THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 50, No. 2

*Printed in U.S.A.*

# METABOLIC STUDIES ON SKIN

## III. LIPID METABOLISM IN MOUSE SKIN DURING THE HAIR GROWTH CYCLE*

S. C. BROOKS, PH.D., L. K. LANGS, B.S. AND V. C. GODEFROI, M.S.

Extensive morphological, histochemical, and chemical studies have been carried out with mouse or rat skin in the various phases of the hair growth cycle (1–11). These studies have shown extensive cellular changes in the skin throughout the 22-day cycle. For example, the number of epidermal cells increases two or three times during the first seven days of the growth phase (anagen). Simultaneously the hair germ develops into a bulb which descends through the corium into the subcutaneous fat. On the sixth or seventh day of anagen, hair growth begins followed by a concurrent reduction of epidermis to its previous thickness. When the hair growth ceases (catagen, 19th day) the follicle shortens, coming to rest high in the corium and thus entering the resting phase (telogen, 22 days).

Significant increases in the enzymes cytochrome oxidase, succinic dehydrogenase, and esterase have been shown to occur on the fourth day of the cycle (3–5). Concurrent increases in the epidermal ribonucleic acid (5), phospholipids (6), and cholesterol (6) have been reported. A thorough study of the lipid content of mouse skin throughout the hair cycle has been carried out by Carruthers (6–10). In addition, changes in the number of mast cells during the cycle is reflected in the varying concentrations of histamine and serotonin in the skin (11).

An insight into the fluctuating metabolic patterns during this 22-day period would be of interest especially in view of these dramatic cellular and enzymatic changes. Through these studies it is hoped that some knowledge may be gained concerning the endogenous control of the hair growth cycle.

The experiments presented here have been designed to investigate the lipid metabolism of mouse skin in the various stages of the hair cycle.

This investigation was supported in part by USPHS Research Grant No. CA 02903 from the National Cancer Institute, General Research Support Grant No. FR 05529 from the National Institutes of Health, and an institutional grant to the Detroit Institute of Cancer Research Division of the Michigan Cancer Foundation from the United Foundation of Greater Detroit.

Accepted for publication July 20, 1967.

* From the Detroit Institute of Cancer Research Division of the Michigan Cancer Foundation, and Wayne State University School of Medicine, Department of Biochemistry, Detroit, Michigan 48201.

### METHODS

Female $A_f$/Sp mice six to eight weeks of age were used for all experiments. The mice were maintained at constant temperature and humidity, kept in stainless steel cages, and fed Purina chow *ad libitum*. In order to minimize the possibility of seasonal effects, the data were pooled from four separate experiments, which were conducted over a period of 12 months.

Synchronization of the hair growth cycle of the experimental mice was accomplished by first plucking the hair from the dorsal region. Mice whose dorsal skin was in the telogen phase, as determined by the painless ease with which the hair was removed, were kept for experimentation. After 22 days, the hair was again plucked from the dorsal region to initiate the hair cycle to be investigated.

At various times during the experimental hair growth cycle, the mice were over-anesthetized with ether and the dorsal skin was shaved with an electric clipper if necessary. The skin was removed, cut into rectangular patches, measured, and the panniculus adiposis and panniculus carnosis were scraped off with a dull scalpel. The skins from two mice were pooled for incubation (ca. 0.25 g), cut into small pieces and placed in Warburg flasks. In addition to the skin, each flask contained 2 ml Krebs-Ringer phosphate buffer, 10 mg of glucose, and acetate-1-$C^{14}$ (10.0 mC/mM). The incubations were carried out for 3 hours at 37° C under oxygen.

The $CO_2$ produced during the incubation was collected on an alkaline filter paper placed in the center well and prepared for radioactive assay according to the procedure of Popjak and Tietz (12).

The contents of the incubation flasks were saponified by refluxing under nitrogen with 15% KOH in 50% aqueous ethanol for 8 hours. The nonsaponifiable lipids were obtained with 4 petroleum ether extractions of the alkaline mixture and the radioactivity measured. These lipids were

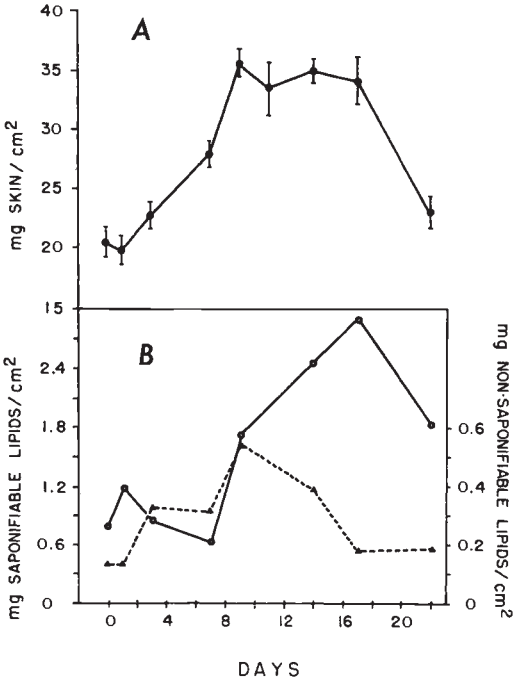


FIG. 1A. Weight of one square centimeter patches of mouse dorsal skin at various days of the hair growth cycle. Brackets over each point indicate the standard error of the determinations on 5 groups of 4 mice each.

FIG. 1B. Mass of saponifiable lipids (○——○) and nonsaponifiable lipids (▲- - -▲) contained in one square centimeter patches of mouse skin during the hair growth cycle. Each point represents the data from the pooled skins of six mice.

converted to their trimethylsilyl (TMS) ether derivatives in preparation for analysis on gas-liquid chromatography (GLC) (13).

