Hepatic cirrhosis—a collagen formative disease?

J. O'D. McGEE AND A. FALLON

From the University of Oxford Department of Pathology, Radcliffe Infirmary, Oxford

In the 1940s Klemperer grouped together as 'collagen diseases' a number of conditions such as systemic lupus erythematosus, scleroderma, and dermatomyositis. This idea was founded on the fact that a pathological lesion in these conditions was discernible in the connective tissues of the body. It is now clear, however, that there is no significant integral change in collagen in systemic lupus or in dermatomyositis although there is an apparent increase in the collagen content of some connective tissues in scleroderma. The designation 'collagen diseases' for this group of conditions is therefore inappropriate. If the term 'collagen disease', even in modified form, is to be retained it should more properly be applied to diseases in which measurable qualitative or quantitative abnormalities in collagen are a significant feature of the disorders in question.

Those diseases in which there is a qualitative change in the collagen molecule, as in some inherited disorders of collagen metabolism (see F. M. Pope and A. C. Nicholls (Pope and Nicholls, 1978) at page 95) could be conceived of as collagenopathies by analogy with the inherited disorders of haemoglobin metabolism, the haemoglobinopathies. Although those collagenopathies due to an enzyme deficiency are not strictly analogous to the qualitative haemoglobinopathies (where there is an amino-acid substitution in haemoglobin), the end result of the deficiencies is that the amino-acid sequence of collagen is altered. On the other hand, there are a number of diseases in which quantitative changes in the collagen content of tissue is of paramount importance in the destruction of organ architecture and functions. These diseases can collectively be termed collagen formative diseases. Some of the diseases which fit into this category are chronic valvular disease of the heart, atherosclerosis, dust diseases of the lungs, chronic nephritis, and cirrhosis.

Effects of collagen deposition

In all these diseases collagen is deposited in large quantities. The morphological residue of this process is obliteration of normal organ architecture. In some instances the functional significance of the process is

also clear. Valve distortion and malfunction in chronic rheumatic heart disease is mainly due to excessive collagen deposition. Intimal fibrosis in atherosclerosis, particularly of medium sized arteries such as those of the coronary circulation, contributes significantly to arterial narrowing and consequential ischaemia. Respiratory reserve is compromised in lung dust diseases by the fibrous obliteration of alveolar air spaces. The functional significance of collagen deposition is less clear cut in the nephritides and hepatic cirrhosis. However, the functional significance of collagen in the normal and abnormal glomerulus cannot be trivial when it is appreciated that a major protein component of basement membrane is type IV collagen (Kefalides 1971) and that the hyalinised glomeruli of the end-stage kidney are conglomerates of types IV collagen and interstitial collagen (Nagle *et al.*, 1960).

Deposition of collagen in the liver in cirrhosis has two functional effects. Firstly, it completely disrupts the normal hepatic architecture so that the normal relationship between vascular inflow and outflow is destroyed. This leads to portal/hepatic venous and portal/hepatic arterial shunting of blood. Much of the vascular inflow therefore bypasses the nodular parenchyma (Hase, 1968). Intrahepatic shunts also contribute to the development of portal hypertension. Secondly, the normal collagen content around hepatic sinusoids in regenerating nodules becomes modified. In the normal liver the sinusoids form an open system where plasma has free access to the sinusoidal villous border of the hepatocyte (Fig. 1). In regenerating nodules, however, this open system becomes a closed one in which sinusoids are converted to capillaries with a subjacent basement membrane (Schaffner and Popper, 1963). In addition to the probable deposition of type IV collagen as part of these basement membrane complexes, large amounts of interstitial type I or type III collagen with a 680 Å periodicity are deposited in the space of Disse (Fig. 2). At present we can only speculate on the effect a basement membrane and increased amounts of interstitial collagen in the space of Disse have on plasma hepatocyte interchange but it seems reasonable to suppose that they have an effect.

