Vitamin D Metabolism in the Hooded Seal (*Cystophora cristata*)[1]

K. M. KEIVER, H. H. DRAPER[*2] *AND K. RONALD*

*Department of Zoology and *Department of Nutritional Sciences, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1*

ABSTRACT Fish-eating mammals, such as seals, appear to ingest levels of vitamin D that are toxic to most mammals. To determine how seals cope with high vitamin D intakes, the metabolism of tritiated cholecalciferol ($[^3H]D_3$) was investigated in hooded seal (*Cystophora cristata*) pups during their postweaning fast and pups and adults consuming herring alone or supplemented with 400,000 IU D_3 daily. $[^3H]D_3$ was metabolized to 25-$[^3H]OHD_3$ and 24,25-$[^3H](OH)_2D_3$. 1,25-$[^3H](OH)_2D_3$ was not detected, but plasma levels of 1,25-$(OH)_2D$ were similar to those in other mammals and were not affected by vitamin D intake. Plasma vitamin D, 25-OHD and 24,25-$(OH)_2D$ increased with vitamin D intake, but 25-OHD did not increase to the extent seen in other mammals. The supplemented seals showed no evidence of toxicity. Levels of 24,25-$(OH)_2D$ were higher in the unsupplemented seals (4 to 33 ng/mL) than reported in other mammals with similar 25-OHD levels and did not decrease with 25-OHD. High levels of 24,25-$(OH)_2D$ relative to 25-OHD have also been found in hooded seals in the wild. The half-lives of vitamin D, 25-OHD and 24,25-$(OH)_2D$ were shorter than those reported for most other mammals. Increased conversion of 25-OHD to 24,25-$(OH)_2D$ and a high capacity for vitamin D storage in their large blubber mass appeared to be factors in the resistance of seals to vitamin D toxicity. *J. Nutr.* 118: 332–341, 1988.

INDEXING KEY WORDS:

- *vitamin D metabolism*
- *vitamin D toxicity*
- *pinnipeds*

Fish-eating mammals, such as seals, appear to ingest vitamin D at levels that are toxic to most mammals. Seals, however, do not appear to suffer from vitamin D toxicity even though their principal food, fish, is the highest known natural source of the vitamin. Vitamin D metabolism was investigated in the hooded seal (*Cystophora cristata*) to determine how seals cope with high vitamin D intakes.

The hooded seal is a migratory pelagic seal of the North Atlantic and Arctic oceans. As hooded seals are relatively inaccessible during most of the year, little is known about their feeding habits. During the breeding and moult periods, when the seals are accessible, they are fasting. Known prey items include a variety of fish and invertebrate species (1).

Vitamin D metabolism was investigated in weaned pups during the postweaning fast and in pups and adults ingesting fish with and without cholecalciferol (D_3) supplements. The study was designed to examine the following hypotheses concerning the tolerance of seals to vitamin D: *1*) their intestinal absorption of vitamin D is decreased when intake is high; *2*) they have a high capacity to convert 25-OHD to less toxic metabolites; *3*) they rapidly excrete vitamin D and/or its metabolites; *4*) they have a large capacity for storage of vitamin D and/or its metabolites in their large blubber mass.

MATERIALS AND METHODS

Seven weaned pups (age 2–3 wk) and three adults were captured in the Gulf of St. Lawrence. The seals, except for two pups that were fasted, were fed whole herring (*Clupea harengus*) supplemented with NaCl and vitamins,[3] excluding vitamin D. The herring contained

[1]Financial support for this study was provided by operating grants (to K. R. and H. H. D.) from the Natural Sciences and Engineering Research Council of Canada and scholarships (University of Guelph, Ontario Graduate and Norman James Aquatic Mammalogy) to K. M. K.

[2]To whom reprint requests should be sent.

[3]Weekly intake: 1400 mg vitamin B-1, 4116 mg vitamin E (ICN Canada, Toronto, Ont.), 22.5 mg retinol and 45 mg β-carotene (Novopharm, Toronto, Ont.), 14 Novo-B tablets (Novopharm) each containing 10 μg vitamin B-12, 35 mg vitamin B-1, 15 mg vitamin B-2, 5 mg vitamin B-6, 50 mg niacinamide, 20 mg Ca *d*-pantothenate, 300 mg vitamin C and 4 (adults) or 2 (pups) Stresstabs (Cyanamid Canada, Montreal, Que.) each containing 600 mg vitamin C, 30 mg vitamin E, 4 mg folic acid, 15 mg vitamin B-1, 15 mg vitamin B-2, 100 mg niacinamide, 5 mg vitamin B-6, 12 μg vitamin B-12, 45 μg biotin, 20 mg pantothenic acid and 27 mg ferrous fumarate.

0022-3166/88 $3.00 Received 27 May 1987. Accepted 2 October 1987.

0.38% Ca and 0.43% P (pups) or 0.43% Ca and 0.54% P (adults). The seals were held in continuous flow (132 L/min) freshwater tanks with access to dry ledges and received their natural photoperiod. All sources of ultraviolet (UV) radiation, as measured by a Blak-ray ultraviolet meter (Ultra-violet Products, San Gabriel, CA), were eliminated 6 mo prior to the experiments. Body mass was measured weekly with dynamometers.

The maximum vitamin D intake of wild hooded seals, based on the vitamin D content of halibut (*Hippoglossus hippoglossus*) (2), the estimated energy required for maintenance of the captive seals and the energy content of halibut, was estimated at approximately 400,000 IU/d. As the estimated energy requirements of the pups for maintenance (14,644–18,828 kJ/d) overlapped the range of estimates for the adults (17,991–48,116 kJ/d) and included no allowance for growth, the calculated mean energy requirement of the adults (31,798 kJ/d) was used to calculate the intake of halibut by both pups and adults.

Pups were divided into three groups: fasting (n = 2), fed herring (n = 2) and fed herring plus 400,000 IU D_3 daily (n = 3). Supplementation with D_3 began when the pups consistently consumed enough whole herring (approximately 2 kg/d at age 15 wk) to maintain a constant body weight. D_3 (Sigma Chemical, St. Louis, MO) was dissolved in 1 mL corn oil and stored in gelatin capsules at −80°C.

After 1 mo in captivity, the fasting pups were injected with [^{3}H]D_3, specific activity 38.5 Ci/mmol (New England Nuclear, Boston, MA). Fed pups were injected with [^{3}H]D_3 when the D_3 supplements had been fed for 2 wk. [^{3}H]D_3 was injected into the extradural vein in 1.0 mL 100% ethanol followed by 1.0 mL ethanol to rinse the syringe. The [^{3}H]D_3 was >94% pure as determined by high-performance liquid chromatography (HPLC). The amount of [^{3}H]D_3 injected, age, body mass, vitamin D and herring intakes are given in **Table 1**.

Blood samples (10–50 mL) were obtained in heparinized tubes from the hindflipper plexus prior to injection of [^{3}H]D_3 and postinjection at 5, 15 and 30 min and 1, 2, 4, 6, 12 and 24 h, then every 24 h until 168 h. Seals receiving the D_3 supplements were bled prior to and after 1 wk of supplementation. Plasma was stored at −80°C.

