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POSSIBLE TOXIC LIVER DEGENERATION IN BLACK RHINOCEROSES *(Diceros bicornis)*

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Introduction

In a recent extensive review,[7] the disease problems of rhinoceroses were discussed. No report of liver problems was mentioned, indicating at least a lack of documentation of rhinoceros medical problems involving the liver. This report documents the occurrence of a condition affecting two black rhinoceroses with liver lesions.

History

The first case occurred in a 4-5 year old female, black rhinoceros which was ill for 2-3 months, the primary clinical problems being anemia and icterus. The second case involved a mature male black rhinoceros with anemia, depression, and ulcerative skin lesions scattered over the entire body. The clinical signs had been present for 6-7 months.

Both rhinoceroses had been at the zoo for over five years. They were housed and had access to a fenced area that was adjacent to an area containing white rhinoceroses *(Ceratotherium simus)*. The only differences in enclosures was that one side of the black rhinoceroses area was fenced with posts that were old telephone poles.

Clinical Laboratory Results

Table 1 gives the results of hematology and blood chemistry done on the female black rhinoceros over a one month period, and Table 2 gives values for the male black rhinoceros several months before death.

Gross Description

The female black rhinoceros had slightly yellow mucous membranes and a liver that was enlarged and gray-black. Broad fibrous bands were present in the capsule. The male rhinoceros had numerous scattered skin lesion characterized by focal swelling, necrosis, and serum exudation. The liver was enlarged, had well-defined capsular fibrotic streaks, and was gray-black.

Histologic Lesions

The liver lesions were similar in both animals. The lobular architecture was somewhat disrupted, with some individualization of hepatocytes (Figure 1). Most of the hepatocytes contained a green-brown pigment (Figure 2). In some cells the pigment occurred in clumps (Figure 3), while in others it was scattered throughout the cytoplasm (Figure 4). Some of the pigment was acid-fast, positive when stained by the Fontana and Schmorl methods, partly positive with 48 hours oil-Red-O (ORO), and autofluoresced yellow-brown. Other intramcellular pigment was positive for bile by Hall's stain and was not autofluroescent. Ultrastructurally the pigment appeared to be membrane-bound and resembled phagosomes, or had no particular structure (Figure 5). Most hepatocytes had very few normal organelles. The skin lesions of the male rhinoceros had areas of necrosis in the epidermis and dermis which were well-delineated from adjacent tissue (Figure 6). Linear areas of collagen necrosis were deeper in the dermis with the accumulation of marcophages and lymphocytes (Figure 7). In some of these areas were necrotic small blood vessels. At the base of most of the affected areas dermal blood vessels had endothelial proliferation that partly or completely blocked their lumens (Figure 8).

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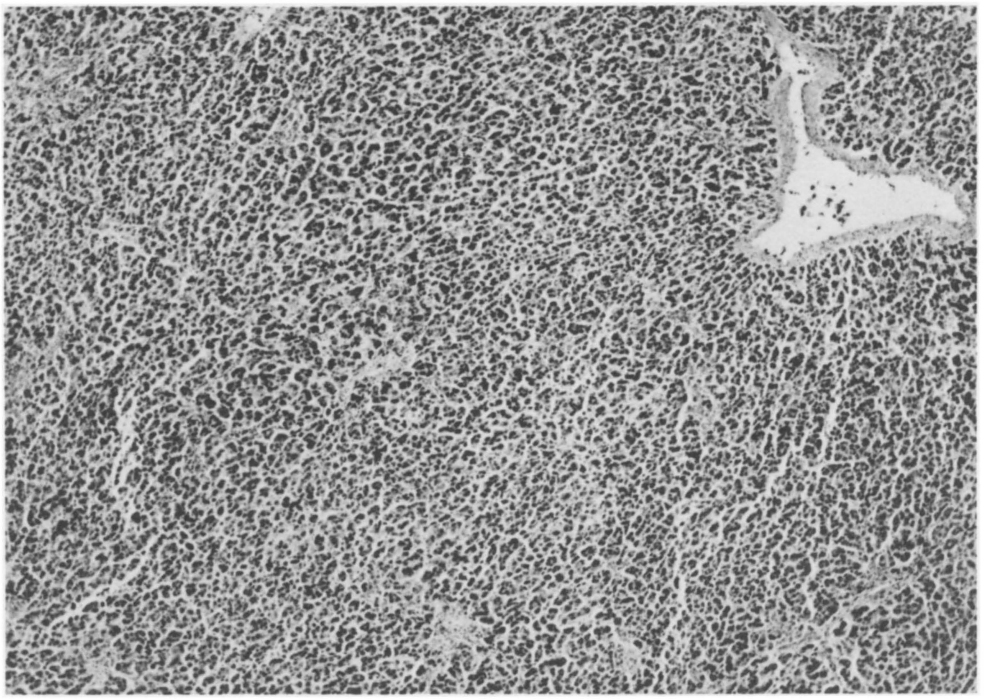

