Biochem. J. (1986) 233, 535–540 (Printed in Great Britain)

Interrelationships in rats of tissue pools of cholecalciferol and 25-hydroxycholecalciferol formed in u.v. light

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1. Vitamin D-deficient rats were irradiated with u.v. light three times weekly for 30 min for several weeks. D_3 (cholecalciferol) and 25(OH)D_3 (25-hydroxycholecalciferol) concentrations in skin, plasma, muscle and adipose tissue were measured. In other experiments, isolated skin or the whole animal was irradiated once and the cholecalciferol response monitored. 2. Only a small fraction of the 7-dehydrocholesterol in skin is converted into D_3 (< 2%), and the presence of fur decreases the proportion converted into 20% of that occurring in shaved rat skin. 3. D_3 formed in the skin disappears relatively slowly, so that about 90% has gone after 7 days. In normal rats 10 μg of D_3 formed over 2 h irradiation only caused a small rise in plasma D_3 concentration over the following week, indicative of a high rate of clearance from this tissue. 4. Irradiation of vitamin D-deficient rats for a prolonged period raised plasma D_3 and 25(OH)D_3 concentrations to a constant value. 5. D_3, but not 25(OH)D_3, could be found in adipose tissue and muscle. Prolonged irradiation of normal rats showed these tissues and plasma could hold very large amounts of D_3. 6. Pharmacokinetic analysis of the changes in D_3 concentration in rats showed that the disposition kinetics of D_3 was explained by a two-compartment model with half-lives of 13.8 and 7.7 days. The volume of distribution of the more-slowly-turning-over compartment was 500 ml, which presumably reflects the large amounts of D_3 that can accumulate in adipose tissue. 7. Rat skin can synthesize about 0.85 ng of D_3/mJ of u.v. light energy, but it seems that not all this is available to the rat. 8. Adipose-tissue D_3 is available for use by the rat, the $t_{\frac{1}{2}}$ being 12.0 days.

Cutaneous synthesis of D_3 in response to u.v. light is considered to be important in maintaining vitamin D status in man (Haddad & Hahn, 1973; Lawson *et al.*, 1979). Vitamin D status is dependent upon the extent of exposure to sunshine, the latitude and the time of year. There is a highly significant positive correlation between summer plasma 25(OH)D_3 values and those reached in the following winter (Lawson & Davie, 1979). Obviously, in summer, pools of D_3 or one of its active metabolites must be built up that can then be drawn upon during the following winter. However, studies on vitamin D metabolism showed that, within a few days of a dose of vitamin D, tissues contained a very small proportion of the original dose (Cruickshank *et al.*, 1954; Norman & DeLuca, 1963; Kodicek, 1961). It seems that the vast majority of the vitamin is excreted in bile. Plasma is known to have the highest concentrations, even if small, of D_3 or other biologically active metabolites in pigs and rats (Quaterman *et al.*, 1964; Rosenstreich *et al.*, 1971). The presence of D_3 or 25(OH)D_3 has also been reported in rat adipose tissue (Rosenstreich *et al.*, 1971), human adipose tissue and muscle (Mawer *et al.*, 1972) and bovine muscle (Koshy & Van Der Slik, 1977). The conclusion from these studies is that D_3 and 25(OH)D_3 are rapidly metabolized and excreted and that no tissue contains a large store of vitamin D. Nevertheless, even though the concentrations of D_3 and 25(OH)D_3 in adipose tissue and muscle are small, since these tissues can account for a substantial proportion of total body mass, their pools of these substances may be significant in meeting vitamin D needs during periods of deprivation.

There is little information on the size, nature and availability of these tissue pools of vitamin D and its metabolites. We report here the use of an h.p.l.c. system for measuring tissue D_3 and 25(OH)D_3 concentrations and its application to the assessment of cutaneous synthesis of D_3 at various energy levels of u.v. light. We have also measured the resulting changes in the tissue levels of these substances and assessed the availability of adipose-tissue D_3 pools.

METHODS

Animals

Piebald weanling rats of either sex born from females with a low-vitamin D status were fed a vitamin D-deficient diet having a Ca:P ratio of 4:1 from weaning and throughout all experiments. After 2–3 weeks, plasma 25(OH)D_3 was undetectable and rickets had developed, as shown by X-ray examination of the tibia. When vitamin D-replete rats were required, the animals were given cholecalciferol orally in arachis oil (0.25 μg/day).

