

Regulation of Collagen Production in Freshly Isolated Cell Populations from Normal and Cirrhotic Rat Liver: Effect of Lactate

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Previous work has shown that lactic acid, and to a lesser extent pyruvic acid, is able to increase collagen synthesis significantly in liver slices of CCl₄-treated rats but not normal rats. The purpose of this report is to document which cells in the cirrhotic liver are responsible for the lactate-stimulated increase in collagen synthesis. It was found that (a) incorporation of ³H-proline into protein-bound ³H-hydroxyproline is increased threefold to fourfold in hepatocytes from CCl₄-treated rats as compared with normal rat hepatocytes; (b) neither the hepatocytes from normal nor those from CCl₄-treated rats modify their collagen synthesizing capacity when 30 mmol/L lactic acid was added to the incubation medium; (c) nonparenchymal cells obtained from livers of CCl₄-treated rats synthesize much less collagen than hepatocytes, but their synthesis is stimulated twofold by lactic acid; (d) from the different nonparenchymal cells, only fat-storing (Ito) cells increase collagen synthesis when lactic acid is present in the incubation medium. These results suggest that the increased lactic acid levels observed in patients with alcoholic hepatic cirrhosis may play an important role in the development of fibrosis by stimulating collagen production by fat-storing (Ito) cells. (HEPATOLOGY 1991;13:551-556.)

The normal response of the liver to an acute injury is to regenerate (1). If regulation of the regenerative process is altered, as occurs in chronic injury, fibrous scar tissue accumulates. The factors that participate in the transformation of the hepatic response from regeneration to scar formation remain unclear (2). Various cells within the normal liver have been shown to produce collagen. Fat-storing (Ito) cells (FSC) produce collagen type I and type III (3). Additional collagen-synthesizing cells, such as myofibroblasts, could be recruited from the vessels during injury (4) or could be derived from FSC

(5, 6). Hepatocytes have the capacity to produce collagen *in vitro* (7) and have been shown to produce collagen *in vivo* (8-10). However, recent studies have suggested that FSC (Ito) cells, which contaminate hepatocyte cultures, are the primary producers of collagen in those cultures (11). Fibrosis may result from excessive deposition of collagen by cells from nonmesodermal origin and from connective tissue cells (2). The relative role of each cell class in collagen production in cirrhosis still remains to be determined.

Previous work from our laboratory (12) has shown that lactic and pyruvic acids are able to increase collagen synthesis by liver slices from CCl₄-treated rats but not normal rats. However, with tissue slices it is not possible to determine which liver cell populations are involved in the process. Lactic acid increases collagen synthesis in cultured fibroblasts (13) and myofibroblasts (14), although the mechanism by which this occurs remains to be determined. This work was undertaken to establish which cell types in fibrotic livers increase their collagen production in response to lactic acid. Cells isolated from normal rat liver were used as controls.

MATERIALS AND METHODS

Universal liquid scintillation counter (Aquasol) and ³H-(2,3) L-proline (27.2 Ci/mmol) were purchased from New England Nuclear (Boston, MA). The following reagents were purchased from Sigma Chemical Co. (St. Louis, MO): chloramine T, L-proline, D-(+) glucose, mineral oil (Nujol), pyruvic and L-(+) lactic acids, albumin (fraction V), protease (type XIV), collagenase (type IV) and metrizamide (grade I). Dowex-norite and trichloroacetic acid (TCA) were from Merck (Darmstadt, Germany); carbon tetrachloride (CCl₄) was obtained from J.T. Baker (Mexico). Cation exchange resin (AG50W-X8), 200 to 400 mesh, was from BioRad Laboratories (Richmond, CA).

Animals. Adult male Wistar rats weighing (80 to 100 gm) were used. Groups of five animals each were injected intraperitoneally (0.15 ml) with carbon tetrachloride diluted in mineral oil three times a week during 3 wk, as described by Ehrnpreis, Giambrone and Rojkind (15).

