EXPERIMENTS ON THE EPIDERMIS OF ANIMALS WHICH MAY BE OF VALUE TO THE COSMETIC CHEMIST*

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THE SKIN OF most laboratory animals is devoid of sweat glands and provides no opportunity for making studies on the control of perspiration. The hair coat has a cyclic growth during which time the hair grows actively for a period and then rests for an interval. The cycle in the rat (1) lasts 34 days equally divided into resting and growing periods. Further studies have shown that the metabolism of the skin (2) is low during the resting period (0.92 cmm. O_2 mg./hr.) and that it increases greatly just prior to growth (1.26 cmm. O_2 mg./hr.). Likewise the fluid content of the skin is 55 per cent during inactivity of the hair and then increases to 67 per cent prior to the formation of the new hair (3). This growth of hair with associated metabolic and fluid changes in the skin has provided many opportunities for learning to what extent hair growth may be affected both by factors from within the organism and from environmental conditions. In experimental work on hair growth in animals, the animals and the cyclic activity of their hair coat must be well understood to obtain reliable results.

Changes also occur in the epidermis. At the age of 22 days when the hair coat is resting, the stratum germinativum consists of one or two layers, there are occasional granular cells which represent the granulosum, there is no definite lucidum and the corneum consists of four or five layers of very flat cells (Fig. 1). The latter stratum is often lost in histological preparations, indicating that the layer is dry and brittle.

In the 30-day-old rat, when activity begins in the hair coat the stratum germinativum consists of several layers of cells and a very distinct granulosum is present. Even occasional lucidum cells are found. More of the corneum is retained, indicating that it is not as dry as in the 22-day-old rat (Fig. 2).

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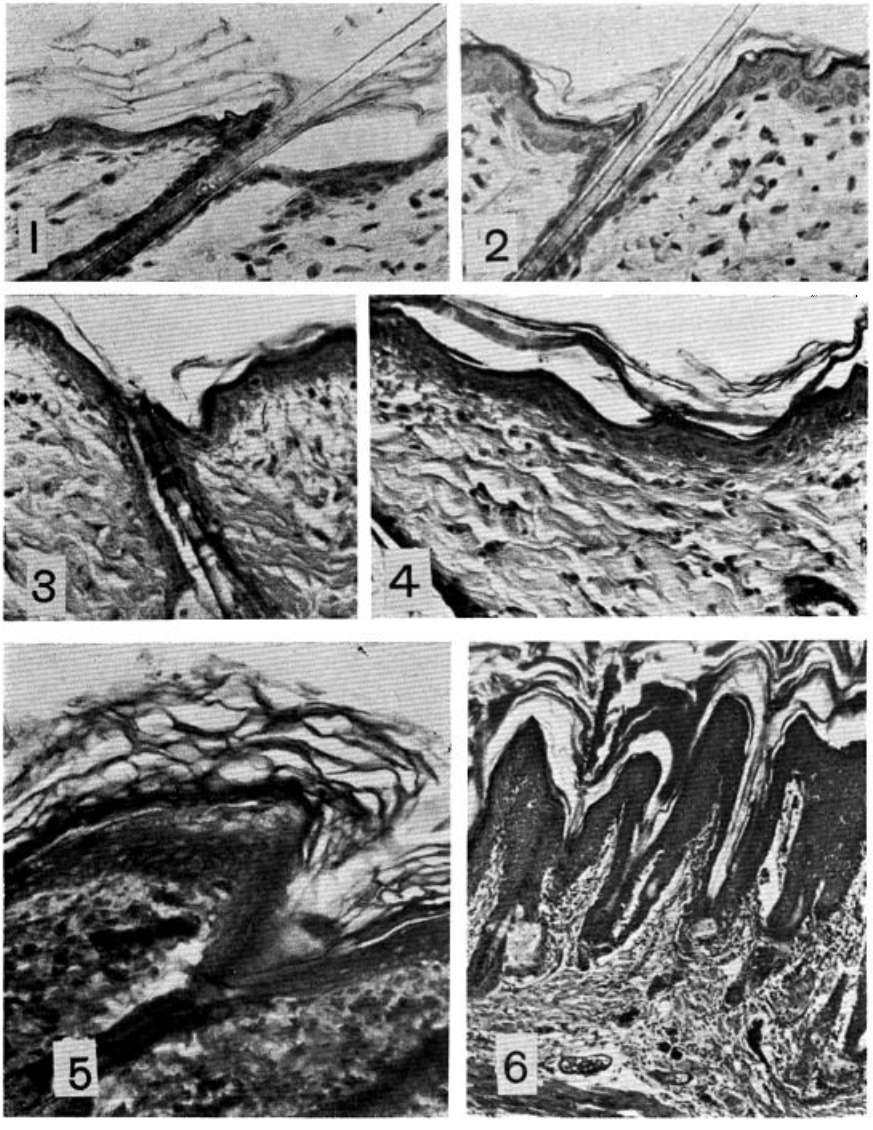


Figure 1.—Skin of 22-day-old rat showing a single-layered germinativum, little indication of a granulosum, and a hard, brittle corneum.

