Growth characteristics of experimental intracerebrally transplanted oral epithelium

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Oral mucosa was transplanted into the brains of 50 baby rats; in 45, viable growth was demonstrated when the animals were sacrificed 1 to 24 months later. The transplants showed stratified squamous, cuboidal, and transitional-like epithelium plus secondary changes such as cholesterol clefts, calcification, and in one case, lamellar bone. The tumors had an unpredictable growth rate. The histological characteristics were those of epidermoids, dermoids, craniopharyngiomas, or Rathke cleft cysts, and suggested a common histogenesis.

KEY WORDS • oral mucosa • brain implantation • growth characteristics • epidermoid • craniopharyngioma

DERMOIDS and epidermoids of the central nervous system have been attributed to disordered embryogenic investment of cutaneous epithelium by closing medullary folds during the third and fifth fetal week; the resulting specific tumor would then depend upon the degree of differentiation of the trapped cutaneous ectoderm.3 Craniopharyngiomas have been assumed to be derived from squamous epithelial rests of the pars tuberalis, remnants of the embryonal stomatodeum evagination (Rathke's pouch).28 The logic of separating epidermoids from craniopharyngiomas is debatable, as they are structurally very similar and their derivation from Rathke's pouch is not firmly established as is often assumed.11,12,15 Intrasellar cysts, also considered to be derived from Rathke's cleft have either simple cuboidal or ciliated epithelial lining and have been differentiated from the suprasellar craniopharyngioma.2,8,22,25,26 thus further separating these tumors.

This study was undertaken to determine if oral epithelium implanted intracranially produced any or all of these tumors. Previous studies have demonstrated that implantation of dermal tegmentum into the spinal cord, cranium, and subarachnoid space results in growth of epidermoids and/or dermoids in rats,29 dogs,18 and cats.28 In the present study, oral epithelium was implanted into both cerebral hemispheres or the hypothalamus of young rats, and the growth characteristics of the transplanted mucosa examined histologically from 1 month up to 2 years later.

Methods

Oral epithelial transplantation was performed in 50 white baby rats between the ages of 8 to 10 days. The animals were
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anesthetized with sodium pentobarbital, and under sterile conditions a $2 \times 2$ mm biopsy of epithelium was taken from the posterior roof of the oral cavity. The specimen was placed in sterile saline, and under the dissecting microscope the biopsy was diced into pieces less than 0.2 mm in diameter. Craniotomy was performed, and one small segment of epithelium was placed indiscriminately in the cerebrum of each of 15 animals. In the remaining 35 rats, the epithelium was placed in the hypothalamic area.

The rats were sacrificed at intervals from 1 to 24 months following implantation in order to study the characteristics of both long and short periods of implant growth. At the time of sacrifice, the animals were anesthetized with sodium pentobarbital and the brain perfused with physiological saline followed by 10% formalin solution. The calvarium was opened, the brain with its leptomeninges intact removed and fixed in 20% formaldehyde for 3 weeks. Following fixation, the brain was divided into three equal parts, embedded in paraffin, and sectioned in the coronal plane. After the

![Figure 1](image1.png)

**Fig. 1.** Photomicrograph of oral epithelium biopsy prior to implantation, illustrating squamous epithelium (a) with keratohyaline granules (arrow). Mucous glands (b) are numerous in the dermis. H & E, X 250.

![Figure 2](image2.png)

**Fig. 2.** Left: Photograph of rat frontal lobe illustrating growth 24 months following implantation. H & E, X 8.75. Right: Photomicrograph of the squamous epithelium living the wall of growth. Arrow denotes the collagenous basement membrane between the epithelium and brain. H & E, X 220.
epithelial growth was located, serial sections 6 μ thick were cut at 25 μ intervals throughout the tumor to study its configuration in three dimensions. The histological sections were stained routinely with hematoxylin and eosin (H & E), Masson trichrome, Luxol fast blue-periodic acid-Schiff (LFB-PAS), Holzer stain for glial fibers, and Von Kossa stain for calcium.

The histological characteristics of the original oral implant are illustrated in Fig. 1. Stratified squamous epithelium lines the oral cavity characterized by a prominent basal cell layer with large ovoid nuclei and acidophilic cytoplasm. There is flattening of the nuclear elements toward the periphery with a thin layer of keratin on the outer margin. Keratohyaline granules are easily identified in the more superficial epithelial layers. Loose connective tissue containing mucous glands underlies the epidermis. Thus, a source for growth of epithelium glandular elements and connective tissue is present in the implant.

Results

At the time of sacrifice none of the 50 animals showed neurological deficits.

