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Topological Analysis of the Morphogenesis of Liver Cirrhosis

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Summary. Cirrhotic and precirrhotic livers consist of three-dimensional parenchymal networks. Topological analysis has been carried out using the total connectivity number p_1 on the livers from 6 autopsy cases of chronic liver diseases of different types. The total p_1 amounts to 6,100,000 in chronic hepatitis of periportal type and 6,350,000 in portal cirrhosis, but to only 100,000 in posthepatitic cirrhosis of coarse-nodular type. In view of the similarity of values for p_1 in chronic hepatitis and portal cirrhosis, the former is considered to give rise to the latter by continuous change in structure or through "piecemeal" progression of the periportal lesion. Development of posthepatitic cirrhosis from chronic hepatitis is possible only through a substantial reduction in p_1, which is brought about by parenchymal necrosis sufficient in extent to cause multiple disconnection of the network.

Key words: Liver cirrhosis – Chronic hepatitis – Subacute hepatitis – Morphogenesis – Topology.

Introduction

In a previous reconstruction study (Takahashi, 1978) it was found that the nodules of cirrhotic livers made an interconnected three-dimensional network in spite of their apparent separation on routine histological examination (Fig. 1). The basic pattern of this network had already been established in some precirrhotic conditions, and it was expected that the development of cirrhosis would be interpretable in terms of a possible structural transformation of the network system. Probable morphogenetic relationships between different forms of cirrhotic and precirrhotic livers can be properly evaluated if these conditions are related to topological isomorphism or heteromorphism of the network, if we consider this to be the structural framework of the pattern.

The basic principle of structural transformation may be summarized as follows. Imagine that a cirrhotic liver is only produced by regenerative hyperplasia

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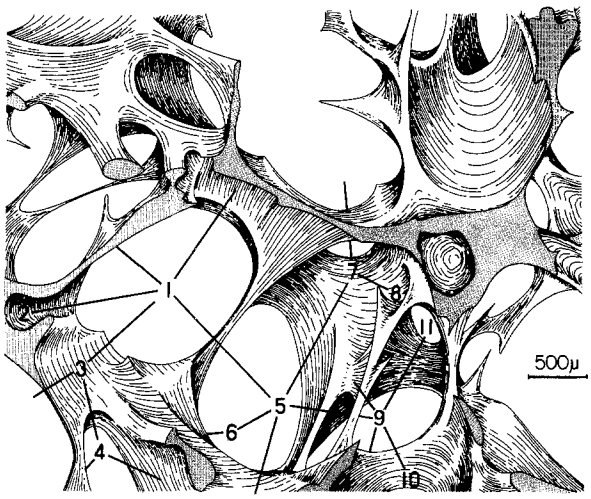


Fig. 1. Graphic reconstruction of the interstitial shell in Case 3 (posthepatitic cirrhosis) showing the connecting relation of the "nodular network". The nodules correspond to the round spaces and are registered by consecutive numbers. The lines indicate the internodular connections

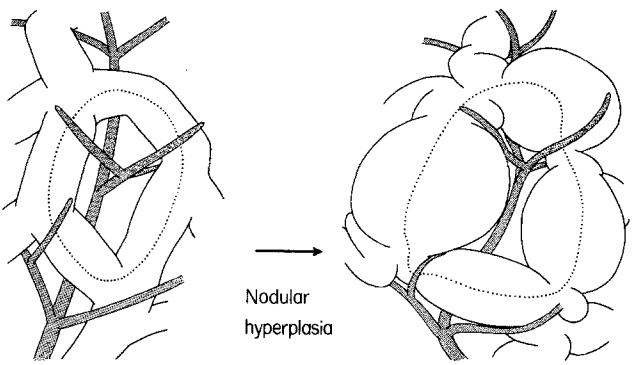


Fig. 2. A schema illustrating continuous transformation of the network system from a parenchymal linkage into a nodular linkage. The parenchymal loop is kept intact through the process of continuous transformation (*dotted line*)

of parenchymal masses which survive initial injury. Regeneration continuously transforms the parenchymal network into a nodular network, while the internal connections of the network are left unchanged (Fig. 2). Preservation of these connections may be demonstrated by taking the loop in the network as a mark (see figure), which will persist in such a transformation. This type of structural change can be described as "continuous transformation". However, cirrhotic or pre-cirrhotic livers frequently undergo secondary parenchymal necrosis due to recurrent hepatitis or some circulatory disturbance, and when the extent of the necrosis is enough to cause multiple destruction of nodules, all their connections with the adjacent nodules are inevitably destroyed (Fig. 3). If the nodule has united with others to form a loop in the network, its loss breaks the loop open, and thus necrosis leads to a radical transformation of

