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PLASMA RETINOL AND ALPHA-TOCOPHEROL LEVELS IN CAPTIVE WILD ANIMALS

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Abstract—1. The plasma retinol and alpha-tocopherol levels of a number of captive wild animals on defined vitamins A and E intake were determined.

2. In all the species examined they appeared to be low in relation to intake and as compared with recorded values of some domesticated species.

INTRODUCTION

Diseases associated with vitamins A and E deficiency have been reported in captive wild animals.

Vitamin A deficiency during pregnancy is regarded as a possible cause of foetal abnormalities among captive monkeys (Lapin and Yakovleva, 1963). Ramalingaswami *et al.* (1955) reported xerophthalmia and patchy loss of conjuctival pigment in primates. Polar bears with persistent dermatitis responded favourably to very high levels of vitamin A, after other therapeutic measures had failed (Foster, 1981). Halliwel and Graham (1976) observed deficiency in raptorial birds fed on unsupplemented all-meat diet. Semi-aquatic turtles, particularly juveniles tend to be sensitive to vitamin A deficiency (Frye, 1986). Carnivora cannot synthesise vitamin A from the pro-vitamin A carotenoids (Gershoff *et al.*, 1957), as a result they succumb to deficiency-induced diseases if fed on carcass meat without liver or supplementation. Ullery and Allen (1986) reported that levels 50–100 times of requirement can be toxic.

Vitamin E and selenium-responsive muscular dystrophy syndrome were observed in zoos especially in Eastern North America (Rechcigl, 1977). In the macropods, muscular dystrophy was occasionally associated with vitamin E deficiency; the quokka appeared to be particularly susceptible (Kakulas, 1963). Day and Dinning (1956) reported progressive muscular weakness in non-human primates which could be successfully treated with alpha-tocopherol. Both muscle and erythroid series are affected in Rhesus monkeys; and the exact requirement for this vitamin depends on the level of unsaturated fat in the diet (Portman, 1970). Other susceptible species include, goodfellow tree kangaroos (MacKenzie and Fletcher, 1980), lesser kudus (Rudi *et al.*, 1980) and brown pelicans (Campbell and Montali, 1980). Excess vitamin E reduces blood platelets and depresses both the iodine uptake by the thyroid gland and the growth rate (Ullery and Allen, 1986).

Because of the multiple physiological functions of vitamins A and E, it was felt important to assess the plasma retinol and alpha-tocopherol levels of captive animals of known sex and fed known amounts of vitamins A and E.

MATERIALS AND METHODS

Animals and diets

The animals were non-breeding adults resident in the zoo for over two years. Their energy and protein intake were adequate for maintenance and moderate activity. Supplements of vitamins and trace elements were given in order to counteract the stress of confinement and unfamiliar climate. Irrespective of body size, stress-susceptible species were allowed an adequate supply of vitamins A and E, after due regard to toxicity. The free ranging were given variable vitamin supplementation occasionally.

Sample preparation and extraction

Whole blood samples were collected from healthy animals into heparinized tubes during routine veterinary examination. The plasma was separated from the cells by centrifugation of the whole blood at 800 *g* for 15 min (Chilspin bench centrifuge, MSE, Crawley, Sussex, UK) and stored at −70°C until required. The extraction method was similar to that of Leenheer *et al.* (1979). The thawed and well mixed plasma sample 1 ml was transferred to a capped glass tube (120 × 16 mm). To it were added 1 ml of absolute ethanol (Burroughs Ltd, London), 1 ml of 0.1% ascorbic acid in water (BDH Chemicals, Poole, UK) and 5 μl of 9.24 μg/μl alpha-tocopheryl acetate (Sigma Chemicals, Poole, UK). After thorough mixing extraction was carried out with 5 ml *n*-hexane (BDH, Poole, UK) for 5 min on a Rotamixer shaker (Baird and Tatlock, Romford, Essex, England); and after centrifugation at 800 *g* for 15 min the upper organic layer containing the vitamins was transferred to brown glass tubes (65 × 16 mm). The organic layer was evaporated to dryness on a water bath at 40°C under a stream of nitrogen. The residue was redissolved in 50 μl of HPLC grade methanol (Rathburn Chemicals, Wakeburn, Scotland) and 25 μl were taken for analysis. The procedure was carried out under subdued light.

