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Potentiation of Ethanol-Induced Pancreatic Injury by Dietary Fat

Induction of Chronic Pancreatitis by Alcohol in Rats

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Effects of sustained ethanol intoxication and dietary fat content on pancreatic morphology were investigated in the rat model implanted with gastrostomy catheters, which permitted continuous intragastric infusion of ethanol plus liquid diet containing one of three levels of corn oil: 5% (low-fat), 25% (high-fat), and 35% (extra-high-fat) of total calories. After various durations of infusion ranging from 30 to 160 days, the pancreatic histology was examined. Mean blood alcohol levels achieved in the low, high, and extra-high fat diet groups were similarly high: 210 ± 120, 224 ± 122, and 289 ± 110 mg/dl. The average weight gain of these ethanol-fed groups during the first 8 weeks of experiments was 15.4 ± 1.9, 19.6 ± 8.0, and 14.9 ± 5.2 g/wk, respectively, and was not statistically different from that of pair-fed controls infused with isocaloric amount of dextrose and respective diet, nor from that of age-matched animals given the regular chow. None of control animals showed abnormal pancreatic morphologic features except occasional mild steatosis in those fed the extra-high-fat diet. With the low dietary intake of unsaturated fat, chronic ethanol intoxication produced only mild pancreatic pathology such as steatosis and interstitial edema. Administration of ethanol and the high-fat and extra-high-fat diets caused hypogranulation and apoptosis of acinar cells. Focal lesions of chronic pancreatitis were also observed in 20% or 30% of ethanol-fed animals given the high-fat or extra-high-fat diet. These lesions were characterized by fat necrosis, mononuclear cell infiltration, fibrosis, acinar atrophy, ductal dilatation, and intraductal mucious or proteinacious plugs. The incidence of focal acute pancreatitis was less (7–20%) but appeared increased with higher dietary fat content. Induction of either acute or chronic pancreatitis was not correlated with plasma levels of triglycerides or cholesterol. These results demonstrate potentiation by dietary unsaturated fat of ethanol-induced pancreatic injury. This model possesses many features analogous to those seen in alcoholic pancreatic injury in man. The hyperlipidemia does not appear to be an important pathogenetic factor for ethanol-induced pancreatitis produced in this model. (Am J Pathol 1988, 131:246–257)

ALCOHOLISM IS THE most common etiologic factor for acute and chronic pancreatitis.[1–7] The lack of vital information regarding the early pathophysiologic steps involved in pathogenesis of alcoholic pancreatic injury may have been attributable to the unavailability of an easily reproducible animal model of this disease. In the early 1970s, Sarles et al described focal lesions of chronic pancreatitis in more than half of Wistar rats that ingested ethanol for 20–30

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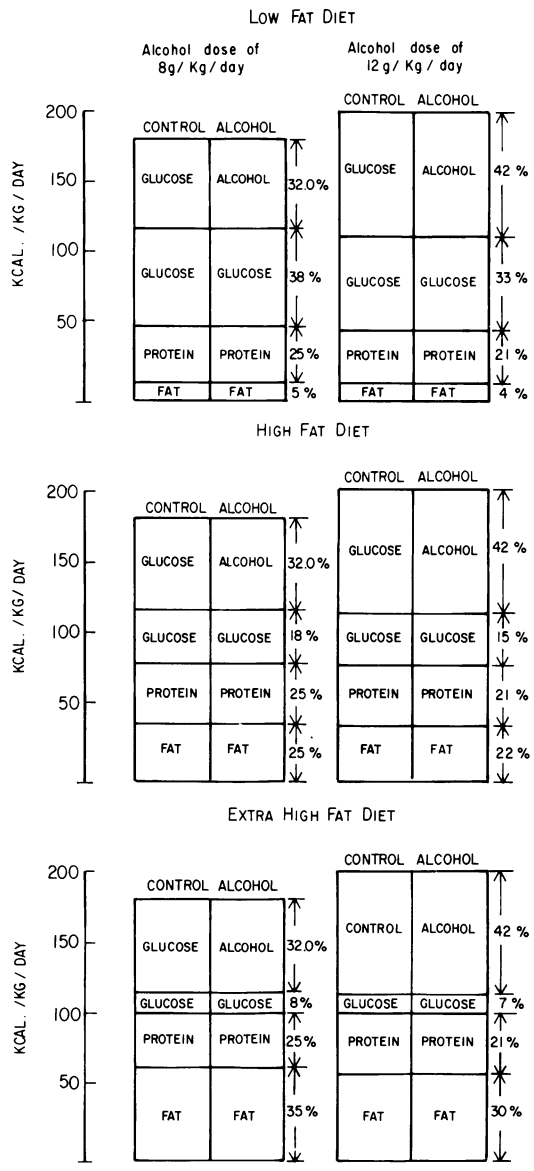


Figure 1—Proportions of the caloric sources in low-, high-, and extra-high-fat diets.

months.[8] Pancreatic juice from these animals contained significantly higher protein concentrations than that from controls and even spontaneously secreted precipitates. These conditions appeared to be very similar to those observed in humans with calcifying pancreatitis.[9] However, subsequent studies in an attempt to confirm Sarles' findings have yield conflicting results,[9-12] and the ductal plugs observed in ethanol-fed rats have also been reported to occur in many control animals.[13] One of the major factors responsible for inconsistent reproduction of alcoholic pancreatic injury in animal models could have been lack of methodology to assure high ethanol intake and sustained intoxication as seen in human alcoholics. Indeed, this has recently been shown to be the key factor for induction of progressive alcoholic liver injury in the animal model.[14]

Epidemiologic studies have shown a linear relationship between the mean daily consumption of ethanol and the log of the relative risk for developing pancreatitis.[15] However, there was no statistically obtainable overall threshold dose of ethanol for development of acute pancreatitis because individual threshold doses were different from one another, which suggested a genetic component responsible for this different susceptibility.[15,16] Studies in France have shown that patients with chronic calcifying pancreatitis drank more ethanol and had greater intake of fat and protein than matched controls.[17] This finding was confirmed by a similar study in Brazil[18] and also supported by experimental observations with animal models.[16]

We have recently developed a rat model of chronic ethanol intoxication which achieved sustained blood ethanol levels as well as independent control over ethanol and nutrient intake.[14,19,20] Because of these unique features, we were able to induce, for the first time in the rat, advanced ethanol-induced liver injury progressing from steatosis to necrosis and fibrosis.[20] Furthermore, maximal controlability of nutrient intake in this model enabled us to carefully study effects of dietary levels of fat on the severity of ethanol-induced liver injury.[20] In the present study, this model was employed for investigation of 1) the effects of sustained ethanol intoxication on pancreatic histology and 2) the effects of dietary levels of unsaturated fat on ethanol-induced pancreatic injury.