Figure 1: Section of rhinoceros liver illustrating minimal architectural disruption and pigment accumulation. H&E X 34.

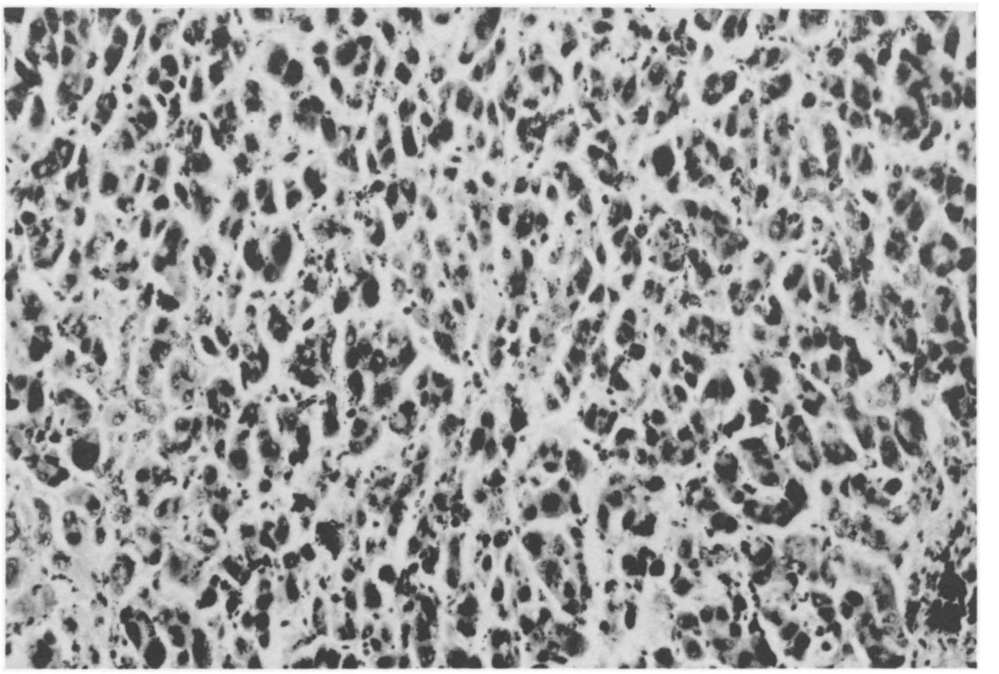

Figure 2: Higher magnification of pigment-containing hepatocytes. H&E X 140.

