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GASTROENTEROLOGY

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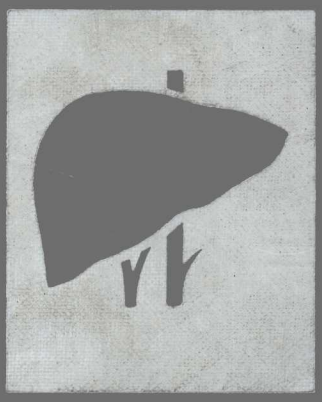

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GASTROENTEROLOGY

Fourth Edition

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Liver

SECTION 13. THE LIVER

Chapter 145

Structure of the Liver

Fenton Schaffner • Hans Popper

Historical Background

The ancients recognized that the liver received and delivered blood via the portal and hepatic veins and secreted bile via the common bile duct. They also coined the term "parenchyma." The teachings of Galen about the liver and the belief that it was the site of blood formation stood until the 17th century.

The concept of the liver lobule as a unit of hepatic structure related to function is credited to Malpighi. The orientation of this lobule in modern terms was proposed by Kiernan, who suggested that the lobule was supplied by portal blood from the periphery and drained by hepatic vein tributaries in its center. Mall disagreed with this concept and proposed, on the basis of bile drainage, that the portal tracts were functionally central structures. The 2 men who must be credited with the development of the modern concepts of hepatic structure are Elias and Rappaport. The former indicated, on the basis of 3-dimensional reconstructions, that the liver cells were arranged in plates 1 cell thick. The latter provided proof based on histochemical as well as cinematographic studies that the portal tracts are indeed central and that the basis of structure should be pie- or pear-shaped acini with portal tracts as bases and "central" veins as apices. The bile canaliculi were shown to be connected with the large bile ducts by Gerlach, while the connecting piece, now known as the bile ductule, was described by Hering and further clarified by Eppinger.

These fundamental contributions, as well as subsequent ones made to the description and naming of the structure of the liver by early students of the structure of the body (Da Vinci, Vesalius, Glisson, Muller, Krukenberg, Disse, Purkinje, Henle, and Schwann) and more modern pioneer ultrastructural descriptions by Palade, Novikoff, Wachstein, Fawcett, Berhard, and Rouillier, are noted without special references purely for their historical interest.

Present trends are in the areas of identification of specific portions of membranes by histochemical or immunocytochemical techniques utilizing the transmission and scanning electron microscopes. The combination of such morphologic studies with biochemical ones on the same models provides basic structural-functional correlations. Many of these studies use the hepatocyte or the Kupffer cell as a convenient model in which to examine a general biologic phenomenon. Others are concerned with the reaction of the livers of animals to biophysical and biochemical alterations that have no relation to human physiology or pathology. Still other studies are directed toward human problems, either in experimental animals or in diseases in man that serve as experiments of nature. The literature in this field has burgeoned so greatly that only a small sampling of the most recent contributions can be offered with the hope that general principles are covered. Greater detail can be found in modern texts of anatomy and histology as well as in the newer atlases of ultrastructure of the liver.[1–3]

Topography of the Liver

The liver, the largest unpaired organ in the body, is pyramidal in shape with its apex to the left and base to the right. It occupies all of the right upper quadrant of the abdomen above the costal margin, except for a small portion of the epigastrium that is not protected by the bony cage. It extends 5 to 10 cm to the left of the midline. In adult males, the liver weighs about 1500 g, and in females 1300 g. The newborn liver weighs less than 80 g.

The shape of the liver varies somewhat with the habitus of the individual. The dome extends to the fourth interspace or fifth rib in the right midclavicular line. Flatness on percussion begins at the sixth rib or sixth interspace laterally as well as anteriorly. The size (anterior surface area) of the liver can be estimated by percussion, scanning, ultrasound, or x-ray examination. Each technique has its drawbacks and advantages, but percussion is simplest and is always available.

The human liver is divided into right and left lobes of almost equal size by a left longitudinal groove on the inferior surface of the organ. This groove contains the ligamentum teres and the remnant of the ductus venosus. The right longitudinal groove contains part of the gallbladder bed anteriorly and the groove in which the vena cava lies posteriorly. The 2 longitudinal grooves are connected in the middle by a transverse groove that contains the porta hepatis or hepatic hilum. A small quadrate lobe, not clearly demarcated, is below the hilum, and a small caudate lobe is cephalad to the hilum on the inferior surface. The right kidney and hepatic flexure of the colon make impressions on the inferior surface. The anterior surface is smooth and the junction of the inferior and anterior surfaces is the sharp edge normally palpated. A sagittal ligament, the falciform ligament, traverses the anterior surface of the left lobe from the diaphragm to the free edge. From here it continues as the round ligament to the umbilicus. The falciform ligament does not demarcate the right and left lobes, but it does contain the paraumbilical veins.

The superior surface facing the diaphragm is connected to the diaphragm by a transverse peritoneal duplication, the coronary ligaments, with lateral extensions in the right and left triangular ligaments. In addition, a fibrous appendix fixes the edge of the left lobe to the diaphragm. The peritoneal duplication does not entirely cover the dome of the right lobe, and this area is in direct contact with the right diaphragm. The liver has a great deal of plasticity and rib grooves may indent its anterior lateral surface. This is particularly true if any deformity of the chest is present.

The surfaces of the liver can be divided into segments: the right lobe into posterior, anterior superior, and inferior areas; the left lobe into medial, lateral superior, and inferior areas. In general, these segments are similar to vascular segments supplied by hepatic artery and portal vein branches.[4]

The Hepatocyte

Although the liver is the largest single organ in the body, is irregular in shape, and has a complex set of fluid fluxes, many of its functions can be best understood in terms of a single cell, the hepatocyte. The hepatocyte, in effect almost an entire liver in miniature, is a 12-sided figure (dodecahedron), about 25 μ in average diameter. Structural modifications from the basic or primitive animal cell prototype are few, despite the complex and sophisticated functional capability of the hepatocyte.