Figure 2.—Skin of 30-day-old rat showing the many-layered germinativum, a granulosum, and a moist corneum.

Figure 3.—Normal skin of a 31-day-old rat.

Figure 4.—Skin of rat receiving wool fat applications (twice daily) from 21st to 31st day of life.

Figure 5.—Skin to which mineral oil was applied (twice daily) from the 21st to 33rd day of life. Biopsy taken at age of 35 days.

Figure 6.—Oleic acid applications from 21st to 27th day (twice daily) caused this effect.

The skin of the rat provides excellent material for studying the effects of various substances on the epidermis. Just what will stimulate the skin to have an ideal condition without irritating it, causing parakeratosis, an acanthosis, or affecting it in some adverse way is the chief interest of the cosmetic chemist.

Histological Effects of Substances on the Epidermis

A few years ago several substances were tested on the epidermis. Among the substances causing little or no effect was lanolin which has long been known to be beneficial (Fig. 4). The skin of the treated animal often felt slightly softer and upon histological examination the epidermis was similar to the epidermis of the control animal (4).

Among the substances causing a mild effect were stearic acid, castor oil, and mineral oil. Much to my surprise, mineral oil produced a hypertrophy of the entire epidermis (Fig. 5). This hypertrophy involved the prickle cell layer, granulosum, and imperfect cornification. Perlman (5) found that mineral oil added to the diet of the rat caused hypertrophy of the gingiva of the mandibular region.

Olive oil and xylene extensively affected the entire epidermis. Following xylene applications imperfect cornification was particularly noted. The cells were swollen, loosely united with air, and fluid between them. Upon cessation of the applications the corneum was shed in great quantities.

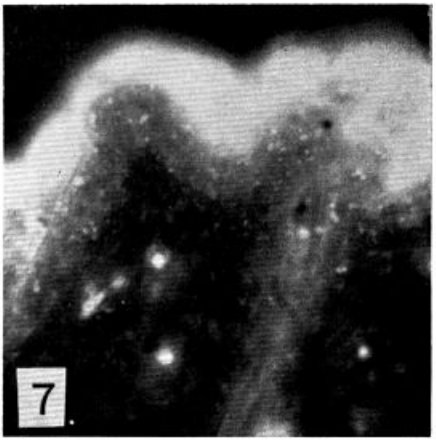
Olive oil produced the most consistent and marked changes. Great hypertrophy took place in the prickle cell stratum and the granulosum became seven or eight layers in thickness. Parakeratosis was very marked. One might suspect that the effect of olive oil was due to free fatty acids present in it. Accordingly, oleic acid was applied and not only did this acid affect the epidermis but it penetrated down into the hair follicles and greatly affected their epithelial lining (Fig. 6).

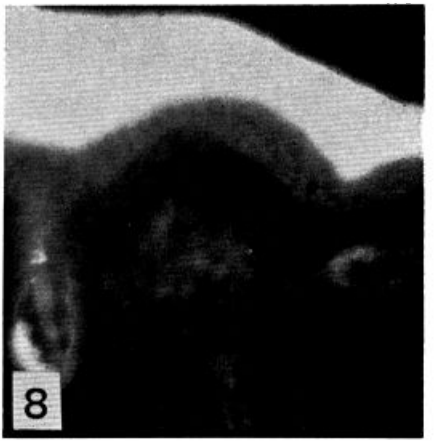
Ethylene glycol and propylene glycol applications caused little effect.

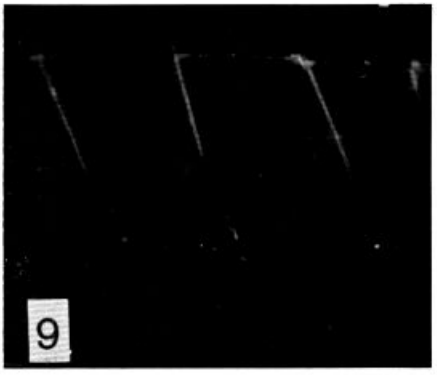
Oils did not cause the parakeratosis by retarding the desiccation of the cells for the wool fat would have the same effect. Where oils were administered the cells of the corneum must have been altered, permitting them to retain their fluid content. The granulosum is also affected. It is thicker, contains more granules, and mitotic figures are more frequent in it. Stimulation of the granulosum cells must have resulted from the contact of the cells with the olive oil or oleic acid which necessitates their penetration. This possibility stimulated a study on the extent of penetration of substances.

