Early Lesions of Pancreatic Ductal Carcinoma in the Hamster Model

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Syrian golden hamsters were treated weekly with 10 mg/kg body weight *N*-nitrosobis(2-oxopropyl)amine for life (Group 1) or 6 weeks and were sacrificed at biweekly intervals from 2 weeks (Group 1) and 8 weeks (Group 2) after initiation of the experiment. The pancreas was examined in step sections, and the sequential alterations noted for each interval were recorded. Lesions were found in intrapancreatic and extrapancreatic ducts. Equivalent alterations consisting of hyperplasia, metaplasia, atypia, and lesions characteristic of carcinoma *in situ* developed ubiquitously and simultaneously in pancreatic ducts of different sizes and in ductules, but not in acinar cells. Among the most significant findings were intrainsular ductular formations, their proliferation, and sequential malignant alteration comparable to the involved preexisting ductules. Differences between the two experimental groups were of a quantitative rather than qualitative nature. The incidence and multiplicity of neoplastic lesions at each interval according to group, sex, and anatomic locations of adenocarcinomas are outlined. Predilected areas for some lesions were found. Results indicate a common origin of all induced tumors from a pluripotent cell populating the pancreatic ductal system. (Am J Pathol 88:291–308, 1977)

THE HISTOGENESIS of exocrine pancreatic cancer in man is still subject to diverse opinions with regard to classification of these lesions. Although most neoplasms are believed to derive from ducts, no conclusion has been reached regarding other epithelial tumors.[1-6] This difficulty has been compounded by a failure to detect and examine pancreatic cancer at its early developmental stages. Since pancreatic exocrine cell tumors resembling those in man may now be experimentally induced in a high incidence,[7] the present investigation attempted to clarify the site of tumor origin by analyzing the sequence of events in pancreatic carcinogenesis.

Materials and Methods

Three groups of randomly bred, 8-week-old Syrian golden hamsters from the Eppley colony were used. They were kept under standard conditions in plastic cages in groups of 5 by sex and given Wayne pelleted diet and water *ad libitum*. Each group of 27 females and 27 males received weekly subcutaneous injections of N-nitrosobis(2-oxopropyl)amine in physiologic saline for life (Group 1) or 6 weeks (Group 2). Controls (Group 3) were given solvent only. Animals were sacrificed at 2-week intervals, from 2 (Groups 1 and 3) and 8 weeks (Group 2) after the first injection. The pancreas, with attached extrahepatic bile

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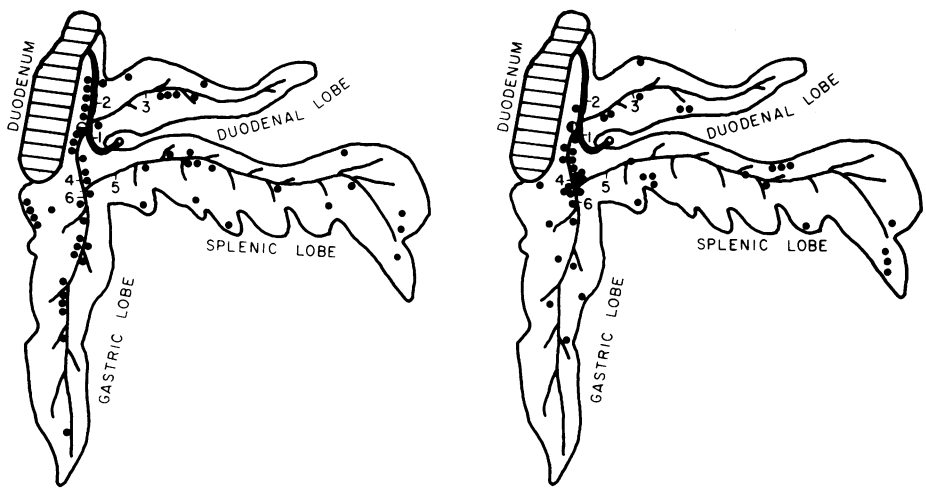
ducts, was completely fixed in 10% buffered formalin, processed for histology by conventional methods, cut into step sections (seven sections from each pancreatic segment), and stained with hematoxylin and eosin. Induced lesions from each animal were schematized on forms (Text-figure 1) for proper orientation and comparison. The following findings of number and location in available sections were recorded: hyperplasia (unilayer, multilayer, papillary, atypical), proliferation (simple, adenomatous, atypical), metaplasia, carcinoma *in situ*, and carcinoma. Using this method, these findings in 93 hamsters (51 females and 42 males) were evaluated with sufficient accuracy (Table 1). Lesions having diverse patterns within the same or proximal section were classified by the most advanced change recorded. The common bile duct was included in this study since its alteration during carcinogenesis paralleled that in the pancreatic ducts. The durations stated for the latencies are from the beginning of treatment. Differences noted among groups and sexes were mentioned.

Anatomic Considerations

Unlike that of most laboratory animals, the hamster pancreas is composed of three well-defined segments or lobes forming a λ-shaped organ. The short segment, the duodenal lobe, is located laterally to the descending duodenum and the two larger shanks posteriorly (splenic lobe) and anteriorly (gastric lobe) to the stomach. These three shanks join mediodorsally at the proximal duodenum and form the head of the organ. Accordingly, the hamster pancreas has three bodies, three tails, and one head. Each lobe usually has one main duct, except the gastric lobe, which sometimes presents two main ducts. The duodenal duct enters the common bile duct directly, whereas the gastric and splenic ducts join in the head region to form a pancreatic common duct, which opens to the common bile duct proximally to the duodenal duct (Text-figure 1). The common bile duct enters the duodenum dorsolaterally and passes a relatively long distance through the pancreas head.



TEXT-FIGURE 1—Anatomic location of pancreatic ductal adenocarcinomas in hamsters of Group 1 (**left**) and Group 2 (**right**). Figures approximate the original size of hamster pancreas. Duodenal lobe, which normally has dorsolateral position to duodenum, has been dislocated in these drawings. *1* = common bile duct, *2* = common duct, *3* = duodenal duct, *4* = common pancreatic duct, *5* = splenic duct, and *6* = gastric duct. Significant numbers of adenocarcinomas had periductal locations and only a few (Group 2) involved the main duct. Ten tumors in Group 1 (◓) and 7 of those in Group 2 (◐) developed in the angle between common pancreatic duct and common duct.

Table 1—Average Number of Pancreatic Lesions in Syrian Hamsters Induced by *N*-nitrosobis(2-oxopropyl)amine After Continuous and Limited Treatment

	No. of animals				Ductular proliferation				Adenoma				Intraductal carcinoma				Ductular carcinoma *in situ*				Adenocarcinoma			
	G1		G2		G1		G2		G1		G2		G1		G2		G1		G2		G1		G2	
Week	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
6	3	3	0	0	0	0	0	0	0	0	—	—	0	0	0	0	0	0.3	0	0	0	0	0	0
8	3	0	3	5	13	—	7	2	0	—	0	—	0	0	2	0	0	0	1	0	0	0	0	0
10	3	4	4	6	11	9	11	6	0	0	0	0.3	2	3	1	1	0.3	1	0.3	0.3	1	0	0.3	0.2
12	1	6	4	6	49	17	29	14	1	1	0	0.3	1	0.3	1	2	6	1	1	1	1	0.3	0.3	1
14	2	3	3	3	25	9	53	18	0	1	1	0.3	3	0.3	3	1	6	1	1	1	3	0.3	1	0.3
16	3	1	2	4	41	28	86	40	1	3	2	1	4	0	2	2	2	0	3	4	4	0	3	1
18	2	0	2	1	49	0	61	57	2	0	1	0	11	0	2	0	8	0	3	2	13*	0	2	0
20	1	0	3	0	14	0	65	0	2	0	5	0	13	0	5	—	19	0	2	0	14	0	2	0
22	—	—	3	0	—	—	79	0	—	—	4		—	—	3	—	—	—	3	—	—	—	3	—
24	—	—	3	0	—	—	150	0	—	—	15		—	—	4	—	—	—	4	—	—	—	1*	—
26	—	—	3	0	—	—	54	0	—	—	4		—	—	2	—	—	—	3	—	—	—	2	—
28	—	—	3	0	—	—	76	0	—	—	8		—	—	3	—	—	—	5	—	—	—	2	—

G1 = Continuous treament (Group 1) and G2 = limited treatment (Group 2).
* One periampullary adenocarcinoma.

