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Occurrence of pancreatic ductal cell dysplasia in rats fed with a high fat diet and ethanol

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Summary. The effects of alcohol and diet on acute pancreatitis were studied in 192 male Wistar rats. The animals were fed with standard laboratory food up to three months of age and, after that, were divided into four groups of 48 animals, each group receiving a different diet: standard, fat-rich, protein-rich or carbohydrate-rich. In each diet group, 24 animals obtained 15% (v/v) ethanol in their drinking solution while the other 24 rats had water ad libitum. The diet period lasted for 12 weeks, after which acute experimental pancreatitis was induced under diethyl ether anesthesia by ductal injection of rat bile into the pancreatic ducts. Moderate or severe ductal cell dysplasia developed in three of the 15 survivors in the group fed with a high-fat diet and 15% ethanol in their drinking solution. Mild acute pancreatitis was histologically found in 13 rats and moderate pancreatitis in one rat in this group. One rat did not show any pancreatic parenchymal changes. Two of the rats with ductal cell dysplasia had mild pancreatitis and the pancreas of the third rat was normal in this respect. Dysplastic changes were not found in any other experimental group used in the study. The observation is statistically significant at $p < 0.025$ level. The results indicate that alcohol and a high fat diet together might have a carcinogenic effect on pancreatic ductal epithelium in rats.

Key words: Pancreas - Ductal cell dysplasia - Rat - Ethanol - Diet

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Introduction

Spontaneous pancreatic neoplasms originating from ductal epithelium are rare in mammals (Rowlatt, 1967), but they occur spontaneously in Syrian Golden hamsters; consequently, carcinogenesis of these tumours has been thoroughly experimentally investigated in this species (Pour et al., 1982, 1983a, 1983b, 1983c). Rats are prone to spontaneous acinar cell tumours and it has been claimed that only those types of pancreatic neo-plasms that occur spontaneously in a given species are also inducible experimentally (Pour, 1980).

In this article, we report a significant occurrence of pancreatic ductal cell dysplasia in male Wistar rats fed with a fat-alcohol diet. This observation was made in a series of experiments designed to investigate the effects of alcohol and diet on experimental pancreatitis.

Materials and methods

Experimental animals

192 male Wistar rats were fed with standard laboratory food (Astra-Ewos, Sweden) up to three months of age and, after that, were divided randomly into four groups of 48 animals, each group receiving a different diet: standard, fat-rich, protein-rich or carbohydrate-rich. In each diet group of 48 animals, 24 rats obtained 15% (v/v) ethanol in their drinking solution while the other 24 rats had water *ad libitum.* The diet period lasted for 12 weeks. Two rats were housed in each cage and were kept in an air-conditioned room where temperature and artificial light were controlled (+ 20°C, 24 h circadian cycle: 12 h light, 12 h dark). All animals had free access to food and drinking solution according to their group.

Diets

The diets used in this experiment were produced for this project by Special Diet Services, Essex, Great Britain, apart from the standard diet mentioned above. The composition of the standard and fat-rich diets are shown in Table 1.

In our standard diet, all fibre came from grain which in the state of starch, was also the main source of carbohydrates. Other raw materials used for producing this diet were cereals, fish protein concentrate, roasted soya meal, fodder yeast and both animal and vegetable fat.

The fat-rich diet was essentially a mixture of vegetable and animal proteins, together with beef tallow and cereals. The fat contribution was provided by meat and soya bean together with beef tallow. Fibre was essentially cellulose.

The protein content in the protein-rich diet was achieved using casein, whole egg and a soya bean concentrate. The fat content was achieved by the use of whole egg. Fibre was essentially cellulose.

The high carbohydrate level in the carbohydrate-rich diet was achieved by keeping protein low (6.8%) and utilizing pure cornflour starch, although this diet also contained approximately 6.5% total sugar. Essential trace elements and vitamins were added to all diets (Table 1).

Experimental design

After the diet period, experimental pancreatitis was induced under diethyl ether anesthesia by ductal injection of rat bile (0.2 ml/100 g of rat weight) into the common pancreatic duct. 24 hours later, the animals were anesthesized again and killed by exanguination. Previous to this, the animals fasted 24 hours, obtaining only water (alcohol was also substituted with water). The specimens for the histological study were taken from the same place in each animal, 2 cm proximal to the splenic hilus, fixed in 10% buffered formalin, sectioned and stained with routine hematoxylin and eosin stain.