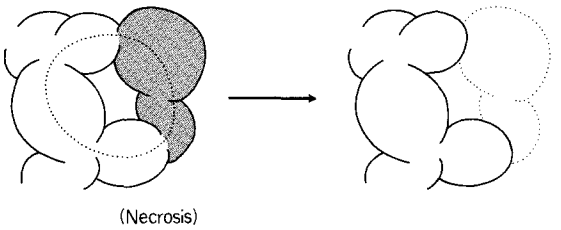


Fig. 3. Discontinuous transformation of the network induced by necrosis of the parenchymal region. Destruction of nodules breaks the loop in which the nodules have been linked

the network (see figure); that can be described as "discontinuous transformation".

The manner in which the network is reformed by "discontinuous transformation" can be measured precisely by several topological parameters, since such parameters express the inner connectivity of a network and describe the difference between different networks. In the present problem, it is relevant to examine the connectivity with the connectivity number p_1[1] after the parenchymal or nodular network has been simplified in the form of a one-dimensional complex (node-branch network). Here we replace nodules by nodes and inter-nodal connections by branches. $p_1 s$, or the total connectivity numbers of the livers from selected autopsy cases of different chronic liver diseases have been determined by reconstruction with semi-serial sections. The results are here considered, together with morphogenetic analysis of different types of liver cirrhosis.

Methods and Material

A test space of sufficient volume was taken from each liver specimen, and the cumulative numbers of nodules (n), branches as internodular connections (b) and connectivity number (p_1) in the test space were determined from serial sections (see Appendix). The results were converted to values for the whole liver after the volume ratio of the organ to the test space was estimated.

In practice, an area of rectangular shape was delineated as the test area on the first slide of the serial sections. The same area was then examined in each of the serial sections, thus the test space submitted to scanning was rectangular in shape. It is evident that the larger the volume of tissue scanned, the smaller is the error of total p_1. In this study, the test space was set in all cases so as to contain 500 to 600 nodules, and its volume amounted to about 600 mm^3 for coarse-nodular cirrhosis and about 25 mm^3 for fine-granular lesions, such as portal cirrhosis.

Most of the specimens from the previous report were also useful in the present study. They had been sectioned semiserially at an interval of one to five sections, a single section being 6 μ in thickness.

The test area of the first slide of the serial sections was projected onto a sheet of thin tracing paper using a profile projector (Nikon, model V-16-C) at a magnification of 20 to 50×, and the borderline between the parenchymal and interstitial areas was delineated (Fig. 4). The procedure was repeated at each step of the serial sections, until the entire test space was covered. Counting of p_1 and the other parameters was performed after the reproduction of the histologic profiles in every slide in a drawing displaying the borders between the two areas.

The technique of counting was as follows. On the first drawing, all nodules were coded with serial numbers, as shown in Figure 4. At the same time, inter-nodal connections were given the numbers of another series after being entered on the drawing in the form of linear branches connecting the nodules. On the next drawing nodules and connections corresponding to the foregoing

[1] The precise mathematical meaning of p_1 is explained in an Appendix of this paper

