Vol. 52: 191–198, 2002 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published December 10

Exocrine pancreatic carcinogenesis in the guppy *Poecilia reticulata*

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ABSTRACT: Exocrine pancreatic neoplasms developed in the guppy *Poecilia reticulata* following exposure to the direct-acting carcinogen methylazoxymethanol acetate (MAM-Ac). Fish 6 to 10 d old were exposed to nominal, non-toxic concentrations of 4 and 10 mg MAM-Ac l^{-1} for 2 h and then transferred to carcinogen-free water for grow-out. Whole specimens were sampled monthly up to 9 mo post-exposure to follow the histologic progression of the lesions. No neoplasms occurred in 119 control specimens examined. Pancreatic acinar cell adenomas and carcinomas occurred in 42 of 243 (17%) of the specimens exposed to MAM-Ac. As in earlier studies, specimens exposed to the low MAM-Ac concentration exhibited a higher pancreatic neoplasm incidence (27.8%) than those exposed to the high concentration (7.8%). Acinar cell adenomas accounted for 27 of the 42 neoplasms. Adenomas exhibited a high degree of acinar cell differentiation and some contained foci of atypical acinar cells that were less differentiated and more basophilic than were surrounding adenoma cells. Carcinomas occurred in 15 specimens and exhibited a range of cellular patterns. Although no distant metastases were found, carcinomas tended to invade neighboring tissues and organs. The occurrence of carcinogen-induced pancreatic neoplasms in guppies strengthens the usefulness of small fish species in carcinogen testing and provides an additional model for studying pancreatic neoplasia.

KEY WORDS: Fish · Exocrine pancreas · Neoplasia · Carcinogenesis · Guppy

INTRODUCTION

Exocrine pancreatic neoplasia is uncommon in fishes. Most reports of exocrine pancreatic neoplasia in fishes have been isolated cases in diverse species with no clear evidence of etiology (Nigrelli & Gordon 1951, Otte 1964, Fournie et al. 1988, Thiyagarajah & Bender 1988, Heidel et al. 1992, Fournie & Hawkins in press). However, an epizootic of exocrine pancreatic neoplasms was reported from a population of mummichog *Fundulus heteroclitus* from a creosote-contaminated site in the Elizabeth River, Virginia, USA (Fournie & Vogelbein 1994). It was suggested that the pancreatic neoplasms as well as an array of hepatic neoplasms resulted from chronic exposure to carcinogenic polynuclear aromatic hydrocarbons (Vogelbein et al. 1990).

Although pancreatic neoplasms occur in fishes exposed to potent chemical carcinogens, little is known about the initiation and progression of the lesions. Exocrine pancreatic neoplasms of duct cell origin were induced in the mangrove rivulus *Rivulus marmoratus* by exposure to diethylnitrosamine (DEN) (Thiyagarajah & Grizzle 1986). A pancreatic acinar cell carcinoma developed in a gulf killifish *Fundulus grandis*, injected as an embryo with N-methyl-N′-nitro-N- nitrosoguanidine (MNNG) (Grizzle et al. 1988). Exocrine pancreatic neoplasms including acinar cell adenomas, carcinomas, and adenocarcinomas were induced in the guppy *Poecilia reticulata* by exposure to methylazoxymethanol acetate (MAM-Ac), a direct-acting carcinogen

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(Fournie et al. 1987). That study employed high, toxic concentrations of MAM-Ac mainly to examine the hepatocarcinogenic sensitivity of several species to MAM-Ac (Hawkins et al. 1985).

The present study was designed to investigate the initiation and progression of exocrine pancreatic carcinogenesis in the guppy following exposure to non-toxic levels of MAM-Ac and to examine the histogenesis and cells of origin of the lesions. A low and a high MAM-Ac concentration were used to re-examine the atypical dose response seen in the initial report in which higher incidences of the lesions occurred at lower exposure concentrations.

MATERIALS AND METHODS

Specimens of 6 to 10 d old king cobra strain guppies *Poecilia reticulata* were exposed to methylazoxymethanol acetate (MAM-Ac; CAS:592-62-1) in well water for 2 h in 1 l beakers situated in a vented carcinogen glovebox. Each of the 3 beakers contained 200 specimens in 855 ml of water. Nominal exposure concentrations of MAM-Ac were 4 and 10 mg l^{-1}. After exposure, specimens were rinsed in clean water and then transferred to each of three 57 l aquaria containing recirculating well water. Each test group was maintained in a single aquarium. Fish were fed a commercial flake food supplemented with newly hatched brine shrimp.

Regular monthly samples of 5 to 7 fish were taken from each of the 3 test groups for histopathological examination. Moribund specimens and those exhibiting gross signs of disease were sampled when first observed. Whole fish, or visceral masses only for large specimens, were fixed in Bouin's solution (Luna 1968) and processed for routine paraffin histology. Sections were cut at 6 µm and stained with Harris' hematoxylin and eosin for microscopic evaluation. The study was terminated 270 d following exposure because of a systemic bacterial infection in all 3 test groups. To help control the infection and keep fish alive for as long as possible, aquaria were treated with 4 mg l^{-1} nifurpirinol on 3 consecutive days beginning on Day 130 post-exposure. This treatment partially arrested the mortalities but did not eliminate the infection.

