ISOTOPE KINETICS OF HUMAN SKIN CHOLESTEROL SECRETION*

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From 59 to 113 mg cholesterol is secreted daily by human skin, making up 1.5–9% of total skin surface lipids (1, 2). Part of the cholesterol originates from local de novo synthesis by the epidermis and the sebaceous glands (3), and since radioactive cholesterols appears on the skin surface after its intravenous administration (1, footnote 1), it is possible that another part results from secretion of plasma cholesterol through the skin. However, the relative contributions of these two potential sources to total skin surface cholesterol secretion are unknown. The purpose of the present study was to describe the kinetics of skin surface cholesterol after intravenous administration of radioactive cholesterol, and to develop methods for estimating the fraction of skin surface cholesterol that originates in the general circulation.

Materials and Methods

Patients and Their Diets (Table I). The studies were carried out on 10 patients during hospitalization on the metabolic ward at The Rockefeller University Hospital, New York. Clinical details, as well as diets and caloric intakes required for energy balance, are listed in Table I. The patients, one female and nine males, aged 39–72 yr, all had abnormal lipoprotein patterns except for patient 7. None had any skin disorder or history of serious skin disease. Eight of the patients (nos. 1–8) participated in a previous study (2) of sterol and squalene secretion by human skin. The diets consisted solely of liquid formula feedings (5); the detailed composition have been reported in our earlier paper (2).

Isotopic Cholesterol. From 65 to 160 uCi of either [4-^{14}C]- or [1,2-^{3}H]cholesterol or both were administered intravenously to each patient (Table I). These radioactive compounds, purchased from New England Nuclear Corp., Boston, Mass., were purified by thin-layer chromatography before use. 1 ml of ethanol solution of the isotope was dispersed in 150 ml of physiologic saline. The mixture was administered immediately after preparation.

Determination of Specific Radioactivities of Cholesterol. Venous blood for the determination of the specific radioactivity of cholesterol (SA)[2] was drawn biweekly. Plasma cholesterol was quantified (6) on the Auto Analyzer (Technicon Instruments Corp., Tarrytown, N. Y.) Radioactivities of aliquots of the isopropanol extracts of plasmas were counted in a Packard Tri-Carb (Model 3380) liquid scintillation spectrometer equipped with an automatic absolute activity analyzer (Model 544) (Packard Instrument Co., Downers Grove, Ill.).

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[1] Ahrens, E. H., Jr. 1965. Unpublished observations.

[2] *Abbreviations used in this paper:* f, skin surface cholesterol; SA, specific radioactivity.

TABLE I

Clinical Data

Patient no.	Initials, age, and sex	Height and weight	Calories*	Isotopic cholesterol	Formula diet‡ and inclusive dates of its feeding period	Diagnosis
		cm/kg		*dose/μCi*		
1	FG, 62, F	157/67	1,800	^{14}C, 95 ^{3}H, 106	I 0–80 days, III 81–147 days I 0–19 days, III 20–86 days	Hyperlipoproteinemia, type IV, § IHD‖
2	ThN, 39, M	180/85	2,700	^{14}C, 118	I 0–58 days, III 59–122 days	Hyperlipoproteinemia, type IIa, tendinous xanthomatosis
3	JG, 55, M	176/63	2,560	^{14}C, 96 ^{3}H, 97	I 0–59 days, III 60–145 days I 0–17 days, III 18–103 days	Hyperlipoproteinemia, type IIa, IHD, xanthelasma, hypertension
4	RH, 42, M	175/89	3,250	^{14}C, 102 ^{3}H, 131	I 0–66 days, III 67–148 days III 0–67 days	Hyperlipoproteinemia, type III
5	HS, 56, M	169/83	2,650	^{14}C, 65 ^{3}H, 113	I 0–45 days, III 46–99 days III 0–44 days	Hyperlipoproteinemia, type IV, IHD, hypertension
6	AG, 53, M	169/72	2,350	^{14}C, 143 ^{3}H, 85	IV 0–107 days, V 108–158 days IV 0–20 days, V 21–71 days	Hyperlipoproteinemia, type IV, generalized atherosclerotic vascular disease, xanthomatosis
7	AJ, 72, M	172/63	1,700	^{3}H, 130	VI 0–119 days	Normocholesterolemia, cerebrovascular disease, chronic obstructive pulmonary disease
8	SH, 46, M	178/75	2,100	^{14}C, 115	III 0–65 days	Hyperlipoproteinemia, type V, gouty arthritis
9	AM, 44, M	175/74	2,500	^{3}H, 105	II 0–105 days	Hyperlipoproteinemia, type II
10	SB, 52, M	171/86	3,000	^{3}H, 160	VI 0–98 days	Hyperlipoproteinemia, type IV

* Calories required to maintain constant body weight (for at least a month).

‡ Formula diets and time of diet change, dating from day of administration of isotopic cholesterol: I = 70% cottonseed oil (70% of total calories as fat); II = 40% cottonseed oil; III = 20% cottonseed oil; IV = 45% olive oil; V = fat-free, VI = 40% lard, cholesterol 400/mg/day.

§ Typing of serum lipoprotein pattern according to Fredrickson et al. (4).

‖ IHD, ischemic heart disease.

Skin surface lipids were collected by acetone swabs, from clothing, or by showering as described previously (2). Samples from the forehead, middle of the upper back, and anterior forearms were obtained by acetone swabs. Samples from the head were collected (*a*) by showering the head 24 h after a previous shower or (*b*) from caps worn by the patient and from pieces of cotton cloth used by the patient to wipe his face or neck. Samples from the upper and lower trunk (including arms and legs, respectively) were obtained by extracting shirts and bottoms, respectively. Methods for the fractionation of free and esterified cholesterol by digitonin precipitation, quantification of cholesterol by gas chromatography, and the determination of SA of skin surface cholesterol have been described previously (2).

