American Journal of Pathology, Vol. 132, No. 1, July 1988

Role of the Ito Cell in Liver Parenchymal Fibrosis in Rats Fed Alcohol and a High Fat–Low Protein Diet

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Eight pairs of young adult rats were pair-fed a high fat–low protein diet and ethanol or isocaloric glucose by permanent intragastric cannula for up to 6 months. Biopsies of the liver were taken monthly and the fibrosis was quantitated morphometrically using the sirius red polarization method of collagen visualization by light microscopy. Morphometric analysis of the sinusoids and scars were performed on electron micrographs made from the liver biopsies. An increase in the collagen in both the central and portal areas was found when the livers of the alcohol-fed rats were compared with controls. The predominant cell in the scars was the Ito cell. An increase in the percentage of the total Ito cell square area made up of rough endoplasmic reticulum (RER) was noted when the sinusoids of the liver of the ethanol-fed rats were compared with controls. No difference in the RER was found when the sinusoidal Ito cells were compared with the Ito cells located within the scars of the ethanol-fed rats. It was concluded that Ito cell "activation" by chronic ethanol feeding in the sinusoids of rats accurately predicts "activation" of the Ito cells within scars. The Ito cells are diffusely activated even though the scarring is localized. This implies that local factors as well as Ito cell activation are necessary for scar formation. In the case of alcoholic liver disease, scar formation may be initiated by centrilobular necrosis. (Am J Pathol 1988, 132:73–85)

HEALING BY FIBROSIS in the liver parenchyma involves Ito cells regardless of the initiating cause. This includes liver damage caused by focal heat or cold,[1-2] alcoholic liver disease (ALD),[3-8] carbon tetrachloride poisoning,[9-11] choline deficiency cirrhosis,[12] the fatty liver of obesity,[13] and occurs in patients treated with etretinate or methotrexate for psoriasis.[14] Changes correlating with fibrosis include an increase or a decrease in lipid content of Ito cells and/or an increase in rough endoplasmic reticulum or ribosomes.[1-5,7,8,12,14] Transformation of Ito cells to fibroblasts or myofibroblasts is thought to explain increased collagen synthesis that culminates in fibrosis.[3-6,9,10,15-23] This change is referred to as "Ito cell activation" or "transformation."[3-5] In ALD, changes in the Ito cells have been evaluated in their normal perisinusoidal location but not within the scarred areas.[3,4] It is possible, however, that the changes seen in sinusoidal Ito cells do not reflect those seen within the scars. It would seem that evaluation of the Ito cells within the scars may be more relevant to the scarring process. Therefore, we compared morphometrically the changes in Ito cells located in scars with those located within the sinusoids. Ito cells are located throughout the lobule with a centrilobular predominance,[22,24,25] whereas the scarring is primarily located perivenular in ALD.[7,8,18,21,26-29] Morphometric quantitation of the collagen in these livers was also performed for establishment of the presence and the location of the scarring process.

Materials and Methods

Experimental Animals and Diet Administration

Data for this study are derived from liver tissue removed as a part of a large experiment. Some of the results have already been reported.[30] Eight pairs of Charles River Wistar male rats weighing approxi-

Supported by the Medical Research Council of Canada and NIAAA 4571.

Accepted for publication February 18, 1988.

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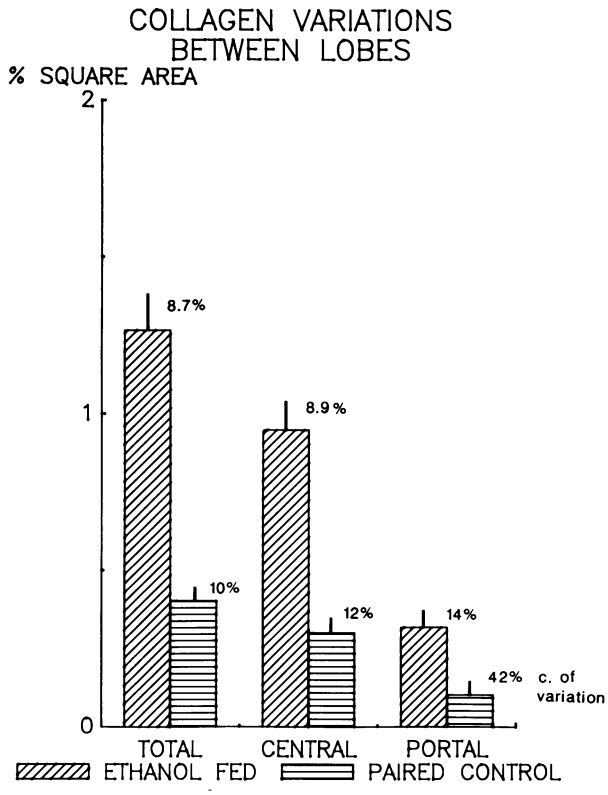

Figure 1—The variation in collagen content between the lobes of the liver of a single ethanol-fed rat and its pair-fed control is shown. The variation (coefficient or variation) is greatest in the portal tract collagen.

mately 380 g were implanted with single gastrostomy cannulas as described previously.[31] Animals were pair-fed by continuous infusion of the high fat–low protein diet plus either ethanol or isocaloric dextrose solution for 3 weeks to 6 months. The diet of Thompson-Reitz[32,33] was modified by increasing the proportion of dextrose to reduce the protein, vitamins, and minerals by 29%. The final concentration of choline chloride was 213 mg/liter. The low content of choline and protein in the diet made it borderline in lipotropes.[34] The diet resembles that reported by patients with ALD.[35–37]

The liquid diet was started at 12 ml/100 g body weight (1 ml = 1.2 Cal) with a volume not exceeding 50 ml/day, so that the relative percentage of each nutrient gradually decreased relative to the ethanol consumed as the rats gained weight. This is because the amount of ethanol given was dependent on the maintenance of a high blood alcohol level (BAL), so, as the metabolic tolerance to ethanol increased and the body weight increased, the dose of ethanol required to maintain high BAL progressively replaced the percent calories derived from the diet. The initial caloric distribution at an ethanol dose level of 8 g/kg body weight/day was 14% of the total calories as protein, 37% as fat, 20% as dextrose, and 29% as ethanol. By 6 months of feeding, the caloric contributions of protein, fat, dextrose, and ethanol were 10, 28, 16, and 46%, respectively, when the ethanol dose level was 12 g/kg body weight/day.

The rats had free access to water and nonnutrient fiber (alpha-cell, ICN Nutritional Biochemicals, Cleveland, OH). They underwent liver biopsy monthly starting with the first operation, when the cannula was inserted so that a baseline pretreatment biopsy was available as a type of control for lab chow. Ketamine HCl anesthesia, was used intraperitoneally. The biopsies were taken from different lobes. They were split for formalin fixation for light microscopy and glutaraldehyde fixation for electron microscopy examination. For light microscopy, hematoxylin and eosin, Masson's trichrome, and silver reticulum stain were used. Sirus red for polarization and morphometric quantitation of collagen were performed using the Sirus Red F3BA in saturated aqueous picric acid.[38] The quantitation of collagen morphometrically using this method correlates well with biochemical measurements of collagen in the livers of rat and man.[38–41] Liver biopsies (25 total) and all lobes of the liver were analyzed after the rats were killed. Blood was taken from the tail vein at the time of liver biopsy, including baseline. BAL was determined using the TDX in the clinical laboratory. Serum alanine aminotransferase (ALT) was measured using the ACA Dupont automatic clinical analyzer. Serum ALT was significantly higher ($P < 0.001$) in the ethanol-fed rats (171 ± 83 U/l) compared with controls (55 ± 26 U/l) or baseline chow-fed (48 ± 26 U/l). This indicates that the control diet regimen did not increase the ALT. There was no difference in the liver protein levels in the ethanol-fed rats (197 ± 29 mg/g wet wt) compared with the controls (219 ± 34 mg/g wet wt), indicating that ethanol and the low protein diet did not reduce liver protein.