Abbreviations used: D_3, cholecalciferol ('Vitamin D_3'); 25(OH)D_3, 25-hydroxycholecalciferol ('25-hydroxyvitamin D_3').

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Irradiation

U.v. light was obtained from a Hanovia 7A prescription lamp, details of which have been described previously (Davie & Lawson, 1980). At a distance of 50 cm, as used in these experiments, the power was 380 μW/cm^2 over the wavelength range 250–320 nm. The relative power emitted from the lamp expressed in terms of the most effective wavelength, 295 nm, is 245.3 μW/cm^2, calculated from data of Kobayashi & Yasumura (1973). This value is based on the activity spectrum obtained *in vivo*; other activity spectra obtained *in vivo* have been reported: that of Bunker & Harris (1937) would give a value about 30% lower and that of Knudsen & Benford (1938) a value about 45% lower.

Steroid assays

Cholecalciferol and 7-dehydrocholesterol in skin. The procedure used was essentially as described by Takada *et al.* (1981) and involved h.p.l.c. on a Zorbax-Sil column with 0.5% propan-2-ol in hexane as the eluting solvent.

Assay of D_3 and 25(OH)D_3 in other tissues. Tracer quantities of [1,2-^{3}H]D_3 (20 Ci/mmol; Amersham International, Amersham, Bucks., U.K.) and 25(OH)[26,27-*methyl*-^{3}H]D_3 (20 Ci/mmol; Amersham) were added to a known volume of plasma or weighed quantity of tissue and the whole extracted with chloroform/methanol (2:1,v/v). The chloroform extracts were fractionated on a column (1.0 cm × 60 cm) of Sephadex LH-20 with chloroform/hexane (7:3, v/v) as the mobile phase. The D_3 and 25(OH)D_3 were collected separately and the latter substance determined by h.p.l.c. on a Zorbax-Sil column using 4% (v/v) propan-2-ol in hexane as the eluting solvent. The D_3 fraction was further purified by chromatography on a column (1.0 cm × 50 cm) of Lipidex-5000 (Packard Instrument Co.), elution being with chloroform/hexane (1:7, v/v). The fraction containing the D_3 was further subjected to a h.p.l.c. on a column of Zorbax-Sil with 1% (v/v) propan-2-ol in hexane as eluting solvent. The fraction eluted between 1 min before and 1 min after the elution time of D_3 was collected. The D_3 content was measured on a column of Zorbax-ODS with 1% (v/v) water in methanol as the mobile phase. Typical recoveries of D_3 and 25(OH)D_3 were 80% and 50% respectively. The quantities of D_3 and 25(OH)D_3 were estimated by comparison of peak height given by the sample with that of known standards and corrected for recovery.

Experimental procedures

(*a*) In expt. 1, skin was obtained from a group of 24 vitamin D-deficient rats and shaved as appropriate. The isolated skin (50 cm^2) was irradiated with u.v. light for up to 2 h and the D_3 content estimated on the three samples of skin at each time interval.

(*b*) For expt. 2, shaved dorsal skin (50 cm^2) from a group of vitamin D-deficient rats, half of whom had also been given D_3 orally, were irradiated for 30 min three times per week and the animals killed in groups of three 24 h after the last irradiation.

(*c*) Expt. 3 involved the irradiation of 18 vitamin D-replete rats after a single exposure to u.v. light. The shaved skin (50 cm^2) was irradiated for 2 h and the rats killed in groups of three after 1, 2, 3, 5 and 7 days.

(*d*) In expt. 4, a group of vitamin D-deficient rats was irradiated over 50 cm^2 of dorsal skin for 30 min three times per week. The animals were killed in groups of three 24 h after the last irradiation. The results of this study were used to calculate D_3 synthesis in the skin of these rats from the steady-state equation (Goodman & Gilman, 1975) that describes the relationship between dosage of a substance and plasma concentration achieved at plateau levels, thus:

$$C_p = 1.44 \times F \times t_{\frac{1}{2}} \times D/V_d \times T$$

in which C_p = plasma concentration (μg/ml); F = fractional absorption (assumed to be unity); $t_{\frac{1}{2}}$ = half life; V_d = volume of distribution (ml); D = dosage given (ng) and T = dose interval (days).

(*e*) The protocol for experiment 5 was the same as for expt. 4, except that vitamin D-replete rats were used.

