antioxidant capacity compounded by iron overload. *Vet. Pathol.* 37:86-88, 2000.

13. Paglia DE, Dierenfeld ES, Tsu I-H. Pathological iron overloads acquired in captivity by browsing (but not by naturally grazing) rhinoceroses. Abstracts of the International Elephant and Rhino Research Symposium, Vienna, Austria, p. 46, 2001.

14. Paglia DE, Weinberg ED. Iron loading in birds and mammals - A notable, yet often unrecognized initiator of disease and death. Iron Disorders Institute Magazine *i.d.Insight,* 2nd quarter, p. 8, 2001.

15. Paglia DE, Miller CL, Foerster SH, Wynne JE, Tsu I-H, Kenny DE. Evidence for acquired iron overload in captive tapirs (*Tapirus* spp.) *Proc. Annu. Conf. Am. Assoc. Zoo Vet.*, New Orleans LA, pp. 124-126, 2000.

16. Halliwell F, Gutteridge JMC. Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. *Arch. Biochem. Biophys.* 246:501-514, 1986.

17. Britton RS, Tavill AS, Bacon BR. Mechanisms of iron toxicity. In: Brock JH, Halliday JW, Pippard MJ, Powell LW (eds). *Iron Metabolism in Health and Disease.* WB Saunders, Philadelphia, pp. 311-351, 1994.

18. McCord JM. Iron, free radicals, and oxidative injury. *Semin. Hematol.* 35:5-12, 1998.

19. Bothwell TH, Charlton RW, Motulsky AG. Hemochromatosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic and Molecular Bases of Inherited Disease.* McGraw-Hill, New York, 7th ed., pp. 2237-2269, 1995.

20. Weinberg ED. Iron and susceptibility to infectious disease. *Science* 184:952-956, 1974.

21. Payne SM, Finkelstein RA. The critical role of iron in host-bacterial interactions. *J. Clin. Invest.* 61:1428-1440, 1978.

22. Weinberg ED. Iron and infection. *Microbiol. Rev.* 42:45-66, 1978.

23. Paglia DE, Kenny DE, Dierenfeld ES, Tsu I-H. (2001b) Role of excessive maternal iron in the pathogenesis of congenital leukoencephalomalacia in captive black rhinoceroses *(Diceros bicornis). Am J Vet Res* 62:343-349.

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

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

26. Hallberg L. Bioavailability of dietary iron in man. *Ann. Rev. Nutr.* 1:123-147, 1981.

27. Fairweather-Tait SJ. Iron in food and its availability. *Acta Paedeatr.Scand. Suppl.* 361:12-20, 1989.

28. Ghebremeskel K, Williams G, Brett RA, Burek R, Harbige LS. Nutrient composition of plants most favoured by black rhinoceros *(Diceros bicornis)* in the wild. *Comp. Biochem. Physiol.* 98A:529-534, 1991.

29. Dierenfeld ES, duToit R, Bass L. Nutrient composition of selected browses

consumed by black rhinoceroses *(Diceros bicornis)* in the Zambezi Valley, Zimbabwe, and Texas. *J. Zoo Wildl. Med.* 26:220-230, 1992.

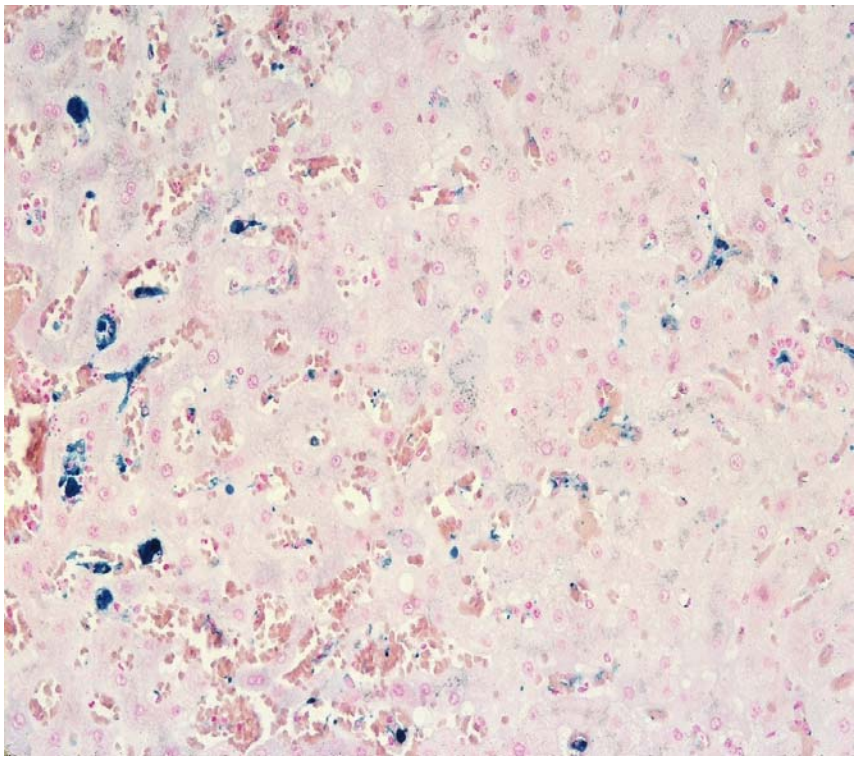
30. Witte DL, Crosby WH, Edwards CQ, Fairbanks VF, Mitros FA. Hereditary hemochromatosis. *Clin. Chim. Acta* 245:139-200, 1996.

