EFFICACY OF ABSORPTION OF VARIOUS VITAMIN E FORMS BY CAPTIVE ELEPHANTS AND BLACK RHINOCEROSES

Andreas M. Papas, Ph.D., Richard C. Cambre, D.V.M., Scott B. Citino, D.V.M., and Ronald J. Sokol, M.D.

Abstract: A biochemical vitamin E deficiency may exist in captive elephants (*Elephas maximus* and *Loxodonta africana*) and black rhinoceroses (*Diceros bicornis*) because plasma α-tocopherol concentrations apparently are lower in these animals than in their free-ranging counterparts. Analysis of serum or plasma from 35 elephants and 11 black rhinoceroses from 11 zoological institutions and one private owner confirmed common occurrence and persistence of low circulating α-tocopherol levels. Concentrations averaged <0.3 μg/ml despite prolonged supplementation with D,L-α-tocopheryl acetate, the most common vitamin E supplement for animal diets. Further experimental work demonstrated that supplementing the diet with D,L- or D-α-tocopheryl acetate or D-α-tocopherol to provide up to 62 IU/kg body weight (BW) in elephants and 23 IU/kg BW in black rhinoceroses increased circulating blood α-tocopherol by <0.2 μg/ml. Apparently, elephants and black rhinoceroses absorbed these fat-soluble or water-dispersible forms of vitamin E poorly. In contrast, the water-soluble form, D-α-tocopheryl polyethylene glycol 1,000 succinate (TPGS), was absorbed well, as indicated by rapid increases in circulating blood α-tocopherol (0.3–1.9 μg/ml) from several-fold lower TPGS doses in the diet (4.8 or 6.6 IU/kg BW in elephants and 1.5 or 3.9 IU/kg BW in black rhinoceroses). There is a marked difference in the bioavailability of TPGS versus other vitamin E forms in captive elephants and black rhinoceroses, suggesting that there are major species differences in the utilization of various forms of vitamin E.

Key words: Nutrition, vitamin E, water-soluble vitamin E, D-α-tocopheryl polyethylene glycol 1,000 succinate, elephant, *Elephas* sp., *Loxodonta* sp., black rhinoceros, *Diceros bicornis*.

INTRODUCTION

Impetus for this work was provided by the death of a 20-yr-old female African elephant (*Loxodonta africana*) at the Denver Zoological Gardens. Postmortem examination revealed acute and chronic severe rhabdomyolysis of skeletal muscle in the hind leg and mild acute degeneration of the heart muscle; bacterial pneumonia developed probably as a secondary complication. Serum creatine phosphokinase and lactate dehydrogenase, measured in the 2 wk preceding death, were significantly elevated.

From the Eastman Chemical Co., P.O. Box 431, Kingsport, Tennessee 37662, USA (Papas); the Denver Zoological Gardens, City Park, Denver, Colorado 80205, USA (Cambre); the National Zoological Park, 3000 Connecticut Avenue N.W., Washington, D.C. 20008, USA (Citino); and the Department of Pediatrics, University of Colorado School of Medicine, P.O. Box C228, 4200 East Ninth Avenue, Denver, Colorado 80262, USA (Sokol).

These findings, coupled with a depressed serum α-tocopherol concentration (0.05 μg/ml) and apparently normal serum selenium (0.18 μg/ml), suggested vitamin E deficiency as a contributory cause. Because the estimated daily vitamin E intake from the feed and supplemental D,L-α-tocopheryl acetate exceeded 1.5 IU/kg BW and was within the range proposed for elephants[5] and above the recommended allowances by the National Research Council for other herbivores,[22] this deficiency could not be explained.

Vitamin E deficiency is known to cause disorders of the reproductive, muscular, circulatory, and nervous system in many animal species.[18,19] Because the circulating blood levels of vitamin E (measured as α-tocopherol) in captive elephants (*Elephas maximus* and *L. africana*) and black rhinoceroses (*Diceros bicornis*) have been reported as substantially lower than those of their free-ranging wild counterparts,[1,3,5,6] a possible biochemical deficiency has been

implicated in incidents of myopathy in elephants.[3] This condition also has been suspected, but not confirmed, as a contributory factor in hemolytic anemia and encephalomalacia in black rhinoceroses.[20,21] In earlier studies of captive black rhinoceroses, the level of dietary supplementation was not quantified. Thus, a biochemical deficiency resulting from low intake could not be determined.[6,9] For elephants, the correlation between dietary intake and blood concentrations of vitamin E observed in one zoological park became statistically nonsignificant when pooled with estimated data from eight other parks.[5] Thus, quantification of dietary levels of vitamin E has been a serious limitation of earlier studies.