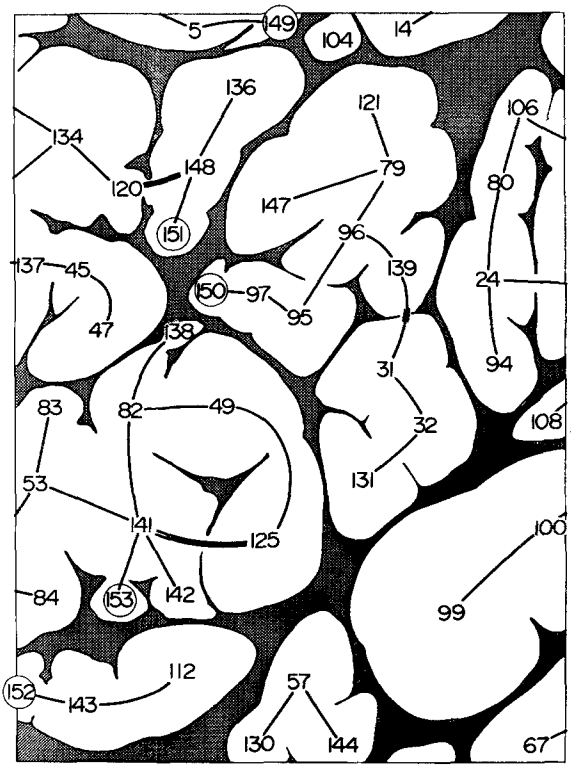


Fig. 4. Graphic treatment of internodular connectivity on a step of the serial sections from Case 4 of portal cirrhosis. All the nodules are registered with serial numbers. The five nodules from 149 to 153 are new ones which emerge for the first time on this level, the others having already been registered at one of the foregoing steps. The registration number of the branches is omitted here for the sake of better perspective. There is a loop 141–125–49–82–141 that is linked on this step with a new branch 141–125. This branch produces a multiple connection and increases p_1 by +1. The branch 120–148 is an additional newly appearing multiple connection and adds another contribution to p_1, although the loop itself is not visualized here because it is formed across several steps so as to penetrate the reference level. 139–31 is a new branch which requires another registration, for it unites two groups of nodules which have been disconnected in the foregoing steps. This branch reduces p_0 by 1

drawing were assessed. If new nodular sections appeared or if new connections were seen they were also registered with their respective consecutive numbers. Multiple connections were carefully identified and counted separately, for they are indicative of the presence of loops made by nodular chains (for the concept of multiple connections see Appendix). When a loop was identified from successive drawings, it increased p_1 by 1, because a closed loop has topologically a connectivity number of 1. Attention was also paid to a connection which joined two separate networks into a united one. Each such connection reduces the number p_0 of separate groups of nodules by 1. At every step of counting, observed n, b, p_0 and p_1 have to satisfy the Euler-Poincaré theorem of $n-b=p_0-p_1$, which expression was therefore useful for assessing the results of the observations. The procedure was repeated until all the nodules and connections in the test space were scanned. n, b and p_1 in the test space were then equivalent to the numbers of nodules, branches and multiple connections respectively.

In this operation it was sometimes difficult to identify individual nodules properly on a single histological slide because of their highly imperfect separation, as pointed out in the previous paper.

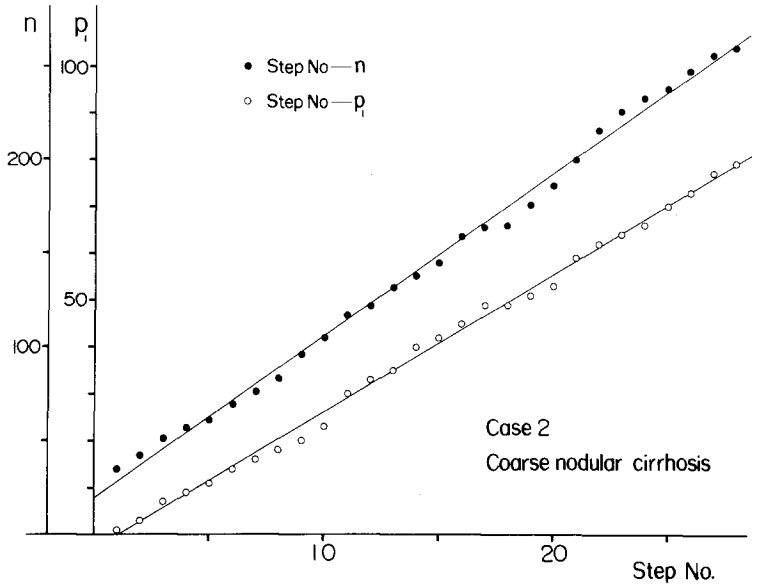


Fig. 5. n as well as p_1 increases with advancing steps in the serial sections

In such cases, successive drawings were placed one upon another and transilluminated. By looking through a sufficiently large number of tracings at the same time, individual nodules were better discriminated and their three-dimensional connections were more readily visualized. The same treatment was also applied to livers with subacute and chronic hepatitis, for there was a certain degree of nodularity in the "parenchymal network" even in these diseases.

