THE MODULATING INFLUENCE OF STROMAL ENVIRONMENT ON EPITHELIAL CELLS STUDIED IN HUMAN AUTOTRANSPLANTS[1]

E. J. VAN SCOTT, M.D. AND R. P. REINERTSON, M.D.

The various patterns of biologic behavior that cells of the epidermal system may assume under normal and pathologic circumstances are well known. This property is referred to as the *pluripotentiality* of the epidermal cell. The necessary basic factors that determine the specific response of the epidermal cell in each circumstance are unknown. In a broad sense, the pluripotential capacity of the epidermal cell is best revealed by comparing its behavior with that of closely related epithelial cells, particularly those of the stratified squamous mucosa at the muco-cutaneous junctions. The various epithelial cell lines of the integument and the mucous membranes differ greatly in behavior and morphologic characteristics depending upon their respective locations. One may tentatively conclude that the structural features and functional capacities assumed by the epithelial cell in each location is determined by the environmental milieu in which it finds itself. More specifically, it might be surmised that the determinants are found in the connective stroma of each location, since this is suggested by evidence such as submitted by Grobstein (1) and Auerbach (2) and others who have shown that the development of epithelial cells in embryonic tissues is influenced differently by environments of mesenchyme derived from different anatomic locations. However, these experiments were performed with embryonic tissue and may pertain only to permanent irreversible changes in cell lines that occur during development, *i.e.* differentiation. It is clear that differentiated adult epithelial cells can undergo changes in structure and function within limits—for example, under certain conditions cells of pilosebaceous follicles may form epidermis (3), and epidermis in turn may produce new pilosebaceous follicles (4, 5). The inference that an influence of connective tissue stroma modulates the behavior and structure of already differentiated, adult epithelial cells however needs confirmation.

The present study was undertaken to ascertain the response of adult epidermal and epidermally-related cells placed in different connective tissue environments in an autologous host.

PATIENTS AND METHODS OF STUDY

Autotransplantation was performed on eleven patients as shown in Tables 1–5. Five patients had a diagnosis of mycosis fungoides; four had multiple basal cell epitheliomas; one had mild psoriasis; and one patient was dermatologically normal. Only areas of skin or oral mucous membrane that appeared clinically normal were used as recipient sites. The patients received no therapy during the time of their participation in the study.

Epithelial tissue and/or connective tissue stroma were transplanted in several ways. Sites of implantation were excised with either a scalpel or cutaneous punch after 1 to 5 weeks. The specimens of excised skin or mucous membrane were fixed in Cajal's uranium nitrate-formalin solution, sectioned serially 6 microns thick and colored with hematoxylin and eosin, or by Gomori's trichrome aldehyde fuchsin method.

OBSERVATIONS

Procedures involving epidermis and oral mucous membrane

Split thickness graft of oral mucous membrane transplanted to skin. It is well known that full or split thickness skin grafts, consisting of epidermis and its underlying connective tissue, retain the characteristics of the donor site rather than assume those of the recipient site. To test whether a split thickness graft of mucous membrane would respond similarly, a 4 mm. punch biopsy specimen was taken from the buccal side of the lower lip and transplanted to the skin of the back of the same patient. The area of implantation was removed two weeks later and histologic examination revealed that the implant had retained its characteristics of mucous membrane: the mucosa had no stratum granulosum, its stratum corneum was a loose network of parakeratotic cells (Fig. 1).

[1] From the Dermatology Service, General Medicine Branch, National Cancer Institute, National Institutes of Health, Public Health Service, Department of Health, Education and Welfare, Bethesda, Maryland.

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TABLE 1

Oral mucosa transplanted into skin

Patient	Implant removed at:	Characteristics of implanted epithelal tissue in: upper corium	mid-corium	lower corium	sub-cutaneous tissue
Oral mucosa *with* sub-mucosal connective tissue (split thikness) grafted into skin					
M. G.	2 wks.	Oral mucosa			
Pure oral mucosa (*devoid* of sub-mucosal connective tissue) implanted into skin					
W. R.	1 wk.	Tonofibrils + Str. granulosum + Keratinization +	Duct-like formations*	Degeneration Giant cells	
W. R.	9 days	Tonofibrils + Str. granulosum + Keratinization +	Duct-like formations*	Degeneration Giant cells	Cells with vesicular cytoplasm
A. G.	2 wks.	Tonofibrils + Str. granulosum + Keratinization +		Degeneration Giant cells	
G. G.				Degeneration Giant cells	Cells with vesicular cytoplasm
W. R.	1 wk.	Tonofibrils + Str. granulosum + Keratinization +		Degeneration Giant Cells	
M. H.	2 wks.	Tonofibrils + Str. granulosum 0 Keratinization 0			
I. W.	8 days		Degeneration		

* See text for description and definition

Pure oral mucosa implanted into skin. Pure oral mucosa, free of underlying connective tissue, was obtained by scraping the buccal mucous surface opposite the 2nd molars with a scalpel, care being taken not to draw blood. The gelatinous appearing tissue was placed immediately in sterile saline in a petri dish. Under a dissecting microscope the tissue was inserted, by means of fine forceps, into the barrel of a styletted 16-gauge needle with stylette partially withdrawn. The needle was then inserted to the depth of subcutaneous tissue in either the back or the upper arm of the patient and the mucosa implanted at various depths of the skin by inserting the stylette as the needle was withdrawn from the skin. The site of implantation was removed with a 4 mm. punch 1 to 2 weeks later. This procedure was performed a total of seven times in five different patients. The histologic findings are recorded in Table 1.

Mucosal tissue was found to have been implanted in the *upper* third of the corium in five of the specimens, and in each case appeared to be histologically viable. (Tissues were considered viable if normal appearing mitotic cells were present, if the stainability of cells was the same as that characteristic for the tissue normally, and if structural changes were present that could have been produced only by viable tissue. Non-viability was apparent by loss of stainability, loss of nuclei in non-keratinizing cells, amorphous cellular debris, and multinucleated giant cells.) In four of these five specimens there were structural changes in the mucosal epithelium that made it resemble epidermis. The mucosal cells were found to be spatially oriented in layers and to have formed a distinct stratum granulosum (Fig. 2). In three instances, attachments between the implanted mucosal cells and the epidermis of the recipient site could be identified; the direction of keratinization in these instances was toward the cutaneous surface. In the one instance where attachment had not occurred, keratinization was directed centrally within nests of mucosal cells, producing small cysts. Keratinization was complete, not parakeratotic (Fig. 3).

Mucosa was found to have been implanted in the *mid*-corium in three instances. In one specimen, complete necrosis of the implanted cells had occurred. In the other two, the implanted mucosa was viable and its reaction was quite different

from that seen in the mucosa implanted in the upper corium. Formation of neither stratum corneum nor stratum granulosum could be detected. Instead, focal areas of cellular degeneration were found, whose further development gave rise to what might be called *ductal structures*. The development of these structures seemed to begin with degeneration of a single cell (Fig. 4), or perhaps a group of a few cells (Fig. 5), centrally located within the mass of mucosal tissue. Larger accumulations of degenerated cells were necrotic in appearance (Fig. 6). By examination of serial sections, it was ascertained that some of these necrotic nests had fallen out during the sectioning or staining process, leaving a space that appeared to be a lumen within the mass of cells (Fig. 7).

