Response of Transplanted Skin of Newborn and Suckling Mice to Application of 20-Methylcholanthrene*

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Subcutaneous transplantation of skin results in the formation of epidermal cysts which ultimately undergo destruction (3, 4). Application of 20-methylcholanthrene to the epidermis of young adult mice previous to transplantation stimulated epithelial growth in cutaneous and subcutaneous grafts made from skin thus treated. These grafts were likewise destroyed, unless the epithelium had, in the course of painting, acquired neoplastic properties (8). Neoplastic growth was, however, readily obtained if skin of mouse embryos was exposed to carcinogens previous to transplantation (6, 10, 11). The change in transplantability following stimulation by a carcinogen seems of interest in view of the role attributed to the sebaceous glands in epidermal carcinogenesis (6, 9—12). We therefore decided to study the transplantability of neonatal skin at various stages of development and following treatment with a carcinogen.

MATERIALS AND METHODS

The experiments were carried out in mice of the Swiss strain, maintained on a stock diet of Purina Laboratory Chow and water ad libitum. Pieces of skin, about 2 mm. square, were transplanted into subcutaneous pockets at each side of the chest wall of the recipient. Newborn and 1-, 2-, 4-, or 7-day-old animals were used as donors and related animals 2–3 months of age as recipients. Untreated skin was used for the control series. In the test series, a 0.3 per cent solution of 20-methylcholanthrene dissolved in benzene was applied with a camel's hair brush No. 6 to the skin of the donors a few minutes before they were killed. The transplants, or remnants thereof, were removed after 2, 4, 7, or 10 days; 2, 3, or 6 weeks; and 3, 6, or 12 months. The tissues were fixed in Bouin's solution, imbedded in paraffin, cut serially, and stained with hematoxylin and eosin. Altogether, 76 control and 156 painted transplants were examined microscopically.

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MICROSCOPIC OBSERVATIONS

CONTROL SERIES

Grafts of skin of newborn mice.—Two days after transplantation, many cells of the surface epithelium and of the developing hair follicles had perished, and keratin was sloughed off (Figs. 1, a, 2, a). The surviving epithelium underwent hypertrophy and mitotic proliferation. The epithelium of the hair follicles migrated toward the surface and furnished most of the new epithelial covering. Within 4 days the surface of the graft was re-epithelialized, and epithelium grew out from the edges of the transplant. After 7 days epidermal cysts began to form, with walls composed of two or three layers of elongated squamous cells and debris and keratin in the center. The corium underwent atrophy or necrobiosis and was infiltrated by leukocytes, phagocytic cells, and fibroblasts. This reaction varied in degree and in the proportion of polymorphonuclear and mononuclear leukocytes. Within 2 or 3 weeks the cysts had closed, while in both the graft and the wall of the cysts hair appendages continued to grow. The cysts were filled with keratin and hair and were surrounded by a connective tissue capsule. Six weeks after transplantation, the epithelial lining of the cysts was atrophic, and the connective tissue capsule had become thicker and denser than before. Two types of reaction could be distinguished in the grafts: Either the pressure of the capsule led to shrinkage of the cyst and loss of lining epithelium and scarring, or a marked foreign-body reaction characterized by a fibroblastic tissue and many multinucleated giant cells developed (Fig. 3, a). This granulation tissue grew into the center of the cyst and destroyed its lining as well as the remnants of the transplant. Six weeks or 3 months after grafting, two of eight transplants had been replaced by a small granuloma containing debris, dead hair, and keratin, while the remaining six transplants still showed epithelium. All eight transplants permitted to stay in the recipient for 6 or 12 months were resorbed.

Grafts of skin of 1- or 2-day-old mice.—The
transplanted epidermis remained somewhat better preserved than that of the newborn mice. On the whole, however, the fate of the grafts and the reaction of the recipient were in kind and intensity comparable to the changes described in the previous group. Six weeks or 3 months after transplantation, complete or fragmented epidermal cysts were identified in eleven of sixteen grafts. After 6 months, remnants of cysts were observed in two of eight transplants. Twelve months after transplantation, none of the grafts was recovered.

