Excretion of Sterols from the Skin of Normal and Hypercholesterolemic Humans

IMPLICATIONS FOR STEROL BALANCE STUDIES

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ABSTRACT The 24 hr sterol excretion from the entire skin surface was determined in six normal and five hypercholesterolemic (Type II) patients fed a controlled, eucaloric diet containing 400 mg of plant sterols. All subjects received radiolabeled cholesterol intravenously in order to measure cholesterol turnover and exchange. The 24 hr skin surface lipids were collected subsequently at intervals of 7–10 days. Sterols were quantified and identified by a combination of thin-layer and gas-liquid chromatographic methods. The mean 24 hr excretion of cholesterol in milligrams was 82.6 in the normal subjects and 82.7 in the hypercholesterolemic patients. Cholesterol constituted 89% of the total sterol excretion through the skin surface in both groups.

The specific radioactivity of cholesterol in the skin surface lipids increased gradually after the intravenous administration of the isotope. Within 4–5 wk the specific activity equaled and then remained higher than that of the plasma up to 10 wk. These specific activity curves suggested that, for at least some of skin surface cholesterol, there was a precursor-product relationship between the plasma cholesterol and the skin cholesterol.

The presence of plant sterols, β-sitosterol, campesterol, and stigmasterol in the skin surface lipids of man has not been reported previously. We identified these sterols in the skin surface lipids of all of our subjects. They constituted about 7% of the total skin surface sterols. The occurrence of plant sterols in the skin surface lipids suggested that plasma sterols were transferred from the plasma into the skin. 1–2% of the skin surface sterols were tentatively identified as lathosterol and lanosterol.

The present study documented that a significant amount of cholesterol was excreted from the skin surface and that probably there was a net transfer of plasma cholesterol into the skin surface lipids. Both normal subjects and hypercholesterolemic patients excreted similar amounts of cholesterol per day into the skin surface lipids. We suggest that this daily loss of cholesterol from the skin surface may need to be considered in sterol balance studies.

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INTRODUCTION

Cholesterol is excreted from the body as a constituent of the skin surface lipids (1). However, in human sterol balance studies the actual daily excretion of sterols has been measured only in the feces under conditions of a metabolic steady state (2–5). The excretion of cholesterol through the skin surface has been omitted in sterol balance studies, although significant amounts of sterols, cholesterol in particular, might be lost via this route. To date, however, the total daily excretion of sterols through the skin surface in man has not been quantified.

As regards the origin of cholesterol in the skin surface lipids, the skin cholesterol is said to be derived primarily from biosynthesis in the keratinizing epidermis of the skin (6). Wilson has shown that in the rat a small amount of squalene, which is synthesized largely in the sebaceous glands (6), is converted to sterol esters in the preputial gland, a modified sebaceous gland (7). Should cholesterol be transferred or exchanged from the plasma to the skin surface lipids of man, this would represent another pathway of excretion of cholesterol.

The abnormality in the metabolism of cholesterol in familial hypercholesterolemia (Type II hyperbetalipoproteinemia [8]) is not clearly understood. Inadequate excretion of cholesterol from the body has been suggested as one of the mechanisms possibly important in the causation of the hypercholesterolemia (9). In several studies no differences have been observed in the turnover of total exchangeable cholesterol in normal men and patients with hypercholesterolemia (10–13). However, Samuel and Perl have reported that both rapidly miscible pool and total exchangeable body mass of cholesterol were significantly higher in hypercholesterolemic patients as compared to normal subjects. They did not observe any difference as regards the input rate of cholesterol which is the sum of dietary and biosynthesized cholesterol (14).

The present investigation was undertaken to determine the total 24 hr excretion of cholesterol and other sterols from the skin surface of normal human subjects and of Type II hypercholesterolemic patients in order to amplify the role of this excretory pathway in cholesterol metabolism and the sterol balance in man. The transfer or exchange of plasma cholesterol to the lipids of the skin surface was also examined. The variety of sterols excreted by the skin was identified and quantified.

METHODS

The 24 hr total sterol excretion from the entire body surface was determined in six normal human subjects and in five hypercholesterolemic patients with Type II hyperbetalipoproteinemia. The clinical data are given in Table I. The subjects were usually fed a cholesterol-free, 40% fat diet containing 350–400 mg plant sterols per day (Table II). Two normal men (subjects 5 and 6) were studied when they were receiving this diet except for the inclusion of either 750 or 1000 mg of cholesterol per day as egg yolk cholesterol. Two hypercholesterolemic patients (numbers 10 and 11) were fed the cholesterol-free diet with only 20% of the calories being provided by fat. All diets were eucaloric and body weight remained constant throughout the experiment.

