

Changes in Collagen and Albumin mRNA in Liver Tissue of Mice Infected with *Schistosoma mansoni* as Determined by In Situ Hybridization

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ABSTRACT We have employed in situ hybridization to evaluate the molecular mechanisms responsible for hypoalbuminemia and increased liver collagen content in murine schistosomiasis. Results were compared using a simplified method of hybridizing isolated hepatocytes from *Schistosoma mansoni*-infected and normal mouse liver with mouse albumin (pMalb-2) and chick pro- $\alpha 2(I)$ collagen (pCg45) probes. Whereas hepatocytes from infected mice showed significantly less albumin mRNA than hepatocytes from control, there were more grains of procollagen mRNA in hepatocytes from infected as compared with control liver. Hybridization of infected liver tissue sections with the collagen probe showed more grains per field in granulomas than in liver regions, whereas with the albumin probe there was more hybridization in liver tissue than in granulomas. These results suggest that in murine schistosomiasis a reduction in albumin mRNA sequence content may be associated with decreased albumin synthesis and ultimately leads to hypoalbuminemia. In addition, although the granuloma seems to be the primary source of type I collagen synthesis, hepatocytes are also capable of synthesizing collagen, especially under fibrogenic stimulation.

Schistosomiasis is a major worldwide infectious disease involving more than 200 million people (1) and is probably the most prevalent cause of chronic liver disease (2). Liver involvement is characterized by an immunological response to schistosome eggs trapped in hepatic venules causing a granulomatous response and marked fibrosis. Although most humans with schistosomiasis have a normal serum albumin level, a significant number of infected individuals have hypoalbuminemia (3). The reason for hypoalbuminemia has remained unclear because the total hepatocyte mass and cell morphology is usually unaffected by this disease (4).

Mice infected with cercariae of *Schistosoma mansoni* have provided a model of the human disease. Dunn et al. (5) found that the enlarged liver of schistosome-infected mice contains 20 times more collagen than the liver from uninfected control mice, and several groups have reported hypoalbuminemia in infected mice (6, 7). The molecular mechanisms responsible for increased collagen synthesis and hypoalbuminemia in murine schistosomiasis have not been explored.

In a previous study, we reported a simplified and sensitive

method of in situ hybridization using isolated hepatocytes (8). In the present investigation, we have used this technique with purified albumin and collagen cDNA probes to evaluate the abundance of the messenger RNA for these two proteins in isolated hepatocytes as well as in liver tissue sections. This method allowed us to identify the cell type(s) involved in the synthesis of collagen and albumin, to determine whether the mRNA levels for these proteins are changed and to distinguish whether the granuloma or liver tissue is responsible for the bulk of collagen synthesis during schistosome infection.

MATERIALS AND METHODS

Preparation of Isolated Hepatocytes: CF1 female mice were infected subcutaneously with 50 cercariae of *S. mansoni*, Puerto Rican strain (received from Case Western Reserve Medical School, Department of Geographic Medicine), and littermate controls were provided by the same source. 8-wk postinfection, animals were anesthetized by intraperitoneal injection of pentobarbital (65 μ g/g body weight) and hepatocytes were isolated. Livers were perfused in situ using the technique of Elliott et al. (9), as described previously (8). Briefly, livers were perfused for 5 min, after which the perfusion medium was supplemented with 0.05% collagenase (Sigma Chemical Co., St. Louis,

MO, Type I). The livers were removed, minced in collagenase-containing buffer, passed through a mesh, washed, and differentially centrifuged to remove nonparenchymal cells and debris. Viability of isolated cells was determined by trypan blue dye exclusion.

Preparation of Liver Sections: Normal and *S. mansoni*-infected livers were perfused in situ as described previously (8). The perfusion medium consisted of 137 mM NaCl, 5.3 mM KCl, 0.8 mM MgSO₄, 0.4 mM Na₂HPO₄, 0.4 mM KH₂PO₄, 5.5 mM glucose, and 5 mM HEPES, pH 7.4 plus heparin at a final concentration of 300 µg/ml as a ribonuclease inhibitor (10). After perfusion, livers were excised rapidly, blotted, and a small piece was placed on a slide and imbedded immediately in Tissue Tek II (Lab Tek Products, Ames Co., Inc., Elkhart, IN) at -20°C. Cryostat sections 5-7 µm in thickness were placed on microscope slides, which had been pretreated according to the procedure of Brahic and Haase (11). The sections were air-dried for 2-3 min and were then fixed in ethanol/acetic acid, 3:1 (vol/vol) for 30 min at room temperature. The slides were treated with 0.2 M HCl for 20 min at room temperature and with 300 mM NaCl-30 mM sodium acetate, pH 7.0, for 30 min at 70°C (11). The slides were washed in distilled water and dehydrated in graded ethanol solutions (50, 70, and 95%).

Preparation and Labeling of Cloned Albumin and Collagen DNA Probes: Plasmid pBR322 containing mouse albumin DNA (pmlb-2) was a kind gift of Dr. S. Tilghman (Fox Chase Cancer Center, Philadelphia, PA) and chicken pro- α 2(I) collagen DNA (pCg45) in plasmid pBR322 was generously provided by Dr. H. Boedtker (Harvard University, Cambridge, MA). Albumin and collagen gene sequences were prepared from their respective recombinant plasmids essentially according to the method of Villa-Kamaroff et al. (12). Briefly, the plasmids were grown in bulk in *Escherichia coli* HB101, isolated by CsCl density gradient centrifugation, purified, ethanol-precipitated, and digested with restriction enzyme *Hind*III. The digests were then electrophoresed in 1% preparative agarose slab gels using Dingman and Peacock buffer (13) to separate the purified inserted gene sequences from the residual linearized plasmid band. The recombinant DNA sequences were reisolated from the gel and were nick-translated with [³H]-*d*-cytidine triphosphate and [³H]thymidine triphosphate to a specific activity 3-5 × 10⁷ cpm/µg DNA using a modification of the procedure of Rigby et al. (14). Nick-translated, purified, recombinant-cloned hepatitis B virus DNA (15), prepared by the same procedures, was used as a control.

