Environmental Health Perspectives
Vol. 56, pp. 213-217, 1984

Proliferative Lesions of the Exocrine Pancreas in Male F344/N Rats

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While the rat pancreas is susceptible to experimental cancer induction, the spontaneous incidence of pancreatic cancer in this species is reported to be very low. However, we observed unusually high incidences of focal acinar hyperplasia and acinar adenoma in vehicle control male F344/N rats of some NCI/NTP 2-year toxicological studies. The vehicle in these studies was corn oil given by gavage.

Focal acinar hyperplasia, acinar adenoma, and acinar carcinoma (found rarely) represent a continuous spectrum of proliferative lesions of the exocrine pancreas. While the carcinomas have clear morphological indications of malignancy, the biological behavior of focal acinar hyperplasia and acinar adenoma is not known. Although induction of acinar carcinomas is considered clear evidence of carcinogenicity of a test chemical, significantly increased incidences in treated rats of acinar adenomas but not carcinomas provides some evidence of carcinogenicity. The association of acinar hyperplasia and adenoma with vegetable oil gavage complicates the interpretation when marginally elevated incidences of these lesions are observed in rats administered the test chemical in vegetable oil vehicle. Studies of the biological behavior of exocrine pancreatic lesions in male rats would be helpful in assessing the significance of their presence when found after test compound administration.

Introduction

While the rat has been used as a model for induction of pancreatic cancer (*1–6*), spontaneous exocrine pancreatic tumors in this species occur infrequently (*7, 8*). The National Toxicology Program's (NTP) interest in pancreatic carcinogenesis was stimulated by observations of unusually high incidences of focal acinar hyperplasia and acinar adenoma in control male F344/N rats receiving corn oil by gavage (vehicle controls of 2-yr toxicological studies). A subsequent microscopic review of pancreata from male F344/N rats comprising vehicle and untreated controls of 37 NCI/NTP 2-yr toxicological studies was performed to evaluate the extent and strength of the association of vegetable oil gavage with proliferative acinar lesions (*9*). Although male rats receiving vegetable oil by gavage had an overall elevated incidence of focal acinar hyperplasia and adenoma compared to untreated rats, the association was inconsistent between groups of vehicle controls. The data indicated that a complex interaction of dietary factors including high lipid intake was likely to influence the incidence of benign proliferative lesions of the exocrine pancreas.

Since the proper classification and interpretation of pathologic lesions in test animals are critical elements in the assessment of chemical toxicity, an ad hoc workgroup of experts in carcinogenesis of the exocrine pancreas was convened by the NTP (*1*) to review the pathology and pathogenesis of chemically induced neoplasia of the exocrine pancreas, (*2*) to consider a classification scheme for proliferative acinar lesions, and (*3*) evaluate the relevance of these lesions in test animals used in 2-yr toxicological studies. This report discusses classification of proliferative acinar lesions adopted for use by NTP pathologists and their possible significance in chronic toxicity tests.

Development of Criteria

Standard criteria for classifying exocrine pancreatic lesions were developed in a pathology review of the NCI/NTP 2-yr study of methylene chloride where exocrine pancreatic lesions were found in male rats. D. Longnecker (Dartmouth Medical School) and J. Reddy (Northwestern Medical School) were instrumental in developing the criteria. Slight modification of criteria occurred during a review of over 2,000 rat pancreata (*9*). Finally, the lesions and criteria were reviewed by the ad hoc workgroup convened by the NTP.

Classification and Criteria: Lesions of the Exocrine Pancreas

The morphological criteria adopted as guidelines for use by NTP pathologists are shown in Table 1.

Focal basophilic cellular change occurs in the pan-

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Table 1. Proliferative lesions of the exocrine pancreas: diagnostic criteria.

