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## **HYPOAMINOACIDEMIA IS NOT ASSOCIATED WITH ULCERATIVE LESIONS IN BLACK RHINOCEROSSES, *DICEROS BICORNIS***

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**Abstract:** Ulcerative oral and skin lesions have become an issue of concern for the health of the managed black rhinoceros (rhino) (*Diceros bicornis*) populations. Lesions exhibited by the black rhino are clinically similar to those observed in other species with superficial necrolytic dermatitis (SND). One biochemical alteration in dogs with SND is severe hypoaminoacidemia, and nearly all cases are fatal. The objective of this study was to determine if black rhinos with analogous lesions exhibit a similar hypoaminoacidemia. Amino acid concentrations were measured in monthly plasma samples collected for 1 yr from black rhinos with ( $n = 4$ ) and without ( $n = 34$ ) lesions clinically consistent with SND. The rhinos with skin and/or oral lesions were zoo born males, ages 2, 6, 17, and 23 yr, from four different facilities. Three rhinos recovered from skin ( $n = 2$ ) and oral lesions ( $n = 1$ ). However, the one male with both skin and oral lesions died with the disease. None of the affected black rhinos exhibited a decrease in any of the amino acids evaluated or for total amino acid concentrations ( $P > 0.05$ ). Based on the absence of hypoaminoacidemia and the comparatively low mortality rate in rhinos with lesions, it appears that this syndrome is not entirely consistent with SND observed in other species. These data will be useful for future assessments of rhino nutritional status and other potential metabolic diseases.

**Key words:** Amino acids, black rhinoceros, *Diceros bicornis*, hypoaminoacidemia, skin lesions, superficial necrolytic dermatitis.

### INTRODUCTION

Superficial necrolytic dermatitis (SND) (also called metabolic epidermal necrosis, hepatocutaneous syndrome, or necrolytic migratory erythema) belongs to a group of syndromes in which cutaneous lesions indicate the presence of a glucagon-secreting pancreatic tumor or hepatic disease. In the domestic dog, it is a particularly severe disorder that has been associated with a variety of concurrent health problems, including glucagonomas,<sup>6</sup> diabetes mellitus,<sup>20</sup> hepatic pathologies,<sup>8,13</sup> hyperadrenocorticism and hypothyroidism,<sup>15</sup> diet, and a possible association with phenobarbital administration.<sup>10</sup> Lesions associated with SND also were reported in the cat,<sup>9</sup> red fox,<sup>19</sup> and human<sup>11</sup> but at much lower incidences.

A decade ago, ulcerative lesions were described in black rhinos, and it was suggested that they

exhibited clinical similarities to SND in domestic dogs.<sup>14</sup> The lesions begin as raised plaques, which progressively lead to vesicles and erosions, and finally to ulcers. Ulcers are usually bilaterally symmetrical and tend to expand peripherally, with most lesions seen on pressure points, such as the back, feet, lateral body wall, tail, head, ears, and vulva and/or prepuce. Lesions can occur suddenly, with remissions, or can progress slowly as prolonged eruptions over time. Oral lesions generally are more persistent than skin lesions. Ulcerative lesions in black rhinos contain no viral, bacterial, or fungal components; show minimal inflammation; and often occur in conjunction with other clinical pathologies. In one report, 23 of 34 affected rhinos had concurrent health conditions, including liver disease, anemia, gastrointestinal diseases, respiratory tract infections, and urinary tract disease.<sup>14</sup> Other associated symptoms can include weight loss, lameness, depression, anorexia, weakness, pregnancy, and estrus and/or breeding. A recent survival analysis performed on 296 of 334 black rhinos (88.6%) in the captive United States population (1930–2001) identified that skin lesions were a significant mortality risk.<sup>4</sup> The frequent occurrence of these lesions, compounded with an unknown etiology and the absence of effective treatments, makes this syndrome a serious health concern for the managed black rhino population.