Buccal mucosa implanted in the lower corium was evidently unable to survive since in no specimen were surviving mucosal cells found in that region. Necrotic remnants of mucous membrane and multinucleated giant cells were present there and were associated with accumulations in the subcutaneous tissue of cells with a vesicular cytoplasm (Fig. 8). The identity of these latter cells is unknown.

Epidermis implanted in the sub-mucosal connective tissue. Attempt was made to obtain pure epidermis by stretching the skin of the arm or back, removing the stratum corneum with repeated applications of cellulose tape, and scraping off the remaining epidermis with a scalpel. The fact that small points of bleeding occurred with this procedure indicated that the corium had been penetrated and that the absolute purity of the epidermal specimens could not be assumed. Such epidermal specimens were implanted sub-mucosally on the buccal side of the lower lip by means of a styletted 16-gauge needle and the site of implantation marked with a small drop of India ink placed in the puncture wound. Implantation was done in three patients and the implantation sites removed at 8, 9 and 21 days respectively following implantation.

The epidermal implants removed on the 8th and 9th days appeared to be viable but to have lost the characteristics of epidermis: the cells were separated from each other; no tonofibrils could be identified; no cells containing granules could be found that might be indicative of an attempt to form a stratum granulosum (Fig. 9). The specimen removed at 21 days showed no evidence of implanted cells. A large nest of multinucleated giant cells was present (Fig. 10) and presumably was the site of the implant.

In one patient mucosa was scraped from the buccal mucous membrane and was immediately implanted submucosally in the lower lip. Sixteen days later it was excised. Only multinucleated giant cells were found histologically in the implant site.

Sub-epidermal corium implanted sub-mucosally. Sub-epidermal corium was obtained from a 4 mm. punch biopsy specimen of skin of the back of one patient. After the epidermis was removed with a scalpel, a thin slice of the upper corium was cut off and implanted sub-mucosally on the buccal aspect of the patient's lower lip. Implantation was done by first undermining the mucosa with a cataract knife and then inserting the piece of corium through the small incision into the sub-mucosal space. The site of insertion was marked with a small drop of India ink. Two weeks later, the area of implantation was removed with a 6 mm. punch.

Upon histologic examination the identity of the implanted corium could not be ascertained, although the implantation tract could be traced from the point of entry at the mucosal surface on one side of the specimen, through the sub-mucosa to a point about midway from either side of the histologic sections. The mucosa over the central portion of the specimen contained numerous keratohyaline granules (Fig. 11), whereas the mucosa at the periphery showed no such change (Fig. 12). The presence of granules in the mucosa overlying the site where corium had been implanted does not necessarily indicate that they had been induced by the implanted corium. It should be noted, however, that keratohyaline granules were not found in the mucosa in the three instances where epidermis had been implanted sub-mucosally.

Transplantation of hair roots

Transplantation of hair roots with connective tissue stroma. In three patients, the lower half of the dermis from a 4 mm. punch biopsy specimen of scalp was implanted intradermally in the back through a small stab wound. The wound was closed with a suture. The implantation sites were removed with a 6 mm. punch at 9, 14 and 24 days respectively.

In each instance the only essential change noted was that all hair roots had converted to the

TABLE 2

Scalp hair roots transplanted into skin

Patient	Implant removed at:	Characteristics of implanted epithelial tissue in: upper corium	mid-corium	lower corium	sub-cutaneous tissue
Hair roots *with* corium and papilla implanted into skin					
M. G.	2 wks.	All roots telogen No other changes			
C. P.	24 days	All roots telogen No other changes			
A. H.	9 days	All roots telogen No other changes			
Epilated hair roots (*devoid* of corium & papilla) implanted into skin					
M. J.	1 wk.	Necrosis of hair bulb Epithelial "pearls" in ext. sheaths Duct-like formations* in ext. sheaths			

* See text for description and definition

telogen (resting) stage (Table 2, Fig. 13). No other significant changes were discernible.

Transplantation of epilated hair roots. In one patient, hairs were manually epilated from the scalp and immersed in saline. Under a dissecting microscope the root ends of anagen (growing) hairs were cut off above the level of the keratogenous zone. The root portions were inserted into a styletted 16-gauge needle, implanted intradermally in the back of the patient, and the site excised at 1 week. Histologic examination revealed that the bulbar portion of all hairs had degenerated. The external sheaths contained areas in which groups of several cells had keratinized to give the appearance of epithelial pearls. In other areas focal degeneration of external root sheath cells occurred without keratinization, producing duct-like structures similar in appearance to those that developed in oral mucosa implanted intradermally (see foregoing). The same kind of structure also occurred when the external sheath was implanted as an isolated tissue (see later).

Implantation of isolated segments of hair roots. Epilated scalp hair roots were immersed in saline and under a dissecting microscope were dissected into separate tissue components by techniques similar to those employed by Crounse and coworkers (6, 7). The microdissection was done as

TABLE 3

Isolated scalp hair root tissues implanted into skin

Patient	Implant removed at:	Characteristics of imp anted epithelial tissue in: upper corium	mid-corium	lower corium	sub-cutaneous tissue
Hair bulbs (*devoid* of corium & papilla) implanted into skin					
M. H.	2 wks.	Degeneration Giant cells	Degeneration Giant cells	Duct-like formations*	
W. R.	1 wk.	Degeneration Giant cells			
G. G.	1 wk.		Degeneration Giant cells		
Pure external sheaths implanted into skin					
W. R.	1 wk.		Partial degeneration Duct-like formations*		
W. R.	9 days	Degeneration	Duct-like formations*	Undifferentiated epithelial cells	

* See text for description and definition

follows: the shaft of a hair was grasped with a fine forceps and with another forceps or microscalpel the external and internal sheaths together were slipped off the hair shaft over the hair bulb. Figure 14 illustrates a hair so divided. The hair bulb was isolated by a simple cross cut below the keratogenous zone. The isolated segments, *i.e.* either the hair bulbs or the hair sheaths, of several hair roots were inserted into the barrel of a styletted 16-gauge needle for intradermal implantation.

In three patients (Table 3) the bulbar portions of scalp hair roots were autologously implanted into the skin of the back and the site of implantation excised either 1 or 2 weeks later. In all three instances the bulbar tissue that had been implanted in either the upper or mid-corium did not survive (Fig. 15). In the one instance where bulbar tissue had been implanted in the lower corium, viable epithelial tissue could be identified. Keratinization had occurred centrally within the mass of tissue, giving the appearance of a large epithelial pearl; in another locus within the same mass of tissue a duct-like structure similar to those already described (see foregoing) was present (Fig. 16).

Isolated hair sheaths were implanted intradermally in the back of one patient on two separate occasions. In both instances, duct-like structures developed in the external sheaths implanted in the mid-corium (Figs. 17 & 18). In one specimen sheaths had been implanted in the upper corium and had degenerated; sheaths implanted in the lower corium appeared as a nest of indifferent, though viable, epithelial cells.

Transplantation of basal cell epithelioma

To explore the apparent dependence of basal cell epithelioma upon its stromal connective tissue, a biologic relationship suggested by Pinkus (4, 8), basal cell tumor tissue with and without its own environmental corium, was autologously transplanted into the skin of the back.