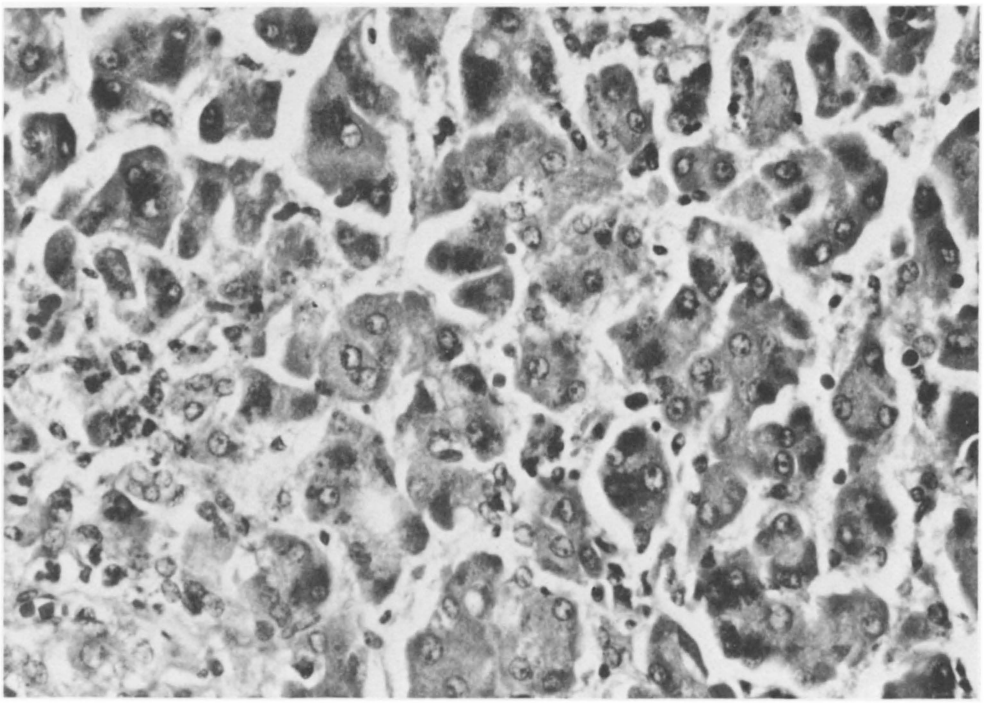

Figure 3: Large pigment accumulations in some hepatocytes. Also note individualization of cells. H&E X 320.

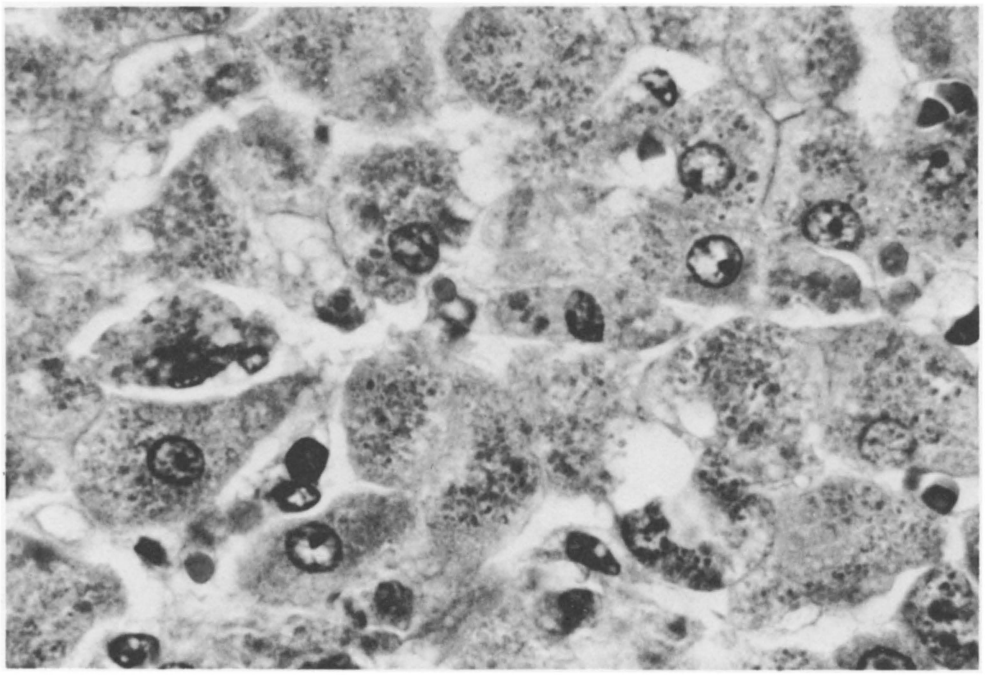

Figure 4: Scattered, granular pigment seen in many hepatocytes. Compare to Figure 3. H&E X 765.