Because of its anatomic location and curved course, often only a small segment(s) of this duct can be obtained in histologic sections and therefore could mistakenly be interpreted as the pancreatic duct. Step sections are essential for appropriate orientation. The short segment of the common bile duct between the openings of the pancreatic ducts and duodenum is termed the common duct. The detailed topographic and anatomic patterns of the hamster pancreas will be described elsewhere. Each main pancreatic duct collects secretions from the secondary ducts, which further subdivide into interlobular and intralobular ducts (ductules). Histologically, the primary and secondary ducts are covered by flat epithelial cells, in which spindle-shaped nuclei are arranged in the direction of flow. Ductular cells show relatively larger nuclei and no defined cytoplasm (Figure 1).

Results

Alterations during carcinogenesis affected the common duct, pancreatic duct and ductules, and to some extent, the islets. Although these changes occurred almost simultaneously in each animal, they will be described individually below.

Common Duct

In many cases, alterations in the common duct were the first and earliest lesions which could be readily identified. All stages of tumor development from hypertrophy, hyperplasia, and atypia to benign and malignant neoplasia could be followed in this duct in a remarkably consequent order. As early as 2 weeks from the beginning of treatment, focal hypertrophy and hyperplasia (unilayer) of the epithelium were observed, often with goblet cell metaplasia. Later the alterations progressed to larger segments of the duct and often encroached on peribiliary pancreatic ducts (Figure 2). Although marked activity of basal cell layers was present, mitotic figures were rare; they were commonly found at the stage of stratification which began as early as 2 weeks from treatment initiation, usually in small areas at the mid-portion of the duct, and extended, during the next 8 weeks, throughout the entire length of the common duct. However, it was sharply demarcated from the adjacent common bile duct segment. Dilation and tortuosity of the common duct, which often accompanied hyperplasia, resulted in a pseudopapillary pattern of epithelium. Due to later true papillary proliferation (at 12 weeks), pseudoadenomatous structures were often obtained in some histologic sections. The stage of papillary hyperplasia (Figure 3) was present in all animals of both groups from 12 weeks on, until termination of the experiment. Benign and malignant tumors were found in 4 hamsters only and consisted of 1 intraductal papilloma (Figure 4), 1 cystadenoma, and 2 periampullary adenocarcinomas, one of which had partially invaded the duodenum. Both carcinomas (contributing to 1.7% of the total malignant neoplasms) showed focal papillary structures comparable to intraductal

papillomas (Figure 5), and communication of the common duct with the duodenum was retained in both.

Pancreatic Ducts

Large Ducts (Main Ducts)

Hypertrophy and unilayer hyperplasia of ductal epithelium were sometimes found concomitantly with alterations of the common duct; however, they usually occurred 2 weeks later. These changes developed locally and initially, often in the common pancreatic ducts and also in the adjoining gastric and splenic ducts. The markedly enlarged and cylindrical epithelial cells took an upright (standing up) position; the often accompanying tortuousity resulted in a focal pseudobridging or narrowing of lumen. The circumscribed piling up of the epithelium (Figure 6), with occasional goblet cell metaplasia, sometimes developed as early as 6 weeks in the same ducts and was often associated with acute inflammation or periductal fibrosis. In later weeks the alteration affected larger segments of the ducts, often in ladder-form fashion (by sparing intervening areas), and encroached on the merging ducts (Figures 7 and 8). The nuclei of hyperplastic epithelium were closely packed, elongated, and hyperchromatic. However, mitoses were few at this stage; they appeared gradually by increasing atypia. Irregular arrangement and stratification of the otherwise uniform cells—glands-within-the-gland formations, budding, and villiform cell proliferation lacking a connective tissue stalk, each characteristic of carcinoma *in situ* or intraductal carcinoma (Figures 9 and 10)—developed around 10 weeks (in one Group 2 female, as early as 8 weeks). These lesions appeared focally or multifocally in different ducts (but primarily in the common pancreatic, gastric, and splenic ducts), in several segments of the same duct, or affected a sizeable length of a duct, thereby extending also into the merging ducts (Figure 9). Loss of nuclear polarity, cell pleomorphism, atypical glandular structures, overt mucus production with occasional rupture of a gland, and a remarkable increase in the mitotic rate were followed by invasion, which occurred at around 12 weeks. However, uniform cell patterns, such as those in cases of carcinoma *in situ*, were often found in the vicinity of adenocarcinomas.

Small Ducts (Secondary Ducts)

Lesions in these ducts resembled those described for large ducts with regard to multiplicity, extent, and degree of the sequential hyperplasia, dysplasia, and atypia (Figures 11 and 12). However, there was no preferred pancreatic segment for small duct involvement, which appeared

concomitantly with or after equivalent changes in larger ducts. At an advanced stage of involvement, the similarity in the neoplastic response of the different-sized ducts made it extremely difficult to determine their actual origin, especially when one lesion overlapped or coalesced with another. However, papillary and cystic-papillary proliferation seemed a preferred alteration of large ducts.

Ductules (Interlobular and Intralobular Ducts)

Multifocal hypertrophy and unilayer hyperplasia of ductules occurred as early as 2 weeks into the experiment. Their further alteration differed from that of ducts by virtue of proliferation (multiplication), which was the most significant morphologic alteration of the pancreas during carcinogenesis and appeared as early as 6 weeks. They developed simultaneously in several areas, predominantly around the ducts, and did not show any predilected pancreas segment. In earlier phases, the proliferated and occasionally distended ductules were lined by a single layer of endothelial-like cells and contained light eosinophilic or inspissated mucus (Figure 13). Their size and numbers later increased (up to 150 per animal) and formed adenomatous patterns (as early as 8 weeks) or adenomas (at 10 weeks) by occupying a larger area (or the entire space) of lobules (or lobes), respectively (Figure 14). This process occurred either by continuous reduplication or coalescence of several neighboring lesions. Ductular proliferation could be observed during the entire carcinogenic process in most tissues, which were not altered by atrophy or tumor invasion. Whereas most proliferated ductules retained their benign uniform appearance, in some changes developed that were comparable to those in ducts as early as 8 weeks in the individual ductules (Figure 15), but more frequently simultaneously in all participating ductules of a lesion. With increasing atypia, they tended to coalesce with neighboring lesions (Figure 16). Isolated foci formed a pseudocapsule or true capsule of varying thicknesses, which was often encircled by a lymphocytic wall. Such foci occasionally reached a lobular or lobar size; focal invasion of the capsule or fusion with smaller adjacent lesions were frequently found in step sections, as early as 10 weeks. Many of these carcinomas developed in close proximity to ducts, which in turn showed advanced alterations.

