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# Fat-rich diet induces inflammatory changes in the intact rat pancreas

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**Summary.** The effects of chronic ethanol ingestion combined with fat-rich, protein-rich or carbohydrate-rich diets on the histology of the intact rat pancreas were studied. 192 male Wistar rats were randomly divided into four different dietary groups. Half of each group received 15% (vlv) ethanol in their drinking solution and the rest were used as controls and given tap water. After a 12-week diet period the pancreas were removed and histological specimens were stained with hematoxylin and eosin. No significant difference was observed between the groups in occurrence of edema, but inflammatory cells were found in (9124) rats in the fat-rich group receiving water ($p<0.01$. In the fat-rich diet group receiving ethanol this finding was observed in 5 of 24 rats. In these groups slight parenchyma1 cell necrosis was also observed in conjuction with the inflammatory cells. All specimens in the other groups were normal. It is suggested that inflammatory changes caused by a fat-rich diet may be due to unknown toxic effects of this diet.

**Key words:** Inflammatory cells - Pancreas - Ethanol - Diet - Rat

## Introduction

Long-term ethanol ingestion has been demonstrated to cause pancreatic histological and ultrastructural lesions in experimental studies. However so far it has not been possible to induce acute pancreatitis in rats solely through the administration of ethanol (Aho et al., 1983). Furthermore, the reported observations of the effects of chronic ethanol administration on the histology of the pancreas have been controversial (Singh et al., 1982; Sarles et al., 1971).

The composition of the consumed diet has also been shown to cause changes both in biochemical and histological levels. Protein malnutrition was associated with loss of cellular cohesion and vacuolation of acinar cell cytoplasm (Haig, 1970). In the same study, however, carbohydrate- or fat-rich diet did not induce any significant histological changes in the pancreas.

The reports concerning the co-effects of long-term ethanol ingestion and different composition of diets are few. In the previous study at this laboratory, we reported that the anterior fat-rich diet intensified acute pancreatitis in rats. Furthermore, when long-term ethanol consumption was combined with the fat-rich diet the mortality was increased (Ramo, 1986). It is thus possible that the effect of long-term ethanol ingestion on the histology of the pancreas is modified by the composition of the consumed diet. The present study was undertaken to study the co-effects of different diets and long-term ethanol ingestion on the morphology of the intact pancreas in rats.

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## Materials and methods

### *Animals*

192 male Wistar rats were used in this study. Animals were reared to three months of age on standard laboratory food (Astra-Ewos, Sweden) and after that they were randomly divided into eight different groups, each consisting of 24 animals. Two rats were housed in each cage and they were kept in air-conditioned rooms where temperature and artificial light were controlled (+20°C, 24 h of circadian cycle: 12 h light, 12 h dark). All animals had free access to food and drinking solution according to their group.

### *Diets*

Special diets used in this study were produced by Special Diet Services, Essex, Great Britain, apart from the standard diet mentioned above. The diets were the

same as used in our previous study and the composition of the diets has been earlier described in detail (Rämö et al., 1986).

*Experimental design*

In each diet group of 48 rats, 24 animals received 15% (v/v) ethanol in their drinking water for 12 weeks. The other 24 were used as controls, drinking tap water. Previous to the sampling all animals were fasted for 24 hr, drinking tap water only; alcohol was also substituted with water.

*Sampling*

Experimental animals were killed by exsanguinating the abdominal aorta under diethyl-ether anesthesia. The specimens were always taken from the same place, 2 cm proximal to the splenic hilus. These specimens were fixed immediately in neutral phosphate-buffered formalin and embedded in paraffin. The 5 micrometer-thick sections were stained with hematoxylin eosion (H-E).

*Histological evaluation*

Three different morphological components were used as the parameters on the basis of evaluating the general description of the specimens: edema, number of inflammatory cells and parenchymal cell necrosis. Furthermore, special attention was paid to the possible existence of protein plugs, lipid droplets, the size and morphology of pancreatic ducts and ductal cells.

*Statistics*

The significance of the differences between the histological findings in the different groups were calculated using Fisher's exact four-fold test.