Integration of the Specific Activity-Time Curves. The areas under the curved parts of the semilogarithmic SA-time plots were integrated manually by adding up the SA's of each individual day as determined from the curves. Areas under the log-linear parts were integrated by the use of the formula:

$$\int_{t_1}^{t_2} \mathrm{SA}\, dt = -\frac{0.434}{a}\,(10^{at_1 + \log b} - 10^{at_2 + \log b}),$$

where $\int_{t_1}^{t_2}\mathrm{SA}\, dt$ is the area under the curve from time t_1 to t_2 (days). a and b are members of the equation: $\log \mathrm{SA} = at + \log b$, depicting the log-linear part of the SA-time curve determined by regression analysis with an Olivetti Programma 101 calculator (Olivetti Underwood Corp., New York). It was assumed that beyond the observed points the curves continued log-linearly to infinite time.

Theoretical Consideration of Skin Cholesterol Secretion. It was the aim of this study to consider possible mechanisms of secretion of cholesterol through the skin and to develop methods for calculation of the fraction of skin surface cholesterol that is derived from plasma cholesterol. For this purpose we constructed a hypothetical model for cholesterol metabolism by the epidermis and sebaceous glands (Fig. 1), taking the following hypotheses and considerations into account:

(*a*) Compartments A and B of Fig. 1 represent the rapidly and slowly turning over pools, respectively, of body cholesterol (7). Pool A, the pool that is labeled with radioactive cholesterol, is assumed to consist of plasma, red cells, and liver, as well as of other viscera (7). Whole skin cholesterol equilibrates with plasma cholesterol only slowly (8), and a minimum of about 22% of skin cholesterol of the baboon has been calculated to belong to pool B (9). It has been assumed that no cholesterol leaves the body from pool B (7, 9). Since the bulk of a skin biopsy specimen is composed of dermis, we presume that it is this connective tissue layer that gives the whole skin the characteristics of pool B.

(*b*) The epidermis and the sebaceous glands are avascular, but the basal cell layers of both are separated from the rich capillary network in the dermal papillae and around the sebaceous glands, respectively, by only a thin basal lamina. Apparently the nutrition of these organs takes place by perfusion from the dermal capillaries. A fraction of plasma radioactive cholesterol carried by the perfusate will be taken up by the epidermis and the sebaceous glands (F_{CA} in Fig. 1). For this, the perfusate has to pass through a small amount of dermis, a part of which possibly belongs to the slowly turning over pool B, and thus the perfusate may carry to C some pool B cholesterol. However, because the amount of dermis that the perfusate has to pass is only a small fraction of the thickness of the total dermis and because only a fraction of cholesterol carried by the perfusate will be taken up by the epidermis and the sebaceous glands, it is likely that the amount of pool B cholesterol incorporated into C is negligible compared to the amount of cholesterol coming directly from plasma. Most of the cholesterol released by the dermis is presumably carried by the venous and lymph capillaries to pool A.

(*c*) Pool C is assumed to consist of all viable cells of the epidermis and the sebaceous glands. These cells arise from the basal cells and move towards the surface of the epidermis to form the horny layer of tightly packed keratinized dead cells (D in Fig. 1); in the sebaceous glands the cells move towards the center of the gland, where D is represented by disintegrated lipid-filled cells (sebum), which reach the skin surface through the sebaceous duct. On the skin surface, the sebum and the loose dead epidermal cells with their contained lipid mix with each other and form compartment E of Fig. 1.

The two main sources of cholesterol in C are (*a*) de novo synthesis (F_{CO}) and (*b*) plasma (F_{CA}). We suppose that cholesterol from both sources is taken up by C through incorporation into cell

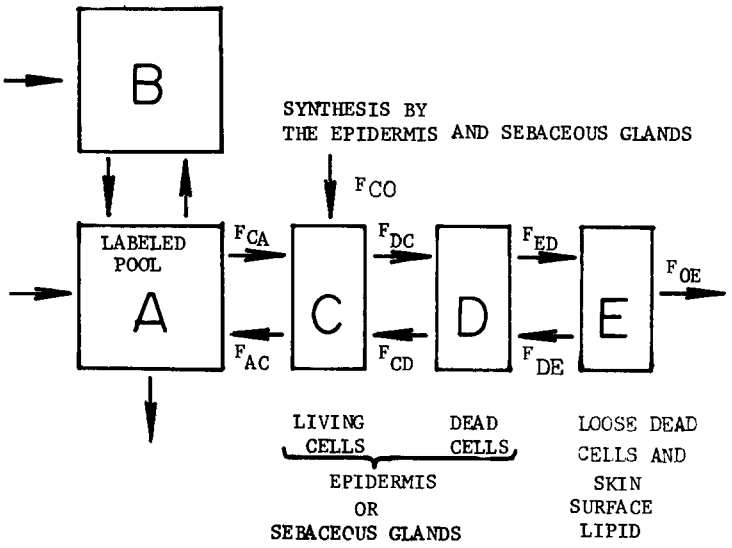

FIG. 1. Hypothetical model for the kinetics of skin surface cholesterol. A and B are rapidly and slowly turning over pools, as conceived by Goodman and Noble (7); a part of dermis is assumed to belong to pool B. Pool A is labeled by intravenous injection of a single dose of radioactive cholesterol, and pools A and E are sampled for the specific activity of cholesterol. In this model F is an absolute rate constant (mg/day). It is assumed a) that F_{DE} and $F_{CD} \ll F_{DC}$ and F_{ED}, and that $F_{CD} \ll (F_{CA} + F_{CD})$; and b) that labeled cholesterol from plasma and cholesterol synthesized de novo by the epidermis or sebaceous glands mix with each other before leaving C.

membranes and that it mixes before leaving C. As the cells move continuously away from the basal cell layer, the main direction of movement of cholesterol will be towards the surface. Since neither the epidermal (10) nor the sebaceous cells (11, 12) move at equal rates, cholesterol molecules starting at the same time at C will not appear in E as a single front but will show a statistical distribution.