Grafts of skin of 4- or 7-day-old mice.—These transplants were fairly resistant to the early injurious influences of grafting, although—in keeping with the advanced development of hair at the time of transplantation—much keratin and hair had been shed from the surface of the grafts. The epithelium of both the hair follicles and the surface showed better survival than that of the younger individuals. There was vigorous epithelial growth inside the grafts and at their margins. Two days after transplantation multicentric regenerative growth originating chiefly in the hair follicles caused early re-epithelization of the graft. After 4 days, marginal outgrowth was in progress, and after 7 days cyst formation was advanced. Here and there, at the junction of the graft and the newly formed epithelium of the cysts, small epithelial pegs extended into the surrounding connective tissue. Three weeks after transplantation most cysts began to disintegrate. No essential difference in the early reaction of the recipients to these transplants, as compared to the younger ones, could be established. However, after the cysts had formed, abundant granulation tissue developed, causing rapid breakdown of the cysts. Six weeks or 3 months after transplantation, only four of sixteen grafts containing epithelial elements were recovered. Six or 12 months after transplantation, the grafts had been resorbed or replaced by a fibrous button showing a center of keratin and debris.

Test Series

Grafts of skin of newborn mice.—During the early stages following transplantation, painted epidermis showed better resistance to the injury of grafting than that of unpainted skin. Within 2 days the epithelium of the hair follicles and of the surface underwent hypertrophy and vigorous mitotic proliferation. The epidermis became thicker, and the appendages developed more rapidly than in the untreated grafts (Figs. 1, b, 2, b). Long epithelial tongues grew out from the margins of the grafts. Sloughed-off keratin was abundant. Four days after transplantation epithelial outgrowth was advanced, and after 7 days large epidermal cysts filled with extruded hair, keratin, and debris were found. The cyst wall was composed of five to six layers of hypertrophic epithelium. Many hair follicles had lost their hair and had replaced it with plugs of keratin. Within the next 2 weeks, the proliferating epithelium of the hair follicles formed numerous pegs, composed of small hyperplastic epithelial cells with frequent mitoses (Fig. 3, b). The epidermal cysts continued to grow; sebaceous glands and abortive hair were present in the cyst wall. Infiltration of the graft by polymorphonuclear and mononuclear leukocytes was more conspicuous than in the nonpainted grafts. Gradually, connective tissue enveloped the transplant and formed a capsule that increased...
Fig. 2.—a. Same experiment as in Fig. 1, a. 
X400. The graft is partly preserved, but there is little active growth. b. Same experiment as in Fig. 1. b X 400. Hyperplastic growth inside the graft is associated with good development of skin appendages; there is marked mitotic proliferation of the epithelium.
FIG. 3. — a. Untreated skin graft of a 1-week-old mouse 3 weeks after transplantation. ×200. A cyst originating from the margin of the graft is in an early stage of disintegration; there is a foreign body reaction with multinucleated giant cells. b. Painted skin graft of a newborn mouse 10 days after transplantation. ×200. The illustration shows the margin of a cyst at its insertion at the graft. Keratinized hair follicles are seen at the edge of the graft, and hyperplastic epithelial pegs are present in the cyst wall.
in thickness and density as time went on. Three weeks after grafting some cysts had been eroded, and a pronounced foreign-body reaction was noted in the vicinity. Other transplants and the attached cysts underwent shrinkage. Six weeks or 3 months after transplantation, epidermal cysts or remnants of such cysts were observed in eleven of sixteen transplants. Six or 12 months after grafting, no epithelial elements were found.

Grafts of skin of 1- or 2-day-old mice.—As a rule, growth and regressive changes in the transplant resembled those described in the preceding paragraph (Fig. 4, b). Six weeks or 3 months after transplantation, epithelial fragments were noted in 15 of 32 grafts, while 17 had been replaced by granulomatous tissue. Six or 12 months after grafting, scar tissue containing debris and keratin indicated the former site of the transplant.