Arrangement

The human liver is a continuum of hepatocytes from one end to the other, interrupted periodically by vessels and ducts (Fig. 145–1). Hepatocytes are arranged in cords in a few lower animals, but such a configuration is not found in the adult human liver. The arrangement of the cells in man is rather like that of a rubber sponge with the spaces representing sinusoids. About four-fifths of the space of the liver is occupied by the hepatocytes and about 15% by sinusoids in the rat and dog[5] as well as in man.[6] About 60% of the total number of cells are hepatocytes, one-third are sinusoidal lining cells, and bile duct cells, connective tissue cells, and blood vessel cells each contribute about 2% to the normal liver.[6] In adult man, the hepatocytes are in plates (i.e., the rubber of the sponge), and these plates are 1 cell thick. The plates are 2 cells thick in the fetus and up to about the fifth year of life. The platelike arrangement ensures maximal exposure to the sinusoids, since at least 2 surfaces of every cell face the space of Disse.

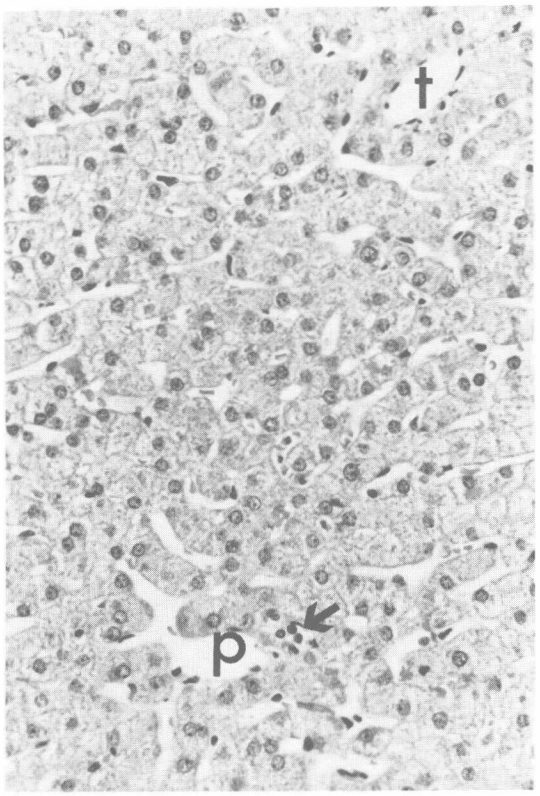

Figure 145–1. Low power light microscopic appearance of the normal liver. Note the similar appearance of all the hepatocytes. The parenchyma between the terminal hepatic venule (t) and the portal tract (p) containing a small duct *(arrow)* measure the radius of a lobule. (Hematoxylin and eosin; × 100.)

The plates of hepatocytes appear to radiate outward from the terminal hepatic or central vein (Fig. 145–2). Indeed, this pattern was responsible for designating the smallest tributaries of the hepatic veins as "central." However, embryologic evidence and histochemical and flow studies indicate that these veins are on the periphery of structurally ill-defined masses of hepatocytes called the hepatic acinus of Rappaport.[7] The veins, hence, are best called terminal hepatic venules. The true centers of these masses are portal tracts (Fig. 145–3), and cell division during growth or regeneration occurs mainly around portal tracts. A single row of hepatocytes, on cross-section called the limiting plate, envelops the portal tract. The hepatocytes in the limiting plate are more basophilic and are particularly vulnerable to injury, especially in chronic liver diseases and in long-standing cholestasis. The cells of the lobular periphery or in acinar zone 1, the apex of the hepatic acinus, store iron; this is especially the case in conditions associated with iron overload. The cells around the central vein or in zone 3 around the terminal hepatic venule contain the most lipofuscin pigment and are most vulnerable to anoxia, cholestasis, and many toxic injuries. They are also subjected to greater venous pressure when passive congestion is present and can virtually be squeezed out of existence in this circumstance.

The mass of hepatocytes grouped around the terminal hepatic venule and delimited by lines drawn between the portal tracts is called the lobule. The pear-shaped mass of cells between one portal tract and one terminal hepatic venule is called an acinus. Both the acinus and the lobule can be divided into zones that, while they do not coincide in the 2 systems, can be looked upon as periportal, intermediate, and central, or zones 1, 2, and 3. The functional implications of these divisions relate to enzymatic and other activities, respectively.[8–10] Glucose release, oxidative metabolism, urea formation, and bilirubin and bile acid excretion are mainly periportal, while glucose uptake, fat synthesis, and biotransformation are mainly central. These in turn relate to the oxygen saturation of the tissues, or at least of the blood perfusing the tissues in these zones. The highest oxygen saturation is in the area next to the small portal tract, and the lowest is in the area around the terminal hepatic venule. The distribution is not perfectly symmetrical and, hence, anoxic or toxic necrosis is more strictly paracentral rather than central. Sinusoidal volume is greater in zone 3, but the surface/volume ratio is greatest in zone 1, suggesting that the transport of water and solutes into the hepatocytes is greater in the periportal area.[11] These observations support the concept of the acinus, rather than the lobule, as the structural unit of the liver. The larger portal tracts are exceptions in that adjacent hepatocytes may not be well saturated with oxygen or well perfused with blood, in as much as these must come from the smaller portal tracts.