By this regimen, the mean ethanol intake was 12.5 g/kg body weight/day. BAL was maintained at 329 ± 109 mg/dl over the test period. The rats increased their body weight at a constant rate. By 6 months, the alcohol rats had gained 105% and the pair-fed controls had gained 150% of their original weight. This difference in weight gain was significant from 8–20 weeks of feeding and is consistent with the known energy wasting that results from metabolizing ethanol.[42] Survival on this dietary regimen was: 8 pairs lived 8 weeks, 6 pairs lived 12 weeks, 5 pairs lived 16 weeks, 3 pairs lived 20 weeks, and 2 pairs lived 24 weeks.

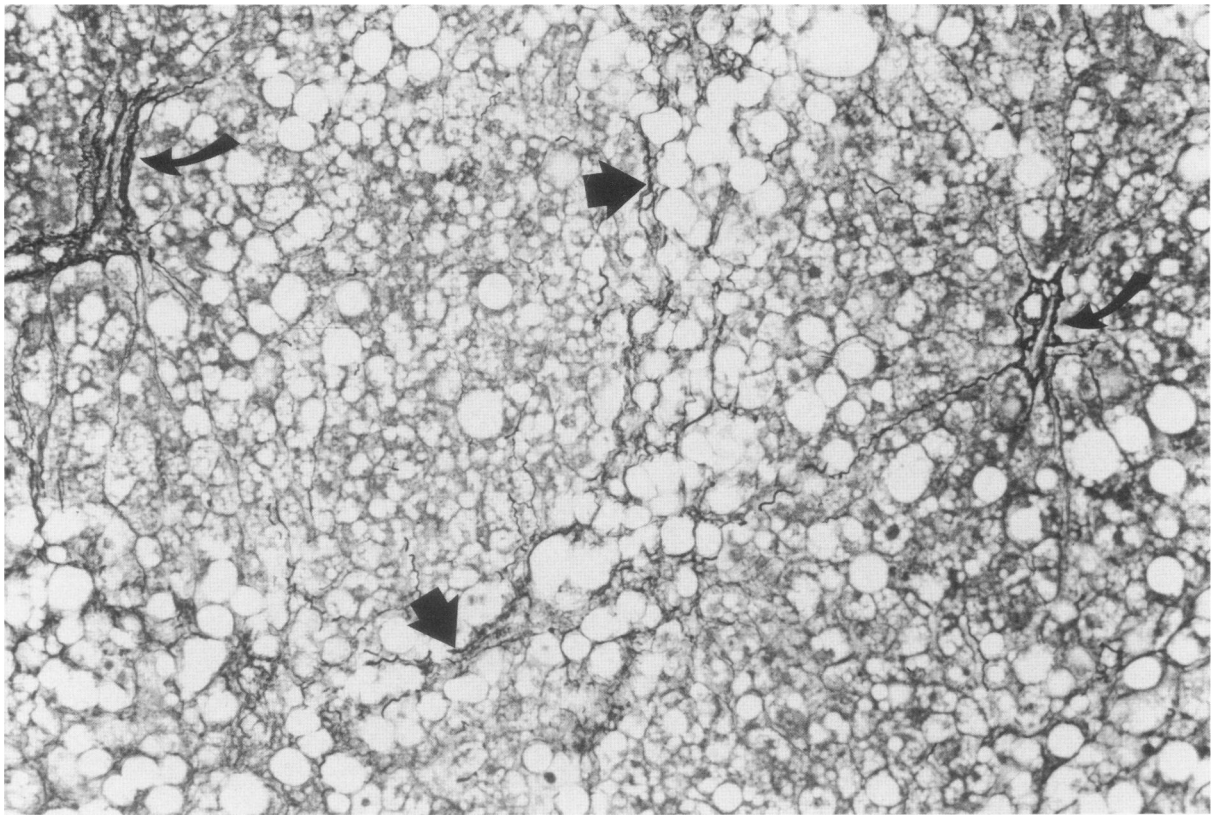

Figure 2—Fatty liver in a rat fed ethanol for 3 weeks. Note also the centrilobular collapse and increase in reticulum fibers (straight arrows). The portal tracts to the left and right (curved arrows) appear normal. Reticulum stain ×100

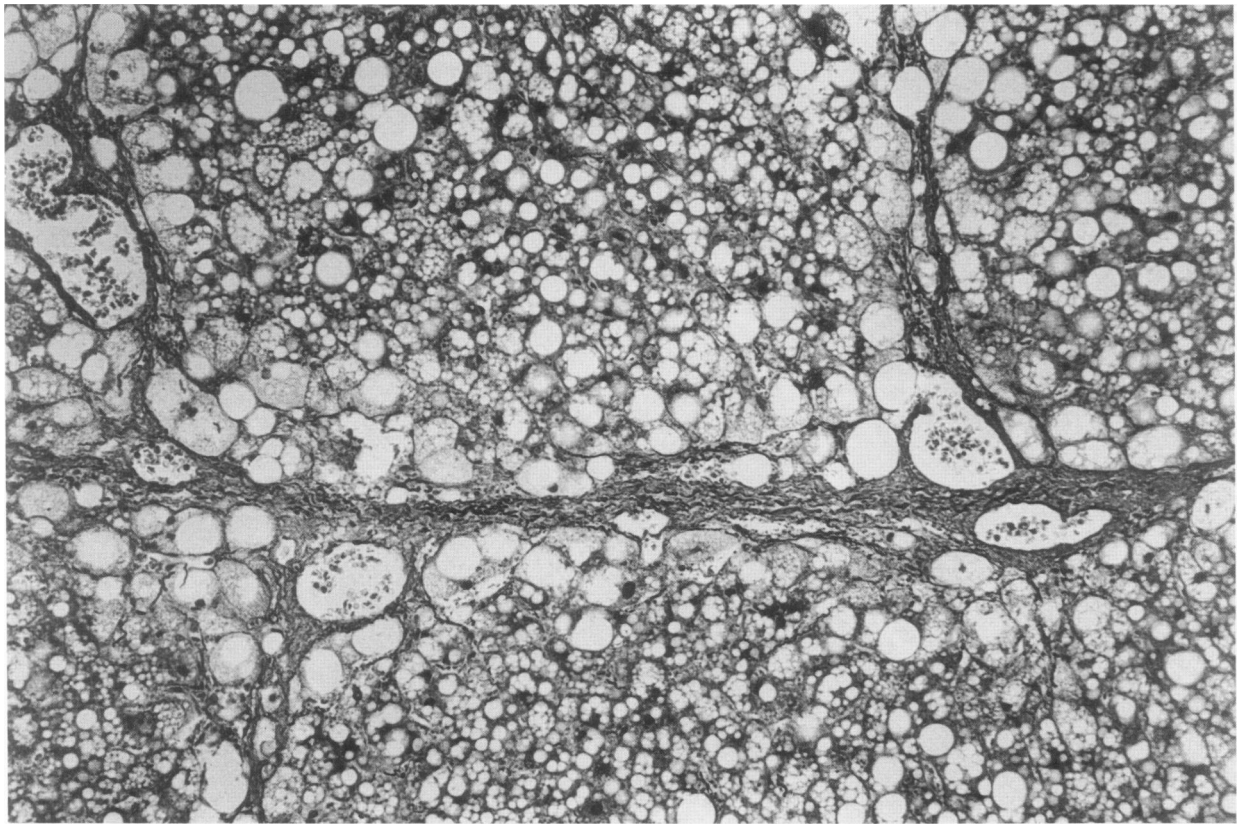

Figure 3—Liver biopsy of a rat fed ethanol for 3.5 months. Note the centrilobular bridging fibrosis, pericellular fibrosis. The hepatocytes near the fibrosis show "balloon degeneration." Reticulum stain ×188

