Intestinal Mucosal Alterations in Experimental Cirrhosis in the Rat: Role of Oxygen Free Radicals

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Cirrhosis is associated with altered gastrointestinal function, and bacterial translocation from the gut plays an important role in the etiology of spontaneous bacterial peritonitis (SBP) seen in this condition. Although alterations in gut motility and intestinal permeability are recognized in cirrhosis, the intestinal damage at the cellular and subcellular levels is not well understood. This study looked at the mucosal alterations in experimental cirrhosis and the role of oxygen free radicals in this process. It was shown that cirrhosis results in oxidative stress in the intestine, as seen by increased xanthine oxidase (XO) activity and altered antioxidant status. Cirrhosis also affects enterocyte mitochondrial function, as assessed by respiratory control ratio, swelling, and calcium flux. Increased lipid peroxidation of the brush border membranes (BBMs) was seen along with altered intestinal transport. In conclusion, this study shows that intestinal mucosal alterations are seen in experimental cirrhosis and are possibly mediated by oxidative stress. (HEPATOLOGY 2002;35:622-629.)

The clinical course of patients with cirrhosis is often affected by a number of complications, including portal hypertension, ascites, and spontaneous bacterial peritonitis (SBP). Bacterial infection is responsible for up to one fourth of the deaths of patients with cirrhosis,[1] and SBP is a major complication in cirrhosis that is common in patients with cirrhosis and ascites.[2] SBP probably develops as a consequence of impaired defense mechanisms against infection seen in cirrhotic patients. Although key steps in the pathogenesis of SBP are yet to be elucidated, it is evident that the gut is a major source of bacteria in SBP. Altered gut motility has been shown in cirrhosis,[3] and this probably facilitates bacterial overgrowth, which is frequent in patients with chronic hepatopathies. This bacterial overgrowth could increase the severity of the hepatopathy and also produce bacterial peritonitis. Cirrhosis reflects irreversible long-term injury of the hepatic parenchyma in association with extensive fibrosis. Cirrhosis can develop in a number of different conditions, including alcoholic liver disease, biliary disease, and viral infection. Oxidative stress plays an important role in the pathogenesis of toxic liver diseases and other hepatic alterations.[4] Protein oxidation may play a role in the pathogenesis of carbon tetrachloride–induced liver injury,[5] and administration of antioxidants also decreases the amount of hepatic fibrosis in CCl_4-induced cirrhosis.[6]

Intestinal permeability is increased in patients with cirrhosis,[7] and alterations in tight junctions have been implicated in the pathogenesis of primary biliary cirrhosis.[8,9] The intestine has cells at various stages of differentiation, and the epithelium undergoes a continuous renewal process. Oxygen free radicals are known to play an important role in gut epithelial damage, which may alter the gut barrier function and facilitate bacterial translocation and release of endotoxin. However, little information is available on the intestinal alterations in cirrhosis; this study looked at the effect of experimental cirrhosis on mucosal changes at the level of different enterocyte populations.

Materials and Methods

Tris, HEPES, bovine serum albumin, p-nitrophenyl phosphate, peroxidase, o-dianisidine, glucose oxidase, thiobarbituric acid, dithio-bis-(2-nitrobenzoicacid), adenosine diphosphate, succinate, ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid, 1,1′,3,3′ tetramethoxypropane, arsenazo III, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma Chemical Co. (St. Louis, MO). Polyethylene glycol 4000 was obtained from Fluka AG (Buchs, Switzerland). ^{14}C-labeled glucose was obtained from BARC (Bombay, India). Millipore membranes (pore size, 0.45μm) were obtained from Millipore (Bangalore, India). All other chemicals and solvents used were of analytic grade.

Animals. Adult Wistar rats of both sexes (150-250 g) exposed to a daily 12-hour light-dark cycle and fed water and rat chow (Hindustan Lever Ltd., Bombay, India) ad libitum were used for this study. This study was approved by the Animal Experimentation Ethics Committee of Christian Medical College and Hospital.

Induction of Cirrhosis. Cirrhosis was developed by intragastric feeding of CCl_4 as described.[10] Briefly, Wistar rats of both sexes (150-250 g) were treated with phenobarbitone (35 mg/dL) in tap

Abbreviations: SBP, spontaneous bacterial peritonitis; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; XO, xanthine oxidase; BBM, brush border membrane.

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water, which was the only source of drinking water, for $2\frac{1}{2}$ weeks before initiation of the experiment. Twice-weekly intragastric instillation of CCl_4 was then begun under light halothane anesthesia using a 2.5-mL syringe attached to Tygon tubing (diameter, 2 mm). The initial dose of CCl_4 was 40 μL/rat. Subsequent doses were adjusted based on the change in body weight. Fully developed micronodular cirrhosis was obvious by the 11th week of this treatment. Control animals were treated with phenobarbitone similar to the test animals but were not given CCl_4. Treatment with phenobarbitone and CCl_4 was stopped at the 11th week, and the animals were killed by decapitation after 10 days and the intestine removed.

