Rat pancreatic B-cells after chronic alcohol feeding. A morphometric and fine structural study

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Summary. Quantitative analysis of the light microscopic and fine structure of rat islet B-cells was carried out in chronic alcoholism. Absolute pancreatic weight and volume were similar in groups C (control) and E (ethanol), but relative pancreatic weight in group E rat was decreased. The results for fasting blood glucose and insulin levels were similar in the two groups of animals. There was a significantly reduced total pancreatic islet volume in E rats. The total number of endocrine cells both per islet and per μm^2 of islet was similar in the two groups of animals. The volume density and number of B-cells per islet and per μm^2 of islet were not changed in ethanol-treated rats as compared with the control. On the other hand, diameter, surface area and volume of the B-cells and their nuclei were found to be statistically significantly decreased. Histological examination revealed that islet blood vessels were dilated in alcoholic rats. Over the 4-month period of ethanol intake a significant decrease in cell profile area, nuclear profile area and volume density of cytoplasmic granules and an increase in the profile area and volume density of endoplasmic reticulum occurred. The gross histological alteration seen in most B-cells of the ethanol-treated rats was irregularity of the nuclear envelope with deep invagination and with margination of heterochromatin and many empty granules or granules without clear electron dense crystals of insulin.

The present results indicate some optical and structural abnormalities of B-cells in chronic alcoholism that may be related to cell dysfunction and may contribute, at least in part, to the endocrine pancreas functional disturbance.

Key words: Ethanol, Langerhans islets, B-cells, Morphometry

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Introduction

Although controversy regarding the definition and different histological types of alcoholic chronic pancreatitis in humans and animal models prevails in the literature (Sarles, 1975; Singh, 1987; Singer et al., 1990; De Angelis et al., 1992), the association between ethanol and chronic pancreatitis is well established. Functional studies have shown that exocrine deficiency has a significant effect on islet cell function, and there are exocrine pancreas- insulin, glucagon, pancreatic polypeptide and somatostatin interactions, as well as enteropancreatic interaction in chronic pancreatitis (Klöpel et al., 1978; Go, 1990; reviewed by Owiang et al., 1991).

To the best of our knowledge, quantitative analysis of the light microscopic and fine structure of islet B-cells has not yet been carried out in chronic alcoholism. Because very little is known about the effect of alcohol on islet B-cells, the purpose of this study was to determine the effect of chronic ethanol consumption on islet morphology, with special attention to B-cells.

Materials and methods

Animals and feeding protocol

Twenty male Wistar rats, 2 months old and weighing approximately 240 g were randomly allocated into control (C) and ethanol-fed (E) groups. Control rats (n=10) were fed on a commercial stock cereal based pelleted die (25% protein) as defined by Bieri et al. (1977). The ethanol group (n=10) was given free access to a hyperprotein cereal based pelleted diet (34% protein) and an aqueous solution of 25% sucrose-32% ethanol as recommended by Hartroft (1971). The amount of feed offered to the control animals was adjusted to the energy intake of the animals receiving alcohol (i.e. the

control rats were pair-fed). Ethanol was replaced by starch in the control diet. The amount of tap water given to the ethanol-treated rats was 25 ml, and mean consumption per rat per day of the alcohol solution was 12 ml. The amount of tap water given to the control group was adjusted so that the rats in both groups received the same amount (approximately 40 ml) of liquid per day.

The experiment lasted for 4 months. At the end of the feeding period the animals were fasted overnight and the abdomen opened under light ether anaesthesia.

Blood ethanol determination

Samples for blood ethanol determination were obtained from the tail vein, and blood ethanol concentrations were determined using the Sigma diagnostic alcohol procedure (No. 322-UV, Sigma chemical Co., St. Louis, Mo.). The mean blood ethanol concentration was determined for all ethanol-treated rats studied based upon multiple blood ethanol determination (at 10 AM on day 1, 15, 30, 45, 60, 75, 90, 105 and 120) performed throughout the 120-day exposure period.

Blood glucose and plasma insulin determination

Fasting blood glucose level was measured by the glucose oxidase method (Hyvarinen and Nikkila, 1962). Plasma insulin was measured by radioimmunoassay using commercial kits in accordance with the instructions (INEP- Diagnostics, Zemun) and rat insulin standard (Novo Industry, Bagsvaerd, Denmark).

Preparation of histological samples

The pancreas was quickly removed, washed in cold 154 mM Nacl and weighed in air. The total volume was determined using an immersion method (Scherle, 1970). Tissue samples were taken from the splenic portion of the pancreas.

Histochemistry:

The tissues were immersed in Bouin's solution for 8 hours and embedded in paraffin according to standard procedures, sectioned and stained with haematoxylin and eosin and Victoria trichrome stain.

Immunocytochemistry:

Immunocytochemistry was performed on 5 µm serial sections from Bouin-fixed paraffin-embedded tissue using a prediluted polyclonal antibody against neuron specific enolase (rabbit anti-neuron specific enolase-NSE; Lot No. 11086663, Seemed, San Francisco, CA) and polyclonal antibody against insulin (guinea pig anti-porcine insulin, 1:1000; Dakopatts A564, DAKO Corp., CA).

For NSE determination, sections were immunostained by the streptavidin-biotin technique using the Histostain-SP kit (Cat NO. 95-9543; Zymed, San Francisco, CA). After deparaffinizing, the endogenous peroxidase was blocked with 3% hydrogen peroxide in methanol. After further dehydration and washes in phosphate-buffered saline (PBS), the slides were incubated with 10% normal goat serum in a humid chamber at room temperature for 10 min. After shaking off the blocking serum, the prediluted polyclonal antibody was applied. Incubation took place at 37 °C for 30 min. After several washes in PBS the secondary biotinylated antibody (goat anti-rabbit IgG) was applied for 10 min at room temperature. After several washes and a 5-min incubation with the streptavidin-biotin-complex at room temperature, the slides were rewashed in PBS, reacted with chromogen (aminoethyl carbazol) I, rewashed, dehydrated and mounted for microscopic evaluation.

For determination of insulin-containing cells, sections were immunostained by the unlabelled peroxidase-antiperoxidase complex (PAP) technique (Sternberger, 1986). After removal of the paraffin wax and the blocking off of endogenous peroxidase, sections were incubated with 10% normal rabbit serum at room temperature for 30 min. After shaking off the blocking serum, the sections were incubated with antisera against insulin guinea pig anti-porcine insulin (1:000; Dakopatts A 564, DAKO Corp., CA), for 45 min at room temperature. Then the sections were washed in PBS and incubated with rabbit anti-guinea pig immunoglobulin-peroxidase conjugate (1:40; Dakopatts P 141, DAKO Corp., CA) for 45 min at room temperature. Visualization of immunoreactive sites was performed in DAB-HCl/H_2O_2 (0.7 mM, 2,2 diaminobenzidine-HCl/0.002% H_2O_2) in Tris-HCl, pH 7.6, for 5 min. The nuclei were counterstained with Mayer's haematoxylin.

Electron microscopy:

The specimens were immediately fixed in 4% cold glutaraldehyde in 0.1M Na-cacodylate buffer at pH 7.4 for 1 h. They were postfixed in 2% osmium tetroxide at 4 °C for 1 h. Following that, they were dehydrated in a graded series of ethanol and embedded in epoxy resin. Semithin sections (1 µm) were stained with toluidine blue and examined under a light microscope. Ultrathin sections, cut using an LKB ultratome II, were contrasted with lead citrate and uranyl acetate prior to vieweing with an Opton 109 electron microscope.

Stereological methods

Light microscopy:

Five pancreatic segments from each animal were used for light microscopy morphometry. Each block was completely serially sectioned, but a single section from the middle area of the tissue was chosen for analysis

from each region. This made a total of 50 sections per group for NSE and insulin-containing cells, i.e. five sections per animal. The Weibel multipurpose test system containing 42 points and 21 lines was used (Weibel et al., 1966). At x200 magnification, using Victoria-stained samples, all islet profiles within a section were counted; their minor and major axes were measured, and their profile area, volume and numerical density and total volume were determined by point-counting. Conventional morphometry and standard stereological equations were used to calculate the following parameters: the total number of specifically immunostained (NSE) endocrine cells, and specifically immunostained B-cells per islet and per μm^2 of islet, as well as the volume and surface density, profile area, diameter and total volume of cell and nucleus of B-cells. The mean diameter of the blood vessels was calculated from the formula $D= Vv/Sv$, and their mean length from the formula $L= 4Vv/pD$.