Materials and Methods

Male Wistar rats weighing 350–400 g were used. The detailed description of gastrostomy catheterization and infusion of ethanol and a diet have been de-

Table 1—Weight Gains and Blood Alcohol Levels of Rats Infused With Low-, High-, or Extra-High-Fat Diet

Diet	Pair-fed control	Ethanol-fed	
	Weight gains* (g/wk)	Weight gains* (g/wk)	Blood alcohol level (mg/dl)
Low-fat	15.6 ± 0.8 (12)	15.4 ± 1.9 (12)	216 ± 120 (14)
High-fat	20.4 ± 7.8 (10)	19.6 ± 8.0 (10)	224 ± 122 (10)
Extra-high-fat	23.2 ± 8.4 (5)	14.9 ± 5.2 (5)	289 ± 110 (6)

Values are expressed as the mean ± 1 SD for the number of animals indicated in parentheses.

* Weight gains were determined during the first 8 weeks of the experiment. The mean weight gain of 6 age-matched rats fed Chow *ad libitum* was 21.3 ± 7.6 g/wk.

Table 2—Pancreatic Histopathology of Rats Infused With Ethanol*

Rat no.	Duration (days)	Steatosis		Acinar necrosis					Fat necrosis	Fibrosis		Acinar atrophy	Ductal dilatation
		Stromal	Intracellular†	Apoptosis	Focal	Edema	Hemorrhage	Inflammation		Interlobular	Periductal		
Low-fat diet													
111	30	—	2+	—	—	1+	—	—	—	—	—	—	—
130	30	—	1+	—	—	—	—	—	—	—	—	—	—
136	30	—	1+	—	—	2+	—	—	—	—	—	—	—
137	30	—	1+	—	—	—	—	—	—	—	—	—	—
148	30	—	—	—	—	—	—	—	—	—	—	—	—
150	30	—	1+	—	—	1+	—	—	—	—	—	—	—
122	45	—	1+	—	—	2+	—	—	—	—	—	—	—
134	45	—	3+	—	—	1+	—	—	—	—	—	—	—
112	85	—	1+	—	—	—	—	—	—	—	—	—	—
115	85	—	3+	—	—	2+	—	—	—	—	—	—	—
124	85	—	1+	—	—	1+	—	—	—	—	—	—	—
140	85	—	3+	—	—	2+	—	—	—	—	—	—	—
142	85	—	2+	—	—	2+	1+	1+	1+	—	—	—	—
143	120	—	3+	—	—	1+	—	—	—	—	—	—	—
144	160	—	3+	—	—	—	—	—	—	—	—	—	—
High-fat diet													
201	30	—	1+	—	—	—	—	—	—	—	—	—	—
217	30	—	1+	—	—	—	—	—	—	—	—	—	—
218	30	—	3+	1+	—	—	—	—	—	—	—	—	—
252	30	2+	2+	—	1+	1+	1+	1+	—	—	—	—	—
213	45	—	2+	2+	—	—	—	—	—	—	—	—	—
210	85	—	2+	1+	1+	—	—	—	—	—	—	—	—
224	85	—	4+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
225	85	—	3+	1+	—	—	—	—	—	1+	—	—	—
233	85	—	1+	1+	—	—	—	—	—	—	—	—	—
238	85	1+	1+	1+	—	—	—	—	—	—	—	—	1+
241	85	—	3+	1+	—	—	—	1+	1+	1+	1+	1+	—
228	100	1+	2+	1+	—	1+	—	1+	1+	1+	1+	1+	1+
207	100	—	1+	1+	—	2+	—	1+	1+	1+	—	—	—
234	100	1+	1+	1+	—	—	—	—	—	—	—	—	—
231	160	1+	3+	1+	—	—	—	1+	—	—	—	—	1+
Extra-high-fat diet													
302	30	2+	1+	1+	—	1+	—	1+	—	1+	1+	1+	1+
304	30	2+	1+	—	—		—	—	—	—	—	—	—
306	30	2+	3+	1+	—	1+	—	—	—	1+	—	—	—
310	30	2+	1+	1+	—	—	—	—	—	—	—	—	—
308	45	1+	1+	—	—	—	—	—	—	—	—	—	—
314	45	1+	1+	—	—	1+	—	—	—	—	—	—	—
305	85	2+	3+	2+	—	—	—	1+	1+	1+	1+	1+	—
309	85	1+	4+	1+	—	—	—	—	—	—	—	—	—
307	120	1+	3+	2+	1+	1+	1+	1+	—	1+	—	—	—
312	160	2+	4+	2+	1+	—	1+	1+	1+	1+	1+	1+	1+

* 1+, ≤1 focus per low power view; 2+, >1 focus except for intracellular steatosis.
† 1+, 0–25% of acinar cells with lipid droplets; 2+, 25–50%; 3+, 50–75%; 4+, 75–100%.

scribed.[14,20] Briefly, animals were aseptically implanted with either single or double gastrostomy catheters as reported earlier.[21] The use of spring coil and swivel allowed the protection of the catheter while permitting the free movement of the animal in an individual metabolism cage. Rats received as their total nutrient intake intragastric infusion of the liquid diet plus ethanol or isocaloric glucose solution. Protein was provided by lactalbumin hydrolysate, fat by corn oil, and carbohydrate by glucose. Caloric contributions of ethanol and macronutrients for low-, high-, and extra-high-fat diets are illustrated in Figure 1. In order to maintain the blood alcohol level, ethanol and the diet were continuously infused, and the dose of ethanol was progressively increased from 8 g/kg/day to 12 g/kg/day in the 4th week and 15 g/kg/day in the 17th week. Animals were closely observed every day for the degree of alcohol intoxication and were never found to be unconscious.

After various durations of intoxication ranging from 30 to 160 days, animals were sacrificed, and the pancreas was obtained and fixed in 10% formalin or 2.5% glutaraldehyde in sodium cacodylate for light and electron microscopy. In addition to the routine

