

Liver Units in Three Dimensions: I. Organization of Argyrophilic Connective Tissue Skeleton in Porcine Liver With Particular Reference to the "Compound Hepatic Lobule"

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ABSTRACT Liver units were investigated in pig livers by means of histologic serial tracing, physical model building, and computer-aided three-dimensional imaging. Observations of the argyrophilic connective tissue skeleton were based mainly on the celloidin-embedded serial sections treated with silver impregnation. The parenchymal mass that clothed the initial segments of hepatic venous radicles was demarcated by fibrous septa which formed isolable units with two basic patterns: the simple hepatic lobule (SHL) and the compound hepatic lobule (CHL). Both lobule types presented regular limiting structures circumscribing each unit. Three-dimensional studies revealed that 25% of the lobules in a section belonged to the SHL type and 75% to the CHL type, the latter being predominant among the surface lobules. When considered in only two dimensions, however, the SHL-like lobules constituted the majority. Polygonal analysis disclosed that the pentagonal lobule was the most typical, instead of the "hexagonal" or "classic" lobule. The CHLs represented a multiaxial unit containing a system of venous tributaries in accordance with intralobular septation, whereas the SHLs were found with one axial vessel having a dendritic tendency at the incipient end; some SHLs were drained eccentrically by separate vessels into a sublobular vein. It was observed that, in dividing CHLs, whereas particular sinusoids were transformed into portal twigs, other sinusoids were changed into central venous tributaries. Fibrous deposition occurred along the septal-line sinusoids, bringing into view the septum-initiating plane. Fibroconnective tissue was supplied from the portal area and central (sublobular) adventitia, where portal triad structures and adventitial arterioles, respectively, were included. The findings of the present study facilitate the understanding of several characters of the lobules that have been reported previously, or occasionally postulated, such as the portal-central bridging tendency, the intralobular arterioles or ductules, the translobular artery or portal vein, the "portal-portal" or "portal-central" anastomoses, and the apposition of pericentral zone close to periportal zone. Based on differences in

argyrophilia of sinusoidal reticulum, in proportion of lobule types, and in vasculature, the anatomic heterogeneity of liver unity was demonstrable in zonality, regionality, and locality.

INTRODUCTION

It was not an accident that led to the reopening of a field thought to be closed. As mentioned by Teutsch (1981), the pioneering discovery by Wepfer in 1664 (cited by Bloch, 1970) of the anatomical unit of the liver followed by Malpighi's (1666) perspicacious discussion, is being continued and extended today; centuries of striving to find a definition of this structural unit have not yet led to general agreement.

An adequate description of the liver unit should provide not only structural but also secretory and microcirculatory unity (Rappaport et al., 1954; Rappaport, 1958), and in the "hexagonal lobule" of Kiernan (1833) there is no microcirculatory unity (Rappaport, 1963, 1973, 1976). The innovative concept of the "simple liver acinus," which has emerged in the literature since 1953 (see Elias, 1953), is competent to satisfy the cited prerequisites, and therefore represents the "structural and functional unit" of the organ (Rappaport et al., 1954).

The acinar concept has been elaborated to account for the many patterns of the histopathologic lesions involved in liver disease (Rappaport et al., 1954; Rappaport and Hiraki, 1958a,b). Its conspicuous feature, the microcirculation-based zonality, has been applied further to explain the hepatocyte heterogeneity in enzymic expression (Rappaport, 1973, 1976). During the past few decades, an enormous number of investigations have been devoted to clarifying the mechanism of the hepatic role in metabolism and the significance of the heterogenous distribution that prevails (for reviews, see Jungermann and Sasse, 1978; Gumucio and Miller, 1981; Teutsch, 1981; Jungermann and Katz, 1982; Jungermann, 1986; Sasse, 1986; Gumucio and Chianale, 1988; Gumucio, 1989). In presenting their findings on metabolic zonation, many investigators conformed their results to fit the acinar context. A few, however, preferred the more anatomical terms of compartmentation, i.e., "periportal" and "pericentral,"

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probably being aware that the concept of the simple liver acinus has not been perfected. For the recognized shortcomings of the concept, one can consult Matsumoto et al. (1979), Teutsch (1984, 1988), Sasse (1986), McCuskey (1988), Lamers et al. (1989), and Quistorff and Rømert (1989).

Current Trends

It seems that the time-honored concept of the lobule has given way to the novel concept of the liver acinus in the literature. In their work on liver biology and pathobiology, Arias et al. (1988) stated that "the microcirculatory unit is the hepatic acinus. . . . This view of the hepatic acinus considers that the hepatic veins drain the periphery of the functional unit, rather than the center, as in the older concept of the hepatic lobule." In a standard textbook, we find the following: "increased emphasis has been given to the acinar concept of liver structure in recognition of the fact that it is fast gaining almost universal acceptance" (Cormack, 1987). A team of ultrastructural pathologists (Phillips et al., 1987) relate in their superb publication that "the concept that the hepatic parenchyma is organized in lobules bounded by portal areas and with the central vein in the middle has largely been replaced by the acinar concept proposed by Rappaport. The acinar organization is based upon a combination of microanatomic, microcirculatory, and metabolic considerations." An international group (Bianchi et al., 1977), on reviewing the classification of acute and chronic hepatitis, agreed that the topography of the different forms of necrosis is easier to explain on the basis of the hepatic acinus than according to the lobular concept. In another published series, it is stated that "Rappaport's studies led to a clear understanding of the hepatic microcirculation. This is the anatomical basis for the distribution of various types of lesions in alcoholic and nonalcoholic chronic active liver diseases and can explain the consequences of developing fibrosis and cirrhosis. . . . The old concepts . . . gave us a misconception of the anatomic unit of the liver" (Galambos, 1979).

Nevertheless, most textbooks on histology still, as before, present students with both concepts of liver units and do not discard either of them. It is explained that these two conceptions "should not be considered as conflicting concepts of liver structure but as complementary ones" (Fawcett, 1986); "should be considered as complementary, not mutually exclusive" (Kelly et al., 1984); "are not mutually exclusive or conflicting" (Rhodin, 1974); "are not mutually irreconcilable" but "are in agreement" and "mutually complementary" (Geneser, 1986); "do not, however, appear to be contradictory nor should they be mutually exclusive" (Wilson, 1958); "are not incompatible. . . . each describes the same design but from a different perspective" (Lemasters et al., 1986); and "are not conflicting, but rather represent different interpretations of certain aspects of hepatic structure and function" (Jones, 1990).

Coming of Age

A group of liver pathologists (Matsumoto et al., 1979), realizing the import of such quotations, began a painstaking work investigating the microangioarchitecture of normal livers, with the aid of graphic reconstructions from thousands of serial sections. They

called attention to the fact that the perfusion sources of the human liver lobules are arranged as planes that are interconnected (the "continuous inflow front"), pervading the entire organ, whereas the acinar concept has the "linear" axis as its principal perfusion center. In addition, their pathological and enzyme histochemical findings did not admit the acinar perfusion pattern. It was noticed that distributions of the glucose- and ketone body-metabolizing enzymes in rat liver lobules deviate from the acinar zonality (Teutsch, 1984). The development of microdissection and microchemical techniques (Lowry and Passonneau, 1972) has facilitated high-resolution and high-precision analyses of quantitative histochemistry. Using these in conjunction with improved qualitative histochemical procedures, Teutsch (1988) could demonstrate, in computer-based tridimensional quantitation, that the glucose-6-phosphate hydrolytic activity in the liver lobule of the rat does not follow the perfusion pattern of the acinar concept; instead, the results confirm the lobular gradient and support the "primary lobule" concept (Matsumoto et al., 1979; Matsumoto and Kawakami, 1982). Most recently, the study of ammonia-metabolizing enzymes by Lamers and colleagues (1989) has shown in computer-aided reconstruction that the results in two and three dimensions do not correlate with predictions on the acinar basis.

Justification of the Present Investigation

As far as morphologic truth is concerned, before a concept can be approved (or disapproved), we must verify (or disprove) whether it is considered from a two- or three-dimensional point of view. As a rule, a generalized concept should not include exception for certain species without scientifically acceptable reasoning.

It is interesting to note that "the pig liver has, so far as we know, never been included in the acinar concept" (Teutsch, 1988). A careful survey of the literature disclosed that the pig has lost its popularity for morphological research on liver units since the midcentury, the period when interest in the field shifted to the configuration of the liver cell plates (muralia), or the "laminae hepatis" (Elias, 1949), and the period of the advent of electron microscopy. Concurrently it was reported that the normal pig liver is an example of subclinical cirrhosis of a purely portal type (Elias et al., 1954).

It was not until the 1980s that the pig's classical liver lobules were reinvestigated by Wünsche (1980, 1981). In her ensuing studies, Wünsche (1982, 1985) showed that all four types of liver lobules (classic, biliary, portal, and acinar) are present in the pig and called for correction of the ideas that regard the pig liver as "exceptional" (Brissaud and Sabourin, 1888), "atypical" (Elias et al., 1954), or "pathologic" (Elias and Sherrick, 1969). Instead, it was demonstrated in meticulously isolated, injected lobules that the existence of the basketlike organization of portal terminal ramifications, called the *corbicula portalis*, substantiates the classical concept of hepatic lobulation (Wünsche and Preuss, 1985, 1986).

It becomes clear then that the hidden third dimension, when unraveled, gives a new insight into the concept of liver units and that the pig is no longer a "taboo" animal for this purpose. Therefore, we under-

took the investigation on liver units in three dimensions, with special reference to the pig liver. As for the unusual distinctness of the lobulation due to fibrous septation, the "structural divergence" (Elias et al., 1954), we have examined the literature in detail and ascertained that, among about 80 mammalian species studied, there exist 8 mammals whose livers share a more or less similar lobular demarcation: the polar bear (Braus, 1924; McIndoe, 1928; Pfuhl, 1932; Elias and Sherrick, 1969; Jones and Spring-Mills, 1984, 1988; Teutsch, 1988; Jones, 1990); the degu, *Octodon cumingii* (Hyrtl, 1864, cited by Oppel, 1900); camel (Turner, 1877; Braus, 1924; Arey, 1932; Elias and Sherrick, 1969; Jones and Spring-Mills, 1984, 1988; Kelly et al., 1984; Teutsch, 1988; Jones, 1990); wild pig (Kostorz, 1936; White, 1939); raccoon (Arey, 1932; Elias and Sherrick, 1969; Jones and Spring-Mills, 1984, 1988; Jones, 1990), and dromedary and American bear (White, 1939).

Although it is greatly supererogatory at this day to discuss the light microscopic structures of normal pig liver lobules, a clear conception of the liver units is best obtained by establishing the basic knowledge on the organization of the hepatic lobules. It is widely realized that fundamental problems persist when the hepatic structures are described in terms of their functional aspects, and these controversies require us to go back to the beginnings of light microscopy (Sasse, 1986). As will be seen in the present paper, several structures that were observed have been neglected or have not been interpreted correctly in relation to the lobule's basic architecture. Such observations remain in the literature and seem unlikely to be reconsidered as contributory to the comprehensive understanding of the functional biology and pathobiology of the liver units. We believe, therefore, that descriptive liver histology demands renewed investigation.

The objective of our studies is to reevaluate the concepts of liver units in three dimensions. Taking advantage of the abundance of fibroconnective tissue in the porcine liver, the investigation was initiated by observing the lobular structures as visualized by the silver-impregnation method. Based on serial sections, many lobules and related structures were reproduced by physical model reconstruction and by computer-graphic tridimensional imaging. The description of the models, the ontogenetic development of hepatic lobules and the dynamic changes in lobule types and lobule subpopulations, and the terminal portal vasculature and microcirculatory reconstruction will be dealt with in subsequent papers.

In the present article we report on the characterization of the two lobule types as viewed from the organization of the argyrophilic connective-tissue skeleton. For descriptive convenience, we employ the designations "simple hepatic lobule" (SHL) and "compound hepatic lobule" (CHL), the latter being referred to with various terminologies by previous observers.

MATERIALS AND METHODS

Liver Acquisition, Block Cutting, and Fixation

Ten nonpathologic pig livers were obtained from a large abattoir in Tokyo and a local slaughter house in its nearby province. The animals, which had been raised for an average of 6 months until attaining a

body weight of approximately 100 kg (liver weight 1.2–2.0 kg), were killed by application of an electric shock to the lateral cervical region. The freshly acquired livers were immediately flushed with normal saline and perfused with 3.7% formaldehyde solution, lobe by lobe, through a cannula inserted into a major branch of the portal vein at the porta hepatis. The perfusion was conducted by pressing a plastic syringe manually or by using an electrically driven rotary pump. Care was taken to employ a moderate pressure that would not alter significantly the liver volume. After the organ became hardened, it was further fixed by immersion in the same fixative and transported to our laboratory; in summer, it was carried in a cooled box. Sometime prior to fixation, the liver was divided into pieces, about one-fifth of a lobe, and perfusion fixation was performed through branches of the portal vein identified on the cut surfaces.

From these perfused specimens, samples were taken preferentially at a free edge of the liver lobe. The tissue was cut into triangular prismatic blocks of about 6 mm × 10 mm × 20 mm. The top and bottom sides of a prism were cut perpendicular to the hepatic capsule as well as to the liver edge. Therefore, the convex (diaphragmic) and the concave (visceral) surfaces, which approximated and met at the tip of the liver edge, formed two lateral sides of each block (Fig. 1). The capsular surfaces were intended to be used as planes of reference in vertical alignment for the solid model reconstruction. In actual practice, we created a pinhole perpendicularly at three separate locations in the block and implanted a horsetail hair into each for use as points of reference.

The blocks underwent immersion fixation at room temperature for at least 2 weeks with the fixative changed a few times. We took special care to secure ample free space in the fixing container to avoid distortion in the tissue blocks and accordingly were able to preserve the normal configuration of the lobules.

To be sure we were not dealing with the product of an accident or of seasonal changes, the pig livers were procured in different seasons of the year. In livers that presented a cirrhotic appearance, this was confirmed histologically and the livers were excluded from the investigation.

Embedding, Sectioning, and Staining

The tissue blocks were subjected to deliberate dehydration through a graded series of ethanol, followed by double changes of anhydrous ethanol twice and of a mixture containing 50% (v/v) diethyl ether in ethanol twice. Next, they were submerged in ascending concentrations of 2%, 4%, 8%, and 10% celloidin, taking a total of 3 months when embedding terminated. Sectioning was accomplished by cutting serially with a Thoma-Jung-type microtome (Yamato, Tokyo, Japan), set at 20 µm thickness, using a microtome knife of grade A (Morimoto, Tokyo, Japan). The celloidin block was accurately oriented with respect to the knife blade so that the plane of section was at right angles to the prismatic block of the tissue, i.e., parallel to the top side of the prism (see Fig. 1). Several blocks were attempted, and from the sections obtained we selected an uninterrupted series, consisting of 244 well-cut sections, for the purpose of three-dimensional studies. Se-

quential numbers were placed on a corner of each celloidin slice by marking with calligraphic ink, which once dried would not disintegrate in any solvent.

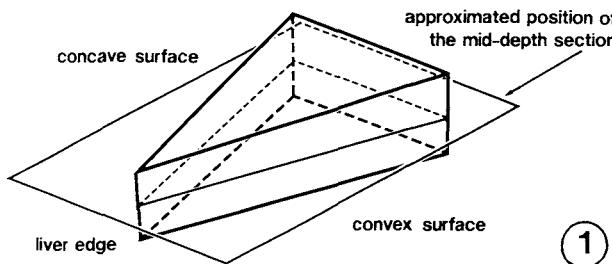
The entire series of the celloidin-embedded sections were stained with a modification of the silver-impregnation method (Ishii and Ishii, 1965). As the reducing agent, a 10% solution of Rochelle salt (sodium potassium tartrate) was applied (Suzuki, 1958), instead of the 1.5% formaldehyde solution, whereby undesired superfluous precipitations of silver could be noticeably suppressed. By using a soft calligraphic brush to sweep slightly but thoroughly, particles of silver that had been deposited unspecifically on both sides of individual sections could be removed satisfactorily. We checked the metal-impregnated sections microscopically before and after brushing and were able to ensure that the fibrous-specific silver precipitates were essentially unaffected by this physical wiping.

A counterstain with hematoxylin facilitated the observation of nuclei under the microscope and, at the same time, technically served to strengthen the thin slices of sections in a way to withstand unpredictable handling-related damage. Finally the sections were reacted in 1% gold chloride solution, at 20°C shielded from light, to yield a more beautiful visualization of argyrophilic fibrous tissue. The sections were carefully dehydrated, cleared in xylol, and mounted in balsam.

Apart from the silver method, the liver tissue was prepared in 10- and 5- μm -thick, paraffin-embedded sections, serial and nonserial, and stained with hematoxylin and eosin, Masson-Gomori trichrome, and Azan stains. These routine preparations were used for general histological study to check pathologic lesions, if present, and for comparative study to assess the stainability of fibroconnective tissue and to confirm the findings.

Abbreviations

A	hepatic artery
a	hepatic arteriole or arteriolar branch
Aa	adventitial arteriole
ac	arterial capillary
Ad	adventitia
B	bile duct
b	bile ductule (ductular epithelium consisting of three or four up to six or seven cells in cross section)
BP, bP	bile duct (ductule) with peribiliary arteriolar (capillary plexus (three or more profiles of vessels accompanying))
C	central vein
cl	circumferential lamina
Cs	centralized sinusoid
cs	circumferential sinusoid
d	ductular formation
H	hepatic capsule
IS	interlobular connective tissue septum
iS	intralobular septum
iv	inlet venule
L	lymphatic vessel
N	nerve bundle
P	portal vein
p	portal venule (diameter < 40 μm ; the largest ever identified as a terminal branch)
pl	perilobular portal branch
Ps	portalized sinusoid
PT	portal tract
R	reticulum
rl	radial lamina
rs	radial sinusoid
S	sublobular vein



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Fig. 1. Orientation of liver tissue for cutting prismatic blocks, and approximation of position of the middepth section. Microtome sectioning was performed parallel to the top and bottom sides, which were cut perpendicular to the convex and concave surfaces and at right angles to the liver edge. The middepth section is represented in Figure 4.

Microscopy and Recording Systems

The specimens were studied under an Olympus microscope BH-2 with the SPlan Apo objective set, and photographed by an Olympus full-automatic photomicrographic system (PM-10AD series), using an orange filter (YA 3, Kenko) to record on the Kodak Panatomic X monochrome film.

A section was selected from the series at about the middle of the depth of the tissue block (Fig. 1). Then the entire picture of the impregnated tissue of this middepth section (preparation number 132; referred to hereafter as a master section) was produced by tracing through a camera lucida. This whole-section drawing was employed to map the lobule-type distribution (see Fig. 4).

For analysis of polygonal lobular fields, montages of five micrographs were prepared of section numbers 52, 72, 92, 112, 132, 152, 172, 192, and 212. Low-magnification micrography was accomplished with an Olympus stereoscopic microscope (SZH) with an automicrographic apparatus (PM-10AK).

Three-Dimensional Reconstructions

The findings obtained from observation of histologic serial sections were partly confirmed in three-dimensional reconstructions. We employed a solid model-building technique and a computer-assisted three-dimensional (3D) imaging technique.

Complete serial profiles of selected lobules and connective tissue septa, consisting of 80, 90, 92, and 119 consecutive sections, were traced onto a modeling medium called Styrenepaper of 2.5 mm thickness. After cutting out with a nichrome-wired cutter, the traced materials were assembled into positive and negative models. At the same time, sequential information on the lobules was inputted via a digitizer (Graphtec KD-5050) connected to a personal computer (NEC PC-9801RX2), and was processed by an application software (Nikon Cosmozone 2SA) into tridimensional images using a more than 1,600,000 color display capacity. More details of the methods will be described in a forthcoming paper.

RESULTS

General Survey

We carefully checked the hematoxylin and eosin-stained preparations in accordance with the guidelines

for a systematic approach to microscopic study of the liver set forth by Grases and Beker (1981), and we found no pathologic lesions in the preparations used. The lobules were distinctly demarcated by the orderly connective-tissue septa pervading the porcine hepatic parenchyma; these fibrous partitions were well visualized in Masson-Gomori trichrome stain and Azan stain. However, those collagenous substances that occurred in small accumulations along the sinusoids, where intralobular septal formation could be anticipated, were not always discernible. Such argyrophilic deposits were brought into full view in the preparations treated with the silver method.

Generally, the silver-impregnated tissue appeared reddish brown where the fibrous substances were bundled and black where the argyrophilic substances were attenuated, due to the specific reaction of ammoniacal silver nitrate on the surface of the collagenous fibers or fibrils (Ishii and Ishii, 1965). The rose-pink hue of the parenchyma was due to the reaction with gold chloride on the celloidin-embedded specimens.

Fibroconnective Tissue Skeleton

The continuum of the fibroconnective tissue skeleton in the porcine liver was recognized with five structures that differed in anatomical disposition. They were the hepatic capsule, the interlobular connective tissue, the intralobular septum, the central venous adventitia, and the sinusoidal reticulum (Fig. 2). The first two were extra- or interlobular, whereas the last three were intralobular in nature.

The Extralobular Skeleton

The hepatic capsule

The hepatic capsule, or the capsula fibrosa Glissonei, that covered the liver had a thickness of 15–25 μm , which was similar on both surfaces¹ of the tissue harvested. Close to the liver edge, the capsule became thinner, 7–12 μm .

The capsula fibrosa Glissonei constituted one side of those lobules lying beneath it, called the surface lobules (see below), and consequently gave them a flattened facet. The capsule contained no sublobular vein,² an indication of the polarized arrangement of the surface lobules showing that none drained toward the outward direction (Fig. 3a,c–e).

The interlobular connective tissue

Collagen fibers were laid along the plane in which the portal venous branches and the hepatic venous radicles were distributed. They formed trabecles or, in three dimensions, septa of 10–25 μm thickness, which were set down straight or slightly curved, delineating the histologic lobules into irregular polygonal areas. These lobules ranged between 0.7–1.2 mm in diameter

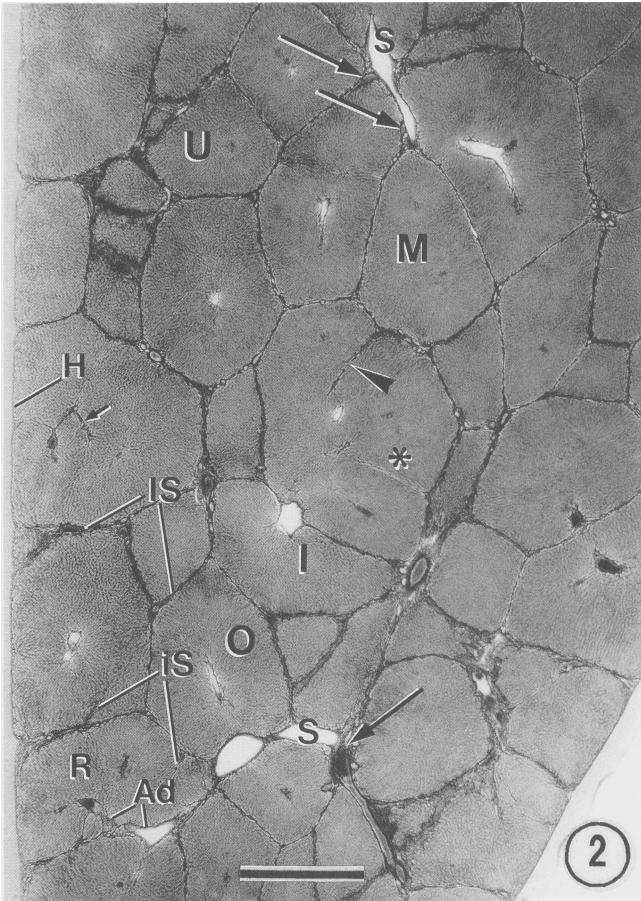


Fig. 2. General survey of a celloidin-embedded specimen (preparation 132) treated with silver impregnation (Ishii and Ishii, 1965). The continuity in the argyrophilic connective tissue skeleton is obvious. The intralobular septa appear as fibrous ingrowths with exceedingly attenuated strands of fibers (asterisk), or with an interruption (arrowhead), or as an isolated septum (short arrow). At some places, branches of the portal vein (long arrows) cross over the sublobular vein. Differing appearances of the SHL-like lobules are depicted; uni-center (U), multi-center (M), uni-exit (O), multi-exit (I). Bar = 1 mm.

but were measured noticeably smaller near the liver edge, about 0.4–0.8 mm (Fig. 4).

Apparently the two venous systems ran independently in the interlobular septa. However, the occurrence of a portal vein crossing over or coming to an immediate apposition with a radicle of hepatic vein was not infrequent (Figs. 2, 3a,b). The portal vein was ensheathed by an abundance of collagenous substances and was accompanied by branches of the hepatic artery and the bile duct, which were enwrapped by a relatively thinner fibrous investment (Fig. 5). These constituted the portal tract, which was generally observed coursing longitudinally at the adjoining corners of three or four lobules that formed the "interlobular space" of Kiernan (1833), thus blunting the very vertex of the angles of the polygonal fields. The perilobular branch (Pfuhl, 1922), originating therefrom, was presented as a smaller vasoductular bundle in the septum between the apposing lobules (see Fig. 8a). In many cases, a transverse section of a portal tract included

¹We have encountered a pig's liver (used in another series) in which, remote from the liver edge, the concave capsule appeared to be quite a few times thicker than the convex capsule, the thicknesses being 70–110 μm and 25–30 μm , respectively. In ox liver, the difference was even more pronounced.

²In certain species, such as the frog and the rat (Wakim and Mann, 1942), significant hepatic vessels are situated in the hepatic capsule. We have confirmed these observations.