RESULTS

Normal anatomy and histology

The guppy pancreas occurred as diffuse masses of glandular acinar cells suspended in the mesentery between folds of the intestine (Leake 1975) and in the liver along the hepatic artery and portal vein. Most of the glandular tissue lay in the duodenal region along with small isolated groups of endocrine pancreatic islets of Langerhans. A recent ultrastructural study of normal and neoplastic exocrine pancreas in the mummichog *Fundulus heteroclitus* demonstrated that the pancreas of small fish species is organized as an anastomosing tubular gland (Vogelbein & Fournie 1994). Exocrine pancreas in the guppy was also arranged as a continuous branching network of anastomosing tubules composed of acinar cells. Although true terminal acini were not identified, some tubules ended in blind oval or round structures resembling classical acini. We use the term 'acinus' to refer to any 2-dimensional cross section of the branching tubular system composed of acinar cells.

Histologically, the guppy exocrine pancreas appeared similar to that of higher vertebrates and other small fish species (see Fournie & Vogelbein 1994). Acinar cells were pyramidal with basally situated nuclei and numerous eosinophilic zymogen granules in the apical cytoplasm (see Fournie et al. 1987). Basal portions of acinar cells were devoid of zymogen granules and stained basophilically due to the presence of rough endoplasmic reticulum. Centroacinar cells appeared as flattened epithelial cells with scant pale-staining cytoplasm that occurred in tubular lumina.

Pancreatic lesions

Several types of neoplastic lesions developed in the mesenteric exocrine pancreas of guppies exposed to MAM-Ac. They included acinar cell adenomas, atypical acinar cell foci, and acinar cell carcinomas (Table 1). No pancreatic lesions were observed in any of the 119 control specimens that were histologically examined. Based on the small size of these fish (adult specimens about 2 cm in standard length), some tumors were quite large, reaching up to 7 mm in greatest dimension. Approximately 17% of the exposed fish that were examined histolog-

Table 1. Pancreatic neoplasms in guppies exposed to MAM-Ac

MAM-Ac conc. (mg l^{-1})	No. of fish examined	No. of fish with neoplasms	No. of fish with adenomas	No. of fish with acinar cell carcinomas
4	115	32	21[a]	11
10	128	10	6[a]	4
Control	119	0	–	–

[a]Nine adenomas contained atypical acinar cell foci

ically developed neoplasms. The test group exposed to the low (4 mg l^{-1}) MAM-Ac concentration had a higher total neoplasm incidence (27.8%) than did the high (10 mg l^{-1}) concentration (7.8%). There was no apparent difference, however, in the proportion of adenomas to carcinomas in the 2 test groups. No distant metastases of any acinar cell carcinomas were found; however, several lesions did invade neighboring tissues.

Acinar cell adenoma

Acinar cell adenomas occurred in 27 specimens. Adenomas first appeared 116 d post-exposure and were detected in specimens through to Day 255. Generally, adenomas consisted of an increased amount of exocrine pancreatic tissue organized into well-defined, irregularly shaped masses that may or may not compress adjacent normal tissue.

Adenomas maintained a high degree of acinar cell differentiation, with constituent cells uniformly exhibiting the characteristic basal basophilia, full complements of zymogen granules, and tubulo-acinar architectural features. Fig. 1 illustrates a well-defined lesion that showed the characteristic compression and sharp demarcation from the adjacent normal parenchyma. Adenomas of 9 specimens, however, contained atypical acinar cell foci. These foci were irregularly shaped and consisted of less differentiated acinar cells with a basophilic cytoplasm, usually devoid of zymogen granules (Fig. 2). The usual acinar arrangement was not apparent, and no mitotic figures were observed.

Acinar cell carcinoma

Acinar cell carcinomas occurred in 15 specimens and developed late in the study with the first lesions identified at Day 234. They tended to invade neighboring mesentery and tissues and ranged from very well-differentiated to more poorly differentiated neoplasms. Well-differentiated carcinomas were composed primarily of pyramidal acinar cells arranged in a typical glandular pattern (Fig. 3) and measured up to 7.0 mm in greatest dimension. Most carcinoma cells retained their characteristic basal basophilia and full complement of zymogen granules. Some cells were distinctly spindle-shaped and some basophilic cells were devoid of zymogen granules (Fig. 4).

