# Influence of Hair Growth Cycle on the Triglyceride Fatty Acid Composition of Mouse Epidermis.* (28015)

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Previous studies have shown that the lipid (phospholipid, neutral lipid, cholesterol, cholesterol esters and triglycerides) composition of Swiss female epidermis at the 12th and 22nd days of the hair growth cycle does not differ appreciably(1). Total lipid content of the 4th day (4 day post plucking) male and female epidermis is much lower than that of the epidermis of the 12th and 22nd days of the hair growth cycle whereas the phospolipid level is highest in the 4th day epidermis of both sexes and lowest in the 22nd day epidermis(2). Female epidermis at days 4, 12 or 22 of the hair growth cycle has twice as much total lipid and about ½ as much phospholipid as male epidermis at comparable stages of the hair growth cycle. Four day post plucking male epidermis is also characterized by a very high cholesterol content, a low percentage of triglycerides and a high level of lipid material which is eluted from silicic acid with cholesterol esters. Other interesting chemical differences in the epidermis undergoing changes induced by the hair growth cycle have been found(2). Since the epidermis undergoes a speedy proliferation within 2 days after plucking(3), this tissue offers excellent material for studies on the biochemistry of mammalian cells in rapid growth. Also between the 10th and 17th day post plucking the epidermis is very thin and becomes somewhat thicker again in the last days of the cycle(3). Therefore it seemed worthwhile to determine whether the triglyceride fatty acid composition of the epidermis of both male and female mice is altered during the cell changes induced by the hair growth cycle.

*Methods.* Procedures for plucking the mice and for separation of epidermis from dermis have been described(4). The lipids were extracted from the epidermis with ethanol and ethanol-ether mixtures and the neutral lipid fraction was separated into triglycerides, cholesterol esters and cholesterol on silicic acid(1). Until sufficient epidermis was ready for extraction it was covered with redistilled ethanol and stored at −25°C. The triglyceride fractions were also stored under $N_2$ at this temperature until ready for use. The triglycerides were transesterified to the methyl esters with methanolic HCl(5). The methyl esters of the fatty acids were then separated from the unsaponifiables or other impurities by chromatography on silicic acid by the method of Luddy *et al.*(6). The fatty acid composition of the methyl esters was determined by gas-liquid chromatography on an Aerograph Hy-Fi, Model 600 Chromatograph with a hydrogen flame ionization detector. The column was 5 ft. × 1/8 in. stainless steel packed with 12% ethylene glycol adipate on 80/100 mesh Gas-Chrom P.† Temperature of the column was 195°C. Usually 2 μl of the esters dissolved in $CS_2$ at a concentration of 0.5 to 1% solutions were employed. Each sample was run 3 times and the average values were used. For more accurate measurement of trace components 5 μl of the $CS_2$ solutions of the methyl esters were employed. Identification of the fatty acid methyl esters was based upon a comparison of relative retention times with known fatty acid methyl esters.‡ These esters at various concentrations and mixtures were also used for calibration of the GLC apparatus. The areas under the curves were measured with a Disc Integrator.

*Results.* The fatty acid composition of 2, 4, 12 and 22 day post plucking male epider-

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TABLE I. Fatty Acid Composition of Male Epidermal Triglycerides.*

| Fatty acid | Days post plucking 2 (3) | 4 (4) | 12 (5) | 22 (4) |
|---|---|---|---|---|
| | (Fatty acids – % of total) | | | |
| Myristic | 1.5 ± .7 | .8 ± .1 | 1.1 ± .3 | .8 ± .1 |
| Palmitic | 17.8 ± 2.1 | 18.0 ± 1.2 | 18.1 ± 1.3 | 17.3 ± .5 |
| Palmitoleic | 3.4 ± .3 | 3.6 ± .4 | 5.8 ± .3 | 4.0 ± .5 |
| Stearic | 2.4 ± .3 | 1.6 ± .4 | 2.0 ± .5 | 1.2 ± .3 |
| Oleic | 32.6 ± .4 | 33.9 ± 1.4 | 40.5 ± 2.9 | 35.0 ± .8 |
| Linoleic | 37.2 ± 3.3 | 39.5 ± 2.4 | 31.7 ± 5.6 | 40.8 ± .8 |
| X | 3.4 ± 1.6 | 1.7 ± 1.2 | 1.2 ± .6 | .9 ± .2 |
| Other | .6 ± .2 | .7 ± .2 | 1.1 ± .3 | .3 ± .2 |

* Mean ± standard deviation of number of samples indicated in parentheses from different groups of mice. Epidermis from 50 to 100 mice were pooled and extracted for each analysis.

