6-1984

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Daniel S. Longnecker
Dartmouth College

Environmental Health Perspectives
Vol. 56, pp. 245-251, 1984

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Focal proliferative changes in the acinar cells of the pancreas of rats have been induced by several systemically administered carcinogens including azaserine, *N*-nitrosobis(2-oxopropyl)amine, *N*-nitroso-(2-hydroxypropyl) (2-oxopropyl)amine, and *N*δ-(*N*-methyl-*N*-nitrosocarbamoyl)-L-ornithine (MNCO). Foci, nodules, and adenomas induced by these carcinogens are usually made up of atypical-appearing acinar cells that maintain a high degree of differentiation, but a minority of these lesions exhibit anaplastic cellular changes that suggest the development of malignant potential. Such anaplasia may occupy the whole of smaller lesions or may occur as a secondary focal change within larger nodules or adenomas. Many foci and nodules per pancreas have been induced by single or multiple exposures to these known genotoxic carcinogens, but relatively few of them develop into carcinomas. Azaserine and MNCO have induced acinar cell carcinomas in rats. Those induced by azaserine have exhibited a broad spectrum of histologic variants, including ductlike, cystic, and undifferentiated patterns. Higher doses of MNCO have induced a second pattern of change in the pancreatic lobules of rats, which includes cystic and tubular ductlike structures that have been called cystic and tubular ductal complexes.

MNCO has also induced focal acinar cell lesions, cystic and tubular ductal complexes, and adenocarcinomas in the pancreas of Syrian golden hamsters. In this species, ductal complexes are much more numerous than are proliferative lesions of acinar cells, and the histologic appearance of the carcinomas is ductlike. Hyperplasia and atypical changes were also seen in the epithelium of the intralobular ducts of hamsters.

The response of rats and hamsters to carcinogens that affect the pancreas in both species differs in a consistent pattern. Proliferative lesions of acinar cells were characteristic of carcinogen-exposed rats whereas ductal complexes and ductlike neoplasms predominated in hamsters given MNCO.

Introduction

Several chemicals have been demonstrated experimentally to induce carcinomas in the rodent pancreas (*1*). Lesions induced by azaserine, *N*-nitrosobis(2-oxopropyl)amine (BOP), *N*-nitroso(2-hydroxypropyl)-(2-oxopropyl)amine (HPOP), and *N*δ-(*N*-methyl-*N*-nitrosocarbamoyl)-L-ornithine (MNCO) in the rat pancreas will be listed and compared. MNCO has also induced carcinomas in the pancreas of hamsters (*2*), and the effect of this carcinogen will be compared in the two species. All of the carcinogens to be discussed are mutagens and have been demonstrated to induce DNA damage in the pancreas by alkaline elution analysis of DNA. Thus all of these carcinogens are genotoxic agents and are classed as initiators.

Azaserine-Induced Lesions

The major experience in our laboratory has been with azaserine which, although it acts without S-9 as a bacterial mutagen in the Ames system, appears to be activated in mammalian cells by a pyridoxal-dependent enzyme system (*3*). *N*-7-Carboxymethylguanine was identified in DNA from cells exposed to azaserine (*4*), a finding consistent with the generation of diazoacetate as the ultimate reactive carcinogenic species derived from azaserine by an α,β-elimination reaction.

Foci of phenotypically altered acinar cells have been identified in the pancreas as early as 1 month after a single injection of azaserine. These foci are about the size of small islets. Some of them have a high mitotic index in routine histologic sections, and an even higher labeling index in autoradiograms done to detect S-phase DNA synthesis (*5*). As expected from this observation, some of the foci grow at variable rates and form grossly visible nodules within 4 to 6 months after azaserine injection. In the vast majority of these lesions the acinar cells retain a high degree of differentiation. Phenotypic changes include reduced zymogen content, increased cytoplasmic basophilia, and altered nuclear size and configuration.