The results of counting in a case of coarse-nodular cirrhosis are shown in Figure 5. The number of registered nodules (n) and p_1 increases in a direct relationship to the number of steps of histological sections examined, i.e., in proportion to the volume scanned. It is clear from the

Table 1

Case No.	Age and sex	Weight of the liver (g)	Histologic appearance of the liver
Case 1	58 M	1030	Cirrhosis, postnecrotic, with medium-sized nodules
Case 2	23 F	780	Cirrhosis, posthepatitic, coarse-nodular. Seemingly completed nodulation
Case 3	57 F	590	Cirrhosis, posthepatitic, coarse-nodular. Uncompleted nodulation
Case 4	3 M	1970	Cirrhosis, portal, related with a congenital metabolic error (lipodystrophic diabetes)
Case 5	51 M	1660	Chronic persistent hepatitis, 2 years' duration. Suicide by leaping from the height
Case 6	36 F	580	Subacute hepatitis, 60 days after the onset. Death from hepatic insufficiency

figure that examination of a volume containing about 300 nodules establishes a sufficiently definite linear regression of p_1 on the volume scanned. The relevant total values for the whole organ were calculated from the volume ratio of the organ to the space examined. The total p_1 estimated in this way corresponds to the "minimum genus" introduced by DeHoff et al. (1972) into the topological analysis of sintering.

Six livers with cirrhosis and precirrhotic disorders were selected from autopsy material and were submitted to topological analysis. The series consists of one case each of four representative types of cirrhosis, subacute hepatitis and chronic hepatitis. All the cases apart from Case 1 of postnecrotic cirrhosis had been used already in three-dimensional reconstruction analysis for the previous paper. The detailed morphology of the livers was described in that work, and here only the outline of the cases is listed in Table 1. The histologic pattern of cirrhosis was described according to the terminology of Popper and Schaffner (1957), Sherlock et al. (1956) and Gall (1960).

Results and Discussion

The results of topological analysis are presented in Figure 6 together with sketches of the histological appearance. There is a wide difference in the estimated total p_1 among the cases. However, the ratio of total p_1 to n is almost the same, being approximately 1:3 without exception. The reason for such a statistical analogy is unknown for the present, but it deserves attention as an empirical rule of nodular connectivity in general.

It is not within the scope of the present study to discuss the general problem of the genesis of cirrhosis; only the morphogenetic relationship of chronic hepatitis to cirrhosis is considered here. It has been widely accepted that chronic hepatitis, in particular the aggressive form, frequently proceeds to cirrhosis (DeGroote et al., 1968). However, there has been and still is much debate about the type of cirrhosis which follows chronic hepatitis and on the mechanism which causes the lesion to progress. Gall (1960) paid attention to the type of cirrhosis which was characterized by relatively large, multilobular nodules and narrow fibrous bands. He designated the type as "posthepatitic" cirrhosis, with the implication that it was the representative type of cirrhosis originating from chronic inflammation of the portal triads, i.e., chronic hepatitis in today's terminology. The extension of connective tissue septa described by Gall has much in common with that due to "piecemeal necrosis" in chronic hepatitis, since he assumed periportal fibrosis of an inflammatory origin, which spread continuously into the parenchymal region. In recent years, piecemeal necrosis has been regarded by many authors to be the basic mechanism which contributes to the transition of chronic hepatitis to cirrhosis. On the other hand, Baggenstoss et al. (1972, 1974) stresses in their "chronic active liver diseases" the importance of bouts of submassive hepatic necrosis which they regard as the decisive factor leading to cirrhosis. The term chronic active liver disease was proposed by them to replace chronic hepatitis as a more generalized category. In addition, some authors regard portal (or Laennec) cirrhosis as one of the chronic conditions that frequently follow hepatitis (Kunkel et al., 1950; Baggenstoss et al., 1952; Klatskin, 1958). All these discussions are closely concerned with the geometry of the morphological pattern, and should be re-examined from that point of view.