Urine and feces were collected for 24 h every second day by placing the seals in wooden boxes situated in a refrigerated room (3). As blood samples could not be obtained from seals in the boxes, collections were not made until 48 h postinjection.

The pups were euthanized with sodium pentobarbital (18 mg/kg) and tissues were collected to determine distribution of radioactivity. Kidneys of fed and two supplemented pups were examined for calcification.

The adults fed herring alone were injected with [^{3}H]D_3 (specific activity 18.3 Ci/mmol, Amersham Canada, Oakville, Ont.) and their blood, urine and feces were collected as for the pups. When plasma radioactivity had declined to undetectable levels (<50 dpm/mL) they were supplemented with 400,000 IU D_3 daily. After 2 wk, they were again injected with [^{3}H]D_3 and blood, urine and feces were collected.

Sample analyses. The analytical procedures used did not distinguish between D_3 and ergocalciferol. It was assumed that only D_3 compounds were present as ergocalciferol has not been found in oils from marine fishes (4, 5). Radioactivity was counted in a Beckman LS 7800 liquid scintillation counter (Beckman Instruments, Fullerton, CA) equipped with automatic quench correction. Plasma Ca was determined with a Corning Ca analyzer (Corning Scientific Instruments, Medfield, MA).

Vitamin D and its metabolites in plasma were separated on a Dupont Zorbax-Sil column (4.6 mm × 25 cm, Maynard Scientific, Weston, Ont.) using a 254-nm interference filter, solvent flow 2 mL/min and absorbance units full scale 0.005. All solvents used were HPLC grade. Reference standards for D_3 (Sigma Chemical), 25-OHD_3, 24,25-$(OH)_2D_3$ and 1,25-$(OH)_2D_3$ (kindly supplied by M. R. Uskokovic, Hoffman-LaRoche, Nutley, NJ) were purified by HPLC and quantitated with a Gilford 250 spectrophotometer (Gilford Instrument Laboratories, Oberlin, OH) at 264 nm.

The percentage of total plasma radioactivity as D_3,

TABLE 1

***Amount of [^{3}H]D_3 injected, age, body mass, vitamin D intake and herring intake of the seals*[1]**

Diet	[^{3}H]D_3 injected	Age	Body mass	Vitamin D intake	Herring intake
	μCi		*kg*	*IU/d*	*g/d*
Pups					
Fasting	25.9, 24.4	1.5 mo	29.0, 34.0	0	0
Herring	30.6, 30.3	4.2 mo	33.0, 42.5	1,001, 990	2,002, 1,980
Herring + D_3 supplement[2]	31.8 ± 0.1	4.2 mo	39.2 ± 1.6	400,965 ± 25	1,930 ± 50
Adults					
Herring	41.3 ± 4.7	>4 yr	192 ± 19	874 ± 265	4,370 ± 1,324
Herring + D_3 supplement[2]	68.4 ± 20.1	>4 yr	248 ± 27	400,609 ± 108	3,047 ± 541

[1]Means ± SEM are given when n = 3.
[2]400,000 IU/d.

25-OHD_3, 24,25-$(OH)_2D_3$ and 1,25-$(OH)_2D_3$ was determined using 0.5–1.0 mL plasma. D_3 was extracted with methanol/methylene chloride (6) and subjected to HPLC (7). All radioactivity in the D_3 extract coeluted with standard D_3.

Extraction of metabolites, prepurification for HPLC and separation by HPLC were accomplished by the method of Fraher et al. (8) with modifications. The method frequently resulted in poor recovery of radioactivity (<50%), believed to be due in part to the high lipid content of the seal plasma (6.8–14.2 mg/mL). To improve recovery, all solvent volumes were doubled and the plasma was reextracted with acetonitrile/water (1:1, vol/vol). Radioactivity was found to coelute with D_3, 25-OHD_3 and 24,25-$(OH)_2D_3$. As expected from the small amounts of 1,25-$(OH)_2D$ present in plasma and of [^{3}H]D_3 injected and the loss of half the tritium label during 1-hydroxylation, no 1,25-[^{3}H]$(OH)_2D_3$ could be detected.

Plasma levels of vitamin D and its metabolites were determined by competitive protein binding assay. Prior to extraction, 9000–12,000 dpm of [^{3}H]D_3, 25-[26,27-^{3}H]OHD_3, 1,25-[26,27-^{3}H]$(OH)_2D_3$ (New England Nuclear) or 24,25-[23,24-^{3}H]$(OH)_2D_3$ (Amersham Canada) was added to the plasma and recovery estimated following purification by HPLC.

Vitamin D in plasma (1.0–6.0 mL) was extracted as previously described. The extract was shaken with 2 volumes of acetonitrile/water (1:1, vol/vol) to remove polar compounds, then chromatographed on a Lipidex column (hydroxyalkoxypropyl-dextran, Sigma Chemical) (9) and purified by HPLC (10). Vitamin D was determined by the method of Horst et al. (11) using rat plasma as a source of binding protein.

Plasma samples (0.5–2.0 mL) were assayed for 25-OHD and 24,25-$(OH)_2D$ by the method of Lambert et al. (9) with modifications as described by Gibson et al. (12). 1,25-$(OH)_2D$ was assayed using the assay system of Amersham Canada.

Possible interference of 25-OHD-26,23-lactone in the assay procedure for 24,25-$(OH)_2D$ was investigated using plasma from three D_3-supplemented, two herring-fed and two fasting seals. The 24,25-$(OH)_2D$ fractions from HPLC were rechromatographed using a 2:98 (vol/vol) isopropanol/methylene chloride solvent system (13) and the fractions that coeluted with standard 25-OHD-26,23-lactone (kindly supplied by R. L. Horst, Ames, IA) and 24,25-$(OH)_2D_3$ were collected and assayed using the 24,25-$(OH)_2D$ assay procedure. No 25-OHD-26,23-lactone was found in the 24,25-$(OH)_2D$ fractions; the possibility that it was present in the plasma before purification was not investigated.

Plasma samples from healthy human adults were assayed for vitamin D (n = 1), 25-OHD (n = 3), 24,25-$(OH)_2D$ (n = 3) and 1,25-$(OH)_2D$ (n = 3) as reference standards. A pooled sample (n = 4) from children (age 4–7 yr) was also assayed for 25-OHD, 24,25-$(OH)_2D$ and 1,25-$(OH)_2D$.

Four Greenland halibut (*Reinhardtius hippoglossoides*, mass 1680–2080 g) and five Atlantic cod (*Gadus morhua*, mass 515–1255 g), caught in the Davis Strait off Greenland, and 20 herring each from those fed to the pups and adults were analyzed for gross energy using an oxygen bomb calorimeter (Parr Instruments, Moline, IL), for Ca by atomic absorption spectrophotometry and for P by a modification of the method of Fiske and Subbarow (14). Samples were pooled for each species and the vitamin D content was determined by chick bioassay, using the ash content of the left tibias (15). The vitamin D content of fresh herring (n = 10) was also measured to determine whether deterioration of vitamin D had occurred during prolonged storage in the herring fed to the seals.

Data analyses. Differences in plasma Ca levels and in plasma levels, half-lives and turnover rates of vitamin D and its metabolites were analyzed using t-tests. Paired t-tests were used to examine differences between animals before and after supplementation. Level of significance used was $P < 0.05$ for all analyses. Values are reported as the mean ± SEM when $n > 2$.