The objective of this work was twofold: 1) to determine whether low circulating blood levels of α-tocopherol in captive elephants and black rhinoceroses persisted despite prolonged supplementation with D,L-α-tocopheryl acetate, the common form added to the diet; and 2) to determine the efficacy of various vitamin E forms in improving vitamin E status.

MATERIALS AND METHODS

Supplemental vitamin E

The vitamin E forms evaluated were as follows: D,L-α-tocopheryl acetate, solid, 500 IU/g; D-α-tocopheryl polyethylene glycol 1,000 succinate (TPGS), 20% solution in water, 77.4 IU/ml; D-α-tocopherol, oil, 1,000 IU/g; and D-α-tocopheryl acetate as oil, 1,100 IU/g or water-dispersible solid, 700 IU/g (embedded in gelatin). (All forms evaluated were from Eastman Chemical Co., Kingsport, Tennessee 37662, USA, except for D,L-α-tocopheryl acetate, which was obtained from several suppliers [Hoffmann-La Roche, Nutley, New Jersey 07110, USA; BASF, Parsippany, New Jersey 07054, USA; and Rhone Poulenc, Monmouth, New Jersey, 08852, USA].) The D,L forms of vitamin E are mixtures of eight stereoisomers and are produced synthetically; D-α-tocopherol is a single stereoisomer derived

from natural sources and modified chemically to produce its esters. The TPGS was prepared by esterification of polyethylene glycol 1,000 to the acid group of D-α-tocopheryl succinate.[8]

Dietary vitamin E supplementation and circulating blood concentrations

Data were obtained for 35 elephants and 11 black rhinoceroses held by 11 zoological institutions and one private owner (Table 1). In all cases, the supplemental form of vitamin E was D,L-α-tocopheryl acetate. Serum or plasma samples were frozen at $-30°C$ or lower until packaged on dry ice and shipped for later determination of α-tocopherol by high performance liquid chromatography (HPLC) with fluorescence detection.[10]

Elephant trials

Trial 1 was conducted at the Denver Zoological Gardens with two female Asian elephants (*E. maximus*) (estimated ages: 34 and 31 yr; weights: 3,264 and 4,855 kg, respectively). Both animals were offered the same diet as was provided for the previous 9 mo, which was fortified with D,L-α-tocopheryl acetate to supply 1.7 IU/kg BW (total computed from feed components and supplement). The daily diet was composed of a grain mixture (8–10 kg), monkey biscuits (0.7 kg), prairie grass hay (48.0 kg), and produce (apples, carrots, and bananas; 3.0 kg). To ensure consumption of the intended amount of the test form of vitamin E, the dose was added to a small portion of the grain mixture. After determining a baseline blood α-tocopherol level (Fig. 1), which reflected vitamin E status from the diet, both elephants were dosed with the following vitamin E forms and concentrations: TPGS, 20% solution in water, 4.8 IU/kg BW; D-α-tocopherol, oil, 5.1 IU/kg BW, mixed with corn oil 1:1 (corn oil added was 16 and 24 g/day for the two elephants) or without corn oil at 5.1 and 10.2 IU/kg BW; and D-α-tocopheryl acetate (oil form), 30 IU/kg BW.

Table 1. Dietary and circulating vitamin E levels in elephants (*Elephas maximus* and *Loxodonta africana*) and black rhinoceroses (*Diceros bicornis*). Data from other sources included for comparison.