(*f*) Half-lives of D_3: [1,2-^{3}H]D_3 was incubated with plasma from vitamin D-deficient rats for 16 h at 4 °C. A group of three rats was each injected intracardially with 0.2 ml containing about 2 μCi of radioactivity. A sample of blood was obtained at time intervals over the following 20 days. The lipid extract of these plasma samples was prepared, fractioned by chromatography on Sep-Paks (Water Associates) and the radioactive D_3 measured.

All radioactivity was measured with a Packard liquid-scintillation spectrometer (no. 2650). Quenching was corrected for by using automatic external standardization and correlation curves for ^{3}H.

Kinetic data were calculated according to the formulae of Greenblatt & Kock-Weser (1975) and Shipley & Clark (1972) for a two-compartment system in which plasma is

Table 1. D_3 response with time of isolated skin irradiated with u.v. light

Each value is the mean of three determinations. The unirradiated skin (50 cm^2) contained no detectable D_3.

Time (min)	Vitamin D response					
	Front shaved		Back shaved		Back unshaved	
	(ng/cm^2)	(ng/min per cm^2)	(ng/cm^2)	(ng/min per cm^2)	(ng/cm)	(ng/min per cm^2)
15	98.7	6.6	132.0	8.8	24.1	1.6
30	152.4	5.1	159.0	5.3	30.6	1.0
60	261.7	4.4	268.7	4.5	51.2	0.9
120	412.1	3.4	275.8	2.3	95.3	0.8

Table 2. Comparison of the vitamin D and 7-dehydrocholesterol concentration of irradiated isolated skin

Skin (50 cm^2) from the back of the rat was shaved and irradiated for 60 min as described in the Methods section. Each value is the mean of eight determinations ±S.D.

	Vitamin D-replete rats	Vitamin D-deficient rats
Vitamin D		
(ng/cm^2)	266.6±46.0	288.3±38.3
(μg/g)	1.25±0.25	1.37±0.19
7-Dehydrocholesterol		
(μg/cm^2)	22.1±3.7	20.7±2.7
(μg/g)	103.9±18.3	98.0±22.3

assumed to be part of compartment one and adipose tissue part of compartment two. Data were plotted as ln [D_3] on the y-axis and time as a linear function on the x-axis. The two-compartment model is supported if the resulting curves approximate to straight lines.

(*g*) In this expt., a group of vitamin D-deficient rats, all 7 weeks of age, were given 6.2 μg of D_3 daily for 3 weeks. At this stage the mean weight of 21 rats was 154±14 (S.D.) g, and no further vitamin D was given. The animals were killed in groups of three.

RESULTS

Irradiation of isolated skin

The exposure of isolated skin to u.v. light results in an increase in the D_3 content according to the length of exposure. Table 1 records the changes in D_3 concentration of skin taken from either the back or the front of vitamin D-deficient rats after exposure to u.v. light for up to 2 h. It seems that removal of the fur allows the formation of the vitamin to increase about 5-fold. The proportion of 7-dehydrocholesterol converted into D_3 after 1 h exposure to u.v light was about 1%; a proportion unaffected by the vitamin D status of the rats from which the skin was obtained (Table 2). The rate at which D_3 formation occurs appears to decline during the 2 h of irradiation, the mean conversion rate during 2 h of irradiation being about half the mean rate observed after 15 min of u.v. light. This decrease may not be real, however, but may reflect the destruction of D_3 known to occur with prolonged irradiation.

Single u.v. irradiation of vitamin D-replete rats

In one series of observations the shaved backs of a group of normal rats were irradiated with u.v. light for 2 h to produce about 250 ng of D_3 per cm^2 of skin or 10 μg for the total area. D_3 and 25(OH)D_3 levels were measured over the succeeding 7 days (Fig. 1). The latter substance was detected only in the plasma and showed a slow decline over this period (Fig. 1*a*). The D_3 content of the skin fell at a steady exponential rate over the observation period, releasing about 50% of the D_3 in skin after 24 h and over 90% by the end of 1 week (Fig. 1*b*). No tissue examined showed a significant increase in either D_3 or 25(OH)D_3 over the experimental period. Only in plasma was a small increase in D_3 detected over the first 48 h after irradiation (Fig. 1*a*), the levels in adipose tissue and muscle remaining effectively unchanged over the 7-day period (Fig. 1*b*).