Each subject was given intravenously 35–40 μCi of either cholesterol-4-^{14}C or cholesterol-1α-^{3}H [1] dissolved in 5 ml ethanol and then suspended in 500 ml of 0.9% sodium chloride solution. In the course of the cholesterol turnover study, venous blood samples were drawn in the fasting state weekly or twice weekly for the next 9 or 10 wk. The serum was separated and stored frozen in glass vials at -20°C for subsequent analysis. The total serum cholesterol was determined by the method of Abell, Levy, Brodie, and Kendall (15). The radioactivity was determined on another portion using 10 ml of scintillation solution (4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-*bis*[2-(5-phenyloxazolyl) benzene] per litre of toluene), in a Packard Tri-Carb liquid scintillation counter (model 3380, Packard Instrument Co., Inc., Downers Grove, Ill.) equipped with external standardization. Results were expressed as cholesterol specific radioactivity (disintegrations per minute per milligram of cholesterol.

[1] Obtained from New England Nuclear Corp., Boston, Mass. (> 98% pure as checked by thin-layer and gas-liquid chromatography).

TABLE I

The Description of the Human Subjects: Clinical Data

Subject	Sex	Age	Weight	Serum		Type of hyperlipoproteinemia*
				Cholesterol	Triglycerides	
		yr	*lb*	*mg/100 ml*	*mg/100 ml*	
1	M	39	164	168	136	Normal
2	M	34	182	173	134	Normal
3	M	34	186	190	231	Normal
4	M	40	160	154	89	Normal
5	M	40	164	180	86	Normal
6	M	43	175	214	154	Normal
7	F	51	123	864	141	Type II
8	F	63	110	297	148	Type II
9	F	69	111	386	214	Type II
10	F	54	127	295	127	Type II
11	M	49	133	473	146	Type II

* Defined by lipoprotein electrophoresis (8).

The skin surface lipids were collected at intervals of 7–10 days during the next 10 wk after the intravenous administration of isotopic cholesterol. In the early morning, each subject took a warm shower bath without soap or shampoo. The subject's entire body surface, including the head and hair, was wiped clean with a towel soaked in acetone in order to remove lipids from the body surface. In the case of patients 7, 8, 9, and 10, the hair was excluded because of personal objections by the patients. In one additional normal subject, the skin surface lipids were collected daily for 6 consecutive days. This subject had stopped the use of any oils, deodorants, or cosmetics for 4 wk before this collection period. All subjects wore specially cleaned standard hospital white cotton pajamas which had been previously laundered many times. This clothing was always extracted three times with boiling acetone before use to render it free of lipids and sterols insofar as possible. The gauze bandages used to cover the head and forehead and also the sponges used to wipe the body surface for collection of the lipids were similarly extracted before use.

We analyzed separately three complete sets of clothing, bandages, and sponges which were later used in the collection of skin surface lipids. Each set of clothing, band-

TABLE II

Composition of the Diet

	As per cent of total calories
Carbohydrate	45 or 65
Protein	15
Fat	40 or 20
Fatty acid content	
Saturated	11
Polyunsaturated	9
Monounsaturated	19
Cholesterol	0, 750, or 1000 mg/day
Plant sterols	350–400 mg/day

ages, and sponges was extracted with boiling acetone as described above. The extracts were evaporated to dryness and were subjected to sterol analysis as described below. Only trace quantities of cholesterol and β-sitosterol (a total of 16.4 and 2.0 μg per set, respectively; average of three determinations) were present in the clothing, bandages, and the sponges used in the study. There were thus no errors introduced artifactually into the results from the use of the clothing.

During the 24 hr collection period the subjects did not use any cosmetics and did not wash any part of their bodies except for the hands. The head and forehead were covered with gauze bandage when going to bed so as to minimize possible loss of lipids onto the bed clothes. The next morning the subject's entire body surface was gently wiped with acetone-soaked sponges to collect the lipid material. Rubber gloves were used during the collection to avoid contamination of lipids from the hands of the investigator.

The lipids were extracted from the sponges and bandages and the clothing worn over the 24 hr collection period by boiling with 2- to 3-liter portions of acetone three times. The acetone extract was brought to a small volume by evaporation and was centrifuged to remove cell debris, hairs, and other insoluble material. The total lipids extracted from the clothing, bandages, and sponges were dried in a vacuum desiccator and the weight of the lipid determined.

The lipids were then dissolved in chloroform:methanol (2:1, v/v) and made up to 100 ml with the solvent. A portion, usually 10 ml, was used for the isolation of free and total digitonin-precipitable sterols. The ester cholesterol was obtained by difference. Free sterols were precipitated before saponification, whereas for the total sterols the procedure involved saponification with alcoholic KOH, extraction of the unsaponifiable lipids with hexane, and precipitation of the sterols with digitonin (16). The precipitate was washed with diethyl ether and dried. The free sterols were recovered from the digitonides by dissolving the precipitate in pyridine and extracting the free sterols with diethyl ether (17). The ether extract was dried under *vacuo* over concentrated H_2SO_4. The free sterols were redissolved in chloroform for thin-layer chromatographic analysis. Another portion of the chloroform:methanol extract of the skin surface lipids was similarly saponified, extracted with hexane, and evaporated to dryness in a counting vial. The radioactivity present in this extract was counted after addition of 10 ml of the scintillation solution described above.

