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Vesicular and Ulcerative Dermatopathy Resembling Superficial Necrolytic Dermatitis in Captive Black Rhinoceroses (*Diceros bicornis*)

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Abstract. The histopathology, clinical presentation, and epidemiology of a cutaneous and oral mucosal disease affecting 40 black rhinoceroses (*Diceros bicornis*) at 21 zoological parks (50% of the captive US population) were investigated. Twenty-seven biopsies were examined from recent lesions, and clinical information was available from 127 episodes. The cutaneous lesions began as plaques that progressed to vesicles, bullae, or ulcers. Lesions waxed and waned in individual cases. Lesions were predominantly bilaterally symmetrical, affecting pressure points, coronary bands, tips of the ears and tail, and along the lateral body wall and dorsum. Oral lesions were first noticed as ulcers and were present on the lateral margins of the tongue, palate, and mucocutaneous junctions of the lips. All recent lesions had similar histopathologic findings of prominent acanthosis, hydropic degeneration of keratinocytes in the stratum spinosum, spongiosis, intraepithelial vesicles, and parakeratosis without dermal inflammation. Chronic lesions were ulcerated. No pathogens were identified by culture or electron microscopy. Most episodes coincided with stress events (transportation, sudden cold temperatures, intraspecific harassment, estrus, advanced pregnancy) or concurrent diseases (toxic hepatopathy, hemolytic anemia, respiratory or urinary tract infections). Affected rhinoceroses usually were lethargic and had weight loss. Affected rhinoceroses also had lower hematocrit, serum albumin, and cholesterol values than captive healthy or wild rhinoceroses. The clinical patterns and histopathologic findings are similar to those of superficial necrolytic dermatitis in dogs and necrolytic migratory erythema in humans. The high prevalence of this skin disease in captive black rhinoceroses under many circumstances suggests that their epidermis is acutely sensitive to any disruption of metabolic homeostasis. We propose that metabolic changes secondary to a stress response from maladaptation or nutritional inadequacy of captive diets may contribute to the development of this disease in rhinoceroses without hepatopathies.

Key words: Black rhinoceros; eosinophilic granulomas; hepatocutaneous syndrome; necrolytic migratory erythema; skin; superficial necrolytic dermatitis; ulcerative skin disease.

Efforts to sustain viable global populations of black rhinoceroses (*Diceros bicornis*) through captive breeding programs have been hindered by the significant health problems of rhinoceroses in captivity.[40] The most prevalent disease afflicting captive black rhinoceroses is a dermatologic and mucosal condition characterized by recurrent plaques, vesicles, and ulcers.[11,23,32,42,43,52] This skin and mucosal disease has not been identified in wild black rhinoceroses and is not associated with *Stephanofilaria dinniki* infestations, as are most ulcers in wild rhinoceroses.[30,48,56] Other species of rhinoceros are only rarely affected (L. Munson and R. E. Miller, personal observation). Because this skin disease causes considerable morbidity and can contribute to mortality in endangered black rhinoceroses, a collaborative study was initiated to better characterize this condition. We report herein that the disease has the clinical and pathologic features of superficial necrolytic dermatitis.

Materials and Methods

Study population

Biopsies were obtained from twenty-one black rhinoceroses that acquired skin plaques, vesicles, or acute ulcers from 1989 to 1994. The clinical and pathology records of black rhinoceroses with a history of similar plaques, vesicles, or ulcers during the period from 1972 (first available clinical records) until 1994 in US zoological parks participating in the American Zoo and Aquarium Association (AZA) Black Rhinoceros Species Survival Plan (SSP) also were reviewed. From historical records, an additional 19 rhinoceroses were selected based on having analogous clinical presentations or similar histopathologic findings in biopsies. Combined, the 21 recently biopsied and 19 historic cases resulted in 40 affected rhinoceroses in the study. These 40 rhinoceroses represent approximately 50% of the US captive population.

The affected population included 19 male and 21 female rhinoceroses, ranging from 1 to 39 years of age (1–5 years, $n = 4$; 6–15 years, $n = 11$; 16–30 years, $n = 21$, >30 years,

n = 4), from 21 zoological parks. Of 36 captive-born rhinoceroses, 21 animals were offspring of wild parents and 15 animals were ≥2 generations removed from the wild.[26] Thirty-one affected rhinoceroses were East African subspecies (*D. bicornis michaeli*), and nine rhinoceroses were southern African subspecies (*D. bicornis minor*). These numbers reflect the approximate proportion of each subspecies in the captive population.

Clinical information on affected rhinoceroses was obtained from the medical records and the responses to a questionnaire that was sent to veterinarians at AZA Black Rhinoceros SSP participating zoos. The character, topographic location, and month of initial appearance of skin lesions, number of disease events, treatment outcome, clinical course, and concurrent diseases were recorded. To evaluate patterns of clinical disease, the clinical data were grouped by episode; a single episode was defined as the occurrence of new skin lesions or the reccurrence of a former lesion at least 1 month after resolution of previous lesions. Treatment outcomes were considered positive if lesions resolved within 14 days. Clinical chemistry and hematologic values from blood drawn within 1 week of an episode were included in population statistics. In cases with serial samples within an episode, the first and last samples from each episode were included. Values from affected rhinoceroses were compared with values from healthy captive or wild rhinocersoses, but no statistical analyses were performed because blood analyses were conducted in different laboratories. Appropriately stored serum or plasma samples from affected rhinoceroses during disease episodes were not available for glucagon or amino acid assays.

Diet information was available from 15 zoos that participate in the Black Rhinoceros SSP.[38] Alfalfa hay was the principal dietary component in 12 zoos, and four zoos fed primarily mixed timothy hay. Two zoos offered browse, including different types of *Acacia* spp. and *Ficus* spp. Supplementation with grain and premixed concentrates varied widely among zoos and within the same zoo over time and consisted of hoofstock or herbivore pellets (eight zoos) or horse chow (five zoos). Other grains, bread, fresh vegetables, and fruit supplemented the hay diets at some zoos. Mineral salt supplements also were offered in 12 zoos.

Biopsies

Twenty-seven skin biopsy samples from 21 rhinoceroses were available for histopathology, and biopsies from eight of these rhinoceroses were examined ultrastructurally. Skin or oral mucosal biopsies were derived from the margins of acute and chronic lesions. Full thickness wedge skin samples were fixed in 10% buffered formalin or 4% glutaraldehyde in 0.1 M sodium cacodylate at pH 7.4. For histopathology, the tissues were embedded in paraffin, sectioned at 7 μm, and stained with hematoxylin and eosin (HE). For electron microscopy, glutaraldehyde-fixed sections were embedded in paraffin, sectioned, and stained with HE to identify the specific areas of affected epithelium by light microscopy. The lesion site then was excised from the paraffin block, deparaffinized, postfixed in 2% OsO_4, embedded in Epon, sectioned at 50 nm, stained with lead citrate and uranyl acetate, and examined on a Phillips 301 electron microscope.

