HISTOLOGIC CHANGES IN THE SKIN OF THE FEMALE GUINEA PIG FOLLOWING PERCUTANEOUS APPLICATION OF ESTROGEN[1]

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THIRTEEN FIGURES

Topically applied estrogens are absorbed through the skin (Zondek, '29) and, if dissolved in a volatile organic solvent, may be as effective systemically as when injected subcutaneously in oil (Zondek, '38). The systemic effects of percutaneous application of estrogen have been studied by Burrows and Kennaway ('34) in the mouse; Loeser ('37) in the human female and baboon; Ito, Hajazu and Kon ('37) in the rat; and by Emmens ('41) in the mouse and capon. Moore, Lamar and Beck ('38) and Zondek ('35) are the only investigators who have studied the effects of percutaneously applied estrogen in the guinea pig, but they gave no attention to the skin. On the basis of these investigations it is established that the systemic changes produced in the guinea pig when estrogen is applied to the skin are identical with, or similar to, those elicited by subcutaneous injection. Information on the local response of the skin itself is not available. The objective of the present study was to investigate in female guinea pigs the histological changes which occur in the skin at the site of application of estrogen. In addition, an attempt was made to find out if these effects are modified by previous pregnancies.

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MATERIALS AND METHODS

An anhydrous cream consisting of 60 gm stearic acid, 6 gm triethanolamine, 124 gm propylene glycol, and 18 gm anhydrous lanolin, was developed as a vehicle for the estrogen. This cream possessed several desirable characteristics. It (a) melted at body temperature, (b) was absorbed rapidly, (c) was non-irritating to the skin, (d) permitted the incorporation of a high concentration of estrogen in a small volume, and (e) was easy to prepare with little possibility of error. Pellets were made in one grain tablet triturate molds and weighed to determine the accuracy of the mold. The average weight of the pellets was 68 mg and a mold was discarded if it produced pellets varying more than 5 mg from this norm.

Gynestrol Crystallizate (S. B. Penick Co.) was the estrogen used. It contained 97.7% estrone, 1 gm being equivalent to 9.3 million I.U. Each pellet contained 245 I.U. In order to insure uniform mixture of the estrogen with the cream vehicle the estrogen was dissolved in propylene glycol over a warm water bath. The mixture was stirred and inspected microscopically until all of the estrogen crystals were dissolved. Then the anhydrous lanolin was added to the estrogenized propylene glycol and stirred. The stearic acid and triethanolamine were heated together over a water bath until a uniform amber-colored solution resulted. The latter solution was combined with the estrogenized propylene glycol and stirred for several minutes over a water bath. This mixture was removed from the water bath and allowed to cool. The cooling mixture was stirred until a semi-solid cream was obtained.

The 35 guinea pigs used in this study included *virgin animals* which were sexually mature and exhibited a normal estrus rhythm and *multiparous animals* which had been bred twice since the onset of sexual maturity but with several months intervening since the weaning of the last litter. They were divided into 4 groups: (a) 5 virgins and 5 multipara controls which received no treatment and whose skins were studied to determine the normal variations in histology, (b) two virgins and three multipara which received the cream vehicle without

estrogen, (c) 10 virgins and (d) 10 multipara, the latter two groups receiving the estrogen cream.

In preparation for inunction, the hair was removed from the left flank of the guinea pig with an electric clipper, care being taken not to injure the skin. A rubber mask with an aperture of approximately 57 cm^2 was placed over the flank in order to limit the area of the skin subjected to inunction. With the animals immobilized, one pellet was placed on the skin of the left flank and rubbed in with the index finger for one and one-half minutes. This volume was just sufficient to cover the exposed area. The inunctions were carried out uniformly on all animals.

Thus the daily dose for each animal receiving estrogen was 245 I.U., the hormone content of one pellet. This was the minimal amount necessary to produce estrus in ovariectomized animals within a period of 48 hours, as determined by preliminary tests. Inunctions were performed daily for periods up to 62 days. Animals from the virgin and multiparous groups were sacrificed successively every 6 to 9 days. Two control animals were sacrificed at the mid-point of the experiment and the remainder at the conclusion of the experiment. The guinea pigs were fed a diet consisting of Purina Chow supplemented with cabbage, milk, carrots and oranges.

