Pancreatic Neoplasms Induced in Syrian Golden Hamsters

I. Scanning Electron Microscopic Observations

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Pancreases from Syrian golden hamsters treated with N-nitrosobis(2-hydroxypropyl)amine for 10 to 25 weeks were examined under scanning electron microscopy (SEM). Findings indicate that the neoplasms originated from the ductal epithelium and developed progressively. Adenomas were lined by epithelium of differing cells types, ranging from a flat singly ciliated form to cuboidal-columnar types, or to mixed cell populations. The epithelial lining of the ductal carcinomas exhibited tubular and papillary cystic spaces, and cell surfaces were similar to the cuboidal and columnar epithelium of adenomas and of ductal epithelial hyperplasia. However, microvilli were dense and of varied lengths. The SEM observations correlated with patterns seen in routine histologic preparations. (Am J Pathol 83:517–530, 1976)

THE UNEXPLAINED INCREASE and unknown etiology of human pancreatic cancer [1-3] indicate a need for experimental models to study these tumor types. Pancreatic carcinogenesis in laboratory animals was recently linked to the effects of certain chemicals.[4,5] Moreover, the cells of origin in pancreatic cancer have been the subject of controversy. Accordingly, further studies using the Syrian golden hamster were carried out to correlate light and electron microscopic structures of these neoplasms and to provide a basis for morphologic comparison of the tumors in experimental models and in man. The present investigation describes the surface ultrastructure of pancreas duct tumors induced by N-nitrosobis(2-hydroxypropyl)amine (BHP) as part of a comparative study. However, SEM not only allows examination of lumina in the pancreas but, in addition, the cut surface permits an estimate of cell patterns and types in a third dimension, by which topographic correlations are readily obtained.

Materials and Methods

Randomly bred, 8-week-old Syrian hamsters from the Eppley colony were kept under standard conditions (room temperature, 21 ±1 C; humidity, 50 ±5%; air change, 10 times per hour). They were housed in plastic cages and given Wayne pelleted diet and water *ad*

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libitum. BHP was synthesized and purified by Dr. L. Wallcave of the Eppley Institute and was injected in 0.09% NaCl subcutaneously at dose levels of 0.5 (Group A) and 0.25 g kg body weight (Group B) once weekly. Controls (Group C) received the solvent only.

Animals were sacrificed (1 to 3 per week) between 10 and 18 weeks from the beginning of treatment (Group A) and between 10 and 26 weeks (Groups B and C). The hamsters were anesthetized by intraperitoneal injection of sodium pentobarbital (20 mg 100 g body weight). Perfusion was performed via the portal vein using 50 ml of a 10% Dextran 40 solution (in 0.09% NaCl) and via the right and left cardiac ventricles with 50 ml of a 1.5% glutaraldehyde solution (in Sorensen's buffer, pH 7.4) at a pressure of 50 cm H_2O (18-gauge hypodermic needle). The pancreas was sliced at intervals of 2 to 3 mm under a stereomicroscope, and areas of interest were selected for transmission and scanning electron microscopic (SEM) studies. After repeated washing in Sorensen's buffer, tissues were further fixed in 2.5% buffered glutaraldehyde and postfixed in buffered 1% osmium tetroxide. Tissues for SEM (two to five pieces per animal) were prepared according to the methods of Malick and Wilson[6] and examined in an ETEC Autoscan with 20 kV accelerating voltage. Remaining tissue and pancreases of animals which died during the observation period underwent routine histopathologic preparation (i.e., hematoxylin and eosin staining, paraplast embedding of graded sections).

Results

Alterations found in the pancreatic duct epithelium are summarized in Table 1. At 10 to 12 weeks the epithelial lining was focally hyperplastic, a change which was found in the intralobular and interlobular ducts. The majority of ductuli (flat to cuboidal epithelium) and ducts (cuboidal to columnar epithelium), however, appeared to remain unaltered and were identical to those of controls (Figure 1). Whereas in normal ducts the lumen is lined by unilayered epithelial cells of uniform height (Figure 2), the lumen of ducts with hyperplastic epithelium was irregularly shaped. Cell surfaces were rounded, and their centers were elevated, compared to the borders adjacent to neighboring cells, and they projected into the lumen at various levels. Each cell showed numerous small microvilli, and cell boundaries were easily distinguished. Concurrently, papillary-polypous structures were found in large ducts (Figure 3). The cells of these lesions exhibited surfaces of varying heights, shapes, and forms. They were lined by small microvilli, and the boundary to the neighboring cell was clearly visible (Figure 4). Eventually, the lumens of the original ducts were filled by epithelial growths, within which additional ducts formed. These new ducts sometimes showed cystic distensions, and the luminal epithelium was cuboidal or resembled glands in cross sections. These lesions were similar to carcinoma *in situ* or intraductal carcinoma and corresponded to the histologic diagnosis (Figure 5).