Immunohistochemistry

Nine biopsy samples that had cytoplasmic inclusions in keratinocytes were stained for cytokeratins. The 7-μm sections were deparaffinized, dehydrated through graded alcohols, incubated with 0.3% hydrogen peroxide in methanol for 10 minutes to block endogenous peroxides, and washed in phosphate-buffered saline. Tissues then were preincubated in 1% normal goat serum to block nonspecific immunoglobin binding and incubated in prediluted mouse anti-pancytokeratins (Biogenex Laboratories, San Ramon, CA) for 30 minutes at room temperature. Antibody binding was visualized with anti-mouse immunoglobulin–strepavidin–biotin–peroxidase (Biogenex Laboratories) according to manufacturer's directions and with diaminobenzidine as a chromogen.

One biopsy of an epidermal vesicle was examined by indirect immunofluorescence for the presence of autoantibody using anti-rhinoceros IgG and whole serum (provided by H. Chaplin, Washington University School of Medicine, St. Louis, MO) and anti-horse IgG and IgM. Another biopsy was examined for autoantibody using anti-porcine IgG, and binding was visualized by fluoresceinated protein G.

Microbial cultures

Primary cell lines of black rhinoceros dermal fibroblasts (provided by O. Ryder) and white rhinoceros (*Cerótotherium simum*) dermal fibroblasts and kidney cell lines (provided by L. Munson) were used for virus isolation. Skin samples or vesicle fluid contents from four recently affected rhinoceroses were shipped on dry ice, minced, and applied to subconfluent cell monolayers. Cells were passaged four times and examined for cytopathic effects, and the final passage was negatively stained with 3% phosphotungstic acid and examined on a Phillips electron microscope for virus particles. Standard aerobic bacterial cultures were performed from cutaneous lesions of 11 rhinoceroses.

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Fig. 1. Skin; black rhinoceros. Gross appearance of the vesicular stage of superficial necrolytic dermatopathy and more chronic expanding ulcerative lesions on the hips, stifles, and hocks.

Fig. 2. Skin; black rhinoceros. Closer view of ruptured and intact epidermal vesicles from Fig. 1.

Fig. 3. Skin; black rhinoceros. Gross appearance of the chronic ulcerative stage of superficial necrolytic dermatopathy bilaterally on the stifles, hocks, and coronary bands.

Fig. 4. Skin, coronary band; black rhinoceros. Closer view of chronic ulcerative and proliferative lesions of superficial necrolytic dermatopathy.

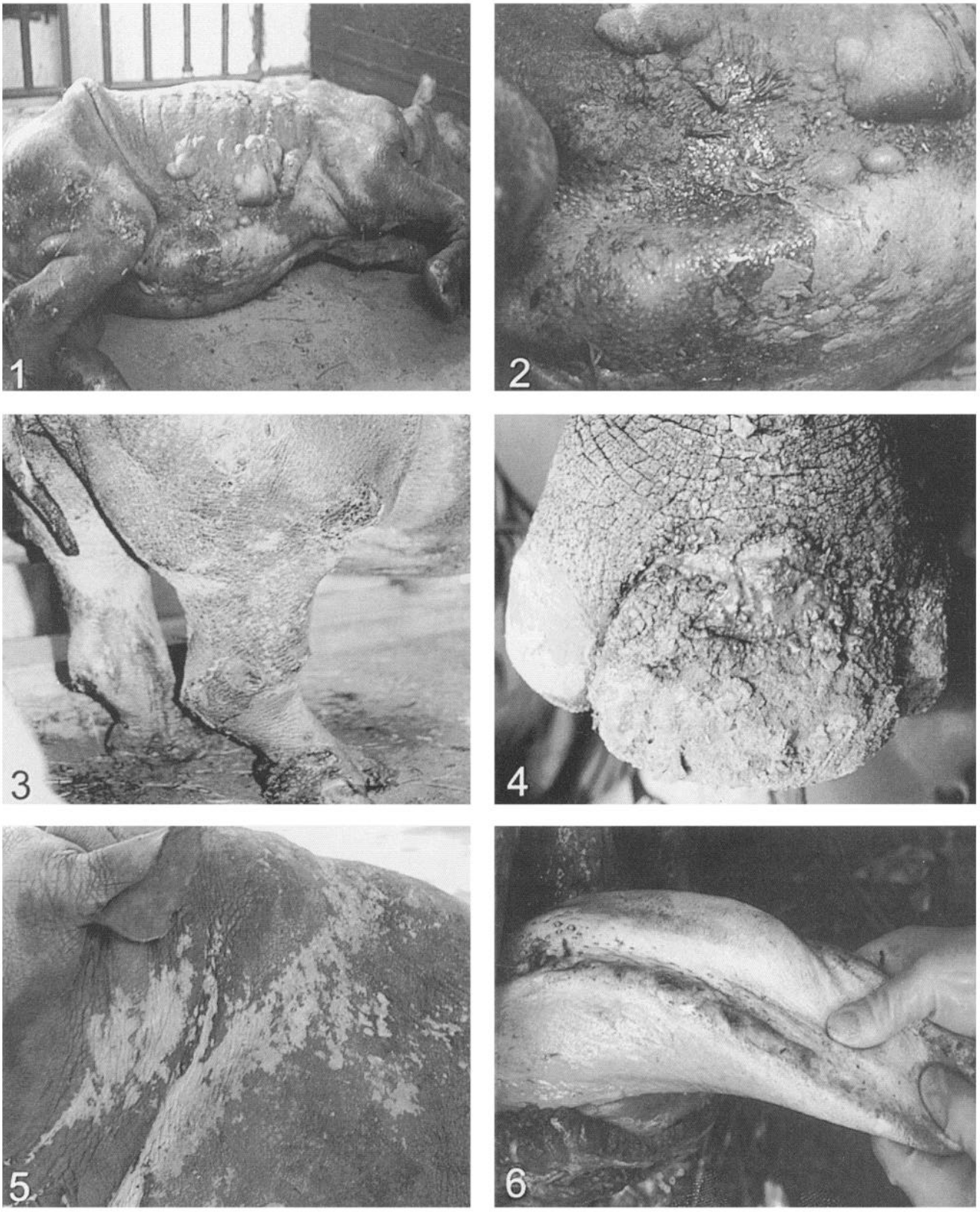

Fig. 5. Skin; black rhinoceros. The exfoliative form of superficial necrolytic dermatopathy in which large sheets of superficial epidermis are sloughed.

Fig. 6. Tongue; black rhinoceros. With the oral pattern of superficial necrolytic dermatitis, the ulcers are located along the lateral margins of the tongue in contact with the teeth and on the prehensile portion of the lips (not shown).

Table 1. Most significant lesions in 23 black rhinoceroses that died with concurrent superficial necrolytic dermatopathy.

Cause of Death	No. Rhinoceroses
Pulmonary or systemic mycosis	6
Toxic hepatopathy	5
Chronic or acute hemolytic anemia	3
Pulmonary tuberculosis	2
Chronic renal disease	1
Bacterial pneumonia	1
Hepatic hemangiosarcoma	1
Leukoencephalomalacia	1
Anemia from uterine adenocarcinoma	1
Salmonellosis	1
Euthanized due to chronic dermatopathy	1

Results

Clinical findings

One hundred twenty-seven episodes of epidermal disease were noted in the 40 rhinoceroses during the study period. Cutaneous lesions evolved through characteristic stages, beginning as raised plaques that progressed to erosions or ulcers (Figs. 1–4). Many, but not all, cases had an intermediate vesicular or bullous stage. In some rhinoceroses, erosions or ulcers were the first clinical sign noted. Some cases had extensive regional superficial epidermal exfoliation (Fig. 5), and a few rhinoceroses had small transient subcorneal pustules. Chronic ulcers expanded peripherally and developed thick rounded edges, described as vegetative or wartlike. The evolution of lesions in the oral and nasal cavities was not observable. However, ulcers present at these sites (Fig. 6) had raised edges that became protuberant, fungate, and/or hemorrhagic in chronic cases.