Samples of skin were fixed in Allen's modification of Bouin's fluid, embedded in paraffin, sectioned at 10 μ, and stained in Harris' hematoxylin and refined eosin, or Ehrlich's elastic tissue stain.

OBSERVATIONS

(a) Non-treated controls

The flank epidermis of the virgin control animals lacked a continuous stratum granulosum. An occasional granulated cell was observed between the stratum corneum and germinativum in some animals and none in others. There was no clearly defined stratum lucidum.

The histology of the multiparous animals differed only slightly from that of the virgins, the epidermis being slightly

higher and the dermal collagenous fibers somewhat more densely arranged. In order to minimize these minor variations, both virgins and multipara were included in the control animals which received the base cream alone.

(b) Base cream-treated controls

The skin of the inuncted flank of the animals, sacrificed at the mid-point of the experiment, was not different from that of the untreated side except for a slight increase in the height of the epidermis and increased condensation of the collagenous tissue of the derma. These changes were observed consistently and were believed to be the result of friction induced during inunction (figs. 1 and 2). Application of the base cream did not modify the texture of the skin or the rate and quality of hair growth.

No variations from normal were observed in the estrus cycle of the control animals. All subsequent references to control animals will deal only with base cream-treated controls.

(c) Estrogen-treated virgin animals

Epithelial structures. The first epithelial change on the estrogen-inuncted flank occurred between 9 and 17 days of treatment. The cells immediately subjacent to the stratum corneum were enlarged (fig. 11) as compared with the base cream-treated control (fig. 3) and the epidermis was thrown up into elevations (fig. 10) apparently as a result of hypertrophy of the epithelial cells rather than the development of underlying dermal ridges. Accelerated mitotic activity probably was not involved because no increase in the number of mitoses was found in the stratum germinativum underlying the elevations. The hypertrophied cells were observed frequently among the superficial desquamating scales. The stratum corneum was thick, the deeper layers being arranged in thin stratified lamellae (fig. 11), this stratification not being evident in the base cream-treated control animals.

Treatment of virgin animals with estrogen for the longer period of 17 to 62 days produced more extensive epidermal alteration. Cellular hypertrophy was more marked and involved the lowermost cells of stratum germinativum. Both nucleus and cytoplasm were enlarged. In superficial areas where this change was most severe, the nuclei were disrupted and nearly indistinguishable from cytoplasm. Estrogen treatment reduced the number of cell layers in the epidermis to as few as four. However, the total epidermal thickness was maintained by the general cellular hypertrophy and a slight increase in the amount of intercellular substance. Thus, the overall epidermal height was comparable to the thinner areas of epidermis in control animals. Coincident with these changes the basement membrane became less distinct and the delicate felted fibers of the subjacent derma seemed to insinuate themselves between the basal cells.

Treatment for 9 to 16 days modified the pilo-sebaceous apparatus. Some sebaceous glands appeared to be hypertrophied. Most of them exhibited more nuclear pycnosis and cellular degeneration (fig. 11) followed by disappearance of glands far in excess of that observed in control animals. All portions of the hair sheaths after 16 days of treatment displayed pycnosis. Many of the hair sheaths appeared to retract radially from the hair shaft, forming deep intervening crypts. This separation of sheath and hair shaft frequently extended throughout the length of the hair to the side of the hair bulb. Loss of hair and depression of hair growth was noted grossly at 16 to 62 days. Empty hair sheaths, degenerating hair shafts and sebaceous alveoli were frequent (fig. 11).

Derma. The initial effect of estrogen treatment on the derma was apparent in the reticular layer. As early as 11 days after the initiation of treatment, the derma appeared to be less fibrous due to an apparent swelling of collagenous fibers. The modification intensified with prolongation of estrogenic action, and involved especially the fibers adjacent to sebaceous glands and hair follicles. The response of elastic fibers to estrogen

was similar in virgins to that in multipara and will be described subsequently.