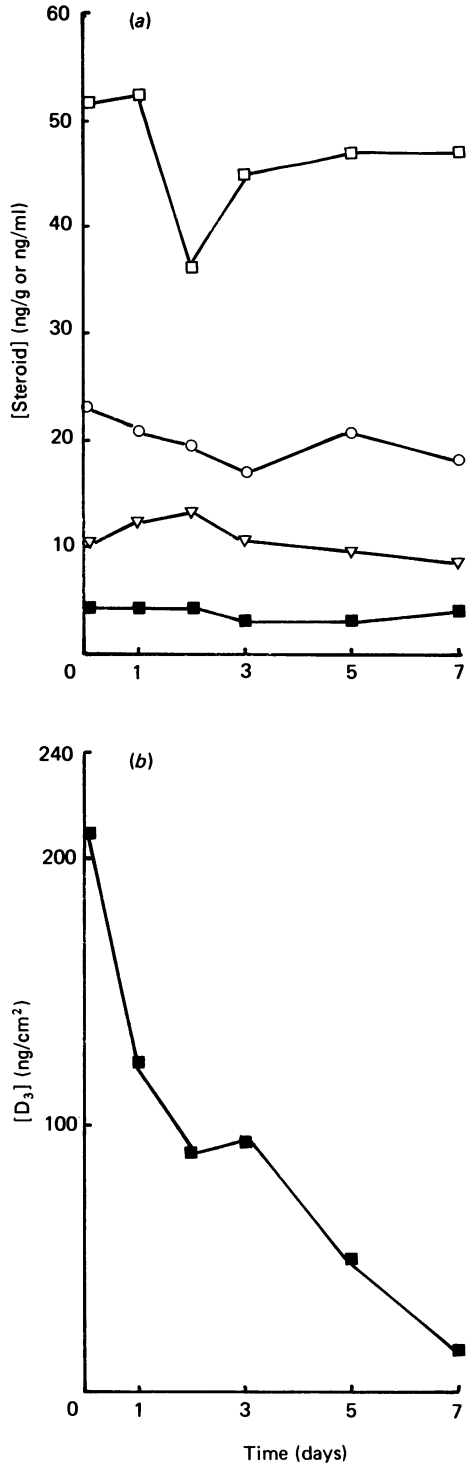

Fig. 1. Changes in the D_3 and 25(OH)D_3 contents of tissues of vitamin D-replete rats after a single exposure to u.v. light

Each point is the mean of three determinations. (*a*) D_3 concentration in adipose tissue (□), plasma (▽) and muscle (■), and 25(OH)D_3 concentration in plasma (○). (*b*) D_3 concentration in skin (■).