Isolation of Villus and Crypt Cells From the Small Intestine. The whole length of the small intestine was washed gently with cold physiologic saline containing 1 mmol/L dithiothreitol. Enterocytes of various stages of maturation (villus to crypt) were isolated by the metal chelation method as described.[11] Briefly, the intestine was filled with solution A (1.5 mmol/L KCl, 96 mmol/L NaCl, 27 mmol/L sodium citrate, 8 mmol/L KH_2PO_4, and 5.6 mmol/L Na_2HPO_4, pH 7.3), clamped at both ends, and incubated at 37°C for 15 minutes. After incubation, the luminal contents were discarded and the intestine was filled with solution B (phosphate-buffered saline, pH 7.3, containing 1.5 mmol/L ethylenediaminetetraacetic acid and 0.5 mmol/L dithiothreitol) and incubated at 37°C for different time intervals of 4, 2, 2, 3, 4, 6, 7, 10, and 15 minutes. At the end of each time period, the incubated solution containing cells was collected in separate tubes. These 9 fractions were pooled into 3, with the first 3 fractions being villus cells, the next 3 fractions middle cells, and the last 3 fractions crypt cells. These cell fractions were centrifuged at 900*g* for 5 minutes and washed with Krebs-Hensleit buffer, pH 7.4, containing 5 mmol/L glucose and 2.5 mmol/L calcium. Separated villus, middle, and crypt cells were identified by assaying the marker enzyme for differentiation (alkaline phosphatase).

Assessment of Cell Viability and Yield. Cell viability was studied by the dye-exclusion method using trypan blue. Cell counts of various fractions were made after isolation. The proportion of the individual cell population was expressed as percentage of total isolated cells.

Biochemical Estimations. Filamentous and unpolymerized actin were quantified by deoxyribonuclease inhibition assay as described.[12] Briefly, the isolated enterocytes were lysed; the lysate was treated with guanidine HCl (monomeric actin plus filamentous actin) and without guanidine HCl (monomeric actin alone) and the activity of deoxyribonuclease I assayed in the presence of substrate DNA. Because the assay measures the ratio of G to F actin, cell number or protein content would not interfere and the data are expressed as the ratio itself. The activity of the alkaline phosphatase[13] was assayed using p-nitrophenyl phosphate as substrate. One unit of the enzyme is defined as the amount of enzyme required to produce 1 μmol of p-nitrophenol in 15 minutes under the assay conditions. Adenosine deaminase activity was measured using adenosine as substrate.[14] One unit gives an initial rate indicated by 0.01 per minute decrease in optical density (260 μm, 1-cm light path) at 25°C. Protein estimation was performed using bovine serum albumin as the standard.[15] Xanthine oxidase (XO) activity was measured spectrophotometrically based on the production of uric acid, which was measured at 295 nm.[16] The assay was performed in the presence and absence of 0.4 mmol/L nicotinamide adenine dinucleotide to assay XO plus xanthine dehydrogenase and XO, respectively. One unit corresponds to 1 μmol/min of urate formed at 25°C. Catalase activity was estimated by measuring changes in absorption at 240 nm using hydrogen peroxide as substrate.[17] One unit is the activity that disproportionates H_2O_2 at the rate of 10^{-3} absorbance/s.

Cell homogenate proteins were precipitated by trichloroacetic acid, and glutathione in the acid supernatant was quantitated using high-performance liquid chromatography after derivatization. The derivatization method was based on the initial conversion of *S*-carboxyl methyl derivatives of free thiols with iodoacetate followed by the conversion of free amino groups to 2,4-dinitrophenyl derivatives. Dinitrophenyl derivatives were separated by high-performance liquid chromatography on an Ultrasil amino column (Shimadzu, Kyoto, Japan) with a gradient of methanol and sodium acetate and detected at 365 nm.[18]

Mitochondrial Preparation and Assessment of Function. Mitochondria were prepared from various enterocyte populations as described.[19] The final mitochondrial pellet was washed twice with a solution containing 250 mmol/L sucrose and 5 mmol/L HEPES, pH 7.4, and suspended in the same solution. Mitochondrial function was assessed by MTT reduction, oxygen uptake, and calcium flux measurements. MTT reduction was performed using a microplate reader as described.[20] In a total volume of 150 μL in each well, mitochondria corresponding to 150 to 200 μg protein were taken, 6 μL of 1.25 mmol/L MTT was added, and the volume was made up with 25 mmol/L phosphate-buffered saline. Plates were incubated at 37°C for 20 minutes followed by addition of 150 μL dimethyl sulfoxide and mixed thoroughly to dissolve the formazan. The plates were read on a multiwell scanning spectrophotometer (enzyme-linked immunosorbent assay reader) using a test wavelength of 570 nm and reference wavelength of 630 nm. The amount of MTT formazan formed was calculated from the standard curve prepared using authentic MTT formazan. Mitochondrial swelling was determined by the decrease in absorbance at 540 nm up to 7 minutes.[21] Oxygen uptake was determined polarographically using a Clark-type electrode in 2 mL of respiration medium (225 mmol/L sucrose, 5 mmol/L $MgCl_2$, 10 mmol/L KH_2PO_4, 20 mmol/L KCl, 10 mmol/L Tris, and 5 mmol/L HEPES, pH 7.4) containing 5 mmol/L succinate as respiratory substrate. A mitochondrial protein of 2 mg/mL was used. Oxygen uptake was stimulated with 0.3 mmol/L adenosine diphosphate. Oxygen uptake during both state 3 and state 4 respiration was measured, and the ratio of state 3/state 4 respiratory rate was used to calculate the respiratory control ratio.[21]

