J. Korean Soc. Food Nutr.
19(6), 583~590(1990)

한국영양식량학회지

Isolation of Free and Esterified Forms of Provitamin D in Rat Skin

Jung-In Kim

Dept. of Food and Nutrition, Inje University, Kimhae, 621-749, Korea

Abstract

Free and esterified forms of provitamin D in 30-day-old male rat skin were isolated and quantitated using silicic acid column chromatography and high-performance liquid chromatography(HPLC) systems. Two forms of free provitamin D(cholesta-5, 7-dien-3β-ol and cholesta-5, 7, 24-trien-3β-ol) and at least thirteen esterified forms of provitamin D were isolated. The average total concentration of provitamin D in the whole skin was 6,056ng/㎠. The skin contained 846 ng/㎠of free provitamin D and 5,209ng/㎠of esterified provitamin D. The proportion of esterified provitamin D in the skin was 86%

Key words : provitamin D · 7-dehydrocholesterol · skin · rat

Introduction

7-dehydrocholesterol(cholesta-5, 7-dien-3β-ol) or provitamin D is essential in synthesis of cutaneous vitamin D[1-5].

In rat, pig, chicken and human skin, 7-dehydrocholesterol has been isolated, localized and quantitated[6-10]. However, all of these studies have focused on the free form of 7-dehydrocholesterol and not the esterified forms of this compound.

Little is known about the quantities of 7-dehydrocholesterol in esterified and free form in the skin. 7-dehydrocholesteryl acetate, isobutyrate and palmitate achieved antirachitic potency after ultraviolet irradiation, suggesting that esters of 7-dehydrocholesterol can be biologically active[11-13]. 7-dehydrocholesterol is a sterol which has double bonds at C_5 and C_7. It is known that esterification of sterols increases during epidermal keratinization[14-16]. It is hypothesized that this process may be responsible for salvaging free fatty acids liberated by the degradation of membrane phospholipids during epidermal differentiation[17]. An enzyme in rat skin has been shown to be active in esterification of sterol intermediates of cholesterol biosynthesis[18]. Furthermore, an intermediate of cholesterol biosynthesis, $\triangle^7$-cholestenol, is mostly esterified in rodent skin[8,19]. The above-mentioned studies have suggested that 7-dehydrocholesterol in rat skin may be esterified. Moore and Baumann [20] reported that rat skin has fast-acting sterols which react with the Schoenheimer-Sperry reagents more rapidly than cholesterol. Only a small proportion of the fast-acting sterols, including $\triangle^7$-cholestenol and 7-dehydrocholesterol were present in free form. Takada et al.[21] reported that 78~93 % of 7-dehydrocholesterol in adult male rat skin was esterified. However, they used indirect methods to quantitate 7-dehydrocholesteryl esters. The concentration of esterified 7-dehydrocholesterol was determined by measuring the content of 7-dehydrocholesterol after saponification. No investigations have been performed to isolate and quantitate esterified forms of provitamin D or to determine how many esterified forms are present in the skin. Therefore, this study was carried out to directly determine if esterified provitamin D is present and to isolate and quantitate free and

esterified forms of provitamin D in rat skin.

Materials and Methods

Reagents and Chemicals

7-dehydrocholesterol was purchased from Aldrich Chemicals Co. (Milwaukee, WI, USA) and 7-dehydrocholesteryl acetate from Steroids Inc. (Wilton, NH, USA). Heptanoyl, lauroyl, myristoyl, palmitoyl, palmitoleoyl, stearoyl, oleoyl, linoleoyl, arachidoyl, 11-eicosenoyl, behenoyl, and erucoyl chloride were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Isopropyl alcohol, n-hexane, dichloromethane, methanol, ethyl acetate, and diethyl ether were of HPLC analysis grade and obtained from Burdick and Jackson Laboratories, Inc. (Muskegon, MI, USA).

