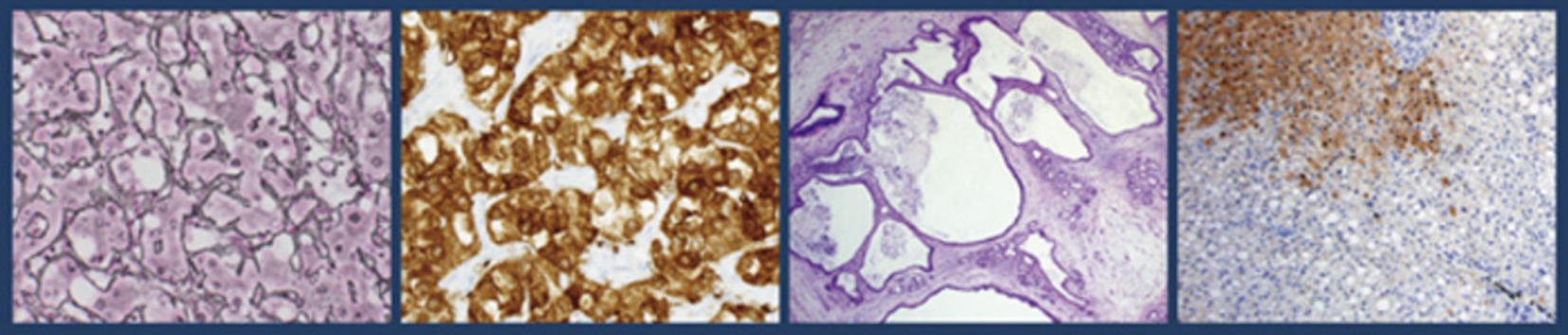
Seventh Edition

MacSween's Pathology of the Liver



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SEVENTH EDITION

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Preface

The three current editors of *MacSween's Pathology of the Liver* wish to take the opportunity to pay tribute to the visionary leadership and inspiration of the main initiator for the very first edition, Roddy MacSween, who sadly died on 11 December 2015 at the age of 80 after a long illness. His legacy to pathology and more broadly in medicine shall never be forgotten. Obituaries to Roddy have been published widely—by *The Herald Scotland*, *The Lancet*, the Royal Philosophical Society of Glasgow, and many others—and our brief words here barely do justice to his immense influence.

Roderick (Roddy) Norman McIver MacSween was born in 1935 on the Isle of Lewis in Scotland. He excelled in secondary education in Skye and Inverness and went on to study at the University of Glasgow, graduating BSc with Honours in Physiology in 1956 and then MBChB three years later.

His postgraduate house officer year was spent in Glasgow's Royal and Western Infirmaries where he subsequently went on to gain experience in clinical medicine and infectious diseases. He joined the University Department of Pathology at the Western Infirmary shortly after that. The then-Professor of Pathology, Dan Cappell, had a vision of sub-specialisation in histopathology, a model that has been widely embraced around the globe. Early in his pathology training, Roddy was stimulated by his postmortem findings of patients with iron overload and chose to develop an interest in liver disease, a field in which he went on to build an international reputation.

After gaining his MRCPath in 1967, he was seconded by Cappell to University of Colorado Medical Center, where he worked closely with clinical gastroenterologists who were developing the new discipline of hepatology. He returned to Glasgow in 1970 as a Wellcome Senior Research Fellow working with Professor John Anderson on clinico-pathological and immunological studies in liver disease. He made outstanding contributions to our understanding of autoimmune liver disorders, in particular the disease that was then known as primary biliary cirrhosis. He was awarded an MD with honours for this work. He progressed through the ranks of Senior Lecturer, Reader, and Titular Professor, and in 1994 succeeded Anderson as the sixth Chair of Pathology at the Western Infirmary and Head of Department, positions he held until retirement in 1999.

He became an outstanding interpreter of liver biopsies with a national and international reputation and a substantial worldwide referral practice. In 1978, he was invited to join a group of distinguished international hepatopathologists that had become established under the aegis of the European Association for the Study of the Liver in 1968. This group was known as the Gnomes, as their first meeting had been held in Zurich (a term coined by the late Dame Shelia Sherlock). Roddy was a very active member of this group, which over the years has made significant contributions to our understanding of the classification and histological assessment of liver disease. He was the original sponsor for two of the current editors (ADB and SGH) for election to the group, and we are both indebted to him for the opportunities that this brought.

He served as President of the Royal College of Pathologists from 1996 to 1999. He was highly regarded by his peers in other medical disciplines, and he served as Chairman of the Academy of Medical Royal Colleges from 1998 to 2000. He retired in 1999 and was knighted in 2000 for services to Medicine and Pathology. He was awarded a DSc from his *alma mater* in 2007, one of several honorary titles bestowed upon him.

Roddy was a wonderful mentor and supervisor to many whose careers have been heavily influenced by him. All three of us feel very fortunate to count ourselves amongst a large number of individuals whom he encouraged and supported in their pursuit of an understanding of liver disease. His enthusiastic professional support was not by any means restricted to those wanting to learn about the liver. Generations of pathologists who had come through the Western either as local trainees or as visitors from elsewhere in the UK or overseas fell under his tutelage.

Readers of this book will of course most likely know him best for establishing it as the key international textbook in its field. In 1979, Roddy published the first edition of *Pathology of the Liver* with Peter Anthony and Peter Scheuer as co-editors. As a young medical student, one of us (ADB) had the privilege of proofreading the entire edition. It rapidly became the standard text for both pathologists and hepatologists worldwide. Its comprehensive nature was reflected in a review that stated, "If it's not in MacSween it's not in the liver". The fifth edition in 2006 was retitled *MacSween's Pathology of the Liver* in recognition of the leadership he had shown in developing it as an enduring concept.

The current edition sees a number of important changes. There is coverage of new technologies, such as next generation sequencing, which are beginning to contribute to the phenotyping and genotyping of hepatic disorders, in particular, hepatic neoplasms. The subjects of inherited and developmental liver disease have been combined into a single chapter, which includes an algorithmic approach to the pathological assessment of paediatric liver disease. New scoring systems and approaches to the assessment of the fatty liver diseases are outlined. Additional outstanding figures have been included in the chapter on non-hepatotropic infections with up-to-date coverage on emerging infections. The chapter on drugs and the liver has been

extensively updated, as has the chapter on vascular disorders. Tumours and tumour-like lesions are more comprehensively covered than ever before and recent developments in clinical management and pathological assessment in the field of hepatic transplantation are reflected in an updated section on this importance topic. As always we have attempted to ensure the use of contemporary terms throughout. In recent years the term "primary biliary cholangitis" has replaced the term "primary biliary cirrhosis". This is a change that we fully support and have therefore adopted the new terminology in this edition.

For each of us, the editing of this book has been a labour of love; we are honoured to be able to continue the fine tradition that Roddy started almost 40 years ago. We are grateful for the ongoing support and encouragement of our respective partners, Alison, Rick, and Dawn, during the preparation of the book. Thanks also to all of the contributors and the team at Elsevier for keeping us on track.

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Contents

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Structure, Function, and Responses to Injury

1

James M. Crawford, Paulette Bioulac-Sage, and Prodromos Hytiroglou

CHAPTER CONTENTS

Keywords

Inflammation
Regeneration
Fibrosis
Vascular Remodeling
Regression
Cirrhosis

The practice of hepatopathology requires a clear understanding of liver anatomy and physiology, as a prelude to understanding the expression of pathological processes in the liver. As an anatomical entity, the liver is deceptively simple. It is large, representing about 2% of the total body mass of an adult human and occupying most of the right upper quadrant of the abdomen. It has a roughly triangular profile, with incomplete clefts helping to define the different 'lobes' of the liver. It has only one point of vascular inflow, the *porta hepatis*. Blood exits through several venous orifices into the inferior vena cava, which traverses a deep groove in the dorsum of the liver. There are no 'moving parts' of the liver, with the exception of daily secretion of several litres of bile into the common hepatic duct, which exits from the porta hepatis.

Belying its macroscopic simplicity, the liver is home to biosynthetic and biodegradative metabolic pathways of unequaled complexity, generating enough metabolic heat to be a prime source of core homeostatic temperature maintenance. This chapter considers the embryology, macroanatomy and microanatomy of the liver and its basic response to injury, concluding with the appearance of 'normal' liver in biopsy and autopsy specimens.

Development of the liver

General features

In human embryos the liver first appears at the end of the third week of development. The liver bud, or *hepatic diverticulum*, arises as a hollow midline outgrowth of endodermal tissue from the ventral wall of the future duodenum. The connective tissue framework of the liver into which the endodermal bud grows is of mesenchymal origin and develops from two sources: (1) the *septum transversum*, a transverse sheet of mesenchymal cells that incompletely separates the pericardial and peritoneal cavities and is the primordium for both the diaphragm and the liver, and (2) cells derived from the mesenchymal lining of the associated coelomic cavity, which actively invade the septum transversum. The confluence of endodermal cells from the hepatic diverticulum growing into the mesenchymal primordium creates the solid organ destined to become the liver (Fig. 1.1).

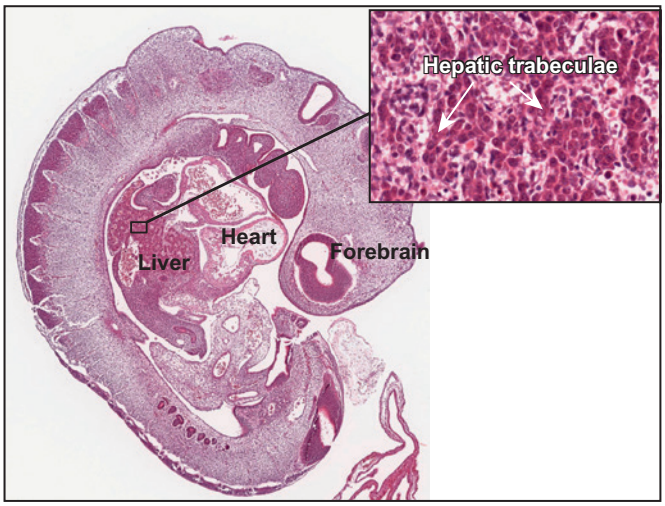

Figure 1.1 Photomicrograph of hepatic primordium in human embryo at 25 days' gestation. The lower-power view shows the organs of the coelomic cavity. The higher-power *inset* shows cords of endodermal cells within the mesenchyme of the septum transversum, thus forming the hepatic primordium.

During the fourth week, buds of epithelial cells within the mesenchymal stroma extend radially out from the hepatic diverticulum. Between the epithelial cords, a plexus of vascular hepatic sinusoids develops. As the epithelial buds grow into the septum transversum, they break up into thick, anastomosing epithelial sheets that meet and enmesh vessels of the hepatic sinusoidal plexus, forming the primitive hepatic sinusoids (Fig. 1.2 A). The intimate relation between hepatocytes and sinusoidal capillaries, so characteristic of the adult organ, is therefore already anticipated in the 4-week-old embryo. The hepatic diverticulum remains as a tether between the developing liver primordium and the duodenum, ultimately becoming the extrahepatic biliary tree.

Once established, the liver grows rapidly during the fetal period, to become the largest single visceral organ (by mass) for the remainder of gestation. It bulges into the peritoneal cavity on each side of the midline, as right and left lobes, which are initially symmetrical. It also grows ventrally and caudally into the mesenchyme of the anterior abdominal wall, extending down to the umbilical ring. Associated with these changes, the stomach and duodenum, which were initially in broad contact with the septum transversum, draw away from it, thus producing a midsagittal sheet of mesoderm, the ventral mesogastrium or future lesser omentum. As the duodenum withdraws from the septum transversum, the stalk of the original hepatic diverticulum is also drawn out to form, within the lesser omentum, the epithelial elements of the extrahepatic bile ducts. Simultaneously, the cephalad aspect of the liver becomes partly freed from its originally broad contact with the septum transversum by extensions of the peritoneal cavity and its visceral and parietal mesothelial surfaces so that, in the adult, direct contact of the liver with the diaphragm persists only as the 'bare area' of the liver. This is bounded by the attachments of peritoneal reflections, which form the coronary and falciform ligaments.

Vascular arrangements

By the fifth week of development (in embryos of 5 mm), the liver parenchyma consists of anastomosing sheets of liver cells, each sheet being several cells in thickness. Coursing between the liver cells is the vascular 'sinusoidal plexus'. Initially, the afferent hepatic blood supply is through the symmetrically arranged vitelline veins returning from the abdominal region of the embryo. Blood is also received from the laterally placed right and left umbilical veins, which run in the body wall and carry oxygenated blood from the placenta directly to the paired horns of the sinus venosus of the cardiac primordium. Both the vitelline and the umbilical sources of blood enter into the hepatic sinusoidal plexus through a developing branching vascular network. Blood draining from the sinusoidal plexus passes through symmetrical right and left hepatocardiac channels, to enter the sinus venosus through this same network[1] (Fig. 1.2 B). This network, along with the mesenchyme through which it passes, constitutes the early portal tract system within the liver parenchyma.

Once these vascular connections are made (Fig. 1.2 C), the circulatory pattern within the liver changes rapidly. The left umbilical vein becomes the principal source of blood entering the liver, partly because it comes to carry all the blood returning from the placenta when the right umbilical vein withers and disappears (generating the 'double-artery/single-vein' umbilical cord of the term fetus), and partly because the initial volume of blood returning from the gut in the vitelline veins is small. The definitive vascular pattern of the fetal liver is already established by the seventh week in embryos, about 17 mm long (Fig. 1.2 D).[2] The originally paired vitelline veins have given way to a single portal vein that, on entering the liver, divides into right and left branches. Blood in the left umbilical vein

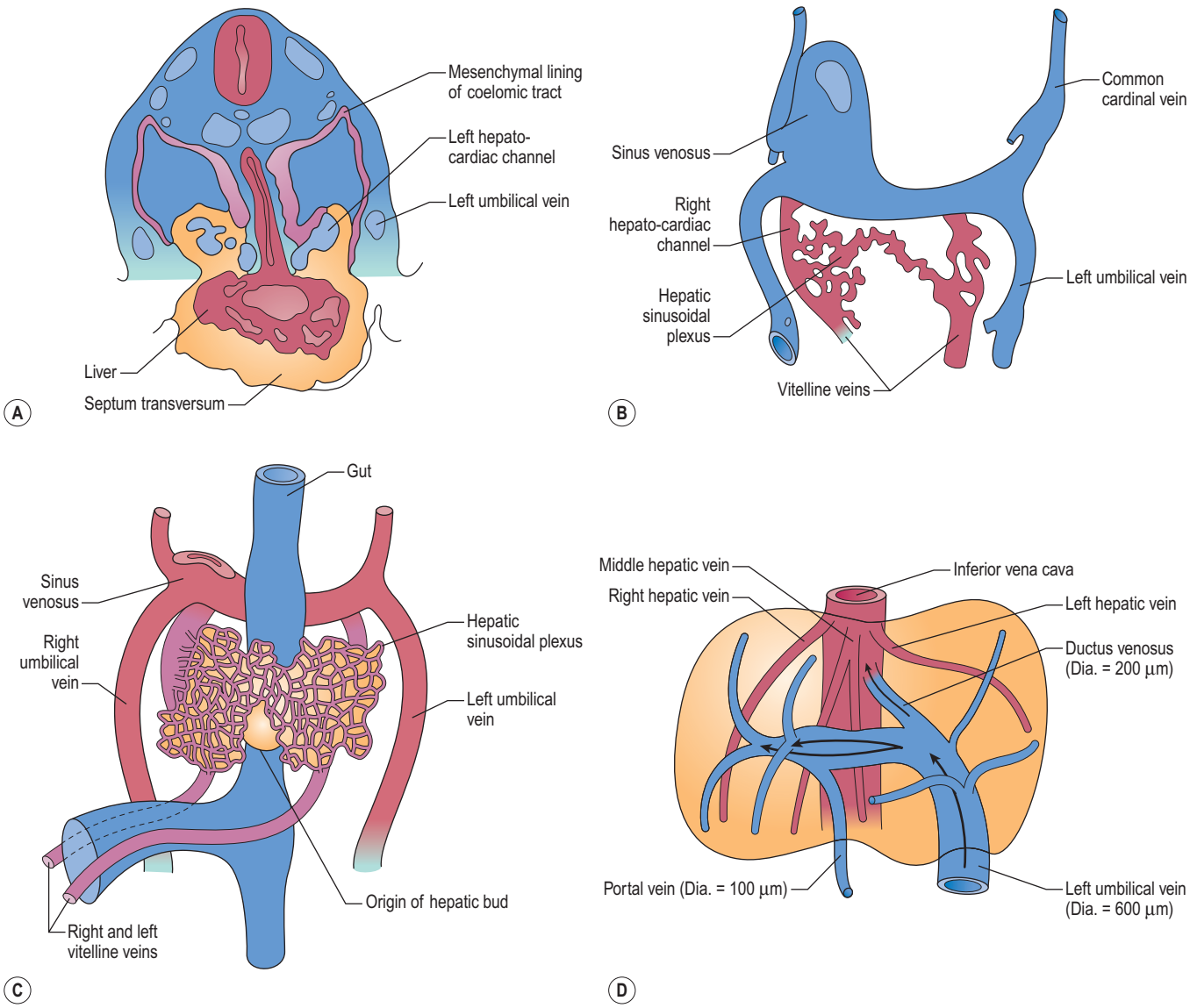

Figure 1.2 **A,** Section through region of the hepatic bud in a human embryo of 25 somites (~26 days). **B,** Vascular channels associated with the developing liver, in a human embryo of 30 somites. **C,** Vascular channels in the human liver at a slightly later stage, showing the further extensive development of the hepatic sinusoidal plexus. **D,** Scheme of the portal hepatic circulation, in a human embryo of 17 mm (~7 weeks). *(**A** and **B** redrawn from and **C** based on Streeter GL. Contributions to embryology of the Carnegie Institution of Washington, 1942;30:213–44; **D** redrawn from Lassau JP, Bastian D. Organogenesis of the venous structure of the human liver: a haemodynamic theory. Anat Clin 1983;5:97–102.)*

traverses a venous extension in the falciform ligament and has a choice of three routes: (1) through the liver in branches that enter the sinusoidal plexus of the left half of the liver; (2) through the sinusoidal plexus of the right half of the liver, by retrograde flow through its connection with the left branch of the portal vein; and (3) through the *ductus venosus* traversing the short space between the porta hepatis to the inferior vena cava, to enter directly into the systemic venous circulation. By these routes, the converging bloodstreams from the definitive portal vein and indirectly from the umbilical vein enter the rapidly enlarging hepatic primordium through the porta hepatis. Intrahepatic mesenchyme condenses around the intrahepatic branching portal venous system, making up the ramifying *portal tracts* of the liver (see later discussion).

The hepatic artery is derived from the celiac axis. Arterial sprouts grow into the hepatic primordium from the porta hepatis along the mesenchyme of the portal tract system, spreading to the organ periphery as the fetal liver enlarges. These arterial sprouts appear to be the organizing element for formation of the intrahepatic biliary tree. The hepatic arterial system continues to proliferate and grow after birth, reaching an adult form only at 15 years of age.[3–6] In the adult, about four arteries supply the largest intrahepatic bile ducts.[7] At the level of the terminal portal tracts, there is a uniform 1:1 pairing of hepatic arteries and terminal bile ducts, and approximately two artery/bile duct pairs per single portal vein.[8] The most terminal portions of the portal tree lose their portal veins, leaving only residual hepatic artery/bile duct dyads, which themselves disappear into the parenchyma.[8,9]

To complete this discussion, the rapid changes in hepatic circulation at birth must be considered. A sphincteric mechanism closes the ductus venosus at its proximal end, blood flow ceases in the umbilical vein, and the left side of the liver receives blood that now flows from right to left through the left branch of the portal vein. The closed segment of the umbilical vein between the umbilicus and the liver regresses to form the *ligamentum teres*; the ductus venosus undergoes fibrosis and becomes the *ligamentum venosum*.

Hepatocyte ontogeny

Primitive hepatocytes are derived exclusively from the endodermal outgrowths of the hepatic diverticulum. Hepatocellular synthesis of alpha fetoprotein (AFP) begins at the earliest stage of liver differentiation, about 25–30 days after conception, and continues until birth. Glycogen granules are present in fetal hepatocytes at 8 weeks; the maximal glycogen reserve is achieved at birth, but the rapid onset of glycogenolysis over 2–3 days postpartum depletes the storage to approximately 10% of term levels. Hepatocellular haemosiderin deposits appear early in development, predominantly in periportal hepatocytes, and become more marked as hepatic haematopoiesis decreases (see later). Hepatocellular bile acid synthesis begins at about 5–9 weeks and bile secretion at about 12 weeks.[10] Canalicular transport and hepatic excretory function, however, are still immature at birth and for 4–6 weeks postpartum, and therefore exchange of biliary solutes across the placenta (especially bilirubin) is important in the fetus. Within the sinusoids, endothelial cells, Kupffer cells and hepatic stellate cells appear at 10–12 weeks.[11] When the adult liver is ultimately formed, hepatocytes constitute 80% of the cells in the normal liver. Of the remaining 20%, bile duct epithelial cells (cholangiocytes) comprise 1–3%, sinusoidal endothelial cells 10%, Kupffer cells 4%, and lymphocytes 5%.[12]

Bile duct system

The extrahepatic and intrahepatic biliary system is best understood if the liver is regarded as an exocrine gland. The endodermal cells of the hepatic primordium give rise not only to the epithelial parenchyma—the future hepatocytes—but also to the epithelial lining of the intrahepatic bile duct system. The extrahepatic bile ducts are derived from the caudal portion of the hepatic diverticulum, the portion that does not invade the septum transversum but remains as a stalk connecting the foregut to the developing liver.[13] The caudal part of this tethering diverticulum forms a secondary bud, constituting the epithelial primordium of the cystic duct and gallbladder. The epithelial lining of the extrahepatic bile ducts is continuous at its caudal end with the duodenal epithelium and at the cephalic end with the primitive hepatic sheets.

The intrahepatic ducts develop from the limiting plate of hepatoblasts that surround the mesenchyme of the primordial portal tracts. This has been known since the 1920s[14] and was confirmed using immunohistochemical methods and monoclonal antibodies to (cyto) keratins and cell surface markers.[15,16] Specifically, normal adult hepatocytes express keratins 8 and 18 (K8 and K18), whereas intrahepatic bile ducts, in addition, express K7 and K19. During the first 7–8 weeks of embryonic development, no intrahepatic bile ducts are evident, and the primordial epithelial cells express K8, K18 and K19. At about 9–10 weeks (27–30 mm embryos), primitive hepatocytes (hepatoblasts) surrounding large portal tracts near the liver hilum express these cytokeratins more intensely and form a layer of cells (Fig. 1.3) that ensheaths the mesenchyme of the primitive portal tracts to form the so-called ductal plate. This is followed by development of a second but discontinuous layer of epithelial cells which show a similar phenotypic change, and thus a network of segmentally double-layered bile duct precursor structures is formed.[13,17] This process is recapitulated centrifugally along the length of the portal tract tree, from the hilum outward to the periphery.