Thin-layer chromatography. Thin-layer chromatography (TLC)[2] was carried out on 20 × 20 cm glass plates coated with Florisil[3] (100–200 mesh); the solvent system was heptane:ethyl ether (45:55, v/v) (18). The lipids were visualized under ultraviolet light after spraying the plate with Rhodamine-6G solution.[4] Two bands were obtained; one, closer to the origin, had an R_f value identical to cholesterol. The R_f of the second band was slightly higher, and it contained lanosterol as identified subsequently by gas-liquid chromatography (GLC). The bands were scraped, eluted with diethyl ether, and subjected to GLC as trimethyl silyl ether derivatives (18).

Gas liquid chromatography. GLC was carried out on a dual column gas chromatograph[5] equipped with hydrogen flame ionization detector and an automatic digital integrator.[6] The column was a 120 cm glass U-tube, 4 mm I.D., packed with Diatoport-S[7] (80–100 mesh) coated with a film (3.8%) of SE-30 (methylsiloxane polymer). Temperatures of column, detector, and the flash heater were 230, 250, and 300°C, respectively. Helium was the carrier gas at a flow rate of 100 ml/min; the inlet pressure was 40 psi. Identification of the sterols of the skin surface lipids was carried out by comparing their retention times with those of the various pure sterols.[8] A standard reference compound, 5α-cholestane, was employed for all analyses under the above-mentioned conditions.

RESULTS

The 24 hr excretion of cholesterol in the skin surface lipids of the normal men was appreciable, an average of 82.6 mg ±21.5 (SD) of which 86.7% was esterified (Table III). The two normal men (subjects 5 and 6) fed a diet containing either 750 or 1000 mg of cholesterol per day excreted 113.1 mg and 80.8 mg of cholesterol per 24 hr, respectively in the lipids of the skin surface. Since these values are little different from those of the other four normal subjects fed a cholesterol-free diet, the results have been grouped together. The 24 hr excretion of cholesterol through the skin surface of the normal men represented 2.84% of the total lipid and 88.8% of the total sterol excretion.

The hypercholesterolemic patients excreted similar amounts of cholesterol (82.7 mg ±6.5) per day through the skin surface as the nomal men. The free and esterified cholesterol contents were also similar to the normal subjects. Two hypercholesterolemic patients (subjects 10 and 11) fed a diet providing 20% of the calories from fat excreted similar amounts of cholesterol in the skin surface lipids as compared to the three hypercholesterolemic patients fed 40% of the calories as fat.

The data showing the reproducibility of the 24 hr excretion of skin lipids in a normal subject over a consecutive 6 day period are presented in Table IV. This patient had stopped the use of any oils or cosmetics on the skin for nearly 4 wk before the beginning of the collection period. He used only one kind of soap for bathing. When analyzed, this soap contained only a trace quantity of cholesterol (less than 1 mg per 100 g) and no plant sterols. After the 6 consecutive days of skin surface lipids collection, the skin of the subject appeared normal with the usual oiliness and no scaling, chafing, or dryness was noted. The mean daily excretions of total lipids, cholesterol, and plant sterols were 2.73±0.65 g,

[2] *Abbreviations used in this paper:* GLC, gas-liquid chromatography; TLC, thin-layer chromatography.

[3] Floridin Co., Tallahassee, Fla.

[4] Applied Science Laboratories, Inc., State College, Pa.

[5] F & M Scientific model 402 high efficiency gas chromatograph; F & M Scientific Corp., Avondale, Pa.

[6] Infotronics CRT model 100 digital integrator; Infotronics Corp., Austin, Tex.

[7] Hewlett-Packard Co., Palo Alto, Calif.

[8] Obtained from Steraloids, Inc., Pawling, N. Y. (>99% pure as checked by us).

TABLE III

Excretion of Cholesterol into the Skin Surface Lipids of Normal Subjects and Hypercholesterolemic Patients

Subject	Diet		No. of collections	Total lipids	Cholesterol		
	Fat	Cholesterol			Total	Ester	Free
	% of total calories	*mg/day*		*g/day*		*mg/day*	
Normal							
1	40	0	4	2.25±0.48*	59.2±12.6	49.7± 9.7	9.5±1.6
2	40	0	5	3.80±1.54	98.5±28.1	82.6±20.0	15.9±6.1
3	40	0	7	2.63±0.52	85.2±17.7	75.9±14.8	9.3±2.6
4	40	0	2	2.78±0.54	58.5± 5.9	49.3± 8.2	9.2±3.3
5	40	750	3	3.54±1.54	113.1±22.0	98.8±14.9	14.3±5.1
6	40	1000	3	2.45±0.07	80.8±18.0	73.1±16.7	7.7±2.8
Mean ±SD				2.91±0.62	82.6±21.5	71.6±18.0	11.0±3.3
Hypercholesterolemic							
7	40	0	7	2.75±1.99	85.5±45.3	74.5±36.2	11.0±1.8
8	40	0	7	2.67±0.55	86.5±14.7	78.9±13.1	7.6±1.4
9	40	0	3	2.08±0.85	88.1±15.5	78.2±17.0	9.9±3.4
10	20	0	2	1.10±0.67	81.6±17.5	72.5±16.1	9.1±0.1
11	20	0	2	2.16±0.61	71.9±14.2	60.4± 9.1	11.5±2.4
Mean ±SD				2.15±0.66	82.7± 6.5	72.9± 7.2	9.8±1.6

* Values are mean ±SD.