Fig. 1 Ultrastructure of a normal sinusoid. Sinusoid lined by flattened sinusoidal cells. Arrow shows gap between adjacent sinusoidal lining cells. Part of a perisinusoidal cell (PSC) and a hepatocyte (H) are also shown. Note floculent plasma protein in vessel lumen and space of Disse. ($\times 38\,000$)

Liver collagen increase in cirrhosis

On theoretical grounds the collagen content of the liver in cirrhosis could increase because of an increase in the rate of collagen synthesis or a decrease in the rate of collagen degradation, or by a combination of both processes. In experimental hepatic fibrosis induced by carbon tetrachloride there is good evidence that the fibrosis in this model is due to increased collagen synthesis (Fig. 3). Not only does the rate of collagen synthesis increase but there is a parallel increase in the level of hepatic prolyl hydroxylase (Table 1). Similarly, in regenerating rat liver after hepatectomy there is a direct correlation

between an increased rate of collagen synthesis and prolyl hydroxylase (Table 2). We may reasonably suppose, therefore, that a rise in hepatic prolyl hydroxylase concentration is a reliable index of increased collagen synthesis, at least in liver. Indeed, raised levels of prolyl hydroxylase (Table 3) have been reported in human liver disease associated with fibrosis (McGee *et al.*, 1974). More recently, in a study of human alcoholic liver disease (Table 4), a direct correlation has been shown between the rate of collagen synthesis measured directly on biopsy tissue and the hepatic level of prolyl hydroxylase (Vidins *et al.*, 1978). There is evidence, therefore, that the increase of collagenous tissue found in human and

Fig. 2 Ultrastructure of cirrhotic sinusoid. Note massive amounts of collagen in space of Disse, a perisinusoidal cell (PSC), part of a hepatocyte (H), and golgi complex in the PSC. ($\times 38\,000$)

experimental liver disease can be explained by an increase in the rate at which the liver synthesises this protein. At present there is no reliable data indicating that decreased collagen degradation plays any part in collagen accumulation in cirrhosis.

Increased collagen production in liver disease could conceivably be due to increased collagen synthesis per 'fibroblast' or to an increase in the number of collagen-producing cells, all of which are functioning at a physiological rate. *A priori*, however, it seems more likely that a combination of both these mechanisms would operate in liver disease. Consider-

ing each mechanism separately, there are factors which may be responsible for the increase in the collagen productivity of individual cells. Chen *et al.* (1974) have claimed that when lymphocytes from patients with alcoholic hepatitis, hepatic schistosomiasis, and chronic active hepatitis are exposed to autologous liver *in vitro* they release macrophage inhibition factor and another 'lymphokine' which stimulate collagen synthesis in fibroblasts up to two-fold. The specificity of this 'lymphokine' effect was not checked, however, since total protein synthesis in the fibroblast test system was not examined.

Fig. 3 Collagen synthesis in acute carbon tetrachloride injury. Collagen synthesis was measured (as described by McGee *et al.*, 1973) in mouse liver at daily intervals after a single dose of oral carbon tetrachloride. Data in injured liver expressed as a ratio of the value in normal control livers.

Collagen-stimulating factors

Other factors extracted from fibrotic livers have been described which have a profound effect on the synthesis of collagen by fibroblasts in culture. These

Table 1 Prolyl hydroxylase and collagen synthesis in mouse liver three days after carbon tetrachloride

Treatment	Prolyl hydroxylase* (CPM $\times 10^{-3}$ /g)	Collagen synthesis† (CPM/g)
Nil	745	510
CCl ₄	2448	1731

*Prolyl hydroxylase measured as described by McGee *et al.*, 1974.

†Collagen synthesis measured as described by McGee *et al.*, 1973.