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In the dog,<sup>15</sup> human,<sup>11</sup> and cat,<sup>9</sup> hypoaminoacidemia (i.e., reduced plasma amino acid concentrations) is often associated with SND outbreaks. Low amino acid concentrations may contribute to disrupted epidermal homeostasis, which results in ulcerative lesions forming at the joints, footpads, and other locations susceptible to stress and injury.<sup>15</sup> Normal concentrations of amino acids are essential for the pliability, strength, and hydrophobic nature of the epidermis. By contrast, hypoaminoacidemia can lead to dermal spongiosis, which increases epidermal fragility. The cause of hypoaminoacidemia in animals afflicted with SND is unknown; however, a retrospective study in dogs concluded hypoaminoacidemia was not related to malnutrition but rather to a metabolic hepatopathy that caused an unexpected, sudden increase in the hepatic catabolism of amino acids.<sup>15</sup>

Because of the clinical and histopathologic similarities between the cutaneous lesions observed in black rhinos and those observed in dogs diagnosed with SNDs, the objective of this study was to determine if affected rhinos had the same dramatic reductions in plasma amino acid concentrations as seen in dogs. If they are not significantly lower in rhinos that are exhibiting lesions, it would suggest that the syndrome is distinct from SND in dogs and other species.

## MATERIALS AND METHODS

### Sample collection

Plasma samples were collected approximately monthly for 1 yr from 23 male and 15 female black rhinos at 16 Association of Zoos and Aquariums accredited zoos. Diet, season, age, and sex differences can all impact amino acid metabolism and circulating concentrations.<sup>22</sup> Thus, monthly samples were collected over the course of a year to provide a more reliable estimate of baseline amino acid values. In addition, rhinos were not subjected to fasting or any kind of specialized or restricted diets. Blood was collected into plastic heparinized tubes, centrifuged, and the plasma (2–4 ml) was stored frozen (–20°C) in 1-ml aliquots for amino acid analysis. After 6 mo, acidic degradation of amino acids may result in altered measurements of plasma amino acid concentrations.<sup>22</sup> Therefore, all samples were analyzed within 6 mo of collection. Samples were not deproteinized with sulfosalicylic acid upon collection, so plasma cysteine concentrations were not determined in this study.<sup>16</sup> Personal communication with zoo

veterinarians at each facility determined whether a rhino exhibited ulcerative skin and oral lesions consistent with those previously described.<sup>14</sup>

### Sample collection and analysis

Plasma amino acid concentrations were measured by an automated analyzer (Biochrom 30, Biochrom, Ltd., Cambridge Science Park, Milton Road, Cambridge, CB4 0FJ, United Kingdom) by using cation-exchange chromatography and spectroscopic determination of a ninhydrin reaction with amino acids. Analyses were conducted at the University of California Amino Acid Laboratory (Department of Molecular Biosciences, School of Veterinary Medicine, Davis, California 95616, USA). Norleucine was used as an internal standard to standardize amino acid concentrations across time.

### Data analysis

Monthly plasma amino acid concentrations were averaged for each individual for each amino acid and for total amino acids. A molar ratio of branched chain amino acids (BCAA) to aromatic amino acids (AAA) was calculated for each rhino by using the formula (valine + leucine + isoleucine)/(phenylalanine + tyrosine), and a mean ratio was calculated for each rhino. The mean  $\pm$  standard deviation was calculated for rhinos with and without lesions. *T*-tests determined if there were statistical differences in individual amino acid and total amino acid means between rhinos with lesions and rhinos without lesions. Statistical analyses were conducted by using Intercooled Stata (v. 9.0, StataCorp, LP, College Station, Texas 77845, USA).

## RESULTS

Details on the black rhinos evaluated in this study are presented in Table 1. Four rhinos developed ulcerative lesions during the collection period: two had skin lesions, one had oral lesions, and one had both types of lesions. All rhinos with lesions were captive-born males from different institutions. Three facilities had rhinos with and without lesions, housed in the same or adjacent enclosures. Three of the rhinos with skin lesions had a history of lesion eruption before this study.

