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SERUM ALPHA-TOCOPHEROL, ALL-TRANS RETINOL, TOTAL LIPIDS AND CHOLESTEROL IN THE BLACK RHINOCEROS (*DICEROS BICORNIS*)

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Abstract—1. Mean concentration of serum alpha-tocopherol (Vitamin E) in 28 free-living black rhinoceroses sedated during translocation in Zimbabwe was 1.92 (SD, 0.43) mg/l.

2. Alpha-tocopherol was not detectable (< 0.15 mg/l) in five captive black rhinoceroses held at London Zoo.

3. Circulating levels of all-trans retinol (Vitamin A) were not different between the two groups.

4. The low level of alpha-tocopherol in captive rhinoceroses suggests a risk of acute haemolytic anaemia.

INTRODUCTION

Requirements for vitamins and trace elements and their physiological concentrations in wild animals have not been fully investigated. Captive exotic animals, particularly herbivore browsers, often suffer from diseases caused or pre-disposed by nutritonal imbalance or deficiency. Of these nutrients, Vitamins A and E are significant because of their multiple physiological roles (Diplock, 1985; Scott, 1986) and the high incidence of deficiency of the two fat soluble vitamins.

Vitamin A deficiency has been reported in captive primates (Ramalingaswami *et al.*, 1955; Lapin and Yakovleva, 1963), polar bears (Foster, 1981), Raptors (Halliwel and Graham, 1976) semi-aquatic turtles (Frye, 1986) and other species.

A muscular dystrophy syndrome responsive to Vitamin E and selenium treatment was observed in zoos in Eastern North America (Rechcigl, 1977). Wallach (1970) reported manifestations of Vitamin E deficiency in reptiles, primates, carnivores, herbivores and birds. Other examples include primates (Liu *et al.*, 1984), camels (Finlayson *et al.*, 1971), macropods (Kakulas, 1963), lesser kudus (Rudi *et al.*, 1980), Mongolian wild horses (Liu *et al.*, 1983), zebra foals (Higginson *et al.*, 1973), waterfowl, ground feeder birds, reindeer, forcas gazelles, greater kudus and dik-diks (Sauer and Zook, 1972).

Fatal haemolytic anaemia has often been observed in captive black rhinoceroses. Miller *et al.* (1986), in their survey of North American, European and Japanese zoos, reported 27 episodes in 21 animals of which 17 died. The aetiology of the disease is not well understood, although red cell parasites, heavy metals, toxins from food sources, auto-immune diseases and deficiency of essential nutrients have been suspected as possible causes. Douglass and Plue (1980) suggested leptospirosis as the cause of fatal haemolytic anaemia in both an 11-year-old female and an imported 9-year-old male black rhinoceros.

The purpose of the study reported here was to investigate the serum levels of alpha-tocopherol and all trans retinol (isomers of Vitamins A and E, respectively) in wild and captive rhinoceroses. The results are discussed in relation to the incidence of haemolytic anaemia in the captive species.

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MATERIALS AND METHODS

Animals

Blood samples were collected from 28 free-ranging and five captive clinically healthy black rhinoceroses. Samples from the wild species browsing on local vegetation were obtained during translocation exercises in Zimbabwe. The samples were stored frozen for transport to London.

Analytical methods

Total lipids and cholesterol were assayed colorimetrically by means of kits supplied by Boehringer Mannheim, GmbH (BCL, Boehringer, Mannheim House, Bell Lane, East Sussex, UK).

Extraction of the alpha-tocopherol and retinol

The method of Leenheer *et al.* (1979) was adopted with the following modifications. Serum (500 μl) was mixed with 500 μl of absolute ethanol and 1 ml of ascorbic acid (0.1% in water); and alpha-tocopheryl acetate 9.25 μg/μl (5 μl) was added as an internal standard. In order to eliminate degradation of the vitamins all the procedures were performed under red light.

Separation of alpha-tocopherol and retinol

The technique employed was a modification of that of Barnett *et al.* (1980). A Varian 5000 HPLC equipped with

a variable wavelength UV-100 detector (Varian Ltd, Palo Alto, CA, USA) and 30 cm × 4 mm column packed with a 5 micron C18 reverse-phase (Varian Micropack MCH-5 Octyldecylsilane) packing was used. The vitamins were isolated by eluting with methanol 90%, water 9% and acetonitrile 1% over 20 min. The flow rate was 1.8 ml/min. Retinol and alpha-tocopherol were detected at 325 and 292 nm, respectively. The column temperature was 40°C.

Quantification

Retinol and alpha-tocopherol values of the samples were computed from their standard curves after correcting for recoveries. Recovery was performed by multiplying the peak area of the samples by the ratio of the expected area: measured area of the internal standard. Area versus concentration standard curves were plotted for the range 30–150 ng/μl for retinol and 0.20–4.0 μg/μl for alpha-tocopherol. The mean recoveries of retinol and alpha-tocopherol from the spiked samples were 98 and 97.6%, respectively.

Data analysis

The unpaired Student's t-test was used to investigate statistical difference in serum all-trans retinol and alpha-tocopherol between the wild and captive groups.