Table 2 Prolyl hydroxylase and collagen synthesis in rat liver 48 hours after partial hepatectomy (data from Benjamin *et al.*, 1978)

Procedure*	Prolyl hydroxylase†	Collagen synthesis‡
Sham operation	1.0	1.0
Partial hepatectomy	1.6	1.8

*Rats either had a sham operation which involved opening the abdomen and handling the liver or a 68% partial hepatectomy.

†Prolyl hydroxylase measured as described by McGee *et al.*, 1974. Data expressed as the ratio of the enzyme level in sham operated to control livers.

‡Collagen synthesis measured as described by McGee *et al.*, 1974. Data expressed as a ratio of collagen synthesis in the regenerating liver (after partial hepatectomy) to the synthesis rate in control normal livers.

Table 3 Prolyl hydroxylase concentrations in normal liver and hepatic cirrhosis

Diagnosis	Prolyl hydroxylase (CPM/mg protein)
Normal	1158 (726, 893, 1459, 1553)
Gilbert's syndrome	1310 (946, 1674)
Cirrhosis	7537 (3585, 6000, 9144, 9366, 9588)

Prolyl hydroxylase was measured in needle biopsies of liver as described by McGee *et al.*, 1974. Normal livers were obtained at laparotomy on patients undergoing surgery for duodenal ulcer.

Table 4 Correlation coefficient for human liver biopsy prolyl hydroxylase activity versus collagen synthesis determined as the amount of ³H-proline incorporated into collagenase digestible protein during a 24-hour incubation (data from Vidins *et al.*, 1978)

N=32	Prolyl hydroxylase (mU/mg protein)	CPM collagen (μ g/DNA)	Correlation coefficient	P
Range	0.236-7.544	14.1-405.7	0.61	<0.005

factors are readily extracted from liver by homogenisation in phosphate buffer and can be partially purified by molecular sieve chromatography on G25 Sephadex (McGee *et al.*, 1973). These collagen-stimulating factors are assayed by their ability to increase collagen synthesis in fibroblasts or by their ability to stimulate prolyl hydroxylase activity in the same cells (McGee *et al.*, 1973). They are readily obtained from experimental models of hepatic fibrosis induced by carbon tetrachloride but not from normal livers. The factors, labelled F1, F2, F3, and F4 in order of elution from G25 Sephadex, increase collagen synthesis up to eightfold (Fig. 4). Similar fractions from normal liver homogenates produce virtually no stimulation (Fig. 4). The factors have a specific effect on collagen synthesis itself; they have no effect on general protein synthesis in the same cultures when fractions from damaged and normal livers are compared (Fig. 5).

All the factors also stimulate prolyl hydroxylase activity four- to fivefold in the same cells (McGee *et al.*, 1973). They are trypsin-sensitive (Table 5) and heat-stable (Fallon and McGee, 1978), therefore they may be peptides. They not only increase the rate of collagen synthesis in cells but also produce a four- to sixfold increase in the amount of collagen secreted by the same cells (Table 6). Similar factors have now been found in human cirrhotic liver and in healing wounds (O'Hare and McGee, 1978) and in experimental pulmonary fibrosis (Thomson and Patrick, 1978) but not in the respective normal tissues. It seems, therefore, that collagen-stimulating factors may have a general role to play in collagen formative diseases as the chemical mediators of increased collagen production.

Fig. 4 G25 chromatogram of collagen-stimulating factors. Extracts of normal liver (●—●) and liver damaged by one dose of CCl_4 (○—○) were chromatographed and 1-ml aliquots of selected fractions assayed for collagen-stimulating activity by incubation with replicate fibroblast cultures. After three hours' incubation each culture was pulsed with 3, 4, ^3H -proline and collagen synthesis measured as previously described (McGee et al., 1973). (Data from Fallon and McGee, 1978.)

Fig. 5 Protein synthesis in L-929 fibroblasts after incubation with 1-ml aliquots of selected fractions from a G25 Sephadex chromatogram of normal (●—●) and CCl_4 -damaged mouse liver (○—○). This is the same chromatogram shown in Fig. 4 but after the culture was pulsed with 3, 4, ^3H -proline as in Fig. 4. Total protein synthesis was measured. (Data from Fallon and McGee, 1978.)

