mediately cephalad to the anterior fold of the hind limb. The graft and its bed were protected from external irritation by attaching 'Perspex' wound-healing chambers[2] to the skin immediately surrounding the grafted area; the grafts were held firmly in position and prevented from drying by packing 'Sofratulle' dressing over the graft before the chambers were closed.

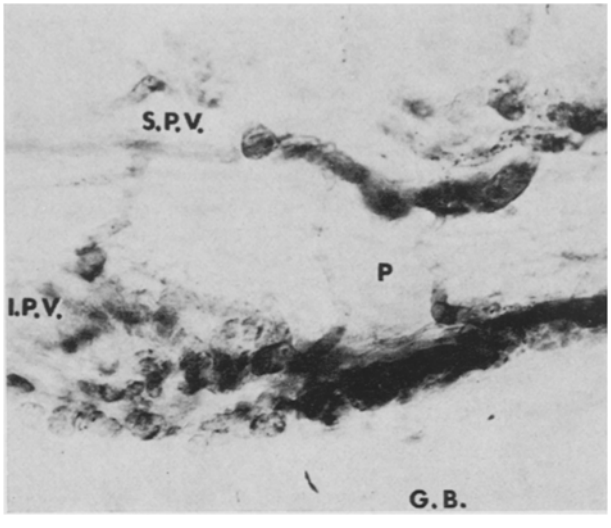
Increased vascular permeability was assessed by injecting colloidal carbon (Gunther Wagner Pelikan Werke, Hanover, Germany: batch C11/1431a) i.v. in a dose of 0.1 ml/100 g body-weight at various times after grafting; 30–60 min later when free circulating carbon had been cleared by the reticuloendothelial system, animals were anaesthetised and killed by exsanguination. The graft and a generous area of host tissue was excised, fixed in buffered 10% formalin and cleared by a modification of the Spalteholz technique[2]. The cleared tissues were examined by means of a Zeiss stereo-dissecting microscope.

Increased vascular permeability is characterized by the deposition of carbon within the walls of vessels exhibiting abnormal permeability[3]. Within 6 h of grafting, the initial traumatic inflammatory response in the host tissues subsides and is followed by a fine punctate carbon labelling of newly formed capillaries at the hostgraft junction. Circulating carbon does not enter the vessels of the allograft in the initial 2 days after grafting, but on days 3 and 4 small intraluminal clumps of carbon are seen in the vessels of the graft indicating a re-establishment of a sluggish allograft circulation. On the 5th and 6th days, venules in the surrounding host tissues and in the graft become heavily demarcated with carbon; the former vessels exhibiting diffuse punctate labelling consistent with increased permeability, whereas the graft vessels show a combination of dense intraluminal sludging of columns of carbon as well as deposition of carbon on and within the endothelium.

At the time of maximum accumulation of carbon within the graft vessels, i.e. on day 6, small and large venules are seen within the graft to be abnormally and generally uniformly dilated (Figure) and venules of the order of 13–40 μm diameter exhibit vesicular dilations reminiscent of micro-aneurysms. Such aneurysmal dilatations are most prominent along the course of venules lying deep to the panniculus carnosus (Figure), i.e. close to the host-graft junction whereas uniform venular ectasia and fewer aneurysms are seen in the suprapannicular vessels. Although fine intraluminal and intramural carbon labelling is seen in both the diffusely ectatic venules and the focal dilatations, grafts with dense columns of sludged carbon within vessels rarely exhibited significant numbers of focal micro-aneurysms.

Vascular dilatation with compaction stasis of erythrocytes has previously been described for both skin[4] and renal[5] allografts; such changes together with the focal aneurysmal dilatations described herein might well be related to the heightened sensitivity exhibited by allograft vessels to humoral vasodilator factors[5] at the time of onset of rejection.

It is concluded that the formation of focal venular microaneurysmal and diffuse venular ectasia is a component of the early inflammatory response heralding the onset of allograft rejection.



Section through an allograft avulsed from the graft bed (GB). Microvenular aneurysms are seen predominantly in the inferior pannicular venules (IPV). The superior pannicular venules (SPV) and the connecting vessels traversing the panniculus carnosus (P) show uniform dilatation and few aneurysmal dilatations (×25).

Résumé. La réaction cutanée de l'immunite de transplantation contre une greffe allogénique est accompagnée d'une forte mais courte augmentation de la perméabilité vasculaire. La réponse vasculaire apparaît rapidement dans le tissue vecteur et est accompagnée d'une dilatation diffuse de la veine et d'un dévelopement des anévrismes milliaires des vénules dans le tissu de la greffe allogénique.

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Estrogen Target Cells in the Skin

Effects of estrogens on various components of the skin have been known for some time. However, the mediation of these effects remains unclear. Despite extensive use in therapy, birth control and cosmetics, the cellular and subcellular sites and the mechanisms of action of estrogens in skin are little understood[1,2].