Whereas the described epithelial alterations were found in preexisting ducts, simultaneous focal duct distensions and proliferations developed into, and were adjacent to, Langerhans islets. These ducts were usually lined by cuboidal or flat epithelium. The cystic lumina were irregularly

Table 1—Number of Pancreatic Tissues Examined and Type of Alterations Found in Specimens Stained With Hematoxylin and Eosin and in Specimens Examined by Scanning Electron Microscopy

	Group A		Group B		Group C	
Weeks	H&E	SEM*	H&E	SEM*	H&E	SEM*
10–12	3(0) 3(H)	1(0) 2(H)	3(0) 1(H)	1(0) 2(H)	6(0)	3(0)
12–15	1(0) 2(H) 2(A) 2(C)	1(A) 1(C)	2(0) 1(H) 1(A)	2(0) 1(A)	6(0)	2(0)
16–18	1(0) 2(A) 4(C)	1(A) 1(C)	1(0) 3(H) 2(A) 4(C)	2(H) 2(A) 2(C)	7(0)	3(0)
19–21	0	0	1(H) 4(A) 6(C)	1(H) 2(A) 2(C)	4(0)	1(0)
22–24	0	0	1(H) 14(C)	1(H) 5(C)	3(0)	2(0)
25–26	0	0	1(0) 3(C)	1(0) 1(C)	2(0)	1(0)

0 = no diagnostic alteration; H = hyperplasia of ductal epithelium, ductal proliferations; A = ductal adenoma; C = ductal adenocarcinoma.
* Number of organs examined in addition by SEM.

shaped, and surface cells projected into the lumen to varying heights. The cell surface was densely covered with small microvilli. In cystic spaces, the surface lining cells appeared flattened. The wall was perforated by branching small ducts, which may have returned to the initial lumen, forming epithelial bridges (Figure 6). There, intrainsular and periinsular, as well as intralobular, duct proliferations frequently exhibited lumina filled with secretions, which indicated a connection to the preexisting duct system (Figure 7). Surface cells in the intralobular duct proliferations appeared flat, irregular in size and shape, and were clearly distinguishable from one another. The luminal surfaces of the cells were covered with small microvilli in varying densities and each cell had a single, centrally located, long cilium (Figure 8). At the cut surface of the specimen, only a few of these lumina were seen. However, they might have extended a considerable distance through the lobulus (adenomatous duct proliferation), but they did not replace the lobule or compress surrounding acini, thereby forming a pseudomembrane. Similar cells with a central

cilium were also seen on some cuboidal cells of ducts in untreated hamsters, as seen in Figure 2. They were not found on columnar epithelium.

Cystic papillary adenomas of pancreatic ducts were first observed during the fourteenth week after beginning treatment and were the size of a lobulus. The surrounding pancreas tissue usually appeared to be compressed (pseudomembrane). Epithelial bridges projected into the cystic lumen of the tumor; cell borders were distinct. In one type of tumor, central parts of the cell surface appeared to be more transparent than peripheral parts, and a centrally located cilium was usually prominent. The overall density of microvilli varied from cell to cell. The cut surface of the adenoma structure showed a flat epithelium, in contrast to another type of cystic papillary adenoma with cuboidal-columnar epithelium. The cell surface in these adenomas was characterized by distinct cell boundaries and elevated central portions. The cell projected into the lumen to varying degrees, and cilia were absent (Figure 9). Adenomas with mixed cell populations (ciliated and nonciliated surface cells) were frequent (Figure 10). The density and length of microvilli, as well as their distribution on the cell surface, demonstrated the differences between the two adenoma cell populations (Figure 11).

Ductal carcinomas were seen after 15 weeks and consisted of closely packed structures which appeared in cross sections as glands (adenocarcinomas) separated by a stroma of a varying width. The epithelium lining the lumen was cuboidal or columnar. Often cystic papillary foci resembling adenomas were found in the carcinoma (Figure 12) or at its periphery. In some cases, malignant and benign ductal neoplasms appeared to be separated, and in others, papillary cystic structures and glandular structures were confluent (Figure 13). The luminal cell surfaces in carcinomas were of varying shapes, projected into the lumen to different degrees, and were covered by microvilli of varying densities and lengths (Figure 14). Lumina were often filled with mucus or secretions, as well as with desquamated and inflammatory cells. Concurrent with the development of ductal adenomas and adenocarcinomas, hyperplastic changes of the ductal epithelium and ductal proliferations were seen multifocally. At later time periods, adenomas and carcinomas also became multicentric, and the whole spectrum of neoplastic and proliferative alterations could be found within a single organ.

Discussion

From routine histologic and SEM examination of BHP-induced pancreas tumors in hamsters, it appears that alterations of the ductal epithe-

lium occur progressively, since epithelial hyperplasia is found prior to neoplasia. In addition, adenomas seem to develop before carcinomas are observed, and adenomas are sometimes still present in carcinomatous areas. The delay of these events by 2 to 3 weeks in animals treated with only 50% of the initial dose also favors this interpretation. Further investigations are needed to determine whether the changes seen before tumor manifestation indicate an autonomous progression without continuous specific stimulation by the carcinogen and are true preneoplasms, or if they are related to other mechanisms and unspecific stimuli. The same is true for the transition from adenoma to carcinoma.

The cells of origin for the hyperplastic changes of pancreas duct epithelium are apparent, since these cells occur in preexisting ducts. Although cells show alterations in size, form, and shape, and cell surfaces exhibit variations in length and density of microvilli, they are similar to those found in normal ducts. Papillary duct proliferations and the lumina of newly formed ducts in intraductal carcinomas (carcinoma *in situ*) have similar cell surface changes. It remains to be determined to what extent such lesions are related to stimuli causing degeneration and regeneration or to spontaneous occurrence in old animals. They were not seen, for example, in the present controls.