RESULTS

As radioactive D_3 levels decreased, 25-[^{3}H]OHD_3 levels increased in both pups (**Fig. 1**) and adults (**Fig 2**). Radioactive 25-OHD_3 was detected in the plasma of all seals by 72 h postinjection, but 24,25-[^{3}H]$(OH)_2D_3$ was detected only in the fasted and herring-fed pups and one D_3-supplemented adult. Radioactive 1,25-$(OH)_2D_3$ was not detected. Recoveries of plasma radioactivity were 87 ± 1% (n = 129) after extraction and 76 ± 1% (n = 129) after HPLC.

The rate constants used to calculate the half-lives and turnover rates were obtained from the relationships between specific activities and time (**Fig. 3**). The decline in specific activity of vitamin D after 12 h postinjection was monophasic except in three pups in which it was biphasic. When it was biphasic, the inflection point occurred at 72 h. The decay of vitamin D from 12 to 72 h was corrected for the decay from 72 to 168 h according to Gurpide (16). The decline in specific activity of 25-OHD and 24,25-$(OH)_2D$ was monophasic.

The half-lives of plasma vitamin D, 25-OHD and 24,25-$(OH)_2D$ are presented in **Table 2**. The half-life of vitamin D in the adults was significantly increased by D_3 supplementation from 12 to 72 h but was not affected in the pups from 12 to 72 h or 72 to 168 h. The half-life of 25-OHD in the pups also was not significantly affected by vitamin D intake. The half-life of 24,25-$(OH)_2D$ appeared to be similar to that of 25-OHD. However, the specific activity of 24,25-$(OH)_2D$ was still increasing in the fasted pups at 120 h; therefore the decline in specific activity of 24,25-$(OH)_2D$ was measurable only in three seals over a short period (herring-fed pups 72–168 h, adult 72–120 h).

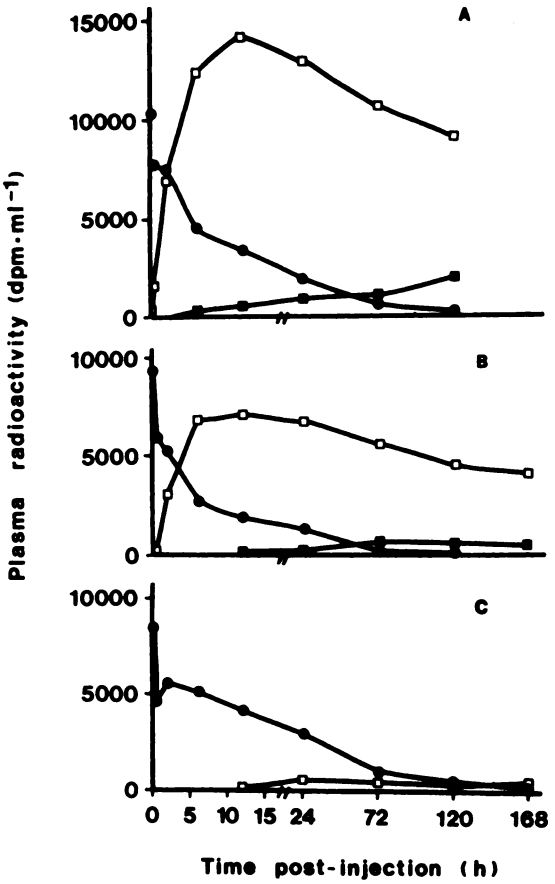

FIGURE 1 Relationships between mean plasma radioactivity as D_3 (●), 25-OHD_3 (□) and 24,25-$(OH)_2D_3$ (■) and time postinjection in pups that were (*A*) fasting (n = 2), (*B*) fed herring alone (n = 2) or (*C*) supplemented with D_3 (n = 3).

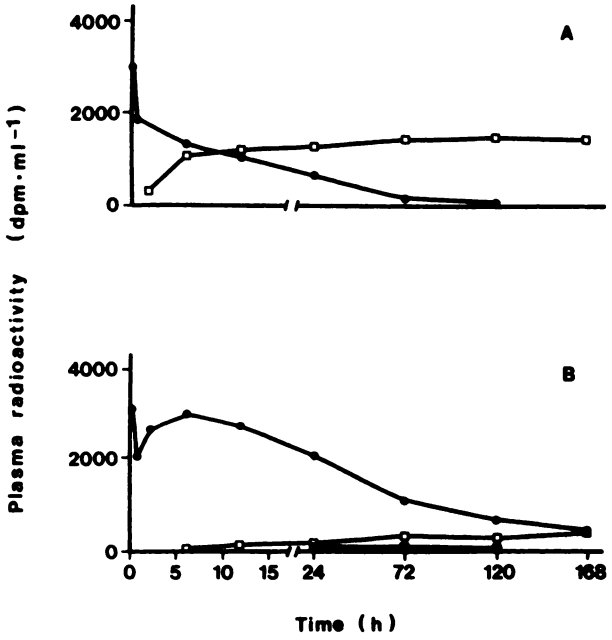

FIGURE 2 Relationships between mean plasma radioactivity as D_3 (●), 25-OHD_3 (□) and 24,25-$(OH)_2D_3$ (■) and time postinjection in the adults (n = 3) (*A*) fed herring alone or (*B*) supplemented with D_3.

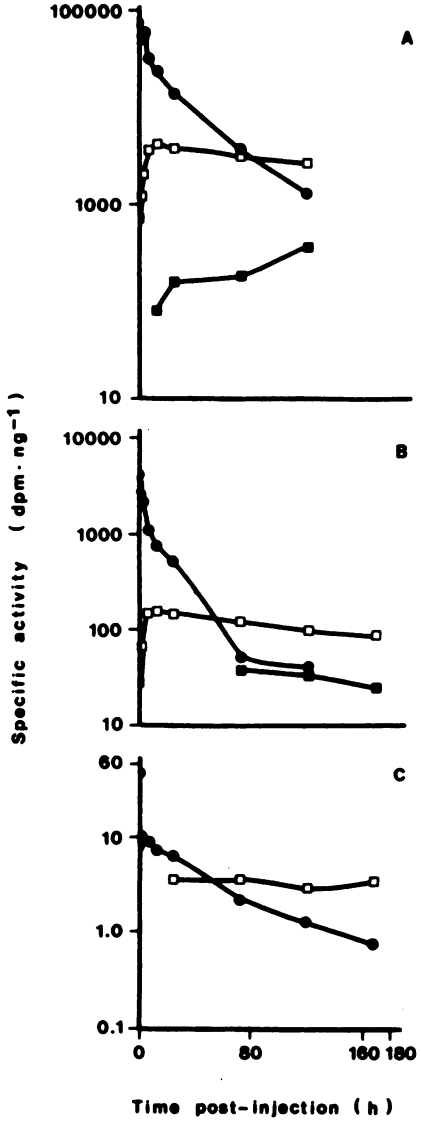

FIGURE 3 Relationships between mean specific activity of vitamin D (●), 25-OHD (□) and 24,25-$(OH)_2D$ (■) and time postinjection, plotted on a semilogarithmic scale, in pups that were (*A*) fasting (n = 2), (*B*) fed herring alone (n = 2) or (*C*) supplemented with D_3 (n = 3).