Electron microscopy:

Prints were made from negatives of nucleated B-cells (4500 times original magnification) at a final magnification of 9000. About 5-6 prints were analyzed from each animal (total of 48 and 64 prints for groups C and E, respectively). For stereological analysis (excluding granular radius) the micrographs were covered with a transparent lattice point counting grid with lattice ratio r= 9 (1:9, 690:6210; 0.9:0.3); 20x29 cm, using the method of Weibel et al. and Weibel and Bolender as described by Aherne and Dunnill (1982). The test grids were used to obtained «hits» on test organelles of interest (coarse points for nucleus and cytoplasm, and fine points for mitochondria, Golgi complexes, endoplasmic reticulum and granules. The following morphometric/stereological parameters were obtained from these prints: for the whole cell, the profile area; for the nucleus, the profile area and volume density; for the mitochondria, the number of profiles per section and volume density; for the endoplasmic reticulum and Golgi apparatus, the volume density; and for the granules, the profile area, volume density, number of profiles per section and number of profiles per μm^2 of cytoplasm.

The mean granular radius was measured using a Kotron MOP-AMO3 semiautomatic analyzer. The test micrograph was placed on the measuring tablet and a light, weighed cursor was used to mark four radii of each granule and to outline each granule. The number of granules outlined was then available in printed form.

Results

Nutrition, blood ethanol, glucose and insulin levels

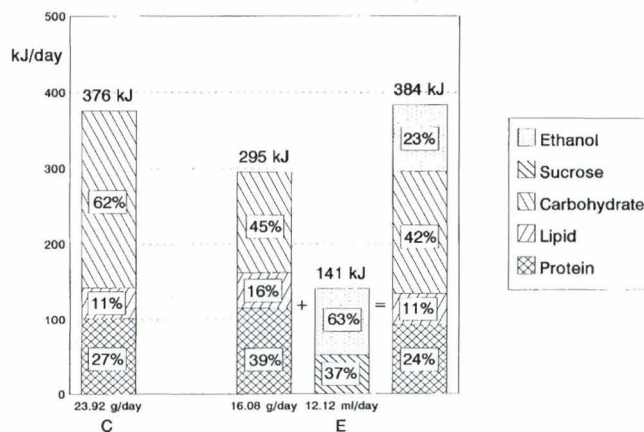The average daily amounts of diet and energy consumed as a whole and separately for ethanol, protein,

Table 1. Body weight, pancreatic weight and volume and fasting blood glucose and insulin levels in the rat (mean±SEM).

	GROUP C (n=10)	GROUP E (n=10)	STATISTICAL SIGNIFICANCE (p)
Initial body weight (g)	232.6±1.27	240.6±2.37	n.s.
Final body weight (g)	359.0±5.61	429.0±17.98	<0.001
Pancreatic weight (g)	1.30±0.038	1.33±0.0071	n.s.
Pancreatic weight as % body weight (g)	0.36±0.012	0.32±0.014	<0.02
Pancreatic volume (cm³)	1.24±0.049	1.28±0.087	n.s.
Glucose (mmol/L)	5.30±0.242	5.34±0.197	n.s.
Insulin (mIU/L)	22.0±1.34	20.9±3.48	n.s.

fat and carbohydrate intakes are presented in Fig. 1. These data show that there was no significant difference between the ethanol-treated rats and controls with regard to daily energy intake and energy from protein and fat. The average ethanol intake of group E was 3.09 g/day (data not showing) which was 23% of the daily energy intake.

The blood ethanol concentration (mean±SEM) was 125±13 mg/ml and ranged from 107 mg/100 ml to 138 mg/100 ml.

Body weight, pancreatic weight and volume, as well as fasting blood glucose and insulin levels are shown in Table 1. Animals in the examined groups had similar weight gains (data not shown) and body mass increased during the experiment continually. Absolute pancreatic weight and volume were similar in groups C and E, but relative pancreatic weight in group E rats was decreased. The results for fasting blood glucose and insulin levels

Fig. 1. Average daily intakes of solid feed and fluid and relative amounts of dietary energy source of the different diets consumed by rats throughout the experiment. Figure shows average daily amounts of basal diet (in g) consumed by the non-ethanol rats of group C (first column) and the average daily amounts (in g) of the hyperprotein diet (second column) plus the amounts (in ml) of sweetened ethanol mixture (third column) consumed by ethanol-treated rats of group E throughout the 4-month experimental period. The fourth column shows the energy proportion of ingredients in the final regimen (hyperprotein diet plus ethanol sucrose mixture) of group E.
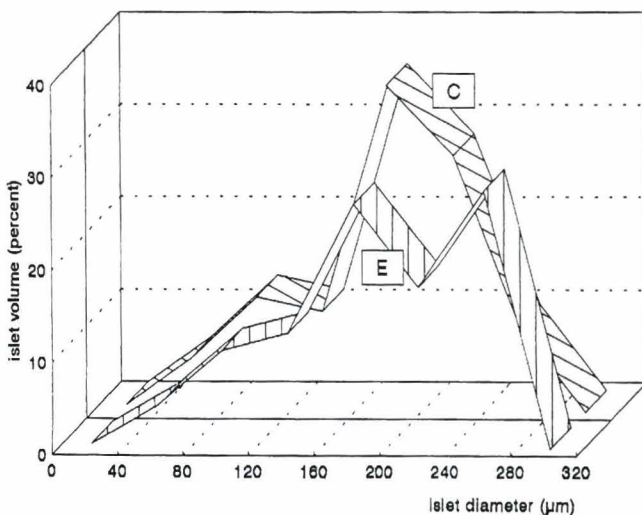
Table 2. Stereological results for the islets of Langehans in the control and experimental rats (mean±SEM).

	GROUP C (n=10)	GROUP E (n=10)	STATISTICAL SIGNIFICANCE (p)
Volume density (mm^0)	0.006±0.012	0.005±0.006	n.s.
Absolute volume (cm^3)	0.009±0.0010	0.006±0.0009	<0.05
Numerical profile density (N/mm^2)	0.873±0.0926	0.742±0.0755	n.s.
Numerical density (N/mm^3)	9804.0±1414.8	8403.0+1456.4	n.s.
Absolute number	12378.3±1959.6	10640.2±2117.6	n.s.
Diameter (mm)	95.3±7.16	98.8±8.26	n.s.
Surface area (mm^2)	5360.4±923.2	5139.7±739.5	n.s.
Total number of endocrine cells per islet	55.8±2.82	60.3±5.56	n.s.
Total number of endocrine cells per mm^2 of islet	0.01259±0.00167	0.01368±0.00193	n.s.

Table 3. Summary of morphometric results for the B-cells of the islets of Langerhans (mean±SEM).

	GROUP C (n=10)	GROUP E (n=10)	STATISTICAL SIGNIFICANCE (p)
Volume density (mm^0)	0.70±0.028	0.66±0.024	n.s.
Number/islet	30.8±1.49	28.4±3.88	n.s.
Number/µm^2 of islet	0.0070±0.00086	0.0068±0.00113	n.s.
Diameter (µm)			
Cell	12.16±0.176	11.24±0.192	<0.01
Nuclei	7.29±0.12	6.50±0.19	<0.01
Surface area (µm^2)			
Cell	116.40±3.41	99.50±3.41	<0.01
Nuclei	42.28±1.55	33.56±1.71	<0.01
Volume (µm^3)			
Cell	940.31±41.89	745.07±38.89	<0.01
Nuclei	203.36±9.74	147.18±9.89	<0.01

were similar in the two groups of animals. There was no significant difference between the ethanol-treated rats and controls with regard to final mean body weight.