Light Microscopy

With conventional light microscopy, the hepatocyte appears with routine stains in sections of paraffin-embedded tissue as a simple cell with a centrally placed nucleus (Fig. 145–1). The appearance of the cyto-

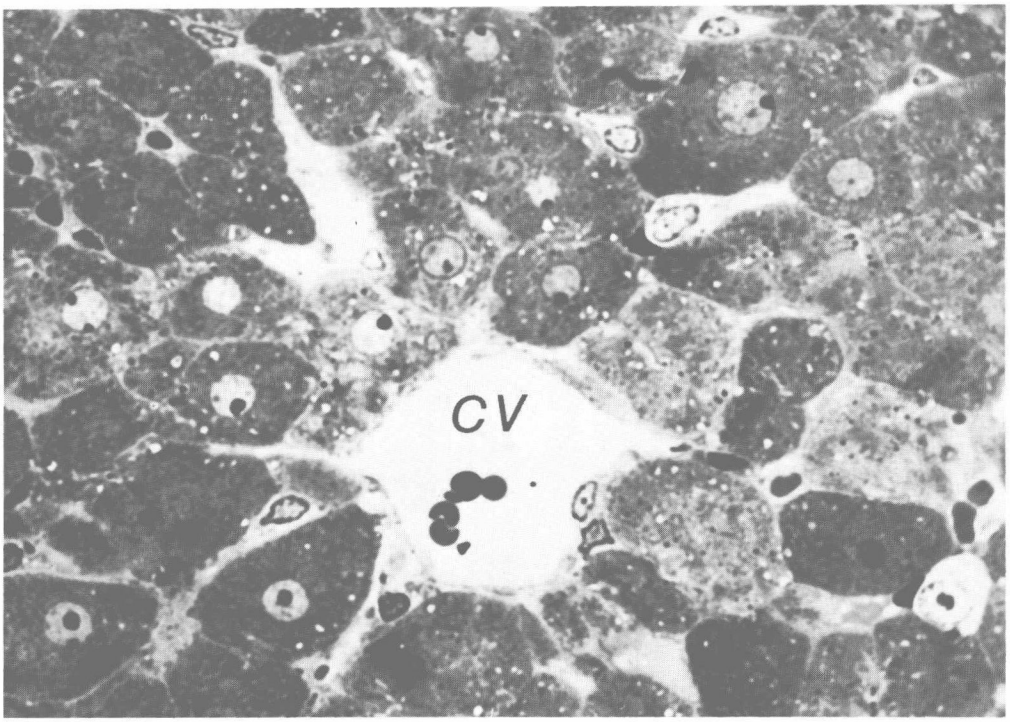

Figure 145–2. Centrilobular zone (zone 3) with terminal hepatic vein (CV) in center. The narrow inlets from the sinusoids are seen on both sides of the vein lumen. (Toluidine blue–PAS of epoxy embedded; × 630.)

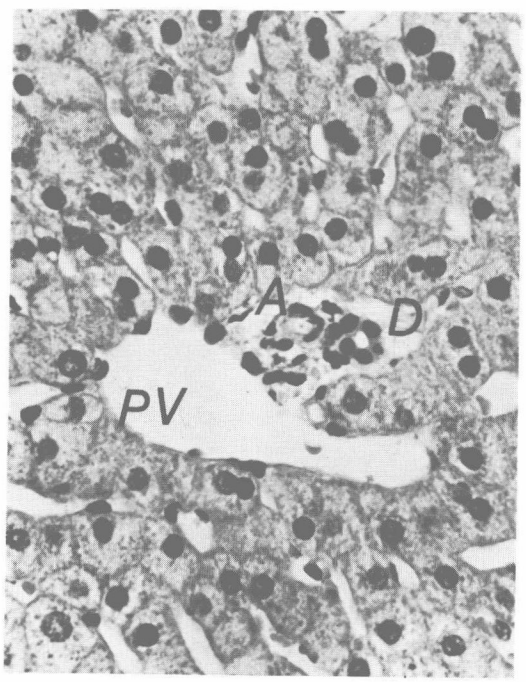

Figure 145–3. A small portal tract showing branches of the portal vein (PV), the hepatic artery (A), and a small bile duct (D). (Hematoxylin and eosin; × 450.)

plasm of the normal hepatocyte varies mainly according to how much water, glycogen, or fat is present. The cytoplasm in the fasting state is dense and homogeneous with a bluish cast with hematoxylin-eosin stains. Glycogen-rich cytoplasm is pale or almost colorless, but the cell borders are dense and distinct. All the cells in any section, especially in the same zone (see later), look alike. The glycogen content of the different zones of the lobule undergoes a diurnal rhythm, with more glycogen periportally during the day. Hydropic cells (the microscopic counterpart of cloudy swelling) also have pale cytoplasm, the extreme being the balloon or the clear cell. Cell borders of hydropic cells are often indistinct. If these cells are numerous and not in small clusters, adjacent cells vary greatly throughout the lobule or zone.

Fat is not seen in conventional sections because it is dissolved in the paraffin embedding medium (Chapter 158). However, the site of the fat droplet remains as a sharply delimited hole. Fat droplets are usually found along the sinusoidal border when the droplets are small. As fat accumulates, the droplets become more numerous and then larger. Sometimes the cell is crowded with small fat droplets and has a central nucleus and distinct cell borders (plant cells). Finally, the droplets may coalesce to form one large drop that occupies the center of the cell and displaces the nucleus to one side. Scattered fat droplets are normal, but fat in many cells is termed steatosis or fatty liver. During fat accumulation in the liver the fat occurs in islands of cells, usually in a given zone. Some conditions, such as obesity, diabetes mellitus, and alcoholism, cause predominantly centrilobular steatosis; others, such as protein-calorie imbalance in animals, are associated with periportal steatosis. Occasionally, fat droplets in adjacent cells in very fatty livers appear to coalesce to form fat cysts. These cysts may persist long after the rest of the fat has been mobilized and serve as an indication of previous steatosis.

The bluish cast of the hepatocytes is caused by hematoxylin (basophilic) staining of cytoplasmic nucleoprotein. Specific staining with such dyes as pyronine, before and after ribonuclease digestion, can be used to prove the ribonucleic acid content of the bluish areas. This nucleoprotein is decreased during starvation and hepatocellular injury; under these circumstances, hepatocytes lose their basophilia and appear eosinophilic. The basic proteins may also increase.