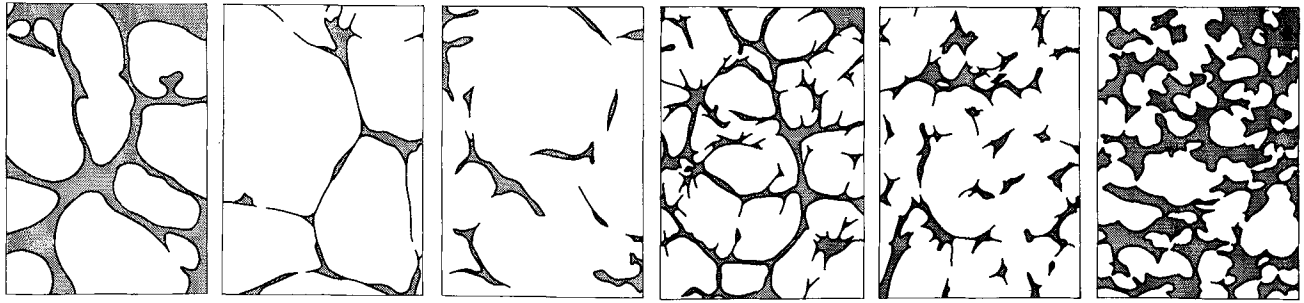
	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
	Cirrhosis postnecrotic	Cirrhosis posthepatitic (A)	Cirrhosis posthepatitic (B)	Cirrhosis portal	Chronic hepatitis	Subacute hepatitis
						
Hepatic weight (g)	1030	780	590	1970	1660	580
Total n ($\times 10^4$)	118	30	68	1859	1728	1421
Total p_1 ($\times 10^4$)	37	10	23	635	610	501

Fig. 6. Sketches of the liver histology with the results of topological analysis. The sketches were prepared under an equal magnification of histological sections. n=number of nodules. $\times 16$

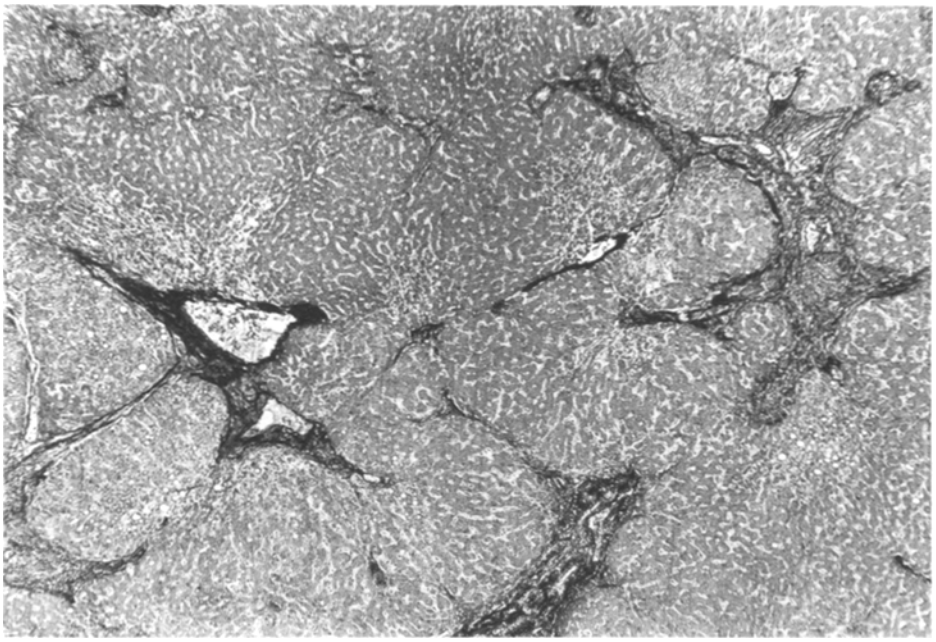


Fig. 7. Histological appearance of the liver in Case 5. Chronic hepatitis in a state of suppressed activity. Azan stain. ×60