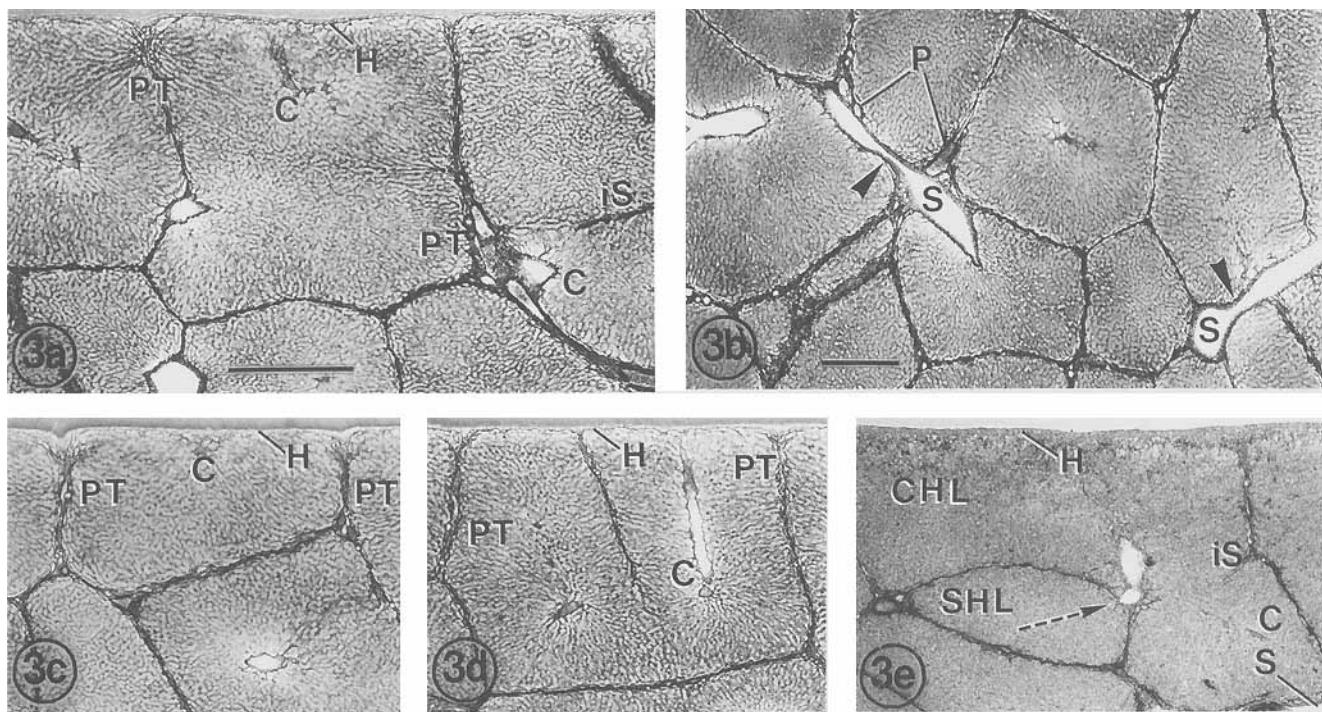


Fig. 3. Surface lobules with a flattened facet adapting to the hepatic capsule (a, c–e) and lobule bases (b). The central veins that originate in the subperitoneal region follow a course parallel to (c) or at right angles with (d) the surface capsule. The portal tracts, on approaching the capsule, radiate fibrous strands into the surrounding sinusoids, which are densely reticulated (a, upper left). These structures fan out laterally, leaving a collagenous ridge that bridges the gap between the interlobular septum and the undersurface of the capsule (a, upper

right, c, d). An SHL may have its central vein (e; dashed arrow) draining into the central vein of a CHL instead of a sublobular vein. Note that in a a portal tract crossing over a central vein as the latter is about to depart from a CHL at its base. In b, part of a lobule's base (arrowheads) impinges on the sublobular vein, resulting in local narrowing of lumen. Note two portal branches crossing over a sublobular vein. Silver impregnation; bars = 500 μm (bar in a applies to c–e).

multiple profiles of the bile duct and hepatic artery but only one of the portal vein (Fig. 5b). The bile duct and hepatic artery, but not other combinations, showed a strong tendency to be placed preferentially close together, so that, if a typical portal area (such as depicted in Fig. 5a) were to be mentally bisected, the pair filled one half and the portal vein took up the other.

When the portal vein in a portal space ramified to give rise to a portal branch of another nearby portal space, it was fairly common that the vessel that communicated between those two portal spaces was unusually restricted in diameter, presenting an appearance that could be mistaken for a portal-portal anastomosis (Fig. 5d–f). The narrowed segment appeared closer to the parent portal area.

The interlobular septa reached the surface capsule at frequent intervals. Where the portal triad structures were included, the fibrous strands spread laterally into individual sinusoids beneath the capsule (Fig. 3a,c,d).

On the other hand, radicles of the hepatic vein coursed apparently unaccompanied. The lumen of the hepatic vein sometimes exhibited local narrowing (Fig. 3b), in adaptation to the surrounding mass of lobules; the latter directed their bases on these vessels (Kiernan, 1833). We could not find localized smooth muscle fibers that protruded into the luminal space, such as is known in the dog, the raccoon, the seal (Årey, 1941) and many other species (see Elias and Sherrick, 1969).

Portal occupancy rate of the interlobular spaces. Where three or more interlobular septa met, they formed an interlobular space, which was usually occupied by the portal triad structures. Smaller interlobular spaces might not be supplied by the arterial components. Occasionally the interlobular space was found to be occupied by branches of the hepatic artery and the biliary duct in the absence of the portal vein. An interlobular space that was empty, without any vasoductular structure, was not infrequent. Sometimes a sublobular vein was encountered.

Based on whether or not they contacted the hepatic capsule at the surface, the lobules could be divided into two populations, namely, the “surface lobule” and the “deeper lobule” (Johnson, 1918a). We have found that the rate at which histologic lobules were equipped at the interlobular spaces with portal ramifications differed among these subpopulations and could be represented in a quantitative form (Table 1). This rate was quantified in a range from 1 to 0. 1, when the portal branches were present in all the interlobular spaces; 0, when none were seen in the section. In the master section, 1 was derived from 2:2, 3:3, 4:4, 5:5, 6:6, and 7:7; 0 was from 0:2, 0:3, 0:4, and 0:5. In obtaining these figures, the Glisson's capsule that established a side of the surface lobules was regarded as an interlobular septum; the intralobular septa of the compound hepatic lobules (see below) were not considered as interlobular septa. The results showed that roughly one-half

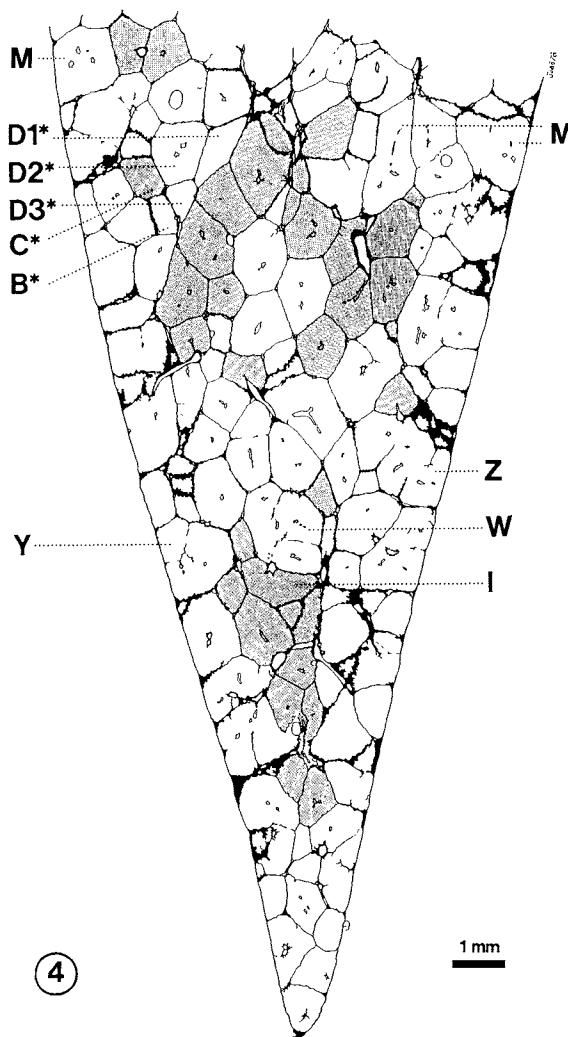


Fig. 4. Camera-lucida drawing of the master section, silver preparation 132, and the lobule mapping: SHL, shaded; CHL, open. In the CHLs W, Y, and Z, apparently the splitting is not a binary fission. The lobular fields with septate profiles are often larger than average. The lobules that constitute the liver edge are on the average small. Some lobules individually represent isolable units (SHL, lobule C*; CHL, lobule B*); some form a unit together with others (group of lobules D1*, D2*, D3*), which can be demonstrated in three-dimensional tracing or reconstruction (also see groups of Figs. 6, 7). I, Multiexit SHL; M, multicenter CHL. Bar = 1 mm.

TABLE 1. Portal occupancy rate of interlobular spaces of histologic lobules in a single section (preparation 132)

Lobule subpopulation	Lobule count	Portal occupancy rate	
		Range	Mean \pm 1 S.D.
Surface lobule	42	0-1	0.501 \pm 0.232
Deeper lobule	102	0-1	0.674 \pm 0.265

and two-thirds of the interlobular spaces in the surface lobules and the deeper lobules, respectively, were occupied by portal venous branches (Table 1). In addition to the surface-associated locations, there was a ten-

TABLE 2. Numbers and percentages of SHL-like lobules of various shapes¹

Lobule form	Lobule count	Percentage
Triangular	3	1
Quadrangular	31	10
Pentagonal	116	39
Hexagonal	98	33
Heptagonal	43	14
Octagonal	8	3
Total	299	100

¹The SHL-like lobules that presented a convex outline with the central vein(s) cut transversely were counted from the preparations (52, 72, 92, 112, 132, 152, 172, 192, 212).

dency for the territory close to large portal canals to provide the lobules with higher portal occupancy rates (quantitation not shown).

The polygonal lobules. Whether or not the conventionally described hexagonal shape is typical among liver units can be evaluated by quantitating the polygonal appearance of the lobules. We had preliminarily examined the transition in lobular forms and found that at every 20 sections (400 μ m), the structural outlines changed so remarkably that it was not easy to match a profile of a lobule in the section under examination with that of the same lobule in the previous or the next twentieth section. The polygonal lobules with the central vein transversely sectioned (White, 1939) were picked up from the montages of preparations 52, 72, 92, 112, 132, 152, 172, 192, and 212 and classified as shown in Table 2. There were about 130–150 histologic lobules in a section, and only 26–40 profiles were qualified for the calculation. As a rule, the convex polygons were counted, and the concave polygons were excluded. The results showed that the hexagons were not the majority; the highest proportion were the pentagons.

Lobule types in three dimensions, the SHL and the CHL. There were two lobule types referred to as the SHL and the CHL. An SHL was defined as a parenchymal unit of simple shape without an intralobular septum(a). Accordingly, the contained axial vessel was usually simple in outline without prominent tributaries. A CHL was defined as a parenchymal unit of compound form, appearing like an agglomeration of SHLs with the interconnected portion remaining in the base. Its central venous system was composed of a number of major tributaries that corresponded with the dividable components. Features of the SHL- and CHL-type units were distinguished in both positive and negative reconstructions of plastic models (Figs. 6, 7a,b). The positive expression of the lobules could be cut open or rendered semitransparent in the computer graphic 3D imaging to demonstrate the intralobular aspect (Fig. 7c-e). A full description on the styrene-plate and computer-aided reconstructions will appear in a future paper.

In histologic preparations, a CHL was recognized by the intralobular septum(a). Its components, the "lobulins" (Debeyre, 1910), could appear as separate units where the septation was complete but were readily demonstrated to belong to an integral unit in serial sections. The findings given below pertain to both lobule types unless stated otherwise.

Lobule mapping. How the lobule types were distrib-

uted could be studied by means of lobule mapping. The procedure was accomplished by tracing every lobule of a master section in both retrograde and anterograde

directions throughout an entire series. To demonstrate, suppose a master section contained 150 lobular fields, and each traversed an average depth of 1,000 μm . To obtain a complete mapping with the 20- μm -thick serial sections, the lobules had to be examined in at least 7,500 ($150 \times 1,000/20$) viewings. The lobular fields that could not be shown to associate, throughout the series, with the intralobular septum(a) were typed as SHL and the others as CHL.

In our master section, there were 144 lobular fields, of which 42 were surface lobules and 102 deeper lobules. Studying this section alone, we found that 25 of the total 144 histologic lobules were associated with intralobular septa. However, after examining the whole series, we found that only 36 lobules were actually not associated with any intralobular septum. They represented the SHLs (Table 3). The SHLs were simple in outline, containing a single central vein, which sometimes appeared slightly dendritic at the incipient end. Not infrequently a multiplicity of short central veins coexisted in an SHL, opening separately in the same sublobular vein (see Figs. 2, 33). The SHLs occurred usually in clusters, occasionally isolated, being scattered randomly in the section (Fig. 4).

The remaining 108 lobules were the CHLs. The merging of their parenchyma with that of their neighbors could be, but usually was not, obvious in a single plane. The parenchyma became united through the interruption of a septum or by an incompletely developed septum, resulting in intralobular septation. If the latter was evident in the section under examination, the CHLs were labeled as septate, if not nonseptate (Table 3). The CHLs were predominant among the surface lobules, and 38% of this subpopulation presented intralobular septa.

As for the size of the lobular fields, there seemed to be no difference between the two lobular types. However, profiles of the septate CHLs, which usually assumed the form of a concave polygon, were, more often than not, larger than the nonseptate and the SHLs (Fig. 4).

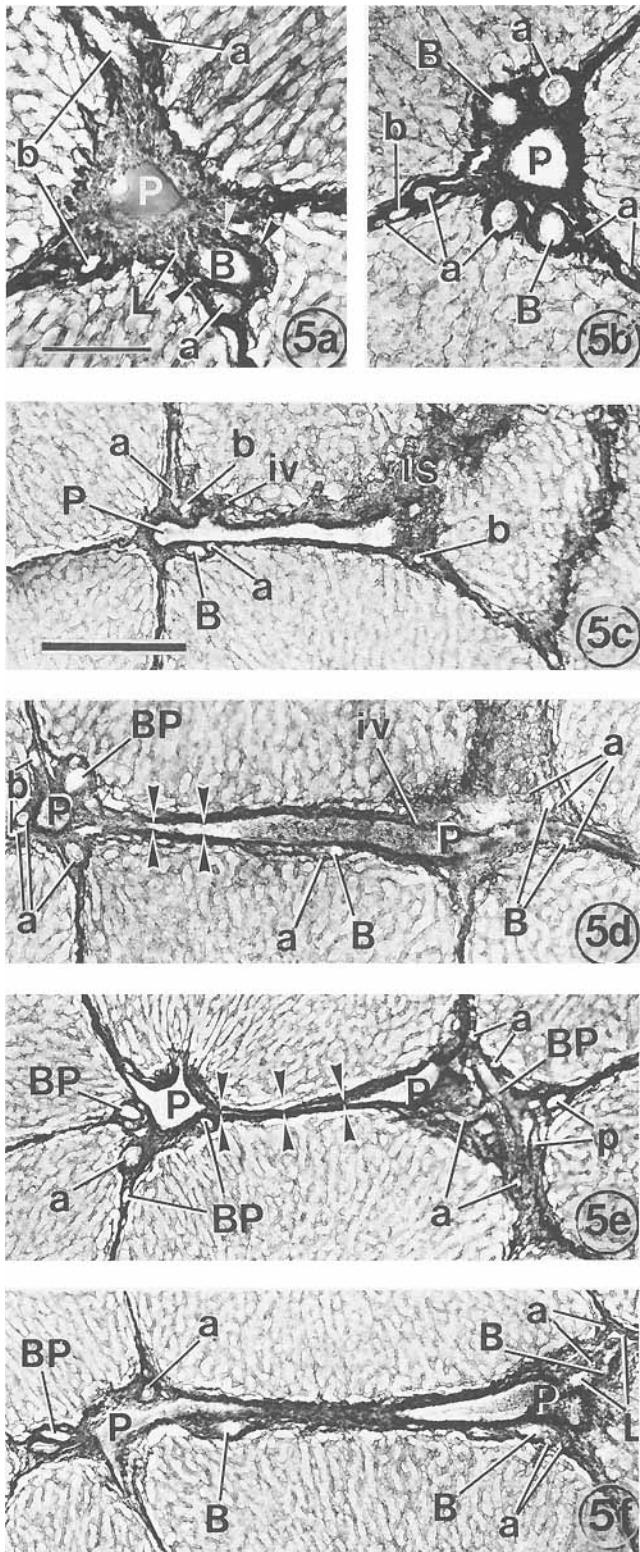
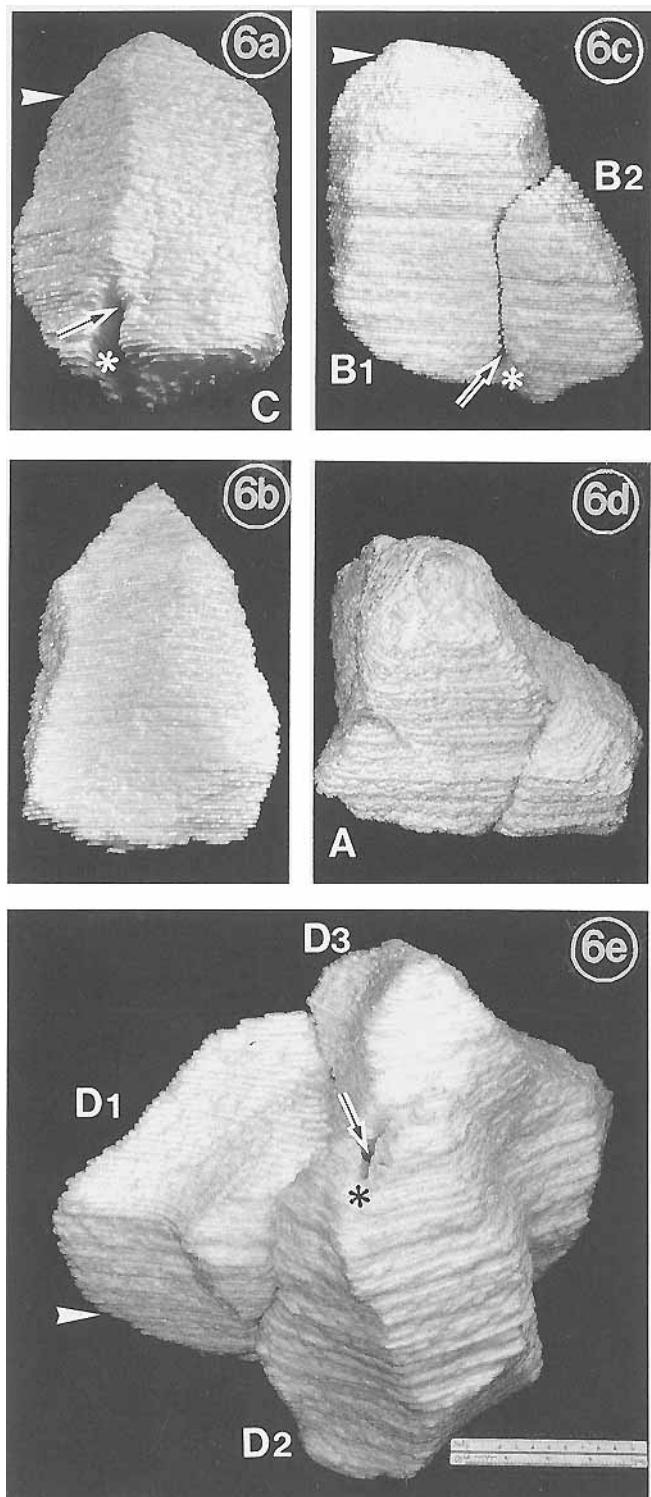


Fig. 5. **a:** In a small portal tract, the portal vein is typically surrounded by a thick fibrous investment, which occupies more than half of the portal space. A hepatic arteriole has formed a peribiliary plexus (arrowheads) around a bile duct. Note that the periportal regions of adjacent lobules are not equally marked with sinusoidal reticulum; note also the continuation of the paraportal fibers into the sinusoids. **b:** Branchings of the biliary ducts and the hepatic arterioles are striking, whereas the portal vein appears in a single profile. The presence of lymphatic vessels (appearing as irregular slits in the portal area) assists in estimating the thickness of the fibrous layer that invests the portal vein. **c:** A side branch emitted laterally by an interlobular portal vein has a much thinner investment compared to that of the proximal vessel. Note the irregular luminal caliber in the portal vein; the interlobular septum on the right is cut tangentially. **d-f:** Variations in a portal ramification emitted from a "parent" portal tract (visible on the left) to a "daughter" portal tract (right). To attain the interlobular position, the portal vessel has to course through the septum and come to reside in the neighboring portal space. The intermediate segment, sandwiched between two lobules, appears constricted (arrowheads) proximally (**d**) or for nearly the whole interval (**e**). Although the ranking of orders of portal arborization was based on serial sections, in a single section, the "parent" portal tract could be correctly identified by the sizes of the contained hepatic arterioles (and the bile ducts); portal lumina were not always reliable. Silver impregnation. Bar in **a** (for **a,b**) = 100 μm ; bar in **c** (for **c-f**) = 200 μm .

The Intralobular Skeleton

Overall organization

Differences between the two lobule types were found predominantly in the intralobular skeleton. The intralobular septa that occurred characteristically in the CHLs were studied best in the surface lobules; such



septa were encountered in only 9% of the deeper lobules (Table 2). They appeared as an elongated fibrous offshoot or as an interrupted septum growing deep into the lobule (see Fig. 2). Morphologically, the intralobular septa represented the immature steps of septal formation, appearing thin in early stages, next thickened but loosely woven, then compacted, and finally consolidated.

The so-called "reticulum" of Mall (1888) or "Gitterfasern" of Kupffer (1899) emanated from the above-described hepatic capsule and inter- and intralobular septa. It formed a network of fine and coarse fibrils, which followed the form of the sinusoidal channels throughout the lobule. In the silver-impregnated celluloid-embedded sections, the lobules exhibited a certain difference in the degree of staining of the sinusoidal reticulum. The sinusoidal reticulum of the peri-central zone was not well visualized, in contrast with that of the peripheral zone (Fig. 8a). The centrilobular sparsity of the impregnated reticulum sometimes extended to cover part of the midzonal sinusoids. Although the reticulum differed in no significant manner between the two lobule types, in those CHLs with multiple central veins, the sinusoidal reticulum of the intervenerular area displayed an increased stainability, similar to that of the peripheral zone, despite its being central in position.

Centrilobularly, the reticulum merged with another fibrous structure, the adventitial coat of the central vein, which continued into that of the sublobular vein. The latter was embedded in the interlobular connective tissue at the efferent end of the lobule, or the "base" of Kiernan (1833). In both lobule types, it was frequently observed that as the central venous adventitia joined the sublobular venous adventitia, the caliber of the central vein lumen diminished to some extent, forming a discernible short isthmic segment (see Figs. 7c,d, 23a).

The sinusoidal reticulum

The sinusoids and hepatic laminae. The general disposition of the argyrophilic reticulum positively defined the sinusoids and negatively outlined the hepatic laminae. The sinusoids and the hepatic laminae were observed in two different arrangements: in a circumferential pattern and a radial pattern, both of which are highly characteristic of the lobular organization in the porcine liver.

The radial sinusoids, interwoven between the hepatic laminae, radiated from the central vein towards the periphery. Most of them could be traced to abut directly on the circumferential sinusoids that lay perpendicular to them. The majority of the radial sinu-

Fig. 6. Positive models of Styrene paper reconstruction of an SHL (a, b; reproduced from lobule C* in Fig. 4), a two-component CHL (c; lobule B*), a three-component CHL (e; lobules D1*, D2*, D3*), and a multicomponent surface lobule (d). The CHLs are characteristically marked with a steep fissure(s), which is not present in the SHL (a and b show opposite sides). In both lobule types, a sublobular vein recess is recognizable (asterisks), being continuous with a central vein exit (arrow). Lobule A (d) presents a flattened facet due to the hepatic capsule (the facing side). The level of the master section is indicated by arrowheads. Scale = 10 cm (models were $\times 125$ in microscopic dimension but were photographed from different angles, hence the nonproportional size perception).