Separation of retinol and alpha-tocopherol

The method was a modification of that used by Barnett *et al.* (1980). The vitamins were separated by high performance liquid chromatography (HPLC) using a Varian 5000 liquid chromatograph equipped with a variable wavelength UV-100 detector (Varian Ltd, Palo Alto, CA, USA). The column used was 30 cm by 4 mm packed with a 5 micron octadecylsilane C18 reverse-phase (Varian Micropack MCH-5 octadecylsilane) packing. Disposable cartridges (MCH-5) were used in the guard column. The vitamins were isolated by gradient elution with HPLC-grade 90% methanol, 9% water and 1% acetonitrile (Rathburn,

Walkerburn, Scotland). The initial flow rate was 1.8 ml/min and the back pressure 270 atm. Retinol was detected at 325 nm and alpha-tocopherol and alpha-tocopheryl acetate at 292 nm. The operating temperature was set at 40°C to counter the baseline drift caused by the fluctuating ambient temperature. The chromatogram was recorded with a Rikadenki Multi Pen Recorder (Rikadenki Kogyo, Tokyo, Japan). Retinol, alpha-tocopherol and alpha-tocopheryl acetate were eluted at 5.2, 16.3 and 19.4 min respectively. Figures 1 and 2 show the solvent system, the flow rate and wavelength employed during the separation.

Quantification

There was a linear relationship for the range covering 30–150 ng/μl (retinol) and 0.80–4.01 μg/μl (alpha-tocopherol). The retinol and alpha-tocopherol concentrations of the unknown samples were computed from the standard curves. Normalization was performed by dividing the peak areas of the standards and unknowns by the peak area of the internal standard for each sample. The mean recoveries of retinol and alpha-tocopherol from the spiked samples were 98 and 97.6% respectively. The lower detection limit was 10 ng for retinol and 230 ng for alpha-tocopherol.

RESULTS AND DISCUSSION

The vitamin intake consisted of dietary vitamin and commercial supplement. Table 1 summarizes the vitamin intake, plasma retinol and alpha-tocopherol levels of various adult exotic species in captivity. Data of pregnant, lactating and growing young an-

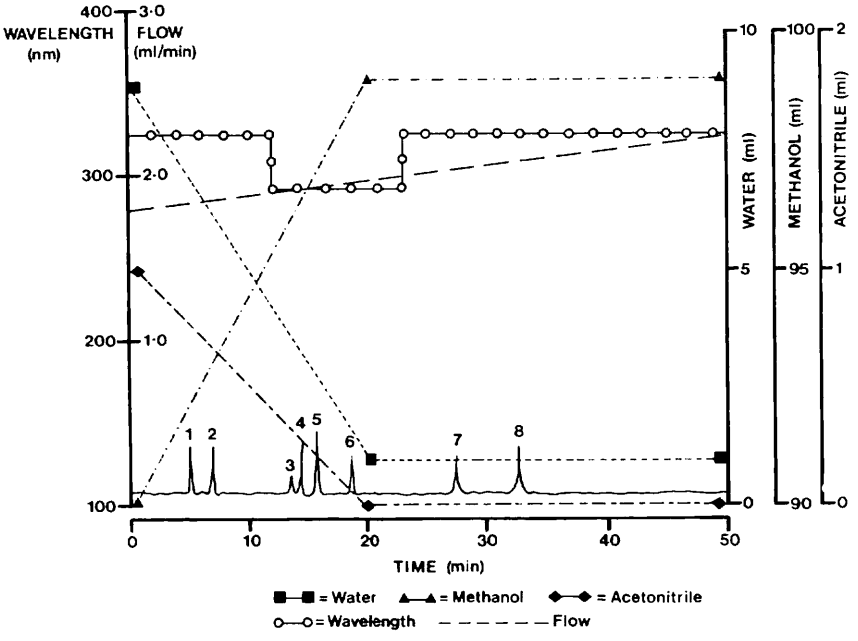

Fig. 1. Solvent system, flow rate and wavelength employed for the separation of the fat soluble vitamins by HPLC on 30 cm × 4 mm, a 5 micron reverse phase column (Varian MCH 5). Inset illustrate peaks 1. Retinol 2. Retinyl acetate 3. Ergocalciferol 4. Cholecalciferol 5. Alpha-tocopherol 6. Alpha-tocopheryl acetate 7. Retinyl palmitate 8. Retinyl stearate.

