EFFECT OF ESTRADIOL LOCALLY APPLIED TO ABNORMAL SKIN

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The fact that natural folliculoids,[1] such as estradiol or estrone, and artificial folliculoids, such as stilbestrol, are effectively absorbed through the skin has been demonstrated by numerous investigators.[2] It has been found, for instance, that in the mouse inunction of the skin with solutions or suspensions of folliculoid compounds causes typical estrous-like changes in the internal sex organs. Although it has often been claimed that in certain cases folliculoids may act as carcinogens, none of the aforementioned investigators were able to produce cutaneous changes resembling cancer by the local application of the folliculoids to the skin.

That folliculoids may elicit some local action on the skin has been shown by Steinach,[3] who found that cutaneous hyperemia may be induced by such compounds in the rat. Argüello [4] observed, in the same species, great diminution in the number of hair follicles and hyperplasia of the cornified layer after subcutaneous injection of estrone. Although these changes were greatest in the region immediately surrounding the site of injection, he did not consider them the direct effect of the endocrine substance, since local application of estrone to the skin had no effect. He expressed the belief that they were more probably due to an alteration in the pituitary. On the other hand, Kun [5] reported that the percutaneous administration of estradiol or its benzoate in an ointment, in addition to causing hyperemia of the skin, stimulates the growth of hair in infantile or senile rats, hair growth in which is normally poor. Histologically there is thickening of the skin, with cellular proliferation in the epidermis and in the connective tissue of the corium. The hair follicles, the sebaceous glands and the cutaneous muscles all show an increase in size.

Previous observations [6] showed that squamous cell metaplasia and "precancerous" changes are most readily produced in rats after local injection of follicu-

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1. The term folliculoid is used to designate substances having an activity resembling that of the ovarian follicles (Selye, H.: Pharmacological Classification of Steroid Hormones, Nature, London **148**:85, 1941).

2. Glimm, E., and Wadehn, F.: Beitrag zur Kenntnis eines Sexualhormons der menschlichen Placenta (Feminin), Biochem. Ztschr. **179**:3, 1926. de Jongh, S. E., and Laqueur, E.: Ueber die percutane Wirkung des Menformons, Acta brev. Neerland. **2**:220, 1932. Burrows, H., and Kennaway, N. M.: On Some Effects Produced by Applying Oestrin to the Skin of Mice, Am. J. Cancer **20**:48, 1934. Emmens, C. W.: The Inunction of Sex Hormones on the Skin, J. Endocrinol. **2**:368, 1941. Palmer, A., and Zuckerman, S.: Further Observations on the Similarity of Stilbestrol and Natural Oestrogenic Agents, Lancet **1**:933, 1939.

3. Steinach, E.: Zur Geschichte des männlichen Sexualhormons und seiner Wirkungen am Säugetier und beim Menschen, Wien. klin. Wchnschr. **49**:161, 1936.

4. Argüello, R. A.: Influence de l'administration prolongée d'oestrone sur la peau annexes du rat blanc, Compt. rend. Soc. de biol. **124**:497, 1937.

5. Kun, H.: Wirkungen des Follikelhormons auf die Haut bei perkutaner Verabreichung. Histologische Untersuchungen an infantilen und senilen Ratten, Wien. klin. Wchnschr. **50**:408, 1937.

6. Selye, H.; Thomson, D. L., and Collip, J. B.: Metaplasia of Uterine Epithelium Produced by Chronic Oestrin Administration, Nature, London **135**:65, 1935.

loids into the uterine lumen, an observation which has repeatedly been confirmed [7] and which implies that such local treatment may also lead to pathologic types of keratinization in the skin. In view of the fact that folliculoids are frequently used as ingredients of ointments for the skin, it appeared desirable to reinvestigate this problem.

A few years ago Howard,[8] working at McGill University, described the so-called rhino mouse, which bears the allele of the gene for the hairless mouse. In this animal the skin is almost completely hairless and greatly thickened, as well as increased in total area. As a result of this, deep, thick folds of skin cover the surface of the animal, so that its appearance justifies the name given it. This cutaneous abnormality is a simple recessive characteristic, heterozygotes being completely normal and the second filial generation showing a 3:1 ratio of normal and rhino rats. The histologic structure of the skin will be described in detail in the doctor's thesis of Mr. F. C. Fraser, of the department of genetics; so I shall limit myself to a few statements which are essential to the understanding of the results obtained. The skin of the heterozygotes is, as was said, macro-

