

Morphogenesis of Cirrhosis from Chronic Hepatitis

There are other examples of morphogenesis into which we can gain insight at least partially by taking a topological viewpoint. To be introduced in this chapter is the developmental process of cirrhotic livers from pre-cirrhotic lesions like chronic hepatitis. Here too, the liver lesion has a clear skeleton that spreads in the 3-D space, and morphogenesis of cirrhosis involves a progressive reformation of skeleton. Let us begin with visualizing what the skeleton of these diseases looks like.

Cirrhosis is the terminal state common to various chronic diseases of the liver. To put it more strictly, while pre-cirrhotic diseases like chronic hepatitis keep advancing toward cirrhosis, we also find in cirrhosis itself a disease continuously advancing to its terminal stage. This may be understood by the fact that there are a number of patients who have cirrhosis and yet are leading a life that is not very much disabled. Why can the condition of such patients deteriorate with time, and why, some day, do fatal events happen, like hepatic insufficiency? All these cannot be understood without assuming that in the entire course from chronic hepatitis to the terminal stage of cirrhosis, we have a slowly but incessantly advancing disease. Then, how do the liver lesions develop and progress? This is what we are going to examine in this chapter.

The cirrhotic liver: microscopic appearance (Figs. 6-1, 6-2)

Shown as an example in Fig. 6-1 is a microscopic appearance of cirrhosis. It contains spherical structures that are called nodules. Their dimension is various, ranging in some cases up to 1 cm in maximum diameter. Stretching between neighboring nodules are narrow belts stained green. They are called the interstitium and consist of fibrous tissue, which may be regarded as a scar that is left after the hepatocytes were destroyed due to, for example, viral hepatitis. The nodules are spherical because in their inside the hepatocytes continue regenerating, and in this sense, nodules are also called the regenerative nodules.

Cirrhosis can also develop from livers with chronic alcoholic injury. Figure 6-2 presents an example and here the intranodular hepatocytes are shown containing vacuoles; these are fat droplets accumulating in the hepatocytes due to impaired metabolism. The basic pattern of hepatic lesion is similar as in the foregoing case. Why can cirrhosis, while developing from diverse pre-cirrhotic diseases, present quite a similar pattern? Despite many assumptions proposed so far (Popper and Elias, 1955; Thaler, 1952), problems still remain about the morphogenesis of cirrhosis, awaiting further studies. To be introduced in the following are some of our studies and attempts (Takahashi, 1978a,b; Takahashi and Suwa, 1978; Endoh and Takahashi, 1997; Takahashi and Endoh 2000).

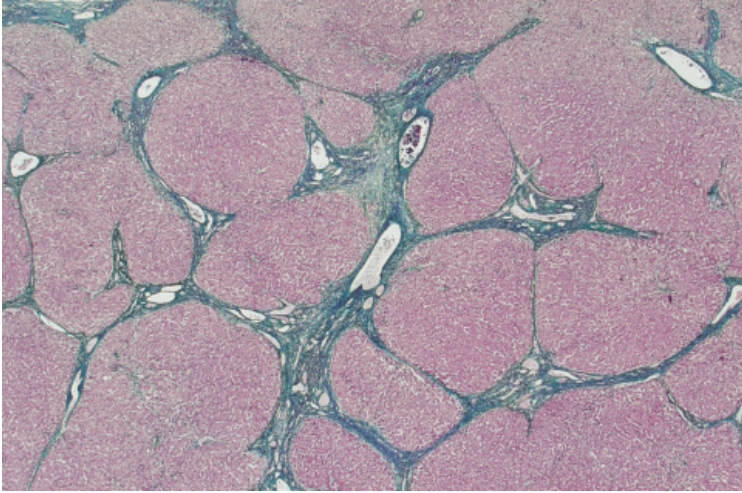


Fig. 6-1. Microscopic appearance of liver cirrhosis presumably resulting from chronic hepatitis. The round masses stained brown are called nodules consisting of hepatocytes that survived injury and are regenerating. The green belts are the interstitium, the scar zones left after hepatocytes were destroyed in the active phase of viral hepatitis. Elastica-Goldner stain.

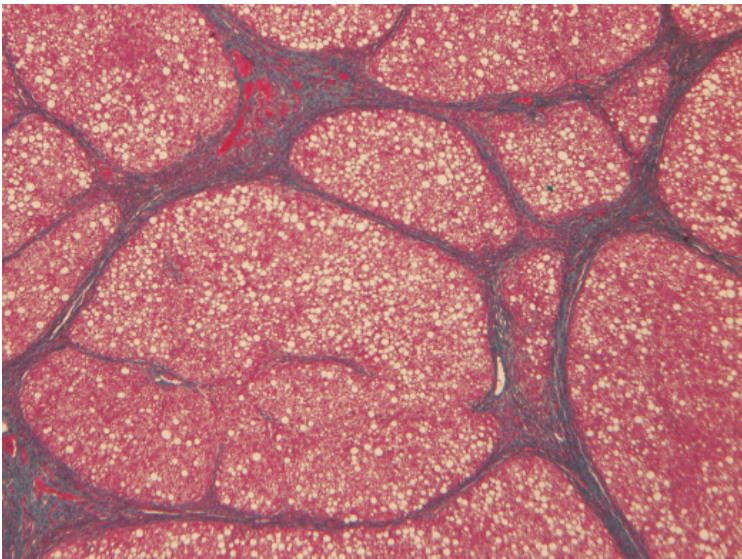


Fig. 6-2. Another cirrhosis, from a patient of chronic liver injury due to alcohol abuse. The microscopic pattern of lesion is basically the same as in the case of Fig. 6-1, except that the hepatocytes in the nodules contain droplets of lipid. Elastica-Goldner stain.

Three-D structure of cirrhotic liver: the skeleton (Figs. 6-3, 6-4, 6-5, 6-6)

Figure 6-3 is a 3-D reconstruction of the cirrhosis shown in Fig. 6-1. Expressed in green wireframes in this figure are the interphasic borders between nodules and interstitium. Both the portal (red) and hepatic veins (blue) are found mostly running within the interstitium, hardly entering the nodules. Indicated by PC are the places of P-C bridging where terminal portal veins communicate with terminal hepatic (or central) veins. All these are quite the same as exhibited in Fig. 3-30 (Chapter 3). As explained there, this vasculature implies that a significant part of portal blood flowing into the liver is shunted away via PC anastomoses without entering the nodules and therefore without being detoxified by hepatocytes. This explains the mechanism of hepatic insufficiency to which cirrhotic livers are particularly susceptible.

However, there is another aspect in the 3-D structure of cirrhosis. Figure 6-4 presents another cirrhosis and in this case it is the interstitium that is reproduced, while the nodules were left as void spaces. Here one may notice that the nodules are not perfectly wrapped by interstitium. There are defects at many places of interstitial septa, through which the regenerating parenchyma continues from a nodule to the neighboring ones. The skeleton of structure may clearly be visualized by registering nodules with consecutive numbers, and expressing the internodular connectivity in the form of linear diagram. Thus, as shown, one can confirm that all the nodules are connected with the adjacent ones, forming a fine 3-D network with many loops (holes). Solitary nodules having no connection with others are few, if any. This skeleton proved to be common to several cirrhotic livers studied, which were seemingly of various microscopic patterns. How can we explain the development of this network skeleton?

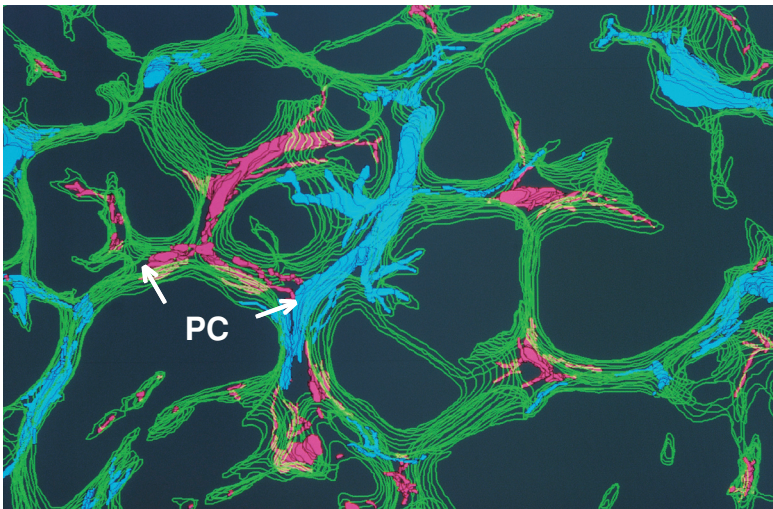


Fig. 6-3. Computer-assisted 3-D reconstruction of cirrhosis shown in Fig. 6-1 to visualize the relation of nodules (green wireframes) with portal (red) and hepatic venules (blue). At several places, anastomoses between portal venules and hepatic (central) venules are found in the form of P-C bridgings.