Hybridization of Hepatocytes: Hepatocytes prepared from *S. mansoni* infected and control mice were hybridized with either ³H-labeled pmlb-2 or ³H-labeled pCg45 probe. The hybridization conditions were as described previously (8). Briefly, in silicone-treated microcentrifuge tubes, isolated hepatocytes were fixed in a 3:1 mixture of ethanol/acetic acid, treated with 0.2 M HCl, 0.3 M NaCl, and 0.03 M sodium citrate, and dehydrated, followed by hybridization with [³H]albumin or collagen probe for 36-48 h. The hybridization medium was essentially that used by Brahic and Haase (11) without added RNA and contained 50% (vol/vol) deionized formamide, 10 mM Tris HCl (pH 7.5), 1 mM EDTA, 600 mM NaCl, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 1 mg of BSA/ml, and 100 µg of sonicated denatured calf thymus DNA/ml. After hybridization, the cells were washed extensively in a 50% formamide, high salt buffer, and mounted on a microscope slide, followed by dehydration and autoradiography with a liquid emulsion for 1-3 wk, as previously described (8).

Hybridization of Liver Sections: The hybridization conditions for liver sections were essentially as described by Brahic and Haase (11). The dehydrated and air-dried sections were hybridized with the mixture as noted above. The hybridizing mixture containing the labeled probe was prehybridized by heating to 100°C for 1 min, cooled quickly to 0°C, and preincubated at 50°C for 1 h. 5 ml of the mixture containing 4-5 ng of [³H]cDNA was then placed on the liver sections and covered with a 12-mm siliconized glass coverslip. After incubation at 37°C for 48 h, the slides were washed twice in the hybridization medium without the ³H-labeled probe, followed by extensive washing in the same buffer for a total of 36 h. The slides were then washed twice in 300 mM NaCl-30 mM sodium citrate, pH 7.0, followed by dehydration in graded ethanol (50, 70, and 95%) containing 300 mM ammonium acetate. The dried slides were autoradiographed.

Autoradiography: Kodak NTB-3 nuclear track emulsion was melted at 45°C and diluted 1:1 with 600 mM ammonium acetate (11). The slides were dipped in melted emulsion for 1-2 s and allowed to dry in an upright position for 1 h at room temperature. They were transferred to light-proof boxes containing silica gel desiccant and exposed at 4°C. The slides were developed in Kodak D-19 developer for 30 min at room temperature, followed by a brief wash in 1% acetic acid, and fixation for 3 min in Kodak fixer. After the slides were washed in distilled water, they were stained for 30-45 min with Giemsa stain diluted 1:50 in 10 mM phosphate buffer.

RESULTS

In situ hybridization of isolated hepatocytes from normal and *S. mansoni*-infected mice using the mouse albumin cDNA probe (pmlb-2) is shown in Fig. 1, A and B. The silver radiographic grains (representing cDNA-RNA hybridization) were imbedded in the liquid emulsion that overlay the cells and were counted at three levels of microscopic focus by two independent observers as described previously (8). Statistical analysis employed the Mann-Whitney U test. There were clearly more albumin mRNA grains in the cells from control as compared with schistosome-infected liver. Fig. 2 shows the distribution profile of the grain counts per cell with 100 cells from infected and control livers plotted as a histogram. A background of one grain per cell at each level of focus, observed in the absence of added radioactivity, was subtracted from each value. Cells containing albumin mRNA were divided into eight groups, ranging from 0-20 to >120 grains per cell. There was a significant decrease ($p < .001$) in the number of grains in infected compared with control hepatocytes (56.9 ± 5.9 SEM, infected vs. 97.2 ± 7.3 SEM, control). This indicated a significant decrease in albumin mRNA sequence content in the cytoplasm of hepatocytes isolated from infected mice as compared with control.

Similarly, isolated hepatocytes from normal and infected mice were hybridized in situ using the nick-translated chicken pro- α 2(I) collagen cDNA probe (pCg45). Fig. 3 shows representative autoradiograms demonstrating that there are more grains in the cells from infected livers (B) than from controls (A). Again, autoradiographic grains were counted in 100 cells at three levels of microscopic focus by two independent observers. The findings, presented in histogram form (Fig. 4), indicated that there is significantly more pro- α 2(I) collagen mRNA in the hepatocytes of infected mice than in control animals (12.6 ± 0.6 SEM, infected vs. 9.37 ± 0.4 SEM, control; [$p < .005$]). With ³H-hepatitis B virus-DNA (a virus that does not infect mouse hepatocytes), one-third of the cells showed no hybridization, and the mean grain count per cell was less than one grain for each level of focus. When the [³H]DNA probes were digested with DNase or when the cells were digested with pancreatic ribonuclease prior to hybridization, no autoradiographic grains above the background were observed. As an additional control, when fibroblasts were hybridized with the [³H]albumin DNA probe, no hybridization was detected above background (data not shown).

Tissue sections from mice infected for 8 wk with *S. mansoni* were hybridized in situ with pmlb-2. Fig. 5A shows an *S. mansoni* egg and granuloma surrounding the egg with few autoradiographic grains. Fig. 5B shows that liver tissue surrounding the granuloma has more albumin mRNA grains than the granuloma itself. When ten different granulomas and surrounding liver tissue were evaluated quantitatively, the liver tissue had significantly more albumin mRNA than did the granuloma tissue (Table I). However, when parallel studies were performed with the collagen cDNA probe (pCg45), there was a marked increase in autoradiographic grains in the granuloma as compared with liver tissue (Table I and Fig. 6).