Results

The results of the whole study concerning acute pancreatitis and the role of ethanol and diet as modifying factors are published elesewhere (Rämö et al., submitted for publication). The experimental pancreatitis caused a mortality of 9 rats in the group receiving the fat-rich diet and ethanol. Among the remaining 15 rats, 13 showed mild acute pancreatitis, one moderate pancreatitis and one was normal in the histologic examination of pancreas samples. An exceptional pancreatic ductal finding was histologically observed in 3 of these 15 animals. Normal ductal cuboidal epithelium (Fig. 1) was altered to columnar. Epithelial cells were arranged partly in pseudopapillary structures and showed enlarged, multilayered and hyperchromatic nuclei and increased mitotic activity (Figs. 2-4). Histologically, the alterations observed corresponded to moderate and severe dysplasia approaching adenocarcinoma in situ (Fig. 4). Two of the rats with dysplastic changes had mild inflammatory alterations in the pancreatic parenchyma, while the third had no pancreatitis. There were no marks of similar dysplastic changes in the pancreatic ducts in the rats of any other groups which also had lower mortality rates than the ethanol-fat-rich diet group. The observation that all 3 animals showing dysplastic changes were from the ethanol-fat-rich diet group is statistically significant ($p<0.025$).

Table 1. Percentage composition of the diets.

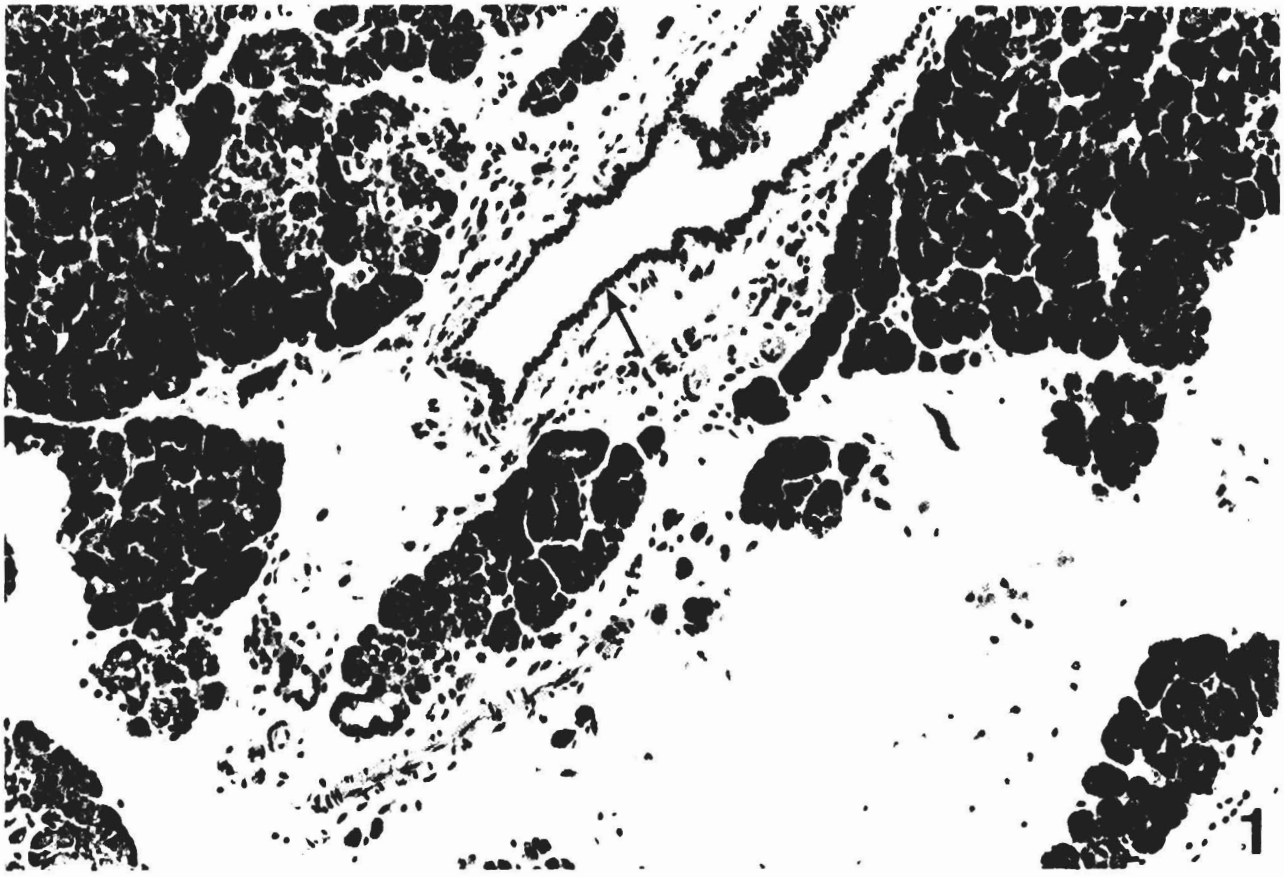
Constituent		Standard	Fat-rich
Crude fat	%	5.40	35.00
Crude protein	%	24.10	22.00
Carbohydrate	%	48.60	7.00
Moisture	%	11.70	10.00
Crude fibre	%	3.90	20.00
Calcium	%	1.16	0.73
Phosphorus	%	1.00	0.60
Magnesium	%	0.30	0.24
Chlorine	%	0.26	0.30
Iron	mg/kg	100	250
Copper	mg/kg	12	10
Cobalt	mg/kg	600	566
Manganese	mg/kg	60	46
Zinc	mg/kg	30	20
Iodine	mcg/kg	800	890
Vitamin A	IU/kg	12000	5100
Vitamin D3	IU/kg	1500	1230
Vitamin E1	mg/kg	20	56.8
Vitamin K1	mg/kg	0.25	0.0
Vitamin B1	mg/kg	3.0	12.5
Vitamin B2	mg/kg	12.0	6.6
Vitamin B6	mg/kg	5.0	7.2
Vitamin B12	mcg/kg	20.0	19.8
Pantothenic acid	mg/kg	10.0	15.8
Choline	mg/kg	1000	1305
pAmino benzoic acid	mg/kg	0.0	0.0
Menadione	mg/kg	0.0	6.70
Vitamin C	mg/kg	0.0	1.00
Niacin	mg/kg	40.000	39.00
Metabolisable energy	MJ/kg	13.00	16.50

