Hepatic Fibrosis in Schistosomiasis

George Y. Wu* and Catherine H. Wu**
*Department of Medicine
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, New York 10461
**Department of Biochemistry
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, New York 10461

Abstract

Schistosomiasis, a disease affecting over 200,000,000 human beings, is caused by infection with one of several species of the Schistosoma trematodes. The infectious form for mammals is the cercaria developed by passage through snails. The major cause of death in those infected results from distortion of the hepatic circulation caused by a unique kind of fibrosis in the liver. The fibrosis occurs in relation to the formation of numerous highly cellular and collagenous granulomata as part of a cell-mediated immune response to eggs deposited by worms in the portal tract. After a period of time, the granulomata disappear as new formation of broad bands of collagen appear in the liver. Because of the nature of those bands the condition is called pipestem fibrosis. The fibrosis distorts liver architecture and with that the circulation of blood in the liver. Prevention of the disease by ecological controls directed largely against the snail vector, would seem to be most important. Failing that, the disease itself is treated largely by use of a number of chemotherapeutic agents directed against the Schistosoma. The present article reviews the modes of treatment now in use, and describes possible means of preventing or reversing the deposition of collagen that constitutes the fibrosis. Research on analogs of proline and lysine to inhibit collagen biosynthesis or stimulate collagen degradation is described. Possible new approaches for prevention of maturation and mating of worms lodged in portal tracts is considered as a means of preventing egg formation and the consequent host-immune response that causes fibrosis.

Schistosomiasis, bilharziasis, is a worldwide problem of immense proportions affecting an estimated 200 million persons (Warren, 1980). It is a tropical disease concentrated among nations of northern and equatorial Africa, South America, Southeast Asia and Japan (Martinez-Baiz, 1953). However, a significant number of cases are appearing in temperate climates of industrialized nations as a result of immigration and foreign travel from endemic areas (Warren et al., 1974).

Three species of Schistosoma cause significant human disease: S. japonicum, S. mansoni and S. hematobium. Among the many signs and symptoms associated with schistosomiasis, hepatosplenic manifestations are largely responsible for the morbidity and mortality of the disease. Approximately 100 million people suffer from liver disease as a complication of S. mansoni and S. japonicum infections. It is no wonder that schistosomiasis ranks as a major cause of liver disease worldwide.

The Parasite

Schistosomes are trematodes that alternate generations between asexual multiplication in intermediate hosts and sexual reproduction in mammalian definitive hosts. The process may be seen to begin with contamination of water by human excretry waste containing schistosome eggs. The eggs hatch into miracidia, a form infectious for certain types of fresh water snails. In this intermediate host, miracidia develop into cercariae, the infective form for mammals. A single miracidium may produce up to 100,000 cercariae. Cercariae emitted by the snails penetrate human skin and migrate to the lungs where they traverse the capillaries and enter the arterial circulation, eventually migrating to the liver. Sexual coupling of the worms occurs in the liver and portal vein. Paired schistosomes then migrate to the mesenteric veins to lay eggs. A single pair can produce from 300 to 3,000 eggs per day. However, more than 50 per cent of that number never leave the body of the host. A large proportion of these eggs appears in the portal blood washed by the flow to the liver and are trapped in the hepatic parenchyma.

Pathogenesis and Pathophysiology

The use of various animal models has contributed greatly to our understanding of how the parasite causes hepatic disease. It is now well established that the initial process in the development of fibrosis is the entrapment of schistosome eggs in the hepatic microcirculation. Microcirculation studies in mice demonstrated that eggs alone do not cause any significant decrease of liver blood flow. However, a granulomatous response involving eosinophils, macrophages, lymphocytes, epithelioid cells and collagen fibers occurs around the eggs (Fig. 1). That results in formation of areas of avascular fibrosis which cause distortion of blood flow. Because the granulomatous response was found to be transferable by injection of lymph node cells or spleen cells but not by serum, the response was proposed to be a cell-mediated reaction (Warren, 1972). Granulomas maintained in vitro were found to elaborate various lymphokines, e.g., macrophage migration inhibitory factor (Boros et al., 1973), eosinophil stimulation promotor (James and Colley, 1975), all of which may be involved in the cell immune regulation. In addition, gran-
ulomas were found to secrete a fibroblast stimulating factor (Wyler et al., 1978).