Focal cellular change
1. Tinctorial variation from rest of pancreas
2. Generally smaller than islet size
3. No compression or displacement
4. Variable mitotic rate
5. Acinar architecture preserved
6. No capsule
7. Little or no nuclear crowding
8. Variable nuclear pleomorphism
Acinar hyperplasia
1. Variable tinctorial change from rest of pancreas
2. Generally larger than islet size
3. Mild compression or displacement
4. Variable mitotic rate; may be high
5. Mild alteration in acinar architecture
6. No capsule
7. Variable nuclear crowding
8. Variable nuclear pleomorphism
9. Usually contiguous with unaffected parenchyma
Acinar adenoma
1. Sharp demarcation from rest of pancreas (usually a discrete rounded or nodular mass that is not contiguous with unaffected acini)
2. Often 3 mm or larger
3. Compression or displacement is usually present
4. Variable mitotic rate
5. Variable alteration in acinar architecture
6. Capsule helps differentiate, if present
7. Mild to moderate nuclear crowding
8. Variable nuclear pleomorphism
Acinar carcinoma; many features of adenoma usually present
1. May show pleomorphic growth pattern
2. Glandular, trabecular, or solid pattern
3. May have scirrhous reaction
4. Variable vascularity
5. Invasion and/or metastasis are definite indications of malignancy

creas of untreated 2-yr old male F344 rats with an incidence of about 5% (*9*). These are sharply defined clusters of acini comprised of hypertrophied cells with altered cytologic features. The cytoplasm of affected acinar cells contains an increased amount of basophilic staining rough endoplasmic reticulum and the zymogen granules are decreased in number and/or show decreased eosinophilia. The acinar cell nuclei are parabasal in location, enlarged, and usually have prominent nucleoli (Fig. 1).

A similar lesion described as "basophilic-atypical acinar cell focus" is seen in pancreata of male rats given 4-hydroxyaminoquinoline-1-oxide (4-HAQO) (*6*). The mitotic index and nuclear uptake of ^{3}H-thymidine by acinar cells in these basophilic foci were not substantially different from normal acinar cells, thus seeming to indicate a low growth potential (*6*). The authors concluded that the "basophilic focus" induced by 4-HAQO would not progress to tumor formation.

Based on our review of pancreata in male F344 rats we also believe that focal basophilic cellular change is not part of a morphological sequence resulting in acinar tumors. The cytologic appearance of these acinar cells is consistent with an altered capacity for, or a defect in, the synthesis of secretory proteins.

Foci of acinar hyperplasia have morphological features indicating cell proliferation and expansive growth. The lesion generally is spherical or oval, circumscribed and causes slight compression of adjacent acini (Fig. 2). Within foci of hyperplasia the affected acini show varied degrees of cellular crowding and/or enlargement of the acini which imparts a more prominent tubular pattern.

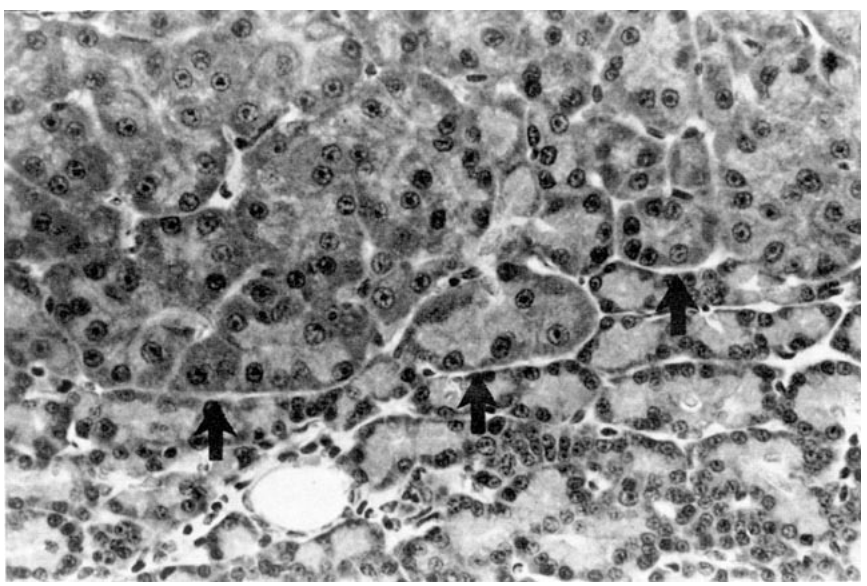

FIGURE 1. Focal basophilic cellular change. Affected cells are in the upper portion of the photomicrograph and the boundary of the focus is delineated by arrows.