Islet

Alterations affecting the islet cells included hyperplasia, which formed a cell aggregrate exceeding the size of regular islets by a factor of four or more. However, islet cell hyperplasia of a lesser extent and frequency was usually also present in control animals. One of the most consistent and

routine alterations during pancreatic carcinogenesis was ductular formation within islets or (more frequently) in the periphery of normal-appearing islets, as early as 8 weeks. These intrainsular and/or periinsular ductules (Figure 17) initially developed as narrow channels, which were lined by endothelial-like cells, and which showed, in appropriate sections, communication to intralobular ductules (Figure 17). In most animals, formation of these ductules preceded the above-described ductular proliferation and seemed to be an impetus for the morphologic neoplastic process. By a means similar to that of interlobular and intralobular ducts, these new ductules began to proliferate rapidly, resulting in adenomatous and cystadenomatous patterns (Figure 18) as early as 10 weeks and especially in Group 2 animals. In advanced cases, the intrainsular origin of the proliferated ductules could be ascertained only in one of the step sections. The cystic, dilated ductules were filled with mucus, cell debris, and occasionally by erythrocytes and were lined with flat epithelial cells. As the size and number of intrainsular ductules increased, the islets gradually atrophied; their remnants could be detected within the thin connective tissue septa projecting into or crossing the lumen (Figure 18), giving rise to multilocular adenomatous patterns. Communication of cystic spaces to the collecting ductules of a usually normal caliber could often be found in step sections. Some of these intrainsular lesions seemed to represent a stationary process, especially in Group 2 animals; in other instances cellular hyperplasia, metaplasia, and atypia developed as early as 10 weeks and were comparable to those observed in small ducts and proliferated ductules (Figures 19–21). Fibrous capsules around the affected islets, some of considerable size, were frequently found. Lesions consistent with carcinoma *in situ* occasionally occurred at 10 weeks, but usually 2 weeks later. In such lesions and in many invasive tumors with similar patterns (Figure 22), which developed around this time, foci of islet cells could still be seen. Ductular patterns were mostly retained in carcinomas; however, some showed partially papillary or cystic papillary patterns, often indistinguishable from comparable ductal neoplasms.

Table 1 summarizes the average numbers of induced lesions in each interval by group and sex. In the final stage, the organ was nearly completely occupied by large numbers of lesions (e.g., at 24 weeks, a Group 2 female had over 200 foci of proliferated ductules—41 showing adenomatous patterns; 8, additional hyperplasia; and 12, atypia of the epithelium; in addition, there were 11 ductular adenomas, 5 intraductal carcinomas, 6 ductular carcinomas *in situ*, and 2 adenocarcinomas). Although the total number of malignant lesions increased at intervals, a direct dose relationship could not be established (Table 1). Group 1

animals displayed more malignant lesions than did Group 2 animals, which had mainly benign lesions. There were also differences in the neoplastic response of individual animals with regard to onset, multiplicity, and degree of alteration, but not with regard to target cells. Females seemed to show a greater response than males, most of which died during the first 16 weeks of the experiment. Text-figure 1 demonstrates the anatomic locations of pancreatic adenocarcinomas. Among 64 carcinomas in the first group and 52 in the second, 38 and 44% were in the head, 25 and 29% in the splenic lobe, 28 and 15% in the gastric lobe, and 9 and 12% in the duodenal lobe. Of these, 77% in Group 1 and 60% in Group 2 developed in close proximity to the main ducts. In the head region, the angle between the common pancreatic duct and common duct and between the splenic and gastric ducts was the preferential site of carcinomas (Text-figure 1). Tumor size varied from 2 to 5 mm in both groups and on the average was larger in Group 1 hamsters. Ductal and ductular lesions often developed in close proximity and coalesced, frequently causing a remarkably mixed cellular pattern. Therefore, the duct or ductule origin of most adenocarcinomas could not be determined, except in the case of tumors of a common duct which showed a distinct papillary pattern.

Alteration of acinar cells was generally of a degenerative nature, as a result of luminal obstruction of the ductules or ducts and compression by neoplasms. Lobular, lobar, or segmental atrophy was seen in peripheral portions of tumors. Often acinar cells were replaced by proliferating ductules or metaplastic cells, such as goblet and mucin-producing cells. Alterations consistent with the neoplastic process were not encountered.

Discussion

Under these experimental conditions, all pancreatic neoplasms were found to have their origin in ductal systems. As indicated in tumors of other organs, hyperplasia and dysplasia preceded neoplasia; however, malignant pancreatic lesions seemed to also develop *per se*.

Although the neoplastic reaction seemed to be shared similarly among ducts of various sizes, certain segments of large pancreatic ducts showed a relatively earlier response, since the first morphologic alteration developed primarily in collecting ducts (common pancreatic and the adjacent gastric and splenic ducts) near the head and in the peribiliary ducts. A relatively higher cell turnover rate, longer contact with pancreatic juice–borne carcinogen (the concentration of which is expected to be higher in collecting ducts), and the bile reflux mechanism are all hypothetical factors which should be investigated. Nevertheless, once initiated,

the ductal alteration represented a progressive process following a remarkable pattern of spreading; the hyperplastic epithelium seemed to creep along the ducts, but often jumped over and involved a considerable length of a duct. This peculiar behavior was retained in the malignant counterpart, the intraductal carcinoma, which filled the lumen before invasion.

Alteration of small ducts did not show preference for any area, except for a frequent secondary involvement by alteration of the large ducts. The morphologic patterns of the sequential neoplastic processes in these small ducts mimicked those of large ducts, an observation which could account for the mutual cell origin of their neoplasms. The neoplastic response of ductules differed from that of ducts in terms of their initial multiplication with subsequent formation of either adenomas or adenocarcinomas; comparison of data from the two experimental groups indicates that the direction for differentiation toward either pattern seems to be dictated by carcinogenic dose. The ubiquitous ductular proliferation in the early phase of the neoplastic process and their absence or sparsity in atrophic pancreatic areas due to ductal obstruction at the more advanced stages may point to their partial regressive nature.

The most interesting and significant finding in this study was the intrainsular ductular formation, their proliferation, and a sequential malignant alteration comparable to other affected ductules. This alteration, which was also observed in one of our previous studies with a related pancreatic carcinogen,[9] is apparently not specific for Syrian hamsters and has been found in other animals under specific experimental conditions.[10-13] According to some reports, similar phenomena may occur in humans, especially in diabetic patients, as well as in the vicinity of or within pancreatic tumors.[4,6,14-18] All these findings indicate a definite relationship between islet and ductal cells, which is not surprising in view of their common origin from a totipotent cell.

It cannot be determined at present whether or not the mutual carcinogenic response of ductal and ductular cells is general or depends on the nature and dose of the causative agents or on other factors. The amount of carcinogen applied in this experiment may have overwhelmed the threshold dose for a specific response. It would be instructive from a conceptual viewpoint to gain information on a possible relationship between dose and the most responsive target cells.

Although specific features of adenocarcinomas arising from ducts and ductules could not be determined, the number of induced carcinomas was low and the time lapse too short to allow such a conclusion. Nevertheless, it was interesting to see that the rate and tendency toward malignancy

differed among intrapancreatic and extrapancreatic ducts. Proliferation of the common duct epithelium, although appearing as the earliest lesion, remained stationary in many animals of this study and showed, in contrast to pancreatic ducts, only a slight trend toward neoplasia. The experimental parameters, however, could have a bearing on these findings. Remarkably, all carcinomas originating from the common duct were confined to the most distal (periampullary) segment. At an advanced stage, such neoplasms may simulate pancreatic cancer and lead to an unrealistically high tumor incidence in the pancreas head, as apparently is the case in humans.[8] The present data, along with the specific biologic behavior of these induced neoplasms which is also comparable to those in man,[7,19] clearly indicate the significant advantages of the hamster model over other models of pancreatic tumor induction [20-24] for investigating various aspects of pancreatic carcinogenesis.