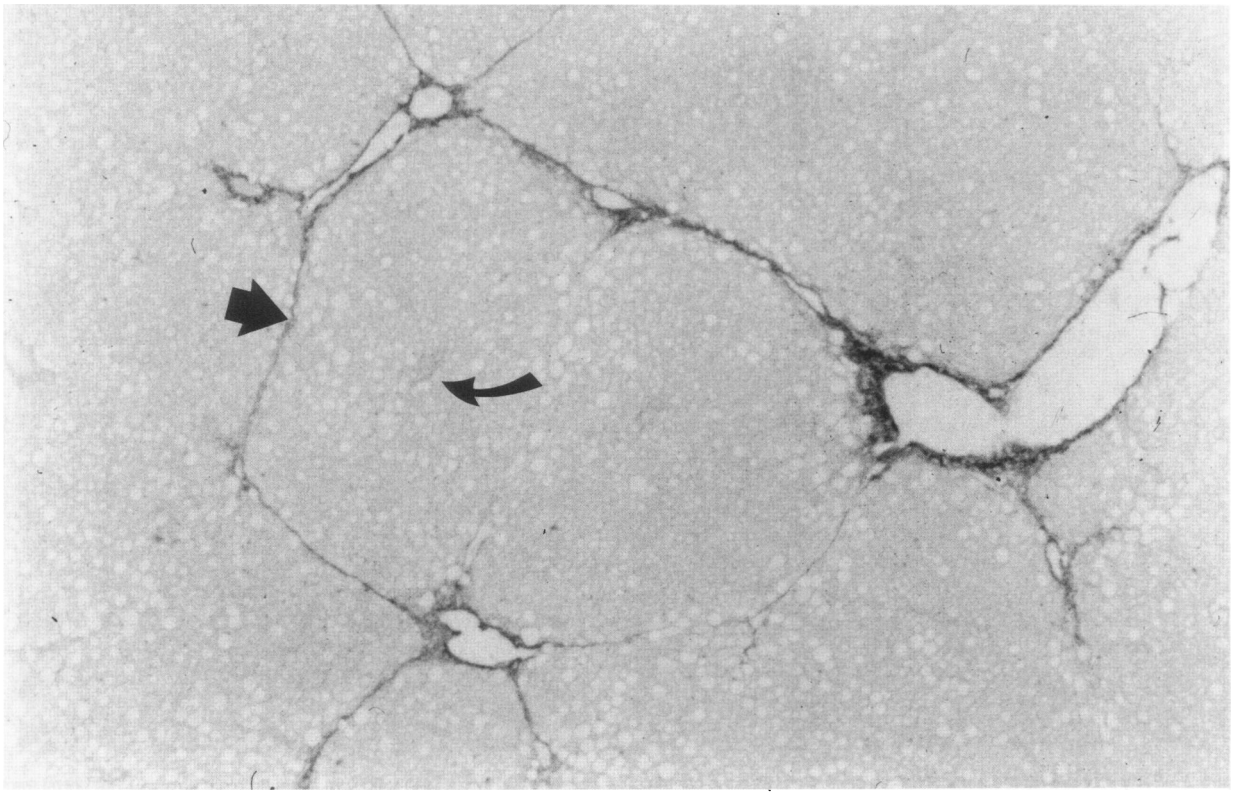

Figure 4—Liver at the time of sacrifice at 6 months of ethanol feeding. Note the central–central bridging of fibrosis (straight arrow) and sparing of the portal tracts (curved arrow). Sirius red stain ×58

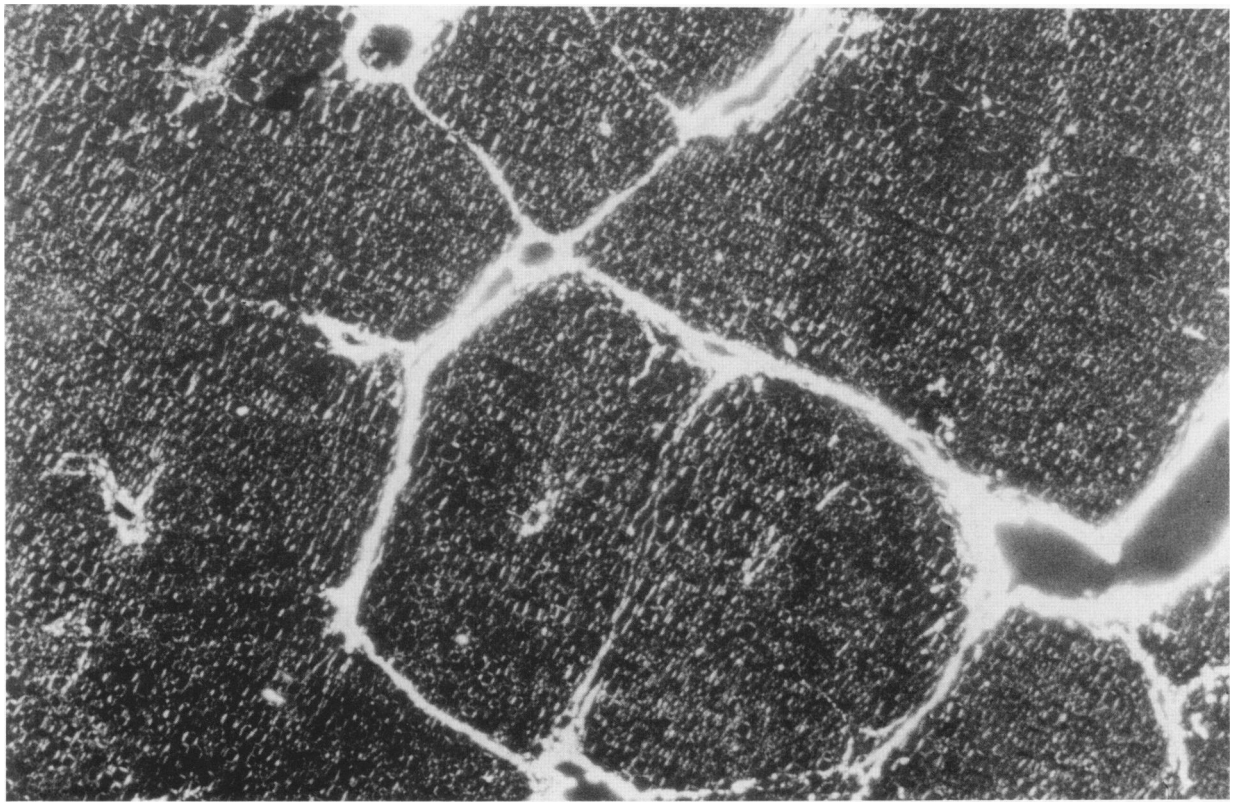

Figure 5—Same liver section as seen in Figure 4 using polarized light. Note that the collagen is more easily visualized and varified. ×58

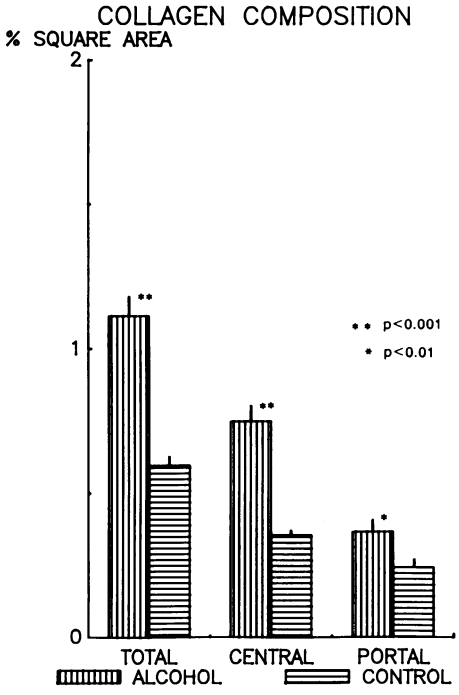

Figure 6—When the data of the biopsies and autopsy livers was pooled, there was a significant increase in collagen in the livers of rats fed ethanol compared to controls whether the data was centrilobular, portal or total collagen.