Clinical episodes were characterized by sudden onset, followed by spontaneous remission and exacerbation of lesions. Most cases had a prolonged clinical course with eruption over time of lesions at multiple sites and slow reepithelialization of ulcers. Seventeen of the 40 rhinoceroses had recurring episodes (2–22 episodes/rhinoceroses; eight of the 17 rhinoceroses had more than seven episodes), and 23 rhinoceroses had a single episode (from 3 days to several months in duration). Thirteen of these 23 rhinoceroses had unresolved lesions at death, and 10 other rhinoceroses died during an episode (Table 1). Two rhinoceroses with the most persistently recurring disease (13 and 22 episodes) had predominantly oral lesions.

Epidermal lesions were located at pressure points (51 episodes), along the back (18 episodes), and on the coronary bands or feet (17 episodes), lateral body (15 episodes), tail (13 episodes), head (seven episodes), ears (eight episodes), and vulva/prepuce (four episodes) (Fig. 7). Lesions at the pressure points and on the ears and feet typically were bilaterally symmetrical in distribution. Twenty-two episodes involved lesions on the lips, and 55 episodes involved the oral cavity. Oral cavity lesions, when visible, were located on the tongue (particularly at the lateral margins; Fig. 6), hard palate, gingiva, and/or buccal mucosa. Lesions were noted in the nasal cavities or nostrils during seven episodes, and five other episodes involved epistaxis without visual identification of a lesion. One rhinoceros had nasal lesions only, whereas all other rhinoceroses with nasal lesions also had lesions at other sites.

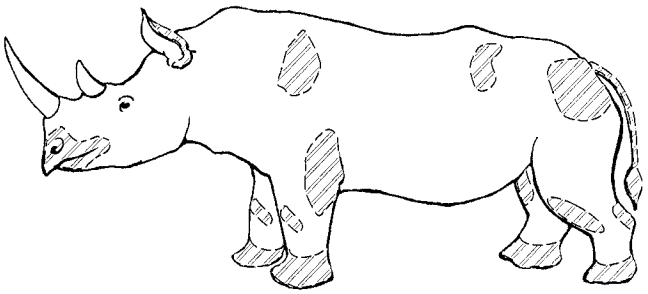

Fig. 7. Distribution of skin lesions; black rhinoceros. Cross-hatched areas indicate most common sites of lesions, which were usually bilaterally symmetrical.

Histopathologic findings

In recently developed lesions and at the margins of chronic ulcerated lesions, the pathologic changes were limited to the epidermis or oral mucosa and were comparable in all affected rhinoceroses. The epithelium had marked hyperplasia with deep, branching, and anastomosing rete pegs (Figs. 8, 9) in comparison with normal rhinoceros skin (Fig. 10). The superficial stratum spinosum had laminar spongiosis and moderate to severe hydropic degeneration of keratinocytes. There was hypogranulosis and regional to diffuse parakeratosis. In cases with severe spongiosis, intraepithelial clefts, vesicles, or bullae formed, many of which contained proteinaceous fluid. The epithelium overlying these vesicles was necrotic and in many cases had exfoliated, resulting in erosions and sometimes ulcers. Most intraepithelial vesicles were acellular or contained a few polymorphonuclear leukocytes. No intravesicular acanthocytes were noted. Also, no autoantibodies were identified by indirect immunofluorescence in the two cases examined.

Additional epidermal and mucosal changes noted in some newly developed lesions were disorganization of the stratum spinosum, dyskeratosis, and rarely indistinct eosinophilic cytoplasmic inclusions in keratinocytes. Individual rhinoceroses sometimes also had prominent superficial crusts consisting of cornified

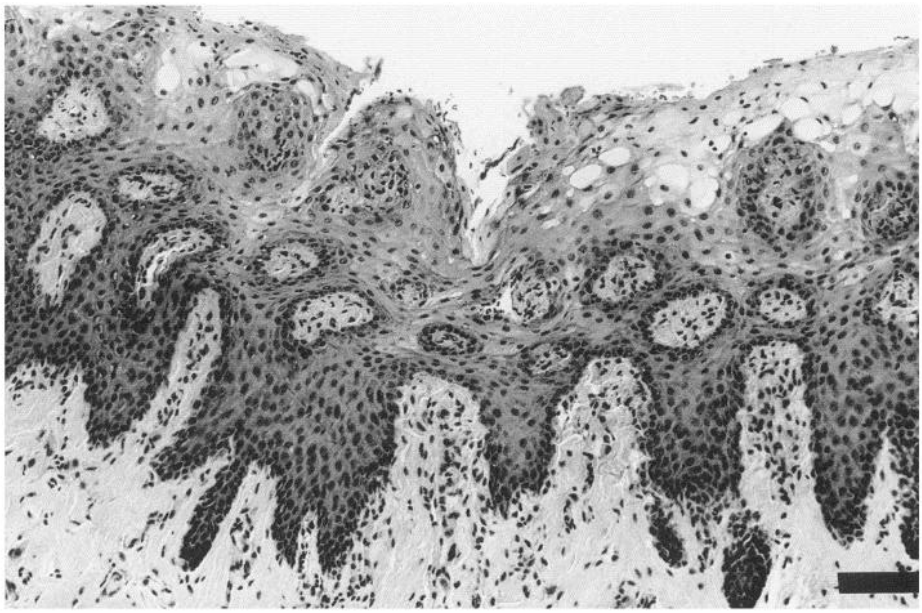

Fig. 8. Skin; black rhinoceroses. Subacute lesions occurred with toxic hepatopathy (secondary disease). The epidermis has acanthosis, hydropic degeneration of the stratum spinosum with early cleft formation, hypogranulosis, and parakeratotic hyperkeratosis, and the dermis has marked neovascularization and no inflammation. HE. Bar = 100 μm.

cells, fibrin, neutrophils, eosinophils, and bacterial colonies or had prominent subcorneal pustules. Biopsies from rhinoceroses with extensive clinical exfoliation were composed of sheets of parakeratotic superficial epithelium.

Pathologic changes in the dermis were minimal or absent in newly developed lesions; the most common findings were edema and hemorrhage, telangiectasia, and neovascularization in the dermal papillae. Inflammatory changes were notably minimal except in the dermis subjacent to ulcers.

Chronic lesions had more extensive ulcers with deep beds of mature granulation tissue in the subjacent dermis or submucosa, but the changes of acanthosis, spongiosis, hydropic degeneration, and parakeratosis were always present at the ulcer margins. In seven cases, chronic ulcers had marked eosinophilic infiltrates and collagen degeneration with mineralization in the adjacent dermis, and four rhinoceroses had eosinophilic granulomas in the deep dermis and subcutis subjacent to their ulcers.