The saponifiable lipids were extracted from the acidified hydrolysate with 3 diethyl ether extractions. The extract was washed with water to remove any extracted acetate-1-$C^{14}$ and the fatty acids were esterified with $BF_3$ in methanol (14). The radioactivity was measured in a liquid scintillation spectrometer.

Gas-liquid chromatography of the lipids was carried out on 6 ft glass U-tubes packed by the filtration method (15) with Chromosorb W (60–80 mesh) coated with diethylene glycol succinate polyester (DEGS). The effluent gas stream was split so that 8% passed to the detector and 92% to the collector. Collections were made at the specified time intervals in 0.6 mm i.d. stainless steel capillaries (16), which were then eluted with the appropriate toluene scintillation solution for monitoring in a liquid scintillation spectrometer. Labeled lipids, which were present in concentrations too low for detection by the mass detector, were located by plotting the radioactivity recovered each minute (17).

Previous investigations in this laboratory have shown that the collection of fatty acid methyl esters and sterol TMS ethers from GLC was consistent and reproducible within a ±5% standard deviation for quantities between 1.5 and 50 μg (17). The efficiency of collection was determined routinely for each experiment by chromatographing standard solutions of labeled methyl palmitate or cholesterol TMS ether. These collection values were used to correct for column loss in calculating the radioactivity present in experimental fatty acid and sterol samples.

The $Sr^{90}$ ionization detector (5 ml) was calibrated with standard fatty acid methyl esters and sterol TMS ethers. The methyl esters were obtained from Applied Science Laboratories, Inc. (State College, Pa.), Lachat Chemicals, Inc. (Chicago, Ill.), and the Lipid Distribution Program of the National Institutes of Health (Bethesda, Md.). Cholesterol and $\Delta^7$-cholestenol were prepared as described previously (16). The 7-dehydrocholesterol was obtained from Nutritional Biochemical Corp. (Cleveland, Ohio) and recrystallized to constant melting point. All solvents were redistilled before use.

## RESULTS AND DISCUSSION

*Skin weight.*—Inasmuch as the extreme morphological changes in skin during the hair cycle are reflected in skin thickness and weight (1), comparisons of metabolic data from day to day are best expressed on an area basis (Reference 18 contains a detailed discussion of the problem of expressing metabolic data derived from skin in different hyperplastic states). Figure 1 depicts the 75% increase in wet weight of one square centimeter patches of mouse skin. This maximum increase is reached after the first week of the cycle and is maintained until catagen beings. Concurrent increases in the dermal collagen and adipose tissue have been reported (1). Prior to this weight plateau, the epidermis has become 2 or 3 times thicker and returned to "normal" thickness. The first week of anagen also is characterized by a 10–20% increase in water content (1).

The total lipid mass of the skin during the growth period has been shown to remain essentially unchanged at the 12th and 22nd day, but decreased at the 4th day (6). Figure 1 presents data on the weight of the saponifiable and nonsaponifiable lipids extracted from one centimeter of skin at the various stages of hair growth. The weight per unit of skin area of the nonsaponifiable lipids is shown to change with hair growth as does the skin's gross weight per unit area (Fig. 1). Saponi-

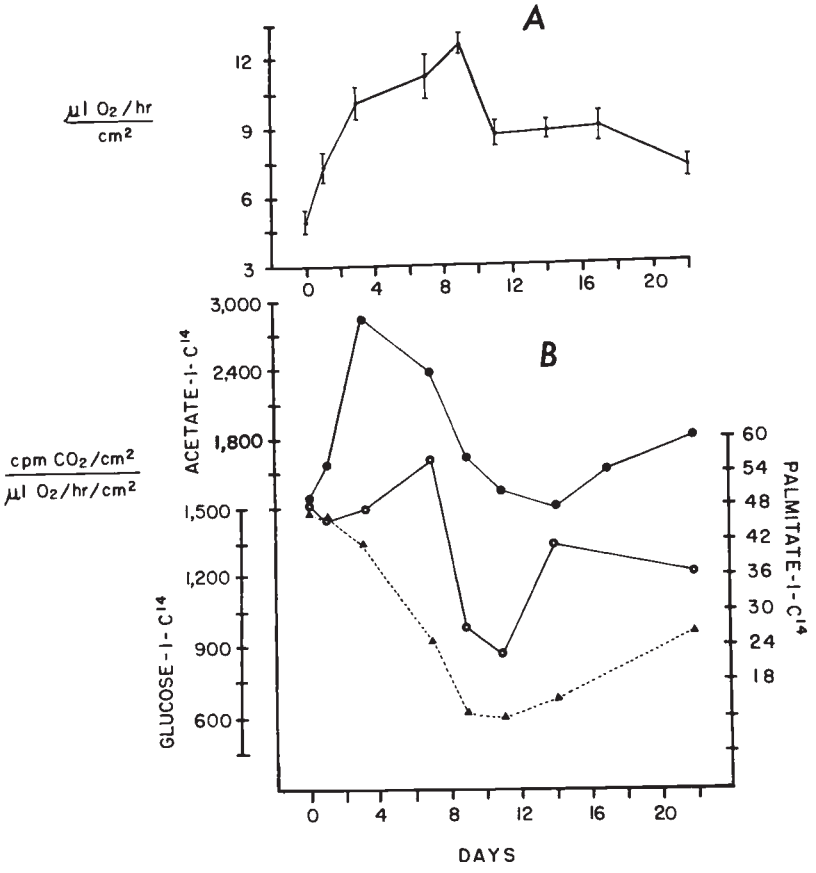


FIG. 2A. Oxygen consumption of one square centimeter patches of mouse dorsal skin incubated at various days of the hair growth cycle. Brackets over each point indicate the standard error of the determinations on 12 groups of 2 mice each.