Deaths occurred among both the ethanol and control-fed rats from a variety of causes, including plugged cannulas, convulsions, postoperative state, and ethanol overdose. No infections were encountered. The pair-fed rats were killed when their pair died.

Morphometric Analysis

Both the amount of collagen on light microscopy and the composition of the Ito cells by electron microscopy were quantitated morphometrically. In the case of morphometry on electron micrographs, sinusoids and scars discovered on thick plastic sections stained by toluidine blue were examined as follows: 1) 13 scars in 5 ethanol rats, 2) 24 photographs of sinusoids with Ito cells in 4 ethanol-fed rats with scars and 1 without scars, 3) 1 scar in 1 control rat, and 4) 11 photographs of sinusoids with Ito cells in 5 control rats, 4 of which were pair-fed with rats that had scars. Sinusoid samples span the entire timeframe of the experiment whereas the scars were sampled when they were found beginning at 2 months and extending through 5.5 months. All electron micrographs were analyzed at the same magnification (×10,000) and the percent of the total square area (sq μm) was determined using a Zeiss IBAS computer of the following parameters: Ito cell, Ito cell fat, Ito cell rough endoplasmic reticulum, Ito cell nucleus, "old" collagen, "new" collagen, macrophages, PMNs, fibroblasts, myofibroblasts, and lymphocytes. EM photographs analyzed were as follows: 105 of scars from ethanol-fed rats, 24 of sinusoids from ethanol-fed rats, 5 of a scar from a control rat, and 11 sinusoids from control rats. A total of 13 scars from 5 ethanol-fed rat livers from 22 blocks and 105 EM photographs, including 370 Ito cells, were examined. In the scars, numerous Ito cells were present but the number could not be accurately counted because of the long slender cellular processes that characterize the Ito cells in scars. In the sinusoids, there was one Ito cell per sinusoid.

When collagen was measured morphometrically by light microscopy using the sirus red stain and polarized light method, tracings of the liver from the 25 biopsies and 4 or 5 lobes taken at autopsy were made by projection of the image on tracing paper at a magnification of ×98. Collagen was designated as portal or central location since portal–central bridging was rarely encountered. Two random fields were traced for every biopsy and lobe examined (256 tracings total). Thus, all 16 livers were examined when biopsied,

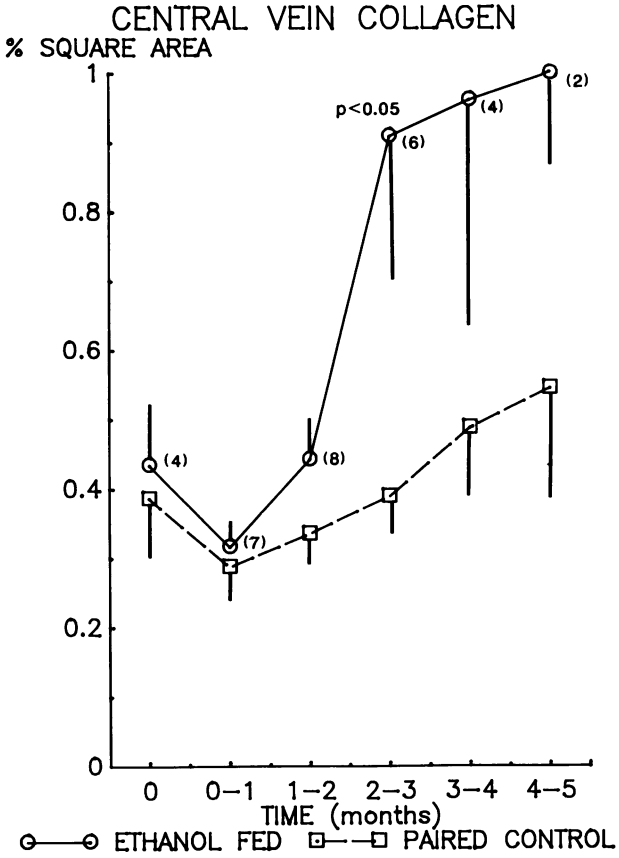

Figure 7—When the amount of centrilobular collagen was measured in liver biopsies over the time period studied, there was a significant increase only after 2–3 months of ethanol feeding.

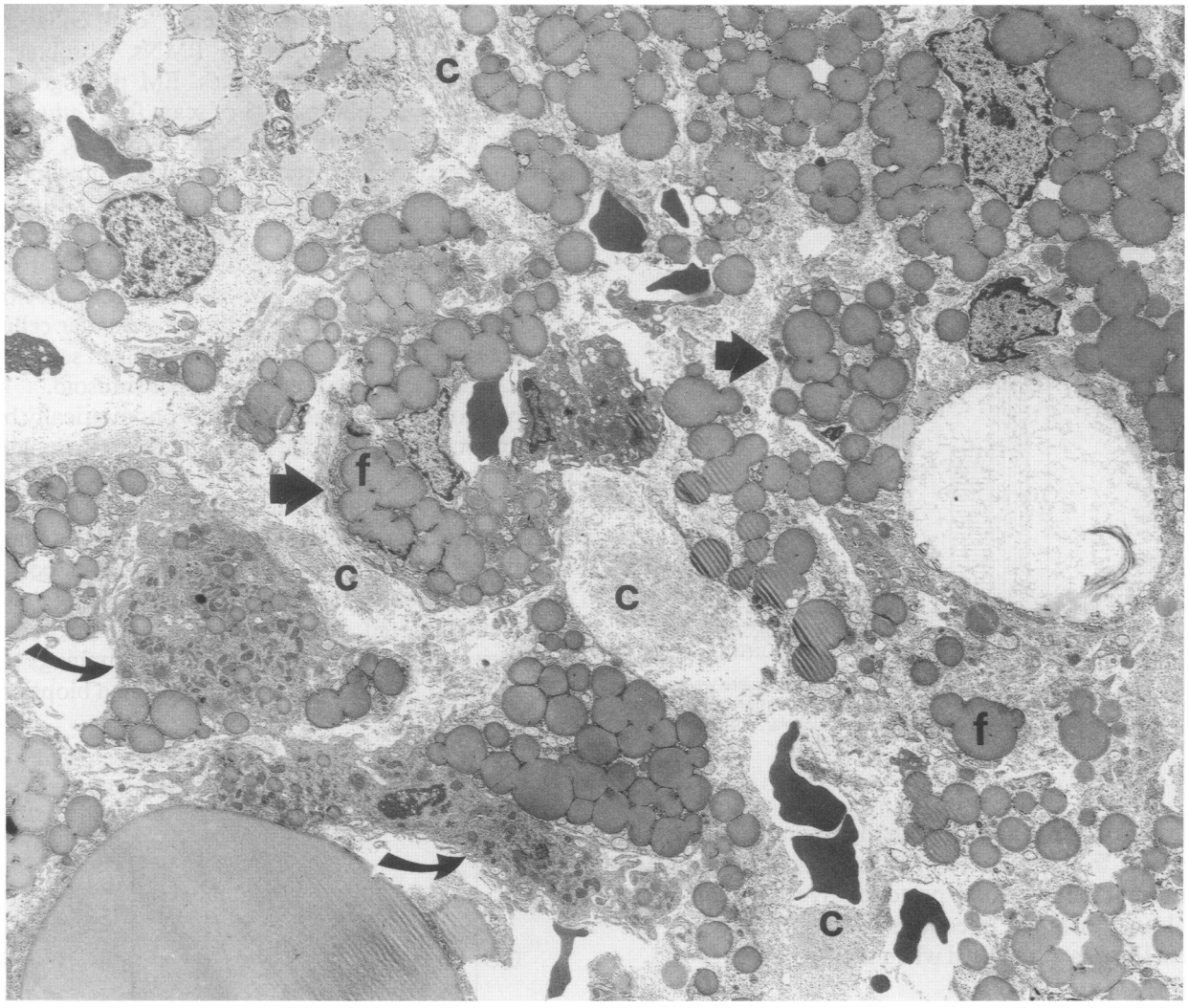

Figure 8—Electron micrograph of an early scar from a liver of a rat fed ethanol for 4 months. Note the collagen (c), fat (f), the fat laden Ito cells (straight arrows), and macrophages (curved arrows). ×3300

including baseline and autopsied livers. The percent total square area was determined morphometrically using a Zeiss IBAS computer.