The Penetration of Substances into the Skin

Various attempts have been made to see if, and by what channels, fats and fatty acids penetrate the skin (6). In other techniques fats have been







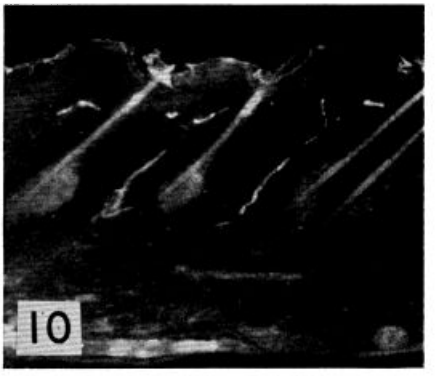


Figure 7.—Skin treated with linoleic acid. Biopsy taken 10 minutes after application.
Figure 8.—Lanolin 10 minutes after application.
Figure 9.—Normal skin of rat.
Figure 10.—Biopsy of skin 20 minutes after application of linoleic acid.
Figure 11.—Applications of iodinated linoleic acid were applied at 9:30, 11:30, and 1:30, and four hours later the biopsy was taken.

applied to the skin and the skin stained after sectioning to obtain the location of the fat (7). In either instance, the dyes spread to adjacent tissues and no one has ever been able to demonstrate fatty acid penetration or destination too clearly (8). Theoretically some lipoid substances such as cholesterol, lecithin, and the fatty acids should be able to penetrate since they are miscible to some extent in both fats and water. The penetration of fats and fatty acids was therefore studied by fluorescence. They possessed or enhanced fluorescence due to substances dissolved in them (9). Applications of the substances were made, biopsies were taken, fixed in formalin, and sections were cut on the freezing microtome.

Linoleic acid penetrates the epithelium rapidly (Fig. 7). Twenty minutes after the application sections show that linoleic acid is present in the blood vessels (Fig. 10). Cross sections of various vascular channels show that linoleic acid adheres as a thin film to their lining.

Oleic acid is also absorbed readily. Droplets of varying sizes can be seen in the epidermal cell layers ten minutes after the application. Only minute amounts are ever found in the blood vessels at any time, indicating that passage into the vessels is slow, not extensive, or there is little retention in the vessels.

Lanolin (Fig. 8) and ricinoleic acid were retained mainly in the outer strata of the epidermis. If they penetrate their absorption must be very slow or in small amounts since they cannot be detected by fluorescence.

The path of penetration of substances through the skin has been thought to be *via* the hair follicles. Since the linoleic acid is found in the horizontal plexus of vessels under the epithelium before it is found in vessels around the sebaceous glands, a great amount must pass directly through the epidermis.

The linoleic acid must affect the epithelial cells and decrease their protective properties since after several applications the acid seems to penetrate faster and in greater quantities. The penetrating fatty acids probably induce growth and repair of the epithelium as does any other injury.

The fact that lanolin was retained by the superficial strata of the epidermis and did not penetrate more deeply is surprising. However, lanolin did not penetrate in the experiments of others (6, 10). Radioactive sodium has also been absorbed from fatty bases (11).

While the present experiments on penetration were quite convincing and it appeared that linoleic acid even entered the blood vessels, this observation needed confirmation. Radioactive linoleic acid was sought with the intention of following its course through the epithelium. However, such acid could not be obtained and linoleic acid was iodinated converting it into mono-iodo-stearic acid.

Applications of the iodinated material were made, biopsies were taken, and slides prepared (12). Great penetration took place into the hair folli-

cles and sebaceous glands and some penetration through the epidermis directly (Fig. 11). The amount in the dermis seemed to depend upon the quantity applied, either by means of concentration or number of applications. The dermis acted as a barrier and restricted the depth of penetration. Several days after the last application, the material in the dermis had diffused and much of the epidermis had shed, the shedding cells carrying with them the radioactive material. There was no evidence that the iodinated material had passed in any quantity into the blood vessels. Iodination of the linoleic acid molecule probably prevented such passage.

The effect of the radioactive material on the N. B. T. plates was so diffuse that one could not determine whether the material passed through or between the cells of the epidermis. Likewise the exact depth of penetration could not be determined. This was disappointing and a better method needs to be devised.

