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FAT SOLUBLE VITAMINS IN BLOOD AND TISSUES OF FREE-RANGING AND CAPTIVE RHINOCEROS

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ABSTRACT. Several disease syndromes in captive rhinoceroses have been linked to low vitamin status. Blood samples from captive and free-ranging black (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*) and tissue samples of captive individuals from four rhinoceros species were analysed for vitamins A and E. Circulating vitamin A levels measured as retinol for free-ranging versus captive black and white rhinoceros were 0.04 (±0.03 SD) vs. 0.08 (±0.08) and 0.07 (±0.04) vs. 0.06 (±0.02) μg/ml, respectively. Circulating vitamin E levels measured as α-tocopherol were 0.58 (±0.30) vs. 0.84 (±0.96) and 0.62 (±0.48) vs. 0.77 (±0.32) μg/ml, respectively. In contrast to earlier findings, there was no significant difference in vitamin E concentration between captive and free-ranging black rhinoceros. When the samples of captive black rhinoceros were grouped into those taken before 1990 and after 1990, however, those collected before 1990 had significantly lower ($P < 0.001$) vitamin E levels (0.46 ± 0.83 μg/ml) and those collected in 1990 or later significantly higher ($P < 0.001$) vitamin E levels (1.03 ± 1.04 μg/ml) than the captive population as a whole. This is probably due to increased dietary supplementation. There were significant differences in circulating vitamin concentrations in black rhinoceroses from different regions in the wild. Serum 25-hydroxy (OH) vitamin D_3 averaged 55.7 ng/ml in free-ranging rhinoceroses; no carotenoids were detected in any blood samples. Captive black and white rhinoceroses appear to be adequately supplemented in vitamin A and E. Captive Indian rhinoceroses (*Rhinoceros unicornis*) had significantly lower vitamin A concentrations in blood ($P < 0.001$) and higher vitamin A concentrations in liver tissue samples ($P < 0.001$) than other rhinoceros species. Equine requirements are not recommended as a model for rhinoceros vitamin requirements.

Key words: *Ceratotherium simum*, *Dicerorhinus sumatrensis*, *Diceros bicornis*, nutrition, retinol, rhinoceros, *Rhinoceros unicornis*, tocopherol, vitamin A, vitamin E.

INTRODUCTION

The vitamin status of zoo animals has been monitored for over a decade, and comparative data for many species have been published. Deficiencies especially in tocopherol (vitamin E) have been recognized in many species (Dierenfeld, 1989; Dierenfeld and Traber, 1992). In early surveys, different species of rhinoceroses seemed to have low levels of vitamin E (Brush and Anderson, 1986; Ghebremeskel and Williams, 1988). A significant difference in serum vitamin E levels between free-ranging and captive black rhinoceroses (*Diceros bicornis*) was demonstrated (Dierenfeld et al., 1988; Ghebremeskel et al., 1988). Low levels of serum vitamin E have been found in captive rhinoceroses (Papas et al., 1991; Taugner et al., 1995). Low vitamin E status in captivity was suspected to contribute to several disease syndromes peculiar in black rhinoceroses (Miller et al., 1986, 1987; Ghebremeskel et al., 1988). Vitamin analyses of forages consumed by free-ranging and captive black rhinoceroses suggested that forages in captivity supply lower amounts of vitamin E (Dierenfeld et al., 1990, 1995), and that diets in captivity can only attain comparable levels when supplemented (Ghebremeskel et al., 1991). Accordingly, much effort has been made to supplement captive rhinoceroses with vitamin E and to

TABLE 1. Plasma or serum samples from adult rhinoceroses used for this study.