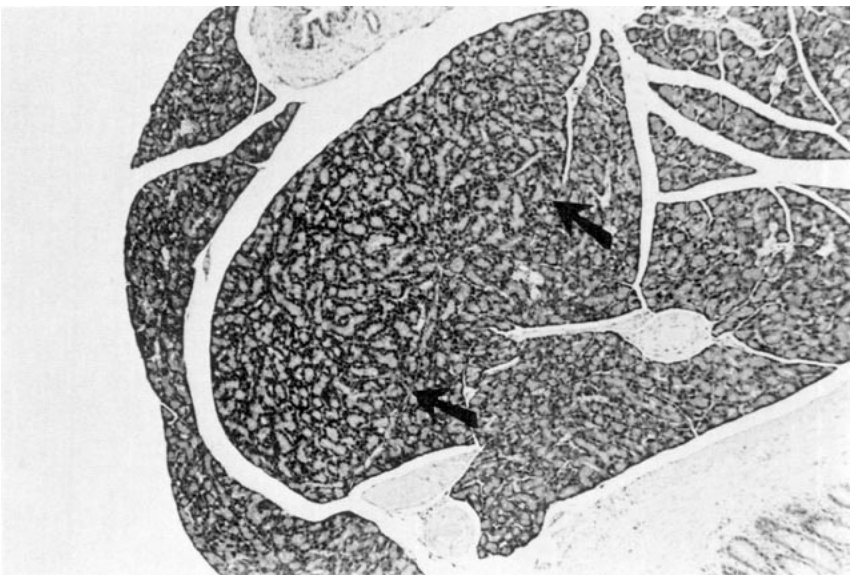

FIGURE 2. Focal acinar hyperplasia. Focus is delineated by arrows.

The acinar cells may be similar in size or larger than normal cells, and frequently have large nuclei with prominent nucleoli. The relative amount of basophilic cytoplasm in the basal portion of the cells is similar to normal acinar cells but the apical cytoplasm often contains an increased amount of zymogen granules. Cells in mitosis usually are more frequent in foci of hyperplasia than unaffected parenchyma and there may be necrosis of scattered, individual cells which show nuclear pyknosis and karyorrhexis. The degree and extent of the cytologic alterations vary among foci of hyperplasia which presumably reflect differing rates of cells proliferation and turnover.

Similar lesions occur as part of the sequence of morphological changes which precede the development of acinar cell carcinomas in the rat induced by azaserine (*1,2*) and 4-HAQO (*6*). These have been called atypical acinar cell foci and nodules (*10,11*) and acidophilic foci and nodules (*6*). In the azaserine model less than 1% of these lesions was estimated to become neoplasms (*1*). Because of the high rate of regression we prefer the more committal term (relative to biological behavior) of hyperplasia to atypical acinar cell focus or nodule.

There are no clear-cut histological features to distinguish acinar adenoma from focal hyperplasia. These comprise a spectrum of proliferative lesions from foci less than 1 mm in diameter to multilobulated tumors greater than 5 mm in diameter. To provide consistency among the pathologists that evaluate toxicity studies for the NTP, we have adopted size (greater than 3 mm in diameter) as a major criterium for diagnosis of adenoma. Adenomas are composed of well-differentiated acinar cells which maintain a relatively high but variable degree of organization as a compound acinar gland (Fig. 3). As adenomas increase in size the acinar cells show progressively greater deviation in structural arrangement from normal. This consists of a branching tubular pattern with minimal lumenal space. Although centroacinar cells and small ducts are present in small adenomas, they are less frequent or absent in large ones.

Acinar carcinomas are infrequent in the F344 rat (*7,9*), but have histologic features of malignancy including heterogeneous growth pattern, loss of acinar architecture, and cellular anaplasia and pleomorphism. Within a single carcinoma the acinar cells may be arranged in glandular and trabecular patterns, or in solid sheets (Fig. 4). Acinar cells within the solid areas show the greatest degree of anaplasia, but zymogen granules usually are present. There is little disagreement regarding the malignant nature of this lesion. We have not observed ductular carcinomas in the F344 rat (*9*).