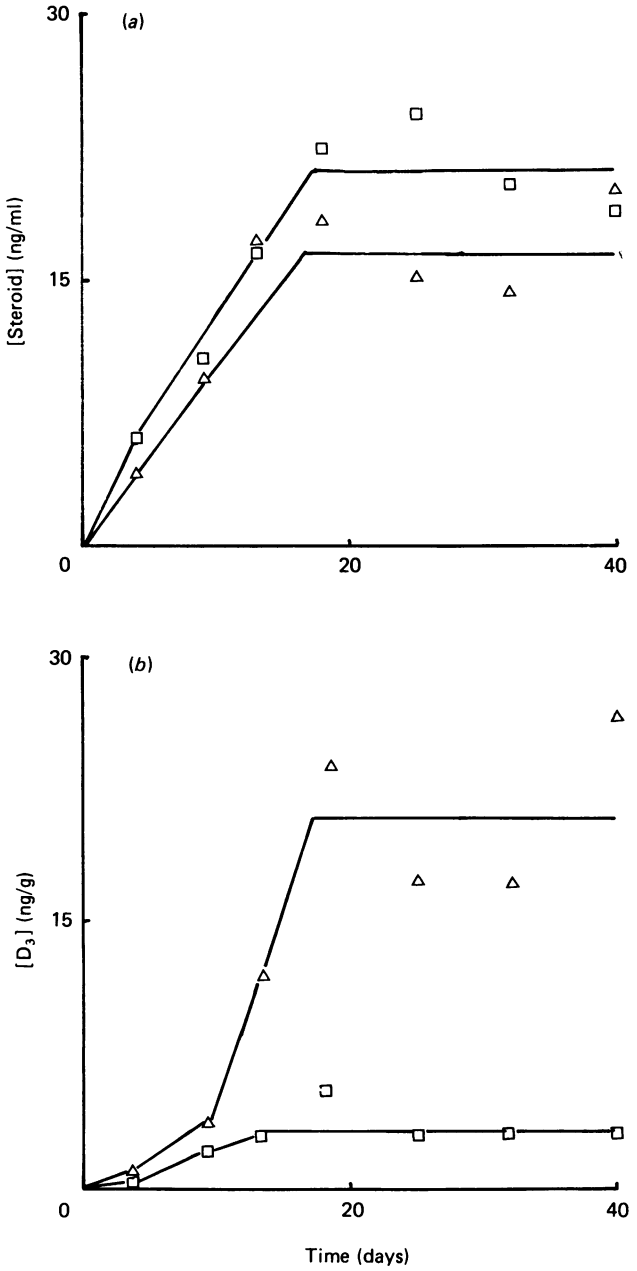

Fig. 2. Changes in D_3 and 25(OH)D_3 concentrations in vitamin D-deficient rats after exposure to u.v. light

Each point is the mean of three determinations. (*a*) Plasma: □, 25(OH)D_3 concn.; △, D_3 concn. (*b*) D_3 concn: △, in adipose tissue; □, in muscle.

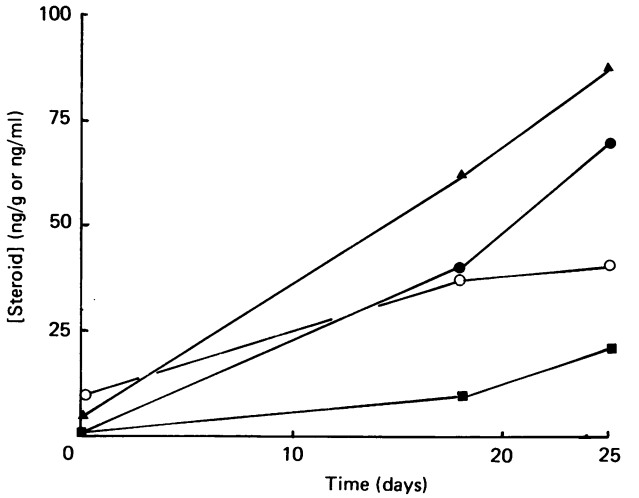

Fig. 3. Changes in the D_3 content of plasma (●), adipose tissue (▲) and muscle (■) and in plasma 25(OH)D_3 (○) concentration of vitamin D-replete rats after irradiation with u.v light

Steroid concentration is given in ng/ml or ng/g as appropriate. Each point is the mean of three determinations.

Prolonged irradiation of vitamin D-deficient rats

The concentration in plasma of vitamin D-deficient rats of both D_3 and 25(OH)D_3 rose soon after beginning the irradiation and reached steady values after about 3 h of exposure over about 2 weeks (Fig. 2). Plasma 25(OH)D_3 values were higher than those of D_3, but the difference was not significant. The tissues, by contrast, only accumulated D_3, since 25(OH)D_3 could not be detected in adipose tissue at any time intervals, and less than 1.5 ng/g was found in muscle on a few occasions. The presence of 25(OH)D_3 in muscle of these rats is probably due to contaminating amounts of plasma remaining in this tissue.

The D_3 concentration in adipose tissue and muscle increased more slowly than in plasma, with less than 1.0 ng/g present after 1 h of exposure over 4 days (Fig. 2). After 2 h exposure, adipose-tissue D_3 concentration had reached about 4.0 ng/g, and subsequent irradiations increased the concentration rapidly to reach similar values as those found in plasma after 3–4 h of u.v light. Muscle D_3 concentration was always very much less than the level found in plasma. D_3 and 25(OH)D_3 was not detectable in the plasma or tissues of these rats before beginning the series of irradiations.

In other experiments vitamin D-replete rats were irradiated over a prolonged period with u.v. light. At the beginning of the experiment the plasma 25(OH)D_3 concentration was about 9.0 ng/ml, but this steroid could not be detected in adipose tissue and muscle. D_3 was not detectable in plasma and muscle of these rats, but small amounts were found in adipose tissue. After exposure to u.v. light the plasma 25(OH)D_3 concentration in the rats rose at about the same rate as in the vitamin D-deficient animals. Again, 25(OH)D_3 was not detected in muscle and adipose tissue. In contrast, a more rapid increase was seen in the D_3 concentration in plasma, muscle and adipose tissue of vitamin D-replete rats compared with the increase seen with vitamin D-deficient rats. Over the time scale of this experiment the D_3 concentrations did not seem to be reaching a steady-state level (Fig. 3).