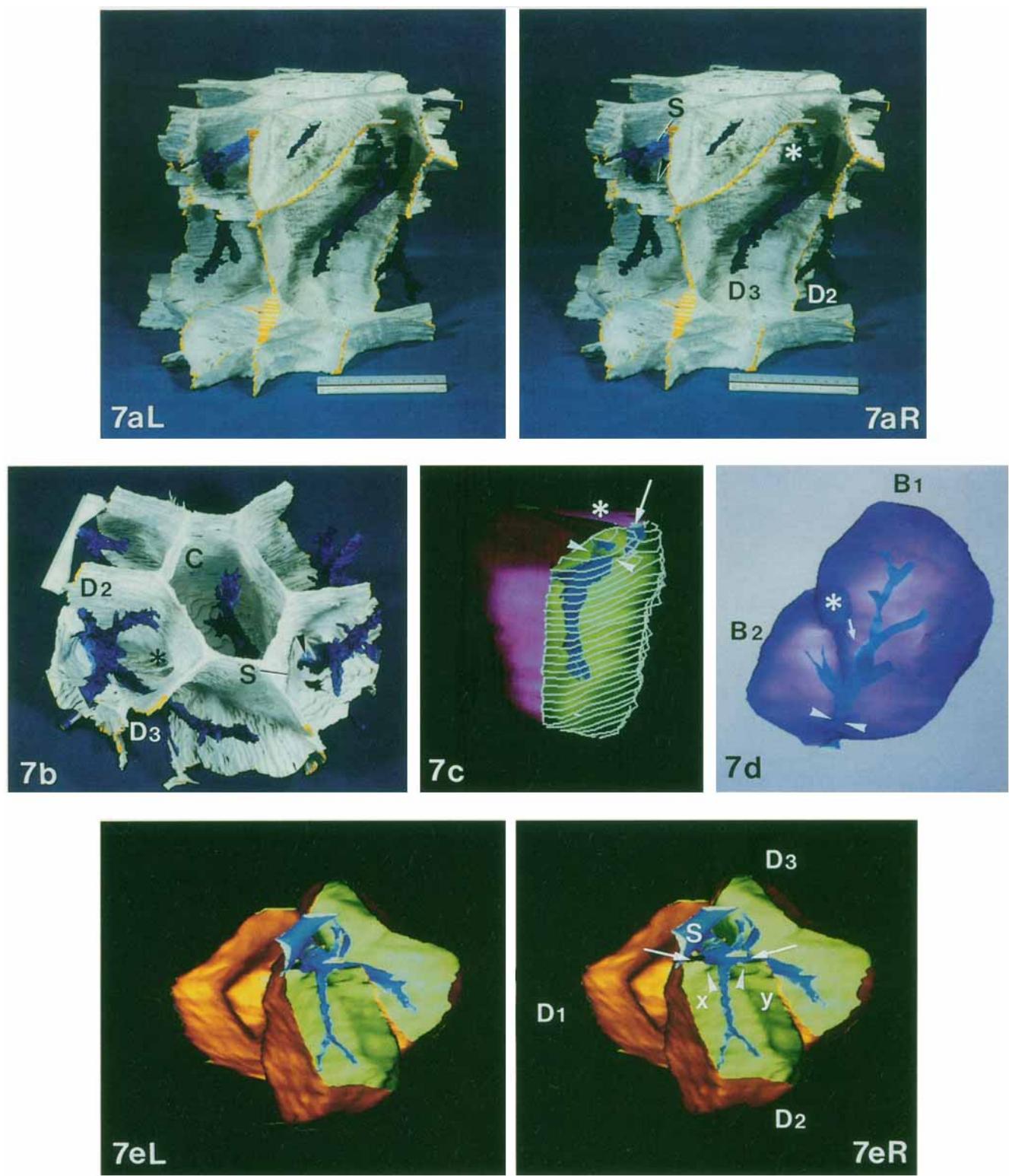


Fig. 7.

TABLE 3. Quantitative analysis of the lobules in a single section (the middepth section, 132) with respect to subpopulation, based on locality, and to lobule type, based on three-dimensional studies¹

Lobule type	Lobule count		
	Subpopulation		Total
	Surface lobule	Deeper lobule	
SHL	0 (0)	36 (35)	36 (25)
CHL	42 (100)	66 (65)	108 (75)
Septate	16 (38)	9 (9)	25 (17)
Nonseptate	26 (62)	57 (56)	83 (58)
Total	42 (100)	102 (100)	144 (100)

¹Percentages are in parentheses. Classification as septate or nonseptate defines the appearance in two dimensions. SHL, simple hepatic lobule; CHL, compound hepatic lobule.

sinoids followed a straight course. However, crooked sinusoids were not infrequent, appearing more often in the peripheral zone than in the central zone. The corners of a lobular field were usually found with curvilinear sinusoids but were sometimes found with rectilinear sinusoids (Fig. 9).

The radial sinusoids communicated with each other. Occasionally, adjacent columns of sinusoidal reticulum were linked by aberrant fibrils that penetrated straight across the intervening hepatic lamina without forming any patent channel; they were also seen between the septum and the circumferential sinusoid (Fig. 8c,e). The circumferential sinusoids exhibited a

Fig. 7. **a:** Stereo pair (L,R) demonstrating a negative model of Styrene paper reconstruction. Cut edges of the fibroconnective tissue septa and portal areas are painted in luminous yellow, central veins in deep blue, and a sublobular vein in light blue (projected on the septum). Compartments D2 and D3 are interconnected through a defect (asterisk) in the septum, indicating an incompletely formed septal structure. Likewise, another defect is discernible on the left (arrowhead). Bars = 10 cm. **b:** A block of bonded plates had been removed to exhibit the interior of lobule C in the negative model. The lobular space is completely walled off, having a central vein with some tributaries lying in its axial position. Labeling is in accordance with the previous figure. **c:** Lobule C in computer-assisted 3D imaging of the positive expression. Part of the lobule has been cut out and filled with sectional contour lines to demonstrate the central vein (blue) and its exit (arrow), which opened in the sublobular vein lodged in the sublobular vein recess (asterisk). Note a somewhat isthmic appearance in the central vein (arrowheads). **d:** Lobule B, a two-component CHL, as displayed in semitransparent modality based on computer-graphic reconstruction. The efferent system is dendritic. The axial vessel of component B2 joins the venous trunk of component B1 as it approaches the base, forming a central vein stem. The two components are separated apically by a splitting septum (asterisk), which gradually shifts (arrow) towards the major stream of component B1 and bridges over the distance between the vein and lobule's side wall. There is an isthmus in the central vein stem (arrowheads). This figure shows the side opposite to that in Fig. 6c. **e:** Stereo-paired computer-written images of lobule D, a three-component CHL. The components D2 and D3 have been largely cut open on this side and the component D1 on the other side, so that the viewer can appreciate the unfinished septation (arrows). The partitions D1-D2 and D2-D3 (indicated as x and y, respectively) seem to be directed toward the venous forks but are finally bent against the larger venous trunks (arrowheads). The isthmic segment of central vein stem is strikingly augmented in this CHL by advanced septal formation, but is not clearly perceived from this angle. Axis rotation; X, -60°; Y, 0°; Z, -10° (in L), and X, -60°; Y, 0°; Z, -3° (in R).

condensation of reticulum and tended to incorporate thick fibrils or fibers that extended from the septum (Fig. 8c). They formed a layer of paraseptal labyrinth, which was especially appreciated in tangential sections (Fig. 8b).

The outermost tunic of the lobule was circumscribed by a sheet of small hepatocytes arranged in a decidedly single-cell layer, i.e., the circumferential laminae. In the CHLs, their cellular continuation proceeded deeply sandwiching the intralobular septum (Fig. 10). The circumferential laminae were perforated in some places by twigs of inlet vessels (Fig. 8a). Such perforations in the laminae were easily found about the corners (Fig. 8d, also discernible at corner 5 in Fig. 9) and along their arms (Fig. 8c,e); very rarely was a perforation encountered at the middle of a side between two corners. In the lobule's base, the circumferential laminae continued as a sheath surrounding the central vein stem and its major tributaries (Fig. 10). We will refer to this part of the circumferential laminae as the limiting sheath.³

The lobular field was occupied primarily by arrays of the radial laminae that stretched outwards from the central vessel. They were not simple columns in form but included sites of interruption, bifurcation, bridging, and coalescing. Although the radial laminae were generally one cell thick, occasional multiple-cell plates or clusters of hepatocytes were intermingled here and there, occurring more often periportally than otherwise (Fig. 8c).

The parenchymal cells of the circumferential and the radial laminae tended to react differently to the gold-chloride solution. In the radial laminae, the hepatocytes had a homogenous reddish-pink hue, whereas, in the circumferential laminae, the cells remained unstained and clear, highlighting the presentation of the septa and the central veins that they flanked or surrounded. In the hematoxylin-eosin preparations, the circumferential laminae sometimes stained strongly eosinophilic.

In addition to the circumferential and radial patterns, which occurred commonly in both the SHLs and the CHLs, there was another mode of arrangement called the septal-line pattern. This pattern was observed particularly in the CHLs and was related closely to the formation of the intralobular septum. The septal-line pattern appeared to extend the circumferential pattern into the lobule. The most frequent sites of the septal-line structures occurred where the sinusoids and the hepatic laminae were seen to extend into the lobular field from one side of the lobule, passing midway between two centered vessels, rather than toward the central vein as usual (see Figs. 17a, 18a, below). Despite being disposed centrilobularly, the septal-line sinusoids, together with the neighboring radial

³The so-called lamina limitans, or the limiting plate (lamina), of Elias (1949) defines the single layer of small hepatocytes that surrounds the portal spaces and the hepatic canals, including the canals for the sublobular veins, but not the central veins. The limiting plate forms a continuity throughout the liver lobe and is continuous with the thin sheet of hepatocytes immediately beneath the hepatic capsule. Therefore, there are the external (subcapsular) limiting plate, the periportal limiting plate, and the perihepatic limiting plate (Elias and Sherrick, 1969).

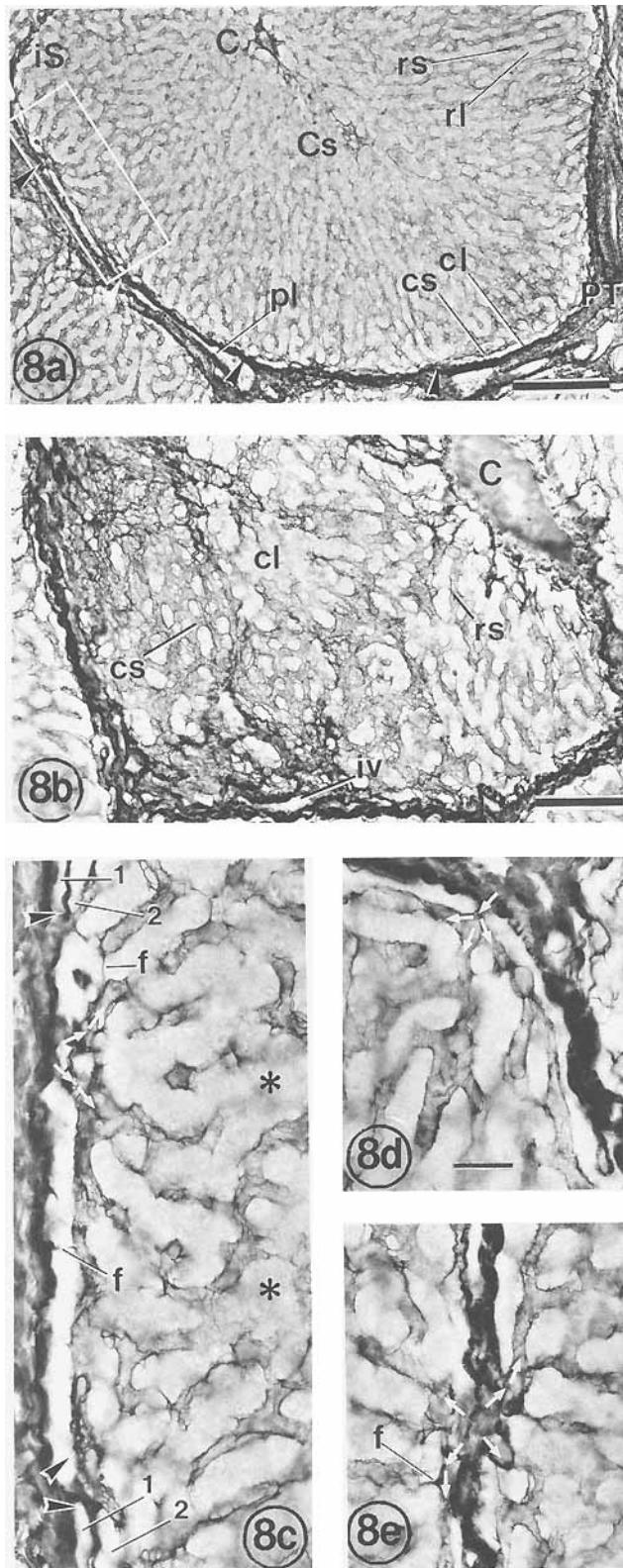


Fig. 8.

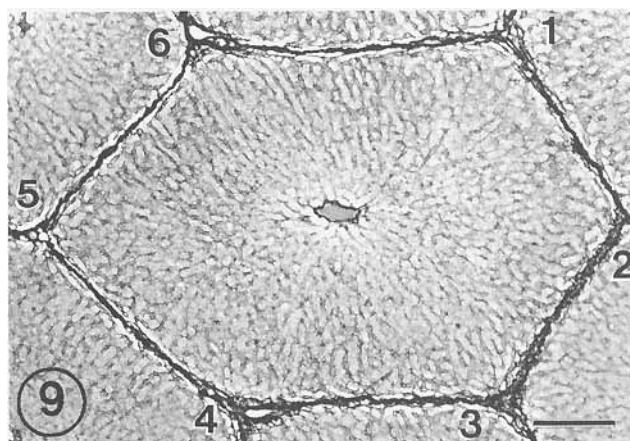


Fig. 9. Seemingly homogenous hexagonal lobule. Five out of six interlobular spaces are occupied by portal tracts. Corner 2 is apparently "empty" of afferent vasculature. Intralobularly, most corners are dominated by plexiform sinusoids, but one, corner 6, is observed with rectilinear sinusoids. Note also that the reticulum is more argyrophilic in the peripheral zone, less in the central zone. Silver impregnation. Bar = 200 μ m.

sinusoids, were impregnated markedly. The septal-line laminae were also noticed with marked changes (see below).

The sinusoidal reticulum and portal tract. In the vicinity of a portal tract, the lobules that surrounded the interlobular space were not equally reticulated. The lobule (or part of the lobule) that lay immediately next to the portal vein, without the hepatic artery or the bile duct intervening, was marked with sinusoids of dense reticulum. On the other hand, the lobule (or part of the lobule) that lay close to the hepatic artery or the bile duct had a less dense sinusoidal reticulum. The tendency for uneven staining of the reticulum was consistent in small, medium, and large portal tracts (Figs. 5a, 11, 12, respectively; note also sizes of the arterial branches; the layering of their media is of reference value).

When a portal tract was longitudinally sectioned, the portal vein appeared in close relation to the prominently reticulated sinusoids along its side (Fig. 10). Especially where the inlet venules entered the lobule, the sinusoids were marked by conspicuous bundles of fibers (Fig. 13).

The reticulum and inlet venules of CHLs. Distinctive in-

Fig. 8. a: Transverse section of a lobular field showed gross differences in the argyrophilia of the sinusoidal reticulum in central and peripheral zones. At the periphery are found the circumferential sinusoids and circumferential laminae; the latter are perforated by inlet venules at several sites (arrowheads). The rectangle outlined is enlarged in c. b: In a tangential section, circumferential sinusoids appear as a layer of labyrinth with dense reticulum. c-e: The inlet venules (white arrows) give afferent supply unilaterally (c) or bilaterally (e). Angular inlet venule(s) supply the lobular corner (d). Thick fibers (arrowheads in c) grow from the septum into the circumferential sinusoids. Sometimes, two layers of circumferential laminae (1, 2 in c) are obvious with an intermediate fibrous membrane, and the outer layer appears attenuated. Occasionally slender fibrils (f in c, e) penetrate across the laminae. Clusters of hepatocytes (asterisks) are encountered near the periphery. Silver impregnation: a, Bar = 200 μ m; b, bar = 100 μ m; d, bar = 25 μ m (for c-e).

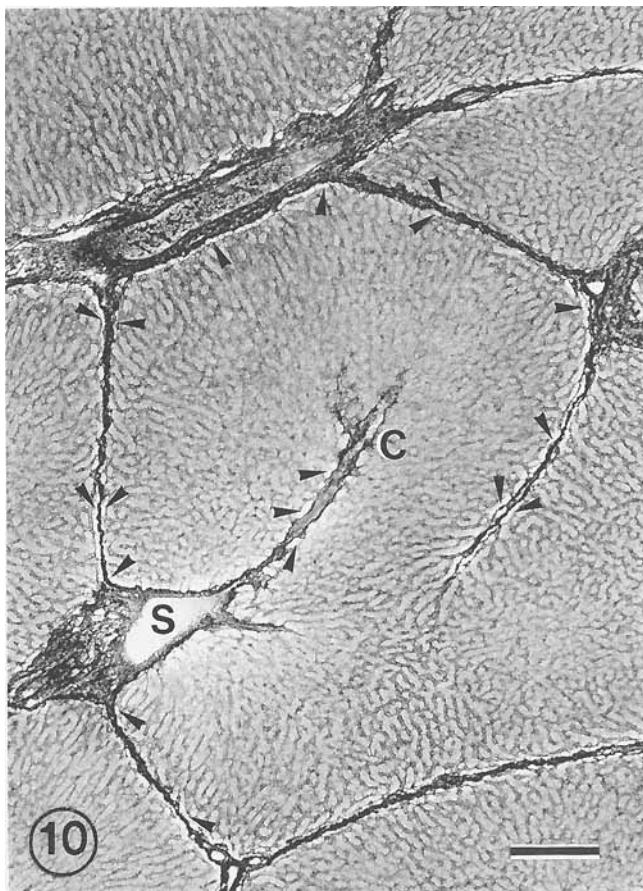


Fig. 10. Circumferential laminæ are continuous with the layer of fiber-contacting hepatocytes along the intralobular septum and the sublobular central adventitia. In most places the laminæ are well defined (arrowheads). Note the marked disparity in the reticulated appearance of the peripheral-zone sinusoids, especially near a portal tract (upper left) and of the central-zone sinusoids. Silver impregnation. Bar = 200 μ m.

corporation of the fibrous substances into the sinusoidal reticulum was obvious along the septal-line sinusoids of the CHLs. Findings from many CHLs with various stages of septal formation suggested strongly that the transformation of sinusoids into portal venous twigs was among the earliest changes observed of the septal-line events. This structural alteration was studied to advantage by observing the reaction in the reticulum and argyrophilic substances.

Figure 14 illustrates a sinusoid that communicated directly with the inlet venule; its lumen was slightly enlarged but its wall was strongly fibrosed.⁴ By serially tracing the whole course of this peculiar sinusoid in many lobules, we were able to determine that it reached as far as about two-thirds, or more, of the dis-

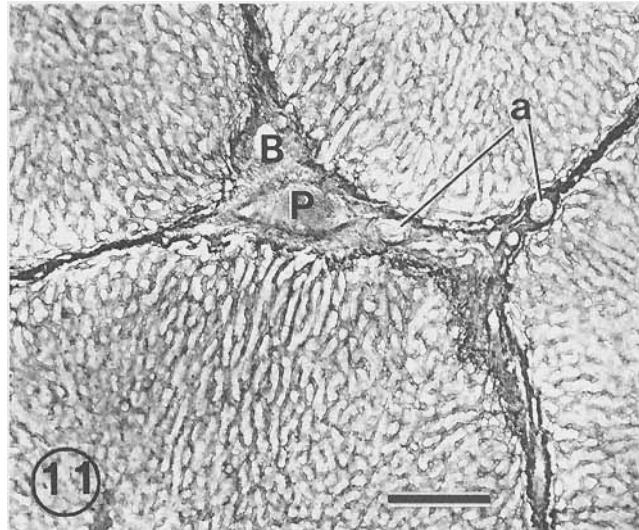


Fig. 11. Medium-sized portal tract with arterioles of moderate to large size (a few layers in tunica media). The lobules circumjacent to the portal space are not equally reticulated. Even in a single lobule the reticular response was uneven; that part close to the portal vein harbored a more dense reticulum in the sinusoids. Silver impregnation. Bar = 100 μ m.

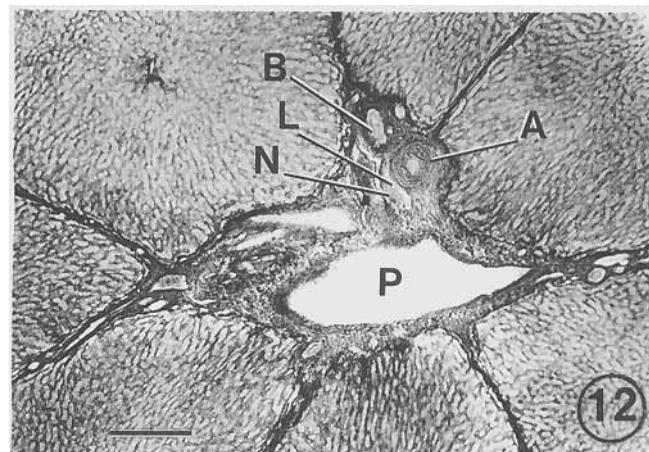


Fig. 12. Large portal tract with the hepatic artery. The adjoining lobules are by no means homogenous in the reaction pattern of the reticulum. Smaller orders of portal triad structures are not labeled. Silver impregnation. Bar = 200 μ m.

tance from the portal space to the central vein (Fig. 14b) or to the line connecting two central veins; it never passed beyond this imaginary line (Fig. 15; see also Fig. 20a).

The fiber-invested sinusoid appeared sclerotic. The surrounding hepatic laminæ were attached to its fibrous coat (Figs. 16a-r). The leading portions of these laminæ, gathered around the thick-walled sinusoid, were noted with clear hepatocytes that tended to be reduced in size, resembling those cells of the circumferential laminæ. Collagenous fibers radiated from the fibrotic sinusoid, passed between the clear cells, and

⁴We observed that the hepatic arterioles or "arterial capillaries" (Elias and Sherrick, 1969), which opened into the circumferential sinusoids at the lobule's periphery or along the intralobular septum, were not related to changes in reticulum (see Figs. 14c, 22d) as remarkable as those of the portal inlet venules.

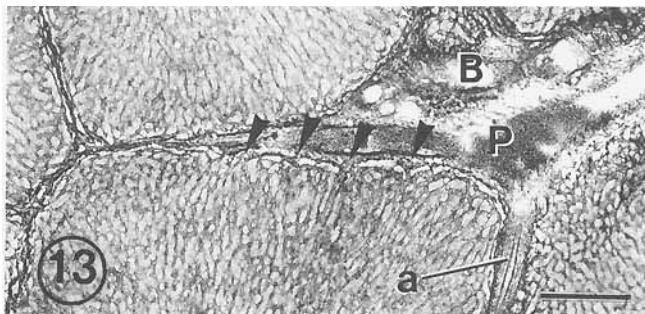


Fig. 13. Longitudinal section through a portal vein reveals densely appearing reticulum related with the inlet venules (arrowheads). Silver impregnation. Bar = 200 μm .

dispersed parasinusoidally or penetrated intercellularly. When clusters of hepatocytes at different depths were encased by the fibers, the cells assumed a rosette arrangement, each cell having a very small size with clear cytoplasm and a light ovoid nucleus, reminiscent of the ductular profile of Hering's canal (Fig. 16a,f,h,k,n).

The neighboring sinusoids were seen with various degrees of reticulum condensation, increasing as they lay close to the portal tract, decreasing as they became remote from the fibrotic sinusoid (Fig. 14a-d). The aberrant fibrils of His (Aterman, 1981) presented a striking appearance (Fig. 16s-x). In due course, the thick-walled sinusoid became invested with a compacted fibrous layer and now appeared like a venous twig that continued from the portal inlet venule and penetrated deeply into the lobule. The caliber of this "portalized" sinusoid was about two to three times the diameter of the average radial sinusoid.

These silver-detected changes of sinusoidal portalization were observed at certain sites of the lobular field and at different levels of the lobule's length (Figs. 15, 17b,c,f-h). They formed a structural landmark of the septal-line pattern and appeared to represent a focus for the further deposition of septal substances.

The intralobular septum

The septum-initiating sites. We agree with Johnson (1919) that the various stages of septal forming can be appreciated in any single preparation. However, to understand tridimensionally the formation of intralobular septa, we studied the process in many CHLs by following them serially.

Septal formation did not occur in any randomized direction, nor did it occur sporadically in the lobules. The phenomenon was observed to take place along the septal-line sinusoids, in the presence of what we refer to comprehensively as septum-initiating sites. These sites included the portal tracts (or some portal triad structures), the central venous adventitia, the sublobular venous adventitia, and the portalized sinusoids. Not all portal tracts induced septal production, nor did the central and sublobular adventitia. Only when the septal-line sinusoids and laminae stretched between such sites did they come to be recognized as septum-initiating sites; these sites constituted a three-dimensional linkage throughout the CHL.

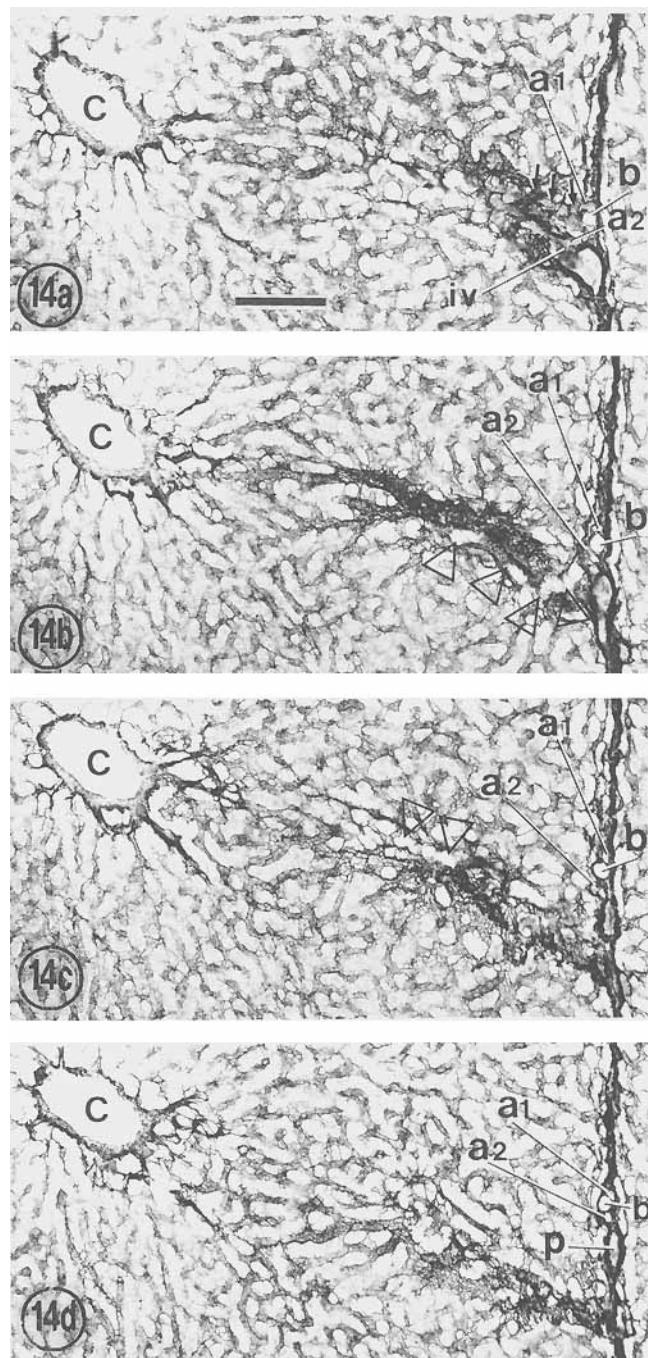


Fig. 14. Serial sections depicting a portalized sinusoid that reaches from an inlet venule of a small portal tract in an interlobular septum to about two-thirds of the radial (portal-central) distance. Ductular transformation of adjacent hepatic laminae (at open arrowheads in b, c), tends to establish connection with the extralobular ductule. The central vein has reacted with adventitial outgrowths on the corresponding side. Although the magnification in these photomicrographs is not sufficient to demonstrate them convincingly, two arterioles (a1, a2) were easily identified under oil immersion; a1 invades intralobularly (arrows in a), and a2 forms an anastomosis with the inlet venule. Note the differences in fibrous appearance: a condensed reticular pattern with numerous transverse wavy fibrils on portal side and stout bundles lacking transverse fibrils on central side. Silver impregnation. Bar = 100 μm .