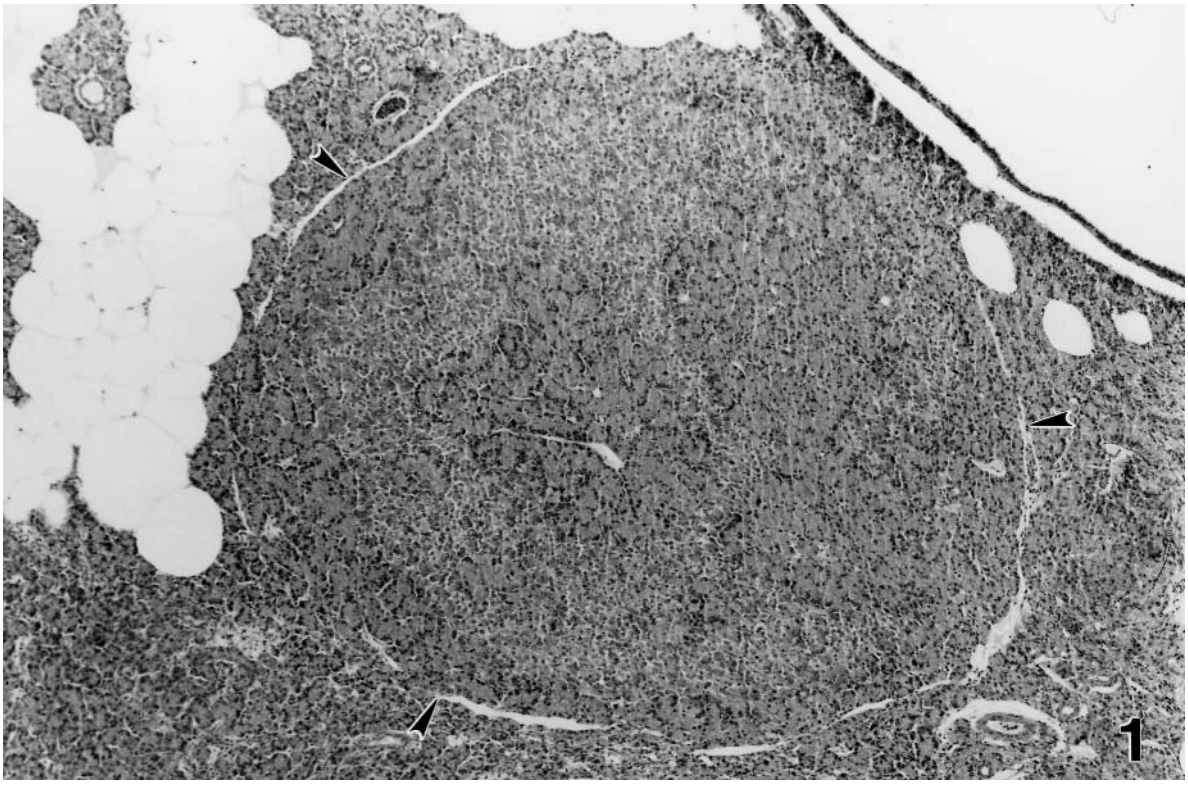

Fig. 1. *Poecilia reticulata.* Acinar cell adenoma in the mesenteric pancreas showing sharp demarcation from the adjacent parenchyma (arrowheads). H&E. ×110

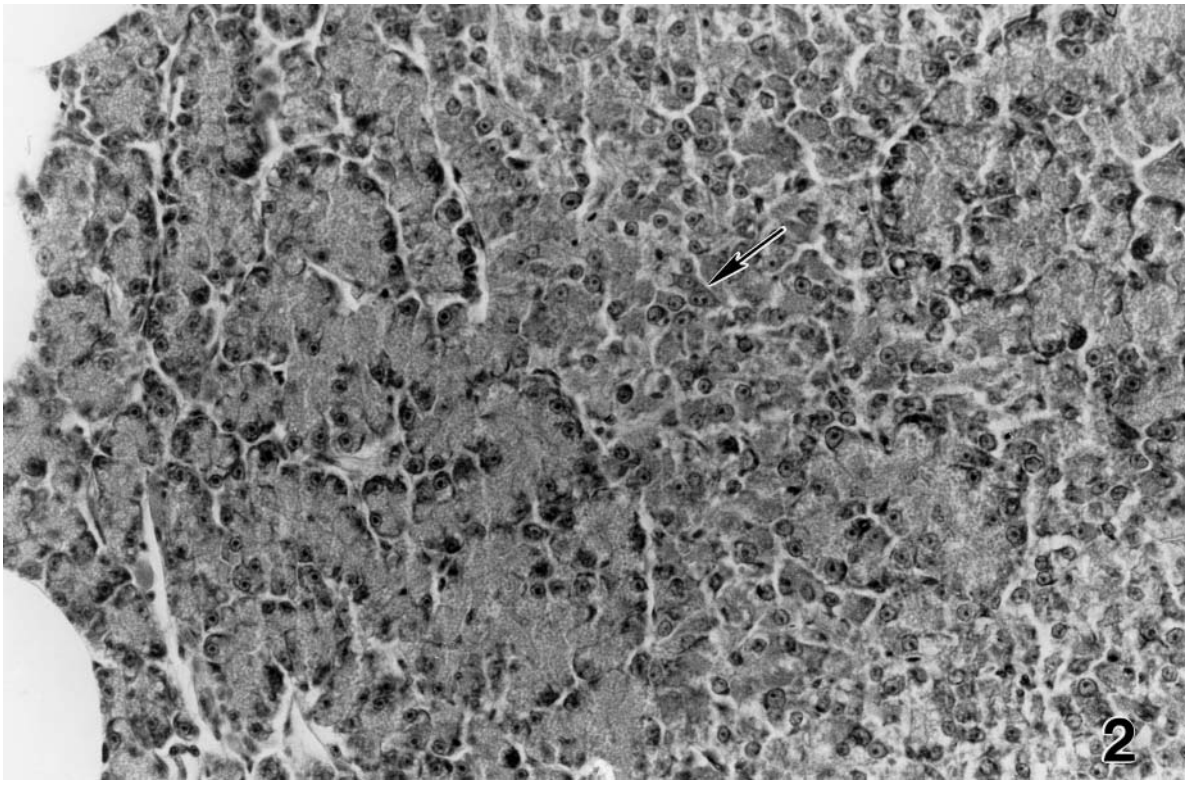

Fig. 2. *Poecilia reticulata.* Higher magnification of an atypical acinar cell focus within an adenoma showing less differentiated, irregularly shaped acinar cells (arrow). H&E. ×440

