Proceedings of the Eighth DR. SCHOLL CONFERENCE ON THE NUTRITION OF CAPTIVE WILD ANIMALS

Edited by

Thomas P. Meehan, D.V.M.
Steven D. Thompson, Ph.D.
Mary E. Allen, Ph.D.

Copy Editor

Barbara E. Royal

December 8 and 9, 1989
Lincoln Park Zoological Gardens
Chicago, Illinois

TABLE OF CONTENTS

Eighth Dr. Scholl Nutrition Conference - 1989

VITAMIN E: CONSIDERATIONS IN PRACTICAL ANIMAL FEEDING AND CASE STUDIES WITH ELEPHANTS AND RHINOCEROS

Andreas M. Papas, Ph.D.
Eastman Chemicals Division
P.O. Box 431
Kingsport, TN 37662

Richard C. Cambre, D.V.M.
Denver Zoological Gardens
City Park
Denver, CO 80205

Scott B. Citino, D.V.M.
Miami Metro Zoo
12400 Southwest 152 Street
Miami, FL 33177

INTRODUCTION

Renewed interest has been focused recently on the vitamin E nutrition of farm and captive wild animals. For example, the National Research Council (NRC, 13, 15) increased this year the recommended allowances for horses several fold over the 1978 levels. The recommendations for other species are the subject of considerable debate in light of better understanding of the functions of vitamin E such as its role in enhancing the immune system and preventing disease (14).

Scientists involved in the nutrition and health of captive wild animals have long been aware of the low circulating levels in several species and the potential role of vitamin E deficiency in the incidence of myopathy, hemolytic anemia and other diseases (2-5, 11). In some species, such as black rhinoceros and elephants, supplementation with the commonly used form dl-a-tocopheryl acetate either failed (unpublished data) or required a long period before increasing the circulating levels (3). New research with elephants and with black rhinoceros reported in this paper shows strikingly poor absorption by both species of the fat-soluble and water-dispersible forms of vitamin E. In contrast, both species exhibited good absorption of TPGS (d-a-tocopheryl polyethylene glycol 1000 succinate) a water soluble form (6). This is a vivid example of the factors that need to be considered in the vitamin E nutrition of animals and particularly captive wild animals for which research data are limited.

We will review in this paper some of the factors to be considered in practical nutrition programs and report preliminary results of studies with elephants conducted at the Denver Zoological Gardens and with black rhinos conducted at the Miami Metro Zoo.

CHEMISTRY

In most cases, vitamin E has become synonymous with the most active form, a-tocopherol and its esters (7,12). Natural foods and feeds, however, contain, in addition to alpha-, the beta-, gamma-

and delta- tocopherols (Fig. 1) with some vitamin E activity (the NRC concluded that the non alpha tocopherols in a mixed diet contribute about 20% of the vitamin E activity indicated by the a-tocopherol content). Later, four additional compounds, analogous to tocopherols (tocotrienols) were characterized.

The tocopherols and tocotrienols are widely distributed in foods and feeds. The prevalent tocopherol forms also vary widely. For example, the alpha form is dominant in safflower oil while gamma and delta are more abundant forms in black berries and soybean oil. Latex from the Hevea rubber tree contains several of the tocotrienols. In animal tissues, the alpha form is by far the predominant form, but the role of the other forms in sparing alpha or in other biological functions is not well understood.

In their habitat, carnivores receive primarily d-a-tocopherol (natural source, also designated as RRR, see below) while all other species receive a mixture of all natural tocopherols and tocotrienols. Most commercial feeds today are supplemented with dl-a-tocopheryl acetate only.

NATURAL VERSUS SYNTHETIC

The tocopherol molecule has three asymmetric carbons marked by asterisks in Fig. 1. Only the RRR diastereoisomer is found in nature (RRR denotes that the methyl groups are positioned to the right). Synthetic a-tocopherol, a condensation product of trimethylhydroquinone and racemic isophytol, is a mixture of the eight diastereoisomers (Fig. 2; two possible positions R=right, S=left for three asymmetric carbons produce 2 x 2 x 2 = 8 diastereoisomers). Natural tocopherols are isolated from vegetable oil distillates (primary soybean oil). The non-alpha tocopherols can be converted to alpha by methylation. Alternatively, the tocopherols are isolated and are commercially available as the mixture of all forms (7,12).