DISCUSSION

The cell(s) responsible for the production of collagen in normal and fibrotic liver has been controversial for several years

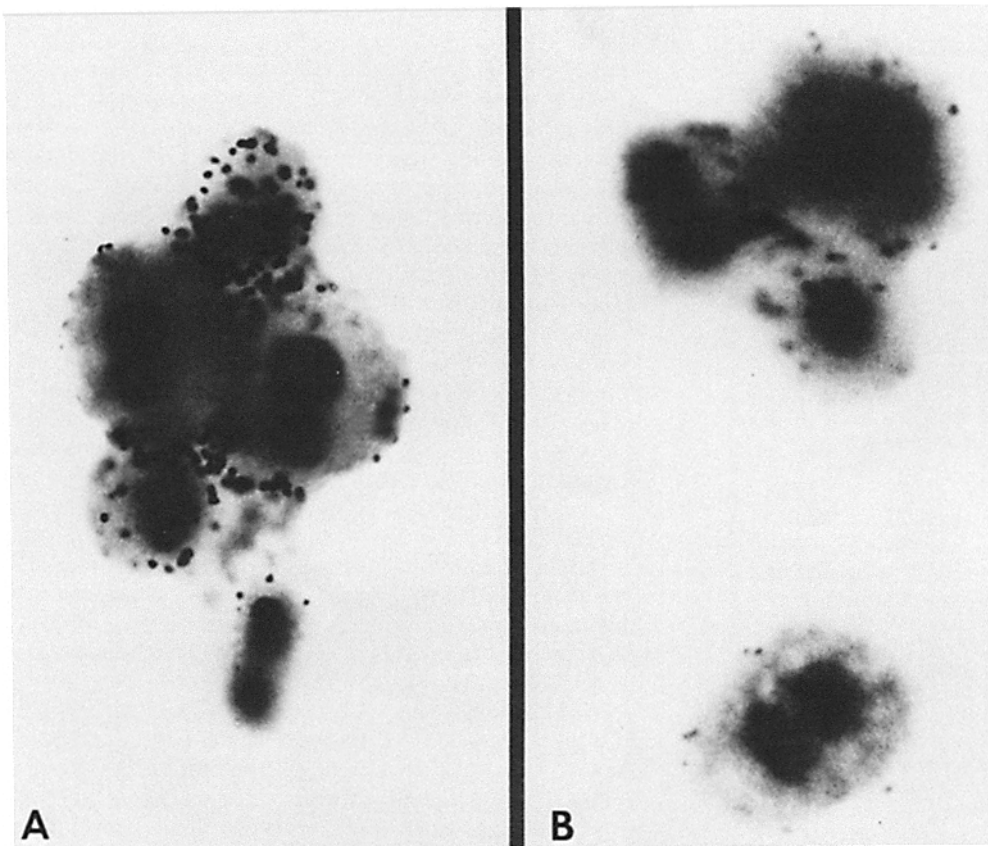


FIGURE 1 In situ hybridization of isolated mouse hepatocytes with ^3H -labeled cloned albumin cDNA. Hepatocytes were isolated from mouse livers after in situ perfusion by collagenase, digestion, sedimentation, and resuspension, as described in Materials and Methods. In microcentrifuge tubes, cells were fixed in a 3:1 mixture of ethanol/acetic acid, treated with 0.2 M HCl, 0.3 M NaCl, and 0.03 M sodium citrate and dehydrated prior to hybridization with a [^3H]albumin probe for 36–48 h. After hybridization, the cells were washed extensively in a 50% (vol/vol) formamide, high salt buffer and were mounted on a microscope slide prior to dehydration and evaluation by autoradiography. (A) Hepatocytes from liver of normal mouse and (B) hepatocytes from liver of schistosomiasis-infected mouse (original magnification, $\times 400$).

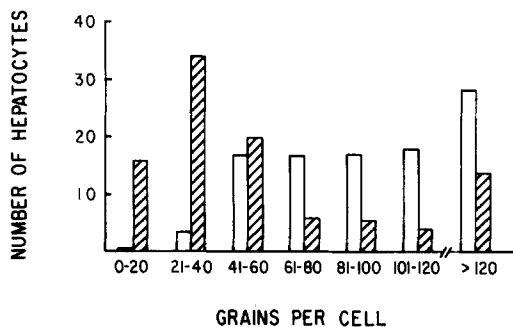


FIGURE 2 Histogram of hepatocytes hybridized with ^3H -labeled cloned albumin cDNA. Cells were isolated, hybridized, and autoradiographed as described in Materials and Methods. The number of radiographic grains per cell (a semiquantitative assessment of albumin mRNA sequence content) was determined at three levels of microscopic focus. Hepatocytes from liver of control animals are represented by open bars; hepatocytes from liver of infected animals are represented by striped bars.

(10–31). Fibroblasts have been demonstrated to synthesize collagens in many tissues, and this appears to be the case in the liver (16, 17). However, several investigators believe that lipocytes are precursors of fibroblasts in the liver and that these cells may synthesize collagen (18, 19). Lipocytes have also been shown to proliferate in states of hepatic injury, and the activation of these cells has been proposed to be a primary mechanism for increased collagen formation (19). Intermediate cellular forms also exist that have capabilities of both fat storage and collagen synthesis (18). The myofibroblast has been found recently in biopsy studies of alcoholic cirrhosis to

proliferate (20), and smooth muscle-like cells similar to those found in blood vessel walls have been determined to proliferate and produce collagen types I and III in explants of fibrotic liver (21). Immunofluorescence studies have indicated that isolated Kupffer cells also produce type I collagen (22).