A single layer of flattened epithelium is found in the cystic glandular spaces formed in lobuli within Langerhans islets, or in adjacent areas and in some cystadenomas. These lesions occur in areas without preexisting ducts or in areas where the original architecture has been destroyed or replaced. The presence of cells with a single, centrally located cilium is evidence of a derivation from intralobular ductal epithelium, since cells with a single cilium are common in the normal pancreatic ducts of hamsters. Cells with multiple cilia are occasionally observed in the pancreatic ducts of human fetuses and in ducts of humans with aberrant pancreas tissues. Adenocarcinomas with multiciliated epithelium found in man[6,7] have not been observed in these investigations. Papillary projections into the gland or cystic lumina of duct proliferations and adenomas, present in histologic cross sections, may partially correspond to the epithelial bridges seen under SEM. Mixed cell populations (ciliated flat surface cells, nonciliated cells) in adenomatous duct proliferations and ductal adenomas indicate that the neoplastic duct epithelium is apparently able to undergo a varying differentiation and maturation.

Another indication that neoplastic and possible preneoplastic epithelial alterations originate from ductal epithelium is the presence of secretions, which often fill newly formed ducts, glands, and cystic spaces. When

mucus droplets cannot be found directly ejected from the cellular surface, the presence of mucus suggests a connection between the common ductal system, proliferative, cystic and adenomatous alterations, and tumors.

Findings from this investigation indicate that pancreas tumors induced by BHP in Syrian hamsters show cellular characteristics similar to those found in pancreatic duct epithelium. The alterations are to some extent similar to those described in man. Transmission electron microscopy may supply further evidence of similarity since it provides information concerning cellular organelles and cell differentiation. In addition, transmission electron microscopy may elucidate accompanying changes of insular and acinar cells.

References

1. Silverberg E, Holleb AI: Cancer statistics, 1974—worldwide epidemiology. CA 24:2–21, 1974
2. Levin DL, Connelly RR: Cancer of the pancreas: Available epidemiologic information and its implications. Cancer 31:1231–1236, 1973
3. Wynder EL, Mabuchi K, Maruchi N, Fortner JG: Epidemiology of cancer of the pancreas. J Natl Cancer Inst 50:645–667, 1973
4. Pour P, Krüger FW, Althoff J, Cardesa A, Mohr U: Cancer of the pancreas induced in Syrian golden hamsters. Am J Pathol 76:349–358, 1974
5. Reddy JK, Svoboda DJ, Rao MS: Susceptibility of an inbred strain of guinea pigs to the induction of pancreatic adenocarcinoma by N-methyl-N-nitrosourea. J Natl Cancer Inst 52:991–993, 1974
6. Malick LE, Wilson RB: Evaluation of a modified technique for SEM examination of vertebrate specimens without evaporated metal layers. Proceedings of the Eighth Scanning Electron Microscopy Symposium, IIT Research Institute, 1975, pp 259–266
7. Sommers SC, Meissner WA: Unusual carcinomas of the pancreas. Arch Pathol 58: 101–111, 1954
8. Frantz VK: Tumors of the pancreas. Atlas of Tumor Pathology, Section VII, Fascicles 27 and 28. Washington, DC, Armed Forces Institute of Pathology, 1959

Acknowledgments

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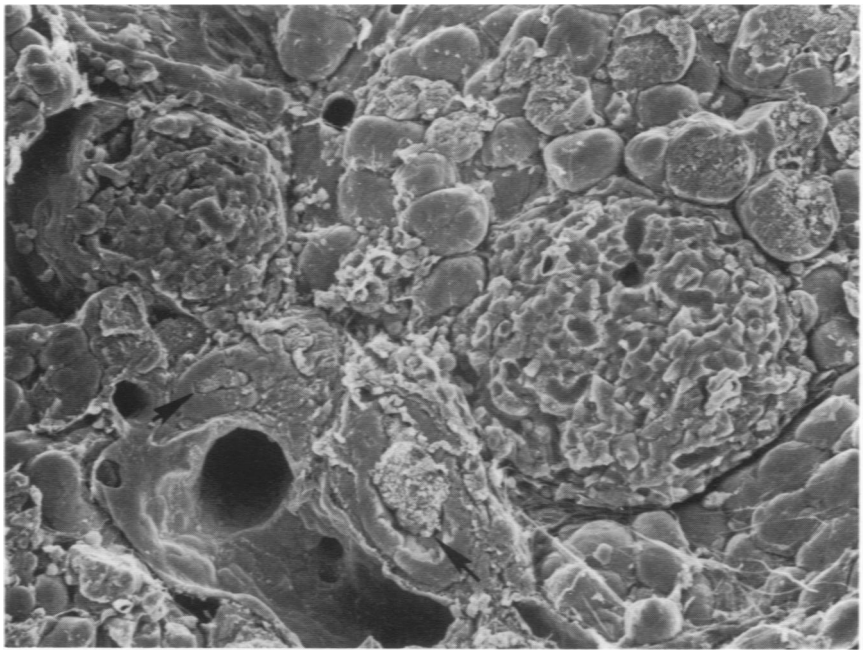