## Results

The results of the histological evaluation are summarized in Table 1. The first of the parameters used to evaluate the degree of inflammation was edema. No significant variation in the occurrence of this parameter was detected between the groups, but slight or moderate edema of the pancreas was found in all groups. The occurrence of inflammatory cells and parenchymal cell necrosis were related to each other, and these changes were found in two of the experimental groups. In 9 out of 24 rats in the fat-rich/water receiving group and 5 out of 24 rats in fat rich/ethanol receiving group slight infiltration of inflammatory cells in conjuncion with parenchymal cell necrosis ($p<0.01$, when compared to the groups receiving standard diet was

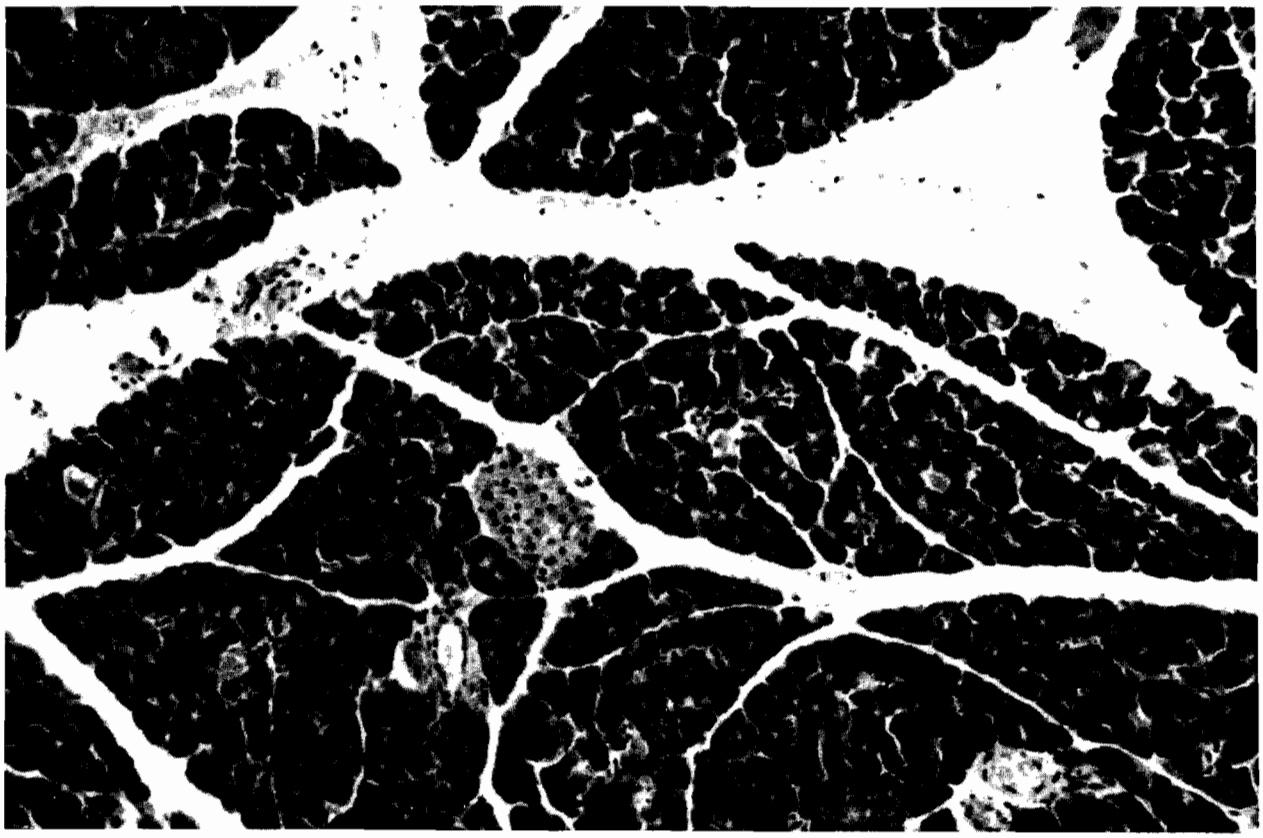

**Fig. 1.** Slight pancreatic parenchymal cell necrosis and mixed diffuse infiltration of lymphatic cells were found in addition to interlobular edema in rats of the fat-rich diet group. ×300