A great deal of investigation has been undertaken to determine whether parenchymal cells (i.e., hepatocytes) are capable of synthesizing collagen. Many of these studies have been performed with liver-derived epithelial cell lines (23–26). However, primary cultures of hepatocytes have also been reported to be collagen-producing (27, 28, 31). Yet at least two studies have not been able to show collagen synthesis in hepatocytes (17, 29).

The controversy regarding the cell that produces specific collagens in liver has persisted in part because different techniques have been utilized to identify the various cell types responsible for synthesis of this protein. In examination of tissue samples, the implicated cell is often shown to be spatially related to collagen fibers or to proliferate during increased collagen production (19, 20). However, proximity does not necessarily mean synthesis; it can mean that the cell is attracted to the connective tissue components. The hepatocyte has been reported to synthesize collagen in several studies employing cell culture (23–28, 31). However, certain problems remain with this methodology because fibroblasts often contaminate parenchymal cell preparations. In addition, since cells dedifferentiate with changes in function and morphology when grown in culture (30), a cell's ability to produce a given protein under in vitro conditions does not guarantee that this capacity for synthesis exists under in vivo conditions.

In situ hybridization is a useful approach to investigate

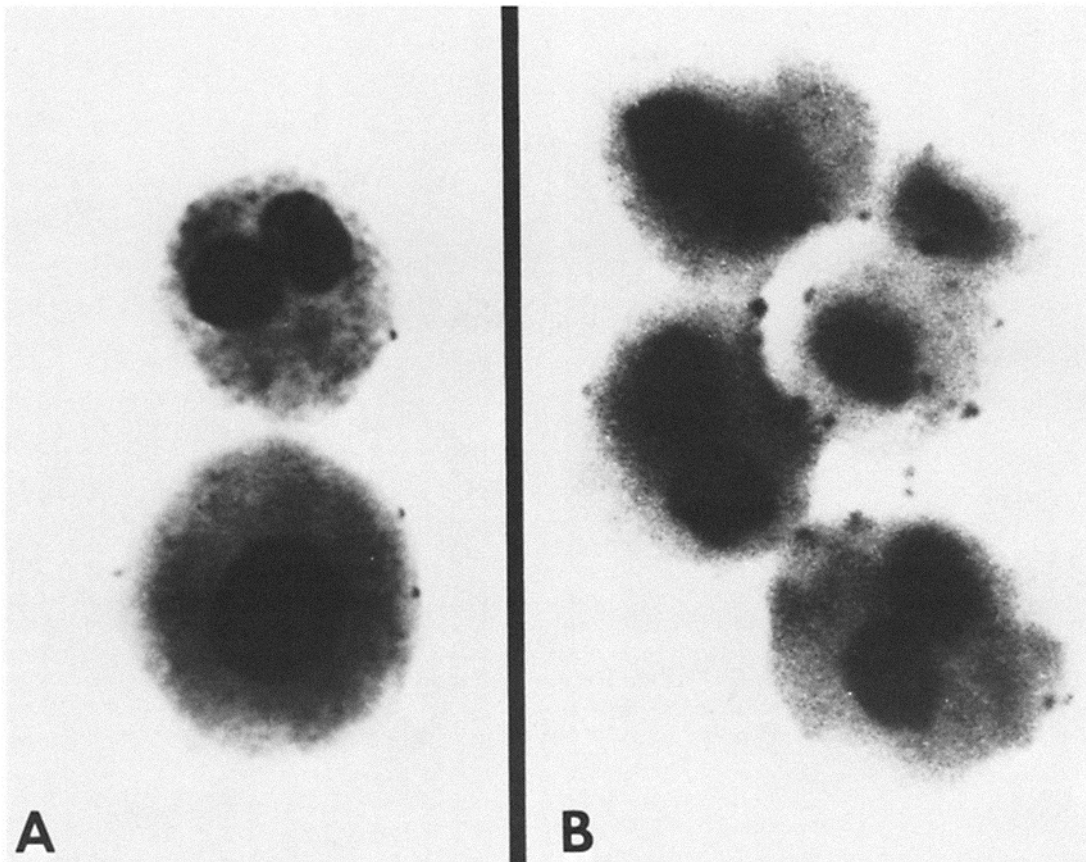


FIGURE 3 In situ hybridization of isolated mouse hepatocytes with a ^3H -labeled pro- $\alpha 2(\text{I})$ collagen cDNA probe. Hepatocytes were isolated, treated, hybridized, washed, and autoradiographed as described in Materials and Methods. Cells from control mouse liver (A) demonstrate fewer radiographic grains than do cells from infected mouse liver (B; original magnification, $\times 400$).

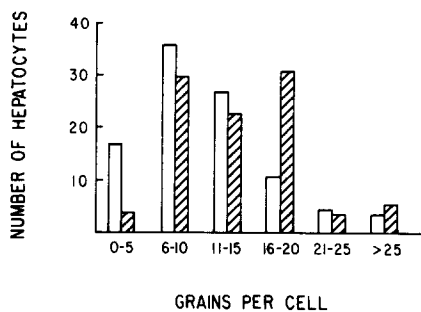


FIGURE 4 Histogram of hepatocytes hybridized with a ^3H -labeled chicken pro- $\alpha 2(\text{I})$ collagen cDNA probe. Cells were isolated and treated as described in Materials and Methods. A semiquantitative determination of type I collagen sequence content was accomplished by counting the radiographic grains. Open bars represent control hepatocytes; striped bars represent *Schistosoma mansoni*-infected cells.

sequence content was accomplished by counting the radiographic grains. Open bars represent control hepatocytes; striped bars represent *Schistosoma mansoni*-infected cells.

mRNA levels for specific proteins because it evaluates mRNA presence in the intact tissue or in cells at the time of isolation before alterations in subcellular mechanisms or dedifferentiation can occur. Also, it measures the synthetic capacity of a cell for a specific protein, not merely the cell's proximity to a given product. A limitation of the technique is that the specific mRNA is localized within a cell, not the synthesis of the actual protein. However, aside from specialized cell types in unique stages of development, differentiation, or transformation (32–34), in most eucaryotic systems there is a direct correlation between mRNA content and the synthesis of the specific protein (35).