Grafts of skin of 4- or 7-day-old mice.—During the early stages following transplantation many cells of the basal layer and of the hair follicles remained intact. The surviving epithelium gave rise to regenerative growth, which was more vigorous than in the corresponding nonpainted grafts. Re-epithelialization of the transplant was thus accelerated, but less conspicuously so than in the painted grafts of skin of the younger mice. The outgrowing epithelial tongues were shorter but thicker than those seen in grafts of painted skin 1 or 2 days old at transplantation. Within 2 weeks large epidermal cysts were found filled with much keratin and hair. The cyst walls consisted of several layers of keratinizing epithelium. There were few appendages; if present at all, they were seen at the border between the original transplant and the outgrowing epithelium. Likewise in this area, but also in the transplant itself, numerous, large, proliferating epithelial pegs with keratotic centers were observed. Leukocytic and lymphocytic infiltration of the grafts was heavy. Three weeks after transplantation these changes were far advanced (Fig. 4, a), and a thick connective tissue capsule surrounded the epidermal cysts. Six weeks after grafting most cysts were broken up, and an abundant connective tissue growth with multinucleated giant cells was noticeable in the vicinity of bulky accumulations of keratin and debris (Fig. 4, c). Six weeks or 3 months after transplantation 9 of 10 grafts still showed remnants of cysts; others consisted of small granulomas surrounding inspissated debris, hair, and keratin, while still others had been absorbed. After 6 or 12 months, no epithelial elements could be detected. One mouse killed at the age of 12 months had developed a tumor at the site of transplantation. This growth proved to be a spindle-cell sarcoma, not arising in the transplant itself, but in the subcutaneous tissue of the recipient.

DISCUSSION

In syngenesiotransplants1 of skin of newborn and sucking mice, epithelial growth increased with increasing age of the donor and as the hair follicles developed. The older the tissue at the time of grafting, the less pronounced was the new formation of appendages, the larger was the amount of hair and keratin within the cysts, and the more vigorous was the reaction of the recipient. Consequently, cysts derived from 7-day-old skin disintegrated earlier than those derived from skin 4 days old and younger at the time of transplantation.

The less active growth of grafted skin of newborn and of 1- or 2-day-old mice, as compared with that of 4- or 7-day-old mice seems contrary to expectation, since young tissues are considered to be endowed with greater growth capacity than older ones (4, 5). However, regeneration as well as outgrowth of the epithelium from the graft are not only contingent upon the inherent growth capacity of the regenerating tissue but also on the number of cells available. In mice below the age of 1 week, hair follicles are small and not yet fully developed. As the skin approaches full development, more cells are on hand which will respond to the stimulus of transplantation than in the epidermis of the newborn. Moreover, in the latter, the inherent tendencies to grow and to develop may possibly outbalance the requirements of regenerative growth created by experimental interference with the physiologic processes. As observed also in autogenous grafts, these developmental potencies seem to be pronounced (10, 11). The epithelium growing out from a graft of newborn skin had the ability to form hair follicles, sebaceous glands, and hair, although these potencies are lost with increasing age of the donor. Since hair will not develop without participation of dermal papillae, some equivalents of the latter presumably arise from small amounts of corium growing out with the epithelium.

4-0-Methylcholanthrene applied to the skin of mice immediately preceding transplantation stimulated growth of the epithelium and promoted the development of hair follicles and sebaceous glands. Epithelial migration was most marked in the epidermis of the newborn, and the epidermis was

1 These grafts belong to the group of homologous transplants, but the relationship between host and donor is closer than in the usual type of homoiotransplantation, host and donor being closely related individuals—e.g., brother and sister.
FIG. 4.—a. Painted skin graft of a 7-day-old mouse 3 weeks after transplantation. X 100. At the margin of the cyst deep epithelial pegs show marked leukocytic infiltration. b. Painted skin graft of a 1-day-old mouse 10 days after transplantation. X 400. Part of the cyst wall showing new formation of sebaceous glands and abortive hair. c. Painted skin graft of a 7-day-old mouse 6 weeks after transplantation. X 400. The cyst wall shows advanced foreign body reaction near deposits of keratin.
of 1-, 2-, or 4-day-old mice reacted with more vigorous growth than that of 7-day-old mice. The maximum of growth stimulation was represented by hyperplastic epithelial pegs arising from hair follicles and extending into the corium of the transplant; however, no carcinomas were found. The older the skin at the time of transplantation, the more intensive was the response of the recipient leading to destruction of the graft.