Isolation of Parenchymal and Nonparenchymal Cells. Normal or CCl₄-treated rats were fasted overnight. The animals were injected intraperitoneally with 500 IU heparin and anesthetized with ether. For isolation of hepatocytes the procedure described by Berry and Friend (16) with some

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modifications was used. The liver was perfused *in situ* through the portal vein, first with 100 ml of Ca^{++} -free Ringer-Krebs solution at 37° C, pH 7.4 (16 ml/min), and then with collagenase (0.05%) in 100 ml of a solution that contained 5 mmol/L Ca^{++} and 10 mg/ml of albumin at 37° C, pH 7.4 (8 ml/min). The infused solutions were not recirculated. The liver was then removed and placed in Ringer-Krebs solution with Ca^{++} at 37° C. The cells were released by gently combing the perfused liver and filtered through a nylon mesh to remove aggregates and undigested tissue. The hepatocytes were collected by centrifugation at 50 *g* for 2 min. The cells were washed twice with Ringer-Krebs solution with Ca^{++} and collected by centrifugation at 50 *g* for 2 min. The final cell pellet was also suspended in Ringer-Krebs solution with Ca^{++} . The nonparenchymal cells present in the supernatants were discarded. Hepatocytes were counted with a hemocytometer, and viability was determined by trypan blue exclusion (0.3%). If the viability of the cells did not exceed 85%, the cell pellet was suspended in 30% metrizamide and overlaid with 5 ml of a saline buffer (17). After centrifugation for 3 min at 27 *g*, intact hepatocytes (85% viability) were harvested at the phase boundary and then washed once by centrifugation (15 *g* for 1 min). To isolate nonparenchymal cells, the liver was also perfused through the portal vein with 100 ml of Ca^{++} -free Ringer-Krebs solution, pH 7.4 (15 ml/min). Then 50 ml of Ringer-Krebs with Ca^{++} (albumin free) containing protease (1 mg/ml) was infused. The liver was removed, cut into small pieces and the cell suspension filtered through a nylon mesh. The cell suspension was centrifuged at 50 *g* for 2 min, and the cell pellet, which still contained hepatocytes, was discarded. The supernatant that contained nonparenchymal cells was then centrifuged at 400 *g* for 5 min, and the cell pellet was washed twice by centrifugation. The final cell suspension was mixed with metrizamide, and the cells were separated by density gradient centrifugation at 4,300 *g* for 30 min at 4° C (18). The metrizamide gradients for the separation of liver cells were prepared by the method described by Stone (19). The densities of the aqueous solutions of metrizamide were determined in a Baush & Lomb refractometer. The gradient was fractionated manually and fraction 3 ($\delta = 1.04$) and fraction 6 ($\delta = 1.09$) were carefully removed. Both fractions were diluted with Ringer-Krebs with Ca^{++} and collected by centrifugation at 400 *g* for 5 min. Cells recovered from fraction 3 appeared under phase microscopy as small rounded cells with refractile droplets. When placed in culture, these cells showed fibroblastic structure and gave vitamin A fluorescence, suggesting that this fraction contained FSC. Fraction 6 also contained rounded cells with a larger diameter (15 μm). When the cells were placed in culture, some of them showed a cobblestone-like structure; others were able to phagocytose latex particles, suggesting the presence of endothelial and Kupffer cells in this fraction. Viability of isolated nonparenchymal cells was determined by trypan blue exclusion before incubations.

Incubation Medium. The incubation medium contained Ringer-Krebs bicarbonate buffer with 2.5 mmol/L calcium supplemented with 0.2% albumin (20). After O_2/CO_2 (95%:5%) was bubbled into the medium, the pH was adjusted to 7.4.

Total Protein Synthesis. Protein synthesis was determined as the incorporation of ^3H -proline into TCA-precipitable proteins. Only fresh hepatocytes and nonparenchymal cells, obtained as described in "Materials and Methods," with a viability greater than 85% were used. Ten million cells were placed in 25 ml siliconized flasks with 3 ml of medium and preincubated at 37° C in a constant shaking bath with an O_2/CO_2 gas mixture (95%:5%). After this, 60 μCi of ^3H -proline