Calcium flux was followed by measuring the change in absorption spectrum of arsenazo III.[21] Mitochondria (1 mg protein/mL) were suspended in reaction medium containing 250 mmol/L sucrose, 5 mmol/L HEPES, 5 mmol/L succinate, and 40 μmol/L arsenazo III (pH 7.4). Calcium (10 μmol/L) was added as indicated in Figure 5D. When calcium is added to the mitochondrial suspension, there is a sudden increase in optical density due to calcium binding by the dye. The dye binding is a reversible, dynamic process. As the mitochondria takes up calcium, the external

level decreases, and this is reflected by the decrease in optical density over time.

Isolation of Brush Border Membranes. Brush border membranes (BBMs) were isolated from the small intestine of overnight fasted control and cirrhotic rats by the polyethylene glycol precipitation method.[22] Briefly, luminal contents were washed thoroughly with ice-cold saline and the mucosa scraped using a glass slide. Approximately 3% homogenate of the mucosa was prepared in 2 mmol/L Tris-HCl containing 50 mmol/L mannitol, pH 7.1, using a Porter-Elvelhem homogenizer for 2 to 3 minutes at full speed. This was allowed to remain at 4°C for 15 minutes and filtered using nylon cloth. To this, 50% polyethylene glycol solution was added to make a final concentration of 10% polyethylene glycol, stirred for 15 minutes, and centrifuged at 7,500*g* for 15 minutes. The pellet was discarded and the supernatant was centrifuged at 27,000*g* for 40 minutes. A total of 30 mL suspension buffer (10 mmol/L Tris-HCl and 300 mmol/L mannitol, pH 7.1) was added to the pellet, which was centrifuged at 27,000*g* for 40 minutes. The pellet was washed twice with the same suspension buffer and finally suspended in 1 mL of the same buffer using a syringe fitted with a 26-gauge needle. Purity of the isolated BBM was checked by enrichment of the marker enzyme alkaline phosphatase, sucrase, and maltase.

Peroxidation Parameters. Malonaldehyde was measured using the thiobarbituric acid method.[23] The amount of malonaldehyde formed was calculated from the standard curve prepared using 1,1′,3,3′ tetramethoxypropane and expressed as nmol/mg protein. For conjugated diene measurement, total lipids from BBMs were extracted, dissolved in 1 mL heptane, and read at 233 nm and calculated using a molar absorption coefficient of 2.52×10^4 and expressed as μmol/mg protein.[24]

Measurement of D-glucose Uptake. Isolated BBMs were assessed for their ability to transport glucose by uptake measurements performed using the rapid filtration technique at room temperature as described.[25] Briefly, 50 μL BBM vesicles corresponding to 100 μg protein was incubated with 150 μL of uptake buffer containing 150 mmol/L sodium thiocyanate, 50 μmol/L D-glucose, 0.8 μCi (^{14}C) D-glucose, and 10 mmol/L HEPES (pH 7.5) at varying time intervals. At the end of incubation, the mixture was diluted with 2 mL ice-cold stop buffer (150 mmol/L NaCl and 10 mmol/L HEPES, pH 7.5) and immediately filtered using a 0.45-μm filter under constant vacuum. The filter was washed 3 times with 5 mL stop buffer and transferred to counting vials. The radioactivity retained in the filter was counted using an LKB Rack-Beta scintillation counter.

Lipid Analysis. BBM lipids were extracted by the method of Bligh and Dyer.[26] Lipids were separated by thin-layer chromatography. Cholesterol,[27] diacylglycerol, and triacylglcerol[28] were quantitated as described. Free fatty acids were methylated and quantitated by gas chromatography. Phospholipids were separated