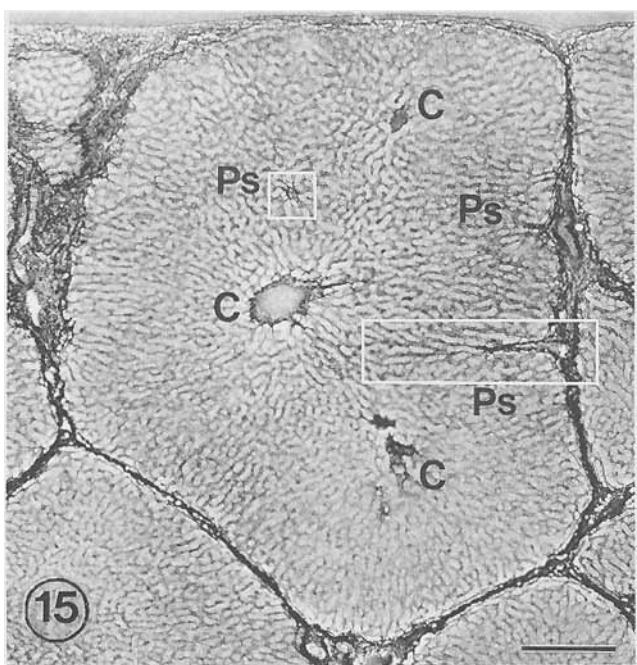


Fig. 15. In this surface lobule, three portalized sinusoids are demonstrated with the central veins (or tributaries) occurring in three regions. One portalized sinusoid appears to divide the line connecting the two nearest central veins. The sinusoids in the outlined square and rectangle are enlarged in Figure 16c and 16u, respectively. Note the adventitial outgrowths (sprouting from the largest central vein) in relation to the direction of the portalized sinusoids. Silver impregnation. Bar = 300 μ m.

In the septum-generating lobules, we observed the septal-line sinusoids and laminae spanning between the septum-initiating sites of the following combinations: 1) two portal tracts that were located at the opposing sides of a CHL (Fig. 17a); 2) a portal tract and a portalized sinusoid (Fig. 17b); 3) two separate portalized sinusoids (Fig. 17c); 4) a portal tract and a central vein (Fig. 17d); 5) a portal tract and a sublobular vein (Fig. 17e); 6) a portalized sinusoid and a central vein (Fig. 14b); or 7) other mixed combinations, such as a portal tract, a portalized sinusoid, and a sublobular vein (Fig. 17f). An intralobular septum might appear in a section in which the septum-initiating sites were not apparent, but, when the structure was examined serially, it became evident that one or more of the enumerated combinations was responsible for the septation.

With the portal tracts situated at the periphery of the lobule, the central vein at the center and the portalized sinusoids interposed between, the septa were set up interconnecting these structures (Fig. 17g). The process of septal formation inevitably involved the conversion as an intralobular region along a septal-line pattern into the peripheral region. In this structural alteration, the septum-initiating sites were always engaged; among them the portalized sinusoids were the newly developed structures.

It appeared that the hepatic arteriole or arterial capillary did not accompany the portalized sinusoid in the earliest stages (Fig. 16a-c,s-x), but was found in

slightly advanced stages (series of Fig. 16d-r and Fig. 14a-d). On becoming a firmly established septum-initiating site, the portalized sinusoid developed fully into a portal twig with the accompaniment of an arteriole and a biliary ductule in an accumulation of collagenous substances (see Fig. 17g). These were continuous with those in the larger portal tract.

Septal deposition. In the CHLs that had established septal-line structures (the septal-line sinusoids and laminae and the septum-initiating sites), septal deposition was carried out in a definite pattern. The few rows of single-cell hepatic laminae that spanned between the septum-initiating sites became unusually straight, with few cross connections. They appeared clear as contrasted with the sinusoidal channels whose definition was strongly enhanced due to reticular thickening (Fig. 18b,c). The two intermediary sinusoids were particularly involved. Their caliber tapered towards the septum-initiating site, where the sinusoids were bound with significant bundles of fibers that headed into the sinusoids and blended with the reticulum. Therefore, in cross section, the septum-initiating sites usually presented a "double-spurred profile," displaying a pair of fibrous spines penetrating from the site into the sinusoids and merging with the reticulum (Fig. 18a-c). Some fibrous strands crossed over to the neighboring channels through the sinusoidal cross connections (Fig. 18d,e). The interposed hepatic lamina was compressed and appeared atrophic. The proximal part of the septal-line sinusoids became obliterated with bundles of fibers, whereas their distal part remained patent yet occupied densely by wavy fibrils (Fig. 18f,g).

As the argyrophilic deposition advanced, the fibrous spurs grew thicker and extended deeper along the sinusoidal walls, spreading throughout the plane of separation. In due course these membrane-like fibrous ingrowths approximated and consequently entrapped those fragments of the hepatic cell plate that became exceedingly attenuated and finally seemed to diminish (Fig. 18g).

The thickness of the forming septum was not uniform throughout its length; as a rule, the nearer to the septum-initiating site, the thicker it was. Minor amounts of fibrous substances were added laterally to the proximal part of the developing septum. The manner of argyrophilic deposition was similar in that the reticulum of adjacent sinusoids inspissated and then combined. Whereas the circumferential sinusoids of the region became thickly reticulated and pervaded by larger fibers, the circumferential laminae seemed to undergo a pressure atrophy and consequently to disappear as this new argyrophilic layer came to incorporate with the existing fibrous mass of the septum-initiating site (Fig. 18h,i). Apparently this additional septal deposition contributed to the rounded appearance of the angle formed by the developing septum and the septum-initiating site.

There were occasions in which septal deposition was not always typical as described, especially in those CHLs that were small or that had not established significant septal-line structures. In the surface lobules, in which there was a predilection for the CHLs, septal deposition was sometimes found to proceed atypically in some way. The double-spurred profile might not be

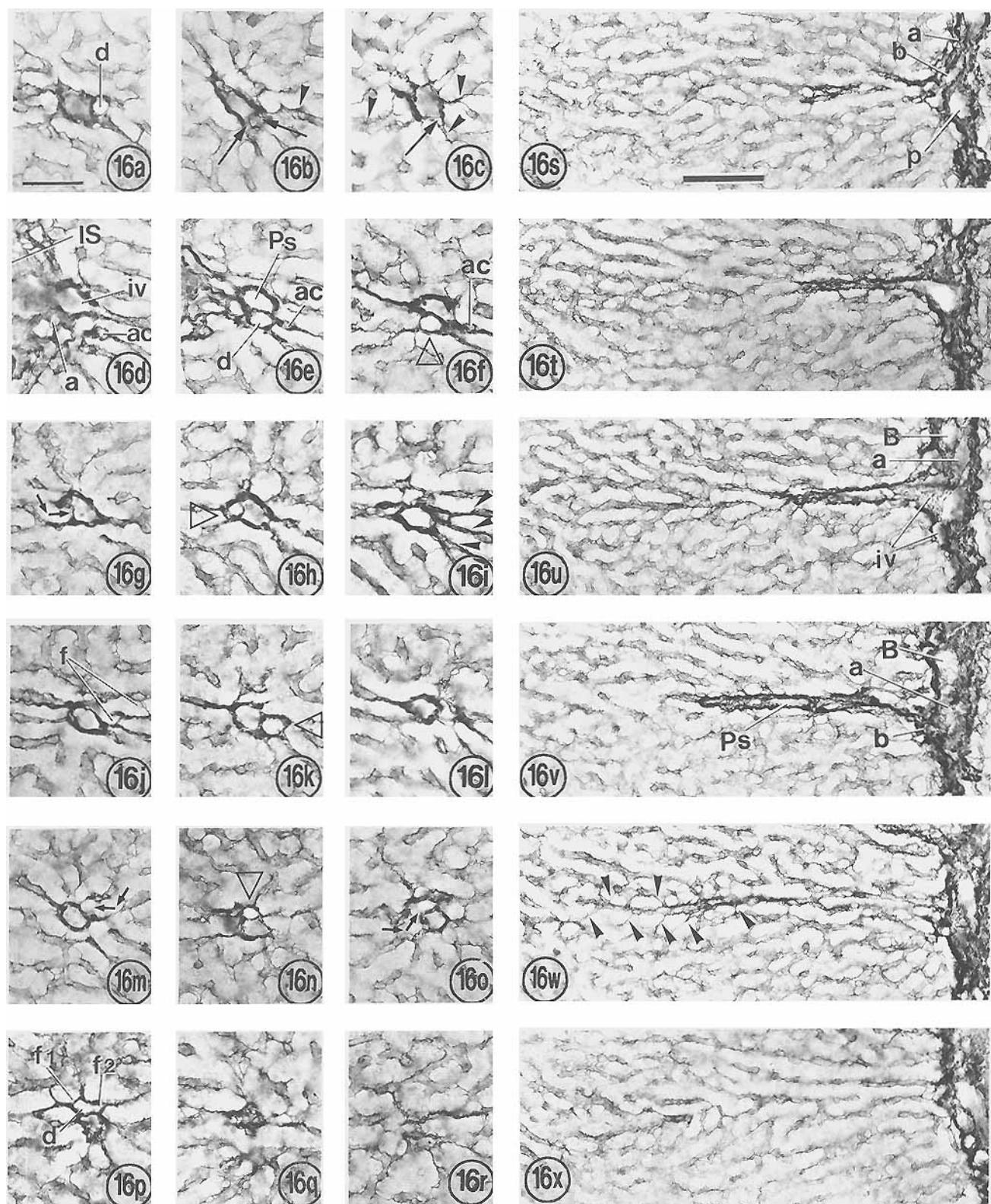


Fig. 16.

clearly perceived, since one of the "spurs" developed more rapidly than the other and the argyrophilic strands were slender (not shown). The silver-detected changes along the septal-line sinusoids were observed to progress more or less alike from both ends of the septal plane. As a consequence, the septum occasionally appeared slightly disconnected about in the middle, which represented the point of septal junction (Fig. 19).

Septal angulation. Generally the developing septa were noncurved and nonangulated, being aligned with the septal-line sinusoids that were stretched between the septum-initiating sites. As the basal end of the central vein was approached, however, the septum shifted towards the vein (Fig. 20a), which, in transverse section, usually presented an angulation of the septum (see Fig. 22a). The septal materials were found to be the bundles of fibers that ran in conformity with the existing sinusoids (Fig. 20a).

As seen in Figure 21, a splitting septum in a CHL sometimes seemed to sever the lobular field by dividing the central venous "fork." In later stages, however, when portal triad structures had largely invaded the growing septum, the latter penetrated close to the fork and finally bent toward the larger vein (Fig. 21a-c). It was observed that the bifurcation of the vein, which was invested thickly by an adventitia, did not admit the entrance of sinusoids, and that the bend in the septum was created primarily by fibrous proliferation along sinusoids, attempting to bridge the shortest distances between the central adventitia and the tip of the septum.

In larger CHLs, the tip of the previously established intralobular septum behaved as a septum-initiating site, and the developing septum might proceed in the same line or might deviate to a different direction (Fig. 17h). A portalized sinusoid sometimes acted as an interposition between two other septum-initiating sites in such a situation that all three were not aligned (Fig. 17g). In these cases, the final septum usually formed an angulation.

Fig. 16. Semiserial sections showing changes of portalization, with ductular transformation, in transverse view (a-r) and in oblique-longitudinal view (s-x). Figures are so arranged that the hepatic capsule is parallel to the top margins; the interlobular septum is at the left margin in the transverse sections (viewable in d) and at the right margin of the oblique views. In an earlier stage (a-c), the affected sinusoid thickened its fibrous walls which radiated fibrils into its surroundings and induced condensation of the reticulum (arrowheads). Ductular transformation (a) occurred proximally. Note the patency in communications with adjacent sinusoids (arrows). In a slightly advanced stage (d-r), as the sinusoid became more fibrotic, it induced convergence of hepatic laminae in the immediate vicinity; the laminae appeared rather clear and their width reduced. Some sinusoids were collapsing and their walls becoming attached to each other (arrowheads) in i. The ductular formation could be seen to take place in all directions (open arrowheads in f, h, k, n) around the portalized sinusoids. The aberrant cross fibrils (f) were also thickened (in j), which sometimes demarcated the segment of the lamina involved in ductular transformation (p). Note in the most distal end (q, r), the appearances are of early changes. The longitudinal view (an earlier stage) confirms that the portalization specifically involved that single sinusoid that received a certain inlet venule (there were two inlet venules in u) and pursued a noncrooked course directed centrally. Note the numerous cross fibrils at the advancing end of the portalized sinusoid (arrowheads in w). Note also there appears to be no convergence of laminae in longitudinal view. Silver impregnation. Bar in a = 50 μm (for a-r); bar in s = 100 μm (for s-x).

The septum-initiating plane. Tracing the complete series of each CHL disclosed that the previously described septum-initiating sites were interconnected in the hidden third dimension, forming a complex that linked them into a continuous plane. With respect to the lobule's longitudinal axis, at the level in which tributaries of the central vein arose, the septum-initiating plane was located midway, splitting the lobule about the central venous fork (see Fig. 21c). At the level in which the central vein stem left the lobule, the septum-initiating plane bridged the distance between the central vein and a portal tract that resided in the interlobular connective tissue on the side that the vein approached (Fig. 22b-d). At the transitional level between these two, the septum-initiating plane gradually shifted towards a major central vessel (Fig. 22a, see also Fig. 21c) which in due course came to lose its center position.

The portal-central bridging plane is worth special mention. It represents one of the most frequently seen planes in CHL units in the normal porcine liver. The fibrous substances supplied from the central adventitia were no less than those that grew from the portal area down the sinusoidal stream (Fig. 22c, see also Fig. 28a). The unbound side of the central vein was surrounded by a large mass of the lobular parenchyma, which was usually arrested without an apparent cleavage. To describe its anatomical disposition, we will refer to this type of intralobular septum as the *mesentery-like septum* (Fig. 22d); its profile resembles more or less the embryologic mesentery.

That the mesentery-like septum was continuous with the splitting septum could be appreciated in computer-based tridimensional imaging (Fig. 7d). In final stages, the splitting septum extended from the top of the lobule downwards to splitting the side opposite to the mesentery-like septum (see Fig. 29).

The central-sublobular (hepatic) adventitia

According to Kiernan (1833), the intralobular segments of the hepatic vein system are designated as the central vein, and the extralobular segments are subdivided into the sublobular vein and the hepatic vein; the latter is confined to those large divisions that receive no drainage from individual central veins. Since our preparations were derived from tissue blocks of an ordinary size, sampled at the free edge of the liver lobe, the hepatic vein proper, in the sense described by Kiernan (1833), was not available for observation in the present study. The description provided below is concerned with the central veins and sublobular veins.

The central vein and sinusoids. The beginning of a central vein was separated from the lobule's perimeter in all directions by the radial sinusoids. The exception was that in the subperitoneal region of the surface lobules, the central vein emerged immediately under Glisson's capsule (Fig. 3a,c,d), giving the appearance of the top part of the lobules having been removed (Kiernan, 1833).

The radial sinusoids converged their long axes on either the central vein or its tributary. We noted very often that the sinusoids near the base did not empty into the basal segment of the central vein stem but rather directed their central ends to unite with the vein at a level more intralobularly located (see Fig. 23a).

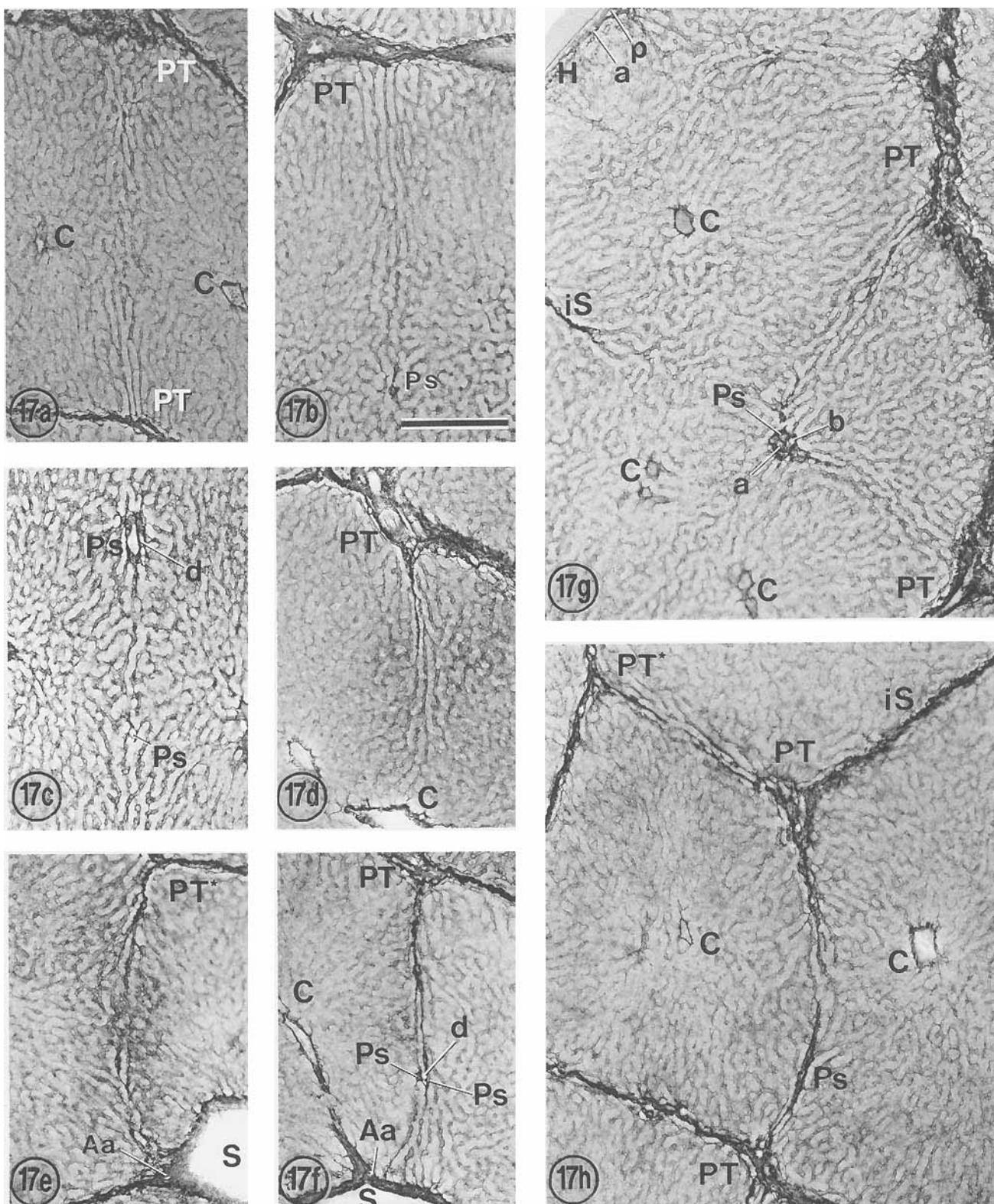


Fig. 17. Varieties of septal-line patterns that occur between the septum-initiating sites. Depicted are combinations of two portal tracts (a), a portal tract and a portalized sinusoid (b); two portalized sinusoids (c); a portal tract and a central vein (d); a portal tract (elongation) and a sublobular vein (e); and mixed combinations of a portal tract, portalized sinusoids, and a sublobular vein (f); and of portal tracts, a portalized sinusoid, an intralobular septum, and a portal

venule in the hepatic capsule (g). Note that the portalized sinusoid in g is well established with accompaniment of a biliary ductule and an arteriole; it therefore represented an intralobular portal tract prior to being incorporated in an intralobular septum. Sometimes the septal-line pattern did not align with the previously formed intralobular septum and resulted in septal angulation (h). Silver impregnation. Bar = 200 μ m.

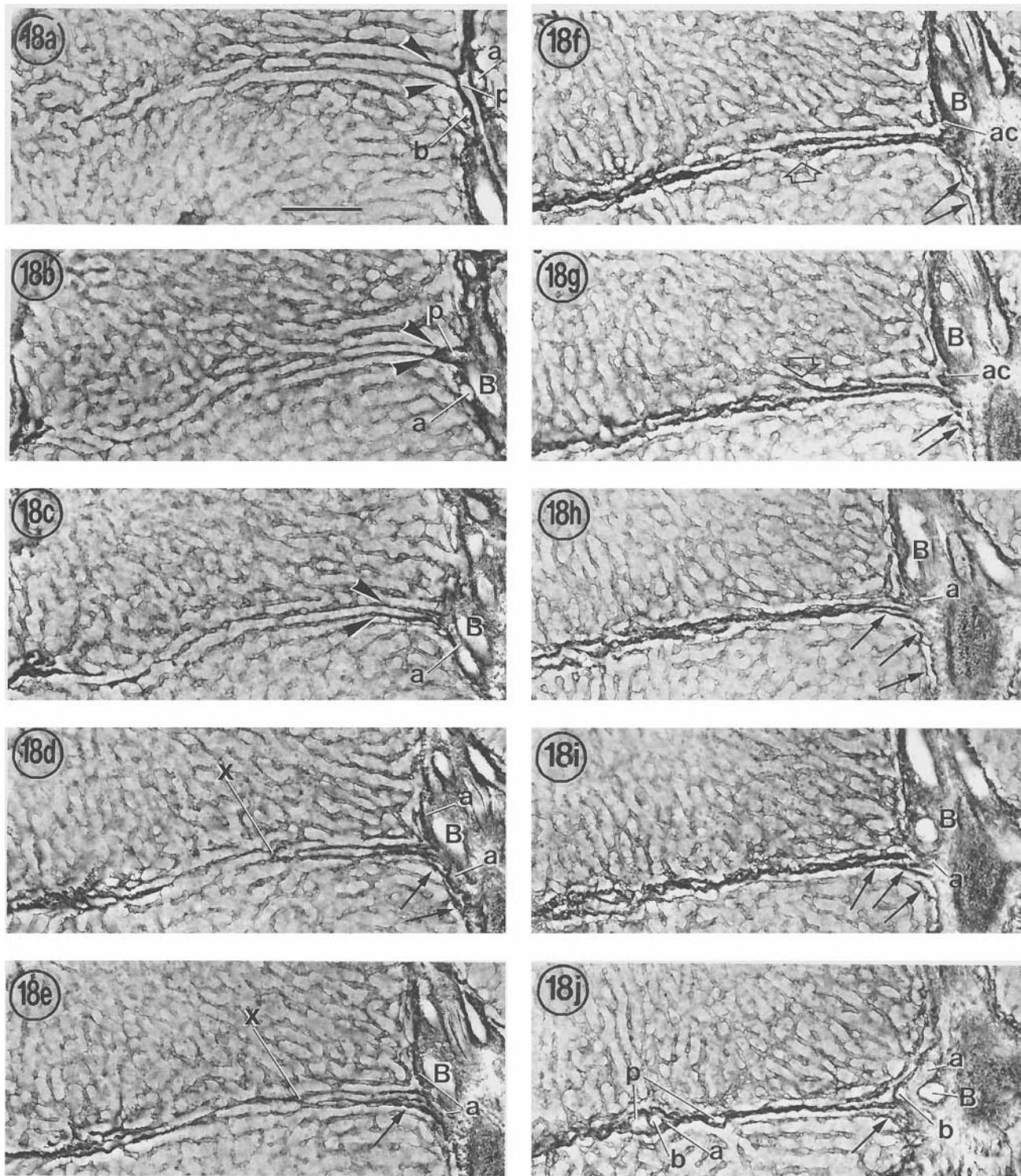


Fig. 18. Septal formation demonstrated, in semiserial sequence, between two septum-initiating sites: a medium-sized portal tract on the right and an intralobular septum on the left, which contains portal triad structures (j). The growing process is better demonstrated on the right. Before significant deposition has occurred, the septal-line laminae appear hypertrophied (a). As the septal-line sinusoids accumulate increasing reticulum, their walls thicken and the laminae appear with reduced width (b, c). As more fibrous substances are supplied from the portal tract, a "double-spurred" profile develops and is grad-

ually elongated into septal-line sinusoids (arrowheads in b, c) and sometimes crossed (X in d, e). The fiber-invaded sinusoids are yet not obliterated in the distal end and are continuous with the radial sinusoids (open arrows in f, g). Additional argyrophilic deposits are found near the proximal end (arrows in d-j). Fragments of laminae with hypotrophic cells are discernible between these layers. Note the intimate topographic relationship between the arterioles (or arterial capillaries) and the peripheral end of the developing septum. Silver impregnation. Bar = 100 μ m.

