Acetaldehyde and Lactate Stimulate Collagen Synthesis of Cultured Baboon Liver Myofibroblasts

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Cells with electron-microscopic characteristics of myofibroblasts were isolated from baboon liver biopsy specimens by collagenase digestion and Percoll density gradient centrifugation and then cultured. The cultures consisted of only one cell type. By immunofluorescence, these cells synthesized collagen types I, III, and IV and laminin. Typical features of myofibroblasts were maintained throughout many passages in the culture. To study the effects of ethanol (and its oxidation product acetaldehyde and associated metabolite lactate) on myofibroblast collagen synthesis, the cell cultures were incubated for 24 h in a medium containing either 50 mM ethanol, 200 μM acetaldehyde, or 5 mM lactate. The cells did not contain significant alcohol dehydrogenase activity. Acetaldehyde stimulated significantly ($p < 0.05$) myofibroblast collagen synthesis without changing noncollagen protein synthesis or proline pools. Lactate caused a significant ($p < 0.02$) increase in intracellular proline pool and collagen synthesis. Ethanol itself did not have any effect on collagen synthesis of myofibroblasts. The stimulation of collagen synthesis of hepatic myofibroblasts by acetaldehyde and lactate may contribute to the development of alcoholic liver fibrosis, as alcohol intake is known to elevate acetaldehyde and lactate in tissues and blood.

In alcoholic liver injury, it has been found that fibrosis around the perivenular area of the hepatic tissue is among the first lesions in the sequence of events leading to alcoholic cirrhosis (1,2). In the presence of perivenular fibrosis, proliferation of mesenchymal cells surrounding the venules occurs (3,4). Around both fibrotic and nonfibrotic venules, myofibroblasts represent the most common type of mesenchymal cells (3,4). Myofibroblast proliferation is commonly associated with deposits of collagen fibers leading to perivenular fibrosis. It is not known what causes the proliferation of myofibroblasts and increases the deposition of collagen around the perivenular areas in alcoholic liver injury.

Myofibroblasts have been found in normal and fibrotic livers in animals and humans (3–8). Their characteristics, revealed by electron microscopy, include bundles of parallel microfilaments with a diameter of 4–6 nm along the axis of the cell; dense bodies among the microfilament bundles; indented, convoluted nuclei; desmosomes connecting adjacent cells; and basal laminalike substance partially surrounding the cells (9,10). Voss and coworkers (11)

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Abbreviations used in this paper: DME, Dulbecco's modified Eagle medium; FITC, fluorescein-isothiocyanate; PBS, phosphate-buffered saline.

observed cells similar to myofibroblasts in vitro in the outgrowth of explants of fibrotic liver. These cells were found to synthesize collagen types I, III, IV, and V.

There are a number of studies describing mechanisms whereby ethanol or associated metabolites, such as lactate, could affect hepatic collagen synthesis in alcoholic liver injury. Increased incorporation of radioactive proline into collagen hydroxyproline has been observed in liver slices of rats fed alcohol (12) and in liver biopsy specimens incubated in a medium containing ethanol (13). Lactate has been shown to stimulate collagen synthesis in mouse 3T3 fibroblasts (14). The hepatic free proline pool size has been incriminated in the regulation of collagen synthesis in some studies (15), whereas in other studies no such effect was found (16,17). Administration of ethanol causes an increase in free liver proline content in vivo (18). Lactate is known to inhibit proline oxidase activity (19), thereby probably also affecting proline pool size. Acetaldehyde is an oxidation product of ethanol that appears in the blood in association with alcohol consumption. Nothing is known so far of the effects of this metabolite on hepatic collagen synthesis in myofibroblasts.

The present study was undertaken to investigate the role of myofibroblasts in the development of alcoholic fibrosis. For this purpose, myofibroblasts were isolated from liver tissue and cultured. The effects of ethanol, acetaldehyde, and lactate on collagen synthesis of cultured myofibroblasts were studied.

Materials

Collagenase type I was purchased from Sigma Chemical Co., St. Louis, Mo., and Percoll density gradient material from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Tissue culture media and fetal bovine serum were obtained from the Grand Island Biological Co., Grand Island, N.Y., and fluorescein-isothiocyanate (FITC)-conjugated antibodies from Cappel Laboratories, West Chester, Pa. L-[u-^{14}C]Proline was purchased from New England Nuclear, Boston, Mass. Antiserum to human factor VIII-associated proteins was purchased from Calbiochem-Behring Co., La Jolla, Calif.