| Species and source | n | Vitamin E | | Circulating level[b] (μg/ml) |
| | | Dietary supplementation[a] | | |
		Level	Duration	
Elephants[c]				
Free-ranging				
Reference no. 6	10	—	—	0.79 ± 0.05
Kruger Natl. Park[d]	15	—	—	0.74
Denver Zoological Gardens, Denver, Colorado	3	1.5–2.0 IU/kg BW	9 mo	0.09 ± 0.02
Miami Metrozoo, Miami, Florida	5	1.6 IU/kg BW	18 mo	0.09 ± 0.01
Elephantastic, Inc., Denver, Colorado	8	0.3 IU/kg BW	>12 mo	0.06 ± 0.01
Reid Park, Tuscon, Arizona	2	4.5 IU/kg BW	>4 yr	0.23 ± 0.08
Lincoln Park Zoo, Chicago, Illinois	3	1,060 IU/day	>12 mo	0.23 ± 0.04
Jacksonville Zoo, Jacksonville, Florida	4	1.2–1.6 IU/kg BW	31 mo	0.16 ± 0.01
Louisville Zoological Park, Louisville, Kentucky	3	140 IU/day	>2 yr	0.22 ± 0.05
Brookfield Zoo, Brookfield, Illinois	2	1,900–3,400 IU/day	>12 mo	0.29 ± 0.03
Fort Worth Zoo, Fort Worth, Texas	3	6,000 IU/day	>2 yr	0.17 ± 0.02
Lee Richardson Zoo, Garden City, Kansas	2	0	—	0.07 ± 0.02
Black rhinoceros				
Free-ranging				
Reference no. 9	28	—	—	1.92 ± 0.08
Reference no. 6	31	—	—	0.77 ± 0.05
Reference no. 4	79[e]	—	—	0.2–0.8
Denver Zoological Gardens	4	2,500 IU/day	10–11 mo	0.13 ± 0.01
Miami Metrozoo	2	5,600 IU/day	18 mo	0.16 ± 0.06
St. Louis Zoological Park, St. Louis, Missouri	3	1,000–6,000 IU/day	9–40 mo	0.13 ± 0.03
Lee Richardson Zoo	1	2,500 IU/day	10 mo	Not detectable
Cincinnati Zoo, Cincinnati, Ohio	1	1,636 IU/day	2 yr	0.23

[a] Supplemental vitamin E was D,L-α-tocopheryl acetate.

[b] Serum or plasma analyzed; reported as $\bar{x} \pm$ SEM.

[c] Captive elephants studied included both African and Asian; free-ranging animals were only African.

[d] Male elephants from Kruger National Park, South Africa; data reported in the Elephant Species Survival Plan, Indianapolis, Indiana, September 1990.

[e] Locations, number of animals, and plasma α-tocopherol (μg/ml) were as follows: South Africa, 44, 0.6; Kenya, 7, 0.2; Namibia, 4, 0.8; and Zimbabwe, 24, 0.5.

Washout periods were allowed between supplements.

Blood samples were drawn by catheterizing an accessible ear vein 2–5× per week during baseline and dosing and at least once per week during the washout periods. Blood samples were aspirated into a disposable syringe, transferred into a serum tube, and permitted to clot and then serum was recovered and frozen at −77°C until analyzed for α-tocopherol.[10] Representative samples from the baseline, TPGS, and D-α-tocopherol periods were analyzed for total lipids and cholesterol using the Ektachem 700 Clinical Analyzer (Eastman Kodak, Rochester, New York 14650, USA). Serum samples from the baseline and midtrial were analyzed for selenium.[30] The paired Student's *t*-test was used to compare the serum concentration for the last week of each dose period with the concentration immediately preceding baseline or washout period, with each animal serving as its own control. The same test was used for comparing vitamin E forms.

Trial 2 was conducted at the Denver Zoological Gardens with the following four elephants: 1) 25-yr-old female Asian, weigh-
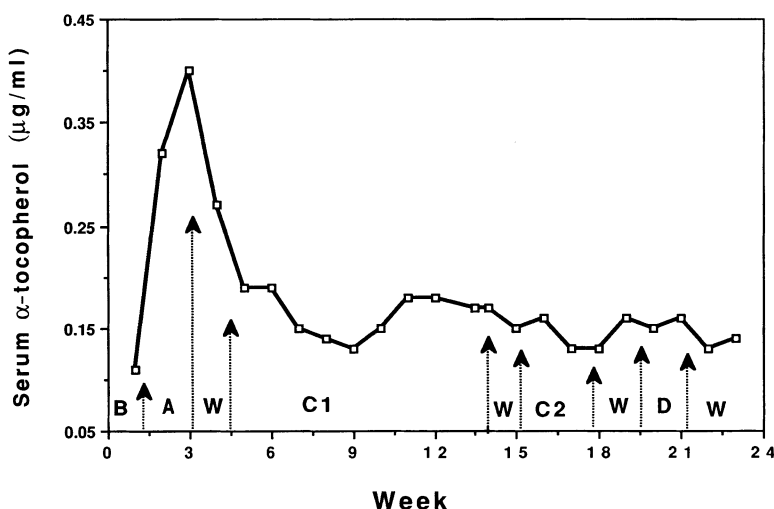
Figure 1. Effect of supplementation with D-α-tocopheryl polyethylene glycol 1,000 succinate (TPGS), fat-soluble, and water-dispersible forms of vitamin E on the circulating serum levels of α-tocopherol of elephants in trial 1. Arrows denote change in dose form or amount. B: base line, SEM = 0.007; A: TPGS, 20% solution in water, 4.8 IU/kg BW, SEM = 0.106; W: washout, SEM = 0.002; C1: D-α-tocopherol, oil, 5.1 IU/kg BW, added as mixture with corn oil 1:1 (weeks 5–11), and following washout (week 12), it was evaluated without corn oil (week 13–14), SEM = 0.045; C2: D-α-tocopherol, oil form, 10.2 IU/kg BW, SEM = 0.033; D: D-α-tocopheryl acetate (oil form), 30 IU/kg BW, SEM = 0.024.