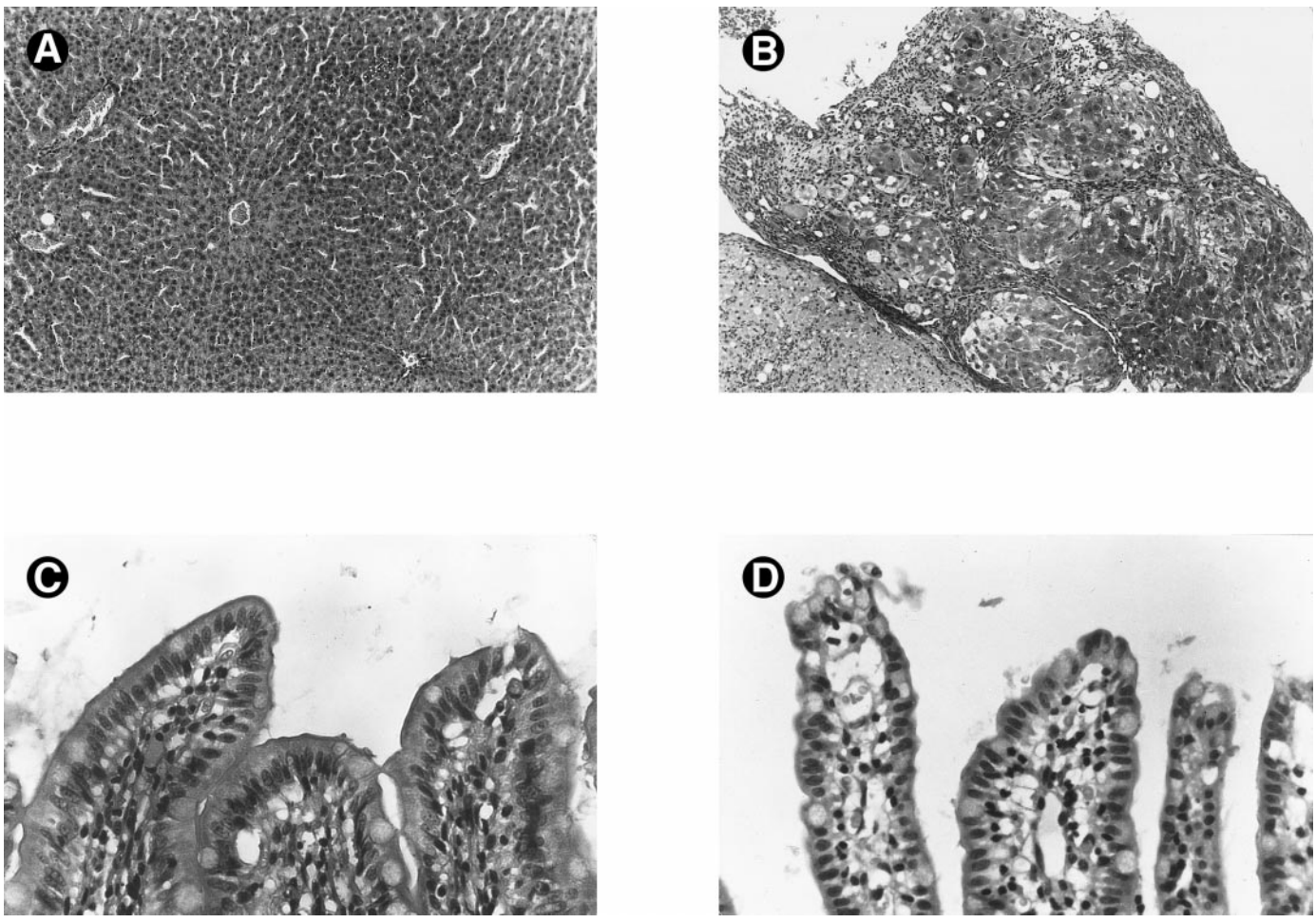

Fig. 1. Light microscopy of liver and intestinal epithelium from control (A and C) and cirrhotic (B and D) rats.

by thin-layer chromatography and quantitated by phosphate estimation after acid hydrolysis.[29,30]

Statistical Analysis. Data are expressed as means ± SD of 3 to 5 separate experiments. Statistical analysis was performed with Student's *t* test.

Results

Cirrhosis was established by histologic analysis of the liver from animals treated with CCl_4 and compared with control liver (Fig. 1A and B). To determine whether the intestine was affected in cirrhosis, light-microscopic studies on intestine were performed. It was found that intestinal samples from control rats and rats killed before the onset of cirrhosis in the second, fourth, and sixth weeks showed normal crypt and villi as seen in Fig. 1C. The lymphatics and blood vessels appeared within normal limits. Intestinal mucosa from fully developed cirrhotic rats showed areas of villous epithelial damage, and the cells covering the villus tips showed shortening, nuclear disarray, and degenerative changes of the cytoplasm, which are highlighted in Fig. 1D. Extrusion of the dead cells was also frequently seen in these villi, unlike the control. The intestine has cells at various stages of differentiation, and the turnover of intestinal epithelial cells is a finely regulated process extending from undifferentiated crypt stem cells to terminally differentiated villus cells. To determine the susceptibility of these different cell populations in cirrhosis, enterocytes were isolated and grouped as villus, middle, and crypt fractions and their viability assessed using trypan blue exclusion. A decrease in viability was seen in all of the cell populations of enterocytes from cirrhotic rats compared with control (Fig. 2A). Cell yield studies in cirrhosis showed a marked decrease in villus fraction compared with control (Fig. 2B), and this was associated with an increase in cell yield in the middle fraction. The cytoskeleton of the cell plays a major role in maintenance of cellular integrity, morphology, and structure. Figure 2C shows the ratio of monomeric actin/monomeric actin plus filamentous actin in various cell populations of control and cirrhotic rats, and it was observed that the ratio of monomeric actin/monomeric actin plus filamentous actin was altered prominently in middle and crypt cells in cirrhotic rats.