Neither the rate of synthesis of cholesterol nor the rate of transfer of plasma cholesterol are necessarily similar in the epidermis and the sebaceous glands. A faster transit of cholesterol through the sebaceous glands is to be expected on the basis of the shorter average renewal time of the sebaceous gland cells (7.4 days, ref. 11) compared with that of the viable cells of epidermis of the upper arm (17.7 days, ref. 11). Moreover, since the thickness of epidermis varies from 3–4 cells to 10–12 cells (13), it is to be expected that the time taken for the plasma cholesterol to traverse the epidermis is different, depending on the body site. Thus, it is obvious that any calculations based on the model of Fig. 1 should be applied to small skin areas, and not to the total body skin surface. In the present study, forehead and head were selected as skin areas rich in sebaceous glands, anterior forearm as an area poor in sebaceous glands and with a thin epidermis, and the feet as an area poor in sebaceous glands and with a thick epidermis. On the trunk, the epidermis and the sebaceous glands can be expected to contribute in varying degrees to total skin surface cholesterol secretion.

(*d*) Since steroid hormones are absorbed percutaneously, an inward movement of cholesterol has to be assumed (F_{DE} and F_{CD} in Fig. 1), although we consider F_{CD} to be much smaller than either F_{CA} or F_{CO}.

Since the cells in C that incorporate and synthesize cholesterol move outwards, it is likely that the rate of transfer of cholesterol from A to C (F_{CA}) is greater than its transfer back to plasma (F_{AC}); in this case there will be net secretion of cholesterol from plasma through the skin onto the skin surface. However, it is also possible that $F_{AC} = F_{CA}$; and, because there is synthesis of cholesterol in C, F_{AC} can even be greater than F_{CA}. This means that there could be net movement of cholesterol from the epidermis or the sebaceous glands to plasma.

There are no methods available for individual determination of F_{CA}, F_{AC}, or F_{CO}. In this paper we will described methods for the estimation of $F_{CA}/(F_{CA} + F_{CO})$, a quantity that is equal to the fraction

of skin surface cholesterol that is derived from plasma (f). Descriptions of the principles involved in calculations based on three different methods by which these calculations were made are given at appropriate points in the Results section.

Results

SA-Time Curves of Plasma and Skin Surface Cholesterol

Measurable SA's were recorded on most skin areas (forehead, upper trunk, forearm) within 6–10 days after the i.v. administration of radioactive cholesterol. In patient 10 this occurred on the 4th day. Representative SA-time course curves are shown in Figs. 2 and 3.

Peak SA's were found on the forehead in 13–24 days (mean 19; n = 10 patients), on the back in 28–35 days (mean 31, n = 2), on the upper trunk in 35–47 days (mean 41, n = 3), on the anterior forearms in 35–40 days (mean 37, n = 2), and on the feet in 68–75 days (mean 71, n = 3). Thus, the SA curves rose more steeply on skin areas rich in sebaceous glands (forehead, back, upper trunk) than on the areas having a sparser distribution of these glands (forearm); they were also steeper on skin areas with a thin epidermis (forearm) than on areas with a thick epidermis (feet). These results suggest that the transit of plasma cholesterol through the skin is faster through sebaceous glands than through epidermis (even thin epidermis); with increasing thickness of epidermis the transit of cholesterol is longer.

After the peak specific activity at each sampling site was attained, which could be either below or above the plasma SA curve, the SA curves of skin surface cholesterol showed a tendency to become paralleled to the plasma curve (Fig. 2) and remain paralleled for the duration of our observation period (up to 203 days in patient 7). When the slope of the plasma SA curve changed due to dietary or drug

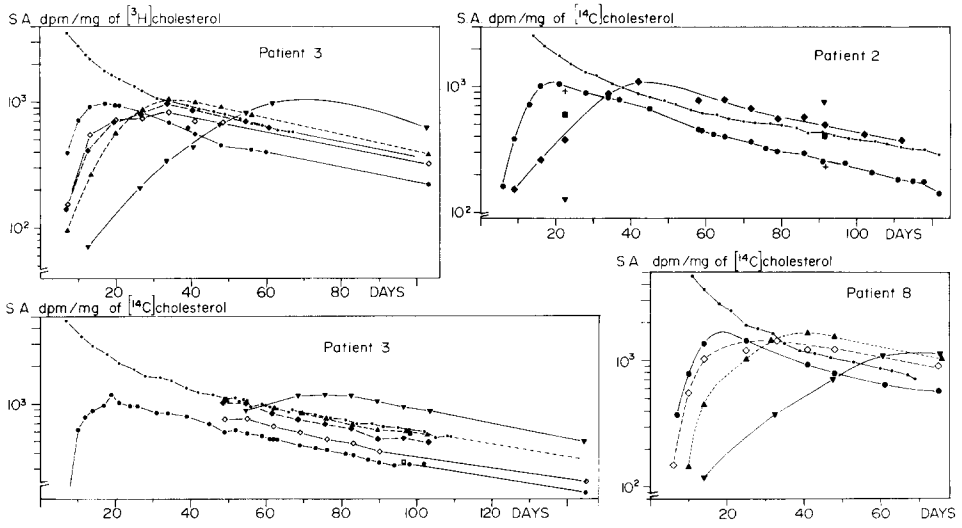

FIG. 2. Specific activities of plasma and skin surface cholesterol after i.v. administration of radioactive cholesterol in patients 2, 3, and 8. •, plasma; ●, forehead; ◇, back; ◆, upper trunk; ▲, forearms; ▼, feet; +□, head; ■, total body.