Preparation of Standard Fatty Acyl Esters of 7-dehydrocholesterol

7-dehydrocholesteryl esters except 7-dehydrocholesteryl acetate were synthesized from 7-dehydrocholesterol and fatty acyl chlorides. 10mg of 7-dehydrocholesterol and 20$\mu\ell$ of heptanoyl, lauroyl, myristoyl, palmitoyl, palmitoleoyl, stearoyl, oleoyl, linoleoyl, arachidoyl, 11-eicosenoyl, behenoyl, or erucoyl chloride were added to 0.2mℓ of pyridine. After being incubated at 22℃ for ten hours, the reaction mixture was brought to pH 3 with HCl and extracted with diethyl ether. Each of 7-dehydrocholesteryl esters was isolated by Silica Gel GH thin-layer chromatography with 3% ethyl acetate in n-hexane as solvent. The isolated 7-dehydrocholesteryl esters were purified by high-performance liquid chromatography(HPLC) consisting of a Waters Associates U6K injector, a Model 6000A pump, a radial compression module containing a column, a Lamda-Max model 480 variable wavelength spectrophotometer set at 281 nm, a Model 730 Data Module(Milford, MA, USA), and a Beckman 165 variable wavelength detector(Berkeley, CA, USA). The purification was performed by straight phase HPLC using a Radial Pak B silica column(Waters Associates) and 0.1% isopropyl alcohol in n-hexane as the mobile solvent. The compounds were further purified by reverse phase HPLC using a Radial Pak C_{18} column(Waters Associates) and isopropyl alcohol : dichloromethane : methanol(55 : 15 : 30) as the mobile phase.

Preparation of Internal Standards

Two internal standards, [3α-^{3}H]-ergosterol and 7-dehydrocholesteryl heptanate, were used to measure the recovery of free and esterified forms of provitamin D, respectively, during the lipid extraction of rat skin and their isolation by chromatography. [3α-^{3}H]-ergosterol was purified by straight phase HPLC with a Radial Pak B silica column and 8% ethyl acetate in n-hexane as the mobile phase and further purified by reverse phase HPLC with a Radial Pak C_{18} column and 100% methanol as the mobile solvent. 7-dehydrocholesteryl heptanate was synthesized, isolated and purified by the above-mentioned methods.

Animals and Sample Preparation

30-day-old Sprague-Dawley male rats were purchased from Chales River Co.(Wilmington, MA, USA) and sacrificed with carbon dioxide. Their hair was shaved from the dorsal side and the underlying skin was removed. Muscle and subcutaneous fat were scraped off of the skin with a scalpel. The skin was stored in a freezer at −20℃ for further analysis.

Lipid Extraction

To minimize the degradation of the provitamin D compounds in rat skin, lipid extractions were carried out at 4℃. The internal standards, 7-dehydrocholesteryl heptanate and [3α-^{3}H]-ergosterol were added to a flask containing the rat skin cut into small pieces. The skin was extracted with 50mℓ

of diethyl ether for 50min and re-extracted by the same procedure. An equal volume of water was added and the ether phase was removed, dried and resuspended in 1mℓ of hexane.