In the present study, we have evaluated the effect of *S.*

mansoni infection on albumin and collagen mRNA in murine liver by in situ hybridization. This model is appropriate to evaluate the regulation of the hepatic synthesis of these proteins because hypoalbuminemia develops after six weeks of infection in conjunction with a marked increase in hepatic collagen synthesis. Schistosome infection caused a significant decrease in the amount of albumin mRNA per hepatocytes, but all cells still appeared to have abundant quantities of albumin mRNA. This suggested that all hepatocytes are capable of synthesizing albumin, consistent with several recent immunohistochemical studies (36–39). However, other studies have reported that albumin is synthesized by only a fraction of normal hepatocytes (40–47). If the latter were true, this would imply a negative regulation of albumin synthesis for which there is presently no evidence.

In situ hybridization of tissue slices from mouse livers infected with *S. mansoni* indicated that most of the pro- $\alpha 2(\text{I})$ collagen mRNA was found in the granulomas, within which fibroblasts are most likely responsible for the synthesis of this protein. In situ hybridization of isolated hepatocytes with the procollagen cDNA probe clearly indicated the presence of procollagen mRNA in these cells, whereas no detectable hybridization above background was found with hepatitis B virus DNA. In addition, there was significantly more procollagen mRNA in hepatocytes isolated from infected mice than from control animals. This hybridization occurred despite utilizing a chicken pro- $\alpha 2(\text{I})$ collagen DNA probe which will tend to underestimate the amount of collagen mRNA in mouse hepatocytes. The chicken pro- $\alpha 2(\text{I})$ collagen mRNA

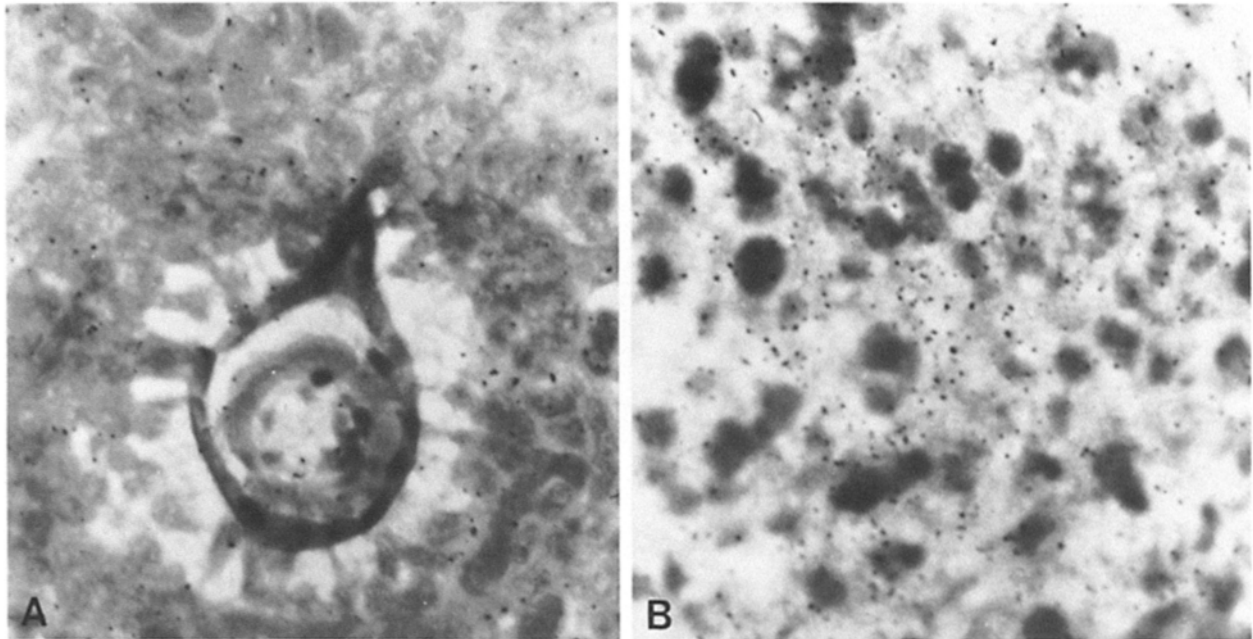


FIGURE 5 In situ hybridization of tissue sections from infected mouse liver with a ^3H -labeled albumin cDNA probe. Sections were prepared by in situ perfusion of the liver and cryostat sectioning. The slides were prepared, hybridized, and autoradiographed according to the procedure of Brahic and Haase (11), as described in Materials and Methods. The granuloma (A) demonstrates few autoradiographic grains in comparison to the liver tissue adjacent to the granuloma (B; original magnification, $\times 100$).