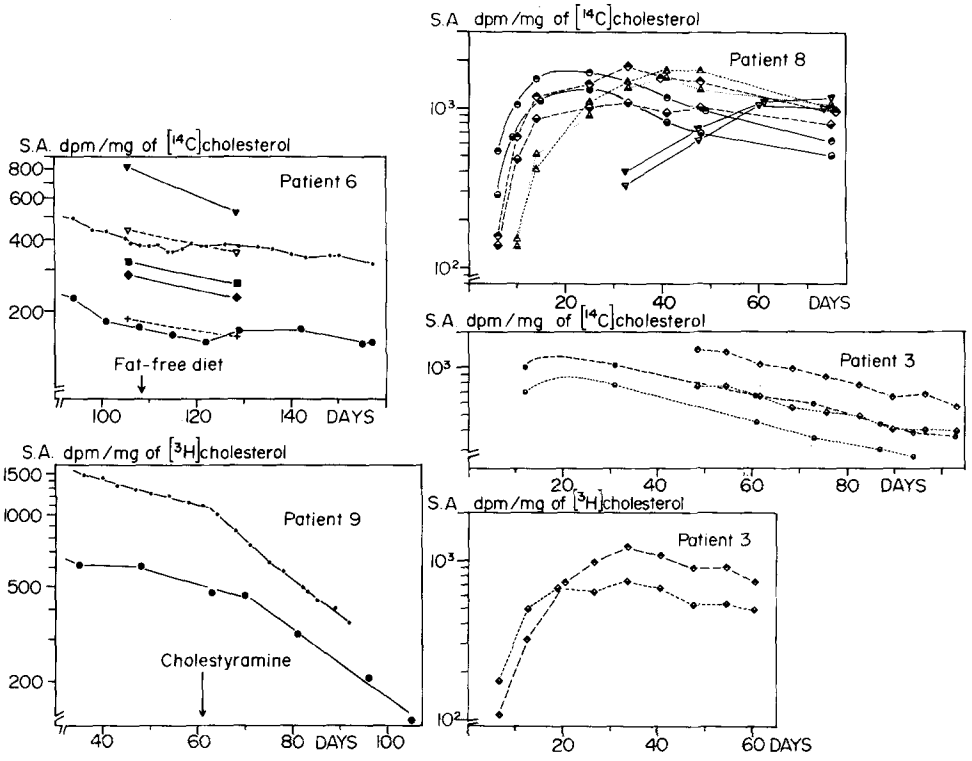

FIG. 3. *Left:* Specific activities of plasma and skin surface cholesterol after i.v. administration of radioactive cholesterol in patients 6 and 9. Patient 6: after change to a fat-free diet on day 108, the curves for plasma and forehead flattened. Patient 9: effect of cholestyramine treatment (20 g/d) begun on 61st day and maintained hereafter. •, plasma; ●, forehead; +, head; ◆, upper trunk; ∇, lower trunk; ▼, feet; ■, total body. *Right:* Specific activities of skin surface free and esterified cholesterol in patients 3 and 8. ⓔ◆△▽, free and ●◆▲▽, esterified cholesterol: ⓔ●, forehead: ◆◆, upper trunk (patient 3) or back (patient 8); △▲, forearms;▽▽, feet.

intervention, a similar change was found on the skin surface, but only after some delay (Fig. 3, patients 6 and 9). These results are in keeping with the assumption that plasma cholesterol is a direct precursor of skin surface cholesterol, bearing in mind that (*a*) it may take several weeks for cholesterol from plasma to pass the thickest epidermis and (*b*) the SA of plasma-derived cholesterol is diluted during its passage through the skin by the newly synthesized epidermal and sebaceous gland cholesterol.

The SA of skin surface free cholesterol was higher, as a rule, and that of esterified cholesterol lower than the SA of total cholesterol on all skin areas. On only three occasions did we record a higher SA in ester cholesterol; these exceptions occurred during the rising part of the SA-time curve (Fig. 3, right).

The difference between the SA's of free and esterified cholesterol was greatest on skin areas rich in sebaceous glands. 74 analyses on the SA's of free and esterified cholesterol were made in five of the patients at different times after the administration of the isotopes. The ratio of the SA of ester cholesterol to the SA

of free choleterol was lowest on the forehead and upper trunk (means of 0.63 and 0.64, respectively), and highest on feet and forearms (means of 0.88 and 0.92, respectively).