The mean concentrations ( $\pm$ SEM) and ranges for all plasma amino acids for rhinos with lesions, without lesions, and for the rhino that had the most severe case of skin and oral lesions are presented in Table 2. There were no differences in

**Table 1.** Summary of the black rhinoceroses, with and without skin and/or oral lesions, evaluated in this study.

	No lesions	Lesions
Eastern subspecies	25	4
Southern subspecies	9	0
No. males, females	19, 15	4, 0
No. zoos	14	4
No. captive born	30	4
No. wild caught	4	0
Mean age (range), yr	16 (2–38)	12 (2–23)
Total no. rhinoceroses	34	4

concentrations of individual plasma amino acids, or total amino acids between rhinos with and without lesions ( $P > 0.05$ ). The amino acid concentrations of the rhino with the most severe case of ulcerative lesions are presented as a visual comparison. There also was no difference ( $P > 0.05$ ) in the BCAA:AAA molar ratio between rhinos with ( $4.4 \pm 0.7$ , mean value  $\pm$  standard deviation [SD]) and without ( $4.5 \pm 0.6$ , mean value SD) lesions. In dogs with SND, the most severe reductions in amino acid concentrations were reported for arginine, glutamine, proline, and threonine ( $<20\%$  of normal).<sup>15</sup> However, neither temporal patterns nor mean concentrations of these amino acids differed between rhinos with and without lesions (e.g., Fig. 1).

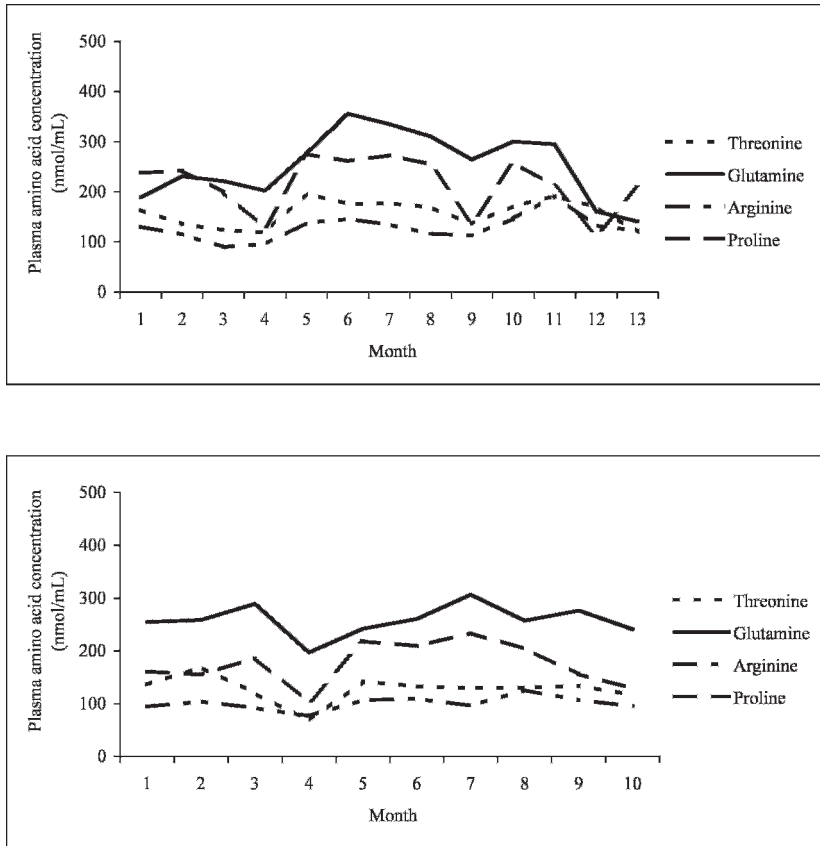
## DISCUSSION

Hypoaminoacidemia was not observed in any of the black rhinos that exhibited ulcerative skin and/or oral lesions during the study period. Individual amino acid concentrations vary greatly among species, which makes direct comparisons difficult. Amino acids in the cat and dog are two to five times higher for some amino acids and two to three times lower for others.<sup>9,15</sup> In the horse (Order: Perissodactyla), a more closely related species to the rhino with similar nutritional requirements and digestive system, plasma concentrations of some amino acids (e.g., asparagine, citrulline, isoleucine, glycine, threonine, tyrosine) were similar to values found in black rhinos, but this was not the case for all amino acids (Amino Acid Laboratory, Department of Veterinary Molecular Biosciences at the University of California, Davis Web page; <http://www.vetmed.ucdavis.edu/vmb/aal/aal.html>). For example, glutamine and serine were approximately double in the horse, whereas glutamic acid and phenylalanine were higher in black rhinos. A study that determined whole blood tyrosine in