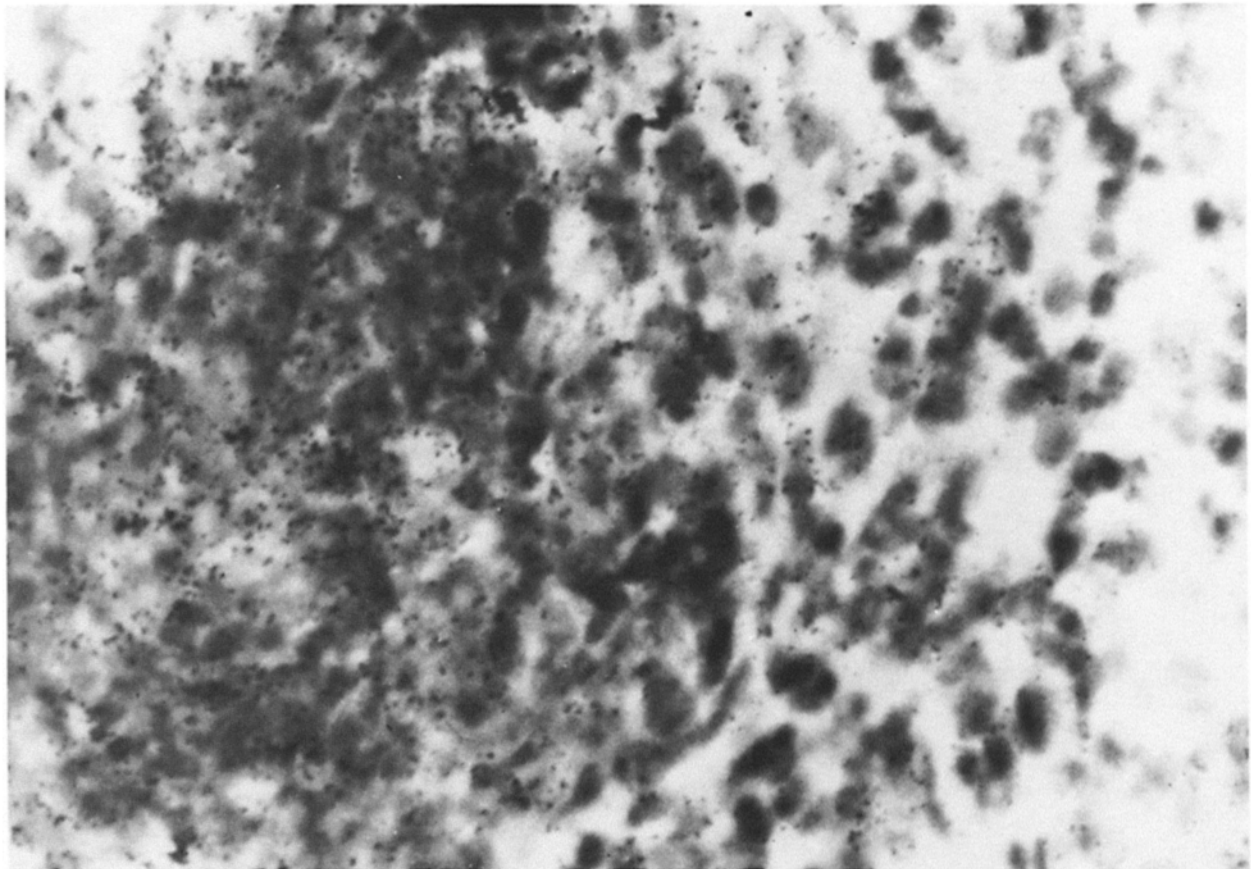


FIGURE 6 In situ hybridization of liver tissue from infected mouse liver with a ^3H -labeled pro- $\alpha 2(\text{I})$ collagen probe. Sections were prepared, hybridized, and autoradiographed as described in Materials and Methods. The left side of the picture shows the granuloma tissue with more silver radiographic grains than are found in liver tissue on the right (original magnification, $\times 100$).

TABLE I
Relative Abundance of Albumin and Collagen mRNA in Liver Sections

cDNA Probe	Tissue	Autoradiographic grains per field*
Albumin (pmlb-2)	Infected	
	granuloma	67.75 ± 5.80 [‡]
	liver tissue	193.25 ± 19.22
	Control	210.53 ± 15.30
Collagen (pCg-45)	Infected	
	granuloma	353.70 ± 60.44 [‡]
	liver tissue	93.44 ± 6.33
	Control liver	85.52 ± 8.25

* Field area = 0.0625 mm²; n = 10 fields; ± SEM.
[‡] P < 0.001.

has been shown previously to cross-hybridize with species as divergent as nematodes (48).

These results do not resolve the controversy as to which cell(s) is primarily responsible for the synthesis of collagen in the normal and fibrotic liver. They suggest that hepatocytes are capable of synthesizing type I collagen at a low level in normal states and that hepatocytes produce more collagen mRNA under fibrogenic stimulation. Non-hepatocytes (presumably fibroblasts) within the granuloma have the greatest collagen mRNA content and appear to be the cell type primarily responsible for collagen synthesis in murine schistosomiasis. This does not mean, however, that the hepatocyte is not responsible for type I collagen synthesis in other forms of hepatic fibrosis or cirrhosis. Hepatocyte injury and subsequent collagen synthesis induced by viruses, hepatotoxins or ischemia may well act by mechanisms significantly different than those occurring in schistosomiasis, where there is a large immunological response and the hepatocyte is relatively spared. Therefore, localization of procollagen mRNA by in situ hybridization seems to be a reasonable approach to address the question as to which cell(s) is responsible for increased collagen production following hepatocellular injury induced by various pathophysiologic mechanisms.