The histological appearance of the liver of chronic hepatitis selected for the present analysis is shown in Figure 7. The picture is characterized by conspicuous fibrosis extending from the portal triads, in which there is rather focal round cell infiltration. Fibrous bands sometimes connect the portal triads with adjacent ones or with central veins, producing at least partial lobular disorganization. Signs of piecemeal necrosis are inconspicuous, and the hepatitic activity is considered to be suppressed.

The total p_1 in this case is estimated at 6,100,000 (Fig. 6), and the case thus belongs to the class of higher p_1 among the disease types examined. Of course there may be different morphological patterns in this category of chronic hepatitis. However, it must be emphasized that a relatively mild form of chronic hepatitis already harbors a vast number of multiple connections in the network structure.

Case 2 presents a typical histologic appearance of "posthepatitic" cirrhosis with apparently completed nodular structure. Total p_1 estimated in this case is only 100,000, which is the smallest among the cases examined. Thus, "posthepatitic cirrhosis" appears to be one of the most distant relatives of chronic hepatitis from a topological viewpoint. Progress of chronic hepatitis to posthepatitic cirrhosis requires of the network a reduction of its multiple connections from 6,100,000 to only 100,000. The latter condition cannot be attained unless the great majority of loop structures in the former are broken. It is a process

of radical, discontinuous transformation of the network structure, which can be brought about only by destruction of network due to parenchymal necrosis.

Generally speaking, hepatic parenchymal necrosis may be quite different in the manner in which it extends. In this case, it must be so extensive as to cut off the branches of parenchymal network and dissociate its connections, and in view of our knowledge of the structure it is necessary to assume necrosis of a more or less massive scale. In this respect, the recent studies of Baggenstoss et al. (1972, 1974) are worthy of special notice. On the basis of follow-up experience, they regard "bridging or multilobular necrosis" as the most decisive prerequisite for advancement of "chronic active liver disease" to cirrhosis. Whether necrosis of this kind is inherent in the progression of chronic hepatitis is still open to debate, but their observation supports the occurrence of frequent bouts of submassive necrosis in this lesion whatever its cause.

There are transitional cases between chronic hepatitis and posthepatitic cirrhosis, classified by Miyake (1960) as the imperfect type of posthepatitic cirrhosis. Case 3 of the present series exhibits an intermediate appearance shown by the presence of incomplete septa between relatively large nodules. Total p_1 in this case is estimated at 230,000, which is somewhat larger than that in the completed type. This case is considered to correspond to an intermediate stage in a series of network transformations from chronic hepatitis to completed cirrhosis.

Another probable method of progress from chronic hepatitis is continuous extension of fibrotic septa following "piecemeal necrosis", which has been regarded as the main route leading to cirrhosis by DeGroote et al. The topological meaning of "piecemeal necrosis" may be given as follows. Firstly, when extension of fibrosis produces an anastomosis between two neighboring portal triads, it penetrates the parenchymal network and adds a new loop to the network, thus, each new anastomosis increases the total p_1 by 1. Secondly, if connective tissue septa continue to stretch without generating anastomoses, the result is an exaggeration of the nodular pattern, but p_1 remains unchanged, as an accentuation of the nodular character of the parenchymal network does not affect its inner connectivity. It may be said that the total p_1 either increases or remains constant, when chronic hepatitis develops into cirrhosis through "piecemeal necrosis" alone.

A comparison of total p_1 in Figure 6 proves that Case 4 of portal cirrhosis is the nearest relative of chronic hepatitis. The synonyms for portal cirrhosis such as Laennec's or primary septal cirrhosis (Popper and Schaffner) will fit the histologic appearance of this case. The total p_1 is 6,350,000, being almost equal to the case of chronic hepatitis. The approximate isomorphism of the two lesions may be appreciated, if the sketches in Figure 6 are compared. Both the livers in Cases 4 and 5 show fine granulation of almost equal dimension, and it can be clearly seen that extension of connective tissue septa in chronic hepatitis will produce the picture of portal cirrhosis.

