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Behavior of Explants from Human Adult Bronchial Epithelium *in vitro*.^{*} (23645)

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During the last few years several strains of normal and carcinomatous human tissues have been maintained continuously in tissue cultures(1,2,3,4). None of these strains have been derived from non-neoplastic bronchial epithelium.

The following paper presents observations on the behavior of explants from adult human bronchial epithelium and reports the establishing of 2 permanent strains of such epithelium.

Material and methods. Samples from the bronchus of patients undergoing removal of a lung or a lobe of the lung were obtained from the operating room under aseptic conditions. The epithelium was stripped from a 1.5-2 cm length of the most proximal part of the bronchus. The small fragments, consisting of bronchial epithelium and submucosal tissue, were treated for 10-15 minutes with 5 ml of a freshly made 0.1% solution of trypsin in Hanks' salt solution containing 20 mg streptomycin and 20,000 units of penicillin. The fragments were then explanted into chicken plasma-chicken extract clot to which 40% human serum medium was added. The human serum was pooled from 2 or 3 donors, diluted with Hanks' solution containing 50 mg streptomycin and 50,000 units of penicillin per 100 ml. From the several methods of tissue-culture tried the use of micro Petri dishes(5) proved to be most satisfactory for the primary explants in our experiments. Perforated cellophane discs were put into these dishes to support the cultures since the grow-

ing cultures liquefied the clots. During 24 to 48 hours the phenol red indicator in the medium turned yellow showing acid production in the growing cultures. The medium was replaced daily or every other day by fresh 40% human serum medium. If a primary explant was established, subcultures in roller tubes were attempted after 7 to 10 days.

Results. Cultures from the bronchial epithelium of 119 adults were made. During the first 24 hours large numbers of round cells wandered to the periphery of the explant. Active movement of the cilia of ciliated epithelium could be observed. In many cultures no further migration of the cells occurred and the explant died in 4-6 days. If the explant became established, sheets of epithelial tissue grew out from the periphery of the cultures from the second day onward. The clones had blunt and rounded periphery, and the adjacent cells were without visible intercellular substance and were polygonal in shape. Many of the outgrowths ceased to increase after a few days, several survived 2 to 3 subcultures up to 40-50 days. Two permanent strains were established. Ciliation was often observed in the second or third subcultures, and both permanent strains contained ciliated epithelial cells up to the 6th generation. Such differentiation was not seen in the subsequent rapidly growing substrains. The microscopic appearance of the first outgrowth did not show any appreciable difference between the strains which ceased to grow and the 2 strains which gave rise to permanent strains.

Table I shows that apparently normal bronchial epithelium derived from persons having

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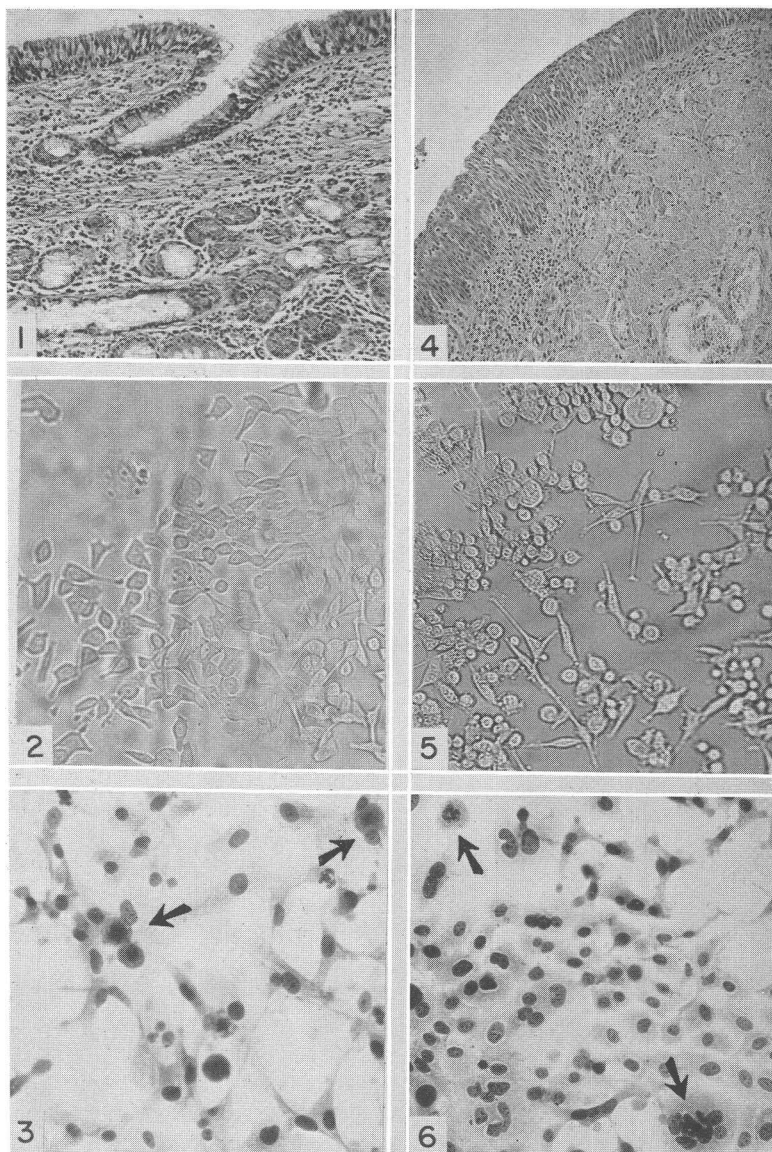


