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Dietary Vitamin D Dependence of Cat and Dog Due to Inadequate Cutaneous Synthesis of Vitamin D

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As in herbivores and omnivores, the biosynthesis of vitamin D_3 in the skin exposed to ultraviolet (uv) light is generally expected to also occur in the dog and the cat. The purpose of this *in vitro* study was to measure the concentrations of vitamin D_3 and its precursor 7-dehydrocholesterol (7DHC) in dog and cat skin before and after a quantitatively and qualitatively standardized exposure to uv light. The results are compared to those obtained by the same method in the skin of the rat. The efficiency of extracting 7DHC and vitamin D_3 from skin was 72 ± 8% and 67 ± 3%, respectively. In dog and cat skin the concentrations of nonesterified 7DHC were below the detection limit of the HPLC system. Therefore, skin extracts were saponified and total 7DHC and vitamin D_3 concentrations were measured by normal-phase HPLC. Before irradiation with uv-B light the total concentrations of 7DHC were 1858 ± 183, 1958 ± 204, and 17,620 ± 2345 ng/cm^2 skin (mean ± SEM; $n = 5$) for the dog, the cat, and the rat, respectively. The corresponding concentrations of vitamin D_3 were 211 ± 44, 193 ± 18, and 161 ± 32 ng/cm^2 skin for the dog, the cat, and the rat, respectively. Irradiation of standard solutions of 7DHC with 0.15 J uv-B light/min resulted in a time-dependent decrease in 7DHC and a concomitant increase in previtamin D_3. After exposure of skin to a total of 2.25 J uv-B light no significant changes in concentrations in vitamin D_3 were found in extracts of the skin of the dog and the cat, whereas a 40-fold increase in the vitamin D_3 concentration occurred in the skin of the rat. It is concluded that in the skin of the dog and the cat only low concentrations of esterified 7DHC are present and that this 7DHC is also inadequately converted to vitamin D_3. As shown previously there is no detectable increase in vitamin D_3 in the dog exposed to uv irradiation *in vivo*. Therefore, these low 7DHC concentrations are not caused by high turnover of 7DHC but are due to restricted availability of this vitamin D_3 precursor in the skin of the dog. Thus, the dog and the cat are, unlike herbivores and omnivores, not able to synthesize vitamin D_3 adequately in the skin and are mainly dependent on its dietary intake, i.e., vitamin D_3 is an essential vitamin for the dog and cat.

Since Mellanby (1918) cured rachitic puppies with vitamin D-rich cod liver oil, the physiologic functions and the metabolic pathways of vitamin D have been established. Vitamin D includes cholecalciferol (vitamin D_3) of animal origin as well as ergocalciferol (vitamin D_2) of plant origin. Vitamin D metabolites are of great importance in calcium metabolism, regulating its active intestinal absorption, renal regulation, and its deposition in and mobilization from the skeleton (DeLuca, 1984).

Vitamin D has long been considered an essential dietary ingredient, but in several species, including sheep, cattle, horses, pigs, rats, and man, it has been demonstrated that vitamin D can be formed in the skin (Chaudhary and Care, 1985; Hidiroglou *et al.*, 1985; El Shorafa *et al.*, 1979; Norman, 1979; Esvelt *et al.*, 1978; Holick *et al.*, 1982). The biological actions of vitamin D depend upon hydroxylation in the liver and kidney to form the biologically active 1,25-dihydroxy vitamin D [1,25$(OH)_2$ vitamin D, calcitriol]. On the basis of its site of synthesis and its target cells, its mode of

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action, its feedback regulation, and its structure, calcitriol is considered to be a steroid hormone (DeLuca, 1984). Consequently, vitamin D can be looked upon as a prohormone, especially when formed in the skin from a cholesterol metabolite, 7-dehydrocholesterol (7DHC).