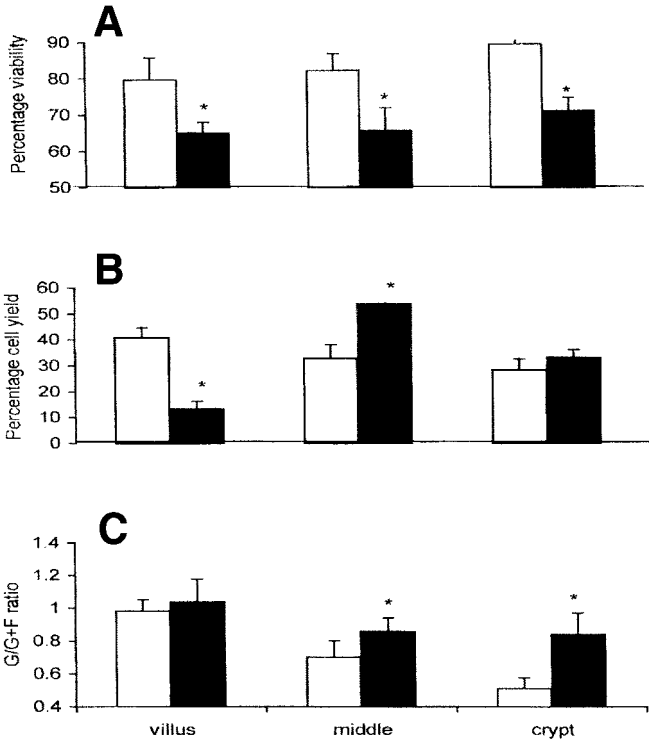

Fig. 2. Cell viability (A), cell yield (B), and actin organization (monomeric actin/monomeric actin plus filamentous actin [G/G+F ratio]) (C) of various populations of enterocytes isolated from control and cirrhotic rats. The assays were performed as described in the text. Each value represents the mean ± SD from 3-5 separate experiments. *$P < .05$ when compared with control.

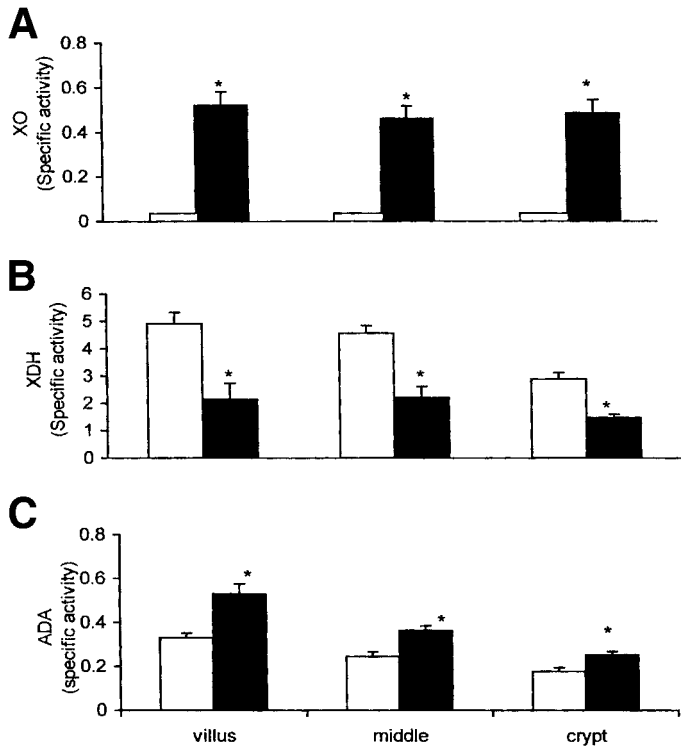

Fig. 3. Activity of XO (A), xanthine dehydrogenase (XDH) (B), and adenosine deaminase (ADA) (C) of various enterocyte populations from control and cirrhotic rats. The assays were performed as described in the text. Each value represents the mean ± SD from 3-5 separate experiments. *$P < .05$ when compared with control. (□) Control; (■) cirrhosis.

The possible biochemical mechanism involved in intestinal damage during cirrhosis was then analyzed. Oxygen free radicals are known to play an important role in gut epithelial damage, which may alter the gut barrier function and facilitate bacterial translocation and release of endotoxin. One of the important sources of free radicals in the intestinal mucosa is the enzyme XO. This enzyme was almost undetectable in the control intestine, whereas it was significantly increased in cirrhotic rats (Fig. 3A). Concomitant with this, xanthine dehydrogenase activity was significantly reduced in these fractions (Fig. 3B). Superoxide generation by XO requires xanthine or hypoxanthine as substrates. Adenosine deaminase provides the substrate for XO, and its activity was also increased significantly in all of the fractions in cirrhotic rats (Fig. 3C).

Oxidative stress parameters were measured in the total enterocytes; as shown in Fig. 4A and B, a significant increase in lipid