The natural a-tocopherol is designated as RRR-a-tocopherol or, more commonly d-a-tocopherol. The synthetic is designated as all-rac-a-tocopherol or, more commonly, dl-a-tocopherol. Their esters are named in a similar manner. Non-alpha tocopherols are named also in a similar manner; only the natural mixed tocopherols are available in commercial quantities.

The differences in biological activity between natural source and synthetic forms are discussed below.

BIOLOGICAL ACTIVITY

The currently accepted biological activities, expressed as International Units (IU), are based primarily on the rat resorption-gestation bioassay. In this test, female rats raised on vitamin E-free diets are mated to normal males; in mid-gestation the test form of vitamin E is supplied and the biological activity of the dose is determined from the number of rats having at least one live offspring (all or none assay). Other bioassays are based on prevention of muscular dystrophy, erythrocyte hemolysis or encephalomalacia (12).

On the basis of the rat resorption-gestation bioassay, 1.0 mg of the synthetic all-rac-a-tocopheryl acetate was designated as 1.0 IU. One mg of the natural RRR-a-tocopheryl acetate was assigned

the value of 1.36 IU. The activities for the natural and synthetic a-tocopherol are 1.49 and 1.1 IU respectively. These activities have been disputed by Ames (1) who proposed relative potencies of .81-.83 IU for the all-rac-a-tocopheryl acetate and 1.66 IU for the RRR-a-tocopheryl acetate. His proposals, however, have not been accepted and the ratio of 1.36:1.0 is used to define the relative potencies of the natural and synthetic forms.

Recent research underscores major factors to be considered in using the IU values.

1. How well does the rat resorption-gestation test relate to other important functions of vitamin E such as a biological antioxidant and enhancer of the immune system of other species? Work by Ingold and his group at the NRC of Canada (10) demonstrated that rat tissues (with the exception of liver) show preference in their uptake of the natural RRR diastereoisomer over the synthetic SRR (over 2:1 by the red blood cells and over 5:1 by the brain).

2. Hidiroglou and his coworkers at the University of Florida (8) compared the natural and synthetic forms of a-tocopherol (as tocopherols or their acetate esters) in cattle. While all experimental cattle received 1,000 IU/day and similar circulating levels would be expected, the circulating levels were higher with the natural form. In addition, the tocopherol form produced an increase over the acetate form. Hidiroglou et al. observed similar trends favoring the natural forms in sheep (9).

3. The IU value does not predict the absorption of various forms by different species. It is important to note that, in general, vitamin E absorption is poor, ranging from 20-40%; a variety of factors such as biliary and pancreatic secretions (16) and polyunsaturated fatty acids affect the absorption. In general aqueous solutions are better absorbed than oil solutions. Differences in absorption between the species and vitamin E form are very well illustrated in the results with elephants and black rhinoceros reported below. Both species absorb very poorly the fat-soluble or water-dispersible forms tested, while they absorb well the water-soluble form, TPGS. The magnitude of the difference, based on the increase of circulating levels, is over 50 fold. These results also underscore the difficulties in extrapolating rat data to other species; for the same reason, we advise against extrapolating the rhinoceros and elephant results to other species.

4. In practical feeding, the non-alpha tocopherols are assigned zero IU value and are not included in feed supplementation because of their low activity in the rat fetal resorption and other bioassays in comparison to a-tocopherol. Yet as an antioxidant in vitro, gamma is more active than alpha; there is evidence that gamma is absorbed (12, 17) and that it may have a sparing effect on alpha. We need to learn more about the function of the non-alpha tocopherols and tocotrienols.

In developing a nutrition program, it is very important to consider the vitamin E form and its absorption by the supplemented animal in addition to the IU value. Failure to do so may lead to deficiency even with very high levels of supplementation as measured by the IU content.

FUNCTION

The function of vitamin E and disorders associated with its deficiency are well known, although its mechanism of action is not clearly understood. Its major function is that of a biological antioxidant with a special role of protecting the cell membrane from free radicals. Deficiency is associated with the following disorders:

Lesions of the reproductive system causing defective embryos or atrophy of the testicles.

