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Intraductal Proliferation in the Pancreas and Its Relationship to Human and Experimental Carcinogenesis

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Summary. In 21 patients who had undergone total pancreatectomy for pancreatic head carcinoma, the uninvolved pancreas was examined with regard to the type, incidence and regional distribution of duct epithelial proliferation. The results were compared with those in 37 operative specimens from patients with chronic pancreatitis, in 46 normal pancreases from autopsies and with findings in experimental pancreatic carcinogenesis.

While the incidence of squamous metaplasia and non-papillary epithelial hypertrophy varied little in the different groups, papillary epithelial hyperplasia was found three times more often in cases of carcinoma, with associated mild duct obstruction. Atypical epithelial proliferation was only detected in the vicinity of carcinomas. Unequivocal transition from papillary hyperplasia to atypical proliferation was not observed. In hamsters treated with dihydroxy-di-n-propylnitrosamine (DHPN) for induction of pancreatic duct carcinomas, the early duct lesions closely resembled atypical epithelial proliferation of human pancreas.

It is concluded that (1) papillary epithelial hyperplasia is probably only indicative of early duct obstruction and/or a general neoplastic stimulus, (2) intraductal epithelial proliferation with atypia is a true precursor of duct carcinoma, and (3) chronic pancreatitis lacks atypical duct lesions.

Key words: Pancreatic duct proliferations – Normal pancreas – Pancreatic carcinoma – Chronic pancreatitis – Pancreatic carcinogenesis in man and animals.

Introduction

Proliferative changes in the duct epithelium of the pancreas are of considerable interest in the study of the development of carcinomas, since by far the majority

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of pancreatic carcinomas derive from ducts and may therefore be preceded by precancerous duct lesions. Indeed, several authors have reported on the frequent association of epithelial hyperplasias of the pancreatic ducts with pancreatic carcinomas (Sommers et al., 1954; Cubilla and Fitzgerald, 1976; Kozuka et al., 1979).

In a preliminary report, we have also found a similar relationship (Klöppel et al., 1979) which was not obvious in chronic pancreatitis. We now have extended this study, not by increasing the absolute number of cases, but by focusing only on those cases from which enough tissue specimens from different regions of the pancreas were available, since it proved to be necessary to know exactly the regional distribution of the lesions. To avoid shedding of duct epithelia into the lumen with early autolysis of the cells, we only used surgical specimens of autopsy material obtained within 2 to 4 h after death.

In its second part the study deals with the question of whether the duct changes most frequently correlated with human pancreatic carcinoma are comparable with those in hamsters treated with dihydroxy-di-n-propylnitrosamine (DHPN) in order to induce pancreatic duct carcinomas, as was first reported by Pour et al. (1974).

Material and Methods

Tissue Collection. From the pancreases of 21 patients (mean age: 56.7 y) who had undergone total pancreatectomy because of pancreatic head carcinoma, 5 to 10 tissue samples were taken from the tumour and another 5 to 10 from the remaining non-tumourous parts of the pancreas. The regional distribution of the samples were indicated according to a scheme proposed by Orci et al. (1978).

From 37 patients (meang age: 43.2 y) who had undergone partial ($n=35$) or total ($n=2$) pancreatectomy because of chronic recurrent pancreatitis, 5 to 10 samples were taken from various parts of the pancreas.

From 46 autopsy cases without pancreatic disease the total pancreas ($n=6$; mean age: 55 y) or parts ($n=40$; mean age: 43.2 y) of it were taken within 2 to 4 h after death. 5 to 10 samples were obtained from the tissue obtained in this way, whereas 16 samples were collected from the whole glands.

Histology. The samples were fixed in Bouin's solution, embedded in paraffin and dehydrated in alcohol. At least 3 sections were prepared from each tissue block and stained with haematoxylin and eosin, periodic acid Schiff (PAS) and aldehyde fuchsin.

Typing of Ductal Changes. In general four main types of epithelial changes can be distinguished in the pancreatic ducts. The terms used to designate these lesions partly follow the classification introduced by Kozuka et al. (1979). (1) Mature squamous metaplasia with its immature variant (basal cell metaplasia; Korpassy, 1939; focal epithelial hyperplasia; Cubilla and Fitzgerald, 1976), (2) non-papillary epithelial hypertrophy and hyperplasia with pyloric gland metaplasia (Roberts, 1974; mucus cell hypertrophy, Cubilla and Fitzgerald, 1976), (3) papillary epithelial hyperplasia with hypertrophy (ductal papillary hyperplasia, Cubilla and Fitzgerald, 1976), (4) atypical epithelial proliferation or intraductal carcinoma (carcinoma in situ, Cubilla and Fitzgerald, 1976). All these lesions overlapped and were often associated with a focal proliferation of ducts (adenomatous duct hyperplasia, Sommers et al., 1954).