No Epithelial Growth

In five of the 50 brains examined, we were unable to find evidence of viable transplanted epithelial growth; two of these specimens contained granulomatous tissue and inflammatory cells at the implant site, suggesting that the failure of the transplanted epithelial cells to proliferate was secondary to inflammation. No evidence of tumor or inflammation was present on histological examination of the remaining three animals; however, small growths may have been missed.

Growth Characteristics of Transplant

Forty-five of the animals showed epithelial growth of the transplant. One of the largest tumors was found in an animal sacrificed at 24 months (Fig. 2 left). Although this tumor occupied almost the entire frontal hemisphere, there was no neurological deficit. The growth had an
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epithelial border between 1 to 8 cells thick (Fig. 2 right), separated from cerebral tissue by a thin collagenous basement membrane. The size of the tumor was not necessarily proportional to the duration of the time between implantation and sacrifice; two other animals sacrificed at 24 months contained much smaller viable growths, one microscopic in size, the other measuring 1.5 mm in diameter.

Epithelial Characteristics Following Cerebral Implantation

Epidermoid. There were 26 typical epidermoid tumors. A typical example of an epidermoid tumor is illustrated in Fig. 3. This growth from an animal sacrificed at 7 months demonstrated the four layers originally described by Bailey. The stratum durum, a thin homogenous layer with no nuclei, is located immediately adjacent to the brain; the brain in this instance shows minimal reactive gliosis, and there is no infiltration of epithelial elements into the cerebral tissue.

The stratum granulosum is several layers thick; its cells contain well-formed nuclei with acidophilic cytoplasm, and have definite cell boundaries. The epithelial cells have ovoid nuclei in the basal cell layer with flattening of the cells toward the center. Keratohyaline granules are evident in the cytoplasm of cells distant from the basal layer.

The stratum granulosum blends into the stratum fibrosum, which consists of layer upon layer of homogeneous fibrous-like material with no cell boundaries or nuclei.

In the next layer or stratum cellulosum, the cell boundaries are quite distinct and nuclear skeletons are present (Fig. 3 right).

The cellular characteristics of the four strata can only be preserved by careful histological preparation. Of the 45 tumors, 26 were characteristic epidermoids, although all did not demonstrate the four strata clearly.

Dermoid. There were 12 dermoid tu-
tumors. A typical dermoid tumor consisting of squamous epithelium with dermal appendages is illustrated in Fig. 4 left. This tumor is from an animal sacrificed 4 months after implantation and contains stratified squamous epithelium with flattening of the epithelial cells toward the interior of the growth. The central portion of the tumor consists of laminated keratin, and keratohyaline granules are present. Loose connective tissue underlies the epithelium surrounding mucous glands that stain with the periodic acid-Schiff reagent. The mucous cells have a circular configuration with flattened nuclei at the periphery (compare with Fig. 1).

Miscellaneous Epithelial Findings. Many of the epithelial transplants demonstrate multiple fingers of epithelium displacing and invading surrounding brain parenchyma. Figure 5 left illustrates multiple epithelial pearls containing desquamated keratin with nuclear remnants in the interior of the cysts. Mononuclear inflammatory cells underlie the epithelium in brain parenchyma. Higher magnification of the same tumor (Fig. 5 right) demonstrates epithelial cell mitosis, evidence that the implanted epithelium is healthy and proliferating.

In contrast, some transplants did not contain the desquamated keratin but consist of a solid sheet of epithelial cells (Fig. 6) in which the nuclei have a homogenous circular configuration with clear cell boundaries and resemble transitional epithelium. Two nests of epithelial cells appear to be isolated from the tumor; however, serial sections of the tumors reveal they are slender protruding epithelial tentacles connecting with the central mass. This animal was sacrificed 10 months following implantation.

Figure 7 top illustrates a cystic tumor comprised of two different lobules, one containing a material staining with periodic acid-Schiff, the other desquamated keratin. Higher magnification (Fig. 7 center and bottom) demonstrate that each cyst is lined by a single cell layer of epithelium separated from the surrounding brain by connective tissue. Although the oral epithelium assumed multiple characteristics following implantation, columnar epithelium characteristic of adamantinoma was never identified.
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**Fig. 8.** Left: Photomicrograph of multilobulated growth illustrating dark-staining calcium. Von Kossa, X 100. Center: Successive steps of epithelial degeneration into calcium. Spherical grouping of normal epithelium (a), beginning coalescence of epithelium (b), and loss of cellular structure and deposition of calcium (c). H & E, X 315. Right: Lamellar bone surrounded by brain and connective tissue. H & E, X 100.

calcium salt has its origin in part from degeneration of epithelial cells, and from degenerative changes in the brain parenchyma and connective tissue. A higher magnification of this tumor demonstrates the process of epithelial degeneration (Fig. 8 center). A circular nest of healthy epithelial cells with distinct nuclei is contrasted to an adjacent area where the epithelial cells manifest early degeneration with loss of clear nuclear structure. In Fig. 8 center, the epithelial cells have degenerated into a homogenous mass with complete loss of cellular structure which stains positive for calcium. There are frequently mononuclear inflammatory cells and fibrocytes intermixed with brain, connective tissue, and epithelium. In one animal, sacrificed at 17 months following implantation, lamellar bone was present (Fig. 8 right).

