

Formation of fatty acid esterified vitamin D₃ in rat skin by exposure to ultraviolet radiation

Katsuji Takada

Laboratory of Hygienic Chemistry, Kobe Women's College of Pharmacy, Higashinada-ku, Kobe, 658, Japan

Abstract The formation of fatty acid esters of vitamin D₃ was demonstrated in rat skin exposed to artificial ultraviolet rays by using multi-dimensional high-performance liquid chromatography, ultraviolet spectrophotometry, and gas-liquid chromatography-mass spectrometry. This result indicated that the fatty acid esters of 7-dehydrocholesterol in rat skin (at least 80% of 7-dehydrocholesterol in rat skin is esterified) is also isomerized into vitamin D₃ ester in vivo. The initial percentage of the esterified form was 84.3% and this did not significantly change up to the time when about half of the skin total vitamin D₃ disappeared (2 days). Consequently, it was speculated that the vitamin D₃ ester was delivered into the blood circulation from skin without having been hydrolyzed. This was supported by the presence of vitamin D₃ ester in rat plasma exposed to ultraviolet radiation. In addition, in connection with the study of the restriction of vitamin D₃ synthesis, distribution of total vitamin D₃ in rat skin exposed to ultraviolet irradiation in vivo was compared with that in isolated skin exposed to ultraviolet radiation. The dermal layer of the isolated skin contained about 4 times more total vitamin D₃ than that of in vivo skin. This finding suggests not only that ultraviolet rays could not penetrate deeply into the in vivo skin, but that the restriction of cutaneous synthesis of vitamin D₃ observed in vivo may arise from this reduced penetration of ultraviolet rays.—**Takada, K.** Formation of fatty acid esterified vitamin D₃ in rat skin by exposure to ultraviolet radiation. *J. Lipid Res.* 1983. **24**: 441-448.

Supplementary key words vitamin D₃ • fatty acid esters • high-performance liquid chromatography • gas-liquid chromatography-mass spectrometry

During the past decade, our understanding of the vitamin D endocrine system has improved enormously. It has been revealed that the major source of the vitamin D₃ is its endogenous photosynthesis in the skin (1-3). The vitamin D₃ generated in the skin is delivered into the blood circulation and metabolized to 25-hydroxyvitamin D₃ in the liver (4) and subsequently to 1,25-dihydroxyvitamin D₃ in the kidney (5) before it can exert its effect on target tissues. The cutaneous synthesis of vitamin D₃ has long been noted in the study of this endocrine system. The skin is not only the site in which vitamin D₃ synthesis occurs but it is also a target tissue of 1,25-dihydroxyvitamin D₃ like other endocrine sys-

tems (6-8). Several investigators have revealed that 7-dehydrocholesterol (7-DHC) in the skin is converted to previtamin D₃ by ultraviolet (UV) radiation (9-11), and that the previtamin D₃ is thermally isomerized into vitamin D₃ in the skin at body temperature (12) in the same manner as demonstrated in organic solvents (13).

7-DHC is a normal metabolic intermediate in the biogenesis of cholesterol (14) and attains a higher concentration in skin than in most other body tissues (15, 16). The 7-DHC content of rat skin is affected by UV radiation (17) and by the action of 1,25-dihydroxyvitamin D₃ (18). The optimum wavelength for the cutaneous synthesis of vitamin D₃ is 305 nm (19), which is longer than that in organic solvents (20), and this affects the yield of by-products, i.e., tachysterol and lumisterol (21). In addition, vitamin D₃ synthesis in rat skin in vivo is restricted to a small extent in order not to induce hypervitaminosis D resulting from excess exposure to UV radiation (19, 22).

As reported in our previous paper (17), the greater part of 7-DHC in rat skin is present as fatty acid esters. The observations reported so far have not taken the esterified form into consideration but have concentrated only on the free form or the total of the free and esterified forms of vitamin D₃. In this report, the formation of esterified vitamin D₃ in rat skin and its delivery from the skin to the blood circulation were investigated. In addition, in the investigation of the factors that restrict the vitamin D₃ synthesis in vivo, the distribution of total vitamin D₃ in rat skin irradiated in vivo and in vitro was examined.