The origin of collagen-stimulating factors is not known, but there are at least three obvious sources in liver disease. They may be derived from necrotic hepatocytes, from the degradation of some preformed inactive precursor in plasma (like the kinins), or they may be generated by macrophages which are active in all the diseases where these factors have been found. In relation to the macrophages, it is interesting to recall the work of Heppleston and Styles

Table 5 Trypsin sensitivity of collagen-stimulating factors*

Addition	Trypsin	Collagen synthesis (CPM $\times 10^{-3}/10^6$ cells)
Nil	—	27
Nil	+	26
Buffer	—	20
Buffer	+	20
F1	—	68
F1	+	25
F2	—	57
F2	+	18

*Collagen-stimulating factors were isolated by molecular sieve chromatography. One ml of F1 or F2 or column buffer was added to replicate cultures without pretreatment or after digestion with trypsin (1 mg/ml) at 37° for 30 minutes. Before addition to the cultures trypsin was inactivated with soya bean inhibitor (final concentration 0.8 mg/ml). Cultures were incubated with normal or trypsin-treated factors for 6 hours and collagen synthesis measured as in legend to Fig. 3. (Data from Fallon and McGee, 1978.)

Table 6 Effect of collagen-stimulating factors on collagen synthesis in fibroblasts*

Addition	Cell layer collagen (CPM $\times 10^{-3}/10^6$ cells)	Medium culture (CPM $\times 10^{-3}/\text{culture}$)
Nil	9.8	2.1
Buffer	7.7	2.4
F1	23.8	13.2
F2	28.0	9.4

*Collagen-stimulating factors (1 ml of F1 or F2) were added to replicable fibroblast cultures and incubated for three hours. Each culture was then pulsed with $10 \mu\text{Ci}$ of 3, 4, ^3H -proline for two hours and the amount of labelled collagen in the cell layer and in the medium measured separately (from Fallon and McGee, 1978).

(1967) who described a factor, released by silica-treated macrophages, that greatly increased hydroxyproline production by fibroblasts. We have tried to repeat this experiment without success. But it remains an attractive idea that the macrophage factor(s) is similar to collagen-stimulating factor isolated from liver and other tissues.

Collagen-producing cells

Collagen synthesis in liver disease might increase via an increase in the number of collagen-producing cells, all of which may function at a physiological rate. It is generally accepted that cells with the typical ultrastructure of fibroblasts are found in portal tracts in normal liver. Nevertheless, there was debate about the existence and type of cell within the liver lobule which is responsible for collagen production. There is now evidence which suggests that the perisinusoidal cell (Ito cell, fat storage cell) is the intralobular fibroblast. This evidence is based mainly on its ultrastructural features (McGee and Patrick, 1972). It contains a well-developed rough endoplasmic

Fig. 6 Perisinusoidal cell in normal liver. Note perisinusoidal cell in space of Disse wedged between two hepatocytes (H) and containing abundant rough endoplasmic reticulum which contains a flocculent material. Lipid vacuole (L) also present. ($\times 17\,000$)

reticulum and a Golgi apparatus, and displays a consistent spatial association with collagen fibrils (Figs 2 and 6).

The cells also contain lipid droplets (Fig. 6)—a feature particularly prominent in man—which contain high concentrations of vitamin A. Although lipid droplets are a frequent finding in collagen-producing cells in a variety of tissues (McGee and Patrick, 1972) the function of the lipid in intercellular matrix development is not known. In fibrogenic liver disease the number of fibroblasts in portal tracts increase. It has also been shown that perisinusoidal cells increase in number in acute (McGee and Patrick, 1972) and chronic carbon tetrachloride

injury (Kent *et al.*, 1976) and that this increase is associated with fibrosis. As yet, nothing is known about the stimulus responsible for this proliferative response.