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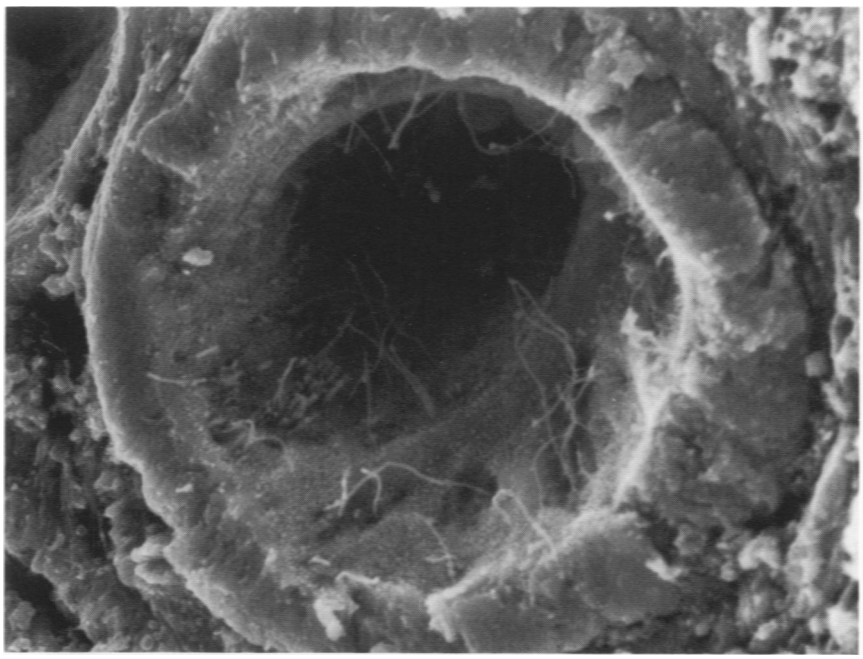

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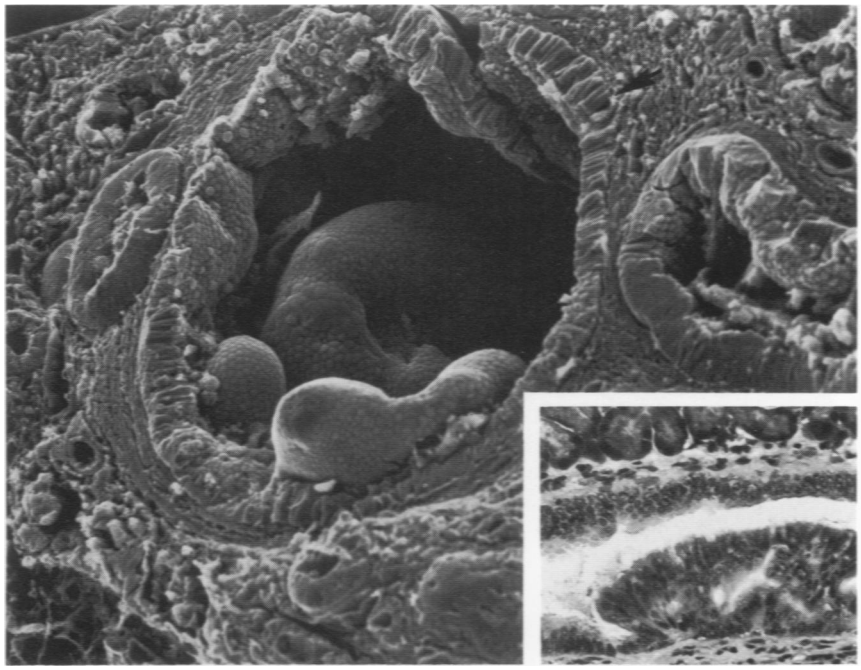
Figure 1—Normal pancreas of Syrian hamster showing two Langerhans islets surrounded by acini (cross sections in upper right corner). In lower left portion, open lumen of a vein. Between vein and islets the cross sections of medium-sized and two smaller-sized ducts filled with secretions (*arrows*). (× 200) **Figure 2**—Interlobular duct lined by flat-cuboidal epithelium showing microvilli and cilia at the surface (× 1500).

Figure 3—Epithelial papillary hyperplasia of intralobular pancreas duct. Nonhyperplastic portions show a high degree of columnar epithelium (*arrow*) (× 150). **Inset**—A similar papillary hyperplasia in a paraplast section is shown (H&E, × 100).

Figure 4—Epithelial papillary hyperplasia of pancreatic intralobular duct. Cell surfaces show varying shapes and are covered with short microvilli. Mucus droplets are visible in right upper corner (*arrow*). (× 500)