Methods

Cell Isolation and Culture

Cells were isolated from the livers of baboons fed either laboratory diet (histologically normal) or alcohol (exhibiting perivenular fibrosis) (3). About 2 g of liver was obtained during surgery and placed in a sterile tube containing Dulbecco's modified Eagle medium (DME). The biopsy specimen was immediately weighed and washed with medium. The liver capsule and big vessels were removed (if present) and the specimen was minced with scissors. The sample was digested with a sterile collagenase solution (80 mg of collagenase in 20 ml of DME) for 1 h at 37°C with shaking. After digestion, the sample was left standing for 5 min to allow for sedimentation of hepatocytes and tissue fragments. Ten milliliters of the supernatant was carefully layered on preformed Percoll density gradient. The gradient was made of 10% and 60% Percoll-DME isoosmotic solutions, with a neutral pH. These solutions were pumped into a sterile tube to form 10%–60% gradient with a final volume of 30 ml. The gradient with layered supernatant was centrifuged at 800 g for 30 min at 4°C (20). After centrifugation, the gradient was fractionated using a Beckman fraction recovery system (Beckman Instrument Co., Geneva, Switzerland). The desired fractions were washed twice with Nutrient Mixture F-12 (Grand Island Biological Co., Grand Island, N.Y.); primary cultures were established in a medium containing Nutrient Mixture F-12, 10% fetal bovine serum, 100 U of penicillin per milliliter of medium, and 100 μg of streptomycin per milliliter of medium. The cells were subcultured after trypsinization. The subcultures were grown in DME containing these same supplements and fresh ascorbate (50 μg/ml medium), if not otherwise stated.

Morphology

For electron microscopy, the cell monolayers were scraped or fixed in situ with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and were postfixed with 1% OsO_4 in 0.1 M cacodylate buffer (2% sucrose was added to both fixatives). The monolayers were dehydrated and embedded in Epon 812. Thin sections were obtained with a LKB IV ultramicrotome (LKB Instruments, Inc., Gaithersburg, Md.), stained with uranyl acetate and lead citrate (Ladd Research Industries, Burlington, Vt.), and examined with a Zeiss EM 10-C electron microscope (Carl Zeiss Inc., Thornwood, N.Y.). For light microscopy, Epon blocks were stained with toluidine blue (10 min) at 37°C.

Indirect Immunofluorescence

Affinity-purified rabbit antibodies to procollagens (type I and type III) and laminin and goat antibodies to collagen type IV were prepared as described previously (21–23). The cells were grown on coverslips without ascorbate in the medium in order to accumulate collagen inside the cells. The cells were washed with phosphate-buffered saline (PBS) and fixed with 3.7% paraformaldehyde for 20 min at 20°C. After fixation, the cells were washed once with 50 mM ammonium chloride solution in PBS and three times with PBS. To demonstrate intracellular collagen, the cell layers were treated with acetone for 10 min at −20°C and washed again with PBS. The cells were incubated with the antibodies at concentrations of 10–30 μg/ml for 30 min at 20°C in a humidified chamber, washed carefully with PBS, incubated again for 30 min with FITC antirabbit or antigoat IgG, and finally washed with PBS. After mounting, the coverslips were observed with a Zeiss fluorescence microscope (Carl Zeiss Inc.) and photographed with Kodak Ektachrome 400 film (Eastman Kodak Co., Rochester, N.Y.)

Biochemical Measurements

For measurements of alcohol dehydrogenase activity, the cells were scraped and the enzyme activity was analyzed as described by Bonnichsen and Brink (24) or by Petersen et al. (25).

Cell cultures were incubated in a medium containing 50 mM ethanol, 200 μM acetaldehyde, or 5 mM lactate. For ethanol and acetaldehyde incubations, airtight culture flasks were used, and the medium was changed every 24 h. Initial and final concentrations of ethanol and acetaldehyde were monitored during every medium change with a head space gas chromatograph model F42 (Perkin Elmer, Norwalk, Conn.). Lactate concentration of the medium was measured according to Gutmann and Wahlefeld (26); pyruvate concentration was measured according to Passonneau and Lowry (27).

Cellular collagen and noncollagen protein syntheses were studied with cells grown to a stationary phase. The experiments were mostly carried out with cells after 5–10 passages, but occasionally older cells were also used. Twenty-four hours before radioactive labeling, the medium containing ethanol, acetaldehyde, or lactate (at the indicated concentrations) was changed. For labeling, the cell cultures were washed twice with Hank's balanced salt solution and incubated for 4 h with [^{14}C]proline containing medium (5 μCi/ml) with ascorbate but without fetal bovine serum. The incorporation was stopped by cooling the flasks; the cells were immediately scraped and homogenized in the medium by sonication for 30 s, heated at 80°C for 15 min, and dialyzed for 3 days against 200 mM NaCl, 50 mM Tris-HCl, pH 7.4, at 4°C with four changes. After dialysis, *N*-ethylmaleimide was added at a final concentration of 2.5 mM. Aliquots of the dialysate were taken to measure [^{14}C]hydroxyproline, total radioactivity incorporated into proteins, and deoxyribonucleic acid (DNA) (28).