128.8±11.4 mg, and 4.9±2.3 mg, respectively. Most of the total daily excreted skin lipids (about 75%) were collected in the clothing worn over the 24 hr collection period. The clothing had been extracted with boiling acetone before use to render it free of lipids and sterols. Hence, any lipid material, including sterols, extracted from the clothing worn over the 24 hr collection period must have come from the skin surface being absorbed as sebum and as the desquamated epithelial cells adhering to it. The collection system, then, combined lipids obtained from the acetone washings plus lipids collected in the clothing worn for the 24 hr period. Some 90% of the sterols (114.6 mg) was collected by the clothing and only 10% (14.2 mg) was obtained through the acetone washes. As is well known, the sebum contains little sterol (19). Thus, our results fit the hypothesis that most of the sterol excretion from the skin is in the form of desquamated epithelial cells shed into the clothing.

The specific activity curves for the plasma and the skin surface cholesterol for three normal subjects (numbers 1, 2, and 3) and two Type II patients (numbers 7 and 8) are shown in Fig. 1. The specific activities of the free and ester fractions of the skin surface cholesterol were also plotted. The specific activity of the skin surface cholesterol, initially much lower, increased gradually and equalled the plasma cholesterol specific activity 4–5 wk after intravenous administration of radiolabeled cholesterol (Table V). Thereafter, the skin sur-

TABLE IV

The Daily Sterol Excretion from the Skin for 6 Consecutive Days in a Normal Subject

Day	Total lipids	Cholesterol			Plant sterols	Other sterols
		Total	Free	Ester		
	g/day		*mg/day*		*mg/day*	*mg/day*
1	3.49	128.1	13.1	115.0	9.1	2.19
2	3.22	119.9	17.5	102.4	5.3	0.96
3	1.79	141.1	21.5	119.6	5.2	2.62
4	3.01	111.2	20.1	91.1	2.9	2.04
5	2.13	136.4	25.4	111.0	2.8	1.92
6	2.71	135.9	27.9	108.0	3.9	0.91
Mean ±SD	2.73±0.65	128.8±11.4	20.9±5.3	107.9±10.1	4.9±2.3	1.77±0.69