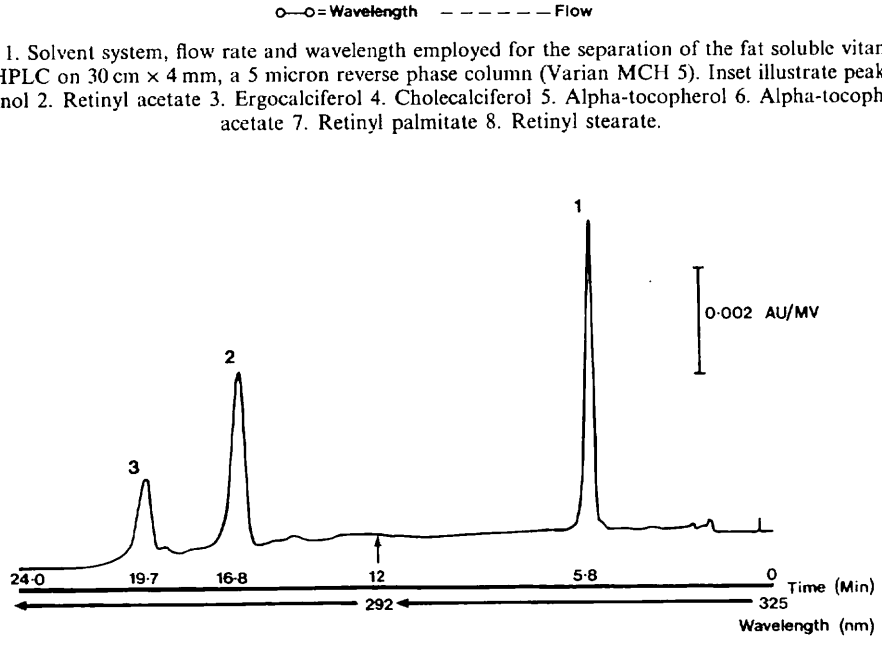

Fig. 2. Profile of plasma 1. Retinol 2. Alpha-tocopherol 3. Alpha-tocopheryl acetate (internal standard), retention times and detection wavelength.

Table 1. Plasma retinol and alpha-tocopherol levels of captive animals on a defined vitamins A and E intake