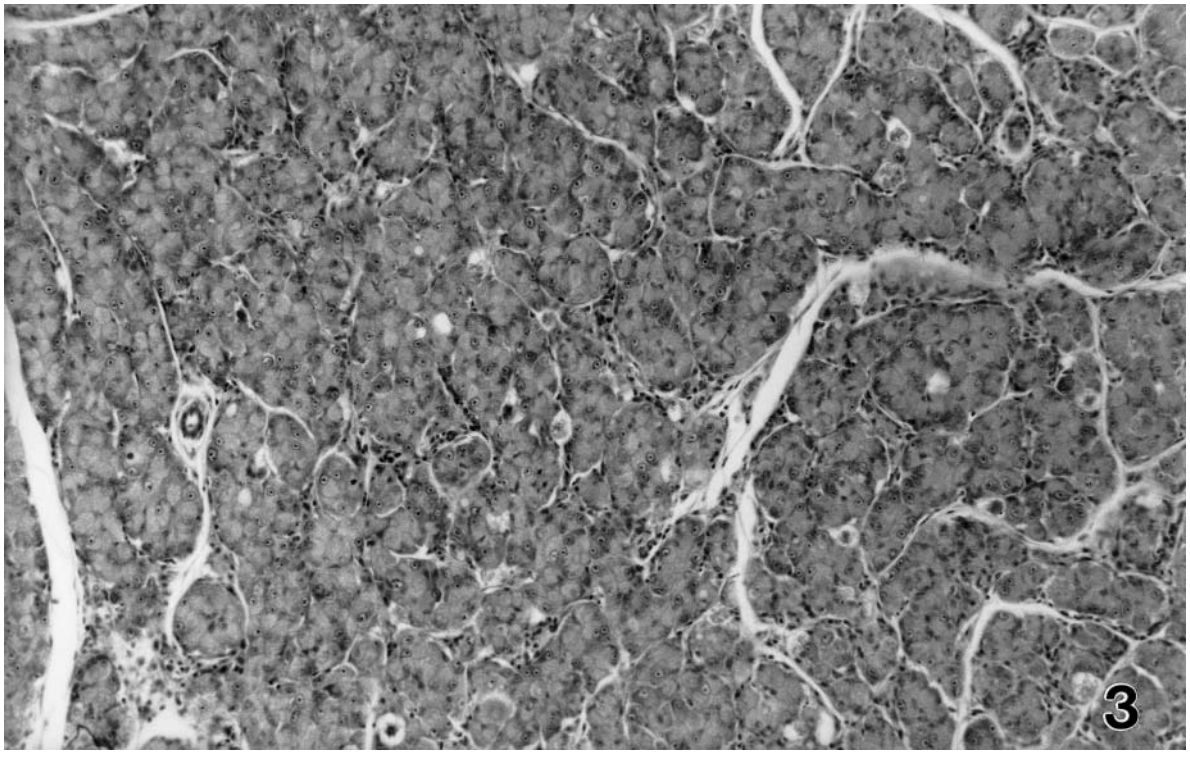

Fig. 3. *Poecilia reticulata.* Well-differentiated acinar cell carcinoma showing neoplastic cells arranged in glandular patterns. H&E. ×220

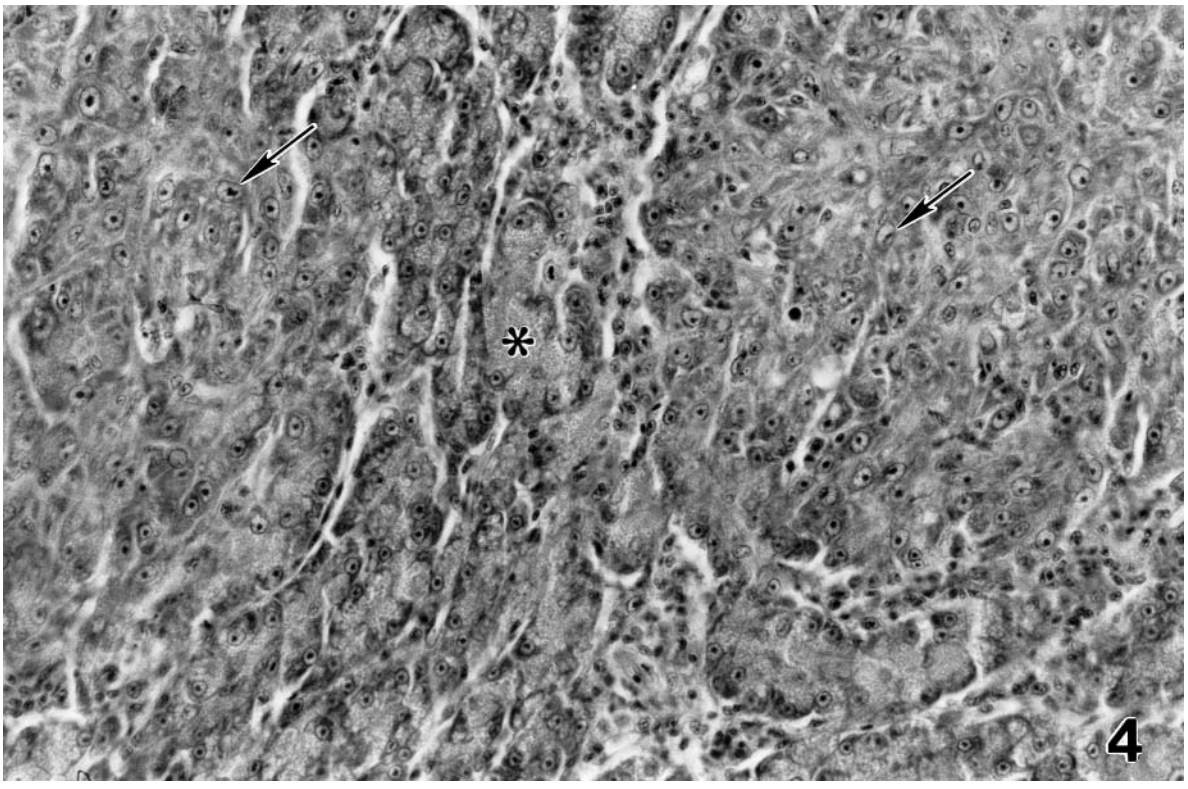

Fig. 4. *Poecilia reticulata.* Acinar cell carcinoma showing both well-differentiated (asterisk) and more poorly differentiated areas (arrows) in the same tumor. H&E. ×440

