10. Rosenfeld, I., Beath, O., Selenium, Academic Press, New York, 1964.
11. Fishbein, W. N., Carbone, P. P., Science, 1963, v142, 1069.
12. Gale, G. R., Biochem. Pharm., 1964, v13, 1377.
13. Gale, G. R., Kendall, S. M., McLain, H. H., DuBois, S., Cancer Res., 1964, v24, 1012.
14. Boyland, E., Nery, R., Nature, 1964, v203, 1379.
15. Kofod, H., Huang, T-Y., Acta Chem. Scand., 1954, v8, 485.

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Explanted Nasal Mucosa: Growth Patterns of Cystic Fibrosis and Control.* (30210)

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While studying nasal mucosa from patients with cystic fibrosis of the pancreas (CF), we observed an organoid appearance in the primary and reaggregated tissue cultures. This report summarizes our observations.

Methods. We obtained nasal polyps(1) directly at elective surgery. The resected polyps were skinned by forcing the mucosa flat against a pair of microdissecting scissors and then snipping the translucent mucosal swatch. Those swatches were cut into 1 mm squares, washed 5 times with Hanks' solution plus antibiotics, and divided into groups of 3-4 squares. The antibiotics included polymyxin, neomycin, streptomycin, and penicillin and mycostatin. Each group of squares was briefly dried in a dish of a 2-piece Carrel flask (Bellco #15-031) and then gently squashed with a coverslip. The coverslip remained in the flask thereafter. The dish was flooded with Parker's CMRL-1066 medium (Microbiological Associates) supplemented with 10% calf serum (Colorado Serum Co.). The lid was sealed with a sterile Dow Silicone High Vacuum Grease and the sidearm was capped with a rubber serum stopper(2). All culture procedures were subsequently carried out in the closed containers, by means of disposable plastic syringes. We maintained an approximate pH 6.9 with CO_2.

Results. Explants were taken from 11 patients. Explants from 4 patients yielded good growth in multiple flasks. Explants from 5 patients yielded unsatisfactory growth due to infection with Candida, Staphylococcus or Pseudomonas, which was harbored in the thick mucus of these chronically infected children. Explants from 2 patients were discarded without further culture when no ciliated epithelium was found on any of the 1 mm squares in the first 3 days. Routine histopathological examination of multiple sections of every polyp disclosed no malignant changes. None of the patients was symptomatic for a viral disease within 2 weeks of surgery. All patients had a positive sweat electrolyte test(3) and complete exocrine pancreatic insufficiency. In addition to these studies, a control polyp from a non-cystic fibrosis patient was cultured.

Dissections carried out by micromanipulation during the first 3 days *in vitro* demonstrated an easily identifiable mucosal layer; this consisted mostly of ciliated cells and interspersed nonsecreting crescentic cells. Pavement-like outgrowths of compact cells occurred in 5-8 days from areas where ciliary activity had spontaneously ceased. We did not follow the growth unless it rose from previously ciliated areas. For a given area, outgrowth did not begin until ciliary activity stopped. In other portions of the same speci-

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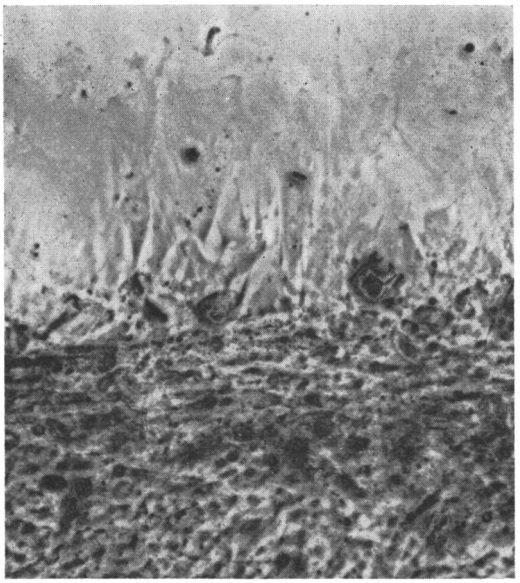

FIG. 1. Edge of fenestrated membrane, with layer of radially oriented elongate cells lining the interior. Occasionally, a similar appearance was noted at the growing edge, so that the most distal cells were oriented along the axis of growth and parallel to each other, but at right angles to the cells packed behind them.

men, ciliary activity might continue to be manifest for days or weeks, however. The outgrowths themselves never contained beating cilia. The compact pavement contained little mucus when tested by a sensitive periodic acid Schiff method(4). In about a week, before the cell sheet covered the coverslip, the outgrowths organized into fenestrated membranes. The fenestra were incompletely lined by a single layer of radially oriented, elongate cells (Fig. 1). Such membranes fixed briefly in cold buffered formalin and then examined by conventional paraffin technic consisted of 3 lamina: 2 outer layers of cells with some hairy excrescences sandwiching an inner layer of more spindly or plate-like cells. The fenestrated appearance and the laminated appearance, 2 aspects of the same cultures, reappeared after cells had been disaggregated in 0.25% trypsin in 0.2 ml Hanks' solution for 20 minutes at 37°C. It took about 3-5 days for the reaggregation to be fully effective. Indeed, Fig. 1 is a culture 5 weeks old, after transfer followed by one disaggregation.