The turnover rates of vitamin D, 25-OHD and 24,25-$(OH)_2D$ are presented in **Table 3**. Vitamin D turnover in the pups was increased 11-fold over the fasting value by feeding herring and a further 154-fold by supplementing with D_3. The turnover of 25-OHD was increased 13- and 7-fold, respectively. In the fasting pups, 25-OHD turnover was higher than that of vitamin D, while in the pups fed herring alone there was no clear difference. Vitamin D turnover in the D_3-supplemented pups was markedly greater than that of 25-OHD. Reflecting their much greater body mass, the increment in turnover of the vitamin following supplementation in the adults was about half that of the pups.

Urinary radioactivity over the 7-d postinjection period accounted for ≤3% of injected radioactivity. Urine and feces were not obtainable from all seals and the relationship between excretion and time could be es-

TABLE 2

Half-lives of vitamin D, 25-OHD and 24,25-$(OH)_2$D in plasma of individual seals

Diet	Half-life[1]			
	Vitamin D			
	12–72 h postinjection	72–168 h postinjection	25-OHD	24,25-$(OH)_2$D
	d			
Pups				
Fasting	1.2, 0.9	1.0, 1.5	6.5, 11.5	
Herring	0.3, 0.6	2.1	6.9, 11.5	8.4, 5.9
Herring + D_3	1.7, 0.9, 1.4	1.5, 2.9, 2.4	3.4, 6.4	
Adults				
Herring	0.6, 0.8, 1.2	0.8		
Herring + D_3	2.2, 2.2, 2.0	2.4, 4.6, 2.6	8.9	10.8

[1]Blank spaces indicate that data were unobtainable.

TABLE 3

Turnover rates of vitamin D, 25-OHD and 24,25-$(OH)_2$D in plasma of individual seals

Diet	Rate of turnover[1]			
	Vitamin D			
	12–72 h postinjection	72–168 h postinjection	25-OHD	24,25-$(OH)_2$D
	ng/(mL·d)			
Pups				
Fasting	0.12, 0.08	0.14, 0.05	0.32, 0.24	
Herring	8.1, 2.5	1.0	4.4, 3.1	1.6, 1.9
Herring + D_3	190, 295, 282	206, 93, 162	35, 15	
Adults				
Herring	2.4, 1.0, 1.1	1.7		
Herring + D_3	97, 97, 127	91, 45, 95	6.9	2.1

[1]Blank spaces indicate that data were unobtainable.

timated individually only for the D_3-supplemented pups. Samples from the other seals were pooled for each treatment. Fecal excretion averaged 44% of the dose in the pups fed herring alone, 40 ± 5% (range 32–50%) in the D_3-supplemented pups and 24 and 26% in the adults fed herring alone and with D_3 supplements, respectively. There was no apparent effect of vitamin D intake on urinary or fecal excretion of radioactivity. Fasting pups produced very little urine and feces and excretion of injected radioactivity was negligible.

Concentrations of radioactivity were highest in the plasma, liver and lung in the fasting pups and pups fed herring alone and highest in the liver and blubber in the D_3-supplemented pups. The plasma volume determined for one D_3-supplemented pup (17) at age 7 mo was used to estimate the radioactivity in the plasma compartment of all pups. As the pups were younger than 7 mo at the time of [^{3}H]D_3 injection, plasma volume was probably overestimated by approximately 15%.

The highest percentages of injected radioactivity were found in the plasma and blubber (**Table 4**). The high concentrations in the fasting pups reflect a lack of excretion. Plasma radioactivity decreased with increased vitamin D intake. Blubber was the predominant site of radioactivity in the D_3-supplemented pups.

Recoveries of vitamin D, 25-OHD, 24,25-$(OH)_2$D and 1,25-$(OH)_2$D in plasma prior to assay were 79 ± 1% (n = 63), 75 ± 1% (n = 88), 65 ± 1% (n = 79) and 67

TABLE 4

Distribution of radioactivity in the tissues of individual pups at 7 d postinjection

Tissue	Fasting	Herring diet	Herring + D_3
		% of dose	
Plasma	60, 41	20, 17	3, 3
Blubber	35, 27	11, 10	24, 23
Liver	9, 3	1, 2	2, 2
Voluntary muscle	9, 3	0, 0	0, 0
Skin and hair	5, 8	3, 3	3, 2
Lung	3, 2	0.8, 0.4	0.5, 0.9
Kidney	0.9, 0.6	0.2, 0.2	0.1, 0.1
Heart	0.5, 0.3	0.1, 0.1	0.1, 0.1

TABLE 5

***Plasma levels of vitamin D, 25-OHD, 24,25-$(OH)_2$D and 1,25-$(OH)_2$D in the pups*[1]**

Diet	Vitamin D	25-OHD	24,25-$(OH)_2$D	1,25-$(OH)_2$D	$\frac{24,25\text{-}(OH)_2D}{25\text{-OHD}}$
	ng/mL	*ng/mL*	*ng/mL*	*pg/mL*	
Fasting					
When captured		7, 11	9, 16		1.3, 1.5
3 wk		3, 4			
4 wk	<0.3, <0.2	3, 3	4, 15	10, 27	1.3, 5.0
Herring					
When captured		9, 17	14, 18		1.6, 1.1
10 wk		42, 48	20, 14		
11 wk	3, 2	45, 54	18, 17	96, 62	0.44, 0.30
Herring + D_3					
When captured		12 ± 4	15 ± 0.9		1.6 ± 0.5
Presupplement (herring)	2 ± 0.3	59 ± 7	18 ± 5	37 ± 10	0.30 ± 0.08
1 wk supplementation	383 ± 58	133 ± 7	46 ± 4		0.35 ± 0.04
2 wk supplementation	580 ± 57	129 ± 7	53 ± 4		0.41 ± 0.01
3 wk supplementation	365 ± 37	164 ± 24	70 ± 7	24 ± 4	0.43 ± 0.03

[1]Means (± SEM) are given when $n = 3$. Blank spaces indicate that no measurements were taken.

± 1% ($n = 20$), respectively. Intra-assay coefficients of variation ($n = 5$) were 13, 5 and 5% and interassay coefficients ($n = 5$) were 12, 5 and 6% for vitamin D, 25-OHD and 24,25-$(OH)_2$D, respectively. Assay values for adult human plasma were 0.7 ng/mL for vitamin D, 13–15 ng/mL for 25-OHD, 1–2 ng/mL for 24,25-$(OH)_2$D and 20–27 pg/mL for 1,25-$(OH)_2$D. Metabolite values for the pooled plasma of four children were 24 ng/mL, 3 ng/mL and 90 pg/mL, respectively. These values are within the range of normal values for young and adult humans (18–20).