Isolation of Free and Esterified Forms of 7-dehydrocholesterol in Rat Skin

Free and esterified forms of 7-dehydrocholesterol in rat skin were isolated by silicic acid column chromatography and HPLC. The lipid extract of rat skin was loaded on a 1.5×13cm column packed with 5gm of silicic acid. The column was eluted with a step-wise gradient mobile phase and 5mℓ fractions were collected from the column. The gradient mobile phases consisted of n-hexane(20mℓ), 1% diethyl ether in hexane(70mℓ), 1% ethyl acetate in hexane(30mℓ), 6% ethyl acetate in hexane (30mℓ), 14% ethyl acetate in hexane(50mℓ), and finally methanol(20mℓ). An aliquot(100μℓ) from each fraction was taken to measure radioactivity. The aliquot was dried and resuspended in Intra-Gel(Packard Instrument, USA) and counted in a Packard Tri-Carb Model 4600 automatic liquid scintillation counter to determine free provitamin D-containing fractions(polar provitamin D fractions). Ultraviolet absorbance spectrum(220~320nm) of each fraction was taken by a Perkin-Elmer 552A UV/VIS spectrophotometer to determine esterified provitamin D-containing fractions(nonpolar provitamin D fractions). Free provitamin D fractions were pooled and applied on straight phase HPLC using a Radial Pak B silica column and 8% ethyl acetate in n-hexane at 1.8mℓ/min as the mobile phase. The peak that migrated in the region of both 7-dehydrocholesterol and ergosterol was collected and then applied on reverse phase HPLC. A Radial Pak C_{18} column was eluted with 100% methanol at 1.4mℓ/min. In this system ergosterol eluted before 7-dehydrocholesterol. The fraction with the same retention time as ergosterol was collected and the radioactivity was counted to calculate sample recovery. Two free forms of provitamin D from rat skin, 7-dehydrocholesterol(cholesta-5, 7-dien-3β-ol) and cholesta-5, 7, 24-trien-3β-ol were isolated. The concentrations of the free forms of provitamin D were calculated by integration of the area under their HPLC peaks and comparison with a standard curve developed using standard 7-dehydrocholesterol. Esterified provitamin D-containing fractions were pooled and applied on straight phase HPLC. A Radial Pak B silica column was eluted with 0.03% isopropyl alcohol in hexane at 1.5mℓ/min. A broad peak with the retention time (Rt) similar to the standard 7-dehydrocholesteryl palmitate(Rt=14.94 min) was collected and applied on reverse phase HPLC. A Radial Pak C_{18} column was eluted with isopropyl alsochol : dichloromethane : methanol(55 : 15 : 30) at 0.6mℓ/min. An ultraviolet absorbance spectrum(220~320 nm) of each peak was taken using the Beckman 165 detector during the HPLC run. To determine the concentration of each compound, each HPLC chromatogram was photocopied and the peaks were cut out and weighed. These weights were compared with those of various amounts of standard 7-dehydrocholesteryl palmitate peaks. Sample recovery was calculated by comparing the known amount of 7-dehydrocholesteryl heptanate added prior to lipid extraction and the amount recovered following reverse phase HPLC.

Results and Discussion

HPLC Separation of Standard 7-dehydrocholesteryl Esters

HPLC chromatographic profile of standard 7-dehydrocholesteryl esters is shown in Fig. 1. A Radial Pak C_{18} column was eluted with isopropyl alcohol : dichloromethane : methanol(55 : 15 : 30) at 0.6mℓ/min. Reverse phase HPLC resolved 7-dehydrocholesteryl acetate(Rt=10.47 min), heptanate (Rt=11.15min), laurate(Rt=13.47min), linoleate

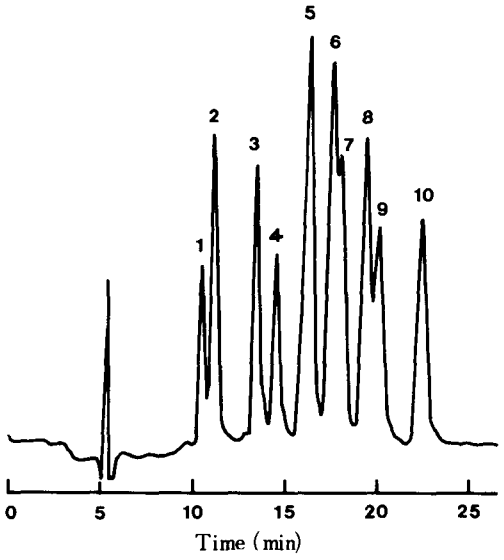

Fig. 1. HPLC chromatogram of standard 7-dehydrocholesteryl esters.
Peaks : 1, 7-dehydrocholesteryl acetate ; 2, 7-dehydrocholesteryl heptanate ; 3, 7-dehydrocholesteryl laurate ; 4, 7-dehydrocholesteryl linoleate, 7-dehydrocholesteryl palmitoleate and 7-dehydrocholesteryl myristate ; 5, 7-dehydrocholesteryl palmitate and 7-dehydrocholesteryl oleate ; 6, 7-dehydrocholesteryl 11-eicosenate : 7, 7-dehydrocholesteryl stearate ; 8, 7-dehydrocholesteryl erucate ; 9, 7-dehydrocholesteryl arachidate ; 10, 7-dehydrocholesteryl behenate.