Basal cell tumors transplanted with intact stromal connective tissue. Punch biopsy specimens of basal cell tumors were placed in saline immediately after excision from the patient. In one instance, the entire biopsy specimen was left intact. In six other instances, the epidermis and upper corium were cut off with a small scalpel. All specimens were implanted into clinically normal skin of the back through a stab wound, which was closed with a suture. The implantation sites were excised 1 to 5 weeks later.

The implanted tumor tissue survived in all seven instances, whether implanted intradermally or even subcutaneously (Table 4, Figs. 19 & 20). No histological alterations were detectable, except for one specimen. In this specimen, numerous duct-like structures, similar to those described earlier (see foregoing), were found in the tumor tissue. A few resembled normal eccrine ducts (Figs. 21 & 22), although no attachments between these structures and indigenous eccrine units could be traced in serial sections. Indeed, each was found to be independent of the others, and each was found to begin and end within the tumor tissue. In addition to these structures, several focal areas of keratinization that had the appearance of epithelial pearls were present (Fig. 23).

Transplantation of pure basal cell tumor tissue, free of native stromal connective tissue. In six instances, punch biopsy specimens of tumors were dissected in saline under a dissecting microscope in order to isolate pure epithelial tumor tissue from the surrounding connective tissue. By means of careful manipulation, minute spherical islands of what appeared to be pure tumor could be shelled out of the fibrous corium. One to several such kernels were inserted into a 16-gauge styletted needle and implanted into normal skin of the back of the autologous patient. The implantation sites were excised 1 to 5 weeks later.

Histologic examination revealed several changes that occurred in the tumor tissue implanted under these conditions (Table 5), in contrast to the general absence of changes in the tumors that were implanted with stromal connective tissue intact. In the three instances where tumor had been implanted into the subcutaneous tissue, the implanted tumor did not survive and could be identified as discrete nests of degenerated tissue (Fig. 24). Where bits of tumor had been inserted at the junction of the lower corium and the subcutaneous tissue, the tumor appeared to have partially degenerated and the remaining tumor cells had assumed a more undifferentiated appearance (Fig. 25). Within the immediate area were many cells with a vesicular cytoplasm (Fig. 26); the origin or nature of these cells is not known.

TABLE 4

Basal cell epithelioma transplanted with stromal connective tissue

Patient	Implant removed at:	Characteristics of implanted epithelial tissue in:			
		upper corium	mid-corium	lower corium	sub-cutaneous tissue
W. R.	2 wks.	Survival with no changes			
W. R.	3 wks.		Survival with no changes		
W. R.	37 days		Survival with no changes		
W. R.	2 wks.		Survival with no changes		
W. R.	1 wk.	Survival with no changes	Duct-like formations* Keratin cysts*		
E. B.	1 wk.	Survival with no changes		Survival with no changes	
E. B.	17 days				Survival with no changes

* See text for description and definition

TABLE 5

Transplanted pure basal cell epithelioma

(*devoid* of native stromal connective tissue)

Patient	Implant removed at:	Characteristics of implanted epithelial tissue in:			
		upper corium	mid-corium	lower corium	sub-cutaneous tissue
W. R.	37 days				Degeneration Giant cells
M. G.	2 wks.		Partial degeneration Epithelial "pearls"		
W. R.	1 wk.			Partial degeneration Giant cells Undifferentiated epithelial cells	
E. B.	1 wk.		Partial degeneration Undifferentiated epithelial cells		
E. B.	1 wk.	Survival with no changes	Survival with no changes		Degeneration Cells with vesicular cytoplasm
E. B.	17 days				Degeneration

In one specimen tumor that had been implanted in the upper and mid-corium survived well and showed no significant changes from the usual appearance of basal cell epitheliomas taken from the patient for diagnostic purposes.

In another specimen where the tumor implant was confined to the mid-corium, the appearance of the implanted tumor tissue had some resemblance to squamous cell carcinoma. The cells of this implant were moderately undifferentiated in

appearance but several sites of focal keratinization were present, appearing as epithelial pearls (Fig. 27).

DISCUSSION

In this study, epithelial tissues transplanted with connective tissue stroma intact survived and retained their original histologic characteristics in the new, though foreign, environments during the 1 to 5 week interval of residence in the new environment. On the other hand, when transplanted as pure epithelium devoid of native connective tissue, they either did not survive at all in their new environment, or they survived and developed morphologic characteristics which in many instances resembled those of epithelium normally resident in the recipient site.

These observations seem to support not only the concept that the epidermal cell is pluripotential, but also the behavior of buccal mucous membrane transplanted into the skin suggests that the mucosal cell may be equipotential with the epidermal cell in regard to the extent that its behavior may be modulated. The cells of the epidermal system, the buccal mucosa, and even the undifferentiated cells of basal cell epithelioma are capable of several different behavioral patterns. In the aggregate, the several patterns which these cells can pursue would seem to be a property of epithelial cells; the *specific* pattern pursued in a given circumstance probably results from an interaction of the cells and their environment and seems to be determined by the environment. The work of Grobstein (1) and Auerbach (2) on the influence of mesenchyme on embryonic epithelia provides evidence for this hypothesis. The work of Fell and Mellanby (9) further indicates that specific chemicals may markedly modulate the behavior of embryonic epithelium. These authors found that high concentrations of vitamin A not only completely suppressed keratinization of chick ectoderm in explants of embryonic whole skin, but that it even caused the ectoderm to "differentiate" into mucous-secreting, often ciliated, epithelium similar to normal nasal mucosa. The change was reversible if the tissue was cultured in medium having a low concentration of vitamin A, when the epithelium again became keratinizing and squamous in type. It is interesting that vitamin A also influences the cartilage matrix of embryonic bone in explant, an effect on connective tissue reported by the same authors (10).

Whether the same factors that modify the structure and function of embryonic epithelium similarly modify adult epithelium is unknown. The stromal environment undoubtedly can yield a complexity of factors to which the epithelium it supports may respond in varying degrees. Such factors may include the effectiveness of the blood supply and nutrients, the temperature, concentration of hydrogen ions, partial pressures of oxygen and carbon dioxide, etc. Of these, blood supply certainly can determine whether transplanted tissue will grow successfully or not. It is well known that in plastic surgery, a full-thickness graft of skin grows less satisfactorily than a split-thickness graft because vascularization of the transplanted full-thickness piece of skin is incomplete. Nourishment of a graft may be impaired by the fibrin clot that surrounds and isolates it from an adequate vascular supply. For this reason very small masses of tissue were used for implantation in the present work. A more intimate contact could be assured between the cells of the implant and the tissue bed of the new host location. Differences in technique, therefore, seem to be important in determining whether implanted tissues grow or fail to grow. This may explain discrepancies between our results and those of Lyles *et al.* (11) who recently reported that curettings from basal cell tumors failed to grow when reimplanted into the skin of the same patient.