The Fraction of Skin Surface Cholesterol Derived From Plasma

The fraction of pool C cholesterol that is derived from plasma cholesterol is $F_{CA}/(F_{CA} + F_{CO})$. This fraction remains the same in all effluents of pool C irrespective of their magnitudes and, since we postulate no synthesis in either pool D or E, it equals the fraction of skin surface cholesterol derived from plasma. Were there no synthesis of cholesterol in pool C, SA of cholesterol in pool E (SA_E) would be equal to that of plasma cholesterol (SA_A). Lowering of SA_A with respect to SA_A will be proportional to the magnitude of F_{CO} as compared with F_{CA}; the fraction of skin surface cholesterol that is derived from plasma (f) is given by the ratio SA_E/SA_A. Were this a constant infusion experiment at isotopic equilibrium, the calculation of the SA ratio and hence f would represent no problems. However, since pool A was pulse labeled and its SA is continuously declining, and since cholesterol starting from A at time does not reach E until t + T (T = transit time), f cannot be calculated from simultaneous measurements of SA_A and SA_E. Rather, SA_A has to be measured in a plasma sample that has been taken T days earlier than the measurement of SA_E in the skin surface lipid sample. However, since T is not known, this poses some difficulty.

In the following paragraphs, three different methods are described that were used for the determination of f from SA_E/SA_A.

Method 1. According to Shipley and Clark (14), the fraction of any pool (i) arising from the labeled pool (a) can be determined from the ratio:

$$\frac{\int_0^\infty SA_i\,dt}{\int_0^\infty SA_a\,dt} \text{ i.e., } \frac{\text{the area under the SA curve of pool } i}{\text{the area under the SA curve of pool } a}$$

Thus, the fraction of the skin surface cholesterol in pool E that is derived from the labeled plasma pool A is obtained from

$$f = \frac{\text{area under the SA curve of pool E}}{\text{area under the SA curve of pool A}}.$$

The following mean values were obtained for f (Table II): forehead 0.36 ± 0.05 (n = 8 patients), back 0.55 (1), upper trunk 0.46 ± 0.11 (3), forearms 0.63 (1) and feet 0.61 (1). The smaller values of f on skin areas rich in sebaceous glands suggest that more of the cholesterol secreted by the sebaceous glands was synthesized de novo than in the epidermis. Furthermore, more of the cholesterol synthesized de novo was esterified cholesterol, whereas cholesterol derived from plasma was preferentially free cholesterol: this is indicated by the higher values of f in free cholesterol (0.48 and 0.41) than in esterified cholesterol (0.33 and 0.27) on the forehead of patient 3 and on the upper trunk of patient 4, respectively (these data are not included in Table II).

The use of Method 1 requires that the specific activity of skin surface cholesterol be determined regularly for long periods of time (3–4 mo). In view of the inconvenience of this under some circumstances, we recognized the need for an alternative approach, and therefore developed a method for the determination of f in which only one sample of skin surface cholesterol was required, provided a plasma SA time curve also was available.

Method 2. In this method a double isotope technique was used for determining the transit time (T) of cholesterol through the skin. With the aid of T a measured value of SA_E could be related to a corresponding value of SA_A, whereafter f could be determined from SA_E/SA_A. The theoretical considerations underlying this method are as follows:

If a single dose of [^{14}C]cholesterol in plasma is followed by a single dose of [^{3}H]cholesterol a few weeks later (Fig. 4, left), the ratio of the two isotopes (^{3}H/^{14}C) will keep declining for long periods and will not become level until

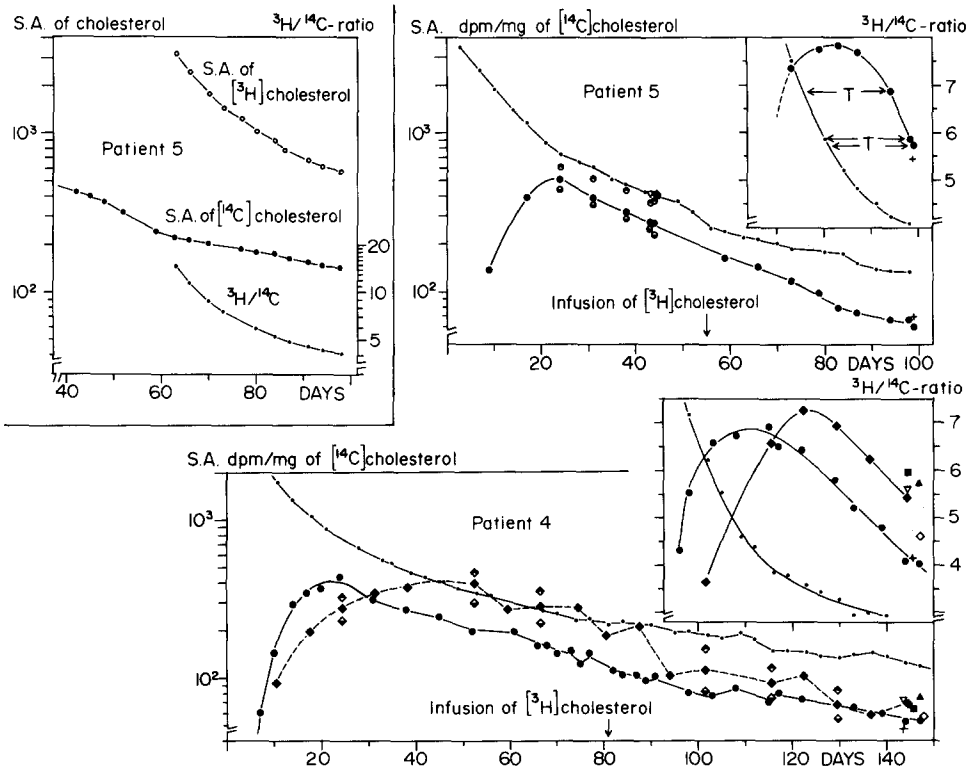

FIG. 4. Left: Specific activities (dpm/mg) and ^{3}H/^{14}C ratios of plasma cholesterol, after a single i.v. dose of [^{14}C]cholesterol at time zero and of [^{3}H]cholesterol at 55 days in patient 5. The ^{3}H/^{14}C ratio is related to the vertical scale on the right. Right: Specific activities of [^{14}C]cholesterol and ^{3}H/^{14}C ratio in plasma and different areas of skin surface after i.v. administration of [^{14}C]cholesterol at time zero and [^{3}H]cholesterol at the indicated times in patients 4 and 5. The inserts show the method for determining the transit time (T) of plasma cholesterol through the skin onto the skin surface. •, plasma; ●, forehead; +, head; ◇, back; ◆, upper trunk; ▽, lower trunk; ▲, arms; ▼, feet; ■, whole body (shower). ⊖◇, free cholesterol and ⊖◆, esterified cholesterol of forehead and upper trunk, respectively.