ing 2,895 kg; 2) 5-yr-old male African, weighing 811 kg; 3) 3-yr-old female African, weighing 650 kg; and 4) 3-yr-old female African, weighing 547 kg. Diets before and during the trial consisted of a grain mixture (2.3 kg [4.1 kg for elephant 1]); protein, mineral, and vitamin supplement (0.3 kg); and prairie grass hay (67, 40, 20, and 20 kg, respectively, for elephants 1–4). Following a baseline period, which reflected vitamin E status from the diet that included D,L-α-tocopheryl acetate at <0.5 IU/kg BW, the following forms and dose levels were tested (Fig. 2): D-α-tocopheryl acetate, solid, water dispersible, 62 IU/kg BW; TPGS, 20% solution in water, 6.6 IU/kg BW; and D-α-tocopherol, oil, 62 IU/kg BW. Analytical methods and statistical comparisons were as in trial 1.

Black rhinoceros trial

The rhinoceros trial was conducted at the Miami Metrozoo with one 12-yr-old male and one 16-yr-old female (estimated BW: 1,200–1,500 kg). During the trial, the ani-

mals continued to receive the same diet they had been receiving for >1 yr, with the exception of the vitamin E supplement, which was replaced with the test forms. Their daily diet consisted of 2.7 kg pelleted feed (ADF 16 Low Fiber Herbivore Diet 9034, HMS, Bluffton, Indiana 46714, USA), 0.8 kg pelleted supplement (Elephant Diet, Cargill, Nutrena Feed Division, Minneapolis, Minnesota, 55440, USA), 9–14 kg timothy hay, and 1.0–1.5 kg alfalfa hay. This diet provided 4.1 IU/kg BW supplemental vitamin E as D,L-α-tocopheryl acetate. The test forms of vitamin E were provided in admixture with grain and water to formulate a moist ball, which was offered by hand to ensure consumption. Following determination of baseline plasma α-tocopherol levels, the following forms and dose levels replaced D,L-α-tocopheryl acetate in the supplement (Fig. 2): D-α-tocopheryl acetate, solid, water-dispersible, 1.5 and 23 IU/kg BW; and TPGS, 20% solution in water, 1.5 and 3.9 IU/kg BW.
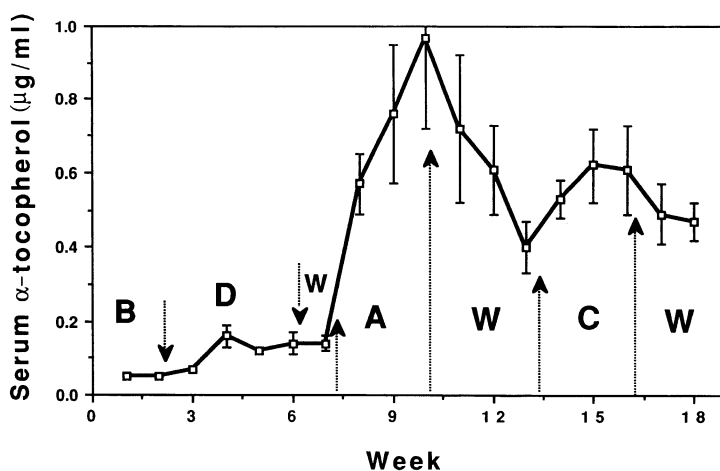
Blood was sampled 1–2× per week from

Figure 2. Effect of supplementation with TPGS, fat-soluble, and water-dispersible forms of vitamin E on the circulating serum levels of α-tocopherol of elephants in trial 2. Arrows denote change in dose form or amount. B: baseline; D: D-α-tocopheryl acetate, solid, water dispersible, 62 IU/kg BW; W: washout; A: TPGS, 20% solution in water, 6.6 IU/kg BW; C: D-α-tocopherol, oil, 62 IU/kg BW.

the radial vein and transferred into a tube containing ethylene diamine tetraacetic acid (EDTA). Plasma was separated by centrifugation, stored at $-70°C$, then packaged on dry ice and shipped to the same laboratory as used for the elephant trials to be analyzed for α-tocopherol. Statistical analyses were as above.