Stereological analysis at light microscopic level

Table 2 summarizes some stereological parameters obtained for the islets of Langerhans in the control and experimental groups of rats. It was found that all parameters investigated were slightly decreased in the ethanol-treated rats, except for the mean islet diameter

and total number of endocrine cells per islet and per mm of islet. However, there were no statistically significant differences between the two groups of rats, except that ethanol-treated rats were found to have a significantly reduced total pancreatic islet volume. This reduction amounted to about 34% of the total islet volume in comparison with controls (p<0.05). The size of islets was very variable (diameter ranged from 0-40 to 280-320 nm) and showed extremely variable distribution within the pancreatic tissue, but the distribution curve of the diameter of islets showed similar frequency distribution of islet diameter in both examined groups (Fig. 2). However, it was found that the distribution of total islet volume among islets of various diameter classes differed between the two groups. Thus, although

Fig. 2. Frequency distribution of islet diameter in control (C) and experimental (E) rats.
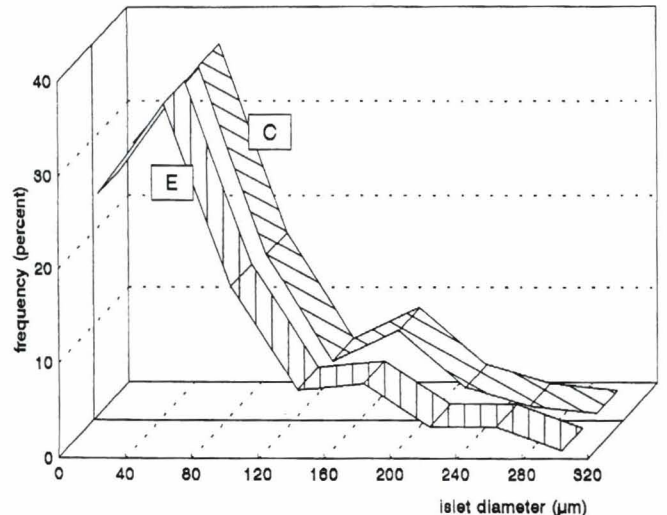
Fig. 3. The distribution of total islet volume among islets of different diameter classes in control (C) and experimental (E) rats.

Table 4. Stereological results for blood vessels in the islets of Langerhans in the control and experimental rats (mean±SEM).

	GROUP C (n=10)	GROUP E (n=10)	STATISTICAL SIGNIFICANCE (p)
Volume density (mm⁰)	0.025±0.03	0.074±0.007	<0.001
Diameter (μm)	9.73±1.23	12.62±1.05	n.s.
Total length (cm/islet)	0.004±0.001	0.008±0.001	<0.001

smaller islets were predominant numerically and the bulk of the endocrine volume consisted of midsized islets in both groups, in the ethanol-treated animals there was a lower frequency in the endocrine volume of midsize islets (Fig. 3). The total number of endocrine cells both per islet and per μm^2 of islet was similar in the two groups of animals (Figs. 4, 5).

Morphometric results for B-cells are presented in Table 3. The volume density and number of B-cells per islet μm^2 of islet were not changed in ethanol-treated rats as compared with the control (Figs. 6, 7). On the other hand, diameter, surface area and volume of the B-cells and their nuclei were found to be statistically significantly decreased.

Histological examination revealed that islet blood vessels were dilated in alcoholic rats. This was confirmed by stereological analysis (Table 4). Thus, in ethanol-treated rats the volume density, diameter and total length of islet blood vessels was increased, the differences for volume density (p<0.001) and for total length of blood vessels (p<0.001) being statistically significant.

Stereological analysis at electron microscopy level

A summary of the quantitative analysis of the ultrastructural morphometry of B-cells is shown in Table 5. There were no significant differences between the results for controls and ethanol-treated animals with respect to any of the following parameters studied: volume density of the nucleus; profile area and volume density of the cytoplasm; profile area, volume density and number of mitochondria per cell profile; profile area and volume density of the Golgi system; number of total cytoplasmic granules per cell profile and per μm^2 of cytoplasm, mature cytoplasmic granules and immature cytoplasmic granules as well as mature to immature granule ratio. Over the 4-month period of ethanol intake, a significant decrease in cell profile area, nuclear profile area (Figs. 8, 9) and volume density of cytoplasmic granules (Figs. 10, 11) and an increase in the profile area and volume density of endoplasmic reticulum occurred (Fig. 12).

The gross histological alteration seen in most B-cells of the ethanol-treated rats was irregularity of the nuclear envelope with deep invaginations (Fig. 9) and with margination of heterochromatin and many empty granules or granules without clear electrondense crystals

Table 5. Ultrastructural morphometry of B-cells of the islets of Langerhans, summary of quantitative analysis (mean±SEM).

	GROUP C (n=10)	GROUP E (n=10)	STATISTICAL SIGNIFICANCE (p)
Cell			
No. of cells analyzed	48	64	
Profile area (μm²)	133.±3.55	115.5±4.78	0.05
Nucleus			
Profile area (μm²)	32.1±1.98	26.1±1.92	0.05
Volume density (μm⁰)	0.25±0.014	0.22±0.018	n.s.
Cytoplasm			
Profile area (μm²)	101.5±5.82	89.1±4.62	n.s.
Volume density (μm⁰)	0.75±0.014	0.78±0.018	n.s.
Mitochondria			
No. per cell profile	24.9±5.19	24.6±2.94	n.s.
Profile area (μm²)	5.39±1.09	5.52±0.69	n.s.
Volume density (% of cytoplasm)	5.30±0.85	6.10±1.00	n.s.
Golgi system			
Profile area (μm²)	2.09±0.547	1.90±0.697	n.s.
Volume density (% of cytoplasm)	1.80±0.420	1.80±0.640	n.s.
Rough endoplasmic reticulum			
Profile area (μm²)	5.43±1.170	10.19±1.74	<0.05
Volume density (% of cytoplasm)	6.20±1.400	11.50±1.580	<0.05
Cytoplasmic granules			
Volume density (% of cytoplasm)	29.20±1.600	23.30±2.200	<0.05
No. per cell profile	216.0±25.1	215.7±31.13	n.s.
No. per μm² of cytoplasm	2.10±0.154	2.42±0.181	n.s.
Mature to immature granule ratio	1.50±0.22	2.50±0.66	n.s.
Mature «electrondense» granules			
No. per cell profile	109.1±14.05	119.7±18.91	n.s.
No. per μm² of cytoplasm	1.07±0.107	1.34±0.149	n.s.
Immature «pale» granules			
No. per cell profile	72.70±10.62	62.70±10.64	n.s.
No. per μm² of cytoplasm	0.74±0.080	0.62±0.068	n.s.
Mean diameter (nm)	437±7.6	366±5.5	<0.01

of insulin (Fig. 11).

Discussion

In the present study the effect of long-term ethanol consumption with maximal controllability of nutrient intake enabled us to carefully study the effects of alcohol per se on B-cells in the islet of Langerhans.