The more poorly differentiated lesions measured up to 5.0 mm in greatest dimension and many consisted of neoplastic cells arranged in distinct lobular patterns (Fig. 5). Some acinar cells in these tumor masses appeared normal and contained full complements of zymogen granules. However, the major portion of the masses consisted of variably shaped cells with basophilic cytoplasm, large pleomorphic nuclei containing prominent nucleoli, and a reduced amount or total lack of zymogen (Fig. 6). Mitotic figures were common in these lesions and foci of tumor necrosis were present in some neoplasms.

DISCUSSION

Pancreatic carcinogenesis in small fishes appears to be unusual in that a typical dose response pattern does not occur. In this study, guppies exposed to 4 mg MAM-Ac l^{-1} had a higher incidence of adenomas and carcinomas than fish exposed to 10 mg MAM-Ac l^{-1}. Further, the latency period for pancreatic adenomas was shorter in fish exposed to the lower concentration. There was no clear difference, however, in the proportion of adenomas to carcinomas in the 2 test groups. Fournie et al. (1987) reported that MAM-Ac-exposed guppies did not show a clear dose response for the induction of exocrine pancreatic carcinogenesis. In that study, which employed toxic MAM-Ac concentrations up to 100 mg l^{-1}, pancreatic neoplasms occurred only in fish exposed to low concentrations. A similar dose response was reported for pancreatic adenomas in larval mangrove rivulus exposed to DEN (Thiyagarajah & Grizzle 1986). In both studies, fishes exposed to higher concentrations had lower incidences of pancreatic lesions than those exposed to lower concentrations. Further studies will be necessary to determine the cytologic basis for this response.

The apparently high sensitivity of the guppy exocrine pancreas to the carcinogenic effects of MAM-Ac (Fournie et al. 1987, this study) contrasts with the overall low incidence of exocrine pancreatic neoplasms in the medaka *Oryzias latipes* (Hawkins et al. 1991). Only 8 cases of carcinoma of the exocrine pancreas were reported in medaka from a pool of about 10 000 specimens used in carcinogenesis tests of a variety of compounds (Hawkins et al. 1991). The medaka pancreatic carcinomas were poorly differentiated, aggressive neoplasms as evidenced by their large size, elevated mitotic activity, invasive growth pattern, and metasta-

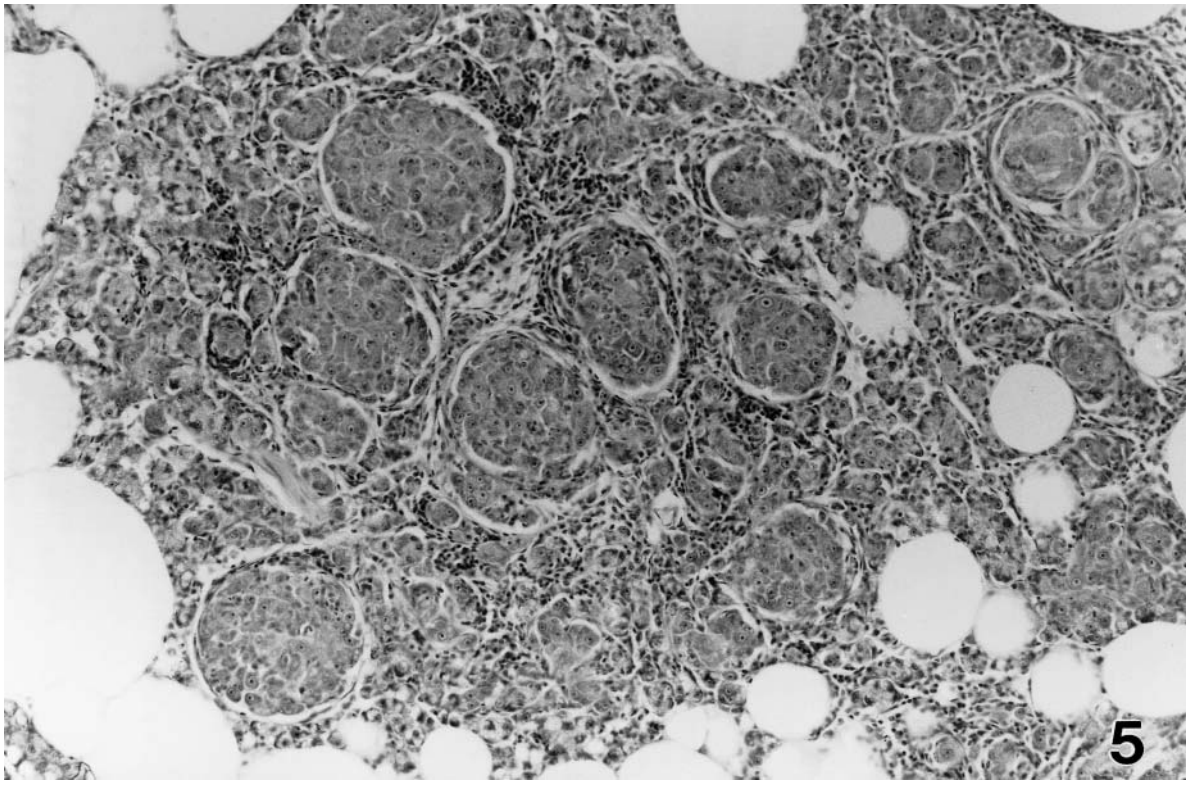

Fig. 5. *Poecilia reticulata.* Acinar cell carcinoma showing prominent lobular arrangement of neoplastic cells. H&E. ×220