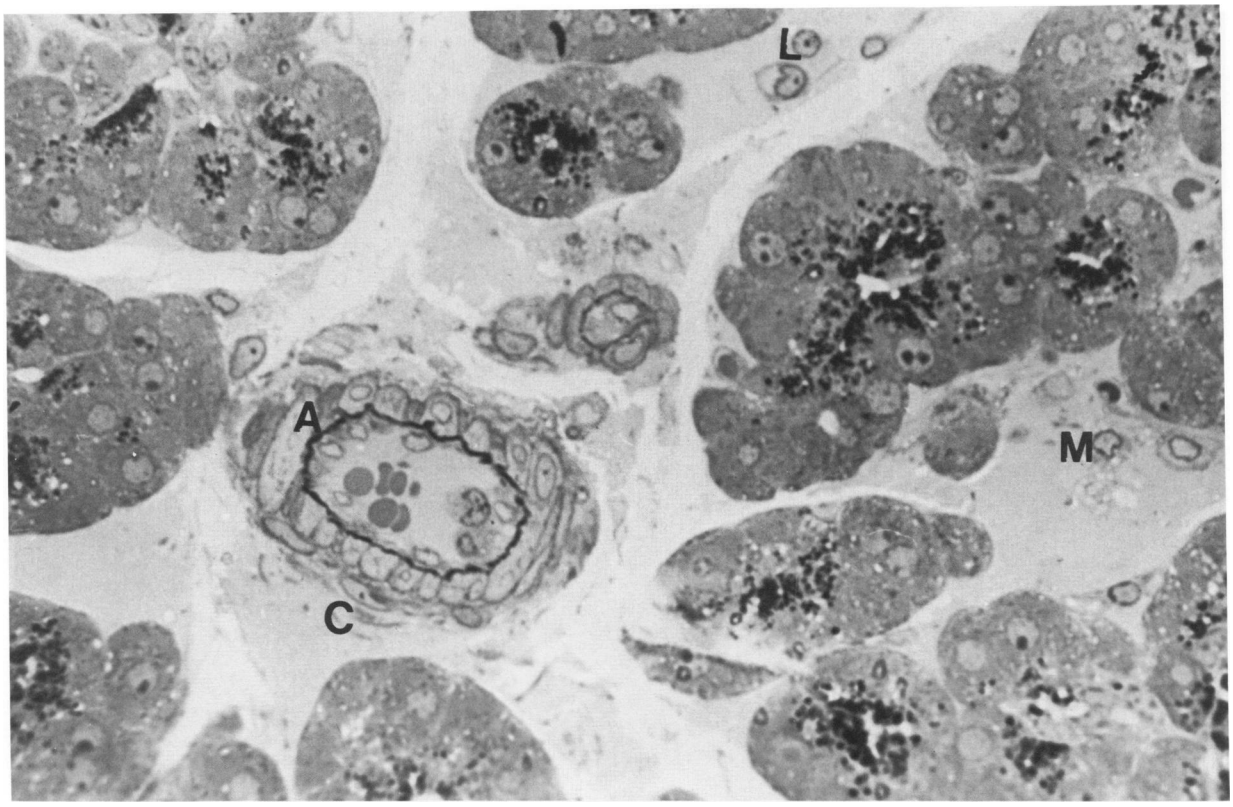


Figure 2—Pancreas from Rat 115, fed ethanol and the low-fat diet for 85 days. The edematous fluid between the separated acini is evident. Lymphocytes (*L*) and macrophages (*M*) can also be seen within the edematous interstitial space. A small artery (*A*) is surrounded by loose bundles of collagen (*C*). (Plastic-embedded, stained with toluidine blue, final magnification ×3120)

hematoxylin and eosin stain, Wilder's reticulin and Mason's trichrome stains were employed for identification of reticulin and collagen. Histologic evaluation of light microscopy consisted of the blind and systematic grading of steatosis, acinar necrosis, edema, hemorrhage, inflammation, fat necrosis, fibrosis, acinar atrophy, and ductal dilatation. For instance, the degree of steatosis was graded by estimating the percent acinar cells containing fat droplets: ie, <25%, 1+; <50%, 2+; <75%, 3+; >75%, 4+. Other pathologic criteria were graded as follows: one or fewer focus per low-power view, 1+; more than one focus, 2+.

Blood alcohol levels were determined with Dupont Automatic Clinical Analyzer in representative venous blood samples obtained from the inferior vena cava of rats prior to the tissue collection. Plasma levels of cationic trypsinogen were measured by a specific radioimmunoassay[22] in representative samples obtained randomly from the low- and high-fat dietary groups. Plasma levels of glucose, cholesterol, and triglycerides were also determined in these samples with the use of a Technicon SMAC high-speed computer-controlled biochemical analyzer (Technicon Instruments Corp., Tarrytown, NY). The significance of differences between the groups was assessed by either the paired or the standard *t* test.

Results

Continuous intragastric infusion of a diet plus ethanol or glucose resulted in steady growth rates during the first 8 weeks of experiments (Table 1). Growth rates of ethanol-fed animals given three different diets were not statistically different either from those of cor responding controls or from that of age-matched rats given chow *ad libitum*. However, the mean weight gain of ethanol-fed rats given the extra-high-fat diet was 37% lower than that of pair-fed controls, and this reduction was the largest among comparisons between three pairs of groups. Mean blood alcohol levels determined in representative samples were also shown in Table 1. These values were not statistically different from one another and verified that ethanol-fed animals were subjected to a high degree of sustained intoxication. Large standard deviations were probably due to the fact that these animals constantly infused

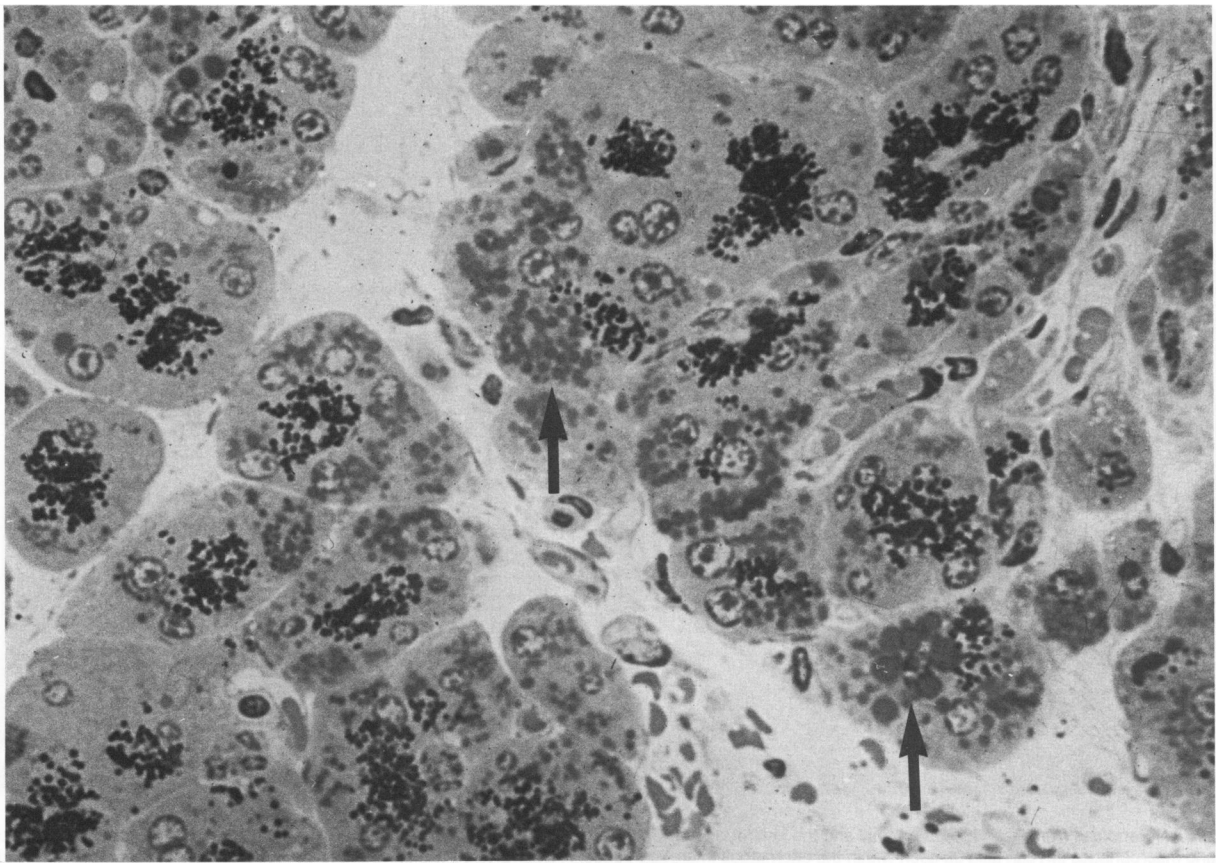


Figure 3—Plastic-embedded pancreas from a rat fed ethanol and high-fat diet illustrates the fat globules in the acinar cells (*arrows*). The fat is stored at the pole opposite the zymogen granules. (Toluidine blue and osmium stain, final magnification ×2000)

with ethanol exhibit the cyclical pattern of blood alcohol levels as previously reported.[21]