Statistical Analysis

When the constituents of scars were compared with sinusoids, a possible bias where the number of raw observations for some rats was much larger than that for other animals because some livers had many scars to sample and others had few or none, had to be overcome. Sampling of sinusoids over time of sampling and the age of the scars were also variables that could bias the analysis. For avoidance of excessive weighting of the results, the computer program for randomly limiting the number of observations to a sample size of 5 or 6 was used. Descriptive statistics (mean, standard deviation) were computed. Pearson correlation coefficients were also calculated to evaluate associations of the dependent variables within groups. All statistics were computed using standard statistical packages, SPSS-X, Release 2.2 (SPSS Inc., Chicago, IL), or SAS Version 5 (SAS Institute, Cary, NC) and run on the Amdahl V8 470 at the University of Ottawa. Student's *t*-tests involving the comparison of various means were done under the hypothesis of no difference between the groups, and probabilities were calculated from two-tailed tests. When the F test for homogeneity of variance indicated significant differences in the variances for the two groups, the *t* values and associated probabilities were computed using a separate rather than a pooled variance estimate. Additional descriptive statistics were generated to assess the possible effect of time on the amounts of the constituents analyzed, but the results indicated no clear trends. Pearson correlation coefficients for within-group data

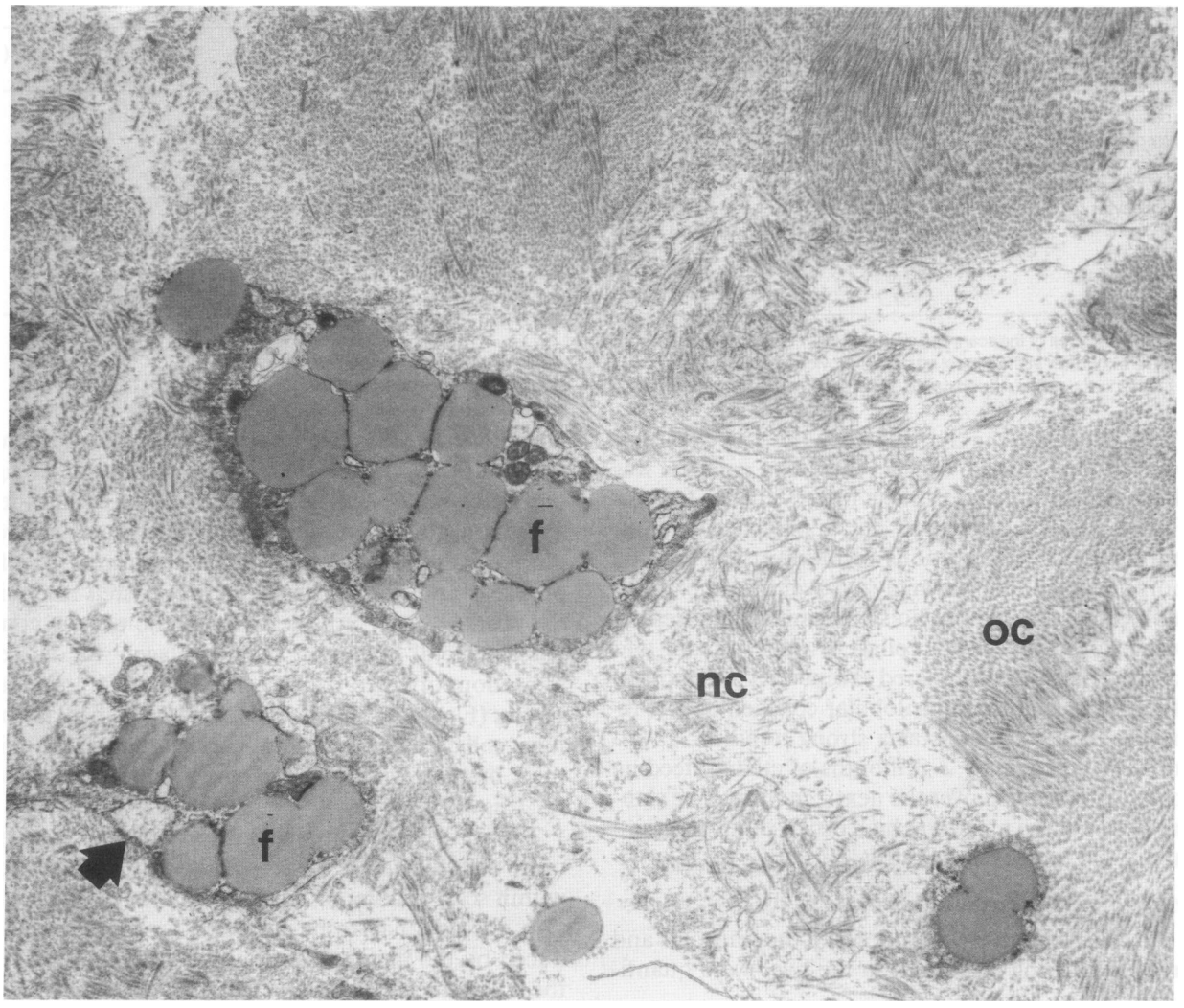

Figure 9—Electron micrograph of a liver scar from a rat fed ethanol for 4 months. Portions of Ito cells containing fat (f) and rough endoplasmic reticulum (arrow) are seen. Note the areas of "new" collagen (nc) and "old" collagen (oc). ×9200

revealed few or no significant correlations among the elements, so evaluations were concentrated on the effect of the independent variable (ethanol) on the composition of scars and sinusoids.

Statistical analysis of collagen percent total square area was performed using a Wang program for mean, standard deviation, standard error of the mean, and Student's *t*-test. The coefficient of variation was high because some ethanol-fed rat livers did not have fibrosis, and the amount of scarring was variable from lobe to lobe at autopsy (Figure 1). There was good agreement between controls, however, with little fluctuation over time including baseline values.

Results

Morphologic Findings

The biopsied livers of the ethanol-fed rats showed severe fatty change (more than 75% of hepatocytes contained fat stores) (Figure 2). Fatty change was less severe in controls (under 50% of livers contained fat except for 2 biopsies). Baseline livers were not fatty. Multiple foci of inflammation that included macrophages, mononuclears, and PMNs were seen in 4 biopsies of ethanol-fed rats. Multifocal centrilobular spotty necrosis was seen in 4 biopsies from ethanol-fed rats beginning after 3 weeks of ethanol feeding (Figure 2). Fibrosis was observed in 5 of the ethanol-fed rats (9 biopsies). It was centrilobular, pericellular or bridging in character (Figure 3). Two control rats showed questionable thickening of the collagen around one central vein.