free-ranging black rhinos reported concentrations 50 times higher than previously reported in plasma of the same species.<sup>21</sup> This same study reported that whole blood tyrosine concentrations in rhinos were higher than whole blood tyrosine concentrations in horses. The high red blood cell tyrosine concentrations were not reflected in the plasma concentrations of the rhinos in this study.

The lack of hypoaminoacidemia observed in rhinos is in stark contrast to findings in dogs. Dogs diagnosed with SND exhibit marked reductions ( $\leq 60\%$  of normal) in all but four measured amino acids (glutamic acid, phenylalanine, tryptophan, and ornithine), and in the total amino acid concentration (only approximately 30% of normal) compared with healthy individuals.<sup>10,15</sup> The four amino acids associated with the most severe hypoaminoacidemia in dogs (arginine, glutamine, proline, and threonine) also showed no quantitative or qualitative differences between rhinos with and without lesions, which further emphasized the lack of a relationship between amino acid metabolism and the outbreak of ulcerative lesions in rhinos. Affected rhinos exhibited lesions intermittently throughout the collection period, with manifestations that ranged from 1 mo to 1 yr, with no changes in plasma amino acid concentrations as symptoms progressed.

One major difference between rhinos and dogs diagnosed with lesions, is that SND in dogs is nearly always fatal (mean survival, 6.43 mo; range, 2–32 mo).<sup>15</sup> Partial recovery and short-term survivability was achieved only in dogs that were fed additional protein or amino acid supplements, or that were given parenteral amino acid infusions.<sup>15</sup> By contrast, many rhinos with either type of lesion frequently recover from episodes without treatment, although recurrence is common.<sup>14</sup> Of the four affected black rhinos in this study, three had lesions historically, and three have since entered remission. The male with oral lesions exhibited concurrent severe weight loss, fatigue, and anorexia before recovering. Unfortunately, the rhino with both skin and oral lesions died during the study. Before death, this animal displayed neurologic symptoms, including abnormal behavior and confusion. Also noted were intermittent ulcerative lesions on the pressure points, tail, and tongue; alternating foreleg lameness of unknown etiology; arrested growth for the age attained; and bouts of lethargy and mild anorexia. This rhino had persistent jaundice, with increases in serum bilirubin, total protein,



**Figure 1.** Profiles of the four amino acids that exhibit the greatest hypoaminoacidemia in dogs with superficial necrolytic dermatitis<sup>14</sup> for (A) a black rhinoceros that never exhibited lesions and (B) a black rhinoceros with lesions.

serum glutamic oxaloacetic transaminase, alkaline phosphatase, iron, and copper concentrations. The postmortem revealed extensive subcutaneous hemorrhage and edema. The adrenal glands had a focus of subcortical mineralization, with a small number of lymphocytes and plasma cells. Extensive areas of coagulative necrosis in the deep adrenal cortex were surrounded by a rim of congestion, hemorrhage, and degenerate neutrophils and cortical epithelial cells. Upon liver examination, the hepatic cords were disrupted and the hepatocytes were swollen, with marked amounts of perinuclear brown pigment determined to be lipofusion. Several small hematomas were found in the brain and pituitary tissues, and were attributed to the rhino falling before final collapse. The kidney and heart tissues were unremarkable (Nelson, pers. comm.).