Total [^{14}C]hydroxyproline was quantitated by the procedure of Juva and Prockop (29). Incorporation of radioactivity into total proteins was assayed after precipitating the proteins with trichloroacetic acid and counting the pellet. Deoxyribonucleic acid in the dialysate was determined by the method of Burton (30). Collagen formation as percentage of protein production was calculated according to Breul et al. (28).

For the measurements of free intracellular proline pools, the cells were labeled as discussed previously. Free radioactivity was rinsed away by washing the cell layers 10 times with Hank's salt solution at 4°C. The cells were then harvested by scraping them into 20% sulfosalicylic acid solution. The mixture was suspended by hand with a Teflon pestle and let stand at 4°C for 30 min. The precipitate was removed by centrifugation at 12,800 g for 10 min. Aliquots of the supernatant were fractionated on a Beckman Instruments (Palo Alto, Calif.) amino acid analyzer to quantitate the intracellular free proline pool and its specific activity.

The number of cells was counted after trypsinization in a hemocytometer and viability tested using trypan blue. Phagocytic activity in vitro was tested by uptake of latex particles (1.1 μm in diameter). A suspension of latex particles was added on cell layers for 2.5 h at 37°C, after which the cultures were washed twice with medium and examined by phase contrast microscopy (31).

The statistical significances of the differences between two means were calculated by Student's *t*-test, and the correlation was evaluated by linear regression analysis.

Results

Characterization of Cultured Cells

The 20% Percoll gradient fraction was found to contain myofibroblasts. Four to five days after the initiation of the culture, cell growth was clearly observed. Most growing cells had a fibroblastlike appearance. Occasionally during the first days of culture some more rounded cells were also seen; they did not multiply and disappeared, usually within 1–2 wk. The cells which continued to grow consisted of homogenous populations (Figures 1*A* and 1*B*). By electron microscopy, they had the typical appearance of myofibroblasts (Figure 1*C*). Their cytoplasm contained numerous microfilaments with dense bodies among them. Microfilaments were oriented along the long axis of the cells and had an average diameter of 6 nm. The nuclei were convoluted. Basal laminalike material surrounded the cells (Figure 2), and cells were occasionally seen to attach to each other with intercellular adherens-type junctions (Figure 3). The same myofibroblast features were seen in secondary cultures of the cells (Figures 4 and 5). The cells did not show any phagocytic activity.

Collagen types I, III, and IV were found to be synthesized by the cells (Figures 6*A*–6*C*). The cells were also capable of producing laminin (Figure 6*D*). Production of the latter and a collagen type IV is consistent with the observation of a basal lamina around most of the cells. Factor VIII appeared absent, as judged by negative immunofluorescence.

Alcohol Dehydrogenase Activity

Alcohol dehydrogenase activity of the myofibroblasts as determined by two different methods is reported in Table 1. With an ethanol concentration of 50 mM and a nicotinamide adenine dinucleotide concentration of 1.5 mM, 200 μM of 4-methylpyrazole was capable of inhibiting 61% of the enzyme activity (Table 1). In some experiments the activity was inhibited completely, whereas in other experiments half of the activity persisted. The variability might be due to the fact that the activity of the enzyme was extremely low, at the limit of detection of the method.