The number of fibrotic lesions in the liver of an infected person progressively increases with the daily deposition of eggs. Collagen content is increased 10–20 fold over normal (Warren, 1966). Eventually adjacent granulomatous foci coalesce and a "clay pipestem" fibrosis develops. As a consequence of the deposition of the eggs in the portal radicals, the portal vasculature in particular is constricted and portions obliterated. The result is a presinusoidal blockade with splenomegaly and dilated collateral vessels without significant hepatocellular damage. Thus, hepatic schistosomiasis is one of the few chronic liver diseases in which the initial insult is not associated with parenchymal cell necrosis. Parenchymal function remains

Figure 1. A section of a liver from a mouse infected with Schistosoma mansoni. This specimen was taken eight weeks after administration of fifty cercariae. Note the eggs (with lateral spines) grouped in the center of a large granuloma.
Hepatic fibrosis is a consequence of an excessive accumulation of collagen in the liver. Collagens are a heterogeneous group of polypeptides that have certain common properties: 1) An amino acid composition consisting of about 33% glycine, 24% imino acids (proline and hydroxyproline), and the presence of two characteristic hydroxylated amino acids (hydroxyproline and hydroxylysine); 2) amino acid sequence in helical regions with glycine appearing as every third residue; and 3) each collagen molecule consists of a helix of three individual chains wound together to form a molecule of approximately 300,000 daltons.

At present, five relatively well described types of collagen are known (Types I-V) (Bornstein and Sage, 1980). Each collagen contains genetically distinct α chains and has characteristic tissue distribution and properties. In human liver, Types I, III, IV and V are present normally (Popper and Udenfriend, 1970; Chen and Leevy, 1975). By immunofluorescent techniques, Type I is mainly found in the broad bands of collagen in the portal triads; Types III and V are found in the reticulin fibers of the sinusoids (Biem-pica et al., 1980). Normal liver contains approximately equal amounts of Types I, III and V (Rojkind et al., 1979). Type IV is present in small amounts only. The proportions of the various constituent types of collagen have been observed to change with the development of fibrotic disease (Chen and Leevy 1975, Rojkind and Martinez-Palomó, 1976; Rojkind et al., 1979). For example, in mild fibrosis the ratio of Type I to Type III collagens was found to be nearly normal, but with severe cirrhosis due to alcohol, hemochromatosis or Wilson's disease, Type I and the ratio of Type I to Type III were found to increase significantly (Seyer et al., 1977; Rojkind et al., 1979). In experimental schistosomiasis in mice, the ratio of collagen Type I to Type III was found to change from the normal of 1:1 to 2:1 in the late stages of the disease (Wu et al., 1982). Apparently, a temporal pattern of collagen-type composition may be characteristic for a particular etiology of hepatic fibrosis.

Collagen Biosynthesis

1) The Collagen Gene and Transcription

In the past few years, recombinant DNA technology has been used to elucidate the structure of collagen genes. In particular the entire procollagen α2 (pro-α2) gene of Type I collagen (Sobel et al., 1978; Lehrach et al., 1979) and its promoter region (Vogeli et al., 1981) have been sequenced from chicken and sheep. Human Type I pro-α2 genes have been also partially purified (Myers et al., 1981).