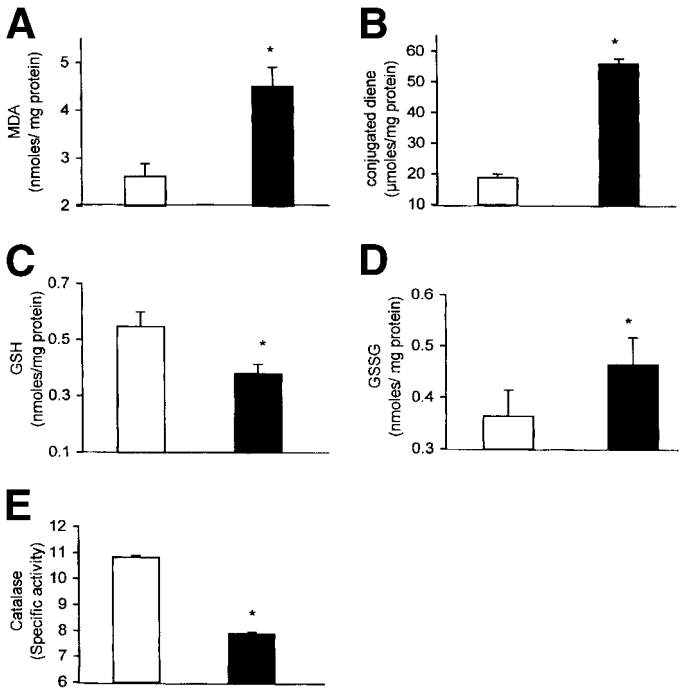

Fig. 4. Malonaldehyde (MDA) (A), conjugated diene (B), reduced glutathione (GSH) (C), oxidized glutathione (GSSG) (D), and catalase activity (E) of enterocytes isolated from control and cirrhotic rats. The assays were performed as described in the text. Each value represents the mean ± SD from 3-5 separate experiments. *$P < .05$ when compared with control. (□) Control; (■) cirrhosis.

peroxidation was seen in the cirrhotic rats compared with control. The defense against oxygen free radicals is important for normal functioning of the cells, and the intracellular nonenzymatic antioxidant tripeptide glutathione maintains the thiol-redox status of the cell. The level of reduced and oxidized form of glutathione is shown in Fig. 4C and D. Oxidized glutathione level was increased in cirrhotic rats compared with control; concomitant with this, a decrease in the reduced form of glutathione was seen. The antioxidant enzyme catalase showed a decrease in the mucosa of cirrhotic rats compared with control (Fig. 4E).

To look at the subcellular changes, enterocyte mitochondria from various stages of differentiation were isolated and examined for functional and biochemical changes. It was found that intestinal mitochondria from cirrhotic rats showed an increase in superoxide production as measured by MTT reduction (Fig. 5A), accompanied by a decrease in the mitochondrial respiratory control ratio (Fig. 5B). Swelling studies on mitochondria showed an induction of permeability transition pore in cirrhotic rats (Fig. 5C). Cirrhosis also resulted in impairment of normal calcium flux in the intestinal mitochondria (Fig. 5D).

The BBM is an important functional component of the intestine and is in direct contact with the luminal contents, which include metabolites from the liver. A decrease in activity of the BBM enzyme alkaline phosphatase was seen in cirrhotic rats (Fig. 6A), whereas the activity of sucrase and maltase was unaltered (data not shown). Lipid peroxidation parameters such as malondialdehyde (Fig. 6B) and conjugated diene (Fig. 6C) were found to be increased in BBMs of cirrhotic rats when compared with control. Functional integrity of the BBM was assessed by glucose transport, and cirrhosis resulted in decreased glucose transport by BBMs (Fig. 6D). BBM lipid analysis showed alterations in phospholipids, a decrease in phosphatidyl choline and phosphatidyl ethanolamine, and an increase in lysophosphatidyl choline, lysophosphatidyl ethanolamine, and phosphatidic acid. There was also an increase in total free fatty acids in BBMs from cirrhotic rats, specifically a significant increase in the level of linoleic and arachidonic acids (Fig. 7).

Discussion

It is known that cirrhosis is associated with altered gastrointestinal functions such as gut motility and barrier function, and mucosal abnormalities secondary to portal hypertension exist. These intestinal alterations may facilitate bacterial translocation, leading to complications such as SBP.[3,7] Histopathologic studies have shown translocation of bacteria such as *Candida albicans* and *Escherichia coli* by direct penetration of enterocytes associated with disruption of basal membrane.[31] Little is known about the alterations at the cellular level in the intestine in cirrhosis, and this study looked at the intestinal mucosal alterations in experimental cirrhosis in rats.

Intragastric feeding of CCl_4 is a recognized model for development of cirrhosis,[9] and 11 weeks of this treatment resulted in full development of micronodular cirrhosis in treated animals with