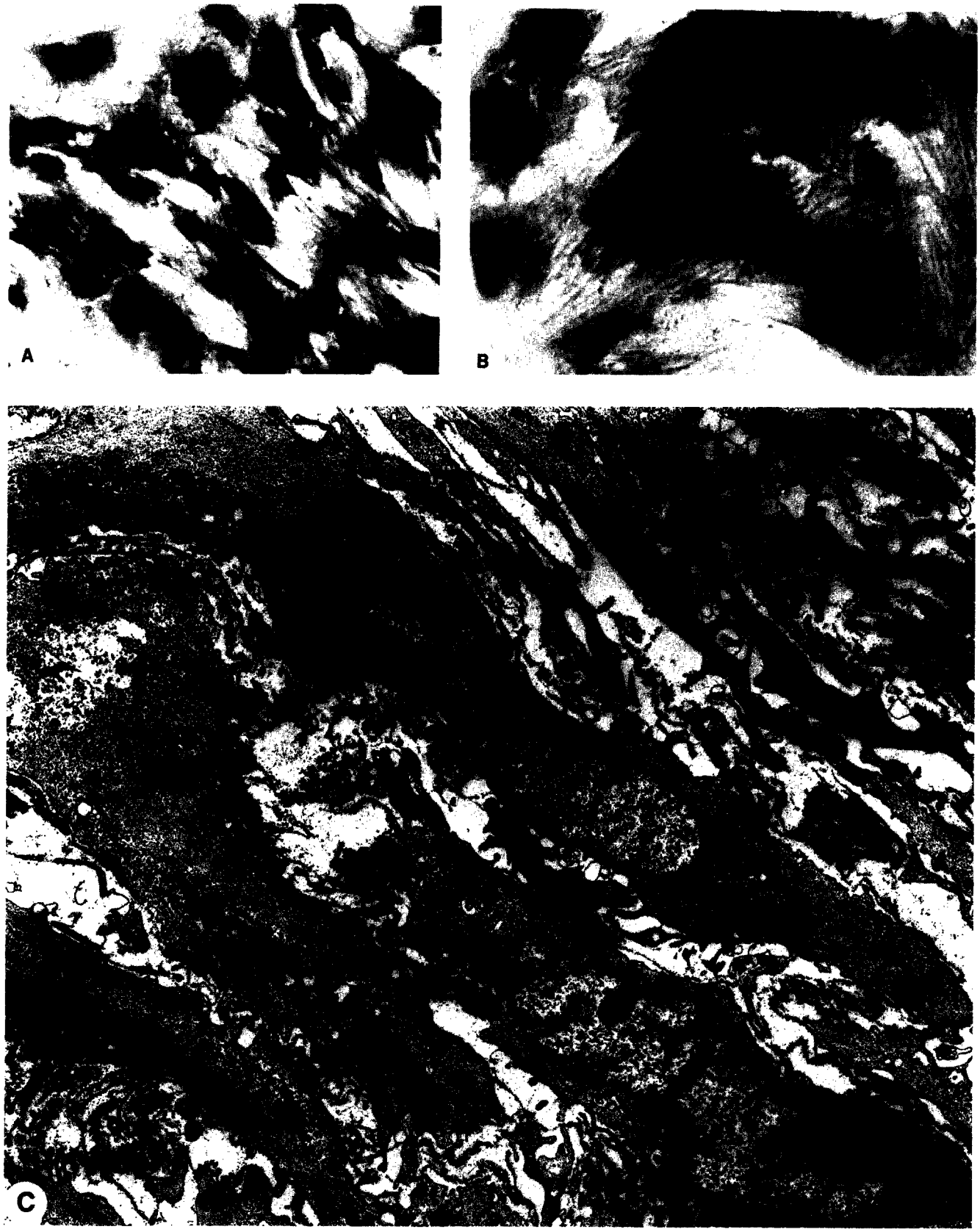

Figure 1. Myofibroblasts in primary culture. Myofibroblasts were obtained from the liver of a baboon fed alcohol for 9 yr and were cultured as described in Methods. Only one cell type is present, as evidenced by light microscopy (toluidine blue: *A*, ×180; *B*, ×410) and electron microscopy (C: uranyl acetate and lead citrate, ×7500). The cells have characteristic indented nuclei, abundant microfilaments (*MF*), and are surrounded by a basal laminalike structure.

Figure 2. Detail of Figure 1C showing higher magnification of basal lamina (*BL*), microfilaments (*MF*) with dense bodies (*DB*) and pinocytic vesicles (*arrow*). (Uranyl acetate and lead citrate, ×58,000.)

Maintenance of Ethanol, Acetaldehyde, and Lactate in Cell Culture Medium During the Incubation Period

Because acetaldehyde is highly volatile at 37°C, the culture flasks were tightly capped during the incubation to avoid disappearance by evaporation. Fifteen minutes after adding acetaldehyde (200 μM final concentration) to the flasks, the medium concentration had dropped to 170 μM, probably because of equilibration with the air space of the flask. After 24 h, this concentration dropped to 82 ± 33 μM (mean ± SD) when incubated with the cells but remained at the level of 154 ± 16 μM when incubated without the cells. There was no variation in the concentration of 50 mM ethanol in the medium during the 24-h incubation. Medium with 50 mM ethanol contained no more than 2 μM acetaldehyde and medium with 200 μM acetaldehyde contained no more than 70 μM ethanol.

The initial cell culture medium (DME with 1000 mg/L glucose) containing 10% fetal bovine serum had a 2 mM lactate content. After incubation with confluent myofibroblasts for 24 h, 8.7 ± 1.7 mM (mean ± SD) lactate was found in the medium. Similarly, when 5 mM lactate was added to the

Figure 3. Myofibroblasts in primary culture. Adherens-type junction (*arrow*) between adjacent cell projections. (Uranyl acetate and lead citrate, ×24,000.)