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Legends for Figures

Figure 1—Normal histologic pattern of hamster pancreas. Main pancreatic duct is lined by flat epithelial cells with spindle-form nuclei having moderate chromatin content. Secondary ducts (*left, upper corner*) usually show same cellular pattern and can be distinguished from large ducts by smaller lumen and thinner layer of periductal connective tissue. Interlobular and intralobular ducts (ductules) have relatively long, stretched, and vesicular nuclei, the size of which may exceed that of ducts by factors of two and three (*upper* and *lower right*). Only a delicate connective tissue can be seen around interlobular ducts. Indistinct cell cytoplasm is lightly eosinophilic. Nuclei of terminal ductules (*lower middle*) are smaller and often cone-shaped. (H&E, × 100)

Figures 2–5 show common duct alterations during carcinogenesis with *N*-nitrosobis (2-oxopropyl) amine in hamsters. All were stained with hematoxylin and eosin.

Figure 2—Hypertrophy and focal hyperplasia of epithelium (*right*) with scattered goblet cell metaplasia extend into peribiliary pancreatic duct. Here altered epithelium has apparently involved a duct of secondary nature; such ducts occur in the head of the pancreas in large numbers and open directly into the common duct. In advanced cases, alteration extends along interlobular ducts (*middle*), and distinction between large and small ducts cannot be made. Periductal fibrosis and plasmolymphocellular reaction are found equally around all involved ducts. Many activated basal cells with characteristic perinuclear halo are seen at this stage, but no mitoses are encountered. Atrophy of acini, apparently due to luminal obstruction, and replacement by ductular cells are seen in the *lower middle*. Female, 8 weeks, Group 2. (× 50)

Figure 3—Distention, papillary proliferation, and goblet cell metaplasia (intestinalization) with pseudoadenomatous pattern of the common duct (*left*) and the common pancreatic duct (*right*). Ramification, excess mucus production, and subsequent obliteration of communicating branch may result in formation of peribiliary mucous lake (*lower right*). The angle between common duct and common pancreatic duct is usually a predilected area for carcinomas of pancreatic origin. Female, 10 weeks, Group 1. (× 50) **Inset**—High magnification of same lesion showing stratification, cell pleomorphism, and mitotic figures. The alteration has somewhat mimicked intestinal villi. This intestinalization is confined to common duct, whereas common bile duct and duodenal epithelium are unchanged. (× 100)

Figure 4—Intraductal papillary polyp in midportion of common duct. There is a thick layer of periductal connective tissue and partial atrophy of adjacent pancreatic tissue. Female, 26 weeks, Group 2. (× 40) **Inset**—Higher magnification of same tumor showing uniform cellular pattern and fibrous connective tissue stalks. Numerous activated basal cells and only a few mitotic figures are found. (× 100)

Figure 5—A periampullary adenocarcinoma, with basic structure mimicking that of intraductal papillary polyp (see Figure 4). Upper portion of photo corresponds to lumen of duct, in which connection to duodenum is retained. In this section, invasion of original periductal fibrous capsule is seen. The actual tumor mass (4 mm in diameter) is located below this lesion and has invaded duodenal wall. Note focal mineralization of dense connective stroma (*lower left*) and lymphocellular infiltrate (*right lower*). Female, 24 weeks, Group 2. (× 65)

Figures 6-10 show alteration of pancreatic large duct during carcinogenesis with *N*-nitrosobis(2-oxopropyl)amine (all stained with hematoxylin and eosin).

Figure 6—Circumscribed hypertrophy and hyperplasia of epithelium in splenic duct. Nuclei are elongated and closely packed. Basal cell activities are seen. Adjacent acini have been replaced by ductal cells (*upper right* and *left*). A few mitoses can be seen at this stage (*upper right*). Female, 10 weeks, Group 1. (× 65)

Figures 7 and 8—These two sections derive from same common pancreatic duct but are taken about one-half millimeter apart from one another. Cellular hyperplasia with piling up is circumscribed in **7**, whereas in **8** it involves the entire circumference of lumen. Such a ladder-form distribution pattern of altered epithelium is a frequent finding. Compared to Figure 6, there are irregularities in cell arrangements and a marked hyperchromasia of crowded nuclei. Mitosis could often be seen at this stage. Involvement of a secondary duct and replacement of acini by regular ductular cells (Figure 7, *right middle*) are seen. Male, 16 weeks, Group 1. (× 65)

Figure 9—Extended villiform or filament-like hyperplasia of gastric duct epithelium in region between body and head, affecting also several secondary ducts; alteration seems to encroach along dividing interlobular ducts (*upper right*). Periductal fibrosis and scattered round cell infiltration are seen. Female, 14 weeks, Group 1. (× 25) **Inset**—Higher magnification of intraductal lesion showing filamentous cell proliferation lacking connective tissue stalk, characteristic of one type of intraductal carcinoma. Focally overcrowded cells have usually uniform nuclei, but with varying chromatin content. Mitotic figures were present. (× 65)

Figure 10—Another, more common type of intraductal carcinoma showing predominantly glands-within-a-gland structure. Such a pattern can be occasionally retained in adenocarcinomas deriving from these lesions. This is a section from common pancreatic duct. Despite partial luminal obstruction, marked alterations in peripheric pancreatic segments did not occur in many intraductal lesions. Female, 20 weeks, Group 2. (× 65)

Figures 11-14 show alteration of small pancreatic ducts and ductules during carcinogenesis with *N*-nitrosobis(2-oxopropyl)amine (all stained with hematoxylin and eosin).

Figure 11—Hypertrophy and circumscribed goblet cell metaplasia of secondary duct in body of gastric lobe. Upright position of cells, focal crowding, and pleomorphism of nuclei are present. Note variation in luminal caliber and the increased amount of periductal connective tissue and delicate inflammatory reaction. Male, 12 weeks, Group 2. (× 65)

Figure 12—Alteration of a secondary duct, which has attained the size of large duct. Marked cellular hyperplasia, pleomorphism, atypia, glands-within-a-gland formation, and numerous mitotic figures. At this carcinoma *in situ* stage, inflammatory reaction around and within altered duct is frequent. This lesion was found far from the large duct in splenic lobe of a female, 24 weeks, Group 2. (× 65)

Figure 13—Ductular proliferation and mild luminal distension. Cellular patterns are uniform, some stretched and endothelial-like. Proliferated ductular cells attenuate into acini (*upper left*) leading to replacement and gradual enlargement of lesion. Mild inflammatory reaction is present. Female, 8 weeks. (× 65)

Figure 14—Adenoma composed of ductules of various calibers. Some ductules show ramification and pseudopapillary formation. Epithelial lining is flat and uniform. An islet is enclosed in this lesion (*upper middle*). The size of this adenoma exceeds that of the pancreatic lobule. Female, 18 weeks, Group 2. (× 25)

Figure 15—Ductular proliferation with focal atypical pattern. Cellular pleomorphism, loss of cell polarity, goblet cell metaplasia, and focal enlarged nucleoli are present. Cell cytoplasm is pink. Such lesions seem to extend by affecting adjacent tissue (*upper* and *lower left*). A few mitoses can be seen at this stage of hyperplasia. Female, 20 weeks, Group 2. (× 65)

Figure 16—Isolated and encapsulated lesion composed of irregular ductular structures, at this stage representing carcinoma *in situ*. Mitotic figures are encountered; note the peripheral lymphocytic reaction. Female, 18 weeks, Group 1. (× 65)