FIG. 2B. Ratio of the counts/min in $CO_2$ to the oxygen consumption of one square centimeter patches of dorsal mouse skin incubated at various days of the hair growth cycle. The labeled substrates in each experiment were: 1 $\mu$C acetate-1-$C^{14}$, 10.0 mC/mM, ●——●; 0.5 $\mu$C glucose-1-$C^{14}$, 2.9 mC/mM, ▲-----▲; and 0.23 $\mu$C palmitate-1-$C^{14}$, 2.4 mC/mM, ○——○. Radioactivity was measured as $BaCO_3$ on planchets using a gas-flow Geiger tube (efficiency 25%) and the values corrected to an infinitely thin layer.

fiable lipids, on the other hand, display a strikingly different response to the growth cycle by decreasing in total weight during the first week of the cycle followed by an increase paralleling the growth of new hair. The greater concentration of fatty acids at the end of the growth cycle reflects the 10% excess of skin weight at 22 days over 0 day and may be representative of the hair shaft and bulb which remain in the follicle after clipping.

*Lipid metabolism.*—The oxygen consumption of the skin patches increased 2.6 times over the period of the first 9 days of hair growth (Fig. 2). The microliters of oxygen utilized by the skin decreased sharply between 9 and 11 days and are shown to be approximately 30% greater at 22 days than at the start of the cycle. Based on the incorporation of labeled acetate, the synthesis of both saponifiable and nonsaponifiable lipids, on the other hand, increased 19 times by the third to ninth day (Fig. 3). Lipid synthesis, therefore, responds to the initiation of hair growth to a much greater extent than does oxygen consumption, a parameter of the general metabolism. It is noteworthy that the period of the greatest synthesis of saponifiable lipids corresponds with the lowest concentration of these lipids during hair growth.

*Fatty acid synthesis.*—Gas-liquid chromatographic analysis of the cutaneous fatty acids extracted from the skin following incubation

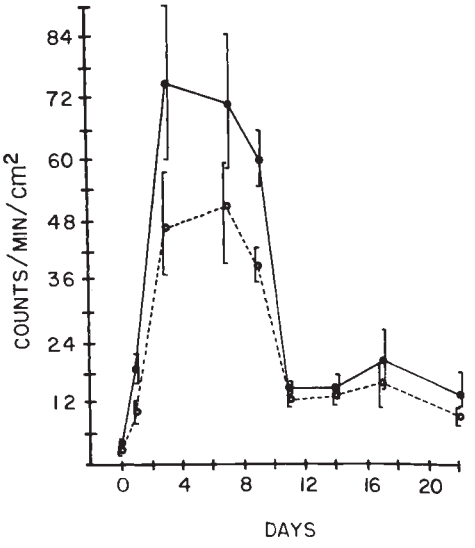


FIG. 3. Incorporation of labeled acetate into saponifiable (●——●) and nonsaponifiable (○- - -○) lipids by one square centimeter patches of dorsal mouse skin incubated at various days of the hair growth cycle. One microcurie of acetate-1-$C^{14}$ (10.0 mC/mM) was added to each incubation tube. Brackets over each point indicate the standard error of the determinations on 10 groups of 2 mice each.

showed that all the acids studied decreased in concentration between the first and seventh day after initiation of the hair growth cycle, and then increased to a level at the start of telogen which was approximately twice as great as the concentration at 0 day. As mentioned above, this difference in fatty acid content in the telogen stage was representative in part of the hair shaft and bulb which were present at 22 days but which had been removed at 0 day. Figure 4 shows the changes in concentration of the major acids in skin. The line graphs in Figure 4 illustrate the depletion of the acids at 7 days after an initial increase in concentration during the first 24 hours. The concentration of each fatty acid rose sharply at 9 days, reached a maximum between 14 and 17 days, and diminished by the start of telogen. The loss of fatty acids in the latter stages of anagen was reflected in a final rise in specific radioactivity and may have been caused by the removal of lipids with the clipped hair. Significant decreases in the skin content of palmitic (16:0), oleic (18:1), and linoleic (18:2) acids occurred by the seventh day. Lesser changes in the concentration of myristic (14:0) and stearic (18:0) acids are shown.

Table I records the specific radioactivities of the fatty acids isolated from the skin incubations at the various stages of hair growth. Each acid reached its maximum specific activity between 3 and 7 days of the growth cycle. Specific radioactivities increased from 10 to 30 times by the third day for the saturated acids: myristic, palmitic, eicosanoic (20:0), heneicosanoic (21:0), and docosanoic (22:0). Stearic acid displayed an unusually great increase in specific radioactivity (40 times) as compared with the other saturated acids. The unusually high specific radioactivity of eicosanoic acid reported previously (17) is maintained throughout the hair cycle. Certain unsaturated acids, however, responded to hair growth by incorporating acetate-1-$C^{14}$ at a much greater rate, i.e. palmitoleic (16:1), 60 times; oleic, 100 times; and the mixture of arachidonic (20:4) and erucic (22:1), 100 times. The octadecadienoic acid peak has previously been shown to be a mixture of unlabeled linoleic and an actively synthesized octadecadienoic acid (17). Therefore, the specific radioactivity of these mixed acids was a value considerably below that of the synthesized acid. Since the total radioactivity incorporated into saponifiable lipids increases 19 times at this stage of hair growth, the 10–20 time increase in specific radioactivity of the saturated acids is to be expected, especially in view of the concentration decreases. However, the extreme increase in specific activity of certain unsaturated acids is far above the overall stimulation of labeled acetate incorporation into the saponifiable lipids. Inspection of the changing concentration of the identified acids (Fig. 4) helps to explain, in some cases, the sharp rise in specific radioactivity; however, the concentration variance of the oleic acid at 0 day (196 $\mu g/cm^2$) and at 3 days (228 $\mu g/cm^2$) would not explain an increase in specific radioactivity of 100 times.