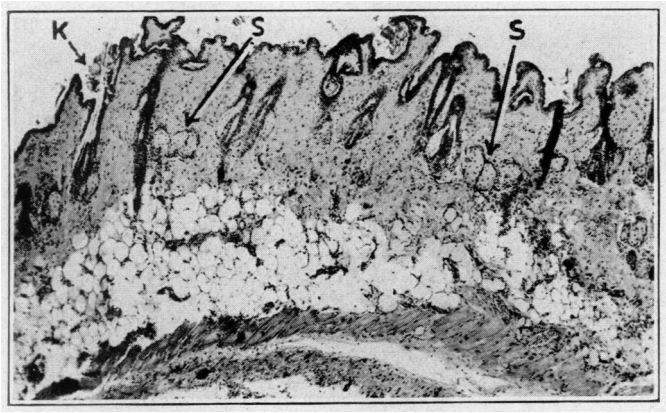

Fig. 1.—Section through the skin of a heterozygote mouse in which the rhino type of skin was not manifest. The general histologic appearance of the skin is also normal, although an abortive beginning of excessive keratinization can be distinguished in occasional hair follicles, such as that marked *K*. The sebaceous glands are indicated by *S*.

scopically normal and covered with fur. Histologic examination does not reveal any major abnormality, although occasional hair follicles are filled with more or less irregular clumps of keratinized squamous epithelium. In contrast, the skin of rhino mice contains almost no normal follicles, and the derma is filled with large cystic spaces, most of which contain amorphous detritus. Few of these cysts are filled with well preserved cornified squamous cells, and some of them have close topographic connections with sebaceous glands, the secretions of which are discharged into the cysts. By following the evolution of these changes, especially in young animals, in which the condition is not as yet fully developed,

7. Arnold, O. H.; Grumbrecht, P., and Loeser, A.: Organveränderungen und Allgemeinreaktion bei intrauteriner Anwendung von oestrogenen Substanzen, Arch. f. exper. Path. u. Pharmakol. **191**:192, 1938. Grumbrecht, P., and Loeser, A.: Aufbau der Uterusschleimhaut bei intrauteriner Einwirkung von oestrogenen Substanzen, Arch. f. Gynäk. **167**:373, 1938; Die innersekretorische Bedeutung des Uterus, ibid. **168**:889, 1939.

8. Howard, A.: "Rhino," an Allele of Hairless in the House Mouse, J. Hered. **31**:467, 1940.

one may easily ascertain that the cysts are actually distended hair follicles in which the hair itself has undergone almost complete degeneration. The wall of the cyst continues to form amorphous material, which is only incompletely extruded and, together with the sebaceous secretion, causes progressive enlargement of the central cavity. The sebaceous glands connected with each hair follicle remain practically normal. These hair follicle cysts become so large that if the skin is separated from the underlying tissue one can clearly distinguish by inspection with the naked eye numerous small white granules, which are visible through the subcutaneous tissue (figs. 1 and 2). The resemblance of these changes to those seen in ichthyosis follicularis (keratosis pilaris or lichen pilaris) is noteworthy.

It was felt that it would be of interest to determine the local action of estradiol on a skin in which keratinization and hair formation are seriously disturbed as a result of a genetic abnormality. Through the cooperation of the department of genetics, I obtained 8 postpubertal rhino mice, weighing 20 to 29 Gm. (average 26 Gm.) at the onset of the experiment. Four of these (1 male and 3 females) were treated with a solution of 250 micrograms of estradiol per cubic centimeter of peanut oil, which was rubbed into their backs by means of a cotton pad once

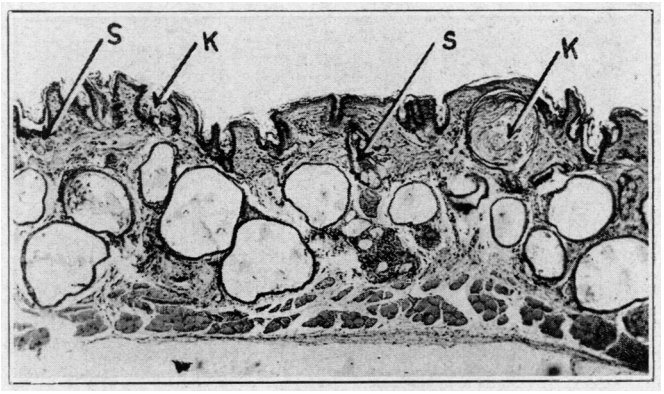

Fig. 2.—Section through the skin of a mouse in which the rhino type of skin was manifest. Note the absence of normal hair follicles. Follicles indicated by *K* are keratinized, the one on the right being the largest keratinized follicle in a series of sections through the skin of untreated rhino mice. The light cysts in the lower layers of the derma contained an amorphous material, perhaps partly sebaceous secretion, which was dissolved in the process of fixation, dehydration and embedding. The sebaceous glands, marked *S*, are well preserved.

daily for four months. The remaining 4 mice (2 males and 2 females) served as controls and were treated with pure peanut oil in the same manner as the animals of the experimental group.

Within a few weeks after the initiation of the treatment the skin of the estradiol-treated animals became covered with a comparatively thick layer of brownish, cornified epidermal scales, which readily sloughed off when the area was rubbed; after this the thickness of the derma increased slowly, but constantly, throughout the entire period of treatment. During the final stages the difference between the treated and the untreated animals could easily be recognized by taking up a fold of skin between two fingers.