The pro-α2 gene is approximately 38-40 kilo base pairs (bp) in length; about eight times longer than the cytoplasmic pro-α2 mRNA that is translated. Sequences of collagen genes have shown that, as in genes of many other proteins, structural regions (exons) exist separated by spacer regions (introns) (Boyd et al., 1980). The coding information in the pro-α2 gene is subdivided into more than 50 exons that are interspersed with introns of various lengths. The pro-α2 gene is unique in that seven out of eight of the exons that code for the helical region of the polypeptide have identical lengths (54 bp). The sequences within these exons vary except for the glycine codon that occurs at every third triplet. It has been suggested that repeated 54 bp coding units imply that an ancestral collagen gene arose from multiple duplications or amplifications of a single genetic unit containing an exon of 54 bp (Yamada et al., 1980). The sizes of the introns vary from 100 bp to 3000 bp. The sequences of the introns also vary considerably except at their 5' and 3' ends (Avvedimento et al., 1980). Sequences at the 5' end have been called "donors" and those at the 3' end "acceptors" (Lerner et al., 1980). Recently, there has been experimental evidence suggesting that a nuclear RNA, U1 RNA, is involved in the mRNA splicing reaction. The 5' end of U1 RNA is complementary with both acceptor and donor ends of introns (Rogers, 1980). This brings the coding sequences that are to be spliced in juxtaposition.

2) Translation

In ways similar to those for other secretory proteins, pro-alpha chains of collagen are synthesized on membrane bound ribosomes. A short hydrophobic sequence, the signal peptide at the amino terminus of the chain, allows the newly synthesized polypeptides to traverse the microsomal membrane into the cisternal space of the rough endoplasmic reticulum (Blobel and Dobberstein, 1975). The signal peptide is removed by cleavage before synthesis of the chains is complete.

There are at present several factors that are considered active in controlling translation of collagen: 1) Pools of prolyl and glycylytRNAs; thus in experimental liver fibrosis induced by CCl4, increased levels of glycylyl and prolyl tRNAs have been detected (Diaz de Leon, 1975). 2) In-
tracellular proline pool; in vitro collagen synthesis in slices obtained from livers of mice infected with schistosomes and from livers of rats made fibrotic by administration of CCI\textsubscript{4} showed a linear correlation between increased collagen synthesis and extracellular proline concentration up to a limiting value (Ehrinpreis et al., 1980; Dunn et al., 1977). In livers of animals and humans with alcoholic liver cirrhosis, increased intracellular levels of free proline were obtained (Kershenobich et al., 1970). In mice with schistosomiasis, arginine was found to be the main-precursor of proline in the liver (Dunn et al., 1978b). Another contributory factor to elevated levels of free proline may be decreased degradation as reflected by decreased levels of proline oxidase (Ehrinpreis et al., 1980).

3) Post-translational Modifications

Procollagen \(\alpha\) chains undergo several rare kinds of post-translational modifications. Certain proline and lysine residues are hydroxylated. This is accomplished respectively by prolyl and lysyl hydroxylases. Lysyl hydroxylase requires molecular oxygen and \(\alpha\)-ketoglutarate as co-substrates, and ascorbic acid and ferrous iron as co-factors (Kivirikko and Prockop, 1972). The substrate is a lysine residue linked to the amino group of a glycine residue in the molecule. There are at least two prolyl hydroxylases, both of which require ferrous iron, molecular oxygen, L-ascorbic acid, and \(\alpha\)-ketoglutarate (Cardinale and Udenfriend, 1974). The proline residue in the third position (Y) of the Gly-X-Y sequences is preferentially hydroxylated. Underhydroxylated polypeptides, such as could occur in ascorbic acid deficiency, cannot form proper procollagen helices and are not secreted. Galactosyl and glucosyl transferases, using UDPgalactose and UDPglucose, transfer the respective sugar moieties. Galactose is linked directly to the hydroxyl group of certain hydroxylsine residues (Spiro and Spiro, 1971); the glucose residues, when present, are always attached to a galactose unit. After hydroxylation and addition of sugars, the procollagen peptide chains spontaneously form a triple helix prior to secretion from the cells.