Ultrastructural changes in the epidermis of recently developed lesions were nonspecific. Keratinocytes from the superficial stratum spinosum had mild to marked hydropic degeneration, disorganization and aggregation of microtubules and microfilaments, and intercellular edema (Fig. 11). Keratinocytes in areas of severe spongiosis and overlying the intraepithelial vesicles were necrotic. Some keratinocytes had small irregular clusters of keratin filaments. The larger eosinophilic cytoplasmic inclusions noted in keratinocytes by light microscopy had ultrastructural characteristics of intermediate filament aggregates and were confirmed by immunohistochemistry to be cytokeratins.

Microbiologic findings

All viral cultures were negative for cytopathic effects, and no viral particles were identified in either cell cultures or degenerate keratinocytes by electron microscopy. Aerobic bacteria isolated from lesions included *Staphylococcus* spp. (five rhinoceroses), *Streptococcus* spp. (five rhinoceroses), *Escherichia coli* (three rhinoceroses), and *Pseudomonas aeruginosa, Enterobacter cloacae, Aeromonas hydrophila, Peptostreptococcus* sp., and *Bacillus* sp. from single episodes. *Cephalosporium* sp. was cultured from one lesion, but no fungi were identified in the lesions by light microscopy.

Clinical pathology

Serum chemistry, electrolytes, and hematology values for affected rhinoceroses are compared with values for unaffected captive[22] and wild[28] black rhinoceroses in Tables 2 and 3. Affected rhinoceroses had lower hematocrit, serum albumin, and cholesterol values and lower calcium concentrations than did healthy captive or wild rhinoceroses. Affected rhinoceroses had higher white blood cell counts and higher chloride, serum urea nitrogen, globulin, total bilirubin, gamma glutamyl transpeptidase (GGT), and aspartate aminotransferase (AST) values than did healthy captive or wild rhinoceroses and higher lactate dehydrogenase (LDH)

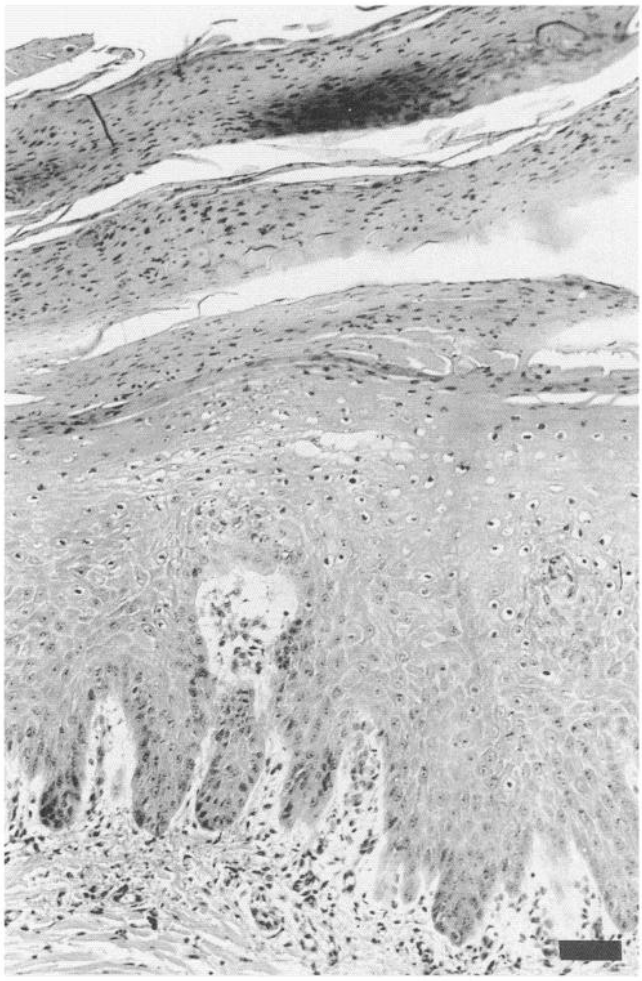

Fig. 9. Skin; black rhinoceros. Chronic recurrent lesions were present during sudden cold temperatures (primary disease). The epidermal changes of lesions arising without hepatopathy are similar to those in Fig. 8, although they are more severe and chronic. HE. Bar = 100 μm.

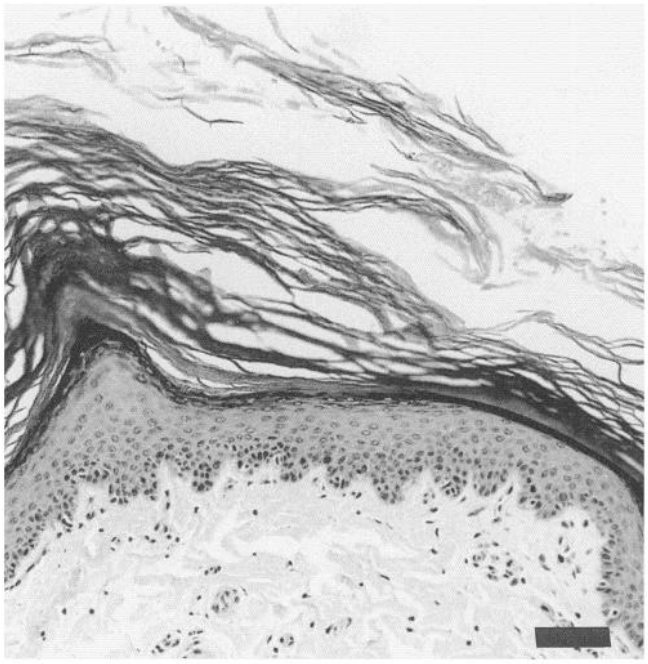

Fig. 10. Skin; black rhinoceros. This animal had no current or previous skin disease. HE. Bar = 100 μm.

and alanine aminotransferase than did healthy captive rhinoceroses. When toxic hepatopathy cases were excluded from analyses, only LDH, GGT, and AST were higher than mean values for healthy captive rhinoceroses, but all were within ranges considered normal for captive rhinoceroses. Five affected rhinoceroses had serum phosphorus levels of <3.0 mg/dl during one or more episodes, but these rhinoceroses also had serum phosphorus levels within the reference ranges during other episodes. The mean level of serum phosphorus for the population of affected rhinoceroses was slightly below values for unaffected captives, but higher than values for healthy free-ranging rhinoceroses.

Concurrent diseases or events

Twenty-three of the 34 rhinoceroses with detailed clinical histories had systemic disease or other notable clinical conditions coincident with the appearance of the epidermal or oral lesions. Concurrent diseases included anemia (17 rhinoceroses), gastrointestinal diseases (14 rhinoceroses, including seven rhinoceroses with gastrointestinal ulcers), liver disease (eight rhinoceroses, including five rhinoceroses with severe toxic hepatopathy), respiratory tract infections (eight rhinoceroses, including five rhinoceroses with mycotic pneumonia), and urinary tract diseases (six rhinoceroses). The most significant lesion of the 23 rhinoceroses that died or were euthanatized during an episode are outlined in Table 1. Also reported were notable weight loss (12 rhinoceroses), unexplained lameness (12 rhinoceroses), depressed attitude (11 rhinoceroses), anorexia (seven rhinoceroses), and weakness (three rhinoceroses). Eight rhinoceroses were pregnant at the time of lesion development, and disease episodes also occurred concurrently with estrus or breeding in two other rhinoceroses. Six rhinoceroses developed cutaneous disease immediately after transportation or introduction of a new rhinoceros into the enclosure, which resulted in intraspecies aggression.