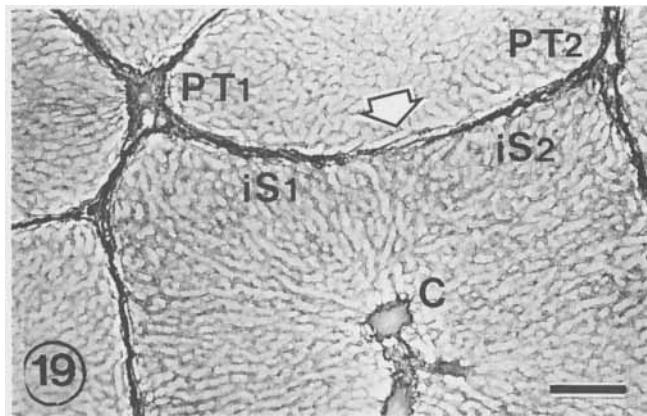


Fig. 19. Most typically the intralobular septa grow inward from two opposite portal tracts and, about midway (open arrow), merge imperceptibly into each other. Silver impregnation. Bar = 100 μ m.

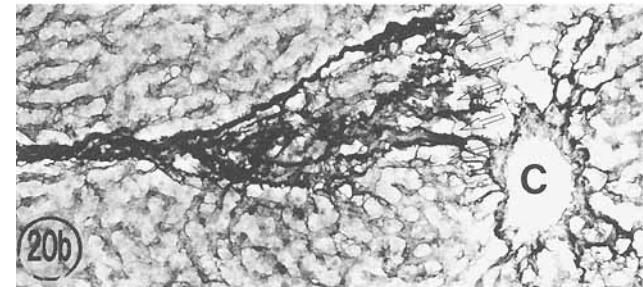
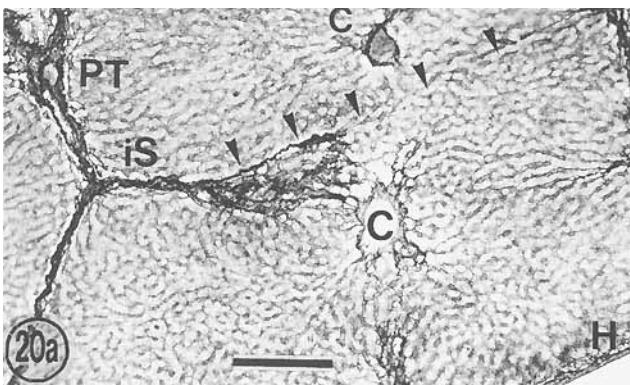


Fig. 20. "Centropetal" shift of a splitting septum. a: The intralobular septum was about to split the lobular field along the septal-line pattern (arrowheads) between two central veins but now has "shifted" toward the larger central vein. In the area of shifting, which is cut tangentially (enlarged in b), tough bundles of fibers alternate with laminae (open arrows). These septal materials appear without transverse fibrils, a characteristic compatible with adventitial outgrowths. In adjacent sections, we confirmed that they sprouted from the central vein. Silver impregnation. Bar in a = 200 μ m.

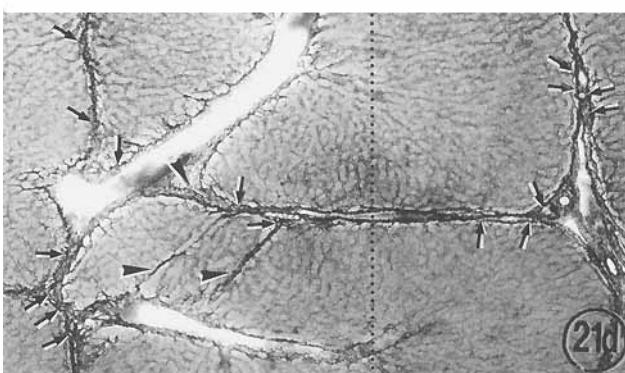
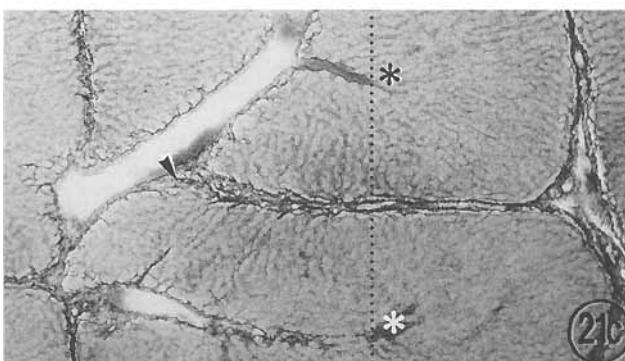
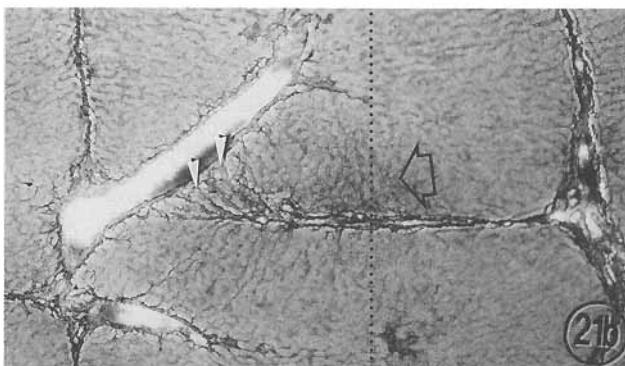
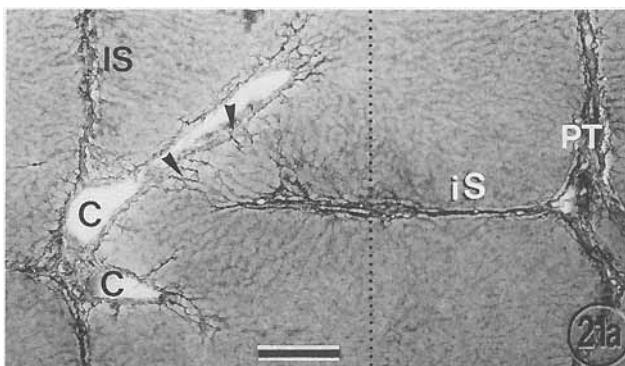


Fig. 21. Longitudinal serial profiles of a splitting septum (with portal triad structures) in its later stages. Although apically (to the right of dotted line), at the level about the beginning of the central veins (asterisks in c), the septum is midway between the veins, basally (to the left) it is directed towards the larger central vein. The sinusoids that drain those regions are more densely reticulated (open arrow in b), contrasting with those of the other side. The adventitial outgrowths from the central vein that ascended the sinusoidal channels are obvious (arrowheads). Arterioles and arterial capillaries were identified (under oil immersion) and are indicated in d (arrows). Note their pervading ability whether in the inter- and intralobular septa or in the adventitia. Silver impregnation. Bar = 200 μ m.

Fig. 21.

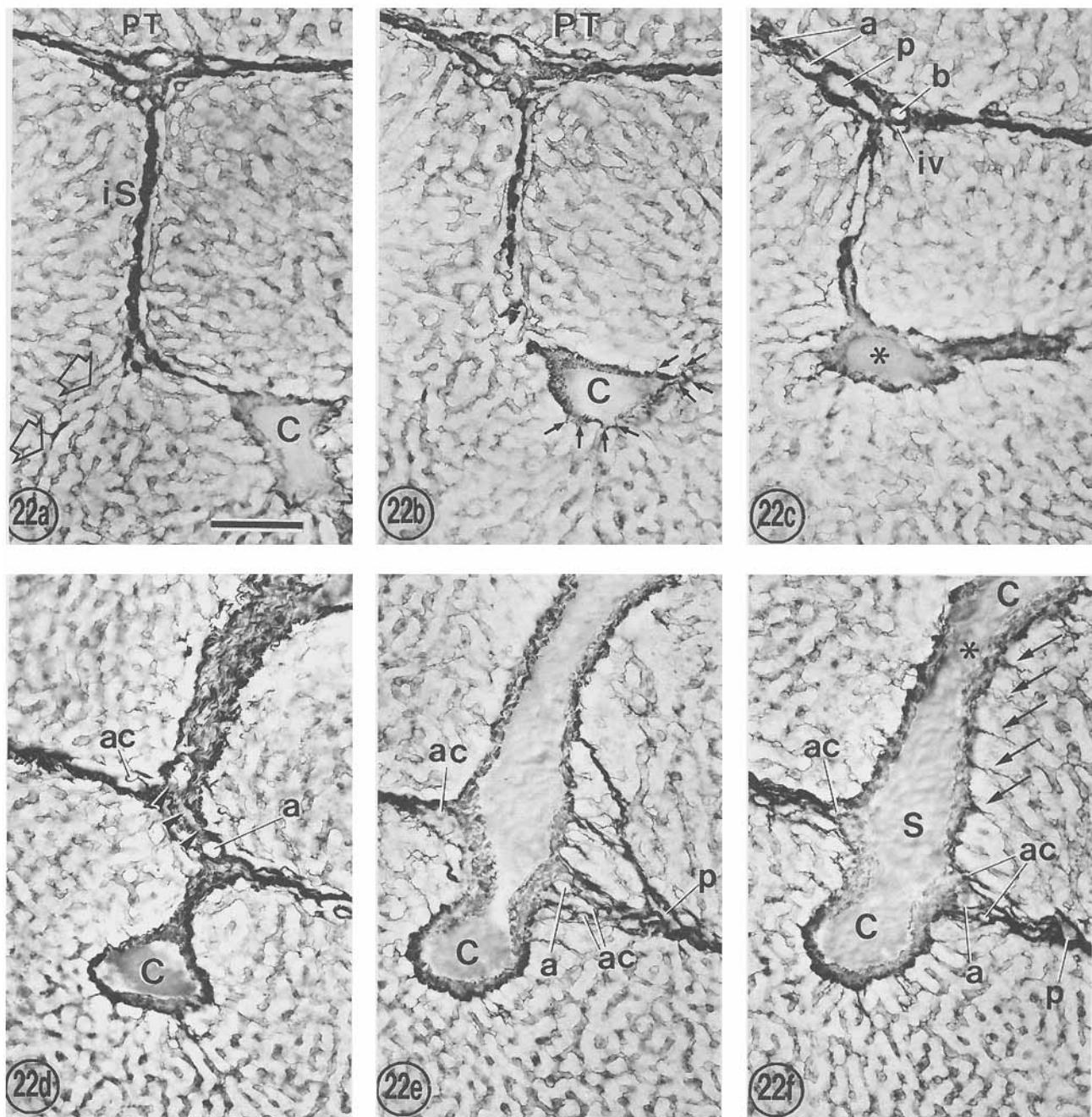


Fig. 22. Transitional changes in the formation of a mesentery-like septum on approaching the base of a CHL (lobule B in Fig. 6c). As the central vein approaches the base, it deviates from being the center of the lobular field (portal venule to central vein distance decreasing). There is another, smaller, central vein to the left (not shown in views a-c), which becomes united into a venous stem (d). The intralobular septum splits the field along a septal-line pattern (open arrows in a), which continues to the other side of the lobule, bends toward the central vein (a) in the presence of adventitial outgrowths and comes to take the form of a mesenterylike septum (d). The thickening of ad-

ventitia related closely with the loss of sinusoidal orifices (small arrows in b) in the walls, the narrowing of lumen in the vein (asterisks), and the release of some fibrils into those seemingly obliterated sinusoids (arrows in f). Note the prevalence of arterioles and arterial capillaries; the arteriole in d represents a branch combined of those coming from the left and the right sides along the septum (arrowheads indicate the emission of an arterial capillary). In d, also note the wavy bundles consisting exclusively of longitudinal (or oblique) fibers in tangential cut through a neighboring central vein. Silver impregnation. Bar = 100 μ m.

At their termination at the central vein, the sinusoids gave evidence of various degrees of circulatory activity (compare Fig. 23 with observations in transil-

lumination studies by Wakim and Mann, 1942; Knisely et al., 1948). Some sinusoids opened widely; some appeared to terminate with a narrowed mouth as

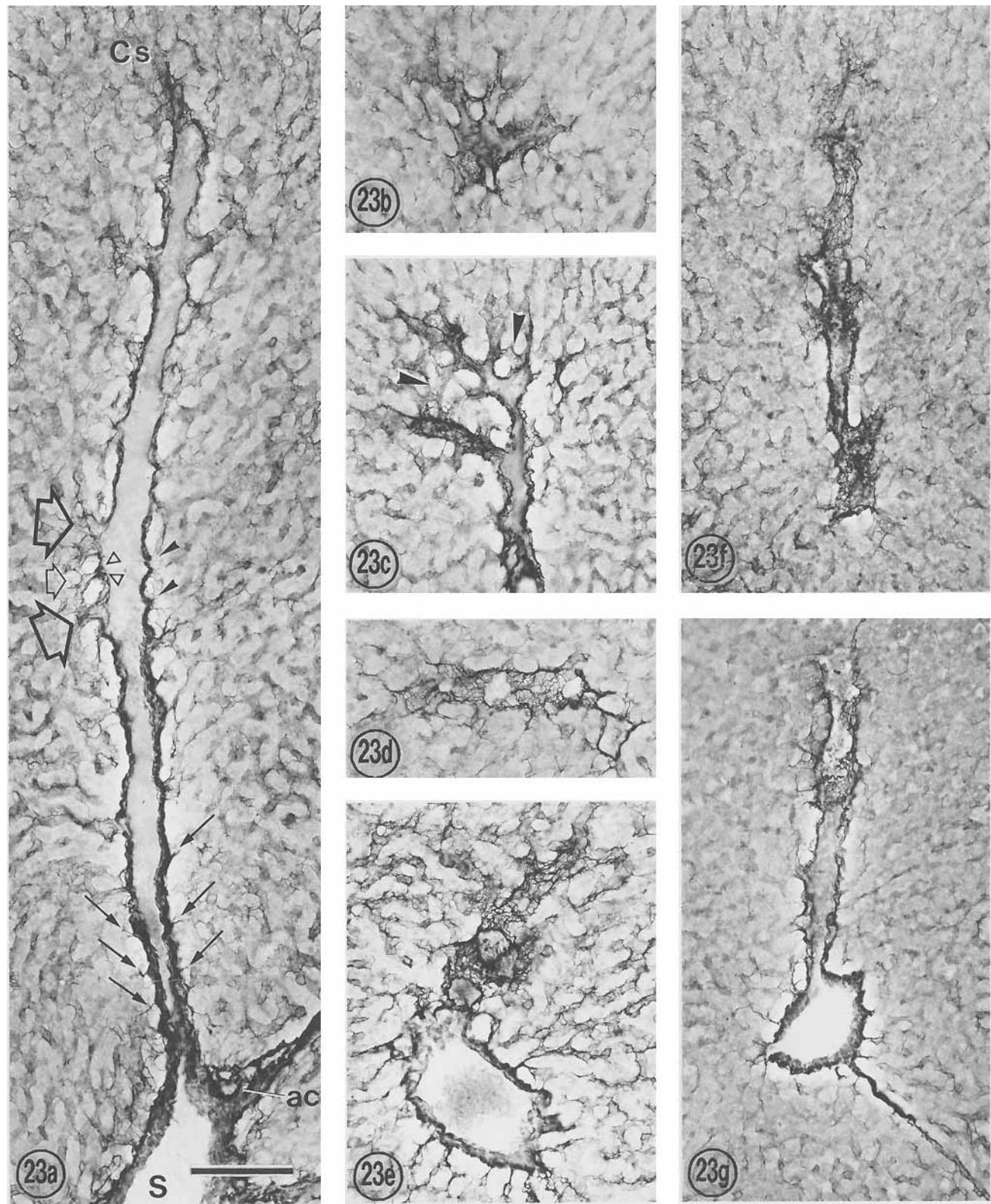


Fig. 23.

a slit in the adventitia, passing between the pericentral hepatocytes of the limiting sheath (Fig. 23a).

Some sinusoids opened directly into the centered vessel; some ended in a cul-de-sac at the limiting sheath but drained via a bend to join the neighboring sinusoid (Fig. 23a). The reticulum converged and terminated as a minute point at the central adventitia, giving an appearance of a fibrillary spike (compare with the "Radiärfasern" of Kupffer, 1899). The latter occurred frequently in the stem segment of the central vein, which contained reduced numbers of sinusoidal orifices (Figs. 22e,f, 23a). The decrement in the outflow paths could be depicted by the ratio between the afferent and efferent sinusoids (at the peripheral emanation and central termination, respectively) studied at various levels. In a transversely sectioned SHL (of Fig. 9), the ratio was found to range from 10:1 to 12:1 in a downstream direction.

The central vein and "centralized" sinusoids. The profiles of intralobular central veins were heterogeneous, presenting various stages of development and differentiation (Fig. 24). Serial study of the silver-impregnated preparations showed that it was difficult to draw a borderline between the sinusoids and the central vein at the latter's beginning. At the commencement, some sinusoids appeared whose walls were reinforced by dense reticulum stained strongly positive in the silver preparations. These "centralized" sinusoids occurred in the center of the lobular field and contrasted with the poorly impregnated background of the pericentral zone reticulum (Figs. 23a, 24). Joining with each other, the centralized sinusoids became wider and tended to form a lacuna or an ampulla (Figs. 23b-d). The argyrophilic layer was then thickened with unbundled fibrils or sparse fibers. The adjacent hepatocytes were usually not cleared.

The centralized sinusoids represented a transition from the sinusoids into the central vessel. They could occur at the most distal end of an intralobular central vein (Figs. 23a-c, 25) or at other sites along the vein (Fig. 23d-g). Where the transitions were gradual (Fig. 25), it could be seen that, as the luminal diameter grew, the collagenous substances in the wall gradually organized into larger, wavy bundles arranged around the central vein longitudinally or obliquely, but not circularly, forming an apparent adventitial coat. The

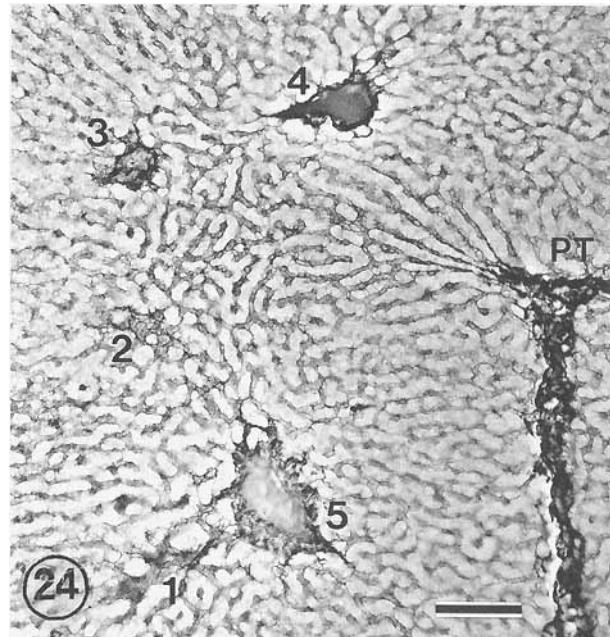


Fig. 24. Structural "age" of the intralobular central veins as ranked by the accumulation of argyrophilic deposits. Considering the disparate degrees of impregnation, the central veins of a CHL were not homogenous in transverse profiles. The numbers indicate a tentative ranking, with 1 being the most recently developed one. The 1 and 2 are actually centralized sinusoids. Note in the figure there is a septal-line pattern between 2 and 3. Note also the tapering of peripheral ends of those laminae approaching a portal tract. Silver impregnation. Bar = 100 μ m.

hepatocytes that contacted the adventitial substances appeared clear.

Where centralized sinusoids occurred near the trunk of a central vein, since the latter was equipped with an adventitia, the communication into the central vein was via a narrow connection through the thick fibrous layer. This resulted in an appearance of a club-shaped vessel, which represented a tributary of the central vein (Figs. 22c, 23g; an earlier stage is seen in Fig. 23f).

The central vein and intralobular septation of CHLs. In the CHLs, transverse sections at various levels of the central vein revealed an obvious irregularity in the accumulations of the fibrous substances in its wall. The adventitial mass appeared to vary according to the orientation of the septal-line pattern and the developing septum(a). Localized thickening of the wall was evident on the part that corresponded with the septal-line structures. The intervening sinusoids appeared with condensing reticulum. The sinusoid-associated porosity in the central vein was remarkably reduced on that side (Figs. 22b, 25). The thickened mass mounted into distinct outgrowths and ascended the sinusoidal channels to incorporate with the forming septum (Figs. 22c, 25, see also Fig. 28a).

The manner in which argyrophilic substances accumulated in the central end of the sinusoids was different, in certain aspects, from that observed at the peripheral end. Centrally, adventitial fibers extended into a large group of sinusoids that reacted with some-

Fig. 23. **a:** A truncal central vein is revealed in an apicobasal transaxial cut of an SHL with a typical centralized sinusoid at its tip. Openings in the venous walls suggest various states of circulatory activities of the sinusoids: wide (large open arrows), narrow (small open arrow), and blind endings (arrowheads); see text for detailed descriptions. Note the isthmus, the gradual loss of sinusoidal connections, and the fibrillary spines (arrows) in the basal segment. Note also the paired fibrous bundles (open arrowheads) that "guard" the narrow orifice of the sinusoid marked with the small open arrow. **b-g:** Variations in development of centralized sinusoids: **b**, short dendritic lacunae; **c**, network of lacunae (arrowheads indicate intervening channels that gradually give way to those that develop into main channels); **d**, side branch of a minor tributary; **e**, side branch of a major trunk; **f, g**, club-shaped vessels annexed to a minor tributary (**f**) or to a major trunk (**g**). In all cases, the reticulum formed by wavy fibrils was finer and more pronounced distal to the major trunk; in the latter case, the circular arrangement disappeared. Note the clearing of hepatocytes contacting fibrous material. Silver impregnation. Bar = 100 μ m.

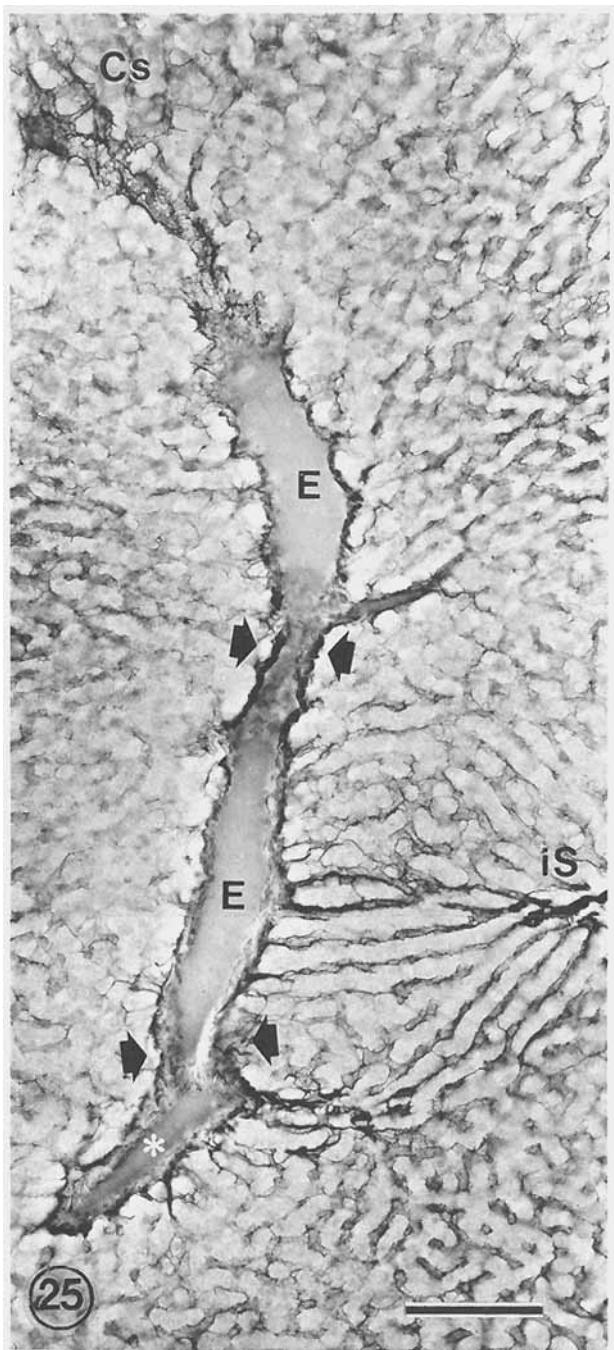


Fig. 25. Major tributary of the central vein in a CHL. Constrictions in walls due to adventitial thickening (arrows) and ampullary enlargements of the lumen (E) defined by the constrictions are obvious. The part of venous wall that corresponds with the intralobular septum is particularly associated with fibrous outgrowths that follow the sinusoidal walls upstream. Note the strikingly narrowed luminal space (asterisk) in the most basally oriented segment. Silver impregnation. Bar = 100 μ m.

what increased reticulum, and the radial laminae did not appear to become as attenuated centrally as they did peripherally (Fig. 25). The previously described double-spurred profile with the atrophic layer of hepa-

tocytes sandwiched in between was very rarely seen in the centrilobular zone. In the vicinity of the central adventitia, the growing fibrous strands sometimes surrounded clusters of local hepatocytes that appeared hypotrophic but did not form a tubular configuration (see Fig. 28a). The adventitial outgrowths were directed as bundles without any accompanying portal vessel; they ascended the septal-line sinusoids towards the growing septum. In later stages, however, it could be demonstrated that small branches of the hepatic arterioles were in intimate relation with the adventitial outgrowths (Figs. 26l, 28b).

The central vein and sublobular vein. The serial tracing of a large number of lobules revealed that occasionally the central vein of an SHL might drain into the central vein of a CHL, instead of the sublobular vein (Fig. 3e). Changes in the size of the lumina of the central-sublobular veins were not always regular. Along the axis of the lobule, an ampullary enlargement usually developed; it was easily appreciated in lengthwise sections of the vein, especially in the CHLs (Fig. 25).

