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Levels of plasma alpha-tocopherol (vitamin E) in zoo animals

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Little information is available concerning the levels of vitamin E in zoo animals, its requirement or its degree of physiological importance. In domestic animals deficiency of this vitamin has been associated with myodegenerative disorders and cardiac myopathies (Blaxter, 1962; Van Vleet & Ruth, 1977), as well as impaired reproductive capacity (Trinder *et al.*, 1969). Vitamin E may also play a role in the immune response to infection and in prostaglandin synthesis. Reported manifestations of vitamin E deficiency amongst exotic animals include cardiomyopathy in camels *Camelus* spp (Finlayson *et al.*, 1971) and Nyalas *Tragelaphus angasi* (Liu *et al.*, 1982), steatitis in reptiles, fish-eating birds and African lions *Panthera leo*, myodegenerative disorders in birds, primates, ruminants, zebras *Equus burchelli* ssp, and hepatic necrosis in large carnivores (Wallach, 1970).

In order to provide a wider basis for analysis of vitamin E levels in zoo animals, and in view of the many possible manifestations of a deficiency in the vitamin, alpha-tocopherol levels were determined in the plasma of a wide variety of exotic species.

Until recently methods for the estimation of plasma alpha-tocopherol levels were time consuming and insufficiently sensitive for animal monitoring studies. The high-performance liquid chromatographic fluorescence method of McMurray & Blanchflower (1979), however, combines high sensitivity with relative simplicity and has been used successfully in the investigation of field cases of suspected vitamin E- and selenium-associated disorders in cows, sheep and pigs. We have now extended its usefulness to other species.

MATERIALS AND METHODS

The data are based on a collation of results from samples received between September 1979 and June 1983 from three zoos in the southeast of England which requested plasma vitamin E determinations. The ages of the animals ranged from neonates to 'aged' adults, and most of them appeared clinically normal although some samples were taken from sick animals. The degree of illness varied; some animals could not be considered clinically ill but had, for example, a foot infection while others showed definite abnormalities. Most of the animals were tested only once, but in the cases when more than one test was made a mean value was taken.

Heparinised blood samples were obtained by venepuncture, usually after sedation, and posted to the Central Veterinary Laboratory at Weybridge, Great Britain, where they normally arrived the next day. Plasma was removed by centrifugation and was frozen if not extracted immediately. This procedure is satisfactory because, using calf whole blood kept at room temperature, we have found no significant differences in plasma alpha-tocopherol levels at 0, 5, 24 and 48 hours after venepuncture. Plasma alpha-tocopherol was measured in duplicate 1·0 ml samples using the method given by McMurray & Blanchflower (1979). Analar grade ethanol and nanograde hexane were used without further treatment. Methanol (HPLC grade) was degassed before use by filtering through a Millipore filter under vacuum. The M-45 solvent delivery system, Micro-Bondapak-C18 HPLC column, guard column and Bondapak-C18/Corasil packing for the guard column were all obtained from Waters Associates. Aliquots (20 µl) of the hexane