Species	Total samples	Individuals sampled	Sex (males/females/unknown sex)	Individuals of known age	Years samples collected
Black rhinoceros					
Captive	321	85	42/43/0	81	1980–2000
Free-ranging (vitamin A analysis)	136	136	27/31/78	0	1988–1992
Free-ranging (vitamin E analysis)	167	167	40/49/78	0	1986–1992
White rhinoceros					
Captive	86	57	21/53/2	28	1976–2000
Free-ranging	5	5	2/3/0	4	1996
Indian rhinoceros (captive)	20	10	3/3/4	3	1985–1999
Sumatran rhinoceros (captive)	6	4	1/3/0	0	1994–1996

find suitable vitamin E formulations that allow supplement absorption (Dierenfeld and Citino, 1989; Lewis and Kirkwood, 1990; Kirkwood et al., 1991; Papas et al., 1991).

Deficiencies of vitamin A have been suspected in captive rhinoceros (McCulloch and Archard, 1969; Jones, 1979; Goeltenboth, 1986). Slifka et al. (1999) did not detect circulating carotenoids in three captive black rhinoceroses. In this study, we investigated the vitamin A and E content of blood and tissues of captive and free-ranging rhinoceroses. Additionally, we analyzed for circulating levels of 25-hydroxy vitamin D ($25(OH)D_3$) to measure vitamin D status and carotenoids to provide baseline information for nutritional assessment.

MATERIALS AND METHODS

Frozen plasma or serum samples from rhinoceroses living in North American zoological institutions between 1982 and 2000 were obtained from the American Zoo and Aquarium Association (AZA) Rhinoceros Taxon Advisory Group (TAG) tissue bank or were shipped directly to the Wildlife Nutrition Laboratory (Wildlife Conservation Society, Bronx, New York, USA) (Table 1). Samples from free-ranging black rhinoceroses were obtained during translocation operations in Zimbabwe (Kock and Morkel, 1994). In the free-ranging animals, blood collection took place after chemical immobilization, whereas a majority of the captives were trained to tolerate blood sampling. Data from Dierenfeld et al. (1988; $n = 31$ free-ranging and 11 captive black rhinoceroses) were included in the data set evaluated in this study.

Liver, adipose, heart, and skeletal muscle tissue samples collected at necropsy and stored frozen (−70 C; <10 yr) as part of the AZA Rhinoceros TAG research protocol (AZA, unpublished) were utilized. No discrimination according to cause of death was made in any samples.

For blood samples from captive individuals, vitamin E measured as α- and γ-tocopherol was extracted and measured using standardized techniques and instrumentation (Storer, 1974). Vitamin A measured as retinol was quantified simultaneously using methods and equipment described by Dierenfeld and Jessup (1990). Circulating carotenoids (α- and β-carotene, cryptoxanthin, lutein/zeaxanthin, and lycopene), in addition to retinol, and α- and γ-tocopherol, were measured on samples from free-ranging rhinoceroses using reversed-phase high performance liquid chromatography (Sowell et al., 1994), and $25(OH)D_3$ was measured by vitamin D-binding protein assay (Chen et al., 1990). Other tissues were saponified prior to extraction (Taylor et al., 1976), and concentrations of vitamins A and E were measured via modifications of the general method of Taylor et al. (1976; see Barker et al., 1998 for details).

Data on vitamin suplementation of captive animals were not available, but since about 1990, awareness of a possible vitamin E deficiency in captive black rhinoceroses increased greatly. However, because circulating vitamin levels reflect dietary intake (Dierenfeld and Traber, 1992) speculations on the degree of supplementation were based on measured parameters.

Vitamin concentrations potentially depend on species, sex, age, and origin (free-ranging/captive) of an individual. Complete information was not available for all animals (Table 1) and no free-living Indian and Sumatran rhinoceros was included in the study. Because vitamin A and E blood levels could not be transformed to normality, the influence of multiple factors was not tested by ANOVA for blood data. Instead,

TABLE 2. Mean (±SD) fat soluble vitamin concentrations in serum or plasma from captive and free-ranging black rhinoceros (*Diceros bicornis*) and reference values for domestic horses.