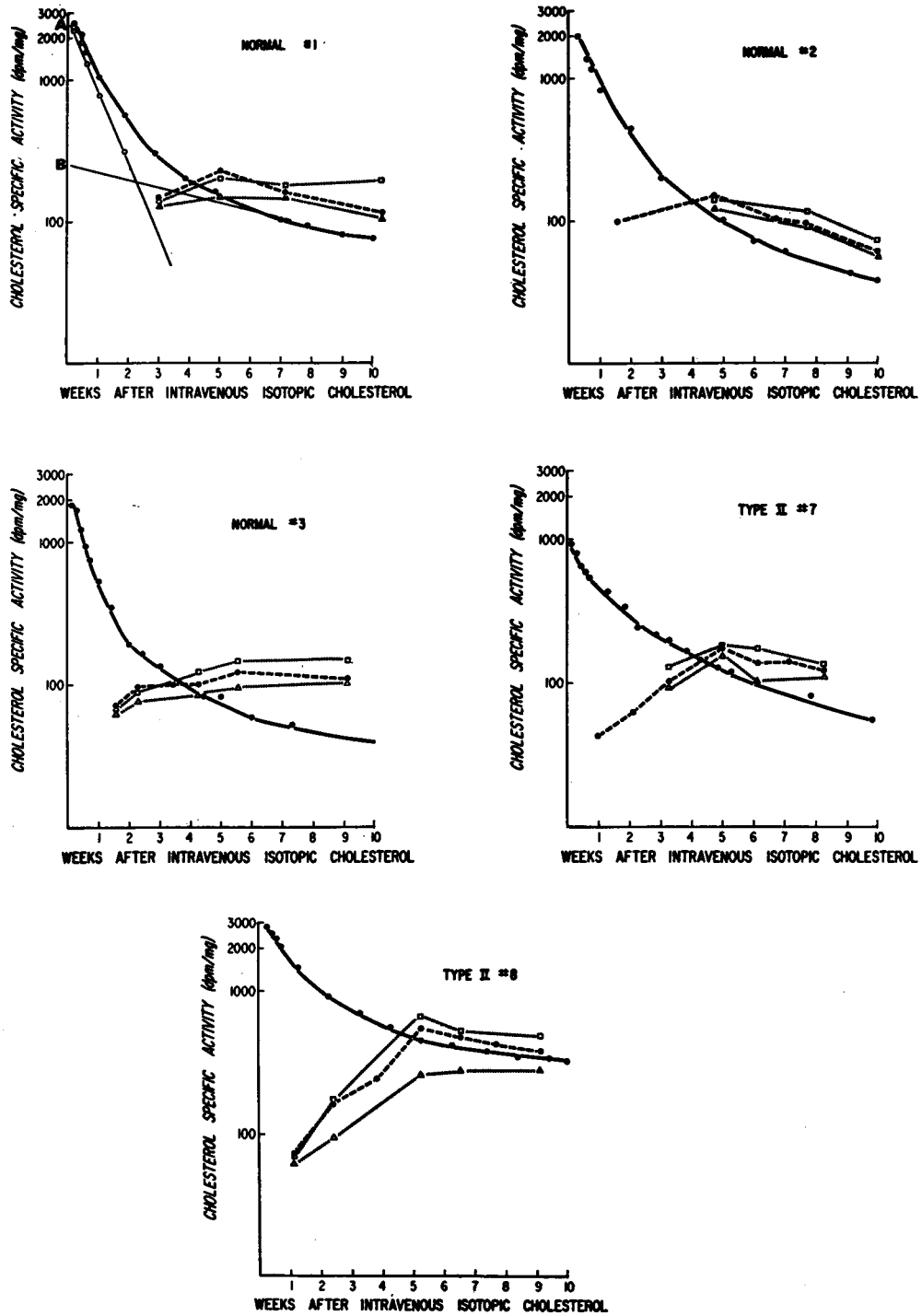

FIGURE 1 The specific activities in dpm/mg of the total (● - — -), free (△ - - - -), and esterified cholesterol (□ - - - -) of the skin surface lipids and the plasma cholesterol (●——) of three normal subjects and two Type II hypercholesterolemic patients plotted against time on semilogarithm graph paper. The plasma cholesterol turnover conformed to the two pool model in all the subjects (13). However, the intercepts C_A and C_B have only been drawn for one normal subject (number 1) in the top left curve.

TABLE V

Ratio of Cholesterol Specific Activity of Skin Surface Lipids and Plasma Expressed as Per Cent of Plasma Cholesterol Specific Activity

	Wk after intravenous isotopic cholesterol								
Subjects	1	2	3	4	5	6	7	8	9
Normal									
1		48		141			258		155
2	16			110		157	160		162
3	27	55		121	174	223		216	210
4				111		168			
5		22			111		111		
6				126		133			198
Hypercholesterolemic									
7	9	26	53		133	140	167	162	
8	4	18		96	123	105	108		110
9							122	123	116
10								110	106
11								109	135

face cholesterol specific activity remained higher than that of the plasma cholesterol in all subjects throughout the experimental period. The specific activity of the esterified cholesterol was always higher than that of the free fraction. The plasma cholesterol turnover in all of our subjects conformed to a two pool model as described by Goodman and Noble (13). The crossing of the specific activity curves of the skin surface cholesterol and the plasma cholesterol occurred at about the time when the former reached its maximum value.

Besides cholesterol, other sterols—lathosterol, lanosterol, and the plant sterols, campesterol, stigmasterol, and β-sitosterol—were tentatively identified by gas-liquid chromatography in the skin surface lipids of all the subjects studied. Fig. 2 depicts a typical gas chromatogram. These sterols moved together on the thin-layer plate and migrated close to the origin. This band had an R_f value identical to cholesterol. Addition of a known amount of pure β-sitosterol to the eluate of this band accentuated the peak proportionately which we identified as β-sitosterol in the gas chromatogram on the basis of retention time relative to 5α-cholestane. The recovery of the added amount of β-sitosterol was 99.6%. Comparison of the relative retention time (relative to 5α-cholestane) of the peak labeled as β-sitosterol (Fig. 2) to that of the pure β-sitosterol either as free sterol or as the trimethyl silyl ether derivative using SE-30 (3.8%) and QF-1 (2%) columns under similar conditions also supported the identification. The other band which moved slightly ahead of the cholesterol-containing band on the thin-layer plate contained lanosterol as tentatively identified by gas-liquid chromatography. The band which had an R_f value identical to cholesterol contained all the radioactivity. No radioactivity could be detected on the rest of the plate. In order to determine whether the radioactivity was present in the cholesterol molecule, cholesterol from this band was isolated as the dibromide following the method of Fieser (20). The precipitate was recovered and counted for radioactivity. The results indicated that all the radioactivity was present in cholesterol only.

The average 24 hr skin excretion of sterols other than cholesterol is shown in Table VI. The plant sterols, β-sitosterol, stigmasterol, and campesterol excretion in the skin surface lipids of normal men were 6.02, 0.35, and 0.24 mg/day. They constituted a total of 7.6% of the total skin sterol excretion. In hypercholesterolemic patients, the respective values were 7.39, 0.41, and 0.41 mg/day or a total of 9.0% of the total skin sterols. Lathosterol constituted 2.17 and 1.11% of the total sterols of the skin surface lipids of the normal and hypercholesterolemic patients, respectively. Similarly, lanosterol represented 1.16 and 0.91% of the total sterols in the respective groups. None of the differences in the sterol excretion between the groups were statistically significant. The unidentified sterols constituted 0.59 and 0.53% of the total sterols in normal subjects and Type II hypercholesterolemic patients, respectively.