Myopathy of the skeletal, cardiac and smooth muscles.

Lesions of the central nervous system manifested as encephalomalacia or axonal dystrophy.

Lesions of the cardiovascular system.

Erythrocyte hemolysis and anemia.

Liver necrosis, kidney nephrosis and discolored adipose tissues.

The incidence of similar disorders in captive wild animals and the possible contributory role of vitamin E deficiency has been well documented, and therefore, the selenium status should also be considered (18).

Of particular interest is the rapidly developing evidence of the role of vitamin E and other biological antioxidants (beta-carotene, vitamin C) in enhancing the immune system and preventing disease (12, 14). The role of vitamin E and other biological antioxidants in the prevention of cancer and in delaying the onset or alleviating the symptoms of diseases of the central nervous system is being actively investigated.

COMMERCIAL FORMS

As indicated earlier, the synthetic dl-a-tocopherol acetate (all-rac) is by far the most commonly used commercial supplement in animal feeds. In light of the evidence showing major species and vitamin E form differences in absorption and utilization, it is important to be aware of other commercial forms of interest to animal nutrition. These vitamin E forms can be classified as follows:

I. Fat Soluble

1. Mixed tocopherols. Commercially available in the natural RRR diastereoisomer, this oil product is a mixture of the alpha-, beta-, gamma- and delta-tocopherols. Currently used primarily as food antioxidant, it may be of interest in supplying a mixture of tocopherols to approximate those available to the animals in their natural habitat.

2. a-Tocopherol, in oil form is available both as natural source (d, or RRR) or synthetic (dl or all-rac). Its use in animal feeds is limited because it is not stable, especially when added to commercial feeds. It is used in injectable preparations and other special applications.

3. a Tocopheryl acetate, in oil form, available both as natural source and synthetic.

The above oil forms can be formulated into solid products.

II. Water-Dispersible

The most common product consists of the a-tocopheryl acetate formulated in solid form with gelatin. It is available

commercially both as the natural and the synthetic forms. Micelle formulations of the oil forms are currently being evaluated. It is important to differentiate the water-dispersible from the water-soluble TPGS below, because their absorption characteristics in some species (elephants and black rhinoceros for example) are markedly different.

III. Water-soluble

TPGS (d-a-tocopheryl polyethylene glycol 1000 succinate) is an ester of the natural form. A waxy solid, it forms clear solutions at concentrations up to 20% (6). TPGS was shown to be absorbed well by elephants and rhinoceros, while the fat-soluble and water-dispersible forms were very poorly absorbed.

In selecting a commercial vitamin E form, it is very important to consider its absorption and bioavailability for the supplemented animals. This is of particular importance for animals having low circulating levels or showing signs of deficiency despite supplementation with the commonly used form dl-a-tocopheryl acetate. For elephants and black rhinoceros, the TPGS is the preferred form. The importance of TPGS in other species is being investigated.

STABILITY

Two aspects of stability are of interest, namely stability of vitamin E naturally occurring in feeds and stability of commercial forms.

The vitamin E content of feeds is highly variable and, more important, processing and storage causes large losses as illustrated in the following examples (7, 12, 15).

1. The a-tocopherol in green grasses decreases dramatically (up to 90%) from early stages to maturity.

2. Even simple processing of forage may cause significant losses. Drying of alfalfa causes losses up to 60%; artificial air drying causes less loss than sun curing. Storage of alfalfa hay for 12 weeks reduced vitamin E content by 73%. For both forage and grains, high moisture storage, ensiling or preservation with organic acids may cause total loss of tocopherols.

The stability of commercial products can be summarized as follows: the tocopherol forms are generally unstable, especially in vitamin-mineral premixes, basemixes or complete feeds. The esters such as the acetate are substantially more stable than the tocopherols. TPGS, as an ester, is believed to be stable in the solid form. As a solution, it should be refrigerated or preserved with a small amount of ethanol (5% of the total solution by volume). For all forms, stability is reduced by high temperature and relative humidity, presence of some minerals and other factors.

Because the content of feeds varies widely and processing, preservation or storage can cause total loss of vitamin E, the values shown in nutritional tables should be used with extreme care and allowance made for potential losses.