Figure 17—Slit-form ductules within one islet, the connection of which to merging ductule can be demonstrated in other sections. Ductular cells are hypertrophic and show focal upright position, and the caliber of lumen varies. There is an increased amount of periductular fibrotic tissue. Male, 12 weeks, Group 2. (× 65)

Figure 18—Proliferation and cystic distension of intrainsular and extrainsular ductules. Gradual atrophy of involved islets may be visualized in this figure. The final stage of islet cell atrophy is the formation of trabeculae extending into cystic spaces. Cystic adenomatous patterns are the result of such an alteration. The presence of erythrocytes in ductular lumen is probably due to rupture of insular vessels. Female, 18 weeks, Group 2. (× 25)

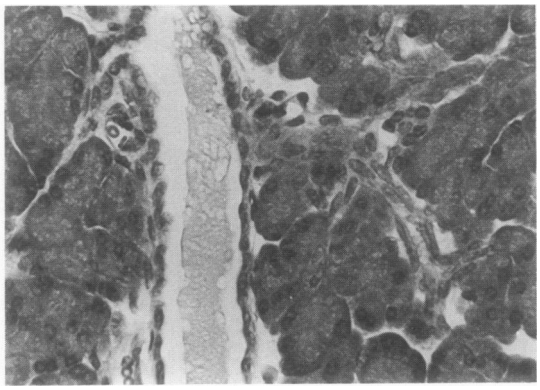
Figures 19-22 show intrainsular ductular formation and their proliferation during pancreatic carcinogenesis by *N*-nitrosobis(2-oxopropyl)amine (hematoxylin and eosin staining).

Figure 19—Hypertrophy and focal hyperplasia of a ductule which extends into and branches within an islet. Focal dilation of intrainsular segment of ductule seemed to result from unilayer hyperplasia and excess cellular secretion rather than mechanical cause (for example, by peripheral ductular obliteration). Mitotic figures are seen in hyperplastic areas. Note dense amorphous material around islet, which borders another islet (*upper right*). Pale, eosinophilic, amorphous material is found frequently at this stage, around or within some islets as well as in adenocarcinomas deriving from such lesions. Male, 14 weeks, Group 1. (× 65)

Figure 20—Hypertrophy and hyperplasia of intrainsular and extrainsular ductules; the similar cellular patterns are indicative of their intimate relationship. Mitotic figures are increased at this stage. Note the beginning of papillary formation in extrainsular ductule, which is now indistinguishable from equivalently changed duct; note thickened periinsular connective tissue membrane. Male, 16 weeks, Group 1. (× 65)

Figure 21—Pleomorphic cellular patterns of intrainsular ductules. Stratification, goblet cell metaplasia, and few mitoses are seen. Adenocarcinomas deriving from these lesions can hardly be distinguished from primary ductal neoplasms by absence of islet cell component. (× 65)

Figure 22—Small adenocarcinoma (in body of duodenal lobe) located near main duct (*left midportion*), as in majority of adenocarcinomas. This tumor has invaded the surrounding capsule. Islets can be readily seen at this magnification at edge of lesion. The similar patterns of proliferated ductules and hyperplastic main duct are striking and account for the difficulty in distinguishing between ductal and ductular adenocarcinomas at advanced stages. The presence of islets between tumor cells favors ductular origin. Female, 18 weeks, Group 1. (× 25)



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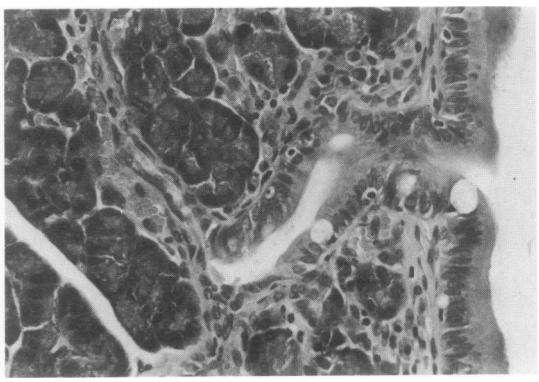

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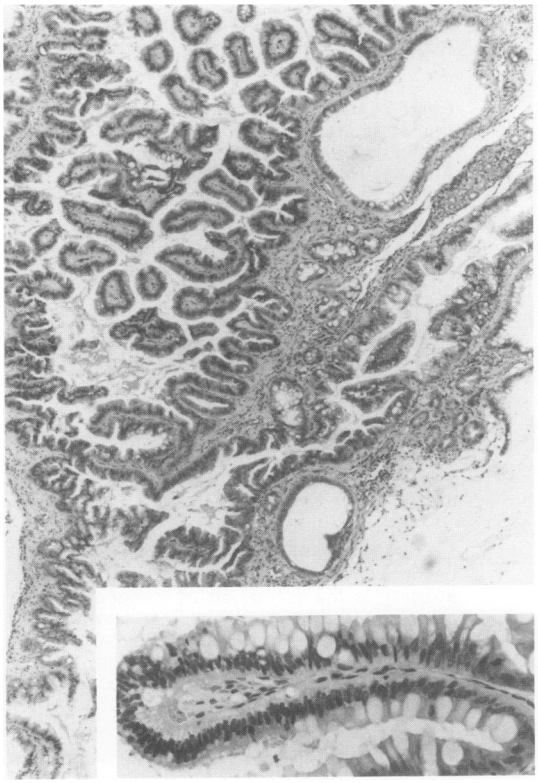

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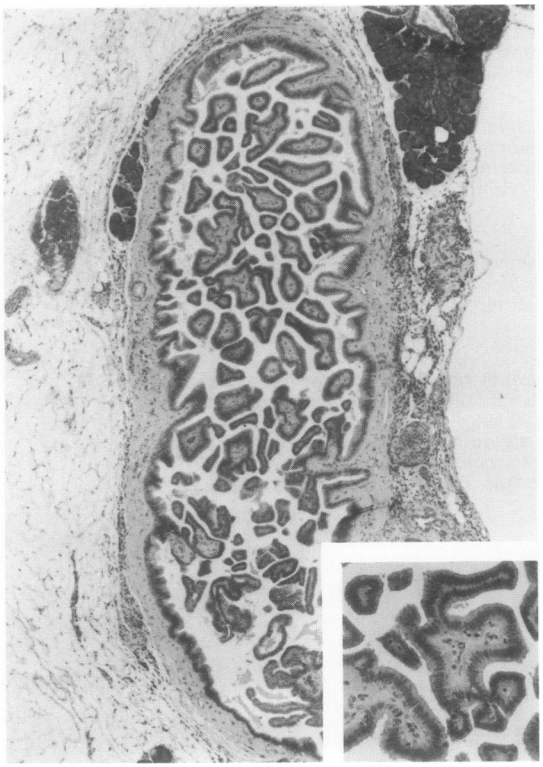

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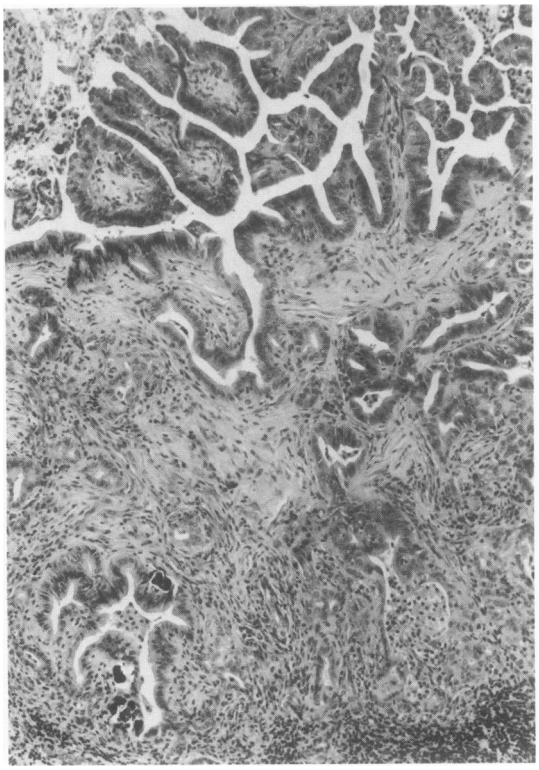