Fig. 6-4. Manually performed 3-D reconstruction of interstitial septa in a cirrhotic liver, visualizing the skeleton of cirrhosis. Regenerative nodules are not independent, but are continuing with the neighboring ones through the holes opening at many places in the septa. Thus nodules are united one with another into a vast 3-D network, as shown by a node-branch system where the individual nodules are identified with consecutive numbers and internodular connections with lines. Reproduced from Takahashi (1978): *Virchows Arch A Path Anat Hist* 377, pp. 190.

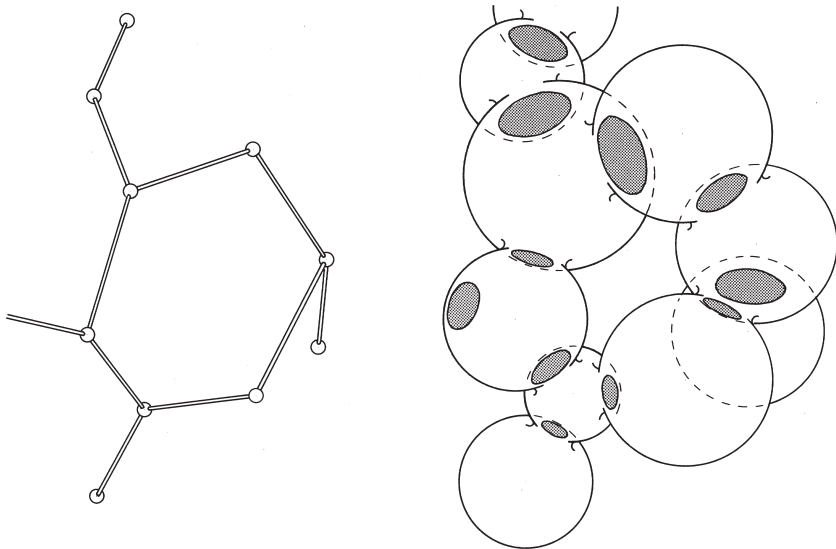


Fig. 6-5. A schema of nodular network. Nodules are adhered into a chain that forms a 3-D network with loops (holes).

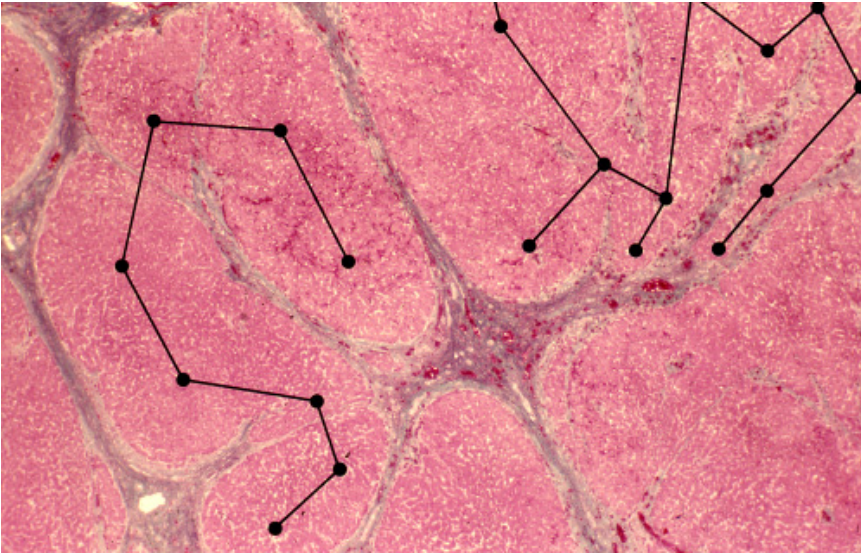


Fig. 6-6. Microphotograph of cirrhosis with a pattern produced by sectioning a nodular network. Note the complex indentation of mutually adhered nodules, as shown by the nodes and branches. Elastica-Goldner stain. Reproduced from Takahashi (1978): *Virchows Arch A Path Anat Hist* 377, pp. 98.

It seems to be improbable that in the creation of this skeleton, the intranodular parenchyma had a leading role. Instead, it is quite likely to be the interstitium that laid the basic mold. The parenchymal tissue forms a network, but it does so only passively, being cast by the interstitium that has already spread a frame in the space.

The nodular network as the skeleton of cirrhotic liver is schematized in Fig. 6-5. Nodules are connected into chains, and the chains are forming a network having many loops. The network character of internodular connection is often visible even in a 2-D microscopic picture. In Fig. 6-6 which is from a cirrhosis, complicated flexions of nodular chains are produced by deeply indented connective tissue septa. Obviously this corresponds to a 2-D section of nodular chains.

Skeleton formation in precirrhosis (Figs. 6-7, 6-8)

The network skeleton shown here is not only common to various cirrhosis. It is found in various precirrhotic diseases and in various stages of development during which they smolder, recur, progress and finally end up in cirrhosis (Takahashi, 1978a). Therefore the basic requirement for a liver with chronic disease to advance to cirrhosis seems likely that somehow, it comes to harbor this network skeleton. Thus, the most essential question in the morphogenesis of cirrhosis is how, in precirrhotic diseases, interstitium emerges so as to form itself into a 3-D network.

Figure 6-7 is microscopic appearance of the liver from a patient dying of fulminant hepatitis on the 28th day. One can find broad areas of necrosis extending in a belt-like fashion, with the branches of hepatic vein dispersed along the axis of necrotic

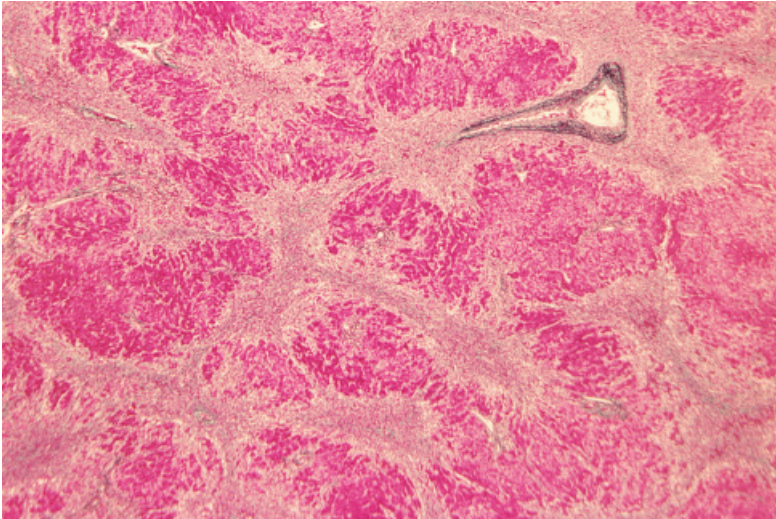


Fig. 6-7. Microscopic appearance of fulminant hepatitis, a potentially cirrhotic lesion. From a patient who died of hepatic insufficiency about one month after the onset of disease. The masses stained reddish are the zone of hepatocytes surviving injury and vigorously regenerating. The belts extending between the regenerating masses are the necrotic areas. Elastica-Goldner stain.

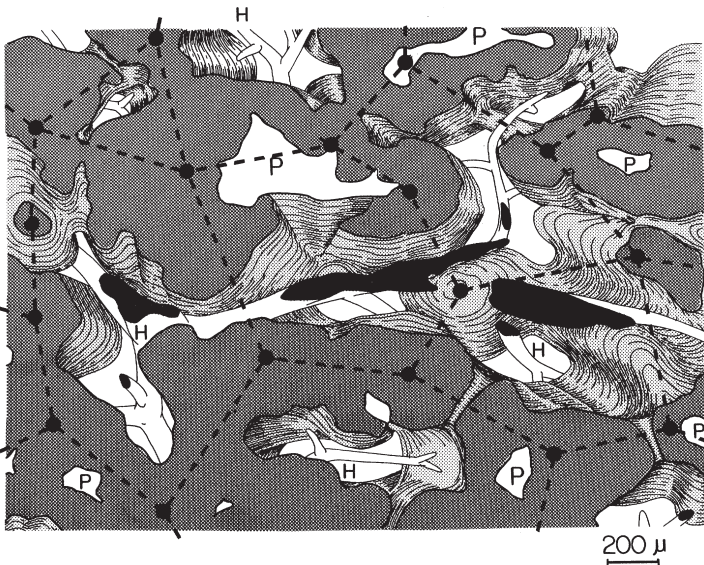


Fig. 6-8. Manual 3-D reconstruction of the liver with fulminant hepatitis shown in Fig. 6-7. The regenerating parenchymal zones form a network as shown by the node-branch diagram. One can see that the necrosis involved the parenchymal zone around the hepatic veins (H). P: portal tract. Reproduced from Takahashi (1978): *Virchows Arch A Path Anat Hist* 377, pp. 105.

belts, suggesting that this is a sort of “zonal necrosis” mainly involving the central zone of lobules (see the next chapter).

Figure 6-8 is a 3-D reconstruction of the liver with fulminant hepatitis shown in the foregoing figure. Here the zones of massive hepatocellular necrosis are shown as void spaces, leaving only the parenchymal zones consisting of hepatocytes that survived necrosis and are regenerating. P indicates the portal tracts and H, the hepatic veins. The result confirms that the necrosis took place in the parenchymal zone around the hepatic (central) veins. A linear diagram is entered in the figure so as to illustrate the connecting relation of the viable parenchymal zones, and it may be clear that here, a parenchymal network has already been created. Here we understand that it is the zonal necrosis that occurred first, and it is the 3-D pattern of necrotic zones that cast the parenchyma into a network. It seems likely that if this patient survived the acute stage, the liver would have started a process toward cirrhotic changes.