Morphometric Quantitation of Collagen

There was centrilobular scarring by morphometric quantitation in 5 of 8 ethanol-fed rats (Figures 4 and

Table 1—Composition* of Liver Scars and Sinusoids in Alcohol-Fed and Control Rats (Percent Total Square Area [μ^2])

Constituents	Alcohol scars (13) (% ± SD)	Alcohol sinusoids (24) (% ± SD)	Control sinusoids (11) (% ± SD)	Control† scar (1) (% ± SD)
Ito cell	23.8 ± 15.2	27.9 ± 15.2	32.0 ± 19.3	11.3 ± 4.5
Macrophages	17.0 ± 18.9	15.1 ± 19.5	16.5 ± 23.5	3.5 ± 6.5
"New" collagen	13.8 ± 15.1	0.2 ± 1.1	-	8.6 ± 7.5
"Old" collagen	5.7 ± 12.6	1.7 ± 3.4	4.7 ± 9	11.9 ± 20.8
Lymphocytes	1.0 ± 2.6	4.2 ± 12.2	3.9 ± 10.6	-
Fibroblasts	0.5 ± 2.0	0.4 ± 2.0	-	-
PMNs	0.3 ± 2.4	0.6 ± 2.2	-	-
Myofibroblasts	0.2 ± 1.4	-	-	-
Other‡	37.7	49.9	42.9	64.7

* The entire area of the scars and sinusoids was included in the data analyzed.
† The single scar in the control rat did not provide sufficient data for comparison with the scars in the alcohol animals.
‡ Other includes ground substance, RBC, endothelium, basement membrane, sinusoidal and capillary lumens, and miscellaneous.

5) and in none of the controls when the data were analyzed in total (Figure 6). The increased collagen was also noted in the portal tracts in the ethanol-fed rats (Figure 6). The latter was not appreciated without morphometric observations of the livers. When the morphometric data was analyzed over the time of ethanol feeding, there were no significant differences between portal or central collagen when ethanol and control livers were compared except after 2–3 months of feeding using the centrilobular collagen data (Figure 7). Even so, there was a strong trend for an increase in collagen by 2–3 months of ethanol feeding that then plateaued over the next 2 months. The standard error of the mean was high because 3 experimental rats did not develop fibrosis.

Morphometric Quantitation of Ito Cells

Ito cells and other constituents of the sinusoids and areas of scarring (Figure 8) in the liver were quantitated as assessed by morphometry of electron micrographs (Table 1). The constituents did not differ significantly in the sinusoids and scars of the ethanol-fed and control rats. Ito cells predominated and macrophages were the second most common. Myofibroblasts and fibroblasts were encountered rarely in the scars. "New" collagen was predominant compared to "old" collagen. "New" collagen was defined as random oriented collagen fibrils in an amorphous matrix (Figure 9). "Old" collagen was defined as uniform collagen fibrils that ran parallel to each other in thick bundles without intervening amorphous material (Figure 9).

The main point of this study was the comparison of Ito cells located at the sinusoids with those located within scars (Table 2). As stated in the Methods section, the data had to be corrected for the bias introduced by the unequal distribution of scars found in the ethanol-fed rats. By this analysis, Ito cell area was relatively greater in the sinusoids compared to the scars (Table 2). On the other hand, the percent total Ito cell square area (sq μm) composed of fat was in-

Table 2—Comparison of Constituents in Sinusoids (24) and Scars (25) of Ethanol-Fed Rats: Results of Student's *t*-test

Constituents	Location	Percent area	SD	*P* value
Ito cell	Sinusoid	27.9	15.2	<0.001
	Scar	13.5	9.9	
Ito cell fat	Sinusoid	15.5	12.2	<0.001
	Scar	32.3	17.6	
Ito cell RER	Sinusoid	4.1	3.7	NS
	Scar	5.3	3.1	
Ito cell nuclei	Sinusoid	28.2	19.1	<0.002
	Scar	11.0	16.8	
"Old" collagen	Sinusoid	1.7	3.4	<0.006
	Scar	8.7	11.3	
"New" collagen	Sinusoid	0.2	1.1	<0.001
	Scar	12.9	16.9	
Macrophage	Sinusoid	15.1	19.5	NS
	Scar	19.3	22.8	
PMN	Sinsoid	0.6	2.2	NS
	Scar	1.3	4.9	
Fibroblast	Sinusoid	0.4	2.0	NS
	Scar	0.5	1.6	
Myofibroblast	Sinusoid	0.0	0.0	-
	Scar	0.3	1.5	
Lymphocyte	Sinusoid	4.2	12.2	NS
	Scar	1.7	3.8	

Table 3—Comparison of Constituents in Sinusoids of Ethanol-Fed (24) and Control Rats (11) (Percent Total Area)

Constituents	Diet	Percent area	*P* value
Ito cell	Ethanol	27.9 ± 15.0	NS
	Control	32.0 ± 19.0	
Ito cell fat	Ethanol	15.5 ± 12.0	NS
	Control	17.6 ± 9.7	
Ito cell RER	Ethanol	4.1 ± 3.7	<0.008
	Control	1.6 ± 1.5	
Ito cell nuclei	Ethanol	28.1 ± 19.0	NS
	Control	37.6 ± 16.0	
"Old" collagen	Ethanol	1.7 ± 3.4	NS
	Control	4.7 ± 9.0	
Macrophage	Ethanol	15.1 ± 19.5	NS
	Control	16.5 ± 23.5	
PMN	Ethanol	0.6 ± 2.2	NS
	Control	-	
Lymphocytes	Ethanol	4.2 ± 12.2	NS
	Control	3.9 ± 10.6	