Cholecalciferol turnover

Fig. 4(*a*) records the concentrations of [^{3}H]cholecalciferol present in the plasma of vitamin D-deficient rats at time intervals after an intracardial dose of this substance. The radioactivity values for the time intervals 12–80 h and 120–385 h were on a straight line, with correlation coefficients of 0.93 and 0.97 respectively, implying that the vitamin is distributed within two

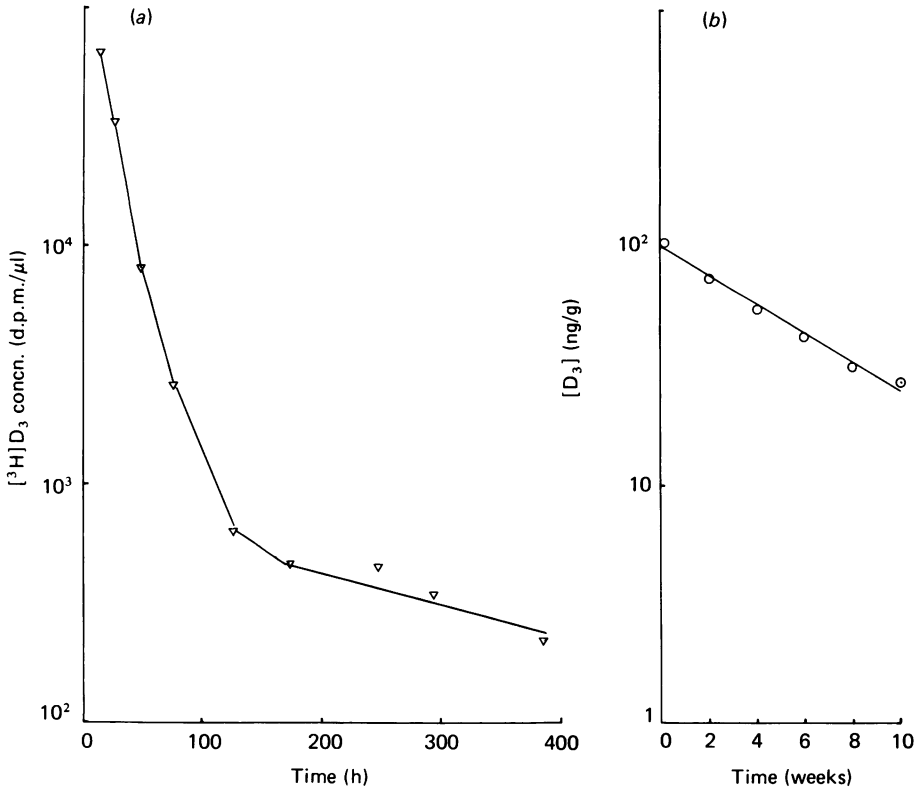

Fig. 4. (*a*) Concentration of D_3 in plasma as a function of time after dosing a group of rats with [^{3}H]D_3 and (*b*) D_3 concentration in adipose tissue of vitamin D-replete rats on a rachitogenic diet with time

Each point is the mean of at least three determinations. Results have been plotted on semilogarithmic paper.

compartments. The half-life of the clearance of cholecalciferol from the first compartment is 13.8 h and from the second one it is 7.75 days. It is the compartment with the slower turnover that governs the rate of utilization of D_3 and, in particular, its capacity. The volume of distribution of compartment two is about 500 ml, which reflects the ability of adipose tissue to accumulate D_3.

Availability of adipose-tissue D_3

The D_3 concentration in adipose tissue of vitamin D-replete rats placed on a vitamin D-deficient diet declined over 10 weeks by an amount that could not be explained by the rat's growth and consequent increase in adipose-tissue weight. For example, after 10 weeks the mean rat body weight had doubled, so that if no D_3 were lost from the tissue, the D_3 concentration would decline by 50%. The actual D_3 concentration at the end of the experiment was 20% of the starting value (Fig. 4*b*). Plotting the data as ln (D_3 concentration) against time showed all the points to fall on a straight line ($r = 0.99$) (Fig. 4*b*). The D_3 in adipose tissue is therefore in a single pool whose half-life, calculated from the slope of the straight line, was 12.0 days.