Study	Retinol		$25(OH)D_3$		α-tocopherol	
	n	μg/ml	*n*	Ng/ml	*n*	μg/ml
Captive rhinoceros						
This study (all samples)	85	0.08[a] (±0.08)			85	0.84 (±0.96)
This study (samples before 1990)	26	0.09[a] (±0.16)			26	0.46[a] (±0.83)
This study (samples from 1990)	59	0.08[a] (±0.06)			59	1.03[a] (±1.04)
Hamilton, 1999	2	0.05 (±0.66)	2	0.096 (±0)	2	0.07 (±0)
Taugner et al., 1995					3	0.04 (±0.04)
Papas et al., 1991						0–0.23 (range)
Dierenfeld et al., 1988					11	0.18 (±0.03)
Ghebremeskel et al., 1988	5	0.06 (±0.02)			5	0
Brush and Anderson, 1986					2	0
Vahala, 1990		0.13 (±0.18)				1.64 (±0.94)
Jones, 1979		0.17 (±0.02)				
Free-ranging rhinoceros						
This study	136	0.04[b] (±0.03)	28	55.7 (±34.2)	167	0.58[b] (±0.30)
Dierenfeld et al., 1988					31	0.77 (±0.05)
Gebremeskel et al., 1988	28	0.05 (±0.01)			28	1.92 (±0.43)
Normal horse values						
(Puls, 1994)						
Deficient				<2		
Adequate		0.18–0.35		2.0–7.0		2.0–10.0
Toxic				18–600		

[a] Values within a column with different superscripts differ significantly.

nonparametric tests (Kruskal-Wallis test and subsequent post-hoc tests, *U*-test, Spearman correlation coefficient) were used in order to compare circulating vitamin concentrations of different groups. All 12 tissue vitamin variables (three vitamins for each of four tissues), however, could be transformed to normality by a logarithmic transformation. Each of the vitamin levels in each tissue was analysed separately. In a first step, still- and newborn animals were compared with adults for black rhinoceros only using a *t*-test. Afterwards, still- and newborns were excluded and ANOVA was used to test the potential influence of species (with subsequent post-hoc tests), sex, and age. The Sumatran rhinoceroses were excluded from the latter analysis because age was known for one individual only. Correlations of vitamin levels between tissues were tested for non-transformed variables using the nonparametric Spearman correlation coefficient. In the case of post-hoc tests only significance information is provided. The significance level was $\alpha = 0.05$. The SPSS 9.0 (SPSS Inc., Chicago, Illinois) and SYSTAT 10 (SPSS Inc.) programs were used for the statistical calculations.

RESULTS

Blood values for vitamins in black rhinoceroses are given in Table 2. In contrast to earlier findings (Dierenfeld et al., 1988; Ghebremeskel et al., 1988), captive animals had significantly higher retinol levels (*U*-test, $P < 0.001$, $n = 221$) and did not differ significantly from free-ranging individuals in α–tocopherol levels (*U*-test, $P = 0.656$, $n = 252$). No carotenoids were detected in serum of any free-ranging animals. Serum $25(OH)D_3$ levels of free-ranging animals were higher than in the two samples of captive animals reported by Hamilton (1999). Free-ranging black rhinoceroses from different regions had significant differences in blood vitamin levels (Kruskal-Wallis test; vitamin A: $P = 0.002$, $n = 136$; vitamin E: $P < 0.001$, $n = 167$; Table 3). Four new- or stillborn animals did not differ significantly in blood vitamin A (0.08 ± 0.03 μg/ml) or vitamin E (1.03 ± 0.57 μg/ml) concentrations from adult animals. Average α-tocopherol levels tended to increase over the years (Fig. 1). In order to test if feeding supplementation led to higher blood vitamin levels, the vitamin concentrations before and after

TABLE 3. Circulating concentrations of vitamins A (retinol) and E (α-tocopherol) in free-ranging black rhinoceros (*Diceros bicornis*).