The modulating influence of the connective tissue stroma on the structure and function of epithelial cells seems to be paramount to understanding both normal and pathologic behavior of epidermal and epithelial cells. Why, for example, is the linear division between mucosal cells and epidermal cells at the muco-cutaneous junction of the lip so remarkably constant? If the behavior of the cells of either tissue is entirely inherent in the cell itself, might we not expect an ever changing line of division between mucous membrane and skin to result from migration of cells in either direction across this border line? Studies on feather and hair suggest that the connective tissue stroma intimately regulates the growth of these epidermal appendages: Lillie and Wang (12) have shown that the structure of feathers is predictably altered by specific alterations of the connective tissue feather papilla; hair roots cultured in a

millipore chamber in the abdomen of mice survive only if their papilla is intact, as shown by Crounse and Stengle (6); the studies of Van Scott and Ekel (13) on geometric relationships between the matrix and papilla of the hair indicate that the size of a hair and the mitotic activity in the hair matrix is proportional to the size of the hair papilla and the number of cells in the papilla. It seems reasonable to tentatively conclude that the population of epithelial cells, continuously mobile and in a state of flux, is under the control of the more stable underlying connective tissue.

A controlling influence of connective tissue on epithelium would seem to explain certain reactions of epidermis under pathological conditions. Billingham *et al.* (14) and Marchant and Orr (15) demonstrated that carcinogenesis of the epidermis in mice is primarily dependent upon the corium and upon changes therein resulting from topically applied carcinogen. The finding of Little (16) and of Gibson and Norris (17) that injection needles frequently contain bits of skin epithelium indicates that such epithelial fragments may be commonly implanted into blood vessels as well as subcutaneously. The conclusion must be that these epithelial fragments do not survive in their new environments. Our data support the hypotheses proposed by Pinkus (4, 8) that basal cell tumors are stroma dependent and that, as a consequence, they do not metastasize to foreign tissue beds beyond the skin unless they take along their stroma. In this regard, cells of basal cell tumors seem to be no different from normal epithelium. It is of interest, therefore, to re-evaluate what is represented by the different morphologic appearances of basal cell tumors. Since the structure of epithelium seems to be so governed by the supporting stroma, may not the different histomorphologic types of basal cell tumors reflect the influence of a particular stroma, rather than indicate that the tumor originated from any particular epithelial tissue such as hair roots or epidermis?

Conditions requisite for orderly degeneration should be thoroughly investigated instead of dismissing the subject by focusing attention only on the conditions requisite for the survival of cells. It should be recalled that orderly and regulated degeneration of cells plays a definite role in the formative stages of embryonic organs. The formation of lumina of embryonic ducts appears to occur by the degeneration of cells located in the center of cords of epithelium (18). Even in explants of fetal salivary gland tissue of the mouse, ducts appear to form by this process (19). Epidermal keratinization is but another example of a process primarily dependent upon orderly degeneration of cells. Indeed, both duct formation and keratinization would appear to result from only variances in the basic scheme of cellular degeneration. The essential similarity between duct formation and keratinization can be seen by comparing the duct-like structures and epithelial pearls that developed in the transplanted epithelia of the present experiments. In some instances, these structures could not be classified definitely as duct-like or pearl-like. In this connection, the observations of Stewart and Lorenz (20) on the histopathogenesis of carcinoma of the forestomach of the mouse during chemical carcinogenesis are interesting. Under the conditions of their experiment, both keratinized "pearls" and glandular structures appeared simultaneously in the mucosal epithelium; the former, by liquefaction, were believed to transform into the latter. Strangeways and Fell (21), studying embryonic limb buds of chicks transplanted into sub-cutaneous tissue of post-embryonic chicks, observed the formation of epithelial tubules by a similar process of central keratinization and degeneration in cords of epithelium. Identification of conditions requisite for degeneration and keratinization of cells of epidermal system may have practical importance, for if the bulk of cells of basal cell tumors were to keratinize most of these tumors should be self curative.

Epithelium is a covering tissue; it possesses no blood supply of its own. Its survival necessarily depends upon its supporting stroma. Even though it may place demands on its stroma, it must be emphasized that its final reaction is dependent upon what the stroma provides.

SUMMARY

Epidermal and epidermally-related tissues, with and without native connective tissue stroma, were autologously transplanted into skin and submucosally into buccal mucous membrane. The implantation sites were removed 1 to 5 weeks later.

Epithelium transplanted with connective tissue stroma intact survived and retained its original histologic characteristics.

Epithelium transplanted as a pure tissue, de-

void of native connective tissue, either did not survive at all, or survived and developed morphologic characteristics which in many instances resembled those of the epithelium normally resident in the recipient site.

The influence of stromal environment on epithelial tissues, normally and under pathological conditions, is discussed.

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PLATE I

FIG. 1. Oral mucosa with sub-mucosal connective tissue (split thickness graft) transplanted to skin of back, removed at 2 weeks. Implant has retained characteristics of mucous membrane. × 42

FIG. 2. Pure oral mucosa (devoid of sub-mucosal connective tissue) implanted into sub-epidermal corium of skin of the back, removed at 1 week. Implanted mucosa has developed kerato-hyaline granules characteristic of epidermis. × 420

FIG. 3. Pure oral mucosa implanted in upper corium of skin of the back, removed at 9 days. Central keratinization has occurred to produce a cyst. Stratum granulosum is present. Stratum corneum indicates that keratinization has been complete, not parakeratotic. × 260

FIG. 4. Pure oral mucosa implanted in mid-corium of skin of the back, removed at 1 week. Arrow indicates swollen, degenerating single cell. × 435