Discussion

The NTP interprets pathology data from chronic toxicity studies conducted by many laboratories. Since the histopathological evaluations are performed by more than 50 pathologists, standard nomenclature and uniformly applied morphological criteria are essential for accurate assessment of chemical toxicity and meaningful comparison of studies conducted at different laboratories. As additional knowledge concerning the pathology and pathogenesis of chemically induced lesions is gained, it should be applied to refining the morphological criteria already in use. Substantial information concerning chemical carcinogenesis of the exo-

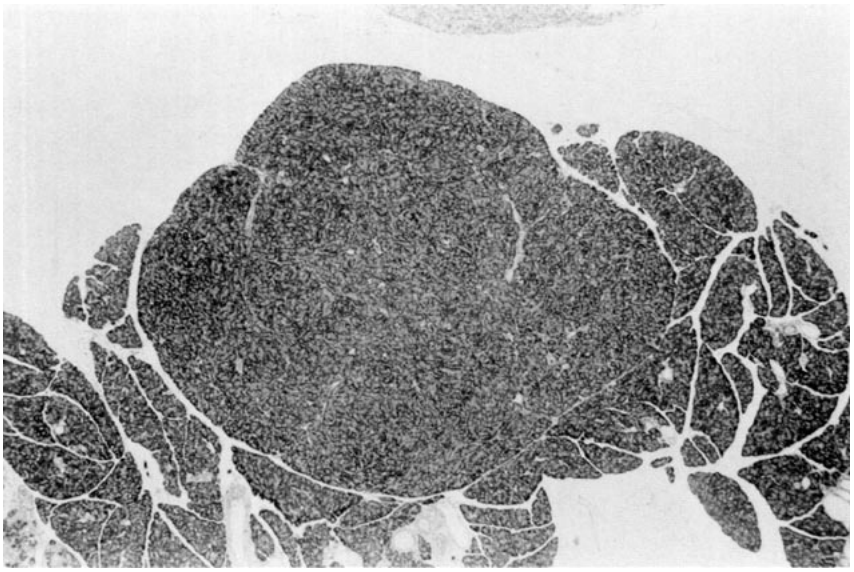

FIGURE 3. Acinar adenoma. Tumor consists of well-differentiated acinar cells which maintain a high degree of organization.

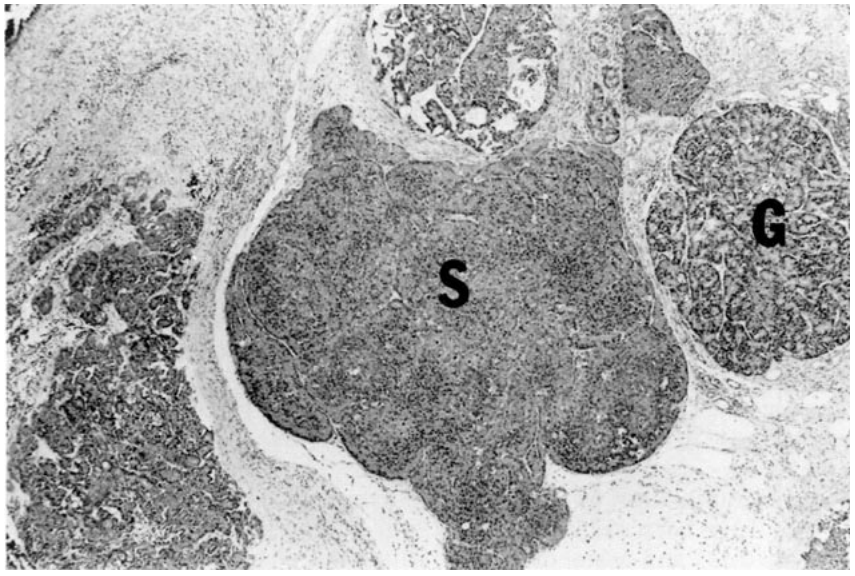

FIGURE 4. Acinar carcinoma. Note pleomorphic growth pattern with solid (S) and glandular (G) areas.

crine pancreas has been accumulated in recent years with the use of rodent models, and we have attempted to define criteria for proliferative lesions of the exocrine pancreas (of the rat) that is consistent with this information.

The morphological criteria for diagnosis of acinar carcinoma are similar to those for diagnosis of malignant neoplasms in other organs. Since any degree of anaplasia is rare in spontaneous proliferative acinar lesions, a chemical that is associated with a significantly increased incidence of acinar carcinoma in test rats is considered carcinogenic for the pancreas.

While the biological potential of acinar hyperplasia and acinar adenoma is uncertain, it is apparent from research in rodent models that at some point (ill-defined by morphological criteria) some hyperplastic lesions progress to adenomas, and some adenomas progress to carcinomas. In the azaserine model the frequency and size of atypical acinar-cell nodules observed early correlated positively with the incidence of carcinoma which appeared between 1 and 2 yr after beginning treatment (*1*). These authors estimated that less than 1% progressed to neoplasia. Thus, a significantly increased incidence of acinar adenomas in chemically treated rats is considered to provide some evidence of carcinogenicity.

