SKIN SURFACE LIPID COMPOSITION, ACNE, PUBERTAL DEVELOPMENT, AND URINARY EXCRETION OF TESTOSTERONE AND 17-KETOSTEROIDS IN CHILDREN

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Fifty-two children, age 5–10, from acne-prone families, were studied for a period of 1 year to examine the interrelationship between sebum, acne, pubertal development, and urinary steroid excretion.

In each of the subjects, 30 boys and 22 girls, the composition of forehead skin lipid was determined 4 times yearly by thin-layer chromatography, with measurement of triglycerides, diglycerides, free fatty acids, wax esters, squalene, cholesterol, and cholesteryl esters. Twice yearly, examination was made of the presence or absence of acne, pubertal maturation and the 24-hour urinary excretion of testosterone as determined by radioimmunoassay, and of total 17-ketosteroids, dehydroepiandrosterone, and etiocholanolone, as determined by paper chromatography.

The relative amount of sebaceous lipids was positively correlated with age of the subjects (wax esters p < .001, squalene p < .05), as was the triglyceride-diglyceride component (p < .05). No significant correlation was seen with the fatty acids. Acne, primarily comedonal, occurred in 27/52 subjects (15 girls, 12 boys) and was associated with higher sebum values. One-half of the children with acne had no signs of pubertal development. A significantly positive correlation was observed between the relative amount of sebaceous lipid and the urinary excretion of 17-ketosteroids, androsterone, and etiocholanolone in both sexes, and of testosterone and dehydroepiandrosterone in boys.

The development of acne in children is an early pubertal event, often evident before other signs of pubertal maturation, and it is associated with an increase in sebum and in the urinary excretion of androgenic steroids.

Epidemiologic surveys have revealed that acne, although chiefly a disorder of adolescence, may be detected in 25% or more of children before the age of 10 [1–4], by which time sebaceous gland development has already begun [5]. This suggests that the occurrence of acne is related to this early sebarche. While other lines of evidence indicate an interrelationship between sebaceous glands and acne [6], there is no proof that sebaceous gland development is indispensable for its occurrence.

To provide further insight into this relationship, we undertook the present study in which children were examined, as part of a long-term prospective survey, for the presence of acne and its relation to the degree of sebaceous gland and pubertal maturation. In addition, urinary androgens were measured to observe whether the sebaceous glands are dependent for their early development on androgen.

MATERIALS AND METHODS

Fifty-two children, 30 boys and 22 girls, from acne-prone families were examined periodically for a period of 1 year. The subjects’ average age of entry into the study was 7.6 years (range 5.0 to 9.9) for the boys and 8.1 years (range 5.4 to 10.8) for the girls.

Four times yearly, skin surface lipid was collected from the forehead of each subject. Collection was made by rubbing a soft hexane-soaked polyurethane sponge over the forehead surface for a few seconds. Determination of the individual components of the skin surface lipid was made by thin-layer chromatographic separation and photodensitometric quantitation [7]. The lipid classes identified included triglycerides, diglycerides, free fatty acids, wax esters, squalene, cholesterol, and cholesteryl esters. Skin surface lipid is a mixture of sebaceous and epidermal lipid, the compositions of which differ from one another [8]. As sebum secretion begins to increase in childhood, the relative amount of wax esters and squalene, which are of sebaceous origin, increases, whereas the proportion of sterols and sterol esters, which are epidermal lipids, shows a relative decrement [5].

Twice yearly, the children were examined for the
presence or absence of acne. The distribution was noted (face, back, and chest) and the type of acne recorded as either comedonal, inflammatory, or both, with each type graded separately on a scale of 1 to 5, based on lesion counts, viz:

- grade 1: fewer than 6 lesions
- grade 2: 6–15 lesions
- grade 3: 16–30 lesions
- grade 4: 31–50 lesions
- grade 5: greater than 50 lesions

Twice yearly, the subjects were also examined for signs of pubertal development, according to the classification of Tanner [9]. The following characteristics were recorded: axillary hair, pubic hair, genital development (males only) and breast development (females only). Except for axillary hair, which was listed as either present or absent, the physical signs were graded on Tanner's scale of 1 to 5. Stage 1 designates the individual characteristics as being infantile or absent, with stages 2 to 5 indicating progressive pubertal development.

Twenty-four hour urine specimens were also collected twice yearly for analysis of testosterone and free fatty acids. Total and individual 17-ketosteroids were determined in each sample by paper chromatography, as described by Roberts, Bush, and Gibree [10].