PLATE I

FIG. 1-3: Culture 130, derived from seemingly normal bronchial epithelium of a case of epidermoid carcinoma of bronchus:

FIG. 1. Segment of bronchus adjacent distally to explant; normal epithelium. Formalin-fixed, H & E, $\times 78$.

FIG. 2. Roller tube culture, 22nd generation; sheets of epithelial cells. Living, unstained, $\times 93$.

FIG. 3. "Flying" coverslip culture, 20th generation, 4 days old, showing many mitoses. Bouin's fixed, H & E, $\times 138$.

FIG. 4-6: Culture 134, derived from metaplastic bronchial epithelium of a case of epidermoid carcinoma of bronchus:

FIG. 4. Segment of bronchus adjacent distally to explant; squamous metaplasia of epithelium. Formalin-fixed, H & E, $\times 78$.

FIG. 5. Roller tube culture, 11th generation; many large cells with bizarre nuclei. Living, unstained, $\times 93$.

FIG. 6. "Flying" coverslip culture, 13th generation, 4 days old showing atypical mitosis and multinucleated giant cell. Bouin's fixed, H & E, $\times 141$.

TABLE I. Growth of Tissue Cultures of Bronchial Epithelium.

Disease of donor	Growth of explant		
	Good	Poor	None
Carcinoma of bronchus	14	3	2
Pulmonary tuberculosis	11	18	51
Bronchiectasis and unresolved pneumonia	9	3	6
Blastomycosis	1		1

carcinoma of the lung grew generally better than analogous tissue from patients suffering from tuberculosis. Cases with unresolved pneumonia and bronchiectasis behaved in an intermediate fashion.

The segment of bronchus from which the epithelium for explant was taken was at a varying distance from the lung lesion and even in cases of bronchial carcinoma arising in a large bronchus several centimeters of grossly normal epithelium separated the tumor from the site of the explant. The microscopical examination of sections from the bronchus immediately distal from the site of the explant or of the bronchi in the whole resected lung gave no indication by which the quality of growth of the explant could be predicted. The number of epithelial layers did not differ significantly in the bronchial mucosa of tuberculous or carcinomatous patients nor in the bronchi from patients suffering from the same pathological state which did or did not give well-growing explants. In some instances, however, in the bronchial epithelium of patients with carcinoma of the lung, even in distant localization, atypical cells with large hyperchromatic nuclei and small foci of intra-epithelial carcinoma were found in conformity with previous observations(6). In 2 cases of bronchiectasis the bronchial epithelium showed marked squamous metaplasia. The influence of the metaplastic or atypical changes in the epithelium on the success of the explant is difficult to evaluate since explants of 4 epidermoid carcinomas of the bronchus did grow for only a few days.

Two explants of bronchial epithelium gave rise to permanent strains. Both of these explants were derived from patients with carcinoma of the bronchus only one of whom (U. Va. 134) had demonstrable metastases.