Species	No. animals	Vitamin intake (mg/d) A	E	Plasma All trans retinol (mg/l) Av.	Range	Alphatocopherol (mg/l) Av.	Range
A. (Artiodactyla)							
Arabian oryx (*Oryx leucoryx*)	2	8.9	516	0.34	0.26–0.47	3.4	2.6–4.3
Axis deer (*Axis axis*)	2	Free ranging		0.52	0.49–0.56	1.7	1.6–1.7
Bactrian camel (*Camelus bactrianus*)	12	57.8	762	0.66	0.33–0.94	3.4	1.9–5.4
Blackbuck (*Antilope cervicapra*)	2	12.0	509	1.0	0.70–1.3	1.3	1.2–1.4
Bongo (*Boocercus eurycerus*)	3	25.1	499	0.39	0.29–0.47	3.1	1.9–3.9
Cape buffalo (*Syncerus caffer*)	1	Free ranging		0.28	–	2.7	–
European bison (*Bison bonasus*)	1	Free ranging		0.84	–	1.5	–
Formosan sika (*Cervus nippon*)	1	Free ranging		0.25	–	1.7	–
Gemsbok (*Oryx gazella*)	4	26.5	232	0.35	0.20–0.45	1.2	0.90–1.4
Greater kudu (*Tragelaphus stepsiceros*)	1	20.0	456	0.36	–	3.6	–
Giraffe (*Giraffe camelopardalis*)	1	90.1	705	0.50	–	4.1	–
Hippopotamus—pygmy (*Choeropsis liberiensis*)	1	14.1	56.6	0.13	–	1.3	–
Llama (*Llama glama*)	2	39.8	657	0.30	0.29–0.30	2.2	2.0–2.5
Muntjac (*Muntiacus muntjak*)	4	Free ranging		0.47	0.36–0.56	4.8	3.9–5.0
Musk ox (*Ovibos moschatus*)	3	Free ranging		0.61	0.52–0.75	1.6	1.5–2.7
Pere David deer (*Elaphurus davidianus*)	5	Free ranging		0.32	0.30–0.34	2.6	2.1–3.7
Reindeer (*Rangifer tarandus*)	1	16.2	344	0.25	–	2.8	–
Roan antelope (*Hippotragus equinus*)	3	15.9	853	0.33	0.32–0.35	3.0	2.3–3.3
Scimitar horned oryx (*Oryx tao*)	6	33.9	762	0.36	0.20–0.72	1.8	1.3–2.2
Sitatunga (*Tragelaphus spekii*)	1	15.2	200	0.21	–	1.8	–
Swamp deer (*Cervus duvauceli*)	2	38.0	802	0.18	0.11–0.26	2.4	2.2–2.6
Thomson's gazelle (*Gazella thomsonii*)	3	Free ranging		0.50	0.34–0.67	2.5	2.0–3.1
Vicuna (*Vicagna vicugna*)	1	12.2	350	0.98	–	5.2	–
Waterbuck (*Kobus ellipsiprymnus*)	3	12.0	509	0.70	0.43–0.88	1.8	1.7–2.1
B. (Carnivora)							
Cape hunting dog (*Lycaon pictus*)	2	14.5	91.2	0.37	0.23–0.51	5.8	4.3–7.2
Cheetah (*Acinonyx jubatus*)	4	25.5	208	0.52	0.47–0.57	6.7	6.6–7.1
Fennec fox (*Fennecus zerda*)	2	0.86	5.8	1.7	1.4–2.1	6.7	5.8–7.7
Jaguar (*Panthera onca*)	2	38.2	768	0.19	0.19–0.20	8.0	6.9–9.1
Leopard (*Panthera pardus*)	2	38.2	768	0.41	0.40–0.41	6.6	6.5–6.7
Lion (*Panthera leo*)	6	38.2	768	0.17	0.10–0.21	5.2	4.1–6.1
Polar bear (*Thalarctos maritmus*)	3	313	1023	0.30	0.26–0.32	7.3	6.5–8.4
Tasmanian devil (*Sarcophilus harrisi*)	1	2.8	19.2	0.16	–	7.7	–
Tiger (*Panthera tigris*)	3	38.2	768	0.23	0.17–0.36	8.8	8.6–11.6
C. (Perissodactyla)							
Donkey (*Equus asinus*)	1	31.9	697	0.25	–	2.4	–
Onagar (*Equus hemionus*)	4	31.9	697	0.36	0.19–0.64	1.6	1.4–1.8
Rhino—white (*Ceratotherium simum*)	1	91.9	1597	0.12	–	<.46	–
Wild horse (*Equus przewalskii*)	4	53.6	920	0.29	0.22–0.38	2.9	2.1–3.2
Zebra grevy's (*Hippotigris grevyi*)	3	53.6	920	0.23	0.20–0.28	3.2	3.0–3.3
D. (Proboscidea)							
Elephant—african (*Loxodonta africana*)	2	101	970	0.11	0.10–0.12	1.4	1.3–1.4
E. (Primates)							
Chimpanzee (*Pan troglodytes*)	2	6.4	20.2	0.68	0.67–0.69	5.1	5.0–5.1
Gorilla (*Gorilla gorilla*)	1	8.9	25.3	0.80	–	6.0	–
Orang-utan (*Pongo pygmaeus*)	2	7.4	27.0	0.69	0.53–0.84	7.2	5.7–8.7

mals were not included as they may not have been representative of the species. Since there was a wide scatter of values within species and, between species of comparable sizes and vitamin intake, the figures were not analyzed statistically. The sex of an animal did not appear to affect the concentration of retinol and alpha-tocopherol in plasma.