DISCUSSION

It has been well documented that the skin can synthesize cholesterol from acetate (21–23). The cholesterol found in the skin surface lipids is believed to be derived primarily from biosynthesis by the keratinizing epithelium of the skin (6). Our finding of isotopic cholesterol in the skin surface lipids demonstrated that this labeled cholesterol was derived from the plasma cholesterol. The specific activity of the skin surface cholesterol gradually rose from zero as the plasma cholesterol specific activity was declining. The crossing of these curves at about the time that the specific activity of the skin surface cholesterol had reached its maximum value suggested a precursor-product relationship (24), i.e., the skin surface cholesterol was a product of the plasma cholesterol.

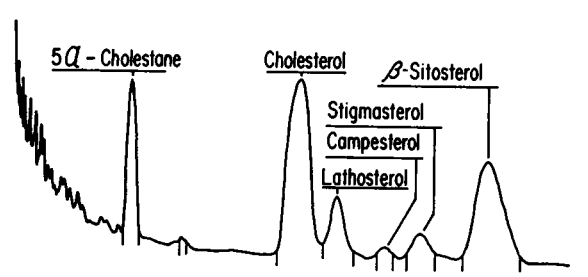

FIGURE 2 The gas-liquid chromatographic pattern of the band which had an R_f value identical to cholesterol on thin-layer plate. 5α-Cholestane was used as the internal standard.

TABLE VI

Excretion of Sterols, Other Than Cholesterol, in the Skin Surface Lipids of Normal Subjects and Hypercholesterolemic Patients

Subject	Lathosterol	Lanosterol	Plant sterols			Unidentified sterols
			Campesterol	Stigmasterol	β-Sitosterol	
Normal						
1	1.85±0.15	0.72±0.25	0.26±0.15	0.26±0.23	7.43±2.17	0.74±0.31
2	2.21±0.94	1.15±0.32	0.17±0.08	0.39±0.24	8.47±4.15	0.60±0.27
3	1.16±0.33	0.92±0.29	0.21±0.08	0.44±0.42	4.69±0.31	0.42±0.15
4	1.97±0.33	1.01±0.22	0.20±0.03	0.31±0.16	5.47±0.58	0.60±0.27
5	2.62±1.23	1.15±0.95	0.37±0.04	0.42±0.08	6.89±0.90	0.43±0.20
6	1.96±1.11	1.27±1.01	0.25±0.13	0.27±0.11	3.19±0.96	0.74±0.33
Mean ±SD	1.96±0.48	1.04±0.20	0.24±0.07	0.35±0.08	6.02±1.94	0.59±0.14
Hypercholesterolemic						
7	1.39±1.17	1.00±0.68	0.24±0.15	0.49±0.26	5.21±3.07	0.51±0.20
8	1.55±1.00	0.96±0.65	0.24±0.15	0.29±0.20	6.91±2.95	0.59±0.27
9	0.70±0.18	1.21±0.89	0.27±0.29	0.38±0.34	8.92±5.32	0.62±0.30
10	0.91±0.30	0.99±0.31	0.33±0.34	0.24±0.30	4.72±0.43	0.50±0.19
11	0.75±0.33	0.89±0.45	0.59±0.05	0.65±0.10	11.19±3.22	0.45±0.13
Mean ±SD	1.06±0.39	1.01±0.12	0.33±0.15	0.41±0.16	7.39±2.69	0.53±0.07

Values are mean ±SD in milligrams per day.

The latter could contribute to the former by either a process of exchange or by net transfer or by both. The identification of plant sterols in the skin surface lipids indicated that plasma sterols were transferred into the skin; this was also clearly net transfer of these sterols since they are not synthesized by mammalian tissues. Thus, the origin of cholesterol in the skin surface lipids is now considered to be from two possible sources: (*a*) from biosynthesis in the skin, and (*b*) from the plasma cholesterol. It is, however, difficult to quantify the exact amounts contributed by each source.

The precise amount of plasma cholesterol transferred to the skin surface and subsequently lost from the body pool could not be directly determined. However, the rate of sterol synthesis in the rat skin was only 3% of the hepatic incorporation rate (25). If the human skin has a similar rate of cholesterol synthesis, relative to that occurring in liver, it may synthesize only a small quantity of cholesterol, 20–30 mg per day. This comparison fits our hypothesis that the plasma cholesterol is a contributor to the skin surface lipids.

The amount of esterified cholesterol in the 24 hr skin surface lipids was higher than the free cholesterol content both in normal men and hypercholesterolemic patients. The ester cholesterol was about 87% of the total cholesterol content. A similar high content of esterified cholesterol in the skin surface lipids has been reported previously (26). Esterification of cholesterol has been shown to occur in the epidermis as well as in the skin surface film (27). The specific radioactivity of the ester cholesterol was higher than that of the free cholesterol at all times (Table VII). Also, the ester/free cholesterol ratio in the skin surface lipids was higher than that of the plasma in all subjects studied. These results suggest that plasma cholesterol is transferred or exchanged into the epidermal cells as either free cholesterol which is esterified later within the cells, or as esterified cholesterol directly. Maca and Connor found increased cholesterol ester deposition within cultured fibroblasts when the culture medium contained β-lipoprotein loaded with esterified cholesterol. They concluded that ester cholesterol accumulated in the fibroblasts both by direct transfer and by esterification of transported free cholesterol (28). Studies of the lipid composition of the xanthoma and xanthelasma suggest a similar view (29).