Further remodelling of the plate then occurs. Invading connective tissue separates it from the liver parenchyma, and the tubular structures become incorporated into the mesenchyme surrounding the portal vein branches. The patterning of intrahepatic biliary tree development is harmonized with that of hepatic arterial development,[18] suggesting that molecular signals emanating from the ductal plate help drive hepatic arterial growth. These signals include expression by cholangiocyte precursors of hepatocyte nuclear factor 6 (HNF6) or HNF1β growth factors, and vascular endothelial growth factor A (VEGF-A).[19,20] This molecular coordination of bile duct formation and hepatic artery formation[21] explains the pairing of hepatic arteries and terminal bile ducts observed in the adult human liver.[8]

The endodermal liver cells that are not contiguous to portal tracts lose K19 expression, instead maturing into hepatocytes within the parenchymal liver cell plates. Expression of the SRY-related HMG

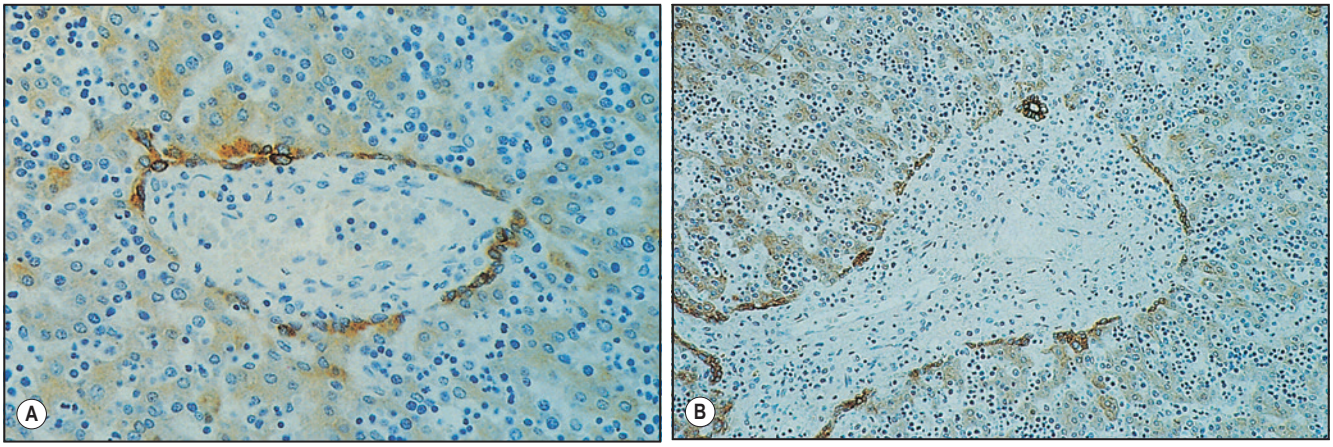

Figure 1.3 Development of the ductal plate and of intrahepatic bile ducts. **A,** Increased expression of keratins in primitive hepatocytes at the interface with the mesenchyme of the primitive portal tracts; human fetus of 12 weeks' gestation. **B,** Later stage showing a discontinuous double-layered plate of epithelial cells at the mesenchymal interface; note the formation of tubular structures *(upper right)* within this plate. Human fetus of 14 weeks' gestation (immunoperoxidase staining); antibody (5D3) to low-molecular-weight keratins.

box transcription factor 9 (SOX9) in a subset of ductal plate cells marks their differentiation into biliary precursor cells,[22] with cholangiocyte precursors on the portal side of primitive ductal structures expressing SOX9, and those on the parenchymal side being negative for SOX9. These primitive ductal structures become surrounded by extracellular matrix and mesenchyme,[23] in the process acquiring mature cholangiocyte morphology and function around their full circumference. Ductal plate cells not expressing SOX9 transdifferentiate into periportal hepatocytes (forming the 'limiting plate', hepatocytes abutting portal tracts) and to hepatocytes lining the canals of Hering.[24] The timing of biliary differentiation and morphogenesis is coordinated by SOX9, with SOX4 also playing a role.[25] Key downstream molecular signals guiding cholangiocyte differentiation then include Notch, Wnt, transcription growth factor β, Hippo-Yap and fibroblast growth factor (FGF) signalling pathways.[25,26]

As the terminal bile ducts mature within the portal tract mesenchyme, epithelial tethers remain as a connection with the hepatic parenchyma.[13] While traversing the portal tract mesenchyme, these structures are *bile ductules*, circumferentially lined by bile duct epithelial cells (cholangiocytes) derived from the ductal plate. As these channels impale the parenchymal interface, they become hemilunar, with half the circumference as bile duct epithelial cells and the other half as hepatocytes. These are the *canals of Hering*, which may penetrate the parenchyma for up to one-third the zonal distance to the terminal hepatic vein or may skirt the portal tract interface as a residual short canal.[27] The canals of Hering are thought to harbor resident stem cells throughout life, serving as a source for a robust proliferative response after liver injury[28,29] (Fig. 1.4).

Bile canaliculi between hepatocytes are first seen in human embryos at the sixth week, long before bile production begins at 12 weeks. They develop from membrane foldings between junctional complexes and appear as intercellular spaces within sheets of presumptive hepatocytes, thereby constituting an 'apical' luminal channel between hepatocytes. The bile canaliculi drain centripetally from the perivenous zonal region toward the periportal zone, discharging their fluid into the hemilunar canals of Hering, where the hepatocyte:bile duct epithelial cell connection occurs.[13] At the level of hepatocytes, and in addition to the fluid pressure generated by active secretion of biliary solutes and fluid,[30] contraction of the hepatocellular subapical pericanalicular actin network provides a contractile mechanical force for propulsion of newly formed bile downstream.[31]

The entire process of intrahepatic bile duct development progresses centrifugally from the porta hepatis and also from the larger to the smaller portal tracts. However, this process may not be complete at 40 weeks' gestation, and full expression of K7 is not found until about 1 month postpartum. Thus the intrahepatic bile duct system is still immature at birth.[13] Indeed, the liver doubles in size in the first year of life and continues to grow incrementally until adulthood, arriving at a mass 10 times that at birth. Formation of the intrahepatic biliary tree is therefore not fully complete until many years after birth. Failure of remodelling and resorption during fetal liver development produces the *ductal plate malformation*,[32,33] which is significant in the production of various congenital malformations of the intrahepatic biliary tree. Failure of remodelling has been observed in HNF6 and HNF1β knockout mice, indicating that these transcription factors in tandem play a role in normal remodelling of the ductal plate[34] (see later). Furthermore, agenesis, injury to or destruction of the ductal plate *in utero* may be a factor in the development of intrahepatic biliary atresia[32] (see Chapter 3).

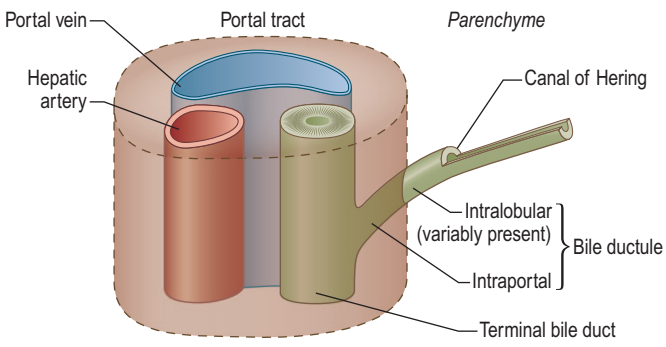

Figure 1.4 Schematic diagram of relationships among bile ducts, ductules and canals of Hering. *(Data from Roskams TA et al. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. Hepatology. 2004;39:1739–45.)*

Despite their common ancestry, mature hepatocytes and cholangiocytes of ductal epithelium in the adult liver are considered as distinct cell types. The epithelium of the terminal twigs of the biliary tree—the canals of Hering—includes typical hepatocytes and typical ductal cells, but no forms intermediate between the two.[35] However, the adult liver retains multiple stem cell niches, including hepatic stem/progenitor cells in or near canals of Hering and biliary tree stem/progenitor cells within peribiliary glands along the larger intrahepatic bile ducts.[36] When there is significant injury to either hepatocytes or cholangiocytes at the interface between portal tracts and parenchyma, a profound ductular reaction can occur.[35] On repair of the injury, hepatocytes and cholangiocytes will return to expressing their normal respective keratins (K8, K18 for hepatocytes; K7, K19 for cholangiocytes).[37]

The limiting plate merits further comment.[35,38,39] Referring back to embryological development, once the ductal plate has involuted, only canals of Hering remain at the portal tract–parenchymal interface as elements containing bile duct epithelial cells. The remainder of the interface is rimmed by mature hepatocytes, directly abutting the portal tract mesenchyme and representing the limiting plate. When liver injury occurs at the interface, involving destruction of hepatocytes and influx of inflammatory cells, the limiting plate is compromised or destroyed; this process is termed *interface hepatitis*. The canal of Hering–bile ductular compartment proliferates in response, giving rise to ductular reaction at the interface.

Haematopoiesis

Hepatic haematopoiesis is a feature of the embryonic and fetal liver of mammals, including humans. The yolk sac is the initial site of haematopoiesis from primitive progenitor cells. Colonization of the liver by definitive erythroid-myeloid progenitor cells begins at about 6 weeks (10 mm embryo).[40,41] Foci of haematopoietic cells appear extravascularly alongside the sheets of hepatocytes, and by the 12th week the liver is the main site of haematopoiesis, having superseded the yolk sac. Hepatic haematopoietic activity begins to subside in the fifth month of gestation, when the bone marrow becomes haematopoietic, and has normally ceased within a few weeks after birth. Parenchymal haematopoiesis is largely erythropoietic; haematopoiesis within portal tracts tends more toward granulocytes, megakaryocytes and monocytes.

Molecular control of liver development

As previously noted, the first morphological indication of development of the liver is an endodermal proliferation in the ventral part of the foregut, just caudad to the cardiac mesoderm and septum transversum, at the 18th postfertilization day in the human embryo. Ventral foregut endoderm is marked by expression of the homeobox transcription factor gene, *HHEX*[42], and over the ensuing days is subdivided into

hepatic (HNF1β)-expressing or pancreatic homeobox factor 1 (PDX1)-expressing progenitor domains, a process called *specification*.[43] Beginning about the 23rd day of gestation, the cardiogenic mesoderm provides an FGF signal that is important for proliferation of the precursor endodermal cells[44]; both FGF1 and FGF2 appear to be involved.[45] These precursor cells proliferate and invade as hepatoblasts into the surrounding septum transversum, forming the liver bud proper. Liver bud outgrowth occurs through about the 56th day of gestation, without further differentiation of the hepatoblasts. Beginning about days 56–58 in the fetal liver, terminal differentiation of hepatoblasts begins, continuing for the remainder of pregnancy as the mature structure of the liver is laid down.

Many of the molecules and receptors involved in regulation of the hepatoblasts and subsequent hepatocyte and cholangiolar differentiation have now been identified.[46–49] It has become increasingly apparent that cellular interactions with nonparenchymal cells play a key role in early hepatic development.[47,50,51] The sheets of hepatoblasts that invade the septum transversum in the developing mouse liver express the transcription factors HNF1β and HNF4α, while the surrounding mesenchyme expresses GATA4[47]; the migratory properties of the hepatoblasts appear to require a homeobox gene *PROX1*. GATA6 also appears to be essential in the formation of the early liver bud, as do FoxA1 and FoxA2 (under the induction of HNF-1β).[52–54] Some of these factors (e.g. GATA4) appear to be important in early stimulation of hepatocyte-specific gene expression, including AFP, transthyretin and albumin; this occurs before morphological change toward a hepatocyte or a cholangiocyte phenotype.[47]

Expression of bone morphogenic protein (BMP) by the septum transversum activates the expression of GATA4.[47,55] Wnt signalling is now also known to play a role in liver induction; liver development requires its repression by secretion of Wnt inhibitors by the endoderm.[56] Studies utilizing embryonic stem cells and RNA technology have reinforced the role of FoxA2 in hepatocytic differentiation.[57] The myriad of transcription factors and signalling molecules identified thus far that may be involved in early liver induction are summarized elsewhere.[46–48] Vasculogenic cells (angioblasts) are also critical for these earliest stages of organogenesis, before blood vessel formation. In the mouse embryo, angioblasts were found as a loose necklace of cells interceding between the thickening, hepatically specified endoderm and the mesenchyme of the septum transversum. This mesenchymal-epithelial interaction precedes the emergence of the liver bud and persists throughout further liver development.

During later fetal liver development there is continued expansion of the parenchymal cell mass. This involves both stimulatory signals and protection from tumour necrosis factor alpha (TNFα)-mediated apoptosis; these phenomena involve the AP-1 transcription factor c-Jun, hepatoma-derived growth factor (HDGF), the Wnt signalling pathway, the nuclear factor-κB pathway and the hepatocyte growth factor (HGF)–c-met pathway, among others.[47,58] In the final step of differentiation, PROX1 and HNF4α direct hepatoblasts to a hepatocyte phenotype, whereas HNF6, SOX9 and HNF1β guide the hepatoblast to a cholangiocyte phenotype.[48] Notch signalling is important for creating the proper balance in the numbers of hepatocytes and cholangiocytes.[59,60] Development and maintenance of hepatocytic differentiation and function are under the control of HNF4α.[61] Maturation of primitive bile ductular structures into mature bile ducts is promoted by LKB1, a tumor suppressor encoded by the *STK11* gene,[62] and by the transcription factors SOX4 and SOX9.[63]

Macroanatomy of the liver

The liver lies almost completely under the protection of the rib cage, projecting below it and coming into contact with the anterior abdominal wall only below the right costal margin and the xiphisternum. The liver is moulded to the undersurface of the diaphragm, the muscular part of which separates it on each side from the corresponding lung and pleural sac. The liver is separated by the central tendon of the diaphragm from the pericardium and the heart. The anterior dome of the liver and its medial, ventral and lateral aspects are covered by the *Glisson capsule,* the connective tissue sheath of the liver with its glistening peritoneal surface. The posterior surface of the liver is the least accessible, and its relationships are of some clinical importance. It includes the following, from right to left:

1. The 'bare area', which is surrounded by the reflections of peritoneum that form the superior and inferior layers of the coronary ligaments. It lies in direct contact with the diaphragm, except where the inferior vena cava (IVC), the right adrenal gland and the upper part of the right kidney intervene.
2. The caudate lobe, which lies between the IVC on the right and, on the left, the fissure of the ligamentum venosum and the attachment of the lesser omentum. The caudate lobe projects into the right side of the superior recess of the lesser sac and is covered by peritoneum; behind it lies the right crus of the diaphragm, between the IVC and the aorta.
3. A small area on the left posterior surface, covered by peritoneum and apposed to the abdominal oesophagus.

The traditional division of hepatic anatomy into right and left lobes (delineated by the midline falciform ligament) and caudate and quadrate lobes is of purely topographical significance. A more useful and important subdivision is made on the basis of the *branching pattern* of the hepatic artery, portal vein and bile ducts. As these are followed into the liver from the porta hepatis, each branches in corresponding fashion, accompanied by a branching tree of connective tissue, derived from the original mesenchyme of the developing liver. On this basis of vascular anatomy, the liver is divided into right and left 'physiological' lobes of about equal size. The plane of separation between these two 'hemilivers' corresponds, on the visceral surface of the liver, to a line extending from the left side of the sulcus for the IVC superiorly, to the middle of the fossa for the gallbladder inferiorly. This parasagittal plane lies approximately 2–3 cm right of the midline. Each lobe has been further subdivided into portobiliary-arterial segments.

Within each hemiliver, the primary branches of the portal vein divide to supply two main portal segments, each of which is further divided horizontally into superior and inferior segments. According to this scheme, there are thus eight segments, or nine if the dorsal bulge of the liver between the groove of the IVC and midline—the caudate lobe—is separately designated. Using the Couinaud system for designating segments[64] (Fig. 1.5), the numerical assignments are *caudate lobe* (I); *left lobe:* medio-superior (II), medio-inferior (III), latero-superior (IVa) and latero-inferior (IVb); and *right lobe:* medio-inferior (V), latero-inferior (VI), latero-superior (VII) and medio-superior (VIII). Because segment IVb lies between the falciform ligament medially and the gallbladder fossa and groove for the inferior vena cava laterally, this region also is designated the *quadrate lobe.* Numerology aside, the caudate lobe stands at the watershed between right and left vascular and ductal territories; its right portion in particular may be served by right or left vessels and ducts, although its left part is almost invariably supplied by the transverse portion of the left branch of the portal vein.

These nine hepatic segments are separate in the sense that each has its own vascular pedicle (arterial, portal venous and lymphatic) and biliary drainage. Although there is substantial opportunity for vascular anastomoses between the different hepatic segments, there are no major intrahepatic vascular connections between the right and left hepatic arteries or portal vein systems. The virtually

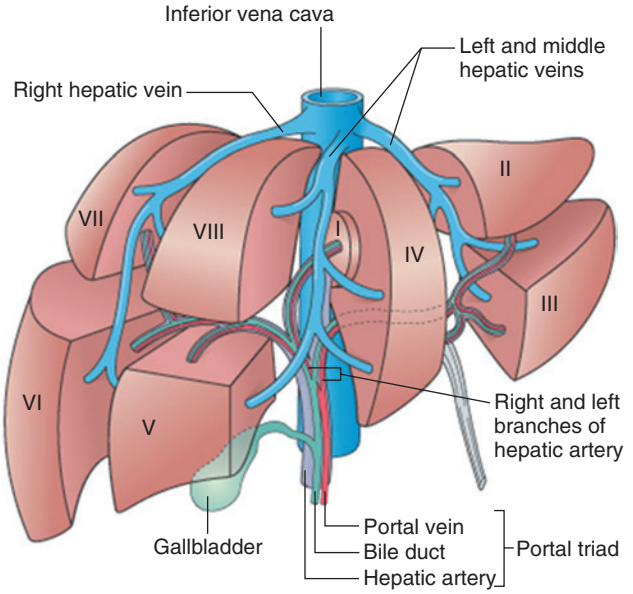

Figure 1.5 Segmentation of the liver based on principal divisions of the portal vein and hepatic artery (see text for numbers). *(Modified from Moore KL, Dalley AF. Clinically oriented anatomy, 4th ed. Philadelphia: Lippincott Williams & Wilkins; 1999, p. 268, copyright 1999 from Lippincott Williams & Wilkins [http://lww.com].)*

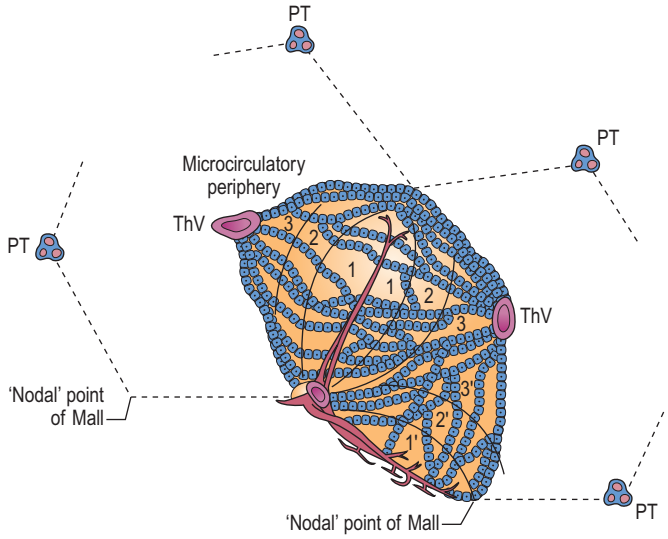

Figure 1.6 Diagrammatic representation of the simple acinus and the zonal arrangement of hepatocytes. Two neighbouring 'classic lobules' are outlined by *dashed lines*, and the acinus occupies adjacent sectors of these. Although only one channel is shown as forming the central core of the acinus, the acinus is arranged round the terminal branches of the portal vein, hepatic artery and bile ductule. Zones 1, 2 and 3 represent areas which receive blood progressively poorer in nutrients and oxygen; zone 3 thus represents the microcirculatory periphery; and the most peripheral portions of zone 3 from adjacent acini form the perivenular area. The nodal points of Mall represent vascular watershed areas where the terminal afferent vessels from neighbouring acini meet. *PT*, Portal tract; *ThV*, terminal hepatic vein (central vein of 'classic lobule'); *1*, *2*, *3*, microcirculatory zones; *1′*, *2′*, *3′*, microcirculatory zones of neighbouring acinus. *(Data from Rappaport AM et al. Subdivision of hexagonal liver lobules into a structural and functional unit: role in hepatic physiology and pathology. Anat Rec 1954;119:11–34.)*

independent vascular supply for each segment has been shown by studies in living humans, using computed tomography (CT), magnetic resonance imaging (MRI) and ultrasonography (US), together with intravenous (IV) contrast injections, which allow ready recognition of the liver's major vascular structures.[65] This vascular anatomy underpins the surgeon's ability to perform a partial hepatectomy and achieve haemostasis for the residual liver, despite the absence of defining connective tissue septa between segments.[66]

While the branches of the hepatic artery and portal vein and the tributaries of the hepatic ducts run together and serve segments of liver, the hepatic veins run independently and are intersegmental. As with the portal vein, they lack valves. The three major hepatic veins—the right, intermediate and left (the intermediate and left often forming a common trunk)—enter the upper end of the retrohepatic segment of the IVC. The terminal portion of each hepatic vein frequently is at least partially exposed above the posterior surface of the liver, where the veins are vulnerable to being severed by blunt abdominal trauma. In addition to these major hepatic veins, several accessory hepatic veins (about five per liver) open into the lower part of the hepatic segment of the IVC.[67] Because the caudate lobe regularly drains directly into the IVC, it may escape injury from venous outflow block.

Microanatomy of the liver

Definition of the fundamental structural and functional 'unit' of the liver has been an elusive goal since the first description of liver lobules by Weppler in 1665 (cited by Bloch[68]). Over the years, several concepts of the basic structural organization of the liver have been operative. The hexagonal 'lobule' described by Kiernan[69] in 1833 remains the standard by which hepatic microarchitecture is named (Fig. 1.6). 'Portal tracts,' containing a portal vein, hepatic artery and bile duct, constitute the periphery of the hexagonal lobule, occupying three of the six apices of the hexagon. The effluent hepatic vein is at the centre of the lobule, thus its name 'central vein'.[70] To this day, regions of the parenchyma are referred to as 'periportal' or 'pericentral'.

More useful are subdivisions of the classic hexagonal lobule into smaller functional physiological units. The most robust concept is the 'liver acinus' (see Fig. 1.6), defined by Rappaport et al.[71] in 1954. In this formulation the portal tract is at one point of the base of a triangle, and the effluent vein is at the sharp apex of the triangle. The vein is regarded as the 'terminal hepatic venule'. In the idealized hexagonal lobule, there are six acinar units. However, actual liver microanatomy is not ideal, and there are ever-variable relationships between portal tracts and terminal hepatic venules across the two- and three-dimensional anatomy of the liver parenchyma. Regardless, a key element of the Rappaport 'acinus' is portal venules derived from portal veins of various sizes, which penetrate the parenchyma and traverse the base of the triangle. Blood emanating from these venules perfuses the parenchyma across a broad zonal front, converging at the apex of the terminal hepatic vein. The most proximal zone, defined by the blood flow, is *zone 1*, with a midzonal *zone 2* and a terminal *zone 3* recapitulating the 'pericentral zone' of the hexagonal lobule. Importantly, zone 1 is not 'pericentral'; it is an ellipsoid region of higher-oxygenated parenchyma, with its long axis being the base of the isoceles triangle (remembering that acini emanate symmetrically on both sides from the penetrating venule). Likewise, the septal venule is orthogonal to a line drawn between parallel terminal hepatic veins coursing through the parenchyma. In three dimensions the perfect geometry is an interlocking system of triangular acini that can be viewed as contributing to the larger hexagonal lobules.

Other formulations for liver microanatomy have been proposed.[72–75] In the 'primary hepatic lobule' model, Matsumoto et al.[72] regarded the 'vascular septum' emanating from the portal tract (the 'penetrating venule' of the liver acinus) as being at the origin of the unit's blood flow. Later, the 'cholehepaton'[76] was viewed as similar to the nephron and synthesized concepts of a 'choleon'[70] and 'hepatic microcirculatory unit'.[77] A group of hepatocytes, again a triangle with the terminal hepatic vein at its apex, is drained by a single bile ductule–canal of Hering (discussed later) at the base of the triangle.[75] In this way a countercurrent cholehepaton unit is described, with blood moving through sinusoids from the base to the apex of the triangle and bile moving from apex to base in return.