	Retinol		α-tocopherol	
Sample source	*n*	μg/ml	*n*	μg/ml
Zimbabwe	55	0.04[a] (±0.03)	86	0.60[a] (±0.23)
Namibia	3	0.04 (±0.01)	3	0.80[a] (±0.05)
Kenya	7	0.04[a] (±0.03)	7	0.24[b] (±0.07)
South Africa	45	0.06[b] (±0.03)	45	0.62[a] (±0.39)

[a] Values within a column with different superscripts differ significantly.

1990 were compared using a *U*-test involving one averaged value per animal per year. Data were not independent because most animals contributed test results for more than 1 yr. There was a significant increase in vitamins A ($P = 0.024$, $n = 165$) and E ($P < 0.001$, $n = 165$) after 1990. Similarly, the data set of circulating vitamin E measurements in captive individuals was divided into samples taken before 1990 and from 1990 and after and compared with free-ranging animals. Animals sampled in both periods were excluded from the latter period to ensure independence. Significant differences among the three groups were found (vitamin A: Kruskal-Wallis-test, $P < 0.001$, $n = 221$; vitamin E: Kruskal-Wallis-test, $P < 0.001$, $n = 252$). As post-hoc tests revealed, both captive groups had significantly higher vitamin A levels than free-ranging animals. However, the group of samples from before 1990 was significantly lower in vitamin E than the free-ranging population, whereas the group of samples from 1990 onwards was significantly higher. While male free-ranging black rhinoceros had significantly higher vitamin A levels than females (*U*-test, $P = 0.008$, $n = 58$), there was no difference in vitamin E levels between the sexes (*U*-test, $P = 0.621$, $n = 89$). No sex differences were detected for the captive black rhinoceros (*U*-test; vitamin A: $P = 0.931$, $n = 85$; vitamin E: $P = 0.984$, $n = 85$). A weak correlation was found in captive animals for age and vitamin A level (Spearman's $\rho = 0.448$, $P < 0.001$, $n = 81$), but not between age and vitamin E levels ($\rho = 0.103$, $P = 0.229$, $n = 81$).

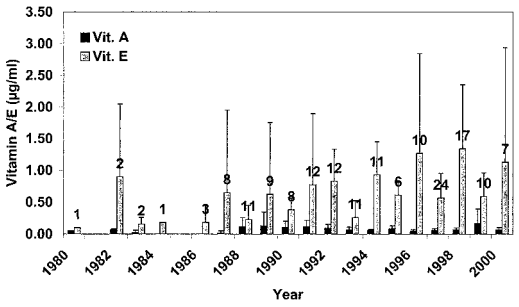

FIGURE 1. Annual average concentration of circulating vitamins A and E in captive black rhinoceros in North America (*n*, SD).

Differences were detected in circulating vitamin levels among four species of captive rhinoceros (Kruskal-Wallis-test; vitamin A: $P < 0.001$, $n = 156$; vitamin E: $P = 0.021$, $n = 156$). Pair-wise post-hoc tests revealed that Indian rhinoceroses had significantly lower vitamin A levels (Table 4) than black and white rhinoceroses and lower vitamin E levels than white rhinoceroses.

Captive white rhinoceroses had higher circulating concentrations of vitamins A and E than previously reported (Table 5). There was no significant difference between values of captive and free-ranging animals (*U*-test; vitamin A: $P = 0.881$, $n = 62$; vitamin E: $P = 0.194$, $n = 62$). The difference in both circulating vitamin A and E levels between captive white and black rhinoceroses was not significant, and no differences were found between free-living white and black rhinoceroses (*U*-test; vitamin A: $P = 0.054$, $n = 141$; vitamin E: $P = 0.158$, $n = 172$). Circulating

TABLE 4. Circulating concentrations of vitamins A (retinol) and E (α-tocopherol) in captive Sumatran (*Dicerorhinus sumatrensis*) and Indian rhinoceros (*Rhinoceros unicornis*).