Many dermal cellular elements were affected. Fibroblasts showed progressive signs of degeneration throughout all levels of the derma. Degeneration of these cells was most marked immediately adjacent to the hair sheaths. The nuclei of most fibroblasts were dark and shrunken. All intergrades from normal fibroblasts to ones in terminal stages of degeneration were observed depending on their proximity to the hair sheaths.

A further intensification of the changes described above occurred in the estrogen-treated flank of animals inuncted for 17 to 62 days. The apparent swelling of collagenous fiber bundles was more pronounced, tending towards a "homogeneous" appearance. Interfibrous vacuoles developed in the delicate subepidermal connective tissue and subsequently throughout the denser layers of the derma (fig. 11). Degenerating nuclei were present in the vacuolar spaces. The majority of these nuclei were situated centrally within the vacuoles, although some were in a marginal position. It is believed that these nuclei belong to fibroblasts and possibly some macrophages. These changes were most prominent in one virgin guinea pig, treated with estrogen for 28 days, in which some areas of the derma approached a nearly homogeneous condition. The absence of the vacuoles in the derma of the control animals (fig. 3) shows that their appearance resulted from the action of estrogen and was not a response to absorption of the base cream.

With prolongation of the period of treatment, certain slight but constant alterations were observed in the cutaneous blood vessels. Endothelial pycnosis, especially in the capillaries, became increasingly apparent. Many of the larger vessels exhibited an abnormally thick tunica adventitia.

(d) Estrogen-treated multiparous animals

Epithelial structures. Epidermal changes in the estrogen-treated area of multiparous animals were more variable than in the virgins. The epidermis of the treated flank in some

cases was thicker than that on the untreated flank of the same animal (figs. 5 and 6) or that of either flank of the control animals (figs. 1 and 2) which were treated with the base cream only. On the other hand, in several animals treated for 17 to 34 days, the epidermis was characterized by thinning, pycnosis, flattening of the remaining cells, and absence of mitosis. The epidermis of these animals was thrown up into high "operculoid" folds (fig. 8) which contained a core of connective tissue and seemed to flank the hair groups at their emergence from the skin surface. These folds were sufficiently large to be faintly visible grossly during inunction and are not to be confused with the epidermal elevations previously described on the treated flank of virgin animals.

The great variability in response is emphasized by the failure of one animal treated for the longest period of time (62 days) to show any change.[2] There was no correlation between duration of treatment and type and extent of the modification induced.

In those cases showing a thickened epidermis, no differences were observed in mitotic activity as compared with base cream-treated controls. The thickened epidermis was marked by the presence of a clearly defined stratum granulosum and a very compact stratum corneum (fig. 7). Cells in which a slight amount of hypertrophy had occurred were found scattered throughout the stratum germinativum of the multiparous animals. The number and distribution of these cells, however, varied widely from one animal to another.

When the epidermis was thinned by estrogen treatment, the more superficial part of all of the epithelial hair sheaths exhibited pycnosis if this change occurred also in the surface epidermis. Deep to the sebaceous alveoli, however, pycnosis was less extensive and the hair sheaths showed little or no change. Depression of hair growth was less pronounced than in the estrogen-treated flank of the virgin animals and hair loss

[2] In spite of the failure of this animal to show a cutaneous response, the fact that the estrogen applications were effective was indicated by marked changes in the liver. The nature of such changes in the liver is the subject of current study.

was no greater than in the base cream-treated flank of the controls.

Derma. The response of the derma was variable, there being no correlation between the duration of treatment and the degree of histological change. The derma of 6 animals inuncted for 9, 28, 34, 45, 55 and 62 days, respectively, showed a loss in fibrous texture which gave it a somewhat homogeneous appearance (fig. 9), which was similar to that which occurred in virgin guinea pigs. Here also, the change seemed to be due to a swelling of collagenous fibers which was evident in all layers of the derma and in the subcutis. As a result, compression of fibroblasts was marked. The cellular elements were otherwise normal. In only 4 guinea pigs was the change sufficiently intense to compare in degree with that described for the virgin animal inuncted with estrogen for 28 days. Complete homogeneity did not occur, some evidence of the original fiber structure always remaining. Vacuolar spaces were present (fig. 8). The subepidermal area of connective tissue, together with the cores of the "operculoid" folds, showed a greater vacuolation than that occurring in a virgin animal treated with estrogen (fig. 10).