Most of these concepts represent different ways of looking at an organ of deceptive structural complexity and with a multitude of functions. However, a basic difference between the liver acinus and the other 'lobule-based' concepts makes them mutually exclusive. Early editions of this text stated that the concept of the 'liver acinus' was proving to be of the greatest value to the pathologist in the interpretation of disordered structure and function. It represented the structural and functional liver unit concept that allowed for an explanation of important histopathological features, such as portal-central bridging, hepatic necrosis and fibrosis. However, the concept of the liver acinus was based on vascular injection of coloured gelatin-based infusion fluids. In 1979 and 1982, Matsumoto et al.[72,78] used meticulous vascular reconstruction of the liver to conceptualize the primary hepatic lobule. Among devoted hepatic anatomists, this last concept has gradually gained attention, and several other concepts (e.g. choleon, hepatic microcirculatory unit, choleohepaton, single sinusoid–hepatic functional unit) can be considered as variants of or existing within the primary lobule.

Given all these considerations, the terminology of hepatic microanatomy is somewhat fluid and certainly mixed. The practice of liver histopathology exists mostly in the two worlds of the classic 'lobule' and Rappaport 'acinus'. We thus ask the reader to understand the use of these mixed terminologies in subsequent chapters.

Anatomy of hepatic microcirculation

Portal circulation

The portal tract derives its name from the ramifications of the portal venous system through the liver. The portal system also provides the tracts along which the hepatic artery system and biliary tree travel, giving rise to the alternate term 'portal triad'. However, as the terminal portal tracts reach their own terminus, the portal vein system ends, ironically leaving a final portal tract 'dyad' lacking a terminal portal vein but containing hepatic artery–bile duct pairs before these also end after a short distance. In the periphery of the normal adult liver sampled by percutaneous liver biopsy, portal 'triads' containing portal vein, hepatic artery and bile duct profiles constitute only 70% of portal tracts. Careful histological examination also reveals an additional 30% of diminutive 'portal dyads' containing only hepatic artery and bile duct profiles.[8] Lastly, the portal system ramifies through approximately 17–20 orders of branches to supply the entire corpus of the liver.[79] The subdivisions are not strictly dichotomous, however, in that one branch may ultimately have fewer subbranches than another. Thus the liver ultimately has an irregular lobular organization at the microscopic level.

Beginning at the porta hepatis, the portal vein divides into successive generations of distributing or *conducting* veins, so called because they do not directly feed the sinusoidal circulation. The vein diameters remain in the macroscopic range, introducing negligible vascular resistance. According to their diminishing position in the hierarchy of branching, they may be classified as *interlobar*, *segmental* and *interlobular*. The portal venous system branches in a *nondichotomous* manner, such that smaller portal tracts may branch off conducting portal tracts without the latter losing their conducting capability.

These further branches of the portal system are those that produce the smallest portal veins, which do distribute their blood into the sinusoids. These succeeding branches are (1) the preterminal portal venules, microscopically found in portal tracts of triangular cross section, and (2) the terminal portal venules, which taper to about 20–30 μm in diameter and are surrounded by scanty connective tissue in portal tracts of circular rather than triangular cross section. From both the preterminal and terminal portal venules arise very short side branches, designated *inlet venules*, which have an endothelial lining with a basement membrane and scanty adventitial fibrous connective tissue but no smooth muscle in their walls. They pass through the periportal connective tissue sheath and enter into the parenchyma to open into the sinusoids. These inlets are reported to be guarded by sphincters composed of sinusoidal lining cells—the afferent or inlet sphincters.[80] Some early branching of the smallest conducting veins may produce more than one portal vein within some portal tracts, and these supply only those sinusoids which abut the canal.

Drawing on the anatomical studies of Matsumoto et al.,[72,78] the conducting portal system and the terminal portal branches both give rise directly to penetrating inlet venules. The inflow-front for portal venous blood thus has a sickle shape. This is at variance with the concept of the acinus, in which portal blood flow exhibits radial symmetry only around the inlet venule arising from terminal portal vein branches. Moreover, the inlet venules do not run the entire length of the distance between two portal tracts, but rather taper off into a sinusoidal configuration near the middle of the interportal distance. In other words, the vascular watershed areas are not located at the nodal points of Mall (see Fig. 1.6), but at the midpoint of the interportal distance (midseptum M of Zou et al.[81]). Zonal gradients of metabolic activities and of enzyme histochemical staining patterns also conform with the sickle-zone pattern, not with the acinus diagram.[71] The sickle-zone pattern has been confirmed by others.[82–84] It is also important to note that the septal venules are not orthogonal to the portal tract, but in three dimensions follow a somewhat arcuate course into the parenchyma.

The importance of keeping this more sophisticated anatomical perspective becomes clear when examining the damaged liver. For example, according to the acinar concept, with severe hepatic congestion involving destruction of hepatocytes in acinar zone 3 and potentially in zone 2, the surviving parenchyma of acinar zone 1 would appear clover shaped, with the portal tract at its centre, but still damaged. However, Matsumoto et al.[72] claim that immediate periportal damage actually never occurs in vascular insufficiency or chronic venous congestion, and that typically the liver biopsy always shows sickle zones standing in relief.

Arterial circulation

The hepatic artery supplies branches throughout the portal tract system. In the largest interlobar portal tracts, there are typically four hepatic arteries accompanying the interlobar bile ducts.[7] The hepatic artery/bile duct ratio drops until, in the most distal portal tracts, hepatic arteries are paired in close proximity with interlobular bile ducts in a 1 : 1 ratio. Even at this level, there are typically two hepatic artery–bile duct pairs per portal vein in a peripheral portal 'triad'.[8]

The terminal blood flow of the arteries is by three routes: into a plexus around the portal vein, into a plexus around a bile duct, and into terminal hepatic arterioles.[79] The perivenous plexus is characteristically distributed around interlobar, segmental and interlobular portal vein branches within the portal area; it drains into hepatic sinusoids. Occasional arterioportal anastomoses between perivenous arterioles

and terminal portal venules have been observed, but the frequency of these in normal human livers is uncertain.[79,80] By the level of the terminal portal veins, the arteriolar plexus is absent.

A peribiliary arterial-derived plexus supplies all the intrahepatic bile ducts. Around the larger ducts the peribiliary plexus is two layered, with a rich inner, subepithelial layer of fine capillaries and an outer, periductular venous network which receives blood from the inner layer. The smallest, terminal bile ducts have only a single layer of fine capillaries. Ultrastructural studies have shown that the capillaries are lined by fenestrated endothelium.[85] The peribiliary plexus drains principally into hepatic sinusoids. The peribiliary plexus develops in parallel with the development of the intrahepatic bile ducts, spreading from the hepatic hilum to the peripheral area of the liver and becoming fully developed with the full maturation of the biliary system.[86] In addition to providing the vascular supply to bile ducts, the peribiliary vascular plexus is thought to be involved in reabsorption of bile constituents (including bile acids) taken up apically by bile duct epithelial cells and secreted across their basal plasma membrane into the portal tract interstitium, constituting a 'cholehepatic' circulation.[87] The cholehepatic reuptake of biliary substances may play a key adaptive role during times of downstream bile duct obstruction, because these solutes may be 'dumped' into the systemic circulation for disposal by the kidneys.[88]

Terminal hepatic arterioles have an internal elastic lamina and a layer of smooth muscle cells, and they open into periportal sinusoids via arteriosinus twigs. Although some mammalian species exhibit hepatic arterioles that penetrate deeply into the parenchyma before entering sinusoids near to the hepatic veins, reports of such penetrating arterioles in humans have been disputed.[89] Regardless, isolated parenchymal arterioles may sometimes be seen in liver biopsies. Ekataksin[77] has suggested that these vessels supply isolated vascular beds in the parenchyma.

Venous drainage

Having perfused the parenchyma through the sinusoids, blood enters the terminal hepatic venules ('central veins' of classic lobules). Scanning electron microscopy (SEM) has clearly demonstrated in the walls of terminal hepatic veins the fenestrations through which the sinusoids open,[90] and the astute observer can see these sinusoidal openings into terminal hepatic veins in histological sections. The terminal vein branches unite to form intercalated veins, which in turn form larger hepatic vein branches, whose macroanatomy is described earlier. The venous anatomy does not strictly parallel the distribution of the portal system, because hepatic veins traverse between portal system-defined lobules as the venous system exits the liver. This is understandable; ultimately the hepatic veins need to exit through the dorsum of the liver, whereas the portal system enters the liver ventrally. The hepatic venous system also does not ramify as extensively as the portal system, so there is a slight preponderance of terminal portal tracts to terminal hepatic veins throughout the liver.

Regulation of hepatic microcirculation

Total hepatic blood flow in normal adults under resting conditions is between 1500 and 1900 mL/min, or approximately 25% of cardiac output.[91] Of this, about two-thirds is supplied by the portal vein and the remainder by the hepatic artery. Because of variations in splanchnic blood flow, portal vein blood flow increases after feeding and decreases during exercise and sleep,[92] the so-called hepatic arterial buffer response (HABR).[93] Direct external regulation of hepatic blood flow is mediated primarily through the hepatic artery, influenced by such vascular mediators as angiotensin II.[94] During times of reduced portal venous blood flow, as during acute occlusion or in cirrhosis, the HABR also helps maintain hepatic perfusion so as to support its core metabolic functions, although it cannot fully compensate for lost portal perfusion.[95]

Intrinsic regulation of blood flow within the liver is quite complex. This account is based on the work of McCuskey[96] but derives ultimately from the pioneering studies of Knisely et al.,[97] using quartz rod transillumination of living liver. Anatomical arterioportal relationships are summarized in Fig. 1.7, which shows a terminal (penetrating) portal venule from which a series of sinusoids originates, as well as a hypothetical accompanying terminal hepatic arteriole (internal diameter ~10 μm). As noted, whether this arteriole actually penetrates the parenchyma is disputed. Regardless, various connections exist between arteriole and sinusoid, with all found in the periportal areas and all of internal diameter no greater than the diameter of an erythrocyte.

In accordance with macroscopic blood flow, approximately two-thirds of the intrahepatic blood supply ultimately comes from the portal venules, whose inlets are controlled by sphincters—afferent or inlet sphincters—composed of sinusoidal endothelial cells. For the remaining third of blood supply, arterial blood flow to the sinusoids is intermittent and determined by independently contractile smooth muscle sphincters in the walls of hepatic arterioles and their arteriolosinusoidal branches. Blood flowing into a group of sinusoids could therefore be arterial, venous or mixed, depending on sphincteric activity of entering venular and arteriolar channels and the distance of the originating sinusoid from the portal tract along the penetrating venule.

There also is heterogeneity in the blood flow through the sinusoids. In the upstream zone the sinusoids form an interconnecting polygonal network. Downstream, however, the sinusoids become organized as convergent parallel channels which drain into the terminal hepatic venule, a convergent architecture that is evident histologically at medium power. In this downstream region, short intersinusoidal channels connect adjacent parallel sinusoids. Blood entering the hepatic venules passes through efferent or outlet sphincters which, like the inlet sphincters, are composed of sinusoidal endothelial cells.

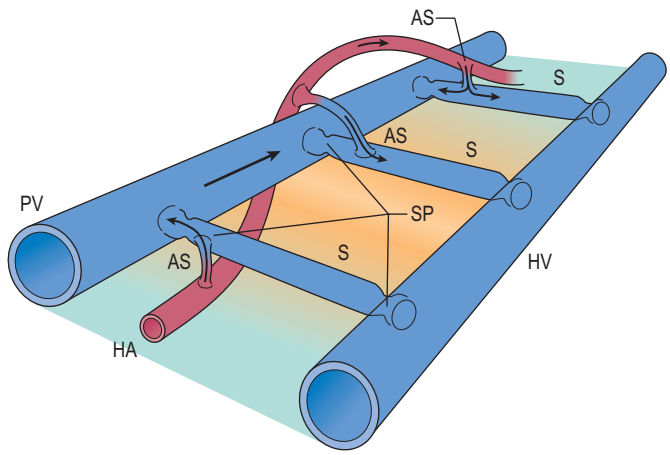

Figure 1.7 Schematic showing relationships of hepatic arteriole *(HA)*, portal venule *(PV)*, sinusoids *(S)* and hepatic venule *(HV)*. Arteriolosinusoidal branches *(AS)* may open in various ways into the sinusoids, and blood flowing to a group of sinusoids could therefore be arterial, venous or mixed. Blood flow through the sinusoids is determined by the activity of 'sphincters' *(SP)* in the arteriolar wall and of sphincteric mechanisms at the inlet and outlet of the sinusoids. *(Based on McCuskey RS. Hepatic microcirculation. In: Bioulac-Sage P, Balabaud C, editors. Sinusoids in human liver: health and disease. Rijswijk: Kupffer Cell Foundation, 1988.)*

The precise mechanisms that regulate the hepatic microcirculation remain controversial.[96,98] The potential morphological sites for regulating blood flow through sinusoids, with secretion of chemokines that influence hepatic stellate cells. The portal and hepatic venules and the hepatic arterioles contain some smooth muscle cells in their wall and are therefore contractile. However, the principal site of regulation is thought to reside in the sinusoids themselves. The sinusoidal endothelial cells respond to a variety of vasoactive substances, and by contracting or swelling, they may vary the diameter of the sinusoid lumen. Thus, blood flow through individual sinusoids is variable. Where the lumen is narrowed, blood flow may be impeded by leukocytes that transiently plug the vessel, a feature that is more common in the narrower, more tortuous periportal sinusoids.[99] It seems likely that flow through some sinusoids may be intermittent, whereas others have relatively constant rates of blood flow. Arterial blood flowing into an individual sinusoid through a dilated arteriosinusoid may increase the rate of blood flow.

Kupffer cells could affect the rate of blood flow through sinusoids, with particular attention to a role for hepatic stellate cells. HSCs are responsive to vasoconstrictors such as endothelin-1.[100] Given their contractile function (see later), HSCs have been implicated in regulation of sinusoidal blood flow.[101] In addition, reduction of the portal blood flow has been shown by *in vivo* microscopy to reduce sinusoidal blood flow, with a considerable decrease in sinusoidal diameter, changes that were reversed on restoring the portal blood flow.[102] It has been suggested that the stellate cells, whose long, slender processes surround the sinusoids, may be responsible for producing these changes.

Functional heterogeneity in the liver

Hepatocyte heterogeneity was recognized as early as the 1850s,[103] when the heterogeneous contribution of different hepatocytes to bile secretion was described. Many aspects of liver physiology and metabolism exhibit a heterogeneous distribution along the portocentric axis. Hepatocytes in the periportal zone have a higher capacity for gluconeogenesis and fatty acid metabolism, whereas perivenous hepatocytes have a higher capacity for detoxification.[104] This heterogeneity is reflected in hepatocyte ultrastructure, including increased smooth endoplasmic reticulum with its biotransforming enzymes in the perivenular region, the hepatocyte complement of mitochondria with their attendant oxidative capacity and gene expression.[105] The concept of *metabolic zonation* is therefore operative.[106,107] As first proposed for carbohydrate metabolism, opposite metabolic pathways such as gluconeogenesis and glycolysis can be carried out simultaneously by hepatocytes in the periportal and the centrilobular region, respectively. Although this possibility for 'futile cycles' may seem at first to be inefficient, the presence of dynamically opposed, interlocking metabolic pathways within individual hepatocytes and between hepatocytes in different positions in the lobule allows for exquisitely rapid regulation of metabolic function, including rapid reversal of the metabolic output of the organ.[108] The presence of metabolic zonation for essentially all liver functions reflects a potentially important level of overall metabolic control. Dysregulation of hepatic zonation may contribute in part to the pathophysiology of metabolic liver diseases such as the metabolic syndrome.[109]

Two types of zonal heterogeneity in the liver are (1) the gradient versus compartment type of zonation and (2) the dynamic versus stable type of zonation.[110] In the *gradient* type, all hepatocytes are able to express a particular gene, but the level of expression depends on the position of the hepatocyte along the portocentral radius. Examples include key regulatory enzymes of carbohydrate metabolism, cytosolic phosphoenol-pyruvate carboxykinase I (PCK) and glucokinase. In the *compartment* type of zonation the expression of genes is thought to be restricted to either the periportal or the perivenular compartment. A striking example of compartmental zonation is a key enzyme of ammonia metabolism, glutamine synthase (GS), which in normal liver exhibits strict localization to hepatocytes rimming the terminal hepatic vein. The *dynamic* type of zonation is characterized by adaptive changes in expression in response to changes in the metabolic or hormonal state, regardless of where the enzyme is zonally located (e.g. PCK, tyrosine transaminase, CPS, ornithine aminotransferase). The *stable* type of zonation, on the other hand, is characterized by the virtual absence of dynamic adaptive change (e.g. fructose-1,6-biphosphatase, GS).

The molecular mechanisms regulating metabolic zonation are varied. In the case of bile formation, physiological availability of biliary solutes in periportal blood is a major determinant, independent of hepatocellular expression of relevant transporters.[110] Fatty acid β-oxidation also is periportal under normal conditions, but the zonation can be affected by nutritional state and oxygen supply from blood.[111] Likewise, gradients of oxygen and hormones across the lobule are thought to play key roles influencing expression of metabolic enzymes.[104]

The Wnt/β-catenin signalling pathway plays a key role in regulating liver zonation.[112–116] Specifically, the precise zonal localization of several β-catenin-regulated liver-specific genes, including GS, transporter 1 of glutamate (GLT1) and ornithine aminotransferase (OAT), are under *APC* control. Targeted disruption of *APC* inhibition, or constitutive activation of the Wnt/β-catenin signalling pathway, leads to panlobular expression of these genes involved in ammonia metabolism. The resultant severe metabolic perturbation is lethal in experimental conditions. Conversely, experimental deletion of β-catenin results in loss of GS expression irrespective of whether *APC* is present.[114] The zonation of bile acid metabolism and transport is the most heavily targeted by β-catenin, partly through the xenobiotic nuclear receptors involved in bile acid metabolism.[116] Lastly, hormones and the sympathetic and parasympathetic nervous systems also influence zonation of hepatic metabolism, including their influence on the hepatic microcirculation.[117,118] However, differences in hepatic innervation between humans and experimental animals hinder an understanding of the importance of innervation in humans.

Zonal heterogeneity is a feature that characterizes not only hepatocytes, but other components of liver tissue as well. Sinusoids have a more tortuous course, more frequent intersinusoidal anastomoses and a narrower lumen in the periportal area, whereas they appear straighter with less intersinusoidal sinusoids and a broader lumen in centrilobular areas.[119] The sinusoidal endothelial cells have higher porosity (by fenestrae) in the centrilobular region, display different wheat-germ agglutinin-binding patterns in periportal versus centrilobular endothelial cells and show portocentral gradients in mannose receptor-mediated endocytosis and in production of reactive oxygen metabolites.[120] In a three-dimensional reconstruction study of parenchymal units in rat liver, Teutsch et al.[121] emphasized the importance of considering three-dimensionality for an adequate functional interpretation of the metabolic heterogeneity of hepatocytes. If the three-dimensionality of the parenchymal units is not taken into consideration, calculations show that changes at the origin of sinusoids are likely underestimated, whereas changes at the termination of sinusoids are overestimated. This should also apply in the interpretation of sections from pathologically altered liver tissue.[121]

The hepatic stellate cells display marked heterogeneity in structure and function based on their zonal (portal-central) and regional (portal versus septal sinusoidal) distribution in the hepatic lobules.[122] They have more cytoplasmic processes with thorn-like microprojections

in the centrilobular zone, whereas their desmin immunoreactivity and vitamin A storage are greater in the periportal zone.

Kupffer cells are located preferentially in periportal regions, and some functional and morphological heterogeneity has been ascribed to their location. Thus, Kupffer cells in periportal zones are larger, contain more heterogeneous lysosomes and are more active in phagocytosis than their centrilobular counterparts.[123] In contrast, in areas around centrilobular veins, Kupffer cells are smaller and more active in terms of cytokine production and cytotoxicity.[124]

The extracellular matrix components in the space of Disse may vary along the portal-central axis. Collagens IV and V and laminin predominate in the area of transition between the bile ductule–canal of Hering units and hepatocytes at the periportal lobular periphery. Fibronectin and collagens III, IV and VI are the predominant components along the portovenous parenchymal axis.[125]

Ultrastructural anatomy of hepatocyte

Hepatocytes are polyhedral cells approximately 20 to 30 μm in diameter, with a volume of approximately 5000 μm^3. Their organization into anastomosing plates of the parenchyma is best illustrated by SEM (Fig. 1.8). The liver cell plates anastomose extensively in the periportal region but become more simplified and radially oriented as they approach the terminal hepatic vein.