	Retinol		α-tocopherol	
	n	μg/ml	*n*	μg/ml
Sumatran rhinoceros				
This study	4	0.04 (±0.01)	4	1.09 (±0.58)
Indian rhinoceros				
This study	10	0.03 (±0.02)	10	0.34 (±0.36)
Taugner et al., 1995			1	0.16
Jones, 1979	6	0.08 (0–0.17)		
Brush and Anderson, 1986			3	0–0.09

vitamin E (*U*-test, $P = 0.007$, $n = 61$) but not vitamin A (*U*-test, $P = 0.988$, $n = 61$), levels differed significantly for the annual average vitamin levels before and after 1990. No sex differences were detected in captive white rhinoceros (*U*-test; vitamin A: $P = 0.243$, $n = 55$; vitamin E: $P = 0.952$, $n = 55$). The effect of sex of free-ranging white rhinoceros was not tested due to the small sample size. In captive individuals, age was correlated with vitamin E levels ($\rho = 0.606$, $P < 0.001$, $n = 27$) but not with vitamin A levels ($\rho = 0.103$, $P = 0.610$, $n = 27$).

From the species comparisons of tissue vitamin levels, only the post-hoc tests are reported. Black rhinoceroses had significantly lower γ-tocopherol levels in all tissues than white rhinoceroses (Table 6). Indian rhinoceroses had significantly more vitamin A in liver compared to black and white rhinoceros, but they had consistently lower vitamin A levels in other tissues; however, the small sample size probably precluded the finding of significance in the other tissues. Both γ- and α-tocopherol levels were consistently positively correlated between all tissues, and both forms of tocopherol were positively correlated with each other in heart, liver, and skeletal muscle ($\rho = 0.419$–0.555, $P = 0.001$–0.021, $n = 30$–41) but not in adipose tissue (Table 7). Vitamin A levels of liver tissue were not correlated to levels in any other tissue; vitamin A levels in heart muscle tissue were correlated to levels in skeletal muscle and adipose tissue ($\rho = 0.443$–0.480, $P = 0.003$–0.004, $n = 35$–43). Stillborn and newborn black rhinoceroses had significantly lower levels of tocopherol in nearly all tissues and lower levels of retinol in liver tissues (Table 6). There was no significant influence of sex or age on the vitamin content of any tissue.

DISCUSSION

Physiologic data on free-ranging animals should provide baseline data against which

TABLE 5. Circulating concentrations of vitamins A (retinol) and E (α-tocopherol) in white rhinoceros (*Ceratotherium simum*).

	Retinol		α-tocopherol	
	n	μg/ml	*n*	μg/ml
Captive rhinoceros				
This study	57	0.07 (±0.04)	57	0.62 (±0.48)
Brush and Anderson, 1986			5	0.09 (0–0.17)
Jones 1979	21	0.16 (0.04–0.36)		
Ghebremeskel and Williams, 1988	1	0.12	1	0
Free-ranging rhinoceros				
This study	5	0.06 (±0.02)	5	0.77 (±0.32)

TABLE 6. Tissue levels of vitamins A (retinol) and E (γ- and α-tocopherol) in captive rhinoceros and horse normal values from Dierenfeld and Traber (1992) and Puls (1994).