There are hints in other collagen formative disorders that macrophages release a factor(s) which may induce fibroblast proliferation (Leibovich and Ross, 1976). It has been postulated also that platelets release a factor(s) which induces arterial smooth muscle cells to proliferate and grow into atherosclerotic plaques (Ross and Glomset, 1973). Probably smooth muscle cells are responsible for intercellular matrix development in normal arteries and in atherosclerotic plaques; the intercellular matrix may

account for the major part of the mass of many plaques. We do not know whether specific action of the macrophage and platelet 'proliferation factors' is restricted to switching on the proliferation of collagen-productive cells, as opposed to other cell types, nor is there negative or affirmative evidence that they are involved in the cell proliferative response in collagen-productive liver disease.

Some recent studies have purported to show that normal hepatocytes synthesise collagen and that this function may be important in generating the excess collagen matrix formed in disease. This hypothesis is based on the finding that populations of hepatocytes that are 95% pure contain prolyl hydroxylase, the enzyme which catalyses the formation of hydroxyproline in collagen (Ohuchi and Tsurukuji, 1972). However, mesenchymal cells isolated from liver, uncontaminated by hepatocytes, also contain this enzyme (Shaba *et al.*, 1973). More recently it has been shown that hepatocytes *in vivo* contain prolyl hydroxylase (Ooshima, 1977). Tissue culture experiments also show that hepatocytes produce collagen *in vitro* (Sakakibara *et al.*, 1976). This last type of *in vitro* study is difficult to interpret since it seems that virtually every cultured cell expresses a collagen gene (Langness and Udenfriend, 1974). Perhaps both mesenchymal (for example, perisinusoidal) cells and hepatocytes make a contribution to the formation of the collagen matrix in liver.

Any hypotheses offered to explain the regulation of collagen production in liver disease must take account not only of the increased quantities of this protein formed but also of the qualitative change in the collagen type produced. In human cirrhosis there is a relative increase in the ratio of type I to type III collagen (Seyer *et al.*, 1977) but there are no clues to how this switch occurs at a cellular or molecular level.

Conclusion

Many diseases in man (for example, hepatic cirrhosis) are characterised by a quantitative disturbance in collagen formation. Such diseases may be grouped together as 'collagen-formative diseases'. In hepatic cirrhosis increased collagen accumulation is probably mainly due to increased collagen synthesis. This increase is partially mediated by the release of collagen-stimulating factors into the liver and by other undiscovered stimuli which cause collagen-producing cells to proliferate. The 'collagen-formative disease' concept could be rejected on the grounds that collagen accumulation in cirrhosis is secondary to hepatocyte death, etc. However, there is no creditable evidence for this view. An advantage of the concept is that it may focus more attention on

the destructive effects of collagen on organ function and may lead to the identification of therapeutic agents that will block collagen production in disease.