Another difference between rhinos and dogs is the age of lesion onset. In dogs, it is primarily older individuals that are afflicted with SND. Thus, age-related hepatic pathologies may be

responsible for the observed hypoaminoacidemia.<sup>6</sup> By contrast, skin and oral lesions occur in black rhinos of all ages,<sup>14</sup> and, in this study, the animals were 2, 6, 17, and 23 yr of age. Some dogs with SND and humans with necrolytic migratory erythema, a syndrome clinically similar to SND, have hyperglucagonemia secondary to a glucagon-secreting pancreatic tumor.<sup>1,2,18</sup> Increased glucagon can stimulate gluconeogenesis and result in increased amino acid catabolism. Plasma glucagon has not been measured in black rhinos, but the lack of hypoaminoacidemia observed in this study suggests that pancreatic tumors are not a likely cause of these lesions. Taken together, the absence of hypoaminoacidemia, reduced mortality, and the different array of concurrent health conditions suggest that the constellation of clinical and biochemical signs observed in black rhinos are different from SND as described in dogs.

In other species, amino acid concentrations can be a valuable diagnostic tool for monitoring or

**Table 2.** Mean plasma amino acid concentrations (nmol/ml)  $\pm$  SEM (range) in black rhinoceroses with and without ulcerative skin and/or oral lesions, and in the rhinoceros that died and that had exhibited the most severe skin and oral lesions.<sup>a</sup>

Amino acid <sup>b</sup>	Rhinoceros without lesions ( <i>n</i> = 34), mean $\pm$ SEM (range)	Rhinoceroses with lesions ( <i>n</i> = 4), mean $\pm$ SEM (range)	Severe lesions ( <i>n</i> = 1), mean $\pm$ SEM (range)
Alanine	262.9 $\pm$ 9.9 (148.0–386.3)	264.0 $\pm$ 33.4 (163.8–301.1)	290.9 $\pm$ 11.1 (269.0–333.7)
Arginine	142.4 $\pm$ 7.4 (93.6–311.2)	134.5 $\pm$ 13.78 (101.0–158.7)	158.7 $\pm$ 9.7 (140.8–189.4)
Asparagine	46.2 $\pm$ 4.5 (6.8–101.6)	79.8 $\pm$ 20.2 (35.6–117.7)	109.8 $\pm$ 9.0 (84.6–133.4)
Aspartic acid	24.4 $\pm$ 2.7 (2.5–67.1)	23.8 $\pm$ 7.7 (6.3–40.6)	40.6 $\pm$ 4.4 (31.2–54.5)
Citrulline	37.1 $\pm$ 1.9 (20.4–63.0)	37.8 $\pm$ 12.6 (15.6–72.2)	15.6 $\pm$ 1.7 (11.1–19.7)
Glutamic acid	149.6 $\pm$ 5.1 (89.4–205.0)	132.5 $\pm$ 20.4 (72.4–160.4)	152.5 $\pm$ 7.2 (131.3–173.6)
Glutamine	265.4 $\pm$ 8.8 (162.4–352.5)	339.5 $\pm$ 38.1 (258.6–435.7)	304.0 $\pm$ 15.8 (275.0–361.1)
Glycine	462.8 $\pm$ 12.9 (333.7–608.9)	427.3 $\pm$ 34.4 (364.3–522.5)	395.2 $\pm$ 18.9 (353.5–448.5)
1-Methyl histidine	6.2 $\pm$ 0.3 (2.6–9.7)	7.3 $\pm$ 0.9 (5.3–8.6)	8.4 $\pm$ 1.2 (5.8–11.4)
3-Methyl histidine	29.6 $\pm$ 18.0 (10.2–91.5)	26.5 $\pm$ 17.6 (10.2–50.3)	25.0 $\pm$ 4.5 (14.1–38.4)
Histidine	92.8 $\pm$ 2.4 (63.3–120.2)	87.0 $\pm$ 5.3 (78.0–102.0)	102.0 $\pm$ 4.3 (90.0–113.6)
Isoleucine	121.3 $\pm$ 3.9 (83.7–172.3)	120.3 $\pm$ 16.1 (95.5–138.0)	138.0 $\pm$ 5.9 (119.5–154.8)
Leucine	206.9 $\pm$ 38.6 (145.6–312.6)	204.0 $\pm$ 32.2 (156.2–223.8)	223.8 $\pm$ 24.7 (188.1–321.3)
Lysine	132.8 $\pm$ 5.7 (83.7–255.3)	125.5 $\pm$ 12.7 (98.9–158.4)	158.4 $\pm$ 9.3 (134.9–189.3)
Methionine	27.0 $\pm$ 1.0 (15.6–38.6)	29.0 $\pm$ 3.8 (22.3–36.1)	36.1 $\pm$ 2.9 (30.8–46.7)
Ornithine	115.2 $\pm$ 11.8 (47.3–466.7)	110.5 $\pm$ 11.4 (90.3–142.3)	111.2 $\pm$ 5.9 (97.0–130.1)
Phenylalanine	103.2 $\pm$ 4.5 (62.3–178.4)	99.5 $\pm$ 7.1 (84.5–115.7)	115.7 $\pm$ 9.5 (100.8–150.6)
Proline	199.5 $\pm$ 10.6 (105.8–336.7)	220.5 $\pm$ 16.8 (175.8–254.8)	254.8 $\pm$ 15.9 (221.5–313.5)
Serine	147.5 $\pm$ 5.5 (74.7–229.3)	175.0 $\pm$ 18.8 (125.2–206.4)	202.2 $\pm$ 9.3 (180.3–233.4)
Taurine	39.4 $\pm$ 2.8 (20.0–97.2)	26.3 $\pm$ 3.3 (18.0–33.7)	28.0 $\pm$ 6.7 (16.7–51.8)
Threonine	163.9 $\pm$ 5.4 (93.6–224.7)	162.5 $\pm$ 17.0 (128.1–208.5)	208.5 $\pm$ 7.8 (194.8–237.3)
Tryptophan	82.6 $\pm$ 3.0 (42.4–111.2)	81.5 $\pm$ 6.1 (67.3–94.6)	87.0 $\pm$ 2.9 (81.8–98.5)
Tyrosine	65.3 $\pm$ 2.6 (41.8–103.7)	68.5 $\pm$ 7.9 (59.0–92.2)	92.2 $\pm$ 6.7 (73.1–109.8)
Valine	411.9 $\pm$ 12.1 (276.7–552.3)	395.8 $\pm$ 27.0 (329.1–459.3)	410.3 $\pm$ 9.0 (394.8–442.7)
Total amino acid values	3344.1 $\pm$ 70.9 (2624.6–4465.3)	3381.0 $\pm$ 153.8 (2962.6–3670.6)	3670.6 $\pm$ 154.4 (3368.4–4252.6)