isotopic equilibrium has been reached between all exchangeable cholesterol pools of body. The fraction of plasma cholesterol leaving plasma on day t will have a certain $^3H/^{14}C$ ratio that is characteristic of that particular day, and during its travel through the epidermis and sebaceous glands the plasma fraction will maintain its characteristic $^3H/^{14}C$ ratio, provided F_{DE} and F_{CD} are negligible as it is assumed. Obviously, this ratio will not be changed by cholesterol arising in pool C from de novo synthesis. Thus, upon arrival at the skin surface (pool E) this ratio will still be the same as it was when the plasma fraction was incorporated into C some time (T) earlier. After having reached its maximum, the skin surface $^3H/^{14}C$ curve will run approximately parallel with the plasma $^3H/^{14}C$ curve and will be separated from it by T days (Fig. 4, right). Based on this principle, f can be determined in any skin surface cholesterol sample in which the $^3H/^{14}C$ ratio is clearly on the declining part of the skin surface $^3H/^{14}C$ curve: from the plasma $^3H/^{14}C$ curve and SA curve, the SA of plasma cholesterol (SA_A) having the same $^3H/^{14}C$ ratio as that in any single sample of skin surface cholesterol is derived graphically. Having thus determined the appropriate SA_A to relate to the determined SA_E, f is obtained as the ratio SA_E/SA_A.

The mean values for f as determined by Method 2 (Table II) were: forehead 0.39 ± 0.05 (n = 5 patients), head 0.36 ± 0.05 (4), back 0.43 (2), upper trunk 0.48 ± 0.15 (3), lower trunk 0.56 ± 0.14 (3), forearms 0.58 ± 0.15 (3), and feet 0.60 (1). The values for f obtained using Method 2 generally agree quite well with those determined by the Method 1 (Table II). Also, the f values of free (0.45) and esterified cholesterol (0.29) detemined by Method 2 for the upper trunk of patient 4 are similar to the corresponding values determined by Method 1 (0.41 and 0.27, respectively). In the same patient, f of free and esterified cholesterol in shower water was 0.39 and 0.27, respectively, and in samples from the head in three patients 0.47 and 0.30, respectively.

The mean values for transit time (T) of plasma cholesterol through the skin were: forehead 24 days, head 22 days, back 27 days, upper trunk 30 days, lower trunk 33 days, forearms 38 days, and feet 72 days. The transit time of free cholesterol was 1–4 days longer than that of esterified cholesterol in the six samples measured.

Method 3. In patient 6 there was flattening of the plasma SA time curve due to a change from 45% olive oil to fat-free formula (Fig. 3). This was followed by flattening of the skin surface (forehead) cholesterol curve about 10 days later. The situation resembled that during a constant infusion experiment: the SA's of the plateaus were then directly comparable, and f could be calculated as a ratio of the specific activities. The value of f obtained by this method (0.44) agrees well with that determined by Method 2 (0.41).

The Amount of Plasma Cholesterol That Is Secreted Through The Skin Onto The Skin Surface (F)

In patients 3 and 4 it was possible to calculate the fraction of total skin surface cholesterol that is derived from plasma; it was 0.49 and 0.37, respectively (Table II). The daily secretion of cholesterol by the total skin, according to the previous study (2) was 59 mg in both patients. Thus, the amounts of skin surface

TABLE II

*The Fraction of Skin Surface Cholesterol Derived from Plasma Cholesterol (f)**

Patient no.	Method‡	Forehead	Head	Back	Upper trunk	Lower trunk	Forearms	Feet	Whole body§
1	1	0.43	—	—	—	—	—	—	—
	2	0.46 ± 0.03 (6)	—	—	0.68, 0.59	0.68, 0.64	0.70 ± 0.05 (4)	—	—
2	1	0.58	—	—	0.52	—	—	—	—
3	1	0.41	—	0.55	0.53	—	0.63	0.61	—
	2	0.41 ± 0.02 (7)	0.34, 0.32	0.53	0.46	0.62	0.64	0.60	0.49
4	1	0.29	—	—	0.33	—	—	—	—
	2	0.33 ± 0.02 (6)	0.29	0.33	0.35 ± 0.04 (3)	0.40	0.41	—	0.37
5	1	0.32	—	—	—	—	—	—	—
	2	0.36 ± 0.02 (4)	0.40, 0.41	—	—	—	—	—	—
6	2	0.41 ± 0.04 (5)	0.41	—	—	—	—	—	—
	3	0.44	—	—	—	—	—	—	—
7	1	0.35	—	—	—	—	—	—	—
9	1	0.34	—	—	—	—	—	—	—
10	1	0.37	—	—	—	—	—	—	—
Mean	1	0.36 ± 0.05 (8)	—	0.55 (1)	0.46 ± 0.11 (3)	—	0.63 (1)	0.61 (1)	—
	2	0.39 ± 0.05 (5)	0.36 ± 0.05 (4)	0.43 (2)	0.40 ± 0.15 (3)	0.56 ± 0.14 (3)	0.58 ± 0.15 (3)	0.60 (1)	0.43 (2)

* Means ± standard deviations (n in parentheses).