Plasma vitamin D, 25-OHD, 24,25-$(OH)_2$D and 1,25-$(OH)_2$D levels in the pups are presented in **Table 5**. When they were captured, their levels of 25-OHD and 24,25-$(OH)_2$D were 11 ± 2 and 15 ± 1 ng/mL, respectively. After 4 wk of fasting, 25-OHD levels had decreased to 3 ng/mL and vitamin D levels were undetectable. After feeding herring, 25-OHD levels increased significantly. Plasma 24,25-$(OH)_2$D levels showed no consistent change when the seals were fasted or fed herring alone. Ratios of 24,25-$(OH)_2$D to 25-OHD were higher when the pups were captured and during fasting than when they were fed herring. Plasma 1,25-$(OH)_2$D levels were not significantly affected by vitamin D intake.

D_3 supplementation for 1 wk resulted in a significant increase in plasma vitamin D and 25-OHD in the pups (Table 5). No further increases were seen at 3 wk. Plasma 24,25-$(OH)_2$D levels increased significantly over the 3-wk course of supplementation.

The plasma levels of vitamin D, 25-OHD and 24,25-$(OH)_2$D in the adults fed herring (**Table 6**) were similar to those of the pups and also increased with D_3 supplementation. As in the pups, D_3 supplementation had no effect on plasma 1,25-$(OH)_2$D levels.

The kidneys of the D_3-supplemented pups showed no evidence of calcification. Plasma Ca levels in the D_3-supplemented pups and adults were not significantly different from those of the seals fed herring alone (range 2.1–2.6 mmol/L).

The vitamin D contents of Greenland halibut and Atlantic cod were 2.0 and 0.7 IU/g wet tissue, respectively. The herring fed to the pups contained 0.5 IU vitamin D/g wet tissue and that fed to the adults 0.2

TABLE 6

***Plasma levels of vitamin D, 25-OHD, 24,25-$(OH)_2$D and 1,25-$(OH)_2$D in the adults*[1]**

Diet	Vitamin D	25-OHD	24,25-$(OH)_2$D	1,25-$(OH)_2$D	$\frac{24,25\text{-}(OH)_2D}{25\text{-OHD}}$
	ng/mL	*ng/mL*	*ng/mL*	*pg/mL*	
Herring	2 ± 0.3	48 ± 7	21 ± 6		0.44 ± 0.13
Herring + D_3					
Presupplement (herring)	0.3 ± 0.1	43 ± 8	17 ± 4	30 ± 6	0.41 ± 0.08
1 wk supplementation	267 ± 12	54 ± 10	22 ± 7		0.47 ± 0.27
2 wk supplementation	353 ± 29	74 ± 11	17 ± 3		0.24 ± 0.07
3 wk supplementation	297 ± 20	220 ± 13	26 ± 7	47 ± 5	0.23 ± 0.04

[1]Values are means ± SEM.

IU/g). The vitamin D content of fresh herring (1.2 IU/g) was only slightly higher than that of stored herring, indicating that the vitamin had not deteriorated significantly.

DISCUSSION

The same major metabolites of vitamin D were found in seal plasma as have been found in other mammals, although in somewhat different proportions. Conversion of vitamin D to 25-OHD was reflected in an increase in 25-[^{3}H]OHD$_3$ levels as [^{3}H]D$_3$ levels declined. The rebound in [^{3}H]D$_3$ that occurred in the D$_3$-supplemented seals is similar to that observed in humans with normal D status (21, 22).

The specific activity of vitamin D initially declined rapidly, then in a monophasic or biphasic manner. The biphasic curve may result from disequilibrium of [^{3}H]D$_3$ with vitamin D in the tissues. Mawer et al. (23) observed that radioactive vitamin D can take up to 4 d to equilibrate in some tissues. The biological half-life of vitamin D (Table 2) was calculated, when possible, after 72 h.

The half-lives of vitamin D and 25-OHD in plasma usually increase with vitamin D status (24, 25). The half-life of vitamin D increased with vitamin D intake in the adult seals but not in the pups, and vitamin D intake had no effect on the half-life of 25-OHD. Half-lives of 0.8–7.9 d have been observed in vitamin D–deficient humans and rats (24, 26) and of 3–36 d in humans in normal D status (24, 27). Regardless of vitamin D intake, the half-life of vitamin D in the seals after 72 h postinjection (0.8–4.6 d) was similar to that in vitamin D–deficient humans and rats.

The half-life of plasma 25-OHD in the seals was also similar to that of mammals with vitamin D deficiency. A half-life of 10.5–12 d has been found in vitamin D–deficient humans (24), 15–36 d in humans when D status was normal (24, 28, 29) and 25–68 d in humans and cows during vitamin D toxicity (25, 30).

The half-lives of 24,25-(OH)$_2$D in the plasma of the three seals in which they could be determined (5.9–10.8 d) were similar to those of 25-OHD. A half-life of approximately 40 d has been reported for humans (31). The shorter half-lives of plasma vitamin D, 25-OHD and perhaps 24,25-(OH)$_2$D in the seals indicate that these compounds are more actively metabolized, excreted or stored in this species than in other mammals.

The turnover rates of vitamin D and 25-OHD increased markedly with vitamin D intake. The higher turnover of 25-OHD than of vitamin D in the fasting pups indicates that 25-OHD levels were decreasing. This indication was consistent with 25-OHD values obtained over time. The higher turnover rate of 25-OHD than of 24,25-(OH)$_2$D presumably reflects a difference in rates of production, as their half-lives were similar. Some 25-OHD may have been excreted or stored.

Since feeding herring increased the turnover rate of vitamin D in the pup plasma 11-fold and that of 25-OHD 13-fold with no change in the half-life of 25-OHD, the fractional conversion of vitamin D to 25-OHD must have been similar in the fasting and fed pups. The much greater increase in the turnover of vitamin D than of 25-OHD resulting from D$_3$ supplementation indicates that the fractional conversion of vitamin D to 25-OHD decreased in the D$_3$-supplemented pups, even when allowances are made for the increase in their plasma 25-OHD levels (Table 5). This conclusion is consistent with similar findings on other mammals (32, 33). As the large turnover of vitamin D in the plasma of the D$_3$-supplemented pups was not accounted for by conversion to 25-OHD, the vitamin D must have been excreted or stored.

As in other mammals (21, 34, 35), the primary route of excretion of vitamin D and its metabolites in the seals appeared to be through the feces via the bile and was unaffected by vitamin D intake. The excretion of radioactivity in the feces over 7 d (24–50%) was higher than that found for most mammals. Biliary excretion ranges from 3 to 16.6% of injected radioactivity over 1 to 3 d in humans and rats (21, 23, 34–36). Most of the radioactivity is excreted in the first 2 d (21, 35). The data suggest that seals may excrete a higher proportion of vitamin D and/or its metabolites through bile than do other mammals.

The concentration of radioactivity in the tissues of the seals indicated that, as in other mammals, vitamin D was not actively stored in any tissue. Accumulation of vitamin D and its metabolites occurs by passive processes rather than by active storage (37). Fatty tissues accumulate a higher concentration of vitamin D than nonfatty tissues and vitamin D is more readily accumulated than 25-OHD (23, 26). Blubber therefore would be expected to accumulate radioactivity in proportion to the amount of radioactive vitamin D in the plasma, and this was apparently the case in the supplemented pups versus those fed herring alone (Fig. 1, Table 4). The high concentration of radioactivity in blubber of the fasting pups suggests that their blubber represented a greater proportion of their total body fat than in the other pups. Seal pups that fast on ice or in water, such as hooded seal pups, appear to deplete visceral fat depots in preference to blubber for thermoregulatory reasons (38). The visceral fat depots of the fasting pups therefore were probably depleted. The higher percentage of total body mass as blubber in the fasting pups (36–50%) compared to the other pups (28–30%) is consistent with this conclusion.