Abuse of ethanol is known to be associated with pancreatitis in humans (Geokas, 1984; De Angelis et al., 1992) and animals (Sarles et al., 1971; Sarles, 1975; Singer et al., 1990), but the role of diet and other environmental factors has remained obscure. It has been suggested that alcohol alone may not make the pancreas more vulnerable to inflammation and that some additional factor is needed (Goslin et al., 1965; Maki et
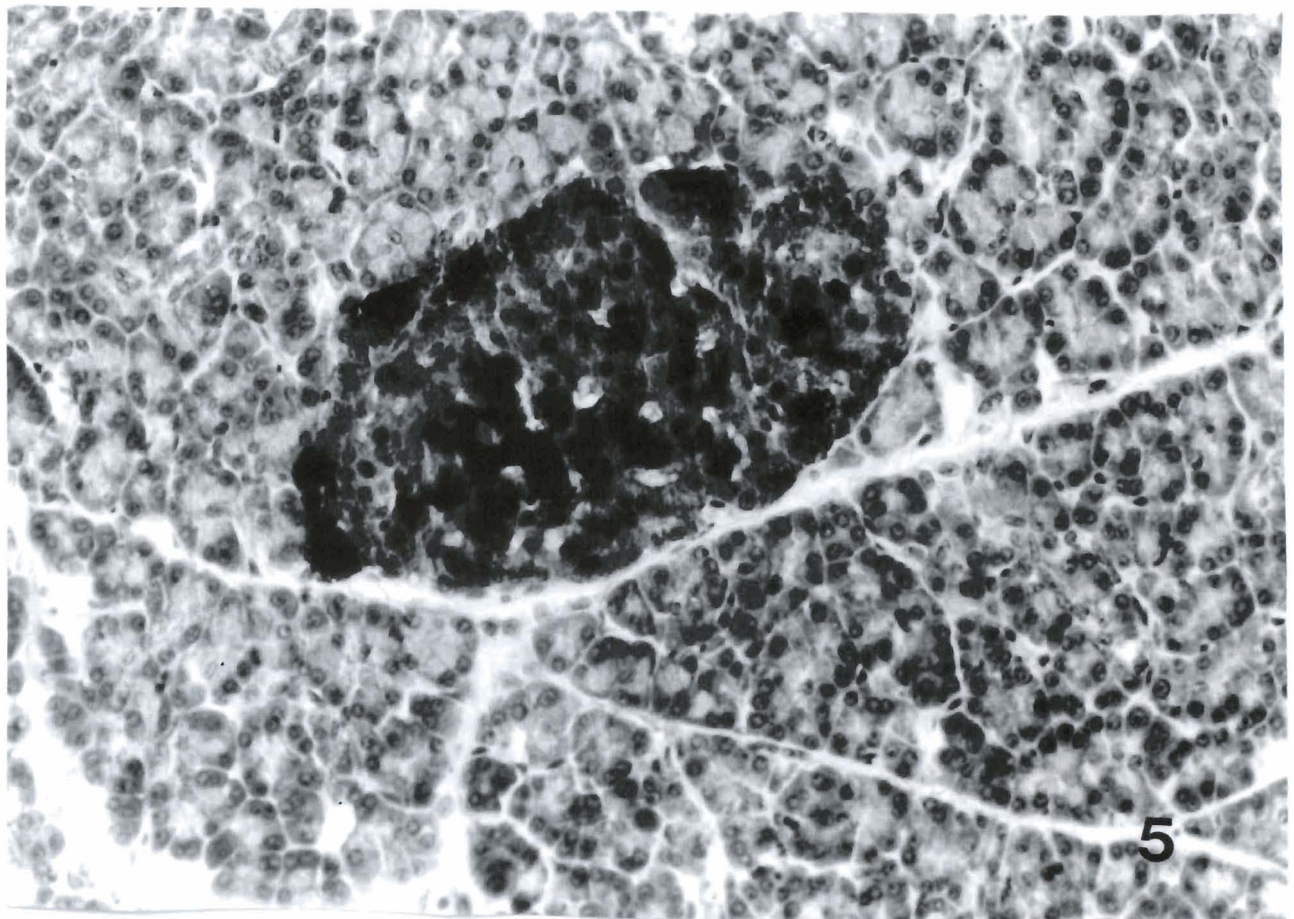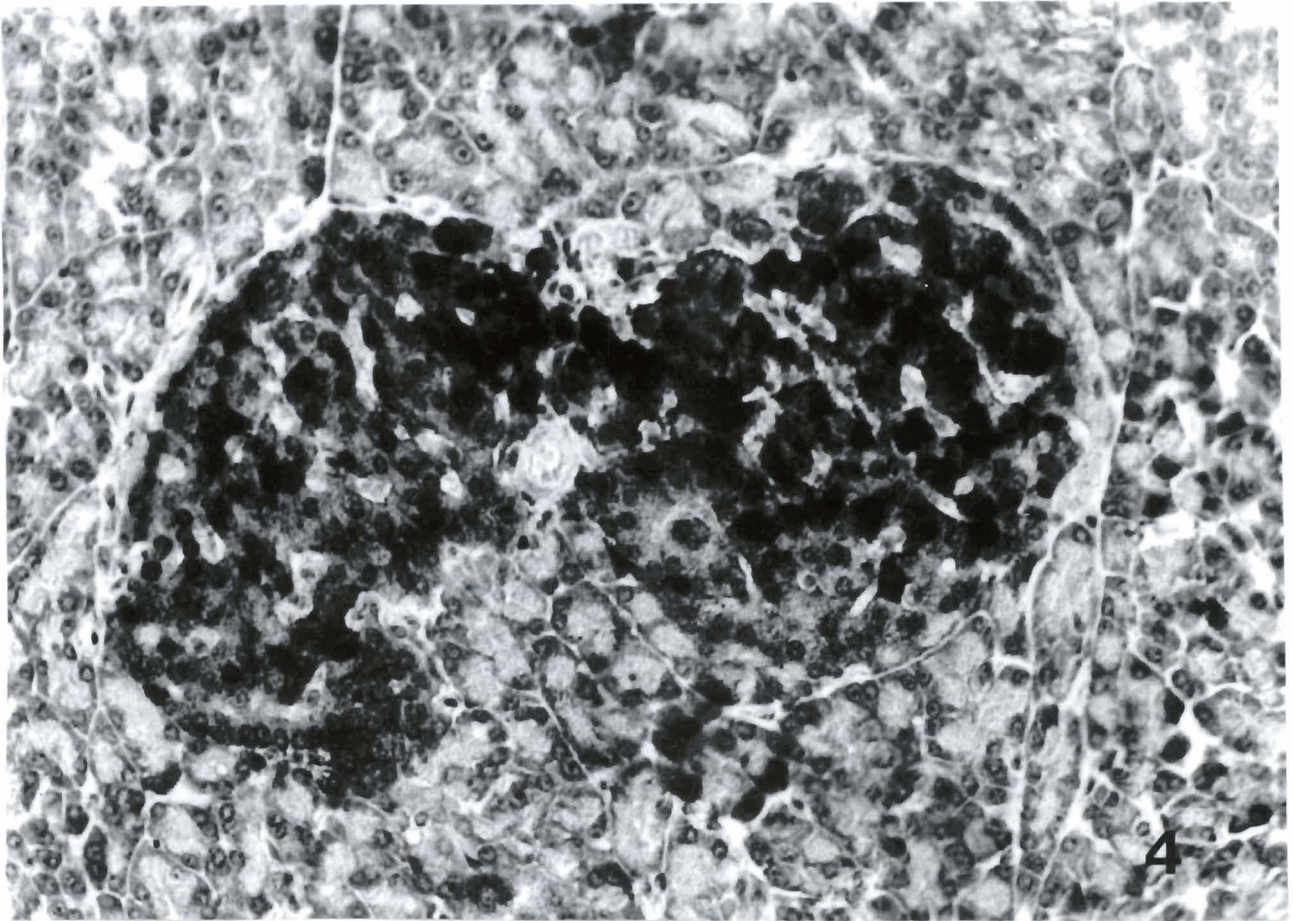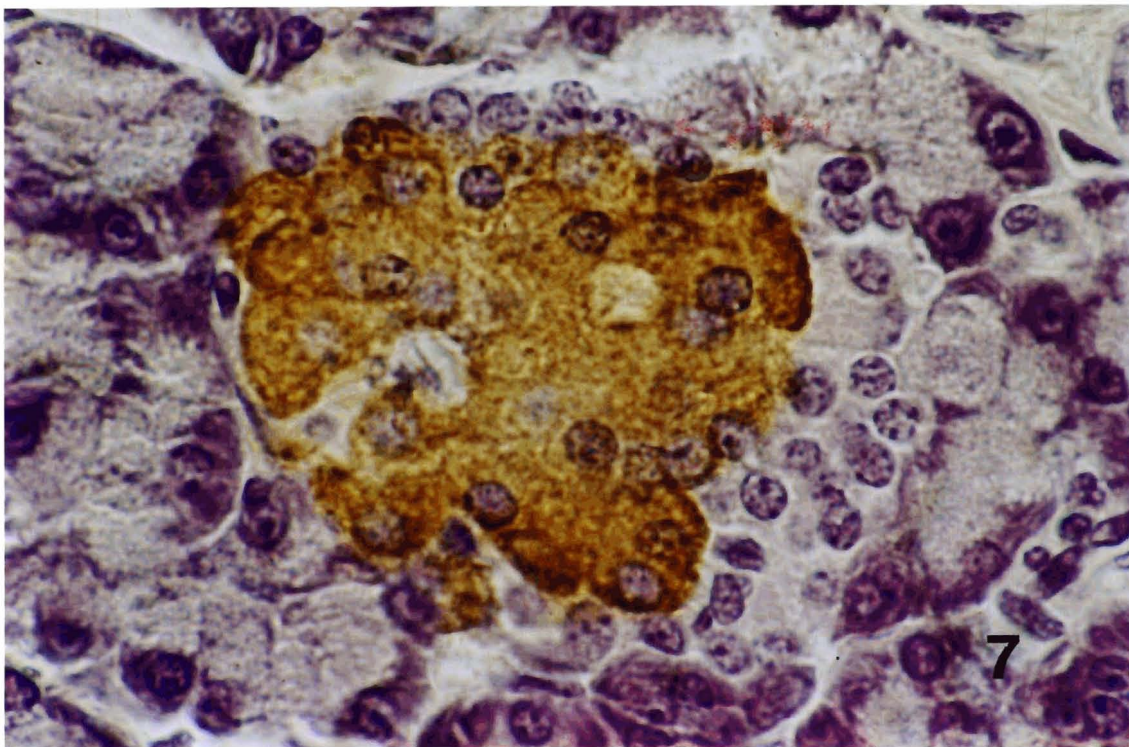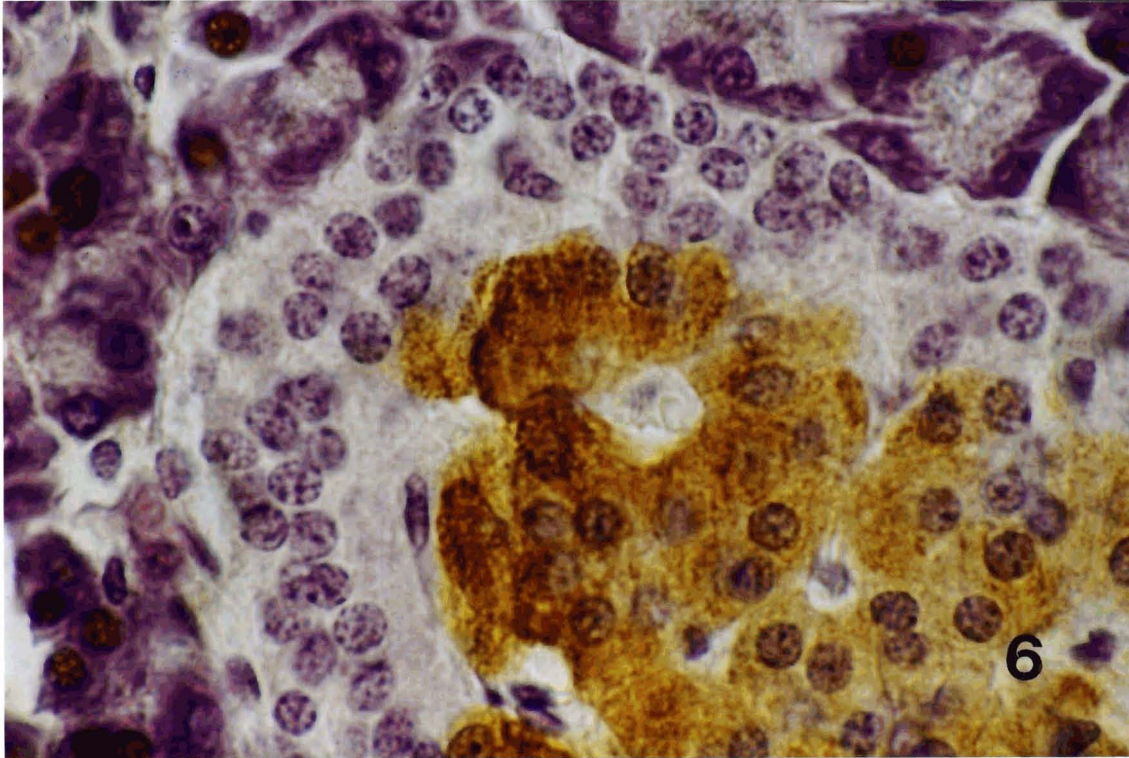
Figs. 4, 5. Immunocytochemical staining for NSE. Normal islet from control (4) and from ethanol-treated rats (5). The number of neuroendocrine cells of islets and their staining intensity are similar in both groups. x 400