<sup>a</sup>SEM, standard error of the mean.

<sup>b</sup>*P* > 0.05 for all amino acids.

studying liver or renal diseases,<sup>7</sup> nutritional imbalances,<sup>12</sup> and genetic defects that affect amino acid metabolism.<sup>3</sup> Branched chain amino acids are catabolized in a variety of tissues, whereas the aromatic amino acids are catabolized solely in the liver. As a result, the BCAA:AAA ratio has been used in research and clinical patients to evaluate and monitor some hepatic pathologies, with the ratio decreasing as the severity of hepatic dysfunction or portal-systemic shunting increases.<sup>7,17</sup> There were no differences in the mean concentrations of the BCAA:AAA ratio between the normal and affected animals in this study. Similarly, the mean value for the molar ratio in dogs with SND was 2.6, which did not indicate severe hepatic dysfunction (normal, 3.0–4.0).<sup>15</sup> Although there are no previous data for rhinos, the lack of a difference between the molar ratios of rhino with and without lesions suggests that the lesions were not caused by hepatic pathologies.

Maintaining a sustainable black rhino population and identifying the etiology and developing treatments for the disease syndromes observed in this species (e.g., idiopathic hemorrhagic vasculopathy syndrome, hepatopathy, hemolytic anemia, ulcerative lesions)<sup>5</sup> is currently a high priority of the Association of Zoos and Aquariums Rhinoceros Species Survival Plan Program. Based on the results of this study, ulcerative lesions observed in black rhinos are not consistent with SND described in dogs. Thus, further research is needed to elucidate the physiologic mechanism(s) and pathogenesis of this disease.

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