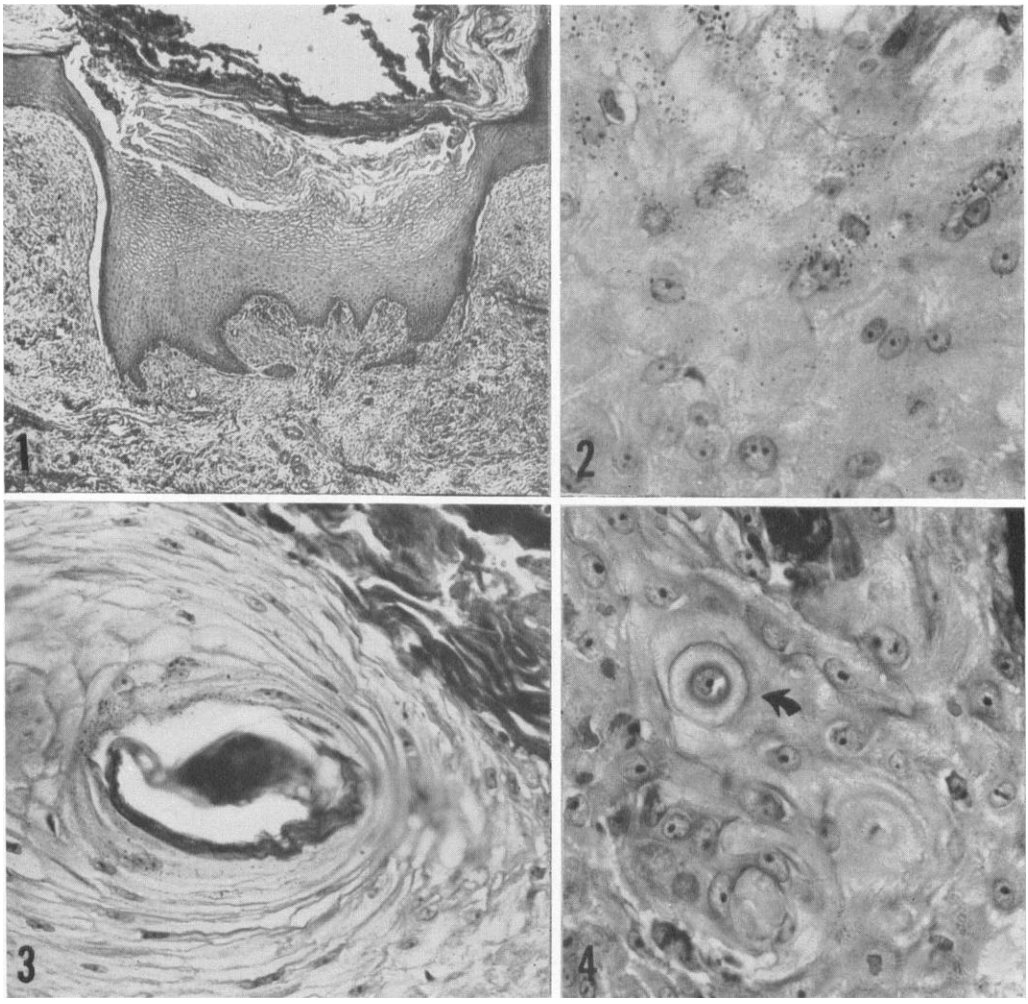
1
2
3
4

PLATE II

(Figures 5, 6 and 7 are representative of successive stages of focal degeneration of cells in the development of duct-like structures)

FIG. 5. Pure oral mucosa in mid-corium of skin of the back for 1 week. Note nest of several degenerating cells surrounded by laminated cells. × 435

FIG. 6. Same specimen as Fig. 5. Larger accumulations of degenerated cells. × 205

FIG. 7. Pure oral mucosa in mid-corium of skin of the upper arm, removed at 9 days. Duct-like structure (arrow) has resulted from nest of degenerated, necrotic cells (such as in corners of photograph) having fallen out during sectioning and staining process. × 250

FIG. 8. Cells with vesicular cytoplasm in subcutaneous tissue associated with degeneration of oral mucosa implanted in this region. × 335

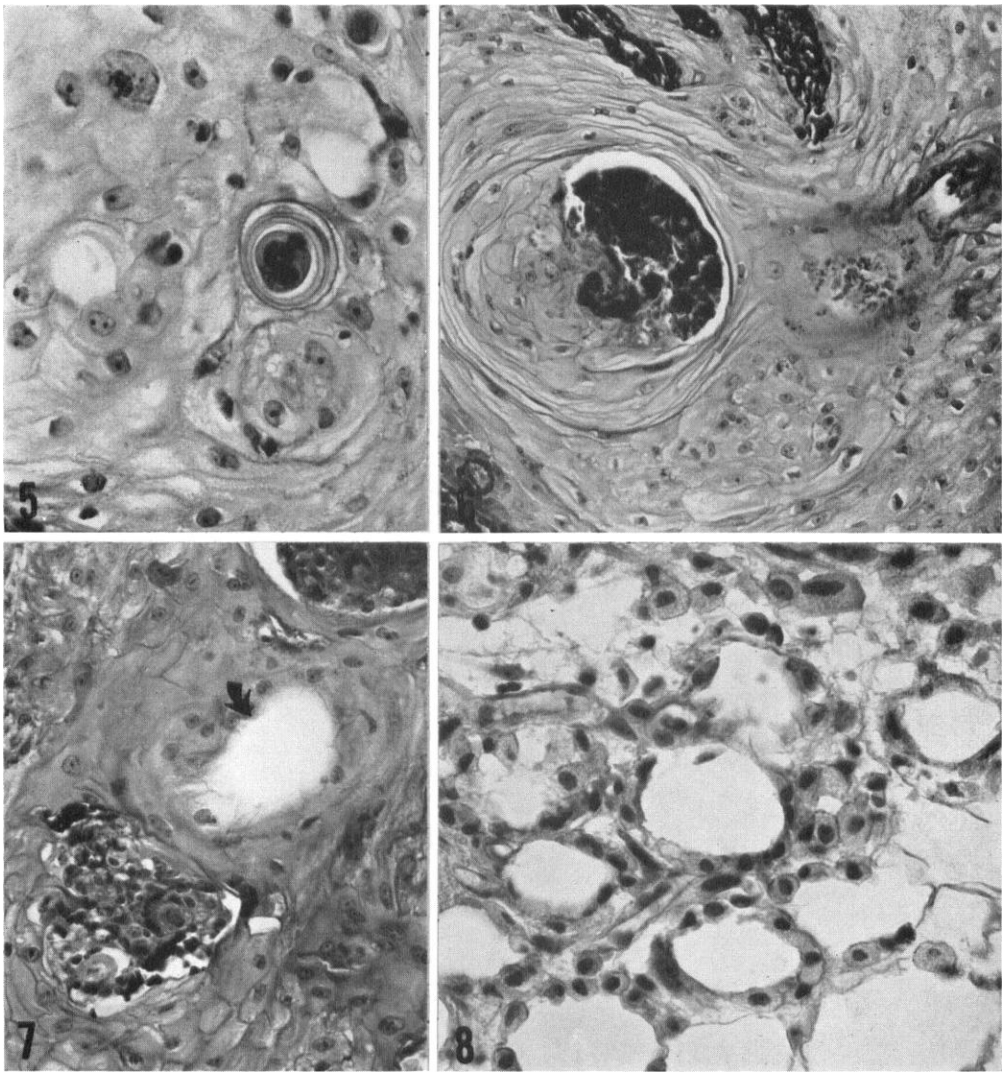
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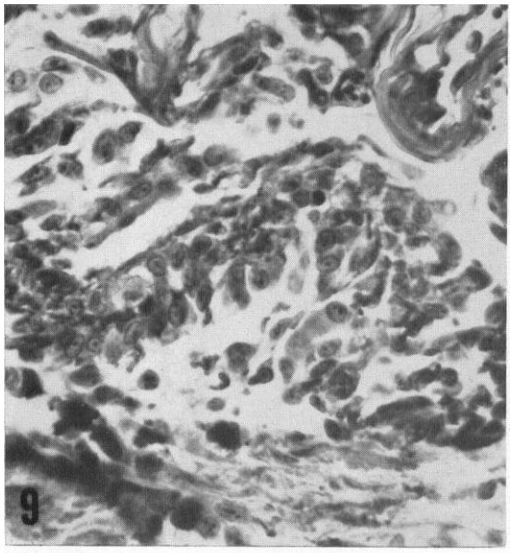
PLATE III

FIG. 9. Epidermis implanted in sub-mucosal connective tissue of lower lip, removed at 8 days. Cells have lost intercellular bridges and have separated from each other. No evidence of stratum granulosum. × 415

FIG. 10.—Nest of multinucleated giant cells sub-mucosally in lower lip indicative of death of epidermal tissue implanted 3 weeks previous. × 235

