HEPATOCYTOGENESIS AND COLLAGEN METABOLISM

The effect of hepatotoxic agents

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INTRODUCTION

The liver arises from the distal end of the foregut as a solid bud of entoderm cells. The hepatic bud grows anteriorly into the mass of splanchnic mesoderm called the septum transversum. The end of the hepatic bud divides into right and left branches, from which columns of entodermal cells grow out into the vascular mesoderm. The columns of cells continue to grow and branch and anastomose with one another. The paired vitelline veins and umbilical veins that course through the septum transversum become broken up by the invading columns of liver cells and form the liver sinusoids. Thus it is seen that the columns of entodermal cells form the parenchymatous tissue of the liver; the liver sinusoids are formed from parts of the vitelline and umbilical veins; and the fibrous capsule of the liver and the connective tissue develop from the mesoderm of the septum transversum. Some of the cells making up the lining of the sinusoids become differentiated into large macrophages, the Kupffer cells. Collections of proliferating mesenchymal cells that have a hematopoietic function are seen between the liver cells and the blood sinusoids. The newly formed erythrocytes and leukocytes enter the circulation by passing through the wall of the sinusoids (1,2).

The main hepatic bud and its right and left terminal branches become canalized to form the common hepatic duct and the right and left hepatic ducts. Further canalization of the columns of cells within the liver takes place so that the duct system eventually joins the bile capillaries. The liver cells start to secrete bile during the fifth month of development. The liver continues to grow rapidly in size and comes to occupy the greater part of the abdominal cavity. In the early stage, the right and left lobes, which correspond to the right and left branches of hepatic bud, are of equal size, later the right lobe becomes much larger than the left lobe (1,2).

LIVER CELLS

About 78-84% of the total liver volume is occupied by the different cellular components and only 16-22% by the elements of extracellular space. Hepatocytes account for 92% of the cellular volume of mature liver and the remaining volume is occupied by the non-parenchymal cells (3).

There are four types of the mesenchymal cells present in the normal liver: endothelial cells, Kupffer cells, Ito cells, and fibroblasts. The first three mentioned are closely related to the structure of the sinusoid capillaries, whereas the latter is present
in the connective tissue of the capsule, the portal spaces, and the adventitia of the hepatic veins (3).

LIVER COLLAGEN

The extracellular matrix of the liver is composed of a large variety of macromolecules. These include collagen, non-collagenous glycoproteins, glycosaminoglycans, and proteoglycans. Connective tissue passes between lobes and is distributed throughout the liver as interlobular septa. These partially outline to small hepatic lobules. Reticulin fibers form most of the supporting connective tissue of the liver. Inside the liver parenchyma they form the very fine framework of the tissue. They line the sinusoids in part, where they are between the liver cells and the incomplete lining of reticuloendothelial cells. They form a dense network around the central veins. The collagenous fibers may be seen in the dense irregular connective tissue of the interlobular septa. They stain in black or dark brown with silver stains. Reticular fibers merge with these.

Total collagen content of the liver varies with different animal species, but it is maintained constant within a single species through the life span of the animal. In human liver the concentration of collagen is 5.5±1.6 mg per gram of fresh tissue whereas in the rat the concentration of this protein is only 0.9±0.15 mg per gram of fresh liver tissue (3,4). The four genetic types of collagen (I,III,IV and V) have been detected both in human and in rat livers. Types I and III are the most abundant of the liver collagens. They constitute about 80% of total collagen in this organ.

Type I collagen corresponds to the thick collagen bundles of the liver and forms the dense connective tissue. It is present in the liver capsule, in the stroma of the portal tracts and large vessels, around terminal venule areas and occasionally type I collagen fibres are also seen inside the liver lobule (3,4).

Type III collagen corresponds to some but not all the reticulin fibers of the liver. It is mixed with type I collagen in the stroma of large vessels and portal triads. It forms reticulin fibers inside the liver parenchyma (3,4).

Type IV collagen is present in sites containing the basement membranes. It has been found around arteries and lymphatic vessels, bile ducts and ductules and nerve ending. It is also present between the endothelial lining of the sinusoid and the liver plates (3,4).

Type V collagen is present in many areas of the liver but has special distribution around vessels and along the sinusoidal surface of the hepatocytes (3,4).

Although the cell types producing collagen in the liver have not been exactly clarified, it is believed that both mesenchymal cells (4,5) and hepatocytes (6,7) may synthesize this protein. Liver myofibroblasts produce in culture the four types of collagen present in the liver (5). Hepatocytes isolated from rat liver are able to synthesize collagens, including types I,III and IV (8). It has been suggested that type III collagen is produced in vivo by Ito cells (9).