Animal Experiments. Twenty 10 to 12 weeks old female Syrian golden hamsters, weighing 140–160 g, received 2,2′-dihydroxy-di-n-propylnitrosamine (DHPN; synonyma: BHP-n-nitroso-bis(2-hydroxy-

propylamine)-diisopropanolnitrosamine) by weekly i.g., administration for 15 weeks (125 mg/kg body weight). The DHPN was suspended in olive oil. The animals were sacrificed 20 weeks after the first application. The pancreas, attached to duodenum and the spleen was completely removed and fixed in Bouin's solution. The whole organ was processed for conventional histology. 6 and more step sections were either stained with haematoxylin and eosin or with PAS.

Results

Normal Pancreases

The normal duct epithelium, which was well preserved, consisted of cuboidal cells with an apical mucin layer (Fig. 1 a). Focal squamous metaplasia occurred in some medium sized ducts. Its occurrence was independent of any ductal scarring. Non-papillary epithelial hypertrophy (Fig. 1 b) characterized by the appearance of tall columnar cells filled with mucin in their apical portion, was found in medium sized and small ducts. It proved to be by far the most frequent change of the epithelium and was seen in almost every pancreas when material from all regions was available. Papillary epithelial hyperplasia was characterized by papillary folds of hypertrophied epithelium with extensive mucus production. They were most often seen in large and medium sized ducts. All lesions were located predominantly in the head of the pancreas, followed by the tail and the body. No atypical epithelial proliferations were observed. The frequency of the epithelial changes are listed in Table 1, in which the values from the total pancreases are given in parentheses.

Pancreases with Pancreatic Carcinomas

In all 21 cases the pancreatic head carcinomas occluded the main pancreatic duct to a variable extent. The histological finding was ductal adenocarcinoma, showing tubular structures of varying sizes. The cuboidal tumour cells showed scanty mucus production. These proliferative cells occasionally formed papillary and cribriform intraluminal projections. The carcionomatous glands invaded the surrounding pancreatic tissue mainly along the interlobular septa, but also by spreading along the pancreatic ducts or occasionally by encroaching on the bile duct.

The non-tumourous parts of the pancreas, i.e., the body and tail, showed a chronic obstructive pancreatitis of varying severity as a result of the duct obstruction. In the body and the tail, squamous metaplasia and non-papillary epithelial hypertrophy were as frequent as in controls. Papillary epithelial hyperplasia, however, was more often encountered than in controls. When it was related to the degree of the obstructive pancreatitis, papillary hyperplasia appeared to be rather more associated with mild to moderate pancreatitis than with the severe form (Fig. 2). In 6 out of 21 pancreases single ducts contained atypical epithelial proliferations, immitating papillary hyperplasia (Table 1) (Fig. 3). The proliferating cells showed nuclei of varying sizes, hyperchromatism and location (Fig. 4). These cells formed small buds and folds with occasional