As the lobule's base was approached, sinusoidal openings in the central vein stem decreased, and there remained none when the vein reached its exit in the sublobular vein (Figs. 23a, 26m,o,q). No individual sinusoid was drained beyond this segment (Fig. 26s). Fibrous substances continued from the sublobular adventitia into the central adventitia and imparted a substantial fibrous coat to the basal end of the vein, where the isthmic segment was usually recognized (Figs. 23a, 27b).

The adventitia and hepatic arterioles. The lobule's base provided a route by which the efferent and afferent vessels could gain access to each other. In many CHLs, the lobules were invaded from the base by small arterioles that could be traced to their origins in the portal tract(s) that coursed alongside or came into close apposition with the corresponding sublobular vein (Fig. 26t). As the hepatic arterioles approached the sublobular vein, they anastomosed and came to pervade its adventitia (Fig. 26n,r; see also Fig. 27d). In the larger sublobular vein, the presence of a capillary plexus in the adventitial layer was obvious (Fig. 27a). The hepatic arterioles were supplied from the local portal tracts (Figs. 27c, 27d). Occasionally, however, a relatively large arteriole was encountered; we could not be certain whether it was "hepatic" in origin (Fig. 27e).

Unlike the portal vein, the hepatic arterioles were very pervasive. On one hand, as the intralobular septa penetrated close to the central veins, the septal planes were widely vascularized by arteriolar vessels (Fig. 21d). On the other hand, the adventitial tissue was permeated by other sets of arterioles that invaded from the base and, while ascending the venous trunk, anastomosed with the septal arterioles through fibrous connections (Fig. 26a-d). Where the arteriole was located in the adventitia, the latter became thickened, and bundles of fibers grew out into the sinusoids of the septal-line pattern (Fig. 26e-l). The stromal spaces in the adventitia were discernible. They could be traced to be continuous with the centrilobular sinusoids; where they communicated, strands of fibrils were always noticed (Fig. 26l). It should be noted that the central adventitia sent argyrophilic fibrous offshoots into the septal-line sinusoids prior to the stage at which the

adventitial arteriole appeared with a recognizable size (Fig. 28a).

Appositional growth of adventitia. Layering of argyrophilic substances around the central-sublobular adventitia occurred in a pattern basically similar to that found at the central end of the later-stage splitting septum as earlier described. Proliferating fibers were guided according to the existing sinusoids that contained increased reticulum (Figs. 22e, 29). The perisublobular parenchyma acquired its vascular supplies from both portal and arterial sources; the latter was found to be derived from the adventitial layer as well as the portal area. It was in accordance with these supplies that the appositional growth of the adventitia was observed.

When a sublobular vein happened to cross over a portal tract, the latter emitted abundant branches to cover the region occluded by the sublobular vein. The circumferential sinusoids that faced the vein were heavily impregnated. These argyrophilic substances came to be incorporated with the adventitia through the slender cross fibers that arose at intervals, leaving small fragments of the circumferential laminae with hypotrophic hepatocytes entrapped in between them (Fig. 30a). Where the arterioles approached the sublobular vein, they were found embedded in an abundance of fibrils and fibers that coursed towards the adventitia (Fig. 30b). Sometimes the adventitial arteriole opened into the periphery of the lobule. The local reticulum exhibited prominent condensation and fused with the fibrous coat of the sublobular vein (Fig. 30c). In remote areas, such as those regions located between two sublobular veins where there appeared to be no responsible portal tract, the adventitial arterioles seemed to play the role. The arterioles escaped the adventitia and came to supply the region. Accumulations of fibers and condensed reticulum were found along their course and in the local sinusoids, adding to the thickening of the adventitial mass (Fig. 30d).

The adventitia and septum-initiating site. Apparently where the combination of septum-initiating sites involved the central adventitia, the portal veins, which represented the major afferent vessels, could invade the septum-initiating plane only unilaterally (Fig. 31a). Once established, the plane came to act as a source of perfusion that could generate newer septal-line structures (Fig. 31b), to add argyrophilic substances to the adventitia (Fig. 31c), and to extend portal triad structures to the neighborhood of the sublobular vein (Fig. 31d, see also Fig. 3b). In the latter case, the elongations of the portal branch that reached the sublobular (hepatic) canal usually exhibited enlarged lumina, placing them in such intimate contact with the sublobular adventitia that casual observation might assume it as possible evidence of the portal-sublobular (central) "anastomosis."

DISCUSSION

Since the term *lobulus* was used by Malpighi in 1666, two years after its first discovery by Wepfer, to designate the liver units of vertebrates, the subject has been studied with particular interest (for historical detail, see Kiernan, 1833; Oppel, 1900; Mall, 1906; Lichtman, 1953; Child, 1954). It was not until 1833, however, that a precise description of the hepatic lobules was made by

Kiernan, who coined many terms in describing these anatomical units and has been regarded by some authors (Mall, 1906; Johnson, 1918a; Cameron and Mayes, 1930; Andrews et al., 1949; among others) as the founder of the basis of our present knowledge of the liver.

Unlike many investigators, such as Johnson (1918a), Pfuhl (1921a,b, 1922, 1932), Kopsch (1936), Elias and colleagues (1954), Hargreaves (1968), Elias and Sherick (1969), Rappaport (1976), Motta and associates (1978), Chen and Chen (1984), Beresford and Henninger (1986), and Sasse (1986), to name but a few, we view it as a mere accident that Kiernan's lobular concept culminates in the liver of the pig. Perusal of his original paper discloses obviously that the pig⁵ was not investigated. As an unexpected result, investigations on liver units thereafter were concentrated mainly on the porcine liver, which has subsequently come to serve as the "classical example" (White, 1939) or the "paradigm" (Elias et al., 1954) of liver histology.

Differences in Liver Unit Concepts

The validity and usefulness of the traditional definition of a hepatic lobule have been questioned repeatedly (Brissaud and Sabourin, 1888; Kelly, 1905; Eppen, 1922; McIndoe, 1928; Rappaport et al., 1954; Ham and Leeson, 1961; Cormack, 1987). A tremendous number of studies have been conducted in search of a universal concept of the liver unit. These efforts have resulted in various proposed interpretations, none of which has so far attained general agreement. According to a textbook (Jones and Spring-Mills, 1988), "three primary schemata have been developed to describe the histological and functional units of the liver. The names classic lobule, portal lobule, and liver acinus have been assigned to the three interpretations. However, only the classic lobule and liver acinus concepts have stood the test of time." Although the microcirculation-based concept of the liver acinus is widely accepted (for the latest reviews, see Jungermann, 1986; Sasse, 1986; Lautt and Greenway, 1987; McCuskey, 1988; Gumucio, 1989), in view of recent evidence, several discrepancies have been pointed out (Matsumoto et al., 1979; Teutsch, 1984, 1988; Sasse, 1986; McCuskey, 1988; Lamers et al., 1989; Quistorff and Rømert, 1989).

It is very interesting to note that every time a "new" unit concept emerges, the idea remains within or not remote from the realm of the hexagon. The portal or structural unit of Mall (1906) was conceptualized on the hexagonal plan (reproduced in Fig. 32). The functional unit of Knisely and coworkers (1948) and the modified functional units of McCuskey (1966) and of Bloch (1970) were parts of the classical lobule. The liver acinus of Rappaport and colleagues (1954) was designed on a subdivision of the hexagonal lobule with

⁵We are not alone in this opinion. Lichtman (1953) stated that "Francis Kiernan first accurately described the structure of the human liver and the relationship of its blood vessels to the constituent lobules." Wünsche (1985) called attention to the fact that Kiernan did not study the pig liver as mistaken by many authors. Likewise, Schaffner and Sieratzki (1987) regarded Kiernan as the first who correctly documented "the real architecture of the liver," which contributed significantly to the understanding of cirrhosis.

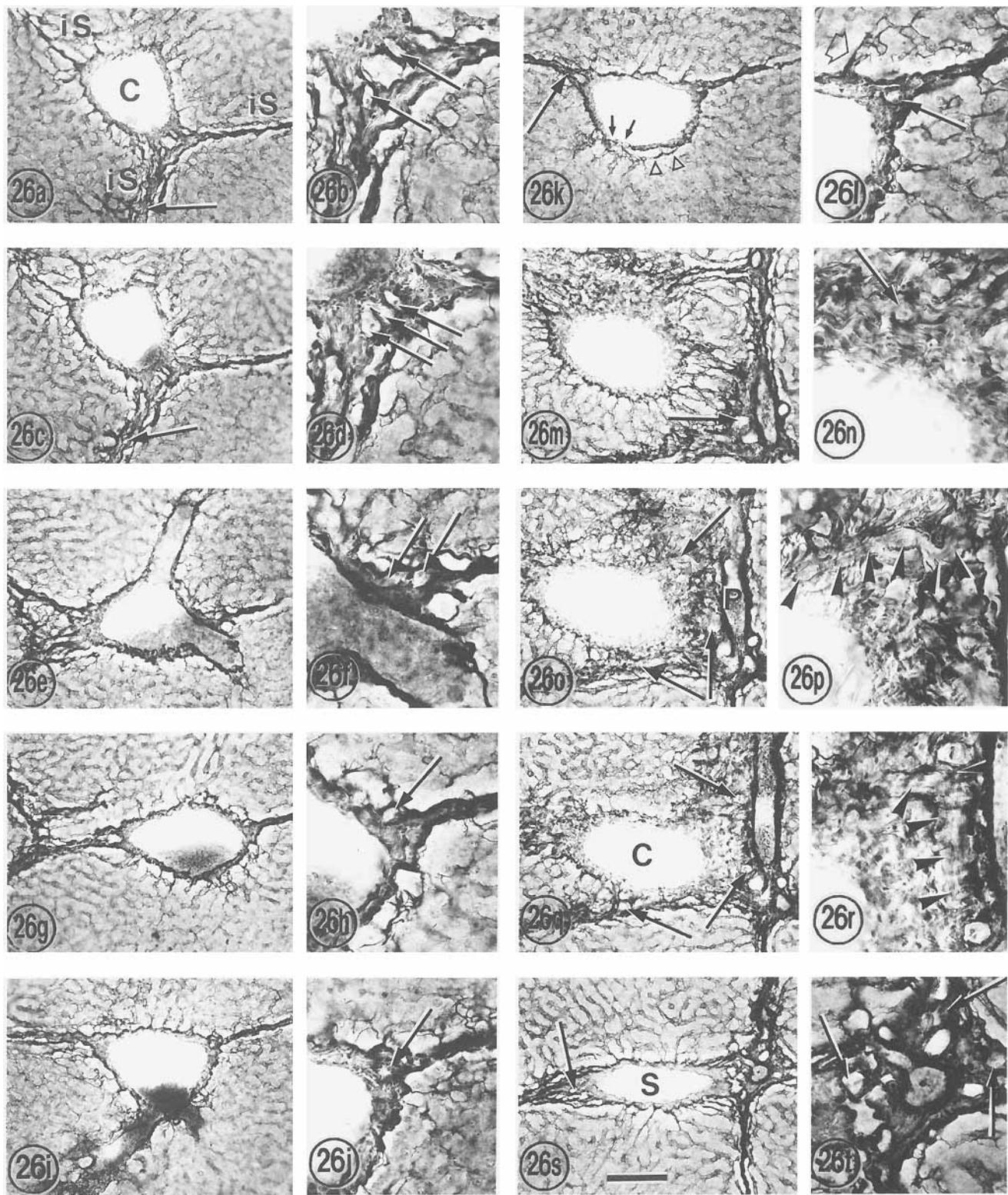


Fig. 26.

interpolated zonation lines (see Fig. 32). The comprehensive schemata of the hexagonal, triangular, and rhomboidal lobules by Preuss and Fricke (1979) were

formulated upon the hexagonal columns. The primary lobule of Matsumoto and associates (1979) was adapted to a corner sector of the classic lobule (see Fig. 32).

Fig. 27. Sublobular adventitia and the artery-derived intramural capillary plexus. The minute perforations in the fibrous walls of a sublobular vein cut longitudinally (**a**) are shown at higher magnification (**b** and **c** for the upper and lower blocks outlined in **a**) to be the capillary plexus (arrowheads). Near the central vein exit (asterisk in **b**), an arteriole is about to invade its adventitia. In a nearby portal tract (**c**), profiles of the arterioles are most numerous and are closer to the adventitia. **d:** The portal tract in the vicinity of the sublobular vein appears to send arteriolar branches towards the adventitia. **e:** Occasionally a relatively large arteriole (arrow) 40 μm in external diameter, courses "on" the sublobular adventitia; the responsible portal tract was unidentified. Paraffin sections, 10 μm with Masson-Gomori trichrome (in **a-c**), 5 μm with hematoxylin and eosin (in **d, e**). Bar in **a** = 500 μm ; bars in **d, e** = 50 μm .

Most recently a modification of the acinar concept was offered, based on the three-dimensional enzymic distribution patterns (Lamers et al., 1989). The proposed scheme, unintentionally it appears, was brought back to the scope of the hexagonal archetype with the traditional three concentric layers (compare Fig. 1a of Lamers et al., 1989; with Fig. 5 of Noël, 1923; or with Fig. 4.1A of Zimmerman, 1978; or with Fig. 6 of Sasse, 1986). Another modified acinar model on a similar hexagonal plan was suggested by Quistorff and Rømert (1989).

As far as hepatic lobulation is concerned, its architecture is not uniform. The several designations referred to as the *double lobules* (Kiernan, 1833; Mall, 1906), *anastomosing lobules* (Mall, 1906), *complex lobules* (Mall, 1906; Debeyre, 1910; Wünsche, 1981), *twin lobules* (Kretzschmar, 1914; cited by White, 1939), *compound lobules* (Johnson, 1918a,b; Kaman, 1966), *Doppeläppchen* (Pfuhl, 1921b), *zusammengesetzte Läppchen* (Pfuhl, 1921b; Kaman, 1966), *Sammeläppchen* (Braus, 1924; Pfuhl, 1932; Bargmann, 1967; Sasse, 1986), *Zwillingläppchen* (Clara, 1931), *composite lobules* (Braus, 1921, 1924; Goerttler, 1959; Tamura, 1952; Kaman, 1966), *aggregated lobules* (Wünsche, 1985), and *collecting lobule* (Sasse, 1986; Leonhardt et al., 1987), indicate unmistakably that in addition to the so-called classic lobule, there exists another type of the lobule that most likely has been ignored and rarely has been considered equal in importance to the "hexagonal" type for the understanding of the basic structure of the liver. It is difficult, therefore, to conceive how the "classic" or "hexagonal" lobule alone could accommodate the variability in chemomorphology of the liver and could explain the complex histopathology of this multiple-function organ.

Fig. 26. Arteriolar "loop" and central adventitia. A central vein is traced (first and third vertical columns) from the middle to the base of a CHL; enlargements of adventitial arterioles (arrows) from each are presented in the second and fourth columns; beyond the base a sublobular vein and a portal tract are demonstrated (**s, t**). The arteriolar courses are continuous basally with arterioles in a portal tract (**o-r**; arrowheads indicate longitudinal profiles), and apically with other arterioles in the intralobular septum (**a-d**). Where the arteriole(s) is located, the adventitia extended into the intralobular septum (**a**) (**a-l**). No direct opening of an arteriole into the sinusoids or the central vein was observed, but an indirect communication is seen through the stromal spaces in the adventitia (**I**). In the latter case, the adventitia released some fibrils, which continue into the reticulum (open arrow in **I**). **k** represents the most basal segment of the central vein to allow sinusoidal orifices (small arrows). Note that there is a sinusoid (at open arrowheads) coming from the thickened adventitia. Note also that the sublobular vein (**s**) has a lumen narrower than that of the central vein. Silver impregnation. Bar = 100 μm .

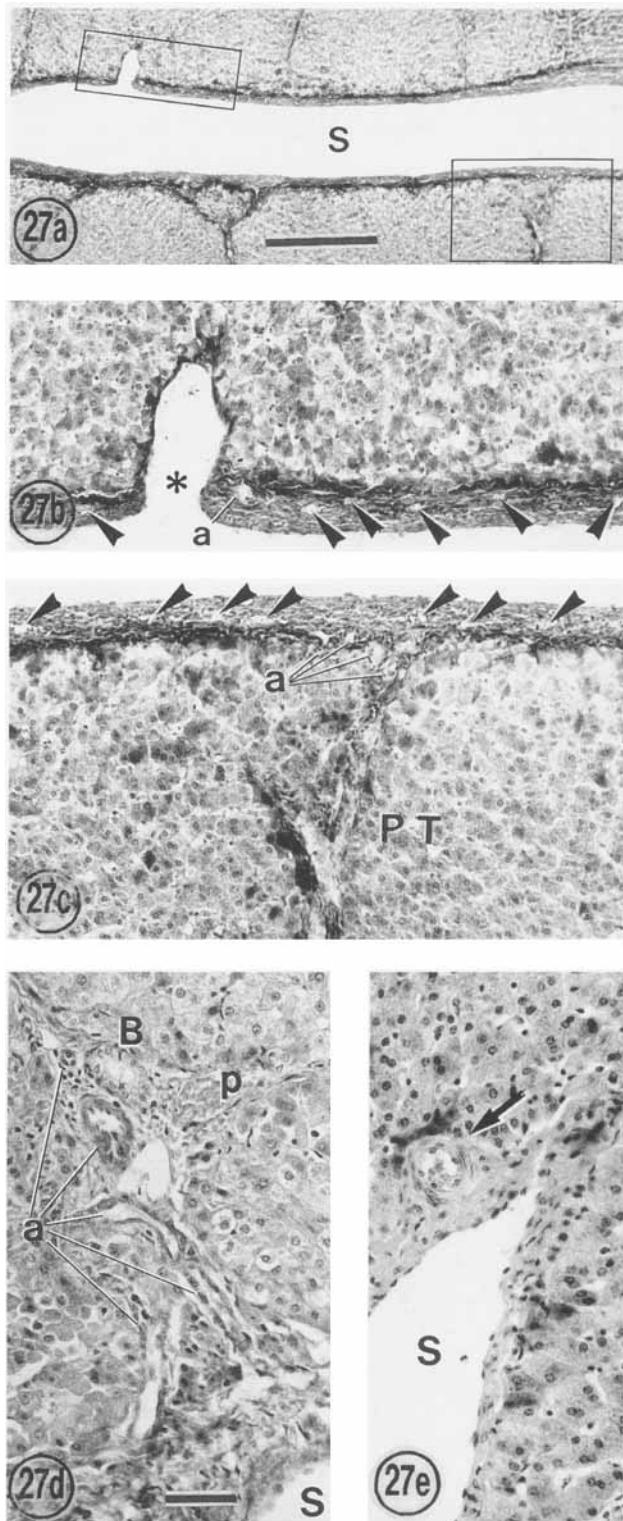


Fig. 27

The many attempts to find the "real" elementary unit of the liver imply that the preexisting unit concepts cannot satisfactorily serve the investigators as a standard model. As a matter of fact, their frame of

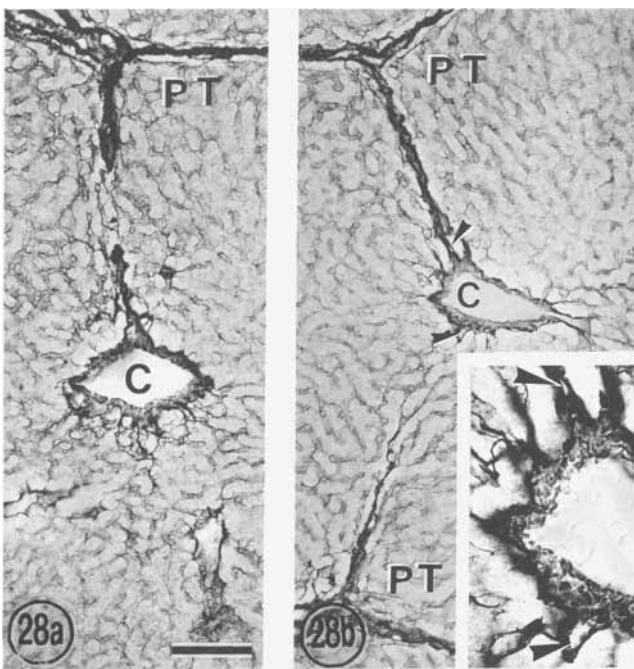


Fig. 28. Formation of primary and secondary, central adventitia-associated, intralobular septa. **a:** The mesentery-like septum is constituted by the portal tract and the active appearing central adventitia; the extent of their contributions appeared to be more or less equal. Note that, in this early stage of the CHL, the adventitia does not always contain capillaries with discernible lumina. Note also the clusters of fiber-trapped hepatocytes, which appear hypotrophic and lacking any ductular formation. **b:** In another CHL of a later stage, a splitting septum is dividing the lobular field on the opposite side, tending to maintain a centripetal direction. Two vessels in the adventitia can be identified (through the serial sections) at the septum-initiating sites (arrowheads, and see inset for enlargement). Silver impregnation. Bar = 100 μm .

reference has been confined to the textbook-derived hexagonal model, notwithstanding the two fundamental patterns of liver unit structure.

Our study shows that only a small part of the lobular population could be represented by the classical, or hexagonal, form of the SHL type. Not represented are the other forms of the SHLs, and the CHLs, which have been reported frequently in the past but have been heretofore overlooked by most textbook authors.⁶ Therefore, the hexagonal lobule is not the norm, and, in view of the lack of basic knowledge concerning the structural differences in liver units, the significance of these neglected structures can be profitably emphasized.

SHL-Like Lobules With Variable Forms

Based on the outline in sections, the forms of the SHL-like lobules, or the nonseptate lobular fields (in two-dimensional histologic preparations), can be divided into convex polygons and concave polygons. Concerning the convex polygonal shapes, White (1939) showed that the pentagonal form is rather more com-

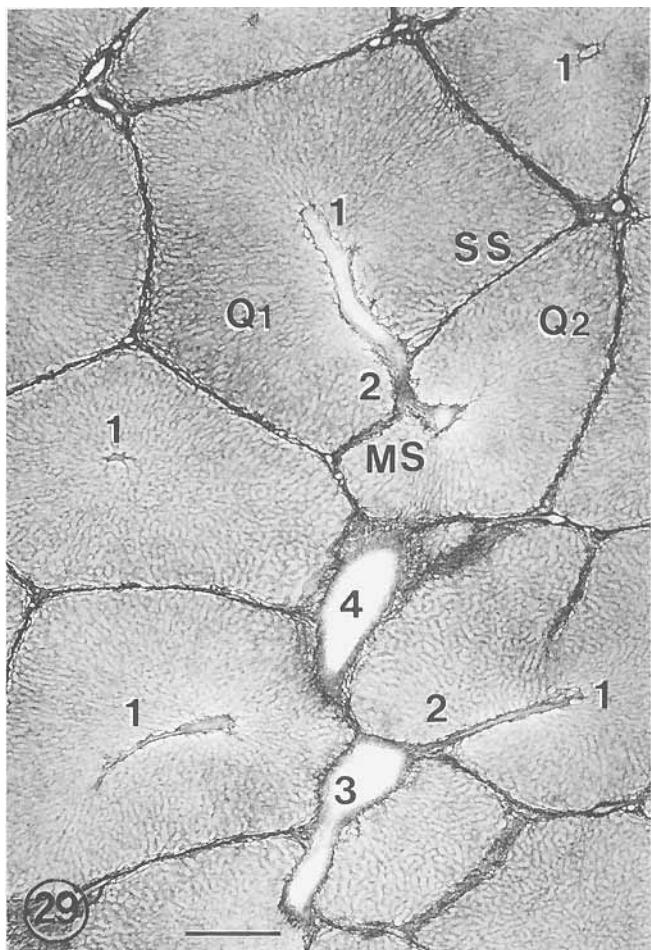


Fig. 29. Argyrophilia-based appearance of pericentral and perisublobular regions. Density of the sinusoidal reticulum in these regions tends to increase more or less as the draining vessels acquire substantial adventitia; vessels have been numbered according to the flowing order. The histologic lobule Q1 and Q2 are isogenous, each being a component of a single CHL; through this series, it was found that the septum MS changes into a mesentery-like septum and septum SS into a splitting septum (the central vein ranked 2 here represents, therefore, a stem segment). The pericentral regions (1) are light, whereas the perisublobular regions (4) are notably dense. Note the appearance of proliferating fibers near the central end of septum SS. Silver impregnation. Bar = 300 μm .

mon; he found that the triangular were less than 1%, quadrangular 10%, pentagonal 47%, hexagonal 39%, heptagonal 4%, and octagonal less than 1%. Our findings were closely comparable (Table 2). Therefore it is more correct to state that the most "typical" form of the SHL is the pentagon, not the hexagon. It is not surprising that some investigators did not describe the hexagonal lobules, as one might expect; instead, what they illustrated very often included pentagonal lobules (Chrząszczewsky, 1866; Johnson, 1918a; Pfuhl, 1922).

We agree that the hexagon is idealized but should not be assumed as representative for liver units. In fact, pentagonal lobules are more numerous. This should be taken into consideration when a new modification of the unit concept is to be designed. Figure 32 demonstrates the possible appearance of major unit

⁶Illustrations of the SHL and CHL in three dimensions can be encountered in Pfuhl (1921b).