Some atypical acinar cell nodules (AACN) reach diameters of 2 to 3 mm; still fewer, the 3 to 7 mm size range. Lesions in the latter group that retain a high

*Department of Pathology, Dartmouth Medical School, Hanover, NH 03756.

degree of differentiation are designated as adenomas. An expansile growth pattern may be evidenced by the compression of adjacent tissue. Foci, nodules, and adenomas form a size continuum of acinar cell lesions. Only a small fraction of these lesions appear to have the potential to develop into neoplasms.

In our experience, neoplasms larger than 7 mm in diameter usually have histologic evidence of anaplastic cellular changes suggesting malignancy. Smaller lesions may also show anaplasia—either partial, in the form of a focal change within a nodule or adenoma, or total, where the entire lesion seems to consist of anaplastic cells. When such changes occur in the absence of local invasion or metastasis, these lesions have been called localized carcinomas or carcinoma *in situ*.

Azaserine-induced carcinomas exhibit a spectrum of histologic patterns (*6*). Most show evidence of acinar cell differentiation. Table 1 presents the classification for 332 azaserine-induced carcinomas that have been collected over a period of 8 years. Most of these patterns have been described previously, although we have recently identified a carcinoma with extensive squamous-appearing areas which has prompted us to add an adenosquamous type to the list. Some portion of each carcinoma typically retained acinar cell differentiation, but about 2% of the neoplasms were uniformly undifferentiated. The existence of such diverse histologic types of carcinoma in the azaserine/rat model is significant because of the evidence that all of these neoplasms have originated through the transformation of acinar cells. No hyperplastic or atypical changes have been identified within the epithelium of the ductal system of azaserine-treated rats.

A characteristic of the azaserine model that deserves emphasis is the fact that a single exposure to azaserine can induce dozens of AACN per pancreas. AACN have been defined by usage to include both small foci and larger nodules of atypical acinar cells, and are apparently the early precursors to azaserine-induced neoplasms. Multiple-dose regimens of azaserine can induce more than 100 such lesions per pancreas (*7*).

The incidence of pancreatic cancer has consistently been higher in male rats than in female rats by a ratio of approximately 2:1. This difference in sensitivity to azaserine between the two sexes is also evident in nodule-induction studies in which we have demonstrated that 1.4 to 4 times as many nodules appear in male rats as in female rats after equivalent regimens of azaserine treatment (*7*). The basis for this sex difference in sensitivity to azaserine is not known. These studies have been done mainly in Wistar and Lewis strain rats. We have shown that Fischer 344 rats of either sex develop fewer acinar cell lesions in response to azaserine (*7*).

Diet has been shown to influence the rate of progression of azaserine-induced lesions. Purified diets high (20%) in unsaturated fats have been shown to enhance the rate of progression of azaserine-induced lesions in comparison to diets that contain the standard (5%) level of corn oil or a high level of saturated fat (18% coconut oil plus 2% corn oil). In the azaserine model, pancreatic neoplasms have increased in incidence and number both when the diet was fed continuously during a prolonged period of exposure to azaserine (*8*), and when, in one experiment, exposure to azaserine was completed before the test diets were fed (*9*). The number of tumors per pancreas has been three to four times as great in the animals fed diets high in unsaturated fats as in their controls.

Table 1. Histologic types of azaserine-induced pancreatic carcinoma in rats. A series of 332 such carcinomas have been classified and the distribution of types is shown.

Histologic type	*n*	%
Acinar cell carcinoma, pure		
Well-differentiated	100	30
Poorly differentiated	143	43
Acinar cell carcinoma mixed with		
Ductlike carcinoma	59	18
Cystic adenocarcinoma	4	1
Microadenocarcinoma	4	1
Undifferentiated carcinoma	15	5
Adenosquamous carcinoma	1	<1
Undifferentiated carcinoma, pure	6	2
Total	332	100