References

- Benjamin, I. S., Than-Than, Ryan S., Rodger, M. C., McGee, J. O'D., and Blumgart, L. H. (1978). Prolyl hydroxylase and collagen biosynthesis in rat liver following varying degrees of partial hepatectomy. *British Journal of Experimental Pathology*, in press.
- Chen, T., Zetterman, R., and Leevy, C. M. (1974). Sensitized lymphocyte and hepatic fibrogenesis (Abstract). *Gastroenterology*, **65**, 532.
- Fallon, A., and McGee, J. O'D. (1978). Collagen stimulating factors in hepatic fibrogenesis. (Submitted for publication.)
- Hase, T. (1968). Development of portal hepatic venous shunts and cirrhosis in carbon tetrachloride poisoning in rats. *American Journal of Pathology*, **53**, 83-98.
- Heppleston, A. G., and Styles, J. A. (1967). Activity of a macrophage factor in collagen formation by silica. *Nature*, **214**, 521-522.
- Kefalides, N. A. (1971). Isolation of a collagen from basement membranes containing three identical α -chains. *Biochemical and Biophysical Research Communications*, **45**, 226-234.
- Kent, G., Gay, S., Inouye, T., Bahu, R., Minick, O. T., and Popper, H. (1976). Vitamin A-containing lipocytes and formation of type III collagen in liver injury. *Proceedings of the National Academy of Sciences*, **73**, 3719-3722.
- Langness, U., and Udenfriend, S. (1974). Collagen synthesis in nonfibroblastic cell lines. *Proceedings of the National Academy of Sciences*, **71**, 50-51.
- Leibovich, S. J., and Ross, R. (1976). A macrophage-dependent factor that stimulates the proliferation of fibroblasts *in vitro*. *American Journal of Pathology*, **84**, 501-514.
- McGee, J. O'D., O'Hare, R. P., and Patrick, R. S. (1973). Stimulation of the collagen biosynthetic pathway by factors isolated from experimentally-injured liver. *Nature (New Biology)*, **243**, 121-122.
- McGee, J. O'D., and Patrick, R. S. (1972). The role of perisinusoidal cells in hepatic fibrogenesis. *Laboratory Investigation*, **26**, 429-440.
- McGee, J. O'D., Patrick, R. S., Rodger, M. C., and Luty, C. M. (1974). Collagen proline hydroxylase activity and ^{35}S -sulphate uptake in human liver biopsies. *Gut*, **15**, 260-267.
- Nagle, R. B., Kohonen, P. W., Bulger, R. E., Striker, G. E., and Benditt, E. P. (1960). Ultrastructure of human renal obsolescent glomeruli. *Laboratory Investigation*, **21**, 519-526.
- O'Hare, R. P., and McGee, J. O'D. (1978). Collagen stimulating factors in healing skin wounds. (Submitted for publication.)
- Ohuchi, K., and Tsurukuji, S. (1972). Protocollagen proline hydroxylase in isolated rat liver cells. *Biochimica et Biophysica Acta*, **258**, 731-740.
- Ooshima, A. (1977). Immunohistochemical localization

- of prolyl hydroxylase in rat tissues. *Journal of Histochemistry and Cytochemistry*, **25**, 1297-1302.
- Pope, F. M., and Nicholls, A. C. (1978). Molecular abnormalities of collagen. *Journal of Clinical Pathology*, **31** Supplement (Royal College of Pathologists) **12**, 95-104.
- Ross, R., and Glomset, J. A. (1973). Atherosclerosis and the arterial smooth muscle cell: proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis. *Science*, **180**, 1332-1339.
- Sakakibara, K., Saito, M., Umeda, M., Enaka, K., and Tsukada, Y. (1976). Native collagen formation by liver parenchymal cells in culture. *Nature*, **262**, 316-318.
- Schaffner, F., and Popper, H. (1963). Capillarization of hepatic sinusoids in man. *Gastroenterology*, **44**, 239-242.
- Seyer, J. M., Hutcheson, E. T., and Kang, A. H. (1977). Collagen polymorphism in normal and cirrhotic human liver. *Journal of Clinical Investigation*, **59**, 241-248.
- Shaba, J. K., Patrick, R. S., and McGee, J. O'D. (1973). Collagen synthesis by mesenchymal cells isolated from normal and acutely-damaged mouse liver. *British Journal of Experimental Pathology*, **54**, 110-116.
- Thomson, D., and Patrick, R. S. (1978). Prolyl hydroxylase in pulmonary fibrosis induced by paraquat. *British Journal of Experimental Pathology*, in press.
- Vidins, E. I., Fox, S., Fuller, G. C., Kuttan, R., Mann, S. W., and Rodil, J. V. (1978). Hepatic prolyl hydroxylase activity as a parameter of collagen synthesis at initial and subsequent biopsy of patients with alcoholic liver disease. *Gastroenterology*, in press.