MATERIALS AND METHODS

Chemicals

Crystalline vitamin D₃ was purchased from Philips-Duphar Co. Ltd. (Amsterdam, The Netherlands) and

Abbreviations: 7-DHC, 7-dehydrocholesterol; UV, ultraviolet; HPLC, high-performance liquid chromatography; N₂, nitrogen; GLC-MS, gas-liquid chromatography-mass spectrometry.

was recrystallized from acetone–water 4:1. Crystalline 7-DHC was obtained from Sigma Chemical Co. Ltd. (St. Louis, MO) and was recrystallized from diethyl ether. The palmityl ester of vitamin D₃ was synthesized according to the method described by Swell and Treadwell (23).

All organic solvents used were analytical reagent grade and were redistilled before use. Other reagents were of analytical reagent grade.

Apparatus

Two high-performance liquid chromatography (HPLC) systems were used. The system used for the preparative chromatography of skin or plasma lipid extracts consisted of a Shimadzu-DuPont LC-841 high-performance liquid chromatograph including a UV detector (254 nm), a SIL-1A sample injector (Shimadzu Co. Ltd., Kyoto, Japan) and a Zorbax SIL column (6.2 mm × 25 cm, DuPont Instruments, Wilmington, DE). The other system used for the analysis of vitamin D₃ in unsaponifiable matter obtained from rat skin or plasma consisted of a Shimadzu LC-3A high-performance liquid chromatograph, a Shimadzu UVD-2 fixed wavelength UV detector (254 nm), a SIL-1A sample injector, and a Zorbax SIL column (4.6 mm × 25 cm). In the reversed phase HPLC system used for the purification and identification of vitamin D₃, a Nucleosil 5C₁₈ column (7.5 mm × 30 cm, Nakarai Chem. Co. Ltd., Kyoto, Japan) was used with this latter HPLC system instead of the Zorbax SIL column.

Animals

Male rats of the Wistar strain (8 weeks old, weighing 150–160 g) were used for the isolation and identification of esterified vitamin D₃ and the analyses of the distribution of vitamin D₃ in rat skin. Two vitamin D-deficient rats were used for the isolation and identification of esterified vitamin D₃ in rat plasma according to the method described by Suda, DeLuca, and Tanaka (24). Male weanling rats of the Wistar strain (4 weeks old, weighing 50–60 g) were fed a purified vitamin D-deficient diet (0.47% calcium, 0.3% phosphorus) supplemented with fat-soluble vitamins A, E, and K for 6 weeks; water was provided ad libitum. After 6 weeks, they weighed about 180 g.

Irradiation procedures

In vivo irradiation. The dorsal hair of rats was removed with an electric animal clipper just prior to UV irradiation and the animals were exposed to an erythematous UV lamp (Matsushita Electric Co. Ltd., Osaka, Japan; main wavelength was 280–360 nm, λ max 305 nm) at the distance of 25 cm for 2 hr (radiation: 88 μ W/cm²).

In vitro irradiation. The dorsal skins were removed

from the backs prior to UV irradiation and then placed on a glass plate. The subcutaneous adipose tissue was not removed from the skin. The irradiation conditions were identical with those described above.

Lipid extraction

After the UV exposure, the skin tissues, from which adipose tissue was removed, were cut into small pieces and they were homogenized in 70 ml of a mixture of methanol–chloroform–saturated KCl solution for 90 sec (30 sec × 3; cooling period, 2 min) at 0–4°C. To separate phases, 20 ml of chloroform and 20 ml of saturated KCl were added to the homogenate and the homogenate was centrifuged at 1800 g for 5 min at 4°C. After the removal of the chloroform layer, the aqueous phase was re-extracted with 50 ml of chloroform. The two chloroform phases were filtered through a Whatman 1PS phase-separating paper. The filtrates were combined and dried under a stream of nitrogen gas.