Species		Age at death (yr)	Liver			Adipose tissue			Heart			Skeletal muscle		
			γ-t.[a] μg/g	α-t.[b] μg/g	Retinol μg/g	γ-t. μg/g	α-t. μg/g	Retinol μg/g	γ-t. μg/g	α-t. μg/g	Retinol μg/g	γ-t. μg/g	α-t. μg/g	Retinol μg/g
Black rhinoceros														
Adults	mean	16.9	0.79[A,c]	23.69	193.40[A]	1.21[A]	22.29	0.67	0.56[A]	20.71	0.35	0.39[A]	8.94	0.21
	SD	10.7	0.91	26.72	248.45	1.37	29.79	0.66	0.55	19.04	0.34	0.37	8.84	0.21
	n	28	23	28	28	14	17	17	21	28	28	18	26	26
New-stillborn	mean		0.00*[d]	3.42*	0.39	0.00	3.49*	0.47	0.00*	3.51*	48.03*	0.00	2.71	0.22
	SD		0.00	2.63	0.80	0.00	3.81	0.48	0.00	2.62	85.47	—	2.04	0.20
	n		2	6	5	2	7	6	3	9	9	1	7	6
Sumatran rhinoceros	mean	8	0.50	19.48	266.35	0.49	8.15	0.38	0.72	34.72	0.27	0.48	16.57	0.37
	SD	—	0.06	1.70	273.76	0.22	4.15	0.03	0.25	8.61	0.14	0.24	8.25	0.21
	n	1	3	3	3	3	3	3	3	3	3	3	3	3
Indian rhinoceros	mean	21.8	0.93	24.91	503.04[B]	1.39	9.91	0.09	0.79	32.84	0.18	0.82	36.62	0.09
	SD	11.3	0.60	20.42	231.17	1.38	9.32	0.01	0.70	33.24	0.04	—	—	—
	n	3	4	4	4	2	2	2	2	2	2	1	1	1
White rhinoceros	mean	24.7	3.13[B]	8.98	149.25[A]	11.23[B]	14.83	0.54	4.13[B]	11.33	0.33	2.46[B]	4.67	0.10
	SD	9.1	2.97	8.40	113.42	9.86	12.58	0.41	2.81	9.21	0.66	1.70	3.67	0.10
	n	11	7	11	10	6	9	8	6	10	9	6	10	9
Horse normal values				4.3–8.3	66–166		25						8–12	

[a] γ-t. = γ-tocopherol.
[b] α-t. = α-tocopherol.
[c] Values within a column with different superscripts differ significantly.
[d] Values for stillborn black rhinoceroses marked with an asterisk differ significantly from adult animals.

TABLE 7. Correlations (ρ; *P*; *n*) between different tissues for γ-tocopherol (in bold) and α-tocopherol (in italics) content in four captive rhinoceros species.

	Heart	Adipose	Liver	Skeletal muscle
Heart		**0.513**	**0.802**	**0.739**
		0.007	**<0.001**	**<0.001**
		26	**35**	**28**
Adipose	*0.477*		**0.499**	**0.555**
	0.002		**0.007**	**0.005**
	38		**28**	**24**
Liver	*0.857*	*0.474*		**0.796**
	<0.001	*0.002*		**<0.001**
	50	*39*		**29**
Skeletal muscle	*0.875*	*0.463*	*0.861*	
	<0.001	*0.004*	*<0.001*	
	45	*36*	*47*	

values of captive animals can be compared. While domestic horses provide a reasonable model for macronutrient digestion (Dierenfeld et al., 2000) and mineral metabolism (unpubl. data) in rhinoceroses, this does not appear to be the case for fat soluble vitamins. Free-ranging black and white rhinoceroses, and captive rhinoceroses of all species, had lower circulating vitamin A and E levels compared with normal horse values. In this respect, rhinoceroses resemble elephants that also display remarkably low circulating levels of these vitamins compared to perissodactyls (Dierenfeld and Traber, 1992; Dierenfeld et al., 1998). Additionally, similar to the findings of Slifka et al. (1999), we did not detect circulating carotenoids in free-ranging and captive black rhinoceroses. In contrast, levels of 0.60 ± 0.24 μg/ml were detected in semi-free-ranging Przewalski horses (*Equus caballus*), which were approximately five times those reported in stabled domestic horses used as a comparative model (Dierenfeld et al., 1997). The comparatively high levels of circulating $25(OH)D_3$ in free-ranging black rhinoceroses are of a magnitude that would be considered toxic for horses according to Puls (1994), but they are within the upper normal range of this nutrient for humans, and they do not seem to cause intoxication. The low levels of this vitamin measured in two captive individuals (Hamilton, 1999) are difficult to explain.