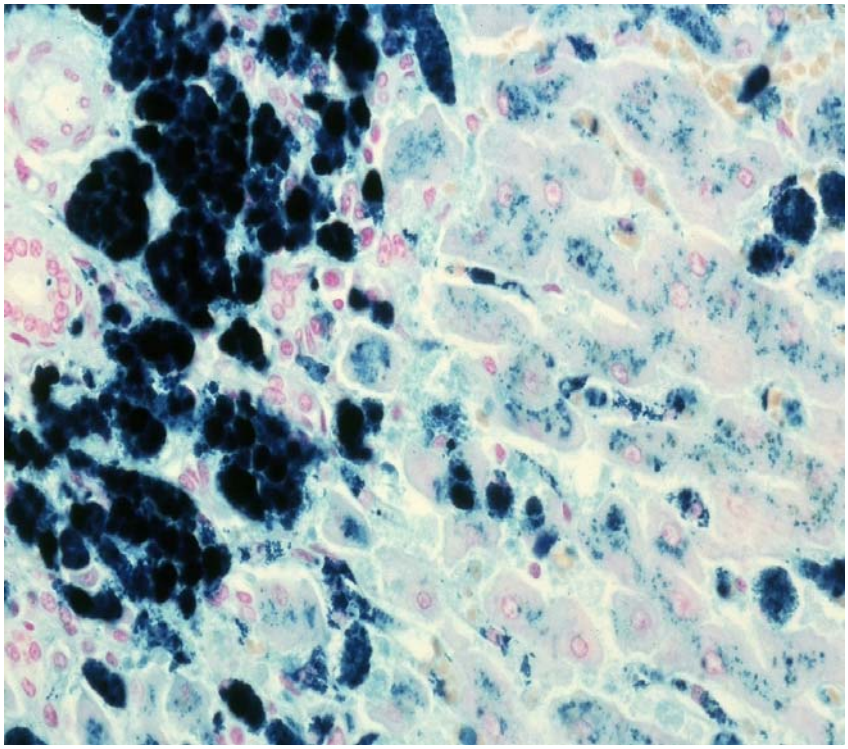
31. Thomas S, Snyder D. Centers for Disease Control (CDC) Guidelines for Hereditary Hemochromatosis. American Hemochromatosis Society, CDC Website, 1998.

32. Paglia DE. Recommended phlebotomy protocol for prevention and therapy of chronic progressive iron toxicity in captive rhinoceroses and tapirs. (Unpublished, 2002).

FIGURE LEGEND

Figure 1. Liver histopathology in a white rhino (first panel) compared to a black rhino (second panel) at necropsy after approximately two decades in captivity. These tissues were treated with Perl's Prussian blue stain to identify ferric iron compounds such as hemosiderin. In the white rhino liver, iron-positive granules are present only in scattered macrophages lining the hepatic sinusoids as they normally phagocytize senescent red blood cells and reprocess their hemoglobin. The uniformly pink liver cells themselves remain free of stainable iron. By contrast, the black rhino liver exhibits dense deposits of iron pigments not only in the sinusoidal and periportal macrophages, but in the hepatic cells themselves, all of which contain abnormal granules and particles of stainable iron.





TABLES

Table 1. Conditions commonly affecting African black rhinoceroses in captivity.

Acute episodic and chronic hemolytic anemia (shortened lifespan of red blood cells)
Superficial necrolytic dematopathy (chronic mucous membrane and skin ulcers)
Susceptibility to common and exotic infections, particularly affecting the lungs (*Salmonella, Leptospira, Mycobacteria, Aspergillus)*
Leukoencephalomalacia (congenital degeneration of central nervous system tissues)
Primary idiopathic or toxic hepatopathy (hepatic cell degeneration and liver failure)
Stress-induced sudden death
Altered phosphorus and calcium metabolism (with possible autoimmune component)
Chronic anemia with cachexia (impaired red blood cell production and body wasting)
Idiopathic hemorrhagic vasculopathy (possible autoimmune disease of microvessels)
Hemosiderosis (extensive deposition of iron pigments in multiple organs)

Table 2. Potential mechanisms by which iron excess might contribute to various disorders affecting African black rhinos in captivity.

PRIMARY EFFECT:

Excess Iron → Increased free radical production → **Cellular oxidative damage** to structural and enzymatic proteins, membranes, organelles, DNA/RNA, etc.

SECONDARY EFFECTS:

Damage to: **Liver Hepatocytes** → Impaired capacity to neutralize toxins, decreased production of specialized proteins, eventual liver failure and death

Red Blood Cells → Acute and/or chronic hemolytic anemia

White Blood Cells → Increased susceptibility to infections (compounded by availability of host iron to invading microorganisms → increased virulence)

Endocrine Cells → Stress intolerance

Heart (Myocardium, Conduction System) → Stress intolerance, sudden death

Blood Vessel Endothelial Cells → Idiopathic hemorrhagic vasculopathy (?)

Embryonic Tissues → Congenital leukoencephalomalacia, gender disparity (?)