After exposure to an adequate amount of ultraviolet (uv) light in the B-range (290–320 nm), photolysis of 7DHC to previtamin D_3 takes place in the skin, mainly the epidermis. Previtamin D_3 can undergo either photoconversion to lumisterol or tachysterol or back to 7DHC in the case of prolonged exposure, or it can isomerize to vitamin D_3 (Holick *et al.*, 1981). The isomerization velocity and the previtamin D_3/vitamin D_3 equilibrium is dependent on the temperature and the presence of cellular lipids and/or proteins of the skin. Without the presence of skin material 80% of vitamin D_3 is obtained after 2 days at 37°C and 65% within 7 min at 120°C (Holick, 1990; Verhaelen-Fasté and Verhaelen, 1981), whereas 50% of vitamin D_3 is obtained after 2.5 hr at 37°C in human skin (Tian *et al.*, 1993). The newly formed vitamin D_3 (and not previtamin D_3 and its photoisomers) is transported by vitamin D-binding protein (DBP) into the circulation which allows further isomerization of previtamin D_3 to vitamin D_3 (Holick *et al.*, 1981).

Mechanisms of calcium retention are essential for all terrestrial animals, including carnivores, whose natural prey is often low in calcium. Vitamin D metabolism in carnivores has not been extensively examined. Wheatly and Sher (1961) demonstrated that 7DHC content in dog skin is low, whereas dogs raised on a balanced dog food without added vitamin D developed clinical, biochemical, and histological signs of hypovitaminosis D even when exposed to uv-B light daily (Hazewinkel *et al.*, 1987). Cutaneous vitamin D_3 biosynthesis in cats has not been reported.

The present study measures 7DHC and vitamin D_3 in the skin of the cat and the dog and investigates the effect of exposure of the skin to uv light on biosynthesis of vitamin D_3. The results are compared with findings in the rat, which is known to synthesize vitamin D_3 in the skin after exposure to uv-B light.

MATERIALS AND METHODS

Skin of adequately nourished, healthy, and lightly pigmented adult cats ($n = 5$), dogs ($n = 5$) (both of mixed breeds and both sexes), and rats ($n = 5$) (Wistar, female; Cpb/Hsd, Zeist, The Netherlands) was collected from animals that were euthanized (with an overdose of barbiturate) for other purposes. The back of each animal was shaved and two pieces of skin (3 × 3 cm each) were excised and freed of subcutaneous tissues. One piece of skin of each animal was used for direct determination of 7DHC and vitamin D_3 contents and the other piece was analyzed after irradiation.

Irradiation of the skin and of a standard solution containing 7DHC (Sigma Chemie, Bruxelles, Belgium) in ethanol (30 μg/ml) was performed with two fluorescent sunlamps (Philips TL 40W/12, Eindhoven, The Netherlands) emitting in the uv-B range between 280 and 340 nm (with the peak at 305 nm). The pieces of skin were irradiated once with these uv-B lamps to a total exposure of 2.25 J per cm^2 skin in 15 min as assessed with a Waldmann uv-B meter (H. Waldmann, Swellingen, Germany). 7DHC was irradiated for 0, 1, 2.5, 5, and 10 min and then analyzed for conversion to previtamin D_3.

Unesterified vitamin D_3 precursors were isolated from the skin by extraction with diethylether following Holick *et al.* (1985). Both free and esterified vitamin D_3 precursors from the skin were extracted (Takada *et al.*, 1981, 1983). Briefly, the skin was homogenized for 90 sec (4°C) in a mixture of methanol:chloroform:saturated KCl solution (2:1:1.5 by volume) using a polytron. After the addition of 4 ml chloroform and 4 ml saturated KCl the homogenate was centrifuged for 5 min at 1800*g*. The chloroform fraction was isolated and dried under nitrogen, then redissolved in a mixture of 25 ml ethanol, 4 ml KOH, and 10 ml pyrogallol (20% w/v in ethanol) and saponified by refluxing for 2 hr. The lipid fraction was isolated after the addition of 20 ml H_2O and 20 ml *n*-hexane. The *n*-hexane phase was dried under nitrogen and redissolved in 1 ml *n*-hexane. This fraction was analyzed by HPLC using a normal-phase LiChrospher Si-60 column and 0.8% (v/v) isopropanol in *n*-hexane as the mobile phase at a flow rate of 1 ml/min. Ultraviolet absorbance was measured at 254 nm. The retention times of 7DHC and vitamin D_3 on the HPLC were determined by the addition of standard 7DHC and vitamin D_3 (Sigma Chemie) to the skin

extracts. The concentration of vitamin D_3 of the saponified skin extracts was measured after injection of 10 times concentrated sample volumes than those presented in Fig. 1. Calibration of the HPLC analysis was performed by the application of increasing concentrations (1–250 μg/ml in *n*-hexane) of crystalline 7DHC and vitamin D_3 standards. The efficiency of the extractions was 72 ± 8% and 67 ± 3% for 7DHC and vitamin D_3, respectively.