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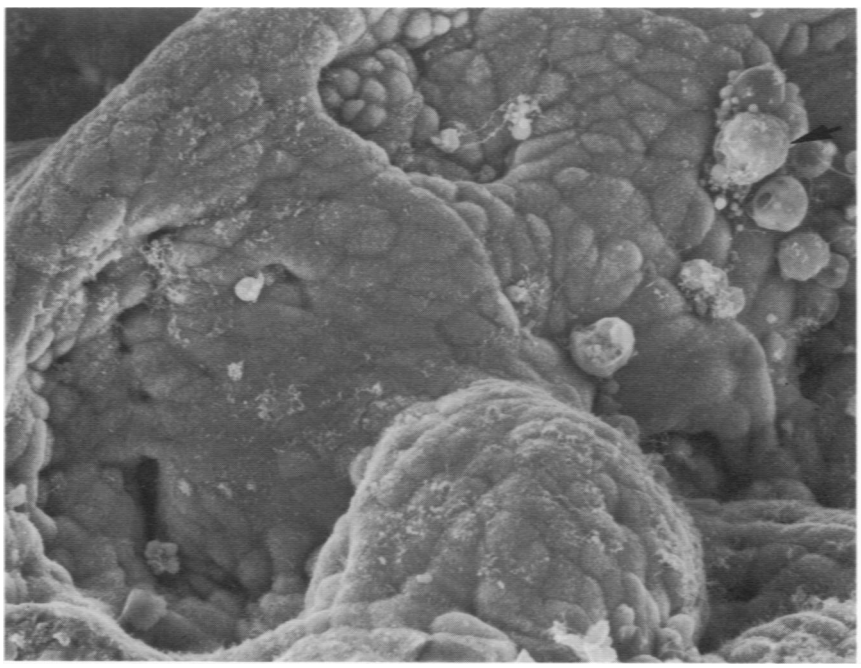

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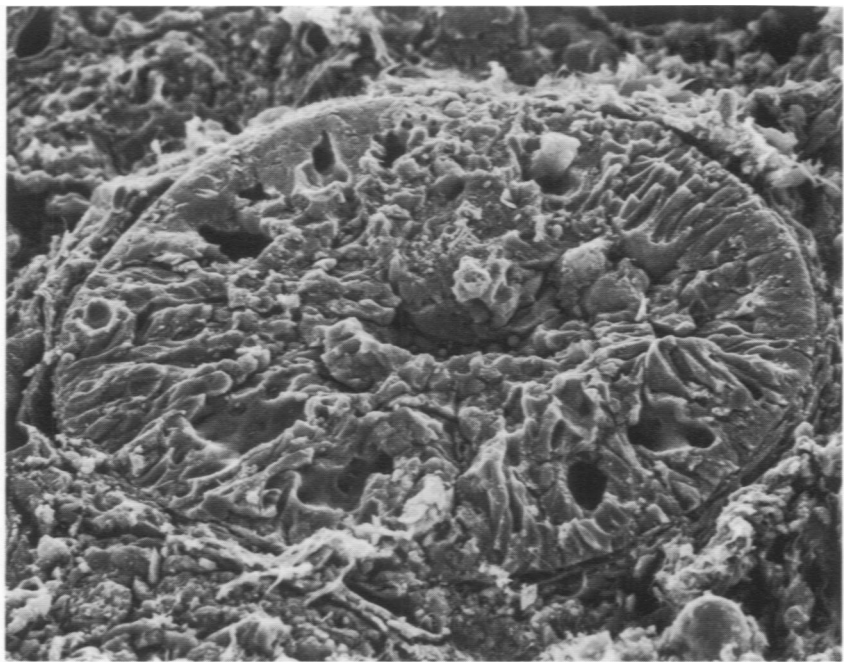


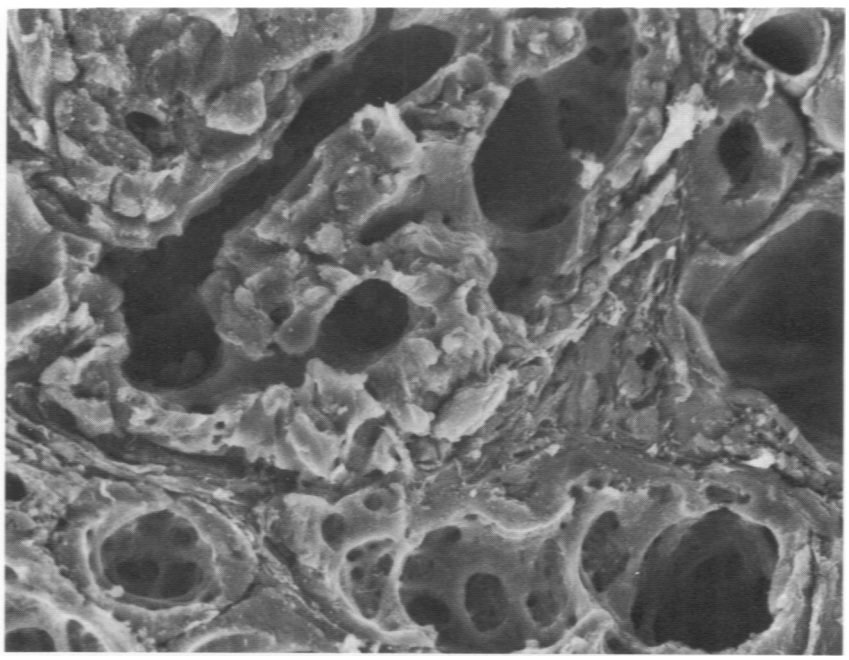
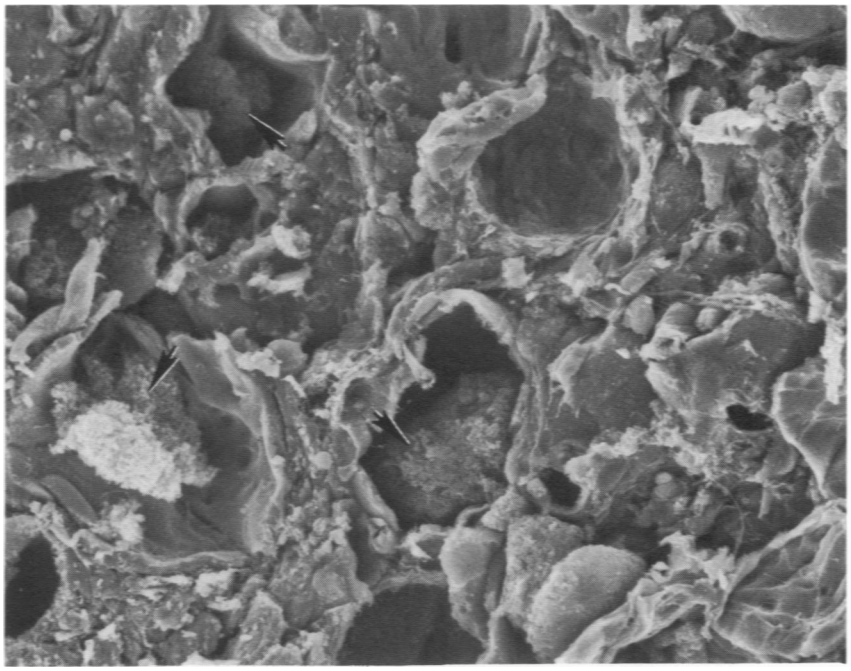