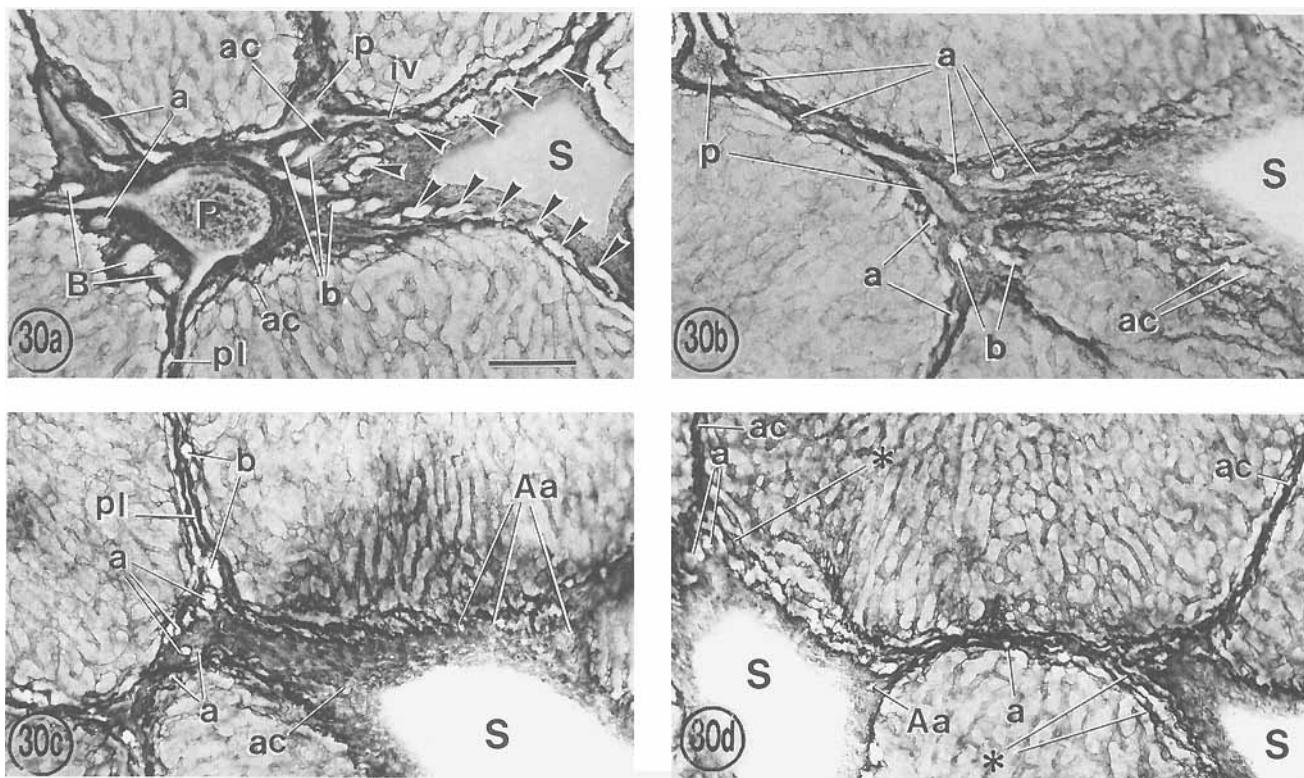


Fig 30. Condensation of reticulum in various perisinusoidal regions. Demonstrated are areas close to portal tracts: A medium-sized portal tract crossing over (a), and a small portal tract approaching (b), and the areas remote from portal tracts: with distant branching of a portal tract (c) and without portal involvement in the intervenuous area (d); see text for detailed descriptions. Note the fragmented lam-

inae that appear as para-adventitial layers in the embedding of fibers (arrowheads in a). Note also those thick-walled sinusoids, appearing like double-railed tracks (asterisks in d), in the flow bed of arterioles; presumably they might represent a capillarized or arterialized sinusoid. Silver impregnation. Bar = 100 μm .

concepts when applied to a pentagonal plan compared with the usually preferred hexagonal plan.

Based on the central vein exit, the SHL-like lobules can be described as either uniexit units or multiexit units. The multiexit SHL (the lobule marked "I" in Fig. 2) conforms with the "type I" lobule according to the drainage pattern classified by Matsumoto and colleagues (1979). The multiexit SHL bears some resemblances with the "parallel lobules" or the "half a lobule" of Kiernan (1833). Although quantitative data on the multiexit lobules are not available, it appears from Figure 24b of Matsumoto and coworkers (1979), from the description of Kiernan (1833), and from our own observations that their occurrence is fairly common.

Considering the lobular gradient of the multiexit SHL, we found an eccentric pattern, instead of the conventional concentric zonation (Fig. 33). The layout suggests a propensity for this type of the SHL-like lobules to possess the centrilobular zone in apposition to the peripheral zone. Interestingly, a more or less similar descriptive feature is obvious here, if one recalls one of several characteristics expressed in the acinar concept, that is, that the acra or the outskirts (i.e., the pericentral zone 3) of the simple liver acini abut on the periportal zone. For detailed descriptions of the acinar concept, the reader is referred to Rappaport (1958, 1963, 1973, 1976, 1987).

Proportions of CHLS

Unfortunately, the manifestation of CHLs in single sections has not been impressive enough to attract their observers to pursue a careful quantitative study. Kretschmar (1914, cited by White, 1939) recorded the occurrence of compound type lobules in neonate pigs and stated that these were seen less frequently in the adult. White (1939) observed that 3–7% of the lobules are found to be compound in the liver sections of pigs more than 30 days old. We have demonstrated that in the 6-month-old pig's liver, 17%⁷ of the lobular fields could be identified as CHLs by one section and that, when the surface lobules were excluded, the CHL prevalence became 9% (Table 2). The latter may account for the relatively rare incidence of the CHLs in the deeper lobules.

In three dimensions, however, serial tracing revealed that the CHLs occupied 75% of the lobular fields. Therefore, the presence of the CHLs is by no means negligible. The result also implies that about

⁷The figure should not be assumed here as contradictory to the observation of Kretschmar (1914, cited by White, 1939), since we have found that the CHL proportion can be demonstrated to decrease constantly with age (unpublished data).

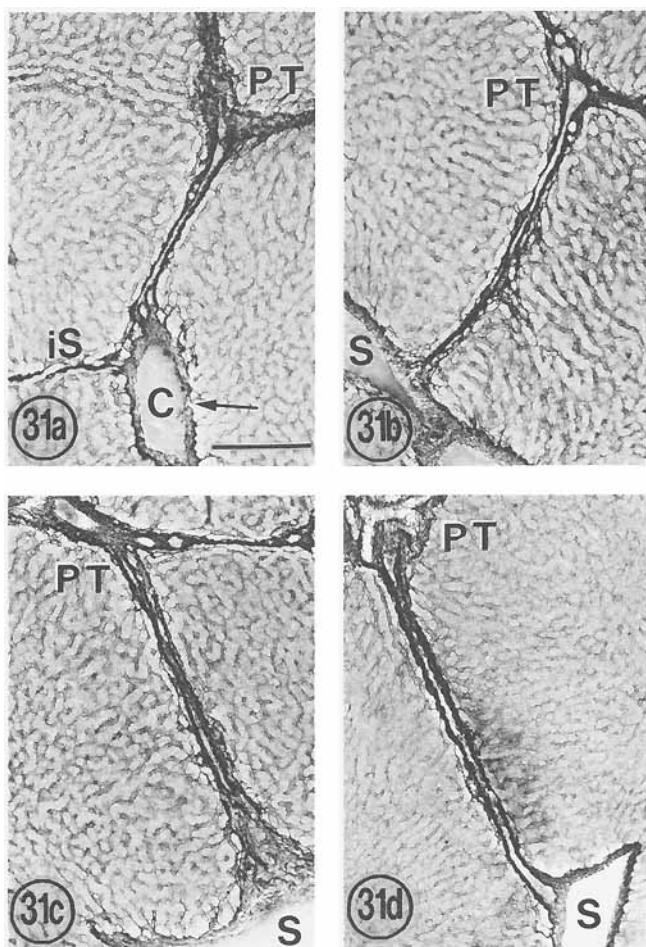


Fig. 31. "One side-one branch"-type portal venules. In contrast to the usually seen "one side-two branches" or "half side-one branch"-type portal venules, there are occasions in which a perilobular portal vein (venule) occupies the whole length of a side of a lobular field. They were encountered frequently in the perisublobular regions. a: A case of a central vein with thick adventitia in stem segment; arrow indicates sinusoidal orifices; the septum to the left had not been finished. See text for other descriptions. Silver impregnation. Bar = 200 μ m.

58% of the CHLs mimicked SHLs; in other words, they were two-dimensionally the SHL-like lobules (Table 2).

The reconstruction studies by Debeyre (1910) and by Pfuhl (1921b) have added significantly to the knowledge of the CHLs but seem to have given little impetus to the field. It appeared to us that the large proportion of CHLs underlies the questionable structures or phenomena that have been observed frequently in liver tissue often discussed but with little unanimity of opinion. Convincing quantitative data were needed, therefore, to show that the CHLs are predominant. The practice of lobule mapping was useful for this purpose, and, to our knowledge, the procedure has not been conducted by others who have studied the porcine liver.

Comparisons of SHLs and CHLs

Although most of the findings presented were based on the silver-treated serial sections derived from a small tissue block of one pig liver, the general histo-

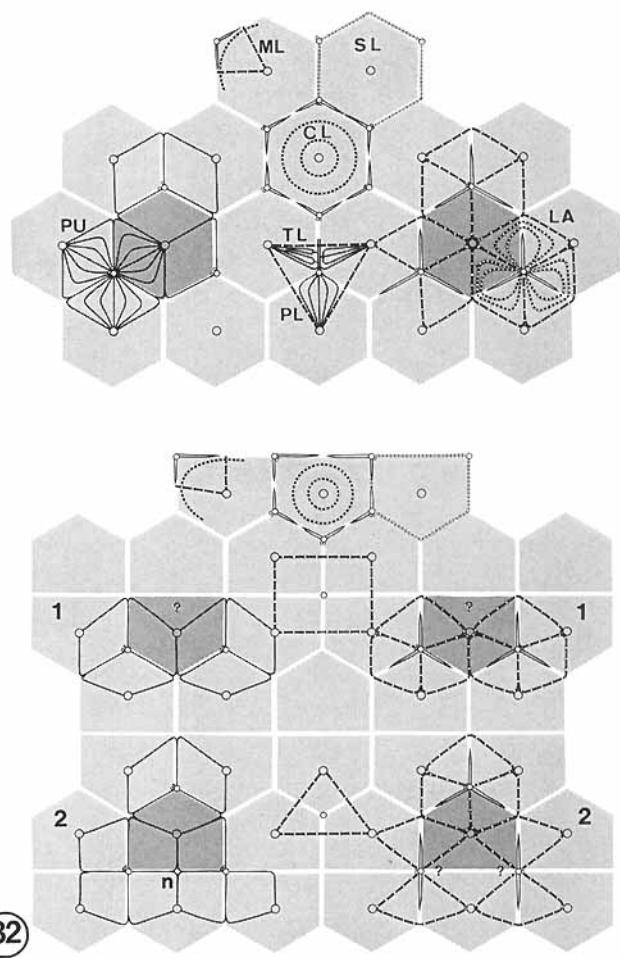


Fig. 32. Possible appearances of some unit concepts when applied to a pentagonal plan (lower half) compared with the stereotypical hexagonal design (upper half). On the hexagonal plan, the classic lobule (CL) is depicted according to the classical descriptions, with a central vein and portal tracts at the interlobular spaces; its lobular field is subdivided into three concentric intralobular gradients, being supplied by the perilobular branches at the periphery ("one side-two branches" or "half side-one branch"). The suine lobule (SL) is demonstrated with fibrous septation, the primary lobule (ML) with the sickle zone. The portal unit (PU) is delineated by the sinusoidal courses (uncongested liver) and the liver acinus (LA) by microcirculatory zonation lines. The portal unit is modified by connecting the nearest central veins into the portal lobule (PL; see Cormack, 1987) or the triangular lobule (TL; see Preuss and Fricke, 1979). The portal unit and the liver acinus are conceptualized with a portal occupancy rate of 0.5; the liver acinus further assigns the "one side-one branch" character to the hexagonal field. When the polygons are formed by an odd number of sides, such as pentagons in the lower part of the figure, it is not easy to deal with the concepts of the portal unit (left) or the liver acinus (right). Two possibilities (1, 2) are attempted in which there occur difficulties (indicated by ? in the figure) in filling the lobular fields (dark tone). The portal unit is compensable, provided a nodal point (n) is established halfway on one side.

logic structures as perceived in the routinely stained preparations were confirmed in at least nine pigs. In fact, we have ascertained that both lobule types coexist throughout the liver, irrespective of locality, from the thickest part in the porta hepatis, through the four major and two minor lobes, up to the thinnest sleeve of hepatic parenchyma that surrounds the vena hepatica

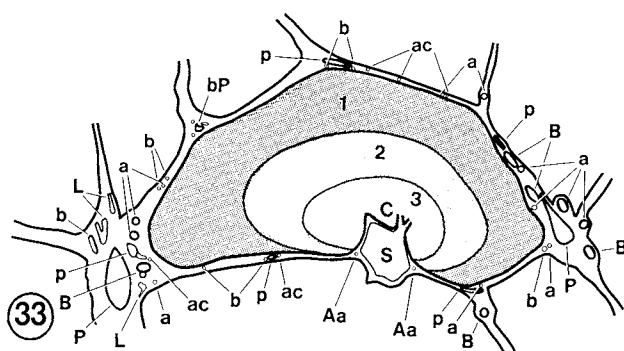


Fig. 33. When the classical description of lobular gradient is applied to a multiexit lobule (lobule I in Fig. 2), interestingly it is found that the zonation creates an eccentric pattern rather than the usual three concentric zones. Peripheral porto-bilio-arteriolar structures were identified (under an oil-immersion, high-power objective), and demonstration of their topography was attempted. Note the remarkably shortened distance between the peripheral zone (1) and the pericentral zone (3) on the basal side. Note also the sites of adventitial arterioles and the abutting septa.

at its opening in the vena cava caudalis (unpublished observations by the authors). The intermingling of the two lobule types was found consistently in about 30 pigs of various strains raised in different geographic locations and ranging in postnatal age from 3 weeks to 3 years (unpublished findings).

Whether or not the organization of argyrophilic substances that form the regular structures characteristic of each lobule type is subject to individual variation or to seasonal changes cannot be known with certainty until studies with the silver method can be conducted in many subjects. We know from earlier studies that seasonal variation is related somehow to changes in the intrahepatic circulation of blood and the sinusoidal activities in amphibians (Wakim and Mann, 1942).

Although the unity of the hepatic lobules is determined by the incipient segment,⁸ i.e., the intralobular division, of the hepatic vein (hence the term *hepatic lobule*), the dendritic pattern of their axial vessels does not necessarily distinguish the SHL from the CHL. These findings agree with previous observations that intermediate forms are always present in porcine lobules (Wünsche, 1981). Mall (1906) studied the livers predominantly in man, dog, rabbit, and cat, as well as pig, and stated that there are all gradations between the two lobule types. This agreement suggests that the basic architectures of the "nonsuine liver" and the "suine liver" (to use the differentiating terms of Elias, 1949) are formed in essentially identical patterns, despite the lack of obvious fibrous septation in the lobulation of nonsuine livers.

Whereas the CHLs were invariably equipped with a branching system of central venous tributaries, an SHL might be found with a (less) dendritic central vein. This feature has been beautifully demonstrated

in the wax-plate reconstruction of an SHL by Braus (1924, his Fig. 162). Sizes of the lobular fields were not always distinguishable. The only structures peculiar to the CHLs were the intralobular septum(a) and the septal-line structures (the septal-line sinusoids and laminae and the septum-initiating sites). Theoretically, however, it is conceivable that the SHL with a dendritic central vein could have established septal-line structures without septal deposition; we were not able to determine whether such a unit existed in the porcine liver.

For practical purposes, with regard to the axial vessels, it is not incorrect to envisage that the intralobular draining veins are dendritic, for two reasons. First, the majority of lobular fields were the CHLs, and even the SHLs can possess an axis with some tributaries. Second, since the routine laboratory animals are of non-suine species, from their microscopic appearance their livers might be considered, although not correctly, as a large CHL.

This view should be beneficial in interpreting the centrilobular distribution of, e.g., morphological changes under certain pathomorphy. The diseased area may possibly appear as a somehow angulated lesion around the central vein, especially when cut obliquely about the confluence of the vessels. Such a case is evidenced in Figure 10 of Rappaport (1976), where the affected hepatocytes are confined to the central veins with two oblique sectional profiles but were interpreted as a triangular necrotic patch specific to the extension of "zone 3."

Significance of the Coexistence of Two Lobule Types, the Ongoing Lobulation

Concerning microcirculatory unity, sectional profiles of the SHL-like lobules can be regarded as unicenter fields (the lobule that is usually seen) or as multicenter fields (Figs. 2, 4). The latter are worth discussing, since the criteria of acinar zonation do not cover the intervenular areas of these two-dimensional units. According to Johnson (1919), the "splitting septum" later develops deeper into the lobule and severs it, carrying with it the afferent vessels to supply the remote region; the latter vessels locate farther than "zone 3" and beyond the "terminal hepatic venule." This process produces the septate CHLs (Table 2). However, as concerns the SHL-like lobules (non-septate fields) with multiple centers, there had been no explanation of how lobular microcirculation is carried out.

It is evident that the lobular fields are not occupied exclusively by the radial sinusoids. Our observations on the septal-line structures and the septum-initiating plane in three dimensions have confirmed and extended the studies of Johnson (1917, 1918b, 1919) on the events that take place in the formation of new lobules. They are shown schematically in Figure 34. The scheme assists us in understanding the interrelationships among those findings involved in the lobuloproliferative process; it is not intended to represent the complete chain of phenomena, nor is it adequate to account for the underlying mechanism. The beginning of a higher order event does not imply the cessation of the previous one; they act together simultaneously. Some observations can be made concerning this sequence.

⁸Although the intralobular segment of the hepatic vein has been labeled as "terminal" by Mall (1906) and Rappaport et al. (1954) among others, we agree with Teutsch (1984) that this should be avoided as far as the anatomical rule is to be respected; according to its flow direction, the vessel is not the termination but the initiation.

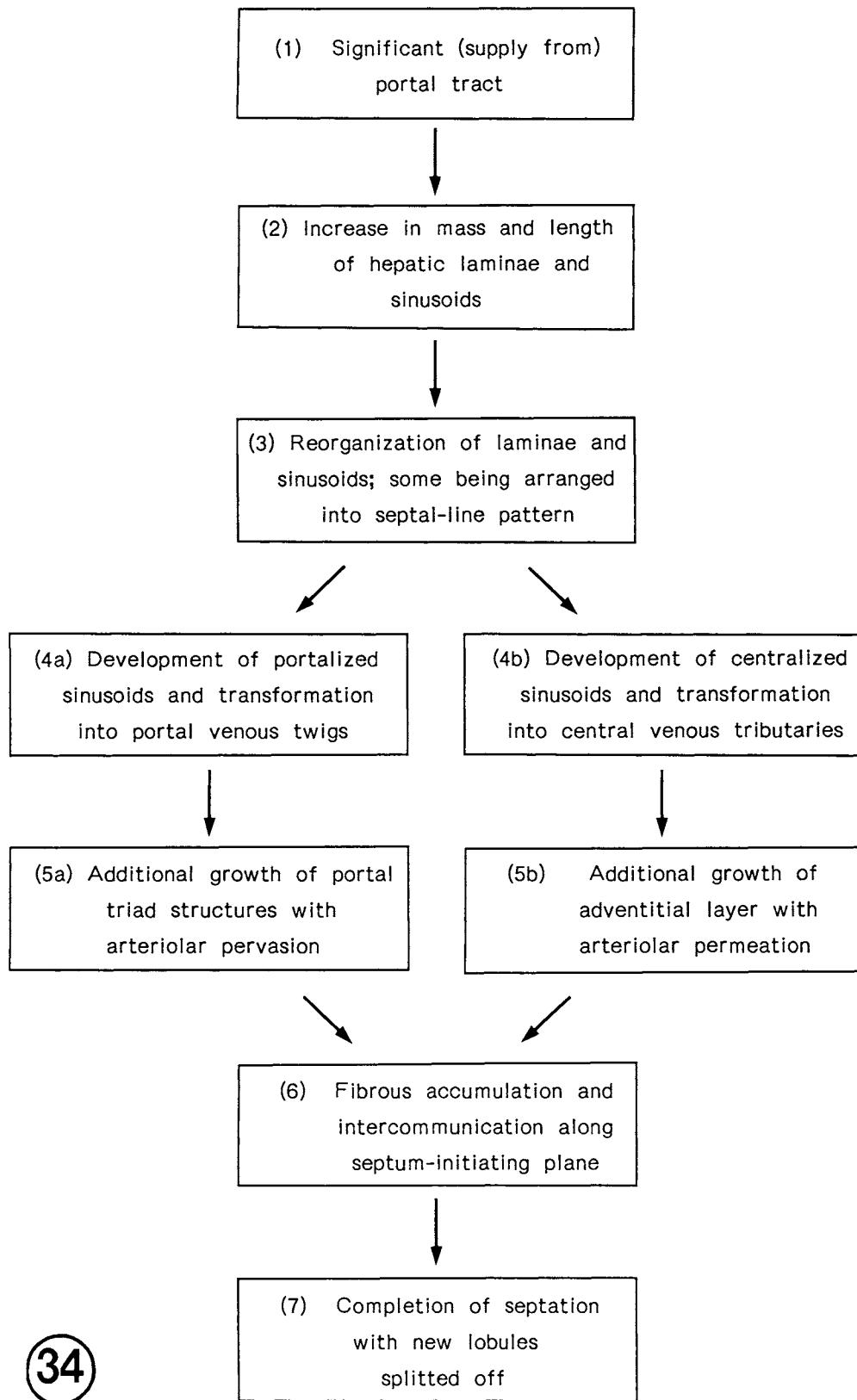


Fig. 34. Probable flow of some major events involved in the forming of new lobules in the pig liver.

1. The primary events in lobular morphogenesis were those of the portal and the central-sublobular vasculature; new ductular formation was found to accompany the sinusoidal portalization, and the appearance of hepatic arterioles in the plane of septation was not always obvious in early stages.

The spatial configuration of the portal-central venous systems, rather than that of the arterial or biliary systems or otherwise, influences significantly the lobulogenesis of the hepatic parenchyma. In the developing embryonic livers, as demonstrated by Mall (1906), the involvement of the afferent and efferent venous vasculature is so representative that, by describing consecutive changes in their relationship, nearly the whole story can be grasped. Mall was of the opinion that the development of the hepatic arterial system is subordinate and is better understood together with that of the biliary system. In cineangiography, the hepatic artery is poorly visualized during fetal life and appears to contribute negligibly to the total hepatic blood flow (Peltonen and Hirvonen, 1965).

Pfuhl (1921a) explained the segmentation of hepatic tissue into lobules by depicting the portal-hepatic venous relationship. The parenchymal unit as the "primary lobule" of Matsumoto and colleagues (1979) is actually based on the two venous vasculatures with the sinusoids intervening; they found that the overall image of the portal angioarchitecture predetermines the biliary and the arterial systems.

2. Neovascular formation was essential in lobular multiplication. It was Mall (1906) who explained that "some of the main feeding capillaries (i.e., the sinusoids) should be converted into veins," although he arrived at the conclusion by deductive reasoning rather than inference from having seen actual evidence. By the silver impregnation method, we could demonstrate the "portalized" sinusoids in serial sections. The conversion of certain sinusoids into portal twigs is comprehensible under the histomechanic law (the first principle) of Thoma (1896, cited by Mall, 1906). Accordingly, hand in hand with the growth in thickness of the vascular wall and in diameter, there exists tension (the second principle). This tension might account for the hypotrophic appearance of the fiber-contacting hepatocytes around the thick-walled sinusoid and for the convergence of regional hepatic laminae on the fibrotic sinusoid; in the latter case, the appearance resembles more or less a sclerotic gastric ulcer upon which the mucosal folds (flexible structures) converge. One can make a similar deduction that the main draining sinusoids should be converted into veins (venules) and there we found the "centralized" sinusoids.

3. The course of the newly formed portal twig was subject to the course of the septal-line sinusoids in the septum-initiating plane. This gives a probable reason for the sometimes reported "portal-central" and "portal-portal" anastomoses. The portalized sinusoid, when it occurs close to the central vein, as seen in Figure 14b, can be perceived as a portal-central anastomosis. If two portalized sinusoids happen to develop along a line that stretches between two portal tracts, in a manner like that depicted in Figure 19, they possibly appear as a portal-portal anastomosing twig.

The length of the portalized sinusoids was subject to the effective perfusion pressure that prevailed. That the portalized sinusoids did not proceed beyond the midpoint suggests that locus as a watershed zone.

4. In the septum-initiating plane, the vascularization (into portal twigs, presumably after capillarization) preceded the fibroconnective-tissue septal formation. This finding suggests that the appearance of the septa was secondary to the appearance of the vascularized plane, or in other words the septation was an accompaniment and was determined in conformity with the demarcation into lobules. According to previous studies (Debeyre, 1912; Johnson, 1917, 1919), the pig's hepatic tissue shows the lobular pattern in late fetuses and postnatally displays the radial arrangement, which gradually increases in definition. The septa appear during the first few weeks after birth and become fully formed in about 2 months. The principal steps in these structural changes were recapitulated repeatedly in the segmentation of the growing lobules of the 6-month-old pigs observed in the present study.

5. The "splitting" might follow the "binary fission" as described by Johnson (1919), or it might not. There could be any division into three or four or more units depending upon the developing major centered vessels. The lobules marked with W, Y, and Z in Figure 4 and the three-component CHL in color Figure 7e are self-evident.

6. If the process were arrested just after steps (5a) and (5b), the outcome would be similar to the "cloison vasculaire" of Debeyre (1910), the "vasculare Grenzschichte (Septum vasculare)" of Pfuhl (1922), and the "continental divide" of Epplen (1922) and would be closely comparable to the hemodynamic surface "inflow-front" with the "septal branch" of Matsumoto and colleagues (1979). These comparisons imply that the lobulation of the parenchyma in suine and nonsuine livers are based on essentially identical designs. The subsidiary differences due to the fibrous septation with some related structural modifications should not be argued so as to ignore the underlying architecture.

7. The definitive septa were mosaic in nature. Upon the groundwork of condensing reticulum in the septal-line sinusoids, fibers were laid down. The fibers were supplied from two sources, the portal area and the central (sublobular) adventitia; morphologically, the topographical characteristics of fibrous reactions were not identical. Based on the argyrophilic appearance, the portal side was characterized by 1) stronger reticulum condensation, 2) induction of ductular transformation, and 3) emergence of all portal triad structures within the fibrous ingrowths. The central side was characterized by 1) weaker reticulum response, thus seemingly lacking transverse wavy fibrils (inherently constituting the sinusoidal walls); 2) entrapped hepatocytes without rosette formation; and 3) invasion of arterioles into the fibrous outgrowths.