REQUIREMENTS

Information on the requirements of some species is limited and the recommended allowances for others (horses for example) have

been revised (13, 15) or are the subject of considerable debate. For captive wild animals, information is even more limited. A reasonable approach, followed by some scientists, is to try to achieve in captive wild animals similar circulating levels as those observed in their natural habitat. It is of interest to consider, however, whether a higher circulating level is advisable for captive animals in order to increase their ability to overcome environmental stress and enhance their immune system (12, 14).

The proposed allowances for vitamin E vary considerably and for some species are in the range of .3 to 2.5 IU/kg body weight. At least part of the variation is the result of differences in the absorption and utilization of various forms by different species.

In practical supplementation, the total IU added to the diet is not meaningful without consideration of the absorption and utilization characteristics of the form used by the supplemented animals. This is very well illustrated by the case studies below.

CASE STUDY I: VITAMIN E NUTRITION OF ELEPHANTS

This study comprised two trials and was conducted at the Denver Zoological Gardens under the supervision of Dr. R. Cambre. Serum samples were assayed for a-tocopherol by the laboratory of Dr. R. Sokol at the University of Colorado School of Medicine. The objective was to determine the effectiveness of several vitamin E forms in elevating the circulating levels.

TRIAL 1

Two female Asian elephants (estimated ages of 34 and 31 years and weights 3264 and 4855 kg) were fed diets supplemented with the vitamin E forms in the sequence shown below (washout periods were allowed between forms and levels):

SUPPLEMENTAL VITAMIN E	DOSE, IU/KG BW	COMMENT
Baseline (dl-a-tocopheryl acetate)	1.8	Basal feed level for whole trial
TPGS	4.8	Water-Soluble form
d-a-tocopherol, dil. with corn oil	5.1	Tocopherol in oil
d-a-tocopherol	5.1	Not diluted
d-a-tocopherol	10.2	As above at 2x level
d-a-tocopheryl acetate	30.0	Acetate in oil form, high dose

The results showed that TPGS was by far the most effective form by increasing the circulating levels from the baseline of .11 to over .40 mcg/ml within five days of dosing. In contrast, the other forms tested, even at 6 times the dose, produced very small or no measurable increases in the circulating levels of vitamin E indicating very poor absorption.

TRIAL 2

The objective was to confirm the results of trial 1 and to determine whether very high levels of the tocopherol or a water-dispersible form of its acetate ester, added to the feed, would be

effective in elevating the circulating levels. One Asian (female 25 years old, 2895 kg) and 3 African elephants (male 5 years old, 811 kg; female 3 years old, 650 kg; female 3 years old, 547 kg) were assigned to the following protocol:

DAYS	SUPPLEMENTAL VITAMIN E		COMMENTS
1-4	Baseline (dl-a-tocopheryl acetate)		basal feed for whole trial
15-40	d-a-tocopheryl acetate	62 IU/kg BW	Water dispersible solid, high dose
41-47	None		Washout
48-68	TPGS	6.6 IU/kg BW	Water solution 20%
69-85	None		Washout
86-106	d-a-tocopherol	62 IU/kg BW	Oil form, high dose
107-120	None		Washout

The results are summarized in Fig. 3 and confirm that, as in Trial 1, the water soluble for TPGS was very effective in rapidly increasing the circulating levels from the baseline of .05 mcg/ml to .50 within 2 days and to over 1.0 mcg/ml within 20 days. In contrast, the acetate at almost 6x higher dose, produced a very small increase compared with TPGS. The tocopherol form also in the same very high dose as the acetate was much less effective than TPGS. The tocopherol appeared to be more effective than the acetate form, but due to the high residual levels from TPGS, it is difficult to draw final conclusions.

The two trials showed conclusively that TPGS was effective in rapidly increasing the circulating levels of vitamin E in elephants. The fat-soluble and water-dispersible forms tested produced very small increases suggesting that the elephants absorb these forms very poorly.