The lipid extract was dissolved in 1.0 ml of n-hexane, and then 100- μ l volumes of the solution were successively chromatographed (preparative HPLC) on a Zorbax SIL column (6.2 mm × 25 cm) to separate the esterified fraction. A solvent system consisting of 1.0% isopropanol in n-hexane was used as a mobile phase and the flow rate was 1.6 ml/min. After discarding the first eluate (5.0 ml), the esterified sterol fraction (5.0–16.7 ml) was collected.

Saponification

The esterified fraction was evaporated to dryness under reduced pressure. The residue was redissolved in 25 ml of ethanol and was saponified by refluxing for 2 hr with a mixture of 10 ml of 20% pyrogallol in ethanol and 4 ml of 90% (w/v) KOH solution. After cooling immediately the unsaponifiable matter was isolated by using 50 ml of benzene according to the method described by Mulder (25). The separated benzene layer was filtered through a Whatman 1PS phase-separating paper to remove water. During this saponification process, the fatty acid esters were hydrolyzed and the previtamin D₃ was isomerized into vitamin D₃ simultaneously (isomerization coefficient: K = 1.25).

Chromatography

The unsaponifiable matter obtained from the esterified fraction was dissolved in 500 μ l of n-hexane and 100- μ l volumes were applied to a Zorbax SIL column (4.6 mm × 25 cm) by using 0.5% isopropanol in n-hexane as an eluent. The flow rate was 1.5 ml/min at a pressure of 60–80 kg/cm². As Fig. 1A shows, the vitamin D₃ peak, which is considered to result from the esterified previtamin D₃ and vitamin D₃, was detected. The eluates corresponding to the vitamin D₃ peak were

collected. After drying the solvent the residue was re-dissolved in methanol and the solution was applied to a Nucleosil 5C₁₈ column (7.5 mm × 30 cm) with a flow rate of 1.7 ml/min of methanol–isopropanol–water 4:2:1. As Fig. 1B shows, a single peak corresponding to vitamin D₃ was clearly separated from other peaks (unknown material). The vitamin D₃ fraction was collected and further purified by chromatography on a Zorbax SIL column (4.6 mm × 25 cm). The finally purified vitamin D₃ fraction was analyzed by UV spectrophotometry and gas–liquid chromatography–mass spectrometry (GLC–MS).

Mass spectrometry

GLC–MS was carried out with a Model JMS D300 combined with a Model JMA-2000 data processing system (Japan Electric Optics Laboratory Co. Ltd., Tokyo, Japan). The GLC was performed on a 1.5% OV-17 column (Gaschrom Q, 80–100 mesh, 2.0 mm × 1.0 m). Helium carrier gas flow rate was 20 ml/min and the column temperature was 260°C. After the sample had been run through the GLC–MS, the mass spectra (mass range: 50–400 m/e, scan interval: 6 sec) of all components' peaks were on file. Mass chromatograms were illustrated by plotting the ion intensity of characteristic fragment ions in the mass spectra of pyrovitamin D₃ and isopyrovitamin D₃ against retention time.

Separative assay of three vitamin D₃ forms in skin

Each dorsal skin sample (1.5 g) was cut into small pieces on dry ice, and was homogenized in 14 ml of methanol–chloroform–saturated KCl 2:1:0.5. To separate phases, 4 ml of chloroform and 4 ml of saturated KCl were added to the homogenate, and it was centrifuged at 1800 g for 5 min. After the removal of the chloroform layer, the aqueous phase was re-extracted with 10 ml of chloroform. The two chloroform phases were filtered through a Whatman 1PS phase-separating paper and the filtrates were combined and dried under a stream of N₂ gas.

The lipid extract was dissolved in 250 μl of n-hexane, and then a 100-μl volume of the solution was chromatographed (HPLC) on a Zorbax SIL column (6.2 mm × 25 cm) with a flow rate of 1.6 ml/min of 1.0% isopropanol in n-hexane at a pressure of 60 kg/cm². The esterified potential vitamin D₃ fraction (5.0–16.7 ml), the free previtamin D₃ fraction (16.7–25.0 ml), and the free vitamin D₃ fraction (25.0–37.5 ml) were fractionated and then saponified. The unsaponifiable matter was analyzed by straight-phase HPLC on a Zorbax SIL column (4.6 mm × 25 cm) with a flow rate of 1.5 ml/min of 0.5% isopropanol in n-hexane. Peak identification was determined by reversed-phase HPLC and GLC–MS. The overall recovery by this assay method