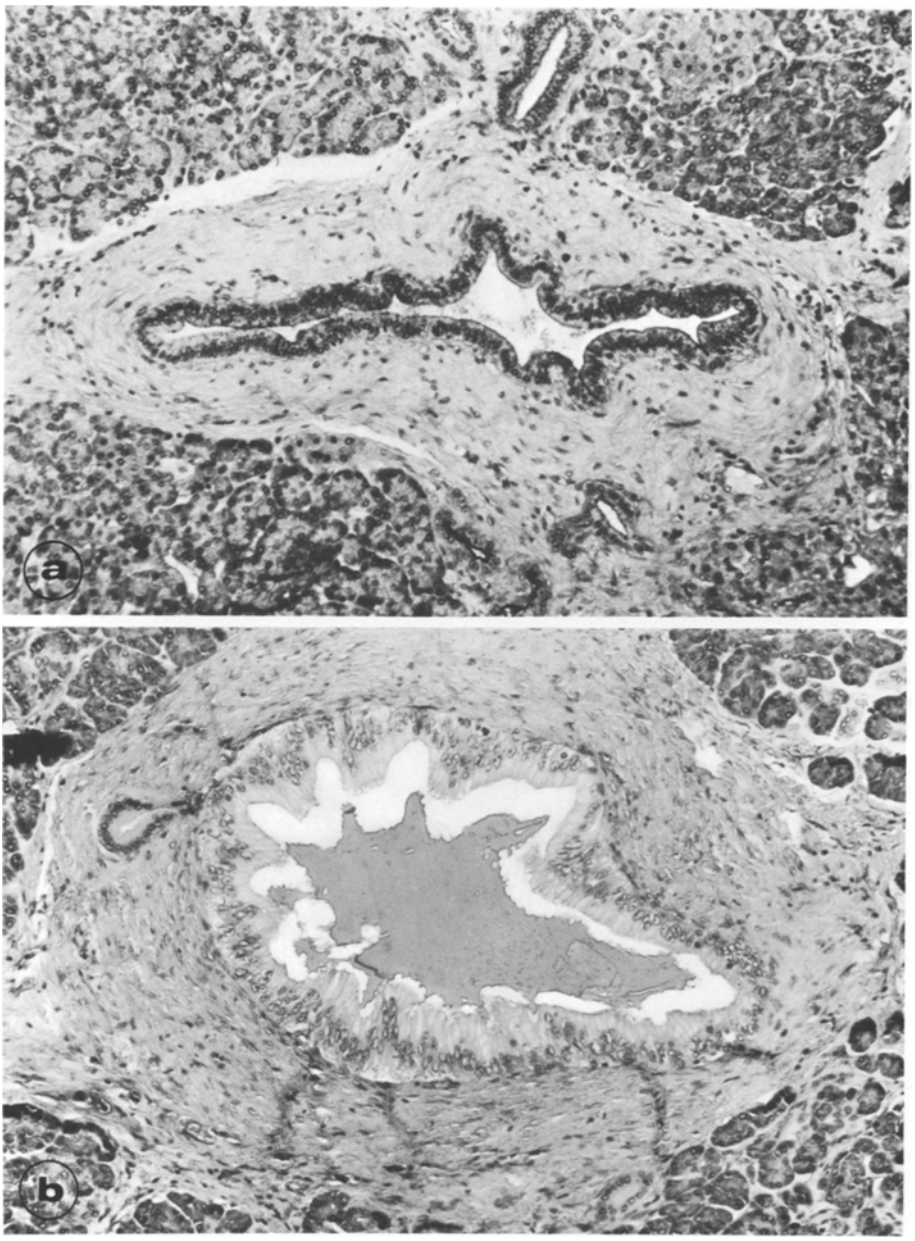

Fig. 1 a and b. Normal pancreas. **a** Medium sized duct lined by cuboidal cells with a small apical mucin layer. PAS. × 140. **b** Medium sized duct with non-papillary epithelial hypertrophy. HE. × 140

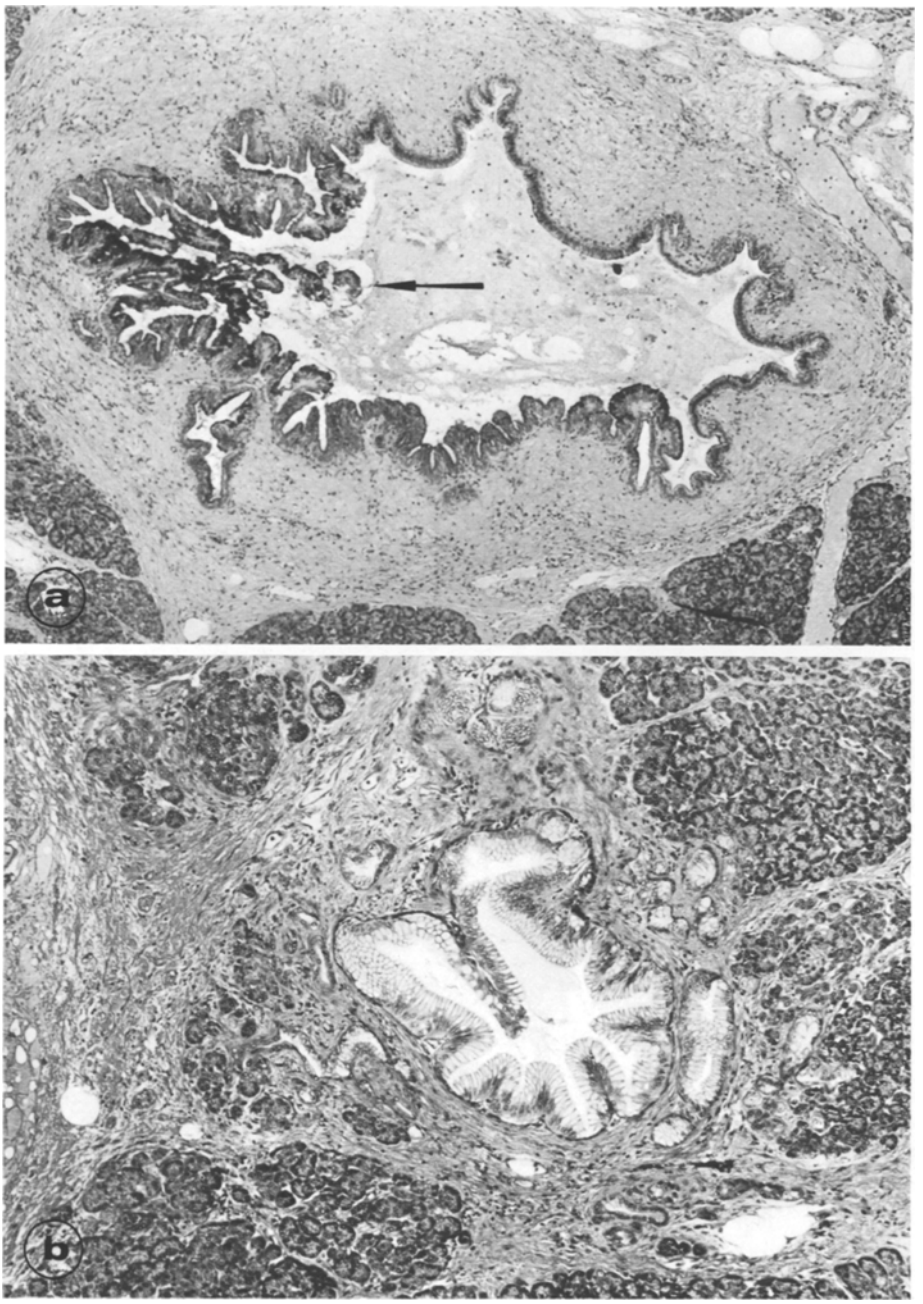

Fig. 2a and b. Mild (**a**) to moderate (**b**) chronic obstructive pancreatitis secondary to carcinoma. **a** Main duct from pancreatic tail with papillary epithelial hyperplasia (*arrow*). PAS. ×58. **b** Medium sized duct from pancreatic body with epithelial hypertrophy and papillary hyperplasia. HE. ×115