Figure 5—Carcinoma *in situ* (intraductal carcinoma) of pancreas almost entirely fills duct lumen. Gland and "duct in duct" formations. (× 300) **Figure 6**—Area of pancreas with intra- and periinsular duct proliferations. Lumina are distended, epithelial lining cells are flat. Luminal walls appear perforated by multiple small ducts resulting in formation of epithelial bridges. (× 600)



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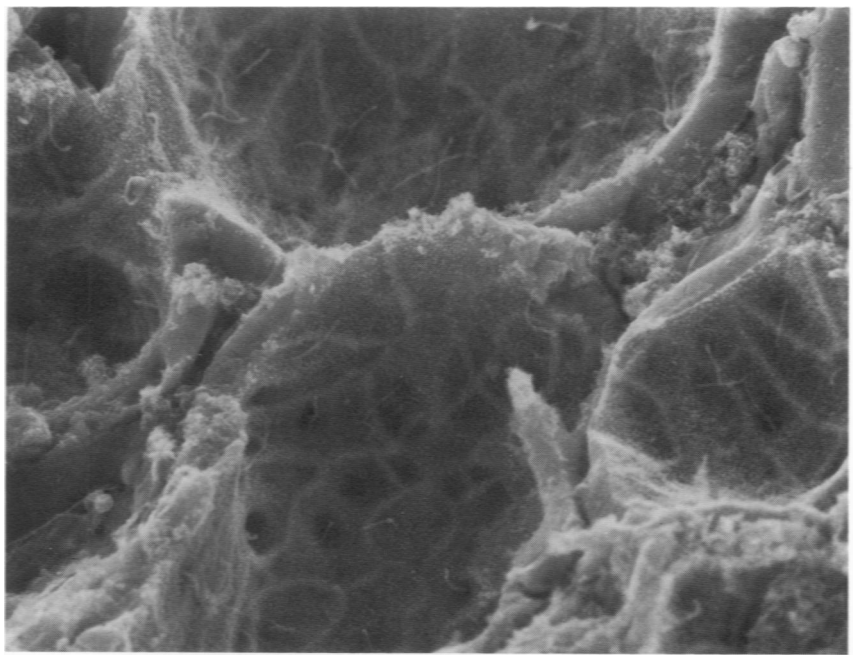

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Figure 7—Intralobular pancreatic duct proliferation. Lumina are filled with mucus or secretions (*arrows*). Epithelial lining is flat. (× 500) **Figure 8**—Intralobular (adenomatous) duct proliferation of pancreas. Lumina are lined by flat epithelial cells which show a single, generally central cilium projecting into the lumen. (× 1500)

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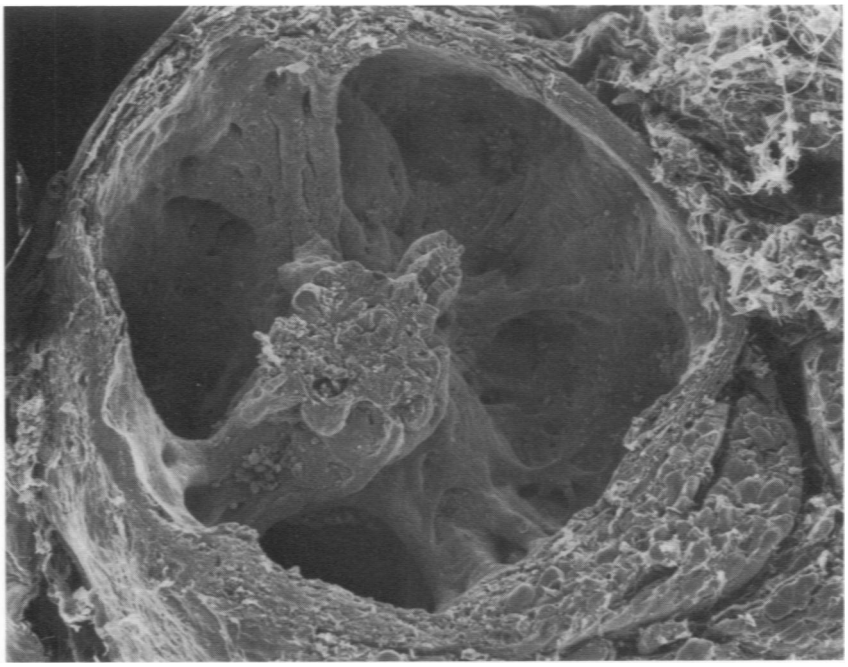