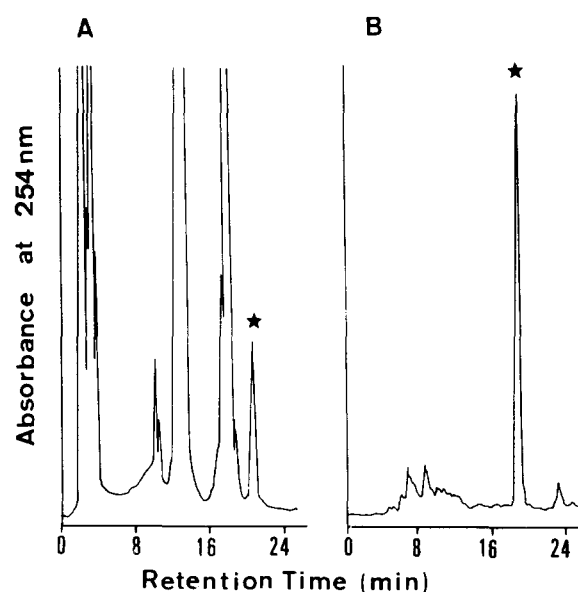


Fig. 1. A: Straight-phase HPLC-chromatogram of the unsaponifiable matter obtained from esterified vitamin D₃ fraction of UV-exposed rat skin. The unsaponifiable matter obtained from esterified sterol fraction was applied to a straight-phase HPLC on a Zorbax SIL column (4.6 mm × 25 cm) with a flow rate of 1.5 ml/min of 0.5% isopropanol in n-hexane. B: Rechromatography of the vitamin D₃ fraction eluted from the preceding straight-phase column on a reversed-phase Nucleosil 5C₁₈ column. The collected eluate corresponding to the vitamin D₃ peak in the preceding straight-phase HPLC was dried under a stream of N₂ gas. The residue was redissolved in methanol and then applied to a reversed-phase HPLC Nucleosil 5C₁₈ column (7.5 mm × 30 cm) with a flow rate of 1.7 ml/min (methanol–isopropanol–water 4:2:1).

calculated by the addition of authentic vitamin D₃ palmitate and vitamin D₃ was 80.1 ± 5.6% and 83.6 ± 4.5% (mean ± S.D., n = 6), respectively.

Identification of plasma esterified vitamin D₃

Two vitamin D-deficient rats were exposed daily to an erythematous UV lamp (at the distance of 50 cm, for 1 hr) for 3 weeks. The rats were maintained on vitamin D-deficient diet throughout the UV exposure period so that the only source of vitamin D₃ was photo-generated vitamin D₃. Lipid was extracted from 8 ml of plasma by the method of Bligh and Dyer (26). The lipid extract was treated by the two-step HPLC in the same manner as the skin lipid extracts in order to identify the vitamin D₃ resulting from esterified vitamin D₃. For further identification, reversed-phase HPLC was carried out on a Nucleosil 5C₁₈ column (7.5 mm × 30 cm) with a flow rate of 1.7 ml/min of methanol–isopropanol–water 4:2:1.

Distribution of vitamin D₃ in rat skin

Rat skin is composed of three main layers, i.e., keratin layer, epidermal layer, and dermal layer. Distribution of total vitamin D₃ in the skin was estimated by remov-