The basic structural framework: cirrhosis and precirrhosis (Fig. 6-9)

From the above, the 3-D structure of cirrhosis may be schematized as in Fig. 6-9. The nodules are connected in the space in the form of network with loops (holes). This network of nodules is intertwined with another one, the interstitial network, which also has loops and is geometrically “conjugate” with the nodular network. The portal

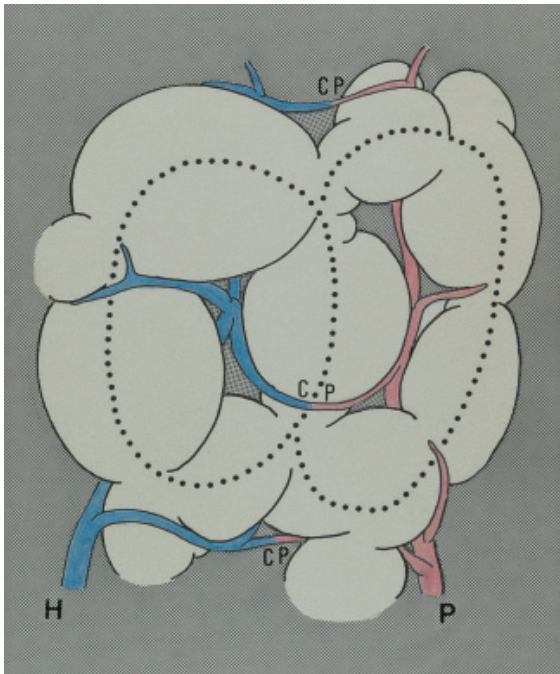


Fig. 6-9. A schema illustrating the basic 3-D structure of cirrhotic liver. The interstitium, the scar zone containing portal and hepatic veins, constitutes the skeleton which presents as a 3-D network. In this network, portal and hepatic venules anastomose at many places in the form of P-C bridgings. Also the nodules are continuous and form another network that is molded by, and intertwined with, the interstitial network.

(P) and hepatic (central: C) veins are running in the interstitium and as the result, they form a vascular network with PC bridgings. This is the basic structural plan of cirrhotic liver, and the problem of cirrhogenesis may be summarized in how, the skeleton of interstitial network develops so that at least partially, formation of its loops may result in PC bridging. Let us consider about this, taking as an example livers with chronic viral hepatitis.

Chronic hepatitis: focal necrosis and its 3-D distribution (Figs. 6-10, 6-11)

Figure 6-10 is a microscopic picture of liver with chronic hepatitis B. There are focal interstitial areas that are stained green (arrows) and dispersed in the section. One can find in such foci various stages of inflammatory changes. Some present as collapsed areas relatively fresh from hepatocellular necrosis, while others are old scarrings leaving little inflammatory signs. Thus the interstitial areas represent foci of hepatocellular necrosis combined with variously advanced fibrotic changes. Often the areas assume star-like shape due to compression by the nearby parenchymal tissue where

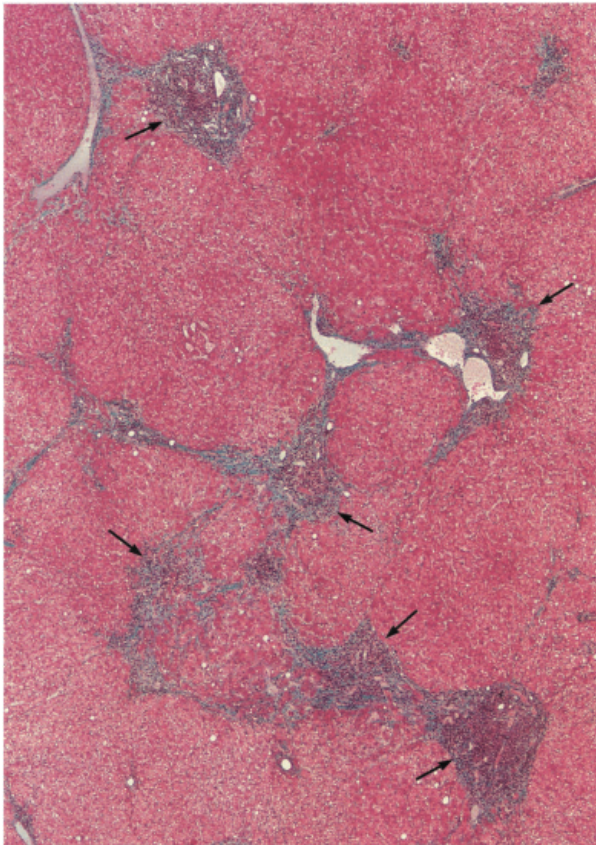


Fig. 6-10. Microphotograph of chronic hepatitis B with the lobular structure moderately reorganized. There are focal necroses dispersed in the hepatic parenchyma (arrows). Elastica-Goldner stain.

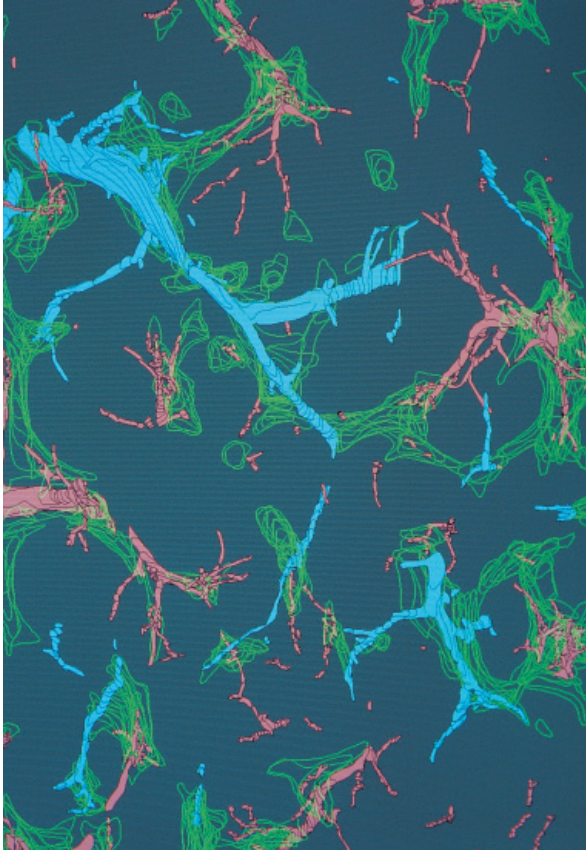


Fig. 6-11. Computer-assisted 3-D reconstruction of chronic hepatitis shown in Fig. 6-10. The green wireframes denote the profiles of focal necroses. Note that the necrotic foci, including both fresh and fibrotic lesions, are distributed randomly: some around the hepatic veins (blue) and some bridging the portal (red) and hepatic veins, although in general, there seems to be some predilection for the periportal zone.

regeneration of hepatocytes has already started. Yet, regeneration has not advanced so far as to transform the entire parenchymal zones into nodules, and the liver has yet to reach the state of cirrhosis.

The liver of Fig. 6-10 with chronic hepatitis is reconstructed in Fig. 6-11. The green wireframes denote the surface contours of the interstitial (or necrotic) areas, shown red are portal veins and blue are hepatic veins. A number of necrotic areas are found dispersed in the parenchyma. Their dimension is various but most are showing sublobular extension occupying a space ranging in volume from a quarter to 1/20 of lobule, though not accessible to precise evaluation because many of them look more or less flattened, which means that parenchymal regeneration in the surroundings has advanced to some degree, compressing the foci of necrosis. The distribution of these foci appears random, with some of them positioned close to the portal vein, with some of the others around the hepatic vein and also with some situated at a place distant from both portal and hepatic veins. As a whole, however, there seems to be a slight

tendency for the focal necroses to occur in periportal zone of lobule. It seems possible that the sort of necrosis described here may correspond at least partially to some types of necrosis which Baggenstoss *et al.* (1972) reported to occur in what they called “chronic active liver diseases.” However, at least from a 3-D distribution point of view, the necrosis is essentially focal and distributed randomly, and from this viewpoint, they do not appear fitting the term “subacute necrosis with bridging” or “subacute hepatitis with multilobular necrosis,” wordings proposed by them. We performed 3-D reconstruction of six livers with chronic hepatitis, using surgical biopsy or autopsy specimens, and obtained results that were essentially the same. Two of the cases were Type B, one was Type C, and the remaining three had been diagnosed as non-A non-B in the 1980s.

It may be confirmed in the figure that at several places, foci are so formed as to involve a portal vein and at the same time a neighboring hepatic vein. As necrotic areas are collapsed and scarring advances, such foci will change into an interstitium harboring a PC bridging. However, it has to be reiterated that the necrosis is essentially focal and randomly distributed. PC bridgings do occur, but their position and distribution suggest that bridging is not inherent in this lesion, arising only as the result of stochastic hitting process. None of the necrotic areas is extensive enough to involve multiple hepatic lobules.