According to the present results, the epidermis of the newborn mouse is susceptible to stimulation by 20-methylcholanthrene. The fact that carcinomas did not develop cannot serve as proof to the contrary, since grafts of older epidermis did not show cancerous growth either. One reason for this failure may be the comparative weakness of the stimulus applied. In addition, factors inherent in the nature of the present experiment and related to the poor transplantability of skin in general opposed continuous growth of the grafts, both painted and unpainted. In this connection, the behavior of tarred skin transplanted into the anterior chamber of the eye is of interest (1). This environment is more favorable to successful grafting than the subcutaneous tissue, because here the reactive processes of the recipient are at a minimum (2). Nevertheless, growth of these transplants was only moderately stimulated by the application of tar, and in the end the grafts were destroyed (1).

Our observations of increased growth in the painted transplants associated with intensification of development of hair follicles and sebaceous glands may be compared to those made in grafts of embryonal skin treated previous to transplantation with a 1 per cent solution of methylcholanthrene. Under these conditions, the development of skin appendages was of the same order as in the controls, if small amounts of the carcinogen had become encysted by the outgrowing epithelium, while encystment of large amounts of the carcinogen caused destruction of the sebaceous glands (6, 10, 11). In our experiments, the development of sebaceous glands was even stimulated by the carcinogen. As also seen in guinea pigs (7), a weak solution of the carcinogen may stimulate the development of appendages, while strong solutions exert a harmful effect. In addition, the response of a tissue to a stimulus depends upon its physiologic age (5). Those processes which are in progress or impending at the time when the stimulus is applied are promoted most conspicuously. Correspondingly, the marked growth tendency of embryonal epidermis becomes accentuated if a graft of such skin is exposed to a carcinogenic agent.

This growth stimulation may, under favorable conditions, lead to neoplastic growth (6, 10, 11). Conversely, the ability of the outgrowing epithelium to form hair follicles and sebaceous glands decreased with advancing age of the skin at the time of grafting. The reaction of the recipient was more pronounced against transplants of older skin than against those of younger skin. However, widespread keratinization, occurring in the transplanted epidermis under the influence of the carcinogen, may have inhibited the movement of the epithelium. Previously, we reported a similar inhibition of epithelial migration over the base of wounds made in skin painted with a carcinogen (7). This inhibition might have been due to changes in the wound-base, to specific injurious effects of the carcinogen on the epithelial cells, or to increased keratinization of the epithelium. In the light of the present observations, the last-mentioned mechanism seems largely responsible for the decreased movement of epithelium painted with carcinogenic agents.

SUMMARY

In subcutaneous syngenesiografts of skin of newborn or suckling mice, epithelial growth and migration were intensified, and cyst formation was accelerated, as the age of the donor increased. Conversely, the ability of the outgrowing epithelium to form hair follicles and sebaceous glands decreased with advancing age of the skin at the time of grafting. The reaction of the recipient was more pronounced against transplants of older skin than against those of younger skin, but ultimately all grafts were destroyed.

A solution of 0.3 per cent of 20-methylcholanthrene applied to the skin of newborn mice previous to grafting stimulated epithelial proliferation and migration and the development of appendages. These effects decreased with the increasing age of the donor. In grafts of 7-day-old skin, pronounced keratinization interfered with
the migration of the epithelium and the ensuing cyst formation. The reaction of the recipient was more vigorous against painted than against non-painted tissue. No carcinomas developed in the painted transplants, and the grafts were finally destroyed.

REFERENCES
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