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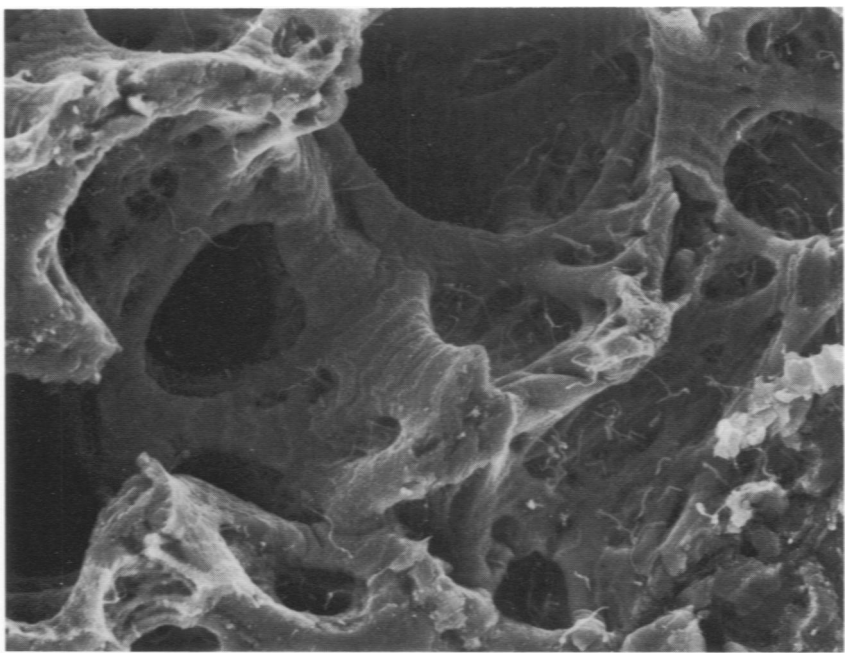
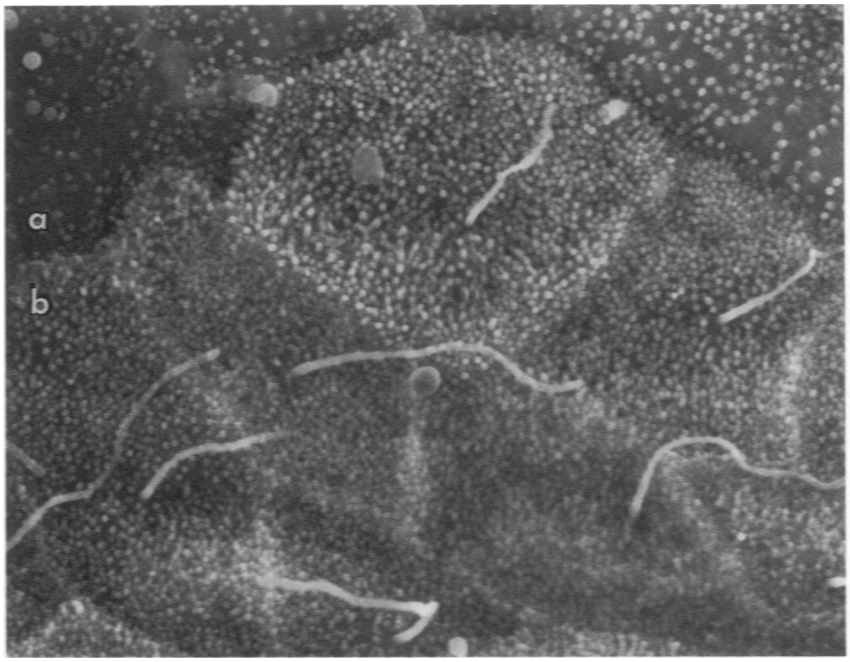


Figure 9—Large cystic papillary adenoma of pancreas. At the center, the cross section of the columnar epithelium is recognizable. Surface cells do not show cilia (× 60) **Figure 10**—Cystic papillary adenoma of pancreas possessing two cell populations (ciliated and nonciliated luminal surface cells) (× 800).



