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A STEREOLOGICAL INVESTIGATION OF THE RAT EXOCRINE PANCREAS AFTER LONG-TERM ALCOHOL CONSUMPTION

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ABSTRACT

A stereological analysis of the rat exocrine pancreas after 6 months of ethanol ingestion was carried out. Thirty male Wistar rats, aged two months at the beginning of the experiment, were divided into two groups. Both of them received a standard laboratory diet. The control group of animals were offered drinking water ad libitum. The experimental group of animals were drinking an ethanol solution. Weibel's multipurpose test system was used to determine the volume density of some exocrine pancreas components.

The results presented show that long-term alcohol ingestion decreased the relative density of the acini and increased the relative volume density of intralobular ducts, connective tissue fibres and artificial spaces. The mean radius of the acini was decreased, but the mean radius of the cell nuclei was increased.

INTRODUCTION

The mechanism by which the pancreas is damaged by ethyl alcohol has not been established yet.

Sarles et al.(1971) reported that chronic ethanol ingestion for 2o-3o months caused protein hypersecretion with plug formation in the pancreatic ducts of rats, which led to chronic pan-

creatitis. On the other hand,Dreiling et al. (1973) found that hypersecreation, caused by alcohol, led to ductular reduplication. Bordalo et al.(1977) suggested that fat degeneration of the acinar and ductular cells, with or without fibrosis, was the first response of the pancreas caused by alcohol.

Many histological investigations have shown that alcohol intake led to the lesions mentioned above, to a greater or lesser degree, depending on the time of consumption.

The aim of this work is the stereological investigation of some visible lesions in the rat exocrine pancreas caused by 6 months of alcohol consumption.

MATERIAL AND METHODS

Thirty male Wister rats, aged two months at the beginning of the experiment (Vinča Lab. Yugoslavia), were used. The animals were divided into two groups, both of which received a standard laboratory diet. The control group of animals (group C) was offered drinking water ad libitum. The experimental group of animals (group E) was offered an ethanol solution (32 g ethanol and 25 g sucrose in 100 ml of water), according to Porta and Gomez-Dumm (1968).

The mean daily ethanol consumption in the animals of group E was 11,2 g per kg of body weight.

After an experimental period of 6 months all the animals were sacrified during i.p. Nembutal anaesthesia. The pancreas was rapidly removed and fixed in Bouin's solution, dehydrated and embedded in paraffin. Sections 6 um thick were stained by HE and AZAN methods for histological and stereological examinations.

Stereological analyses were performed to evaluate the volume density of the acini, intralobular ducts, small blood vessels, connective tissue fibres and artificial spaces around the acini according to Weibel's multipurpose test system (1968). Total volumes of these compartments were calculated after total pancreas volu-

mes were determined using immersion method (Scherle, 197o).

Ten different fields were measured for each animal, using an Olympus light microscope at an objective magnification of loo x.

The mean radius of the acini and nuclei was determined according to Bogataj et al. (1977).

The Student t-test was used to determine statistically significant differences.

RESULTS

The histological examination of the rat exocrine pancreas during long-term alcohol ingestion did not demonstrate prominent lesions that could be named as chronic pancreatitis (Figure 1).