Pancreatic Pathology by Ethanol and Low-Fat Diet

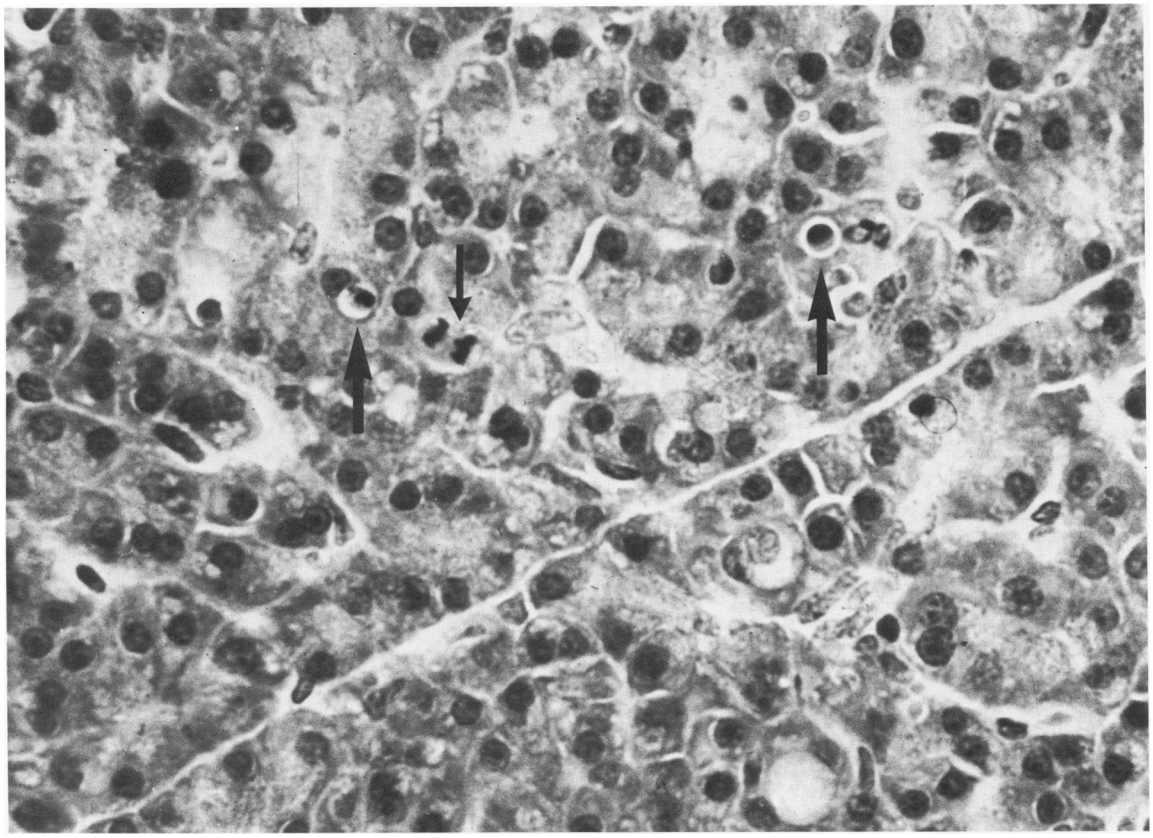
Results of pancreatic histopathology are summarized in Table 2. Infusion of the low-fat diet and ethanol resulted in deposit of fat droplets within acinar cells and interstitial edema (Figure 2) in the majority of animals. In contrast, the pancreas from the control group was normal. Presence of intracellular lipids was confirmed by osmium-stained lipid droplets, and lymphocytes and macrophages were frequently observed in the edematous interstitial space (Figure 2). In one of rats infused with ethanol for 85 days, a focal lesion of acute hemorrhagic pancreatitis was observed (Table 2).

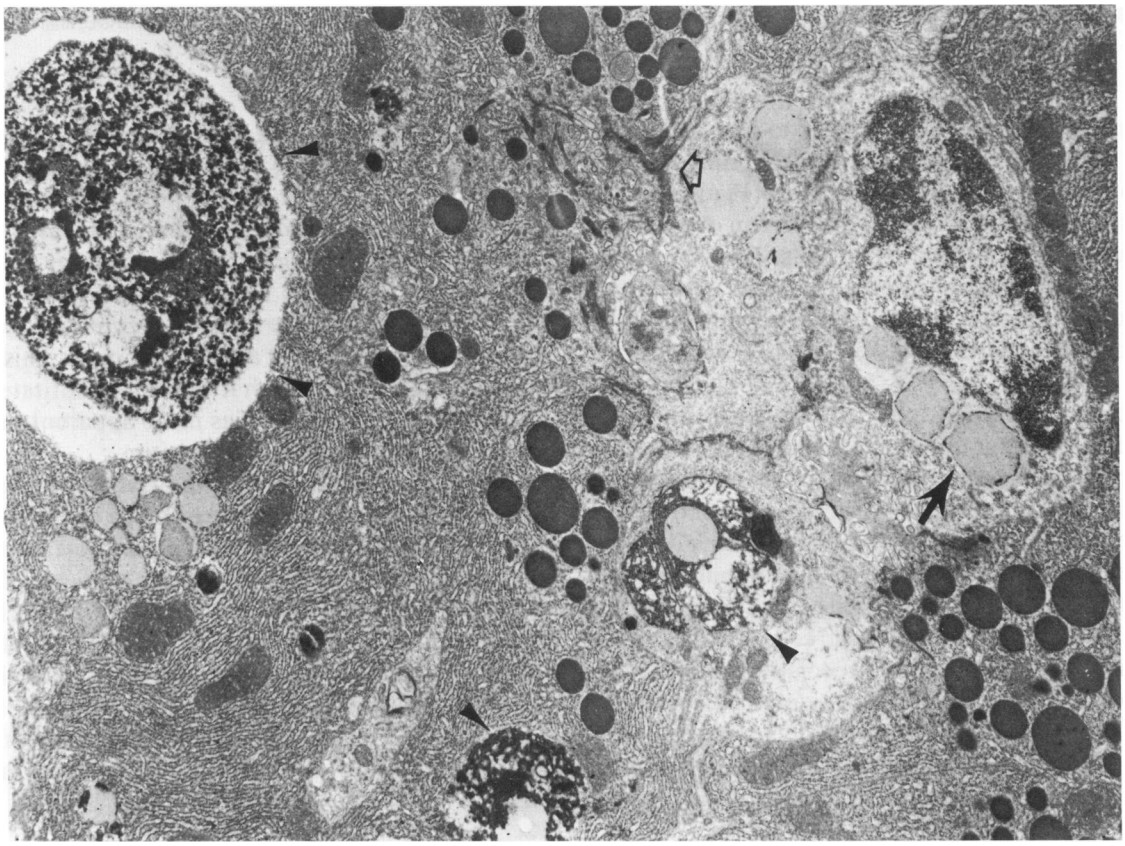
Pancreatic Pathology by Ethanol and High- and Extra-High-Fat Diet