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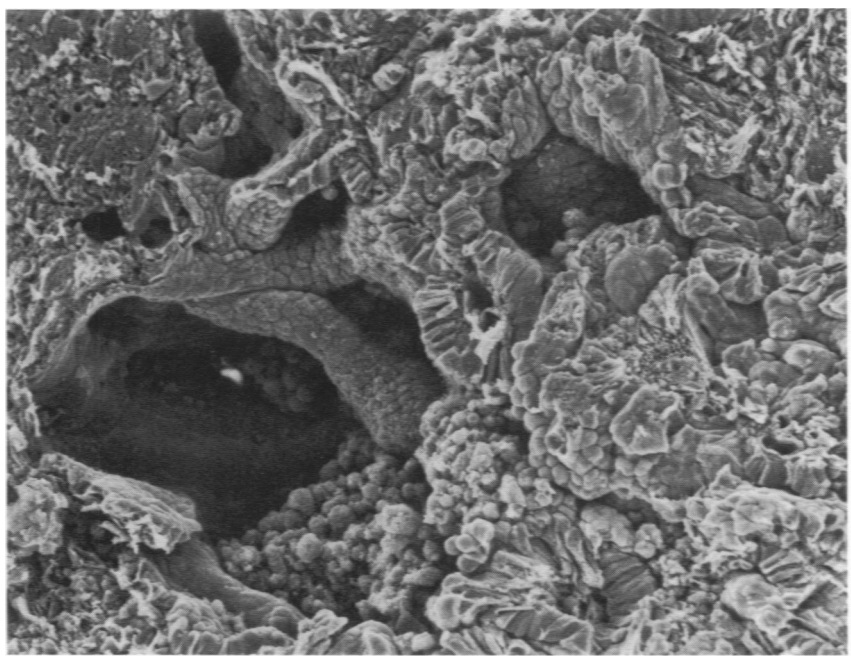

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Figure 11—Cystic papillary adenoma of pancreas showing ciliated cells (*a*) with dense microvilli at the surface. In nonciliated cells (*b*), microvilli are comparatively small and sparse. (× 5000) **Figure 12**—Ductal adenocarcinoma of pancreas with area of cystic papillary structures (× 150).

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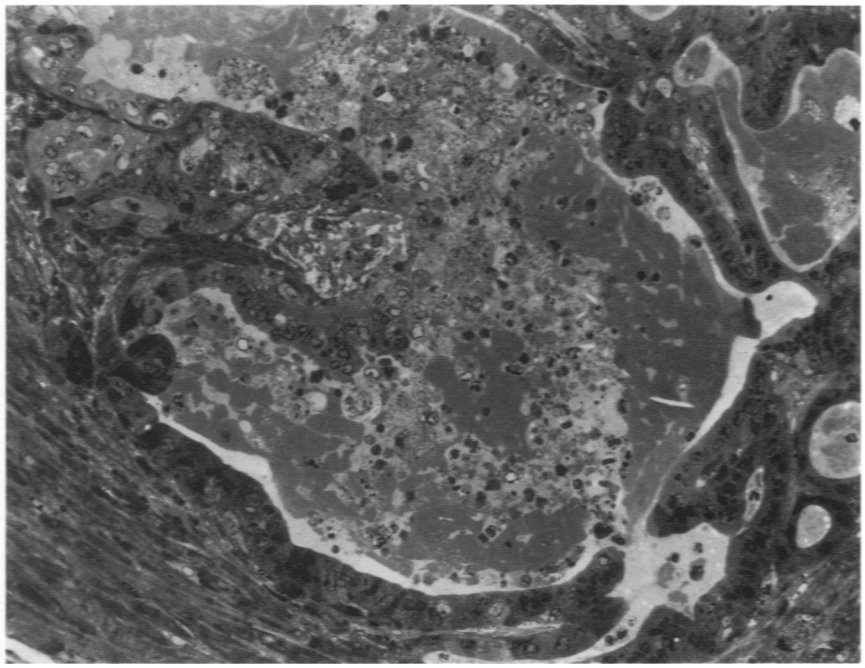

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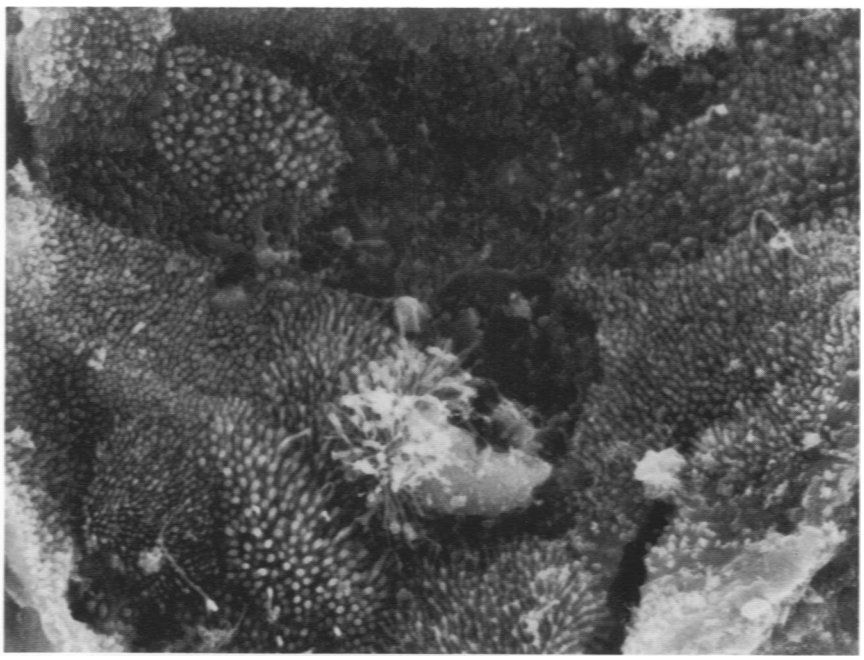

Figure 13—Area from ductal adenocarcinoma with cystic papillary structures (Toluidine blue, × 200). **Figure 14**—Surface from a cystic gland in a ductal pancreatic adenocarcinoma showing cells with microvilli of varying lengths and densities (× 2500).