Analytical methods for the determination of vitamin levels have become more sensitive in recent years; they often differ between laboratories, making direct comparisons difficult. The high concentration of vitamin E reported by Ghebremeskel et al. (1988) for free-ranging animals was not found in this study. The values recorded by Vahala (1990) for three captive animals were also high. Laboratory analytical methods are suspected to underlie differences in the latter example because vitamin A concentrations reported for these individuals are also considerably higher than levels reported by other investigators, except Jones (1979). Additionally, unknown variables associated with storage and handling of plasma, serum, and tissue samples may impact results in a contradictory manner: dehydrated samples may result in artificially elevated concentrations, whereas oxidized or deteriorated samples may contain lower measured concentrations. Plasma samples utilized in this study were known to have been stored appropriately (short-term frozen, unexposed to air or light), and tissue subsamples analyzed were taken from the interior, rather than the periphery of partially thawed samples. Handling and analytical techniques associated with samples in the cur-

rent study, as well as the much larger dataset, support our assumption of representative descriptive data.

Two interpretations for the vitamin E values in rhinoceros blood are offered in the literature. Dierenfeld and Traber (1992) suggested that rhinoceroses lack a certain high-density lipoprotein carrier for vitamin E and/or hepatic tocopherol-transfer protein to incorporate vitamin E into plasma lipoproteins. Ghebremeskel et al. (1988) and Papas et al. (1991) suggested a species difference in bile function and hence vitamin E absorption.

Differences in vitamin metabolism between equids and rhinoceroses are evident when comparing vitamin E values found in horse adipose tissue with values of captive rhinoceroses (Table 6). Clearly vitamin A appears to be stored preferentially in the liver, as it is in other species; however, vitamin E concentrations are equally distributed among tissue types in rhinoceroses, suggesting its role as a general biological antioxidant in all tissues. Dierenfeld (1994) hypothesized that the seasonal environment of temperate equids led to the adaptation of storing vitamins in retrievable tissues for winter periods, which would seem less necessary for animals from warmer climates. In this respect, investigations of other equids, such as free-ranging zebras (*Equus* spp.), would provide an interesting comparison. The remarkable discrepancy in liver and tissue vitamin A levels in Indian rhinoceroses, together with the extremely low circulating vitamin A values in this species, also warrants further investigation, especially with respect to the high incidence of foot lesions in this species in captivity (von Houwald and Flach, 1998).

The contradicting correlations of circulating vitamin concentrations with age in black and white rhinoceros are difficult to interpret. In theory, an increase in stored vitamin A in liver tissue with age would be expected, which was not the case in our data set. Similarly, the cause for the higher circulating vitamin A concentrations in free-ranging male black rhinoceros can only be speculated upon: while it is conceivable that bulls defend territories of higher nutritional quality, information on the social status of the animals sampled is lacking.

When Dierenfeld et al. (1988) and Ghebremeskel et al. (1988) reported lower circulating vitamin E levels in captive versus free-ranging black rhinos, there was speculation on causes and potential negative effects. Native forage consumed by free-ranging black rhinoceros was shown to be higher in vitamin E and fat content than diets for captive animals (Dierenfeld et al., 1990, 1995; Ghebremeskel et al., 1991). It was hypothesized that the higher dietary fat supply in the wild enhanced bile production and hence vitamin E absorption. Because it had been shown that a low supply of vitamin E could lead to erythrocyte hemolysis in humans (Tudhope and Hopkins, 1975), primates (Ausman and Hayes, 1974), rats (Bieri and Poukka, 1970; Peake and Bieri, 1977), and horses (Stowe, 1968), the seemingly low vitamin E levels of black rhinoceroses were regarded as an underlying cause for hemolytic anemia reported in captive individuals (Miller et al., 1986), and dietary supplementation was implemented (Dierenfeld and Traber, 1992). The striking increase in yearly average circulating vitamin E levels (Fig. 1) from 1990 to 2000 may reflect increased supplementations. However, a discrepancy between a high level of dietary vitamin E and low circulating levels in rhioceroses was reported (Lewis and Kirkwood, 1990; Ghebremeskel et al., 1991; Papas et al., 1991), and it was concluded that the supplement formulation is important. Feeding practice concentrated on tocopherol-polyethylene-glycol-succinate (TPGS; Papas et al., 1991). The serum vitamin E levels determined in our study suggest that all vitamin E formulations currently in use appear to be absorbed and reflected in circulating blood levels. Whether these levels correspond to a biochemically active state