ORDER/SPECIES	ACCESS TO GRASS/BROWSE	VIT. E SUPPL. GIVEN	NO. OF ANIMALS	PLASMA α-TOCOPHEROL (μmol/l) INDIV. LEVEL OR MEAN	(RANGE)
Marsupialia					
White-throated wallaby *Macropus parma*; Red-necked wallaby *M. rufogriseus*	yes	to some	16	6·0	(1·8–14·3)
Wombat *Vombatus ursinus*	GP	no	1	8·5	
Primates					
Gelada baboon *Theropithecus gelada*	yes	no	5	0·2	(0–1·2)
Orang-utan *Pongo pygmaeus*	not always	yes	3	15·2	(8·1–20·9)
Rodentia					
Capybara *Hydrochoerus hydrochaeris*	GP	no	1	9·9	
Carnivora					
Spectacled bear *Tremarctos ornatus*	no	yes	1	65·4	
Brown bear *Ursus arctos*	no	yes	2	29·9, 32·3	
Kodiak bear *U. a. middendorffi*	no	yes	3	28·0	(25·5–30·6)
Polar bear *Thalarctos maritimus*	no	yes	2	39·4, 34·8	
Giant panda *Ailuropoda melanoleuca*	yes	yes	2	9·2, 12·0	
Jaguar *Panthera onca*	G	yes	1	56·8	
Cheetah *Acinonyx jubatus*	G	yes	3	18·9	(9·9–36·1)
Proboscidea					
African elephant *Loxodonta africana*	G or E	to some	3	0·5	(0–1·6)
Asian elephant *Elephas maximus*	G or E	to some	2	0·5, 0·7	
Perissodactyla					
Przewalski horse *Equus przewalskii*	yes	no	27	2·6	(0–6·0)
Iranian onager *E. hemionus onager*	yes	no	2	3·0, 0·7	
Grant's zebra *E. burchelli boehmi*; Hartmann's mountain zebra *E. zebra hartmannae*	yes	no	5	3·5	(0–10·1)
Indian rhinoceros *Rhinoceros unicornis*	G or E	to some	3	0·2	(0–0·2)
Southern white rhinoceros *Ceratotherium s. simum*	G or E	to some	5	0·2	(0–0·7)
Black rhinoceros *Diceros bicornis*	G or E	to some	2	0, 0	

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				INDIV. LEVEL OR MEAN	(RANGE)
Artiodactyla					
Wart hog *Phacochoerus aethiopicus*	no	yes	1	4·4	
Collared peccary *Tayassu tajacu*	G or E	to some	4	1·5	(1·2–2·3)
Hippopotamus *Hippopotamus amphibius*	G or E	to some	2	0, 0	
Llama *Lama glama* Guanaco *L. guanicoe*	G or E	to some	2	1·4, 1·6	
Arabian camel *Camelus dromedarius* Bactrian camel *C. ferus (= bactrianus)*	not always	to some	12	1·8	(0·7–3·5)
Chinese water deer *Hydropotes inermis*	G or E		1	2·5	
Indian muntjac *Muntiacus muntjak*	no	yes	2	1·6, 6·0	
Fallow deer *Dama dama* Axis deer *Axis axis* Swamp deer *Cervus duvauceli* Sika deer *C. nippon*	G or E	to some	10	4·8	(1·2–8·8)
Moose *Alces alces*	G or E	to some	6	0·9	(0–2·1)
Reindeer *Rangifer tarandus*	yes	no	1	5·1	
Giraffe *Giraffa camelopardalis*	not always	yes	3	2·1	(0·9–3·7)
Sitatunga *Tragelaphus spekei* Greater kudu *T. strepsiceros*	G or E	to some	8	2·9	(0·7–7·0)
Nilgai *Boselaphus tragocamelus*	G or E	to some	9	1·2	(0·7–1·8)
Gaur *Bos gaurus*	no	yes	3	3·0	(1·2–5·6)
American bison *Bison bison* European bison *B. bonasus*	G or E	to some	7	2·8	(1·4–3·5)
Common waterbuck *Kobus ellipsiprymnus* Lechwe waterbuck *K. leche*	G or E	to some	13	1·0	(0–3·2)
Roan antelope *Hippotragus equinus* Sable antelope *H. niger*	G or E	to some	18	4·4	(0–7·4)