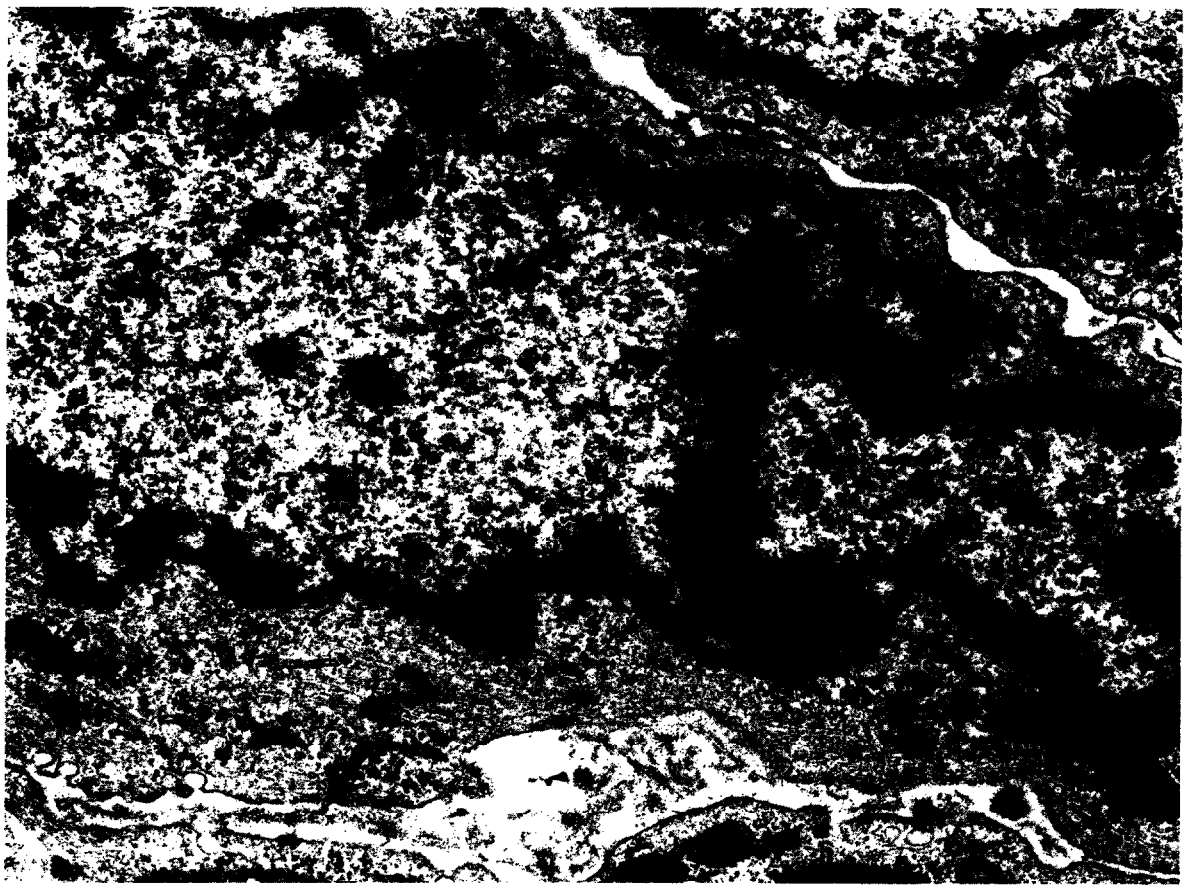

Figure 4. Secondary culture of myofibroblasts showing persistence of basal lamina (*arrow*) microfilaments and dense bodies. (Uranyl acetate and lead citrate, ×30,000.)

culture medium (initial concentration then being 7 mM), 14.1 ± 1.1 mM (mean ± SD) lactate was found at the end of the 24-h incubation.

The pH of the medium remained constant for all incubations. There was no change in the ratio of lactate to pyruvate in the medium between control and 5 mM lactate medium after 24-h incubation during the experiment described in Table 2.

A 24-h exposure to the above-mentioned ethanol or acetaldehyde concentration did not cause any change in the viability of the cells, as determined by the trypan blue exclusion test.

Effects of Ethanol, Acetaldehyde, and Lactate on Collagen Synthesis of Myofibroblasts

The effects of 50 mM ethanol, 200 μM acetaldehyde, and 5 mM lactate on collagen and noncolla-

Figure 5. Detail of Figure 4 showing higher magnification of microfilaments (*MF*) and dense bodies (*DB*). (Uranyl acetate and lead citrate, ×58,000.)