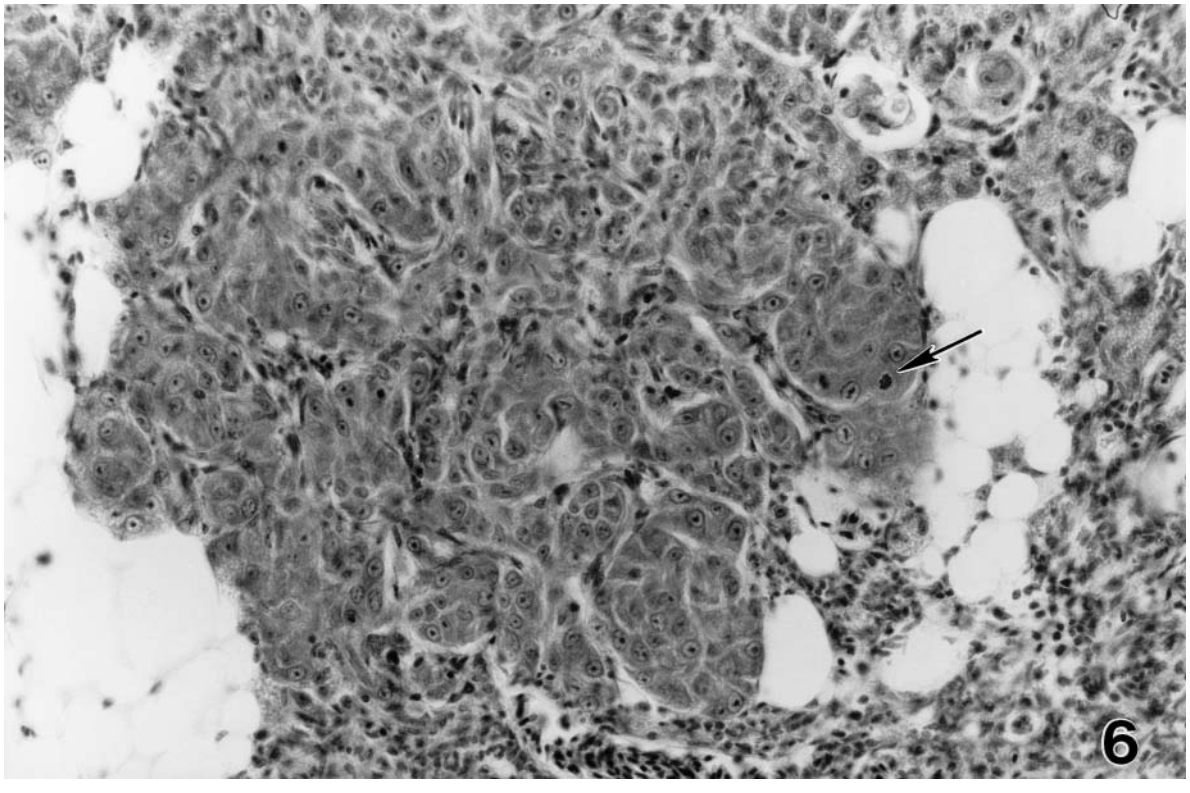

Fig. 6. *Poecilia reticulata.* Higher magnification of a lobular acinar cell carcinoma showing pleomorphic cells with increased cytoplasmic basophilia. Note mitotic figure (arrow). H&E. ×440

tic spread. Based on the occurrence of 3 cases in control specimens, 1 in a non-carcinogen exposure, and the lack of a concentration-response relationship in those developing after exposure to strong carcinogens, the neoplasms in medaka were considered to be spontaneous. On the other hand, the MAM-Ac-induced pancreatic neoplasms in the guppy occurred only in carcinogen-exposed specimens and exhibited a concentration-response relationship, albeit an unusual one. Despite the widespread use of guppies in toxicity and carcinogenicity studies, no exocrine pancreatic neoplasms have been reported from control specimens.

The neoplasms described in this paper were similar to those previously reported from guppies (Fournie et al. 1987) and included both acinar cell adenomas and carcinomas. Guppy adenomas from both studies demonstrated an increased amount of exocrine pancreatic tissue, were composed of well-differentiated acinar cells, and some contained atypical acinar cell foci which are discussed below. In the present study, however, some of the adenomas were more typical, forming discrete masses that compressed adjacent normal pancreatic tissue. Carcinomas also were much larger in specimens, reaching up to 7.0 mm in greatest dimension compared to 2.8 mm in the previous study. The lesions exhibited several cellular patterns, but were mostly rather well-differentiated neoplasms, leaving little doubt as to their cellular origin. Some carcinomas contained areas of cells displaying a range of differentiation. However, the acinar origin of those neoplasms was still apparent. No adenocarcinomas were diagnosed in this study. Fournie et al. (1987) reported only 2 cases of adenocarcinoma and those that occurred 270 d post exposure to MAM-Ac. Neoplasms exhibiting distinct duct-like structures apparently do not typically arise from guppy exocrine pancreas. Because the exocrine pancreas is a gland, all carcinomas of that organ, by definition, could be considered adenocarcinomas. However, we reserve the classification of adenocarcinoma for lesions that strongly reflect duct cell origin or exhibit distinct duct-like structures microscopically.