Plasma membrane

As with other epithelial cells, the hepatocyte is highly polarized (Fig. 1.9). Within its plasma membrane, three specialized regions, or domains, are recognized: *sinusoidal*, which faces the sinusoid and the perisinusoidal space; *lateral*, facing the intercellular space between hepatocytes; and *canalicular*, bounding that specific part of the intercellular space constituting the bile canaliculus.[126,127] Using a more generic terminology, the sinusoidal and lateral domains constitute the *basolateral* plasma membrane of the hepatocyte, and the canalicular domain is the *apical* domain. This polarity is largely maintained by the tight junctions formed between adjacent hepatocytes, which delineate the basolateral domain from the canalicular domain and create a barrier between fluid in the intercellular space and bile in the canaliculus.[128,129] In addition to tight junctions, there are also gap junctions and desmosomes in the lateral domain, and it is across lateral-domain gap junctions that intercellular communication takes place. Stereological studies in the rat have shown that the basolateral, canalicular and lateral domains constitute approximately 70%, 15% and 15%, respectively, of the total cell surface area.[130]

The domains of the hepatocyte plasma membrane are not simply topographical entities. They are specialized to serve different basolateral and apical functions of the hepatocyte. Molecular differences include composition of the lipids in the plasma membrane, the complement of membrane proteins, function of endocytic and exocytic compartments and relationship to the cytoskeleton.[110,122,123,131] Beyond direct regulation of membrane protein function, hepatocytes can dynamically regulate actual membrane lipid content and the concentration of specific proteins in each domain, providing a powerful additional mechanism for functional control of plasma membrane function.[132,133]

Sinusoidal domain

The sinusoidal surface of the hepatocyte faces the perisinusoidal *space of Disse*, the tissue space between hepatocytes and the endothelial sinusoidal lining cells. The hepatocyte sinusoidal surface is covered with abundant microvilli, each measuring 0.5 μm long but not

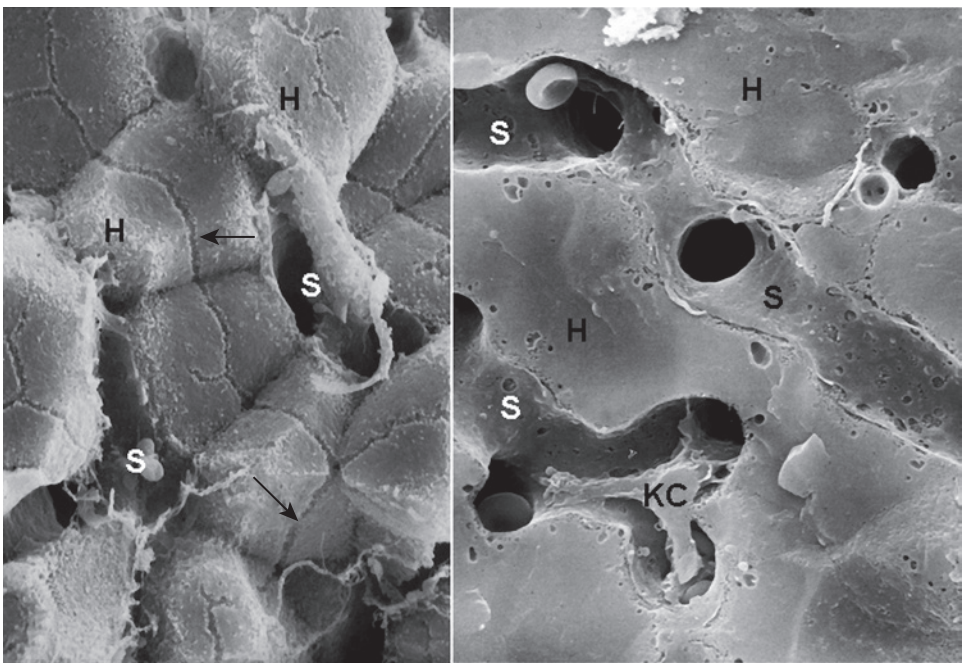

Figure 1.8 Plates of hepatocytes viewed by scanning electron microscopy. **A,** The fracture plane highlights the lateral faces of hepatocytes *(H)*, with bile canaliculi *(arrow)* in view. The labyrinth of sinusoidal spaces *(S)* is evident between hepatocyte plates. **B,** Different image shows the sinusoidal spaces to better advantage. A Kupffer cell *(KC)* is present within the luminal space of the sinusoid. *(Modified from McCuskey RS. Functional morphology of the liver with emphasis on its microvasculature. In: Tavoloni N, Berk PD, editors. Hepatic transport and bile secretion. New York: Raven Press, 1993. copyright 1993 from Lippincott Williams & Wilkins [http://lww.com].)*

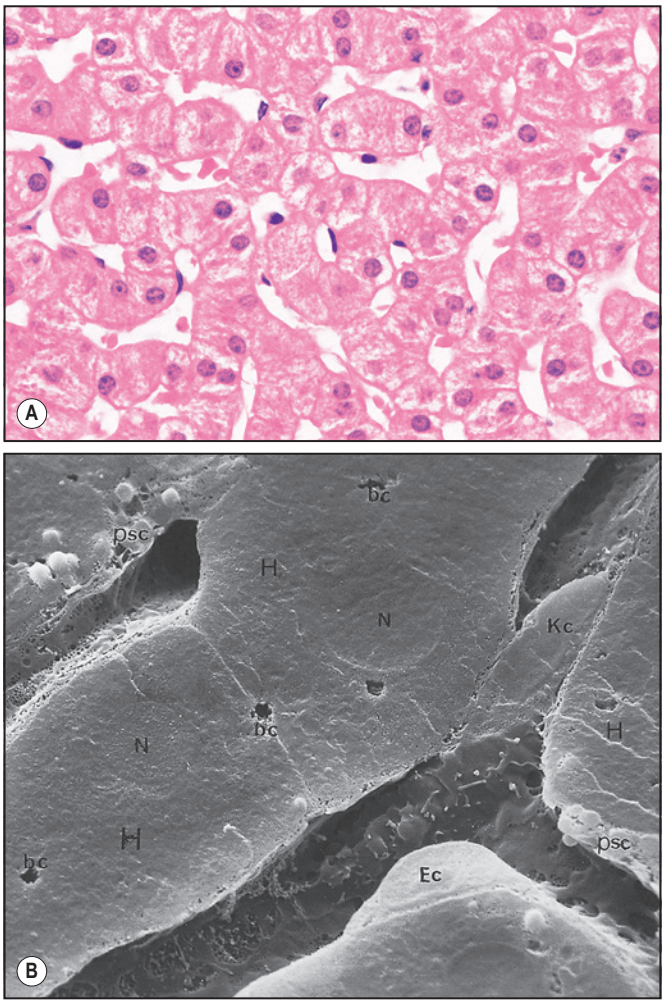

Figure 1.9 Rat liver preparations. **A,** Light microscopy of liver cell plates cut longitudinally showing a centrally placed nucleus and occasional binucleate cells, the sinusoidal surface against which Kupffer cell nuclei are abutting, the canalicular pole and the intercellular surface. **B,** Scanning electron micrograph showing several cell types. Hepatocytes *(H)* contain a nucleus *(N)*, and at the junction between cells the bile canaliculus *(bc)* and the intercellular surface are clearly defined. The sinusoidal surface is seen, and in the sinusoidal area a Kupffer cell *(Kc)*, endothelial cell *(Ec)* and two perisinusoidal cells *(psc)* are present. *(Courtesy of Professor E. Wisse, Brussels.)*

evident, even as a brush or striated border, on optical microscopy. Microvilli may protrude through the fenestrae of the endothelial cells and into the sinusoidal lumen. The surface specialization is related here, as elsewhere, to absorptive and secretory activity; it obviously increases the surface area, but by a factor smaller than might be expected: approximately sixfold, compared with the 40- to 50-fold increase in surface area imparted by the microvilli of the small intestinal absorptive enterocyte. In the plasma membrane between the bases of the microvilli are small, surface indentations or pits.[90] Some of these represent secretory vacuoles discharging fluid into the plasma by a process of exocytosis. Others are clathrin-coated pits involved in selective receptor-mediated endocytosis, termed *caveolae,* invaginated membrane microdomains enriched in cholesterol and sphingolipids and the cholesterol-binding protein caveolin, and responsible for selective membrane trafficking into the interior of the cell.[134]

Hepatocytes along the limiting plate surrounding portal tracts have a surface which abuts the adjacent portal tract mesenchyme. These hepatocytes are irregularly covered with microvilli and may be moulded around connective tissue fibres, producing irregular indentations. The *space of Mall* is conceptualized as the fluid space between hepatocytes along the limiting plate in the periportal area and portal tract fibrous tissue; it is in continuity with the perisinusoidal space of Disse.

Lateral domain

The lateral surface of the hepatocyte extends from the margin of the sinusoidal surface to the bile canaliculus and is specialized for cell attachment and cell–cell communication. Although simple in contour, the lateral surface is not entirely flat; microvilli may extend into it from the sinusoidal surface and protrude into narrow extensions of the space of Disse, and occasional folds (plicae) and round-mouthed openings may represent pinocytic vesicles.[90] There are also knob-like protrusions and corresponding indentations; when fitted into one another, these would form the 'press-stud' or 'snap-fastener' type of intercellular attachment long familiar in transmission electron microscopy (TEM).

The lateral domain also has specialized areas called 'gap junctions' or more accurately, 'communicating junctions'. These are seen on TEM as patches of close approximation of the two membranes and in freeze-fracture preparations as irregularly shaped aggregates of particles on the inner leaflet (P face). The gap between the two membranes is 2–4 nm wide and is bridged by the intramembrane particles (of protein or lipid), which project like 'bobbins' from the external surface of each of the two membranes. Since each 'bobbin' is perforated by a central pore and apposed 'bobbins' are in contact, communications are established which provide for the transfer of ions or metabolites (or both) between hepatocytes.

Canalicular domain

The 'bile canaliculus' is defined as an intercellular space formed by the apposition of the edges of gutter-like hemicanals delimited by tight junctions, on the facing surfaces of adjacent hepatocytes (Fig. 1.10 A and B). Canalicular diameter varies from 0.5 to 1.0 µm in the perivenular area and from 1 to 2.5 µm in the periportal zone, in accordance with flow of bile from the centrilobular region of the lobule toward the portal tract. The canalicular surface is unevenly covered by microvilli, which are more abundant along a 'marginal ridge' at each edge of the hemicanaliculus. In experimental biliary obstruction the canaliculi become dilated and the microvilli disappear, except along the marginal ridges. Intracellular subapical microfilaments are concentrated around the canaliculi, forming distinct, organelle-free pericanalicular sheaths and extending into the microvilli. This pericanalicular microfilament web is contractile, enabling hepatocytes to propel secreted bile along the canalicular channel. The presence of contractile elements in the pericanalicular zone can be demonstrated by indirect immunofluorescence (Fig. 1.10 C).[135] The presence of adenosine triphosphatase (ATPase) can be demonstrated histochemically; CD10 is a reliable immunostain for identifying the bile canaliculus (Fig. 1.10 D). Brown-tinted accumulations of lipofuscin may also outline the canalicular pole of the hepatocyte, reflecting the presence of pericanalicular lysosomes, more evident in the adult liver because of the aging process. When present, lysosomal lipofuscin deposits are more prominent in perivenular hepatocytes.

The canalicular surface is isolated from the rest of the intercellular surface by junctional complexes (see Fig. 1.10 A): desmosomes, intermediate junctions, tight junctions and gap junctions. The 'tight junctions' constitute a permeability barrier to macromolecules between the bile canaliculus and the rest of the intercellular space. 'Tightness',

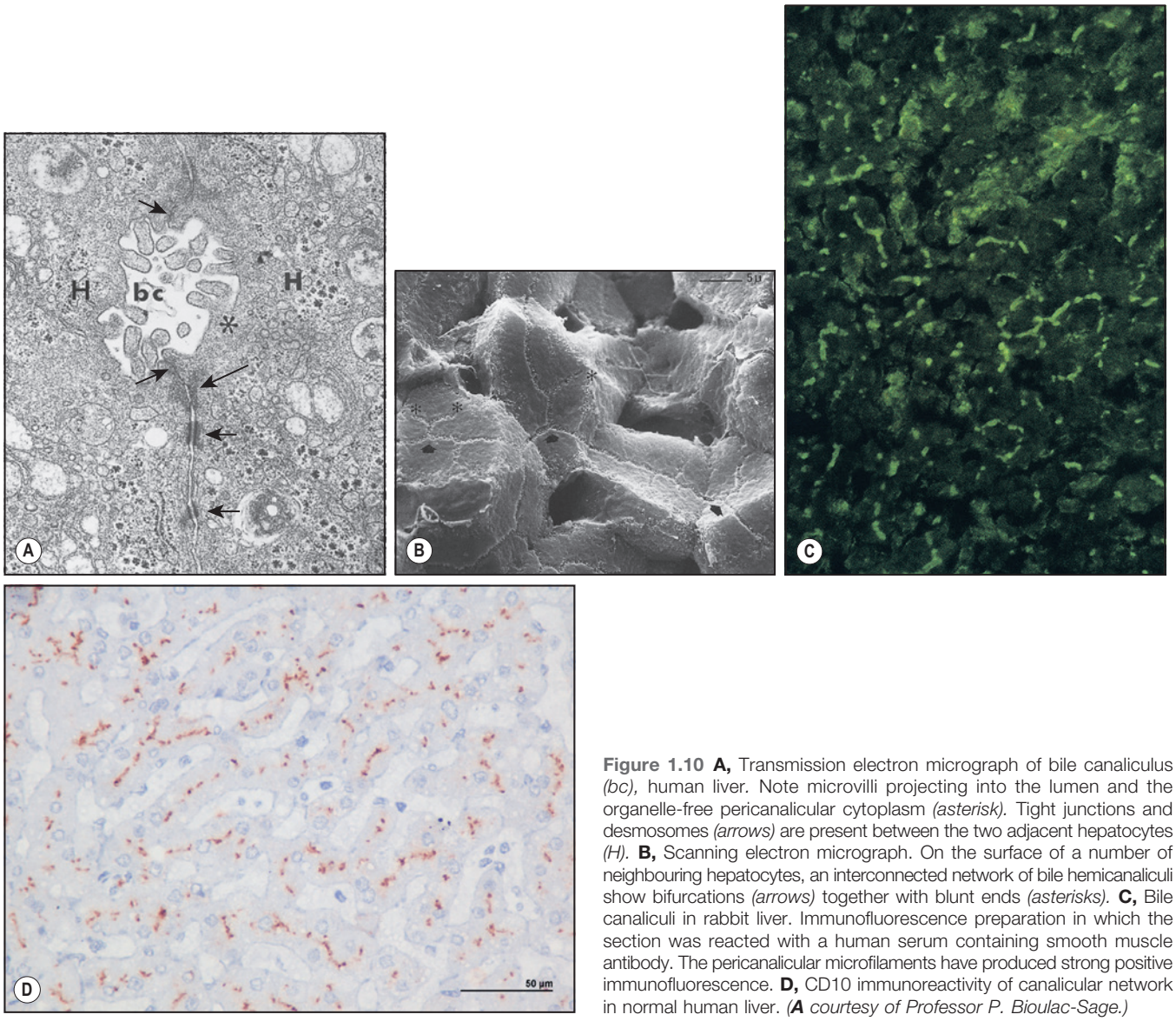

Figure 1.10 **A,** Transmission electron micrograph of bile canaliculus *(bc)*, human liver. Note microvilli projecting into the lumen and the organelle-free pericanalicular cytoplasm *(asterisk).* Tight junctions and desmosomes *(arrows)* are present between the two adjacent hepatocytes *(H).* **B,** Scanning electron micrograph. On the surface of a number of neighbouring hepatocytes, an interconnected network of bile hemicanaliculi show bifurcations *(arrows)* together with blunt ends *(asterisks).* **C,** Bile canaliculi in rabbit liver. Immunofluorescence preparation in which the section was reacted with a human serum containing smooth muscle antibody. The pericanalicular microfilaments have produced strong positive immunofluorescence. **D,** CD10 immunoreactivity of canalicular network in normal human liver. *(**A** courtesy of Professor P. Bioulac-Sage.)*

however, is a relative term; there seems to be a positive correlation between degrees of tightness and the number of strands forming the junction. On this basis, the canalicular tight junctions are comparable to those elsewhere in the body (e.g. rete testis, vasa efferentia) regarded as only 'moderately tight'.

Nucleus

The hepatocyte nucleus shows characteristics expected in the nucleus of a cell actively engaged in protein synthesis: large, occupying 5–10% of the volume of the cell; spherical, with one or more prominent nucleoli; and with scattered chromatin. The nuclear membrane is double layered and contains many pores (Fig. 1.11). At birth, all but a few hepatocytes are mononuclear and of uniform size. In the adult liver there is considerable variation in both number and size of nuclei. About 25% of the adult hepatocytes are binucleate; the two nuclei are similar in size and staining properties. Hepatocyte nuclei fall into various sizes, with volumes in the ratio 1:2:4:8. This variation reflects polyploidy, with the DNA content increasing correspondingly.[136] At birth in humans, almost all hepatocytes are diploid (and mononucleate). From the eighth year, when more than 90% of hepatocytes are diploid, the number of *tetraploid* nuclei (i.e. those with twice the normal DNA content) increases, to reach about 15% in children of 15 years[137] and 40% by middle adulthood.[58,138] Tetraploid cells are thought to arise by mitosis of cells with two diploid nuclei. The DNA content of each nucleus doubles, but the chromosomes are then arranged on a single mitotic spindle, so that division produces two daughter cells, each with a single tetraploid nucleus. The significance of polyploidy in hepatocytes is unknown. Since cell size is proportional to cell ploidy,[139] polyploidy does not provide an increased amount of genetic material per unit volume of cytoplasm.

Hepatocyte mitotic division provides for intrauterine and postnatal growth of the liver, which continues well into childhood. By adulthood the liver has a very low mitotic index, with estimates ranging from one mitosis per 10,000–20,000 cells to up to 2.2 mitoses per 1000 cells. Nevertheless, a high percentage of hepatocyte nuclei are euchromatic, indicating that transcription of most of the genome is occurring continuously. Almost all the DNA is in the extended configuration, and minimal heterochromatin is observed.

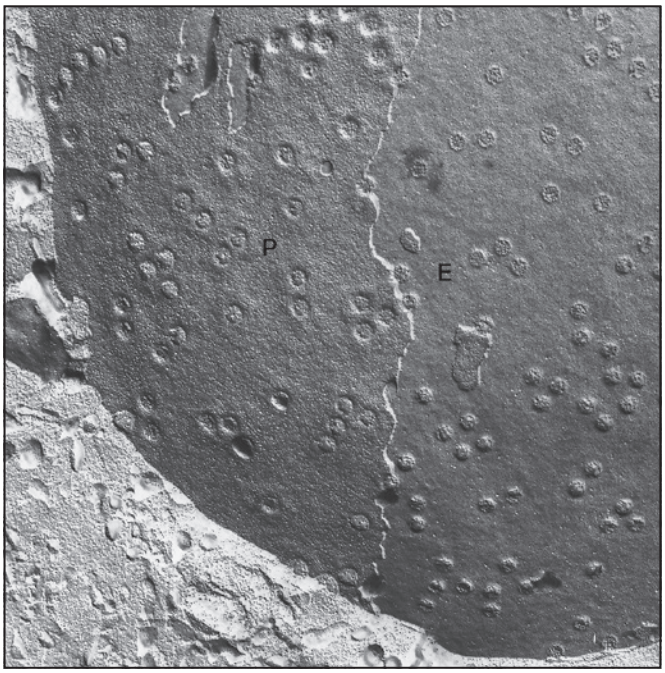

Figure 1.11 Freeze-fracture replica illustrating the inner leaflet (P face) and the outer leaflet (E face) of the nuclear membrane of a hepatocyte. Note the numerous nuclear pores. (Human liver, magnification ×44,800.) *(Courtesy of Professor R. De Vos.)*

Conversely, hepatocytes engaged in protein biosynthesis have a large nucleolus (sometimes several) that can be recognized on light microscopy. The nucleolus is where ribosomal genes are located and where ribosome biogenesis occurs.[140] Electron microscopy (EM) reveals the nucleolus to contain three main components: rounded fibrillar centers composed of thin, loosely distributed fibrils; a dense fibrillar component containing tightly packed fibrils that surround the fibrillar centers; and the granular component, constituted by granules which embed both fibrillar components. Ribosomal genes exist in an extended, ready-to-be-transcribed configuration within the fibrillar centers and partly in the dense fibrillar component. Although the precise location of ribosomal gene transcription remains unclear, newly transcribed RNA molecules undergo early processing and maturation in the dense fibrillar component and are assembled into preribosomes in the granular component. Protein-rich ribosomal subunits then exit the nucleus through pores in the double-membrane nuclear envelope.

Endoplasmic reticulum

The endoplasmic reticulum (ER) is a network of parallel, flattened sacs or cisternae on whose cytoplasmic surfaces may be attached polyribosomes to constitute the rough endoplasmic reticulum (RER) (Fig. 1.12). Clusters of RER are scattered randomly throughout the hepatocyte cytoplasm and constitute approximately 60% of the ER. The remaining 40% is the smooth endoplasmic reticulum (SER), lacking a ribosomal coating. The SER also forms anastomosing networks of tubules and vesicles of varying diameter which are continuous with the cisternae of the RER. The outer nuclear membrane also has attached ribosomes and is continuous with the membrane of the RER. The SER is often found in the region of the Golgi apparatus and communicates with it. The SER frequently has a close topographical relationship with glycogen (Fig. 1.13). The ER occupies 15% of the total cell volume, and its surface area—approximately 60,000 μm^2 per hepatocyte—is more than 35 times the area of the plasma membrane. There is also zonality in the distribution of the ER; the surface area of SER in the centrilobular area is twice that in the periportal zone.[12,141]

The cell functions associated with the ER include (1) protein synthesis, both of secretory proteins and some of the protein constituents of the cell and organelle membranes; (2) metabolism of fatty acids, phospholipids and triglycerides; (3) production and metabolism of cholesterol and possibly production of bile acids; (4) xenobiotic metabolism; (5) ascorbic acid synthesis and (6) haem degradation. In the case of protein synthesis, polypeptides synthesized by RER ribosomes are retained in the membrane or are ejected into the lumen of the RER for folding and post-translational modification. The protein products pass through the SER to the Golgi apparatus for packaging and insertion into membranes throughout the cell (in the case of membrane proteins) or for exocytotic secretion across the basolateral or apical membranes of the hepatocyte.

The cytochrome P-450 system is localized in the ER membrane; this is the system whereby the liver cell metabolizes and detoxifies xenobiotics. This enzyme system can be reversibly induced by certain xenobiotics, such as phenobarbital, and this is accompanied by the synthesis and hypertrophy of the ER; the mechanisms involved in new membrane production are not clear. The preponderance of SER in the centrilobular region of the lobule and the presence of haem in cytochrome P-450 enzymes explain the darker hue of the centrilobular region, which can be observed by visual inspection of cut-liver sections.

Glucose 6-phosphatase is localized on the ER, playing a key role in dephosphorylation of intracellular glucose 6-phosphate before release of glucose into the circulation by hepatocytes. As a correlate, the SER proliferates during synthesis of glycogen and thus is available for hepatocyte glycogenolysis when hepatocellular release of glucose is required for metabolic needs elsewhere.

Golgi complex

Each hepatocyte contains as many as 50 Golgi zones (which may not be separate but rather form a tridimensional continuity) situated most frequently beside the nucleus or in the vicinity of the bile canaliculus.[142] Each complex appears as a stack of four to six curved, flattened parallel sacs, often with dilated bulbous ends containing electron-dense material. The convex or *cis* surface is directed toward the ER, and small vesicles in the *cis*-Golgi transfer synthesized proteins from the ER. The concave or *trans* surface is the origin of secretory vesicles. Vesicles break off from the ends of the sacs and carry the contained secretory proteins, including lipoproteins, for discharge at the sinusoidal surface or less often at the canalicular surface. Membrane proteins destined for insertion into any of the plasma membrane domains also are routed through the Golgi complex. The complex and its associated cytoplasm constitute approximately 2–4% of the cell volume. In addition to its role in the secretion of proteins, the Golgi complex has a large complement of glycosylating enzymes, important in the glycosylation of secretory proteins and in the synthesis and recycling of membrane glycoprotein receptors.[142] The Golgi complex is capable of rapid and reversible structural reorganization into a tubuloglomerular network while maintaining its biosynthetic capabilities.[143] With the SER, RER, lysosomes, other intermediate organelle compartments and even the nuclear and mitochondrial envelope membranes, the Golgi is an integral part of the complex intracellular organelle network involving vesicular trafficking that enables uptake, sorting, degradation, biosynthesis, trafficking and secretion of cellular proteins and lipids.[12,144,145]

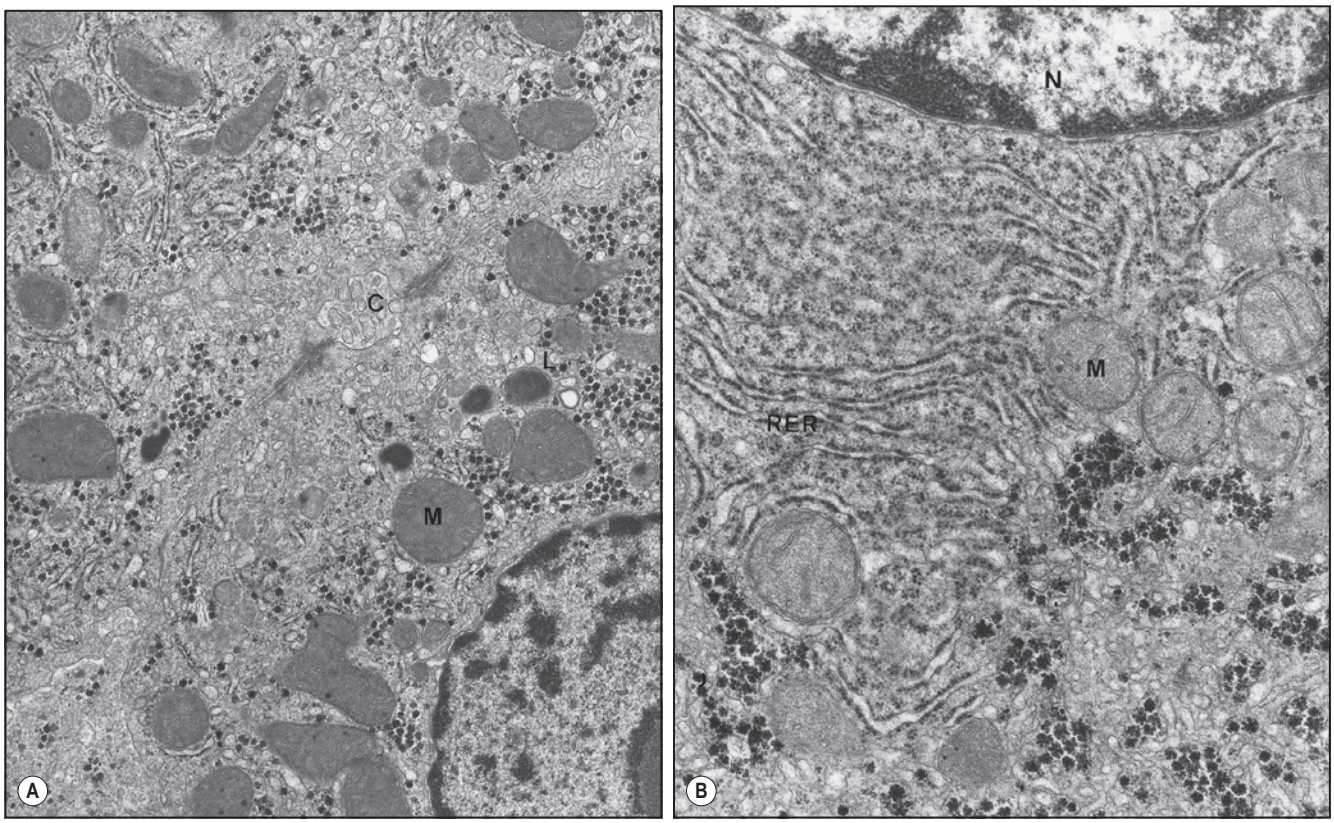

Figure 1.12 **A,** Transmission electron micrograph (TEM) illustrating cytoplasmic organelles, nucleus and bile canaliculus *(C)* between two adjacent hepatocytes. *L*, Lysosomes; *M*, mitochondria. (Human liver; ×23,000.) **B,** TEM illustrating rough endoplasmic reticulum *(RER)* and mitochondria *(M)* with matrix granules. Part of the nucleus *(N)* with inner and outer membrane also shown. (Human liver, ×36,800.) *(Courtesy of Professor R. De Vos.)*

Lysosomes

The existence of lysosomes was first predicted by De Duve[146] on the basis of biochemical studies on liver homogenates. He subsequently identified them as the 'peribiliary dense bodies' found in electron micrographs of liver and established them as a new species of cell organelle. Their functions in health and disease have been reviewed[147,148] and are of particular importance to pathologists because of their involvement in a number of storage diseases (see Chapter 3).