(Rt=14.57min), palmitate(Rt=16.30min), 11-eicosenate(Rt=17.57min), stearate(Rt=18.02min), erucate(Rt = 19.39min), arachidate(Rt = 20.09 min), and behenate(Rt=22.39min)(Fig. 1). The retention times of 7-dehydrocholesteryl linoleate, palmitoleate and myristate were the same, and 7-dehydrocholesteryl oleate comigrated with 7-dehydrocholesteryl palmitate(Table 1).

Free and Esterified Provitamin D Content of 30-Day-Old Male Rat Skin

Fig. 2 shows HPLC chromatogram of standard 7-dehydrocholesterol(Rt=13.12min) and ergosterol(Rt=12.31min). A Radial Pak C_{18} column was

Table 1. Retention times for standard 7-dehydrocholesteryl esters on reverse phase HPLC*

7-dehydrocholesteryl esters	Retention time (min)
7-dehydrocholesteryl acetate	10.47
7-dehydrocholesteryl heptanate	11.15
7-dehydrocholesteryl laurate	13.47
7-dehydrocholesteryl linoleate	14.57
7-dehydrocholesteryl palmitoleate	14.57
7-dehydrocholesteryl myristate	14.57
7-dehydrocholesteryl palmitate	16.30
7-dehydrocholesteryl oleate	16.30
7-dehydrocholesteryl 11-eicosenate	17.57
7-dehydrocholesteryl stearate	18.02
7-dehydrocholesteryl erucate	19.39
7-dehydrocholesteryl arachidate	20.09
7-dehydrocholesteryl behenate	22.39

*Reverse phase HPLC was carried out with a Radial Pak C_{18} column and isopropyl alcohol : dichloromethane : methanol(55 : 15 : 30) at 0.6mℓ/min.

eluted with 100% methanol at 1.4mℓ/min. Reverse phase HPLC chromatogram of free provitamin D in 30-day-old male rat skin is shown in Fig. 3. Two forms of free provitamin D, 7-dehydrocholesterol(cholesta-5, 7-dien-3β-ol)(Rt=13.02min) and cholesta-5, 7, 24-trien-3β-ol(Rt=10.71min), were isolated. The amount of free provitamin D present in rat skin was expressed as ng of provitamin D per cm^2 of surface area(Table 2). 41ng/cm^2 of cholesta-5, 7, 24-trien-3β-ol(4.9% of the average total free provitamin D content) and 805ng/cm^2 of 7-dehydrocholesterol(95.1%) were present in the whole skin.

Many investigators suggested or reported that 7-dehydrocholesterol in rat skin may be esterified [14-21]. However, no investigators used direct methods to isolate esterified forms of 7-dehydrocholesterol. This study directly demonstrated the presence of 7-dehydrocholesteryl esters in rat skin. Fig. 4 shows straight phase HPLC chromatogram