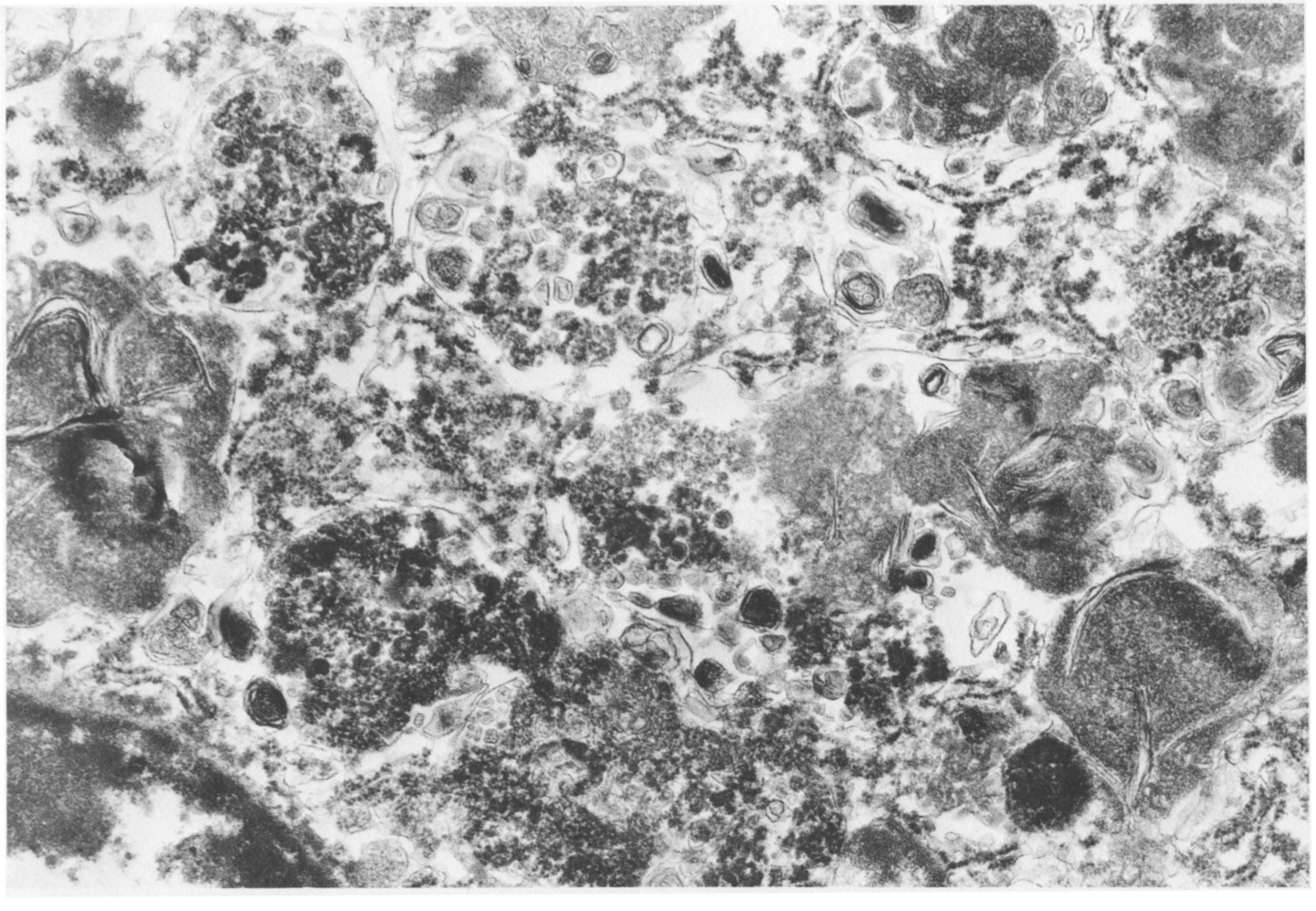

Figure 5: Ultrastructure of typical hepatocyte from affected rhinoceros liver. Membrane-bound structures similar to phagosomes are present, as are areas of amorphous electron density. Few normal organelles are present. Ur. Ac. & Pb. cit. X 12,000.