Fig. 1. A normal cuboidal ductal epithelium of the rat pancreas (arrow). The pancreatic parenchyma contains mild inflammatory changes. Hematoxylin and eosin. x 300

Fig. 2. An altered, mostly cylindrical ductal epithelium of the rat pancreas. Pseudopapillary formations and multilayering of the nuclei are seen as signs of dysplastic change. Hematoxylin and eosin. x 300

Fig. 3. A cylindrical epithelium of an altered rat pancreatic duct. Note enlargement and hyperchromasia of nuclei. Hematoxylin and eosin. x 1,200

Fig. 4. The dysplastic pancreatic ductal epithelial change approaching adenocarcinoma in situ. Note, in addition to nuclear enlargement and hyperchromasia, frequent mitotic figures (arrows). Hematoxylin and eosin. x 1,200



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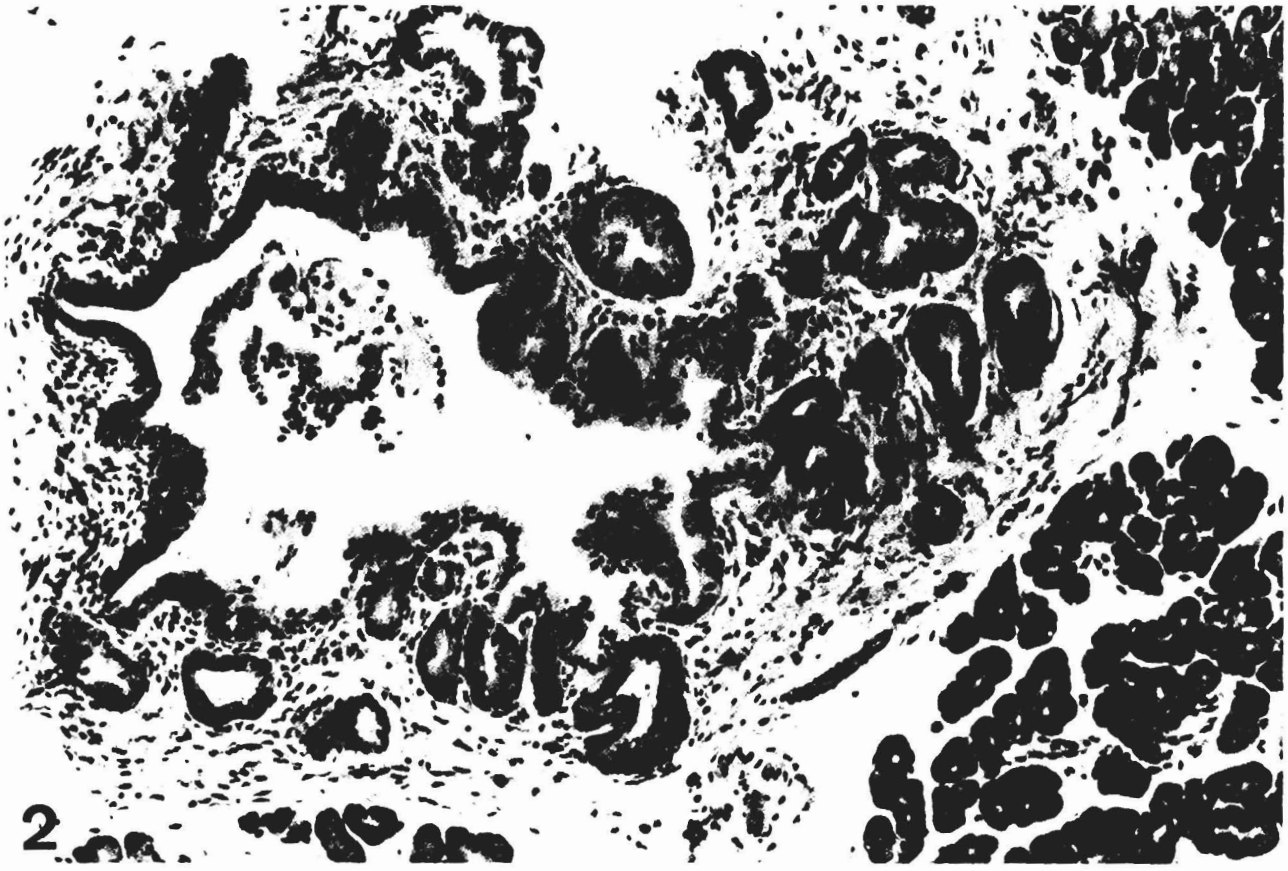

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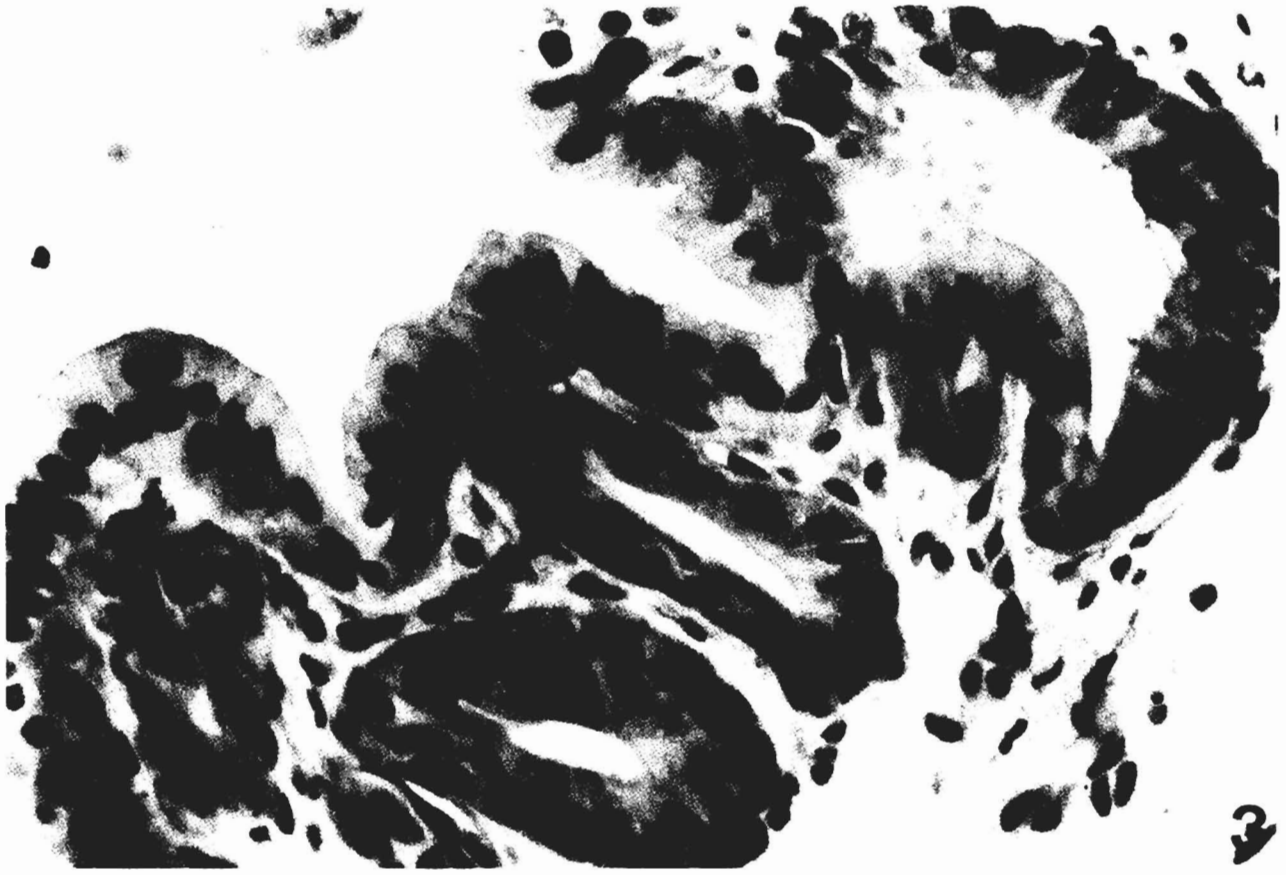