Lesions Induced by Derivatives of Dipropylnitrosamine

The induction of pancreatic carcinomas in hamsters by BOP and HPOP has been well characterized (*10*). In hamsters, the vast majority of carcinomas have been classed as ductal in histologic type, although we have also observed a low incidence of carcinomas with mixtures of malignant ductlike and acinar cells in BOP-treated hamsters. Several investigators have reported failing to induce pancreatic neoplasms in rats treated with BOP or related nitrosamines (*11*). In biochemical studies of the effect of BOP and HPOP in Lewis rats, we observed evidence of damage by means of an alkaline elution analysis of pancreatic DNA from treated rats (*11*). The amount of damage induced by 100 mg/kg of BOP or 40 mg/kg of HPOP appeared to be less than that induced by carcinogenic doses of azaserine, i.e., 10 to 30 mg/kg, but the complete lack of pancreatic carcinogenesis in BOP-treated rats was puzzling. Since we had previously shown that young rats were more sensitive to AACN induction by azaserine than were older or adult rats (*12*), we treated a group of 2 to 3 week-old rats with a single injection of BOP or HPOP. All these rats developed multiple AACN in their pancreases within 4 months after carcinogen injection (Fig. 1) (*11*). We have since observed a group of young rats treated with a single dose of HPOP for a longer period (unpublished). Some of the AACN grew to become adenomas and localized carcinomas, as has

been described in the azaserine model. Ducts have remained normal in these rats. The early lesions are similar in histologic appearance to those that we have observed in azaserine-treated rats. Thus, we believe the BOP and HPOP can serve as pancreatic carcinogens in the rat and our observations to date suggest that the pathway of histogenesis for resulting carcinomas is similar to that observed in the azaserine rat model. As many as 100 AACN and adenomas have been observed in rats given a single dose of 160 mg/kg of HPOP. The fact that a single exposure to a known genotoxic carcinogen yields multiple altered cellular foci should again be emphasized.

Lesions Induced by a Nitrosourea Amino Acid

MNCO is a nitrosourea that appears to be a direct-acting carcinogen. The pancreatropism of this compound has been attributed to the presence of the intact α-amino acid group of ornithine. MNCO is a direct acting mutagen in the Ames assay (*13*), and has damaged pancreatic DNA in rats (*14*). It is an effective carcinogen in long-term studies although the incidence of carcinomas was higher in the kidney, skin, and breast than in the pancreas in a one-year study (*15*). This discussion will focus on the lesions induced in the pancreas by MNCO in Wistar rats. Low doses of MNCO induce AACN, whereas in rats given higher doses there is progressive replacement of the lobular tissue of the pancreas by cystic lesions that we have referred to as cystic ductal complexes. Some of the AACN progress to become acinar cell adenomas, and acinar cell adenocarcinomas.

The cystic lesions in the pancreas consist of multilocular structures with thin fibrous walls lined by a single layer of flattened or cuboidal epithelium (Fig. 2). Such lesions appear to replace acinar cells and may appear around or within islets. The ratio of the diameter of the lumen to the thickness of the wall is high—in the range of 10/1 or higher. The mitotic index of the lining epithelium is low, and most lesions remain smaller than 3 mm in diameter. We have not seen large neoplasms of a comparable histologic appearance. For this reason we believe that the proliferative potential of these lesions is low or nonexistent.

Acinar tissue is less frequently replaced by lesions of a second type that we have designated as tubular ductal complexes. These consist of ductlike structures, usually with a scant fibrous stroma, lined by cuboidal or columnar epithelium. These elements may be intimately