al., 1967; Rämö, 1987). Diet could be such a factor, because fat and protein rich diets are known to modify ethanol metabolism (Tsukamoto et al., 1988). Wilson and colleagues (1988) reported that rats fed on ethanol in combination with a protein-deficient diet developed marked steatosis, whereas alcohol ingestion with a
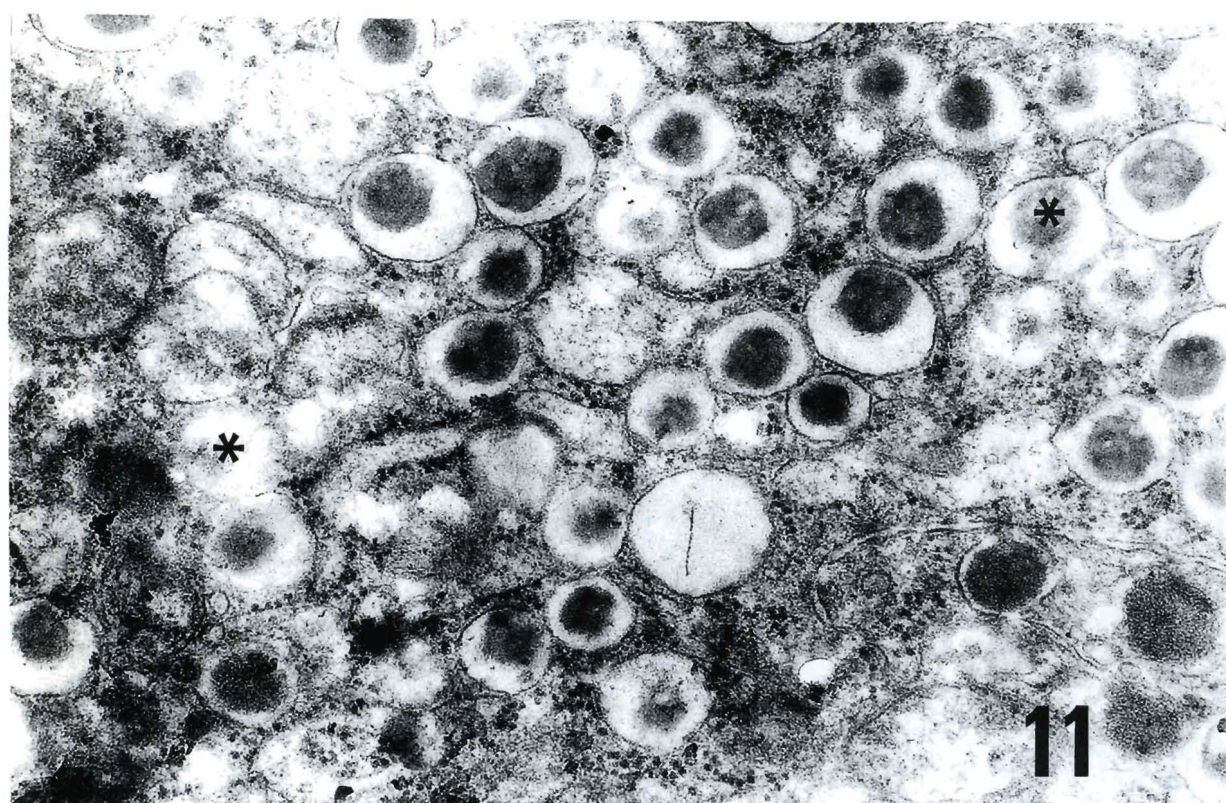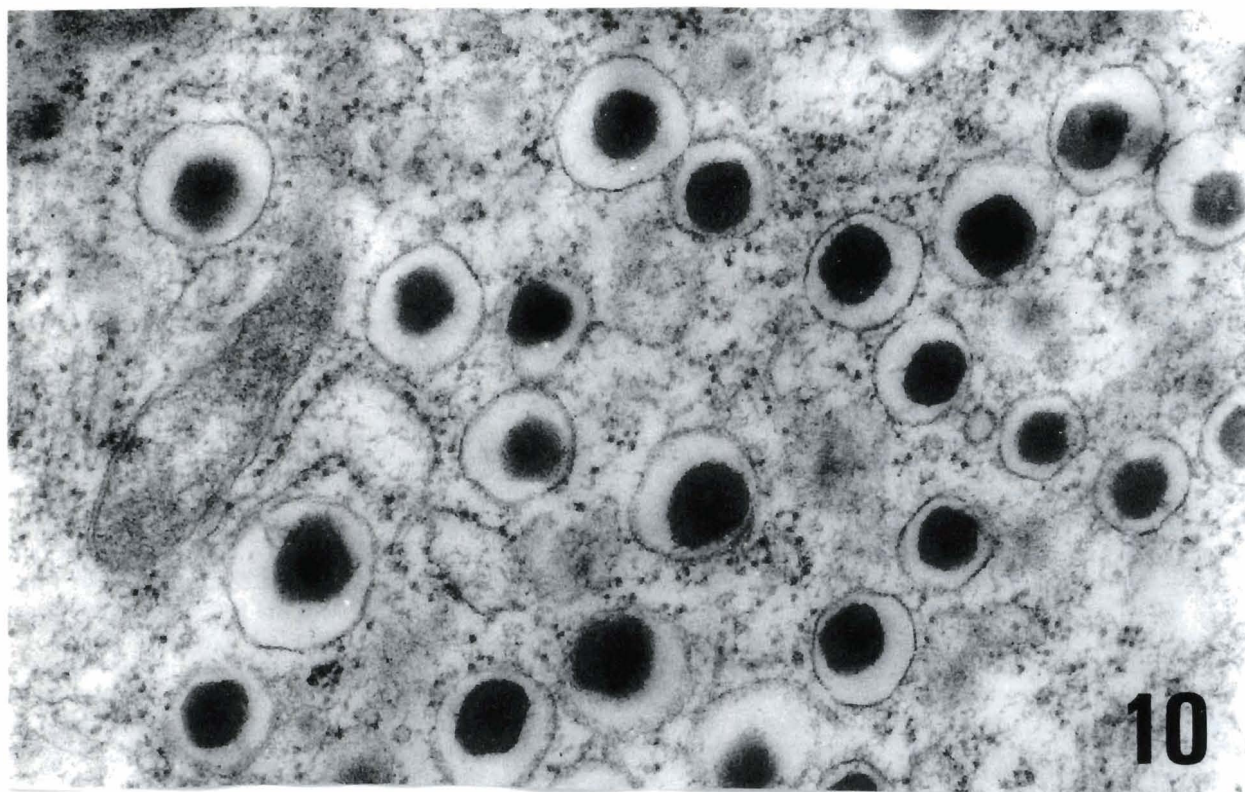