It appears that there is a specific increase in the synthesis of certain unsaturated acids during the early stages of hair growth in mouse skin. The shift of the per cent incorporation of acetate-1-$C^{14}$ from palmitic acid to oleic acid after the initiation of hair

TABLE I

*Concentration and specific radioactivity of fatty acids during hair growth*

| Acid | Day of the hair cycle | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 3 | 7 | 9 | 14 | 17 | 22 |
| | cpm/μg | cpm/μg | cpm/μg | cpm/μg | cpm/μg | cpm/μg | cpm/μg | cpm/μg |
| 14:0 | 2.5 | 16.2 | 44.8 | 33.0 | 30.4 | 7.6 | 4.3 | 9.2 |
| 14:1 + 15:0 | 0.9 | — | 70.1 | 32.2 | 29.9 | 8.6 | 2.1 | 6.4 |
| 16:0 | 1.1 | 4.7 | 16.9 | 16.6 | 6.1 | 2.2 | 1.4 | 3.0 |
| 16:1 | 0.2 | 2.8 | 10.6 | 6.6 | 5.3 | 0.9 | 0.4 | 1.0 |
| 18:0 | 1.5 | 14.4 | 58.0 | 23.3 | 23.6 | 9.1 | 3.1 | 5.9 |
| 18:1 | 0.2 | 2.6 | 20.4 | 5.6 | 5.8 | 1.5 | 0.7 | 1.3 |
| 18:2 | 0.2 | 1.1 | 4.7 | 3.2 | 1.6 | 0.5 | 0.2 | 0.4 |
| 20:0 | 8.4 | 99.3 | 244 | 269 | 149 | 172 | 88.6 | 178 |
| 20:1 + (18:3) | 1.6 | 24.7 | 77.0 | 45.8 | 40.6 | 9.2 | 3.5 | 9.7 |
| 21:0 | 1.0 | 13.3 | 20.4 | 8.0 | 11.3 | 6.9 | 5.6 | 4.8 |
| 22:0 | 2.5 | 9.2 | 26.0 | — | 26.5 | 7.5 | 6.0 | 8.5 |
| 20:4 + 22:1 | 0.3 | 6.1 | 29.8 | — | 20.7 | 5.8 | 1.7 | 7.0 |
| Total cpm† | 1,380 | 15,270 | 41,100 | 46,890 | 50,880 | 32,220 | 21,450 | 19,290 |
| Recovery of radioactivity from GLC | 90% | 89% | 90% | 85% | 100% | 96% | 84% | 90% |

The dorsal skins from six mice were pooled and a ⅓ aliquot (0.10–0.25 g) was incubated with 20 μC of acetate-1-$C^{14}$. The fatty acids from this incubation were analyzed on GLC and the data reported above. Two ⅓ aliquots of the pooled skin were incubated with 1 μC of labeled acetate and the saponifiable lipid data from these experiments were included in Fig. 3. The total saponifiable lipid mass is shown in Fig. 1.

The fatty acids were chromatographed on a 6 ft. × 0.25 in. glass column packed with 18% DEGS on 60–80 mesh Chromosorb W. The GLC temperatures, detection sensitivities, argon flow rates, and collection time intervals were identical to those reported previously from this laboratory (17). The mean of 9 collections of the standard methyl palmitate-1-$C^{14}$ (10 μg; 418 cpm/μg) was 73.8 ± 2.3% SD.

* The carbon numbers used to identify the acids were calculated as in a previous publication in this series (17).

† Total radioactivity in the saponifiable lipids. Aliquots analyzed on GLC contained between $1 \times 10^3$ and $5 \times 10^3$ cpm.

growth is shown in Figure 5. Like palmitic, the other acids of lesser concentration also incorporated a relatively smaller amount of labeled acetate at 3 and 7 days than at the start of growth.

*Fatty acid oxidation.*—The oxidation of exogenous acetate-1-$C^{14}$ to $C^{14}O_2$ (via the Krebs cycle) utilized a greater portion of the consumed oxygen during the first 3 days of hair growth (Fig. 2). Concurrently the oxidation of glucose-1-$C^{14}$ to labeled $CO_2$ (glucose shunt) consumes a decreasing fraction of the oxygen. Both the oxidation of exogenous acetate-1-$C^{14}$ and glucose-1-$C^{14}$ have decreased appreciably by 7 to 9 days of hair growth when the oxygen consumption is highest. Incubation of the skin slices with palmitate-1-$C^{14}$ has shown that the oxidation of this long chain fatty acid reached a maximum at 7 days, at which time the ratio of labeled $CO_2$ produced to oxygen consumed is highest. The palmitate-1-$C^{14}$ used in these experiments would also be expected to serve as a precursor of longer chain saturated and unsaturated acids, which have been shown by Mead *et al.* to be actively oxidized by tissue (19).