4) Secretion

The actual process of secretion requires packaging into Golgi-derived vesicles before exocytosis (Weinstock and Leblond, 1974). Golgi apparatus, secretory vesicles and microtubules are needed for transport. The procollagen that is secreted has propeptide extensions at both the amino and carboxyl termini. The molecular weight of the amino terminus is 15,000–20,000 daltons and that of the carboxyl end is 35,000–40,000 daltons. After secretion, specific peptidases cleave the propeptides from the main molecules (Duksin and Bornstein, 1977). The amino-terminal propeptides have been shown to exert a feed-back inhibition of collagen synthesis in the human fibroblast system (Wiestner et al., 1979) Similar inhibition of collagen synthesis by carboxyl-terminal propeptides has been demonstrated recently (Personal communication, Koda, K., Betheil, J.J., and Seifter, S.). The propeptide extensions appear to serve several functions: 1) Ensure proper secretion; 2) ensure proper alignment of the individual chains to form the triple helix; 3) prevent intracellular fibrillogenesis; and 4) regulate synthesis by feedback control.

5) Collagen Fibril Formation

After cleavage of the terminal propeptides, collagen molecules in the extracellular space aggregate as fibrils stabilized by intra- and intermolecular crosslinks (Gallop et al., 1972). Lysyl oxidase, a copper requiring enzyme (Siegel and Fu, 1976), is responsible for oxidative deamination of the \(\epsilon\)-amino groups of selected lysyl and hydroxylysyl residues. Reactive aldehydes, thus formed, enable covalent crosslinks to occur by formation of Schiff bases and of aldol linkages between chains. Lathyrergic agents such as \(\beta\)-aminopropionitrile inhibit lysyl oxidase, prevent crosslinking and cause collagen of poor tensile strength to be deposited. The condition is known as lathyrism. A genetic equivalent of lathyrism is found in certain types of Ehler–Danlos syndromes.

6) Collagen Degradation

Native triple-helical collagens are cleaved at specific sites by tissue collagenases at physiological pH and temperature. Cleavage occurs in each of the 3 \(\alpha\) chains near the carboxyl terminus and results in 75,000 and 225,000 dalton fragments. Collagenases are usually present in inactive forms and, under experimental conditions, require some kind of activation either dissociative or proteolytic. The mechanism of activation of latent tissue collagenase \textit{in vivo} is not known at present. Liver collagenolytic activity has been studied in normal and fibrotic liver (Takahashi et al., 1980). In the case of hepatic schistosomiasis, collagenolytic activity is markedly increased in experimental animals several months after infection. However, that activity decreases and becomes normal in late stages of the disease.

Assessment of Hepatic Fibrosis

The assessment of liver involvement in schistosomiasis is difficult since, at present, no fool-proof non-invasive method exists for determining the extent of hepatic fibrosis. Ideally, a test for hepatic fibrosis should provide information about both the quantity of collagen present and the rate of collagen accumulation or diminution. In that way, the status and prognosis of the liver disease can be assessed.

Liver biopsy with histological staining for collagen has been used extensively in the past. That kind of estimation is basically descriptive. Use of specific anti-procollagen sera in biopsy specimens has been shown to provide
The activity of prolyl hydroxylase was observed to increase in several conditions associated with increased collagen deposition. It was proposed that assay of the enzymatic activity might reflect the rate of collagen synthesis (Kivirikko and Ristelli, 1976). Prolyl hydroxylase measurements of liver specimens in animal models of hepatic injury as well as in patients with various liver diseases were elevated (Takeuchi et al., 1969; Ristelli and Kivirikko, 1974). Measurement of serum prolyl hydroxy-lase activity has been studied as a means of assessing fibrotic activity of the liver. The activity was significantly elevated in the majority of patients with active hepatic fibrosis and also in those with ongoing hepatic inflammation. Values did not correlate with collagen content of biopsied specimens as measured by hydroxyproline. Nevertheless, this method was considered to indicate hepatic collagen synthesis (Tuderman et al., 1977; Kuutti-Savolainen et al., 1979a).

Another collagen-associated enzyme, galactosyl hydroxy-lysyl glucosyl transferase, was assayed in serum of patients with various liver diseases. The results were similar to those obtained in studies of prolyl hydroxylase in terms of some correlation of elevated activity in fibrotic liver diseases and active parenchymal damage (Kuutti-Savolainen et al., 1979b).