RESULTS

Data on supplemental vitamin E level, length of supplementation, and associated circulating levels in the serum or plasma for captive elephants and black rhinoceroses as well as from other published sources are summarized in Table 1. Circulating serum or plasma α-tocopherol in captive elephants from all sources ($n = 35$) ranged from 0.06 ± 0.01 ($\bar{x} \pm$ SEM) to 0.29 ± 0.03 $\mu g/ml$, values substantially lower than concentrations of 0.79 ± 0.05[6] and 0.74 $\mu g/ml$ reported for free-ranging African elephants. Similarly, for captive black rhinoceroses, the circulating levels ranged from nondetectable to 0.23 $\mu g/ml$, values considerably less than those reported for free-ranging animals.[4,6,9]

Results for elephant trial 1 are summarized in Figure 1. A low baseline level of 0.11 $\mu g/ml$ was observed despite supplementation with D,L-α-tocopheryl acetate at 1.5–2.0 IU/kg BW for 9 mo. Supplementation with TPGS rapidly increased serum α-tocopherol levels to 0.4 $\mu g/ml$ ($P < 0.05$). Following a 1-wk washout, serum α-tocopherol did not return to baseline; therefore, for each subsequent form of vitamin E tested, the serum α-tocopherol was compared with that of the immediately preceding washout period. Following TPGS, D-α-tocopherol supplementation showed no measurable effect, nor did doubling the dose. The D-α-tocopheryl acetate in oil form at a high dose also failed to increase serum α-tocopherol.

Results for elephant trial 2 are summarized in Figure 2. The water-dispersible form of D-α-tocopheryl acetate produced a significant ($P < 0.03$) increase in serum α-tocopherol, from 0.05 to 0.15 $\mu g/ml$; in contrast, TPGS at a lower dose increased the serum level from 0.15 to 0.97 $\mu g/ml$ ($P < 0.04$). D-α-tocopherol at the same high dose as its acetate ester produced a small increase ($P < 0.08$) in serum α-tocopherol over the preceding washout.

Serum cholesterol levels for the six elephants in the two trials decreased from 53

± 3 mg/100 ml during the baseline period to 42 ± 3 mg/100 ml during the last week of TPGS supplementation ($P < 0.01$) and returned to the baseline level (55 ± 8) during dosing with D-α-tocopherol. Similarly, serum total lipids decreased from 163 ± 13 to 147 ± 3 and increased back to 226 ± 29 mg/100 ml during the same periods. Serum selenium concentrations for the baseline and midtrial were 0.130 ± 0.026 and 0.168 ± 0.017 μg/ml, respectively. Differences were not significant.

Results for the black rhinoceros trial are summarized in Figure 3. Despite prolonged supplementation with D,L-α-tocopheryl acetate, plasma α-tocopherol baseline was low (0.16 ± 0.06 μg/ml). The water-dispersible form of D-α-tocopheryl acetate increased plasma α-tocopherol slightly to 0.21 ± 0.11 μg/ml; even at a very high dose, the observed increase to 0.31 ± 0.09 μg/ml was small and not statistically significant. In contrast, TPGS at a several-fold lower dose increased plasma α-tocopherol substantially to >2.0 μg/ml ($P < 0.05$ for TPGS vs. preceding washout; $P < 0.08$ for TPGS vs. D,L-α-tocopheryl acetate).