Cholesterol metabolism in man has been described in terms of two exchangeable pools, A and B (13). The two pool model is a mathematical concept and has no precise physical meaning. However, in general, the plasma, erythrocytes, liver, bile, and intestine comprise the rapidly turning over pool A. The remaining organs and tissues of the body, with the exception of the central nervous system, comprise pool B. Pool B exchanges very slowly with the plasma cholesterol. Synthesis occurs in both pools A and B. Dietary cholesterol enters initially only into pool A, and the loss of cholesterol from the system occurs predominantly through pool A. Our study demonstrated that equilibration of the specific activities of the skin surface cholesterol and the plasma cholesterol occurred 4–5 wk after the intravenous ad-

TABLE VII

Specific Activity of Free and Ester Cholesterol of the Skin Surface Lipids

	Wk after intravenous isotopic cholesterol								
	1	2	3	4	5	6	7	8	9
Normal									
1		139 (137)		148 (200)			145 (180)		104 (194)
2				120 (140)			82 (115)		66 (72)
3	60 (68)	74 (86)		82 (120)	93 (142)				96 (140)
4				142 (158)		109 (170)			
5		116 (149)			102 (168)		146 (172)		
6				129 (150)		107 (184)			122 (182)
Hypercholesterolemic									
7			92 (130)		158 (176)	104 (172)		110 (135)	
8	62 (68)	94 (162)			252 (660)	270 (520)			275 (480)
9							186 (310)		180 (300)
10								136 (168)	104 (120)
11								124 (168)	112 (152)

Values are in disintegrations per minute per milligram cholesterol.
Figures in parentheses indicate the ester cholesterol specific activity; these following the corresponding values for free cholesterol (not in parentheses).

ministration of isotopic cholesterol. This lag time places most of the skin cholesterol as a component of the more slowly turning over pool, pool B. According to the two pool model, the excretion of cholesterol from pool B is considered to be zero (13). In contrast, our results indicated that cholesterol is excreted through the skin surface in appreciable amounts. The calculations of various rate constants for the transfer of cholesterol from pool B to pool A depend upon the validity of the assumption that the direct excretion of cholesterol from pool B is zero. Our finding that the skin (a component of pool B) does excrete cholesterol introduces an additional possible problem in these calculations.

The fact that the specific activity of the skin surface cholesterol remained higher than that of the plasma 5 wk after the intravenous administration of isotopic cholesterol (Fig. 1 and Table V) suggested that the movement of cholesterol from plasma to skin is probably in one direction only for at least a considerable portion of the life cycle of an epidermal cell. In other words, once cholesterol is transferred to the skin from plasma, it may not subsequently exchange with plasma. Cholesterol, then, moves outward to the skin surface with the squamous epithelial cells. Since the epidermal layer of the skin is avascular, it is dependent upon diffusion of nutrients from capillaries of the dermis. The early appearance (by 7 days) of a small amount of radioactive cholesterol in the skin surface lipids indicated that the cholesterol exchanged or transferred from the plasma probably moves outward by diffusion into the epidermal cells. In the normal human skin, 27 days are required for the movement of epidermal cells from the basal layer to the shedding stratum corneum layer (22).

The presence of plant sterols in the skin surface lipids of man has not been previously reported. By gas-liquid chromatography, we have tentatively identified plant sterols in the lipids of the skin surface of man. It is of interest that the three plant sterols found in the skin surface lipids appeared identical in gas-liquid chromatographic patterns (retention times and relative amounts) to the same three plant sterols found in the feces: campesterol, stigmasterol, and β-sitosterol. The possibility that the quantities of these sterols which were found could have been derived from the clothing, the bandages, or the sponges used for the collection of the skin surface lipids or other extraneous sources have been ruled out. Since the body cells do not synthesize plant sterols, our data clearly document that plant sterols of the skin originated from the plasma. These sterols were transferred from the plasma to the skin surface lipids after being absorbed in small quantities from the diet. The disappearance of plant sterols from the skin surface lipids has been observed in a patient fed a plant sterol-free liquid formula diet (stripped lard being used as the sole source of fat providing 40% of the total calories). Furthermore, when this patient was given radioactive β-sitosterol orally, the radioactivity was detected by about 21 days in the skin surface sterols.[9] These results strongly support our view that plant sterols in the skin

[9] Bhattacharyya, A. K., and W. E. Connor. Unpublished observations.

surface lipids originate from the diet. Some 5% of the dietary β-sitosterol is absorbed in man (30). After being absorbed, β-sitosterol is widely distributed in all tissues including skin (31). Plant sterols have also been identified in human breast cancer tissue (32).