Light cells and dark cells may occasionally be present in the same lobule. This is probably a fixation artifact that may indicate some abnormality in cell function. It may also reflect differences in cell water. Cells on the periphery of the biopsy cylinder are smaller because of squeezing during this procedure. More obvious fixation artifacts are clumping of cells and islands of cells stained very lightly or too much by eosin. Much variation in appearance from cell to cell means unrest and indicates organelle pathology.

Pigment. Cells in the perivenular zone may have a brown cast caused by pigment granules (lipofuscin). The pigment has fluorescent properties and appears golden brown when examined under ultraviolet light. The amount of pigment increases with age and during chronic debilitating illness, but is never as much as the amount of similar-appearing pigment found in the Dubin-Johnson syndrome (Chapter 149). The cells surrounding the terminal hepatic vein display the most pigment, and the amount progressively decreases with each row of cells away from the vein.

Pigmentation may also occur in abnormal states. Thus, bilirubin may impart a yellow color to the central zone especially during cholestasis (Chapter 148). This cytoplasmic coloration, which varies in intensity from one cell to the next, is imparted by granular deposits that appear softer than lipofuscin. The cytoplasm of the Kupffer cells and plugs or thrombi in bile canaliculi may also be bile-stained. Peripheral or periportal bile-staining of hepatocellular cytoplasm occurs in long-standing cholestasis and in primary biliary cirrhosis. The only other consistent periportal pigmentation is iron, which occurs in the form of rust-brown granules of hemosiderin. Hemosiderin is concentrated mostly in the cells of the limiting plate of hepatocytes surrounding the portal tract. The normal liver does not react to iron stains, such as Prussian blue or Turnbull's blue. In persons with a high iron intake or who have defective erythropoiesis, however, stainable iron may be demonstrated in the periportal hepatocytes (Chapter 166). When iron overload is excessive, hemosiderin accumulations may be found in macrophages in the portal tracts and in ductular cells. Iron also accumulates in the Kupffer cells when there is hemolysis

or when red cell survival is greatly shortened. Prussian blue staining correlates well with the hemosiderin content of hepatocytes rather than with total hepatic iron;[12] it does not correlate well when the iron excess is in the Kupffer cells.

Still other pigmented substances may be found in the liver. These are mainly exogenous in origin and accumulate principally in macrophages in the sinusoids, in areas of necrosis, and in the portal tracts. Among these pigments are colloidal thorium dioxide, residues of digestion of blood by malaria parasites, and partially oxidized and polymerized fatty acids after infusion of IV fat emulsion.

Nucleus. The nucleus of the hepatocyte is spherical, centrally placed, and about 8 to 10 μ in diameter. It is similar in appearance to that of any eukaryotic cell. In health, the nuclei are uniform so that variation in size and staining quality from one cell to the next (nuclear unrest) is a sign of hepatocellular injury. Mitosis, normally uncommon in the adult liver, also develops soon after injury (Fig. 145–4). Binucleated hepatocytes are common normally.

Chromatin is most dense (i.e., basophilic in hematoxylin and eosin–stained sections) around the periphery of the nucleus, and this condensation is exaggerated after death. The margination of the chromatin is most striking in nuclei that contain a large central collection of glycogen. Such glycogen "degeneration" of nuclei is mainly periportal. The glycogen is a monoparticulate form instead of rosettes as it is in hepatocellular cytoplasm. Glycogen nuclei are most common in diabetes mellitus, although they are also seen in older persons, particularly in those with passive congestion. They are also seen in children, especially around puberty. The nucleus contains 1 to 4 deeply basophilic nucleoli. Inclusions of bits of cytoplasm can occasionally be seen in scattered nuclei. The significance of such inclusions is unknown but it probably indicates irritability.

Nuclear size varies with ploidy.[13] The fetal and neonatal cells are diploid, as are newly regenerated cells. Adult hepatocytes are tetraploid. After injury, binucleated (each diploid) cells are common in younger individuals, while polyploidy (8, 16, and 32 ploid) is common in older persons. Between the ages of 30 and 60 years, regeneration is characterized by an almost equal mixture of polyploid and binucleated cells. Multinucleated hepatocytes are not normally seen and probably result from cellular fusion.

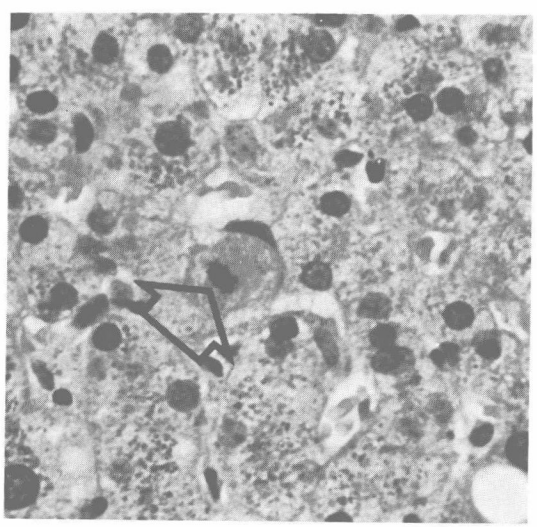

Figure 145–4. Hepatocellular nucleus in metaphase of mitotic cycle *(arrow)*. Note the relatively round outline and uniform size of the normal nuclei and the rounded and ground glass appearance of the mitotic cell. (Hematoxylin and eosin; × 450.)