DISCUSSION

Despite substantial and prolonged supplementation with D,L-α-tocopheryl acetate, captive elephants and black rhinoceroses continued to produce low circulating α-tocopherol concentrations. Because data from free-ranging animals are limited and seasonal variations associated with location, vegetation, and other factors are likely,[4,7] the significance of the difference cannot be determined with certainty. The levels of α-tocopherol observed, however, for captive elephant and black rhinoceros were significantly lower than those reported for other species in captivity[1,3] and for farm animals.[11,12,14] Circulating blood concentrations reflect the amount absorbed,[19] indicating that D-α-tocopherol and D, or D,L-α-tocopheryl acetate (in both the oil and water-dispersible solid forms) were absorbed poorly. In contrast, TPGS was absorbed rapidly and produced a substantial increase in the blood α-tocopherol, even in doses 10-fold lower than other forms. Because serum vitamin E levels are proportional to the lipid concentration in humans,[13] it was important that the increases in vitamin E levels in these species, observed during TPGS supplementation, were not caused by increased lipids. In fact, serum cholesterol and total lipids decreased during TPGS supplementation and returned to the baseline level during D-α-tocopherol dosing. This phenomenon may be similar to what is seen in vitamin E-deficient children with chronic cholestasis. These individuals experience severe malabsorption of fat-soluble and water-dispersible vitamin E forms because of insufficient bile salt secretion for solubilization of dietary fat, yet they absorb TPGS quite well.[27,28] Unlike the cholestatic children, however, where a disease condition may explain the malabsorption, this phenomenon occurred in healthy elephants and black rhinoceroses. When compared with other species, these two species appear to offer dramatic examples of differences in bioavailability of various forms of vitamin E.

Rat studies indicate a preferential uptake by the blood and other tissues of the RRR- (also designated as D-) compared with SRR-α-tocopheryl, one of the eight stereoisomers in D,L-α-tocopheryl acetate.[15] Furthermore, recent work with cattle and sheep demonstrated that the D form is more potent than the D,L form, and the tocopherol form appears to increase circulating levels more than an equimolar amount of acetate ester of vitamin E supplement.[11,12] Despite the apparent advantage of the D form over other stereoisomers, animal diets are supplemented almost exclusively with D,L-α-tocopheryl acetate primarily because of lower cost.

In their natural habitat, elephants and black rhinoceroses feed on plants containing primarily D-α-tocopherol and other D-tocopherols. The acetate ester, which is added to the diets because it increases stability during storage, requires hydrolysis
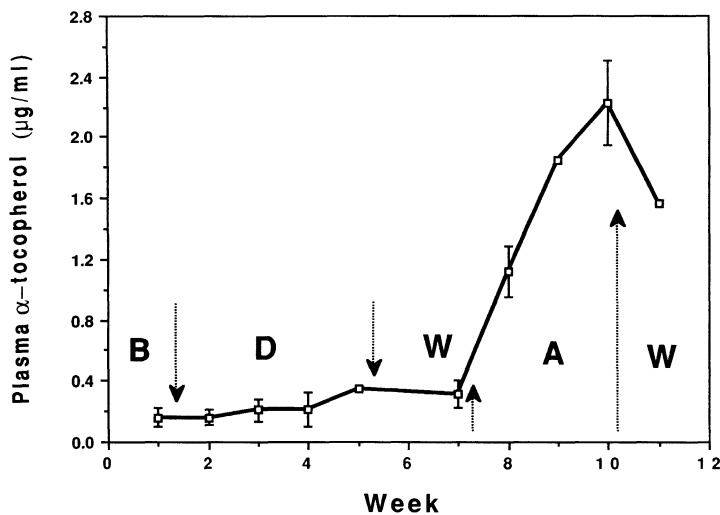
Figure 3. Effect of supplementation with TPGS, fat-soluble, and water-dispersible forms of vitamin E on the circulating plasma levels of α-tocopherol of black rhinoceroses. Data are shown as $\bar{x} \pm$ SEM. The following vitamin E forms and dose levels were evaluated in the sequence and duration shown. B: baseline; D: 1.5 IU/kg BW D-α-tocopheryl acetate, solid, water-dispersible replaced D,L-α-tocopheryl acetate (weeks 2–4), followed by 23 IU/kg BW (week 5); W: washout; A: TPGS, 20% solution in water, 1.5 IU/kg BW (weeks 8–9) followed by 3.9 IU/kg BW (week 10).

prior to absorption of the tocopherol moiety. Absorption of D-α-tocopherol appeared to be only marginally enhanced over its acetate ester, thus indicating that insufficient esterase activity could not fully explain this phenomenon. A general lipid malabsorption condition could not fully account for these differences because there was no apparent serious deficiency of other fat-soluble vitamins. The poor absorption of D-α-tocopherol is particularly puzzling because this form is believed to be the predominant one in natural foods. It is possible that the tocopherol molecule in fresh food exists in a physical or chemical configuration that is more conducive to absorption. Alternatively, other dietary components might stimulate improved pancreatic or biliary secretions that would enhance vitamin E absorption. Species differences in the composition of bile have been reported,[17] and the absence of bile acids may account for the observed differences in the absorption of supplemental vitamin E by elephants and black rhinoceroses (A. H. Hofmann, pers. comm.).