Finally, lysyl oxidase, which initiates the extracellular crosslinking of collagen molecules, has been studied in experimental hepatic fibrosis. Its activity correlated with the formation of early connective tissue septa as determined histologically. The increase in lysyl oxidase activity was greater in magnitude than prolyl or lysyl hydroxylases (Siegel et al., 1978; Ristelli and Kivirikko, 1976). Similar findings in human liver tissue were noted (Fuller et al., 1976).

Recently, antibodies to the non-helical peptide extensions of the termini of procollagen have been obtained and a radioimmunoassay developed for their measurement. Although the collagens used were from animals, significant cross-reactivity of the antisera with human procollagens permits detection of propeptides in human serum (Taubman et al., 1974; Rohde et al., 1976). Radioimmunoassay has been performed using sera of persons with liver dys-

function. The majority of patients with active liver disease of various etiologies had elevated amino-terminal pro-peptide levels in serum. Inflammation and necrosis in biopsies from patients with alcoholic liver disease correlated best with elevation of propeptides (Rohde et al., 1978). The presence of increased levels of collagen propeptides is taken as evidence of increased synthesis and secretion of collagen as part of a fibrotic process.

### Therapy

#### 1) Schistosomicides

The major objective of therapy has been the killing and elimination of adult worms to stop further deposition of eggs. A selection of drugs and their actions is shown in Table I. Results of several clinical trials have been reviewed recently (Katz, 1980; Pieron, 1980). The effectiveness of a particular chemotherapeutic regimen depends on the drug, parasite characteristics and host factors. These topics cannot be discussed in detail in this article. However, several interesting aspects deserve comment.

Although many drugs are called schistosomicides, some experts think that, in most instances, the parasite is not killed by the action of the drug alone. For example, in one study antimonials and oxamniquine, or their active metabolites, never reached levels in the blood known to be sufficient to kill the parasite in vitro (Foster and Cheetham, 1973). A mechanism by which therapeutic agents may cause destruction of worms without directly killing them was noted in early studies with antimonials. Following injection of the drugs intravenously in man, a rapid displacement of worms from the mesenteric veins to the portal venules was observed. Most of the parasites were trapped within 30 min. (Goldsmit et al., 1967). Following the shift, a tissue reaction was noted surrounding worms in the liver in experimental mouse infections. That reaction consisted of leukocytic infiltration that then destroyed female schistosomes. A connective tissue reaction characterized the response to the trapped male worms (Streibel and Kradolfer, 1966). Thus, the drug effects that cause the hepatic shift as well as the host tissue reaction appear to be factors in the therapeutic response of many schistosomicidal agents.

In general, to be effective, schistosomicidal drugs apparently must be absorbed by the parasite. Considerable variability exists in terms of absorption of various agents among the different species of schistosomes, and even between different sexes of the same species. For example, antimonials, niridazole (Hess et al., 1966), and hycanthone (Yanisky et al., 1970) showed greater uptake by female as compared to male worms. That was considered to have contributed to the relative ease with which females succumbed to drugs in early experiments.
There is evidence that the stage of maturity of the worms is also an important factor in determining susceptibility to drugs. Experiments using antimonials demonstrated a lack of activity in animals infected with immature worms up to 28 days after exposure. The activity became 100% at 49-56 days. Furthermore, in unisexual infections, sexual maturity was not achieved, and none of the worms was noted to be susceptible even after an exposure of 173 days. Since children are more frequently exposed to re-infection than adults, and therefore more likely to harbor an immature worm population, a maturity-related sensitivity might contribute to the relative resistance of infections in children.

Differences in susceptibility of different Schistosoma species to different agents have been noted but not well explained. In addition to interspecies variability, various strains within the same species also appear to differ in their susceptibility to the same drug (Nelson and Saoud, 1968), perhaps accounting for some of the reported variable clinical responses among patients infected with the same species of parasite.