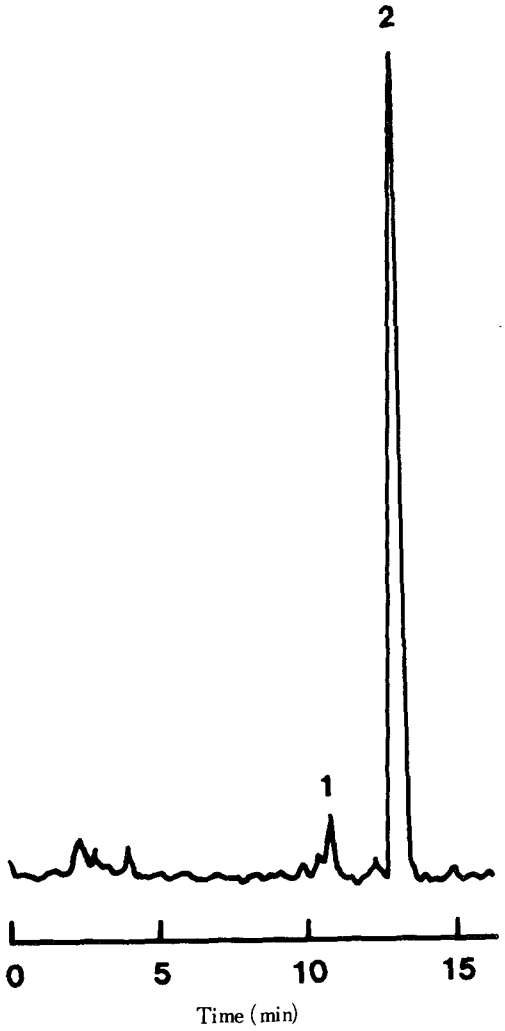

Fig. 2. Reverse phase HPLC chromatogram of free provitamin D in 30-day-old male rat skin. Peaks : 1, cholesta-5, 7, 24-trien-3β-ol : 2, cholesta-5, 7-dien-3β-ol.

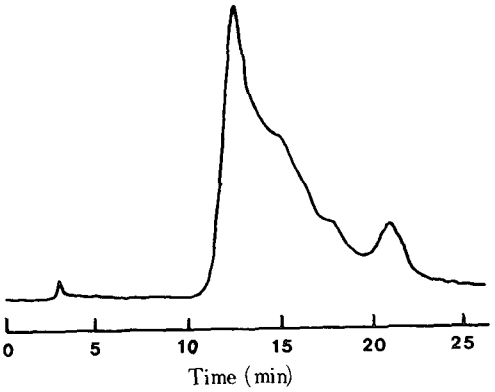

Fig. 3. Straight phase HPLC chromatogram of the nonpolar provitamin D fraction from 30-day-old male rat skin.

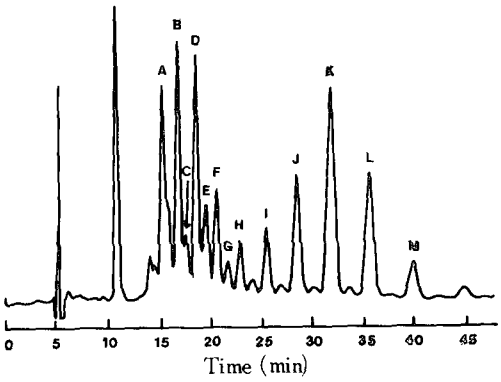

Fig. 4. Reverse phase HPLC chromatogram of esterified forms of provitamin D in 30-day-old male rat skin.

of the nonpolar provitamin D fraction obtained from silicic acid column chromatography. One broad peak was eluted from 11 min to 24 min after injection. In this system the retention times of standard 7-dehydrocholesteryl palmitate and heptanate were 14.94min and 19.40min, respectively. At least thirteen forms of esterified provitamin D were isolated by reverse phase HPLC. The rat skin contained trace quantities of peak C(Rt=18.00min) and peak G(Rt=21.94min). Larger quantities of peak A(Rt=15.54min), peak B(Rt=17.05 min), peak D(Rt=18.82min), peak E(Rt=19.79 min), peak F(Rt=20.82min), peak H(Rt=23.10 min), peak I(Rt=25.70min), peak J(Rt=28.69 min), peak K(Rt=32.05min), peak L(Rt=35.82 min), and peak M(Rt=40.12min) were present in the samples (Table 2)(Fig. 5). All of the peaks exhibited characteristic 5, 7-diene ultraviolet absorbance spectra(λ^{max} 295, 282, and 271nm) except for strong absorbance near 220nm(Fig. 6). Peak K(15.6% of the average total provitamin D content) and peak B(10.7%) were predominant peaks.