β-Sitosterol is excreted preferentially into bile and is also converted to bile acids. The major excretory pathway of β-sitosterol is considered to be the feces (30). The present investigation indicated that the excretion of β-sitosterol through the skin surface is a second excretory pathway. β-Sitosterol constituted about 7% of the total sterol excreted from the skin surface and was the second largest sterol component of the skin lipids. Furthermore, on the basis of 5% intestinal absorption of plant sterols, our subjects absorbed about 20 mg of plant sterols each day. The excretion of β-sitosterol through the skin was about 7 mg per 24 hr. Thus 33% of the calculated daily absorbed β-sitosterol was excreted into the lipids of the skin surface.

The finding of lathosterol in the skin surface lipids suggests cholesterol biosynthesis in the human skin occurs by the Kandutsch-Russell pathway (33, 34). Wilson pointed out that the fundamental difference between this pathway and that described by Bloch and colleagues (23) is that the former pathway utilizes only esterified intermediates whereas the Bloch pathway can utilize free intermediates (7). He demonstrated that in the rat both these pathways of the biosynthesis of cholesterol occur in the skin as well as in the preputial gland (7). The present study does not lead us to conclude, however, that the Kandutsch-Russell pathway is the only biosynthetic pathway present in the human skin. The presence of trace quantities of dihydrocholesterol and 7-dehydrocholesterol has been reported previously in the skin surface lipids of man (35, 36). The presence of the former has recently been shown by using a combination of silver ion-TLC and GLC methods (35), whereas the latter's presence was shown by ultraviolet absorption spectrum of the digitonin-precipitable sterols of the human skin surface lipids (36). Despite our use of the same technique, we could not identify these two sterols. Haati also could not detect these sterols in the human skin surface lipids (37). However, as mentioned earlier, only 0.59 and 0.53% of the total sterol excreted through the skin surface in the normal and hypercholesterolemic patients remained unidentified. These represented two peaks in the gas chromatogram obtained from the band which moved slightly ahead of the cholesterol band on the thin-layer plate and did not correspond to either dihydrocholesterol or 7-dehydrocholesterol.

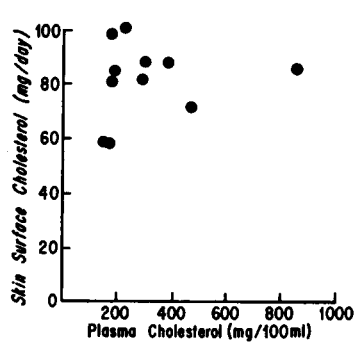

FIGURE 3 The relationship between plasma cholesterol level and amount of cholesterol excreted per day in the skin surface lipids of man.

The amount of cholesterol excreted per day through the skin had no apparent relationship to the level of plasma cholesterol (Fig. 3). Nestel, Whyte, and Goodman suggested that inadequate excretion of cholesterol could be of importance in the development of hypercholesterolemia (9). The excretion of cholesterol per day through the skin surface was similar in both normal and Type II patients. This indicated that inadequate excretion of cholesterol through the skin was not a factor in the hypercholesterolemia in Type II patients. The similarity in the skin surface cholesterol specific activities in normal subjects and hypercholesterolemic patients indicated no difference in the rate of biosynthesis of cholesterol in the skin between the groups. A higher rate of cholesterol biosynthesis in the hypercholesterolemic patients might have lowered the skin cholesterol specific activity relative to that of the plasma.

The daily loss of cholesterol from the skin surface was 1/4 to 1/6 of the daily total fecal neutral steroid excretion in man (2–5). The mean daily turnover of cholesterol determined by the sterol balance method was 876± 304 mg/day in a study by Grundy and Ahrens (2). This was lower by an average of 15% as compared to the values obtained by the kinetic analysis of the plasma cholesterol specific activity curves according to the two pool model in their same group of patients (2). Assuming an average loss of 82 mg of cholesterol per day through the skin surface in these patients the turnover of cholesterol becomes 958 mg/day. This average value is much closer to 1017 mg/day obtained by kinetic analysis of the two pool model in the same group of patients. In the two pool model, the overall metabolism of body cholesterol is taken into consideration. Hence, the cholesterol turnover data obtained by kinetic analysis of the two pool model includes the amount of cholesterol transferred to the skin from the plasma. This transfer of cholesterol from the plasma to the skin affects the slope of the plasma cholesterol specific activity curve. In the kinetic analysis of the plasma cholesterol specific activity curve by the two pool model, the slope of the curve determines the intercepts of the first and second exponents, C_A and C_B, respectively, in the equation:

$SA = C_A e^{-\alpha t} + C_B e^{-\beta t}$ (13). In sterol balance studies, the loss of cholesterol from the skin is not accounted for, although a significant amount is excreted and subsequently lost from the surface of the body as documented in the present investigation. In view of this and the possibility that the plasma cholesterol contributes to the skin surface cholesterol, we suggest that the daily loss of cholesterol from the skin surface may need to be considered in the sterol balance studies.

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