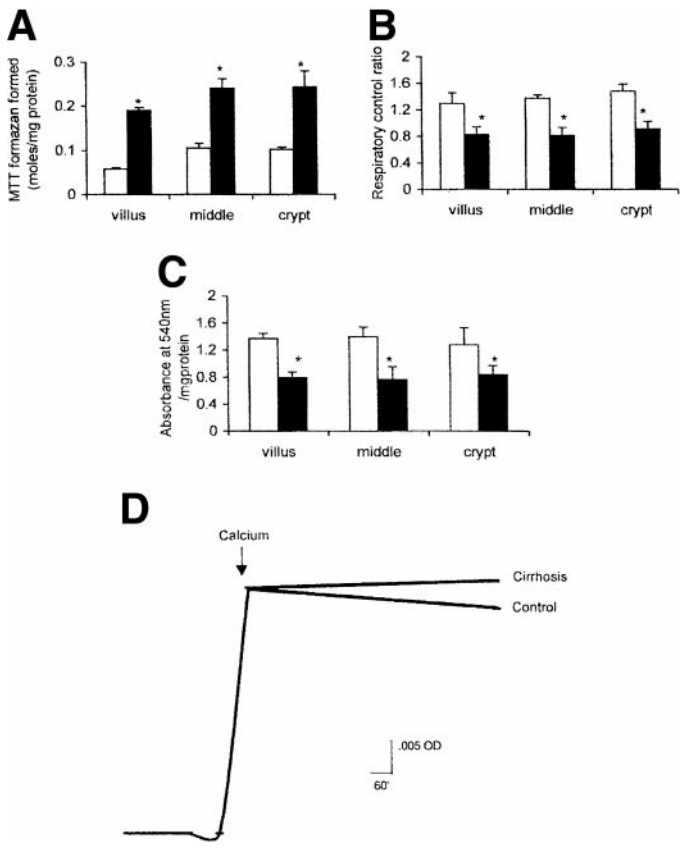

Fig. 5. MTT reduction (A), respiratory control ratio (B), swelling (C), and calcium flux (D) in mitochondria of various cell populations of enterocytes from control and cirrhotic rats. The assays were performed as described in the text. Each value represents the mean ± SD from 3-5 separate experiments. *$P < .05$ when compared with control.

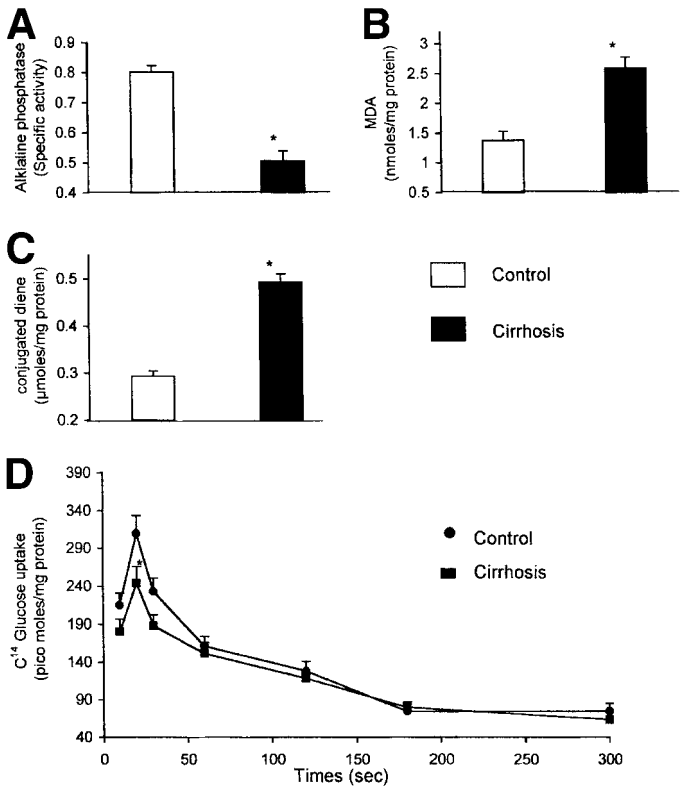

Fig. 6. Alkaline phosphatase activity (A), malonaldehyde (MDA) (B), conjugated diene (C), and glucose transport (D) in BBMs isolated from control and cirrhotic rats. The assays were performed as described in the text. Each value represents the mean ± SD from 3-5 separate experiments. *$P < .05$ when compared with control.

disruption of normal liver architecture, fibrosis, and fatty changes. Oxidative stress has been implicated in the process of fibrogenesis, and many etiologic agents of fibrogenesis stimulate free radical reactions either directly or through inflammatory stimuli.[32] However, the mechanism involved in intestinal mucosal alterations and the role of oxygen free radicals in this process has not been examined. Oxidative stress in the intestine may develop either by the generation of reactive oxygen species in the mucosa or possible inducers generated in the liver and transported to the intestinal lumen through bile once cirrhosis has developed. XO is abundantly present in the intestinal mucosa, and the superoxides generated by XO may contribute to much of the free radical–induced intestinal damage.[33] In this study, an increase in xanthine (produced by the increased adenosine deaminase activity) coupled with increased XO activity *per se* contributed to increased reactive oxygen species generation and oxidative stress in the intestine during cirrhosis.

One of the important consequences of oxidative stress is lipid peroxidation, which results in generation of conjugated diene and malondialdehyde by oxidation of membrane lipids,[34] and these lipid peroxidation products were increased in the intestine of cirrhotic rats. This was associated with reduced antioxidant status. An increase in plasma lipid peroxide and ascorbic acid levels and a decrease in reduced glutathione level and superoxide dismutase activity in hemolysate has been shown in cirrhotic patients.[35] Increased lipid peroxidation accompanied by decreased glutathione level were observed both in the liver homogenate and in the hepatic mitochondria of bile duct–ligated rats.[36] Free radicals have been identified in the bile of rats treated long-term with alcohol,[37] and it has been shown that the major part of the electron spin resonance free radical signal arises from protein-bound bilirubin.[38] Deoxycholate has also been shown to stimulate superoxide production in colonic mucosal scrapings or crypt epithelium.[39] Endotoxin, along with ethanol treatment, also induces a 3-fold increase in radical adducts in the bile.[40] The present study shows that there is an increased generation of free radicals via the xanthine-XO system in cirrhotic rats, resulting in peroxidative damage to the intestinal mucosa.