In addition, a brief comment on the development of postnecrotic cirrhosis from subacute hepatitis (submassive hepatic necrosis) is appropriate. Case 1 of postnecrotic cirrhosis with medium-sized nodules is compared to Case 6 of subacute hepatitis. The transition from the pattern of Case 6 to that of

Case 1 also requires a discontinuous transformation of the network as revealed by a drastic fall of total p_1 from 5,010,000 to 370,000. It will drop to a much lower value when the lesion proceeds further to the coarse-nodular type of postnecrotic cirrhosis.

Topological analysis only reveals geometrical conditions which may be imposed on the possible morphological developments of one hepatic disease from others. Thus topological isomorphism between two hepatic diseases does not always mean a ready transition of the one into the other in terms of the actual histopathological process. For instance, the total p_1 in the case of subacute hepatitis does not differ very much from that in the case of portal cirrhosis, showing that both types are topologically similar. In reality, however, transition from subacute hepatitis to portal cirrhosis is improbable, since the interstitial areas of subacute hepatitis are too wide to permit its progression to portal cirrhosis. Apart from the inter-connections of the network there are, of course, many structural factors that determine the fate of hepatic disease.

Appendix

Application of Network Topology to the Structure of Liver Cirrhosis

We examine the geometrical character of the limiting surface of parenchymal (or nodular) network, which demarcates the network from interstitial septa. This forms a closed surface with a tremendous number of "holes" (see the previous paper; Takahashi, 1978). The topological property of a closed surface is analyzed on the following principle; Imagine the surface of a torus with one hole as shown in the left middle part of Figure 8. Suppose that the surface is infinitely plastic and can be continuously transformed into another shape, for instance into a cup with a handle. However, its transformation into a spherical surface (Fig. 8, upper left) is impossible because the latter has no hole. Similarly, the surface of a disc with two holes (lower left) cannot be derived from a torus surface. Thus an equivalence relation is defined among different types of closed surfaces according to *topological (homological) isomorphism or heteromorphism.* Two closed surfaces are isomorphic ($\cong$) if one can be produced from the other through continuous transformation alone, and are heteromorphic if it is impossible ($\ncong$). Based upon this principle, hepatic lesions are classified according to the geometry of the network they produce. Lesions which belong to the same isomorphic group are susceptible of mutual continuous transformation, which is impossible among heteromorphic groups.

Heteromorphic surfaces are discriminated by means of a topological invariable, the *genus,* which is a measure of connectivity. The genus G of a closed surface is defined as the maximum number of non-self-intersecting closed curves which can be depicted on the surface *without* dividing it into two separate parts. On the spherical surface, no closed curve can be drawn without isolating a part from the surface (Fig. 9). Accordingly, $G=0$ in this case. On the torus surface $G=1$, because it is possible to draw one and only one closed curve which does not generate a separate part (see figure). On the surface of a disc