The development of P-C bridging (Figs. 6-12, 6-13, 6-14)

The liver from another patient with chronic hepatitis is shown in Fig. 6-12, a non-A non-B case. Some of the portal tracts appear widened with fibrosis extending into

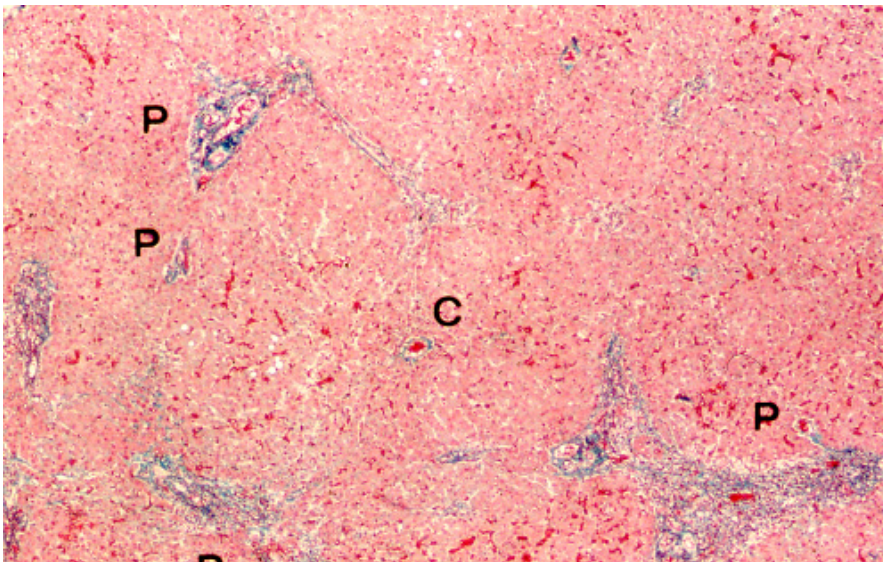


Fig. 6-12. Another case of chronic hepatitis (non-A non-B; the material was obtained in 1980s). Portal (P) and central (C) veins are discernible, and as yet the original lobular structure seems to be retained, a picture that would possibly have been classified as chronic persistent hepatitis in the previous classification. Elastica-Goldner stain.

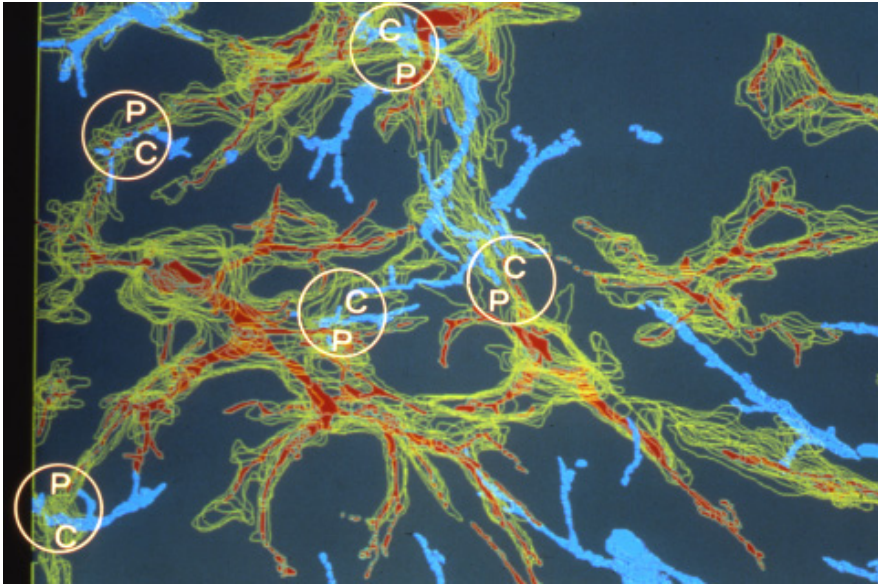


Fig. 6-13. Computer-aided 3-D reconstruction of the liver of Fig. 6-12. The yellow wireframes denote the profiles of fibrotic areas. Despite the impression of not so much advanced disease on the 2-D microscopic picture, there are already fibrosis with P-C bridging at several places.

the surroundings, but seemingly, the lobular structure looks well sustained with portal tract (P) and central veins (C) positioned at a distance. The picture may correspond to what previously was classified as chronic persistent hepatitis (CPH), a type of chronic hepatitis considered to take a comparatively benign course (DeGroot *et al.*, 1968).

Figure 6-13 is a 3-D reconstruction of the liver shown above (Fig. 6-12) and betrays the impression given by the microscopic picture. While in the 2-D section the lobular structure seemed likely to be retained, in this 3-D picture one can find fibrosis, as denoted by yellow wireframes, extending mostly around the portal veins but sometimes involving the hepatic veins. As the result, P-C bridging is demonstrated at several places, suggesting that already, lobular disorganization has started and advanced to a certain degree. Why such a discrepancy can occur between the 2-D and 3-D findings will be understood in the next figure.

Figure 6-14 is a microscopic picture taken from one of the serial sections of the above liver (Figs. 6-12 and 6-13). It demonstrates a picture corresponding to an area of fibrosis possibly resulting from organized focal necrosis, where on 3-D reconstruction, a PC bridging was demonstrated to exist. One can see a portal tract having a small portal vein (P), and this portal tract appears widened due to what has been regarded as inflammatory reaction and fibrosis. Conventionally, this sort of picture has been interpreted as a small fibrosis resulting from piecemeal necrosis which advanced so as to injure the periportal hepatocytes in a piecemeal fashion. However, the fibrosis that seems to be only a minute “periportal fibrosis” has, in reality, a far more massive extension. It contains a small vessel, denoted by C, which is a central (hepatic) vein. Therefore, the seemingly “periportal” fibrosis has to be considered a sectional picture

of focal necrosis occupying an area extending over a half of hepatic lobule from the portal tract to beyond the central vein. Its extension is apt to be underestimated because of shrinkage after postnecrotic collapse. That the small vessel is certainly a central vein has been confirmed while visualizing the 3-D vasculature by reconstruction. It should be stressed that without performing 3-D analysis, differentiation is impossible between small portal and hepatic veins in such fibrotic areas.

This sort of fibrosis is found very often in biopsies of patients having chronic hepatitis. After making reconstruction analysis on several cases of chronic hepatitis, we are convinced that in fact, such “periportal” fibrosis are sequelae of focal necrosis that is collapsed, shrunk, and presenting on section as a tiny mass of collagen.

Skeleton formation and transformation (Figs. 6-15, 6-16, 6-17)

As focal necroses occur in increasing number, more and more they come into contact with the neighboring ones, while at the same time they are collapsed into fibrous interstitial zones. Thus, with time, the skeleton of interstitium is constructed in the form of 3-D network. As explained, the parenchymal tissue that was exempted from necrosis comes to be molded into another network that is intertwined with the interstitial network.

Now, based on this, let us consider how we can explain the morphogenesis of cirrhosis and its progression. While having a skeleton common to cirrhosis, the structural pattern of chronic hepatitis is different from cirrhosis. Before the liver reaches the state of cirrhosis, some secondary changes have to be added, modifying the picture. The first such changes may be nodular regeneration that the parenchymal zones

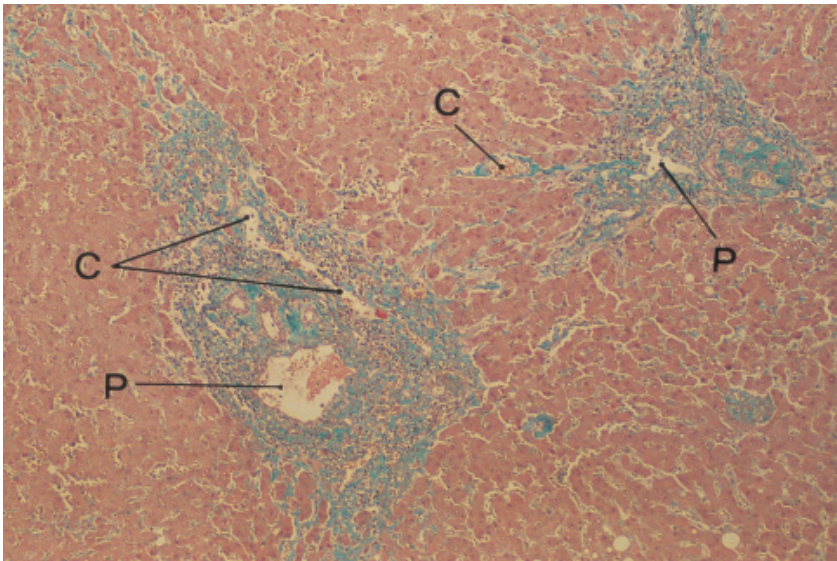


Fig. 6-14. A focal necrosis leaving PC bridging, in the liver shown in Figs. 6-12 and 6-13. There is fibrosis that may seem, at first glance, mild fibrous extension of portal tract (P: portal vein). However, this fibrosis is considered to be the product of focal necrosis that must have been far more massive than expected from this picture, because it contains a central vein (C). That this is indeed a central vein has been confirmed since the picture is a part of serial sections reconstructed in Fig. 6-13.