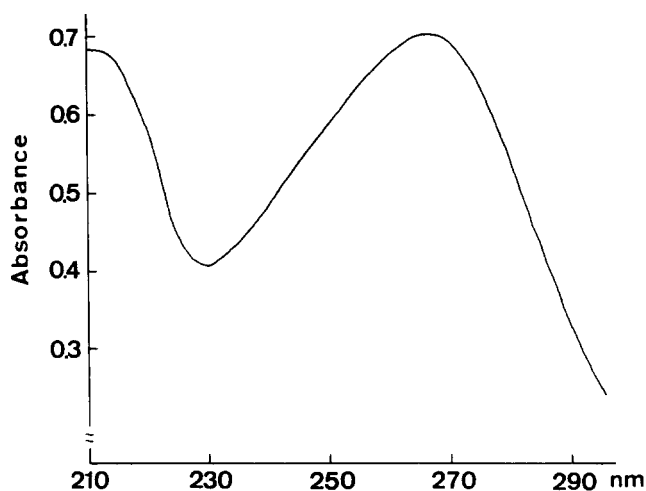


Fig. 2. UV spectrum of the purified vitamin D₃ fraction obtained from esterified vitamin D₃ fraction.

ing the external layers of the skin in order. The keratin layer was removed by shaving. The epidermal layer was removed according to the method described by Baumberger, Suntzeff, and Cowdry (27). The amounts of total vitamin D₃ in the skin were assayed by HPLC as already reported in our previous paper (20). Areas (6 cm²) of rat skin that were exposed to UV irradiation *in vivo* or *in vitro* were treated as described above. The skin preparations were cut into small pieces and directly saponified. The unsaponifiable matter was analyzed by HPLC on a Zorbax SIL column (4.6 mm × 25 cm) with a flow rate of 1.5 ml/min of 0.5% isopropanol in n-

hexane. The amounts of vitamin D₃ were calculated by correcting the peak height of vitamin D₃ with the equilibrium coefficient between previtamin D₃ and vitamin D₃ ($k = 1.25$). This corrected value, therefore, gave the total vitamin D₃ (sum of free and esterified vitamin D₃).

RESULTS

Identification of esterified vitamin D₃ in rat skin

As shown in Fig. 1, the unsaponifiable matter obtained from the esterified fraction of rat skin lipids contained a substance which had the same chromatographic properties in both straight-phase and reversed-phase HPLC as those of authentic vitamin D₃. The peaks are indicated by asterisks in the chromatograms. The retention times were 22.4 min and 20.3 min, respectively. For further identification of this substance as vitamin D₃, which was generated from esterified previtamin D₃ or vitamin D₃ by saponification, the eluate corresponding to the vitamin D₃ peak in the final straight-phase HPLC was collected and then subjected to UV spectrophotometry and GLC-MS.

The UV absorption spectrum shown in Fig. 2 clearly revealed the presence of characteristic 5,6-*cis* triene chromophore as vitamin D₃ (λ max at 265 nm, λ min at 228 nm). Vitamin D₃ is known to be isomerized into two cyclized products, i.e., pyrovitamin D₃ and isopyrovitamin D₃ at a temperature above 150°C. Consequently, vitamin D₃ was identified by demonstrating the formation of the two characteristic thermoisomers. Fig.