The pups accumulated 10–35% of injected radioactivity in their blubber alone. Adipose tissue has been reported to accumulate 5 to 12% of injected radioactive vitamin D in the human and rat (23, 37). The large amount of adipose tissue in seals and its high lipid content (39) may enable seals to accumulate more vitamin D than other mammals, thereby lowering plasma levels of vi-

tamin D and 25-OHD in response to vitamin D intake and reducing the half-life of plasma vitamin D.

Fasting for 4 wk in the absence of UV radiation resulted in undetectable levels of vitamin D in the plasma of the pups and a decrease in 25-OHD to levels that would indicate suboptimal D status in other mammals (40). Plasma 1,25-$(OH)_2$D levels in the fasting pups were similar to those found in 2- to 12-yr-old children (19, 20). Vitamin D, 25-OHD and 1,25-$(OH)_2$D levels in the plasma of the seals fed herring were comparable to those of wild hooded seals (41) and to levels considered normal for other mammals (7, 19, 42, 43).

Plasma 24,25-$(OH)_2$D levels in the fasting pups (4–16 ng/mL) and the seals fed herring alone (8–33 ng/mL) were higher than those found in most mammals (0.2–10.8 ng/mL) with similar 25-OHD levels (18, 33, 40, 42). The ratios of plasma 24,25-$(OH)_2$D to 25-OHD in the seals (0.23–5.0) were higher than those (0.05–0.27) found in other species (33, 40, 43). Since the half-life of 24,25-$(OH)_2$D in the seal plasma was not longer than that in other mammals, these high ratios may have resulted from a greater specific activity or synthesis of the 24-hydroxylase enzyme. It is also possible that pathways for the metabolism of 25-OHD to compounds such as 25,26-$(OH)_2$D or 25-OHD-26,23-lactone are lacking in seals, resulting in an increase in the amount of substrate available for 24-hydroxylation.

The very high ratios of 24,25-$(OH)_2$D to 25-OHD observed in the fasting pups are similar to those found in the plasma of fasting gray (*Halichoerus grypus*) and harp (*Phoca groenlandica*) seal pups (41) but not in the young of nonpinniped species (44). Also in contrast to other mammals (18, 40, 45), the reduction in 25-OHD levels during fasting was not accompanied by a decrease in plasma 24,25-$(OH)_2$D in the seal pups. Information on vitamin D metabolite levels in fasting mammals other than pinnipeds appears to be unavailable. Accumulation of 24,25-$(OH)_2$D caused by decreased bile flow during fasting is not indicated as the half-life of 25-OHD was not different between fasting and fed pups.

On a body mass basis, the D_3-supplemented seals received 10,275 IU/(kg·d) (pups) or 1648 IU/(kg·d) (adults) for 3 wk. Human studies (46, 47) indicate that vitamin D intakes greater than 2000 IU/(kg·d) would produce toxic symptoms in 3 wk. Calcification of soft tissues within 6–30 d has been observed in animals receiving 7300–100,000 IU/(kg·d) (48–51). These studies indicate that the level of D_3 supplementation used in the present study should have produced toxicity within 3 wk in the pups (but not necessarily in the adults) if the seals did not have greater tolerance than other mammals for high vitamin D intakes.

Plasma levels of vitamin D and its metabolites, except 1,25-$(OH)_2$D, increased with D_3 supplementation, indicating that the tolerance of seals to high vitamin D intakes is not due to decreased intestinal absorption. Plasma 25-OHD reached levels (>100 ng/mL) that have been associated with toxicity in humans (47); however, toxicity in mammals is more often associated with levels >200 ng/mL (25, 52).

Vitamin D toxicity is generally believed to be caused by high levels of 25-OHD in the plasma (53, 54). Plasma 25-OHD in the seals did not increase in response to D_3 supplementation to the extent observed in other mammals. Young pigs receiving 12,480 IU/(kg·d) (an intake similar to that of the D_3-supplemented pups) exhibited reduced growth, hypercalcemia and plasma 25-OHD levels of approximately 340 ng/mL within 28 d (52). Quarterman et al. (48) found reduced food intakes in young pigs receiving 11,013 IU/(kg·d) after 15 d and hypercalcemia, calcification of kidneys and lungs and aortic lesions by 4 wk. Humans receiving 533 IU/(kg·d) of vitamin D for 3 wk (55, 56) showed plasma 25-OHD levels of 90 to 110 ng/mL. The D_3-supplemented adult seals had similar plasma 25-OHD levels after 3 wk of supplementation, but were receiving three times the intake of vitamin D on a body mass basis. The results indicate that the smaller response of plasma 25-OHD to vitamin D intake in the seals may be the result of greater conversion of 25-OHD to 24,25-$(OH)_2$D, increased excretion of vitamin D and/or its metabolites in the feces and greater storage of vitamin D in adipose tissue.

The vitamin D contents of Greenland halibut, Atlantic cod and Atlantic herring were low compared to reported values (2, 57, 58) and may be due to the small size of the fish analyzed (<2.1 kg). Vitamin D content of fish usually increases with age (59, 60). The vitamin D content of fish of a size likely to be consumed by seals ranges from 1.4 to 10.0 IU/g wet tissue for halibut up to 11 kg (61), 0.42 to 4.01 IU/g for Atlantic cod up to 7 kg (60) and 3.3 to 21.6 IU/g for herring (2, 57, 58).

Based on the highest reported value for vitamin D in herring of 21.6 IU/g (58), the adult seals would have ingested ~600 IU/(kg·d) and the pups ~3000 IU/(kg·d) (115,000 IU/d). These levels, at least in the case of the pups, are above the intake considered toxic for other mammals on a body mass basis. However, the much lower values for the vitamin D content of the herring fed in this study and the values reported for various species of fish by other investigators indicate that the usual intake of vitamin D by seals does not exceed the amount tolerated by other mammals. The resistance of the seals in this experiment to a supplement of 400,000 IU/d nevertheless indicates a tolerance for much higher intakes than may normally be consumed. The results also suggest that this tolerance may be due to a capacity of seals for innocuous storage of vitamin D in their large blubber mass and for rapid conversion of excess 25-OHD to catabolic metabolites.

ACKNOWLEDGMENTS

We wish to thank H. Pedersen, R. Frank, M. Hadley and N. Laing for their assistance with this project. We

are grateful to G. A. Robinson for his aid in the interpretation of the specific activity curves, B. Hicks for examining the kidneys and M. Berry for supplying human plasma. The comments of G. Jones, R. L. Horst, G. Worthy and S. Innes were greatly appreciated.