‡ Method 1: Area of the skin surface cholesterol SA-time curve as fraction of the area of the plasma cholesterol SA-time curve.
Method 2: SA of skin surface cholesterol at a given $^{3}H/^{14}C$-ratio as fraction of the SA of plasma cholesterol having the same $^{3}H/^{14}C$-ratio (see Fig. 4).
Method 3: Mean SA of a plateau in the skin surface cholesterol SA curve as fraction of the mean SA of a corresponding plateau in the plasma cholesterol SA curve (see Fig. 3, patient 6).

§ Calculated by combining the results for mass of skin surface cholesterol collected from different areas of the skin (ref. 2) and the values of f recorded in the present study for the same areas by method 2.

cholesterol that originated in plasma (F) were 0.49 × 59 mg = 29 mg and 0.37 × 59 mg = 22 mg, respectively. If it is assumed that there was no reflux to plasma of cholesterol from the epidermis or the sebaceous glands (i.e. F_{AC} in Fig. 1 is equal to zero), the values of F equal the daily net secretion of plasma cholesterol through the skin onto the skin surface. However, since we do not know the magnitude of F_{AC}, the above figures give only the maximum daily net secretion rate of plasma cholesterol through the skin. For instance, if $F_{AC} = 0$, the net secretion of plasma cholesterol in patient 4 will be 22 mg/day (the synthesis by the skin contributing another 27 mg). If $F_{AC} = F_{CA}$ and both are equal to 35 mg/day, the net secretion of plasma cholesterol through the skin will be equal to zero, although the daily secretion of cholesterol by the total skin (59 mg), and the values of f (0.37) and F (22 mg) remain the same (F_{CO} will be 59 mg in this case).

Discussion

The model shown in Fig. 1 was constructed to facilitate the discussion of mechanisms of cholesterol secretion through the skin rather than to give a purely kinetic model. The two potential routes of cholesterol secretion by the skin, i.e., the epidermis and the sebaceous glands, were included in the same model for two reasons. (*a*) The two mechanisms are basically similar in starting with dividing basal cells, which, while differentiating and synthesizing cholesterol, move towards the skin surface and finally die, forming the two major sources of skin surface lipids: the sebum and the desquamating epidermal cells. (*b*) Since only plasma and skin surface can be sampled without surgical procedures, it is not possible to differentiate between cholesterol traveling through the epidermis on one hand and through the sebaceous glands on the other.

After intravenous injection of radioactive cholesterol in our 10 patients the earliest detectable radioactivities were found on the skin surface as early as 4–6 days. Since the minimum time for a sebaceous basal cell to reach skin surface is 4–7 days (12) and the minimum transit time for an epidermal basal cell is 24 days (15), it is probable that the earliest radioactivities on the skin surface were due to cholesterol passing through the sebaceous glands. This also was suggested by the earlier peaks of cholesterol SA and shorter transit times from areas rich in sebaceous glands. Transit time must also be a function of skin thickness. The average transit time of cholesterol through the assumingly four to five cell layers thick anterior forearm was 38 days, which agrees with the values (40–56 days) predicted by Halprin (15) as the time taken by an epidermal basal cell to travel to the surface of an epidermis six to seven cells thick. The long transit time of 72 days of cholesterol in the feet would similarly be caused by its travel through a cell layer eight to nine cells thick, which indeed may represent an average for the feet, since the soles have 10–12 cell layers (13). It is theoretically possible that a fraction of cholesterol may be able to penetrate the skin without having been taken up by the cells, but this seems unlikely because of the tight junctions of the horny layer cells in the epidermis.

Although the shapes of the skin surface cholesterol SA-time curves suggest that plasma cholesterol is a direct precursor of skin surface cholesterol, in only a few cases did the skin surface SA-time curves actually intersect the corresponding

plasma curve at its maximum, a criterion frequently used for precursor-product relationships according to Zilversmit (16). Indeed, the forehead curves never crossed the plasma curves. The failure of our curves to obey the Zilversmit rule is apparently due (*a*) to the fact that skin surface cholesterol has two precursors, plasma cholesterol and cholesterol synthesized de novo locally, in which case the rule does not apply (16), and (*b*) to the delay between pools A and E.

Our method 1 for the determination of the fraction of skin surface cholesterol that is derived from plasma cholesterol (f) is theoretically sound and is not dependent on any consideration of routes of cholesterol secretion through the skin, whether through pool B or directly from plasma, or on the way cholesterol is transported through the skin, whether largely incorporated into the cells or diffusing freely. The use of method 2, on the other hand, ideally requires that cholesterol molecules starting at the same time from plasma move through the skin as a single front. This does not occur, however, because: (*a*) the transit of cholesterol through the sebaceous glands is shorter than through epidermis and (*b*) neither in the sebaceous glands nor in the epidermis do the cells move "as soldiers in a row but rather as a band of stragglers" (10, 11). The inhomogeneity of the skin is the probable reason for the tendency of the transit times, as determined by method 2, to lengthen somewhat with time (as in Fig. 4) on areas that contain both sebaceous glands and epidremis: to begin with, the transit time apparently reflects the transit of cholesterol through the sebaceous glands, but later, its transit across the epidermis. Despite these theoretical weaknesses, method 2 gave quite similar values for f to those obtained by the more reliable method 1. We suggest, then, that method 2 is useful and reliable when method 1 can not be applied because of its tediousness. The use of method 3 requires that there is plateauing of the plasma SA curve that is followed by a similar leveling of the skin surface curve. This resembles the situation created by a constant infusion of radioactive cholesterol. Despite the danger that metabolic steady state assumption may have been violated by the intervention responsible for the plateauing, the value obtained by this method agreed well with that obtained by method 2.