Ultrastructure

The development of histochemistry, cytochemistry, and electron microscopy with electron histochemistry and immunocytochemistry has made it possible to visualize precisely the sites where most specific hepatic functions occur. The transmission electron microscope is but an extension of the light microscope with streams of electrons instead of rays of light and electromagnetic fields instead of glass lenses. Specimens must be rapidly fixed, often with substances that increase contrast. Glutaraldehyde and osmium tetroxide, alone or in combination, are the most widely used fixatives. Heavy metal staining of sections made with diamond "knives" after plastic embedding further adds to contrast. With the use of these techniques, the structure of the normal hepatocyte has been carefully scrutinized during the past 3 decades. Indeed, the volume and surface areas of cell components have been quantitated by morphometric or stereologic analysis.[14] Electron immunocytochemistry permits even specific proteins to be identified. Scanning electron microscopy allows the various surfaces of hepatocytes and sinusoidal cells, as well as the relationships

between the different cells, to be studied in detail.[3, 15, 16]

Hepatocellular Surfaces

Sinusoidal Surface and Uptake. At least 2 of the 12 surfaces face sinusoids. These surfaces differ from the others in that numerous microvilli extend from them in a haphazard fashion (Figs. 145–5 and 145–6). On cross-section, these 2 surfaces represent about 40% of the perimeter. The microvilli vary from 0.1 to over 1.0 μ in length. They are often more like ameboid extensions than microvilli and may be branched or fused with their neighbors. Sometimes the cell surface is in the form of a bleb or blister, which may represent an edematous microvillus or several microns of surface. Pieces of the cell surface, from 0.1 to more than 1.0 μ in diameter, may be shed into the perisinusoidal spaces where they are engulfed by macrophages. Shedding is common in liver cell injury from any cause and has also been seen in normal liver.[17] It has been considered a means of adaption to unfavorable circumstances[18] or an artifact.[17]

Material moves into the hepatocytes across the sinusoidal membrane in several ways. These include Na^+,K^+-ATPase–dependent active transport, carrier-mediated transport, specific receptor–dependent endocytosis and non-specific pinocytosis. At the same time, secretion out of the hepatocyte is occurring, at least in part, by the same processes in the reverse direction as uptake. Evans[19] described the sinusoidal plasma membrane as a receptor-rich, metabolically dynamic conglomerate that handles heavy traffic. Separation of sinusoidal plasma membrane from that of lateral surfaces and bile canaliculi, although not complete, has permitted biochemical study of the composition, structure, and function of this important cellular domain. For instance, transport ATPase is in the sinusoidal membrane[20] and its structure and organization within the membrane has been studied.[21] The turnover rate of some of the plasma membrane proteins is much longer than that for cellular proteins in general,[22] although variations are great under different circumstances. The membrane proteins are necessary for maintaining the surface conformation; treatment of isolated cells bearing numerous microvilli with papain results in the loss of all microvilli and a smooth surface.[23] The membrane proteins have carbohydrate extensions from the external surface. This makes up the carbohydrate coat, which contains glycosaminoglycans and several sugars like mannose, galactose, fucose, *N*-acetyl glucosamine, *N*-acetyl galactosa-

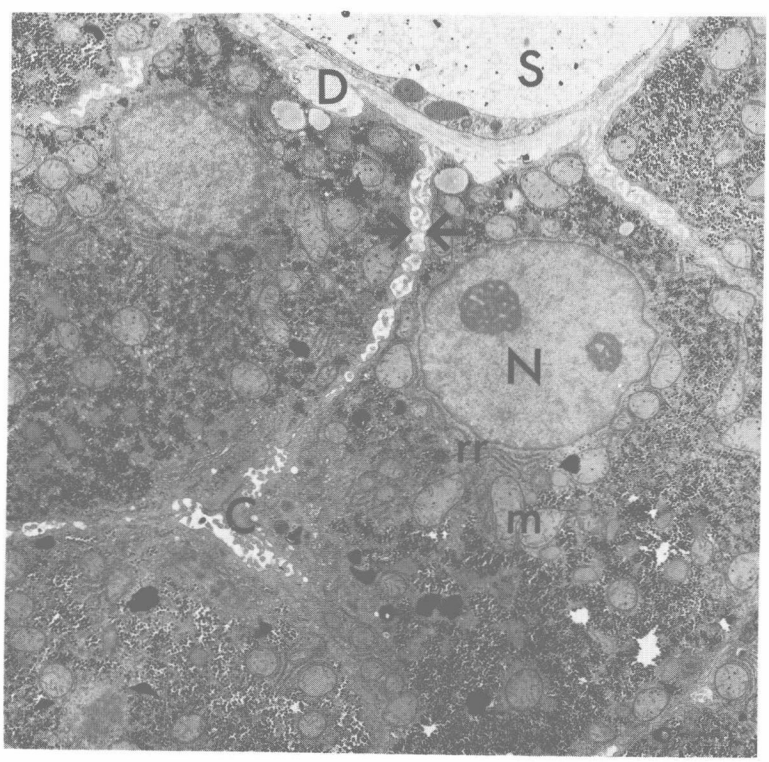

Figure 145–5. Low power electron micrograph of hepatocyte showing nucleus (N), numerous small black glycogen granules, rough endoplasmic reticulum (rr), a group of mitochondria (m), and a bile canaliculus (C), with nearby black lysosomes. The sinusoid (S) is separated from the hepatocyte by a thin layer of the sinusoidal lining cell, which forms the upper border of the space of Disse (D). Note the irregular microvilli extending from the hepatocyte into the space and into recesses between cells extending toward the canaliculus *(arrows)*. (Glutaraldehyde-osmium fixed, lead-uranyl stained; × 6000.)