LITERATURE CITED

1. KING, J. E. (1983) *Seals of the World*, pp. 98–101, Oxford Univ. Press, London.
2. HOLMES, A. D., TRIPP, F. & SATTERFIELD, G. H. (1941) Fish liver and body oils. *Ind. Eng. Chem.* 33: 944–949.
3. KEIVER, K. M., RONALD, K. & BEAMISH, F. W. H. (1984) Metabolizable energy requirements for maintenance and faecal and urinary losses of juvenile harp seals (*Phoca groenlandica*). *Can. J. Zool.* 62: 769–776.
4. EGAAS, E. & LAMBERTSEN, G. (1979) Naturally occurring vitamin D_3 in fish products analysed by HPLC, using vitamin D_2 as an international standard. *Int. J. Vitam. Nutr. Res.* 49: 35–42.
5. TAKEUCHI, A., OKANO, T., AYARNE, M., YOSHIKAWA, H., TERAOKA, S., MURAKAMI, Y. & KOBAYASHI, T. (1984) High-performance liquid chromatographic determination of vitamin D_3 in fish liver oils and eel body oils. *J. Nutr. Sci. Vitaminol.* 30: 421–430.
6. HORST, R. L., SHEPARD, R. M., JORGENSEN, N. A. & DELUCA, H. F. (1979) The determination of the vitamin D metabolites on a single plasma sample: changes during parturition in dairy cows. *Arch. Biochem. Biophys.* 192: 512–523.
7. SHEPARD, R. M., HORST, R. L., HAMSTRA, A. J. & DELUCA, H. F. (1979) Determination of vitamin D and its metabolites in plasma from normal and anephric man. *Biochem. J.* 182: 55–69.
8. FRAHER, L. J., ADAMI, S., CLEMENS, T. L., JONES, G. & O'RIORDAN, J. L. H. (1983) Radioimmunoassay of 1,25-dihydroxy vitamin D_2: studies on the metabolism of vitamin D_2 in man. *Clin. Endocrinol.* 18: 151–165.
9. LAMBERT, P. W., DEOREO, P. B., HOLLIS, B. W., FU, I. Y., GINSBERG, D. J. & ROOS, B. A. (1981) Concurrent measurement of plasma levels of vitamin D_3 and five of its metabolites in normal humans, chronic renal failure patients, and anephric subjects. *J. Lab. Clin. Med.* 98: 536–548.
10. HOLLIS, B. W., ROOS, B. A. & LAMBERT, P. W. (1981) Vitamin D in plasma: quantitation by a nonequilibrium ligand binding assay. *Steroids* 37: 609–619.
11. HORST, R. L., REINHARDT, T. A., BEITZ, D. C. & LITTLEDIKE, E. T. (1981) A sensitive competitive protein binding assay for vitamin D in plasma. *Steroids* 37: 581–591.
12. GIBSON, R. S., DRAPER, H. H., MCGIRR, L. G., NIZAN, P. & MARTINEZ, O. B. (1986) The vitamin D status of a cohort of postmenopausal non-institutionalized Canadian women. *Nutr. Res.* 6: 1179–1187.
13. HORST, R. L., LITTLEDIKE, E. T., GRAY, R. W. & NAPOLI, J. L. (1981) Impaired 24,25-dihydroxyvitamin D production in anephric human and pig. *J. Clin. Invest.* 67: 274–280.
14. FISKE, C. H. & SUBBAROW, Y. (1925) The colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375–400.
15. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (1975) *Methods of Analysis*, pp. 856–857, AOAC, Washington, DC.
16. GURPIDE, E. (1975) *Tracer Methods in Hormone Research*, pp. 1–188, Springer-Verlag, Berlin.
17. KEIVER, K. M., CHANDLER, M., FRANK, R. J. & RONALD, K. (1987) Plasma and blood volumes of the hooded seal (*cystophora cristata*). *Can. J. Zool.* 65: 1866–1867.
18. TAYLOR, C. M., HUGHES, S. E. & DE SILVA, P. (1976) Competitive protein binding assay for 24,25-dihydroxycholecalciferol. *Biochem. Biophys. Res. Commun.* 70: 1243–1249.
19. KREAM, B. E., EISMAN, J. A. & DELUCA, H. F. (1977) Intestinal cytosol binders for 1,25-dihydroxyvitamin D_3: use in a competitive binding protein assay. In: *Vitamin D. Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism* (Norman, A. W., Schaefer, K., Coburn, J. W., DeLuca, H. F., Fraser, D., Grigoleit, H. G. & Herrath, D. V., eds.), pp. 501–510, de Gruyter, Berlin.
20. REINHARDT, T. A., HORST, R. L., ORF, J. W. & HOLLIS, B. W. (1984) A microassay for 1,25-dihydroxyvitamin D not requiring high performance liquid chromatography: application to clinical studies. *J. Clin. Endocrinol. Metab.* 58: 91–98.
21. AVIOLI, L. V., LEE, S. W., MCDONALD, J. E., LUND, J. & DELUCA, H. F. (1967) Metabolism of vitamin D_3-^{3}H in human subjects: distribution in blood, bile, feces, and urine. *J. Clin. Invest.* 46: 983–992.
22. BARRAGRY, J. M., FRANCE, M. W., BOUCHER, B. J. & COHEN, R. D. (1979) Metabolism of intravenously administered cholecalciferol in man. *Clin. Endocrinol.* 11: 491–495.
23. MAWER, E. B., BACKHOUSE, J., HOLMAN, C. A., LUMB, G. A. & STANBURY, S. W. (1972) The distribution and storage of vitamin D and its metabolites in human tissues. *Clin. Sci. (London)* 43: 413–431.
24. MAWER, E. B., LUMB, G. A., SCHAEFER, K. & STANBURY, S. W. (1971) The metabolism of isotopically labelled vitamin D_3 in man: the influence of the state of vitamin D nutrition. *Clin. Sci. (London)* 40: 39–53.
25. MAWER, E. B., HANN, J. T., BERRY, J. L. & DAVIES, M. (1985) Vitamin D metabolism in patients intoxicated with ergocalciferol. *Clin. Sci. (London)* 68: 135–141.
26. LAWSON, D. E. M., SEDRANI, S. H. & DOUGLAS, J. (1986) Interrelationships in rats of tissue pools of cholecalciferol and 25-hydroxycholecalciferol formed in U.V. light. *Biochem. J.* 233: 535–540.
27. MAWER, E. B., LUMB, G. A. & STANBURY, S. W. (1969) Long biological half-life of vitamin D_3 and its polar metabolites in human serum. *Nature (London)* 222: 482–483.
28. SMITH, J. E. & GOODMAN, D. S. (1971) The turnover and transport of vitamin D and of a polar metabolite with the properties of 25-hydroxycholecalciferol in human plasma. *J. Clin. Invest.* 50: 2159–2167.
29. BATCHELOR, A. J. & COMPSTON, J. E. (1983) Reduced plasma half-life of radio-labelled 25-hydroxyvitamin D_3 in subjects receiving a high-fibre diet. *Br. J. Nutr.* 49: 213–216.
30. HOLLIS, B. W., CONRAD, H. R. & HIBBS, J. W. (1977) Changes in plasma 25-hydroxycholecalciferol and selected blood parameters after injection of massive doses of cholecalciferol or 25-hydroxycholecalciferol in nonlactating dairy cows. *J. Nutr.* 107: 606–613.
31. STANBURY, S. W. (1977) The role of vitamin D in renal bone disease. *Clin. Endocrinol.* 7 (Suppl.): 25s–30s.
32. MAWER, E. B. & REEVE, A. (1977) The use of an isolated perfused liver to study the control of cholecalciferol-25-hydroxylase activity in the rat. *Calcif. Tissue Res.* 22 (Suppl.): 24–28.
33. GASCON-BARRE, M. & HUET, P. (1982) Role of the liver in the homeostasis of calciferol metabolism in the dog. *Endocrinology* 110: 563–570.
34. CALLOW, R. K., KODICEK, E. & THOMPSON, G. A. (1966) Metabolism of tritiated vitamin D. *Proc. R. Soc. London B Biol. Sci.* 164: 1–20.
35. CLEMENTS, M. R., CHALMERS, T. M. & FRASER, D. R. (1984) Enterohepatic circulation of vitamin D: a reappraisal of the hypothesis. *Lancet* 1: 1376–1379.
36. PONCHON, G. & DELUCA, H. F. (1969) Ethanol-induced artifacts in the metabolism of ^{3}H-vitamin D_3. *Proc. Soc. Exp. Biol. Med.* 131: 727–731.
37. ROSENSTREICH, S. J., RICH, C. & VOLWILER, W. (1971) Deposition in and release of vitamin D_3 from body fat: evidence for a storage site in the rat. *J. Clin. Invest.* 50: 679–687.
38. WORTHY, G. A. J. & LAVIGNE, D. M. (1988) Mass loss, metabolic rate and energy utilization by harp and gray seal pups during the post-weaning fast. *Physiol. Zool.* In press.
39. WORTHY, G. A. J. & LAVIGNE, D. M. (1983) Changes in energy