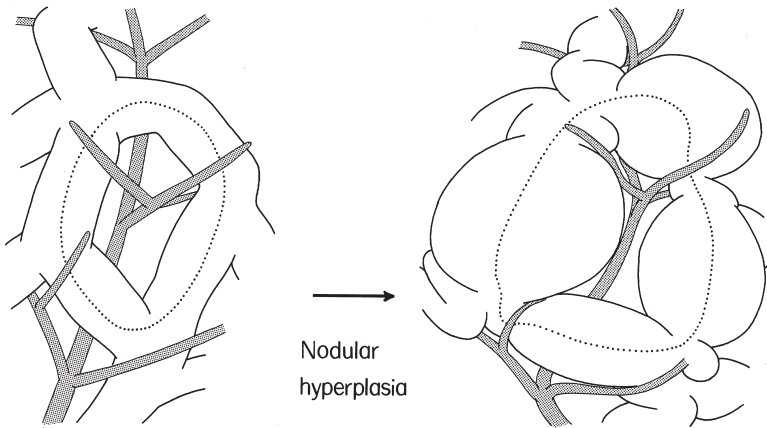


Fig. 6-15. Continuous transformation of network by nodular hyperplasia. With advancing regeneration of hepatocytes that survived necrosis, the parenchyma is gradually transformed into nodular network, but the skeleton maintains its topological properties with the holes of network retained as they were. Reproduced from Takahashi (1978); *Virchows Arch A Path Anat Hist* 377, pp. 190.

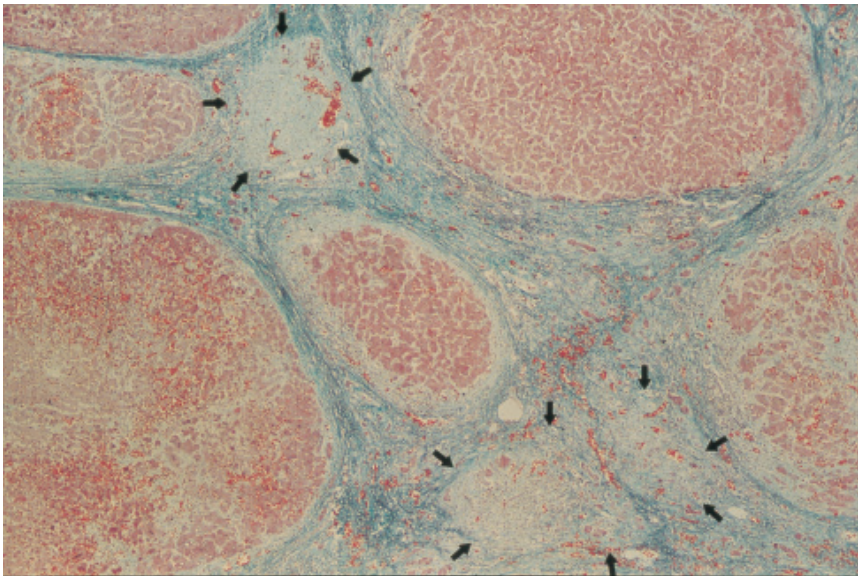


Fig. 6-16. Necrosis of hepatocytes involving whole regenerative nodules (arrows). Liver injury of this scale can sever the continuity of network, resulting in a radical reorganization of skeleton. Elastica-Goldner stain.

undergo. Let us assume therefore that as schematized in Fig. 6-15, cirrhosis may develop only through nodular swelling of parenchyma due to hepatocellular regeneration, while the network skeleton is sustained throughout the process. This is what may be called continuous transformation, with the whole geometric (topological) proper-

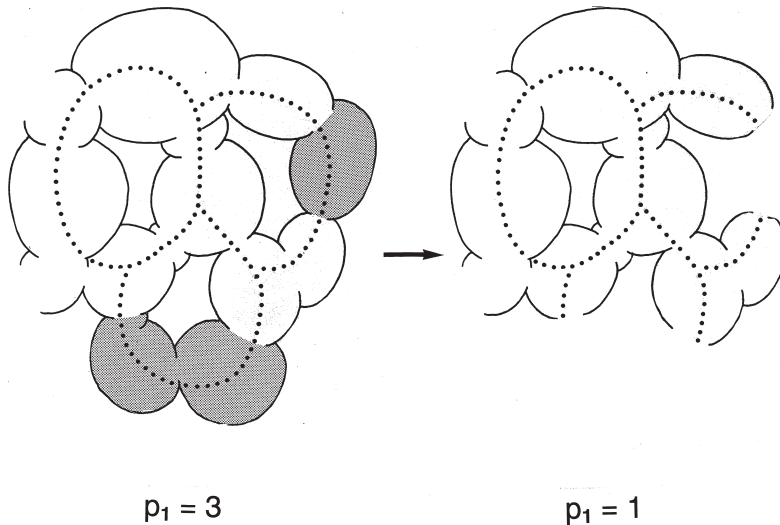


Fig. 6-17. Dehiscence (crack) of loop due to hepatocellular necrosis of such an extension as shown in Fig. 6-16. The consequence is an reorganization of network skeleton, with the number of originally three holes reduced by two.

ties of network maintained. The skeleton might be subjected to distortion through the process, but the whole inner connecting relation would be preserved exactly as it has been.

The second factor that can affect the form of lesion is the hepatocellular necrosis that takes various extension. In Fig. 6-16, necrosis is shown developing in a cirrhotic liver, destroying some of the small nodules (arrows). This can happen in the form of recurrent focal necrosis, when the host immune system is reactivated against hepatocytes expressing virus-related antigens. However, one can also find necrosis attributable to parenchymal ischemia because, as shown above, parenchymal tissue becomes increasingly susceptible to impaired portal blood flow with the advancement of cirrhotic changes. In livers with alcoholic injury, alcohol abuse can also produce hepatocellular necrosis of sizable scale.

If parenchymal network is involved in necrosis of sufficiently large scale, the involved part of network is severed off, bringing about a dehiscence (crack) of loop as in Fig. 6-17. Consequently, at every bout of necrosis, the network skeleton can undergo radical transformation. If so, the topological properties of the whole skeleton can gradually change with accumulation of cracks.

Topological description of cirrhogenesis (Fig. 6-18)

In the foregoing chapter, a method was introduced with which we can compare the geometric properties among various networks. That was to quantify the inner connectivity of network with genus, a topological parameter, but its significance has to be explained in more accurate geometric terms, together with the method of its measurement.

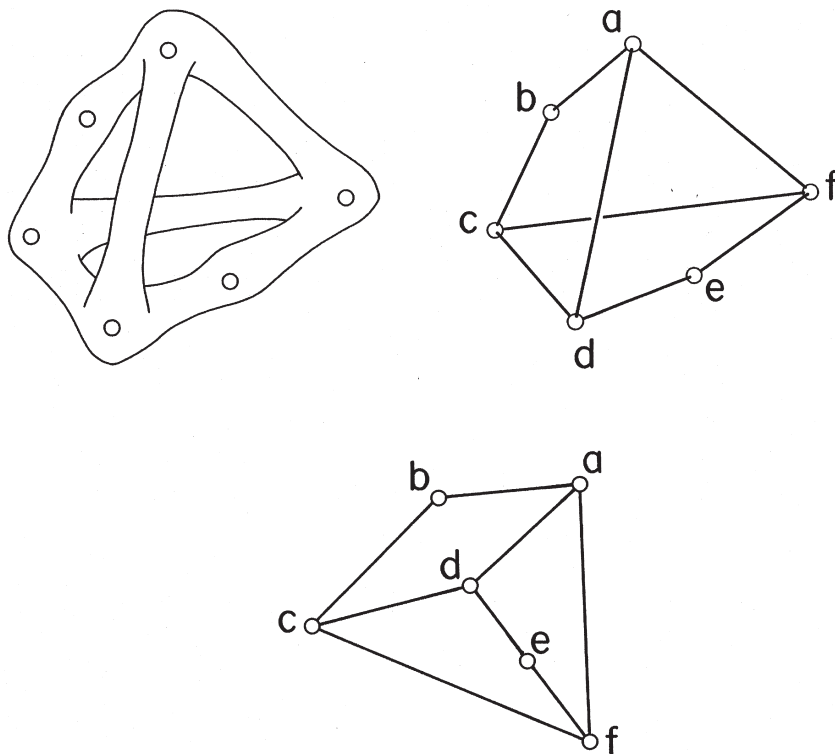


Fig. 6-18. Topological description of 3-D network with genus (p_1). The inner connecting relation of a 3-D network made of closed surface (left) is simplified by replacing it with a node-branch system without changing the connectivity. Here, the number of nodes (n) is six and that of branches (b) is eight. The number of separate parts of network (p_0) is one because the network is united. Then, on account of Euler-Poincaré formula $n - b = p_0 - p_1$ we obtain that p_1 , the number of holes the network contains (the genus), is three, as one can confirm in the lower figure.