It therefore appears that the oxidation of fatty acids to acetate may provide significant quantities of the Krebs cycle substrates during the early phases of hair growth. This would aid in explaining the decrease in fatty acid concentration in skin during a phase of hair growth when these acids are most actively synthesized. Indirect evidence reported by Cruickshank *et al.* has indicated that lipids may be the main energy source for the

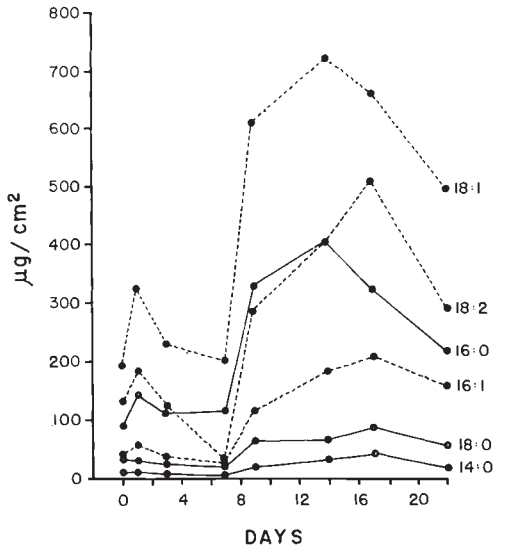


FIG. 4. Concentrations of certain fatty acids in one square centimeter patches of dorsal mouse skin during the hair growth cycle. The acids were extracted from the pooled skins of six mice and analyzed on GLC (see Table I).

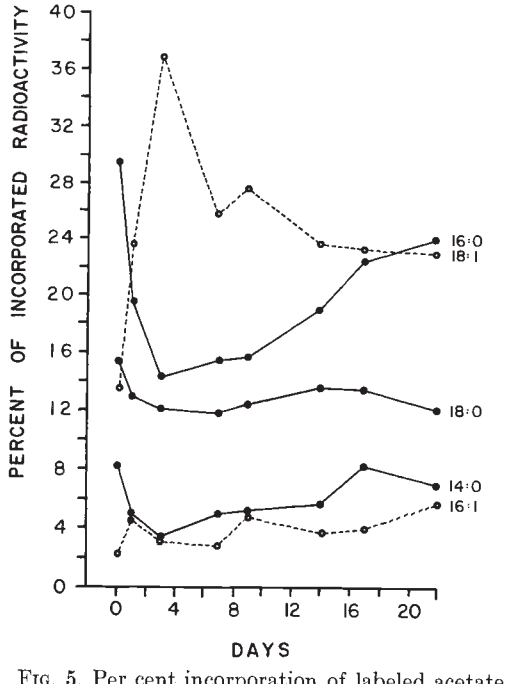


FIG. 5. Per cent incorporation of labeled acetate into certain fatty acids in one square centimeter patches of dorsal mouse skin during the hair growth cycle. The acids were extracted from the pooled skins of six mice and analyzed on GLC (see Table I). Percentages were calculated from the total radioactivity recovered from GLC.

endogenous respiration of skin (20). This finding was extended by Yardley and Godfrey to involve particularly the phospholipids (21).

Further investigation is required to elucidate which acids are most actively oxidized and it must yet be established if the utilized acids are free or if they are esterified to glycerol, phospholipids, or sterols. However, these experiments point to the utilization of the unsaturated acids, especially oleic.

*Sterol synthesis.*—The dramatic increase in the incorporation of labeled acetate into nonsaponifiable lipids is shown in Figure 3. Analysis of this mixture on gas-liquid chromatography produced a variety of radioactive peaks (Fig. 6). The retention times of the labeled peaks recovered from the effluent correspond to those obtained for cholesterol, $\Delta^7$-cholestenol, and 7-dehydrocholesterol, respectively. Radioactive peaks collected before cholesterol [shown in a previous publication (17)] are made up of squalene and shorter chain hydrocarbons or alcohols, which have not been identified. A substantial part of the label was not recovered from GLC (Table II). This material has previously been resolved on deactivated alumina columns and shown to be comprised of compounds other than squalene or sterols (17). Synthesis of this material from acetate-1-$C^{14}$ increased at the expense of sterols during the first 9 days of the cycle.

Since the retention times of the sterols were not sufficiently different to allow for the quantitative analysis of the relatively smaller amounts of the $\Delta^7$-sterols, it was necessary to make several collections of each sterol and rechromatograph each pooled sterol separately in order to obtain accurate specific radioactivities (Fig. 6). The data from these studies are presented in Table II.

A variety of sterols has been isolated from rodent skin utilizing GLC conditions similar to those employed herein (22). Certain of these sterol ethers have retention times approximating those of cholesterol, $\Delta^7$-cholestenol, and 7-dehydrocholesterol. In particular, the cholesterol peak may contain traces of $\Delta^8$-cholestenol and 4$\alpha$-methyl-$\Delta^8$-cholestenol, the $\Delta^7$-cholestenol peak may also include 4$\alpha$-methyl-$\Delta^7$-cholestenol, and the 7-dehydrocholesterol peak may be contaminated with desmosterol.