The association of acinar hyperplasia and adenoma with corn oil gavage may complicate interpretation when marginally elevated incidences of these lesions are observed in rats administered the test chemical in vegetable oil vehicle. A high dietary level of fat has been reported to enhance chemically induced carcinogenesis of the pancreas (*1*) as well as the mammary gland (*12,13*) and colon (*14,15*). In the azaserine-rat model 20% corn oil or 20% safflower oil increased the incidence of pancreatic neoplasms when fed during the post-initiation phase (*11*). Although it was suggested that a high dietary level of unsaturated fat may act as a promotor, additional research to determine the dose-response effect of essential fatty acid intake on tumor incidence is necessary before these conclusions can be confirmed.

The authors thank Ms. Beth deBrito for secretarial assistance.

REFERENCES

1. Longnecker, D. S., Roebuck, B. D., Yager, J. D., Jr., Lilja, H. S., and Siegmund, B. Pancreatic carcinoma in azaserine-treated rats: induction, classification, and dietary modulation of incidence. *Cancer* 47: 1562–1572 (1981).
2. Konishi, Y., Denda, A., Marugama, H., Yoshimura, H., Nobuoka, J., and Sunagawa, M. Pancreatic tumors induced by a single intraperitoneal injection of azaserine in partial pancreatectomized rats. Cancer Letters 9: 43–46 (1980).
3. Dissin, J., Mills, L. R., Mainz, D. L., Black, O., Jr., and Webster, P. D. Experimental induction of pancreatic adenocarcinoma in rats. J. Natl. Cancer Inst. 55: 857–864 (1975).
4. Hayashi, Y., and Hasegawa, T. Experimental pancreatic tumor in rats after intravenous injection of 4-hydroxy-aminoquinoline-1-oxide. Gann 62: 329–330 (1971).
5. Hayashi, Y., Furukawa, H., and Hasegawa, T. Pancreatic tumors in rats induced by 4-nitroquinoline-1-oxide derivatives. In: Topics *in Chemical Carcinogenesis (W. Nakahara, S. Takayama,* T. Sugimura and S. Odashima, Eds.), University Park Press, Baltimore, 1972, pp. 53–65.
6. Rao, M. S., Upton, M. P., Subbaro, W., and Scarpelli, D. G. 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 (1982).
7. Rowlatt, U., and Roe, F. J. Epithelial tumors of the rat pancreas. J. Natl. Cancer Inst. 39: 17–32 (1967).
8. Milman, H. A., Ward, J. M., and Chu, K. C. Pancreatic carcinogenesis and naturally occurring pancreatic neoplasms of *rats and mice in the NCI carcinogenesis testing program.* J. Environ. Pathol. Toxicol. 1: 829–840 (1978).
9. Eustis, S. L., and Boorman, G. A. Proliferative lesions of the exocrine pancreas. Relationship to corn oil gavage in the NTP carcinogenesis testing program. In preparation.
10. Longnecker, D. S., Lilja, H. S., French, J. I., Kuhlman, E., and Noll, W. W. Transplantation of azaserine-induced carcinomas of pancreas in rats. Cancer Letters 7: 197–202 (1979).
11. Roebuck, D., Yager, J. D., Jr., Longnecker, D. S., and Wilpone, S. A. Promotion by unsaturated fat of azaserine-induced pancreatic carcinogenesis in the rat. Cancer Res. 41: 3961–3966 (1981).
12. Carroll, K. K. Lipids and carcinogenesis. J. Environ. Pathol. Toxicol. 3: 253–271 (1980).
13. Hopkins, G. J., and West, C. E. Possible roles of dietary fats in *carcinogenesis. Life Sci. 19: 1103–1116 (1976).*
14. Bull, A. W., Soullier, B. K., Wilson, P. S., Hayden, M. T., and Nigro, N. D. Promotion of azoxymethane-induced intestinal cancer by high-fat diet in rats. Cancer Res. 39: 4956–4959 (1979).
15. Reddy, B. S., Narisawa, T., Maronpot, R., Weisburger, J. H., and Wynder, E. L. Animal models for the study of dietary factors and cancer of the large bowel. Cancer Res. 35: 3421–3426 (1975).