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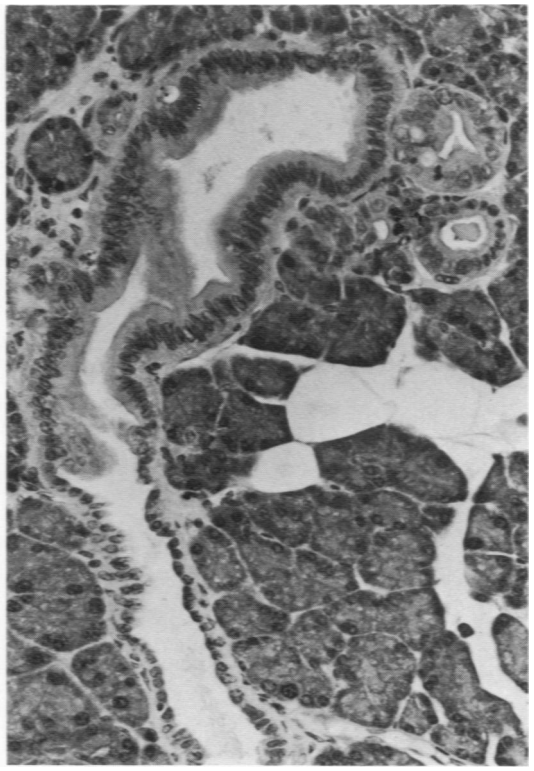

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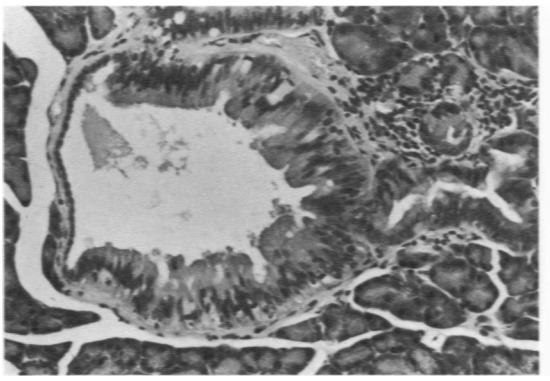

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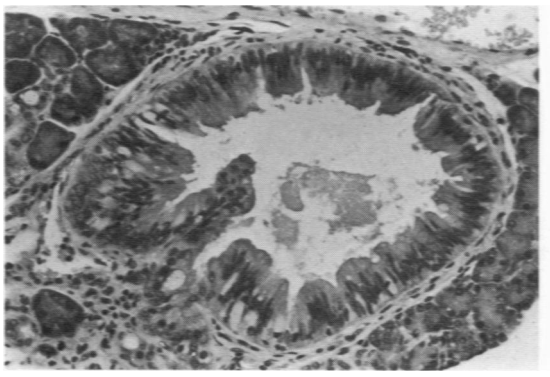

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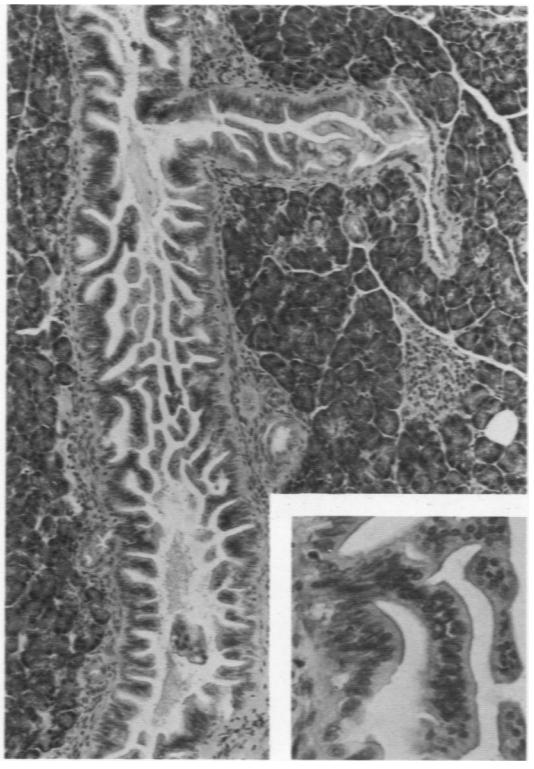

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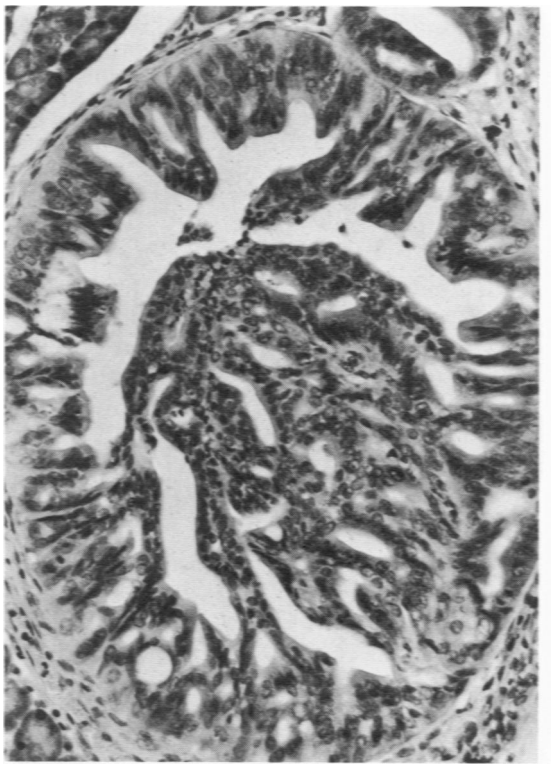

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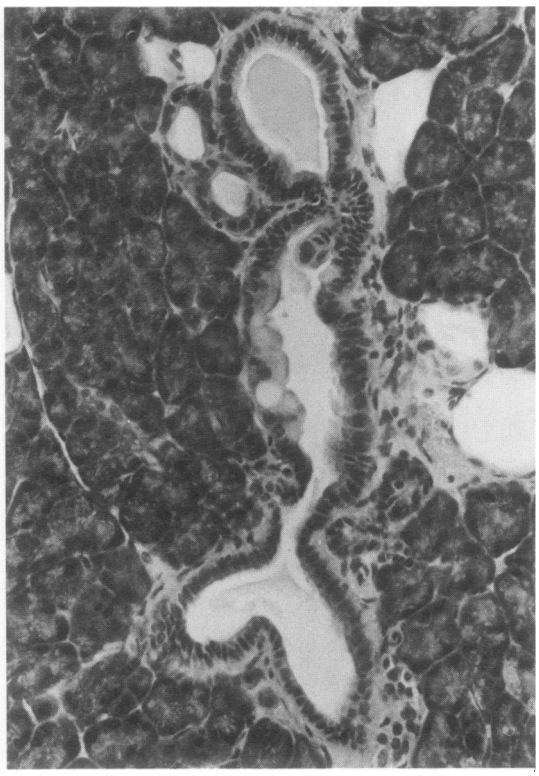