Age and weight of patients have also been reported to affect the outcome of therapeutic regimens. Children, as mentioned previously, have been found to be less responsive to treatment than adults (Newsome, 1956). The mechanism of that difference is undoubtedly complex and may involve differences in immune status, intensity of infection, re-infection, as well as status of hepatic metabolic function.

2) Anti-fibrotic Agents

Although anti-schistosomal drugs are designed to prevent further damage to the liver, they do not directly address the fibrosis and inflammatory processes already existing. On the other hand, anti-fibrotic agents are designed to reduce fibrosis and reverse the fibrotic process. Such agents are meant to take advantage of requirements and processes that are relatively specific for biosynthesis or degradation of collagen. Thus, potential anti-fibrotic agents may be directed against pro-a chain biosynthesis, hydroxylation and glycosylation reactions that are intracellular post-translational modifications, secretion of procollagen, processing of procollagen to collagen, and extracellular fiber formation and crosslinking. Another aspect of anti-fibrotic strategy is development of agents that may stimulate production or activation of collagenases. Experimentation with potential anti-fibrotic agents has been

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**Table I. Anti-Schistosomal Drugs**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Actions on schistosomes</th>
<th>Susceptible species</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium antimony tartrate</td>
<td>inhibition of schistosome glycolysis (Mansour and Bueding, 1954)</td>
<td>S. hematobium</td>
<td>nausea and vomiting; dyspnea; arthralgia; EKG changes</td>
</tr>
<tr>
<td>Stibophen</td>
<td>rapid inhibition of attachment to venous walls with passive shift to liver (Buttle and Khayyal, 1962)</td>
<td>S. mansoni</td>
<td></td>
</tr>
<tr>
<td>Antimony lithium thiomalate</td>
<td>inhibition of monoamine oxidase (Kim et al., 1981)</td>
<td>S. mansoni</td>
<td>mutagenesis (experimentally)</td>
</tr>
<tr>
<td>Sodium antimony gluconate</td>
<td>interference with normal neuromuscular activity (Senft and Hillman, 1973)</td>
<td>S. hematobium</td>
<td></td>
</tr>
<tr>
<td>Antimony dimercaptosuccinate</td>
<td>inhibition of monoamine oxidase (Kim et al., 1981)</td>
<td>S. mansoni</td>
<td></td>
</tr>
<tr>
<td>Hycanthone</td>
<td>stimulation of glycogenolysis (Bueding and Mansour, 1957)</td>
<td>S. hematobium</td>
<td>neuropyschic disturbances: confusion, psychosis, hallucinations, convulsions</td>
</tr>
<tr>
<td>Niridazole</td>
<td>damage to the female reproductive system</td>
<td>S. japonicum</td>
<td></td>
</tr>
<tr>
<td>Oxamniquine</td>
<td>motor paralysis (Woolhouse, 1979)</td>
<td>S. mansoni</td>
<td>dizziness; nausea; headache; drowsiness</td>
</tr>
<tr>
<td>Praziquantel*</td>
<td>inhibition of cholinesterases (Kim et al., 1981)</td>
<td>S. hematobium</td>
<td>dizziness; nausea; drowsiness; headache; diarrhea</td>
</tr>
<tr>
<td>Amoscanate*</td>
<td>not determined</td>
<td>S. hematobium</td>
<td></td>
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*Experimental*
fairly extensive using several models of hepatic fibrosis. Few have been applied to experimental schistosomiasis.

In studies using the CCl₄-induced hepatic fibrosis model in rats, administration of 3,4-dehydroproline was found to reduce the formation of collagen in the liver. In addition, the hepatic pool of free proline was observed to be reduced in relation to non-treated specimens. Non-collagenous protein synthesis was found to be normal (Rojkind, 1973). Administration of azetidine 2-carboxylic acid to schistosome-infected mice caused a concentration-dependent decrease in hepatic protein-bound hydroxyproline. In that case, also, a reduced concentration of free intracellular proline was measured (Dunn et al., 1977).

Table II summarizes properties of selected anti-fibrotic agents.