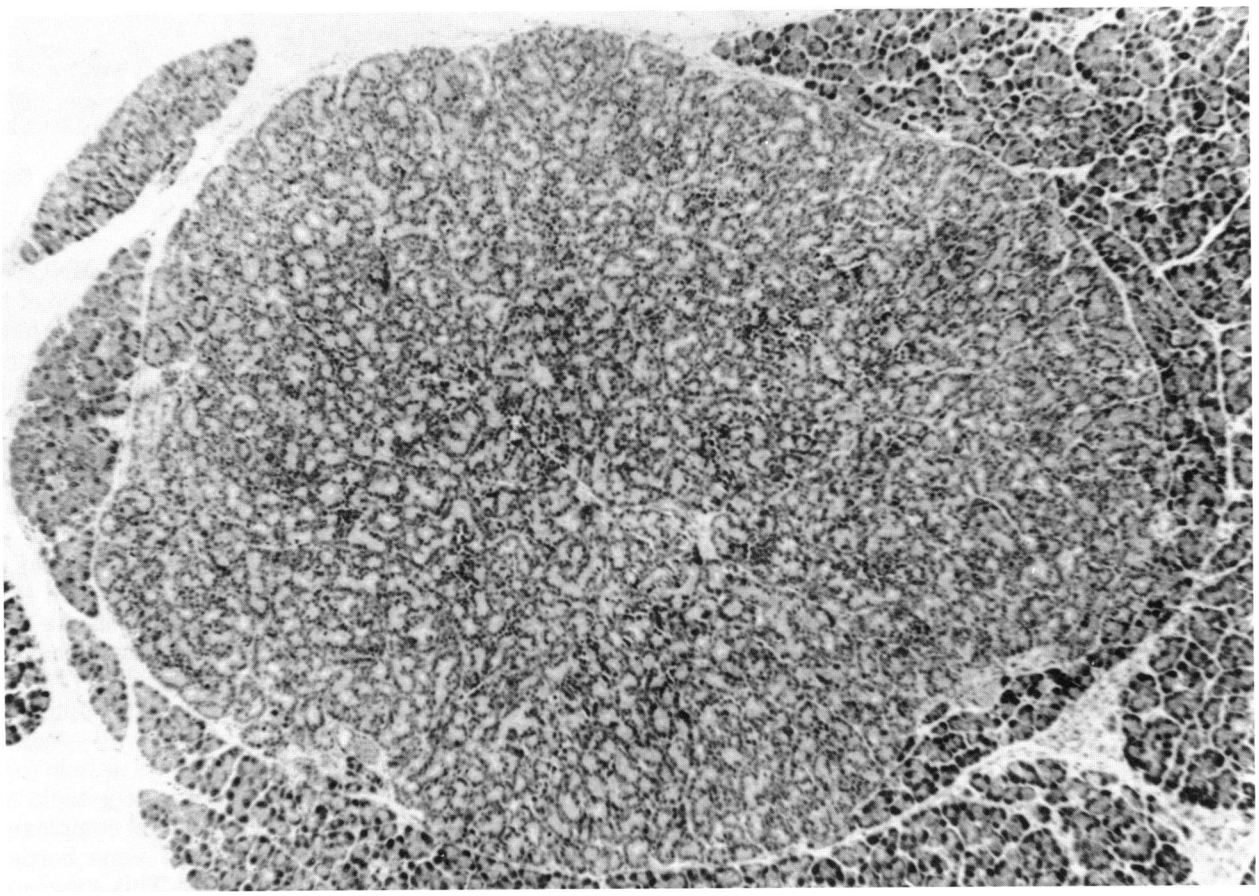

FIGURE 1. Nodule of well-differentiated atypical acinar cells from the pancreas of a 4.5–month-old Lewis rat that received a single 100 mg/kg injection of BOP at 14 days of age. The mitotic index was greater in the nodule than in the adjacent pancreas. Hematoxylin and eosin, ×50.