ORDER/SPECIES	ACCESS TO GRASS/BROWSE	VIT. E SUPPL. GIVEN	NO. OF ANIMALS	PLASMA α-TOCOPHEROL (μmol/l) INDIV. LEVEL OR MEAN	(RANGE)
Scimitar-horned oryx *Oryx dammah*; Gemsbok *O. g. gazella*	G or E	to some	66	2·3	(0·5–8·1)
Addax *Addax nasomaculatus*	G or E	to some	8	1·9	(0·2–4·9)
Blesbok *Damaliscus dorcas phillipsi*	G or E	to some	7	4·6	(2·1–5·8)
Blackbuck *Antilope cervicapra*	not always	to some	4	3·0	(0–6·9)
Dama gazelle *Gazella dama*; Arabian gazelle *G. gazella arabica*	no	yes	3	3·0	(2·3–3·5)
Musk ox *Ovibos moschatus*	G or E	to some	4	2·8	(0·7–4·1)
Markhor *Capra falconeri*	no	yes	4	0·7	(0·7–0·7)
Barbary sheep *Ammotragus lervia*	no	yes	11	0·6	(0–1·8)
American bighorn sheep *Ovis canadensis*; Mouflon *O. musimon*	no	yes	67	2·8	(0–10·6)

Key: G. grassed enclosure, but probably little grass eaten; GP. grass pellets given occasionally; G or E. vitamin E given only if grass is not available; 'not always' signifies that the animals' access to grass is limited or sporadic (e.g. they are kept indoors for part of the year, or part of the day; or animals in one zoo are given access to grass while those in another are not); 'to some' indicates that vitamin E supplement was given to some of the animals at some times (and in the cases where only two animals are involved, to either one at some times).

N.B. The amounts of vitamin E supplement given varied enormously depending on the species (e.g. wallabies, Jaguars *Panthera onca*, Cheetahs *Acinonyx jubatus* and the Artiodactyla: 1 mg/kg body weight daily; Orang-utans *Pongo pygmaeus:* 95 mg/kg diet plus one 9 mg tablet daily; Giant pandas *Ailuropoda melanoleuca:* 10–20 mg daily). Any attempt to relate these figures to the plasma alpha-tocopherol levels would not be valid because of differences in access to grass or browse even amongst individuals of the same species.

Table 1. Levels of plasma alpha-tocopherol in 60 animal taxa found in three zoos in Great Britain.

extract (equivalent to 0·2 ml plasma) were introduced onto the HPLC column via a Rheodyne 7120 sample injector. The eluate from the column was passed through a flow cell (nominal volume 20 μl) held in a Baird-Atomic Fluoripoint FP 100 spectrofluorimeter set at wavelengths 295 nm (excitation) and 330 nm (emission). Concentrations of plasma alpha-tocopherol were proportional to peak height recorded on a 10 millivolt recorder with reference standards of DL alpha-tocopherol.

RESULTS

Table 1 shows the mean levels of plasma alpha-tocopherol for 60 animal taxa representing 18 families and seven orders. More than half of the species examined including most of the Artiodactyla had mean alpha-tocopherol levels of less than 3·0 μmol/l.

DISCUSSION

The results obtained in this survey indicate that levels of plasma alpha-tocopherol in many zoo animals are relatively low. Values obtained for the Artiodactyla, and particularly the Bovidae, are significantly lower than those obtained for farm animals; in concurrent investigations we found levels of <0·2–8·4 μmol/l for sheep, <0·4–6·0 μmol/l for pigs and <0·2–31·1 μmol/l for cattle, and these values agree well with those of other workers (Hidiroglou *et al.*, 1973; Storer, 1974). The highest plasma alpha-tocopherol levels, ranging from 9·2–65·4 μmol/l were found in the Carnivora (Table 1).

A discrepancy of values was found between the two species of primates studied: in four out of the five Gelada baboons *Theropithecus gelada* levels of plasma alpha-tocopherol were undetectable (<0·2 μmol/l), whereas in the Orang-utans *Pongo pygmaeus* the range of values found (8·1–20·9 μmol/l) was the highest for any species outside the Carnivora and compares well with the range of 11·8–39·2 μmol/l which we have recently found for women in as yet unpublished investigations.

Alpha-tocopherol is a plant product and, although present in meat and fish, it is synthesised only in plant tissue. Plasma alpha-tocopherol levels usually reflect dietary intake of the vitamin; all the animals studied were either receiving vitamin E supplements or had access to grass, grass pellets or browse (Table 1). With regard to the animals in the sample which were ill, it is impossible to say whether or not the levels found departed from the norm because for most of the species examined this is the first report on vitamin E levels, and there is a wide variety of reported manifestations of vitamin E deficiency.