DISCUSSION

The prolonged irradiation of vitamin D-deficient rats causes a rapid conversion of D_3 into 25(OH)D_3, with further exposure causing D_3 to accumulate first in the plasma and subsequently in adipose tissue and muscle. As in the irradiation of British people (Davie *et al.*, 1982), the rat plasma 25(OH)D_3 levels rose until reaching a steady-state value, but, in contrast with man, D_3 was also found in plasma of these rats at all concentrations of 25(OH)D_3 measured. Irradiation of rats given small amounts of D_3 resulted in its rapid and extensive accumulation in adipose tissue, and once plasma 25(OH)D_3 concentrations had reached their maximum values, the D_3 concentrations in plasma and muscle also began to increase rapidly. It seems that adipose tissue in particular can hold substantial amounts of D_3, i.e. $V_d = 500$ ml, which is about four times the weight of the rats. It is not clear, however, that adipose tissue stores D_3, since in the present study (Fig. 3) and in a previous one using labelled vitamin (Rosenstreich *et al.*, 1971), the concentration in adipose tissue was in general of the same order as in plasma.

D_3 synthesis in the skin calculated from the steady-state equation gave a value of 46.5 ng of D_3/cm^2 or 0.25 ng/mJ. This is significantly below that calculated for the D_3 concentration in isolated skin after 30 min irradiation (Table 1), namely 0.85 ng/mJ. The latter value agrees well with other values for rat skin reported previously (Bekemeier, 1966; Okano *et al.*, 1978; Esvelt *et al.*, 1978), and in any event the actual amount produced in these cases may differ according to the pattern of light emission by the lamp used (MacLaughlin *et al.*, 1982). The explanation for the difference in D_3 synthesis calculated by these two methods probably lies in F, the fractional absorption, not being unity. All D_3 formed in the skin would seem not to be available for utilization or storage by other tissues; this may be due in part to

destruction of the vitamin remaining in the skin at the time of the next irradiation.

The rapid appearance of D_3 in skin after u.v. irradiation first observed by Bekemeier (1966) and confirmed in other reports including the present one, is perhaps not surprising considering that the formation of D_3 is a physico-chemical event. In skin, as in organic solvents, the conversion of 7-dehydrocholesterol into D_3 increases with time and energy, but the proportion converted in skin is much less, in the present studies being $<2\%$. Light of 295 nm produces maximum yield of pre-D_3 (65% of original 7-dehydrocholesterol in both skin and organic solvents), which can be almost quantitatively converted into D_3 (Abillon & Mermet-Bouvier, 1973; Kobayashi & Yasumara, 1973; Havinga *et al.*, 1960). The nature of the products formed from irradiation of 7-dehydrocholesterol depends upon the wavelength of light used and its intensity (MacLaughlin *et al.*, 1982), thereby accounting for the difference between observed D_3 formation and the theoretical value, since the light from the lamp used in the present studies was not optimum for converting 7-dehydrocholesterol into D_3. Even so, in normal rats, as in man, a single exposure to u.v light results in a small rise in D_3 levels in plasma but not in other tissues, and there is no change in plasma 25(OH)D_3 concentration. In these experiments it was shown that 10 μg of D_3 were formed, of which 5 μg were apparently released into the circulation over the first 24 h, which is equivalent to an average input into the circulation of 20 ng of D_3/h per ml of plasma. It is only if this level of input is at least maintained that plasma and tissue D_3 concentrations increase.

The findings reported here have implications for our understanding of factors involved in the maintenance of vitamin D status. The changes seen in response to u.v light are dependent upon the vitamin D status of the animal, which may explain the difference between these results and others recently reported (Adams *et al.*, 1982). In vitamin D-deficient animals any u.v. light exposure results in a rise in plasma 25(OH)D_3 concentration. However, the extent of this rise is ultimately limited, and eventually the rate of 25-hydroxylation is reduced and the vitamin begins to accumulate in adipose tissue and muscle. D_3 has been suggested as a possible regulatory factor of the liver 25-hydroxylase (reviewed by Fraser, 1980). Plasma seems to be the only site at which significant amounts of 25(OH)D_3 are found. In the rat, once the plasma concentration of this latter metabolite rises above about 20 ng/ml, D_3 accumulates in adipose tissue, muscle and plasma. The data in Fig. 5 confirm that adipose-tissue D_3 is available for the rat during periods of u.v. light deprivation, since the decrease in D_3 concentrations was much greater than the increase in adipose-tissue weight. The recovery of D_3 in these tissues was less than 5% of the amount produced by the irradiation, providing another measure of the amounts of D_3 metabolized by liver, probably for excretion.