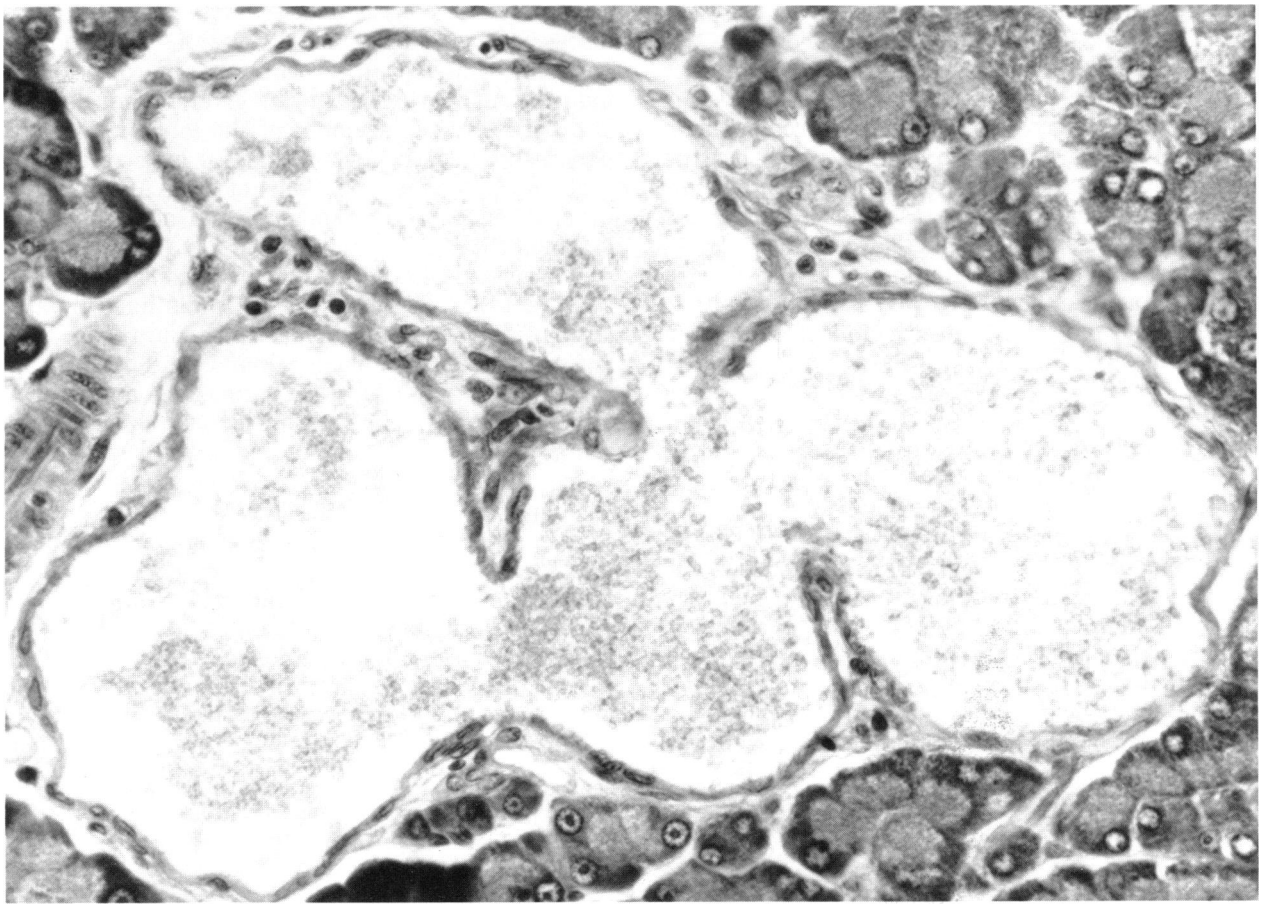

FIGURE 2. Cystic ductal complex from the pancreas of a rat killed 3 months after injection of MNCO. The cystic spaces are lined by flattened epithelium. H & E, ×325.

interspersed with acini. The ratio of the diameter of the lumen to the height of the epithelium is in the range of 1:1. These lesions occur less frequently than either AACN or cystic ductal complexes.

The pancreas of MNCO-treated rats contains a larger spectrum of lesions than are usually seen after azaserine treatment, but we have seen a low incidence and small number of both cystic and tubular ductal complexes in azaserine-treated rats. The lesions that appear to have the greatest potential for progression to neoplasia appear to be the AACN which are presumed to be the precursors to acinar cell adenomas and carcinomas. More than 100 such foci have been counted in a pancreas from a rat given multiple doses of MNCO. It is apparent that this genotoxic initiator, like azaserine, BOP, and HPOP, can induce many potentially preneoplastic foci in the rat pancreas.