RESULTS

In a pilot experiment skin pieces of one rat, one dog, and one cat were extracted with diethyl ether (Holick *et al.*, 1985). Normal-phase HPLC revealed the presence of 1900 ng 7DHC/cm^2 skin of the rat, whereas no 7DHC could be detected in the canine and feline samples. Because most of the 7DHC is present in the skin of the rat in a fatty acid esterified form, the 7DHC contents were analyzed after saponification of the initial extracts (Takada *et al.*, 1981).

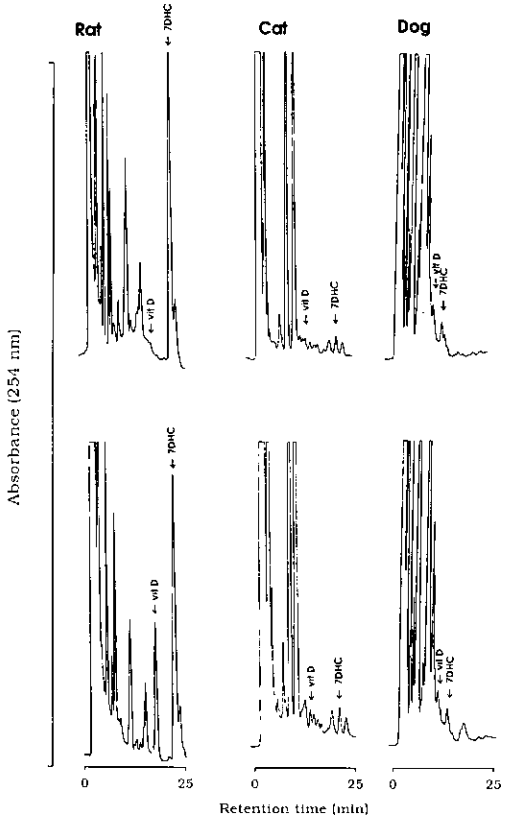

FIG. 1. Normal-phase HPLC analysis of skin extracts from rats, cats, and dogs before (top) and after irradiation (bottom) of the skin with 2.25 J uv-B/cm^2. Skin extracts were prepared as described under Materials and Methods and saponified to obtain the total fraction of free and fatty acid esterified 7DHC and vitamin D_3. Arrows indicate the retention times of crystalline 7DHC and vitamin D_3 after addition to the same extracts. Representative chromatograms are shown. The concentration of vitamin D_3 was assessed using a 10 times more concentrated sample volume.

HPLC analysis of both the free and the fatty acid esterified fraction yielded detectable 7DHC concentrations in the rat, canine, and feline skins (Fig. 1). The mean concentration of total 7DHC in the skin of the rat was almost 10 times higher than those in the skins of the dog and cat (Table 1). The identity of the vitamin D_3 peak on HPLC analysis, performed by adding crystalline vitamin D_3 standard to extracts and by varying isopropanol concentrations in the mobile phase, appeared to comigrate with the crystalline standard in all circumstances.

Irradiation of a standard solution of 7DHC resulted in a time-dependent decrease in 7DHC with a concomitant increase in previtamin D_3 (Fig. 2). Irradiation of the skin of the cat and the dog did not change the concentrations of vitamin D_3 significantly, whereas a significant increase of vitamin D_3 was found in the rat (Fig. 1).

