Biochemical Aspects of Cirrhosis of the Liver

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Cirrhosis of the liver is a disease of man, the end result of many different kinds of prolonged liver injury. It is a complex whose prime characteristics are: (1) destruction of the liver lobule structure and its replacement by nodules, whose hepatocytes, blood vessels, bile ducts and Kupfer cells are no longer in the correct spatial and functional relationship to each other; (2) an increase of fibrous tissue (collagen), which forms bands (septa) between the nodules and penetrates as strands between cells into the nodules; (3) a disturbance of blood flow, leading to an increase of portal venous pressure and formation of porto-systemic shunts, so that blood from the splanchnic circulation is no longer processed by liver cells before entering the general circulation.

As a result of these changes patients with cirrhosis have a short expectation of life. They get jaundice and ascites, and die, usually either of bleeding from the portal venous system or in the coma of liver cell failure, although 10% live long enough to get liver cancers.

In acute hepatic injury after viral, chemical or surgical assault, a small amount, perhaps 10%, of surviving liver is enough to keep the patient going and to act as a base for the regeneration of a normal liver. But the cirrhotic liver may be so irregularly perfused with blood that liver failure, jaundice, coma and death occur in spite of the survival of large amounts of liver tissue in the nodules.

All the normal functions of the liver can be disturbed in cirrhosis. In particular, albumin concentrations fall, bilirubin concentrations rise and, as cirrhosis tends to be part of a continuing process, possibly self-perpetuating, there are signs of liver cell death, in the form of increased activities of transaminases and other enzymes in the blood. Detoxication and excretion of drugs, hormones and bilirubin fail when the disease is severe. In the liver itself there are few changes in enzyme activity once the measurements are corrected for the increased fat, water and collagen contents of the cirrhotic liver (Schmidt & Schmidt, 1970).

Cirrhosis of the liver in man is thought to be caused by a variety of factors, in particular alcoholism, an abnormal, perhaps prolonged, infective hepatitis, bile-duct infection, exposure to a number of toxic substances such as pyrrolizidine alkaloids and some autoimmune disease process.

In a recent Swedish study 3% of autopsies showed cirrhosis of the liver. Half of these were mild and unrecognized until death. Of the remainder half were associated with alcoholism, defined as the consumption of more than 150ml of ethanol/day, and for the rest the cause was essentially unknown (Hållén & Linné, 1970).

In England cirrhosis of the liver accounts for about 0.3% of deaths, i.e. about 1600 people/year [Registrar General’s Statistical Review of England and Wales for 1969 (1971) Part I, Tables Medical, p. 33, H.M.S.O., London]. There seems to be a basal incidence of cirrhosis of the liver in any population, perhaps caused by individual susceptibility to the ubiquitous hepatitis virus. On top of this come the cases caused by ethanol, or whatever toxic factor is prevalent in the local environment, such as bush tea in Jamaica (McLean, 1970) (see Table 1).

It seems that it takes 10–20 years of drinking to produce a good cirrhosis, and, although some increase of prevalence of cirrhosis may be found in people consuming 80ml of ethanol/day, a really striking increase comes with alcoholics (150ml or more/day). In Western countries 40–80% of patients with cirrhosis have a history of high ethanol intake. Perhaps one in 12 alcoholics gets cirrhosis, and once he has developed
Table 1. Ethanol and deaths from cirrhosis of the liver (from de Lint & Schmidt, 1971)

<table>
<thead>
<tr>
<th>Country</th>
<th>Volume of ethanol consumed by drinking population (L/year)</th>
<th>Estimated persons consuming 150ml of ethanol/day (=alcoholics) (% of population)</th>
<th>Cirrhosis death rate (per 100000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>26</td>
<td>9.4</td>
<td>45</td>
</tr>
<tr>
<td>Germany</td>
<td>16</td>
<td>3.9</td>
<td>27</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>12</td>
<td>2.2</td>
<td>18</td>
</tr>
<tr>
<td>England</td>
<td>11</td>
<td>1.9</td>
<td>3</td>
</tr>
<tr>
<td>England Publicans</td>
<td>--</td>
<td>--</td>
<td>26 (total mortality rate = 1200)</td>
</tr>
</tbody>
</table>

the signs of cirrhosis the expectation of life is short: 50% are dead in 2 years, if they do not stop drinking; about 10% of patients develop cancer of the liver (de Lint & Schmidt, 1971; Martini & Bode, 1970).