At the end of the fourth month of treatment all the animals were killed and the skin was carefully dissected from the underlying tissue. With the naked eye it was easy to see that the fine white granules, which are readily visible through the subcutaneous tissue of rhino mice, were greatly enlarged in the estradiol-

treated animals, so that, viewed from the subcutaneous tissue side, the skin of the treated mice exhibited a coarse granular appearance (fig. 3).

On histologic examination the most outstanding change induced by estradiol proved to be an enormous increase in the production of keratin and its accumulation within the abnormal hair follicles. The sebaceous glands disappeared almost

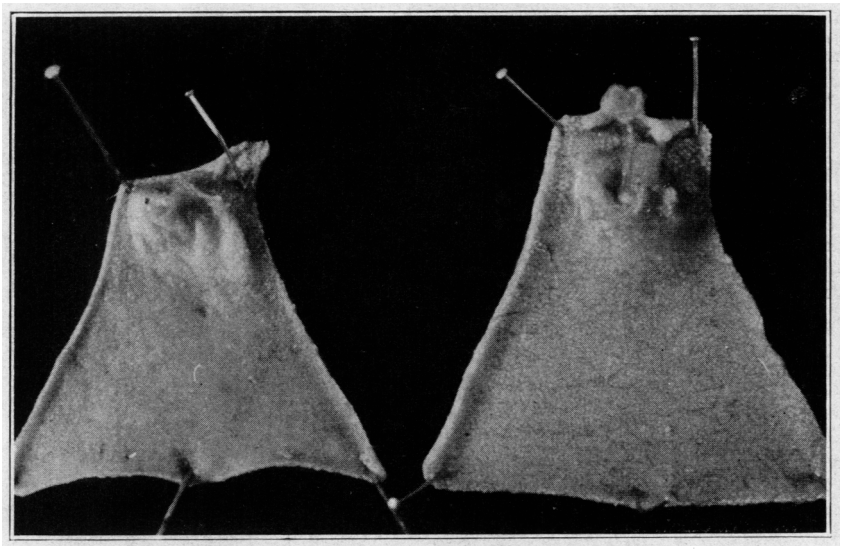

Fig. 3.—Macroscopic view of the caudal part of the skin of the back taken from an untreated and from an estradiol-treated rhino mouse. The dermal side of the skin is uppermost. Through the subcutaneous tissue one may readily distinguish the fine granules in the skin of the untreated mouse (left) and the coarse granules in that of the estradiol-treated mouse (right).

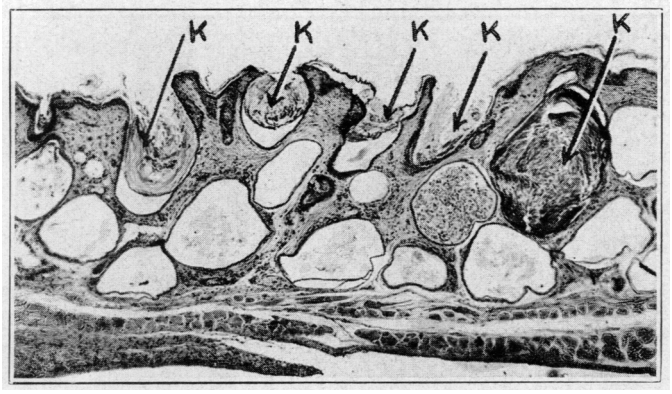

Fig. 4.—Section through the skin of a rhino mouse treated with estradiol. Note the large number of keratinized follicles, indicated by *K*. These follicles are greatly dilated with cornified squamous cells, and most of them are in the process of discharging their contents onto the surface of the skin. Sebaceous glands have entirely disappeared.

completely, perhaps as a result of pressure from the cysts. The surface epithelium was not significantly thickened, although it was perhaps somewhat more stratified than in untreated controls. The blood vessels of the corium were greatly dilated in all estradiol-treated animals. From these histologic observations it appears that

the thickening of the skin in the estradiol-treated mice was mainly due to the fact that under the influence of this substance the hair follicles produced an abnormally great amount of keratinized material, which distended the follicular cavities and thus increased the thickness and the granular appearance of the skin. Since many of these follicles discharge their cornified cells onto the free surface of the skin, the presence of the abundant scaly material could also be explained (figs. 1, 2 and 4). It should be emphasized that in spite of the local application of large quantities of estradiol to the naked skin of these animals, malignant changes have not been induced during the period of observation.

SUMMARY

The main histologic characteristics of the cutaneous structure of the rhino mouse are described, and their resemblance to the changes in ichthyosis follicularis is emphasized.

Chronic treatment by local application of estradiol to the skin of such animals caused increased keratinization of the abnormal hair follicles, hyperemia of the skin and involution of the cutaneous sebaceous glands. Malignant transformation of the congenitally abnormal skin was not observed during the four month period of observation.

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