The increases of dietary fat by fivefold or sevenfold resulted in striking potentiation of ethanol-induced pancreatic injury. First, intracellular accumulation of lipid droplets was observed in all ethanol-fed animals (Figure 3), and stromal fatty infiltration was also evident in one-third or all of ethanol-fed animals given the high-fat or extra-high-fat diet, respectively (Table 2). Secondly, apoptosis of acinar cells was frequently seen in ethanol-fed rats given higher levels of fat (Table 2). The apoptosis was characterized by degeneration of a single cell within a vacuole in a normal acinar cell (Figure 4) and confirmed by electron microscopy (Figure 5). Focal lesions of acute hemorrhagic pancreatitis were seen in 2 alcoholic rats given the high-fat

Figure 4—Pancreas from Rat 213, fed ethanol and high-fat diet for 45 days. Scattered single necrotic cells (apoptosis) were evident (*arrows*). Note the mitosis (*small arrow*). (H&E, final magnification ×2750)

Figure 5—Electron micrograph of pancreas shown in Figure 3, showing necrotic cells within vacuoles of acinar cells (*arrowheads*). Also note the fat in a centroacinar cell and acinar cells (*arrow*). Tonofilaments are prominent (*open arrow*). (Electron micrograph, final magnification ×27,540)





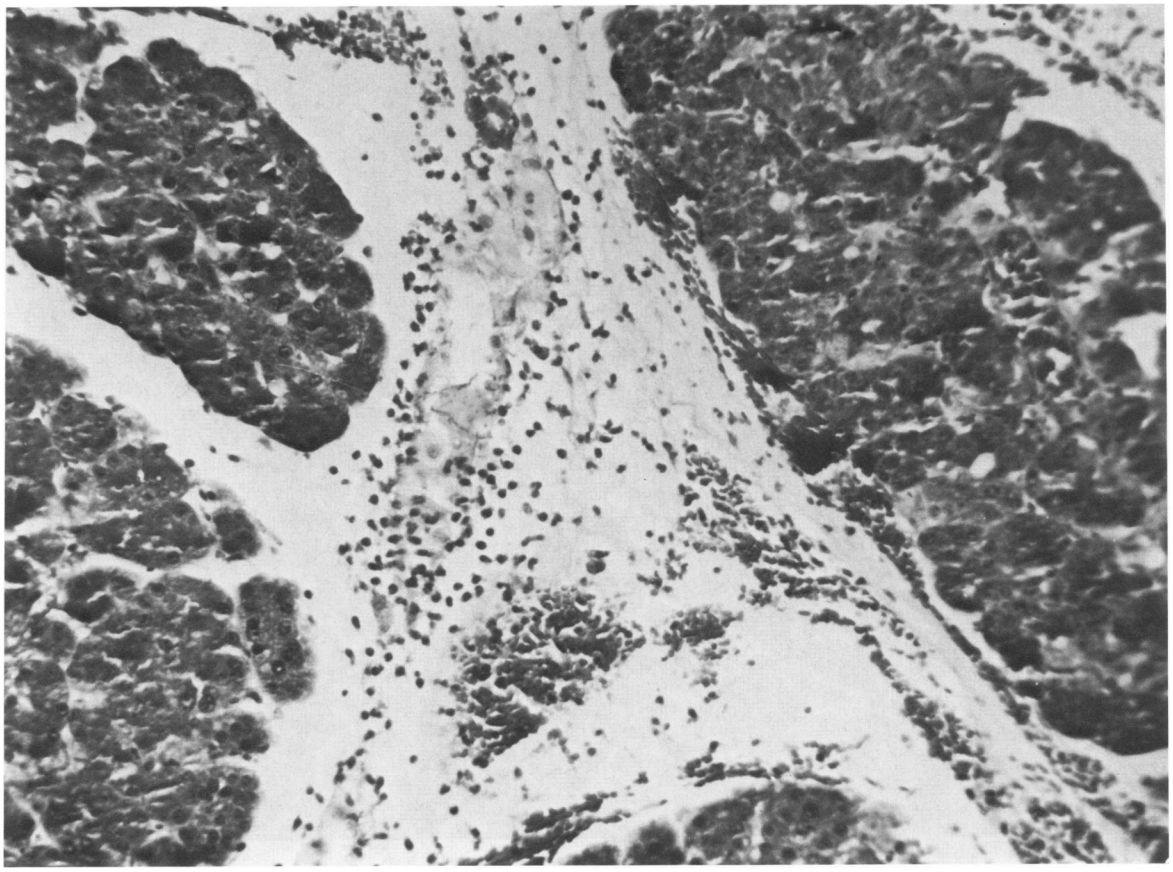


Figure 6—Pancreas from Rat 224, fed ethanol and high-fat diet for 85 days. A focus of acute hemorrhagic pancreatitis is shown. (H&E, final magnification ×550)

diet and another animal fed the extra-high-fat diet. In these lesions, necrosis of acini was accompanied by hemorrhage (Figure 6), fat necrosis (Figure 7), and interstitial edema, as well as infiltration of polymorphonuclear and mononuclear cells. There appeared to be no relationship of ethanol doses with induction of acute pancreatitis, because durations required to induce these lesions ranged widely from 30 to 160 days.

Another prominent effect of higher levels of dietary fat was induction of focal chronic pancreatitis. This chronic change was characterized by interstitial fibrosis, acinar atrophy, fat necrosis, mononuclear cell infiltration, and ductal and ductular dilatation (Table 2). Both interlobular and intralobular fibrosis was observed around atrophied acini with dilated lumens (Figure 8). Mononuclear cell infiltration was prominent in these lesions, especially around the dilated ducts or ductules with mucinous or proteinacious plugs (Figure 9). Those ducts with proteinacious plugs tended to exhibit atrophied epithelial cells (Figure 9). Induction of these chronic changes was only seen after 85 days in ethanol-fed rats given the high-fat diet. However, with the extra-high-fat diet, the similar lesion was not only induced in one rat by 30 days but also was observed at a higher incidence rate (30%, Tables 2 and 3). In ethanol-fed animals given either the high-fat or the extra-high-fat diet, the decrease in the number of zymogen granules was also noted in about a half of animals. This hypogranulation, even though it was not quantitated by a morphometric method, was more apparent in those with lesions of chronic pancreatitis.