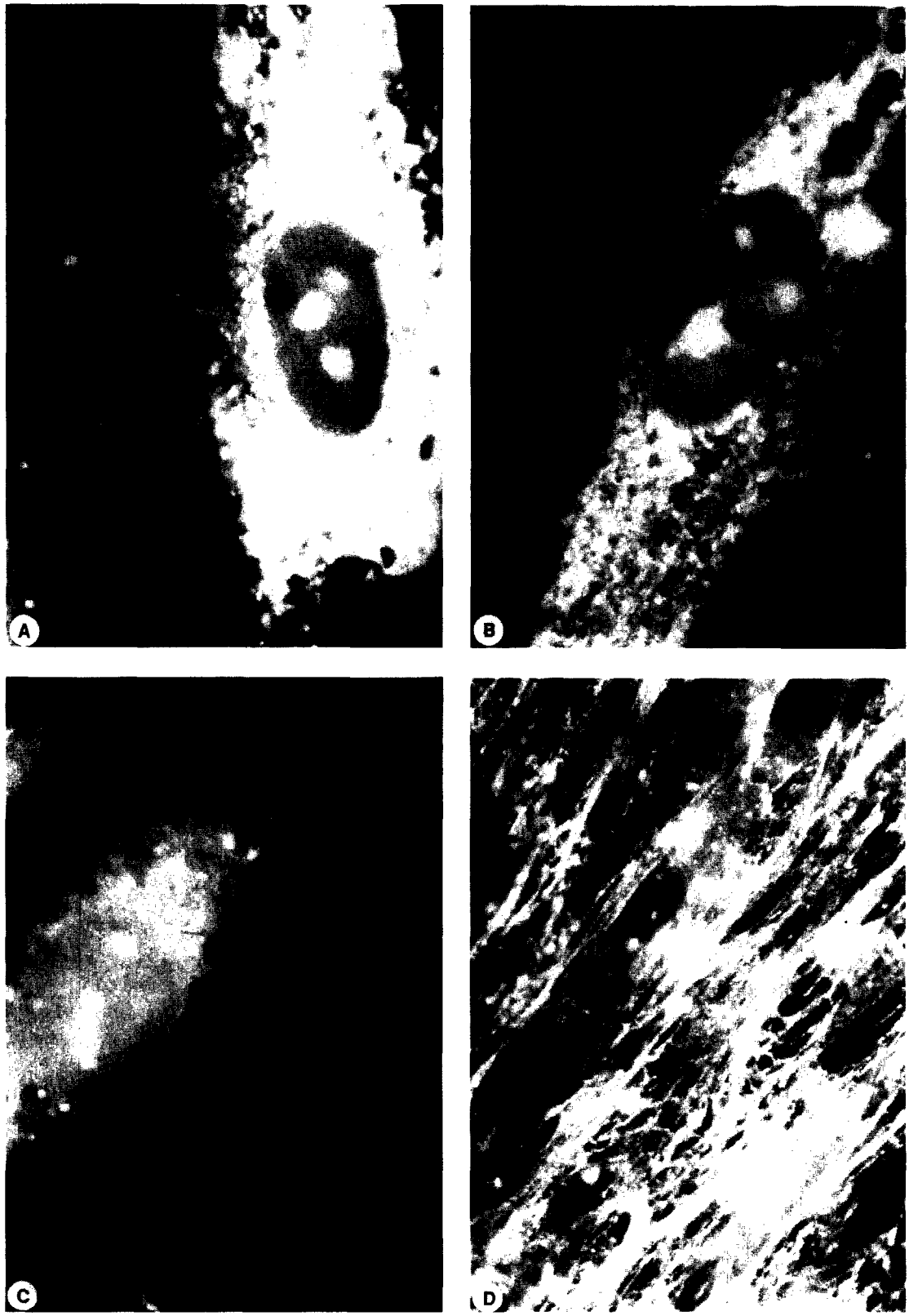

Figure 6. Detection by immunofluorescence of collagen and laminin syntheses by cultured myofibroblast. *A*. Procollagen type I, ×1825. *B*. Procollagen type III, ×1825. *C*. Type IV collagen, ×1825. *D*. Laminin, ×457. Most of the collagen is found intracellularly (due to inhibition of secretion by lack of ascorbate), whereas laminin is mainly found in the extracellular space.

Table 1. Alcohol Dehydrogenase Activity of Myofibroblasts[a]

	Nanomoles of NADH formed per minute	
	per milligram of protein	per microgram of DNA
Forward reaction, pH 9.6	0.480 ± 0.120 (4)	0.070 ± 0.017 (4)
Forward reaction, pH 9.6 with 4-methylpyrazole	0.185 ± 0.135 (4)	0.027 ± 0.007 (4)
Forward reaction, pH 7.3	0.095 ± 0.030 (4)	0.014 ± 0.001 (4)

DNA, deoxyribonucleic acid; NADH, nicotinamide adenine dinucleotide, reduced form. [a] 16×10^6 cells (derived from the livers of alcohol-fed baboons) were harvested by scraping at the stationary phase of growth. They were then homogenized and enzyme activities were determined (see Methods). Values are expressed as mean ± SD; number of experiments is given in parentheses.

gen protein synthesis are presented in Table 2. Myofibroblasts produced 4%–5% collagen of the total protein synthesis. Acetaldehyde caused a significant increase in the incorporation of [^{14}C]proline into [^{14}C]hydroxyproline ($p < 0.05$) and also in the percentage of collagen produced ($p < 0.05$). Supplementation of medium by 5 mM lactate caused even more pronounced increase in hydroxyproline labeling ($p < 0.001$) and collagen production ($p < 0.02$). Ethanol did not have any significant effect on collagen production of myofibroblasts. None of the media supplements caused any significant change in the noncollagen protein synthesis.