Suppose, as in the left upper part of Fig. 6-18, a network made of closed surface and having several loops, or holes. It may serve as the simplest model of parenchymal (or interstitial) network of hepatic lesion. Now assume that arbitrary number of points (nodes) are placed in the trabecular network and, as in the right upper figure, are connected by branches, so that the network may be replaced with a node-branch system without changing the connecting relation. This procedure of replacement corresponds to the construction of the “deformation retract” of DeHoff *et al.* (1972). In this situation, let the number of nodes contained in the system be n , and that of the branches b . If the number of holes is p_1 and that of the separate parts of network is p_0 , then

$$n - b = p_0 - p_1$$

according to Euler-Poincaré principle, where p_1 is called the genus. Let us attempt to apply this to the network of the figure. Then we obtain that $n = 6$ and $b = 8$. The

number of separate parts of network is 1 because the figure is united as a whole. Then,

$$6 - 8 = 1 - p_1$$

and we obtain that $p_1 = 3$, which means that the network contains three holes. That there are three holes may be visualized if we transform the right upper, 3-D network into the lower, 2-D network. This is done only by transforming the holes and stretching and twisting the branches, which does not influence the connectivity. Now assume that a branch (for example ad) is taken away from the network. In this case $b = 7$, with n and p_0 remaining as they have been. Then the number of holes p_1 is calculated at 2, as confirmed in the figure. Accordingly, if we apply the formula to the parenchymal (or interstitial) network of the liver and compare the value of p_1 among livers with different lesions, we will be able to know whether during cirrhogenesis, the skeleton of the lesion is maintained, or undergoes a radical reorganization.

Measurement of genus on serial sections (Figs. 6-19, 6-20)

In measuring the p_1 value contained in a certain volume of liver, we cannot rely on stereology, as explained in the foregoing chapter. This is because p_1 is a quantity expressing the property of form which is non-metric and therefore not influenced by

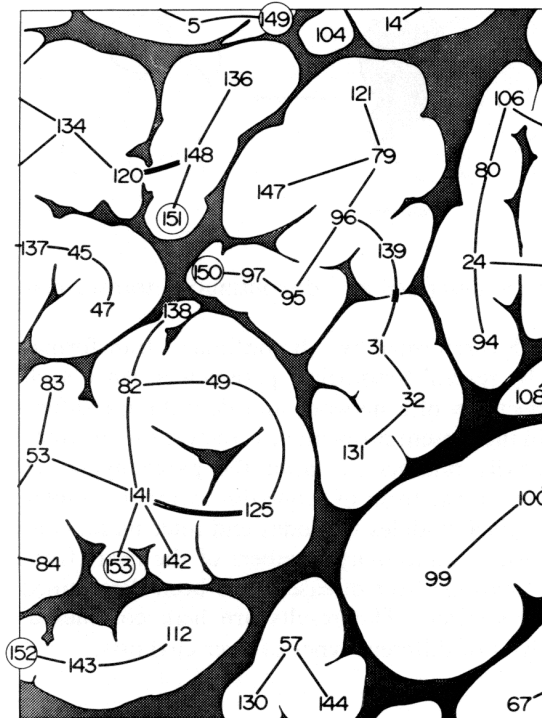


Fig. 6-19. Topological analysis of nodular network on serial sections of cirrhosis aimed at counting the number of genres (p_1) in a certain volume. Nodules are registered with consecutive numbers. Also the internodular connections are checked at every step of serial sections. Reproduced from Takahashi (1978): *Virchows Arch A Path Anat Hist* 377, pp. 192.

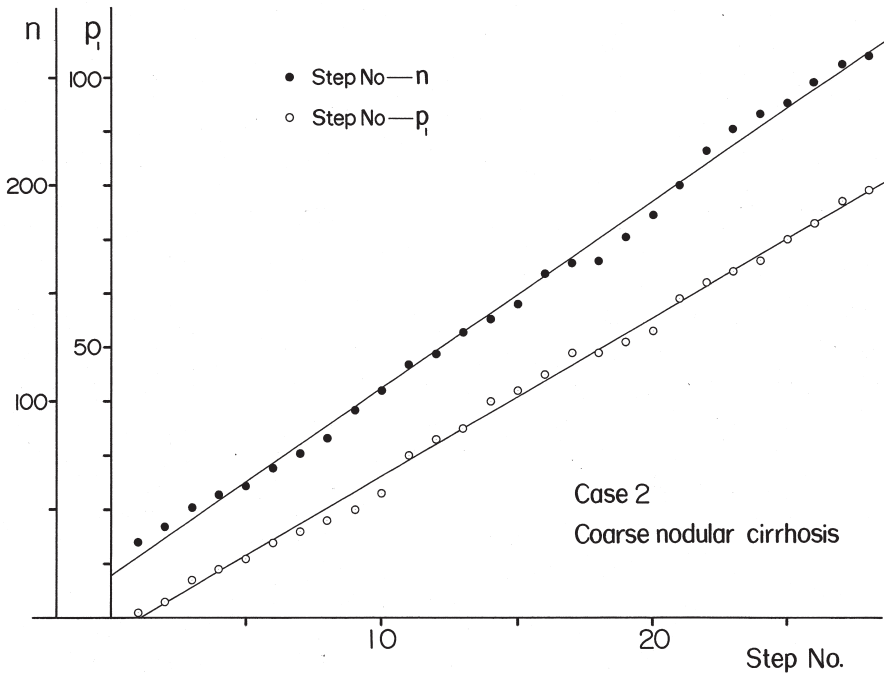


Fig. 6-20. The linear relation between the tissue volume (scanned with serial sections) and the number of nodules contained. The value of p_1 increases with the advancement of serial sections treatment. The total p_1 contained in the whole liver can be estimated by extending the regression line until it reaches the point corresponding to the liver volume. Reproduced from Takahashi (1978): *Virchows Arch A Path Anat Hist* 377, pp. 193.

distorsion, swelling or shrinkage. For this reason, manual 3-D measurement had to be performed to obtain the value of p_1 contained in a certain volume of the liver. A sampling area was defined in one of the semiserial sections, where all the nodules were numbered and internodular connections were checked as in Fig. 6-19. This was done consecutively with semiserial sections, thereby checking the connectivity of nodules and counting the values of n , b and p_0 . The interval of semi-serial sections was set as large as $50 \mu\text{m}$, a coarseness which however gives no influence on the internodular connectivity obtained, because of the semi-gross nature of the object.

As increasing number of steps are processed, the number of nodules and that of holes accumulate, as in Fig. 6-20. This means that the larger the volume analyzed, the more do the holes emerge. In this way we obtain a regression between p_1 and the volume of the tissue scanned. Thus, by extending the regression line to the volume of the whole liver, we obtain the total number of holes, or meshes, contained in the parenchymal network.

Livers with chronic diseases: topological difference and its interpretation (Fig. 6-21)

The values of p_1 thus obtained were compared among six livers with chronic diseases (Takahashi, 1978b) as in Fig. 6-21. In chronic hepatitis as well as subacute

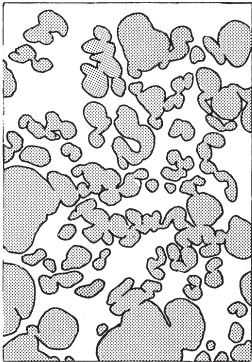
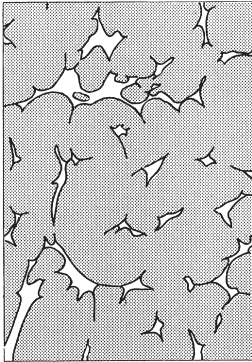
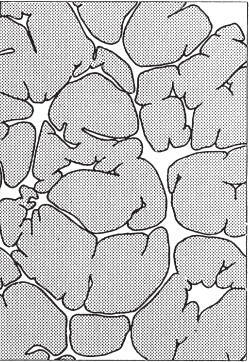
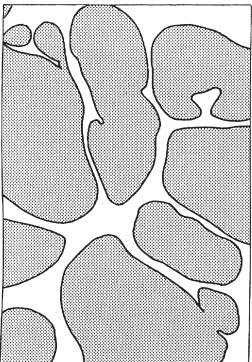
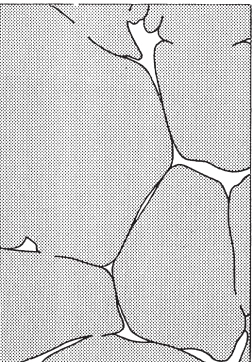

			
Type	Subacute Hepatitis	Chronic Hepatitis	C(alcoholic)
Weight (g)	580	1270	1970
Total p_1 ($\times 10^4$)	501	610	635
			
Type	A'	B	B'
Weight (g)	1030	780	590
Total p_1 ($\times 10^4$)	37	10	23

Fig. 6-21. The total number of genuses estimated in six autopsy livers. In the upper row are livers representing pre-cirrhotic and early cirrhotic lesions, including subacute hepatitis, chronic hepatitis and early alcoholic (portal) cirrhosis. In contrast, all the three cases arranged in the lower row exhibit advanced cirrhoses. Noteworthy is that in all the pre-cirrhoses and early cirrhosis, the total p_1 is within about the same range, 5 to 6 million, whereas in the three advanced cirrhoses, the value is incomparably lower. Reproduced from Takahashi (1978): *Virchows Arch A Path Anat Hist* 377, pp. 195.

hepatitis, i.e., diseases that potentially can progress into cirrhosis, the total p_1 of the liver is about the same and ranges from 5 to 6×10^6 . In an early stage of alcoholic cirrhosis, it remains at about the same level. The other three livers entered in the lower frames are all cirrhosis, either Type A', Type B or Type B' of the classification used in Japan (Nagayo, 1914; Miyake, 1960). Of these, Type A' corresponds approximately to

the postnecrotic type and Types B and B' to the posthepatic type of Gall's classification (1960). It is shown that the total p_1 of the liver in cirrhosis is much lower than the early lesions of the upper frames, ranging from 1/20 to 1/60 of the latter.