RESULTS

Table 1 shows the results of the investigation. There was no significant sex difference ($P > 0.05$) in any of the parameters measured. Since the ages were approximate, the figures were not statistically tested for age dependency.

The concentrations (mean ± SD) of serum alpha-tocopherol, total lipids, total cholesterol and all-trans retinol of the wild rhinos were 1.92 ± 0.43 mg/l, 2.48 ± 0.62 g/l, 0.76 ± 0.26 g/l and 51.54 ± 11.24 μg/l, respectively. Alpha-tocopherol was not detectable in the serum of the captive rhinos (< 0.15 mg/l), and was significantly lower ($P < 0.001$) compared to that of their wild counterparts. Mean serum concentration of all-trans retinol in the captive animals was 58.59 ± 21.80 μg/μl: not significantly different ($P > 0.05$) from that of the wild rhinoceroses.

Since the serum alpha-tocopherol concentration varies in direct relation to cholesterol, phospholipids and triglycerides (Horwitt *et al.*, 1972), alpha-tocopherol/total lipids and alpha-tocopherol/total cholesterol ratios of the wild rhinos were calculated. The mean alpha-tocopherol/total cholesterol ratio was 2.96 ± 1.55 mg/g and the alpha-tocopherol/total lipid ratio was 0.82 ± 0.25 mg/g.

DISCUSSION

The alpha-tocopherol values of the wild rhinos were significantly greater ($P < 0.001$) than those of the captive species. Brush and Anderson (1986) were unable to detect alpha-tocopherol in serum of captive black rhinoceroses. The mean alpha-tocopherol/total lipid ratio of the free-ranging species was marginally greater than 0.8 mg/g, a value regarded as a minimum threshold of Vitamin E status in the human adult (Horwitt *et al.*, 1972).

The discrepancy in serum alpha-tocopherol between wild and captive species is most likely due to differences in dietary intake. Free living black rhinoceros would have unrestricted access to selected woodland vegetation, which has a high nutrient density and is rich in polyunsaturated fatty acids and Vitamin E. In captivity, however, because their digestive system is similar to that of the equids, their dietary intake is formulated based on the requirement of a domestic horse. Consequently, their ration often comprises mainly grass hay (sudan, timothy, coastal bermuda), horse pellets and mineral supplements. The weakness of this approach is the feeding of a grazer diet to a browser. It also fails to recognize the subtle digestive physiology (morphological and functional) differences between the black rhinos and the equids. Hoffman and Stewart (1972) reported substantial differences in diet, stomach structure, physiology and adaptability in ruminants, and concluded that the failure to appreciate the differences would lead to translocation failures or to unsuccessful management of captive animals. Their assertion must equally be applicable to perissodactyla.

The low level of serum alpha-tocopherol observed in supplemented captive rhinocerotidae and particularly in the black rhinoceros is possibly due to reduced hydrolysis of the short chain tocopheryl esters. Machlin and Gabriel (1982) reported that in humans, blood levels of alpha-tocopherol were consistently higher when the free tocopherol was administered rather than tocopheryl acetate suggesting that hydrolysis was a limiting factor. The observation need not be surprising since in nature vitamin E exists only as free tocopherols and tocotrienols. The sprinkling of Vitamin E supplement, a lipid nutrient, on feed lot is imprudent in that it would not ensure complete ingestion, or emulsification in the absence of associated fat.

Because serum alpha-tocopherol in captive black rhinoceros is low and very often undetectable it must

Table 1. Serum alpha-tocopherol, all-trans retinol, and total lipids and cholesterol in black rhinoceroses

	Wild ($n = 28$)		Captive ($n = 5$)	
	Range	Mean	Range	Mean
Alpha-tocopherol (mg/l)	1.06–2.90	1.92	—	<0.15
Total lipid (g/l)	0.92–3.94	2.48	—	—
Total cholesterol (g/l)	0.14–1.30	0.76	—	—
All-trans retinol (μg/l)	21.78–72.46	51.54	42.60–93.10	58.59
Alpha-tocopherol/ total lipid	0.37–1.34	0.82	—	—
Alpha-tocopherol/ total cholesterol	0.90–8.43	2.96	—	—

be a significant element in the aetiology of haemolytic anaemia. In addition to providing protection against oxidative damage (Diplock, 1985) and endotoxin attack that may be mediated through lipid peroxidation (Wicken and Knox, 1980), Vitamin E enhances the host resistance to diseases by stimulating the immune responses (Tengerdy *et al.*, 1984). If Vitamin E is to provide effective cover, the blood level needs to be maintained. Machlin *et al.* (1979) reported that the rate of release from adipose tissue is slow and, unless the blood levels are maintained, animals develop a myopathy even though adipose tissue stores are still high. Excess iron is also known to increase erythrocyte lipid peroxidation when antiperoxidant mechanisms of the red cells are deficient (Gross and Melhorn, 1972).

As the first step to tackling the problem of haemolytic anaemia, effort should be made to raise the serum alpha-tocopherol level to that of the wild species. Moreover, research needs to be undertaken to determine the most effective Vitamin E supplement and methods of administration.

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