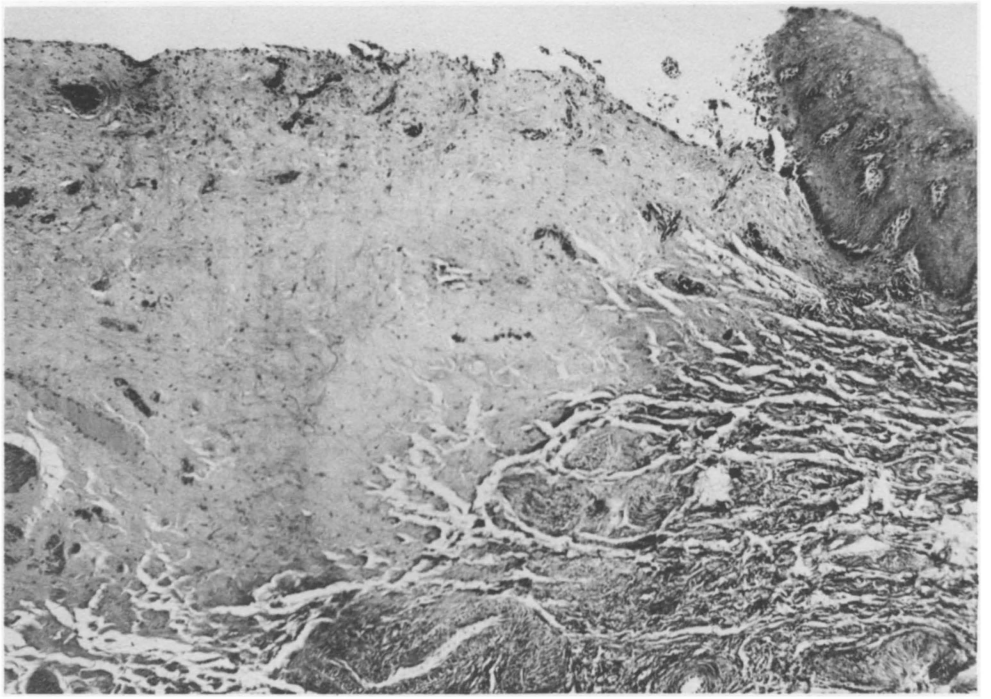

Figure 6: Skin lesion from male rhinoceros. Note well-defined area of epidermal and dermal necrosis. H&E X 140.