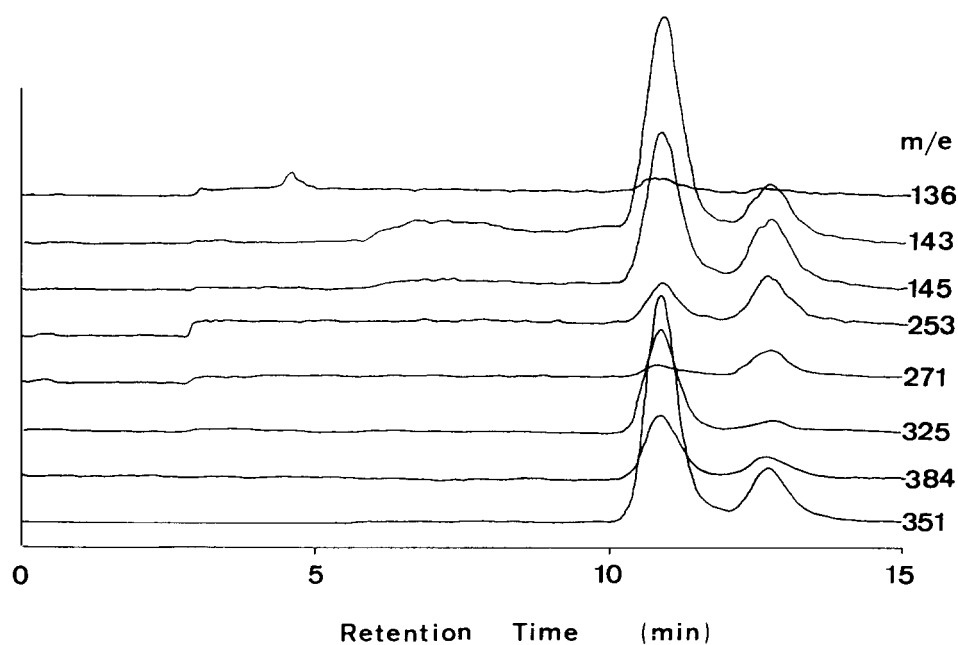


Fig. 3. Mass chromatogram of the purified vitamin D₃ fraction obtained from esterified vitamin D₃ fraction.

TABLE 1. Changes in the total vitamin D₃ and the percentage of three vitamin D₃ forms in the skin after UV exposure

Days after UV Exposure	Total vitamin D ₃	Esterified Potential Vitamin D ₃	Previtamin D ₃	Vitamin D ₃
	μg/g		%	
0	1.56 ± 0.12	84.3 ± 5.8	11.2 ± 4.3	4.6 ± 3.5
1	1.33 ± 0.15	84.7 ± 1.5	5.9 ± 2.3	9.4 ± 2.5
2	0.82 ± 0.06	82.4 ± 5.2	3.2 ± 1.8	14.5 ± 3.7
3	0.49 ± 0.05	75.6 ± 4.0	5.7 ± 4.6	18.7 ± 0.8

All values are the means of four rats ± S.D.

3 shows the mass chromatogram made by plotting the ion intensities of the molecular ion *m/e* 384 and fragment ions (*m/e* 366, 351, 325, 271, 145, 143) against retention time. Two peaks corresponding to pyrovitamin D₃ and isopyrovitamin D₃ were observed at the retention times of 10.8 min and 12.7 min, respectively. The relative ion intensities of the plotted ions were identical with those of authentic pyrovitamin D₃ and isopyrovitamin D₃.

Translocation of esterified vitamin D₃ from skin to blood circulation

Table 1 shows the changes in the contents of total vitamin D₃, esterified potential vitamin D₃, free previtamin D₃, and free vitamin D₃ in the skin up to 3 days after UV irradiation. There was no significant change in the relative amount of esterified potential vitamin D₃ by 2 days, despite the disappearance of about half amount of the initial content of skin total vitamin D₃. This fact suggests that almost all of the esterified form of vitamin D₃ is not hydrolyzed prior to delivery from the skin to the blood circulation. In the period from 2 days to 3 days after UV irradiation, however, a slight decrease in the percentage of the esterified form to total vitamin D₃ was observed ($0.025 < P < 0.05$; by Student's *t*-test). This slightly reduced amount of the esterified form may represent the difference in the delivery rate from the skin to blood circulation between the esterified form and free form, or of the hydrolysis of the ester linkage. Even so, the difference in delivery rate or the extent of the hydrolysis would be small.

As already described above, the esterified vitamin D₃ may be translocated to the blood circulation without cleavage of the ester linkage. Therefore, the presence of the translocated esterified vitamin D₃ in UV-exposed rat plasma was investigated 7 days after UV irradiation. However, the amount of total vitamin D₃ generated by a single UV exposure was so small that the esterified vitamin D₃ was not detected in that plasma. Accordingly, vitamin D-deficient rats were exposed to UV radiation for 3 weeks and then the plasma was analyzed by HPLC as described under Materials and Methods. The rate of esterified potential vitamin D₃ to total vi-

tamin D₃ in the skin at the end of this UV exposure treatment was $72.0 \pm 3.9\%$ (mean ± S.D., *n* = 4) of that in the skin after UV exposure treatment. It is possible to regard the vitamin D-deficient rats as being repleted mainly with esterified vitamin D₃ for 3 weeks. As Fig. 4(a) shows, a peak corresponding to vitamin D₃ was observed in the straight-phase HPLC chromatogram of the esterified fraction. The peak was collected and rechromatographed on a reversed-phase HPLC. A peak that comigrated with authentic vitamin D₃ was observed (Fig. 4(b)). Since the plasma obtained from the rat supplemented with vitamin D₃ orally for 2 weeks did not exhibit the presence of the esterified vitamin D₃, the esterified vitamin D₃ in UV-exposed rat plasma was considered to be translocated from the skin.