FIGS. 11 and 12. Keratohyaline granules (Fig. 11) in mucosa overlying site where sub-epidermal corium had been implanted sub-mucosally 2 weeks previous. Compare to absence of granules in normal mucosa (Fig. 12) at periphery of the same biopsy specimen. × 435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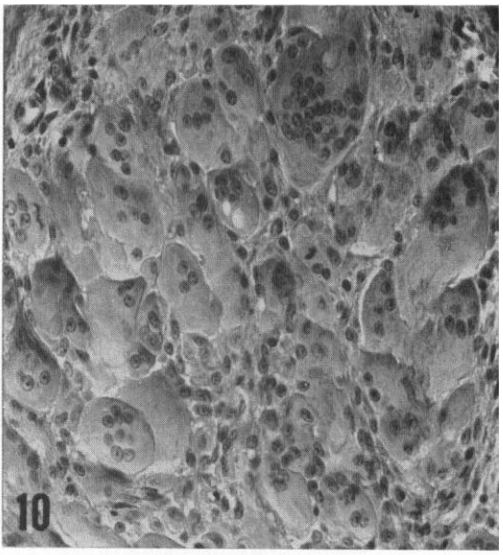
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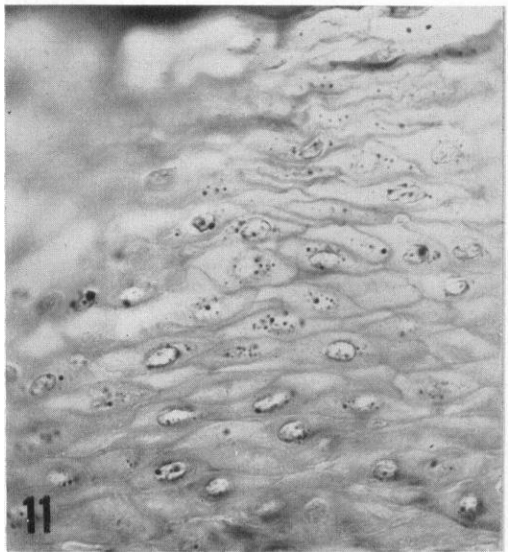
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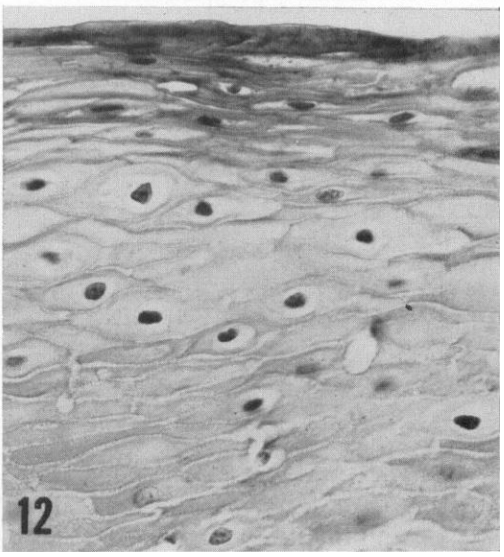
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PLATE IV

FIG. 13. Scalp hair root with intact connective tissue implanted into skin of back, removed 24 days later. No changes in hair root except to telogen state. × 105

FIG. 14. Dissected scalp hair root. Combined external and internal sheaths (right) removed from hair (left). Arrow points to keratogenous zone of hair, above which is the hair shaft and below which is the hair bulb. × 12

FIG. 15. Pure hair bulbs implanted in upper corium of the skin of the back for 1 week. Degeneration of all implanted tissue has occurred. × 105

FIG. 16. Pure hair bulbs implanted in lower-corium of the skin of the back, removed at 2 weeks. Duct-like structure present in upper left. Large keratinizing pearl present in lower right. × 205

FIGS. 17 and 18. Pure external sheaths implanted in mid-corium of the skin of the back, removed at 7 and 9 days respectively. Note duct-like structures. × 235 and × 355

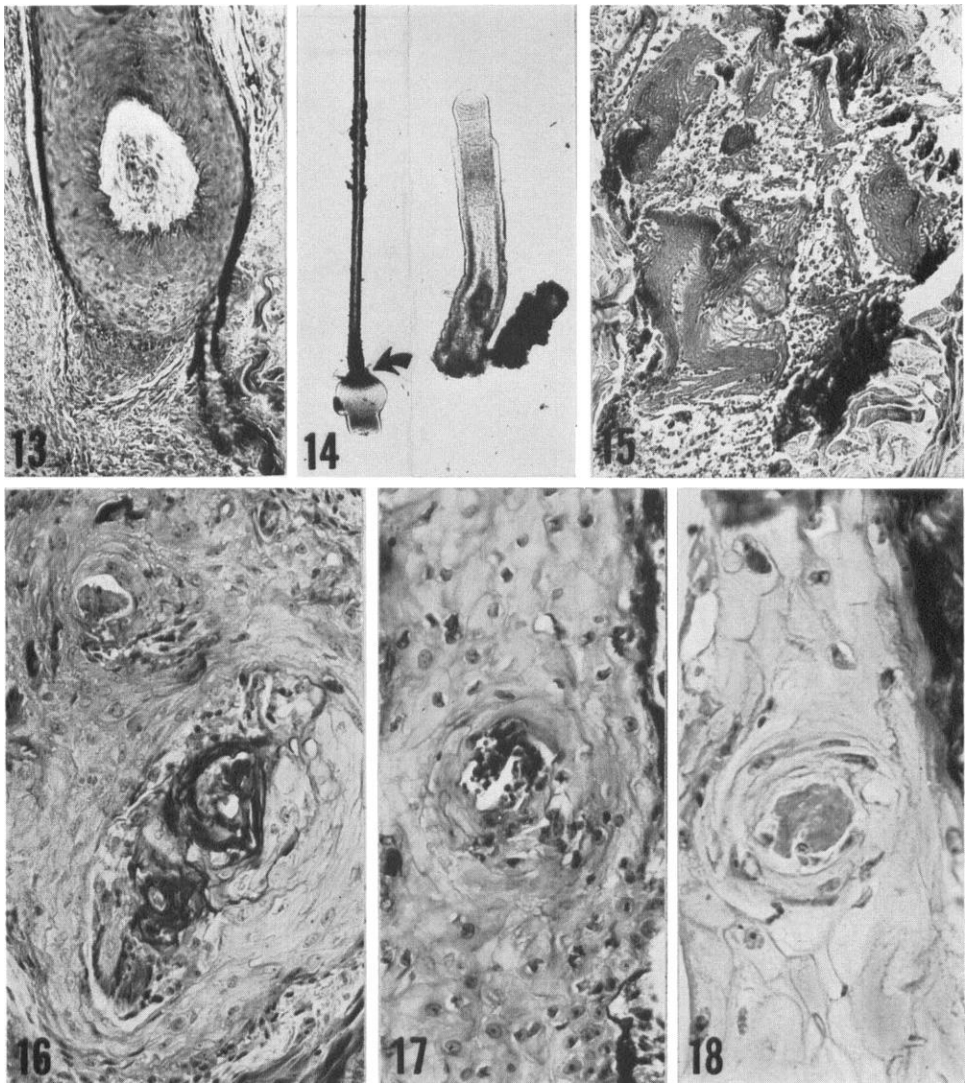
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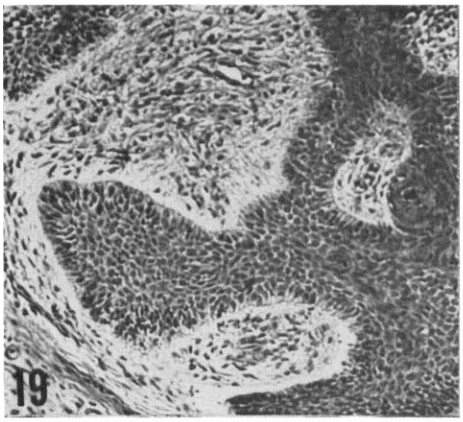
PLATE V