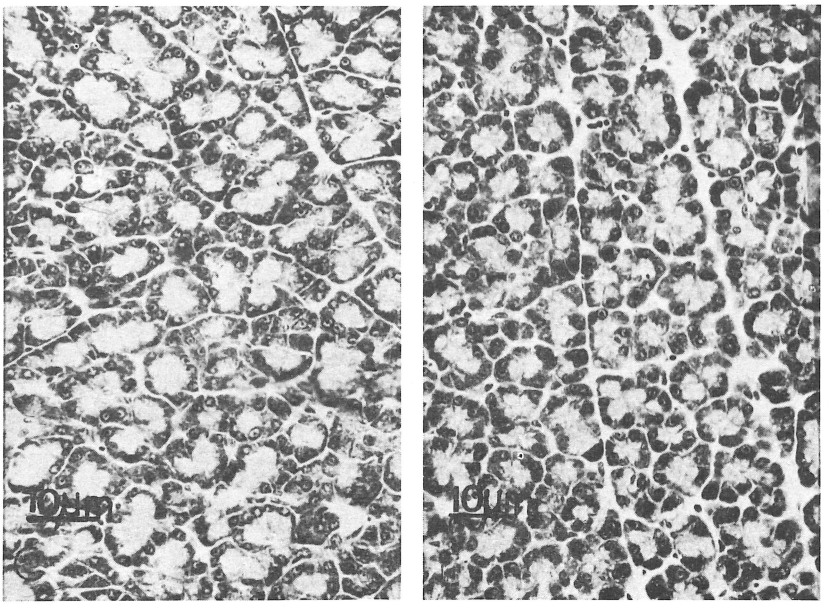

Figure 1. The exocrine pancreas in control (C) and experimental (E) groups of rats (HE;865 x)

Nevertheless, it was found that the acini of the animals in group E were smaller compared to those in the control group of animals.

The existence of acinar atrophy was confirmed by the stereological analysis (Figure 2).

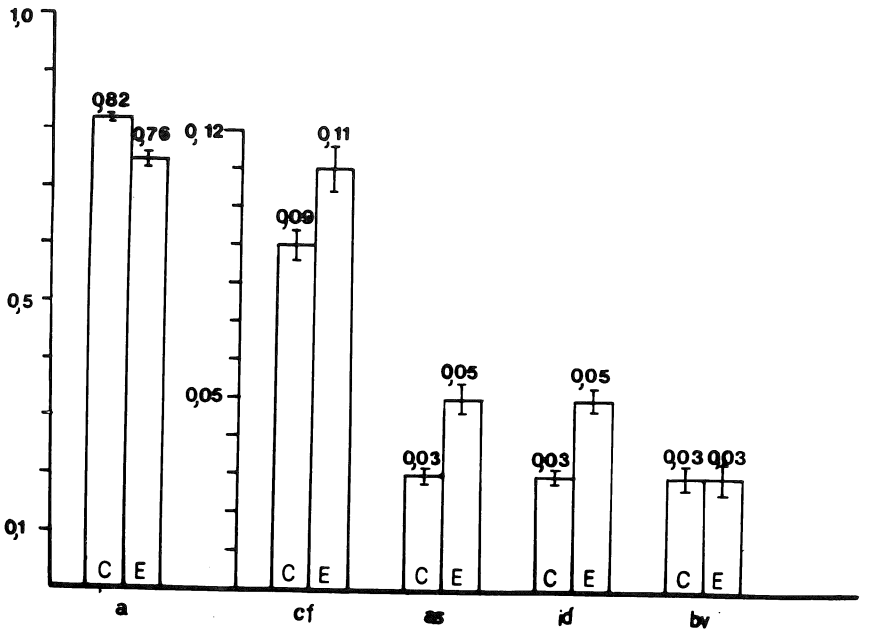

Figure 2. The volume density of the acini (a), intralobular ducts (id), small blood vessels (bv), connective tissue fibres (cf) and artificial spaces around the acini (as) (mean ± SEM)

It was found that the volume density of the acini in animals of group E, which had drunk the alcohol solution, decreased by about 7.3%. The difference was statistically significant ($t=5.46; p < 0.001$). The total volume of the acini was 0.75 ± 0.096 cm^3 (mean±SEM) in controls, and 0.71 ± 0.002 cm^3, in animals of group E.

Large spaces around the acini were found, as a result of atrophy. Dissociation of the acini and acinar cells was often observed suggesting the presence of oedema fluid. The stereological analysis also indicated that the artificial spaces were increased. The difference was statistically significant ($t=4.06$; $p < 0.001$). The total volume of the artificial spaces was 0.02 ± 0.002 sm^3 in controls, and 0.05 ± 0.004 cm^3 in animals of group E.