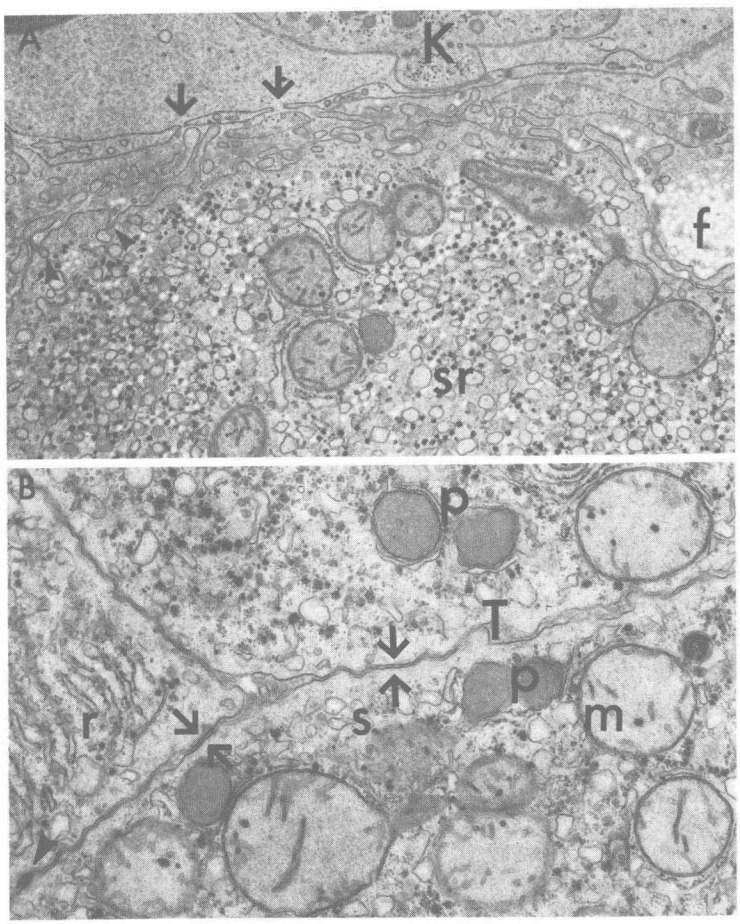

Figure 145–6. *A,* Sinusoid containing Kupffer cell (K) lined by thin endothelial cell layer with fenestrations *(arrows).* The space between the hepatocyte below and the sinusoidal lining cell is the space of Disse. This space contains many irregular microvilli projecting from the hepatocyte, pits *(arrowheads)* extending into the hepatocyte, small bundles of collagen fibrils (f) constituting the stroma, and duplications of lining cells in places. Note the absence of a continuous basement membrane. The cytoplasm of the hepatocyte contains many vesicles of smooth endoplasmic reticulum (sr). (Glutaraldehyde-osmium fixed, lead-uranyl stained; × 8000.) *B,* Cell borders of 3 cells. Some areas *(between arrows)* are straight and represent gap junctions. The arrowhead on the left indicates a desmosome and (T) a tongue and groove. Mitochondria (m), peroxisomes (p), rough (r), and smooth (s) endoplasmic reticulum can be seen. (Glutaraldehyde-osmium fixed, lead-uranyl stained; × 30,000.)

mine and sialic acid.[24] At the base of microvilli, small pits or indentations of the plasma membrane can be seen (Fig. 145–6*A*), although less frequently than in other cells. Some pits have a fuzzy or clathrin coating that contains carbohydrate receptors for specific proteins like insulin,[25] alpha-2-macroglobulin,[26] epidermal growth factor, dimeric immuloglobulin A and apolipoprotein B.[27] The pits are internalized to form small vesicles about 100 nm in diameter. This process is called endocytosis and the vesicles are receptosomes that may still contain the receptor material.[25, 27] With some ligands (e.g., IgA and possibly bile acids), vesicles are shuttled to bile canaliculi directly, with or without intravesicular metabolism; with others, like asialoglycoproteins, they move to the complex composed of the Golgi zone, endoplasmic reticulum, and lysosomes (GERL)[27–29] where the ligand is metabolized. Asialotransferrin is returned to the blood via vesicles called diacytosomes after partial resialylation.[30] The fate of the receptors varies; some are recycled.

Other ligands act on cellular metabolism. The membrane components, including the receptors, are controlled by cytoplasmic functions[31] and some of the components, like fucose, rapidly move from the blood into the Golgi zone of the cell from where it is delivered to the sinusoidal plasma membranes—all within 30 minutes.[32] The manner of internalization of receptor-hormone complexes may differ for various hormones.[33] Mathematical models of interactions between the receptors and the various ligands that may bind to them have been proposed.[34] These reflect the complexity of the uptake process. While the uptake of most protein hormones occurs by receptor-mediated absorptive endocytosis,[24] lipids, organic anions, and possibily bile salts depend on binding in serum to albumin.[35] The sinusoidal membrane has receptors for this protein, but the albumin is apparently not internalized when the ligand

is, and the process is accomplished by some form of molecular transport. Some hormones, such as insulin, seem to exert their effect by inducing formation during or just after transport across the plasma membrane of a mediator. The latter, in turn, releases secondary effectors that influence various kinase and phosphatase activities in the cytoplasm.[36]

When the hepatocytes are isolated, the specialized lateral and canalicular portions are replaced by a surface bearing microvilli, and the entire membrane appears to be converted into a sinusoidal-like membrane[37, 38]

Secretory products of the cell, especially lipoproteins, can also be visualized by radioautography after administration of labeled precursors. Under conditions of anoxia and other forms of cellular injury, large pinocytotic vesicles are seen forming on the sinusoidal surface.[39, 40] These may be more than 1 μ in diameter. Several of these larger vesicles can fuse in the cytoplasm to form large hydropic vacuoles containing fibrin or polysaccharide recognizable by electron microscopy as diastase-resistant, PAS-positive droplets. The small vesicles and the vacuoles eventually become lysosomes or become incorporated into pre-existing ones. Acid phosphatase activity of the vacuoles, characteristic of the latter organelles (see later), may appear as early as the stage of formation on the sinusoidal surface or may not become manifest until incorporation into already formed lysosomes occurs. The sinusoidal surface of the hepatocyte has considerable activity of alkaline phosphatase and 5′-nucleotidase in some species. Glycoproteins and proteoglycans are in the space of Disse over the sinusoidal surface of the hepatocyte. The special glycoprotein, fibronectin, binds to ganglioside receptors on hepatocytic surfaces and may anchor the collagen stroma and keep adjacent cells together.[24, 42] Laminin is found around ductular cells and regenerating or altered hepatocytes.