In the epidermis, estrogens increase the mitotic rate in rodents and man[3], but reduce the size of the sebaceous glands[4]. Also, the secretion of sebum, which is stimulated by androgen, can be diminished by estrogens[5]. Hair growth, in general, is retarded after estrogen treatment in animals that have been gonadectomized, adrenalecto-

mized and hypophysectomized, except for growth of fine sparse hair[6,2]. Estrogens effect the vascularization of the skin[7]. Furthermore, the collagen content of the dermis is changed as reflected in mucopolysaccharide incorporation, hydroxy proline turnover and alterations of the ground substance[8]. These hormonal influences are relevant to various skin conditions as seen in baldness, acne, pregnancy, menopause and aging.

In the present study, dry-autoradiography[9] is utilized in order to search for and identify components of the skin which are targets for estradiol. Autoradiographic studies were performed with 6 Swiss albino mice (Charles River), 2 months old and weighing 25 g. 24 h after ovariectomy, the mice were injected s. c. with 1.0 μg per 100 g body weight of 2,4,6,7-^{3}H-estradiol-17β (New England Nuclear), specific activity 95 Ci/mM, dissolved in 10% ethanol in isotonic saline. 2 animals each were decapitated at 10, 60, and 120 min after the injection. Pieces of skin, about 2 mm^3, were excised from 3 regions: dorsal thorax, inguinal mammary gland, and perineum. The tissue was frozen in liquefied propane at −180 °C. 2 to 4 μm thick sections were cut in a Wide Range Cryostat (Harris Mfg. C., North Billerica, Mass.) and freeze-dried in a Cryo-pump (Thermovac Ind., Copiague, L.I., N.Y.).