were added and the cells were incubated for 3 hr under the same conditions. As established for liver slices (12), maximum incorporation of ^3H -proline into collagen ^3H -hydroxyproline was obtained at this time. In some experiments either 10 mmol/L glucose, 30 mmol/L lactate or 30 mmol/L pyruvate (final concentration) was added to the incubation medium. In preliminary experiments, we found that maximal incorporation of ^3H -proline into collagen ^3H -hydroxyproline in nonparenchymal liver cells was obtained with 30 mmol/L lactic acid (data not shown). In control samples the equivalent volume of distilled water was added. The reaction was terminated by adding a 10-fold excess of cold proline and chilling the samples. Cells and medium were precipitated with 10% TCA and collected by centrifugation at 1,000 *g* for 20 min. The precipitated proteins were then hydrolyzed with 2 ml of 6 N HCl for 20 hr at 104° C. The hydrolyzed samples were vacuum-dried and suspended in 1 or 2 ml of 2 N HCl. For determining total protein synthesis, aliquots (1/10) from each suspension were drawn and radioactivity was counted with 10 ml of Aquasol. Results were expressed as disintegrations per minute of ^3H -proline/ 10^7 viable cells. The procedure described above was followed when working with hepatocytes and total nonparenchymal populations and as with isolated nonparenchymal cells.

Collagen Synthesis. Collagen synthesis was determined as ^3H -proline incorporated into peptide-bound ^3H -hydroxyproline (21). The hydrolyzed samples were resuspended in 2 N HCl, mixed with Dowex-norite to remove humin and centrifuged for 15 min at 1,500 *g* (22). The clear supernatant was used for proline and hydroxyproline measurements. ^3H -proline and ^3H -hydroxyproline were resolved using a cation exchange column (12). The pooled fractions containing ^3H -hydroxyproline were neutralized to pH 5-7, and proper aliquots were used for quantification of radioactivity and concentration after oxidation with chloramine T (21). Results were expressed as disintegrations per minute of ^3H -hydroxyproline/ 10^7 viable cells. Collagen synthesis also was expressed as the relative rate of total protein production. This value was calculated according to the formula of Diegelmann, which considers the different content of iminoacids in collagen with respect to other proteins (23).

RESULTS

Collagen and Total Protein Production by Hepatocytes. Basal incorporation of ^3H -proline into protein-bound ^3H -hydroxyproline was always threefold to fourfold higher in hepatocytes obtained from CCl_4 -treated rats than in normal hepatocytes (Tables 1 and 2). Experiments performed with freshly isolated hepatocytes from normal rat liver showed that the addition of glucose, lactate or pyruvate to the incubation medium did not significantly change the production of protein-bound ^3H -hydroxyproline. Only a slight increase (27% and 12%, respectively) was observed when glucose and lactate were added (Table 1). Glucose and lactate were able to increase the incorporation of ^3H -proline into TCA-precipitable proteins by 11% and 36%, respectively (Table 1). The relative rate of collagen obtained with these metabolites was similar to the control values (Table 1). Accordingly, glucose and lactate appeared to stimulate in a nonspecific manner total protein synthesis, including collagen production.

All the metabolites studied stimulated protein syn-

TABLE 1. Incorporation of ^3H -proline into TCA-precipitable protein and into protein-bound ^3H -hydroxyproline by hepatocytes from normal rats

	dpm/ 1×10^7 cells			
	Control	With lactate	With glucose	With pyruvate
^3H -proline in protein (dpm $\times 10^3$)	5,550 \pm 130	7,560 \pm 730 (36%) ^a	6,180 \pm 130 (11%)	5,690 \pm 410 (2%)
Protein-bound ^3H -hydroxyproline	15,540 \pm 1,851	17,388 \pm 2,365 (12%)	19,776 \pm 2,227 (27%)	16,501 \pm 5,185 (6%)
Collagen synthesis ^b (% of total protein)	0.116	0.094	0.131	0.118

Values within parentheses represent the percentage above control value.

^aSignificant ($p < 0.01$).

^bValues calculated according to the formula of Diegelmann and Peterkofsky (23).

TABLE 2. Incorporation of ^3H -proline into TCA-precipitable protein and into protein-bound ^3H -hydroxyproline by hepatocytes from CCl_4 -treated rats

	dpm/ 1×10^7 cells			
	Control	With lactate	With glucose	With pyruvate
^3H -proline in protein (dpm $\times 10^3$)	3,770 \pm 290	4,930 \pm 200 (31%) ^a	6,430 \pm 980 (70%) ^a	4,530 \pm 230 (20%) ^a
Protein-bound ^3H -hydroxyproline	46,748 \pm 2,518	58,178 \pm 2,370 (24%) ^a	46,939 \pm 2,592 (1%)	39,411 \pm 2,222 (-15%)
Collagen synthesis ^b (% of total protein)	0.512	0.487	0.300	0.358

Values within parentheses represent the percentage above control value.