The intestinal BBM is important in the transport of nutrients, and cirrhosis was found to decrease transport of glucose. Absorption of D-galactose has been shown to be reduced in cirrhotic rats, and this is accompanied by a significant elongation of enterocyte microvilli.[41] It has been shown that intestinal sugar and amino acid transport are disturbed in experimental cirrhosis.[42,43] Cirrhosis was found to decrease the activity of alkaline phosphatase in the BBM, whereas sucrase and maltase activities were not affected. This preferential decrease in alkaline phosphatase activity indicates that oxidative stress induced damage, because this enzyme is susceptible to free radical attack.[44] This was confirmed by the lipid peroxidation parameters assessed in the BBM, which again showed an increase in conjugated diene and malondialdehyde levels, indicative of oxidative stress. Lipids are important constituents of the membrane, and any alteration in their composition might affect membrane function. Cirrhosis was associated with altered phospholipid composition and generation of free fatty acids in the BBM. Biliary cirrhosis induces changes in BBM composition in rats, including decreased structural proteins, microvillous enzymes, and triglycer-

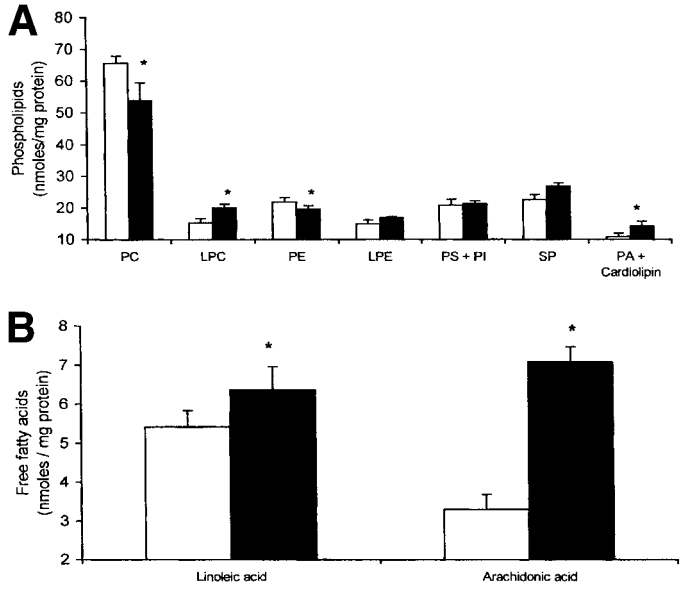

Fig. 7. Mucosal BBM phospholipids (A) and free fatty acids (B) of control and cirrhotic rats. PC, phosphatidyl choline; LPC, lysophosphatidyl choline; PE, phosphatidyl ethanolamine; LPE, lysophosphatidyl ethanolamine; PS, phosphatidyl serine; PI, phosphatidyl inositol; PA, phosphatidic acid; SP, sphingomyelin. The assays were performed as described in the text. Each value represents the mean ± SD from 3-5 separate experiments. *$P < .05$ when compared with control. (□) Control; (■) cirrhosis.

ide content. This is accompanied by a 3-fold decrease in calcium transport in the duodenojejunum, probably due to a lower content of BBM calmodulin.[45]

The cellular composition of the intestinal brush border is dependent on the migration and turnover of cells in the epithelium, a process essential for maintaining normal cell balance. The intestinal epithelium contributing to the brush border is a self-renewing monolayer arising from stem cells located at or near the base of the crypts. Any damage to the epithelium can alter the proliferation and migration of enterocytes. The decrease in cell number in the villus region accompanied by an increase in the middle region reflects alterations in the cell migration pattern during cirrhosis. It is possible that the senescent cells are shed off from the villus region and the cells migrate from the crypt toward the villus to compensate for this cell loss. The cellular cytoskeleton plays an important role in cell migration in the intestine, and actin is an important component of the cytoskeleton.[46] Cirrhosis induced dynamic changes in the state of actin polymerization, which was reflected in the change in monomeric actin/monomeric actin plus filamentous actin ratios. This was prominent in the middle and crypt regions and probably explains the altered cell yield, because it has been shown that the migrating cells undergo alterations in the actin cytoskeletal protein.[47] Hence, oxidative stress induced by cirrhosis might influence functional damage to the intestine by interfering with normal cellular migration along the crypt-villus axis and compromising BBM function.

Organelle integrity is important for normal cellular function, and mitochondria are considered the "powerhouse" of the cell due to their role in energy generation. A prominent feature of cell damage is impairment of mitochondrial function, and cirrhosis was found to induce mitochondrial dysfunction in the intestine. Cytokines such as tumor necrosis factor α have been shown to mediate intestinal injury after transport from the liver through bile in endotoxemia.[48] Mitochondria also play an important role in maintenance of cellular calcium homeostasis, acting as a "sink" to take up calcium from the cytosol to maintain cytosolic calcium levels. Cirrhosis was found to alter enterocyte mitochondrial calcium flux, which could affect cellular calcium homeostasis. This is probably due to the damaged respiratory function, which is essential for normal calcium transport in mitochondria.[49]

In conclusion, these studies have shown that oxidative stress occurs in the enterocytes in cirrhosis, and this may be responsible for structural and functional alterations seen in the small intestine. To our knowledge, this is probably the first detailed study of the biochemical alterations in the intestine in cirrhosis. These changes in enterocytes may lead to gut barrier alterations resulting in bacterial translocation and development of complications such as SBP.

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