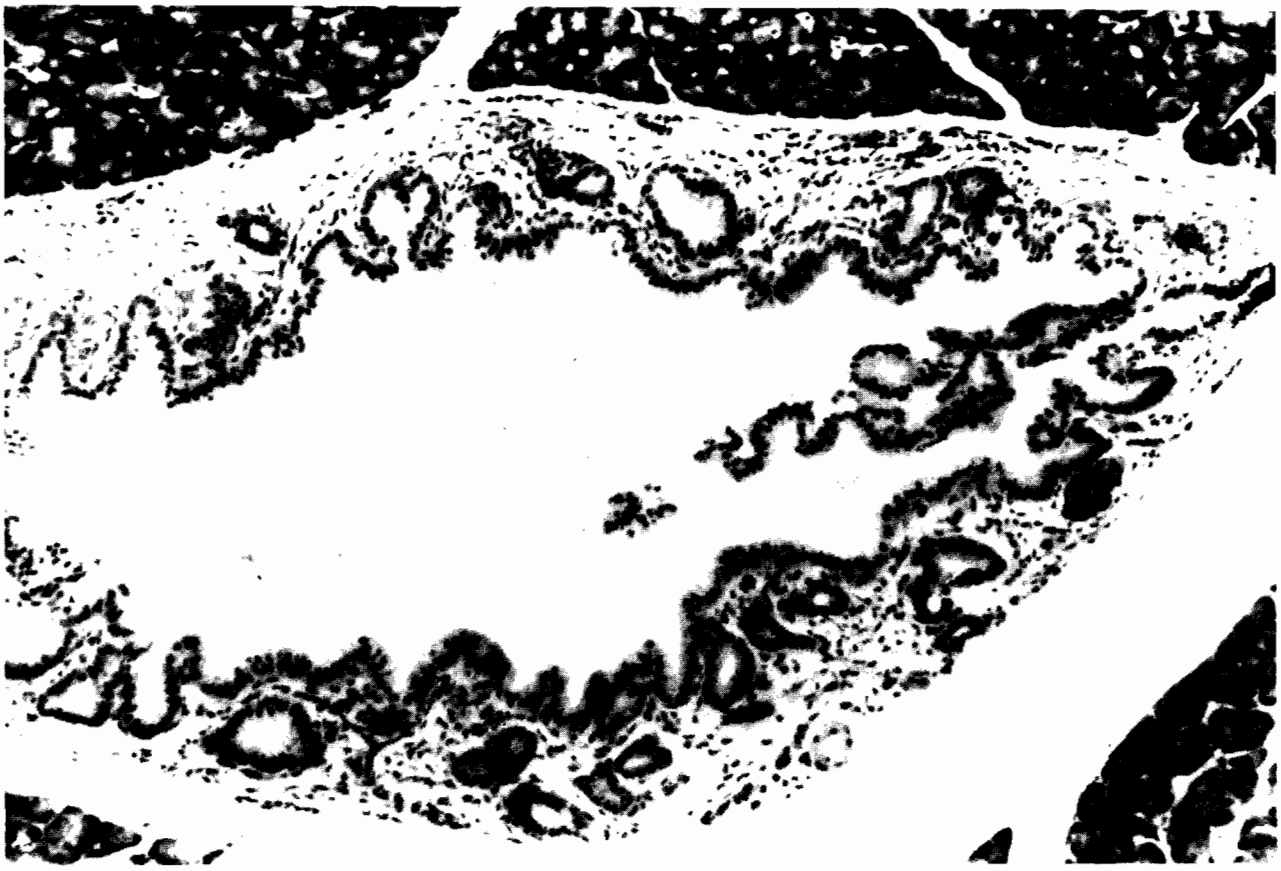

**Fig. 2.** The epithelium of a large pancreatic duct shows regular stratification without cellular atypias. ×300

**Table 1.** Histological findings in different groups.

| Group | Edema | | Inflammatory cells | Slight cell necrosis |
|---|---|---|---|---|
| | slight | medium | | |
| SW (N = 24) | 5 | – | – | 0 |
| SA (N = 24) | 6 | 1 | – | 0 |
| FW (N = 24) | 7 | 2 | 9 | 5 |
| FA (N = 24) | 8 | 1 | 5 | 4 |
| PW (N = 24) | 3 | 2 | – | 0 |
| PA (N = 24) | 6 | – | – | 0 |
| CW (N = 24) | 2 | 1 | – | 0 |
| CA (N = 24) | 6 | 2 | – | 0 |

SW = standard + water, SA = standard + ethanol
FW = fat-rich + water, FA = fat-rich + ethanol
PW = protein-rich + water, PA = protein-rich + ethanol
CW = carbohydrate-rich + water, CA = carbohydrate + ethanol

revealed. Inflammatory cells consisted of a mixed infiltration of lymphocytes, polymorphonuclear leucocytes and monocytes distributed diffusely (Fig. 1). In no other groups were these changes found. The size and morphology of the pancreatic ducts were normal in all groups (Fig. 2). No protein plugs and lipid droplets were found in the pancreas.