Figs. 6, 7. Immunocytochemical staining for insulin. Normal islet from control is predominantly composed of well granulated B (insulin)-cells (6); islet from ethanol-treated rat shows slight reduction of staining intensity (7). x 2,000
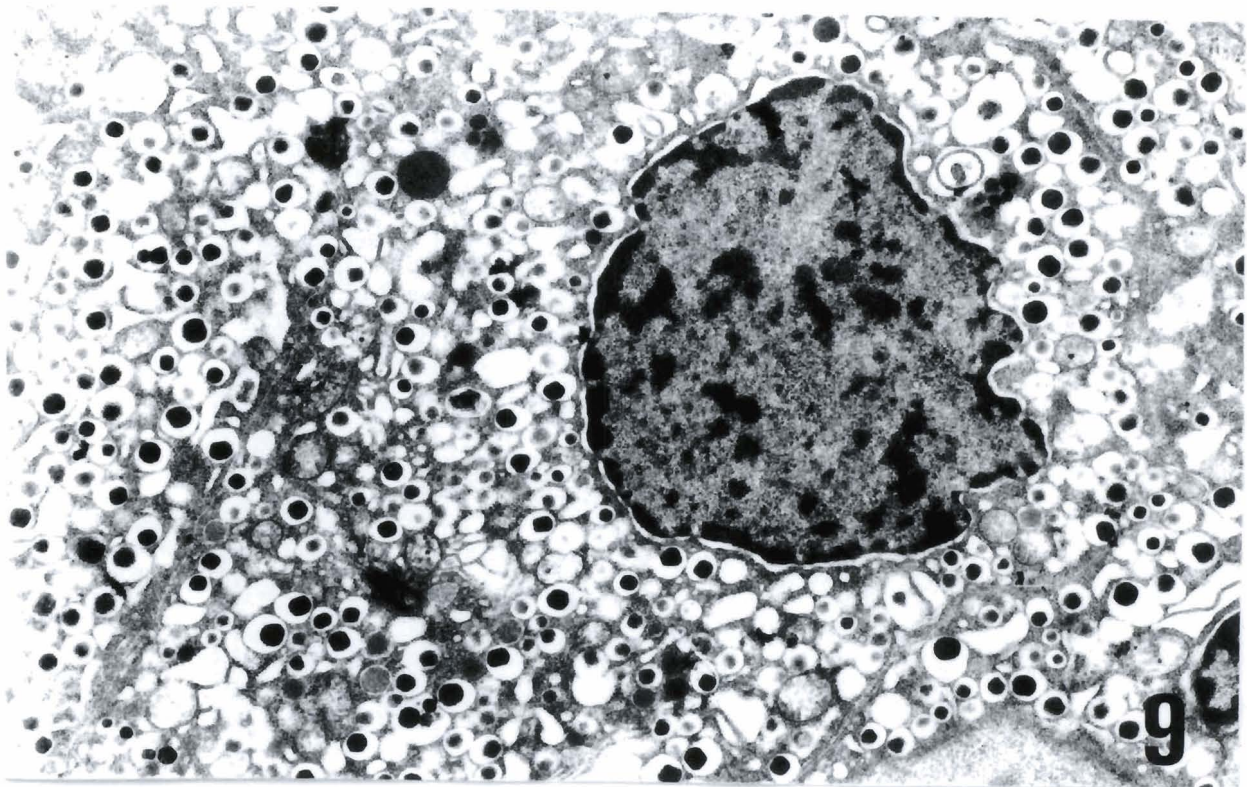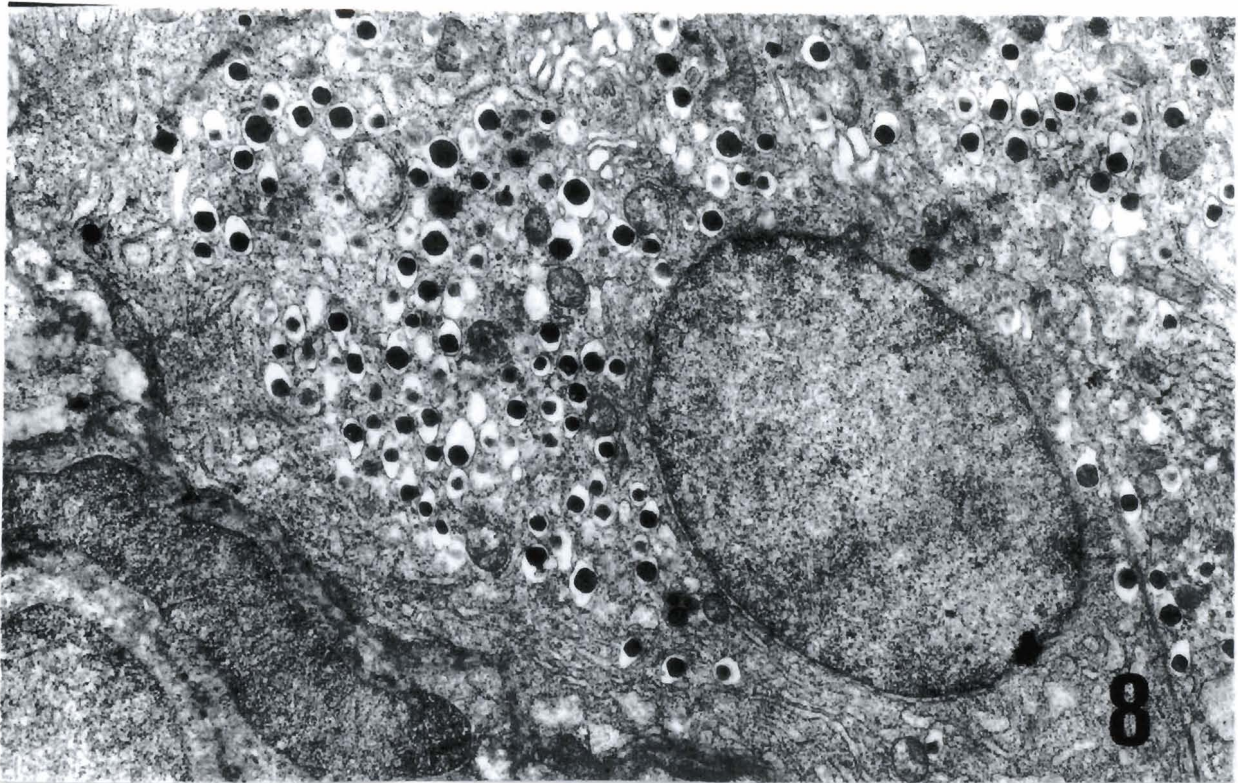
Figs. 8, 9. Electron micrographs of the gross subcellular appearance of B-cells of control (8) and ethanol-treated (9) rats. x 12,000