Lateral Surfaces. The appearance of the lateral surfaces of hepatocytes changes as the distance from the sinusoidal surface increases. The perisinusoidal space extends between neighboring hepatocytes for distances of less than 1.0 to more than 5.0 μ with the majority falling within this range (Fig. 145–5). The surface of the hepatocytes bordering these extensions has microvilli that are shorter and more sparse than on the sinusoidal surface itself. The space is wider with more microvilli in cirrhosis and after estrogen treatment.[41] Where these recesses end, the cell surfaces become closely apposed with a clear space between them of about 22 nm in width. The surfaces are straight, although between occasional cells a tongue of one surface fits into a groove in its neighbor (Fig. 145–6*B*). This space between cells can be penetrated by marker substances containing lanthanum or horseradish peroxidase. The functional significance of this narrow cleft containing stainable polysaccharide is probably related to the paracellular pathway of water secretion into bile (see later). The remainder of the lateral surface is occupied by the junctional complex and the gap junctions, which deserve separate descriptions.

Tight Junction (Junctional Complex). Hepatocytes are attached to one another by modifications of their lateral borders or attachment zones, collectively known as the tight junction or junctional complex. This area of membrane is similar but not identical in all epithelial cells. It consists of a desmosome or macula adherens, an intermediate junction or zona adherens, and a tight junction or zona occludens (Fig. 145–7). The desmosome is a dense, short, thick strip of cell membrane about 0.33 μ in length. At high magnification, it consists of a series of parallel plates. Nearby, and often attached to the desmosome, is a mitochondrion. Also arising from the desmosome and fanning out into the cytoplasm are microfilaments, part of the cytoskeleton of the hepatocyte (see later). These are not as numerous as in some ductular cells (see later). The zona adherens contains an additional fine membrane between the individual cell membranes. The latter are fused and the space between the cells is obliterated in the tight junctions. Lanthanum salts or horseradish peroxidase will not stain the area of the junctional complex. With scanning electron microscopy of specimens prepared by freeze-fracture, the tight junction appears as a continuous belt along both sides of the canaliculus.[44] The belt is composed of 2 to 6 or more nearly parallel strands that have a few discontinuities and some connections between the strands. Discontinuities have not been seen in normal human livers.[45] The tighter a junction is between epithelial cells, the more numerous the parallel strands; the leakier the junction, the fewer the strands, to the point that only

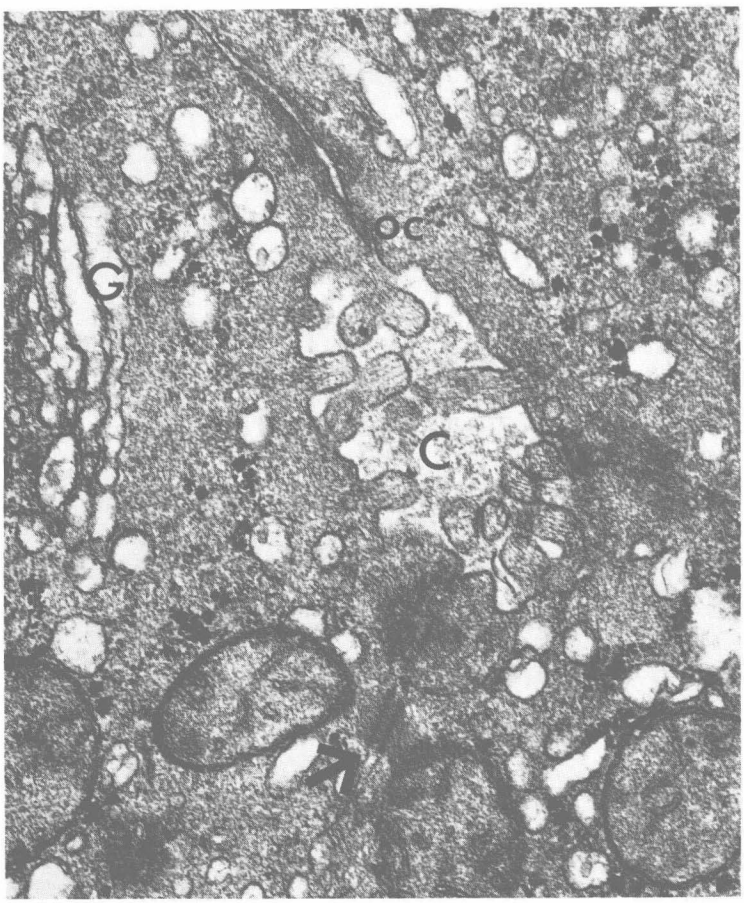

Figure 145–7. Portion of 3 neighboring hepatocytes with a bile canaliculus (C). Note the cell borders and the tight junction, which seals the canaliculus. The dense area is the zona occludens (oc); the lighter portion of the border above is the zona obscurens; and the dense area in the complex below is the desmosome *(arrow)*. A mitochondrion in each cell is near the desmosome and seems attached to it by a dense bundle of fibrils. The black particles are glycogen rosettes. Note the microvilli also containing fibrils almost filling the canaliculus and the Golgi zone (G) nearby in each cell. The Golgi apparatus in the cell shows severe parallel lamellae and large vesicles budding off their ends. Several small vesicles are in the area. The lamellae characteristically curve away from the canaliculus. (Osmium fixed, lead stained; × 24,000.)

one may be present. The junction may actually be a tortous channel through which material moves like a microbe "crawling through a pile of spaghetti."[43] The extent of sealing or leaking is determined by the resistance to flow resulting from the tortuosity itself. Other factors, such as ionic charge, the presence of calcium ions, and the pressure of bile inside the canaliculus, influence the appearance and leakiness of the tight junction. The junction is the gate or sluice of the paracellular pathway of bile formation. In cholestasis, both intrahepatic as well as extrahepatic, flow may be in the opposite direction, directly back to blood[44–46] (Chapter 148). The appearance and number of strands of the tight junction normally, as well as under abnormal circumstances, vary from one cell to another and even in the same belt of fibrils. This suggests that flow through the paracellular pathway is not uniform.