Table 3 summarizes the overall incidence rates of ethanol-induced pancreatic pathology observed in the three dietary groups. It demonstrates potentiation of ethanol-induced pancreatic injury by dietary fat, resulting in induction of acinar necrosis and chronic pancreatitis. Hypogranulation was also a distinct pathologic feature that accompanied induction of chronic pancreatitis in ethanol-fed rats with the higher dietary intake of unsaturated fat. Incidence of acute pancreatitis also appeared affected by dietary fat content.

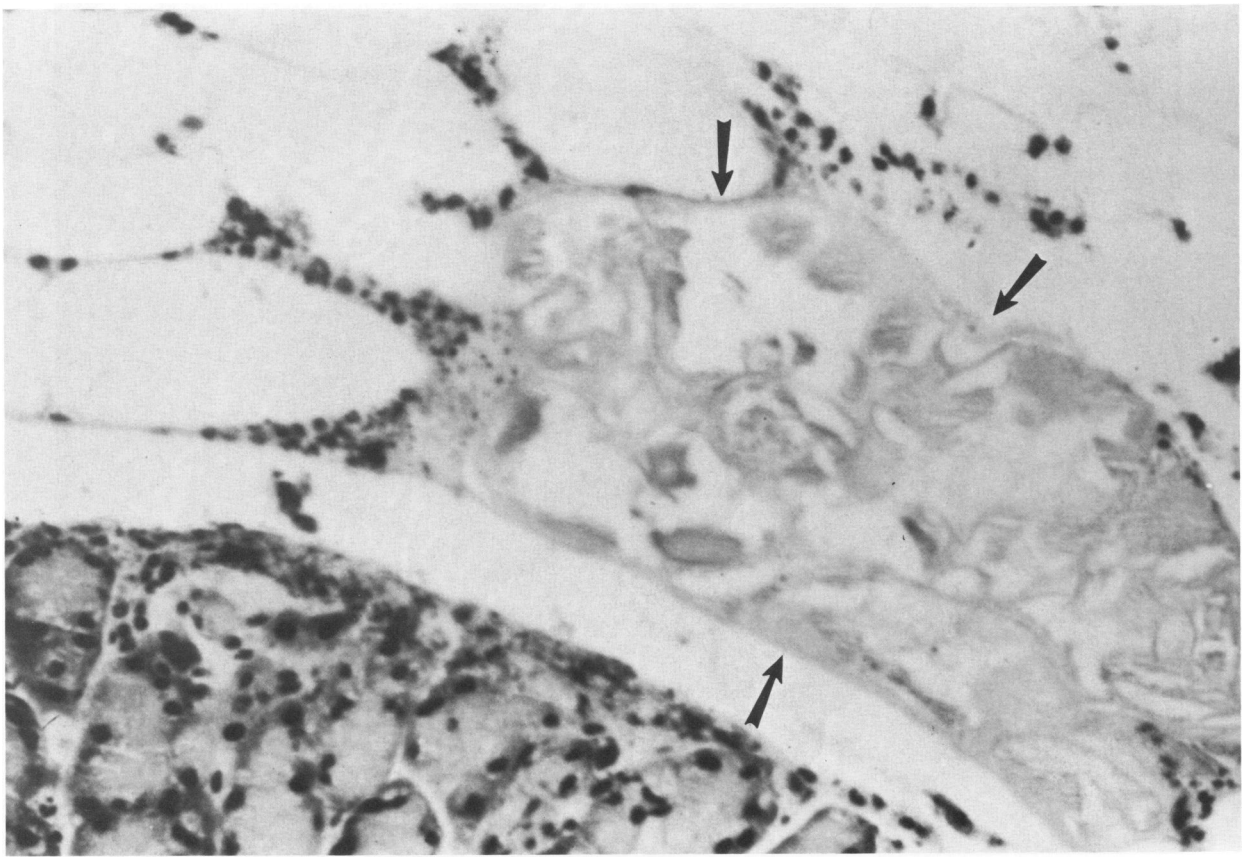


Figure 7—Pancreas from Rat 207, fed ethanol and high-fat diet for 100 days. Note that the fat necrosis (*arrows*) is surrounded by inflammatory cells. Adjacent acini are also necrotic. (H&E, final magnification ×2750)

Plasma Levels of Cationic Trypsinogen

Plasma levels of cationic trypsinogen determined in the low-fat and high-fat dietary groups are summarized in Table 4. The circulating levels of this proenzyme among the control groups were similar regardless of durations or dietary levels of fat ($P > 0.05$) and did not differ from those determined in normal rats fed chow *ad libitum*.[23] In Rats 142 and 252, with focal lesions of acute hemorrhagic pancreatitis (Table 2), plasma levels of cationic trypsinogen were highly elevated to 315 and 250 ng/ml, respectively (thus they were not included in the data in Table 4). In the low-fat diet groups, ethanol administration significantly reduced the plasma levels of catinoic trypsinogen to 32–46% of those measured in corresponding controls. However, the ethanol-fed rats given the high-fat diet did not show any significant changes in this parameter, compared with the pair-fed controls. The animals with lesions of chronic pancreatitis, for example, Rats 224 and 228, did not show any obvious changes in the plasma level of this proenzyme (7.3 and 9.1 ng/ml, respectively).

Plasma Levels of Glucose, Cholesterol, and Triglycerides

Plasma glucose levels were not significantly altered by ethanol intoxication in either the low-fat group or the high-fat group (Table 5). However, in Rat 224, which has been shown to have lesions of both acute and chronic pancreatitis (Table 2), the plasma glucose level was elevated to 421 mg/dl. Because of this high value, the mean glucose level for the high-fat ethanol group was slightly elevated (184 mg/dl), with a wider standard deviation (Table 5). Plasma cholesterol levels in rats given the low-fat diet were similar. On the other hand, this parameter showed a significant increase in ethanol-fed animals given the high-fat diet, compared with the pair-fed controls (Table 5). Plasma levels of triglycerides were not statistically different among the four groups, even though the mean value for this parameter was lowest in the low-fat control group. Representative data on individual ethanol-fed rats are also shown in relation to pancreatic pathology observed in these animals (Table 5). Induction of focal acute pancreatitis was not accompanied by obvious