A clear pattern of histogenesis appears to occur in the MAM-Ac-induced exocrine pancreatic neoplasms in the guppy. Lesions are all of acinar cell origin and probably progress from adenomas consisting of well-differentiated acinar cells to acinar cell carcinomas in which acinar cells are capable of extreme transformation. The atypical acinar cell foci that occur in some adenomas (33%) may be precursor lesions to carcinomas. These foci appear to be phenotypically altered clones of cells that are histologically similar to the atypical acinar cell foci described from azaserine-treated rats (Longnecker et al. 1981, Longnecker 1983) and areas of focal basophilic cytoplasmic change in F344/N rats (Eustis & Boorman 1985). In rodents, these focal lesions have been considered to be either preneoplastic foci (Longnecker 1983) or endstage lesions that do not participate in acinar cell carcinogenesis (Scarpelli et al. 1984, Longnecker 1987, Rao et al. 1987). Because we have seen these atypical foci only within adenomas, they probably do not represent a preneoplastic stage leading to adenoma but rather the progression of adenoma to acinar cell carcinoma. No lesions resembling the rodent atypical acinar cell foci were seen in the guppy. Acidophilic foci are now considered to be the cancer precursor-lesions in the azaserine-induced rat model of pancreatic carcinogenesis (Longnecker 1987, Longnecker et al. 1991) and in the 4-hydroxyaminoquinoline-1-oxide-induced acinar cell lesions of the rat pancreas (Rao et al. 1982, 1987). A single acidophilic focus was reported from a mummichog by Fournie & Vogelbein (1994); however, it is not known whether this represents a similar preneoplastic stage in the mummichog.

A stem cell or stem cell equivalent has not been identified in fish exocrine pancreas. Such cells, however, could play important roles in the initiation and progression of exocrine pancreatic neoplasms in mammals and possibly in fishes. Rao et al. (1989) reported findings that support the presence of a stem cell in the pancreas of adult rats maintained on a copper-deficient diet. Their results suggest that ductular and periductular cells in pancreas are capable of multidirectional differentiation and may be considered to be stem cell equivalents. Possibly, similarly uncommitted and multipotent cells also occur in adult fish pancreas. The range of cellular patterns exhibited by the guppy acinar cell carcinomas could be the result of carcinogen damage of stem cells in various stages of differentiation.

The MAM-Ac-induced guppy neoplasms might provide a model for studying the initiation and progression of exocrine pancreatic tumors. Even though several animal models exist for the study of pancreatic carcinogenesis, the cellular origin and oncogenesis of pancreatic neoplasms are not completely understood. Most pancreatic carcinomas in humans (Cubilla & Fitzgerald 1979, 1984, Lack 1989) as well as in carcinogen-exposed guinea pigs and Syrian golden hamsters (Pour 1984, 1985, Scarpelli et al. 1984) are reported to be of ductal origin. However, studies on several animal models suggest that acinar cells are involved in pancreatic carcinogenesis in those species (Reddy & Rao 1975, Bockman 1981, Longnecker et al. 1981). Evidence exists that some of the neoplasms that display a prominent ductal appearance may arise from alterations in acinar cells or through acinar cell dedifferentiation (Bockman 1981, 1987). Studies utilizing exposures to the guppy with known rodent pancreatic carcinogens might aid development of that fish species as a model for pancreatic carcinogenesis.

Acknowledgements. We thank Lee Courtney for printing the figures and Valerie A. Coseo for typing the manuscript. This paper is contribution number 1125 of the U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, Florida 32561, USA.

LITERATURE CITED

Bockman DE (1981) Cells of origin of pancreatic cancer: experimental animal tumors related to human pancreas. Cancer 47:1528–1534

Bockman DS (1987) Alteration in pancreatic structure associated with adenocarcinoma. In: Scarpelli DG, Reddy JK, Longnecker DS (eds) Experimental pancreatic carcinogenesis. CRC Press, Boca Raton, FL, p 131–144

Cubilla AL, Fitzgerald PJ (1979) Classification of pancreatic cancer (nonendocrine). Mayo Clin Proc 54:449–458

Cubilla AL, Fitzgerald PJ (1984) Tumors of the exocrine pancreas. In: Hartmann WH (ed) Atlas of tumor pathology, 2nd Series, Fascicle 19. Armed Forces Institute of Pathology, Washington, DC, p 1–283

Eustis SL, Boorman GA (1985) Proliferative lesions of the exocrine pancreas: relationship to corn oil gavage in the national toxicology program. J Natl Cancer Inst 75: 1067–1073

Fournie JW, Hawkins WE (in press) Neoplasms of the exocrine pancreas. In: Dawe CJ, Harshbarger JC, Wellings SR, Strandberg JD (eds) Pathology of spontaneous and induced neoplasms in fishes: comparative characterization, nomenclature, and literature. Academic Press, San Diego, CA

Fournie JW, Vogelbein WK (1994) Exocrine pancreatic neoplasms in the mummichog (*Fundulus heteroclitus*) from a creosote-contaminated site. Toxicol Pathol 22:237–247