MNCO-Induced Lesions in Hamster Pancreas

We have recently reported the induction of ductlike carcinomas in the pancreas of Syrian golden hamsters that were treated with MNCO (*2*). We had earlier reported the effect of smaller doses of MNCO in a 6-month study (*16*). The histologic appearance of these carcinomas was similar to those that have been induced by BOP and related compounds. MNCO induced a spectrum of lesions in the hamster pancreas similar to that already described in the rat. Several foci of atypical acinar cells were observed (Fig. 3). The incidence was related to the total dose of MNCO, but it was always less than 100%, and there were usually only one or two such lesions per pancreas. We also observed a dose-related incidence of cystic ductal complexes and occasional tubular ductal complexes that replaced lobular acinar tissue (Fig. 4). The latter structures are similar to the BHP-induced lesions in the hamster that Flaks has called "pseudoductular transformation" (*17*). Some lesions had characteristics of both cystic ductal complexes and tubular ductal complexes while occasional AACN had tubular units similar to those seen in tubular ductal complexes. Thus, while most lesions could easily be categorized as AACN, tubular ductal complexes, or cystic ductal complexes, there were some borderline lesions that were difficult to classify. This suggests the possibility that tubular ductal complexes and cystic ductal complexes might have been derived from carcinogen-altered acinar cells. We also saw a dose-related

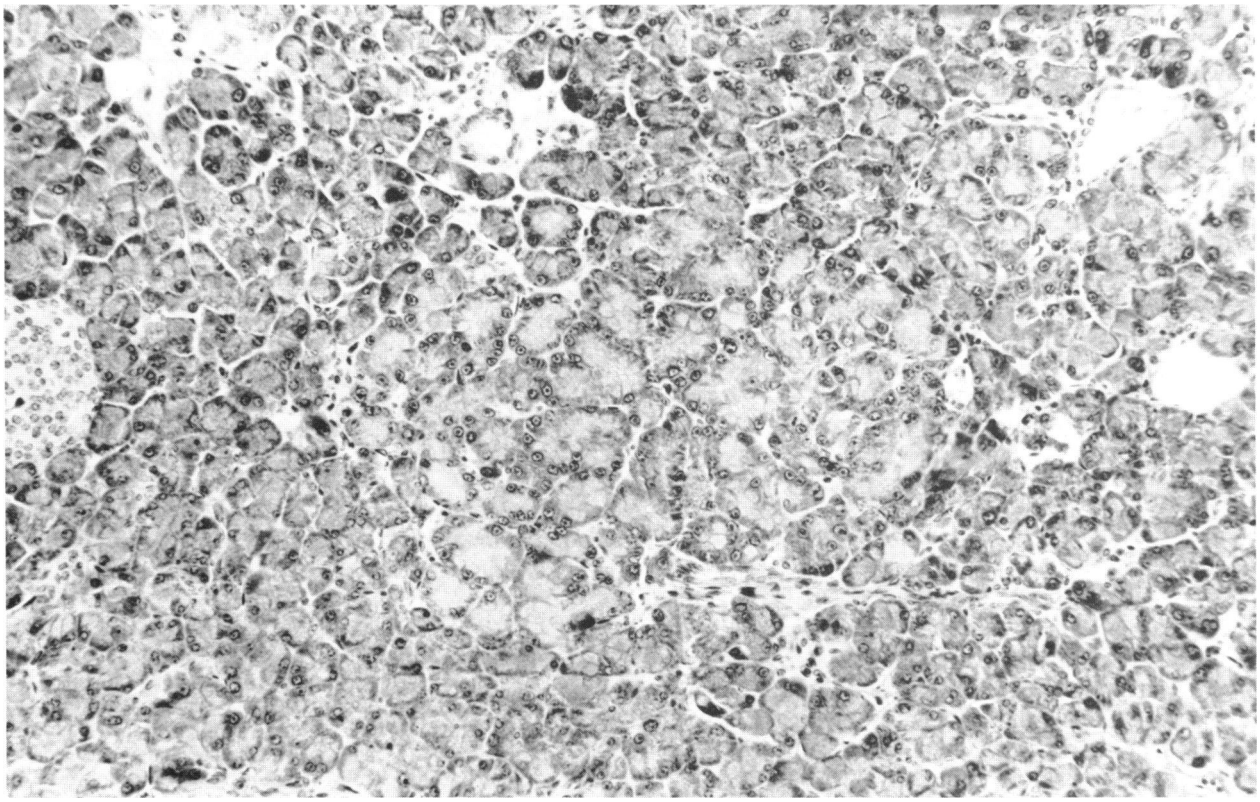

FIGURE 3. Focus of atypical acinar cells from the pancreas of a hamster that was autopsied 32 weeks after initial MNCO treatment. H & E, ×130.