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REFERENCES

- Warren, K. S. 1980. The relevance of schistosomiasis. *N. Engl. J. Med.* 303:203-206.
- Warren, K. S. 1973. Regulation of the prevalence and intensity of schistosomiasis in man. Immunology or ecology? *J. Infect. Dis.* 127:595-609.
- Cook, J. A., S. T. Baker, K. S. Warren, and P. Jordon. 1974. A controlled study of morbidity of Schistosomiasis mansoni in St. Lucian children, based on quantitative egg excretion. *Am. J. Trop. Med. Hyg.* 23:625-637.
- Dunn, M. A., and R. Kamel. 1981. Hepatic Schistosomiasis. *Hepatology.* 1:653-661.
- Dunn, M. A., M. Rojkind, K. S. Warren, P. K. Hait, L. Rifas, and S. Seifter. 1977. Liver collagen synthesis in murine schistosomiasis. *J. Clin. Invest.* 59:666-674.
- Knauff, R. F., and K. S. Warren. 1969. The effect of calorie and protein malnutrition on both the parasite and the host in acute murine Schistosomiasis mansoni. *J. Infect. Dis.* 120:560-568.
- Page, C. R., F. J. Eges, and J. D. Ogle. 1972. Experimental prepatent Schistosomiasis mansoni: quantitative analysis of proteins, enzyme activity and free amino acids in mouse serum. *Exper. Parasitol.* 31:341-349.
- Saber, M. A., M. A. Zern, and D. A. Shafritz. Use of in situ hybridization to identify the presence of collagen and albumin mRNAs in isolated mouse hepatocytes. *Proc. Natl. Acad. Sci. USA.* 80: 4017-4020.
- Elliott, K. R. F., R. Ash, C. I. Pogson, S. A. Smith, and D. M. Crisp. 1976. Comparative aspects of the preparation and biochemistry of liver cells from various species. In Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies. J. M. Tager, H. D. Soling, and J. R. Williamson, editors. North Holland Publishing Company, Amsterdam. 139-147.
- Berger, S. L., and B. Birkenmeier. 1979. Inhibition of intractable nucleases with ribonucleoside-vanadyl complexes: isolation of messenger ribonucleic acid from resting lymphocytes. *Biochemistry.* 18:5143-5149.
- Brahic, M., and A. Haase. 1978. Detection of viral sequences of low reiteration frequency by in situ hybridization. *Proc. Natl. Acad. Sci. USA.* 75:6125-6129.
- Villa-Komaroff, L., A. Efstratiadis, S. Broome, P. Lomedico, F. Tizard, S. P. Naber, W. L. Chick, and W. Gilbert. 1978. A bacterial clone synthesizing proinsulin. *Proc. Natl. Acad. Sci. USA.* 75:3727-3731.
- Dingman, C. W., and A. C. Peacock. 1968. Analytical studies on nuclear ribonucleic acid using polyacrylamide gel electrophoresis. *Biochemistry.* 7:659-667.
- Rigby, P. W., M. A. Dieckmann, C. Rhodes, and P. Berg. 1977. Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. *J. Mol. Biol.* 113:237-251.
- Chakraborty, P. R., N. Ruiz-Opazo, D. Shouval, and D. A. Shafritz. 1980. Identification of integrated hepatitis B virus DNA and expression of viral RNA in an HBsAg-producing human hepatocellular carcinoma cell line. *Nature Lond.* 286:531-533.
- Galambos, J. T., M. A. Hollingsworth, A. Falek, and W. D. Warren. 1977. The rate of synthesis of glycosaminoglycans and collagen by fibroblasts cultured from adult human liver biopsies. *J. Clin. Invest.* 60:107-114.
- Kamegaya, K., M. Oda, and M. Tsuchiya. 1980. Induction of collagen fiber formation by hepatocytes-biochemical and morphologic observation in tissue culture. *Gastroenterology.* 78:1118a. (Abstr.)
- Kent, G., S. Gay, T. Inoué, R. Bahu, O. T. Minick, and H. Popper. 1976. Vitamin A-containing lipocytes and formation of Type III collagen in liver injury. *Proc. Natl. Acad. Sci. USA.* 75:3719-3722.
- Inoué, T., O. T. Minick, and G. Kent. 1979. The role of lipocytes in portal and intralobular fibrogenesis. *Gastroenterology.* 77:18a. (Abstr.)
- Hahn, E. G., R. Timpl, M. Nakano, and C. S. Lieber. 1980. Distribution of hepatic collagens, elastin and structural glycoproteins during the development of alcoholic liver injury in baboons. *Gastroenterology* 79:1204a. (Abstr.)
- Voss, B., J. Rauterberg, G. Pott, V. Bredmer, S. Allam, R. Tedmann, and D. B. V. Bassewitz. 1982. Nonparenchymal cells cultivated from explants of fibrotic liver resemble endothelial and smooth muscle cells from blood vessel walls. *Hepatology.* 2:19-28.
- Voss, B., S. Allam, J. Rauterberg, G. Pott, and V. Bredmer. 1982. Liver derived macrophages synthesize collagen and fibronectin. In Connective Tissue of the Normal and Fibrotic Human Liver. U. Gerlach, G. Pott, J. Rauterberg, and B. Voss, editors. Georg Thieme Verlag, Stuttgart. 97-99.
- Hata, R., Y. Ninomiya, and Y. Tsukada. 1980. Biosynthesis of interstitial types of collagen by albumin-producing rat liver parenchymal cell (hepatocyte) clones in culture. *Biochemistry.* 19:169-176.
- Fordart, J.-M., J. J. Berman, L. Paglia, S. Rennard, S. Abe, A. Perantoni, and G. R. Martin. 1980. Synthesis of fibronectin, laminin, and several collagens by a liver-derived epithelial line. *Lab. Invest.* 42:525-532.
- Niles, R., and B. D. Smith. 1980. Characterization of collagen synthesized by normal and chemically transformed rat liver epithelial cell lines. *Biochemistry.* 19:1820-1825.
- Kumegawa, M., M. Hiramatsu, T. Yagima, K. Hatakeyama, S. Hosoda, and M. Namba. 1982. Effect of epidermal growth factor on collagen formation in liver-derived epithelial clone cells. *Endocrinology.* 110:607-610.
- Guzelian, P. S., G. D. Tureshi, and R. F. Diegelmann. 1981. Collagen synthesis by the hepatocyte: studies in primary cultures of parenchymal cells from adult rat liver. *Collagen Research.* 1:83-93.
- Tseng, S. C. G., P. C. Lee, P. F. Ells, D. M. Bissell, E. A. Smuckler, and R. Stern. 1982. Collagen production by rat hepatocytes and sinusoidal cells in primary monolayer culture. *Hepatology.* 2:13-18.
- Allam, S., B. Voss, J. Rauterberg, P. R. Sudhakaram, and K. V. Figura. 1982. Initial absence of collagen synthesis in primary cultures of rat hepatocytes. In Connective Tissue of the Normal and Fibrotic Human Liver. U. Gerlach, G. Pott, J. Rauterberg, and B. Voss, editors. Georg Thieme Verlag, Stuttgart. 86-88.
- Rauterberg, J., B. Voss, G. Pott, and S. Allam. 1982. Synthesis of connective tissue components by cells grown from explants of fibrotic human liver. In Connective Tissue of the Normal and Fibrotic Human Liver. U. Gerlach, G. Pott, J. Rauterberg, and B. Voss, editors. Georg Thieme Verlag, Stuttgart. 73-77.
- Diegelmann, R. F., P. S. Guzelian, R. Gay, and S. Gay. 1983. Collagen formation by the hepatocyte in primary monolayer culture and in vivo. *Science (Wash. D. C.)* 219:1343-1345.
- Hofer, E., and J. E. Darnell. 1981. The primary transcription unit of the mouse B-major globin gene. *Cell.* 23:585-593.
- Alton, T. H., and H. F. Lodish. 1977. Translational control of protein synthesis during the early stages of differentiation of the slime mold Dictyostelium discoideum. *Cell.* 12:301-310.
- Adams, S. L., D. Boettiger, R. J. Focht, H. Holtzer, and M. Pacifici. 1982. Regulation of the synthesis of extra-cellular matrix components in chondroblasts transformed by a temperature-sensitive mutant of Rous Sarcoma Virus. *Cell.* 30:373-384.
- Darnell, J. E. 1982. Variety in the level of gene control in eukaryotic cells. *Nature (Lond.)* 297:365-371.
- LeRumeur, E., C. Beaumont, C. Guillozo, M. Rissel, M. Bourel, and A. Guillozo. 1981. All normal rat hepatocytes produce albumin at a rate related to their degree of ploidy. *Biochem. Biophys. Res. Commun.* 101:1038-1046.
- Bervav, D., G. Feldmann, and E. Rogier. 1981. Analysis of albumin secretion by single hepatocytes in normal rat using the hemolytic plaque test. *Biol. Cell.* 40:17-22.
- Yokota, S., and H. D. Fahimi. 1981. Immunocytochemical localization of albumin in the secretory apparatus of rat liver parenchymal cells. *Proc. Natl. Acad. Sci. USA.* 78:4970-4974.
- Guillozo, A., C. Beaumont, E. Le Rumeur, M. Rissel, M.-F. Latinier, C. Guen-Guillozo, and M. Bourel. 1982. New findings on immunolocalization of albumin in rat hepatocytes. *Biol. Cell.* 43:163-172.
- Feldmann, G., J. Penaud-Laurencin, J. Crassous, and J. P. Benhamou. 1972. Albumin