We can draw a conclusion from this. During the development of cirrhosis from precirrhotic lesions like chronic hepatitis, the skeleton once formed as a densely meshed 3-D network undergoes radical transformation where most of the segments are cut by hepatocellular necrosis. Perhaps the bout of necrosis recurs many times during a long course of disease, each time creating small number of connectivity changes. This assumption seems to match the actual clinical picture of chronic hepatitis, where, during a course of ten or more years, bouts of hepatocellular necrosis recur many times, gradually leading to the terminal stage of cirrhosis.

Morphogenesis and advancement of cirrhosis (Fig. 6-22)

Figure 6-22 presents a schema of cirrhogenesis based on the above assumption. In an early pre-cirrhotic stage, the liver has a closely knit network with larger p_1 . How-

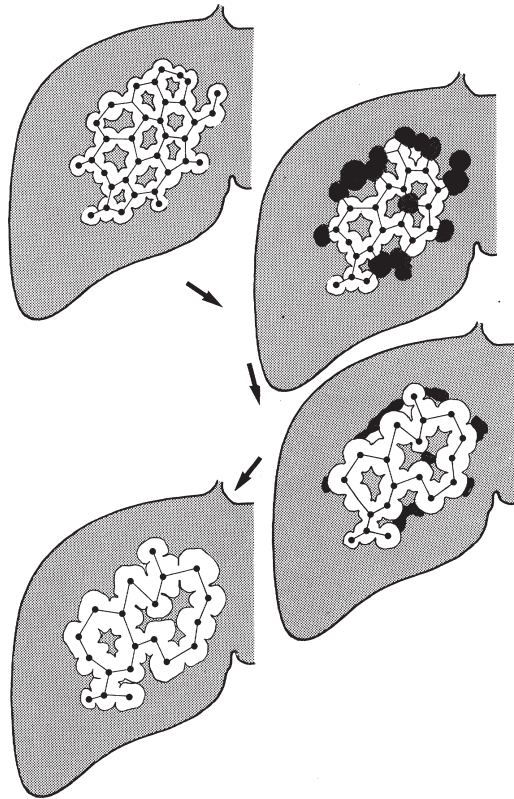


Fig. 6-22. A schema of morphogenesis from precirrhotic (or early cirrhosis) to advanced cirrhosis. In early lesions, the skeleton forms as a fine network with larger genres. With a bout of massive or submassive hepatocellular necrosis that frequently intervenes in the course of disease (black), the parenchymal network comes to be severed at several places, and the total genus number is reduced. Such an event recurs many times, reducing the p_1 value at every recurrence, gradually transforming the skeleton into a network significantly coarser than the original one.

ever, with the bouts of parenchymal necrosis recurring again and again, the skeleton gradually changes into growingly coarser network. With time, the nodules that have survived necrosis will decrease in number while individual nodules enlarge due to continuing regeneration. This assumption seems giving support to the prospective study of Danish group reporting that in the alcoholics, the pattern of cirrhosis tends to be increasingly coarser with the advancement of disease (Fauerholdt *et al.*, 1983).

Viral antigen and its distribution (Figs. 6-23, 6-24, 6-25)

We analyzed the origin and development of focal hepatocellular necrosis in livers with chronic hepatitis B (Endoh and Takahashi, 1997). As well documented, hepatocellular necrosis in this disease is attributable to host immune reaction against hepatocytes that have membrane-bound expression of HBcAg, the HB core antigen (Hsu *et al.*, 1987). Fig. 6-23 presents a liver section with chronic hepatitis B, immunostained for HBcAg. One can see that hepatocytes expressing HBcAg are not distributed diffusely or randomly, but clearly are forming foci, various in dimension but mostly less than 800 μm in the largest diameter, the diameter of a single hepatic lobule.

On closer look, an HBcAg-positive focus contains positively reacting hepatocytes in mosaic fashion and the reaction is taking place in either the nuclei, cytoplasm or cell membrane (Fig. 6-24). Around the positive hepatocytes there are lymphocytes probably engaged in immune activities. There are also antigen-free hepatocytes among the antigen-positive ones, co-existing in “piecemeal” distribution. If such a focus is located in the periportal zone of lobule, a typical picture of so-called piecemeal necrosis can appear in a 2-D microscopic section. It seems to us that the piecemeal necrosis may correspond to a sectional picture of what has been described in the above as focal necrosis. Particularly when this sort of change takes place in the close vicinity of portal tract, it would present on 2-D section a typical picture of piecemeal necrosis.

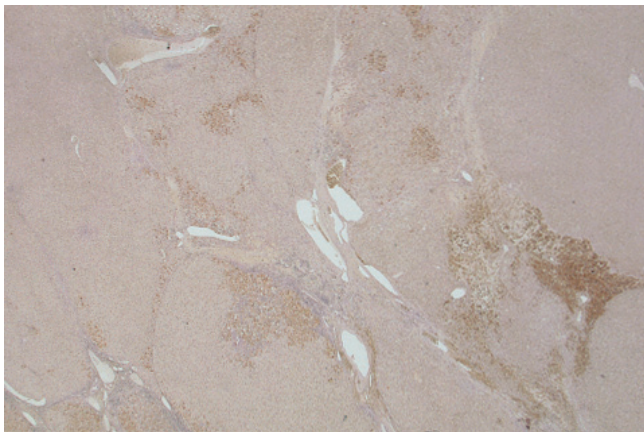


Fig. 6-23. Distribution of hepatitis B core antigen (HBcAg) in Type B chronic hepatitis. Note that the viral antigen is not uniformly distributed, but clearly forming positive areas. Immunostain for HBcAg.

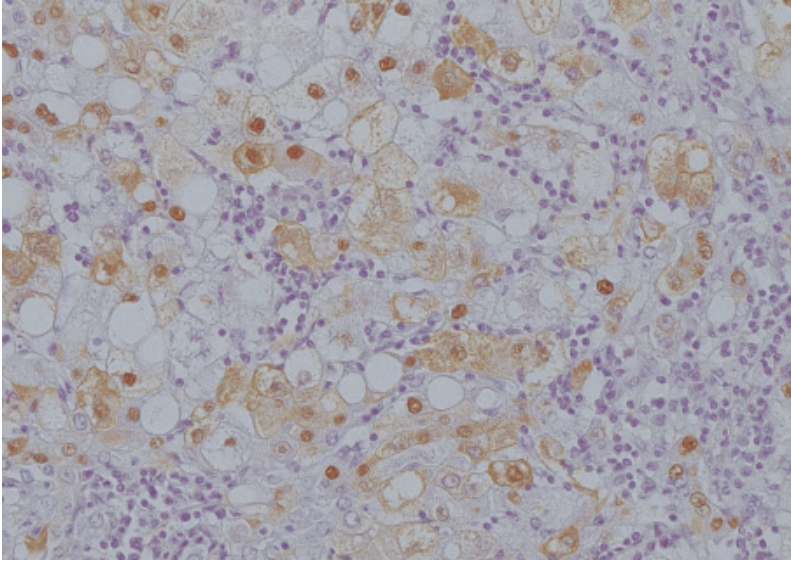


Fig. 6-24. High power microphotograph of HBcAg positive focus. There are positively reacting hepatocytes but the reaction is expressed variously: either upon the cellular membrane, or in the cytoplasm or in the nucleus. Dispersed among these are hepatocytes expressing no reaction. Also, lymphoid cells (presumably T-lymphocytes) are infiltrating in and around this positive area. If positioned around a portal tract, this would present as a typical "piecemeal necrosis." Immunostain for HBcAg.



Fig. 6-25. Cirrhosis developing from chronic hepatitis B. Low power microphotograph of section immunostained for HBcAg. HBcAg+ hepatocytes are not distributed uniformly but forming a clearly demarcated foci. It may be intriguing that without exception, the foci are distributed along the interstitial septa. Immunostain for HBcAg.