Lysosomes present a variety of appearances in electron micrographs of liver and may be part of the intracellular membranous network known as the 'GERL' (Golgi-SER-lysosome). The GERL is involved with endocytosis and exocytosis, serving as a site for sorting of secretory proteins for secretion and for trafficking of endocytosed proteins to lysosomes for degradation. Indeed, the GERL is the site where the lysosomal enzyme acid phosphatase makes its first appearance, most likely playing a role in formation of lysosomes.

Several classes of lysosomes can be identified within the hepatocyte cytoplasm: (1) *primary lysosomes,* small in size, are considered to be functionally in a resting phase; (2) *secondary lysosomes* are functionally activated and delimited by a single membrane (see Fig. 1.12); (3) *autophagic vacuoles* contain parts of the degrading cytoplasmic organelles and are often delimited by a double membrane; and (4) *residual bodies* are larger than primary and secondary lysosomes and are usually more numerous in older organisms. The residual bodies contain the residues of nondigested material or pigments such as lipofuscins (considered undigestible permanent residues). Lipofuscin granules are the most numerous lysosomal bodies present in human hepatocytes.[141]

Lysosomes are frequently found near the plasma membrane proximal to the bile canaliculus and are capable of discharging their contents into the biliary space.[149] The lysosomes in periportal hepatocytes are often larger and more positive for acid phosphatase than those in centrilobular hepatocytes.[12,141]

Lysosomal pleomorphism reflects a variety of functions. First, although the liver cell is long-lived, there is evidence for turnover of its cytoplasm and organelles. Cytoplasmic constituents may be incorporated within and digested by the primary lysosome, forming an autophagic vacuole, then forming a secondary lysosome. Autophagic vacuoles therefore show fragments of organelles or cell inclusions in various stages of digestion. Second, lysosomes also incorporate lipofuscin pigment, which may accumulate undigested over long periods, forming residual bodies; material of exogenous origin, including iron, stored as ferritin, which accumulates in large quantities in iron overload states; and copper, which accumulates in copper-overload conditions and cholestasis. Third, coated vesicles and multivesicular bodies result from receptor-mediated endocytosis.[150] In a complicated sequence of intracellular events, ligand-receptor complexes in clathrin-coated pits on the basolateral cell plasma membrane are internalized to form endocytic vesicles, or *endosomes*. Soluble ligands which are internalized in this way include insulin, low-density lipoproteins, transferrin, immunoglobulin A (IgA) and asialoglycoproteins. Fusion of endosomes occurs to form multivesicular bodies. Some of these vesicles are responsible for transcytosis

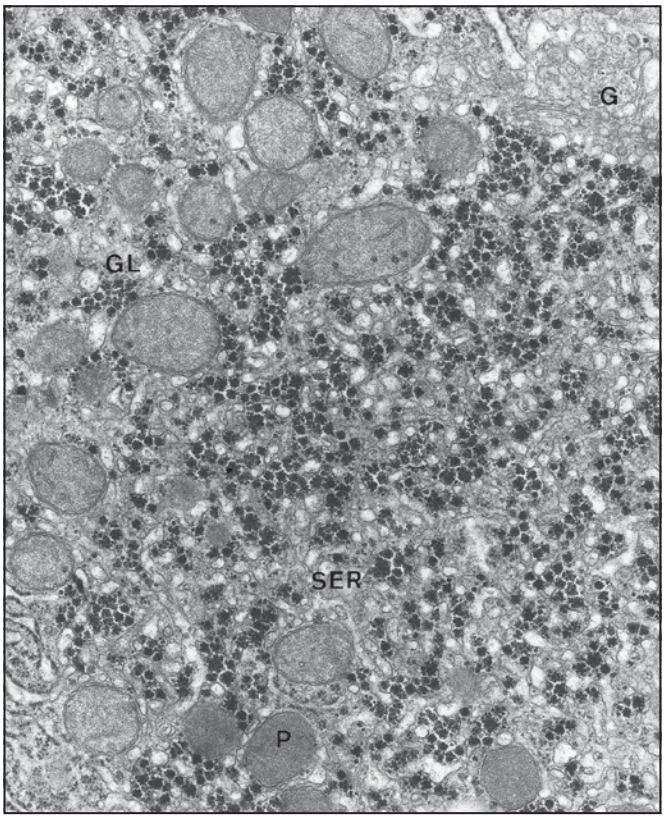

Figure 1.13 Transmission electron micrograph illustrating networks of smooth endoplasmic reticulum *(SER)*; Golgi apparatus *(G)* and peroxisomes *(P)*. Note also glycogen rosettes *(GL)*. (Human liver, ×28,800.) *(Courtesy of Professor R. De Vos.)*

or intracellular transport from the basolateral domain to the canalicular domain. Others fuse with primary lysosomes, and their contents undergo partial degradation before being exocytosed at the canalicular or basolateral domain. Still other vesicles undergo complete degradation and become increasingly electron dense with the formation of dense bodies.[148,150] Microtubules appear to have an important role in sorting the pathways along which endocytic vesicles move within the hepatocyte.[151]

Peroxisomes

Peroxisomes are single membrane-bound ovoid granules 0.2–1.0 µm in diameter (see Fig. 1.13). They were first described as 'microbodies' by Rouiller and Bernhard[152] in 1956. The properties of peroxisomes in liver have been reviewed elsewhere.[153–155] Each hepatocyte may contain 300–600 peroxisomes, and they comprise 1.5–2% of cell volume. Peroxisomes may be more numerous in perivenular hepatocytes, but they are generally homogeneously distributed within the hepatic lobular.[12,141] There is morphological heterogeneity between species; in the rat, peroxisomes contain a paracrystalline striated core or nucleoid in which urate oxidase is concentrated; human peroxisomes lack a core.[156] Peroxisomes contain oxidases that use molecular oxygen to oxidize a number of substrates with the production of hydrogen peroxide (thus the name of the organelle), which in turn is hydrolysed by peroxisomal catalase. Approximately 20% of the oxygen consumption of the liver is used in peroxisomal activity. The energy produced by this oxidation is dissipated as heat. An alcohol overload may be metabolized in the liver by peroxisomal catalase. Drugs, such as clofibrate, which lower blood lipids cause a proliferation of peroxisomes, an increase that has been causally linked to the hypolipidaemic action.[157] Alterations in hepatocyte perixosomes have been reported in bacterial infections, viral hepatitis, Wilson disease and alcoholic liver diseases.[158,159] Various metabolic disorders have been described in which there is either an absence of peroxisomes or a deficiency of peroxisomal enzymes[155,158] (see Chapter 3).

Mitochondria

Mitochondria are large organelles (1.5 µm in diameter and up to 4 µm long) that number approximately 1000–2000 per hepatocyte and constitute about 20% of the cytoplasmic volume of hepatocytes.[160] Mitochondria are the site of oxidative phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP), constituting the source of cellular aerobic metabolism.[161] Longer (up to 4 µm) and larger (up to 1.5 µm in diameter) mitochondria are more numerous in periportal hepatocytes.[160] Mitochondria are bounded by an outer and an inner membrane, each 5–7 nm thick (see Fig. 1.12). The outer membrane possesses special pores that allow the passage of molecules smaller than approximately 2000 daltons. The inner membrane's surface area is greatly increased by the presence of numerous cristae, which fold within the mitochondrial matrix. The space between inner and outer membranes presents a low-density matrix and ranges from about 7 to 10 nm in thickness. Mitochondria have a relatively low-density matrix in which lamellar or tubular cristae and a variable amount of small, dense granules can be observed; these granules may represent a concentration of calcium ions (Ca^{2+}), a small circular DNA and ribosomes.. The dense granules have a diameter of 20 to 50 nm. In addition, filaments of circular mitochondrial DNA about 3–5 nm in width and granules approximately 12 nm in diameter containing mitochondrial RNA are also present. The DNA codes for some of the mitochondrial proteins that are synthesized in ribosomes within the organelle,[162] but most of the mitochondrial protein is encoded by nuclear DNA. Mitochondria are self-replicating and have a half-life of approximately 10 days. Mitochondria may fuse and are remarkably mobile organelles, moving about in the cytoplasm and closely associated with microtubules.

Mutations in the mitochondrial genome account for various mitochondrial myopathies.[163] More recently it has become apparent that specific biochemical abnormalities of mitochondria may play an important role in the pathogenesis of certain liver diseases, and that genetic defects in mitochondrial proteins and enzyme systems may be the underlying cause of other liver and metabolic diseases. Because mitochondria possess a distinct and unique extranuclear genome, a new class of maternally, or mitochondrially, inherited diseases has emerged (see Chapter 3).

The structural compartmentation of mitochondria provides for topographical localization of various enzyme systems, the details of which form almost a science in themselves. It need only be said here that the outer membrane is relatively unimportant as a locus for enzymes; it keeps the inner membrane together and contains *porin*, a transport protein that forms channels permeable to molecules of less than 2 kilodaltons (kD).[160] The inner membrane and cristal lamellae support the respiratory chain enzymes involved in oxidative phosphorylation, which generates ATP. The matrix contains most of the components of the citric acid cycle and the enzymes involved in β-oxidation of fatty acids and in the urea cycle. Mitochondria are randomly distributed within individual hepatocytes but are smaller and less numerous in centrilobular than in periportal cells,[164] the zonal implications of which are beyond the scope of this chapter.

Cytoplasm

The cytoplasm of any cell is a highly concentrated matrix of proteins and microfilaments, within which organic and inorganic solutes diffuse.[165,166] Movement of larger components through the matrix, especially membrane-bound vesicles, involves directed transport along the cytoskeletal fibres,[167] the composition of which is described in the next section. A hepatocyte is extremely rich in non-membrane-bound cytoplasmic inclusions, including glycogen granules, lipid droplets and various pigments.[161] Glycogen granules are the most abundant and on EM may occur in the monoparticulate form (β particles, 15–30 nm in size) or more frequently as aggregates of smaller particles arranged to form 'rosettes' (α particles; see Fig. 1.13). Glycogen granules are dispersed in the cytoplasm but are often associated with the SER. Intranuclear glycogen usually appears as β particles. Glycogen is depleted during fasting, disappearing first from periportal hepatocytes and then from perivenular cells. On refeeding the sequence reverses. In this manner, hepatocytes constitute a major metabolic energy reserve during fasting, thus supporting systemic glucose homeostasis.

Lipid inclusions appear as empty vacuoles in histological sections or as osmiophilic droplets on TEM and usually are not surrounded by membranes. Lipid droplets consist of triglycerides in their interior and are coated with a monolayer of phospholipids.[160] Small lipid droplets have a high surface/volume ratio and are accessible to cytoplasmic lipases, which may degrade the retained triglyceride quickly.[168] Large lipid droplets have a low surface/volume ratio and may reside in hepatocytes long after the metabolic processes in their deposition have subsided.

A variable amount of iron-containing granules is often present within the hepatocyte cytoplasm, depending heavily on the iron status of the host.[169] These are usually in the form of ferritin particles. With an approximately spherical shape, the iron-containing protein *ferritin* consists of a protein shell (apoferritin) 11 nm in diameter and an iron-containing central core approximately 5 nm in diameter. Hepatocyte iron deposits may also occur as single membrane-bound lysosomal bodies (residual bodies), forming aggregates of iron-containing electron-dense particles (siderosomes, haemosiderin granules). In addition to hepatocytes, liver endothelial cells and Kupffer cells[170] also accumulate intracellular iron under conditions of iron overload.

Cytoskeleton

The major components of the cytoskeleton of most eukaryotic cells comprise 6 nm diameter *microfilaments*, 8–10 nm diameter *intermediate filaments* and 20 nm diameter *microtubules*; the definitive review of their structure and function in the hepatocyte remains that of Feldmann.[171] These are structurally, chemically and functionally distinct, linear macromolecules coursing through the cytoplasm. Intermediate filaments are relatively stable macromolecules capable of modulation measured in minutes and longer intervals. Microfilaments and microtubules are highly dynamic structures capable of rapid polymerization and depolymerization on a second-to-second basis and thus rapid adaptation in response to functional demands. For all these macromolecules, polymerization and depolymerization of their constituent molecules are under the influence of various intracellular factors, including free Ca^{2+} ions, high-energy compounds and associated proteins. In addition, various accessory proteins modulate these components and link them to one another, to cell organelles and to the cell membrane; these are part of a microtrabecular lattice, or *cytomatrix*. These structures interact to regulate internal organization, cell shape, movement, division, secretion, metabolism and intercellular communication.[171–176]

Microfilaments

Microfilaments are double-stranded molecules of polymerized fibrous (F) actin; the monomeric form of the protein is globular (G) actin; and these two forms exist in equilibrium in the cell. The microfilaments are present in bundles and form a three-dimensional (3D) intracellular meshwork. There is extensive intracellular binding and cross-linking with other intracellular proteins, such as myosin, lamin and spectrin. The filaments are mainly located at the cell periphery; they attach to the plasma membrane and extend into microvilli. They are particularly concentrated in the pericanalicular zone, forming a pericanalicular web,[177] and attach to the junctional complexes which limit the canaliculus. Four main functions are postulated for the contractile microfilaments of the hepatocyte: (1) translocation of intracellular vesicles implicated in bile secretion, especially by insertion and removal of canalicular plasma membrane transport proteins; (2) coordinated contraction, producing peristaltic movement in the canaliculus;[178] (3) with microtubules, transmembrane control over the topography of intrinsic proteins in the phospholipid bilayer of the cell membrane, thus influencing the protein mosaic and functional differentiation of a particular membrane domain;[179] and (4) possible modulation of the structure and tightness of the 'tight junction', thus regulating the permeability of the paracellular pathway.[180,181] The functional roles for microfilaments involve cell membrane motility, endo- and exocytosis, secretion and vesicle transfer.

Microtubules

Microtubules are a family of unbranched rigid tubules of variable length that are structurally similar in all cells. These polymers are composed of two subunits of tubulin, α and β. Polymerization and growth take place from organizing centres, including centrioles. Microtubules are part of the mitotic apparatus and are therefore important in cell division. They are also present in cell cilia. As with the microfilaments, microtubules attach to and cross-link a number of proteins. Microtubules are involved in the blood-bound secretion of several liver cell products, including lipoprotein, albumin, retinol-binding proteins, secretory component, fibrinogen and other glycoproteins.[182,183] As a cytoskeletal framework, microtubules play a role in the intracellular translocation of vesicles containing IgA and horseradish peroxidase.[183–185]

Intermediate filaments

Intermediate filaments are a family of self-assembling protein fibres.[185] They are structurally similar in all cells, with component polypeptides arranged in an α-helical coiled-coil arrangement which confers tensile strength. These filaments comprise the central scaffold of cells, imparting structural stability to the 3D intracellular architecture. Unlike the microtubules and microfilaments, which are specifically composed of their respective subunit classes, intermediate filaments are strikingly heterogeneous in subunit composition and antigenicity. Intermediate filaments are grouped into five general types[186]—keratin, desmin, vimentin, glial fibrillary acidic protein and neurofilaments—the distribution and expression of which are highly specific to cell type.

Keratins (or cytokeratins) are the intermediate filaments of epithelial cells and are present in hepatocytes and, in greater amounts, in bile duct epithelium. In hepatocytes, keratins are located just inside the plasma membrane and are particularly condensed as a pericanalicular sheath that extends into desmosomes. They are linked to desmosomes on the lateral plasma membrane of hepatocytes, providing scaffolding for the bile canalicular region. Keratins also attach to other components of the cytoskeleton, which includes serving as anchors for the contractile activities of microfilaments. Keratins also attach to organelles such as the RER and vesicles.

Cytokeratins function as 'the mechanical integrators of cellular space', since their firm attachment to desmosomes (which in turn attach to desmosomes on adjacent hepatocytes) provides an integrated continuity of stable 3D architecture across a multicellular region.[172] In the hepatocyte, keratins maintain structural polarity, provide a scaffolding for the bile canaliculus and provide a framework for the distribution of actin and endocytotic vesicles along the plasma membrane.[185,187] In disease states the hepatocellular keratin network, in particular, may become highly disrupted, as discussed later.

Hepatic sinusoid and sinusoidal cells

Sinusoids form a complex vascular network, transporting mixed, nutrient-rich venous and oxygen-rich arterial blood from the terminal portal vein to the hepatic vein branches. The sinusoids have an average diameter of about 5–10 μm (Fig. 1.14), but they may distend to about 30 μm. Periportal sinusoids (zone 1) are more tortuous than the perivenular ones (zone 3).[188] These are special vascular channels that pass between each hepatocyte plate, maximizing contact of blood and its contents with the parenchymal elements of the liver (Fig. 1.15 A). Sinusoids differ from other capillaries in several features because they possess (1) a fenestrated endothelium, without basal lamina; (2) resident macrophages, the Kupffer cells, which bulge into the sinusoidal lumen; (3) special intraluminal resident lymphocytes; and (4) hepatic stellate cells, considered pericytes, lying in the space of Disse subjacent to the sinusoidal endothelium, which store vitamin A and are able to transform into myofibroblasts. The space of Disse also contains sparse, loose extracellular matrix in normal conditions and a few nerve endings, all elements in close

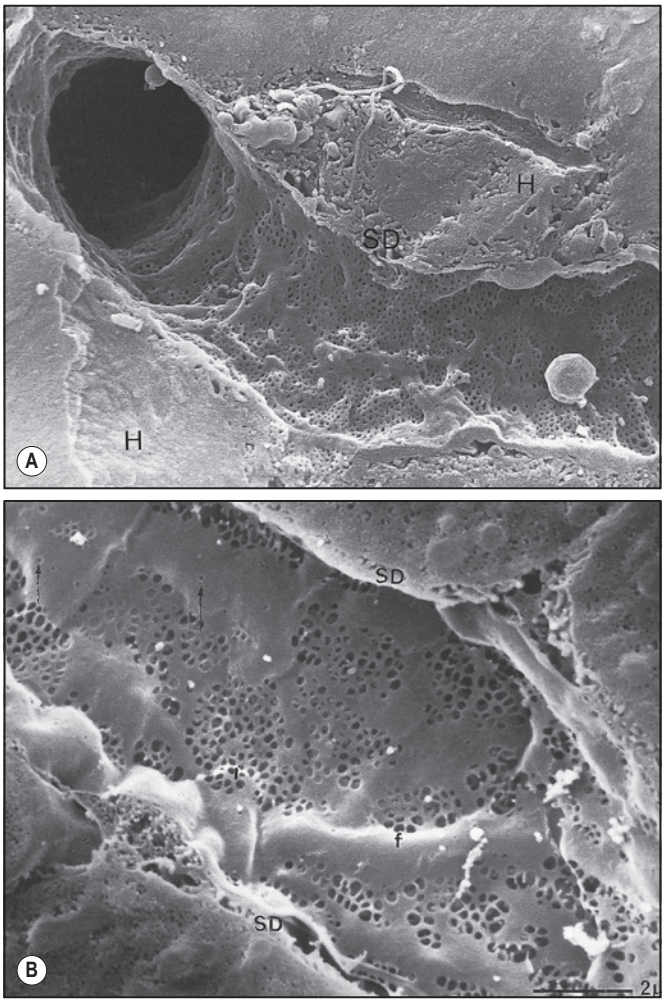

Figure 1.14 Scanning electron microscopy. **A,** Normal hepatic sinusoid in rat liver. Note the regular distribution of fenestrae in the sieve plates, which are separated by intervening cytoplasmic processes. *H,* Hepatocyte. **B,** Endothelial fenestrations *(f)* of about 0.1 μm are grouped together in sieve plates. Processes of endothelial cells show small holes, most probably representing the pinching off of micropinocytotic vesicles *(arrows). SD,* Space of Disse.