DISCUSSION

The concentration of 7DHC in the skin of dogs and cats was only 10% of that in the skin of rats. *In vitro* irradiation caused no

TABLE 1
TOTAL CONCENTRATIONS OF 7-DEHYDROCHOLESTEROL (7DHC) AND VITAMIN D_3 IN SAPONIFIED SKIN EXTRACTS OF THE RAT, THE DOG, AND THE CAT

Species (n = 5)	7DHC (ng/cm^2)	Vitamin D_3 (ng/cm^2)
Rat	17,620 ± 2,345	161 ± 32
Dog	1,858 ± 183	211 ± 44
Cat	1,958 ± 204	193 ± 18

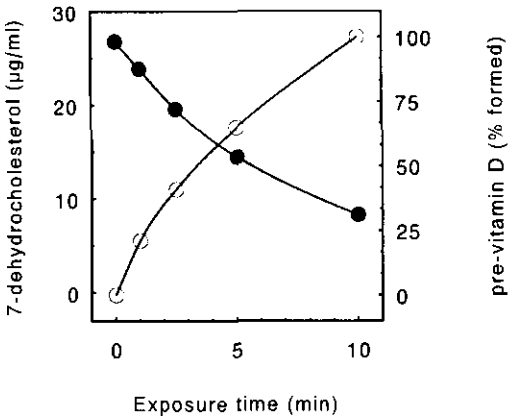

FIG. 2. Time-dependent conversion of 7-dehydrocholesterol (7DHC) to previtamin D_3 during exposure of 30 μg 7DHC per milliliter ethanol to 0.15 J uv-B light/min. The concentrations of 7DHC and previtamin D_3 were measured by normal-phase HPLC. Because no standard previtamin D_3 was available, results are expressed as relative peak height as calculated using an HP-3396A integrator.

increase in the vitamin D concentration in dog or cat skin, but in the rat skin a considerable increase occurred.

Many factors affect the biosynthesis of vitamin D_3 in the skin, including quantity and quality of the uv light, hair coat, and pigmentation. Under monochromatic irradiation the wavelength at which previtamin D formation is maximized is 295 ± 2 nm (Holick *et al.*, 1982), an area covered by the uv-B-emitting lamp in this study. The efficacy of the light tubes used in this study was proved in this and in other studies in sheep and in man (Chaudhary and Care, 1985; Lo and Paris, 1985). In unshorn animals biosynthesis of vitamin D_3 occurs after exposure to artificial uv-B light, although significantly higher increases in vitamin D_3 synthesis after the same exposure with uv-B light were found in shorn sheep (Chaudhary and Care, 1985). To exclude any influence of the hair coat, the samples of skin used in this study were shaved. In more pigmented skin longer exposure times are needed to maximize previtamin D formation, but in all cases only 15% of the 7DHC concentration present in the skin can be converted to previtamin D (Holick *et al.*, 1981); further irradiation may result in photoconversion of 7DHC, lumisterol, or tachysterol to their parent compounds (Fig. 1) (Webb and Holick, 1988), provided that the appropriate wavelength is applied (i.e., 295, 310, and 260 nm, respectively) (Holick *et al.*, 1982). In this study lightly pigmented skin and uv-B light of the correct wavelength for photoconversion of 7DHC, as revealed by the results of irradiation of a solution of 7DHC, were used. A single exposure to uv-B radiation of one to four times the minimal erythema dose (MED; i.e., the lowest dose which causes redness of the skin within 12 hr after irradiation) increases vitamin D_3 synthesis in man with increasing doses of uv-radiation (Adams *et al.*, 1982). Exposure of dogs to artificial uv-B light showed that the MED in dogs can be as high as 0.65 J per cm^2 skin (Hazewinkel *et al.*, 1987): the exposure of 2.25 J used in this study thus being within that range of one to four times MED. The vitamin D_3 in the extract of skin not exposed to uv-B light was of the same magnitude in dogs, cats, and rats.

Based on the finding that no detectable amount of vitamin D_3 was formed in the skin of the dog and the cat, vitamin D_3 is probably obtained from the diet. It is carried by DBP in the circulation to most tissues, including the skin, where it may influence hair follicle development and maturation after further hydroxylation in the living animal (Holick, 1990).