Ethanol and acetaldehyde did not have any effect on the intracellular proline pools or specific activities. Lactate, however, increased significantly ($p < 0.02$) the intracellular free proline pool (Table 3).

There was a significant positive correlation ($n = 16$, $r = 0.60782$, $p < 0.05$) between the incorporation of radioactive proline into hydroxyproline and the lactate concentration of the medium (Figure 7).

Discussion

It has been previously reported that myofibroblasts represent the most common type of mesenchymal cell around the perivenular areas both in normal and alcohol-injured liver (3,4). In addition, the outgrowth of smooth muscle cell-like cells (together with vascular endothelial cells) from fibrotic livers has been described (11). The present study demonstrates for the first time that homogenous populations of myofibroblasts can be cultured from baboon liver and that acetaldehyde and lactate stimulate collagen synthesis in these cells.

Under electron microscopy, the cultured cells looked like the myofibroblasts seen in tissue sections. The presence of microfilaments in these cells has been associated with a capacity for motility and contractility (9). It is known that, in cultures, normal fibroblasts may also take on characteristics similar to those of myofibroblasts. Microfilaments have been observed in cultured chick embryo fibroblasts (32); in human skin fibroblasts, their amount was dependent on the growth phase of the cells (33). In our study, the cells isolated from the liver had features typical of myofibroblasts both in primary and in secondary cultures, and their occurrence was not dependent on the growth phase of the cells. In in vivo studies in the liver of the baboons, both fibroblasts and myofibroblasts can be seen. It is not possible to state whether the isolated cells were initially myofibroblasts or a mixture of myofibroblasts and fibroblasts, which in culture can acquire myofibroblastlike features. The capacity of these cells, however, to synthesize type IV collagen and laminin indicates that they differed from normal skin fibroblasts, which do not synthesize these basement membrane components (34).

The myofibroblasts had a low alcohol dehydroge-

Table 2. Effect of Ethanol, Acetaldehyde, and Lactate on Collagen Synthesis of Myofibroblasts[a]

Medium	Concentration	Hydroxyproline (dpm × 10^{-3}/μg DNA)	Noncollagen protein (dpm × 10^{-3}/μg DNA)	Collagen (% of total protein)
Ethanol (n = 8)	50 mM	3.58 ± 0.85	48.5 ± 15.0	4.2 ± 1.0
Controls (n = 9)		3.57 ± 1.18	49.3 ± 18.1	4.1 ± 1.6
Acetaldehyde (n = 6)	170–82 μM	7.79 ± 2.71[b]	57.1 ± 11.8	6.5 ± 2.4[b]
Controls (n = 6)		4.93 ± 1.23	61.0 ± 13.3	4.2 ± 0.7
Lactate (n = 8)	5 mM	7.68 ± 1.79[c]	57.2 ± 10.3	7.9 ± 2.5[d]
Controls (n = 8)		4.77 ± 0.72	51.2 ± 12.1	5.2 ± 1.0

DNA, deoxyribonucleic acid; dpm, disintegrations per minute. [a] Cells (derived from the livers of alcohol-fed baboons) were preincubated with the metabolites for 24 h, followed by a 4-h incubation with [^{14}C]proline and measurement of the incorporation of the label in hydroxyproline and noncollagen protein. Values are expressed as mean ± SD. [b] $p < 0.05$. [c] $p < 0.001$. [d] $p < 0.02$.

Table 3. Effect of Lactate on the Intracellular Free Proline Pool[a]

Medium	Proline/DNA (nmol/μg)	Specific activity (dpm × 10^{-3}/nmol)
Control	0.26 ± 0.05	4.1 ± 0.2
5 mM lactate	0.35 ± 0.03[b]	3.6 ± 0.2[c]

DNA, deoxyribonucleic acid. [a] Cells were incubated as described in Table 2, then carefully washed and harvested by scraping. The intracellular free proline pool and its specific activity were then quantitated by amino acid analysis (see Methods). Values are expressed as mean ± SD of four sets of cell culture each. [b] $p < 0.02$. [c] $p < 0.01$.

nase activity. Petersen et al. (25) also demonstrated low alcohol dehydrogenase activity in cultured skin fibroblasts. Alcohol dehydrogenase activity found in myofibroblasts was of the same order of magnitude as that of skin fibroblasts (25) and was 1/100 of that found in baboon liver tissue (35). By using immunohistochemistry, alcohol dehydrogenase has also been demonstrated in human skin and lung fibroblasts (36).