**Cholesterol Clefts.** In many growths, cholesterol clefts were evident with nests of epithelium, blood vessels, and connective tissue (Fig. 9). The clefts were found at the periphery of the tumor close to cerebral tissue and not in the interior of any tumor, suggesting that they are formed secondary to degeneration of cerebral tissue rather than keratin.

**Discussion**

Small and moderate-sized epidermoids grow in an elliptical or circular fashion, like epithelial explants studied in tissue culture. They have a central core of keratin surrounded by squamous epithelium that is separated from cerebral tissue by a basement membrane of connective tissue. In tissue culture, the basal layer of the explant epithelium proliferates and migrates to
encircle the rest of the explant; prickle cells then appear, followed by a granular layer, and keratohyaline granules are formed in the stratum corneum. Transplants of oral epithelium into cerebral tissue appear to grow in a comparable manner.

In our experiments, the oral epithelium that grew after transplantation contained stratified squamous and cuboidal epithelium plus solid sheets resembling a transitional type. In many transplants there were viable mucous glands and epithelial ducts. These epithelial elements possessed no blood supply, and their survival must have depended on the supporting stroma. The different types of epithelium in these tumors suggest that the final configuration is determined by the interaction of the transplant and environment rather than by time. The multiple potential characteristics of epithelium are well documented by previous reports of skin and oral epithelium transplants into subcutaneous tissue, and by studies of epithelial changes following anthramine-induced carcinogenesis.

Enlargement of the implants is secondary to proliferation of epithelial elements and connective tissue, in addition to accumulation of desquamated keratin in the center of the growth. There is minimal reactive gliosis in the brain adjacent to the implants. The growths appear to displace and invade the cerebral tissue with multiple fingers of epithelium protruding from the central mass. The cytology of these buds does not suggest malignant change.

The size of the implant did not correlate with the duration of growth, nor did the area of the brain where the implants were placed influence this disparity of growth. Similarly, although dermoids, epidermoids, and craniopharyngiomas show clinical manifestations at certain age peaks, they may appear anytime in the extremes of age depending on the growth rate.

Calcium deposits were found in transplants between 4 and 24 months after implantation. The formation of calcium is secondary to the fact that both the epithelium and connective tissue have outgrown their nutrient supply with degeneration, invasion by fibrocytes and inflammatory cells, and deposition of calcium. In addition to calcium, viable lamellar bone was present in a tumor 17 months following implantation. Nicholson has postulated the formation of bone secondary to epithelial degeneration; granulation tissue proliferates and fibrocytes differentiate into osteoblasts. Lamellar bone has been described in craniopharyngiomas.

Cholesterol clefs were frequently encountered in the growths from oral mucosal implants. This did not occur with skin implants in cerebral tissue. However, the cholesterol clefs were found in the connective tissue adjacent to the brain, not in the central keratin-containing areas of the tumor. This lends support to Love and Kernohan’s opinion that cholesterol is not formed by keratin degeneration but from cerebral tissue.

The cyst containing PAS-positive material illustrated in Fig. 7 is not unlike Rathke’s cleft cyst although ciliated epithelium was not present. The cyst is lined with cuboidal epithelium and contains a mucinous-like material. Similar bilobulated tumors have been described in humans; the intrasellar portion is cuboidal epithelium while the suprasellar portion is squamous epithelial lining, suggesting that epithelial metaplasia is the most likely explanation.

Stratified squamous, cuboidal, and transitional-like epithelium, keratohyaline granules, mucous glands, mucoid-containing cysts, and secondary changes of calcification, cholesterol clefs, and lamellar bone have all been described as constituents of epidermoids, dermoids, Rathke’s cleft cysts, and craniopharyngiomas. However, a single palisade of columnar cells in the shape of an epithelial tree characteristic of adamantinomata was not seen. The many potential characteristics of newborn oral epithelial growth after transplantation into the brain suggest the concept that the origin of these epithelial tumors is a continuum of disordered embryogenesis.

References
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This investigation was supported in part by the Allen P. and Josephine B. Green Foundation.

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