The exact role of vitamin E in animals remains to be elucidated. Its role as an anti-oxidant has been recognised for nearly 40 years and has more recently been shown to be complementary with that of selenium. Vitamin E probably acts as an anti-oxidant by inhibiting peroxidation of unsaturated fatty acids presumably within the cell membrane, whereas selenium constitutes an integral part of the enzyme glutathione peroxidase which catalyses the degradation of peroxides formed within tissues (Hoekstra, 1975). Indirect evidence for the effect of vitamin E on peroxide formation has been found in human patients with low vitamin E levels caused by chronic pancreatitis, a significant negative correlation having been found between serum lipid peroxide levels and vitamin E concentration (Matsumoto *et al.*, 1981).

Vitamin E deficiency and selenium deficiency in animals both cause degenerative lesions in tissues, the nature and location of the lesion depending partly on the species and partly on the nutritional status of the individual. Some of the deficiency diseases, such as necrosis of the liver in rats, exudative diathesis in domestic chicks and white muscle disease in calves, can be prevented by supplementing the diet with either micronutrient (Combs, 1981). It appears, however, that vitamin E is specifically required for the prevention of encephalomalacia in poultry (Cheville, 1966) and steatitis in cats (Cordy, 1954), selenium being ineffective, and experimental young White-tailed deer *Odocoileus virginianus* showed increased mortality with lesions of white muscle disease when the parental diet lacked vitamin E, even when selenium was present (Brady *et al.*, 1978). Vitamin E deficiency has often been unknowingly induced in a number of zoo animals by feeding them large amounts of oily fish-meal protein, resulting in the development of white muscle disease and muscular dystrophy in herbivores, carnivores such as the Jaguar *Panthera onca*, primates and birds (Wallach, 1970).

Deficiencies of vitamin E and selenium alone do not necessarily result in disease (Arthur *et al.*, 1979); an extra 'stress' factor seems to be required. In calves and lambs nutritional myopathy is commonly associated with the time when they are turned out to pasture in the spring after having been held indoors for most of the winter. At this time the sudden challenge of plants rich in unsaturated fatty acids may overwhelm the protective vitamin E-selenium anti-oxidant systems (McMurray & Rice, 1982). Pigs also develop clear symptoms of vitamin E deficiency when fed diets high in unsaturated fatty acids (Grant, 1961). Sudden deaths associated with cardiomyopathy and some skeletal myodegeneration occurred in a group of Nyalas at New York Zoo (Bronx) in the USA; all the animals had low plasma alpha-tocopherol levels but the deaths occurred only when the antelope were subjected to minor physical stress such as restraint or release from a stall (Liu *et al.*, 1982). The results of the investigations described in this paper show that many zoo animals, although clinically normal, are vulnerable to stress-related conditions because of low levels of circulating alpha-tocopherol.

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PRODUCTS MENTIONED IN THE TEXT

Baird-Atomic Fluoripoint FP 100 spectrofluorimeter: manufactured by Baird Atomic Ltd, East Street, Braintree, Essex, Great Britain.
Bondapak-C18/Corasil packing: manufactured by Waters Associates (Inst.) Ltd, 324 Chester Road, Hartford, Northwich, Cheshire, Great Britain.
DL alpha-tocopherol: obtained from Sigma Ltd, Fancy Road, Poole, Dorset, Great Britain.
Micro-Bondapak-C18 HPLC column and guard column: manufactured by Waters Associates (Inst.) Ltd.
Millipore filter: obtained from Millipore House, 11–15 Peterborough Road, Harrow, Middlesex, Great Britain.
Rheodyne 7120 sample injector: manufactured by Altex, Anachem House, 20 Charles Street, Luton, Bedfordshire, Great Britain.

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