Throughout these penetration studies, it was noted that many of the fatty acids were much more effective at a certain time in the hair cycle. For instance, when the hair coat is resting, there is much penetration, and as the time of growth is approached, applications of the fatty acids are less irritating. This led to a detailed study of the skin and the cause or causes of these different effects.

Fluid Passage Through the Skin

Since the application of fatty acids was more irritating at some intervals than others, the fluid condition of the epidermis was suspected as making these effects possible. One means of investigating the fluid aspect was to determine the fluid passage through the epidermis.

For determining fluid loss, skin of different aged animals was stretched across diffusion chambers (13) containing 10 cc. normal saline. The chambers were inverted and left on a screen in an oven maintained at 35°C. and at a humidity of 28–32 per cent. The fluid loss through the skin of rats 22 days old averaged 1.302 mg./sq. cm. for the first hour. The loss gradually increased and by the 29th day of life the average loss per square centimeter was 2.922 mg. for the first hour.

Fluid loss through the epidermis is therefore least when the epidermis is thinnest, there is no distinct granulosum and the corneum is dry, hard, and brittle. There is less fluid in the skin as shown by a previous investigation (3) and more is evaporated than is supplied by the underlying tissues. This tends to dry out the corneum which aids in retarding and reducing the fluid loss. In the 30-day-old rat the epidermis is thicker, and a distinct granulosum is present. At this age the fluid content of the skin is greater. The ratio of the fluid supplied the epidermis by the underlying tissues in respect to evaporation is greater than in a 22-day-old rat and thus a moist corneum exists.

Linoleic acid was gently applied in the morning and in the evening to 22-day-old rats. On the following morning, skin was removed and placed on two diffusion chambers. The first hour the loss was 18.237 mg./sq. cm. By the 29th day of life, as shown by skin from animals of this age, the linoleic acid was less effective and the loss was only 3.239 mg./sq. cm. Thus when the corneum is dry, linoleic acid penetrates more rapidly. By this penetration the epidermis is altered and the water loss is tremendous.

Discussion

Hair growth, changes in the epidermis, fluid content, and metabolism of the skin are closely correlated in the rat. One may ask if we have conditions comparable to this in the human. Undoubtedly there are intervals when the metabolism of the human skin is low. The greater shedding of dry epidermal cells during the winter months is in all probability quite similar to the conditions in the low stage of the skin in the rat.

When such a low exists, then applications of many substances may be irritative, resulting in parakeratosis and shedding of moist cellular material. If coolness does favor the formation of unsaturated acids (14), more of the latter may be found than usual during the winter months and enhance the shedding of epidermal cells.

The fact that mineral oil caused parakeratosis and was not conducive to an improvement of the skin is somewhat disappointing for mineral oil is used widely in the cosmetic industry. According to O'Brien (15), daily applications of kerosene caused parakeratotic plugs in human sweat pores.

The importance of a moist corneum has been emphasized in this review. One may ask if such a condition would not always be present in the human since the presence of sweat glands would supply much moisture to the surface while in the laboratory animals where the skin is devoid of sweat glands, this asset would be entirely lacking. Despite the presence of sweat glands and invisible perspiration in the human, I am still inclined to believe that the scalp and skin become very dry and need additional coverings to prevent water loss and thereby keep the corneum soft and moist.

A moist corneum, active granulosum, high fluid content, and high metabolism of the skin are characteristic of a good skin. In all these experiments, lanolin is the only substance which has not affected the skin adversely. Castor oil also seemed to have little effect. The other substances, in many instances, induced parakeratosis which was not due to the retardation of desiccation for wool fat, would have had the same effect.

Lanolin undoubtedly prevents the loss of moisture content from the skin. What one needs is something which when applied will not only do this but also increase the metabolism and fluid content of the skin as a whole without causing parakeratosis. The development of this substance is the challenge for the cosmetic chemist.

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THE USE OF THE PENETROMETER IN THE DETERMINATION OF CONSISTENCY OF PETROLEUM JELLY*

By R. T. Dobson

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The object of this short talk is to explain to those not familiar with the penetrometer the working method to be employed with this apparatus and precautions which should be undertaken to ensure that repeatable and comparable results may be obtained which are indicative of differences in consistency between petroleum jellies from different sources. As many of you will be aware, the consistency of petroleum jelly can vary sufficiently to cause subtle differences in the finished product in which it is used and, although a Yellow B.P. W/A Grade 45 or a White B.P. W/A Grade 40A or 40B may be specified, you may not always receive supplies derived from

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