<table>
<thead>
<tr>
<th>Table II. Anti-fibrotic Agents</th>
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<tbody>
<tr>
<td><strong>Hydroxylase Inhibitor</strong></td>
</tr>
<tr>
<td>8,9-dihydroxy-7-methylbenzoquinodizinium bromide</td>
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<tr>
<td><strong>Proline Analogs</strong></td>
</tr>
<tr>
<td>Azetidine 2-carboxylic acid</td>
</tr>
<tr>
<td>3,4-Dehydroproline</td>
</tr>
<tr>
<td>cis fluoroproline, cis bromoproline, cis hydroxyproline</td>
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<tr>
<td><strong>Lysine Analogs</strong></td>
</tr>
<tr>
<td>4,5-Dehydrolysine</td>
</tr>
<tr>
<td>Aminopenthycysteine</td>
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<tr>
<td><strong>Croslink Inhibitors</strong></td>
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<tr>
<td>β-Aminopropionitrile</td>
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<tr>
<td>Penicillamine</td>
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<tr>
<td><strong>Anti-Inflammatory Agents</strong></td>
</tr>
<tr>
<td>Colchicine</td>
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<tr>
<td>Corticosteroids</td>
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</tbody>
</table>

**Mechanism of actions**
- iron chelation
- competition with proline for transport and for prolyl tRNA
- competition with proline for prolyl tRNA
- competition with lysine for lysyl tRNA
- competitive inhibition of lysyl oxidase
- combines with free aldehydes in collagen; bound by lysyl oxidase; inhibits lysyl oxidase at high doses
- interferes with microtubule assembly; inhibits transcellular movement; may increase production and release of collagenase

**Effects**
- reduced conversion of proline to hydroxyproline
- reduced hydroxylation of lysine and proline residues, reduced glycosylation of hydroxylysine residues
- reduced collagen extrusion
- decreased collagen synthesis and extrusion
- increased collagen solubility and susceptibility to collagenase
- increased collagen solubility and susceptibility to collagenase
- reduced collagen extrusion

**Selected references**
- Dunn et al., 1978a
- Fowden and Richmond, 1963
- Dashman et al., 1979
- Uitto and Prockop, 1974
- Christner and Rosenbloom, 1971
- Jimenez et al., 1979
- Orbison, 1975
- Siegel et al., 1977
- Diegelmann and Peterkofsky, 1972
- Ehrlich et al., 1974
- Oikaninen, 1977

1) **Amino Acid Metabolic Alterations in Hepatic Fibrosis**

Because of the importance of proline as a structural component of collagen and the evidence relating proline concentrations in cells to collagen synthetic rates, the precursors of proline and their relative contribution to regulation of the proline reservoir in fibrogenesis is important to determine. Our laboratory, in collaboration with Dr. Sheila Cohen at Merck Laboratories, is examining the metabolic fluxes of amino acids in intact livers, normal and fibrotic, by using 13C NMR spectroscopy. While there are difficulties involved in older techniques requiring isolation of free amino acids and their quantification, the NMR method possesses significant advantages in permitting the examination of intact liver by maintaining the organ in a continuous closed perfusion system while the sample is held in a specially designed probe. Precursors enriched with 13C at particular positions in the molecule are perfused and metabolic conversions of compounds are followed by changes in chemical shifts of the specific label.

Using a similar, more conventional approach, Dr. Sam Seifter is using specifically radiolabelled precursors to applied biochemistry, pharmacology and clinical medicine.
study proline biosynthesis. Thus far, our most recent results confirm earlier in vitro studies in that arginine and ornithine can serve as substrates for the formation of proline. Labelled glucogenic substrates were found to be converted to glutamic acid and glutamine but not to proline suggesting that glutamic acid has little if any direct role in proline biosynthesis in fibrotic liver. The evidence indicates that the only detectable intrahepatic amino acid source of ornithine, the most immediate stable proline precursor, is arginine. Since arginine is an essential amino acid, the biosynthesis of proline in fibrotic liver appears to be exclusively dependent on delivery of arginine or ornithine from extrahepatic sources. Considering the tremendous requirement for proline in fibrogenesis, the dependence of the fibrotic liver on such a limited number of precursors is a remarkable phenomenon.