Table 1. Frequency of epithelial proliferations in normal pancreas, in association with pancreatic carcinoma and in severe chronic pancreatitis

Epithelial duct proliferations	Normal pancreas	Associated with carcinoma	Chronic pancreatitis
Squamous metaplasia	14% (21%)	19%	11%
Non-papillary epithelial hypertrophy	56% (83%)	58%	68%
Papillary epithelial hyperplasia	10% (20%)	49%	11%
Atypical epithelial proliferation	– (–)	28%	–

The results from 6 total normal pancreases are given in parentheses

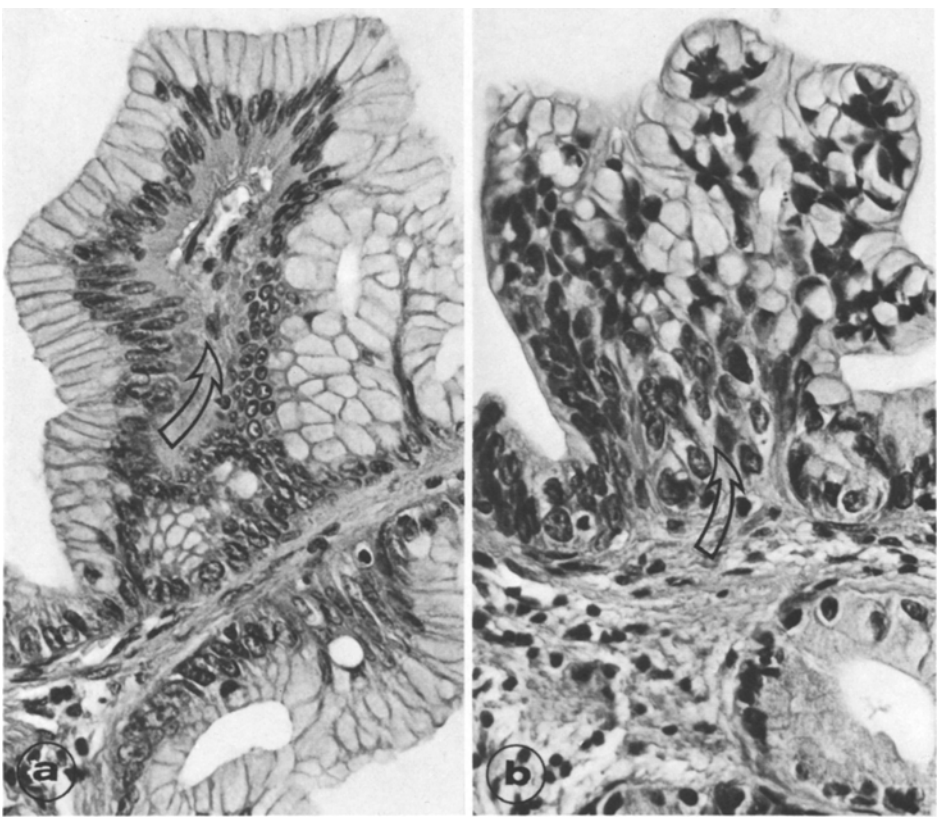

Fig. 3a and b. Comparison of paillary epithelial hyperplasia (**a**) and atypical epithelial proliferation immitating papillary hyperplasia (**b**). Note the regularly arranged epithelium supported by a connective tissue stalk (*arrow*) in **a** and the marked cell atypia (*arrow*) in **b**. HE. ×350

fibrous stalk or a sometimes multilayered epithelium with a cribriform pattern (Fig. 4). When these proliferations with unequivocal atypia were analysed with regard to their regional location in the pancreas, they were found exclusively within a distance of 1 to 3 cm of the tumour edge. On step sections the atypical proliferations appeared to be directly connected with the tumour through the