Since the extraction procedure included saponification for 2 hr at approximately 80°C, almost all previtamin D_3 which might be formed from photoconversion of 7DHC will be converted to vitamin D_3 (Verhaelen-Fasté and Verhaelen, 1981). From the results in rat skin it may be concluded that the extraction and exposure procedures make it possible to detect 7DHC, both originally

present and newly formed. The concentration of 7DHC found in the skin of rats in this study was higher than that reported elsewhere (Holick, 1990) in rat skin extracted without saponification. This step was included in the procedure after a pilot study revealed extremely low 7DHC concentrations in dog and cat skin. However, even after saponification 7DHC concentrations were extremely low in the skin of dogs and cats. This agrees with a previous report on the dog (Wheatly and Sher, 1961). The high 7DHC concentration in the skin of the rats of this study, when compared with other reports (Holick, 1990) and with the skin of dogs and cats of this study, can be caused by the fact that these rats were laboratory animals not exposed to uv-B light before euthanasia and skin collection.

It is possible that a provitamin other than 7DHC plays a role in cutaneous synthesis of vitamin D_3 in dogs and cats, as in amphibians and reptiles (Holick, 1990). Extremely high binding capacity or affinity of circulating DBP could cause rapid translocation of vitamin D_3 into the circulation. Based on the findings of the HPLC profiles of the extracts of the skin of dogs and cats there is no evidence for another provitamin. Although from experience with competitive protein binding assays for the determination of 25(OH) vitamin D and $24,25(OH)_2$ vitamin D (Hazewinkel *et al.*, 1987), a capacity or affinity higher than that in man can be ruled out because a separate binding protein or one with a high affinity for vitamin D_3 and less for other metabolites can be present. In addition, the finding of this study, i.e., the inability to demonstrate biosynthesis of vitamin D_3 in the skin of dogs, is in accordance with previous findings that dogs with nutritionally induced hypovitaminosis D were not cured by daily exposure to uv-B light at 70% of the MED during 3 months (Hazewinkel *et al.*, 1987). From that *in vivo* study it can also be concluded that, even when 7DHC in the skin of dog and cat is biosynthesized in other derivates which are not detectable by the techniques used in this study, these derivates will not be biologically effective.

The concentrations of 7DHC in the skin of dog and cat did not differ significantly from each other but differed considerably from values in herbivores (including sheep, cattle, and horses) able to "photosynthesize" vitamin D (Chaudhary and Care, 1985; Hidiroglou *et al.*, 1985; El Shorafa *et al.*, 1979). Under natural circumstances herbivores have limited access to sources of vitamin D_3, which might explain natural selection for the ability to synthesize vitamin D_3 in the skin under the influence of sunlight. Omnivores (including pig, rat, and man) also evolved with the ability to synthesize vitamin D_3 in the skin (Norman, 1979; Esvelt *et al.*, 1978; Holick, 1990) even though they were able to ingest sufficient vitamin D. Dogs and cats are both Fissiped Carnivores and members of the superfamilies of Canoidea and Feloidea, respectively. Both superfamilies diverged in the early Oligocene period, about 35 million years ago: the Canoidea include strict herbivores, such as the panda, and omnivores, such as dogs, whereas Feloidea include only strict carnivores (Morris and Rogers, 1989). Evolution has not required strict carnivores to provide their own vitamin D, since natural foods fulfill their requirements of this vitamin which is present in the body fat, liver, and blood of their prey. The dog, although an omnivore, does not differ in this respect from the cat, a strict carnivore. Until now, vitamin D_3 was considered to be a prohormone in animals and man (DeLuca, 1984). Based on the findings of this study, vitamin D_3 can be considered to be an essential vitamin for the dog and the cat.

In the published reports, which are often quoted as reference for manufacturers and owners in the preparation of rations for dogs and cats (Halle, 1992; McDowell, 1989; NRC, 1978a, 1986; Lewis *et al.*, 1987;

Wills *et al.*, 1992), biosynthesis of vitamin D is presumed to occur in dogs and cats. There are no published data available to support this assumption. The requirement for vitamin D in food for dogs and cats does not differ from that for herbivores and omnivores (McDowell, 1989) but is half the requirement of vitamin D for laboratory rats not exposed to uv-B light (NRC, 1978b). The present study suggests that requirements for vitamin D in the dog, cat, and rat should be reassessed.

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