^aSignificant ($p < 0.01$).

^bValues calculated according to the formula of Diegelmann and Peterkofsky (23).

thesis by hepatocytes obtained from CCl_4 -treated rats (Table 2). The increase in protein synthesis induced by glucose, lactate and pyruvate was higher than that observed in normal hepatocytes. This was more apparent when glucose was added to the incubation medium (70%). The production of protein-bound ^3H -hydroxyproline when glucose or pyruvate was added to the incubation medium did not change significantly (Table 2). A slight increase of 24% in the formation of protein-bound ^3H -hydroxyproline was observed when lactate was added to the medium. Since only total protein synthesis was increased in the presence of the three metabolites tested, the results suggest a non-specific induction of protein synthesis.

Collagen and Total Protein Production by Nonparenchymal Cells. Basal protein synthesis by nonparenchymal cells from CCl_4 -treated rats was reduced by 30% when compared with total protein synthesis by nonparenchymal cells isolated from normal rat liver (Tables 3 and 4). Contrary to this finding, increased incorporation of ^3H -proline into proteins has been observed in nonparenchymal cells from CCl_4 -treated rats (24). Nevertheless, a different CCl_4 intoxication schedule was used in this study. The addition of glucose, lactate and pyruvate to the incubation medium of nonparenchymal cells from normal rat liver did not significantly modify the production of protein-bound ^3H -hydroxyproline (Table 3). Similar to the results obtained with isolated hepatocytes, glucose and pyruvate increased total protein synthesis (Table 3). The increase in protein synthesis was similar or higher than the increase observed in the formation of protein-bound ^3H -hydroxyproline. In contrast to these results, the formation of protein-bound ^3H -hydroxyproline by non-

parenchymal cells from CCl_4 -treated animals increased significantly in the presence of the three metabolites studied (Table 4). The maximal increase in the formation of protein-bound ^3H -hydroxyproline was observed when lactic acid was added to the incubation medium (110%). When pyruvic acid was added, the increase obtained was only 27%. Glucose, lactate and pyruvate were able to increase protein synthesis by nonparenchymal cells from CCl_4 -treated animals (Table 4). The increase in protein production induced by glucose or pyruvate was similar to the increase observed in protein-bound ^3H -hydroxyproline. Only lactic acid specifically increased the formation of protein-bound ^3H -hydroxyproline ($p < 0.01$). In all the experiments performed, the increase in the formation of protein-bound ^3H -hydroxyproline was twice that observed for total protein synthesis.

Collagen and Total Protein Production by Specific Populations of Nonparenchymal Cells. For these experiments, FSC (Ito cells) were separated from endothelial and Kupffer cells using a metrizamide gradient (18). As illustrated in Table 5, the relative rate of collagen did not increase by lactic acid in endothelial and Kupffer cells from normal or CCl_4 -treated rats. Basal protein synthesis and formation of protein-bound ^3H -hydroxyproline by the endothelial-Kupffer cell fraction from CCl_4 -treated animals were increased threefold compared with cells obtained from normal rat liver. Since Kupffer cells do not appear to produce collagen, these results indicate that endothelial cells may also play an active role in collagen deposition in cirrhosis. When endothelial and Kupffer cells obtained from normal rat liver were incubated with lactate, the increase in protein-bound ^3H -hydroxyproline (78%) was of the same

TABLE 3. Incorporation of ^3H -proline into TCA-precipitable protein and into protein-bound, ^3H -hydroxyproline by nonparenchymal cells from normal rats

	dpm/1 $\times 10^7$ cells			
	Control	With lactate	With glucose	With pyruvate
^3H -proline in protein (dpm $\times 10^3$)	1,040 \times 80	1,040 \pm 230 (1%)	1,360 \pm 30 (20%) ^a	1,390 \pm 130 (33%) ^a
Protein-bound ^3H -hydroxyproline	1,560 \times 333	1,248 \pm 203 (-20%)	1,904 \pm 207 (22%)	1,668 \pm 204 (7%)
Collagen synthesis ^b (% of total protein)	0.061	0.049	0.057	0.049

Values within brackets represent the percentage above control value.

^aSignificant ($p < 0.01$).

^bValues calculated according to the formula of Diegelmann and Peterkofsky (23).