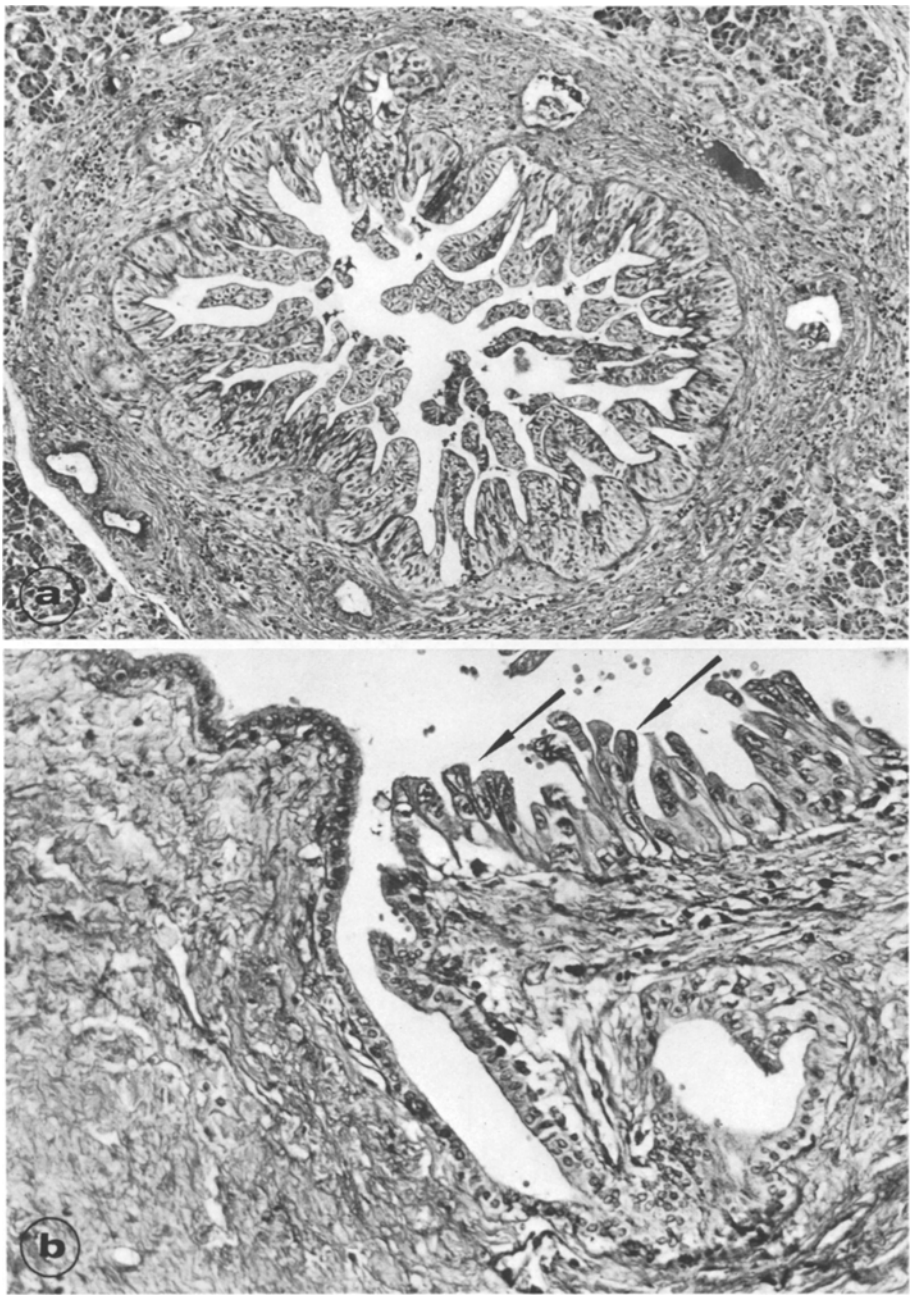

Fig. 4. **a** Atypical epithelial proliferation (intraductal carcinoma) in the vicinity of a pancreatic carcinoma. HE. ×115. **b** Atypical epithelium (*arrows*) encroaching on normal duct cells. HE. ×250

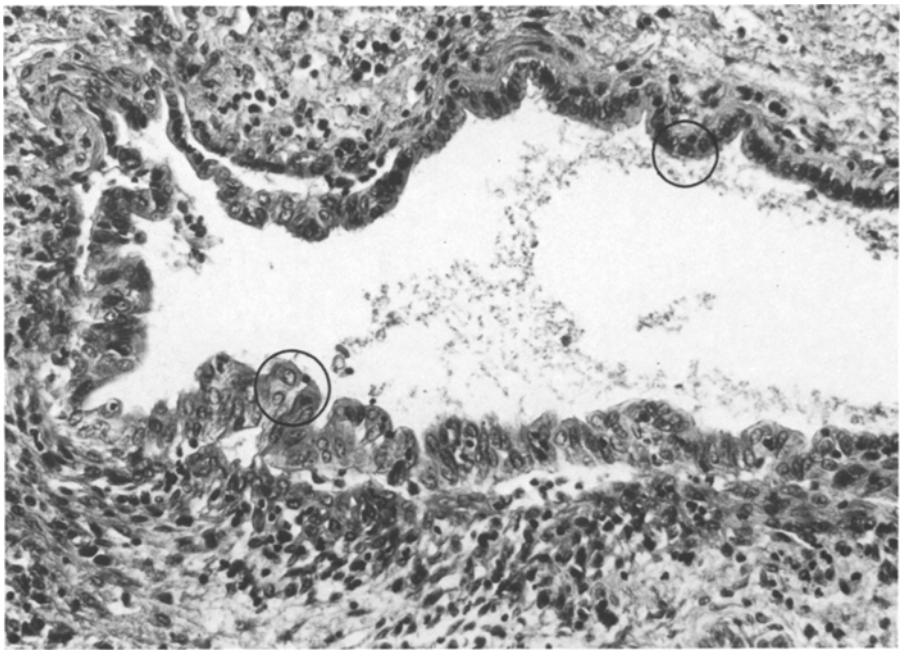

Fig. 5. Pancreatic duct in chronic pancreatitis. An epithelial defect is repaired by a new layer of duct cells (*large circle*). Opposite the regenerating duct cells normal epithelium is seen (*small circle*). PAS. ×250