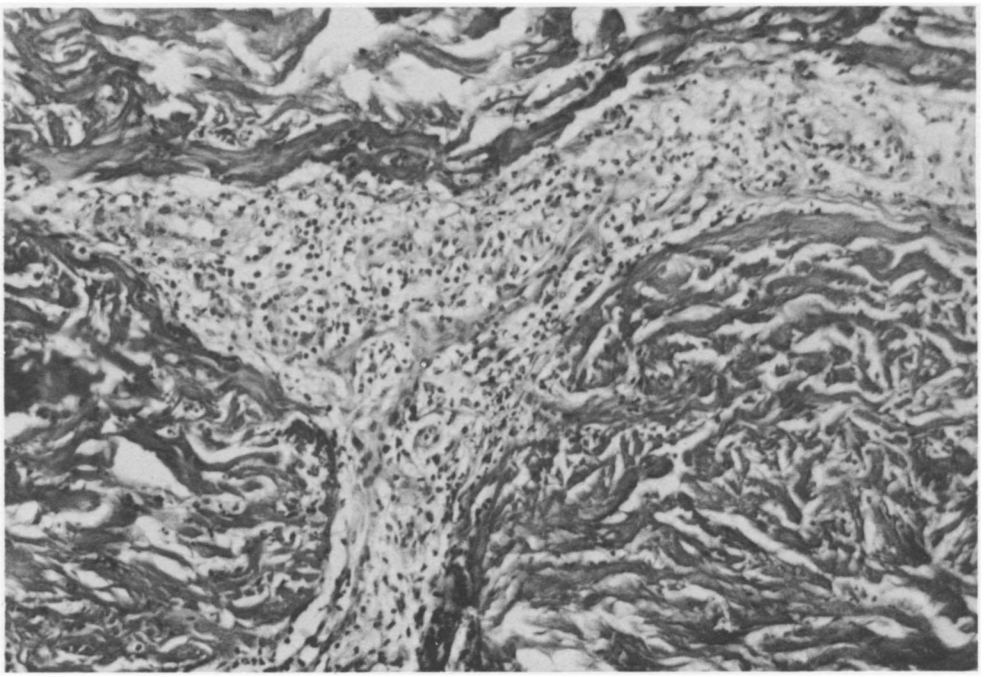

Figure 7: Linear area of dermal necrosis and inflammatory cell infiltration in male rhinoceros. H&E X 140.

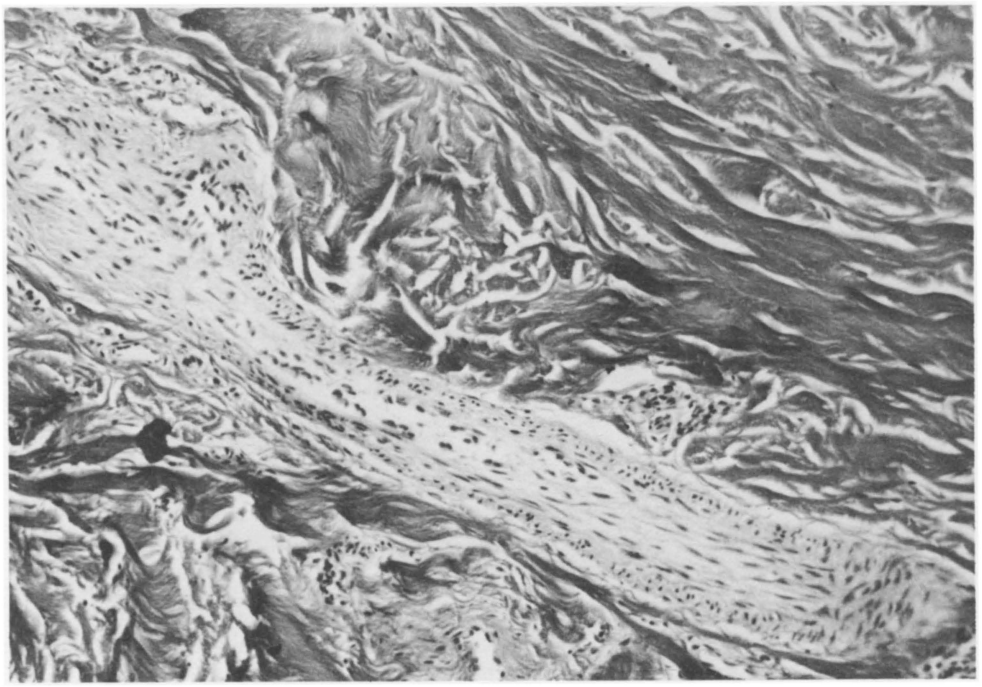

Figure 8: Dermal blood vessel with lumen occlusion due to apparent endothelial proliferation. Dermis of male black rhinoceros. H&E X 140.

Discussion

The morphologic features of the condition seen in the two black rhinoceroses were suggestive of a toxic problem involving the liver. The alterations in serum chemistry reflect this probability, especially the elevated bilirubin, alkaline phosphatase (SAP) and oxalacetate-pyruvate (SGOT) and glutamate-pyruvate (SGPT) transaminases. The elevation of the latter two enzymes coincided with the onset of systemic illness, and may only indicate that there was an acute degenerative process superimposed on a chronic progressive loss of hepatic function. This can only be inferred from the morphologic appearance of the hepatocytes, as no clinical laboratory data was available except that obtained following the onset of clinical signs.

Other possible causes for the condition were considered but ruled out. Blood cultures were negative. Direct Coombs test and LE preps were negative, although their significance as applied to the rhinoceros could be questioned, since test methods for man were used. Leptospirosis, recently reported in black rhinoceroses[4] was not considered since the lack of hemoglobinuria and severe hemosiderosis indicated that the anemia was not hemolytic. The clinical signs and necropsy lesions seen in our animals were different from those reported in leptospirosis. The Coggins test for equine infectious anemia (EIA) was also negative.