CASE STUDY II: VITAMIN E NUTRITION OF BLACK RHINOCEROS (PRELIMINARY RESULTS)

This research was conducted at the Miami Metro Zoo under the supervision of Dr. S. Citino. Two black rhinoceros (one male 12 years old and one female 16 years old) were placed on the following protocol:

Week	Supplemental Vitamin E		Comments
1	dl-a-tocopheryl acetate,	2,000 IU/day	Fed for months before start of trial
2-4	d-a-tocopheryl acetate,	2,100 IU/day	Water dispersible, used as solid
5	d-a-tocopheryl acetate,	31,500 IU/day	As above, 30x higher dose
6	none		Washout
7-8	TPGS	2,100 IU/day	Water solution 20%
9	TPGS	5,250 IU/day	As above, 2.5x higher dose
10	none		Washout

The animals were receiving before and during the trial a diet supplemented with 3,600 IU dl-a-tocopheryl acetate per kg. Blood samples were collected and plasma a-tocopherol was assayed by the laboratory of Dr. R Sokol at the University of Colorado School of Medicine. The preliminary results are shown in Fig. 4.

These results, which are similar to those obtained with elephants, show that the water-soluble form, TPGS, increased the circulating levels several fold while the fat-soluble or water-dispersible forms of the dl- and d-a-tocopheryl acetate respectively, caused a much smaller increase. It is important to note that even at the very large amount of 31,500 IU/day the apparent increase in circulating levels was very small compared to the increase produced by a 15-fold lower dose of TPGS.

These results demonstrate the usefulness of TPGS in meeting the needs of black rhinoceros. They also demonstrate the critical importance of the vitamin E form and the risks involved in basing the supplementation on the IU value alone.

REFERENCES

1. Ames, S.R. 1979. Biopotenties in rats of several forms of alpha-tocopherol. J. Nutr. 109: 2198-2204.

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

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

4. Dierenfeld, E.S., R. duToit and R.E. Miller. 1988. Vitamin E in captive and wild black rhinoceros (Diceros bicornis). J. Wildl. Dis. 24:574-550.

5. Dolensek, E.P. and S.B. Combs. 1985. Vitamin E deficiency in zoo animals. Proceedings of the Fourth Annual Dr. Scholl Conference on the Nutrition of Captive Wild Animals, Chicago, IL, 140 pp.

6. Eastman Vitamin E TPGS, d-alpha-tocopheryl polyethylene glycol 1000 succinate. Eastman Products for the Nutritional Supplement Market. Eastman Chemical Products, Inc., Kingsport, TN. Publication No. V-4 October 2, 1989.

7. Hertig, D.C. 1984. Vitamin E. In the Kirk-Othmer: Encyclopedia of Chemical Technology, Volume 24, 3rd Edition, John Wiley & Sons, Inc.

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

9. Hidiroglou, N., L.R. McDowell and R. Pastrana.1988. Bioavailability of various vitamin E compounds in sheep. Internat. J. Vit. Nutr. Res. 58:189-197.

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

11. Liu, S.K., E.P. Dolensek, D.R.Adams, and J.P. Tappe. 1983. Myelopathy and vitamin E deficiency in six Mongolian horses. J. Amer. Vet. Med. Assoc. 183:1266-1272.

12. Machlin, L,. ed. 1980. Vitamin E: A comprehensive Treatise. Marcel Dekker, Inc. New York.

13. National Research Council. 1989. Nutrient Requirement of Horses, 5th revised edition. Washington, D.C. National Academy Press.

14. Nockels, C.F. 1986. Nutrient modulation of the immune system. In: Recent Advances in Animal Nutrition, W. Haresign and D.J. A. Cole eds. Butterworth, Boston, MA. pp. 172-192.