Geographic distribution and seasonality

Affected rhinoceroses were housed at 21 zoological parks distributed through all geographic regions of the USA that house rhinoceroses (Fig. 12). Twelve zoos had multiple cases, and nine zoos with multiple rhinoceroses had only one rhinoceros affected. Disease episodes occurred throughout the year, with a trend toward more cases during cold months (40% of the

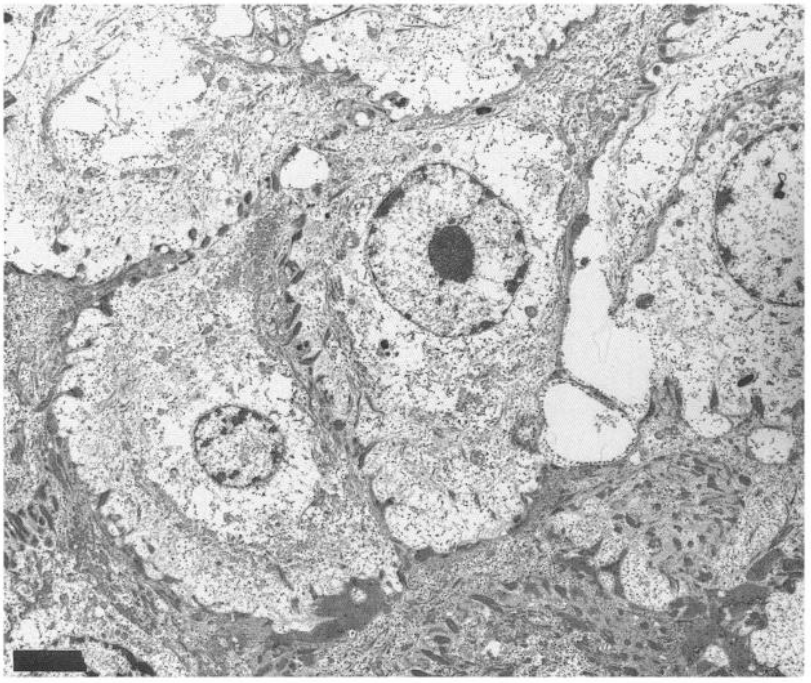

Fig. 11. Transmission electron micrograph. Skin, superficial stratum spinosum; black rhinoceros. The keratinocytes have hydropic degeneration, no keratohyalin granules, and intercellular edema. Lead citrate and uranyl acetate. Bar = 5 μm.

Table 2. Serum chemistry values for captive black rhinoceroses with vesicular and ulcerative dermatopathy (SND), healthy captive rhinoceroses,[22] and healthy wild rhinoceroses.[28]

	SND		Healthy Captive		Healthy Wild	
Serum Component	$\bar{x}$ ± SD	n*	$\bar{x}$ ± SD	n	$\bar{x}$ ± SD	n
Glucose (mg/dl)	86.8 ± 34.8	23	80 ± 29	78	97.3 ± 49.23	54
Sodium (mEq/liter)	130.2 ± 8.6	18	131 ± 3	65	133.5 ± 6.6	35
Potassium (mEq/liter)	4.8 ± 1.2	20	4.6 ± 0.5	71	4.39 ± 0.5	43
Chloride (mEq/liter)	98.1 ± 7.8	20	96 ± 3	70	94 ± 4.5	45
Calcium (mg/dl)	11.1 ± 1.1	22	12.4 ± 0.08	79	11.5 ± 1.1	52
Phosphorus (mg/dl)	4.06 ± 1.5	25	4.5 ± 1.2	79	3.7 ± 1	52
Urea nitrogen (mg/dl)	15.5 ± 6.3	22	13 ± 3	78	9.9 ± 2.2	52
Creatinine (mg/dl)	1.10 ± 0.28	22	1.2 ± 0.2	74	1.17 ± 0.3	53
Uric acid (mg/dl)	0.45 ± 0.27	6	0.6 ± 0.3	34	nd†	
Total protein (g/dl)	8.38 ± 1.5	22	7.9 ± 1	45	8.4 ± 0.6	83
Albumin (g/dl)	2.16 ± 0.5	18	2.7 ± 0.3	33	3.6 ± 0.4	49
Globulin (g/dl)	6.67 ± 1.2	15	5.2 ± 0.6	45	4.6 ± 0.5	26
Cholesterol (mg/dl)	73.8 ± 24.6	16	96 ± 41	73	90 ± 22.9	51
Total bilirubin (mg/dl)	1.77 ± 3.8	22	0.30 ± 0.2	78	0.43 ± 0.2	51
Lactate dehydrogenase (IU/liter)	847.8 ± 969.2	19	364 ± 152	69	1097 ± 371.5	44
γ glutamyl transpeptidase (IU/liter)	50.8 ± 45.6	16	32 ± 20	17	19.4 ± 3.8	41
Alanine aminotransferase (IU/liter)	21.0 ± 18.1	17	13 ± 7	74	24 ± 8.5	54
Aspartate aminotransferase (IU/liter)	137.1 ± 94.9	22	80 ± 37	79	82 ± 23.5	54
Alkaline phosphatase (IU/liter)	117.2 ± 168.8	21	106 ± 174	74	217 ± 251.4	44

* n = number of rhinoceroses.
† nd = not determined.

Table 3. Hematologic values for captive black rhinoceroses with vesicular and ulcerative dermatopathy, healthy captive rhinoceroses,[22] and healthy wild rhinoceroses.[28]

Hematologic Measurement	SND		Healthy Captive		Healthy Wild	
	$\bar{x} \pm$ SD	n*	$\bar{x} \pm$ SD	n	$\bar{x} \pm$ SD	n
White blood cell count $\times 10^3$	12.22 ± 7.27	18	9.84 ± 3.1	85	11.5 ± 4.1	85
Red blood cell count $\times 10^6$	4.79 ± 3.8	17	4.17 ± 1.1	82	5.26 ± 0.6	84
Hemoglobin (g/dl)	12.16 ± 5.04	17	12.6 ± 2.8	82	16.1 ± 1.8	84
Hematocrit (%)	33.32 ± 13.7	18	35.7 ± 7.8	87	43 ± 5.1	87
Mean corpuscular volume (fl)	91.9 ± 11	17	87.1 ± 10.6	82	82.5 ± 7.2	81
Mean corpuscular hemoglobin (pg)	32.38 ± 3.2	15	30.8 ± 3.4	82	30.9 ± 2.3	82
Mean corpuscular hemoglobin concentration (%)	35.57 ± 1.5	15	35.5 ± 1.7	82	37.7 ± 4	83

*n = number of rhinoceroses.

cases occurred between December and March; 13% occurred in December alone).

Response to treatment

Treatments included topical antimicrobials (23 rhinoceroses), systemic antibiotics (15 rhinoceroses), and systemic corticosteroids (seven rhinoceroses), as well as hydrotherapy, moisturizing salves, and topical vitamins. Clinical improvement was variable regardless of treatment, and many lesions resolved without treatment.