In contrast to the fat-soluble and water-dispersible forms, TPGS does not require biliary secretions (i.e., bile acids) for solubilization and absorption,[28,29] and this explains its absorption by cholestatic children. In addition to elephants and black rhinoceroses, horses utilize TPGS well.[24] In contrast, green iguanas (*Iguana iguana*), tapirs (*Tapiris* spp.), deer (*Ococoileus* sp.), and pigs absorb TPGS poorly.[14,24] Further research is required to determine the role of the bile system in the absorption of fat-soluble forms of vitamin E.

Vitamin E enhances the immune system;[23] thus, an adequate status may be important for reducing the incidence of disease, especially under conditions of stress. Animal diets are routinely supplemented with emphasis on adding a target level of IU to meet the recommended allowances or the perceived requirement. This study shows that the dietary vitamin E content is not meaningful without consideration of the absorption and utilization of the form added. The commonly used form, D,L-α-tocopherol acetate, failed to increase the circulating

levels of α-tocopherol in elephants and black rhinoceroses. For these species, TPGS is well absorbed and may be the preferred form. A small amount of the polyethylene glycol 1,000 of TPGS might be absorbed; however, based on animal toxicity studies[2,16,25,26] and investigations in vitamin E deficient cholestatic children,[27-29] the polyethylene glycol appears to be promptly excreted in the urine and lacks any significant toxicity.

CONCLUSIONS

1. Major animal species differences exist in the utilization of various forms of vitamin E. For this reason, vitamin E supplements selected must be bioavailable to the target species.

2. Elephants and black rhinoceroses absorb fat-soluble and water-dispersible forms of D- or D,L-α-tocopheryl acetate and D-α-tocopherol poorly. In contrast, these species rapidly absorb TPGS, a water-soluble form of vitamin E.

3. These findings explain the persistently low circulating blood α-tocopherol concentrations in captive elephants and black rhinoceroses despite dietary supplementation with D,L-α-tocopherol acetate.

LITERATURE CITED

1. Brush, P. J., and P. H. Anderson. 1986. Levels of plasma alpha-tocopherol (vitamin E) in zoo animals. Int. Zoo Yearb. 24/25: 316–321.

2. Carpenter, C. P., and C. B. Shaffer. 1952. Polyethylene glycols as vehicles for intramuscular and subcutaneous injection. J. Am. Pharmacol. Assoc. 41: 27–29.

3. Dierenfeld, E. S. 1989. Vitamin E deficiency in zoo reptiles, birds, and ungulates. J. Zoo Wildl. Med. 20: 3–11.

4. Dierenfeld, E. S. 1990. Vitamin E levels measured in rhino browse plants. Rhino Conserv. Newsl. 1(3): 1–2.

5. Dierenfeld, E. S., and E. P. Dolensek. 1988. Circulating levels of vitamin E in captive Asian elephants (*Elephas maximus*). Zoo Biol. 7: 165–172.

6. Dierenfeld, E. S., R. du Toit, and R. E. Miller. 1988. Vitamin E in captive and wild black rhinoceros (*Diceros bicornis*). J. Wildl. Dis. 24: 547–550.

7. Dierenfeld, E. S., and D. A. Jessup. 1990. Variation in serum α-tocopherol, retinol, cholesterol, and selenium of free-ranging mule deer (*Odocoileus hemionus*). J. Zoo Wildl. Med. 21: 425–432.

8. Eastman Chemical Products. 1989. Publication No. V-4. Eastman Chemical Products, Kingsport, Tennessee.

9. Ghebremeskel, K., G. Williams, J. C. M. Lewis, and R. du Toit. 1988. Serum alpha-tocopherol, all-trans retinol, total lipids, and cholesterol in the black rhinoceros (*Diceros bicornis*). Comp. Biochem. Physiol. 91A: 343–345.

10. Hatam, L. J., and H. J. Kayden. 1979. A high performance liquid chromatographic method for the determination of tocopherol in plasma and cellular elements of the blood. J. Lipid Res. 20: 639–645.

11. Hidiroglou, N., L. F. Laflamme, and L. R. McDowell. 1988. Blood plasma and tissue concentration of vitamin E in beef cattle as influenced by supplementation of various tocopherol compounds. J. Anim. Sci. 66: 3227–3234.

12. Hidiroglou, N., L. R. McDowell, and R. Pastrana. 1988. Bioavailability of various vitamin E compounds in sheep. Int. J. Vitam. Nutr. Res. 58: 189–197.

