PHOTOCHEMICAL CONVERSION OF 7-DEHYDRO-
CHOLESTEROL INTO VITAMIN D₃ IN RAT SKINSToshio OKANO, Mitsue YASUMURA, Kumiko MIZUNO,
and Tadashi KOBAYASHI¹*Department of Hygienic Chemistry, Kobe Women's College of
Pharmacy, Higashinada-ku, Kobe 658, Japan*

(Received February 5, 1977)

It is well-known that there is an intimate relationship between the prevention of rickets and sunlight irradiation on the skin of human beings and animals. A comparatively large number of patients with rickets have been found in areas having insufficient sunshine, such as the Tohoku area in Japan (1). On the other hand, the photochemical conversion mechanism of provitamin D into vitamin D via previtamin D by ultraviolet (UV) irradiation has been almost completely clarified (2). By combining the two ideas, it is believed that provitamin D (mainly 7-dehydrocholesterol) existing in the skin may be photochemically converted into vitamin D by UV rays in sunlight (Dorno rays) and the converted vitamin D may be utilized to prevent rickets. However, these are only speculations and the *in vivo* conversion of provitamin D into vitamin D has never been directly confirmed because the contents of vitamin D in tissues are generally too small to be detected and determined.

In order to solve this problem and to confirm the mechanism, we established a new method for the determination of 7-dehydrocholesterol (7-DHC), previtamin D₃ (pre-D₃) and vitamin D₃ in rat skin. This method includes hydroxyalkoxypropyl (HAP) Sephadex column chromatography and high-pressure liquid chromatography (HPLC) as summarized in the following. Lipids are extracted from small pieces of rat skin with chloroform-methanol (1:1) and the solvent is evaporated under reduced pressure. After refluxing the lipid extracts with ethanolic potassium hydroxide solution for saponification, the unsaponifiable matters are extracted with benzene. The unsaponifiable matters are applied to a HAP Sephadex column prepared according to ELLINGBOE *et al.* (3) [column size, 45 cm × 2.1 cm *i.d.*; mobile phase, *n*-hexane-chloroform (65:35)]. The column chromatogram of the authentic 7-DHC, pre-D₃ and vitamin D₃ gave the two peaks corresponding to vitamin D₃ including pre-D₃ and 7-DHC. Each of the two fractions is individually applied to the HPLC according to TSUKIDA *et al.* (4) [apparatus, Shimadzu-DuPont 841 Liquid Chromatograph equipped with an UV detector (254 nm); column,

¹ 岡野登志夫, 康村満枝, 水野久美子, 小林 正

stainless tube packed with Zorbax Sil (25 cm $\times$ 2.1 mm *i.d.*); pressure, 100 kg/cm²; mobile phase, 0.16% methanol and 2% ethyl ether in pentane; internal standard, α -naphthol]. The contents of 7-DHC, pre-D₃ and vitamin D₃ were estimated by comparing each peak height ratio to the internal standard with that of the corresponding standard. The retention times of α -naphthol, pre-D₃, vitamin D₃ and 7-DHC were 8.04, 11.5, 18.25 and 23.12 min, respectively.

When the compounds in the skin of normal rats [rats of Wistar strain (weighing 100–120 g) fed a normal diet (MF diet of the Oriental Yeast Co.) containing 200 I.U. of vitamin D₃/100 g] were estimated according to the proposed method, the average value of 7-DHC was 84.5 μ g/g while neither pre-D₃ nor vitamin D₃ could be detected. Then, the following two irradiation experiments were carried out.