## Discussion

In the present study, inflammatory cells and slight parenchymal cell necrosis were only seen in the pancreatic tissue specimens of the groups receiving the fat-rich diet. Long-term ethanol ingestion did not cause any significant difference between the groups when compared to the corresponding water receiving group of rats. Slight or moderate edema was seen in all groups and the groups did not differ from each other in this respect.

Long-term ethanol uptake has been reported to cause certain ultrastructural changes in the pancreas, but not pancreatitis (Darle et al., 1970; Singh et al., 1982). Nizze and Broschewitz (1984), however, demonstrated focal lobular pancreatitis in rats after 9 months of ethanol ingestion. Furthermore, they were able to reproduce similar changes in the rat pancreas with ethanol ingestion as those seen after allyl alcohol ingestion (Nizze et al., 1979). This led to the conclusion that ethanol induced pancreatic changes were also due to a primary toxic-metabolic mechanism (Nizze and Broschewitz, 1984). In the present study, no significant changes were observed in the ethanol-and the standard-diet receiving group. We were also unable to detect any previously observed morphological changes suggestive of chronic pancreatitis

(Sarles et al., 1971). The controversial result of the present study may be due to the relatively long period of ethanol uptake in the studies before mentioned. It has been shown, however, that such changes also occur spontaneously in 15-17-month-old Wistar rats (Kendrey and Roe, 1969). Nizze and Broschewitz (1984) suggested that focal lobular pancreatitis was caused by primary protein precipitation plugs in the pancreatic ducts leading secondarily to a «focal chronic-obstructive pancreatitis». Our study, however, did not support this theory, as the pancreatic ducts were normal and protein precipitations were not seen in any of the groups. This is in agreement with a study performed by Singh et al. (1983).

Edema has been shown to be one of the first morphological alterations in experimental pancreatitis (Aho et al., 1983) and it has also been seen in human specimens in acute pancreatitis (Phat et al., 1984). We also used edema as a morphological parameter and it was observed in all groups. Edema can be caused by the inappropriate handling of a specimen and can thus be interpreted as an artificial finding. Hence, it is impossible to draw any conclusions on the basis of this finding. There were no significant differences between any of the groups, however, in this respect.

The most important result in this study was the observed infiltration of inflammatory cells and slight parenchymal cell necrosis in the pancreatic tissue after the fat-rich diet, both in water-and ethanol-receiving groups. The infiltration was slight but evident and seen only in those rats receiving the fat-rich diet. Lymphocytic infiltrations have been shown to be typical of chronic obstructive pancreatitis in humans (Nizze et al., 1984), but, as already mentioned, no signs suggestive of duct obstruction were seen in the present specimes.

Experiments concerning the effects of different diets on pancreatic morphology are few. Furthermore, the different selection of the dietary composition used and the length of the diet period make interpretation of the previous results difficult. In canine models, no significant pancreatic changes after the fat-rich diet have been reported for from six weeks to six moths (Haig, 1970; Kerstein and Neviackas, 1976). Maki et al. (1967) observed, however, that an anterior fat-rich diet caused significant morphological changes in intact pancreas of Wistar rats; after a diet period of four to six moths the same rats were submitted to 15% ethanol as their drinking solution for an additional two moths. Rats that had been maintained on the fat-rich diet showed hydropic changes and vacuolization of the acinar cells. They did not report, however, any infiltration of inflammatory cells. It should be noticed that the experimental design performed by Maki et al. (1967) was different from our's, as the animals did not simultaneously have both a fat-rich diet and long-term ethanol consumption. However, it is also possible that our result could have been different if the diet period had been longer.

The reason for the inflammatory changes in the pancreas after the fat-rich diet is not clear. If the previously reported pancreatic changes caused by ethanol uptake are due to the toxic effects of ethanol metabolities, it is also possible that the inflammatory changes reported here could be of toxic origin. It has been suggested (Braganza, 1983) that polyunsaturated fatty-acids produce some toxic metabolites by aberrant mixed-function oxygensase (MFO) systems in the liver. It is thus tempting to suggest that the effect of the fat-rich diet of the present study may be due to toxic effects of the fat-rich diet on the pancreas. This question is beyond the scope of this study, however, but definitely justifies further investigations.

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