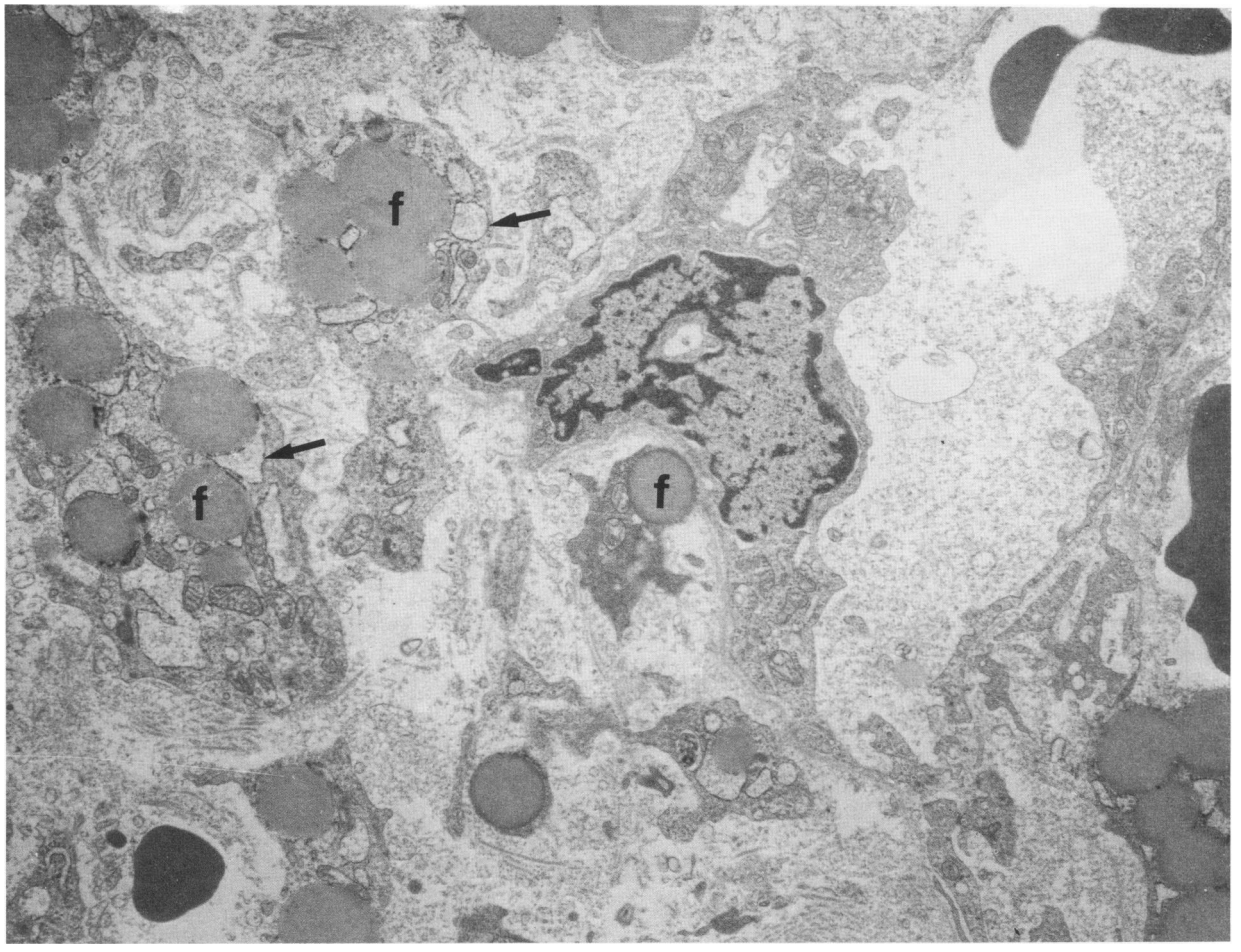

Figure 10—Electron micrograph of a scar in the liver of an ethanol-fed rat. Note that the Ito cells contain prominent dilated RER (arrows) and fat globules (f). The sinusoid is lined by endothelium surrounded by a basement membrane. ×9200

creased in the scar (Figure 9) whereas the RER was not significantly different (Table II). Thus, both the Ito cells in the scars and sinusoids of the livers of the ethanol-fed rats had an increase in the RER compared with the Ito cells in the sinusoids of the control rat livers (Tables 2 and 3). This means that the RER, but not the fat content, can be relied upon to indicate "activation" of Ito cells in the ethanol-fed rats. Both "new" and "old" collagen was increased in the scar (Table 2). The prominent RER and fat of "activated" Ito cells in the scars associated with "new" and "old" collagen is seen in Figure 10. This contrasted with that seen in Ito cells located in the perisinusoidal area of control livers (Figure 11). Macrophages and other cell types did not differ within the scars and sinusoids. Ito cells and macrophages were prominent around central vein fibrosis as well (Figure 12).

When the constituents in the sinusoids of the ethanol-fed and control rats were compared, no differences were noted except that the percent total square area occupied by RER within Ito cells was greater in the ethanol-fed rats (Table 3). The percent total square area of Ito cells occupied by fat was not different. Thus, only the percent of total square area occupied by RER could be relied upon to distinguish the Ito cells within the sinusoids of the ethanol-fed rats.

Discussion

The answer to the question, "can the Ito cell morphology found in the sinusoids of the livers of ethanol-fed rats be relied upon to predict Ito cell activation within liver scars" was assessed using morphometric quantitation of the Ito cell constituents. The RER percent total square area of Ito cells was increased significantly in both the Ito cells located near sinusoids and within scars in the livers of the ethanol-fed rats compared with controls. An association of dilated RER with increased collagen synthesis has been made using liver tissue slices and Ito cells in tissue cul-

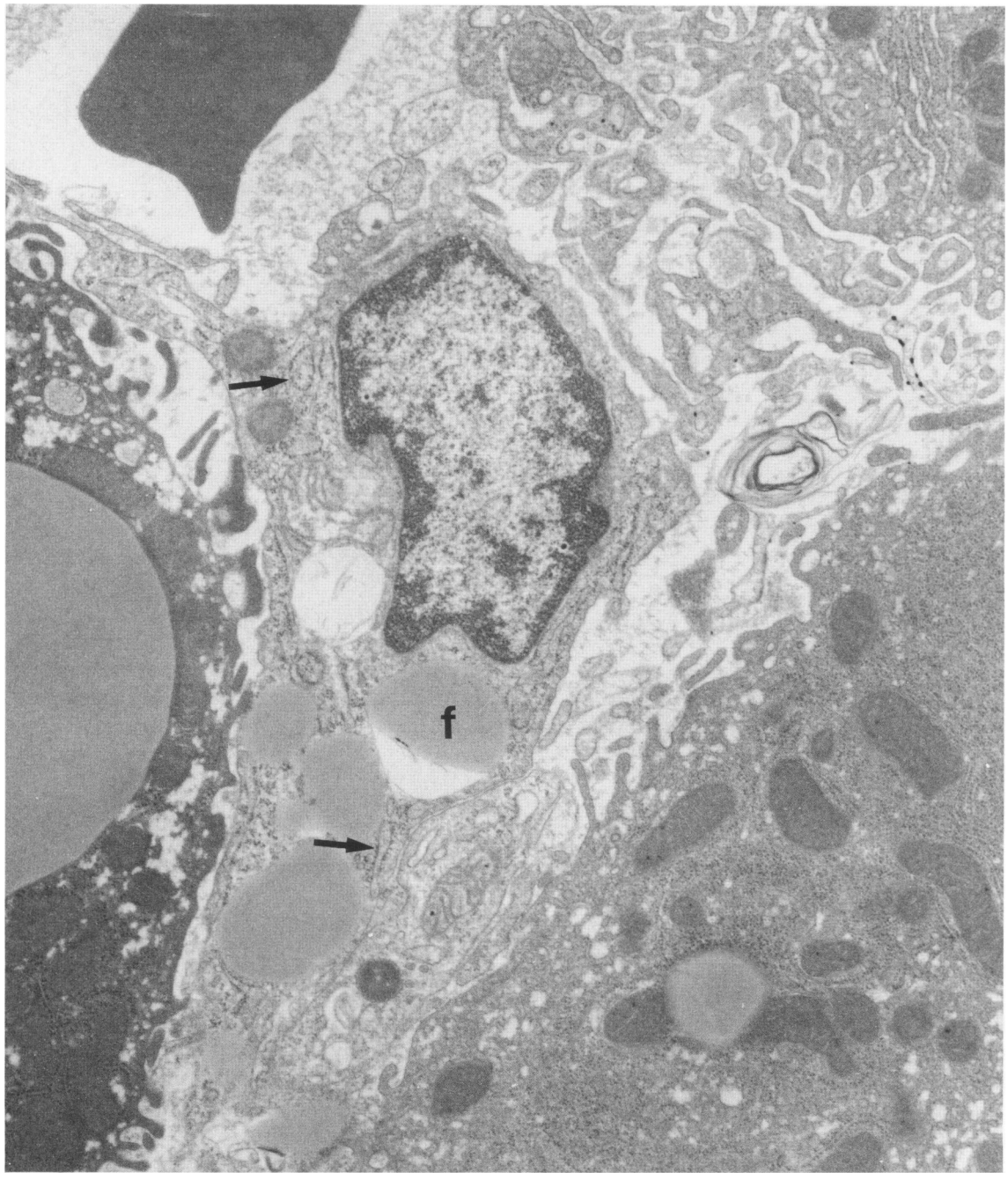

Figure 11—Electron micrograph of the liver perisinusoidal Ito cell from a pair-fed control rat. Note the scant RER (arrows). Fat (f) is prominent in the Ito cell and the adjacent hepatocytes. No collagen is seen and there is no basement membrane under the endothelial cell lining the sinusoid. ×19200

ture.[4,43] Thus, Ito cell "activation" in scars can be predicted by examining the Ito cells in sinusoids for dilatation of the RER. This indicates that Ito cell activation is a diffuse change but does not result in diffuse sinusoidal scarring. Rather, scar formation results from focal pericentral necrosis followed by a local increase in collagen formation by "activated" Ito cells. This implies that local factors, not Ito cell "activation" per se, govern scar formation. The stimulation of collagen synthesis by Ito cells may require necrosis for scar formation but *in vitro* stimulation of collagen secretion results from Ito cells exposed to acetaldehyde, a product of ethanol oxidation, and by interleukin I,[44] a cytokine that may be released by macrophages in the foci of hepatocytic necrosis.