Discussion

The epidermal disease in all rhinoceroses in this study had a similar clinical presentation with 1) a sudden onset of plaques that progressed to an intermediate stage of vesicles or bullae or directly to ulcers, 2) exacerbations and spontaneous remissions of lesions, and 3) a bilaterally symmetrical distribution of lesions at pressure points and peripheral locations of the body. Cutaneous and oral lesions in all biopsied rhinoceroses had similar histopathologic changes of acanthosis, laminar hydropic degeneration of epithelial cells and spongiosis in the stratum spinosum, hypogranulosis, parakeratosis, and no inflammatory response. This clinical presentation and these pathologic findings are distinctive characteristics of superficial necrolytic dermatitis (SND) in dogs[15–17,25,39,57] and necrolytic migratory erythema (NME) in humans.[1,24,35,51] However, the high prevalence of this disease in the captive black rhinoceros population is not typical of either NME or SND. The widespread occurrence of SND in black rhinoceroses may indicate that this disease represents an epidermal sensitivity to a variety of metabolic changes that initiate structural and functional modifications resulting in increased skin fragility.

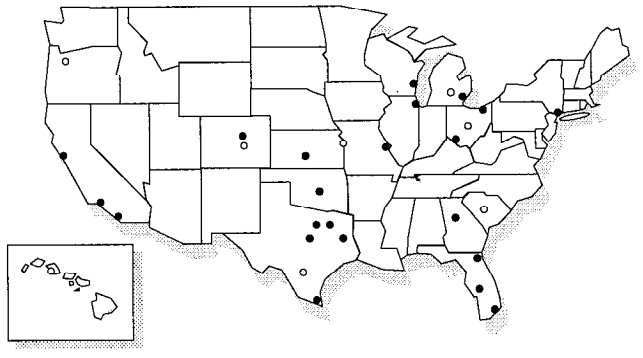

Fig. 12. Geographic distribution of black rhinoceroses with skin disease. Open circles are zoos with unaffected rhinoceroses, and filled circles are zoos with affected rhinoceroses.

In dogs or humans, the metabolic disorders that result in SND or NME are associated with factors that cause abnormal glucose metabolism and hypoaminoacidemia. In dogs, cases of SND have been associated with functional pancreatic islet α-cell neoplasms (glucogonomas),[16] diabetes,[57] liver disease,[39] or poor diets.[4–6] In humans, NME occurs almost exclusively with hyperglucagonemia from a functional glucagonoma, although identical skin lesions have been reported recently in humans with liver disease, pancreatic insufficiency, gluten-sensitive enteropathy, and nutritional deficiencies, such as vitamin B_3 deficiency and kwashiorkor.[35,37,51,54,55] High glucagon levels cause this lesion by inducing hypoaminoacidemia through prolonged gluconeogenesis that depletes serum amino acids.[1,24,51] No rhinoceroses with SND that were necropsied had glucogonomas nor is the high prevalence of this disease in the captive rhinoceros population (50% of US population) consistent with a neoplastic source of glucagon. Unfortunately, we were unable to determine if glucagon or amino acid levels were abnormal because properly handled sera were not available from affected animals.[21]

SND has been noted in dogs with liver disease (hepatocutaneous syndrome),[17,39] and five rhinoceroses in our study and one other rhinoceros[52] had severe toxic

hepatopathy from creosote exposure.[31] Some rhinoceroses with SND without confirmed liver disease had serum levels of LDH, GGT, and AST near the upper limits of reference ranges for captive rhinoceroses, suggesting some hepatic dysfunction. Low serum albumin in affected rhinoceroses also was compatible with liver disease, but these low values also may reflect losses from exudation through skin lesions. Six rhinoceroses had chronic renal disease, which also can alter glucagon metabolism or cause protein loss. However, these confirmed and potential cases of liver and kidney disease do not account for the majority of cases in the black rhinoceros population. A nonhepatic metabolic derangement or dietary inadequacy leading to essential amino acid or fatty acid deficiencies are more probable causes.

Black rhinoceroses have both behavioral and metabolic traits and dietary preferences that distinguish them from white rhinoceroses, a species in which SND is only rarely noted (L. Munson and R.E. Miller, personal observation). For example, black and white rhinoceroses differ markedly in their response to stress,[27,29] and many black rhinoceroses developed epidermal lesions during periods of physical or environmental stress, such as capture, transportation, intraspecific conflicts, or sudden cold temperatures (M. Kock, personal communication).[7,29,32,43,50] A morphologic indicator of chronic stress is adrenal cortical hyperplasia, which has been noted in many captive rhinoceroses (L. Munson, unpublished) in contrast to wild black rhinoceroses (N. Kock, personal communication). Because stress elevates serum catecholamines and glucocorticoids, which then increase the release of glucagon from α-cells,[7,34,36,58] a prolonged endocrine response to stress could result in hyperglucagonemia and the catabolic state that results in SND in other species.[33] Catecholamines also increase during estrus and in late pregnancy in other species,[34] and rhinoceroses developed lesions under these physiologic conditions. Other common findings in rhinoceroses with SND, such as hypocholesterolemia, hypoalbuminemia, normocytic normochromic anemia, depression, cheilitis, anorexia, and weight loss,[8,19,33] are also compatible with the catabolic state induced by hyperglucagonemia, although these abnormalities also could have other causes.

The high prevalence of SND in captive but not wild black rhinoceroses may also implicate captive diets as the cause. Hypoaminoacidemias, essential fatty acid deficiencies, and micronutrient deficiencies that cause SND in other species could occur through inappropriate diets. Black rhinoceroses differ from the rarely affected white rhinoceroses in that they are complex browsers, whereas white rhinoceroses are grazers. Black rhinoceroses have been observed in the wild to browse from more than 200 species of plants representing 49 botanical families and have preferences for specific plants.[10,13,14,41] Diets for captive rhinoceroses consist principally of hay, a diet more appropriate for white rhinoceroses. Black rhinoceroses also have unique digestive and nutrient absorptive characteristics and distinctive red blood cell metabolism,[44,45] and the essential amino acid, fatty acid, and micronutrient requirements needed by black rhinoceroses to compensate for these traits may not be met by captive diets. Poor nutrient bioavailability has been associated with SND in dogs on poor diets (generic dog food dermatosis)[15,53] and in humans with malabsorption of zinc, fatty acid deficiencies, or kwashiokor,[1,24,35,55] indicating that SND can have a dietary basis.

Whether caused by poor diets or hormonally based metabolic derangements, hypoaminoacidemia has been the common denominator underlying SND in dogs and NME in humans[2,17,19] and may be the underlying problem in black rhinoceroses. Low protein has been previously noted in rhinoceroses with these lesions,[43] and serum albumin levels were low in the rhinoceroses in our study, although low albumin may reflect losses from other causes. The epidermis would be a likely tissue to express marginal amino acid deficiencies because of its continuous growth and distinctive requirements of the histidine- and lysine-rich keratohyalin granules in the stratum corneum.[1] Marginal levels of amino acids essential for the pliability, strength, and hydrophobic barrier of the epidermis could result in spongiosis and increased fragility. The distribution of lesions at sites of epidermal tension and trauma is consistent with this hypothesis. Also, the hypogranulosis noted in affected rhinoceroses provides morphologic evidence that the keratohyalin granules (which provide epidermal strength by cross-linking the cytoskeleton with the cell membrane) were not forming. Determining normal amino acid levels in healthy wild rhinoceroses will be essential to ascertain if affected captive rhinoceroses have hypoaminoacidemia.