2) Effects of New Lysine Analogs in Collagen Accumulation in Hepatic Fibrosis

Because of the importance of lysine in hydroxylation, glycosylation and cross-linking functions in collagen, lysine analogs have several possible steps where they might interfere with the formation of a complete functional molecule. If lysine-like compounds could be made such that they are incorporated and act as natural lysine except that collagen-associated functions are inhibited, structural abnormalities could be made relatively specific for collagen, and decreased synthesis or increased degradation might result.

In our laboratory, we have chemically synthesized new lysine analogs that differ from natural lysine only in the substitution of a heteroatom in the α position. These analogs were administered to granuloma cultures derived from livers of schistosome-infected mice. Preliminary results show a significant reduction in collagen biosynthesis as measured by the conversion of proline to hydroxyproline. The effects were found to be directly related to the concentration of analog present in the culture medium. Synthesis of proteins other than collagen was also reduced but to a lesser extent. Further experiments are in progress to determine whether or not changes occur in the structure of proteins and how that affects the relative contribution of early degradation to the overall accumulation of newly formed collagen.

3) Colchicine

Colchicine has been studied in the treatment of hepatic fibrosis due to alcohol (Rojkind et al., 1973). Although the number of patients treated was small, colchicine appeared to have had a beneficial effect in spite of continued alcohol intake. Those promising early studies generated much interest concerning the possible applicability of colchicine in treatment of hepatic fibrosis due to other etiological agents. To answer that question, a large multicenter clinical study is under way; included in the patient population will be a significant number of cases of hepatic fibrosis due to schistosomiasis. Our laboratory, in collaboration with Dr. Michael Dunn at the Walter Reed Hospital, will attempt to study the usefulness of procollagen peptide radioimmunoassay in assessing the severity and activity of the fibrosis and hope to correlate levels of procollagen peptides in serum as a function of response to therapy. Such antibodies have been developed by Drs. Koda, Betheil and Seifer at our institution.

4) Schistosome Biology

We are presently investigating several exciting aspects of schistosome biology that may provide new approaches to therapy. For example, little is presently known about how male and female schistosomes are able to locate each other in the myriad of venules in the liver. There is evidence that pheromone-like attractants are secreted by the worms and such materials stimulate worm migration toward the source (Imperia et al., 1980). In addition, it is known that female Schistosoma do not mature in vivo without the presence of males. The implication is that male parasites transfer a critical substance(s) to the females and, without fulfillment of this requirement, egg production cannot be accomplished.

Our laboratory is interested in determining the nature of the attractant(s), maturation requirements and their mechanism of action with the intent of developing agents that interfere with the normal action in vivo. Prevention of successful mating will preclude egg production and major sequelae of hepatic fibrosis.

Conclusion

Human schistosomiasis is an important cause of disease worldwide. Much of the morbidity and mortality is due to hepatic fibrosis as a result of a host cell-mediated immune reaction to the schistosome eggs trapped in the portal venules.

Significant progress has been made in understanding the events that govern the process of fibrogenesis. However, our state of knowledge of the process still leaves a great deal to be desired. Major unknowns exist such as: The immune factors involved in the stimulation of collagen production, the cell types responsible for the major share of collagen production, the mechanism of collagen gene selection, exon splicing mechanisms, variables regulating the proportions of the different types of collagen during the stages of the disease, and the signals required to guide the molecules from golgi vesicles to the extracellular space.

Clearly, an improved understanding of the various aspects of schistosomal infection and host reaction culmi-
nating in the deposition of collagen is necessary to develop and implement efficacious therapeutic measures. By exploiting processes that are relatively specific for collagen synthesis or degradation, it may be possible to produce agents that specifically inhibit or reverse collagen accumulation and thereby offer the hundreds of millions afflicted with the hepatic fibrosis an effective therapy in the future.

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