Received 7 March 1985/29 July 1985; accepted 8 September 1985

We are grateful to Mr. J. Ashford for skilled technical assistance in this investigation. S.H.S thanks the College of Science at King Saud University for a research grant.

REFERENCES

Abillon, E. & Mermet-Bouvier, R. (1973) J. Pharm. Sci. **62**, 1688–1691

Adams, J. S., Clemens, T. L., Parrish, J. A. & Holick, M. F. (1982) N. Engl. J. Med. **306**, 702–707

Bekemeier, H. (1966) Int. Z. Vitaminforsch. **10**, 80–110

Cruickshank, E. M., Kodicek, E. & Armitage, P. (1954) Biochem. J. **58**, 172–175

Bunker, J. W. M. & Harris, R. S. (1937) N. Engl. J. Med. **21**, 165–169

Davie, M. & Lawson, D. E. M. (1980) Clin. Sci. **58**, 235–242

Davie, M. J. W., Lawson, D. E. M., Emberson, C., Barnes, J. L. C., Roberts, G. E. & Barnes, N. D. (1982) Clin. Sci. **63**, 461–472

Esvelt, R. P., Schnoes, H. K. & DeLuca, H. F. (1978)) Arch. Biochem. Biophys. **18**, 282–290

Fraser, D. R. (1980) Physiol. Rev. **60**, 551–610

Goodman, L. S. & Gilman, A. (1975) Pharmacological Basis of Therapeutics, pp. 18–24, Macmillan, New York

Greenblatt, D. J. & Koch-Weser, J. (1975) N. Engl. J. Med. **29**, 702–705

Haddad, J. G. & Hahn, T. J. (1973) Nature (London) **24**, 515–517

Havinga, E., de Kock, R. J. & Rappoldt, M. P. (1960) Tetrahedron **11**, 267–284

Knudsen, A. & Benford, F. (1938) J. Biol. Chem. **124**, 287–298

Kobayashi, T. & Yasumura, M. (1973) J. Nutr. Sci. Vitaminol. **19**, 123–128

Kodicek, E. (1961) in Symposium on Drugs Affecting Lipid Metabolism (Garatini, S. & Paoletti, R., eds.), pp. 515–519, Elsevier, Amsterdam

Koshy, K. T. & Van Der Slik, A. L. (1977) J. Agric. Food Chem. **25**, 1246–1250

Lawson, D. E. M. & Davie, M. (1979) Vitam. Horm. (N.Y.) **39**, 1–68

Lawson, D. E. M., Paul, A. A., Black, A. E., Cole, T. J., Mandal, A. R. & Davie, M. (1979) Br. Med. J. **ii**, 303–305

MacLaughlin, J. A., Anderson, R. R. & Holick, M. F. (1982) Science **216**, 1001–1003

Mawer, E. B., Blackhouse, J., Holman, C. A., Lumb, G. A. & Stanbury, S. W. (1972) Clin. Sci. **43**, 413–431

Norman, A. W. & DeLuca, H. F. (1963) Biochemistry **2**, 1160–1168

Okano, T., Yasumura, M., Mizuno, K. & Kobayashi, T. (1978) J. Nutr. sci. Vitaminol. **24**, 47–56

Quaterman, J., Dalgarno, A. C., Adam, A., Fell, B. F. & Boyne, R. (1964) Br. J. Nutr. **18**, 65–77

Rosenstreich, S. J., Rich, C. & Volwiler, W. (1971) J. Clin. Invest. **50**, 679–687

Shipley, R. H. & Clark, R. E. (1972) Tracer Methods for *in vivo* Kinetics: Theory and Applications, p. 82, Academic Press, New York

Takada, K., Takashima A., Kobayashi, T. & Shimoi, Y. (1981) Biochem. Biophys. Acta **666**, 307–312