FIG. 19. Basal cell tumor with intact stromal connective tissue implanted in mid-corium of skin of the back, removed at 37 days. Tumor has remained viable, unchanged. × 136

FIG. 20. Basal cell tumor with intact stromal connective tissue implanted sub-cutaneously in the back, removed at 17 days. Tumor has remained viable, with no detectable change. × 63

FIGS. 21 and 22. Basal cell tumor with intact stromal connective tissue implanted in mid-corium of the skin of the back, removed at 1 week. Eccrine duct-like structures present. × 575

FIG. 23. Same specimen as in Figs. 21 and 22. Focal keratinization within tumor tissue appears as epithelial pearl. × 105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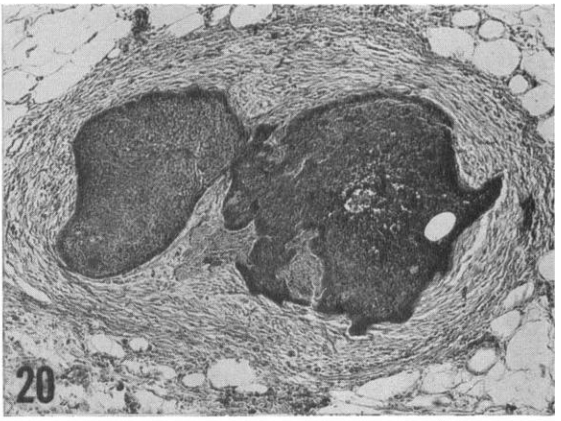
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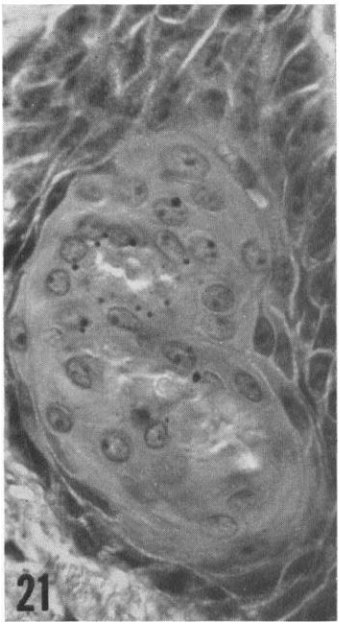
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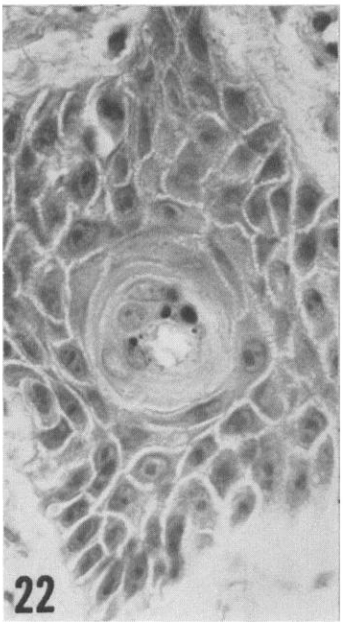
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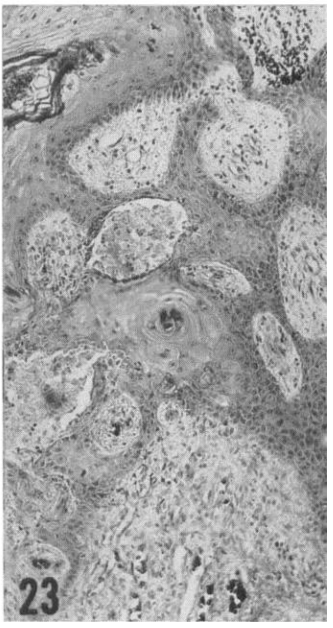
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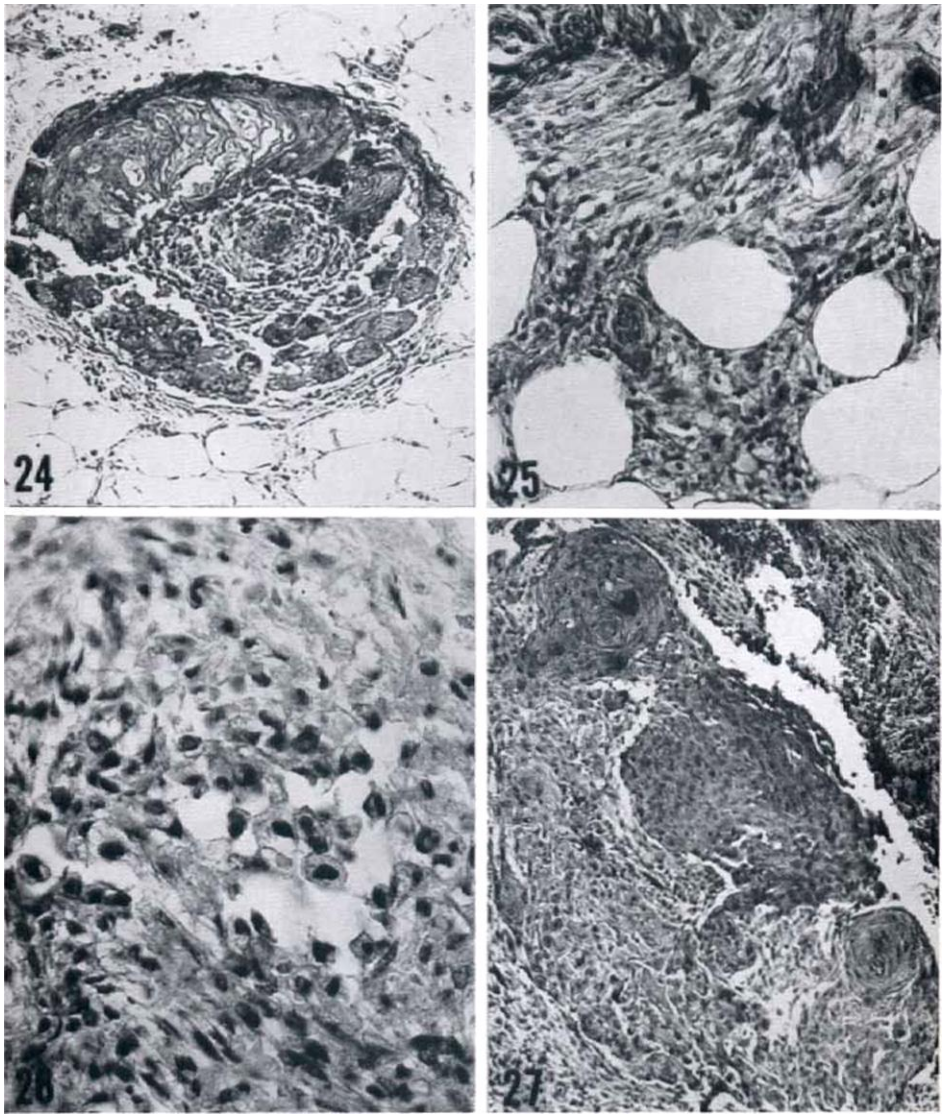
PLATE VI