Other dietary deficiencies, such as those of phosphorus and vitamin E, have been suggested as the basis of these lesions in black rhinoceroses,[6,32,43] although specific proof is lacking. Although captive diets may contain inadequate zinc or biotin levels,[10] the skin lesions in rhinoceroses are not typical of these deficiencies in other species except humans.[24] Neither zinc nor biotin deficiencies cause the hydropic degeneration of keratinocytes and spongiosis that were noted in the black rhinoceroses,[9,12,49] except in humans with acrodermatitis enteropathica (familial zinc malabsorption) or vitamin B_3 deficiency.[20,24,35] Considering the morphologic similarities between rhinoceros and human skin, these potential deficiencies should be further explored.

Differences between our findings and the previous reports of skin disease in captive black rhinoceroses can be explained by the chronicity of the lesions in those reports.[11,23,32,43,52] The acute degenerative changes we noted in the epidermis rapidly progress to reported chronic ulcerative dermatitis and eosinophilic granulomas. Early bullous lesions were not examined in these cases. Although pox virus was identified with skin disease in rhinoceroses from European zoos,[18,46,47] no specific etiologic agents were noted in our cases nor in any previous cases in the United States.[43,52] The cytoplasmic inclusions noted in keratinocytes of some captive US rhinoceroses were confirmed to be keratin aggregations and likely resulted from abnormal keratinocyte maturation. Also, no specific bacterial pathogens were noted in our cases, although *Streptococcus* group L and *Staphylococcus aureus* were thought to be significant isolates from ulcers in previous reports.[3]

The skin lesions in black rhinoceroses from US zoos also differed in distribution, character, and seasonal occurrence from *Stephanofilaria*-induced lesions noted in wild black rhinoceroses. Lesions in African rhinoceroses were predominantly on the ventral neck, forelegs, and shoulders, were highly pruritic, and occurred in summer months.[30,48,56] In contrast, lesions on captive black rhinoceroses occurred year-round, were nonpruritic, and were predominantly at pressure points, in the oral cavity, and on distal extremities (feet, ears, tail). Lesions on African rhinoceroses also are notably inflammatory and associated with filarid larvae and adults,[30,56] whereas lesions in captive rhinoceroses lack parasites and inflammatory changes.

In summary, black rhinoceroses in zoological parks appear uniquely predisposed to a skin disease resembling superficial necrolytic dermatitis in dogs and necrolytic migratory erythema in humans. The high prevalence of SND in this population indicates that diseases with these clinical and histologic characteristics can arise in conjunction with a variety of metabolic disorders. Although no common underlying metabolic disease was specifically identified, stress- or disease-induced hypoaminoacidemia in concert with possibly inappropriate captive diets are suspected as the basis for the high prevalence of these skin problems in captive black rhinoceroses. Future investigations will include in depth diet analysis of captive and wild rhinoceroses, plasma amino acid and essential fatty acid profiles of affected and wild black rhinoceroses, and measurement of plasma glucagon.

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References

1 Blackford S, Wright S, Roberts DL: Necrolytic migratory erythema without glucagonoma: the role of dietary essential fatty acids. Br J Dermatol **125:**460–462, 1991

2 Bloom SR, Polak JM: Glucagonoma syndrome. Am J Med **82:**25–36, 1987

3 Clausen B, Ashford WA: Bacteriologic survey of black rhinoceroses (*Diceros bicornis*). J Wildl Dis **16:**475–480, 1980

4 Clemens ET, Maloiy GMO: Nutrient digestibility and gastrointestinal electrolyte flux in the elephant and rhinoceros. Comp Biochem Physiol **15A:**653–658, 1981

5 Clemens ET, Maloiy GMO: The digestive physiology of three East African herbivores: the elephant, rhinoceros and hippopotamus. J Zool **198:**141–156, 1982

6 Dierenfeld ES, duToit R, Miller RE: Vitamin E in captive and wild black rhinoceroses. J Wildl Dis **24:**547–550, 1988

7 Forichon J, Jomain MJ, Dallevet G, Minaire Y: Effect of cold and epinephrine on glucose kinetics in dogs. J Appl Physiol **43:**230–237, 1977

8 Foster DW, Rubenstein AH: Hypoglycemia, insulinoma, and other hormone-secreting tumors of the pancreas. *In:* Harrison's Principles of Internal Medicine, ed. Isselbacher KJ, Adams RD, Braunwald E, Petersdorf RG, and Wilson JD, 9th ed., pp. 1758–1763. McGraw-Hill, New York, NY, 1980

9 Geyer VH, Schulze J, Streiff K, Tagwerker F, Volker L: Der Einfluss des experimentellen Biotinmangels auf Morphologie und Histochemie von Haut und Klauen des Schweines. Zentralbl Veterinärmed **31:**519–538, 1984

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

11 Gillespe D, Burton M, Kohn C, Gosselin S, Munson L: An unusual case of ulcerative stomatitis and prolonged pregnancy in a black rhinoceros. Proc Am Assoc Zoo Vet **1990:**319–321, 1990