TABLE II. Fatty Acid Composition of Female Epidermal Triglycerides.*

| Fatty acid | Days post plucking 2 (3) | 4 (3) | 12 (4)† | 22 (4)† |
|---|---|---|---|---|
| | (Fatty acids – % of total) | | | |
| Myristic | 1.2 ± .1 | .9 ± .1 | 1.4 ± .4 | 1.2 ± .2 |
| Palmitic | 17.8 ± 1.1 | 16.2 ± .7 | 20.0 ± 1.8 | 19.0 ± .4 |
| Palmitoleic | 3.6 ± .3 | 4.9 ± .8 | 6.4 ± .7 | 6.3 ± .3 |
| Stearic | 1.8 ± .2 | 1.5 ± .4 | 4.0 ± .6 | 3.4 ± .2 |
| Oleic | 34.1 ± .4 | 39.6 ± 1.0 | 54.1 ± 3.4 | 57.6 ± .5 |
| Linoleic | 40.8 ± 1.9 | 34.9 ± 1.3 | 10.0 ± .7 | 10.0 ± .5 |
| X | .6 ± .3 | 1.0 ± .3 | .5 ± .4 | — |
| Other | .3 ± .0 | 1.3 ± 1.2 | 1.6 ± .5 | 1.7 ± .4 |

* Mean ± standard deviation of number of samples indicated in parentheses from different groups of mice. Epidermis from 50 to 100 mice were pooled and extracted for each analysis.
† Part of these data previously published(7).

mis (Table I) does not appear to vary appreciably with each of these stages of the hair growth cycle. The content of oleic and linoleic acids is about the same; these acids represent some 70% of the total whereas palmitic acid accounts for about 18% of total fatty acid content. Of some interest is the observation that a peak (X) from an acid methyl ester, not yet identified, was found in 2 day male epidermis at a concentration of 3.4%, but fell off to less than 1% in 22 day post plucking epidermis. The ratio of the retention times of component X to stearic was 2.05.

The fatty acid composition of 2, 4, 12 and 22 day post plucking female epidermal triglycerides is shown in Table II. The fatty acid composition of the days 2 and 4 of the female triglycerides is similar to that of the males except that component X is present in small amounts. Twelve and 22 day post plucking female epidermis has more oleic and much less linoleic acid than does the 2 and 4 day epidermis. The reason for these differences is not known. At the time of maximum epidermal thickness in either sex (2-4 days) there appears to be no significant change in triglyceride fatty acid composition.

*Summary.* The fatty acid content of 2, 4, 12 and 22 day post plucking male and 2 and 4 day post plucking female epidermal triglycerides is quite similar. Oleic and linoleic acids are present in about equal amounts and represent some 70% of total fatty acid content whereas palmitic acid is some 18% of total fatty acid level. Triglycerides of post plucking days 12 and 22 of female epidermis have more oleic and much less linoleic acids than do the triglycerides of the other samples. At the time of most rapid cell division in the epidermis (2-4 days post plucking) there appears to be no significant change in triglyceride fatty acid composition.

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## A Plasma Card Test for Rapid Serodiagnosis of Schistosomiasis (SPC). (28016)

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Diagnosis of schistosomiasis by examination of the feces or urine is frequently unsatisfactory in light and chronic infections and in cases which are therapeutically affected. To obviate this difficulty numerous immuno-diagnostic tests have been developed. One of these, a fluorescent antibody technic(1) makes possible the use of dried blood collected by finger puncture, instead of serum. This permits rapid collection of specimens under adverse conditions and allows easy transport of specimens(2-3). In spite of these recent advances, the need still exists for an efficient field testing procedure which would permit the prompt collection of data without the time required to mail specimens to a central laboratory and to receive results. This need is particularly acute in many of the endemic areas where health officers frequently lose contact with infected people before they receive the results of serological examinations.

Recently Brewer developed a commercially available plasma collection slide(4). A card test with plasma obtained with the Brewer's collection slide was subsequently developed for field tests for syphilis and other treponematoses(5). This provided the opportunity to attempt the development of a similar technic for rapid field diagnosis of schistosomiasis, utilizing a washed antigen-cholesterol-lecithin complex developed by one of us(6) for the cercarial slide flocculation test.

This report describes the technic for the schistosomiasis plasma card (SPC) test. The procedure was evaluated with patients tested under field conditions and the findings compared to those obtained with the fluorescent antibody (FA) test performed on dried blood samples obtained from the same patients. Additional tests were performed in the laboratory with unheated serum specimens.

*Materials and methods. Antigen. Schistosoma mansoni* cercariae from experimentally infected *Australorbis glabratus* snails (Puerto Rican strain) were used. The cercariae were obtained by exposing infected snails to artificial light and were concentrated by sedimentation at 3 to 6°C. Following sedimentation and washing in distilled water, the cercariae were quickly shell frozen and dehydrated under vacuum from the frozen state. Nonspecific antigenic lipids were removed as described previously(6). An antigen emulsion was prepared by adsorbing the cercarial antigen onto cholesterol lecithin crystals, centrifuging and resuspending the sediment with a solution containing charcoal(5).

*Sources and handling of the blood samples tested.* Finger blood from 189 well documented cases of schistosomiasis was collected in endemic areas from persons in whom the diagnosis had been established by demonstrating the eggs in the stools or urine. To determine the specificity of the reaction samples were tested from individuals living in endemic areas without clinical or parasitological evidence of schistosomiasis and from individuals with prolonged history of hyper-