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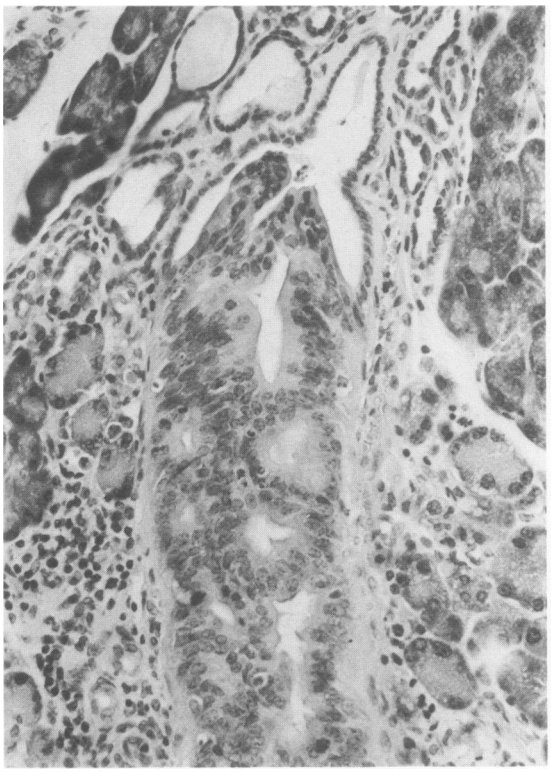

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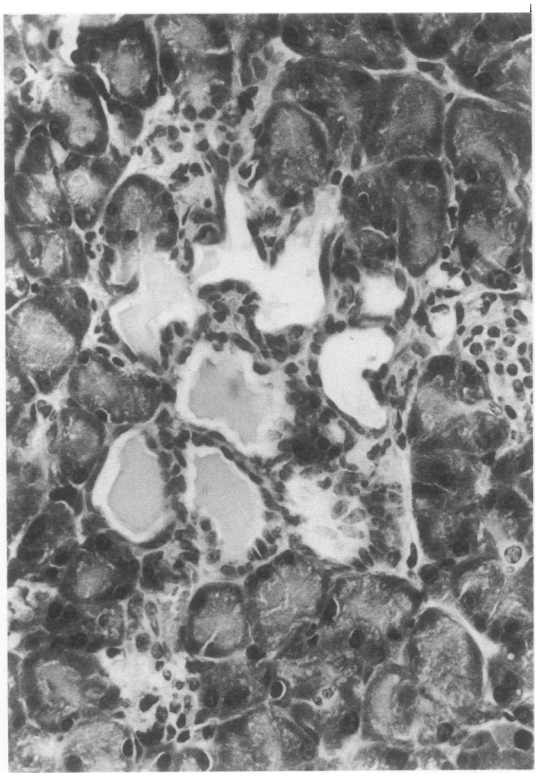

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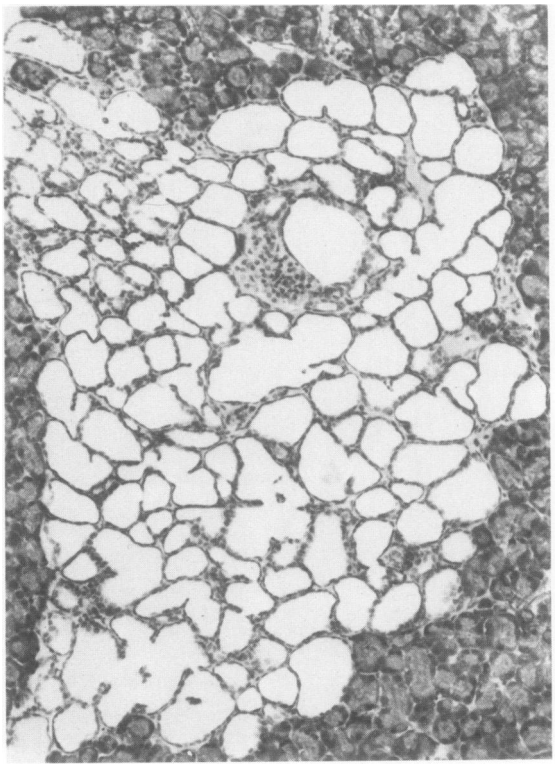

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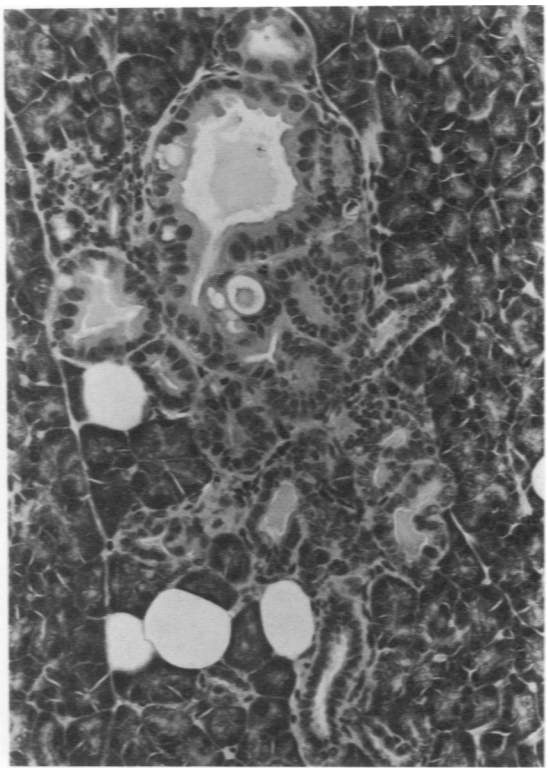

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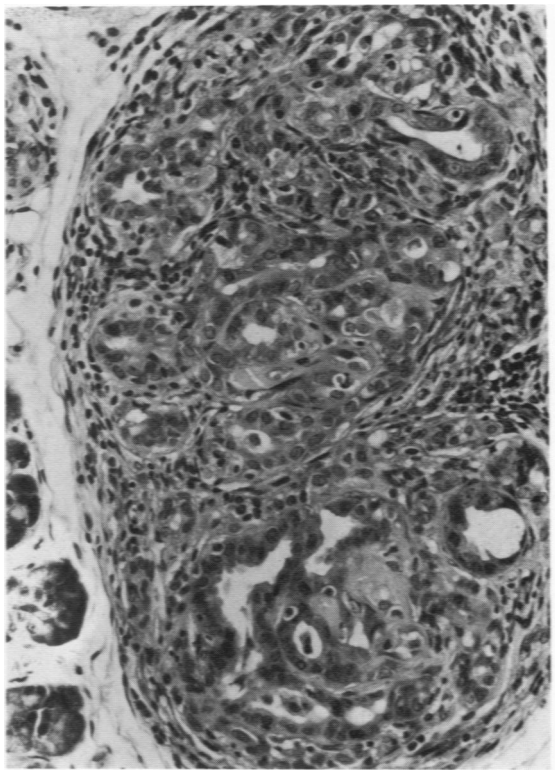

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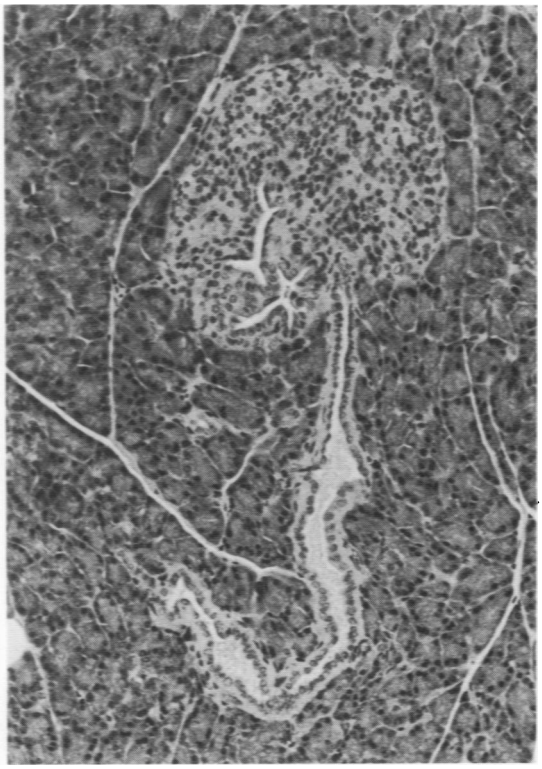