The mean plasma retinol and alpha-tocopherol levels in the Artiodactyla, Perissodactyla and Probscidea were 1.2–5.2 mg/l, <0.46–3.2 mg/l and 1.4 mg/l respectively. The observation supports the findings of Brush and Anderson (1986). On a weight for weight basis the vitamin E intake was much more than that recommended for related domesticated species. However, the plasma alpha-tocopherol values were similar to those in the lower ranges of the spectrum found in domestic animals (Brush and Anderson, 1986; Alderson *et al.*, 1971; Storer, 1974; Hideroglou *et al.*, 1973). The low levels found may be characteristic of these species, or may have been caused by a combination of stress and improper supplementation. It is possible that vitamin E is susceptible to degradation by the microorganisms of the gut. Alderson *et al.* (1971) reported a high degradation of vitamin E in the rumen, increasing with concentrate feeding. In species such as the elephant that lack gall bladder (Reuther, 1977) the low value could have been caused by reduced absorption as a result of incomplete emulsification.

The low level of plasma alpha-tocopherol in relation to the high intake observed in the carnivora was perhaps caused by the reduced hydrolysis of alpha-tocopheryl acetate, the commercial vitamin E supplement. Plasma alpha-tocopherol level is higher when the supplement is given in the form of alpha-tocopherol rather than alpha-tocopheryl acetate, in-

dicating that hydrolysis of the ester may be a limiting factor.

In general, the plasma retinol concentration of the captive Herbivora compared well with that of related domesticated species (Long, 1961), but the relative intake per unit body weight was high (Mitchell, 1964). The values obtained for the rhinoceros and the elephant, show that the plasma retinol level was invariably <0.15 mg/l. Since the trend for alpha-tocopherol was the same there may have been a reduced rate of absorption of the fat soluble vitamins.

In feral ruminants microbial action may explain the reduced dietary vitamin A utilization. There is evidence that the conjugated double bonds of vitamin A are not resistant to hydrogenation as was originally thought. King *et al.* (1962) reported losses of carotene and retinol after *in vitro* fermentation. Moreover, the extent of microbial degradation increases with the feeding of concentrate diet (Warner *et al.*, 1970). In spite of a high intake of vitamin A the plasma retinol levels in the jaguars, lions and the tigers were <0.2 mg/l. The value of 0.3 mg/l for the polar bears should probably be regarded as low since in captivity these animals tend to suffer from vitamin A deficiency-induced diseases. In addition, the vitamin A content of the liver of polar bears from the wild is very high, which may imply high plasma retinol level. Since the vitamin A provided to the Carnivora was substantially higher than the estimated daily requirement, intake could not have been limiting factor. Possibly the rate of hydrolysis of retinyl acetate retarded absorption. It is well established that palmitate, stearate and oleate make-up over 95% of the retinyl esters found in the liver and kidney. The contribution of acetate is negligible.

The small Carnivora, particularly the fennic fox, appeared to have high plasma retinol. Perhaps they were efficient at hydrolyzing retinyl acetate or were being fed on almost toxic amounts.

Plasma retinol and alpha-tocopherol levels of the non-human primates compared well with those of adult human subjects.

Plasma alpha-tocopherol could best be used to assess vitamin E status if related to lipid and selenium plasma levels and per cent haemolysis. This is particularly relevant in captive ruminants, in which unsaturated fatty acids are hydrogenated to a varying degree.

In the form provided the supplementary vitamin A intake was not reflected in the plasma levels. Since captive animals are under constant stress as a result of confinement and unfamiliar environment it may be prudent to increase their vitamin A and E intake substantially, with due regard to toxicity. For comparative purpose it would be desirable to obtain samples from free-living wild animals preferably at different seasons of the year.

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