Prominent cannalicular proliferation was not found on the examined slides, but acinar lumina dilatation was often seen. These histological

alterations suggested the beginning of acinar canalization. Using the stereological analysis it was found that the volume density of small ducts was increased. The difference was statistically significant ($t=4.83$; $p < 0.001$). The total volume of intralobular ducts was 0.03 ± 0.002 cm^3, in animals of group C, and 0.05 ± 0.004 cm^3, in animals of group E.

The histological examination did not demonstrate any increase of connective tissue fibres around the acini, but pericannalicular and perivascular fibrosis was prominent in animals of group E. However, the volume density of connective tissue fibres around the acini was found to be increased in the animals which had drunk the alcohol solution. A statistically significant difference was found ($t=2.71$; $0.02 < p < 0.01$). The total volume of connective tissue fibres was 0.08 ± 0.004 cm^3, for controls, and 0.10 ± 0.005 cm^3, for animals of group E.

There were no differences between small blood vessels in animals of group E, compared with those of the controls. The total volume of small blood vessels was 0.03 ± 0.003 cm^3 for controls, and 0.03 ± 0.003 cm^3, for animals in group E.

The mean radius of the acini was decreased in animals of group E, that had drunk the alcohol solution (table 1).

Table 1. Mean radius of the acini and nuclei of the rat pancreas

	acini (/um)	nuclei (/um)
C (n=15)	13.14 ± 0.57[1]	3.15 ± 0.10
E (n=15)	10.51 ± 0.35[2]	3.43 ± 0.13

1. Mean $\pm$ SEM
2. $p < 0.001$

There was a statistically significant difference between the groups of animals ($t=4.33$; $p < 0.001$).

However, it was found that the mean radius of nuclei was increased in the animals which had drunk the alcohol solution. The difference between the groups was not statistically significant ($t=1.27$; $p=n.s.$).

DISCUSSION

Using stereological analysis we have been able to determine some visible lesions in rat exocrine pancreas which were associated with long-term alcohol consumption.

According to data from the literature alcohol causes alteration to the acinar cells (fatty degeneration), ductular reduplication and proliferation of connective tissue fibres (Sarles et al., 1971; Dreiling et al., 1973; Bordalo et al., 1977; Pirola et al., 1974). We have chosen to examine some intralobular elements of the rat exocrine pancreas as places where the first alterations may be seen in alcoholics.

Some authors found no lesions in the rat pancreas after 6 months of alcohol consumption (Clemente et al., 1979; Singh, LaSure, Bockman,1982). They consider that all alterations present in pancreatic tissue after 6 months of alcohol consumption were caused by aging.

The histological alterations which were seen in our slides were minimal, and would not have been called chronic pancreatitis. However, these minimal lesions appear to be significant after examination by stereological analysis.

Thus, we observed that the acini were smaller in animals of group E, which had drunk the alcohol solution. Moreover as a result of acinar atrophy, there were a strong dissociation of the acini and dilatation of the periacinar spaces. These large spaces around the acini we consider to be oedema fluid, and we find there are statistically significant differences compared with the controls.

Sarles et al. (1971) reported that the atrophic acini were replaced by ducts and fibres after 2o-3o months of alcohol consumption. Although we observed the atrophy of the acini and acinar lumina dilatation, no increase of the ductular mass and fibres around the acini was apparent. However, using the stereological analysis we found a significant increase in small ductuli.

We obtained the same results for connective tissue fibres around the acini. Periductular and perivascular fibrosis was seen in animals that had drunk the alcohol solution for 6 months, but no proliferation of connective fibres around the acini was observed. Using the stereological methods statistically significant differences were found.

The mean radius of the acini was decreased as a result of acinar atrophy.

On the other hand, we found that the mean radius of the nuclei tended to be larger than in the controls. These results indicate that hyperactivity of the nuclei is caused by alcohol ingestion. Similar results were found in all the acinar organelles in alcoholics (Tasso et al., 1973).

The results presented above show that long--term alcohol consumption (6 months) leads to prominent lesions which are not visible in histological examinations. The alterations demonstrated only by stereological methods suggest that the first changes appear in the exocrine pancreas of rats consuming alcohol solutions within 6 months.

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