The elastic fibers were remarkably resistant to the action of estrogen as compared with collagenous fibers. Their number and texture showed only minor changes in animals inuncted with estrogen for progressively longer periods of time. In most multiparous animals the elastic fibers became more widely dispersed (fig. 12) as compared with base cream-treated control animals (fig. 13), in the latter group there being no significant difference between virgins and multipara. Apparently this dispersion resulted from the changes which had occurred in the intervening collagenous fibers. This was particularly noticeable in the immediate vicinity of sebaceous glands and hair follicles where, in non-estrogenized guinea pigs, the elastic fibers were delicate and densely woven (fig. 13). Contrariwise, in estrogen-inuncted areas elastic fibers generally had moved out from their previous close investment of the hair sheaths, probably because of the swelling force of the neighboring col-

lagenous bundles. Some of the elastic fibers in the estrogen-treated skins exhibited frayed and thickened areas.

It was exceedingly difficult to find blood vessels in the derma which had undergone pronounced connective tissue change. Obscure cords of pycnotic nuclei scattered throughout the derma were interpreted as the surviving traces of degenerating vessels.

DISCUSSION

Hypertrophy of epidermal cells under the influence of estrogen is probably an expression of increased hydration. Estrogen is known to increase tissue hydration (Kun, '37; Selye, '44). Since the cream vehicle employed in this study was anhydrous in character and when used alone produced no change in the epidermis of control animals, the evidence clearly points to the estrogen as being the causative agent. Until species differences have been investigated more fully caution should be exercised in making comparisons between the skin responses of widely separated species which have been subjected to estrogen treatment. Yet it is of interest to note that Goldzieher ('46) and Eller and Eller ('49) observed epidermal cell enlargement in aged human female patients which had been treated with estrogen percutaneously.

Excessive hydration may account also for the apparent enlargement of collagenous fibers in estrogen-treated skin. This does not exclude the possibility that the chemical or physical state of the collagen or the ground substance may have been modified. Similar dermal responses were observed by Kun ('37) in infantile and senile rats, Selye ('44) in the hairless strain of mice, Goldzieher ('46) and Eller and Eller ('49) in man, in the latter instance minimal collagenous changes being observed. The vascular dilatory response observed by Kun ('37) did not occur in my animals.

MacKee et al. ('45) postulated the existence of a barrier to skin absorption in the guinea pig "lying somewhere between the horny and non-horny layers." In view of the copious desquamation of the virgin epidermis and the subsequent reduction in the number of cellular strata, it is conceivable that the

absorption barrier which MacKee and his coworkers describe may have been inactivated or removed, thereby allowing direct absorption of estrogen through the skin surface in addition to the usual route by way of the pilo-sebaceous apparatus. This possible loss of an absorption barrier in the virgins may explain the differences in response to estrogen treatment between the virgins and multipara. Whether the lack of a well-defined stratum granulosum in the virgin epidermis is indicative of a less effective absorption barrier cannot be determined from the material studied. In the multiparous animals, in which the stratum granulosum was well defined, there was less consistent evidence of absorption as indicated by variable dermal and epithelial changes.

The point of greatest absorption was thought by MacKee et al. ('45) to be located at the external opening of the hair follicles where the cornified stratum of the epidermis diminishes. In my study retrograde changes occurred in the pilo-sebaceous apparatus and were more severe in the virgins than in multipara and in each group were proportional to the modification of the surface epidermis. The apparent increase in absorption area due to deepening of the peritrichial crypts in virgin animals, associated with epithelial cell degeneration, also may be responsible for some of the differences in epidermal and dermal response observed between the two groups.