The fat in Ito cells did not differ between ethanol-

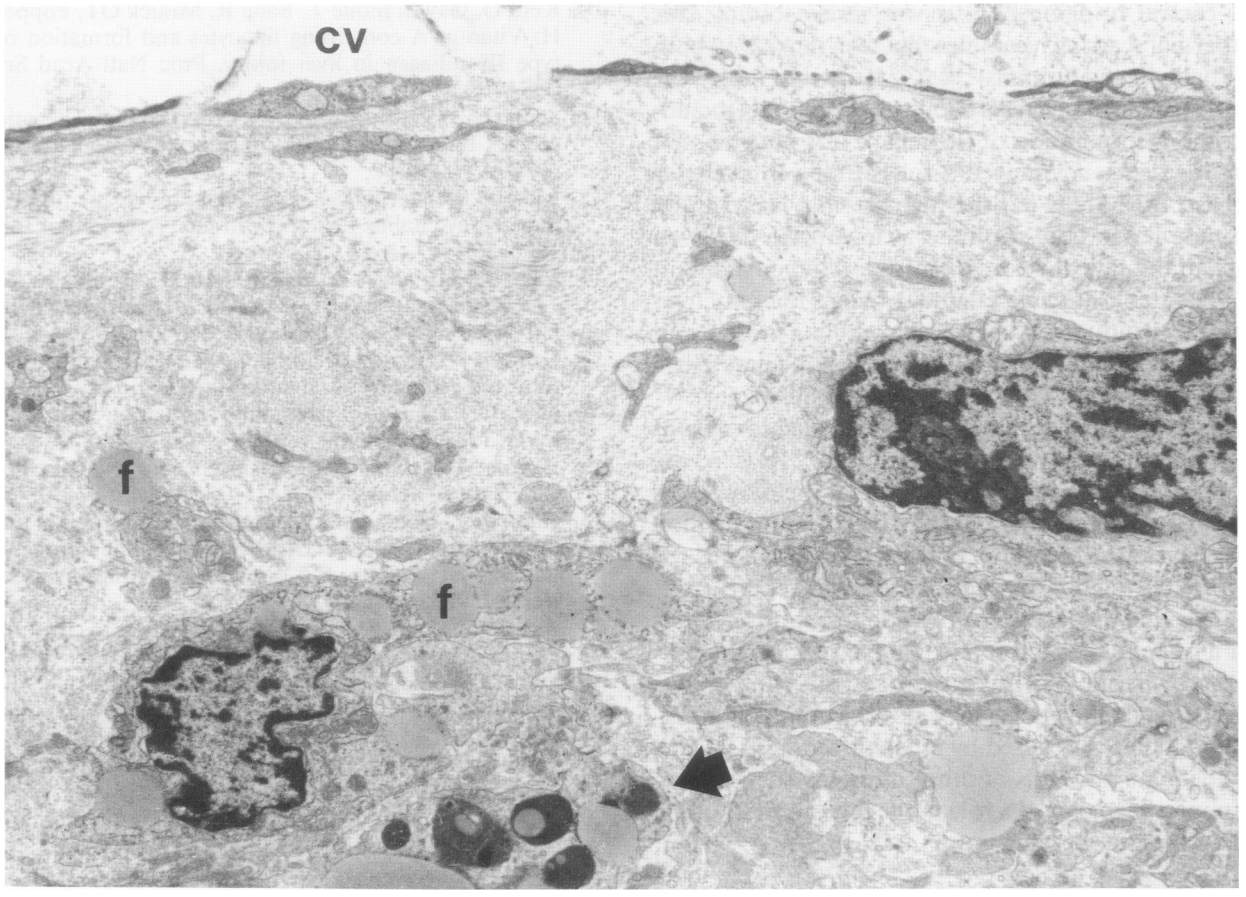

Figure 12—Electron micrograph of a central vein scar showing prominent Ito cells and macrophages (arrow) containing fat (f) from a rat fed ethanol for 2 months. Ito cell processes and free fat are present. The central vein (cv) lumen is at the top. ×9200

fed and controls when the percent total square areas were compared in the sinusoidal location. This is surprising because studies of ALD in man and the baboon showed a decrease in Ito cell fat.[3-5] Even more surprising was the increase in the percent total square area of Ito cell fat located in scars compared with Ito cells in sinusoidal areas in the ethanol-fed rats. Ito cells in this area are probably responsible for producing the collagen in the scars since they are the cell type that predominates within scars. Fat stores are unreliable indicators of Ito cell "activation" in the rat model of ALD.

The fat in normal rat liver Ito cells, like liver parenchymal cells, contains high concentrations of neutral lipids.[45] The Ito cell lipid contains retinyl and cholesterol esters as well as triglycerides (20%). The Ito cell is the main depot of vitamin A in the body. About 90% of the total body storage of retinoids is present in the liver in rats and 90% of liver retinoids are present in the Ito cells in the form of retinyl esters, mainly as retinyl palmitate.[46] Ito cells contain 2 types of fat droplets. Type I has no limiting membrane and constitutes the majority. Type II has a limiting membrane and increases after administration of excess vitamin A.[46] About 25%, or possibly more, of the fat-storing cell lipid droplets consist of triglycerides.[46] Therefore, it is possible that our rats that were fed a high fat diet adequate in vitamin A (306 IU/kg body weight/day) with alcohol have increased Ito cell lipid on the basis of increased triglyceride storage similar to the liver parenchyma. This issue cannot be resolved without isolating the Ito cells and measuring their lipid composition. It is known that ethanol ingestion decreases vitamin A stores in the rat liver and a high fat diet further reduces these stores.[47] Therefore, it is unlikely that the increase in fat stores seen in the Ito cells in the scars or our rats fed ethanol is the result of increased storage of vitamin A or its metabolites.

The sirius red staining method of quantitating liver fibrosis proved useful in documenting significant centrilobular scarring using the high fat–low protein ethanol diet. The scarring was primarily centrilobular, bridging from one central vein to the next, but increase in portal collagen was also observed and por-

tal–central bridging fibrosis was rarely found. This pattern of scarring resembles that seen in ALD in both the baboon and humans.[20,21,39,48] It also resembled the scarring seen in rats fed a choline deficient diet including the incorporation of Ito cells.[12] It is possible that choline deficiency played a role in the centrilobular fibrosis in the rats fed the high fat–low protein diet because the level of choline was low in the diet even though the rats did not go on to develop cirrhosis. It is well established that ethanol worsens the fatty liver pathology induced by diets low in choline protein and vitamins B_{12} and folate.[49–51] However, alcohol ingestion inhibits fibrosis caused by choline deficiency.[52,53] In the baboon, choline supplements failed to prevent liver fibrosis in the ethanol-fed animals.[54] Rats fed the high fat diet and adequate lipotropes, including choline and protein, by the intragastric cannula method also develop comparable fibrosis when ethanol is fed to produce high BAL as in the present report.[7,8] Although this diet may not provide optimum calories for a weanling rat,[34] it is adequate to support the normal growth of 380-g rats used in our study.[8] We conclude that choline deficiency alone is not likely to explain the fibrosis caused by ethanol feeding. The lack of adequate amounts of other nutrients such as protein may have played a role in the pathogenesis of the ethanol-induced scarring.

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Acknowledgment

The authors thank Doug Mauldin and Dr. Dulberg for assistance with the statistical analysis and LeeAnn Perrier for secretarial assistance.