The ethanol concentration in the culture medium could be easily maintained at a constant level as ethanol was not significantly oxidized by the myofibroblasts. The ethanol concentration in the medium corresponded to the blood level of baboons fed ethanol chronically (37), whereas the acetaldehyde concentration used was higher than the blood levels. However, it is known that acetaldehyde concentrations in the liver tissue are higher than those observed in the blood (38), and, therefore, the concentrations used in the cell culture medium may well be close to the intracellular hepatic acetaldehyde concentrations.

The lactate concentration in the cell culture medium increased during the 24-h incubation of the cells. In this experiment, however, the ratio of lactate to pyruvate in the medium did not change within the incubation period, indicating that the cytosolic redox state of the cells was similar in both control cells and those incubated with added lactate. Therefore, the changes in collagen synthesis caused by lactate cannot be attributed to an altered redox state of the cells.

No change was observed in collagen synthesis of myofibroblasts exposed to 50 mM ethanol. Variable results have been obtained before on the effects of ethanol on collagen synthesis in vitro. A stimulation of collagen synthesis with 172 mM ethanol and an inhibition with 345–690 mM ethanol were observed with chick embryo tibia preparations (39). These ethanol concentrations, however, exceed the levels associated with alcohol consumption. In another study, collagen synthesis was depressed after a short treatment with 40 mM ethanol in cultured fetal lung fibroblasts, but the effect was not specific for collagen as noncollagen protein synthesis was decreased as well (40). General inhibition of protein synthesis or decreased secretion due to alcohol or its metabolites have been reported in many studies (41). We did not observe any overall decrease in the noncollagen protein synthesis, perhaps because of the relatively short exposure time of the cells to ethanol or associated metabolites.

Incubation of myofibroblasts with 200 μM acetaldehyde resulted in a significant stimulation of collagen synthesis of the cells. This stimulation was specific for collagen as no change was found in noncollagen protein synthesis. The mechanism of this effect has not been elucidated.

A selective stimulation of myofibroblast collagen synthesis was also observed when the cells were incubated with excess lactate. Similar results have been reported by Green and Goldberg (14) with the mouse 3T3 fibroblast cell line. They observed a gradual increase in collagen synthesis with increasing lactate concentrations up to 40 mM. This change was due to lactate itself and not to pH changes of the medium. Whether the small associated changes in pyruvate concentration play a role is not known. The lactate concentration we used in our experiment has been observed in the blood after ethanol administration (37,42,43); alcoholic patients may even show an increase in lactate without detectable alcohol in the blood (44). It has been suggested that the effect of lactate on collagen synthesis is mediated by activation of prolyl hydroxylase (45), which is the first posttranslational enzyme of collagen biosynthesis

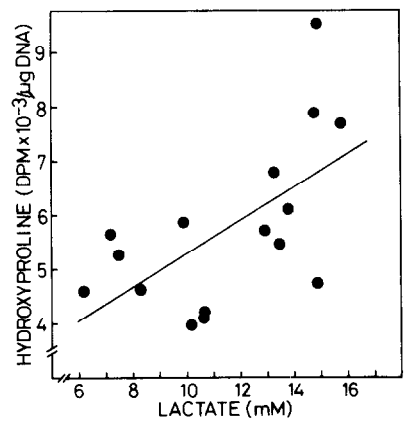

Figure 7. Correlation between lactate concentration in the medium and collagen synthesis as measured by hydroxyproline labeling. Liver myofibroblasts were incubated with [^{14}C]proline with or without lactate as reported in Table 2. At the end of the incubation, lactate concentration and incorporation of the label into hydroxyproline was determined (see Methods) ($n = 16$, $r = 0.60782$, $p < 0.05$).

catalyzing the hydroxylation of prolyl residues in collagen pro-α-chains. How these various in vitro effects of lactate relate to in vivo fibrogenesis is not known. We found that incubation of cells with lactate increased the intracellular free proline pool. Proline availability has been suggested to regulate collagen synthesis in experimental models of fibrosis in vitro (46,47), although its role in vivo has been questioned (16,17).

Our results show that nonparenchymal cells with myofibroblastlike appearance in culture synthesize collagen. Furthermore, collagen synthesis by these cells was stimulated by adding acetaldehyde and lactate to the culture medium. It has been demonstrated that the number of these myofibroblasts increases around the terminal hepatic veins in early alcoholic liver injury (3,4). If the stimulation of collagen synthesis demonstrated in vitro in the present study can be extrapolated to the in vivo situation, these effects could contribute to the development of liver fibrosis associated with alcohol consumption.

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