FIG. 24. Pure basal cell tumor, devoid of native connective tissue, implanted sub-cutaneously in the back for 17 days. Complete degeneration of implanted tumor has occurred. × 120

FIG. 25. Pure basal cell tumor implanted at junction of lower corium and sub-cutaneous tissue for 1 week. Tumor cells (arrows) that remained have undifferentiated appearance. × 100

FIG. 26. Same specimen as in Fig. 25. Cells of unknown identity have appeared within area of tumor implants. × 400

FIG. 27. Pure basal cell tumor implanted in mid-corium for 2 weeks. Numerous keratinized pearls give tumor the appearance of squamous cell carcinoma. × 100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DISCUSSION

Dr. John S. Strauss (Boston, Mass.): I enjoyed this paper by Dr. Van Scott very much, especially since we have been interested in the same problem. Our studies in one patient were stimulated by the presidential address given by Dr. Pinkus at the last annual meeting of the Society for Investigative Dermatology (J. Invest. Dermat. **33:** 171–175, 1959) in which he emphasized the historical distinctions of the stroma surrounding basal cell epitheliomas. We have transplanted some epidermal curettings from a basal cell epithelioma of the cheek in an elderly male patient to his back. These curettings were injected intradermally through a trochar. From histologic study of curettings, we felt that this specimen had a minimum of stroma. When the transplant site was excised after four weeks, there was only a foreign body giant cell reaction as illustrated in the first slide. (Slide) However, when, by the same methods, we transplanted a punch biopsy of the tumor including the stroma as well as the tumor tissue, study of the excised specimen taken at six weeks demonstrated some cellular masses deep in the corium which were histologically basal cell in type. These were surrounded by the characteristic stroma and there were several mitoses (Slide).

To complete the story, the next slide illustrates cyst formation deep in the dermis on the transplantation of normal skin in the same subject (Slide). This has been described before.

I have shown these slides to several individuals who feel that the tumor tissue is probably just surviving and replacing itself, but it is not actively proliferating. I know that Dr. Epstein has done studies along a similar line and has not seen any surviving tumor tissue in specimens removed after a much longer interval. The exact interpretation of the results needs further clarification.

Dr. William L. Epstein (San Francisco, Calif.): We have been studying the same problem of tumor transplantation in man for the past few years. Basal cell epitheliomas and their stroma have been buried deep intradermally in 10 or 12 subjects. Our basal cell implants have remained buried for relatively long periods of time (up to 6 months). None of the buried tumors remained viable for more than a few weeks. A foreign body reaction always appeared and the basal cell tumor cells disappeared. In some cases a keratinous cyst formed similar to those reported after burial of normal skin (Arch. Dermat. **76:** 437, 1957). We believe it develops from the normal tissue that is buried along with the tumor.

Most workers in this field will agree that factors in the corium determine normal epithelial differentiation and perhaps even malignant proliferation, but I don't think Dr. Van Scott has conclusively demonstrated that fact today.

Dr. George F. Wilgram (Boston, Mass.): Dr. Van Scott briefly mentioned in the beginning of his talk changes in the tonofibrils. I wonder whether you would care to elaborate upon the findings concerning tonofibrils.

Dr. Allan L. Lorincz (Chicago, Ill.): I wonder, in any of these experiments involving deep implantation of basal cell epitheliomas where regression was observed, whether some of the original neoplasm was left in situ; and if so, whether the original neoplasm showed any signs of regression as might be expected if the deep implantation procedure would activate immunologic defenses against the tumor.

Dr. John M. Knox (Houston, Texas): We have been interested in this problem from a practical point for we curette most of our basal cell epitheliomas in contrast to employing radiation for treatment. Critics of this technic have mentioned the possibility of a disrupted cell being transplanted and surviving. Therefore, in an attempt to duplicate what might happen clinically, we tried to transplant 18 cases of basal cell epitheliomas by using three different transplantation technics. We were unable to make any of the 18 tumors survive and grow under the conditions we employed for at the end of varying periods of observation, serial sections of the transplantation site revealed no tumor.

Dr. Hermann Pinkus (Detroit, Michigan): This is important work and I hope the authors will pursue it further.

The results with basal cell epithelioma were most interesting to me. I am glad to hear this experimental support of the thesis that the epithelial cells of this tumor are not autonomous and do not survive without their stroma (J. I. D. **33:** 171, Oct. 1959). This seems to be another indication that so-called basal cell cancer is not truly carcinoma, but is a hybrid, a mixture of stroma and epithelium growing together and influencing each other.

Dr. William Montagna (Providence, R. I.):

It is a good sign when dermatologists begin to talk about "modulation" of tissues. When several years ago I talked with Medawar about his observations with Billingham on the transplantation of epidermis to strange sites, Medawar shared with me the doubt that the sheets of epidermis were pure and entirely free of dermis.

We are now just beginning to understand the interrelationship of dermis and epidermis. Dr. Van Scott's observations demonstrate again that the epidermis is a relatively indifferent tissue at the mercy of the inductive potentials of the dermis.

DR. SAUL BLAU (New York, N. Y.): Would Dr. Van Scott care to comment on the paper of Glücksmann ("Local Factors in the Histogenesis of Hypertrophic Scars", Brit. Journ. Plast. Surg., **4:** 88, 1951), wherein it is shown that inclusion of epidermal and keratin debris within the dermis of incised wounds is a direct cause of hypertrophic scars and keloids? And then, could these factors be used to explain the unpredictable hypertrophic scars that sometimes appear after even superficial dermabrasion?

DR. E. J. VAN SCOTT (in closing): I would like to thank the discussers very much and I am glad to see this real interest in the subject with which many of us are concerned.

In answer to Dr. Strauss, a good index of both survival and proliferation of implanted epithelium is indicated by the structures produced by the implant. Also, sufficient numbers of mitoses suggest that the tissue is in fact proliferating as well as surviving.

In answer to Dr. Epstein, I think the size of the implanted specimen is quite critical. For example, if a punch biopsy specimen of the scalp is just rotated 180° and left in place without otherwise removing it, the hair roots therein will degenerate. It seems such a large piece of tissue may be walled off by a fibrin clot so that its nourishment is inadequate. We must recognize, however, that we have not studied our implants for periods longer than five weeks and do not know their fate during longer periods of implantation.

I would not say that the modified behavior of implanted epithelium is only the result of the influence of the corium. It is due to the influence of the environment, but this in turn may be comprised of many factors.

In answer to Dr. Lorincz, our implantation procedures were done in patients with multiple basal cell tumors. We did not specifically look for changes in untouched tumors, but no clinically apparent changes in these tumors come to our attention.

The findings of Dr. Knox are very interesting. Perhaps all implanted basal cell tumors do eventually degenerate. Technics of handling and implanting tissues may account for different results though. For instance, careful handling of tissue to make sure it does not dry out prior to implantation may be critical.

In answer to Dr. Blau, I do not think that our implants were left in place sufficiently long to make observations on keloid formation. From other clinical observations, however, the inclusion of epidermis intradermally would not seem to account for all keloids; some patients develop keloids regularly, even in punch biopsy sites which are not closed with a suture.