The tissues were explanted on the 11 and 30 March, 1957 respectively.

The microscopical appearances of the cultures, U. Va. 130 and 134, are very similar to each other. The 2 cultures grew out from the explants in sheets of epithelial cells 2-3 days after explantation. A fibroblastic-like appearance of the clones was not observed. In roller tubes they formed continuous sheets of cells adherent to the wall. When growing between 2 glass surfaces in plate cultures they had amoeboid movements and diverse cell shapes could be seen. In hanging drop culture many multinuclear cells were formed. Pinocytosis and phagocytosis were observed. In appropriately stained preparations (*e.g.* hematoxylin and eosin, or Wright stain) many bizarre cells with hyperchromatic nuclei and anomalous chromatin arrangement were present (Plate I).

Discussion. The differences found in the explantability of bronchial epithelium of adult humans suffering from carcinoma, tuberculosis or other inflammatory diseases of the lung is thought to be connected with certain properties of the epithelium itself rather than with the treatment which the patient might have undergone prior to the removal of the bronchus. Most of the carcinomatous patients were treated with antibiotics prior to establishment of diagnosis or as a supportive measure. All of the tuberculous patients received treatment with streptomycin with or without addition of isoniazid. Since streptomycin and penicillin were added routinely to every culture without any deleterious effect, it seems unlikely that the antibiotic which the patient received should have had any influence.

Summary. Explants of bronchial epithelium were made from 119 adult human lungs removed by operation. Bronchial epithelium taken from patients with carcinoma of the lung grew more often in primary explants than analogous epithelium from tuberculous patients or from patients with bronchiectasis or unresolved pneumonia. Two explants, both derived from cases of carcinoma of the lung, gave rise to permanent strains. The histological appearance of the bronchial epithelium of the donor, even in a segment immediately distal from the explant, did not give an indi-

cation as to the growth expected in the ex-
plants.

Explantation of carcinoma of the bronchus
was unsuccessful in 4 cases.

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Histopathology of Hydrocephalus Resulting from a Deficiency of Vit. B₁₂.^{*} (23646)

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A high incidence of hydrocephalus and other congenital malformations occurs in infant rats from Vit. B₁₂ deficient mothers(1). The cerebral aqueduct in the hydrocephalic brain is partially or completely occluded and a unique type of tall columnar cell is absent from the roof of the third ventricle and cerebral aqueduct(2). Brains from litter mates of hydrocephalic animals have aqueducts of abnormal size and shape although their gross appearance is normal.

This paper is an extension of previous studies made in our laboratories on the hydrocephalic brain in newborn rats from Vit. B₁₂ deficient dams. The significant observations were that the ependymal lining and choroid plexus were deranged and contained excess lipide.

Methods. Weanling female rats of the Wistar strain were fed a Vit. B₁₂ deficient diet, composed chiefly of soybean meal and glucose(1) until they reached a weight of 160-170 g. During the reproductive phase some of the animals were continued on this diet while control animals received the same

diet supplemented with 30 µg of Vit. B₁₂/kg. Thirty-two Vit. B₁₂ deficient females bore 896 offspring comprising 132 litters. Of this number 92, or about 10%, were hydrocephalic. This abnormality was detected at birth by sectioning the gross brain with a knife blade after fixation in Bouin's solution. In the normal brain there is no grossly detectable distension of the lateral ventricles. Ventricular distension in hydrocephalic brains varied from 1 to 2 mm in mild cases to 6 to 8 mm in severe cases. A higher percentage of mild cases was detected by this method than by the light transmission technic(3) and resulted in approximately doubling of the observed incidence. No hydrocephalus was demonstrated at birth among offspring of control dams. For routine observation 7 µ sections were cut and stained with hematoxylin and eosin. Brains used for fat studies were stained with osmic acid(4) and Herxheimer's fat stains(5). Brains from 34 hydrocephalic offspring, 35 litter mates and 28 controls were examined.

Results. In cases of gross dilatation of the lateral ventricles the cerebral tissue was reduced to the thinness of a membrane. Microscopically, the cortical layers were disrupted and in some areas of the lateral and third ventricles the thinned ependymal lining was folded outward. In other areas, notably in the third ventricle, the ependyma was absent and the brain tissue appeared areolar and

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