Figs. 10,11. Secretory granules of B-cells of control (10) and ethanol-treated (11) rats. Note the secretory granules without clear electrondense crystals of insulin (asterisk) in the experimental animal.x 55,000

nutritionally adequate diet and protein deficiency alone were each associated with a more moderate degree of pancreatic lipid accumulation. However, controlled studies of human dietary habits in connection with acute or chronic pancreatitis are difficult to arrange.

It is clear from our experimental design that rats given a chronic dose of ethanol (about 25% of total calories) did not consume inadequate amounts of other nutrients and had a normal growth rate when compared to pair-fed controls. In this respect, the ethanol diet was nutritionally adequate, and possible effects of alcohol were not confounded with the effects of malnutrition. Previously reported studies from our laboratory, utilizing the same experimental design, showed similar daily growth rates for control and ethanol-treated animals (Koko et al., 1992; Todorovic et al., 1993, 1994).

Morphological studies have revealed both cell-to-cell contact between endocrine islets and exocrine acini (Trimble et al., 1985) and direct connections between the capillaries of the islets and the acini (Bonner-Weir and Orci, 1982; Weaver and Sorenson, 1989). These morphological arrangements may reflect some regulatory roles for islet hormones in the function of the exocrine pancreas and vice versa. Pancreatic insufficiency may result in widespread endocrine changes (Klöppel et al., 1978; Owiang, 1986; Fölsch et al., 1990; Owiang et al., 1991). However, relatively little

is known about the effect of alcohol on endocrine pancreas morphology and the findings are inconsistent to some extent contradictory.

The results of our stereological and morphological investigations indicated that ethanol-treated rats had a significantly reduced absolute pancreatic islet volume. This reduction accounted for about 34% of the total islet volume in comparison with controls. It was also found that the distribution of total islet volume among islets of various diameter classes differed between the two groups. Thus, although smaller islets were predominant numerically and the bulk of the endocrine volume consisted of midsized islets in both groups, in the ethanol-treated animals there was a lower frequency in the endocrine volume of midsize islets. The total number of endocrine cells both per islet and per μm^2 of islet was similar in the two groups of animals. Previously reported studies showed that all islet cells were immunoreactive for NSE (Bishop et al., 1982; Marangos, 1985; Pahlman et al., 1986; Pour et al., 1993). The volume density and number of B-cells per islet and per μm^2 of islet were not changed in ethanol-treated rats. On the other hand, the B-cells and their nuclei were found to be statistically significantly decreased in all the parameters investigated: diameter, surface area and total volume. In addition, relative pancreatic weight (as % body weight) in ethanol-treated rats was decreased. These results
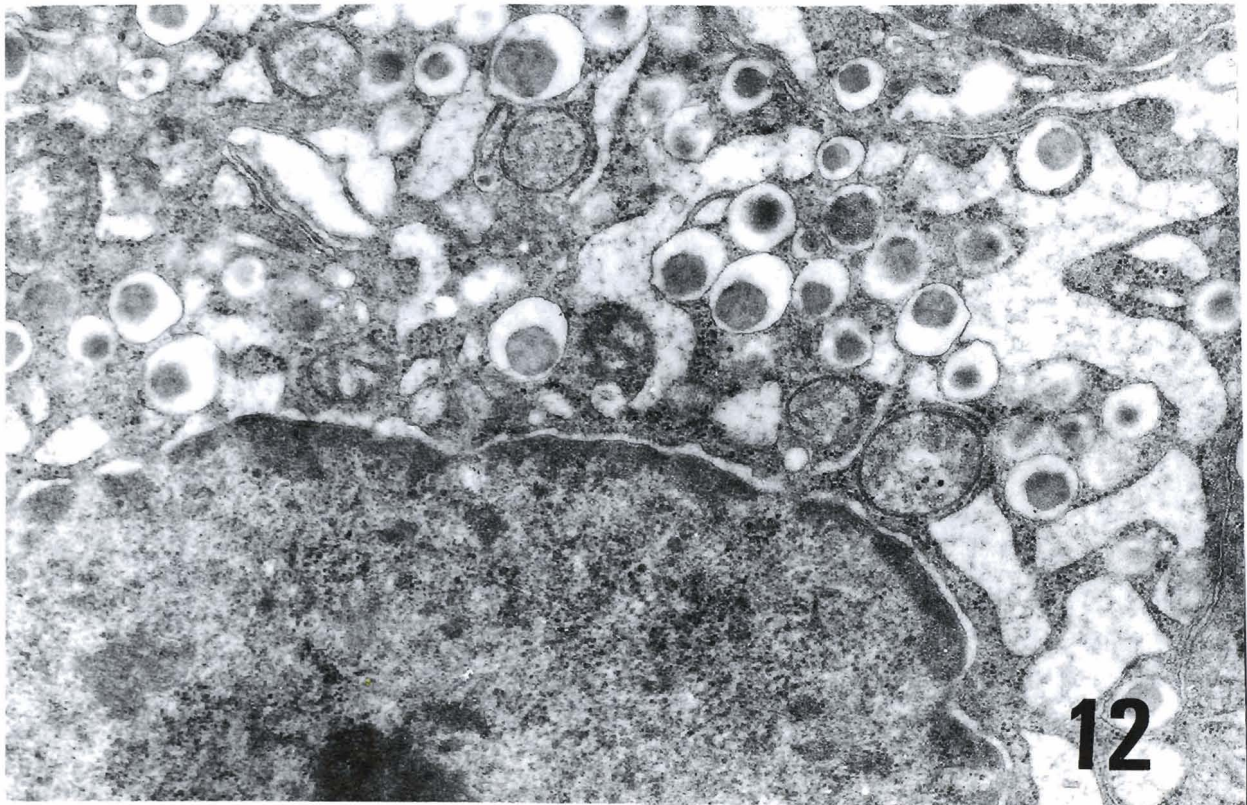
Fig. 12. General distortion of the cisternae of endoplasmic reticulum of B-cells in ethanol-rat. x 22,500

indicated some degree of atrophy of the islets and B-cells in the experimental rats.