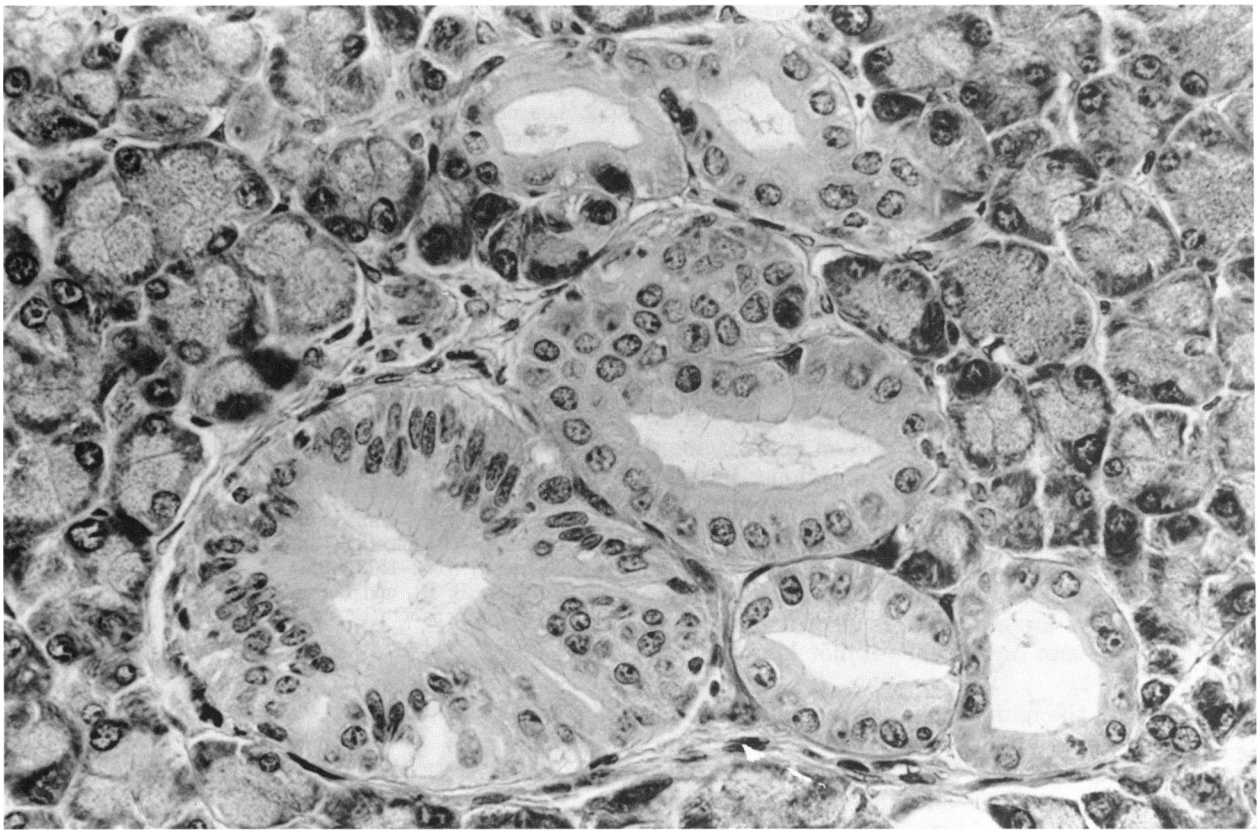

FIGURE 4. Tubular ductal complex from the pancreas of an MNCO-treated hamster that was autopsied after 26 weeks. H & E, ×325.

incidence of focal eosinophilic metaplasia similar to lesions described in aged hamsters by Pour (*18*) and BOP-treated hamsters by Scarpelli et al. (*19*). We did not undertake ultrastructural or histochemical studies to verify hepatocytelike characteristics for these cells; however, their histologic appearance was consistent with this phenotype.