The material present in the hepatocytes was histochemically and ultrastructurally compatible with lipofuscin and bile pigment. Lipofuscin is regarded as derived from the progressive oxidation of lipids and is considered a "wear-and-tear" pigment.[3] The bile pigment can accumulate because of failure to be removed by damaged hepatocytes, but its accumulation does not give any clue to the etiology of the condition. The buildup of pigment in the liver of these rhinoceroses correlated with the high level of indirect bilirubin in the serum of the animals; however, the

TABLE 1

Hematology and Clinical Chemistry of Female Black Rhinoceros *(Diceros bicornis)*

Date:	3/7	3/23	3/30	4/6	4/13	4/17
Hgb g/dl	6.8	8.2	8.7	7.7	9.7	9.8
Hct %	19.6	24.8	26.4	22.4	28.6	27.0
WBC mm^3	14,100	20,500	18,000	14,100	17,000	18,000
RBC mm^3	1.94	2.44	2.48	2.17	2.86	2.64
McV	99	98	103	102	100	102
McH	34.9	33.2	34.4	35.6	34.2	37.4
McHc	35.2	32.8	32.3	34.2	34.0	36.0
Creatinine mg/dl	0.6	0.5	0.6	0.7	0.7	0.6
Ca mg/dl	7.0	10.6	—	—	—	10.3
PO_4 mg/dl	2.8	3.4	—	—	—	2.3
Bil. Dir. mg/dl	2.64	2.52	2.9	3.62	3.2	2.8
Bil. Total mg/dl	9.4	9.34	8.38	9.42	7.9	7.0
SGOT U/L	68	600	—	—	—	214
SGPT U/L	11	82	92	36	21	26
LDH U/L	369	800	500	—	—	411
Alk. Phos. U/L	65	96	—	—	—	52
Albumin g/dl	0.8	1.2	—	—	—	2.0
Total Prot. g/dl	7.2	9.2	8.3	8.4	8.3	6.7

correlation may be fortuitous.

The type of toxin responsible remains a matter of speculation. One report[2] mentions suspected cases of creosote poisoning in black rhinoceroses which were kept in pens made of creosote-treated poles. Skin lesions and hepatic necrosis were mentioned as occurring, but no description or illustrations were given. Anemia and liver damage are described as occurring in animals poisoned by coal tar derivatives;[1,5] however, the descriptions are brief, and it seems that thorough studies of possible chronic effects have not been done, or are not readily available in the literature. The black rhinoceroses described in this report had a yard area that was delineated by a fence made of old telephone poles. It is possible that these poles were treated with creosote or with products containing pentachlorophenol and impurities such as chlorinated dibenzo-p-dioxin or chlorodibenzofurans, chemicals which cause liver and skin lesions in a wide variety of animals.[6,8] Unfortunately, material submitted for laboratory analysis of these compounds was lost at the receiving laboratory and no proof of their involvement is available. After considerable environmental analysis no other source of toxic material was found, and the definitive cause of the condition remains an enigma. White rhinoceroses in an adjacent area that did not have access to the telephone poles have had no disease.

Acknowledgments

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TABLE 2

Selected Hematology and Clinical Chemistry Values for Male Black Rhinoceros *(Diceros bicornis)*

Date:	2/12	3/14	5/09
Hgb g/dl	—	10.0	11.9
Hct %	46.0	29	33.5
WBC mm^3	10.7	11.0	9.24
RBC mm^3	6.09	4.4	4.1
McV	—	—	71
McH	31.6	—	25
McHc	34.5	—	36
Bil. Dir. mg/dl	3.0	—	—
Bil. Ind. mg/dl	10.0	—	—
LDH U/L	—	428	—
SGOT U/L	—	158	—
Alk. Phos. U/L	349	—	—
Albumin g/dl	3.1	1.8	—
Total Prot. g/dl	8.8	8.2	—

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