In this complex situation, where the main variables are time and intensity of liver injury, and where the results are destruction of liver cells followed by liver cell division and regeneration, laying down of fibrous tissue and changes of blood flow, what biochemical approach can be useful?

Experimental studies of cirrhosis

Animals given ethanol develop a mild fatty liver, and a dead liver cell may occasionally be seen, but no cirrhosis.

It is difficult to induce animals to take 40% of their caloric intake as ethanol, as a man consuming 150 ml of ethanol/day is doing, but even when you do so the amount of liver damage is slight, in comparison with the human with acute alcoholic hepatitis.

Cirrhosis of the liver in rats can be produced by causing repeated liver cell necrosis. Cameron & Karunaratne (1936) used CCl₄ for this purpose, and it has been a standard method ever since. The essential procedure is to give a big enough dose to cause necrosis, twice a week, until cirrhosis is produced. The results of biweekly CCl₄ are somewhat irregular and it often takes 15 weeks or more to produce a definite cirrhosis (Rubin et al., 1963; Reuber et al., 1970; Oliver & Sutton, 1966).

We have improved on this method by the addition of phenobarbitone to the rats' drinking water. This stimulates synthesis of cytochrome P-450 and CCl₄ metabolism, and results in a far greater sensitivity to CCl₄ (McLean & McLean, 1965).

In this way we can produce severe disease in all the surviving rats, with cirrhosis, jaundice, ascites and raised portal pressure, after only 8 weeks of CCl₄ treatment (McLean et al., 1969).

Other hepatotoxins can be used to produce cirrhosis, either by repeated dosage or by giving a poison such as a pyrrolizidine alkaloid, which causes a long continued destruction of liver cells. Alternatively a prolonged severe fatty liver caused by feeding on a lipotrope-deficient diet causes cirrhosis in the rat (Rogers & MacDonald, 1965). However, the even more severe fatty liver found in human kwashiorkor and other forms of malnutrition does not lead to cirrhosis. Fibrosis of the liver can also be caused by injection of foreign serum into rats, but no clear-cut 'autoimmune' cirrhosis has been produced experimentally.

In all these methods disruption of liver function by fat or by necrosis is followed first by increased formation of fibrous tissue and then gradually by true nodular cirrhosis. The fibrosis is often reversible, whereas the cirrhosis is not.
Changes in the cirrhotic liver

All the cells in the liver are normally contained in a fine meshwork of fibrils, which undergo a continual turnover of their collagen and mucopolysaccharide molecules. After liver injury there is increased collagen synthesis, and after a single episode of local or diffuse damage the excess of collagen is rapidly removed. Fibrosis of the liver develops after a few more such episodes, but is still reversible. Although enzymic correlates of increased synthesis, such as increased proline hydroxylase activity and increased proline and [35S]sulphate uptake, can be measured, we do not know what causes the fibroblasts to become more active. Nor do we know why collagenase activity then increases until the surplus fibres have been removed. Nor do we know why the process fails in long-continued liver injury (Popper & Udenfriend, 1970; Rojkind et al., 1973).

The end result is that instead of fine fibrils we get broad acellular bands of mature highly insoluble collagenase-resistant fibrous tissue. We have no way of reversing this stage, either in man or in experimental cirrhosis in animals.

It is possible that this growth of fibrous tissue is self-perpetuating in man. Once the liver is broken up into poorly vascularized nodules, collagen synthesis may remain out of step with collagen breakdown.