The content of free and esterified forms of pro-

Table 2. Free and esterified provitamin D content of 30-day-old male rat skin.

Provitamin D Compound	Retention time(min)	Rat I (ng $\triangle^{5,7}$/cm^2)	Rat II (ng $\triangle^{5,7}$/cm^2)	Mean (ng $\triangle^{5,7}$/cm^2)	Mean % of total $\triangle^{5,7}$ content
Cholesta-5, 7, 24-trien-3β-ol	10.71	50.92	31.29	41.11	0.68
Cholesta-5, 7-dien-3β-ol	13.02	902.29	708.26	805.28	13.30
peak A*	15.54	951.61	639.81	795.71	13.14
peak B	17.05	775.12	525.06	650.09	10.74
peak C	18.00	t	t	t	t
peak D	18.82	671.59	508.47	590.03	9.74
peak E	19.79	143.30	137.96	140.63	2.32
peak F	20.82	345.67	254.09	299.88	4.95
peak G	21.94	t	t	t	t
peak H	23.10	185.65	162.85	174.25	2.88
peak I	25.70	266.84	240.27	253.56	4.19
peak J	28.69	576.27	473.91	525.09	8.67
peak K	32.05	971.57	923.21	947.39	15.64
peak L	35.82	651.58	585.89	618.74	10.22
peak M	40.12	225.66	202.94	214.30	3.54

* : Unknown esterified provitamin D compound
t : This compound was present only in trace amount.

vitamin D in the skin is shown in Table 2. 30-day-old male rat skin contained 6,056ng/cm^2 of total provitamin D. This included both 14.0% as free provitamin D(846ng/cm^2) and 86.0% as esterified provitamin D(5,209ng/cm^2). The percentage of esterification was comparable to the study carried out by Takada et al.[21]. It is well known that esters are storage forms of fat soluble vitamins[22]. The cutaneous esterified provitamin D may be a storage form of the provitamin D. 7-dehydrocholesterol is a precursor to both cholesterol and previtamin D in rat skin[1,23]. Therefore, the esterified provitamin D may be a source of cholesterol and previtamin D.

By comparing the reverse phase HPLC retention times of standard free and esterified forms of provitamin D with those found in the lipid extract from rat skin, it is possible to suggest structures of the isolated compounds. Both cholesta-5, 7-dien-3β-ol and cholesta-5, 7, 24-trien-3β-ol may be esterified with fatty acids. Peak A may consist of two or more provitamin D esters since it was not a sharp peak. A small peak within peak A migrated similarly to both 7-dehydrocholesteryl palmitate (16 : 00) and oleate(18 : 1). The retention times of peak B, peak C, and peak D were similar to those of 7-dehydrocholesteryl 11-eicosenate(20 : 1), stearate(18 : 0), and erucate(22 : 1), respectively. In addition, there were HPLC peaks with retention times much longer than the above-mentioned compounds. The peaks correspond to more nonpolar compounds such as provitamin D esters with longer chain fatty acids. Therefore, fatty acid composition of the esterified provitamin D may be both saturated and unsaturated acids with chain lengths longer than or equal to C_{16}. The fatty acid composition of the provitamin D esters differs from that of triglycerides found in rodent skin[24]. In mouse skin triglycerides contained fatty acids ranging from C_{14} to C_{18}. But, it is known that choles-