TABLE 4. Incorporation of ^3H -proline into TCA-precipitable protein and into protein-bound, ^3H -hydroxyproline by nonparenchymal cells from CCl_4 -treated rats

	dpm/1 $\times 10^7$ cells			
	Control	With lactate	With glucose	With pyruvate
^3H -proline in protein (dpm $\times 10^3$)	660 \pm 10	1,090 \pm 20 (65%) ^a	1,110 \pm 70 (68%) ^a	800 \pm 10 (21%) ^a
Protein-bound ^3H -hydroxyproline	1,452 \pm 192	3,052 \pm 962 (100%) ^a	2,553 \pm 577 (75%) ^a	1,840 \pm 196 (27%) ^a
Collagen synthesis ^b (% of total protein)	0.090	0.114	0.094	0.094

Values within parentheses represent the percentage above control value.

^aSignificant ($p < 0.01$).

^bValues calculated according to the formula of Diegelmann and Peterkofsky (23).

TABLE 5. Incorporation of ^3H -proline into TCA-precipitable protein and into protein-bound, ^3H -hydroxyproline by endothelial/Kupffer cell fraction

	dpm/1 $\times 10^6$ cells			
	Normal		CCl_4	
	Without lactate	With lactate	Without lactate	With lactate
^3H -proline in protein (dpm $\times 10^3$)	825 \pm 101	1,387 \pm 113 (68%) ^a	2,655 \pm 331	4,268 \pm 528 (61%) ^a
Protein-bound ^3H -hydroxyproline	18,312 \pm 2,063	32,675 \pm 3,953 (78%) ^a	55,062 \pm 5,559	58,906 \pm 8,657 (7%)
Collagen synthesis ^b (% of total protein)	0.925	0.982	0.863	0.571

Values within parentheses represent the percentage above the value without lactate.

^aSignificant ($p < 0.01$).

^bValues calculated according to the formula of Diegelmann and Peterkofsky (23).

order of magnitude as that observed in total protein synthesis (68%). Kupffer and endothelial cells obtained from CCl_4 -treated animals increased protein synthesis by 61% when lactic acid was present in the incubation medium. However, no significant change in protein-bound ^3H -hydroxyproline formation was observed. Similar to the findings with the endothelial-Kupffer cell fraction, FSC obtained from cirrhotic animals produced more protein-bound ^3H -hydroxyproline than FSC obtained from normal livers. Moreover, lactic acid increased the formation of protein-bound ^3H -hydroxyproline more than threefold. In contrast to these results, lactic acid only increased total protein synthesis by 61% (Table 6). Therefore lactic acid increased twice the relative rate of collagen synthesis in Ito cells from cirrhotic animals (Table 6). With FSC from normal liver, lactic acid stimulated by 93% and 90% the incorporation of ^3H -proline into total protein-bound ^3H -hydroxyproline and into total proteins, respectively.

DISCUSSION

In previous work from our laboratory (12) we have reported increments in collagen synthesis when glucose and lactate were added to liver slices obtained from cirrhotic but not from normal liver. Since in liver slices we cannot assign the effect of these metabolites on collagen synthesis to specific cell populations, we initially separated parenchymal and nonparenchymal cells from normal and cirrhotic rat liver. Hepatocytes from normal or CCl_4 -treated rats did not specifically increase their collagen production in the presence of any of the metabolites studied. Nevertheless, basal collagen production in hepatocytes from cirrhotic rats was three to four times higher than in hepatocytes from normal rats. This difference in collagen production could be related to a change in the phenotypic expression of the injured hepatocytes by CCl_4 . On the other hand, when glucose was present in the incubation medium, total protein synthesis was stimulated significantly in hepatocytes

TABLE 6. Incorporation of ^3H -proline into TCA-precipitable protein and into protein-bound, ^3H -hydroxyproline by fat-storing cells

	dpm/ 1×10^6 cells			
	Normal		CCl_4	
	Without lactate	With lactate	Without lactate	With lactate
^3H -proline in protein (dpm $\times 10^3$)	190 \pm 15	361 \pm 38 (90%) ^a	881 \pm 99	1,483 \pm 153 (51%) ^a
Protein-bound ^3H -hydroxyproline	5,012 \pm 463	9,722 \pm 1,156 (93%)	16,039 \pm 1,480	51,852 \pm 6,258 (223%) ^a
Collagen synthesis ^b (% of total collagen)	1.103	1.126	0.756	1.472

Values within parentheses represent the percentage above the value without lactate.