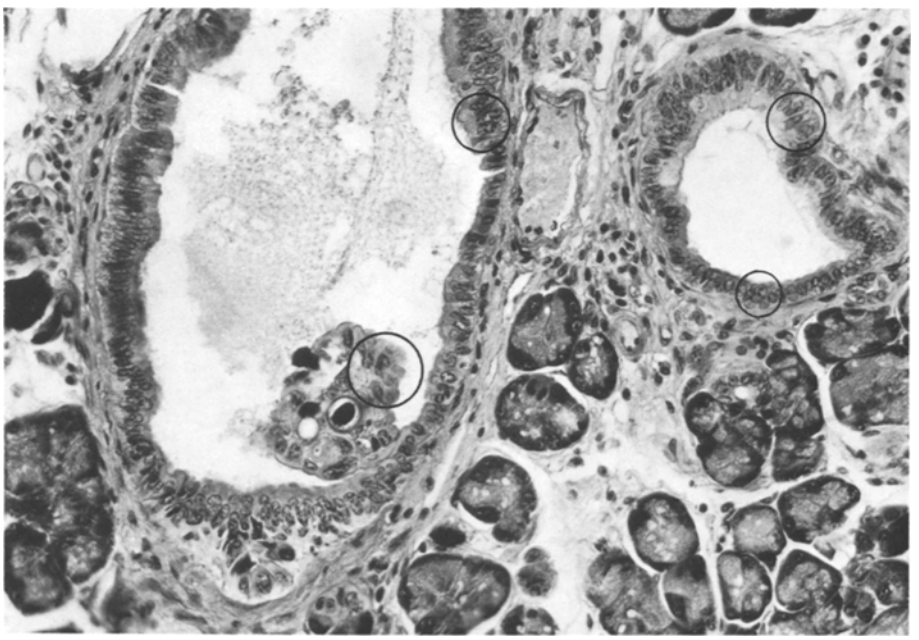

Fig. 6. Precancerous duct lesion in the hamster pancreas after treatment with DHPN. Smooth transitions are seen from normal epithelium (*small circle*) to hypertrophied cells with moderate atypia (*medium circle*) and to a focal intraluminal proliferation of markedly atypical cells (*large circle*). PAS. ×250