Dermal modification in response to estrogen in virgins was related more directly to duration of treatment than was the case with the multipara. The greater variation in the response of multiparous guinea pigs may be due to the successive pregnancies or other associated factors, such as age, epidermal permeability, or integrity of the vascular supply. The composition of collagenous tissue is known to vary with increasing age in the proportion of water and albuminoid which it contains. In the present study, however, age appears not to be a significant variable since the mean age and its variation were similar in the two groups. The difference in the responses of the virgin and the multiparous groups suggests that the

physiological adjustments to pregnancy may have altered the responsivity of the skin to estrogen.

The effect of percutaneously absorbed estrogen on the elastic fibers of the derma was minimal. Eller and Eller ('49) also noted only slight changes in the cutaneous elastic fibers of 5 of 36 patients which had been treated with topical applications of estrogenic ointments. Goldzieher ('46), however, cited an increase in the number of elastic fibers in aged patients similarly treated. In the present study no neogenesis of elastic fibers was observed, but instead the existing fibers became more widely dispersed. Neogenesis of elastic tissue would seem inconsistent with the extensive fibroblast degeneration and other dermal changes cited.

SUMMARY AND CONCLUSIONS

Chronic application of estrogen in a cream vehicle to the flank of female guinea pigs modified the skin at the site of application, these effects being more marked and consistent in virgins than in multipara. The effects included marked hypertrophy of epidermal cells, especially in virgin animals, and various degrees of swelling of the collagenous fiber bundles in both virgins and multipara. The intensity of the changes in virgins was proportional to the duration of treatment. In both groups interfibrous vacuoles containing degenerating fibroblast nuclei appeared regularly between the fiber bundles. In some instances the swollen fibers of the derma produced a nearly homogeneous condition throughout the tissue. In some multiparous females the vacuolated subepidermal connective tissue produced high operculoid folds which flanked the hairs emerging from the skin surface. Elastic fibers were not affected directly by percutaneously applied estrogen, but their arrangement was altered by the swelling of intervening collagenous bundles. There was no indication that estrogen absorbed through the skin is capable of stimulating neogenesis of elastic tissue.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

All specimens illustrated were fixed in modified Bouin's fluid and stained with Harris' hematoxylin and refined eosin except figures 12 and 13 which were stained with Ehrlich's procedure for elastic fibers.

1 Skin of left flank of virgin control animal inuncted for 62 days with base cream only. × 38.

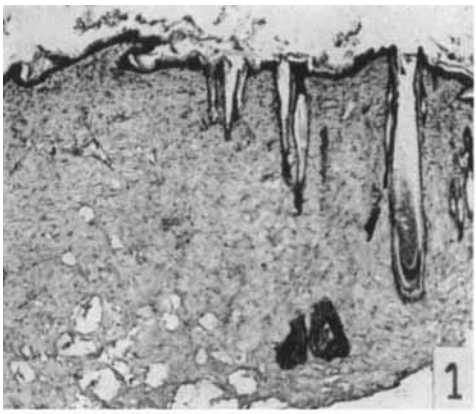
2 Skin from right untreated flank of the control animal illustrated in figure 1. The epidermis is not as thick, and the derma is somewhat less dense than that of the treated flank. × 38.

3 Skin of base cream-treated flank of virgin control animal, showing slightly thickened epidermis. The subepidermal connective tissue shows normal size and arrangement of fibers and distribution of cells. × 200.

4 Reticular layer of derma of the base cream-treated flank of a virgin control. The arrangement of the collagenous fiber bundles and the distribution of cells is normal. × 216.

5 Skin from the flank of a multiparous guinea pig treated with estrogen for 45 days. The epidermis is thicker than on the base cream-treated skin of the controls (fig. 1). The dermis is unchanged. × 38.