Testosterone levels in the urine were measured by radioimmunoassay (RIA) using the New England Nuclear RIA-Pac procedure. Urine samples were hydrolyzed enzymatically at 47°C for 16 hours with β-glucuronidase in a 2 M, pH 4.5 buffer. After addition of 1.2[3H]tritiated testosterone tracer, samples were purified by column chromatography on a Sephadex LH-20 column. Elution with an iso-octane:benzene:methanol mixture, 90:5:5 (V:V:V), effectively separates testosterone from dihydrotestosterone, the latter of which cross-reacts extensively with the antiserum used for this RIA analysis. Aliquots of the testosterone containing fraction were then assayed for recovery studies and quantitation by standard RIA analysis, with the use of antisera prepared in rabbits against testosterone. Dextran-coated charcoal was used to separate bound and free testosterone.

RESULTS

Skin Surface Lipid Studies

Table I shows the forehead surface lipid composition of the children in this study, arranged by age groupings. Shown are the average values of the first two quarterly collections (I) and of the last two quarterly collections (II).* A significantly positive correlation was observed between the age of the subjects and the relative amount of wax esters (p < .001) and of squalene (p < .05) and a significantly negative correlation with cholesterol and cholesterol esters (p < .001). These observations were similar to results obtained previously in a cross-sectional study of a smaller group of children [5]; however, in the present study, a positive correlation was also observed for triglycerides and diglycerides (p < .05). There were no significant differences with age in the relative amounts of the free fatty acids.

Table II indicates the amount of sebaceous lipid relative to epidermal lipid [wax esters/(cholesterol + cholesterol esters)] on the forehead of these children, designated by age and sex. Girls in the 5–6 age group had sebaceous/epidermal lipid ratios that were 4 to 5 times higher, on average, than those of similarly aged boys. In the older age groups, however, there were no definitive differences between the sexes.

Acne

Acne was found to be present at one or both of the twice yearly examinations in 27 of the 52 children, or 52%. It occurred only on the face. Comedones were invariably present, with 20 children showing grade 1, 5 children grade 2, and 2 children grade 3 changes. Small inflammatory papules were detected in 8 children (7 girls, one boy) with all but one showing grade 1 counts, i.e., fewer than 6 lesions. Two of these 8 cases were 6-year-old children (both girls). In Table III the lipid composition values, again arranged by age groups, are compared with the presence or absence of acne. No statistically significant differences could be established between the acne and non-acne subjects for any of the lipid classes examined. However, in each age group, the wax esters/(cholesterol + cholesterol esters) ratio was greater in acne cases than in non-acne cases.

Table I. Skin surface lipid composition (%) by age groups

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Age 5–6</th>
<th>Age 7–8</th>
<th>Age 9–10</th>
</tr>
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<tbody>
<tr>
<td>Triglycerides &amp; diglycerides</td>
<td>I</td>
<td>II</td>
<td>II</td>
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<tr>
<td>Free fatty acids</td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>Wax esters</td>
<td>I</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>Squalene</td>
<td>I</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>Cholesterol &amp; cholesterol esters</td>
<td>I</td>
<td>II</td>
<td></td>
</tr>
</tbody>
</table>

* Average of first 2 quarterly collections.

Table II. Wax esters/(cholesterol + cholesterol esters) ratios by age and sex

<table>
<thead>
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<th>Sex</th>
<th>Age 5–6</th>
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<th>Age 9–10</th>
</tr>
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<tbody>
<tr>
<td>Boys</td>
<td>I</td>
<td>0.25</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0.19</td>
<td>1.49</td>
</tr>
<tr>
<td>Girls</td>
<td>I</td>
<td>0.94</td>
<td>1.54</td>
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<td>1.30</td>
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* Average of first 2 quarterly collections.

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Steroid + cholesterol esters) ratio was higher in the acne subjects, as compared to those without acne, the difference being least for the 5-6 age group and greatest for the 9-10 age group. Failure to show statistical validation of these differences is probably related to the small numbers of subjects compared and to intersubject variability.

**Pubertal Development**

Table IV indicates the stage of pubertal development encountered and comparison with the incidence of acne. Terminal axillary hairs were not present in any subject. Nor was there any degree of pubertal genital development observed in the boys. Pubic hair was noted in 9 of 20 boys and in 10 of 22 girls. Breast development had begun in 8 of 22 girls. Acne was present in 27 of the 52 subjects and could be encountered even when other signs of puberty were lacking.