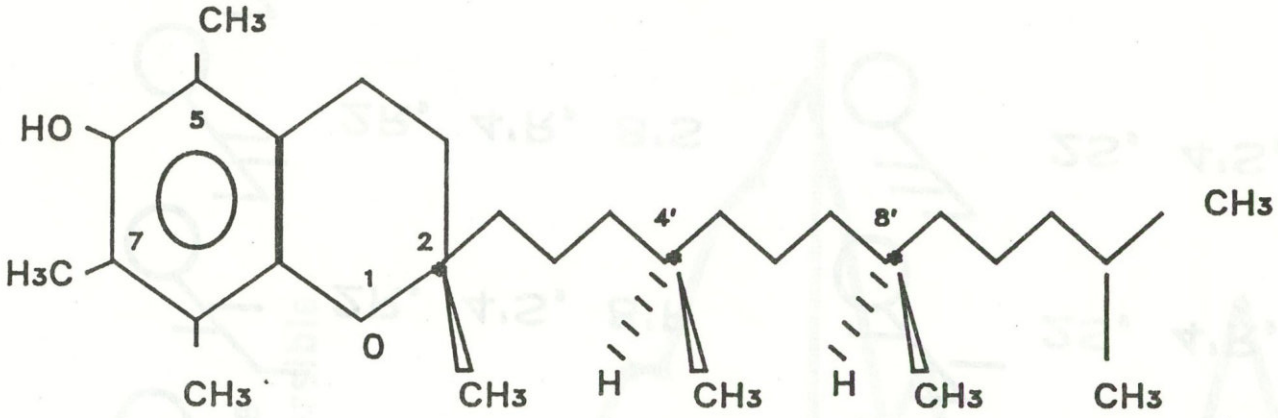
15.Pagan J.D. 1989. The feeding of the horse. The Blood Horse, May 27, 1989.

16. Sokol, R.J., J.E. Heubi, N. Butler-Simon, H. J. McClung, J.R. Lilly, and A. Silverman. 1987. Treatment of vitamin E deficiency during chronic childhood cholestasis with oral d-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS). Intestinal absorption, efficacy, and safety. Gastroenterology 93:975-85.

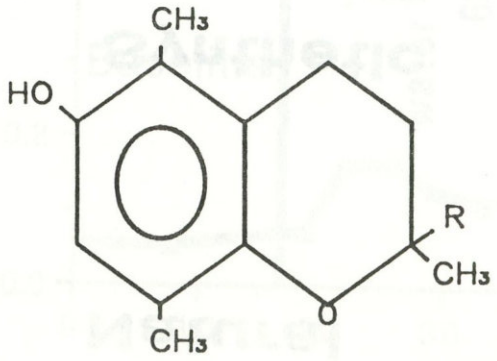
17. Traber, M.G. and H.H. Kayden, 1989. Preferential incorporation of alpha tocopherol compared with gamma tocopherol in human lipoproteins. Am. J. Clin. Nutr. 49:517-526.

18. Ullrey, D.E. 1987. Biochemical and physiological indicators of selenium status in animals. J. Animal Sci. 65:1712-1726.

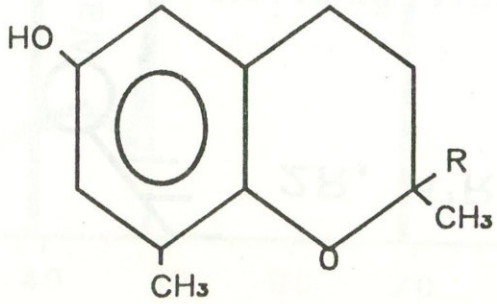
alpha–Tocopherol



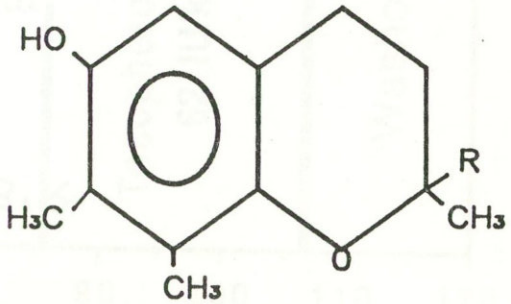
beta–Tocopherol



delta–Tocopherol



gamma–Tocopherol

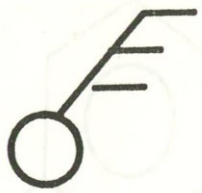


R: Side chain identical for all tocopherols.