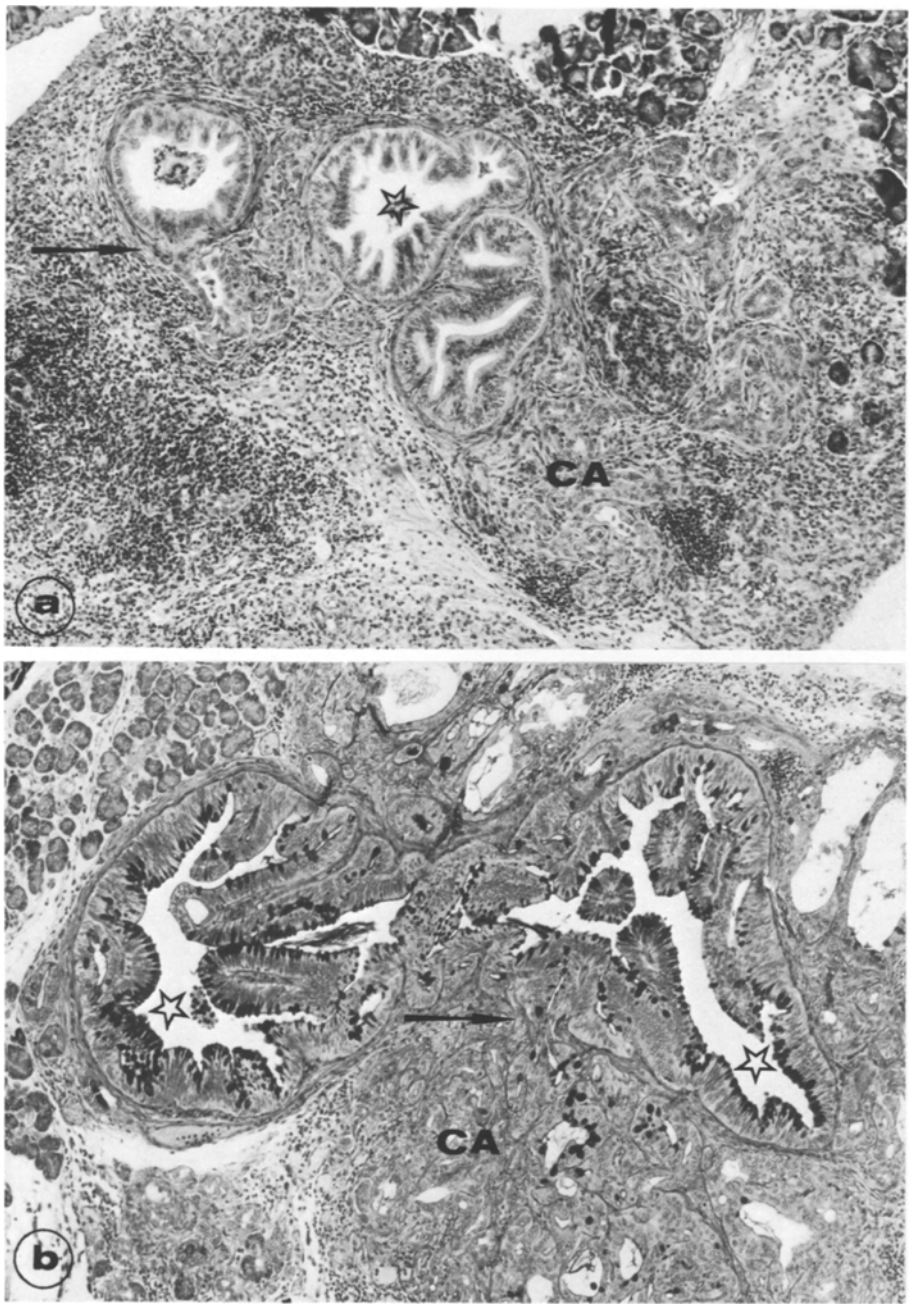

Fig. 7a and b. Atypical, partly papillary epithelial proliferations in a large pancreatic duct (*asterisk*) (**a**) and the common pancreatic duct (*asterisk*) (**b**) of the hamster pancreas after treatment with DHPN. The duct lesions are surrounded by invasive duct carcinomas (*CA*). Note the invasion of atypical epithelium through the basal lamina (*arrows*). HE and PAS. ×115

duct, thus they most likely represented intraductal branches of the tumour. Atypical proliferations at a distance of more than 3 cm, or multifocal intraductal carcinomas without connection with the tumour were not observed in our material.

Pancreases with Chronic Pancreatitis

All of the pancreatic specimens showed a severe chronic sclerosing pancreatitis. The duct epithelium was focally destroyed due to calcified protein plugs in the ducts. The regenerating epithelium formed lines of irregular cells, sometimes resembling the epithelium seen in atypical proliferations (Fig. 5). However, severe atypical alterations as seen in association with carcinomas were not observed (Table 1). The other epithelial changes, i.e., squamous metaplasia, non-papillary epithelial hypertrophy and papillary hyperplasia, were as frequent as in controls. Moreover, it seemed that their frequency decreased with increasing scarring of the tissue.

Hamster Pancreas after DHPN-Treatment

The anatomy of the trilobulated hamster pancreas has been described (Takahashi et al., 1977a). After DHPN-treatment proliferative changes of the duct epithelium (precanceroses) and invasive ductal carcinomas occurred at varying frequency and localization in each animal. A closer topographical analysis revealed that the proliferations originated mainly from the epithelium either of the larger ducts in the three pancreatic lobes, or the common duct in the pancreatic head. The focal precancerous proliferations had a complex pattern (for detailed description see Takahashi et al., 1977b). The most important steps on the way to definite carcinoma were as follows: Focal hypertrophy of duct epithelium with occasional goblet cell metaplasia and mild atypia (Fig. 6), papillary infolding and irregular stratification of epithelium with marked cell atypia (intraductal carcinoma; Fig. 6), encroaching of atypical epithelium on joining interlobular ducts, with breaking through the basal lamina and invasion of periductal areas by atypical tubular glands (Fig. 7). Single carcinomas appeared also to develop from small cystic adenomas, originating from intralobular ductules adjacent to the islets. These carcinomas often had the features of cystadeno-carcinomas.

Discussion

Of the various proliferative changes of pancreatic duct epithelium, papillary epithelial hyperplasia was the type found to occur most frequently in association with pancreatic carcinoma when compared with controls (Sommers et al., 1954; Cubilla and Fitzgerald, 1976; Kozuka et al., 1979). Our data from a series of total pancreatectomy specimens largely confirm these results.

The considerable increase in papillary epithelial hyperplasia associated with pancreatic carcinoma was still apparent when the data were compared with those from six total pancreases of age-matched controls, where the pancreatic regions have been clearly defined. Although papillary epithelial hyperplasia,

which was predominantly detected in the pancreatic head, was increased from 10% to 20%, when compared with the original control series, it did not reach the 58% found in our carcinoma series. Non-papillary epithelial hyperplasia, on the other hand, appeared to be a very common focally accentuated change in the normal pancreas of middle aged man. In controls where the whole gland was available for examination its incidence was 83%, against 56% in the first control series. Thus, within a defined age group, differences in the incidence of non-papillary epithelial hyperplasia (Kozuka et al., 1979) or lesions included under this heading (mucous cell hypertrophy, pyloric gland metaplasia; Cubilla and Fitzgerald, 1976) are most likely due to the different quantitiy of tissue and slides evaluated.