**Urinary Steroid Excretion**

Analysis of the 24-hour urinary excretion of testosterone and of total 17-ketosteroids and individual 17-ketosteroids (dehydroepiandrosterone, androsterone, and etiocholanolone) in 43 subjects showed an increase with age except for dehydroepiandrosterone (Table V). Dehydroepiandrosterone was not detectable in the urines of subjects age 5 and 6 and only in small amounts in older children. By comparison, androsterone and etiocholanolone were excreted in very much larger quantities, with a progressive increase with age. There were no statistically significant sex differences in the excretion of any of the steroids studied; the seemingly higher value, 81.0 μg, for dehydroepiandrosterone in boys age 9 and 10, was unduly weighted by a value of 257.7 μg in the oldest of the five boys in this group.

Table VI shows a statistical comparison between the urinary excretion of these androgenic steroids and the wax esters/(cholesterol + cholesterol esters) ratio (based on the average of the last two quarterly sebum collections obtained in each subject). Statistically significant positive correlations could be established between the excretion of the total 17-ketosteroids, of androsterone, and of etiocholanolone, and the amount of sebum, expressed as the wax esters/(cholesterol + cholesterol esters) ratio. A positive correlation for testosterone and dehydroepiandrosterone was found only for boys.

**DISCUSSION**

The finding of minor degrees of acne in one-half of the children, age 5-10, examined in the present study is in general agreement with published reports of the incidence of acne in children. By the age of 10, 25-60% of children randomly surveyed showed evidence of disease (1-4). Such acne was very minimal in degree and extent and was invariably comedonal; no children were affected with inflammatory lesions before the age of 10 [1,2,4]. In our group of subjects, however, of the 8 children found to have inflammatory acne, 3 were...
under the age of 10, with 2 of them only 6 years of age. Selection of our subjects from acne-prone families may well explain these differences concerning inflammatory acne at this age. Conceivably, some prognostic significance may ultimately become apparent from these observations.

The results of the lipid composition analyses indicated that the relative proportion of sebaceous lipids relative to epidermal lipids on the forehead was higher in the subjects with acne in each age group studied, with the disparity between the acne vs. the normal groups increasing with age. In the youngest age group (age 5 and 6), girls had more sebaceous lipid than boys, but this difference disappeared with increasing age. Of all of the lipid class constituents, only the free fatty acid fraction showed no proportional change with age. Since free fatty acid formation is presumed to result from the intrafollicular hydrolysis of sebum triglycerides by Propionibacterium acnes (P. acnes), the report by Matta [11] that quantitative counts of P. acnes from the forehead did not increase significantly between the ages of 5 and 10 is consistent with our observation of no change in fatty acid content. It is noteworthy that P. acnes organisms were not recoverable in more than 60% of children of these ages [11], although free fatty acids are always present in their surface lipid. This may mean that either bacterial hydrolysis of sebum triglycerides is not the sole mechanism whereby fatty acids are generated or that the technique of surface sampling of bacteria does not detect intrafollicular P. acnes when they are present in very small numbers. The latter explanation is the more likely one. Of interest also is that we could not demonstrate fatty acid differences between the acne and nonacne groups of subjects, suggesting that P. acnes counts in these two groups would not differ either, although Leyden et al demonstrated in older children, age 11-15, a striking increase in P. acnes counts in acne patients, as compared to normal subjects [12].

Pubertal manifestations of genital and breast development and of axillary and pubic hair were frequently absent when sebaceous gland development was evident, even when acne was present. The measurement of surface lipid composition may, therefore afford a potential marker for indicating the very early onset of puberty.

In view of the known androgen-sensitive nature of the sebaceous glands, the stimulus for sebaceous gland development, even at this early stage in childhood is presumed to be androgenic. In the present study we observed in both boys and girls an increase in the urinary output of androgens with increasing age. Correlation analyses disclosed statistically significant positive correlations between the excretion of these steroids and the relative amount of skin surface sebaceous lipids, except for testosterone and dehydroepiandrosterone in girls. As the quantity of testosterone formed at these ages is exceedingly low, its biologic significance at this stage of development is uncertain. The amount of dehydroepiandrosterone excreted at all age levels was lower than had been expected. While studies have reported values similar to those encountered here [13,14], other investigators have found larger amounts of dehydroepiandrosterone [15,16]. The values, however, have been invariably lower by as much as two-thirds of the quantity of either androsterone or etiocholanolone. These latter two 17-ketosteroids, like dehydroepiandrosterone, are principally of adrenocortical origin, even in adults, and their increased secretion in mid- to late childhood, as demonstrated in this study, suggests that the increase in sebaceous gland activity which occurs at this time also, is a consequence of adrenarche. It is tempting to speculate that the higher sebum ratios found in the girls age 5-6 is a reflection of the demonstrated higher levels of androsterone and etiocholanolone. Although these C_{19} steroids are weak androgens, the sebaceous glands at this stage of development may be responsive to the weakest of androgenic stimulation.
REFERENCES