First experiment (in vitro experiment). The normal rats whose hair was shaved with an electric shaver were sacrificed and then about 10 cm² area of the skin was immediately removed from the back of each. After removing the fat tissue from the skin, it was fixed onto filter paper placed on a table at 4°C. The epidermis of the fixed skin was irradiated from a distance of 25 cm with an UV lamp (National Erythral lamp, Matsushita Electric Co.; main wavelength, 280–310 nm; quantum of irradiated energy, 85 μ W/cm²/min) for 30, 60 and 120 min. The irradiated skin was immediately cut into small pieces and then treated according to the proposed method. The estimated values of vitamin D₃ are shown in Table 1 and the HPLC chromatogram of the irradiated skin (120 min) is shown in no. 2 of Fig. 1. The mono peak whose retention time agreed with that of vitamin D₃ was observed besides the peak of the internal standard. Co-chromatography performed by mixing the sample solution with standard vitamin D₃ also gave a mono peak as shown in no. 3 of Fig. 1. From these results and the fact that the estimated values increased according to the increase of irradiation times as shown in Table 1, it was concluded that the observed mono peak on the HPLC chromatogram was due to vitamin D₃ which was photochemically formed from the 7-DHC in the rat skin.

Second experiment (in vivo experiment). The normal rats whose hair was shaved with an electric shaver were put in small cages (A rat was put in a cage) and irradiated from the right upper side (distance, 25 cm) with the same UV lamp mentioned above under the same conditions as described in the first experiment. The rats were immediately sacrificed and then about 10 cm² area of the skin was removed from the back of each. After removing the fat tissue from the skin, it was treated according to the proposed method. The estimated values of vitamin D₃ are shown in Table 1 and the HPLC chromatogram and co-chromatogram obtained from the rat (irradiated 120 min) are shown in nos. 4 and 5 of Fig. 1, respectively. The results were almost identical with the corresponding results of the first experiment and the mono peak observed in no. 4 of Fig. 1 was confirmed as that of vitamin D₃.

Table 1. Yield of vitamin D₃ in rat skin in the first (*in vitro*) and second (*in vivo*) experiments.

Irradiation time (min)	Yield of vitamin D ₃ (ng/g of skin)	
	1st experiment (<i>in vitro</i>)	2nd experiment (<i>in vivo</i>)
0	n.d.	n.d.
30	3.7	4.1
60	7.4	8.2
120	12.5	14.7

Note 1) Values are shown as the mean of duplicate estimations.

2) n.d., not detected (<2 ng/g of skin).

Fig. 1. HPLC chromatograms and co-chromatograms of the vitamin D₃ fractions obtained from the HAP Sephadex column chromatography on the rat skin samples of the first (*in vitro*) and second (*in vivo*) experiments. N: α -naphthol, D₃: vitamin D₃.

It was concluded from the results of the two experiments that the 7-DHC in rat skin was photochemically converted into vitamin D₃ by UV irradiation. It was also deduced that the *in vivo* conversion mechanism might be similar to the *in vitro* one because there were little differences between the corresponding estimated values of vitamin D₃ in the two experiments. The peak due to pre-D₃ was not observed in the HPLC chromatograms of the two experiments. It is well-known that 7-DHC first gives pre-D₃ by UV irradiation and the produced compound is thermally converted into vitamin D₃ (2). Since the presently proposed method included the saponification procedure with refluxing to eliminate interfering substances, the produced pre-D₃ might be thermally converted into vitamin D₃ during the procedure and the mono peak of vitamin D₃ alone was observed in each HPLC chromatogram.

Detailed reports will appear in future. The results presented in this communication was reported at the 164th meeting of the Japan Fat-Soluble Vitamins Research Committee held in Tokyo (1976. 11. 19.).

The authors wish to thank Prof. Dr. N. Hosoya and Dr. S. Moriuchi of the School of Health Sciences, Faculty of Medicine, University of Tokyo, for their helpful advice and discussions.

REFERENCES

- 1) SANO, T. (1966): Clinical investigations on vitamin D. *Vitamins*, **33**, 251-254.
- 2) HAVINGA, E. (1973): Vitamin D, example and challenge. *Experientia*, **29**, 1181-1193.
- 3) ELLINGBOE, J., NYSTRÖM, E., and SJÖVALL, J. (1970): Liquid-gel chromatography on lipophilic-hydrophobic Sephadex derivatives. *J. Lipid Res.*, **11**, 266-273.
- 4) TSUKIDA, K., KODAMA, A., and SAIKI, K. (1976): Simultaneous determination of eight Vitamin D₂ isomers by high-speed liquid chromatography. *J. Nutr. Sci. Vitaminol.*, **22**, 15-20.