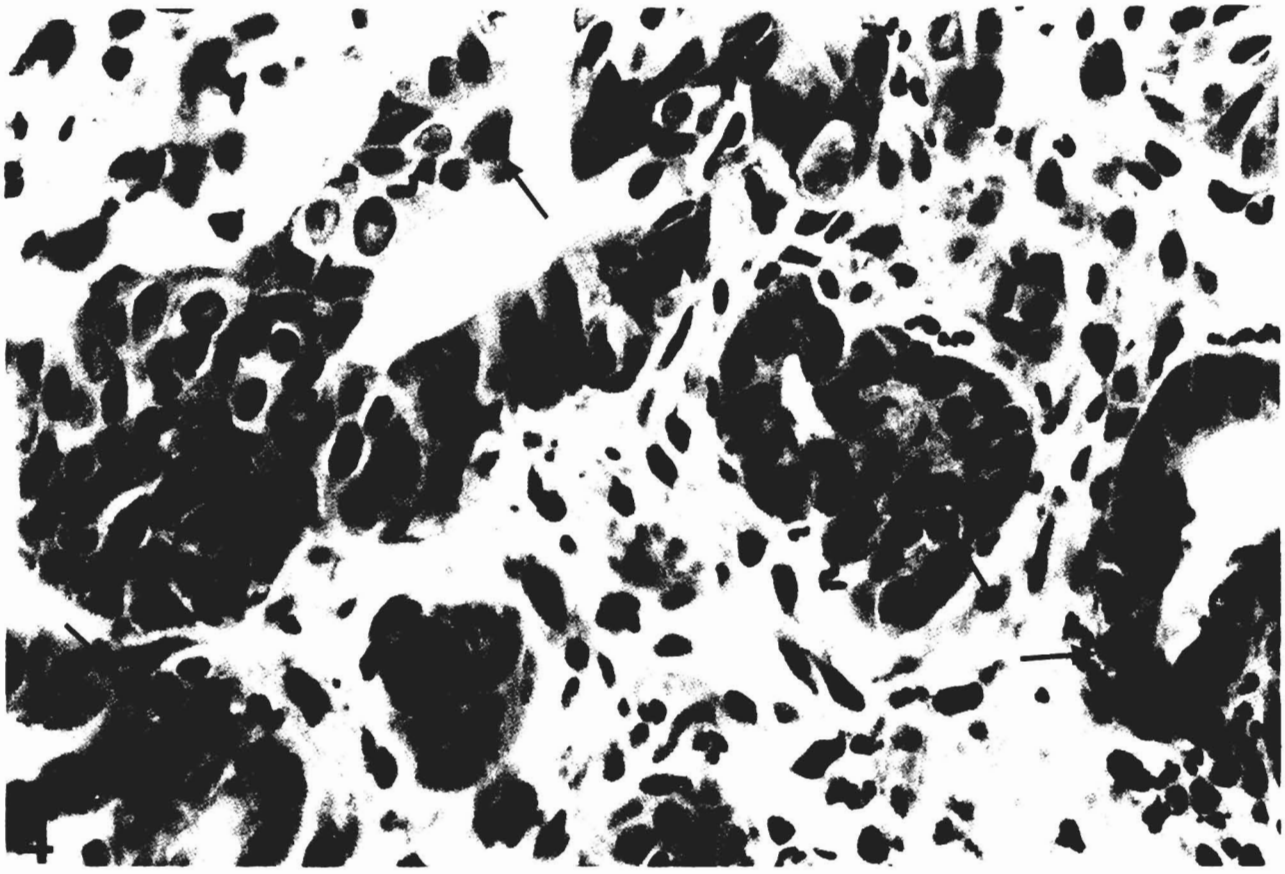

Discussion

Ductal carcinogenesis of the pancreas has been studied principally in Syrian Golden hamsters, which spontaneously develop pancreatic ductal cell neoplasias (Pour et al., 1980, 1982, 1983a, 1983b). It has been found that in this species dietary fat modifies the development of experimental ductular adenocarcinomas by increasing the frequency. The carcinogenetically enhancing effect of the high-fat diets has been suggested to be due to factors other than the fat content of these diets (Birt et al., 1981). On the other hand, the initiation and promotion of pancreatic carcinogenesis in hamsters can be inhibited by a lack of protein in the diet (Pour et al., 1983a). These experimental findigs are supported by epidemiological studies in which a close association between low protein consumption and decreased pancreatic cancer prevalence was revealed (Pawadiwa, 1976; Cordova et al., 1979; Enstrom, 1979; Lyon et al., 1980; James, 1981). In the present study of the rat, it was found that a fat-rich diet promotes the development of ductal dysplasias, but only when combined with alcohol ingestion. The previous experimental and clinical data suggest that diets may interfere with pancreatic carcinogenesis.

Alcohol has been implicated in the etiology of pancreatic cancer in man (Ansari and Burgh, 1968; Hinds et al., 1980; Voirol et al., 1980; Gold and Diener, 1981; Okuda and Ohnishi, 1981). As yet, etiological studies have failed to find a definite correlation between alcohol consumption and pancreatic cancer (Buncher, 1980). It is, however, known that alcoholism leads to chronic pancreatitis, and recurrent pancreatitis has been shown to result in an increased occurrence of pancreatic carcinoma (Pour et al., 1983b). In human chronic pancreatitis, as many as 40% of the patients have a mild or moderate dysplastic change in the pancreatic ducts (Volkholz et al., 1982). We found only one experimental study concerning the effect of ethanol on pancreatic carcinogenesis in past