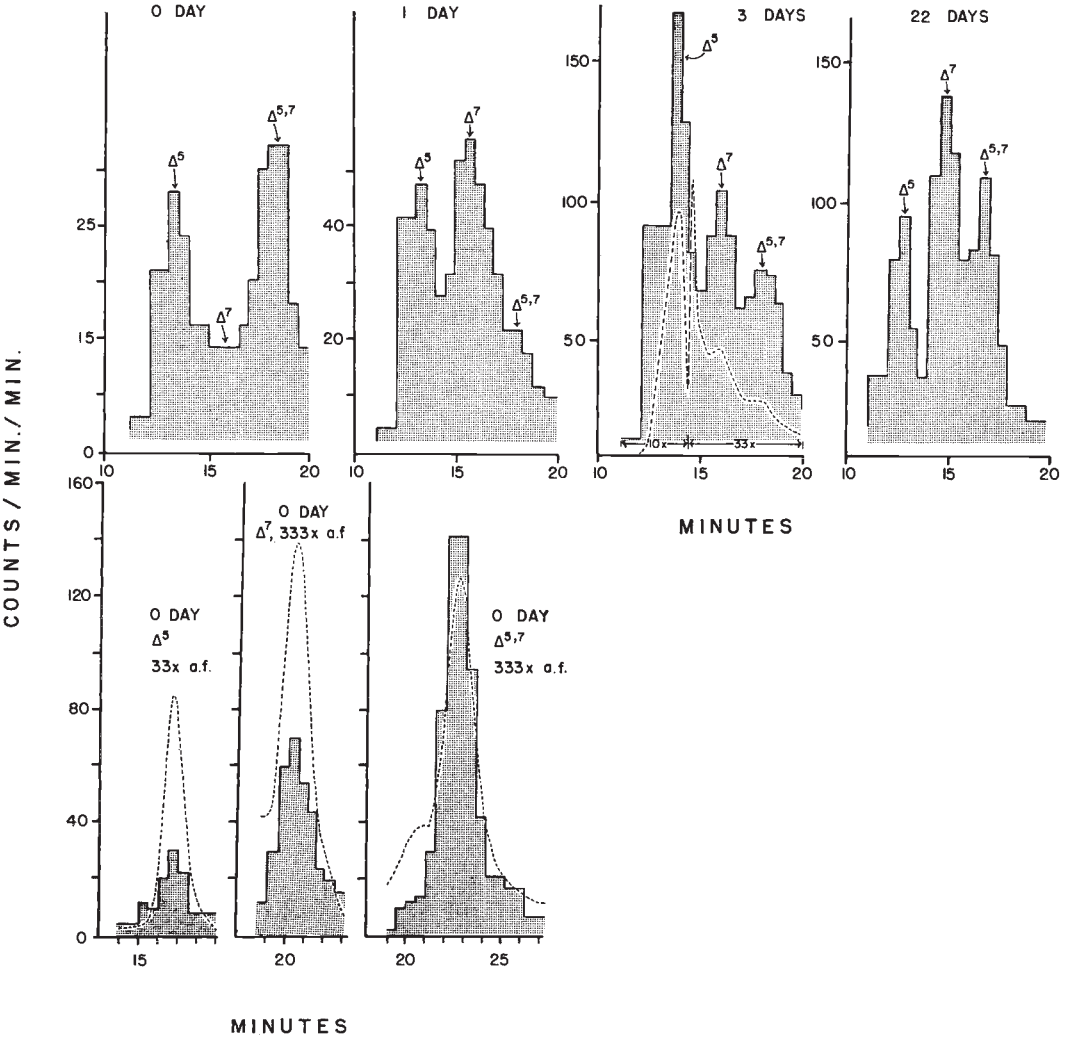


FIG. 6. Bar graphs of the collected radioactivity with superimposed gas-liquid chromatogram of the TMS ethers of the sterols from mouse skin in certain phases of the hair cycle. The figures represent collections of the sterols from the skins of mice incubated with 20 $\mu$C of acetate-1-$C^{14}$ on the various days of hair growth. A sample detector trace of the endogenous sterols is shown superimposed on the collected radioactivity at 3 days. An amplification factor (a.f.) of 10× represents full scale deflection at $2 \times 10^{-2}$ $\mu$amp. The labeled peaks corresponding to cholesterol, $\Delta^5$; $\Delta^7$-cholestenol, $\Delta^7$; and 7-dehydrocholesterol, $\Delta^{5,7}$ are indicated. The relative retention times and column temperatures are given in Table II. An argon flow rate of 181 ml/min was maintained. Note the shift in radioactivity from 7-dehydrocholesterol at 0 day to $\Delta^7$-cholestenol at 1 day, also the relatively high fraction of radioactivity in cholesterol at 3 days compared to 22 days. Similar determinations were carried out at 7, 9, 14, and 17 days of hair growth.

The lower figures depict the collected radioactivity and detected peaks of the endogenous sterols at 0 day of hair growth. The sterol in each chromatogram was obtained from several collections of the individual peaks which were pooled and rechromatographed. Both the radioactive and detected peaks had retention times identical to cholesterol, $\Delta^5$; $\Delta^7$-cholestenol, $\Delta^7$; or 7-dehydrocholesterol, $\Delta^{5,7}$. Similar determinations were carried out at 3, 7, 14, 17, and 22 days of hair growth. The argon flow rate for these chromatograms was 102 ml/min.

TABLE II

*Synthesis of sterols during hair growth*

| Day | Total radioactivity* | Δ⁷-cholestenol | | 7-dehydrocholesterol | | Cholesterol | | | Recovery‡ |
|---|---|---|---|---|---|---|---|---|---|
| | | cpm† | Sp. act. | cpm | Sp. act. | cpm | Sp. act. | μg/cm² | |
| | *cpm* | % | *cpm/μg* | % | *cpm/μg* | % | *cpm/μg* | | % |
| 0§ | 3,240 | 8.8 | 80 | 25.2 | 138 | 20.9 | 4.6 | 47.0 | 67 |
| 1§ | 26,550 | 28.0 | 729 | 6.4 | 208 | 19.4 | 45.0 | 61.1 | 45 |
| 3 | 51,240 | 12.0 | — | 12.9 | — | 21.5 | 32.1 | 60.0 | 44 |
| 7§ | 107,820 | 16.6 | 324 | 9.4 | 120 | 25.3 | 31.0 | 67.9 | 40 |
| 9 | 80,640 | 11.9 | — | 11.1 | — | 19.4 | 35.0 | 42.2 | 37 |
| 14§ | 15,630 | 14.9 | 316 | 9.7 | 42 | 19.4 | 3.1 | 51.6 | 63 |
| 17 | 16,290 | 16.6 | — | 14.3 | — | 13.6 | 3.2 | 68.0 | 63 |
| 22§ | 20,550 | 17.5 | 85 | 16.2 | 85 | 13.6 | 8.9 | 68.1 | 54 |

The dorsal skins from six mice were pooled and a ⅓ aliquot (0.10–0.25 g) was incubated with 20 μC of acetate-1-$C^{14}$. The nonsaponifiable lipids from this incubation were analyzed on GLC and the sterol data reported above. Two ⅓ aliquots of the pooled skin were incubated with 1 μC of labeled acetate and the nonsaponifiable lipid data from these experiments were included in Fig. 3. The total nonsaponifiable lipid mass is shown in Fig. 1.