- synthesis by human liver cells: its morphological demonstration. *Gastroenterology*. 40:17-22.
41. Guillouzo, A., L. Belanger, C. Beaumont, J. P. Valet, H. Briggs, and J. F. Chiu. 1978. Cellular and subcellular immunolocalization of alpha-fetoprotein and albumin in rat liver. Reevaluation of various experimental conditions. *J. Histochem. Cytochem.* 26:948-959.
 42. Guillouzo, A., G. Feldmann, M. Maurice, C. Sapin, and J. P. Benhamou. 1976. Ultrastructural distribution of albumin in rat hepatocytes during postnatal development. *J. Microsc. Biol. Cell.* 26:35-41.
 43. Lane, R. S. 1969. The cellular distribution of albumin in normal rat liver demonstrated by immunofluorescent staining. *Clin. Sci. (Lond.)*. 36:157-159.
 44. Lin, C. T., and J. P. Chang. 1975. Electron microscopy of albumin synthesis. *Science (Wash. D. C.)*. 190:465-467.
 45. Molinaro, G. A., E. Maron, W. C. Eby, and S. Dray. 1975. A general method for enumerating single cells secreting antigen: albumin-secreting hepatocytes detected as plaque-forming cells. *Eur. J. Immunol.* 5:771-774.
 46. Smith, C. I. E., and L. Hammarstrom. 1979. Detection of albumin-secreting hepatocytes at the single cell level by a protein plaque assay. *Eur. J. Immunol.* 9:570-571.
 47. Valet, J. P., N. Marceau, and J. Deschenes. 1981. Restricted specialization of differentiating hepatocytes in terms of albumin and alpha-fetoprotein production. *Cell Biology International Reports*. 5:307-314.
 48. Fuller, F., and H. Boedtker. 1981. Sequence determination analysis of the 3' region of chicken pro- α 1(I) and pro- α 2(I) collagen messenger ribonucleic acids including the carboxy-terminal propeptide sequences. *Biochemistry*. 20:996-1006.