In our experimental cirrhosis model in rats we find that, once the stimulus of cell destruction by CCl₄ is removed, the structure seems to become fairly stable. The small nodules are slowly replaced by large nodules, but the rats neither recover nor do they deteriorate.

Fibrous granulomas full of collagen can be induced by injection of carrageenin or by mechanical damage in the liver or in other parts of the body. The granuloma grows rapidly with much collagen deposition, and then as the stimulating substance or wound is removed or repaired the fibres of collagen are reabsorbed with equal rapidity. Why does this not happen in the cirrhotic liver (Perez Tamayo, 1965, 1970)?

Soon after liver injury there is a sharp increase in the rate of cell division in both the hepatocyte and the other cell populations in the liver. This increased cell turnover continues during nodule formation, but in the later stage of true cirrhosis dies down again (Rubin et al., 1963; Rogers & MacDonald, 1965; Sutton & Spurgeon, 1966).

Since cirrhosis can be produced either by repeated episodes of necrosis or by prolonged fatty infiltration with little or no necrosis, we can eliminate the events of necrosis as such as being essential to the development of cirrhosis (Judah et al., 1970).

Blood flow in the liver is extraordinary in that a spongy anastomatic system of sinusoids is connected both to arterioles at high pressure and to two venous systems at low pressure, but blood flow is maintained in an organized fashion. Very little is known about the growth and regulation of blood vessels in the liver (McLean, 1970).

We have studied cytochrome P-450 and drug metabolism in cirrhosis (Marshall & McLean, 1969) and find that only in very severe cirrhosis, when death from liver failure is threatening, is there failure of cytochrome P-450 synthesis. At this stage the response to phenobarbital also fails, presumably as a result of effective absence of blood supply to the liver.

In view of our profound ignorance of the cell biology of the liver, it is not surprising that we have no effective ways of preventing or treating cirrhosis, except through avoiding those external factors that cause liver injury.
Liver Cancer: Induction and Treatment

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Classification and incidence of liver cancer

Primary malignant tumours of the liver are divided into carcinomas that arise either from the hepatic parenchymal cells (hepatocellular carcinoma) or from the epithelium of the intrahepatic bile ducts (cholangiocarcinoma) and into mesodermal tumours.

In practice, primary carcinoma of the hepatocellular type is the only malignant tumour of the liver that occurs with any degree of frequency and consequently is the only one of clinical importance. Even this tumour is rare in Europe and America, representing only 3% of all carcinomas. In contrast, however, a very high frequency is seen in sharply defined areas such as Mozambique, Africa south of the Sahara and certain areas of the Far East (Table I; Higginson, 1970). Environmental factors rather than inborn genetic or racial factors are suspected of causing this wide geographical variation in incidence.

Carcinogenic factors

Cirrhosis. Between 60 and 70% of patients with hepatocellular carcinoma have a history of cirrhosis, and the association between the two is well documented although the relationship between them is obscure. Agents causing cirrhosis, such as malnutrition, ethanol, viruses and metazoan parasites, have all been implicated as liver carcinogens, but there is not usually a good correlation between the presence of any one of these factors and the incidence of liver cancer.

Environmental carcinogens. The hepatocarcinogenic action of the coumarin aflatoxins has been well documented after research into outbreaks of fatal liver disease in livestock and sudden high incidences of malignant hepatoma in hatchery trout (Schoental, 1967). In each case the agents responsible were traced to related aflatoxins originating from the mould Aspergillus flavus, a contaminant of a variety of foodstuffs used in animal feeds. Other products of fungi and bacteria are also suspected of causing liver cancer. Both griseofulvin isolated from a strain of Penicillium and ethionine produced by a variety of enterobacteria have been shown to be hepatocarcinogenic under the appropriate conditions.

Cycad plants, used in some areas of the world as a source of edible starch, are known to contain a toxic element that may not be completely removed during processing. This toxic material has been identified as cycasin, the glycoside of methylazoxymethanol, and causes liver cancer in the adult rat, but only when administered by mouth (Laqueur & Spatz, 1968). The aglycone is a carcinogen by