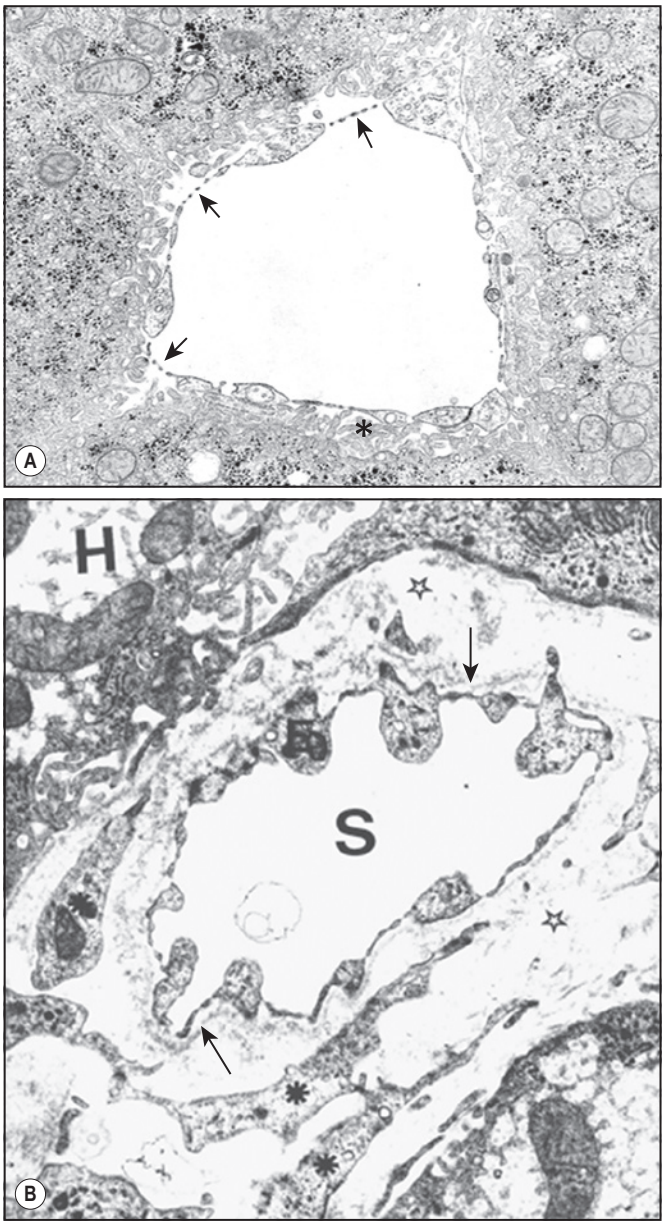

Figure 1.15 Transmission electron microscopy of hepatic sinusoid. **A,** Sinusoid in normal liver. Fenestrations of liver sinusoidal endothelial cells (LSECs) are visible *(arrows)*; hepatocyte microvilli are visible in the narrow space of Disse *(asterisk).* **B,** Capillarized sinusoid *(S)* in an end-stage fibrotic liver. The LSECs are continuous, without fenestrae, and with underlying extracellular matrix comprising a basement membrane *(arrow).* The space of Disse is enlarged *(star)*, containing thick processes of hepatic stellate cells *(asterisks). H,* Hepatocyte.

contact with microvilli of the hepatocyte plasma membrane. The sinusoids can become capillarized in the chronically diseased liver (Fig. 1.15 B),[189] as discussed later.

Four distinct types of sinusoidal cells can be identified (Fig. 1.16), each with its own characteristic morphology, topography and population dynamics. All sinusoidal cells represent about 6% of the lobular parenchyma volume (2.5%, 2.0% and 1.4 % for endothelial, Kupffer and stellate cells, respectively) and 26.5% of all the liver plasma membranes.[190] The sinusoidal lumens occupy approximately 10% of the lobular parenchymal volume. The unique characteristics of sinusoids and sinusoidal cells in the normal liver explain their major role in facilitating exchange between blood and hepatocytes, in intercellular communication, and in extracellular matrix deposition, inflammation and liver immunity. All these functions have major consequences in liver pathology.

Liver sinusoidal endothelial cells

Liver sinusoidal endothelial cells (LSECs) originate from bone marrow[191] and form a highly dynamic barrier between the vascular space and underlying hepatocytes. There is no underlying basal lamina of the sinusoidal endothelium; instead, LSECs rest on a delicate mesh of extracellular matrix that supports the LSECs but does not block fluid or solute movement. The main characteristics of LSECs are (1) their smooth, thin, attenuated cytoplasmic sheet, about 150–170 nm in maximum thickness[192]; (2) the numerous holes (fenestrae) that perforate the cytoplasmic sheet, facilitating exchange between blood and hepatocytes; and (3) their numerous intracytoplasmic pinocytotic vesicles, indicating a high capacity for endocytosis (see Figs 1.14 B and 1.16 A). The cytoplasmic extensions of LSECs may overlap, but there are no intercellular junctions (e.g. endothelial adherence, tight) otherwise found in vascular endothelial cells.[193] Under appropriate stimuli, inflammatory cells can undergo ready diapedesis (migration) from the vascular compartment into the space of Disse.

Endothelial fenestrae

Fenestrae are open transcellular pores without a diaphragm. They are so abundant that on SEM the greater part of the cell has a net-like appearance, forming a tenuous barrier, reinforced where adjacent endothelial cells overlap (see Fig. 1.14 B). The fenestrae are dynamic structures that may vary greatly in size but generally fall into two size categories: small fenestrae (0.1–0.2 μm in diameter) grouped in clusters, forming 'sieve plates', and large fenestrae (up to 1 μm in diameter), which are more numerous at the distal end of the sinusoid. Thus, endothelial cell porosity is higher in the perivenular zone than in the periportal zone.[194] Fenestrae are delineated by fibrillar cytoskeleton elements which could be actin microfilaments. *In vitro* studies have shown that actin-binding drugs, which stabilize or disassemble actin microfilaments, promote an increase in fenestra formation; however, the exact function of this actin ring surrounding fenestrae and its role in fenestra dynamics remain under study.[195] There is evidence that fenestrae are labile, dynamic structures (2–20% of LSEC surface area) whose diameter may change in response to endogenous mediators (e.g. serotonin)[194] and exogenous agents (e.g. alcohol).[196]

This unique porous structure allows the endothelial cells to filter coarsely the sinusoidal blood. Solutes, including macromolecules, pass freely through the fenestrae from the lumen into the space of Disse and come into contact with the basolateral plasma membrane of hepatocytes.[197] Conversely, macromolecules secreted by hepatocytes pass freely into the vascular space. However, large particles (e.g. intact chylomicrons) and formed blood elements are excluded. With the fenestrated, nondiaphragmatic structure of LSECs and the absence of an abluminal basal lamina, the liver sinusoid is the most porous of all endothelial barriers.[198]

The underlying extracellular matrix also modulates the fenestrae of the LSECs, especially under disease conditions. For example, at the earliest stage of liver fibrosis, where extracellular matrix is initially deposited in the space of Disse, a capillarization of sinusoids is observed, corresponding to loss of endothelial fenestrae and formation of a continuous basement membrane (see Fig. 1.15 B).[199] Such phenotypic changes occur also with aging.[200] This decreased porosity impairs the passage of triglycerides from plasma to hepatocytes for degradation, leading to increased risk of systemic atherosclerosis.[201] Thus, alterations in number or diameter of endothelial fenestrae in liver diseases have important implications for hepatic microcirculation and function.

In addition, fenestrae play a role in the formation of hepatic lymph, representing half of the total lymph generated in the body. Filtered plasma entering the space of Disse flows in a retrograde manner back to the portal tracts, entering there into lymphatic channels for drainage through the hepatic hilum en route to the thoracic duct.[202]

Endocytosis

Another striking feature of LSECs is their high endocytotic activity.[196,203] This process appears to be used for uptake and lysosomal degradation of plasma macromolecular solutes, rather than providing an alternative route for their transport from the sinusoidal lumen to the space of Disse. A large number of endogenous compounds may be endocytosed; some are effete molecules and are cleared from the circulation, and others are modified and do appear to undergo transcytosis to hepatocytes, perhaps in a more selective fashion than macromolecular solutes passing only through the fenestrae.[203] This includes removal and degradation of soluble immune complexes, as well as removal of potentially dangerous macromolecules. LSECs have also been shown to store and metabolize serum immunoglobulins and to remove hyaluronic acid/chondroitin sulphate proteoglycans from the circulation.[204–206] Numerous scavenger receptors have been identified (mannose receptor,[207] Fc-γ receptor IIb2, other endocytosis receptors). Thus the LSECs are highly effective scavenger cells. LSEC endocytic uptake of solutes may also stimulate intercellular signalling to hepatocytes, playing a major role in homeostasis and immunity.[198]

Metabolism and molecular phenotype

The LSECs have a predominantly anaerobic metabolism, reflected by a low number of mitochondria and production of high levels of lactate. They have limited but profound biosynthetic activity, producing nitrous oxide (NO), endothelins and prostaglandins and possibly cytokines such as interleukin-1 (IL-1) and IL-6, all of which have potent effects on vascular tone and the functions of nearby cells.[208,209] Through both the generation of NO and the influence of their secreted cytokines on the contractile function of underlying stellate cells (see later), LSECs play a key role in regulation of sinusoidal blood flow.[80] LSECs are subject to damage by some hepatotoxins. For example, LSECs are the initial target of some hepatotoxic drugs (e.g. acetaminophen) and toxins, which occurs before hepatocyte injury. Direct toxic damage to LSECs also is the initiating event in sinusoidal obstruction syndrome (SOS), in which endothelial cell debris occludes the vascular space, with life-threatening consequences.

LSECs show a number of phenotypic differences from other vascular endothelium as well as other types of liver cells,[210] some of which can be used as LSEC markers. Normal LSECs do not bind the lectin *Ulex europaeus* and, in most species, do not express factor VIII-related antigen (von Willebrand factor). Furthermore, LSECs contain absent to low levels of other molecules characteristically

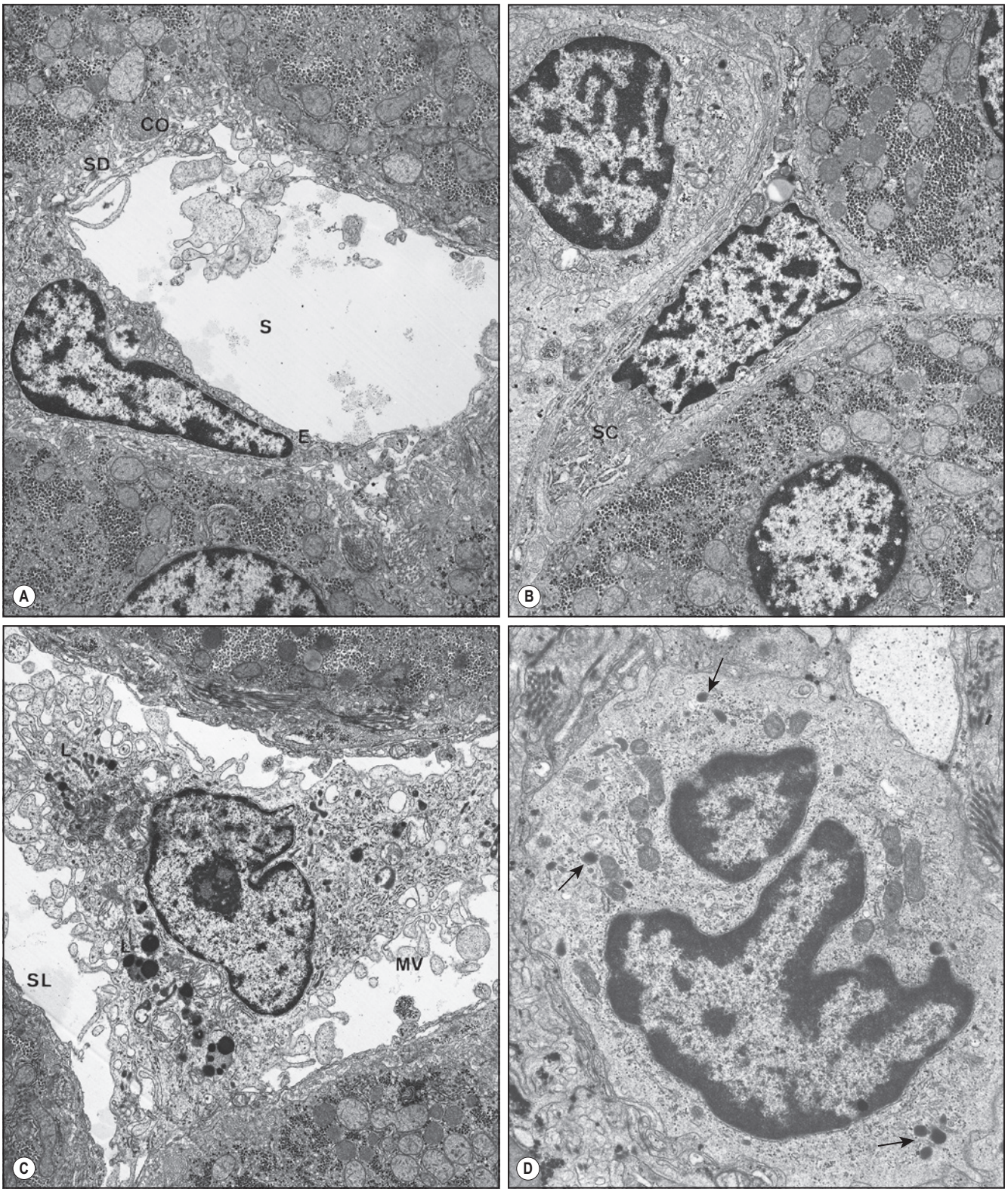

Figure 1.16 Transmission electron microscopy. **A,** Sinusoid *(S)* with its lining endothelial cells *(E)*; *SD,* space of Disse; *CO,* collagen bundles. (Human liver; ×11,500.) **B,** Stellate cell *(SC)* in the space of Disse. Note cell processes, a single small lipid droplet and rough endoplasmic reticulum. (Human liver; ×11,500.) **C,** Kupffer cell with numerous cytoplasmic lysosomes *(L).* Note irregular microvillous projections *(MV)*; sinusoidal lumen *(SL).* Small rims of endothelial lining cells are observed at both sides of the Kupffer cell. (Human liver; ×9200.) **D,** Liver-associated lymphocyte within a sinusoid. Note dense granules in the cytoplasm *(arrows).* (Human liver, ×18,400.) *(Courtesy of Professor R. De Vos.)*

found in vascular endothelium, such as E-selectin, CD31 (PECAM) and CD34,[211] but do express Fc IgG receptors (CD16 and CDw32), CD4, CD14 and aminopeptidase N.[212]

LSECs exhibit membrane immunoreactivity for intercellular adhesion molecule 1 (ICAM1).[212] The natural ligand for this adhesion molecule, lymphocyte-associated antigen 1 (LFA1), is present on Kupffer cells; this LSEC receptor may therefore be involved in adhesion of Kupffer cells to the endothelial lining. Upregulation of ICAM1 expression in LSECs may also be important in 'trapping' LFA1-positive lymphocytes in inflammatory liver diseases.[213]

There is heterogeneity of LSECs along the acinar axis. In addition to the increased porosity in the perivenular zone previously mentioned, variation in cell size, heterogeneous lectin binding and expression of various receptors, cytoplasmic density, endocytic capacity and surface glycosylation have also been demonstrated.[204,212,214,215]

In summary, as a fenestrated barrier, LSECs substantially influence the trafficking of molecules and cells between liver parenchyma and blood. They have major functions in plasma ultrafiltration, in regulation of hepatic microcirculation and in scavenger, innate and adaptive immunity.[198]

Kupffer cells

Kupffer cells, present in the lumen of hepatic sinusoids (see Fig. 1.16 C), can be identified with monoclonal antibodies such as CD68. Kupffer cells are specialized liver macrophages, representing the largest population of resident tissue macrophages. They belong to the mononuclear phagocytic system but manifest phenotypic differences which distinguish them from other macrophages. Kupffer cells are of considerable importance in host defence mechanisms and the innate immune response and can be considered as a main mediator in liver injury and repair. As such, they have an important role in the pathogenesis of various liver diseases.

On SEM, Kupffer cells have an irregular stellate shape, and their luminal surface shows many of the structural features associated with macrophages, such as small microvilli, microplicae and sinuous invaginations of the plasma membrane. Kupffer cells bulge into the sinusoidal lumen. Their cell body rests in contact over a more or less large area on the endothelial lining (Fig. 1.17), but they never form junctional complexes with endothelial cells. However, Kupffer cells may be found in gaps between adjacent endothelial cells, and their protoplasmic processes may extend through the larger endothelial fenestrae into the perisinusoidal space of Disse.

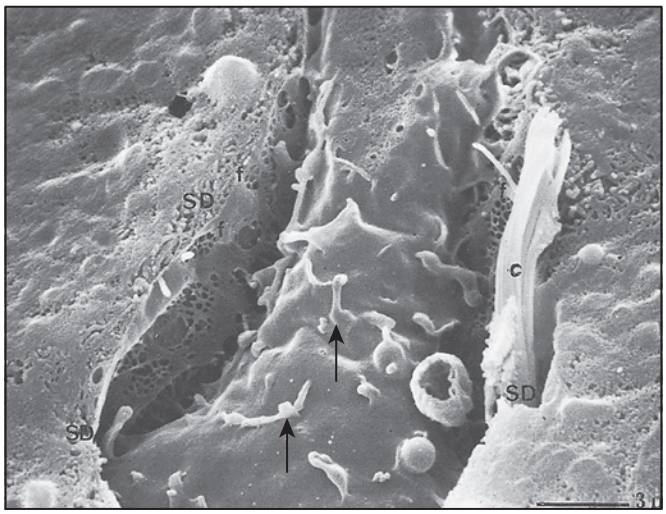

Figure 1.17 Scanning electron microscopy. Part of Kupffer cell lying in a sinusoid and showing characteristic microvilli projecting at the cell surface *(arrows).* Small rims of fenestrated endothelium can be observed at both sides of the Kupffer cell *(f).* A small bundle of collagen fibres *(c)* is situated in the space of Disse *(SD)* on the *right.*

Kupffer cells are more numerous in the periportal sinusoids, and as noted earlier, there is some evidence that, as with hepatocytes and LSECs, Kupffer cells also manifest functional heterogeneity in the lobule,[216] with different subsets distributed through lobular zones.[217] Kupffer cells have been considered to be fixed tissue macrophages, but they appear capable of actively migrating along the sinusoids, both with and against the blood flow. They can migrate into areas of liver injury and into regional lymph nodes.[218] Because of their location and shape, Kupffer cells can interact with blood and cells as they transit.[219]

Kupffer cells contain numerous lysosomes (almost one-quarter of all lysosomes of the liver) and phagosomes, and the cisternae of their endoplasmic reticulum are rich in peroxidase. Their primary functions include the removal, by ingestion and degradation, of particulate and soluble material from the portal blood, in which they discriminate between 'self' and 'non-self' particles. They act as scavengers of microorganisms and degenerate normal cells (e.g. effete erythrocytes), circulating tumour cells and various macromolecules. These functions are in part carried out nonspecifically, but Kupffer cells are also involved in the initiation of immunological responses and the induction of tolerance to antigens absorbed from the gastrointestinal tract. The efficiency of this clearance function is shown by the fact that removal of particulate material is limited only by the magnitude of hepatic blood flow; removal of particles may approach single-pass efficiency.

Kupffer cells play a major role in clearance of gut-derived endotoxin from the portal blood, achieved without the induction of a local inflammatory response. The precise mechanisms involved are not fully understood, but there appears to be finely balanced autoregulation between the release of proinflammatory and inflammatory mediators such as IL-1, IL-6, tumour necrosis factor alpha (TNFα) and interferon-γ (IFN-γ) and mediators such as IL-10 that suppress macrophage activation and inhibit their cytokine secretion.[220] The role of Toll-like receptors (TLRs) in this response is considered in other chapters.

Several cytokines, chemokines and reactive nitrogen and oxygen species released by activated Kupffer cells are thought to have local effects, modulating microvascular responses and the functions of hepatocytes and stellate cells.[221] Although Kupffer cells can express class II histocompatibility antigens[222] and can function *in vitro* as antigen-presenting cells, they appear to be considerably less efficient at this than macrophages at other sites.[223] Their principal roles in the immune response therefore appear to be antigen sequestration by phagocytosis and clearance of immune complexes.[224]

Kupffer cells exhibit an important plasticity, expressing different phenotypes depending on the local metabolic and immune environment. Therefore they can express the proinflammatory M1 phenotype or several M2 phenotypes involved in the resolution of inflammation and wound healing. Kupffer cells can play a protective role through their tolerogenic phenotype, as in some drug- and toxin-induced liver injury, but can also shift to a pathologically activated state and contribute to chronic inflammation in various liver diseases. Impairment of their activity as scavengers of hepatic blood can contribute to pathogens entering the systemic circulation and thus systemic infection.[225]

Strong evidence from bone marrow and liver transplant studies show that Kupffer cells are derived at least in part from circulating monocytes.[226,227] However, Kupffer cells are capable of replication, and their local proliferation accounts for a substantial part of the expansion of this cell population in response to liver injury.[228,229]

Kupffer cells can proliferate in response to T-helper type 2 (Th2) inflammatory signals.[230] Furthermore, Kupffer cells appear in the fetal liver of the mouse before there are circulating monocytes, and evidence indicates that they are derived from primitive macrophages which first appear in the yolk sac.[231] These data suggest that Kupffer cells may have a dual origin.

Liver-associated lymphocytes

Liver lymphocytes include cells of the adaptive immune system (T and B lymphocytes) and innate immune system: natural killer (NK) and natural killer T (NKT) cells.[232] NK cells constitute 31% of hepatic lymphocytes and NKT cells, 26%. The liver is thus particularly enriched with cells of the innate immune system compared with other parenchymal organs. While this has immediate value for the liver dealing with foreign antigens released from the gut into the splanchnic circulation, it also means that the liver is well equipped for an immune response to neoantigens expressed within its substance.[233,234]

It has been estimated that an average normal human liver contains approximately 1×10^{10} lymphoid cells.[235] These lymphocytes are predominantly located within portal tracts but are also found scattered throughout the parenchyma, where they are found in loose luminal contact with Kupffer cells or LSECs. In the peripheral blood, 85% of the lymphocytes are T and B cells, which possess clonotypic antigen-specific receptors. In contrast, up to 65% of lymphocytes within the liver are NK cells, T cells expressing γδ T-cell receptors (TCRs) and T cells expressing NK molecules (NKT cells); clonotypic T and B cells are only a minority of intrahepatic lymphocytes.

Because of their particular location in the space of Disse in the intercellular recess between adjacent hepatocytes, hepatic NK cells were first described as 'pit cells' in the rat liver in the 1970s.[236] Recent studies have shown that two distinct NK-cell subsets are present in mouse and human livers: conventional NK cells and liver-resident NK cells.[237] The latter, which are those appearing morphologically as the pit cells, are large granular lymphocytes, usually also in contact with Kupffer and endothelial cells; some lymphocyte microvilli can protrude into the space of Disse.[234,238] They present characteristic dense granules, visible on EM, as well as a few other organelles usually concentrated on one side of the cell.

The liver contains the largest number of γδ T cells in the body.[235,239] γδ T cells are also found in the skin, gut and respiratory mucosa and in the pregnant uterus and accumulate at sites of infection.[240] They secrete various cytokines and can lyse antigen-bearing target cells. Their precise function in the liver is not yet clear.

Hepatic NKT cells coexpress TCR and NK activating and inhibitory receptors. They can be further subclassified on the basis of their expressing various types of TCRs and various NK receptors. Functional studies have demonstrated that hepatic NKT cells have numerous cytotoxic activities and produce multiple cytokines.[241] Their major role therefore may be to effect local immunological reactions through the production of cytokines.

The relative frequency of the various subpopulations of liver-associated lymphocytes varies between individuals, probably reflecting each individual's immunological status; this in turn is affected by genetic background and both previous and current antigen exposure. The hepatic environment itself also may influence the distribution of the various subsets. The presence of these cells in such large numbers indicates that they must serve important roles in normal hepatic immune responses and immune homeostasis.

Hepatic stellate cells

Originally identified by Boll and von Kupffer in the 1870s, hepatic stellate cells were largely ignored until 1951, when Ito[242] described their morphological features on light microscopy. They were subsequently referred to under a variety of terms—Ito cells, hepatic lipocytes, fat storing cells, stellate cells and para- or perisinusoidal cells.[243,244] The now-accepted nomenclature for them is *hepatic stellate cells* (HSCs).[245] Their pathobiology and their role in regulating microcirculation, vitamin A storage and synthesis of extracellular matrix components, as well as their contribution to liver inflammation and immunology, have been extensively reviewed.[246–250]

HSCs account for about 5–8 % of cells in the normal liver[251] and are regularly spaced along the sinusoids (~40 µm from HSC nucleus to HSC nucleus).[252] There are about 5–20 HSCs per 100 hepatocytes. Despite their relatively sparse distribution, HSCs have long, thin cytoplasmic processes which have a perisinusoidal distribution in the space of Disse[248], organized into a sheath surrounding the sinusoid network. Indeed, the close proximity of HSCs with LSECs resembles that of pericytes in capillaries. The cell body of HSCs is often located in the interhepatocytic recess.