stores during postnatal development of the harp seal, *Phoca groenlandica*. *J. Mammal.* 64: 89–96.

40. TAYLOR, C. M., HANN, J., ST. JOHN, J., WALLACE, J. E. & MAWER, E. B. (1979) 1,25-Dihydroxycholecalciferol in human serum and its relationship with other metabolites of vitamin D-3. *Clin. Chim. Acta* 96: 1–8.
41. KEIVER, K. M., RONALD, K. & DRAPER, H. H. (1988) Plasma levels of vitamin D and some metabolites in marine mammals. *Can. J. Zool.* In press.
42. HORST, R. L., LITTLEDIKE, E. T., RILEY, J. L. & NAPOLI, J. L. (1981) Quantitation of vitamin D and its metabolites and their plasma concentrations in five species of animals. *Anal. Biochem.* 116: 189–203.
43. HORST, R. L. & LITTLEDIKE, E. T. (1982) Comparison of plasma concentrations of vitamin D and its metabolites in young and aged domestic animals. *Comp. Biochem. Physiol. B Comp. Biochem.* 73: 485–489.
44. CHESNEY, R. W., HAMSTRA, A. J. & DELUCA, H. F. (1982) Absence of seasonal fluctuation in serum concentration of 24,25$(OH)_2$-vitamin D in childhood. *Calcif. Tissue Int.* 34: 527–530.
45. HORST, R. L., SHEPARD, R. M., JORGENSEN, N. A. & DELUCA, H. F. (1979) The determination of 24,25-dihydroxyvitamin D and 25,26-dihydroxyvitamin D in plasma from normal and nephrectomized man. *J. Lab. Clin. Med.* 93: 277–285.
46. KANIS, J. A. & RUSSEL, R. G. G. (1977) Rate of reversal of hypercalcaemia and hypercalciuria induced by vitamin D and its 1α-hydroxylated derivative. *Br. Med. J.* 1: 78–81.
47. SILVER, J., SHVIL, Y. & FAINARU, M. (1978) Vitamin D transport in an infant with vitamin D toxicity. *Br. Med. J.* 2: 93.
48. QUARTERMAN, J., DALGARNO, A. C., ADAM, A., FELL, B. F. & BOYNE, R. (1964) The distribution of vitamin D between the blood and the liver in the pig, and observations on the pathology of vitamin D toxicity. *Br. J. Nutr.* 18: 65–77.
49. LITTLEDIKE, E. T. & HORST, R. L. (1982) Vitamin D_3 toxicity in dairy cows. *J. Dairy Sci.* 65: 749–759.
50. MORRISSEY, R. L., COHN, R. M., EMPSON, R. N., JR., GREENE, H. L., TAUNTON, O. D. & ZIPORIN, Z. Z. (1977) Relative toxicity and metabolic effects of cholecalciferol and 25-hydroxycholecalciferol in chicks. *J. Nutr.* 107: 1027–1034.
51. RATZKOWSKI, C., FINE, N. & EDELSTEIN, S. (1982) Metabolism of cholecalciferol in vitamin D intoxicated chicks. *Isr. J. Med. Sci.* 18: 695–700.
52. MURRAY, T. M., CIFUENTES, R. F., JONES, G. & RADDE, I. C. (1979) Effects of graded doses of vitamin D_3 on intestinal CaBP, ^{45}Ca-flux, and Ca-ATPase in the young piglet. In: *Vitamin D. Basic Research and Its Clinical Application* (Norman, A. W., Schaefer, K., Herrath, D. V., Grigoleit, H. G., Coburn, J. W., DeLuca, H. F., Mawer, E. B. & Suda, T., eds.), pp. 655–658, de Gruyter, Berlin.
53. COUNTS, S. J., BAYLINK, D. J., SHEN, F., SHERRARD, D. J. & HICKMAN, R. O. (1975) Vitamin D intoxication in an anephric child. *Ann. Intern. Med.* 82: 196–200.
54. HUGHES, M. R., BAYLINK, D. J., JONES, P. G. & HAUSSLER, M. R. (1976) Radioligand receptor assay for 25-hydroxyvitamin D_2/D_3 and 1α,25-dihydroxyvitamin D_2/D_3. Application to hypervitaminosis D. *J. Clin. Invest.* 58: 61–70.
55. HADDAD, J. G. & STAMP, T. C. B. (1974) Circulating 25-hydroxyvitamin D in man. *Am. J. Med.* 57: 57–62.
56. STAMP, T. C. B., HADDAD, J. G. & TWIGG, C. A. (1977) Comparison of oral 25-hydroxycholecalciferol, vitamin D, and ultraviolet light as determinants of circulating 25-hydroxyvitamin D. *Lancet* 1: 1341–1343.
57. BILLS, C. E. (1935) Physiology of the sterols, including vitamin D. *Physiol. Rev.* 15: 1–97.
58. BAILEY, B. E. (1952) Marine oils with special reference to those of Canada. *Fish. Res. Board Can. Bull.* 89: 1–413.
59. MACPHERSON, N. L. (1933) Vitamin A concentration of cod liver oil correlated with age of cod. *Nature (London)* 132: 26–27.
60. PUGSLEY, L. I., MORRELL, C. A. & KELLY, J. T. (1945) A survey of the vitamins A and D potencies of the liver oil of Atlantic cod (*Gadus morrhua* L.). *Can. J. Res.* 23: 243–252.
61. PUGSLEY, L. I. (1939) Vitamin A and D potencies of liver and intestinal oils of halibut (*Hippoglossus hippoglossus*). *J. Fish. Res. Board Can.* 4: 396–404.