The observation that morphologic fibrogenicity differed with respect to intralobular topography can be interpreted as suggesting a disparity in the kinds of cells responsible for local fibrogenesis. This speculation appears to agree with a previous electron-microscopic study (Tanikawa and Ueno, 1985) suggesting that, in the normal liver, production of fibers within the portal tract, in space of Disse, and in the vicinity of the cen-

tral vein is accomplished by different mesenchymal cell types (i.e., portal fibroblasts, perisinusoidal stellate cells, and perivenous myofibroblasts, respectively).

The mosaic nature of the fibrous septation could imply two potential modes of hepatic lobulation: The "hepatic lobules," formed when the ingrowths from portal supply as a whole are dominant, such as is the case in the normal suine liver, and the "pseudolobules," formed when the outgrowths from central-sublobular adventitia (pericentral fibrous substances) are invasive, such as are found in carbon tetrachloride-induced hepatic fibrosis (rat or mouse), pig serum-induced hepatic fibrosis (rat), and cirrhosis (human).

We are not in a position to state the definite significance of the SHLs and CHLs. From their morphological resemblances, however, it may be safe to think that they represent different developmental stages of the hepatic lobulation found postnatally, which in the pig is characterized by diffuse fibrous septation. We have not obtained evidence of functional differences between the two lobule types. In view of the fact that the fibrous septation follows the established vascular septum, however, it is reasonable to assume that an SHL is more favorably surrounded by vessel-distributing plans, hence the possibility of a more extensive perfusion pattern.

Significance of the Septal-Line Structures

Since rigid structures with an argyrophilic fibrous skeleton were always involved in the septum-initiating sites, to account for the characteristic appearance of the septal-line sinusoids and laminae, which are much more flexible, we adopted the view that "flexible structures such as sinusoids and liver plates can only be straight or plane if they are under tension; if no great tensile force acts upon them, they are crooked"; and that this tension is the result of traction exerted from without and is not caused by active contracton of the laminae, for they are thinner than normal (Elias et al., 1954). The growth and expansion of the lobules, according to those same authors, are the source of external tension. The finding of the septal-line structures in the septum-initiating plane supports us in drawing a conclusion: Where tension exists, fibrous skeleton develops, and vice versa.

On the apical end of the lobule, the septal-line pattern tended to divide the lobular field of the CHL into two (or more) unicenter lobules and on the basal end tended to connect the central vein stem with the portal tract. These configurations led to the formation of the splitting septum and the mesentery-like septum, respectively. The nature of the latter escaped the observation of Elias and coworkers (1954), who concluded that "hepatic veins that run in the septums are sublobular and collecting veins," and that "the septums in the pig's liver do not connect portal with central fields." The formation of the mesentery-like septum suggests that in the normal liver the stem segment of the central vein is prone to be linked to the portal area; this "P-C bridging" did not occur in a random direction but was usually oriented on the side with less intervening parenchyma intervening. The presence of the mesenteroid septum was also encountered by Stöhr (1951, his Fig. 373) and Bergmann (1967, his Fig. 198).

The existence of septum-initiating sites suggests that there is a heterogeneity among like structures, since only some portal tracts reacted in the separation, and the others did not. As for the central veins, only particular sides of the veins were programmed to become involved in the process. Therefore, transverse profiles of the central veins are by no means homogeneous whether considered structurally or functionally. In terms of structure, the adventitia was thicker on the sides predisposed to face the septal-line pattern; in terms of function, those sides became occluded and no longer retain their activity as the "central" vein. Figure 22b shows clearly that the "pericentral" sinusoids on the side close to the septal-line pattern exhibit the reticulum characteristic of the peripheral pattern, and the corresponding half of the central vein permits no sinusoidal opening.

The Limiting Structures

It is generally known that the porcine hepatic stroma is highly developed, inasmuch as lobules are clearly marked off by interlobular connective-tissue septa. On the other hand, that the parenchyma is equally well organized, displaying at least two types of peripheral limiting structures, namely the circumferential laminae and the circumferential sinusoids, delineating each unit, has been poorly described. The limiting structures of the porcine hepatic lobules were first described by Rumjanzev (1927) and confirmed by Kutsuna (1930). Redin (1929, cited by Pfuhl, 1932) observed their presence in both domestic and wild pigs. This knowledge, however, was not familiar to many investigators; presentation of these limiting structures is obviously lacking in the drawings of the pig hepatic lobule in some textbooks (Oppel, 1900, Fig. 558; Szymanowicz, 1924, Fig. 209; Hill, 1937, Fig. 165; Freeman and Bracegirdle, 1967, Fig. 42; Johnson, 1981, Fig. 6.106).

The numerous fibrous strands, which arise from the septum, penetrate through the circumferential laminae, and disperse in the sinusoids, certainly help cement the interface between the lobule and its investment, although they are not analogous to Sharpey's fibers of the periosteum. This anchorage is susceptible to routine histological procedures, however, inasmuch as manmade separations appear in the preparations demonstrated by many authors (Petersen, 1935, Fig. 627; Bremer and Weatherford, 1944, Fig. 330; Jordan, 1947, Figs. 383, 388; Kendall, 1947, Fig. 167; Stöhr, 1951, Fig. 373; Freeman and Bracegirdle, 1967, Fig. 42; Herrath, 1972, Fig. 346; Clara et al., 1974, Fig. 124; Hisauchi, 1976, Fig. 9; Jones and Schmucker, 1977, Fig. 3; Jones and Spring-Mills, 1984, Fig. 20-7, 1988, Fig. 8; Geneser, 1985, Fig. 12-56; Hammersen, 1985, Fig. 331; Geneser, 1986, Fig. 18-61; Jones, 1990, Fig. 1-9 A; Motta, 1990, Plate 8.13A). We suspect that such artifacts have impeded accurate observation at the lobules' margin, a situation that can account for the conspicuous lack of descriptions of the limiting structures.

In the pig liver, Johnson (1919) recognized the "border cells" prior to and after the emergence of separation. In adult pig, Rumjanzev (1927) described the "Randbälkchen" or "Grenzbälkchen" or "Grenzschicht" as a dark-stained, thin layer of liver cells that delineates the lobular perimeter, bordering on the interlob-

ular septa, portal spaces, and sublobular veins. Sometimes the structure is recognized as a sheath surrounding the central vein.⁹

Elias and coworkers (1954), in an attempt to establish that the "normal" liver of the pig is "an abnormal mammalian liver," i.e., representing "subclinical cirrhosis," stated that "in the adult pig, the limiting plate is absent at most places. The limiting plate is present in new-born pigs but is destroyed at a later date; the age at which this occurs is not yet known. . . . Occasionally long sheets of liver cells are seen at the periphery of a septum. These could be mistaken for limiting plates." Our observations are at variance with these. We found that the circumferential laminae occurred as a regular structure. They appeared at the perimeter of each lobular field, running as a thin layer of single cell thickness, whether cut in transverse, longitudinal, or oblique directions. Therefore, they actually formed a continuous sheet covering the entire lobule in three dimensions, presumably (nearly) equal to the positive expression in the 3D images of the parenchyma as displayed in Figure 7c,e.

The other type of limiting structures, the circumferential sinusoids, lay alongside the laminae and were more or less well defined. Their axis of orientation distinguished them from the radial sinusoids, and the thicker reticulum denoted their morphological difference. The injection preparation shown by Rumjanzev (1927, his Fig. 9) clearly indicated this orientation. In some textbook illustrations, although the circumferential laminae are depicted the circumferential sinusoids are absent (Howden, 1926, Fig. 1174; Bargmann, 1967, Fig. 198; Clara et al., 1974, Fig. 124; Preuss and Fricke, 1979, Figs. 1, 2; MacSween et al., 1987, Fig. 1.3; Bucher and Wartenberg, 1989, Fig. 410).

Although we agree with Elias and associates (1954) that the two layers of hepatocytes that flank the septum are hypotrophic, we do not believe that there are "paraseptal sinusoids" in immediate contact with the septum. According to those authors, the "marginal sinusoids" develop into the "paraseptal sinusoids." In view of such transformation, there is a theoretical gap in this explanation because the former are bounded on both sides by the hepatic laminae, whereas the latter, as they described, are the sinusoids interposed between the septum and the lobule's margin.

In our preparations there was no apparent space of fissure on each side of the septum. Electron microscopy of the interlobular septum of adult pig liver reveals that the adjoining hepatocytes rest directly on the septal material (Flaks, 1971). However, the "peripheral sinusoids" in the nonsuine livers of Elias (1949) are, in this sense, equivalent to the part of circumferential sinusoids that are confined to the portal space. Bearing in mind the knowledge of the limiting structures, one may review Figure 17 of Elias (1949) and appreciate the close similarities between the peripheral architecture of the suine and nonsuine liver lobules.

From the morphological point of view, the circumferential sinusoids serve as a distributor of incoming

blood that is delivered through the inlet venules but is shared by a group of radial sinusoids; Figure 8c and d, for instance, are self-explanatory. That this effusion spreads concentrically from the venules and is distributed three dimensionally has been demonstrated in an osmium-ruthenium red injection study (unpublished data).

The Central-Sublobular Adventitia

In the CHLs, the adventitial layer of the central vein emits fibrous offshoots to unite with the developing septum growing in from the portal tracts; its fibrous coat is thickened and its outgrowths are sent off in directions corresponding with the ongoing septation. These findings suggest that the fibrous investment around the central veins of the porcine hepatic lobules plays an active role in differentiating the lobule types.

The central adventitia show a definite pattern of investment. The observations that the centralized sinusoids, sinusoidal openings, and central venous tributaries tend to retain their connections with the central vein with a predilection to the incipient end and that the stem segment of the central vein, on approaching the base, communicates with fewer sinusoids suggest that the vein is polarized. The central-sublobular vein is highly differentiated in terms of function. The extralobular segment does not receive the flow from individual sinusoids; thus it deserves the designation of "sublobular vein" in its proper sense as implied by Kiernan (1833), who studied the livers mainly of man and sheep.

In rat (Gershbein and Elias, 1954; Elias and Popper, 1955; Hase and Brim, 1966), mouse, and other species, but not in man (Elias and Popper, 1955), the functional differentiation of the hepatic veins is poorly defined, for even the very large hepatic veins still receive sinusoidal drainage directly. Those large hepatic veins are not strictly specialized for solely conducting the outflow but behave simultaneously as a functional "central" vein.

The adventitia is thicker near the lobule's base and even thicker in the sublobular veins (and hepatic veins). The narrowing of the basal end of the central vein can be further narrowed in appearance as the vein forces its way through the densely invested sublobular vein. The isthmic segment is comparable to the "bottleneck" nature of the central vein orifice that opens into the sublobular vein as observed (reconstructed) in the human liver (Elias and Popper, 1955).

There were occasions when the argyrophilic investment of the central vein was continuous with the reticulum in the centralized sinusoids and radial sinusoids, with a very gradual transition. These findings support the previous observation that the myofibroblasts of the central adventitia are found to extend deep into the sinusoidal walls, so that it is difficult to draw a line separating the territory of the myofibroblasts from that of the perisinusoidal stellate cells. This has been interpreted as evidence of the uninterrupted transformation of stellate cells into myofibroblasts (Wake, 1988).

In the development of central veins it was found that the circularly oriented fibrils of the centralized sinusoids gradually disappeared and came to be arranged lengthwise along the venous stream. A conduit without

⁹It appears that the "Sammelvene" as demonstrated in Figure 4 of Rumjanzev (1927) is a central vein, probably the stem segment, because there are some sinusoidal openings and the adventitia is rather thick.

circular reinforcement is less resistant to any action exerted transversely. This anatomic disposition might be regarded as allowing for the alterations in luminal spaces, e.g., the ampullary enlargement or the isthmic segment in the central vein and the local narrowing in the sublobular vein.

As far as the argyrophilic structures are concerned, considering the lobular circulatory pathway, the degree of development of the central-sublobular (hepatic) adventitia may be regarded as a determinant of the length of individual lobules, thereby establishing the base. This view is based on the assumption that, the thicker the layer is, the less frequent are the sinusoidal openings in the vein's wall. The sinusoid-associated porosity in the central-sublobular-hepatic vessels indicates their function as a "central" vein, draining the sinusoidal blood that has percolated the intralobular zones.

Concerning the lobule's length, Müller (1843, cited by Oppel, 1906) reported that the separable lobules in the polar bear liver are 1/16-inch wide and 3/8-inch long (approximately 1.6 mm and 9.5 mm, respectively). Pfuhl (1921) recorded the measurements in a 15-month-old pig's lobules, the largest CHL being 4.8 mm × 3.8 mm × 2.3 mm. We have measured many startlingly elongated lobules in a larger series of liver sections prepared from a pig aged 6 months, some reaching 5.6 mm in length (unpublished data).

In rat, the sinusoids enter into almost all branches of the hepatic vein (Gershbein and Elias, 1954; Elias, 1955; Elias and Popper, 1955; Hase and Brim, 1966). The fact may reflect a boundless or ill-defined unit of the hepatic lobulation in terms of lobule length. It has been proposed that "the lobules of the rat liver can be pictured as long cylindrical masses of liver tissue which branch along the tributaries of the hepatic vein" (Hase and Brim, 1966). This view is further supported by the concept of the "hepatic domain" in the rat liver, which is characterized by the centrilobular distribution of hepatocyte-specific enzymes, based on the computer-aided reconstruction of the distribution pattern in three dimensions (Lamers et al., 1989). The concept denotes a spatial continuity of the functional pericentral compartment along the entire course of the hepatic vein.

It is conventionally taught that the portal and the hepatic vasculatures interdigitate and are so arranged that at all points they are separated by a layer of parenchyma (for classical descriptions, the reader is referred to, e.g., Hyrtl, 1878; Rex, 1888; Kelly, 1905; Mall, 1906; Johnson, 1919; Elias, 1949). Since the hepatic lobules are actively developing (growing, reorganizing, proliferating, and expanding), it cannot be assumed that any one spatial arrangement maintains its configuration for any length of time. The findings that the adventitia continue into the interlobular septa are of significance, because they provide the possibility for the two venous systems to approach each other. This fact is evidenced in Figures 2, 3a,b, 26s, 27c,d, and 30a,b. Figure 5 of Lamers and associates (1989) probably includes a similar phenomenon and, therefore, should not be mistaken as an argument for the "periacinar zone 3" with starlike processes that point towards the portal trigone as suggested by those authors.

The "Translobular Artery" and Central Venous Vasa Vasorum

The lobular architecture of CHLs facilitates an understanding of the translobular courses of the arteriolar branches that terminate as the vasa vasorum of the central (sublobular) vein. Cohnheim and Litten (1876) explained that the arterial blood is essential to the hepatic veins. Braus (1924, his Fig. 166) demonstrated that the vasa vasorum of the central vein in human hepatic lobules originate from the hepatic artery, the so-called translobular artery. Three-dimensional demonstration by Vierling (Braus, 1924) indicated the approach of an unaccompanied vessel, probably an arteriole, to the central adventitia through the septum at the base; its presence escaped the notice of some authors (see Elwyn and Strong, 1932, Fig. 321; Smith et al., 1936, Fig. 328; Finerty and Cowdry, 1960, Fig. 282).

Andrews and Maegraith (1953) photographed the translobular arteriole in their study of vascular casts and recorded that "the majority of the arterial vessels run into the base of small sublobular veins." Tajiri (1960) related that the walls of the sublobular and collecting veins are supplied by the arterial branches of the internal thoracic and phrenic arteries, which descend along the hepatic veins and anastomose with the interlobular arteries. In the present study, we observed a capillary plexus of arterial origin pervading the wall of the sublobular veins. The supplying vessels passed in the interlobular septa and reached the veins. In the lobules, the central adventitia was approached from the lobule's base by small arterioles, which, while ascending the venous wall, supplied its fibrous coat. The latter sent out fibers that contributed to the formation of the mesenteroid septum and the splitting septum, which, in turn, facilitated the intercommunication between adventitial arterioles and septal arterioles.

As regards the connection between the arterioles and the sinusoids, we have seen, in the sublobular veins, adventitial arterioles that open into adjacent sinusoids (see Fig. 30c; compare with Fig. 22d). We have not found such direct communication in the central veins, however; only indirect communications, via the interstices among fibrous bundles in the adventitial layer, were observed (see Fig. 26k,l).

If the lobules are not clearly demarcated by septa as in nonsuine livers, an unaccompanied arteriole (or arterial capillary) on its course towards the adventitia could possibly be perceived as an arteriole that traverses the lobular field to the midzonal-centrilobular zone or to the immediate vicinity of the central (sublobular, hepatic) vein. That the venous adventitia is nourished by arterial supply has been observed previously and was reconfirmed in the present study. This anatomic relationship may be partly reflected in cirrhotic livers in which the major blood supply of the organ is converted from portal venous circulation to hepatic arterial perfusion and in which the central veins are found characteristically embedded in the vigorously proliferative collagenized tissue. The physiologic significance of the translobular arterial vessels in the hemodynamic regulation has been suggested experimentally (Andrews and Maegraith, 1953).

The Periphery-to-Center Passage of Arterioles

The question of the arteriolar "shortcut" to the deeper interior of the hepatic lobules has been investigated intensively since the last century but has not been elucidated convincingly relative to lobular architecture. The stereogram based on the many studies conducted by Hans Elias in 1949 (cited by Spellberg, 1954) was an epochal achievement in the morphological study of the liver; its broadening and educational influence was so great that the illustration found its way into many textbooks, even to this day (see, e.g., Fawcett, 1986, Fig. 27-4; Sherlock, 1989, Fig. 1.9; Williams et al., 1989, Fig. 8.159). However, as is still debated (see Matsumoto et al., 1979), it remains obscure whether or not the arterial capillary exists emptying into intralobular sinusoids (and the intralobular cholangiole, which is missing in those modified diagrams as in Williams et al., 1989).

It has been explained that the arterial capillaries penetrate deeply into the parenchyma and, after courses of various lengths, enter into sinusoids at all levels (Elias and Sherrick, 1969). The existence of the intralobular arterioles was supported mainly by Chrzonszczewsky (1866), Braus (1924), Wakim and Mann (1942), Elias (1949), Senevirante (1950), Andrews and Maegraith (1953), Elias and Petty (1953), Riedel and Moravec (1959), Tajiri (1960), and Kardon and Kessel (1980), but many observers did not see or could not confirm their existence (Kiernan, 1833; Mall, 1906; Gilbert and Villaret, 1909; Pfuhl, 1922; Loeffler, 1927, cited by Olds and Stafford, 1930; Olds and Stafford, 1930; Hase and Brim, 1966; Lozano and Andrews, 1966; Mitra, 1966; Block, 1970; Matsumoto et al., 1979; Grisham and Nopanitaya, 1981).

The septum-initiating plane provides the peripheral structures with increased territories that facilitate the access to the intralobular structures. The nature of the intralobular arterioles (and the translobular portal branch reported by Hase and Brim, 1966; Lozano and Andrews, 1966) is easily understood if the lobular architecture is described in connection with the septum-initiating plane. The subtype of the multiexit lobule with the eccentrically located center of drainage (Fig. 33) could be another possibility that allows the peripheral structures to arrive at the central zone by a shortened pathway.

The basal end of most lobules contained the central vein that was not positioned in the center but tended to approach closer to one side than the others. In other words, the periphery-to-center distance was decreasing on one side while the central vein was being directed toward the base; only when a lobule is located on a very large sublobular vein (Fig. 27a) did equidistance to all sides at the base seem to be maintained.

All these situations presumably can result in a passageway shorter than usual that probably can be perceived as indistinguishable from that of the "translobular artery" in nonsuine livers.

Lobule Types and Heterogeneity of the Liver

The heterogenous patterns in the distribution of morphological structures have been well known in hepatic tissue. In addition to the characterization by the generally accepted "zonality" and the recently estab-

lished "regionality," we have observed a relationship to the "locality" of the liver units.

In terms of zonality, the hepatic lobules exhibit nonhomogenous distribution patterns of metabolic functions. The hepatocytes of different zones display certain structural variations (Reith et al., 1968). Likewise, nonhomogenous structural differentiation has been observed in the sinusoidal endothelial cells (Wake et al., 1988) and in the perisinusoidal stellate cells (Wake, 1988).

In the celloidin-embedded liver of the pig, we found that there was a zonality in the extracellular constituents. The argyrophilic reticulum was recognized only sparsely in the central half but was visualized more strongly in the peripheral half of the lobular sinusoids. In the CHLs, the areas of the septal-line pattern were characterized as peripheral, despite the being central in position.

As concerns regionality, it has been meticulously demonstrated that different regions (septal or portal) of the same zone are not identical functionally (Teutsch, 1988). This regionality is ascribed to microangioarchitectural differences with respect to the primary lobule concept of Matsumoto and colleagues (1979).

In the present study, we noted that not all corners of the lobules were occupied by portal veins (portal occupancy rate less than one), that the periportal sinusoids of adjoining lobules were not equally reticulated, that the sinusoidal beds in proximity to the portal tract were not invariably tortuous,¹⁰ and that the basal end of the central vein communicated less readily with the sinusoids compared with the incipient end. These findings suggest that morphological heterogeneity exists when the lobules are considered in terms of regions, or sites, or levels. As regards the portal occupancy of the interlobular spaces (Table 1), apparently it is questionable to state that a hepatic lobule is simply an aggregation of "primary lobules" (Matsumoto et al., 1979); some territories of the lobular parenchyma doubtless would remain outside this; they could not be defined by the interlobular portal vein.

Considering the locality in the liver lobe, the hepatic lobules are by no means homogenous. Based on the topographic location of the central vein in each lobule, Kiernan (1833) differentiated two subpopulations, the "superficial lobules" and the "inner lobules." The term *superficial lobules* denoted those lobules lying in contact with the capsula fibrosa Glissoni and its elongation into large portal canals. Mall (1906) was aware of the subperitoneal lobules and observed their perpendicular disposition. These observations were confirmed by Johnson (1918a, 1919), who referred to the "surface lobules" and the "deeper lobules," and noted that the process of lobular cleavage in the surface lobules is somehow characteristic. Elwyn and Strong (1932) described the uniform apicobasal arrangement of the surface lobules, as contrasted with the variable orientation of the lobules located elsewhere. Hase and Brim (1966) related that the margin of the liver lobe, which has been studied heavily by the transillumination

¹⁰Compare with the plexiform sinusoids that characterize the angle of the primary lobule with the "high potential pool."

TABLE 4. Anatomic and the functional heterogeneity of liver tissue as evaluated in different frames of reference

Unit of liver tissue	Frame of reference	Anatomic heterogeneity	Functional heterogeneity and its morphologic demonstration
Lobule	Zonality	Present	Present and well-established
	Regionality	Present	Present and demonstrable ¹
	Locality	Present	Data unavailable
Lobe	Laterality	Present	Present in perinatal subjects, ² and presumably demonstrable

¹Teutsch (1988).

²Zink (1981).

technique, should not be generalized for its structural peculiarities.

The present study made possible some measurements and quantitations of the lobules grouped by locality, i.e., different locations. The histologic lobules at the liver edge were small on the average. The surface lobules differed from the deeper lobules in the proportion of the constituent lobule types; the surface lobules were predominantly CHLs, and the deeper lobules were preferentially SHLs (Table 3). The interlobular spaces of the surface lobules were supplied less frequently by the portal branches (Table 1), suggesting that the near-surface locations were not as well vascularized by the portal veins as were the deeper locations. We observed also that locations around the large portal canals had high portal occupancy rates.

While anatomic and functional heterogeneity can be demonstrated morphologically with reference to the zonality and the regionality of the hepatic lobules, whether the presented anatomic heterogeneity with respect to locality would reflect any functional significance remains to be investigated (see Table 4). There has been a report suggesting that arterial supply differs between the hilus and the periphery of hepatic lobes (Conway et al., 1985).

In addition to the zonality, regionality, and locality, there is a "laterality" (Table 4). The study conducted by Zink (1981) has suggested a fair possibility of a macroscopic scale of functional heterogeneity in the fetal liver, based on the asymmetrical vascular supply; the umbilical venous blood, which is enriched in oxygen, nutrients, and maternal hormones, preferentially bathes the hepatocytes in the left lobe of the organ.

CONCLUSIONS

There is little doubt that the architectural patterns of porcine hepatic parenchymal units are distinguished as simple hepatic lobules (SHL) and compound hepatic lobules (CHL). In subjects aged 6 months, the CHLs were greater in number, although in a single preparation the SHL-like lobules appeared to compose the majority; among the latter, the pentagonal forms were predominant over the hexagonal profiles. Observations in three dimensions on the organization of the argyrophilic connective-tissue skeleton led to the recognition of the "septum-initiating plane" in the lobular field of the CHL. The septum-initiating plane provided the lobule with a common pathway for peripheral structures to attain access to intralobular or central structures at various depths with respect to the radial length and at

various levels with respect to lobular length. The lobule base of Kiernan (1833) provided another pathway for arterioles that invaded basoapically along the adventitia and came to anastomose with septal arterioles. Our study confirms the findings of Johnson (1919) on the splitting septum and extends the understanding of fibrous septal formation and of de novo development of portal twigs, biliary ductules, and central veins. We were able to demonstrate that zonal heterogeneity exists in extracellular matrix, and structural heterogeneity is recognized at least in terms of zonality, regionality, and locality. It is strongly suggested that one transverse profile of the "hexagonal" hepatic lobule cannot be regarded as an adequate and sufficient representation of the liver units. Whether the profile represents a lobule's apex or its base and whether the profile represents an SHL or a CHL means a difference in the three-dimensional structure, and the positioning of the profile in question needs to be correctly specified with respect to lobular architecture. It seems to us that many controversies about the hepatic lobules in the past have stemmed from the limitations of the available basic knowledge and the ignorance of lobule types and that they may be readily elucidated by renewed investigation on the normal structures.

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