Distribution of total vitamin D₃ in rat skin

In our previous paper (19), the cutaneous synthesis of vitamin D₃ in rat skin *in vivo* was revealed to be restricted within a small range. On the other hand, the synthesis of vitamin D₃ in the isolated rat skin was not restricted. For further investigation of this phenome-

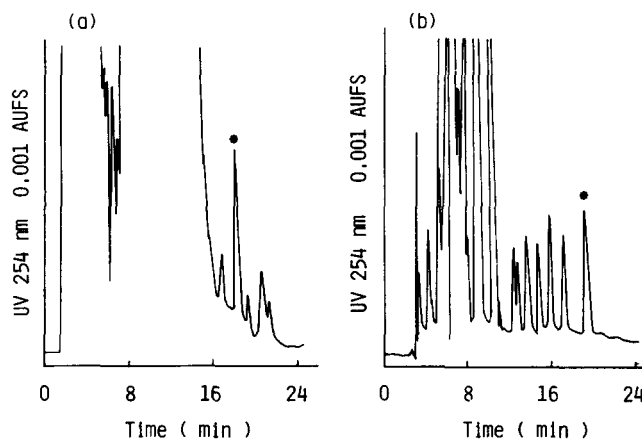


Fig. 4. Chromatographic evidence for the presence of esterified vitamin D₃ in UV-exposed rat plasma. The lipid extract obtained from UV-exposed rat plasma was subjected to two-step HPLC as described in Materials and Methods. (a), Straight-phase HPLC of the unsaponifiable matter obtained from esterified vitamin D₃ fraction. (b), Reversed-phase HPLC of the eluate corresponding to the peak of vitamin D₃ in the straight-phase HPLC.

TABLE 2. Distribution of total vitamin D₃ produced by UV exposure in rat skin in vivo and in isolated skin

	Whole Skin	Shaved Skin	Dermal Layer	Dermal Layer
				Whole Skin
	ng/cm ²			%
In vivo skin	148 ± 29	128 ± 45	12 ± 9	7.9
Isolated skin	375 ± 154	305 ± 154	96 ± 27	25.3

All values are the means of five specimens ± S.D.

non, the distribution of vitamin D₃ produced in the skin in vivo and in isolated skin was analyzed. **Table 2** shows the contents of total vitamin D₃ in the three skin fractions. The shaved skin represents the fraction in which the external keratin layer was removed. The percentages of vitamin D₃ present beneath the keratin layer (epidermal layer plus dermal layer) in both skin samples were not different. However, the percentage in the dermal layer was significantly higher in the isolated skin than in the skin in vivo. These facts indicate that the exposed UV rays penetrate more deeply into the isolated skin than into the skin in vivo.

DISCUSSION

It was reported that the 7-DHC in rat skin is present in two forms different from that in human skin (28). The major form is the fatty acid-esterified form (78–92%) and the minor form is free 7-DHC (17). In the present study, the fraction containing esterified previtamin D₃ and vitamin D₃ was isolated from the UV-exposed rat skin by preparative HPLC and was then saponified. The resulting free vitamin D₃ in the unsaponifiable fraction was isolated and identified by HPLC, UV spectrophotometry, and GLC-MS.

Analyses of three vitamin D₃ forms by a two-step HPLC system revealed that at least 80% of total vitamin D₃ is present in the esterified form and this was not significantly changed after UV exposure up to the time when about half the amount of the skin total vitamin D₃ disappeared. Hence, almost all of the esterified form of vitamin D₃ was considered to be delivered into the blood circulation from skin without ester hydrolysis. This speculation was supported by the chromatographic evidence for the presence of esterified vitamin D₃ in long-term UV-exposed rat plasma.