^aSignificant ($p < 0.01$).

^bValues calculated according to the formula of Diegelmann and Peterkofsky (23).

from CCl_4 -treated rats. Since glycogen levels have been shown to decrease in the liver of CCl_4 -treated rats (25), energy for protein synthesis could be obtained from glucose. If we assume that all the protein-bound ^3H -hydroxyproline is in collagen α -chains, 80% of total collagen produced in the normal liver is made by the hepatocytes. In the cirrhotic liver only 50% of the basal collagen produced is contributed by the hepatocytes. These values are similar to those already reported by Chojkier (9) and Chojkier, Lyche and Filip (10).

The amount of nonparenchymal cells recovered from the livers of CCl_4 -treated rats was two to three times higher when compared with nonparenchymal cells from normal livers (data not shown). When lactic acid was present in the incubation medium, nonparenchymal cells from CCl_4 -treated rats showed a twofold increase in the formation of protein-bound ^3H -hydroxyproline compared with total protein synthesis. On separating the different nonparenchymal cells, we observed that FSC from CCl_4 -treated animals were the only cells that specifically increase the formation of protein-bound ^3H -hydroxyproline in the presence of 30 mmol/L lactic acid. Lactic acid itself increases collagen synthesis in liver myofibroblasts by modifying the intracellular proline pool (14). Nevertheless, in all the experiments carried out in this work, the pool of free extracellular proline was saturated, so the increased production of collagen by lactic acid should not be ascribed to changes in proline concentration. Expressing collagen synthesis as the relative rate of total protein synthesis eliminates the problem of changes in the rate of proline transport and changes in the specific activity of the intracellular proline pool (26). We have already reported that cirrhotic livers differ from normal livers in several parameters (27). Collagen concentration, collagen types, distribution of collagen types, proline pool, actin concentration and synthesis, collagen synthesizing capacity and response to lactic acid are some other parameters in which cirrhotic livers differ from normal livers. We demonstrate here that cells with a FSC phenotype, density of 1.04 and presence of vitamin A, obtained from normal liver differ from those obtained from cirrhotic liver. Greenwel, Saed and Rojkind have recently suggested that FSC from cirrhotic livers are heterogeneous mixtures of clones with variable capacities to produce

type I collagen and fibronectin (28). It is thus possible that the heterogeneous mixture of FSC isolated from cirrhotic liver results from cloning of cells with variable responses to cytokines and growth factors produced by monocytes and Kupffer cells within the liver. It has been shown by Shiratori et al. (29) and by Armendariz-Borunda, Greenwel and Rojkind (30) that Kupffer cells from cirrhotic livers produce FSC growth factors. These cells, which differ in phenotype from FSC of normal liver, produce more collagen and further increase their collagen production when lactic acid is increased.

The mechanism by which lactic acid stimulates collagen production in the cirrhotic, but not in the normal, liver still remains to be investigated. Lactic acid is known to activate prolylhydroxylase by modifying the aggregation of its subunits (31). Recently a substance that can replace ascorbate as a co-factor for prolylhydroxylase activity was found in cultured fibroblasts. When this substance is reduced by NADH, it activates prolylhydroxylase (32). It has been shown that products from ADP-protein ribosylation (poly ADP-ribose and phosphoribosyl-AMP) are inhibitors of prolylhydroxylase activity in cultured fibroblasts (33). Since protein ribosylation is partially dependent on the availability of NAD, hydroxylation of prolyl- and lysyl-residues in collagen could be inhibited by low NAD levels. It is thus possible that NADH formed during the oxidation of lactate to pyruvate may increase the activity of prolylhydroxylase. However, prolylhydroxylase is not rate limiting for collagen biosynthesis and therefore further work is needed to ascertain the mechanism by which lactic acid stimulates collagen production by FSC from cirrhotic liver. Accordingly, alcohol has at least two mechanisms by which it stimulates collagen production. One is through the acetaldehyde (34) formed directly from alcohol metabolism and the other is due to lactic acid formed during NADH accumulation. These *in vitro* effects could have relevance *in vivo*. It has been shown that lactic acid levels are increased after alcohol intake and in patients with severe liver disease (35, 36). Therefore FSC from patients with liver damage may become responsive to lactate and thus produce more collagen. Similarly, lactic acid may increase during muscle wasting and further contribute to liver fibrogenesis.

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