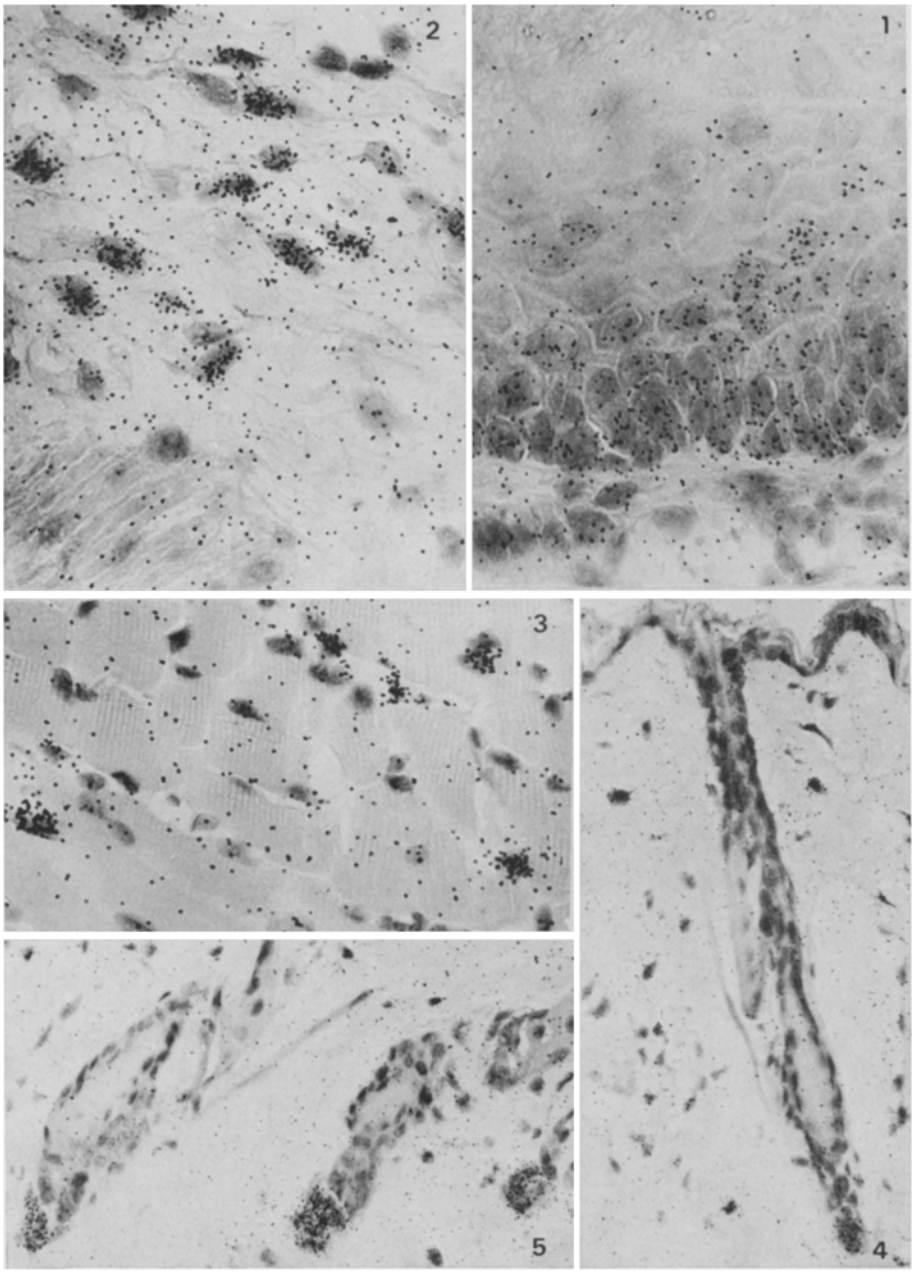

Fig. 1–5. Autoradiograms of mouse skin after injection of ^{3}H-estradiol-17β showing concentration of radioactivity in nuclei of cells of the stratum germinativum of the epidermis (Figure 1); fibroblasts in the dermis (Figure 2) and in striated muscle (Figure 3) as well as in dermal papillae of hair roots (Figures 4 and 5). Stained with methylgreen-pyronin for DNA and RNA. Figures 1–3. Skin from perineal region, 2 h after the injection; exposure time approx. 150 days. ×700. Figures 4 and 5. Skin from dorsum, 1 h after the injection; exposure time 108 days. ×330.

The unfixed and unembedded sections were dry-mounted on desiccated emulsion (Kodak NTB-3) coated slides by pressure with the fingers, using teflon supports for the sections. The section–mounted slides were exposed at −15 °C for 4 to 6 months, then photographically processed and stained with methylgreen-pyronin for DNA and RNA. Throughout the procedure no histological fixation and mounting fluids were applied, and embedding was avoided in order to exclude or minimize redistribution and loss of hormones and tissue constituents. Control autoradiograms were prepared from skin of an untreated mouse in order to exclude chemographic artifacts.

In addition, 10 mice of the same strain and age were used to identify the chemical nature of radioactivity. These animals were treated in the same manner as those used for autoradiography. 1 h after the injection of ^{3}H-estradiol, skin samples from the perineal and dorsal region were pooled from these animals. More than 90% of the tissue radioactivity was extracted as free steroid in the ether soluble fraction. Of the total radioactivity, 64% in the dorsal skin and 63% in the perineal skin was identified as estradiol[10]. These results suggest that the skin, or more specifically, certain structures of the skin, have the capacity to concentrate and retain estradiol in a fashion characteristic of more classical target tissues for estradiol such as uterus, vagina and mammary gland[11]. Therefore, it is likely that the nuclei of certain epidermis cells (Figure 1) and fibroblasts of the dermis (Figures 2–5), as shown in these autoradiograms, represent target sites in estrogen receptor cells.

In autoradiograms of the epidermis of the perineal skin (Figure 1), radioactivity is found to be concentrated in nuclei of basal cell layers, with decreasing intensity toward the cornified surface. While the labeling of cells of the Malpighi layer is intensive in the skin of the perineal-perianal region (Figure 1), the uptake is sparse or undetectable in the skin of the dorsum (Figure 5) and the perimammillary region. These results indicate differences in the estrogen binding affinities in different regions of the epidermis, which merits further investigation. Similar to the epidermis, the cells of the basal layers of the outer root sheath of the hair follicle are radioactively labeled in the perineal region, but not, or only little, in the dorsal region. Cells of the dermal papilla of the hair, however, show distinct nuclear concentration of radioactivity (Figures 4 and 5) in all of the skin areas studied.

In the dermis a certain population of cells, probably fibroblasts as judged by their fusiform shape, show nuclear uptake and retention of the labeled hormone. Such cells are found most abundantly in the deep layer of the dermis (Figure 2) and hypodermis, but also within the endomysium of dermal striated muscle (Figure 3), as well as scattered in the more superficial regions of the dermis (Figures 4 and 5). The sebaceous glands, as can be seen in Figures 4 and 5, are essentially free of radioactivity, except for an occasional weak retention in nuclei of basal cells. No concentration of radioactivity is seen in arrector muscles of the hairs.

The present autoradiographic results are in accord with earlier reported hormone effects of the skin[3–7]. In addition, they help to identify and define the cellular and subcellular sites of action and clarify, whether direct or indirect hormone effects are involved. The possibility to localize estrogen in the mouse skin provides for similar studies in different species and in human skin samples, not only with radioactively labeled estrogen, but also other steroid and polypeptide hormones. Clinical syndromes, such as baldness and acne, believed to be related to the effects of androgens, as well as the changes in pregnancy and aging, can be investigated.

Zusammenfassung. Nach Injektion von ^{3}H-Östradiol wurden Retention und Konzentration des Hormons in Zellen der Keimschicht der Epidermis und in Fibroblasten der Dermis und Hypodermis von Mäusen gefunden. Zellen der Haarpapillen in den Haarwurzeln zeigten starke nukleäre Radioaktivität, während in der basalen Zellschicht der Talgdrüsen keine oder nur schwache Markierung beobachtet werden konnte. Diese Ergebnisse mit der Trocken-Autoradiographie demonstrieren erstmalig die Existenz von verschiedenen Populationen von Östradiol-Target-zellen in der Haut.

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