Quiescent state

HSCs in the normal liver are 'quiescent', having received no stimuli to transform to their 'active' myofibroblastic state. The relationship of HSCs with other cells in the normal sinusoidal environment is closely related to their normal morphological and functional features, as follows:

- The subendothelial processes of HSCs terminate as very thin, thorn-like microprojections which contact the plasma membrane of the hepatocyte microvilli and also the LSECs. Thus the HSCs adhere to the LSECs and hepatocytes, each strongly influencing the behavior of the other cell types in response to hepatic injury and stimulation of hepatic regeneration.[253]
- Autonomic nerve endings in the space of Disse come into contact with HSCs,[117,254] and they respond to α-adrenergic stimulation and contract in response to several vasoactive substances, such as prostaglandin, thromboxane A2, endothelin-1, substance P and angiotensin II. These morphological and functional features support the role of HSCs in the haemodynamic regulation of sinusoidal blood flow.
- In their quiescent phenotype, HSCs store vitamin A (80% of the body's vitamin A) in intracellular lipid droplets. Dietary retinyl esters reach hepatocytes in chylomicron remnants, which pass from the sinusoidal lumen through the endothelial fenestrae and are taken up by hepatocytes. Most of the endocytosed retinol is rapidly transferred to the HSCs for storage.[256] About 75% of HSCs in the normal liver contain lipid droplets, which are intermingled with the usual cytoplasmic organelles and generally abundant cytoplasm.[255] Similar stellate cells, which also share the property of vitamin A storage, exist in the human pancreas,[257] and evidence also indicates a more widespread distribution involving lung, kidney and gut.[252,257]
- In accordance with the needs of the host organism, retinol (vitamin A) is released to peripheral target tissues in the form of retinol-binding protein (RBP) complexes.

In normal conditions, HSCs are not readily visualized on light microscopy (Fig. 1.18 A), but the lipid droplets rich in vitamin A can be demonstrated by fluorescence microscopy of frozen tissue when excitation light of 328 nm is used, or by gold chloride impregnation of tissue during fixation. HSCs can be visualized by glial fibrillary acidic protein (GFAP) in their quiescent state[258,259] (Fig. 1.18 B), but this staining is not always reproducible; cellular retinol-binding protein 1 (CRBP1) immunostaining is the best marker to visualize HSCs in their quiescent as well as activated state.[260] When prominent in some pathological conditions such as hypervitaminosis A,[261–263] HSCs can be visualized in routine histological sections without special stains.

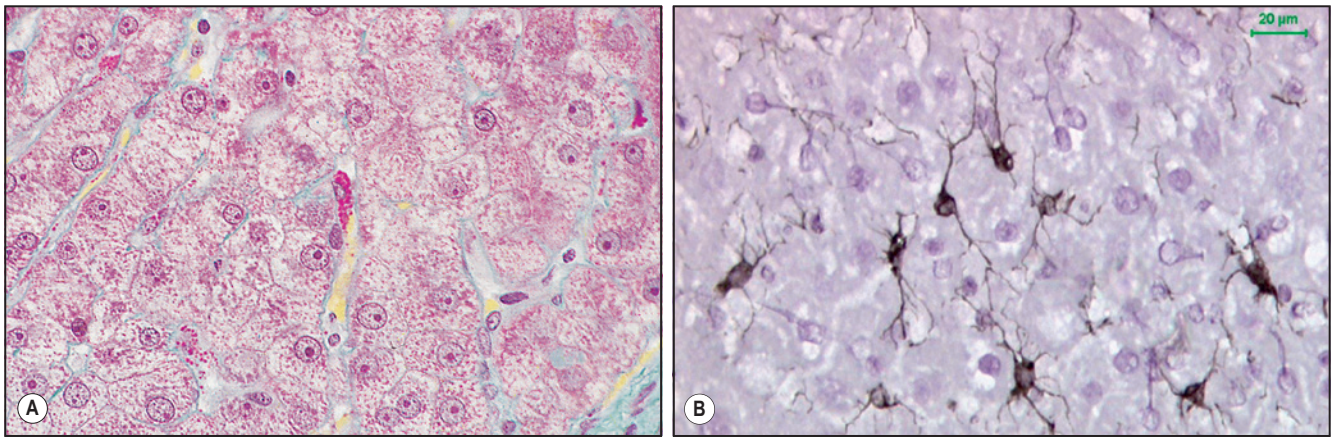

Figure 1.18 Hepatic stellate cells (HSCs). **A,** HSCs may occasionally be seen on optical microscopy; the fat globules are phloxinophilic in this section stained by Masson trichrome, and the perisinusoidal location of the cell is readily appreciated. **B,** GFAP (glial fibrillary acidic protein) immunoreactivity of HSCs in normal rat liver. *(Courtesy of Dr. Liena Zhao.)*

Activated state

HSCs belong to the myofibroblast family. Although in normal conditions, hepatocytes and LSECs are capable of synthesizing types I, III and IV collagen and fibronectin,[249,261] quiescent HSCs are actually the main sinusoidal cells involved in synthesis of both different collagen types and various glycoproteins (e.g. fibronectin, laminin, tenascin, entactin), as well as metalloproteinases and their inhibitors. Under conditions of stress or injury, HSCs change their phenotype by losing their lipid droplets and acquiring a myofibroblastic phenotype. In such activated condition, a key feature of HSCs is upregulation of α-smooth muscle antigen (SMA), imparting to HSCs a contractile phenotype.[249,264] This activation process exhibits marked morphological and functional modifications through a state of 'transitional cells' characterized by the progressive decrease of lipids and increase in RER and filamentous skeleton toward the final state of myofibroblastic cell.[265] Secretions of all the extracellular matrix components are increased by activated HSCs during the process of liver fibrosis. The mechanisms of HSC activation and the consequences are discussed in the later section on fibrosis.

Recent studies have emphasized the major role of HSCs in liver immunology for several reasons: First, vitamin A represents a key factor in regulation of immune responses. Second, HSCs can act as antigen-presenting cells. Third, they produce many soluble inflammatory mediators and promote immunosuppressive responses in homeostasis (induction of regulatory T cells, T-cell apoptosis, inhibition of cytotoxic CD8 T cells). Thus, HSC activation has a major role not only in hepatic fibrogenesis, but also in regulation of hepatic inflammation.

Functions

Given the previous discussion, the five main functions of HSCs in the liver can be summarized as follows[249,266,267]:

1. HSCs are the major site of storage for vitamin A.[249]
2. HSCs play a major role in remodelling of extracellular matrix by production of matrix components and matrix metalloproteinases, both in the normal liver and when activated in the process of liver fibrogenesis.[249,252,267]
3. HSCs act in a pericyte-like manner around the sinusoids and may have a role in the control of microvascular tone in the normal liver.[268–270] Activated HSCs have a definite contractile role in the injured liver and respond to vasoactive agents such as endothelin-1 and nitric oxide.[269,270]
4. HSCs play a role in hepatic regeneration both in the normal liver and in response to liver injury.[247] They express hepatocyte growth factor (HGF),[247,271] which can be enhanced in response to insulin-like growth factor 2 (IGF2).[272]
5. HSCs are central regulators of inflammation and immunity, maintaining homeostasis in the liver.[250]

Lastly, it is important to consider that HSCs are 'plastic cells', since their activated phenotype can revert to quiescence on cessation of proinflammatory stimulation.[273]

Space of Disse

The space of Disse lies subjacent to LSECs and is the fluid space in direct contact with the basal plasma membrane surface of hepatocytes (see Fig. 1.16 A). Abundant hepatocyte microvilli project into the space and may be important in keeping the space open. This space is not normally discernible in biopsy material, but in autopsied liver the hepatocytes shrink from the sinusoids, and the space of Disse is then characteristically evident. Nevertheless, this fluid space represents 2–4% of the volume of the hepatic parenchyma.

Studies with TEM show continuity of the space of Disse with the lateral intercellular space between adjacent hepatocytes, considerably expanding the perisinusoidal fluid compartment[274] (see Fig. 1.16 B). The space of Disse thus forms an extensive and essentially unique extravascular space. As noted earlier, the sinusoidal endothelium not only is discontinuous and extensively fenestrated, but in many species (including humans) lacks a basement membrane. It is thus freely permeable to blood plasma, which enters the space of Disse and comes into direct contact with the hepatocytes and the HSCs. This extravascular plasma constitutes the immediate medium of exchange between blood and hepatocytes, whose surface area of contact is increased by the abundant microvilli. The plasma is then presumed to flow toward the hepatic veins. However, there is evidence that fluid within the space of Disse may also be taken up by lymphatic spaces in the periportal zone in a retrograde fashion, either re-entering the sinusoidal blood or continuing within lymphatic channels to exit via the hilar lymphatics. The nature of the anatomical link between

the periportal ends of the space of Disse and the lymphatics is discussed later.

As mentioned, the *extracellular matrix* (ECM) within the space of Disse is produced predominantly by the HSCs and constitutes the structural 'reticulin' framework of the liver. Hepatocytes may produce some collagen and some proteoglycans. Kupffer cells and LSECs influence the parenchymal ECM primarily through the production of cytokines which modulate the synthetic activity of the HSCs, but may themselves produce small amounts of proteoglycans. There is a gradient in the ECM in the space of Disse, with variation in amount and composition with increasing distance from the portal tracts.[275,276] Thus, laminin, collagen type IV and heparan sulphate predominate in the periportal zone, whereas in the perivenular zones, fibronectin, collagen type III and dermatan sulphate are more abundant.[275] In addition, it has been shown that fibrillin-1 is present in high amounts in the space of Disse in the absence of elastin, contrary to portal zones, where fibrillin-1 is co-localized with elastin to form elastic fibres, as in other sites (e.g. dermis or vessel walls).[277] Fibrillin-1 forms a continuous network of slender fibres in the space of Disse between HSC processes and the sinusoidal membrane of hepatocytes.

The ECM plays a major role in the normal biology of the liver, influencing hepatocyte, LSEC and HSC function. Interaction between the ECM and these cells is of fundamental importance in maintaining their differentiation, growth and function.[278–280] In contrast to activities in other organs, the ECM does not act as a diffusion barrier between the plasma and the hepatocytes.

The ECM components in the space of Disse interact with the hepatocyte and endothelial cell membranes through various surface integrins and other receptors. Hepatocytes have receptors for fibronectin, laminin and types I and IV collagen,[281–284] and they can also bind proteoglycans.[285] The proteoglycans can also act as adhesion and receptor molecules and are an important reservoir for cytokines and growth factors.[286–288]

Unmyelinated nerve fibres are distributed throughout the hepatic lobules, localized mainly in the space of Disse. These nerve endings are oriented toward LSECs, hepatocytes and mainly HSC processes.[117,254,289] Various neurotransmitters (e.g. substance P, noradrenaline, acetylcholine) are present in vesicles of these nerve endings, participating with vasoactive agents (e.g. endothelin-1, prostaglandin, nitric oxide) as well as adrenergic receptors present on HSCs in the haemodynamic regulation of sinusoidal microcirculation.

The biliary system

Architecture

The intrahepatic biliary system, discussed earlier with regard to hepatic embryology, is now examined in detail regarding the architecture of the biliary tree. First, the bile canaliculi that run between adjacent hepatocytes form a complicated intraparenchymal polygonal network. Based on rat studies, canalicular diameter increases gradually from the perivenular region to the periportal region; the diameter enlarges physiologically during periods of high bile flow.[290]

Near the portal tract interface, canaliculi drain into the canals of Hering, partly lined by hepatocytes and partly by cholangiocytes (Fig. 1.19). The canals of Hering drain into bile ductules (also termed *cholangioles*), defined as having a basement membrane and lined entirely by cholangiocytes, usually three to six in circumference. The ductules may run their course for a short distance within the periportal parenchyma or, more often, begin at the limiting plate of the portal/parenchymal interface. Ductules therefore have an obligate portal tract segment and a variable intralobular segment.

In the portal tracts the ductules join the interlobular bile ducts (Fig. 1.20), the smallest branches of which are 15–20 μm in diameter. These interlobular bile ducts are lined by a single layer of flattened cuboidal epithelium, have a basement membrane and are in turn ensheathed in the fibrous tissue of the portal tracts. As the interlobular bile ducts merge downstream, they increase in size and form larger septal ducts more than 100 μm in diameter and lined by a simple, tall, columnar epithelium with basally situated nuclei. Portal tract fibrous tissue shows some condensation around these septal ducts, but there is no well-marked concentric orientation. Table 1.1 summarizes the terminology and heterogeneity of the intrahepatic bile ducts as proposed by Desmet et al.[291] A definitive quantitative 3D study of the human biliary system has been published.[292] The larger ducts further anastomose to form intrahepatic bile ducts, 1–1.5 mm in diameter, which give rise to the main hepatic ducts. As described earlier, the intrahepatic bile ducts are supplied by an anastomosing peribiliary vascular plexus derived from the hepatic artery and draining into periportal sinusoids.

Peribiliary glands

The presence of glandular elements has been demonstrated around the extrahepatic and larger intrahepatic bile ducts[293–296] (Fig. 1.21). These peribiliary glands are of two types: (1) intramural mucous glands, which communicate directly with the bile-duct lumen, and (2) extramural mixed seromucinous glands, which form branching tubuloalveolar lobules and secretory ducts that drain into the bile duct lumen. These glands are relatively dense in the hilar bile ducts, cystic duct and periampullary region. SEM has shown that hilar ducts may also have irregular side branches and pouches in which

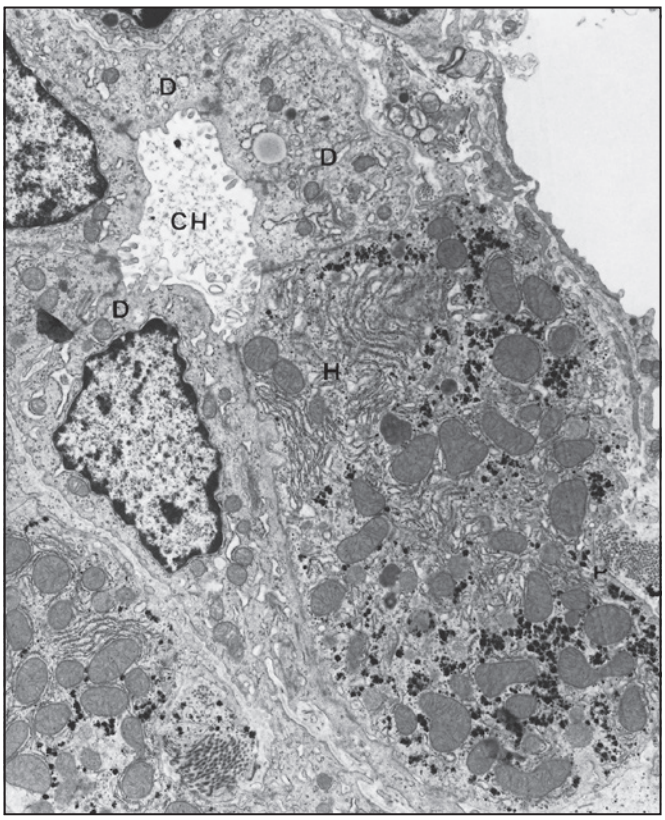

Figure 1.19 Transmission electron microscopy of a section through a canal of Hering *(CH)*, whose wall shows cholangiocytes, (ductal cells, *D*) and a hepatocyte *(H)*. (Human liver, ×9200.) *(Courtesy of Professor R. De Vos.)*

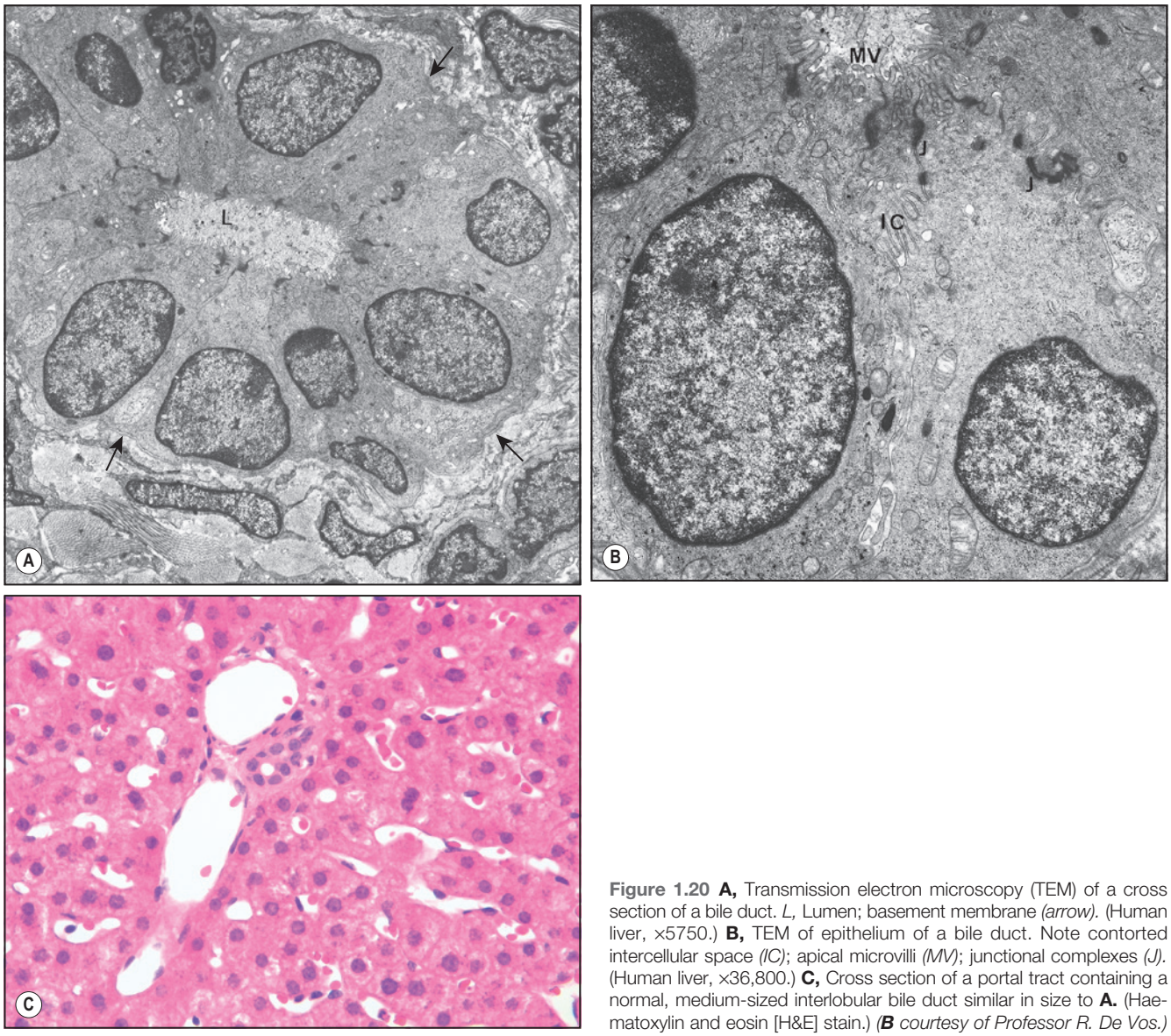

Figure 1.20 **A,** Transmission electron microscopy (TEM) of a cross section of a bile duct. *L,* Lumen; basement membrane *(arrow).* (Human liver, ×5750.) **B,** TEM of epithelium of a bile duct. Note contorted intercellular space *(IC)*; apical microvilli *(MV)*; junctional complexes *(J).* (Human liver, ×36,800.) **C,** Cross section of a portal tract containing a normal, medium-sized interlobular bile duct similar in size to **A.** (Haematoxylin and eosin [H&E] stain.) *(**B** courtesy of Professor R. De Vos.)*

bile may be stored and probably modified.[297] The peribiliary glands secrete several substances, such as lactoferrin and lysozyme. They also are stem cell niches of the biliary tree, capable of differentiating into hepatobiliary and pancreatic endocrine or exocrine cells.[298] These glands have been implicated in pathogenesis of both intrahepatic and extrahepatic cholangiocarcinoma.[295]

Cholangiocytes

Cholangiocytes account for 3–5% of the endogenous liver cell population, lining the intrahepatic and extrahepatic bile duct system. More than cells lining inert conduits, cholangiocytes modify the composition of bile during its transit through bile ducts, involving secretion and absorption of water, electrolytes and other organic solutes.[32,85,299–303] Ultrastructurally, the cholangiocyte has a prominent Golgi complex, numerous cytoplasmic vesicles and short luminal microvilli.[208] Studies in the rat suggest that 10–15% of basal bile flow is produced by ductal epithelium,[304] and the corresponding contribution in humans is estimated at 40%. Secretion is under hormonal control (secretin and somatostatin); secretin is released from the duodenum after vagal stimulation and with the presence of acid in the duodenum and stimulates the secretion of bicarbonate-rich bile.[302] The duct epithelium also secretes IgA and IgM (but not IgG), as shown immunohistochemically on human duct cells.[305] Reabsorption involves water, glucose, glutamate and urate. Bile acids are reabsorbed through biliary epithelium and are recirculated by a cholehepatic shunt pathway through the peribiliary plexus; this recycling promotes bile acid-dependent bile flow in the ducts.[76,306–308]

Extensive studies of cholangiocytes over the past 25 years have increased our understanding of both the normal formation of bile and how bile duct biology is altered during nonobstructive or obstructive cholestasis.[299,301] The following concepts merit emphasis:

- Cholangiocytes exhibit morphological and functional heterogeneity along the length of the intrahepatic biliary tree.[300,309–311] Bile ductules are lined by four to five small cuboidal cholangiocytes. In interlobular bile ducts ranging from 20 to 100 μm in diameter, cholangiocytes become progressively larger and more columnar in shape and ultimately display a primary cilium.

Table 1.1 Terminology of the biliary tree

Generation of branching	Diameter	Terminology	Remarks
Large bile ducts			
First generation	>800 μm	Left and right hepatic duct	Columnar epithelium surrounded by dense fibrous tissue and elastic fibres Peribiliary glands
Second generation	400–800 μm	Segmental ducts	Columnar epithelium surrounded by dense fibrous tissue and elastic fibres Peribiliary glands
Third generation	300–400 μm	Area ducts	Lower columnar epithelium Peribiliary glands
Other bile ducts			
Small intrahepatic bile ducts (not grossly recognizable)	>100 μm 15–100 μm 40–100 μm 15–40 μm	Septal ducts Interlobular ducts Medium sized Small	Located in conducting portal tracts Cuboidal epithelium accompanied by artery
12th generation	<15 μm	Ductules (cholangioles) Canals of Hering	May extend through limiting plate into lobule Lined partly by hepatocytes, and partly by cholangiocytes

Data from Desmet et al.[291] (1997) and Roskams et al.[35] (2004).

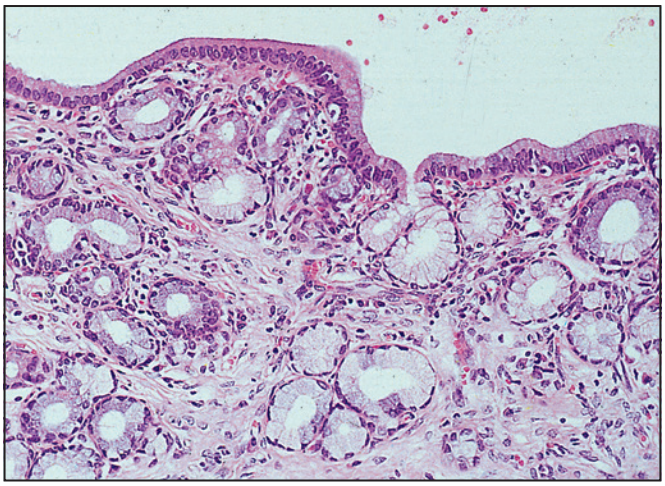

Figure 1.21 Large intrahepatic bile duct near the hilum. Note the surrounding mucous and seromucinous peribiliary glands; one mucous gland opens into the duct lumen. (H&E stain.)