A previously reported study from our laboratory utilizing the same experimental design (Koko, 1986) showed that more than half the ethanol-treated animals developed the following morphological changes: focal areas of parenchymal degeneration with fibrosis; and protein plug formation (sometimes calcified) in the ducts and tubular complexes. In keeping with our pathological results are the experimental experiences of Sarles and colleagues (Sarles et al., 1971, Sarles, 1975), Singh (1987) and Singer et al. (1990), who emphasized that lesions in alcoholic Wistar rats were remarkably similar those in human chronic alcoholic pancreatitis.

Morphological studies have shown that patients with chronic pancreatitis have histological changes in the islet of Langerhans. The study of Klöppel et al. (1978) disclosed qualitative and quantitative changes in the endocrine pancreas in addition to the well known lesions of the exocrine pancreas: focal accumulation of sclerotic islets of variable size; occasional neoformation of islets by ductoinsular proliferation (neosidioblastosis); and perisinusoidal fibrosis in sclerotic islets. These authors hypothesized that the accumulation of sclerotic islets probably resulted from progressive collapse of the exocrine parenchyma. Immunocytochemical quantitation of the distribution of insulin (B), glucagon (A), somatostatin (D) and pancreatic polypeptide (PP) producing cells revealed a significant relative increase in the number of A-cells and a decrease in the number of B-cells of the sclerotic islets in primary chronic pancreatitis as well as in secondary chronic pancreatitis. The number of PP cells was significantly increased in primary chronic pancreatitis only. However, previous results obtained for immunocytochemical quantitation and stereological investigation of individual endocrine cells per islet showed a significant decrease in the surface area of D-cells (Koko et al., 1994). It was suggested that the decreased volume of the islets could be a result of atrophy of those cells. In addition, histological study of alcoholic, nonalcoholic, and obstructive chronic pancreatitis show insular regression, which appears as islets persisting in fibrosis after disappearance of exocrine tissue, in about two thirds of cases with alcoholic and nonalcoholic chronic pancreatitis, but in only one third of cases with obstructive chronic pancreatitis (De Angelis et al., 1992). Geokas (1984) postulated that the islets are relatively resistant to the destructive process in chronic pancreatitis, and their number was variable (hypoplasia, adenoma, hyperplasia) in a report by Sarles et al. (1971).

To the best of our knowledge, no morphometric and ultrastructural study of pancreatic islet B-cells has been undertaken after chronic ethanol intake in either human or experimental animal studies. Our results indicated that over a 4-month period of ethanol intake, a significant decrease in cell profile area, nuclear profile area, volume density and mean diameter of cytoplasmic granules and an increase in the profile area and volume density of the

endoplasmic reticulum occurred. In addition, the gross ultrastructural alteration seen in most B-cells of the ethanol-treated rats was irregularity of the nuclear envelope with deep invagination, margination of heterochromatin and many empty granules or granules without clear electrondense crystals of insulin. However, Klöppel et al. (1978) indicated that electron microscopy revealed no consistent alterations of endocrine cells from patients with severe primary chronic pancreatitis or secondary chronic pancreatitis. In particular, the B-cells did not show any degenerative lesion, although the number of secretory granules appeared to be somewhat reduced.

Our histological examination revealed that islet blood vessels were dilated in alcoholic rats. This was confirmed by stereological analysis. Thus, in ethanol-treated rats the volume density, diameter and total length of islet blood vessels were increased; the last two increases being statistically significant. This result could be explained by the direct effect of ethanol on small blood arterioles. It has been reported that ethanol causes vasodilatation of small gastric arteriolar vessels and vasoconstriction of venules (Oates and Hakkinen, 1988) and this is in accordance with the finding that most islets of Langerhans possessed small arterial blood vessels (Weaver and Sorenson, 1989). Measurements of pancreatic blood flow (PBF) and islet blood flow (IBF) at the lower ethanol concentration revealed a decrease in PBF and a preferential increase in IBF, associated with an increase in insulin release without any change in the serum glucose concentration (Jansson and Petersson, 1988). The mechanism behind the increase in IBF, i.e. dissociation between whole PBF and IBF, may be due to stimulatory effects on the central nervous system. It is indeed known that the IBF to a large extent is dependent on nervous control. A recent study of pancreatic nerves from patients suffering from chronic alcohol-induced pancreatitis revealed an increase in their number and diameter and striking ultrastructural changes consisting mainly of damage to the perineural sheath (Weihe et al., 1990; Büchler et al., 1992). The changes in peptidergic innervation in patients suffering from painful chronic alcohol-induced pancreatitis appeared to be rather selective. They concerned predominantly interlobular, intralobular and paravascular nerve bundles and the transmitter candidates SP and CGRP and, to a lesser extent, VIP/PHI/PHM.

The present results concerning the fasting blood glucose and insulin levels were similar in the control and experimental groups of animals.

Functional studies using various tests for insulin secretion have been performed both in patients and animals with chronic pancreatitis. Fasting insulin levels in chronic pancreatitis were found to be normal or moderately raised (Keller et al., 1965; Peters et al., 1966) or diminished (Go, 1990), and the response to oral glucose was variable (Keller et al., 1965; Fölsch et al., 1990). Fölsch et al. (1990) reported that blood glucose concentrations were higher in patients with chronic

pancreatitis when compared to controls. Clearly, the insulin response was significantly higher to the oral glucose load compared to isoglycemic intravenous glucose administration both in patients with chronic pancreatitis and in healthy volunteers. While the glucose concentrations were higher in the patients with chronic pancreatitis, the insulin response tended to be somewhat smaller. On the other hand, insulin content of the pancreatic tissue was only slightly diminished and did not significantly differ between the examined groups. However, the plasma insulin response following intensive B-cell stimulation with combined intravenous glucagon and tolbutamide 30 min after oral glucose was found to be reduced (Joffe et al., 1968). This suggests that the insulin response in chronic pancreatitis is depleted. On the other hand, serum immuno-reactive insulin (IRI) concentrations were diminished in chronic human alcoholics (Amindzhanov, 1986) or ethanol had no effect on IRI in the ethanol-treated rats (Weesner et al., 1985; Nikolic et al., 1991). Infusion of ethanol at increasing concentrations caused a progressive inhibition of insulin and glucagon secretion (Samols and Stagner, 1980; Singh et al., 1980). However, Jauhonen and Hassinen (1978) reported that ethanol itself produced no marked changes in blood glucose, IRI and glucagon after intravenous infusion. Shah et al. (1977) proposed that ethanol inhibits glucose- and tolbutamide-induced insulin secretion, which in turn, causes glucose intolerance and prevents tolbutamide-induced hypoglycemia. Hyperglucagonemia and hyper-insulinemia have been reported in alcoholics (VanThiel and Gavaler, 1985) and rats after acute and chronic ethanol administration (Simanowski et al., 1989), and both appear to play a role in the pathogenesis of the disturbed glucose metabolism in alcoholic humans (Sereny and Endrenyi, 1978).

In conclusion, the present study offers the following concept for the morphofunctional alterations of rat pancreatic islet B-cells in chronic alcoholism. All investigated parameters indicate that there was atrophy of B-cells and reduction of absolute pancreatic islet volume in the ethanol-treated rats. Our morphometric ultrastructural results indicated marked disturbances of the cytoplasmic granules and endoplasmic reticulum, and these data are in accordance with findings from functional studies demonstrating a reduced insulin reserve and depletion of the insulin response in patients and animals with chronic alcoholic pancreatitis. The absence of ductuloinsular proliferation suggests that some neoformation of endocrine tissue is not taking place, i.e. the B-cell appeared to be sensitive to the direct toxic effect of alcohol and could not regenerate quickly enough.

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