The nonsaponifiable lipids were chromatographed on a 6 ft. × 0.25 in. glass column packed with 6% DEGS on 60–80 mesh Chromosorb W. The column temperatures were identical to those reported previously from this laboratory (17). The argon flow rates are given in Fig. 6. The retention times of the sterols relative to squalene were: cholesterol, 2.45; dihydrolanosterol, 2.70; Δ⁷-cholestenol, 2.90; 7-dehydrocholesterol, 3.41; and lanosterol, 3.66. The mean of 8 collections of the standard cholesterol-4-$C^{14}$ TMS ether (10 μg; 337 cpm/μg) was 61.0 ± 0.7% SD.

* Total radioactivity in the nonsaponifiable lipids. Aliquots analyzed on GLC contained between $1 \times 10^3$ and $5 \times 10^3$ cpm.

† Counts incorporated from labeled acetate into each sterol expressed as a percentage of the total nonsaponifiable lipid counts recovered from GLC.

‡ Percentage of total radioactivity in nonsaponifiable lipids recovered from GLC.

§ In order to determine the specific radioactivity of the Δ⁷-sterols on these days, several collections of each sterol were made and the pooled fractions were rechromatographed (see Fig. 6).

Since the reduction of the unsaturation in the side-chain is the initial step in the conversion of lanosterol to cholesterol in skin (24), the presence of labeled desmosterol in these skin incubations is unlikely. Significant quantities of 4α-methyl-Δ⁸-cholestenol and 4α-methyl-Δ⁷-cholestenol have been isolated from skin incubations (24) and may therefore be contributing to the radioactivity in the cholesterol and Δ⁷-cholestenol peaks, respectively. The coinciding peaks of pooled endogenous sterol and collected radioactivity (Fig. 6) support the assumption that the major portion of the radioactivity is associated with cholesterol, Δ⁷-cholestenol, or 7-dehydrocholesterol.

Appreciable quantities of the cholesterol were lost when the hair was plucked (0 days vs. 22 days). However, this sterol reached its maximum concentration by the seventh day of the hair growth cycle. This response of cholesterol to the increase of epidermal cells in early anagen would be expected in view of similar increases in concentration displayed by cholesterol during epidermal hyperplasia produced by chemicals (18).

It would, therefore, follow that the thinning of the epidermis at the 9th day (1) would be reflected in a decrease in the cholesterol content of the skin (Table II). As the hair shaft lengthened in the last phases of hair growth, the cholesterol content again increased.

The per cent incorporation of labeled acetate into cholesterol followed the cholesterol accumulation during the first week of activated sterol synthesis. The precursor Δ⁷-sterols displayed a wide fluctuation in their incorporation of acetate during the first 24 hours of hair growth (Table II and Fig. 6). At the time of hair removal, the skin incorporated the greatest amount of label into 7-dehydro-

cholesterol while the $\Delta^7$-cholestenol peak contained the least label of the entire period of hair growth. During the first day of growth, this situation was reversed and the $\Delta^7$-cholestenol incorporated the greatest per cent of the labeled acetate at the expense of 7-dehydrocholesterol. Thereafter, the two $\Delta^7$-sterols incorporated nearly equal quantities of the acetate-1-$C^{14}$. It is noteworthy that at the peak of cholesterol synthesis (7 days) the per cent of label incorporated into cholesterol was approximately equal to the total radioactivity found in the $\Delta^7$-sterols. However, at telogen (22 days), the per cent of acetate-1-$C^{14}$ in cholesterol had decreased to one half while the fraction of the label attributed to the $\Delta^7$-sterols had increased. Therefore, at telogen the conversion of the $\Delta^7$-sterol pool to cholesterol had decreased relative to early anagen.

Throughout anagen the specific radioactivities of the sterols decreased in the following order: $\Delta^7$-cholestenol > 7-dehydrocholesterol > cholesterol. This relationship corresponded to the known sequence of sterol precursors in skin (23, 24). Since the specific radioactivity of $\Delta^7$-cholestenol was not greater than that of 7-dehydrocholesterol at telogen (0 day and 22 days), it appeared that there was an accumulation of a metabolically inactive pool of the $\Delta^7$-cholestenol in the resting phase. This accumulation of the $\Delta^7$-sterols appeared to begin earlier at 14 days when the decrease in skin cholesterol was accompanied by a drop in the label incorporated into this sterol during the period of incubation. Concurrently, the specific activity of cholesterol had decreased from approximately 1/10th to 1/100th that of the $\Delta^7$-cholestenol. It would appear, then, that during the latter phases of anagen the conversion of $\Delta^7$-cholestenol to cholesterol was less efficient than during the beginning of hair growth. The resulting accumulation of cholesterol precursors may have some relation to the $\Delta^7$-sterols which have been shown histochemically to be concentrated in the lumen of the sebaceous glands (25).

These experiments have demonstrated that the initiation of hair growth brings about an increase in the synthesis of cholesterol during the first week of growth. This increased production of cholesterol is preceded within 24 hours by a dramatic increase in the capacity of the skin to synthesize the $\Delta^7$-sterols. Diminution of the epidermis at 9 days is accompanied by a decrease in the synthesis of cholesterol which results in the accumulation of a metabolically inactive pool of $\Delta^7$-cholestenol.