Liver fibrosis is accompanied by significant increase of collagen content in this organ. In human cirrhotic liver type I colla-
Excessive deposition of collagen in the liver may result from increased biosynthesis, decreased degradation, or a combination of both processes (4).

There are many causes of liver fibrosis. In some cases it is evoked by toxic agents (4). Several toxic substances such as carbon tetrachloride, D,L-ethionine, dimethylnitrosamine, have been reported to induce liver fibrosis (11) but alcohol seems to be one of the most frequent causes of this process (12). Chronic alcoholism results in a variety of liver changes, almost all of them associate with various degrees of fibrosis (4).

**THE EFFECT OF ETHANOL ON LIVER COLLAGEN**

Extensive studies performed in many laboratories demonstrated stimulatory effect of chronic ethanol-intoxication on collagen biosynthesis in liver (13-27).

We have performed several studies on the effect of ethanol on collagen biosynthesis in rat liver (24-27). The animals were divided into a control group which received drinking water and an investigated group which received 10% (v/v) ethanol throughout the experiment. The rats from each group were killed after 1, 2, 3, 4, 5 and 6 months. Some of them were injected intraperitoneally with $^5$H-proline, 45 minutes before decapitation.

Samples of liver were submitted to histopathological examination. Other samples of this organ were taken for the assay of prolyl hydroxylase. Incorporation of $^5$H-proline into collagen and non-collagenous proteins of liver was evaluated.

It is generally accepted that increased bilirubin concentration and elevated activities of some enzymes (lactate dehydrogenase and its isoenzymes: LD$_2$, alanine aminotransferase, aspartate aminotransferase and gamma-glutamyltranspeptidase) in serum provide a good index of liver damage and are commonly used in the diagnosis of liver diseases. Although ethanol is a well known hepatotoxic agent we did not find any significant evidence for liver damage in rats treated for six months with 10% ethanol. Both bilirubin concentration and the liver enzyme activities were similar to those observed for control animals (27).

In contrast to these observations the activity of prolyl hydroxylase significantly grew beginning from the first month of experiment, both in liver and serum of the investigated animals (24, 25). Following these studies on rats we subsequently attempted to evaluate the activity of prolyl hydroxylase in liver and serum samples obtained from patients with chronic alcoholism, but without symptoms of liver damage. The activity of enzyme from those patients was more than twice that in the control group. It is generally accepted that the enhancement of this enzyme activity parallels the increase of collagen synthesis in many tissues, in various pathological conditions. It allows to conclude that chronic intoxication with ethanol stimulates collagen biosynthesis in the liver.

Such a conclusion has been supported by metabolic studies. We have found that chronic intoxication of rats with ethanol, in the above described conditions, significantly stimulated the incorporation of radioactive proline into liver collagen. The first effects were observed as early as 1-2 months of ethanol administration (26).
It may be concluded from our studies (24-27) and those performed in other laboratories (13,14,20,22) that the increase of collagen production in liver is one of the earliest metabolic changes induced by ethanol in this organ. It precedes morphological (24) and clinical (25) evidences of liver damage induced by this toxin.

On the other hand the collagen content in the rat liver did not increase in the early stage of ethanol-feeding, but increased when ethanol-administration was continued for several months (13, 20). We have found that treatment of rats with 10% ethanol for 6 months resulted in 50% increase of total collagen content in livers of the investigated animals. A change in quantitative relationship between type I, III and V collagens was observed. Proportional amounts of type III and type V collagens were distinctly higher in comparison to those observed in livers of control animals (unpublished results). According to Kato et al (22) this discrepancy may be explained by an increased collagen degradation in the early stage. Although hepatic collagenase activity was not changed in ethanol-intoxicated rats, hepatic collagenolytic cathepsin activity was increased, and correlated with the synthesis of protein-bound hydroxyproline, suggesting an increased turnover of liver collagen in the early stage of ethanol-feeding. Furthermore, Mezey et al (17) observed an increased urinary excretion of collagen-degradation products in ethanol-intoxicated rats.

The mechanism of the stimulatory effect of ethanol on collagen biosynthesis in liver is not known. Holt et al (28) reported that acetaldehyde stimulated collagen biosynthesis by human fibroblasts cultured in vitro. It has been shown that acetaldehyde increases collagen gene transcription in these cells (29). As it is well known that exogenous ethanol increases the level of acetaldehyde in liver (30) it seems possible that this intermediary metabolite may be responsible for the stimulation of collagen biosynthesis in ethanol-damaged liver.

REFERENCES