Fournie JW, Hawkins WE, Overstreet RM, Walker WW (1987) Exocrine pancreatic neoplasms induced by methylazoxymethanol acetate in the guppy (*Poecilia reticulata*). J Natl Cancer Inst 78:715–725

Fournie JW, Black JJ, Vethaak AD (1988) Exocrine pancreatic adenomas in the greater redhorse, *Moxostoma valenciennesi* Jordan, and in the European flounder, *Platichthys flesus* (L). J Fish Dis 11:445–448

Grizzle JM, Putnam MR, Fournie JW, Couch JA (1988) Microinjection of chemical carcinogens into small fish embryos: exocrine pancreatic neoplasm in *Fundulus grandis* exposed to N-methyl-N′-nitro-N-nitrosoguanidine. Dis Aquat Org 5:101–105

Hawkins WE, Overstreet RM, Fournie JW, Walker WW (1985) Development of aquarium fish models for environmental carcinogenesis: tumor induction in seven species. J Appl Toxicol 5:261–264

Hawkins WE, Fournie JW, Battalora MJ, Walker WW (1991) Carcinoma of the exocrine pancreas in medaka (*Oryzias latipes*). J Aquat Anim Health 3:213–220

Heidel JR, Rockey DD, Duimstra JR (1992) Acinar cell carcinoma of the pancreas in coho salmon *Oncorhynchus kisutch*. Dis Aquat Org 13:151–154

Lack EE (1989) Primary tumors of the exocrine pancreas. Am J Surg Pathol 13(Suppl 1):66–68

Leake LD (1975) Comparative histology: an introduction to the microscopic structure of animals. Academic Press, New York

Longnecker DS (1983) Early morphologic markers of carcinogenicity in rat pancreas. In: Milmann HA, Sell SS (eds) Application of biological markers to carcinogen testing. Plenum Press, New York, p 43–60

Longnecker DS (1987) The azaserine-induced model of pancreatic carcinogenesis in rats. In: Scarpelli DC, Reddy JK, Longnecker DS (eds) Experimental pancreatic carcinogenesis. CRC Press, Boca Raton, FL, p 117–130

Longnecker DS, Roebuck BD, Yager JD, Lilja HS, Siegmund B (1981) Pancreatic carcinoma in azaserine-treated rats: induction, classification and dietary modulation of incidence. Cancer 47:1562–1572

Longnecker DS, Chandar N, Shearhan DG, Janosky JE, Lombardi B (1991) Preneoplastic and neoplastic lesions in the pancreas of rats fed choline-devoid or choline-supplemented diets. Toxicol Pathol 19:59–65

Luna LG (1968) Manual of histologic staining methods of the armed forces institute of pathology, 3rd edn. McGraw-Hill, New York

Nigrelli RF, Gordon M (1951) Spontaneous neoplasms in fishes. V. Acinar adenocarcinoma of the pancreas in a hybrid platyfish. Zoologica 36:121–126

Otte E (1964) Eine bösartige Neubildung in der Bauchhöhle eines Goldfisches (*Carassius auratus* L.). Wein Tieraerztl Monatsschr 51:485–488

Pour PM (1984) Histogenesis of exocrine pancreatic cancer in the hamster model. Environ Health Persp 56:229–243

Pour PM (1985) Induction of unusual pancreatic neoplasms, with morphologic similarity to human tumors, and evidence for their ductal/ductular cell origin. Cancer 55:2411–2416

Rao MS, Upton MP, Subbarao V, Scarpelli DG (1982) Two populations of cells with differing proliferative capacities in atypical acinar cell foci induced by 4-hydroxyaminoquinoline-1-oxide in the rat pancreas. Lab Invest 46: 527–534

Rao MS, Subbarao V, Scarpelli DG (1987) 4-hydroxy-aminoquinoline-1-oxide-induced acinar cell lesions of the rat pancreas. In: Scarpelli DG, Reddy JK, Longnecker DS (eds) Experimental pancreatic carcinogenesis. CRC Press, Boca Raton, FL, p 107–116

Rao MS, Dwivedi RS, Yeldandi AV, Subbarao V and 7 others (1989) Role of periductal and ductular epithelial cells of the adult rat pancreas in pancreatic hepatocyte lineage. Am J Pathol 134:1069–1086

Reddy JK, Rao MS (1975) Pancreatic adenocarcinoma in inbred guinea pigs induced by N-methyl-N-nitrosourea. Cancer Res 35:2269–2277

Scarpelli DG, Rao MS, Reddy JK (1984) Studies of pancreatic carcinogenesis in different animal models. Environ Health Persp 56:219–227

Thiyagarajah A, Bender MD (1988) Lesions in the pancreas and liver of an oyster toadfish, *Opsanus tau* (L.), collected from the lower York River, Virginia, USA. J Fish Dis 11:359–264

Thiyagarajah A, Grizzle JM (1986) Diethylnitrosamine-induced pancreatic neoplasms in the fish *Rivulus ocellatus marmoratus*. J Natl Cancer Inst 77:141–147

Vogelbein WK, Fournie JW (1994) Ultrastructural features of normal and neoplastic exocrine pancreas in the mummichog, *Fundulus heteroclitus*. Toxicol Pathol 22:248–260

Vogelbein WK, Fournie JW, Van Veld PA, Huggett RJ (1990) Hepatic neoplasms in the mummichog *Fundulus heteroclitus* from a creosote-contaminated site. Cancer Res 50: 5978–5986

Editorial responsibility: Thomas Lang, Cuxhaven, Germany

Submitted: December 19, 2001; Accepted: June 18, 2002
Proofs received from author(s): December 2, 2002