Many ducts and ductules were normal, but infrequent examples of intraductal papillary epithelial hyperplasia, atypical papillary intraductal hyperplasia, and intraductal carcinoma were seen. The histologic type of most carcinomas was consistent with derivation from either tubular ductal complexes or intraductal proliferative lesions, but no final conclusion regarding the relative importance of these two putative precursors was established.

Conclusions

Our studies of rats and hamsters suggests that exogenous chemical carcinogens can affect acinar cells in both species and ductal cells in hamsters. A composite scheme outlining the possible sequences for the histogenesis of carcinogen-induced lesions in the pancreas of rats and hamsters is depicted in Figure 5. Experience with several carcinogens supports the view that the carcinogen-induced lesions are derived primarily, or exclusively, from acinar cells in the rat pancreas (*5*). HPOP and MNCO are carcinogens that induce acinar cell carcinomas in the rat and ductlike carcinomas in the hamster. Thus, the response of these two species to specific chemical carcinogens differs in a consistent fashion. Pathways have been proposed in both the rat and the hamster whereby ductlike carcinomas might arise from carcinogen-altered acinar tissue. The possibility that the carcinomas induced by these two carcinogens arise from acinar cells in both rats and hamsters should be considered, although the data we have presented is no more than suggestive of this pathway. We also consider the alternate possibility that the carcinomas arise from different cells of the pancreas in the two species, i.e., acinar cells in the rat and ductal cells in the hamster. The pathways outlined in Figure 5 do not reflect the view of Pour that ductal complexes arise by proliferation of ductular and/or centroacinar cells (*20*). The relative importance of lesions derived from the two cell types in regard to the origin of carcinomas in the hamster is unknown. We believe that a similar situation pertains in regard to pancreatic carcinogenesis in the human, i.e., that the relative proportion of tumors arising as a result of transformation of acinar cells and ductal cells is not known with certainty.

Experience with the number and types of lesions that have been induced in the rat pancreas by genotoxic carcinogens such as azaserine, BOP, HPOP, and MNCO provides a basis for comparison and evaluation of the carinogenic potential of additional chemical agents.

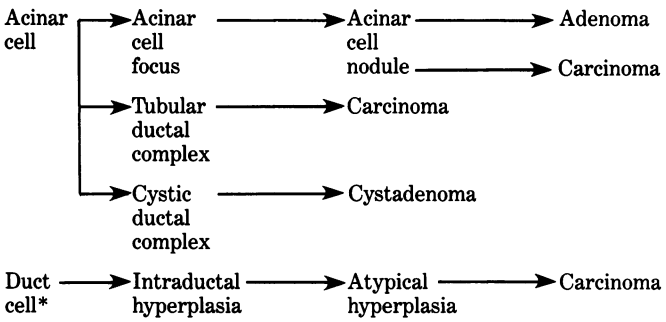

FIGURE 5. Pathways for derivation of carcinogen-induced lesions and carcinomas in rodent pancreas. See the text for a discussion of the histologic types of carcinomas derived from duct cells and acinar cells. The duct cell* is intended to represent ductal, ductular and centroacinar cells.

Proliferative lesions of acinar cells have been the most frequent manifestation of systemic exposure to pancreatic carcinogens in the rat.

These studies were supported by grants CA-19410 (through the National Pancreatic Cancer Project) and CA-30650 from the National Cancer Institute, NIH, Bethesda, MD. The contributions of coinvestigators to the work reviewed here is reflected in the authorship of the references. Nδ-(*N*-methyl-*N*-nitrosocarbamoyl)-L-ornithine was conceived and synthesized by T. J. Curphey for use in these studies. Deborah Solomon of the Norris Cotton Cancer Center provided editorial assistance.

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