As already clarified by Holick et al. (12), the initial photolytic product of free 7-DHC is previtamin D₃ and the isomerization of previtamin D₃ to vitamin D₃ occurs in the skin. The proportion of free previtamin D₃ gradually decreased by 2 days and then plateaued. The initial percentage of vitamin D₃ was 29.3% and this increased to 61.6% in 1 day, and by day 2 and thereafter was

78.3–83.0% of vitamin D₃. This rate of formation of vitamin D₃ at an equilibrium state is identical with the reaction in organic solvents. Since the HPLC method used in this study could not separate the esterified previtamin D₃ from esterified vitamin D₃, it remains uncertain as to whether the initial photolytic product of esterified 7-DHC is esterified previtamin D₃ or not. In organic solvents, however, the photo-reaction of the esterified 7-DHC is identical with that of free 7-DHC. Therefore, this concept might apply to the case in rat skin.

It is known that a small quantity of vitamin D₃ ester is formed during intestinal absorption (29, 30) and it has been detected in the liver, kidney, and adipose tissue (31, 32). It was suggested that esterified vitamin D₃ might be a storage form or a protection form for excessive vitamin D₃. Although the role of esterified vitamin D₃ has not been clarified in detail (33), it must be noted that the major form of vitamin D₃, generated in rat skin, was in the esterified form and the esterified vitamin D₃ may be translocated to blood circulation. Daily UV exposures of vitamin D-deficient rats for 3 weeks raised the plasma 25-hydroxyvitamin D₃ (only the free form) level from a value below the detection limit (1 ng/ml) to a normal level (14.3 ng/ml). The esterified vitamin D₃ must be hydrolyzed in the blood or liver and metabolized to 25-hydroxyvitamin D₃ in the liver. In fact, the daily UV-exposed rat plasma contained free vitamin D₃ as well as esterified vitamin D₃ in about equal amounts.

It has been reported that there are significant differences with respect to the cutaneous synthesis of vitamin D₃ between the skin in vivo and in isolated skin. Our previous experiments had indicated that there was restriction of vitamin D₃ synthesis in rat skin in vivo after repeated UV exposure as well as after a single UV exposure. As shown in Table 1, this restriction may be explained by the reduced penetration of UV rays into the skin in vivo. This result revealed that UV rays could not penetrate deeply into the skin in vivo in comparison with the isolated skin. Although the cause for this reduced penetration of UV rays into the skin in vivo has been unresolved, this phenomenon may relate to the

systematic protective response of skin to UV rays. As a result, the 7-DHC available for the synthesis of vitamin D₃ in vivo is restricted within upper layer of epidermis. This speculation is supported by the fact that the increase in 7-DHC content in rat skin induced by UV irradiation did not affect the restriction level of vitamin D₃ synthesis (21). Newly synthesized 7-DHC under the influence of UV rays is supposed to be present within an unavailable site for the photochemical reaction by UV rays.

Although further investigations are needed concerning the penetration of UV rays into rat skin in vivo, our assumption of the mechanism of vitamin D₃ synthesis in rat skin in vivo can be summarized as follows. 1) Available 7-DHC for the synthesis of vitamin D₃ is restricted within the upper layer of epidermis. Consequently, the synthetic reaction of previtamin D₃ reaches an equilibrium state at the time when about 1–2% of total 7-DHC present in whole skin is isomerized into previtamin D₃. 2) This equilibrium state breaks with the thermal isomerization of previtamin D₃ to vitamin D₃, and then new synthesis of previtamin D₃ from 7-DHC, tachysterol, and lumisterol occurs under the influence of repeated UV exposures.

Recently, Adams et al. (33) reported that vitamin D₃ synthesis in human skin in vivo occurs in proportion to the energy of UV exposure and claimed that there was no mechanism for the restriction of vitamin D₃ synthesis in human skin except for the extent of pigmentation. Although the issue of legitimacy of extrapolating from animal studies to humans is present, their observations in human skin correspond to the range below a restriction level in the case of rat skin. Further investigations for resolving the reduced penetration of UV rays in rat skin in vivo are in progress. ■

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