6 Skin from the untreated flank of the animal utilized for figure 5. The thickness of the epidermis is approximately half as great as that shown in figure 5, and the stratum disjunctum is more characteristic of the type displayed by the control animals. × 38.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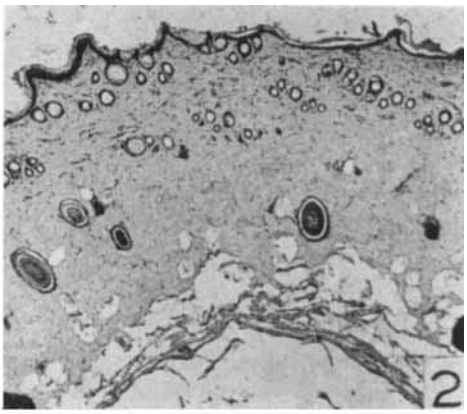
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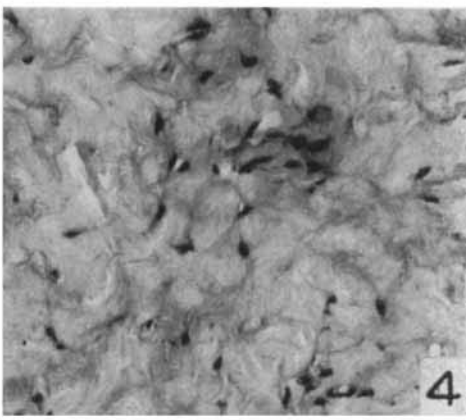
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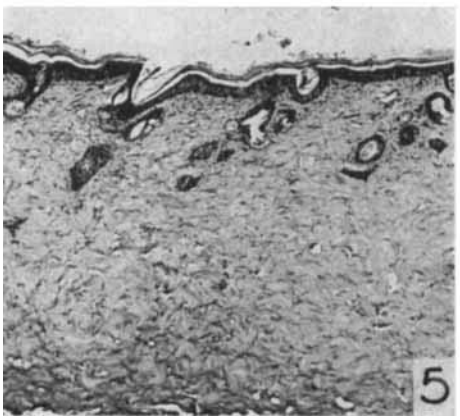
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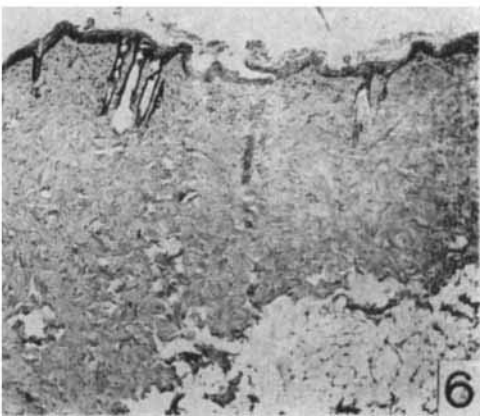
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PLATE 2

EXPLANATION OF FIGURES

7 Epidermis and subepidermal connective tissue from the treated skin of the multiparous animal utilized for figure 5. The stratum disjunctum is dense. Moderately hypertrophic epidermal cells are scattered throughout the stratum germinativum. A few vacuolar spaces containing cells are present in the subepidermal tissue. × 200.

8 Skin of multiparous animal treated in this area for 17 days with estrogen in which epidermal and dermal changes are severe. The epidermis has been thrown into a number of "operculoid" folds. Vacuolar spaces are numerous throughout the dermis. × 56.

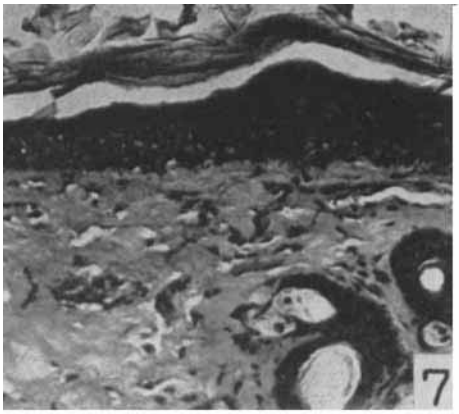
9 Deeper dermis of the same multiparous animal utilized for figure 8. The collagenous tissue is somewhat homogeneous although fibers are detectable. Many vacuolar spaces are evident. × 200.

10 Skin from treated flank of a virgin animal which received estrogen for 17 days. The epidermal elevations are low. Vacuolar spaces are present in the dermis. × 56.

11 Epidermis and subepidermal connective tissue from the animal utilized for figure 10. Hypertrophic cells are located in the epidermal elevations. Vacuolar spaces containing degenerating fibroblasts are present in the connective tissue surrounding hair sheaths and sebaceous alveoli. × 200.