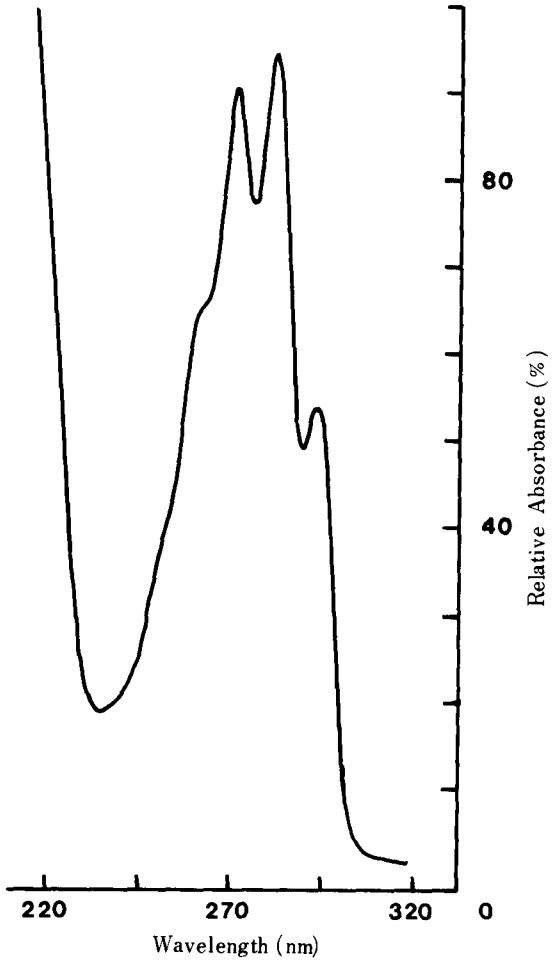

Fig. 5. Ultraviolet absorbance spectrum of provitamin D ester isolated from 30-day-old male rat skin.

teryl esters in the stratum corneum and the stratum granulosum of the new born mouse contain saturated and unsaturated fatty acids with carbon chain lengths of C_{20}, C_{22}, C_{24}[25], and C_{26}. Thus it is very likely that provitamin D may be esterified with long chain fatty acids.

It was demonstrated that upon ultraviolet irradiation some esters of 7-dehydrocholesterol were biologically active[11-13]. The thirteen different provitamin D esters isolated in this investigation may absorb ultraviolet radiation and become bioavailable sources of vitamin D.

Acknowledgement

This research project was supported by Inje Research and Scholarship Foundation in 1989.

References

1. Havinga, E. : Vitamin D, example and challenge. *Experientia,* 29, 1181-1193(1973)
2. Holick, M. F. and Clark, M. B. : The photobiogenesis and metabolism of vitamin D. *Fed. Proc.,* 37, 2567-2574(1978)
3. Haussler, M. R. and McCain, T. A. : Basic and clinical concepts related to vitamin D metabolism and action. *N. Engl. J. Med,* 297, 974-983, 1041-1050(1977)
4. Deluca, H. F. and Schnoes, H. K. : Metabolism and mechanism of action of vitamin D. *Annu. Rev. Biochem.,* 49, 631-666(1976)
5. Norman, A. W. and Henry, H. : 1, 25-dihydroxycholecalciferol-a hormonally active form of vitamin D_3. *Recent Prog. Horm. Res.,* 30, 431-479(1974)
6. Windaus, A. and Bock, F. : Uber das provitamin aus dem sterin der schweineschwarte. Hoppe-seyler's Z. *Physiol. Chem.,* 245, 168-170(1937)
7. Koch, E. M. and Koch, F. C. : The provitamin D of the covering tissue of chickens. *Poultry Sic.,* 20, 35(1941)
8. Wells, W. W. and Baumann, C. A. : Skin sterols. V. Effect of ultraviolet light on the sterols of rat skin. *Arch. Biochem. Biophys.,* 53, 471-478(1954)
9. Wheatley, V. R. and Reinertson, R. P. : The presence of vitamin D precursors in human epidermis. *J. Invest. Dermatol.,* 31, 51(1958)
10. Gaylor, J. L. and Sault, F. M. : Localization and biosynthesis of 7-dehydrocholesterol in rat skin. *J. Lipid Res.,* 5, 422-431(1964)
11. Rosenheim,O. and Webster, T. A. : LXXI. The antirachitic properties of irradiated sterols. *Biochem. J.,* 20, 537-544(1926)
12. Hess, A. F., Weimstock, E., and Sherman, E. : The antirachitic value of irradiated cholesterol and phytosterol. V. Chemical and biological changes. *J. Biol. Chem.* 67, 413-423 (1926)
13. Bills, C. E. and McDonald, F. G : Antiricketic