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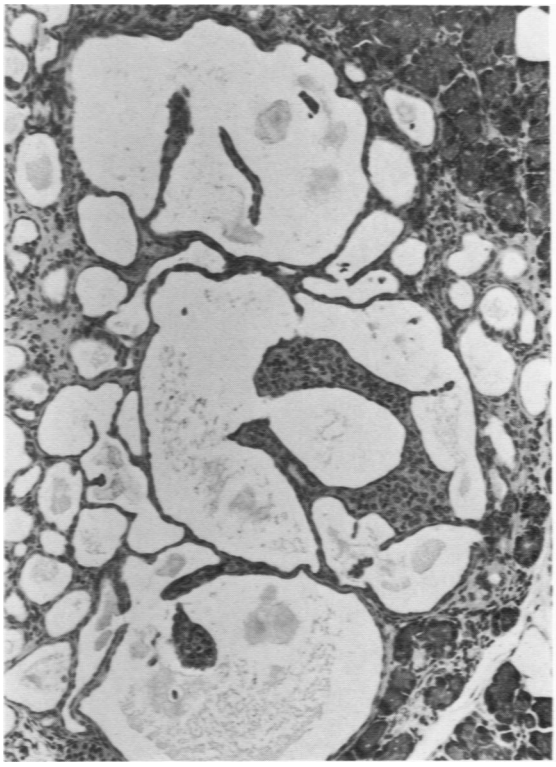

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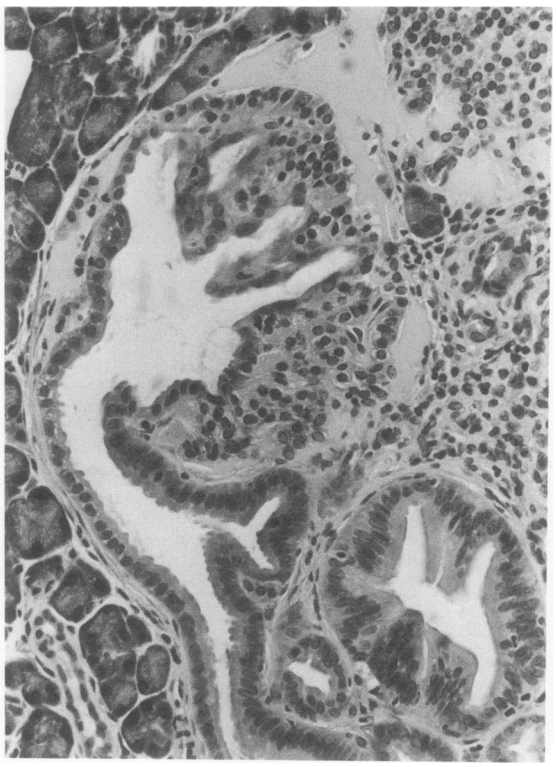

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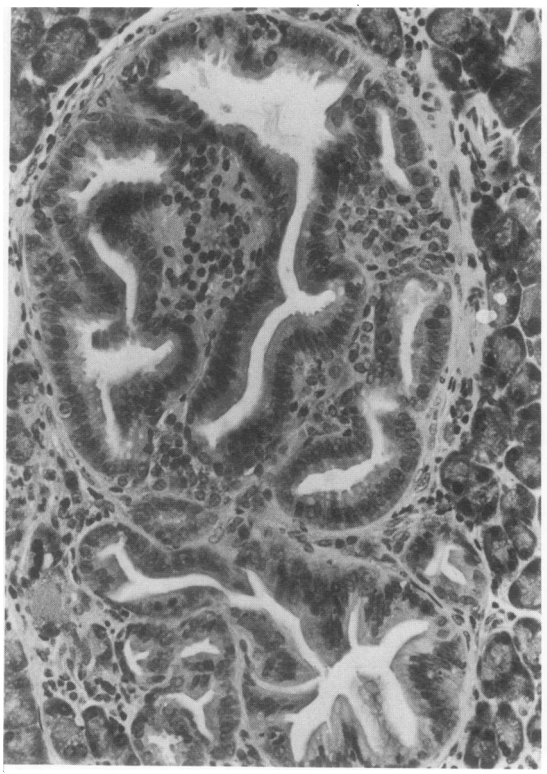

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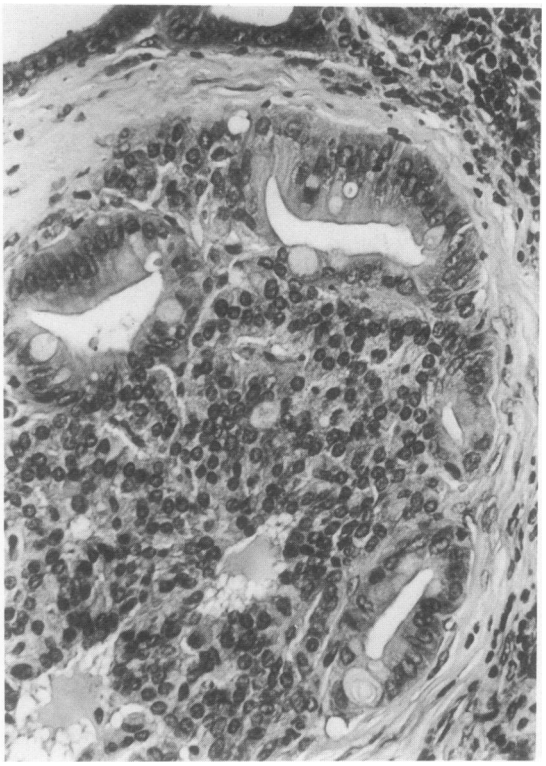

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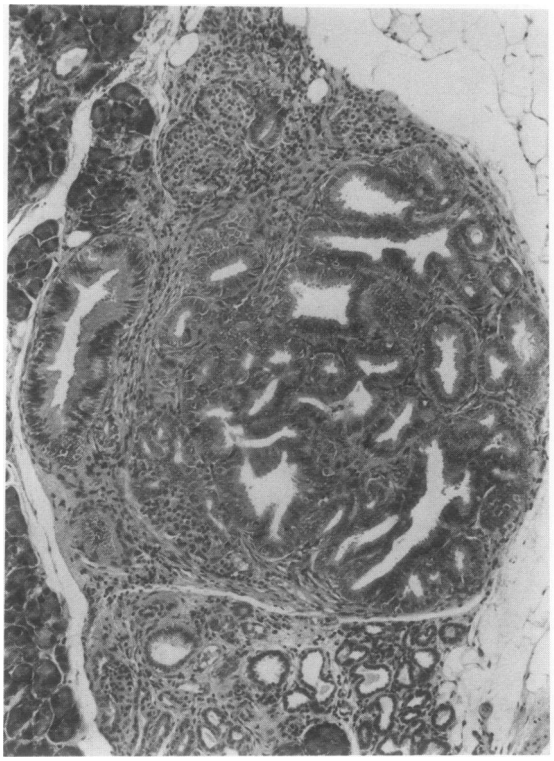

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