Gap Junction and Intercellular Channels. Specialized zones of the lateral border, near the junctional complex where the adjacent cell membranes are straight, are called nexuses or gap junctions[47] (Fig. 145–6*B*). Each junction is a hexagonal lattice about 9 nm from center to center of each hexagon. The gap junctions occupy about 1% of the cell surface in the normal human liver,[48] and are thought to be the pathways by which cells communicate with each other. The gap junction area is greatly reduced during regeneration, and each hepatocyte, which usually has gap junctions with 6 other hepatocytes, has gap junctions with only 1 other cell after hepatectomy.[49] Cholestasis reduces gap junctions about 15% in man.[48] The gap junction is composed of protein with a molecular weight of 28,000 daltons, separated into 2 different polypeptides.[50] Each hexagon of the nexus is a morphologic entity called the connexon and each has a hydrophilic channel along its central axis, 1 to 2 nm in diameter and 4 to 5 nm in length.[51] Channels in other tissues and in other species are about the same size and permit passage of molecules of 250 to 1600 daltons in weight but not

larger.[52] The channel is stable in an open position, but is closed by increases in calcium ion concentration in the hepatocytic cytoplasm. Gap junctions can be internalized and degraded by autophagic sequestration.[53]

Bile Canaliculus. The tight junctions effectively seal a cylindrical space about 1 μ in diameter between 2 or 3 neighboring hepatocytes. On cross-section, the space appears round and is called the bile canaliculus (Figs. 145–5, 145–7, and 145–8). The canaliculus occupies about 6% of the perimeter on section. The arrangement of these spaces in the lobule, the canalicular network, is described later. Each hepatocyte extends fairly uniform microvilli into the space to nearly fill it. Canalicular maturation occurs late in fetal life, but at birth the junctional complex is complete.[54, 55] Microvilli are sparse in the newborn, reflecting the functional immaturity of bile secretion. These microvilli are shorter and more regular than those of the sinusoidal surface. Periportal canaliculi have a diameter about 15% greater than the perivenous or central ones.[56] Enhancing bile secretion by infusion of bile salts widens the perivenous canaliculi, suggesting that intermediate cells are being recruited for bile secretion. The Golgi apparatus and pericanalicular vesicles also increase under these circumstances.[57] Protracted choleretic infusion alters canalicular structure and composition.[58] Diverticula of the canaliculus extend into many hepatocytes.[59] Edematous microvilli or bleb formations are uncommon in the normal liver, but occur in hepatocellular injury, particularly if associated with cholestasis.

The luminal surface has a thin carbohydrate covering (demonstrated by electron microscopy as a fuzzy coat) in livers fixed by perfusion of the portal vein with glutaraldehyde. Nucleotide phosphatase activities, especially that of Mg^{++}-ATPase and alkaline phosphatase, on the canalicular membrane are strong. However, great species variations occur. Mg^{++}-ATPase is almost exclusively on the outer or luminal surface of the canalicular membrane, but some activity also surrounds the pericanalicular microfilaments.[60] By con-

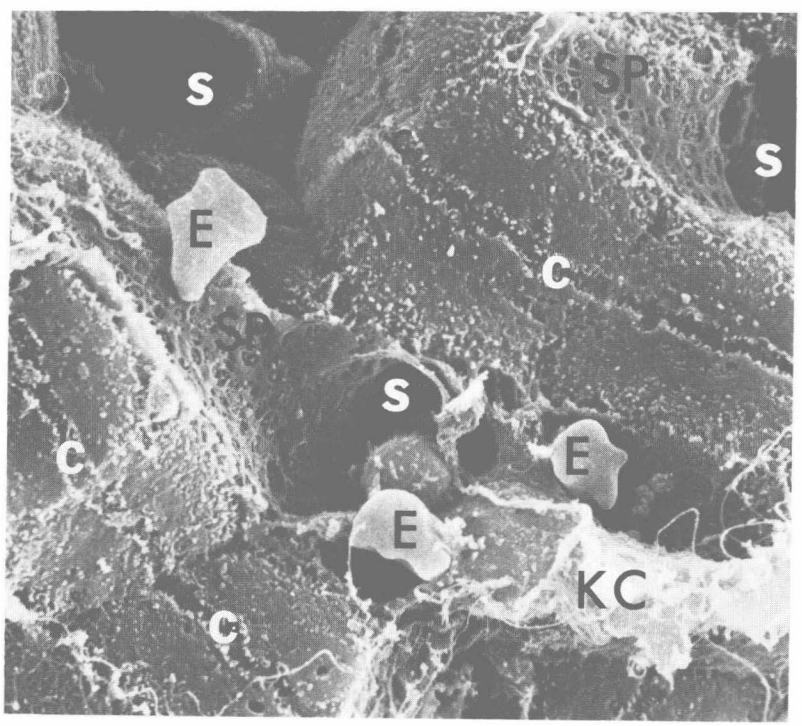

Figure 145–8. Scanning electron micrograph of rat liver showing hemi-canaliculus (c) as grooves in hepatocyte surfaces. The other hemi-canaliculus was in the portion of the specimen fractured off in the freeze-fracture preparation. Note also the thin sinusoidal lining cells with the numerous holes or sieve plate (SP). An ameboid Kupffer cell (KC) is in one sinusoid (s), as are several erythrocytes (E). The canaliculi and the sinusoidal surfaces have numerous short microvilli (× 4000). (Micrograph graciously provided by Prof. Pietro Motta, Chairman, Department of Anatomy, Faculty of Medicine, University of Rome.)