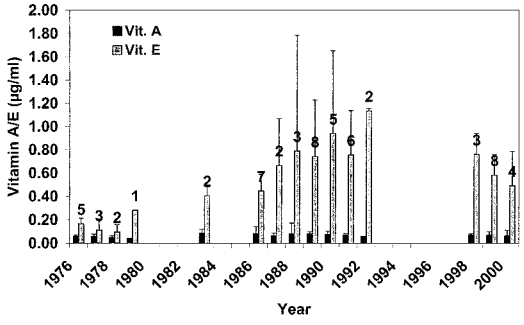

FIGURE 2. Annual average concentration of circulating vitamins A and E in captive white rhinoceros in North America (*n,* SD).

remains to be demonstrated (Dierenfeld, 1999).

The increase in serum vitamin E in captive white rhinoceroses (Fig. 2) suggests that supplementation was also expanded to this species, although the generally lower α-tocopherol levels in blood and tissues suggest that this vitamin is not supplemented to the same extent as in black rhinoceros. The data on γ-tocopherol tissue concentrations support this finding; although most foods contain higher concentrations of γ-tocopherol than α-tocopherol, human and animal tissue concentrations of α-tocopherol are several fold greater than those of γ-tocopherol (Bieri and Evarts, 1974). The proportion of the tocopherols in body tissues reflects their proportion in the diet, with an increased proportion of α-tocopherol leading to a decrease in γ-tocopherol levels (Handelmann et al., 1985; Behrens and Madere, 1987; Traber and Kayden, 1989). Therefore, a higher supplementation with α-tocopherol will lead to lower levels of γ-tocopherol, as in the black rhinoceroses of our study.

Whether the high level of vitamin E supplementation to captive black rhinoceroses had the desired beneficial effect on animal health remains questionable. It has been shown that there is no correlation between black rhinoceros red blood cell hemolysis and circulating vitamin E levels (Ullrey et al., 1989). Animals continued to suffer from hemolytic anemia even after increased supplementation from 1990 onwards (Paglia and Dennis, 1999). Accordingly, an animal suffering from myoglobinuria described by Jarofke and Kloes (1988) showed no signs of vitamin E-deficiency associated muscle lesions but only the usual signs of hemolytic anemia at necropsy (Kulow, 1990). The significantly higher levels of circulating vitamin E since 1990 in captive compared to free-ranging black rhinoceroses (Table 2) suggest a possible oversupplementation in captive animals. However, unless coagulopathies are reported in black rhinoceros (cf. Nichols et al., 1989), negative effects of such a degree of oversupplementation are not to be expected (Farrell, 1980).

Due to high iron levels in tissues of captive black rhinoceroses, these animals are likely to be susceptible to increased oxidative stress (Paglia and Dennis, 1999; Paglia, pers. comm.), and high levels of antioxidant vitamins would appear beneficial. However, Herbert (1994) and Haessig et al. (1999) state that the success of conventional vitamin supplementation is especially questionable in humans with high hepatic iron loads, and the latter authors emphasize the antioxidant effects of polyphenols (flavonoids/tannins) in excessive iron storage. Polyphenolic compounds prevent iron-associated oxidative damage very efficiently (Kuehnau, 1976; Cook and Samman, 1996; Bravo, 1998). Polyphenols occur in native browses consumed by black rhinoceroses but are rare in diets fed to captive animals (Wright, 1998). The potential of such compounds to increase antioxidant status in captive black rhinos, and their sources, should be investigated.

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