Figure 1

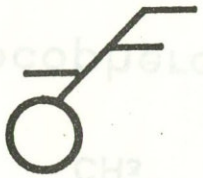
Stereoisomers of a-Tocopherol

Natural

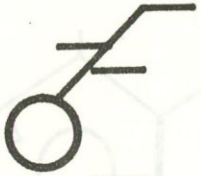


2R, 4'R, 8'R

Synthetic



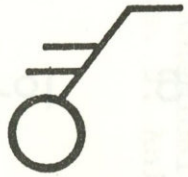
2S, 4'R, 8'R



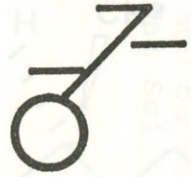
2R, 4'S, 8'R

2R, 4'R, 8'S

2R, 4'S, 8'S



2S, 4'S, 8'R



2S, 4'R, 8'S

2S, 4'S, 8'S

Figure 2

STUDIES ON THE VITAMIN E NUTRITION OF ELEPHANTS

DENVER ZOOLOGICAL GARDENS - TRIAL 2

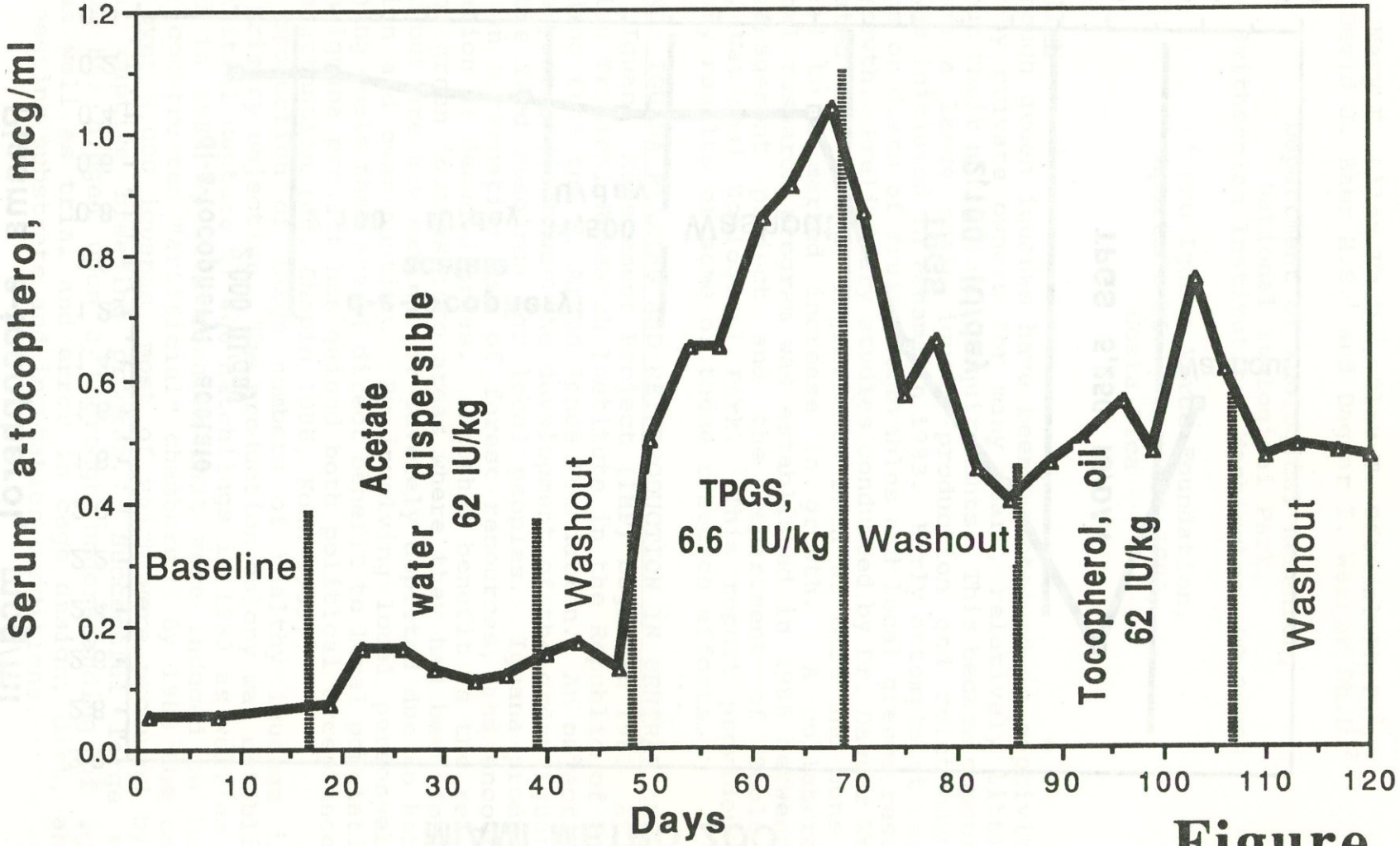


Figure 3

STUDY ON THE VITAMIN E NUTRITION OF THE BLACK RHINO
MIAMI METRO ZOO

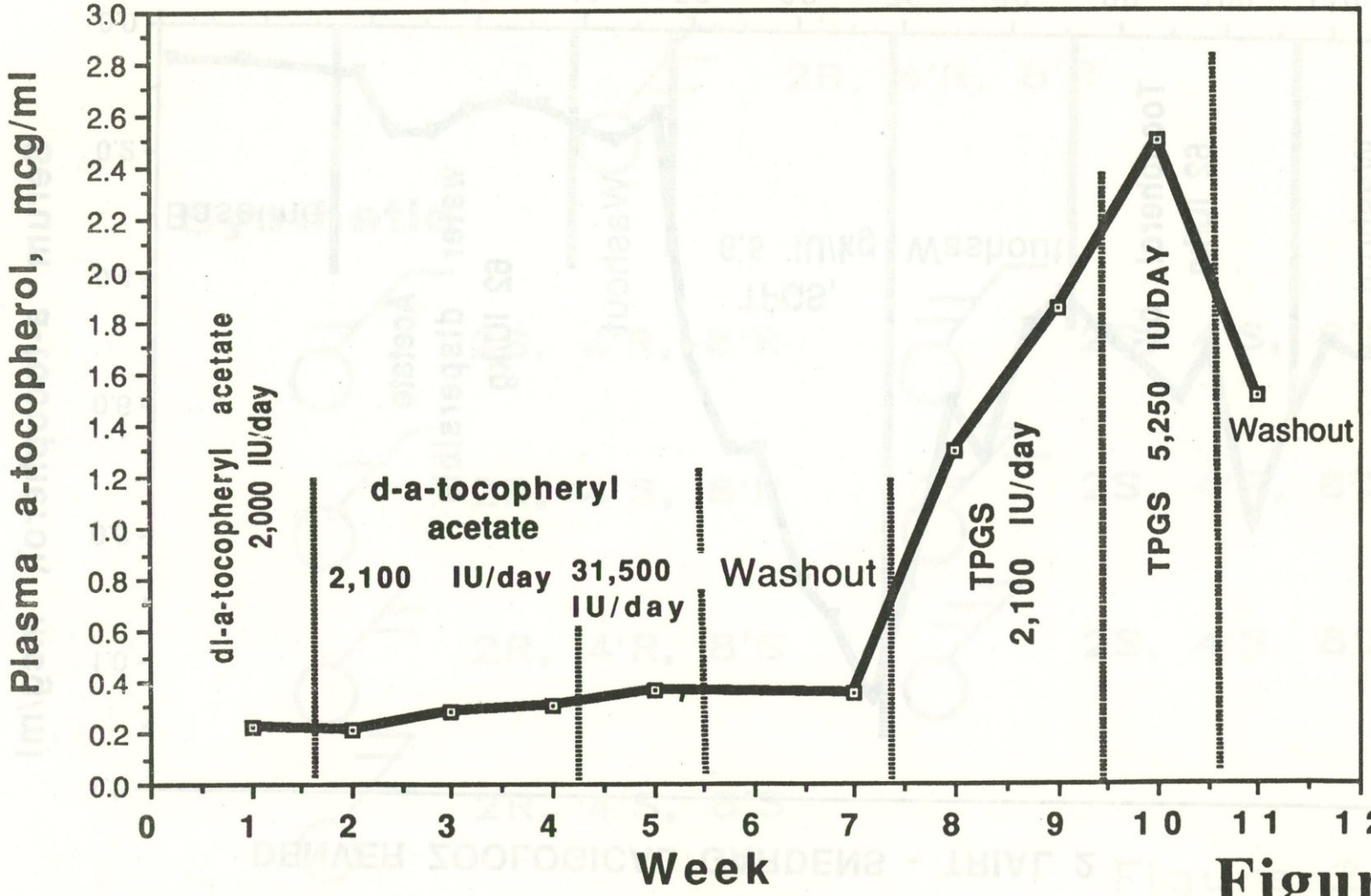


Figure 4