What causes the increased frequency of papillary hyperplasias associated with carcinomas? Papillary hyperplasia could be secondary to the duct occlusion by carcinoma or it might be due to a primary neoplastic change of duct epithelium, which eventually gives rise to the development of a carcinoma. The negative correlation between the severity of obstructive pancreatitis and the occurrence of papillary epithelial hyperplasia, the low incidence of this lesion in severe chronic pancreatitis and its occasional demonstration in otherwise normal pancreases speak in favour of the latter hypothesis. However, it is also remarkable that papillary epithelial hyperplasia is so often associated with mild obstructive pancreatitis, as if it is the first response of the epithelium to a disturbed outflow of pancreatic juice, – a response, which later, if the obstruction persists, slowly recedes. From this point of view papillary epithelial hyperplasia would appear to be secondary to the beginning of obstruction. Since both explanations of the cause of papillary epithelial hyperplasia seem to be possible it is most likely that papillary hyperplasia represents a uniform alteration of the duct epithelium in response to different stimuli, including factors such as obstruction as well as carcinogens. This view was also expressed by Cubilla and Fitzgerald (1976).

It is suggested that pancreatic carcinomas may originate from papillary epithelial hyperplasia and may be multifocal in development. The finding of papillary epithelial hyperplasia, intraductal carcinoma and invasive carcinoma together in the same specimen, suggesting transition from one to another, has been reported by several authors (Sommers et al., 1954; Cubilla and Fitzgerald, 1976; Kozuka et al., 1979). In this study, which was based on strict criteria for atypia, we have not found papillary hyperplasia with true cellular atypia. However, we observed atypical epithelial proliferations with a papillary, partly cribriform pattern which could be clearly distinguished from papillary epithelial hyperplasia by the absence of a fibrous tissue stalk within the epithelial folds. From its histological features, atypical epithelial proliferation represents an intraductal carcinoma. As we were unable to find any unequivocal transition between papillary epithelial hyperplasia and intraductal carcinoma, we believe that the duct carcinoma originates from hypertrophied epithelium, characterized by metaplastic mucus production, and imitates its benign counterpart, papillary epithelial hyperplasia in its early steps of development.

Further evidence along this line came from the study of pancreatic duct carcinomas in Syrian hamsters, treated with DHNP, as introduced by Pour

et al. (1974). Evaluation of the main sequential alterations leading to invasive carcinoma in the hamsters revealed atypical hypertrophy of the epithelium in larger ducts as the initial morphological event in experimental carcinogenesis. The process proceeded to papillary projections and irregular infoldings into the ductal lumen. These features, which characterized this lesion as intraductal carcinoma, closely resembled the atypical epithelial proliferation seen in humans. In the hamsters the atypical epithelium later encroached on adjacent ducts and broke through the basal lamina, thereby becoming an invasive adenocarcinoma.

Multicentricity of the precancerous and cancerous lesions in hamsters was a common finding in both this and other studies (Althoff et al., 1976; Levitt et al., 1977; Pour et al., 1977). In man, similar findings have been reported by several authors (Ross, 1954; Pliam and Remine, 1975; Cubilla and Fitzgerald, 1976; Ihse et al., 1977; Tryka and Brooks, 1979). When we related the atypical epithelial proliferations to the pancreatic region where they were taken from, the lesions could always be located within a distance of 1 to 3 cm to the tumour mass. Moreover, in some cases serial sections clearly showed intraductal connection of the atypical proliferations with the tumour margin, suggesting that the atypical intraductal proliferation represented intraductal branches of the tumour rather than multicentric tumour foci. The intraductal branches of the tumour reached beyond the usual resection line of a Whipple procedure (Brooks and Culebras, 1976; Sato et al., 1978; Hicks and Brooks, 1971). In contrast to other authors however (Ross, 1954; Pliam and Remine, 1975; Cubilla and Fitzgerald, 1976; Kozuka et al., 1979) we did not observe true multifocal tumours in the remaining pancreas. The reason for this discrepancy is currently not known, but should be clarified in larger series.

Chronic pancreatitis has been considered to be forerunner of pancreatic carcinoma (Becker, 1978) and a high frequency of mild to moderate epithelial dysplasia has been found in chronic pancreatitis (Volkholz et al., 1979). We found neither an increased incidence of papillary epithelial hyperplasias nor could we observe any atypical epithelial proliferation in operative specimens with chronic pancreatitis. Thus, from this purely morphological point of view we do not see any causative relationship between chronic pancreatitis and the development of pancreatic carcinoma.

In conclusion, our study confirmed and substantiated the high frequency of papillary epithelial hyperplasia in association with carcinomas, which may be due to early obstruction in concert with a general neoplastic stimulus. While there were smooth transitions from non-papillary to papillary epithelial hyperplasia, unequivocal transformations of papillary hyperplasia to atypical epithelial hyperplasia were lacking. Atypical epithelial proliferation, a lesion by which pancreatic carcinomas often spread along the ducts, seems therefore to be a *de novo* precursor of duct carcinoma. In experimental pancreatic carcinogenesis, almost identical lesions were found to be forerunners of invasive carcinomas. On the basis of those common features it is suggested that pancreatic carcinoma in human originates directly from atypical epithelium proceeding to an intraductal carcinoma before invading the periductal tissue.

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