Substances. V. The action of ultra-violet rays on the ethers and esters of cholesterol. *J. Biol. Chem.*, **72**, 13-19(1927)
14. Kooyman, D. J. : Lipids of the skin : Some changes in the lipids of the epidermis during the process of keratinization. *Arch. Derm. Syph.*, **25**, 444-450(1932)
15. Long, V. J. W. : Variations in lipid composition at different depths in the cow snout epidermis. *J. Invest. Dermatol.*, **55**, 269-273(1970)
16. Yardley, H. J. : Sterols and keratinization. *Br. J. Dermatol.*, **81**, suppl. 2, 29-38(1969)
17. Freinkel, R. K. and Thomas, N. R. : Flux of fatty acids during epidermal differentiation. *J. Invest. Dermatol.*, **69**, 413-418(1977)
18. Brady, D. R. and Gaylor, J. L. : Enzymic formation of esters of methyl sterol precursors of cholesterol. *J. Lipid Red.*, **12**, 270-276 (1971)
19. Kandutsch, A. A. and Baumann, C. A. : Skin sterols. VI. $\triangle^{7}$-cholestenol in tumors and an effect of methycholanthrene on the sterols of mouse skin. *Cancer Res.*, **14**, 667-671(1954)
20. Morre, P. R. and Baumann, C. A. : Skin sterols. I. Colorimetric determination of cholesterol and other sterols in skin. *J. Biol. Chem.*, **195**, 615-621(1952)
21. Takada, K., Takashima, A., Kobayashi, T. and Shimoi, Y. : Application of high-performance liquid chromatography to the study of esterified 7-dehydrocholesterol in rat skin. *Biochim. Biophys Acta.*, **666**, 307-312(1981)
22. Ruth, L. P. and Brown, M. L. : *Nutrition : An integrated approach.* Second edition. John Wiley and Sons, Inc., New York, 142(1975)
23. Dempsey, M. E., Seaton, J. D., Schroepfer, G. J., Jr., and Trockman, R. W. : The intermediary role of $\triangle^{5,7}$-cholestadiene-3β-ol in cholesterol biosynthesis. *J. Biol. Chem.*, **239**, 1381 -1387(1964)
24. Carruthers, C. and Heining, A. : Infleuence of hair growth cycle on the triglyceride fatty acid composition of mouse epidermis. *Proc. Soc. Exp. Biol. Med.*, **112**, 278-280(1963)
25. Elias, P. M., Goerke, J. and Friend, D. S. : Mammalian epidermal barrier layer lipids : composition and intluence on structure. *J. Invest. Dermatol.*, **69**, 535-546(1977)

(Received August 20, 1990)

흰쥐 피부에 존재하는 자유형과 에스테르형의 provitamin D의 분리

김정인

인제대학교 식품영양학과

요 약

생후 30일 된 숫쥐의 피부에 존재하는 자유형과 에스테르형의 provitamin D를 silicic acid column chromatography와 HPLC를 이용하여 분리하였다. 두 종류의 자유형 provitamin D (cholesta-5, 7-dien-3β-ol과 cholesta-5, 7, 24-trien-3β-ol)와 13종 이상의 에스테르형 provitamin D를 분리하였다. 피부에 존재하는 provitamin D의 총량은 6,056ng/㎠이었다. 846ng/㎠의 자유형 provitamin D와 5,209ng/㎠의 에스테르형 provitamin D가 존재하였다. 피부에 존재하는 전체 provitamin D의 86%가 에스테르형으로 존재하였다.