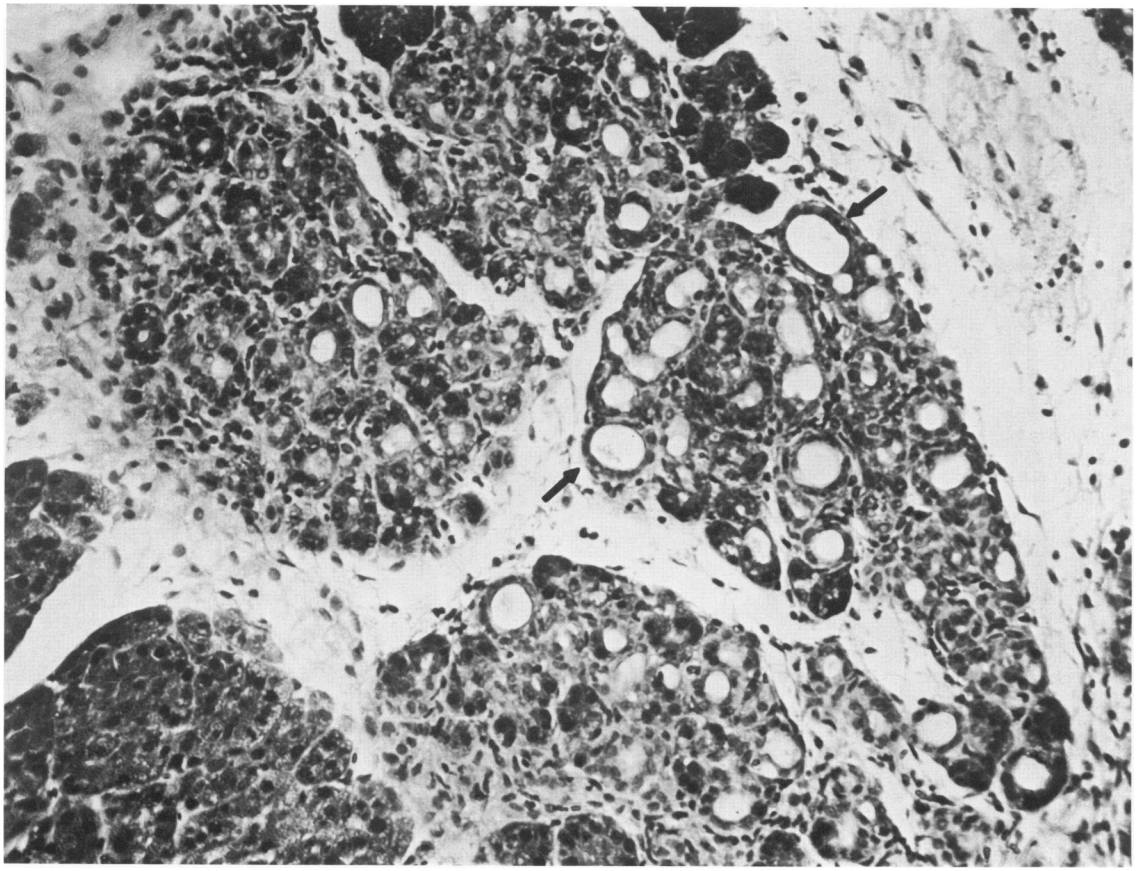


Figure 8—Pancreas from Rat 312, fed ethanol and extra-high-fat diet for 160 days. A focus of lobular atrophy is shown with dilated acinar lumens (*arrows*) (H&E, final magnification ×550)

changes in plasma lipid levels. For example, in Rats 142 and 252, which had focal lesions of acute pancreatitis, the plasma lipid levels were within the normal range or at least were not different from those in animals with milder disease (Table 5). There also appeared to be no correlation between induction of chronic pancreatitis and hyperlipidemia (Table 5).

Discussion

Human alcoholics may consume ethanol in amounts that account for as much as 50% of their total caloric intake and attempt to continue their ethanol intake in order to sustain intoxication. This drinking behavior is pharmacologically enforced by the ability of ethanol to cause physical dependence. Using the intragastric infusion model described in this study, we were able to simulate this condition in rats, which consumed ethanol as up to 47% of total calories by 3 months and exhibited sustained blood alcohol levels.

Despite sustained intoxication, pancreatic pathology in animals infused with the low-fat diet was generally mild and characterized by steatosis and interstitial edema without obvious inflammation. Increased intake of unsaturated fat clearly potentiated ethanol-induced pancreatic injury, resulting in focal lesions of chronic pancreatitis in 20–30% of the animals. These lesions are characterized by acinar atrophy with dilated lumens and ductal dilatation with mucinous or proteinacious plugs and are similar to those described for chronic alcoholic pancreatitis in humans.[8] Anologous changes have also been demonstrated in rats given 20% ethanol as drinking water for 6–12 months.[10,24] These morphologic features are supportive of so-called small-duct theory originally described by Sarles.[25] This hypothesis emphasizes ethanol-induced protein hypersecretion by the pancreas leading eventually to formation of protein plugs and precipitates, ductal dilatation, and periductal fibrosis. In contrast, the "toxic and metabolic hypothesis" described by Darle[26] and Bordalo and Dreiling[27] focuses on changes in acinar cells such as lipid accumulation and enlarged mitochondria as an initial pathologic step. The recent study by Kakizaki et al[24] suggested that

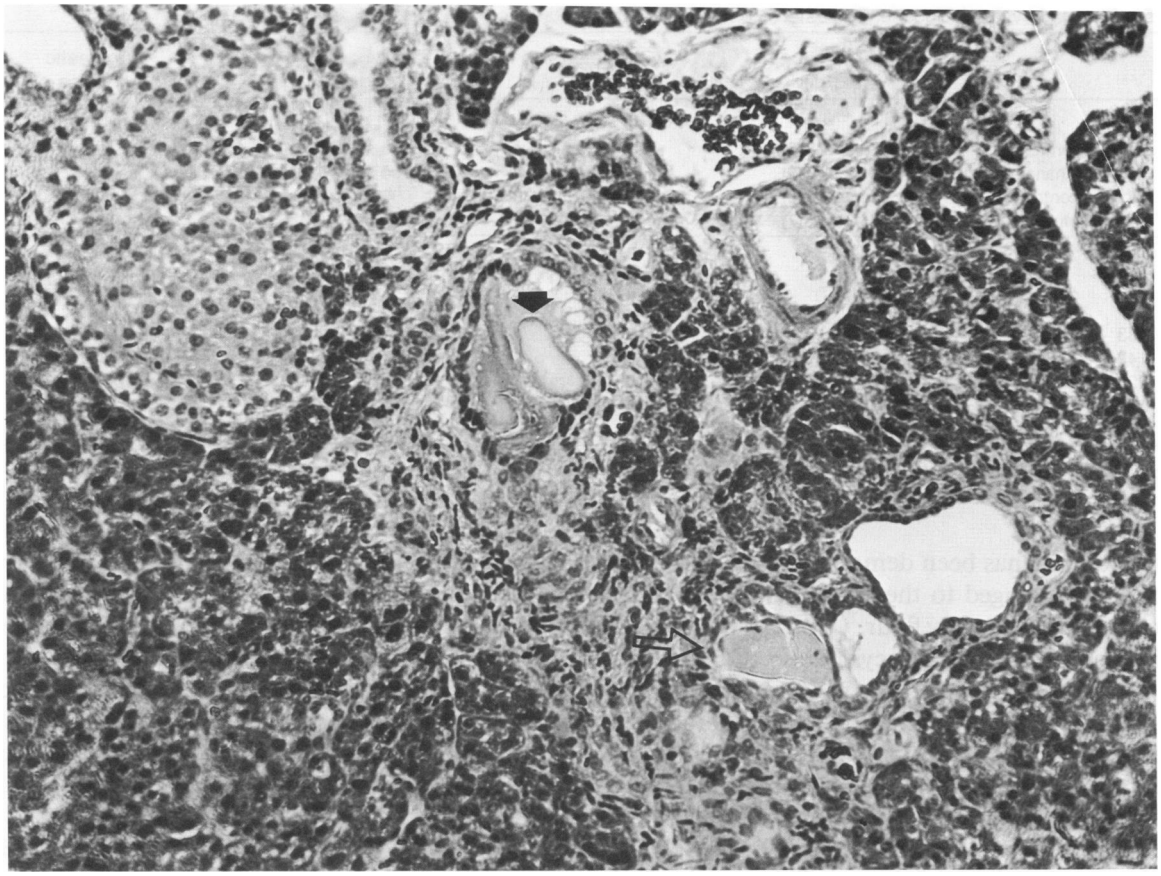


Figure 9—Pancreas from Rat 228, fed ethanol and high-fat diet for 100 days. Ducts are distended with intraluminal mucinous (*solid arrow*) and proteinacious (*open arrow*) plugs. Surrounding fibrosis is also evident. (H&E, final magnification ×550)