13. Horwitt, M. K., C. C. Harvey, C. H. Dahm, Jr., and M. T. Searcy. 1972. Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. Ann. N.Y. Acad. Sci. 203: 223–236.

14. Howard, K. A., S. A. Moore, S. V. Radecki, J. E. Shelle, and D. E. Ullrey. 1990. Relative bioavailability of various sources of vitamin E for white-tailed deer (*Odocoileus virginianus*), swine, and horses. Proc. Annu. Meet. Am. Assoc. Zoo Vet. Pp. 191–195.

15. Ingold, K. U., G. W. Burton, D. O. Foster, L. Hughes, L. A. Lindsay, and A. Webb. 1987. Biokinetics of and discrimination between dietary RRR- and SRR-α-tocopherols in the male rat. Lipids 22: 163–172.

16. Krasavage, W. J., and C. J. Terhaar. 1977. D-α-tocopheryl poly(ethylene glycol) 1000 succinate. Acute toxicity, subchronic feeding, reproduction, and teratologic studies in the rat. Agric. Food Chem. 25: 273–278.

17. Kuroki, S., C. D. Schteingart, L. R. Magey, B. I. Cohen, E. H. Mosbach, S. S. Rossi, A. F. Hofmann, N. Matoba, M. Une, T. Hoshita, and D. K. Odell. 1988. Bile salts of the West Indian manatee, *Trichechus manatus latirostris:* novel bile alcohol sulfates and absence of bile acids. J. Lipid Res. 29: 509–522.

18. Liu, S. K., E. P. Dolensek, and J. P. Tappe. 1985. Cardiomyopathy and vitamin E deficiency in zoo animals and birds. Heart Vessels Suppl. 1: 288–293.

19. Machlin, L. (ed.). 1980. Vitamin E: A Comprehensive Treatise. Marcel Dekker, New York, New York.

20. Miller, R. E., R. Cambre, A. de Lahunta, and W. J. Boever. 1987. Encephalopathy in two black rhinoceros (Diceros bicornis) calves. Proc. 1st Int. Conf. Zool. Avian Med., Oahu, Hawaii. P. 467.

21. Miller, R. E., H. Chaplin, D. E. Paglia, and W. J. Boever. 1986. Hemolytic anemia in the black rhinoceros—an update. Proc. Annu. Meet. Am. Assoc. Zoo Vet. Pp. 7–8.

22. National Research Council. 1989. Nutrient Requirements of Horses, 5th rev. ed. National Academy Press, Washington, D.C.

23. Nockels, C. F. 1986. Stress and disease in cattle. In: Howard, J. L. (ed.). The Veterinary Clinics of North America, Food Animal Practice. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 531–542.

24. Papas, A. M., R. C. Cambre, S. C. Citino, D. J. Baer, and G. R. Wooden. 1990. Species differences in the utilization of various forms of vitamin E. Proc. Annu. Meet. Am. Assoc. Zoo Vet. Pp. 186–190.

25. Smyth, H. F., C. P. Carpenter, and C. S. Weil. 1950. The toxicology of the polyethylene glycols. J. Am. Pharmacol. Assoc. 39: 349–354.

26. Smyth, H. F., C. P. Carpenter, and C. S. Weil. 1955. The chronic oral toxicology of the polyethylene glycols. J. Am. Pharmacol. Assoc. 44: 27–30.

27. Sokol, R. J., N. Butler-Simon, J. E. Heubi, S. T. Iannacone, H. J. McClung, F. Accuzso, K. Hammond, M. Heyman, F. Sinatra, C. Riely, J. Perrault, J. Levy, and A. Silverman. 1989. Vitamin E deficiency neuropathy in children with fat malabsorption. Studies in cystic fibrosis and chronic cholestasis. Ann. N.Y. Acad. Sci. 570: 156–168.

28. Sokol, R. J., J. E. Heubi, N. Butler-Simon, H. J. McLung, J. R. Lilly, and A. Silverman. 1987. Treatment of vitamin E deficiency during chronic cholestasis with oral D-alpha-tocopheryl polyethylene glycol 1000 succinate. Gastroenterology 93: 975–985.

29. Traber, M. G., H. J. Kayden, J. B. Green, and M. H. Green. 1986. Absorption of water-miscible forms of vitamin E in a patient with cholestasis and in thoracic duct-cannulated rats. Am. J. Clin. Nutr. 44: 914–923.

30. Whetter, P. A., and D. E. Ullrey. 1978. Improved fluorometric method for determining selenium. J. Assoc. Off. Anal. Chem. 61: 927–930.