Cell sheets from the 4 patients were studied cytochemically; and from one patient's explant, specimens were prepared for electron microscopy. Modest amounts of formazan-precipitating succinic dehydrogenase were demonstrable(5), corresponding to an ultrastructure in which mitochondria were sparsely distributed. Copious amounts of diphosphopyridine nucleotide (DPN) diaphorase were present. Acid phosphatase stains of cold-formalin fixed cells by a naphthol ester technic(6) showed intracellular variegation of the enzyme without much correlation to electron microscopy. Stains with naphthol yellow S for protein(7) and an oversight solochrome cyanin R-200 stain(8) showed similar variegation without much correlation to ultrastructure. Feulgen stains of osmium-fixed, epoxy-embedded, thin-sectioned nuclei using a fluorescent Feulgen technic(9) demonstrated uniformly fine chromatin patterns with little significant difference between cells. Such epithelial-like features as a prominent nucleolus were not particularly evident. The nuclear ultrastructure was nicely correlative, likewise not typical for the nuclei of epithelial cells. Alterations (transformations) of the cultures apparently did not occur; however, a cytopathogenic change may have occurred in one series of flasks, marked by the agglomeration of cells into clusters without rounding up or detachment. This series was discarded.

Various controls were utilized during these studies. The observed membrane formation did not occur in control cultures of primary human amnion(10), hamster myxosarcoma (11), Chang liver(12) (Microbiological Associates), embryonic lung(13) (Flow Laboratories), or fibroblasts derived from the parenchyma of the polyps discussed in this paper. These controls were carried with the same technic and media, and at the same time, as the polyp studies. Further, none of the above controls showed immediate reactions to vit A in CMRL-1066; but at a dose of 10 μ/ml, cells of the pavement-like plate showed rectangularization, and intense activity in the Golgi region. This appearance persisted after a single pulse of vit A(14). A control polyp from a patient without cystic fibrosis gave an *in vivo* appearance indistinguishable in

morphology and histochemistry from that outlined for the test polyps.

Discussion. The remarkable growth potential of nasal mucosa *in vivo* was known 30 years ago when destructive sinusitis was a frequent disease. The regrowth potential was studied by tissue culture then(15). The questions of what happens to mucosal outgrowths, and what is the origin of the outgrowing cells—these have not been answered satisfactorily by ourselves or others(16). The simple system outlined may provide evidence on how epithelial cells persist in culture. Though the results from preliminary electron microscopy and cytochemistry are not helpful, some kind of influence seems to be present which makes for primitive reaggregation and an organoid appearance. This is uncommon enough in non-embryonic human tissue culture to merit further study. By identifying the mucosal border of the explant with the ciliated marked cells and by time lapse studies, it may become possible to see which cells are orienting which way in the formation of the fenestrated, laminated membrane. We agree that cystic fibrosis cells probably do not show different tissue culture characteristics from similarly derived non-CF cells (17). Yet, we are intrigued by the notion that the CF defect, which has so far been noted only in epithelial cells and not fibroblasts *in vivo,* may be studied some day in tissue culture.

Summary. Explants derived from the mucosa of benign nasal polyps were cultured in closed vessels by a coverslip technic. The polyps had previously been resected from children with cystic fibrosis of the pancreas and a control. The explants grew as fenestrated, multilayered membranes with an appearance suggesting primitive aggregation. The pattern occurred again when the original cell sheets were disaggregated and allowed to grow. Perhaps the mucosal layer was the origin of some of the cells. Use of electron microscopy and cytochemistry was not helpful in differentiating possible cell types.

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1. Shwachman, H., Kulczycki, L. L., Mueller, H. L., Am. J. Dis. Child., 1961, v102, 768.

2. Warren, J., Wittler, R. G., Vincent, M., J. Lab. Clin. Med., 1955, v46, 144.

3. Darling, R. C., di Sant' Agnese, P. A., Perera, G. A., Andersen, D. H., Am. J. Med. Sci., 1953, v225, 67.

4. Ornstein, L., Mautner, W., Davis, B. J., Tamura, R., J. Mt. Sinai Hosp. N. Y., 1957, v24, 1066.

5. Novikoff, A. B., Shin., W. Y., Drucker, J., J. Biophys. Biochem. Cytol., 1961, v9, 47.

6. Burstone, M. S., J. Nat. Cancer Inst., 1958, v21, 523.

7. Deitch, A. D., Lab. Invest., 1955, v4, 324.

8. Pearse, A. G. E. Acta Histochem., 1957, v4, 95.

9. McNary, W. F., Jr., Rosan, R. C., Kerrigan, J. A., J. Histochem. Cytochem., 1964, v12, 216.

10. Rosan, R. C., Nahmias, A. J., Kibrick, S., Kerrigan, J. A., Exp. Cell Res., 1964, v36, 611.

11. Rosan, R. G., Kerrigan, J. A., J. Histochem. Cytochem., 1964, v12, 137.

12. Chang, R. S., Proc. Soc. Exp. Biol. and Med., 1954, v87, 440.

13. Hayflick, L., Moorhead, P. S., Exp. Cell Res., 1961, v25, 585.

14. Aydelotte, M. B., J. Embryol. Exp. Morphol., 1963, v11, 621.

15. Ross, P. J., Trans. Am. Acad. Ophthal. Otolaryng., 1932, 432.

16. Jordan, W. S., Proc. Soc. Exp. Biol. and Med., 1956, v92, 867.

17. Foley, G. E., Drolet, B. P., McCarthy, R. E., Goulet, K. A., Dokos, J. M., Filler, D. A., Cancer Res., 1960, v20, 930.

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