12 Glättli HR, Pohlenz J, Streiff K, Ehrensperger F: Klinische und morphologische Befunde beim experimentellen Biotinmangel. Zentralbl Veterinärmed **22:**102–116, 1975
13 Goddard J: Food preferences of two black rhinoceros populations. East Afr Wildl J **6:**1–18, 1968
14 Goddard J: Food preferences of black rhinoceroses in the Tsavo National Park. East Afr Wildl J **8:**161, 1970
15 Gross TL, Ihrke PJ, Walder EJ: Diseases of the epidermis. *In:* Veterinary Dermatopathology, ed. Cross TL, Ihrke PJ, and Walder EJ, pp. 46–48. Mosby-Year Book, St. Louis, MO, 1993
16 Gross TL, O'Brien TD, Davies AP, Long RE: Glucagon-producing pancreatic endocrine tumors in two dogs with superficial necrolytic dermatitis. J Am Vet Med Assoc **197:**1619–1622, 1990
17 Gross TL, Song MD, Havel PJ, Ihrke PJ: Superficial necrolytic dermatitis (necrolytic migratory erythema) in dogs. Vet Pathol **30:**75–81, 1993
18 Grunberg W, Burtscher H: Über eine pockenartige Krankheit beim Rhinozeros (*Diceros bicornis* L.). Zentralbl Veterinärmed **14:**643–657, 1967
19 Guillausseau PH, Guillausseau C, Villet R: Les glucagonomes. Aspects cliniques, biologiques, anatomopathologiques et therapeutiques (revue generale de 130 cas). Gastroenterol Clin Biol **6:**1029–1041, 1982
20 Hendricks WM: Pellagra and pellagralike dermatoses: etiology, differential diagnosis, dermatopathology, and treatment. Semin Dermatopathol **10:**282–292, 1991
21 Hendriks T: On the stability of immunoreactive glucagon in plasma samples. Diabetologica **20:**553–557, 1981
22 International Species Inventory System: Average physiological values. ISIS, Apple Valley, MN, 1989
23 Jones DM, Thomsett LR: A short review of the diseases of rhinoceros skin with case reports on an exudative dermatitis of the white rhinoceroses (*Ceratotherium simum*). XIV Int Symp Erkrank Zoo **14:**227–231, 1972
24 Kasper CS: Necrolytic migratory erythema: unresolved problems in diagnosis and pathogenesis, a case report and review. Cutis **49:**120–128, 1992
25 Kasper CS, McMurry K: Necrolytic migratory erythema without glucagonoma versus canine superficial necrolytic dermatitis: is hepatic impairment a clue to pathogenesis? J Am Acad Dermatol **25:**534–541, 1991
26 Klos HG: International studbook of African rhinoceroses, pp. 135–160. Zoologischer Garten, Berlin, Germany, 1990
27 Kock MD, duToit R, Kock N, Morton D, Foggin C, Paul B: Effects of capture and translocation on biological parameters in free-ranging black rhinoceroses (*Diceros bicornis*) in Zimbabwe. J Zoo Wildl Med **21:**414–424, 1990
28 Kock MD, duToit R, Morton D, Kock N, Paul B: Baseline biological data collected from chemically immobilized free-ranging black rhinoceroses (*Diceros bicornis*) in Zimbabwe. J Zoo Wildl Med **21:**283–291, 1990
29 Kock MD, Morkel P: Capture and translocation of the free-ranging black rhinoceros: medical and management problems. *In:* Zoo and Wild Animal Medicine, ed. Fowler M, pp. 466–475. WB Saunders, Philadelphia, PA, 1993
30 Kock N, Kock MD: Skin lesions in free-ranging black rhinoceroses (*Diceros bicornis*) in Zimbabwe. J Zoo Wildl Med **21:**447–452, 1990
31 Kock ND, Kock MD, Young KB: Hepatopathy in two black rhinoceroses (*Diceros bicornis*) in Zimbabwe: creosote toxicosis? J Zoo Wildl Med **25:**270–273, 1994
32 Kock RA, Garnier J: Veterinary management of three species of rhinoceros in a zoological collection. Proc Int Rhinoceros Conf **1991:**325–345, 1991
33 Krejs GJ: Non-insulin secreting tumors of the pancreatoc islets. *In:* Williams Textbook of Endocrinology, ed. Wilson JD and Foster DW, 7th ed., pp. 1301–1308. WB Saunders, Philadelphia, PA, 1985
34 Landsberg L, Young JB: Catecholamines and the adrenal medulla. *In:* Williams Textbook of Endocrinology, ed. Wilson JD and Foster DW, 7th ed., pp. 891–965. WB Saunders, Philadelphia, PA, 1985
35 Lever WF, Elder DE: Lever's Histopathology of the Skin, 8th ed., pp. 354–357. Lippincott-Raven, Philadelphia, PA, 1997
36 Marco J, Calle C, Hedo JA: Enhanced glucogon secretion by pancreatic islets from prednisolone-treated mice. Diabetologica **12:**307–311, 1976
37 Marinkovich MP, Botella R, Datloff J, Sangueza OP: Necrolytic migratory erythema without glucagonoma in patients with liver disease. J Am Acad Dermatol **32:**604–609, 1995
38 Maruska EJ, Dresser BL, Barden BD: Black Rhinoceros International Management Survey: American Association of Zoological Parks and Aquariums Species Survival Plan. Cincinnati Zoo and Center for Research on Endangered Species, Cincinnati, OH, 1986
39 Miller WH, Scott DW, Buerger RG, Shanley KJ, Paradis M, McMurdy MA, Angarano DW: Necrolytic migratory erythema in dogs: a hepatocutaneous syndrome. J Am Anim Hosp Assoc **26:**573–581, 1990
40 Mossman HW, Duke KL: General microscopic structure of the mammalian ovary. *In:* Mammalian Ovary, pp. 34–52. Univ. Wisconsin Press, Madison, WI, 1973
41 Mukinya JG: Feeding and drinking habits of the black rhinoceros in the Masai Mara Game Reserve. East Afr Wildl J **15:**125–138, 1977
42 Munson L: Mucosal and cutaneous ulcerative syndrome in black rhinoceroses (*Diceros bicornis*). *In:* Rhinoceros Biology and Conservation, ed. Ryder OA, pp. 354–356. Zoological Society of San Diego, San Diego, CA, 1993
43 Ott JE, McDonald SE, Robinson RT, Wright FH: Ulcerative stomatitis in a black rhinoceros (*Diceros bicornis*). Proc Am Assoc Zoo Vet **1982:**68, 1982
44 Paglia DE, Miller RE: Erythrocytic ATP deficiency and acatalasemia in the black rhinoceros (*Diceros bicornis*) and their pathogenic roles in acute episodic hemolysis and mucocutaneous ulcerations. Proc Am Assoc Zoo Vet **1992:**217–219, 1992
45 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

46 Pilaski J, Schaller K, Matern K, Kloppel B, Mayer H: Outbreaks of pox among elephants and rhinoceroses. Verh Erkrankg Zoot **24:**257–265, 1982
47 Pilaski J, Schaller K, Olberding P, Finke H: Characterization of a poxvirus isolated from white rhinoceroses (*Ceratotherium s. simum*). Zentralbl Bakteriol Mikrobiol Hyg **251:**440, 1982
48 Round MC: A new species of *Stephanofilaria* in skin lesions from the black rhinoceros (*Diceros bicornis*) in Kenya. J Helminthol **38:**87–96, 1964
49 Scott DW: Nutritional diseases. *In:* Large Animal Dermatology, ed. Scott DW, pp. 359–360. WB Saunders, Philadelphia, PA, 1988
50 Seitz HJ, Krone W, Wilke H, Tarnowski W: Rapid rise in plasma glucagon induced by acute cold exposure in man and rat. Pflugers Arch **389:**115–120, 1981
51 Shupack JL, Berczeller PH, Stevens DM: The glucagonoma syndrome. J Dermatol Surg Oncol **4:**242–247, 1978
52 Silberman MS, Fulton RB: Medical problems of captive and wild rhinoceroses—a review of the literature and personal experiences. J Zoo Anim Med **10:**6–16, 1980
53 Sousa CA: Dermatosis associated with feeding generic dog food: 13 cases (1981–1982). J Am Vet Med Assoc **192:**676–680, 1983
54 Tanner M, Brasch J, Christophers E: Erythema necroticans migrans without glucagonoma. Hautarzt **45:**480–483, 1994
55 Thorisdottir K, Camisa C, Tomecki KJ, Bergfeld WF: Necrolytic migratory erythema: a report of three cases. J Am Acad Dermatol **30:**324–329, 1994
56 Tremlett JG: Observations on the pathology of lesions associated with *Stephanofilaria dinniki* Round 1964 from the black rhinoceros (*Diceros bicornis*). J Helminthol **38:** 171–174, 1964
57 Walton DK, Center SA, Scott DW, Collins K: Ulcerative dermatosis associated with diabetes mellitus in the dog: a report of four cases. J Am Anim Hosp Assoc **22:**79–88, 1986
58 Young JB, Landsberg L: Adrenergic influence on peripheral hormone secretion. *In:* Adrenoceptors and Catecholamine Action, ed. Kunos G, pp. 157–217. John Wiley & Sons, New York, NY, 1983

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