ethanol intake initially induced cytotoxic effects on both acinar and ductular cells at the level of the cell membrane, followed by degeneration of ductular cells and atrophy of acinar cells. Our histologic observation on the evolution of chronic pancreatitis in rats suggests that ethanol-induced inhibition in trophic effects on acinar cells is an early, important pathophysiologic event. One of the early but distinct ethanol-induced pathologic changes we observed was induction of apoptosis. This pathologic process is thought to be enhanced in various types of atrophy of many different tissues but is commonly observed during involution of endocrine-dependent tissues in response to changes in circulating levels of trophic hormones.[28] In the pan-

Table 3—Percent Incidence of Pancreatic Pathology Observed in Rats Infused With Ethanol Plus Low-, High-, or Extra-High-Fat Diet*

	Low-fat	High-fat	Extra-high-fat
Steatosis	87%	100%	100%
Edema	67%	27%	40%
Hypogranulation	0%	53%	60%
Apoptosis	0%	80%	70%
Acute pancreatitis†	7%	13%	20%
Chronic pancreatitis‡	0%	20%	30%

* Overall incidence rates were determined on the basis of the data presented in Table 2.

† Acute pancreatitis was diagnosed with histologic evidence of edema and/or hemorrhage with acinar necrosis and acute inflammation.

‡ Chronic pancreatitis was diagnosed with the presence of fibrosis, acinar atrophy, and chronic inflammation. Some animals had lesions of chronic pancreatitis mixed with those of acute pancreatitis.

Table 4—Plasma Levels of Cationic Trypsinogen in Rats Fed Low- or High-Fat Diet*

	Low-fat diet		High-fat diet	
Duration	Control	Ethanol-fed	Control	Ethanol-fed
30 days	8.9 ± 2.5 (5)	4.1 ± 1.5 (5)†	10.8 ± 1.6 (4)	10.2 ± 2.2 (4)
85 days	7.1 ± 4.2 (4)	2.3 ± 1.1 (4)‡	12.1 ± 3.3 (4)	8.2 ± 1.3 (2)
Overall	8.3 ± 2.5 (7)	3.5 ± 1.5 (7)†	10.8 ± 2.4 (13)	9.8 ± 2.0 (10)

* The data from Rats 142 and 252 with acute hemorrhagic pancreatitis were not included.

† $P < 0.01$.

‡ $P < 0.05$.

Table 5—Plasma Levels of Glucose, Cholesterol, and Triglycerides in Rats Fed Low-Fat or High-Fat Diets

	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Pancreatic disease
Diet				
Low-fat control	135 ± 30 (6)	69 ± 25 (6)	58 ± 39 (6)	
Low-fat ethanol	134 ± 43 (9)	72 ± 21 (9)	100 ± 83 (9)	
High-fat control	154 ± 16 (8)	78 ± 9 (8)	99 ± 41 (8)	
High-fat ethanol	184 ± 107 (7)	138 ± 55 (7)*	79 ± 29 (7)	
Rat				
150	181	72	277	Interstitial edema
217	148	237	102	Steatosis
233	132	145	180	Steatosis
142	72	53	128	Acute pancreatitis
224	176	109	85	Acute pancreatitis
228	421	124	51	Chronic pancreatitis
228	100	119	73	Chronic pancreatitis

* Significantly different from pair-fed controls ($P < 0.05$).

creas, apoptosis has been demonstrated in rats when the diet was changed to the regular chow after prolonged feeding of raw soy flour,[29] which is now known to stimulate release of cholecystokinin (CCK), a trophic hormone of pancreatic acini.[30,31] In this respect, we have shown significant inhibition of binding characteristics of CCK receptors on pancreatic acini isolated from rats infused with ethanol and the high-fat diet.[32] However, this inhibitory effect on the CCK receptor was not demonstratable when the low-fat diet was given to the ethanol-fed rats.[32] This negative finding correlates with absence of apoptosis in this group of animals, as shown by the current study (Table 2). In a recent study on hamsters given chronic doses of ethanol and a high-fat diet, the pancreatic lesion similar to apoptosis was also observed.[33] The reduction in acinar sensitivity to CCK, therefore, appears to be an attractive hypothetical mechanism for induction of apoptosis observed in the high-fat ethanol group. Hypogranulation of acini which has preceded or coincided with induction of chronic pancreatitis indicated the gradual loss of protein synthetic capacity, leading eventually to acinar atrophy characterized by dedifferentiative structural changes.[34] This observation is also in support of the hypothesis that the decreased CCK sensitivity of pancreatic acini could be an underlying biochemical mechanism for the ethanol-induced regressive disease.

There is an increasing body of clinical and experimental data suggesting that increased dietary fat, especially of polyunsaturated form, is an important factor in induction of various types of pancreatic diseases, including chronic pancreatitis.[35] Our data showing the increased incidence of chronic alcoholic pancreatitis in the animals fed higher dietary levels of corn oil clearly confirm and support this concept. However, this effect of dietary fat in our model may also be related to the reciprocal decreases in carbohydrate intake in the animals given the high-fat and extra-high-fat diet. This possibility, which is inherent to a pair-feeding technique, cannot be ruled out, because ethanol-induced disturbance in glucose homeostasis, including reduced insulin binding to acinar cells,[36] can be aggravated by the reduced carbohydrate intake.

In our study, induction of acute pancreatitis seemed independent of the duration of intoxication. This corroborates the epidemiologic data by Sarles et al, who have shown no threshold doses for induction of acute alcoholic pancreatitis.[15] Induction of acute or chronic pancreatitis in our model has also been shown unrelated to hyperlipidemia. In those animals with focal lesions of acute pancreatic inflammation, plasma triglycerides or cholesterol levels were not elevated, compared with those in pair-fed controls or in animals with milder disease. This is in contrary to the previous observation of hyperlipidemia in patients with acute pancreatitis[37] and does not support a hypothesis that hypertriglyceridemia is an important intermediary factor in the pathogenesis of acute alcoholic pancreatitis.[37] This discrepancy may be due to the possibility that hyperlipidemia in alcoholic patients can be associated with familial hyperlipoproteinaemia, a genetic abnormality that has been shown to exist in patients with alcoholic pancreatitis[38] but not in Wistar rats, employed in our study.

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