Shown in Fig. 6-25 is a case of cirrhosis derived from chronic hepatitis B, immunostained for HBcAg. Antigen-positive areas are distributed as multiple foci, but it is particularly interesting that the interstitial septa have developed so as to link the positive areas. The message given by this picture may be clear: focal necroses occur in the antigen-positive areas, while new foci arise one after another. In such foci, however, some of the antigen-positive hepatocytes survive and proliferate, paving the way for future recurrence of necrosis in and around the sites. In the meantime, fibrosis is gradually deposited in the antigen⁺ areas that extend with every recurrence and finally join with neighboring necrotic areas. On the other hand, the non-antigen-positive areas keep expanding by hepatocellular regeneration.

Two-D distance distribution to locate the HBcAg⁺ areas (Figs. 6-26, 6-27)

The idea of spatial distance distribution introduced in Chapter 3 was modified to demonstrate the close positional relation between the HBcAg-positive areas and the cirrhotic septa (Endoh and Takahashi, 1997). Figure 6-26 is a 2-D sectional picture of cirrhosis, where sampling points (six points in the figure) are set by tessellation. The points that fell in the interstitium (point No. 4) were omitted. Those falling in the nodules were divided according to whether they fell in an area positive (orange) or negative (white) for HBcAg. In the figure, two points (2, 5) fell in the positive, three (1, 3, 6) in the negative area, respectively. For each of the points, including those hitting the antigen-positive and those hitting the negative areas, the shortest distance from the point to the nearest septal border was measured.

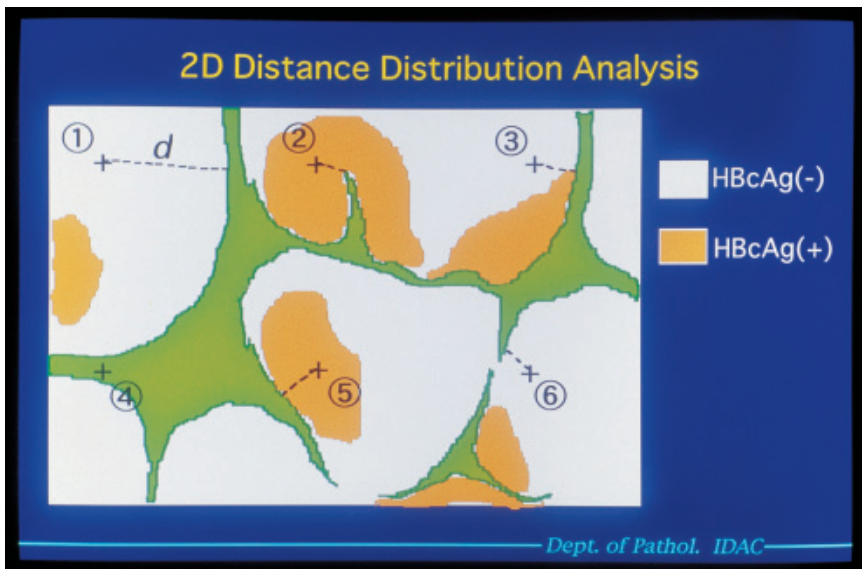


Fig. 6-26. Two-D distance distribution analysis. This is a method to test whether, in terms of position in the tissue, a lesion has affinity with a special structure, in this case HBcAg⁺ areas with the interstitial septa. In the figure, six sampling points are randomly set by tessellation. For details see the text.

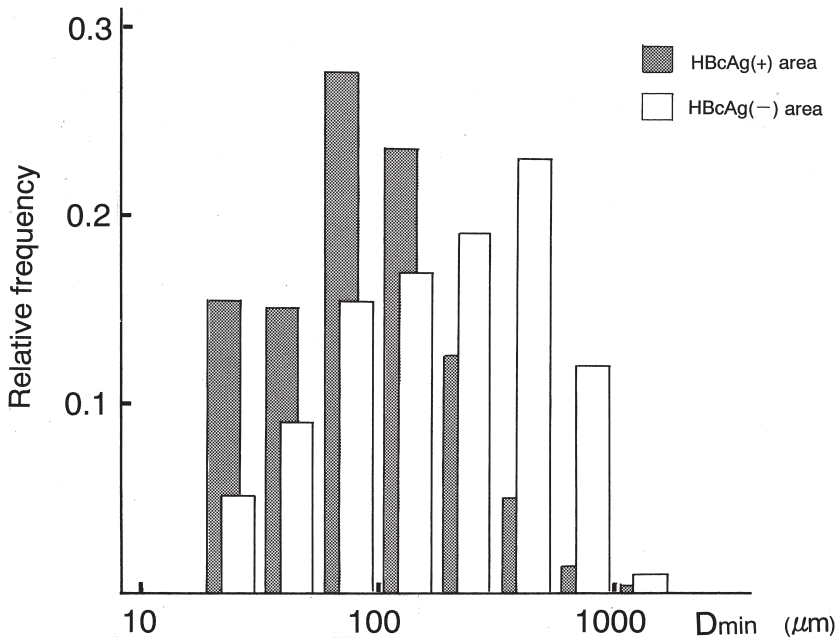


Fig. 6-27. Two-D distance distribution analysis. An example of application to a cirrhotic liver from chronic hepatitis B. D_{min} , the distance from the intranodular test points to the nearest septa, was shown on a log scale. Note that D_{min} from the points hitting HbAg⁺ areas (hatched) is significantly smaller than those from HbAg⁻ areas (non-hatched). Reproduced from Endoh, Takahashi, *et al.* (1997): Tohoku J Exp Med 182, pp. 183.

Liver specimens from 8 patients of cirrhosis developing from chronic hepatitis B were submitted to the 2-D distance distribution analysis. In each case, measurement was performed on 700 to 1,000 sampling points. Figure 6-27 is the result obtained in one of the livers and demonstrates that the distance from the points in the antigen-positive areas is smaller than those in the negative areas. Also in the remaining livers, quite the same results were obtained. In all cases, the difference in the distance proved to be significant on Mann-Whitney's test at $p < 0.001$. This allows us to conclude that the interstitial septa are likely to develop so as to link the necrotic areas.

Model of cirrhogenesis from chronic hepatitis (Fig. 6-28)

From the above, the morphogenesis of cirrhosis from chronic hepatitis B can be schematized as in Fig. 6-28. HbAg-positive hepatocytes are distributed in the hepatic parenchyma forming multiple foci of sublobular dimension. The foci, dispersed in seemingly random fashion, appear to be representing something like viral colonies. In an active phase of hepatitis, antigen-positive hepatocytes in the foci undergo necrosis, while in each of the foci, at least some hepatocytes survive. They proliferate, extending the positive area, which again undergoes necrosis in the next recurrence. Fibrosis advances with the repetition of this cycle, and in the meantime, new necrotic foci add to the previous ones, where old and new foci gradually come to join, creating

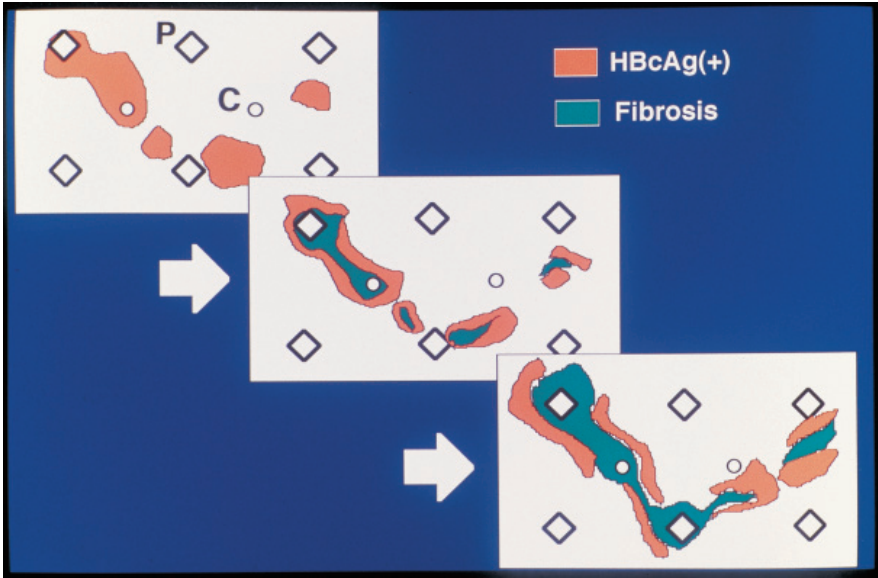


Fig. 6-28. A schema of cirrhogenesis from chronic hepatitis. Viral antigen-positive hepatocytes are distributed as multiple foci (brown) of sublobular dimension. While in an active phase, antigen⁺ hepatocytes in the foci are mostly destroyed, some cells survive. They proliferate, extending the positive area, which again breaks down in the next recurrence. In the meantime, fibrosis (green) is left after every bout of necrosis and is added to the previous scars. While this cycle recurs again and again, there emerge new foci one after another. Thus the fibrotic areas continue spawning, each of which extends and joins with the neighboring ones, finally forming a network skeleton. Thus, slowly the basic pattern of cirrhosis comes into being.

interstitial septa in the form of network. Thus, slowly the basic pattern of cirrhosis comes into being.

Today, most patients of cirrhosis in Japan derive from Type C chronic hepatitis. We expect the same way of thinking may work again in studying the cirrhogenesis in such cases.