12 Skin from the estrogen-treated flank of a multiparous guinea pig showing the distribution of elastic fibers. The connective tissue exclusive of the elastic fibers has a homogeneous appearance. The elastic fibers are not affected greatly but may be more widely separated due to swelling of intervening collagenous fibers. Compare with figure 13. × 200.

13 Skin of a virgin base cream-treated animal, control for guinea pig illustrated in figure 12. The distribution of elastic fibers is shown. × 200.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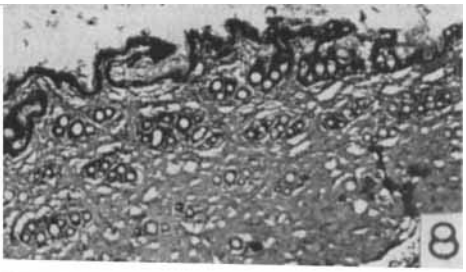
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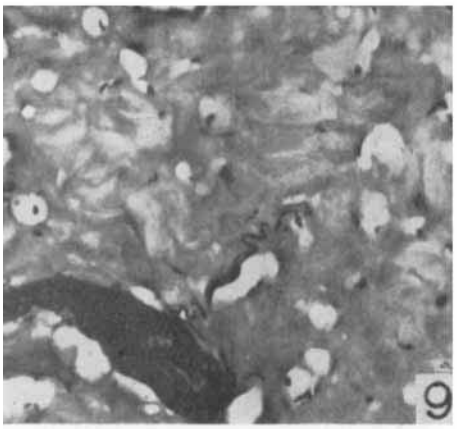
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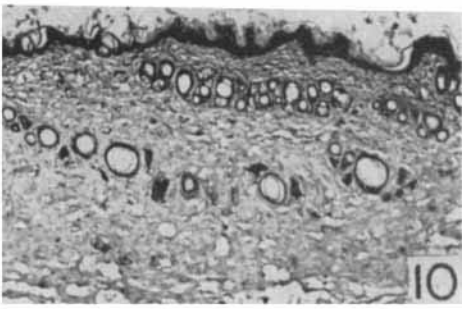
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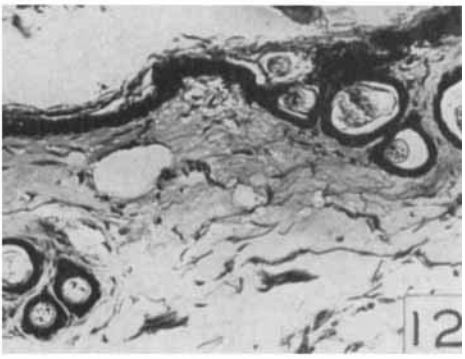
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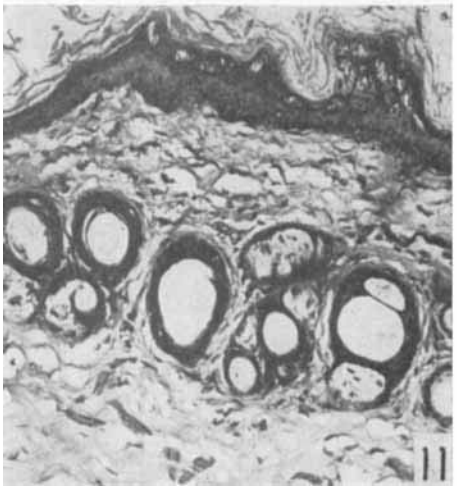
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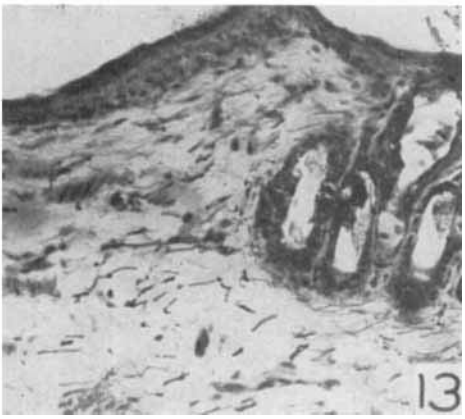
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