SUMMARY

Metabolic studies on mouse skin were undertaken to determine the effect of the hair growth cycle on the metabolism of fatty acids and sterols. Gas-liquid chromatography was employed to analyze and to isolate the lipids from incubations with acetate-1-$C^{14}$.

Substantial increases in the lipid and oxidative metabolism of skin were demonstrated during the first week of hair growth. During this period of anagen there was a decrease in the concentration of certain fatty acids and a concurrent accumulation of cholesterol in the skin. The oxidation of fatty acids was shown to increase; this route appears to be a significant source of energy during the early phases of hair growth.

Thinning of the epidermis at 9 days was accompanied by a decrease in the synthesis of cholesterol and a resultant accumulation of a metabolically inactive pool of $\Delta^7$-sterols. Fatty acids were shown to become more concentrated during the latter phases of hair growth.

REFERENCES

1. Chase, H. B.: Growth of the hair. Physiol. Rev., *34:* 113, 1954.
2. Potter, B.: The physiology of the skin. Ann. Rev. Physiol., *28:* 159, 1966.
3. Argyris, T. S.: The distribution of succinic dehydrogenase and non-specific esterase in mouse skin throughout the hair growth cycle. Anat. Rec., *125:* 105, 1956.
4. Carruthers, C., Quevedo, W. C., Jr. and Woernley, D. L.: Influence of hair growth cycle on cytochrome oxidase and DPNH-cytochrome C reductase in mouse epidermis. Proc. Soc. Exp. Biol. Med., *101:* 374, 1959.
5. Griesemer, R. D.: Change in enzyme activity and ribonucleic acid concentration within the epidermal cell of the rat during the growth stage of the hair cycle. J. Biophys. Biochem. Cytol., *2:* 523, 1956.
6. Carruthers, C.: Influence of sex and hair growth cycle on lipid composition of mouse epidermis. Proc. Soc. Exp. Biol. Med., *109:* 390, 1962.
7. Carruthers, C.: The fatty acid composition of dermal and epidermal triglycerides and phosphatides in mouse skin during normal and abnormal growth. Cancer Res., *22:* 294, 1962.
8. Carruthers, C. and Heining, A.: Influence of hair growth cycle on the triglyceride fatty acid composition of mouse epidermis. Proc. Soc. Exp. Biol. Med., *112:* 278, 1963.
9. Carruthers, C. and Heining, A.: The fatty acid composition of the sterol-wax ester and

mono- and diglyceride fractions of mouse epidermis undergoing normal and abnormal growth changes. Cancer Res., *24:* 1008, 1964.
10. Carruthers, C. and Heining, A.: Phosphatides in mouse epidermis undergoing normal and abnormal growth changes. Cancer Res., *24:* 485, 1964.
11. Moretti, G., Giacometti, C., Boido, V. and Rebora, A.: Histamine, serotonin and mast cells in the skin of the rat during the hair cycle. J. Invest. Derm., *40:* 205, 1963.
12. Popjak, G. and Tietz, A.: Biosynthesis of fatty acids by slices and cell-free suspensions of mammary gland. Biochem. J., *56:* 46, 1954.
13. Luukkainen, T., VandenHeuvel, W. J. A., Haahti, E. O. A. and Horning, E. C.: Gas-chromatographic behavior of trimethylsilyl ethers of steroids. Biochim. Biophys. Acta, *52:* 599, 1961.
14. Metcalfe, L. D. and Schmitz, A. A.: The rapid preparation of fatty acid esters for gas chromatographic analysis. Anal. Chem., *33:* 363, 1961.
15. Fales, H. M., Haahti, E. O. A., Luukkainen, T., VandenHeuvel, W. J. A. and Horning, E. C.: Milligram-scale preparative gas chromatography of steroids and alkaloids. Anal. Biochem., *4:* 296, 1962.
16. Brooks, S. C. and Godefroi, V. C.: Quantitative collection of micro amounts of steroids from gas-liquid chromatography. Anal. Biochem., *7:* 135, 1964.
17. Brooks, S. C., Godefroi, V. C. and Simpson, W. L.: Specific sites of fatty acid and sterol synthesis in isolated skin components. J. Lipid Res., *7:* 95, 1966.
18. Brooks, S. C. and Baumann, C. A.: Skin sterols. X. Studies with certain hyperplasia-inducing hydrocarbons. Cancer Res., *16:* 357, 1956.
19. Mead, J. F., Slaton, W. H., Jr. and Decker, A. B.: Metabolism of the essential fatty acids. II. The metabolism of stearate, oleate, and linoleate by fat-deficient and normal mice. J. Biol. Chem., *218:* 401, 1956.
20. Cruickshank, C. N. D., Trotter, M. D. and Cooper, J. R.: A study of available substrates for the endogenous respiration of skin "in vitro." J. Invest. Derm., *39:* 175, 1962.
21. Yardley, H. J. and Godfrey, G.: Metabolism of phosphate esters and phospholipids in skin maintained *in vitro*. J. Invest. Derm., *43:* 51, 1964.
22. Clayton, R. B., Nelson, A. N. and Frantz, I. D., Jr.: The skin sterols of normal and triparanol-treated rats. J. Lipid Res., *4:* 166, 1963.
23. Gaylor, J. L. and Sault, F. M.: Localization and biosynthesis of 7-dehydrocholesterol in rat skin. J. Lipid Res., *5:* 422, 1964.
24. Kandutsch, A. A. and Russell, A. E.: Preputial gland tumor sterols. III. A metabolic pathway from lanosterol to cholesterol. J. Biol. Chem., *235:* 2256, 1960.
25. Brooks, S. C., Lalich, J. J. and Baumann, C. A.: Skin sterols. XI. A direct demonstration of fast-acting sterols in the sebaceous glands. Amer. J. Path., *32:* 1205, 1956.