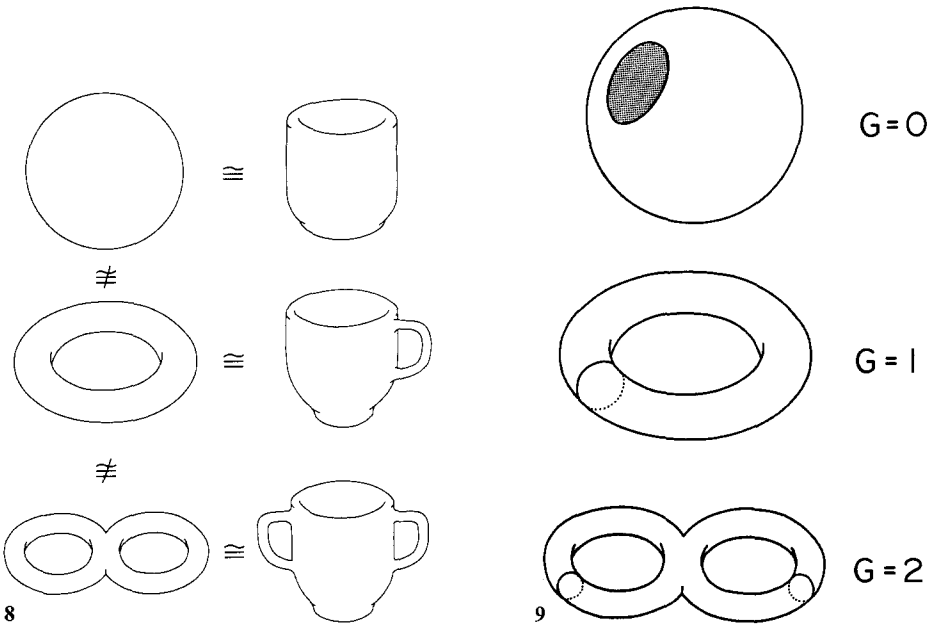


Fig. 8. Different types of closed surface arranged according to the principle of topological isomorphism (≅) and heteromorphism (≇)

Fig. 9. The concept of genus G is illustrated. $G=0$ on the spherical surface (upper), $G=1$ on the torus surface (middle) and $G=2$ on the disc surface with two holes (lower)

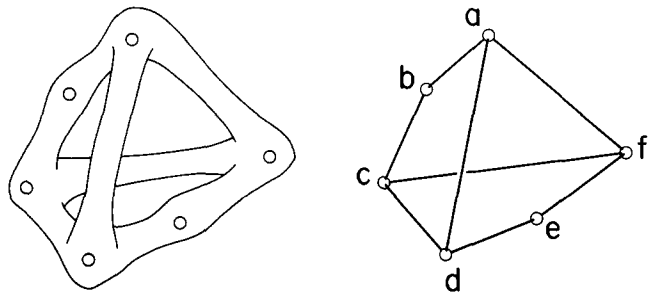


Fig. 10. Construction of a node-branch network (right) which corresponds to a multiply connected closed surface (left)

with two holes, $G=2$ for the same reason. Thus it can be shown that the genus of a closed surface is equal to the number of holes it has.

In diseased livers network analysis by means of genus may not be practical because its determination requires rather laborious processes. The parenchymal (or nodular) network can be treated much more easily, when the closed surface is replaced by a *one-dimensional complex* (node-branch network) of equal connecting relationship. An example is presented in Figure 10, which is drawn according to DeHoff et al. (1968). The figure in the left half is a closed surface with several holes. It may serve as the simplest model of the parenchymal

network of hepatic lesion. Assume now that an arbitrary number of points are placed in the parenchymal mass and are connected by branches, so that the network may be replaced by the one-dimensional complex of equal connectivity (Fig. 10, right). This procedure of replacement corresponds to the construction of the "deformation retract" of DeHoff et al. (1972). In cirrhosis it is, of course, convenient to relate the nodes to nodules and the branches to internodular connections.

After a network surface has been reduced to the corresponding one-dimensional complex, topological analysis can easily be performed by means of another invariable, the *connectivity number* p_1. p_1 may be defined as the maximum number of branches that can be taken away *without* generating a new separate part of the network or without splitting the network into new sub-networks. If the number of nodes in the system is given by n and that of branches by b, p_1 is determined by Euler-Poincaré formula:

$$n-b=p_0-p_1$$

where p_0 denotes the number of separate parts of the network. p_0 and p_1 are the quantities that are called *0th and 1st Betti numbers* respectively, but in this study the expressions "the number of separate sub-networks" and "the connectivity number" are used to substitute for the above concepts. The value of p_1 is exactly the same as the genus of the corresponding closed surface, because the holes in the latter are transferred precisely to a one-dimensional complex in the form of "loops" formed by nodular chains.

In the example given in Figure 10, $n=6$ and $b=8$, and $p_0=1$ because the network is united as a whole, and $p_1=3$ is obtained from the above equation. It will be easily ascertained from the figure that the maximum number of branches that can be removed without splitting the network is 3. It may be clear that the geometrical meaning of G as well as p_1 is the number of *multiple connections* which connect the elements that have already been joined to form a united system. The "hole" as well as the "loop" is a structure that arises as the result of multiple connections.

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