IMMUNOHISTOCHEMICAL IDENTIFICATION OF TYPES OF COLLAGEN SYNTHESIZED BY CULTURED HUMAN LIVER CELL LINE (CHANG LIVER CELLS) UNDER THE INFLUENCE OF ETHANOL OR ITS DERIVATIVES

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Perihepatocellular fibrosis is often encountered in cases of alcoholic liver injury without a remarkable increase in the number of mesenchymal cells. Tissue culture experiments have shown that under certain conditions hepatocytes may produce collagen fibers. On the other hand, there is a proposal that the collagen synthesized in primary hepatocyte cultures derives from lipoocyte, a perisinusoidal cell, mixed with hepatocytes. To clarify whether hepatocytes would produce collagen under certain conditions and to identify the types of collagen produced, ethanol and its oxidation product acetaldehyde were added to the culture fluids of Chang liver cells\(^1\) an established adult human liver cell line with positive ADH, ALDH and albumin, as revealed by immunohistochemical as well as immunoelectron microscopic study\(^2\).

MATERIAL AND METHODS

Chang liver cells were cultured in Falcon tissue culture flasks. The basic fluid consisted of Eagle's MEM with 12% fetal calf serum and ascorbate (50 \(\mu\)g/ml). The cells were cultured in the following fluids: group 1: basic fluid; group 2: basic fluid + ethanol (100 \(\mu\)M); group 3: basic fluid + ethanol and 4-methyloxovrazole (2 mM, an inhibitor of ADH); group 4: basic fluid + acetaldehyde (200 \(\mu\)M). Each group consisted of 50 samples, which were cultured in a 5% CO\(_2\) incubator at 37 degrees centigrade for 28 days. The culture fluid was renewed every three days.

COLLAGEN IMMUNOTYPING: Chang liver cells attached to the bottom of the tissue culture flasks were fixed in 4% paraformaldehyde for 10 min. 1st antibody (antitype I, III, IV or V collagen rabbit IgG, ADVANCE CO., Tokyo) or control rabbit serum was applied for 60 min. at room temperature. Then, 2nd antibody (peroxidase-labelled anti-rabbit IgG goat antibody, TAGO., U.S.A.) was applied for 60 min. The specimens were colored with 3'3'-DAB \(\cdot\) \(\text{H}_2\text{O}_2\) for 5 min. for light microscopy. The specimens were refixed in 2% glutaraldehyde for electron microscopy,
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Fig. 1. Type III collagen fibers (arrows) are observed outside the liver cells. Immunohistochemical identification. x400. Inset: Immunoelectron microscopic picture of type III collagen fibrils, showing cross striation (arrowheads). x30,000.

followed by coloring with 3,3'-DAB·H₂O₂. Finally, the specimen were fixed in 2% OsO₄ and embedded in epon for ultrathin sectioning.

RESULTS AND DISCUSSION
Approximately 16% of the samples cultured in the fluid with ethanol, 11% with acetaldehyde and 82% with the combined application of ethanol and 4-methylpyrazole revealed networks of argentaffine fibers enmeshing liver cells, which were bundles of collagen fibrils with cross striation seen on electron microscopy. Abundant type III and IV collagen (Fig. 1 and 2) as well as a scanty amount of type V collagen were identified outside the cells by immunoperoxidase staining on light and electron microscopy. Type I collagen was not observed in the present experiment, and further study on it is necessary.

The present findings strongly suggest that hepatocytes play an important part in the formation of collagen in alcoholic liver diseases.

REFERENCES