The fraction of skin surface cholesterol that is derived from plasma was always lower on skin areas rich in sebaceous glands than on areas poor in these glands; this indicates that of the cholesterol secreted by the sebaceous glands a higher proportion (at least 61–64%) is synthesized de novo than of the cholesterol secreted by the epidermis (37–42%). On the other hand, f of free cholesterol was always higher than that of esterified cholesterol, which indicates that cholesterol derived from plasma is preferentially in free form and that a higher proportion of the skin surface esterified cholesterol (70–73%) than of free cholesterol (53–61%) is synthesized de novo.

Although the sterol content of the sebaceous secretion is rather low (1.5% on the forehead, ref. 2) compared with that in the lipids of the desquamating epidermal cells (8.8% on feet), the total cholesterol secretion through the sebaceous glands may be equal to or even greater than that by the epidermis. This is indicated by our previous findings (2) in two patients, in whom 35 and 44% of the total daily skin cholesterol secretion was obtained from the head, which forms only 6–9% of the total skin area but is rich in sebaceous glands.

Since we have no means of assessing the magnitude of reflux of cholesterol from the skin back to the general circulation (F_{AC} in Fig. 1), we are unable to reach a definitive estimate of the amount of net secretion of plasma cholesterol through the skin onto the skin surface through the use of measurements of plasma cholesterol radioactivity on the skin surface. Although it is likely that movement of cholesterol through the skin is chiefly unidirectional, it is theoretically possible that the mass of radioactive cholesterol passing to the skin from the plasma is equal to or even smaller than the mass of cholesterol returning from skin to plasma. Since a part of the skin cholesterol returning to plasma would be unlabeled because it was synthesized by the epidermis and sebaceous glands, radioactive cholesterol could appear on the skin surface due to "isotope exchange" even if there were no net secretion of plasma cholesterol. Thus, the skin surface radioactivity data allow us to calculate only the maximum rate of plasma cholesterol secretion through the skin, and for that calculation we must assume that there is no reflux of epidermal or sebaceous gland cholesterol back to pool A (i.e., $F_{AC} = 0$ in Fig. 1).

Using the values for the fraction of total skin surface cholesterol derived from plasma (f), we were able to estimate the maximum rate of net secretion of plasma cholesterol through the skin onto the skin surface in patients 3 and 4; it amounted to 29 and 22 mg/day (mean 25 mg), respectively. In these two patients the f-values for total skin surface agreed within 8% with those of forehead cholesterol obtained by method 1 (Table II). Assuming this correspondence to be generally true for all other patients, we calculated F from the forehead data for f for four patients (nos. 1, 2, 5, and 6) whose secretion rates of total skin surface cholesterol has been determined previously (2): the mean maximum amount of plasma cholesterol secreted daily onto the skin surface was calculated to be 35 ± 8 mg in the six patients. The secretion of neutral and acidic steroids in stools was 1,100 mg/day in patient 3 and 1,290 mg/day in patient 4; in all six patients the mean secretion rate was 1,100 mg/day. Assuming a total daily urinary steroid excretion of 50 mg, we estimate that the maximum amount of steroid secreted through the skin is 2.5% of the total steroid excretion of the body in patient 3, 1.7% in patient 4, and 3.2% in all the six patients.

Summary

Specific radioactivity (SA) time curves of plasma and skin surface cholesterol collected at several skin areas were recorded in 10 patients on formula diets after single intravenous injections of radioactive cholesterol. Earliest detectable radioactivity on skin surface was found in 4–6 days; depending on the skin site, SA's peaked in 13–75 days. SA's of free cholesterol were almost always higher than those of esterified cholesterol.

The general forms of the SA time curves were in keeping with the idea that plasma cholesterol is carried to the skin surface in association with the epidermal and sebaceous cells, whereby (*a*) cholesterol synthesized de novo is mixed with that derived from plasma and (*b*) appearances of plasma cholesterol on the skin surface is delayed by a time factor that corresponds to the movement of epidermal and sebaceous cells from the basal layer to the skin surface. The shorter mean transit times of plasma cholesterol on skin areas rich in sebaceous

glands (22–24 days on the head) than on those poor in these glands (38 days on forearms and 72 days on feet) suggest that cholesterol passes faster through the sebaceous glands than through the epidermis, and faster through thin than thick epidermis.

The fraction of skin surface cholesterol (f) that is derived from plasma cholesterol was estimated by three independent methods, and comparable results were obtained. Values of f were lower on skin areas rich in sebaceous glands (0.29–0.46 on forehead) than on areas poor in these glands (0.41–0.70 for forearms; 0.60 on feet) and lower for esterified (0.27–0.33) than for free (0.39–0.48) cholesterol. These data suggest that higher proportions of sebaceous gland and of esterified cholesterol, respectively, are synthesized de novo than of epidermal and of free cholesterol.

In two patients it was possible to calculate that f of total skin surface cholesterol was 0.49 and 0.37, respectively, and that the maximum amount of plasma cholesterol lost through the skin was 29 and 22 mg/day, respectively. Knowing the total daily excretion of total neutral and acidic steroids in feces in these patients, and assuming a total daily urinary steroid excretion of 50 mg, we estimated that no more than 3.2% of total steroid excretion occurred via the skin.

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