literature. Pour et al. (1983 c) reported that alcohol alone has no effect on the initiation and development of pancreatic neoplasias in hamsters. This finding is supported by the present study in the rat. No pancreatic neoplastic changes were observed in the rats subjected to chronic alcohol consumption and standard, protein-rich or carbohydrate-rich diets. When chronic alcohol ingestion was combined with a fat-rich diet, however, pancreatic dysplasias developed in three animals.

Spontaneous pancreatic tumours in the rat are mostly acinar cell tumours, to date the only type induced in this species (Hoch-Ligetti, 1949; Hayashi and Hasegawa, 1971; Pour, 1980). In our exerimental model, however, the rats with fat-rich diets plus ethanol developed dysplastic changes approaching locally adenocarcinoma in situ in the pancreatic ducts. It seems that a fat diet is necessary for the development of these changes as, in an earlier extensive study (Jalovaara and Apaja, 1978) having a similar experimental design and chronic alcohol administration but without dietary stresses, no similar changes in the ductular epithelium were observed. The induction of experimental pancreatitis by intraductal injection of bile as a causative factor of dysplastic changes has to be taken into account. This experimental model, however, did not lead to any similar phenomenon in any other group than that subjected to the fat diet and alcohol administration. Thus, if the changes were caused by the intraductal bile injection, the alcohol and fat diet had a sensitizing effect. Regurgitation of bile into the pancreas probably occurs in man and has been suggested to be one mechanism leading to acute pancreatitis. It seems more likely, however, that dysplastic changes develop gradually, mimicking the supposed genesis of malignant tumours.

In conclusion, diets, especially alcohol and fat-rich, interfere with the pancreatic ductal carcinogenesis in the rat. Further studies in this field are justified.

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