- Cholangiocytes are capable of proliferating in response to liver injury or surgical reduction, a process enhanced by secretin receptor signalling and secretin-stimulated choleresis.[312] Small cholangiocytes are more resistant to liver injury than large cholangiocytes, and replicate during damage.[300]
- In turn, because of their larger cytoplasmic size, large cholangiocytes exhibit more membrane receptors, transporters and channels and are more responsive to physiologic agonists such as secretin and somatostatin for the modification of secreted bile.[297]
- Cholangiocytes are highly dynamic cells with regard to inter- and intracellular signalling, involving such mechanisms as transmembrane G protein-coupled receptors (TGR5), Farnesoid X receptor (FXR) and sphingosine 1–phosphate receptor 2 (S1PR2).[313] Bile acids are strong modulators of cholangiocyte function. The secretory activity of cholangiocytes is regulated by both cyclic adenosine monophosphate (cAMP) and calcium signalling pathways.[314]
- Cholangiocytes are responsive to innervation, as discussed later.

Accumulation of bile acids during cholestasis (obstructive or non-obstructive) may have profound effects on cholangiocytes, including stimulation of proliferation and increasing secretin responsiveness.[315] However, since cholangiocytes contain an apical sodium-dependent bile acid transporter (ASBT; official symbol NTCP2; gene symbol *Slc10a2*), they are capable of taking up bile salts from the bile duct lumen and returning them to the peribiliary circulatory plexus.[316] While the resultant *cholehepatic shunt* can further stimulate bile secretion by hepatocytes,[317] in the setting of obstructive cholestasis, bile salts can enter the systemic circulation for elimination in urine.

Cholangiocytes express receptors for epidermal growth factor (EGF), secretin and somatostatin.[318] Normally they express class I major histocompatibility complex (MHC) antigens but not class II; cytokine-induced expression of class II antigen is seen in graft-versus-host disease, allograft rejection, primary biliary cirrhosis and primary sclerosing cholangitis and may be important in the pathogenesis of the bile duct injury in these diseases. Indeed, cholangiocytes may themselves secrete proinflammatory cytokines and contribute to the destructive events that occur in autoimmune or secondary inflammatory biliary diseases.[319] Cholangiocytes express more cell–matrix adhesion molecules or integrins than hepatocytes, including $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$ and $\alpha_6\beta_4$, concurring with those expressed by most simple epithelial cells.[320] Bile duct epithelium also expresses glutamyl transpeptidase, carcinoembryonic antigen (CEA) and epithelial membrane antigen, representing other phenotypic differences from hepatocytes, the precise significance of which is as yet uncertain.[310,318] Thus the cells lining the biliary tree clearly have an extremely dynamic biology.[321]

Other constituent tissues

Extracellular matrix

The liver normally has only a small amount of connective tissue in relation to its size. Whereas in the human body, collagen constitutes

about 30% of the total protein, the corresponding figure for the liver is 5–10%. Underlying the visceral peritoneum of the liver, or *serosa*, there is a layer of dense connective tissue admixed with elastic fibres which varies in thickness from 40 to 70 µm. This constitutes the *Glisson capsule*, irregular extensions of which extend as delicate fibrous septa up to 0.5 cm into the superficial parenchyma. These anatomically normal septa produce some architectural distortion and the appearance of subcapsular 'fibrosis', which must not be misinterpreted when wedge liver biopsies are examined. Condensation of the Glisson capsule occurs at the porta hepatis, and the fibrous tissue then extends into the liver, supporting and investing the portal vein, hepatic artery and bile-duct branches, thereby constituting the portal tracts. Some extension of the capsular tissue also accompanies the large hepatic vein branches, but no fibrous sheath surrounds terminal hepatic venules, which are in direct contact with perivenular hepatocytes.

The components of the ECM are collagens, glycoproteins and proteoglycans (Table 1.2). In the normal liver parenchyma, interstitial collagens (types I and III) are concentrated in portal tracts and around terminal hepatic veins, with occasional bundles in the space of Disse. Delicate strands of type IV collagen (reticulin) course alongside hepatocytes in the space of Disse. This so-called reticulin network or framework is usually visualized by silver impregnation staining methods (Fig. 1.22). Far from being inert scaffolding, the intrahepatic ECM is a dynamic tissue compartment that has profound impact on cellular function and the hepatic response to injury.[322,323]

Collagens

Collagens are composed of three identical or similar polypeptide chains folded into a triple helix to give the molecule stability. Numerous types of collagen have been described, and types I, III, IV, V and VI are found in the liver. Types I and III account for more than 95% of the collagen in normal liver, with types IV, V and VI contributing approximately 1%, 2–5% and 0.1%, respectively.[324] The types of collagens fall broadly into two categories: fibrillar and basement membrane. Types I, III and V of the fibrillar collagens and types IV and VI of the basement membrane collagens have been identified in the liver. Types I, III and V are confined mainly to the portal tract and terminal hepatic vein wall. Type I collagen corresponds to the doubly refractile mature collagen in portal tracts and around the walls of hepatic veins and is evident in tissue sections simply by lowering the substage condenser to increase refraction. Type III collagen also is present in the reticulin framework of the sinusoids, associating with type IV collagen to form a two-dimensional (2D) lattice.[325] Type IV collagen is present in the basement membranes around bile ducts, arteries and veins, and forms the reticulin framework present in the space of Disse. Type VI collagen, in contrast, is found in the interstitial matrix of the portal tract. It is absent from basement membranes but is frequently present near blood vessels and may have a role in anchoring vascular tissue to the perivascular matrix.[326]

Table 1.2 Extracellular matrix components

Component	Normal distribution
Collagens	
Type I	Portal tract matrix, hepatic veins, points of inflection in hepatic cords
Type III	Portal tract matrix, space of Disse
Type IV	Portal tract basement membranes, space of Disse
Type V	Portal tract matrix, space of Disse
Type VI	Portal tract matrix, space of Disse
Glycoproteins	
Laminin	Portal tract basement membranes, space of Disse
Fibronectin	Portal tract matrix, space of Disse
Entactin (nidogen)	Portal tract basement membranes
Elastin	Portal tract matrix
Fibrillin	Portal tract matrix
Proteoglycans	
Heparan sulphate	Portal tract basement membranes

From Martinez-Hernandez A, Amenta PS. The hepatic extracellular matrix. I. Components and distribution in normal liver. Virchows Arch A Pathol Anat Histopathol 1993;423:1–11. Copyright Springer Science and Business Media.

Glycoproteins and proteoglycans

The collagens are intimately complexed and interwoven with glycoproteins and the proteoglycans to form the total supporting structure of the liver. The noncollagenous *glycoproteins* include laminin, fibronectin, entactin and elastin. *Laminin* is a large glycoprotein (1000 kD) produced by stellate cells and endothelial cells in the normal liver and in increased quantities by stellate cells and hepatocytes in the diseased liver.[327] Laminin is the major glycoprotein in basement membrane and interacts there with type IV collagen; small amounts are normally present also in the space of Disse.[328] Laminin promotes cell adhesion, migration, differentiation and growth[328-331] and is an important mediator of capillary formation by endothelial cells.[332–335] Laminin also is a critical regulator of the progenitor cell compartment and its response to injury.[336] The *fibronectins* represent a class of large-molecular-weight glycoproteins which exist in plasma and cellular forms. In ECM, fibronectin exists as thin filaments associated with collagen fibres.[337] Fibronectin exists in two isoforms, one of which, plasma fibronectin, is produced by hepatocytes. Fibronectin is also produced by hepatic stellate and sinusoidal endothelial cells. It mediates cell adhesion to collagen. *Entactin*, also referred to as nidogen, is a highly sulphated, dumbbell-shaped glycoprotein restricted to basement membranes and thus is generally absent from the space of Disse. *Elastin* fibres are normally scattered throughout portal tracts. Elastin is deposited in fibrous septa of the cirrhotic liver over time; the presence of elastin in fibrous septa thus provides some indication that the fibrous tissue has not been deposited recently.

The *proteoglycans* are macromolecules consisting of a central protein core to which glycosaminoglycans and oligosaccharide side chains are attached. Proteoglycans are classified according to the type of glycosaminoglycan present. They contain specific functional domains which interact with cell surface receptor molecules. The proteoglycans include heparan sulphate, chondroitin sulphate, dermatan sulphate and hyaluronic acid. Proteoglycans have a core protein with a variable number of unbranched carbohydrate side chains composed of repeating sulphated disaccharide units. For heparan sulphate, these are iduronic acid–*N*-acetylglucosamine; for dermatan sulphate, iduronic acid–*N*-acetylgalactosamine; and for chondroitin sulphate, glucuronic acid–*N*-acetylgalactosamine. Hyaluronic acid is the exception because it lacks a protein core and is formed as a nonsulphated polysaccharide from glucosamine and glucuronic acid. In the liver, heparan sulphate is the most abundant proteoglycan and is present in portal tract basement membranes.

The strong anionic charge on the proteoglycans contributes to their binding to the other constituents of the ECM. Heparan sulphate in particular modulates the proliferative and secretory characteristics of mesenchymal cells[338] and is an essential extracellular component of basement membranes. The proteoglycans can function as adhesion

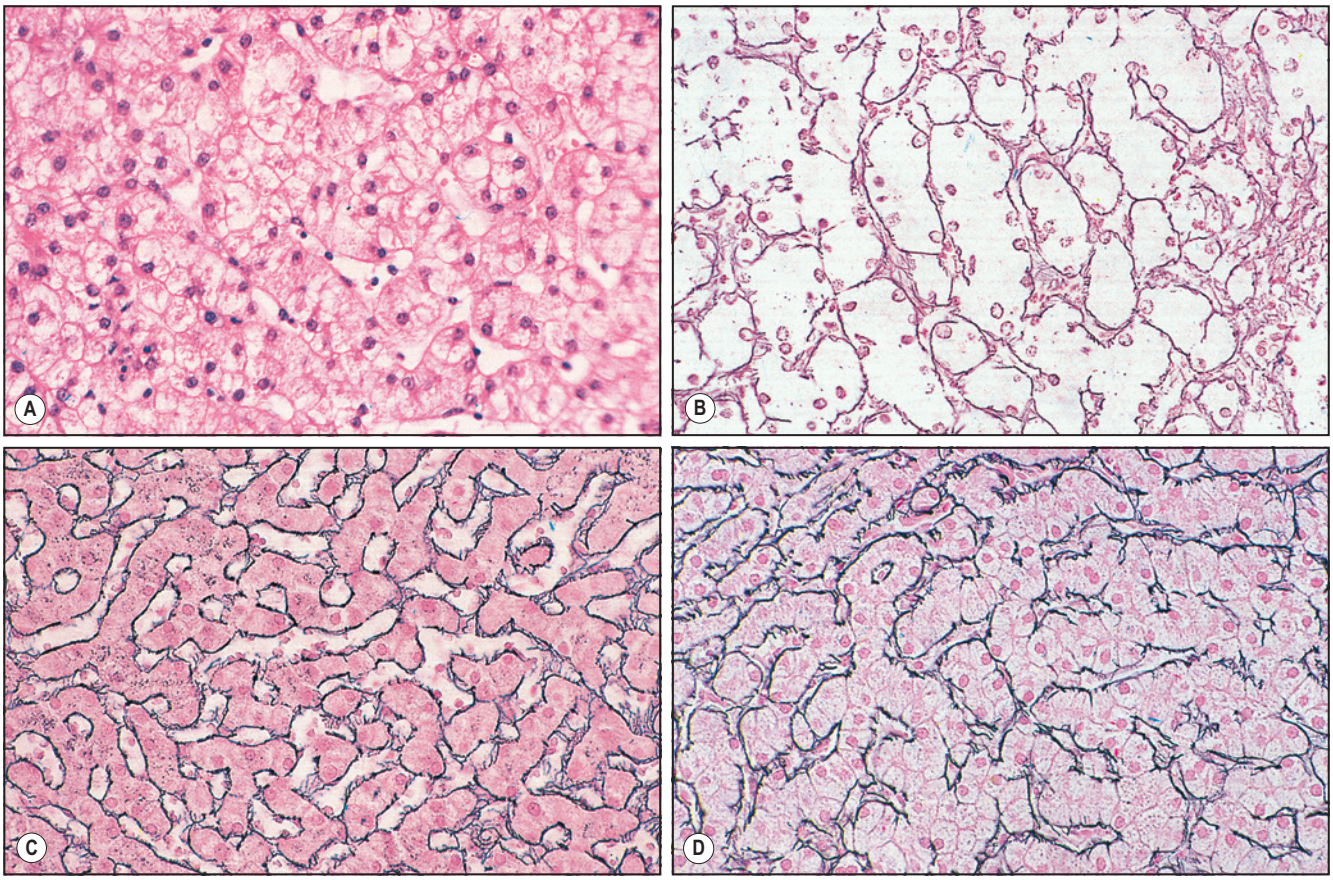

Figure 1.22 **A,** Liver biopsy from a child of 17 months. Note the twin-cell liver plates; these are better shown on a reticulin preparation at the same magnification in **B,** in which the nuclei are seen in a perisinusoidal position. **C,** Adult liver showing normal single-cell liver plates with centrally placed nuclei. **D,** Adult liver at the same magnification as **C,** showing a regenerative response with twin-cell liver plates. As in **B,** the nuclei tend to be in a perisinusoidal position; there is also rosette formation. (**A,** H&E stain. **B, C, D,** Gordon-Sweet reticulin stain.)

molecules[339] and can act as receptor molecules on cell surfaces.[340] As such, they have been identified as an important reservoir for cytokines and growth factors, by binding up these diffusible substances within the ECM. Remodelling of the ECM, as during regeneration, can release substantial quantities of cytokines and growth factors.[341] Conversely, deposition of the ECM during fibrogenesis can increase the reservoir of stored cytokines and growth factors within the liver.

Function of extracellular matrix in the liver

In addition to providing the structural framework of the liver, evidence indicates that the complex matrix in the subendothelial space of Disse is essential for maintaining the integrity and function of hepatocytes and sinusoidal cells. In a general sense, the ECM provides the framework for regulation of cellular polarization, migration, proliferation, differentiation, cell survival and cell death.[322] This occurs through signalling triggered by interaction between the ECM and cellular receptors. For example, hepatocyte differentiation in a polarized state requires an ECM rich in laminin and containing type IV collagen, heparan sulphate and entactin.[342,343] Contact only with collagen matrix leads to loss of hepatocyte polarity and dedifferentiation, as measured by expression of hepatocyte-specific proteins.[330,344,345] Conversely, in liver injury that induces degradation of the collagen matrix by the metalloproteinases, coupled with the production of a laminin-rich ECM, laminin maintains the progenitor cell phenotype and inhibits hepatocyte differentiation.[336] Therefore, liver injury that disrupts the sinusoidal subendothelial matrix could result in loss of differentiated hepatocellular function.

The sinusoidal subendothelial matrix also helps to preserve the functions and activities of endothelial cells and stellate cells. When stellate cells are maintained on a basement membrane-like gel, they remain spherical with extensive filamentous outbranchings and do not proliferate.[346] When cultured on abnormal substrates, stellate cells transform into myofibroblasts and proliferate. Similarly, the fenestrated LSECs lose their fenestrations in association with alterations in the ECM.[332] These events initiate a vicious cycle of reduced porosity of the sinusoidal barrier, impaired movement of solutes and macromolecules into and out of the space of Disse, and thus hepatocellular damage and impaired systemic homeostasis.

Cell–matrix interaction

Receptors for ECM components have been identified on hepatocyte membranes.[347] These include a family of receptors, the *integrins,* which possess a recognition site for molecules containing the tripeptide sequence arginine–glycine–aspartic acid (RGD). Laminin, fibronectin, entactin, tenascin and type I collagen are all known to have RGD sequences. Hepatocytes have integrin receptors for fibronectin and type I collagen[348,349] and nonintegrin receptors for laminin and type IV collagen.[350,351] Hepatocytes also bind proteoglycans, and this binding can be saturated, implying a receptor-mediated interaction.[352] The ECM serves as a binding reservoir for

key fibrogenic cytokines (e.g. TGFβ, TNFα, PDGF, IL-6) and growth factors (e.g. HGF).[353] Release of cytokines by the matrix reservoir facilitates rapid activation of stellate cells, even before cytokine synthesis is upregulated. Exposure of stellate cells to RGD motifs in collagen exposed by the action of matrix metalloproteinase action also stimulates their transition from the quiescent to active state.[354] This is yet another example of the critical interactions between the ECM and the cells within the liver.

Stiffness

The stiffness of the parenchymal ECM has garnered intense interest in recent years, partly because of the correlation between stiffness and stage of hepatic fibrosis. This has promoted the use of noninvasive methods for assessing hepatic stiffness. More fundamental is the influence of matrix stiffness on liver biology. The stiffness of normal liver ECM is approximately 3000 pascals (Pa). Hepatocyte differentiation and function are optimal when in contact with matrix of this stiffness; hepatocytes have difficulty maintaining their biosynthetic function *in vitro* as matrix stiffness increases to 4600 Pa.[355] Hepatic stellate cells are mechanotransductive cells, in that they are anchorage-dependent cells which sense the mechanics of their surroundings by pulling and pushing on the ECM, generating intracellular signals.[356] Alterations in ECM that lead to its 'stiffening' promote HSC myofibroblastic transformation,[357] further promoting intrahepatic fibrogenesis.[358] Alterations in ECM stiffening during hepatic fibrogenesis are nonhomogeneous, differing between periportal and perivenular areas and thus differentially regulating the proliferation of hepatocytes and biliary epithelial cells.[359] This has relevance both for hepatic recovery after damage and for tumorigenesis in the liver.

Lymphatics

The liver is the largest single source of lymph in the body, producing 15–20% of the overall total volume and 25–50% of the thoracic duct flow.[360] Hepatic lymph has an unusually high protein content (85–95% of that in plasma) and a high content of cells, about 80% of which are lymphocytes and the remainder macrophages. Indeed, it has been calculated that in the sheep, more lymphocytes migrate through the liver in the lymph than through any other nonlymphoid organ, and that about 2×10^8 macrophages leave the liver in lymph each day.[361]

The terminal twigs of the intrahepatic lymphatic tree are found in portal tracts as a fine, valved plexus of flattened endothelial tubes, associated with terminal branches of the hepatic artery (Fig. 1.23). Traced centripetally toward the porta hepatis, the lymphatic plexus enlarges and remains primarily periarterial, although in the larger portal tracts it becomes associated also with portal vein branches and bile duct tributaries, adding a fourth element to the traditional 'portal triad'. Similar but much smaller and functionally less important lymphatic plexuses are associated with the hepatic vein branches. A third plexus, found in the capsule, forms significant anastomoses with intrahepatic lymphatics. Most of the collecting lymphatics leave the liver at the porta hepatis and drain into hepatic nodes located along the hepatic artery and then to coeliac nodes. There are other important efferent routes: from the falciform ligament and superior epigastric vessels to the parasternal nodes, from the bare area to posterior mediastinal nodes and from the visceral surface to the left gastric nodes. As efferent collecting lymphatics leave the liver, their walls suddenly thicken through the acquisition of a muscle layer.[362,363] The importance of the anastomoses between intrahepatic and capsular lymphatics is evident when hepatic venous pressure is increased. Production of hepatic lymph then greatly increases, with protein content identical to that of plasma, indicating unrestricted leakage of protein into the space of Disse.[364] The capsular efferent lymphatics

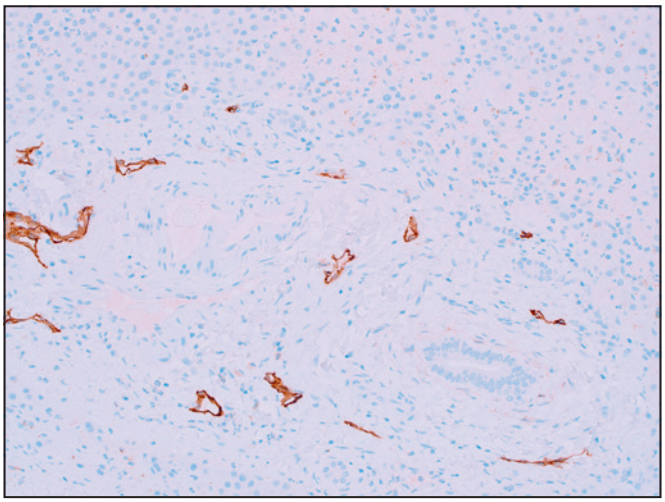

Figure 1.23 Lymphatic vessels in a medium-sized portal tract in human liver. The lymphatic endothelium is selectively labelled by immunohistochemistry using the monoclonal antibody D240.

enlarge in response to the increased lymph flow, and exudation of excess lymph from the capsular plexus forms protein-rich ascitic fluid.[365]

The function of lymphatics is to drain excess fluid and protein from the interstitial spaces of an organ. In the liver the interstitial space of Disse is the most prominent, and it is assumed that hepatic lymph is mainly formed there, with a small supplement from the peribiliary capillary plexus in the portal tracts. A protein-rich filtrate is produced in the space of Disse because of the free permeability of the sinusoidal endothelium and the consequent absence of a colloid osmotic block.[366,367] A protein-poor filtrate is formed by the less permeable peribiliary capillaries, which may dilute the protein of the sinusoidal filtrate.[360]

The route followed by interstitial fluid formed in the space of Disse to its entry into the first-order lymphatic plexus has long been controversial.[360,367,368] It is agreed that lymphatic capillaries are absent within the parenchyma and that there are no direct channels connecting the space of Disse and primary lymphatics within portal tracts. Some have suggested that 'endothelial massaging' by blood cells may be of importance in causing retrograde fluid movement in the space of Disse.[369,370] In mice, Mehal et al.[371] documented retrograde migration of fluorescent-labelled lymphocytes within the space of Disse toward the parenchymal:portal tract interface. Henriksen et al.[366] suggested that, as in other tissues, the terminal lymphatics in the portal tracts had anchoring filaments between opposing endothelial cells which regulated the direction of flow from the interstitial space into the lymphatics.

Electron microscopy studies have established the following pathway using natural markers (precipitated lymph protein and chylomicrons) and artificial tracers injected intravenously (horseradish peroxidase, ferritin, pontamine blue and monastral blue).[368,370] Fluid that formed in the space of Disse escapes at the periphery of the portal tract through gaps between hepatocytes of the limiting plate, either independent of blood vessels or alongside terminal blood vessels penetrating the limiting plate. These gaps contain hepatocyte microvilli, delicate 'wicks' of collagenous fibres and occasional slender processes of portal tract fibroblasts, extending into the parenchyma from the periportal space of Mall. Here, long, flattened processes of fibroblasts form discontinuous linings of 'spaces' which contain tracer material, occasional lymphocytes and macrophages, and

collagen bundles. These 'spaces' are not true lymphatics, since they lack an endothelial lining, but they appear to function as prelymphatic channels, leading fluid toward the terminal twigs of the lymphatic tree. The fluid then enters into portal tract lymphatic vessels, to travel down the portal tree and exit through lymphatic vessels in the porta hepatis.

Hepatic nerves

The liver is innervated by sympathetic and parasympathetic nerve fibres, with innervation of the ductal plate, bile ducts, peribiliary glands, portal veins and hepatic arteries early in human fetal life (Fig. 1.24). The role of hepatic innervation was a source of puzzlement because the orthotopically transplanted liver, which has no innervation, functions well in the recipient.[372,373] Recent studies have now made clear that hepatic innervation has considerable significance under specific conditions.

Autonomic nerve fibres reach the liver in two separate but intercommunicating plexuses around the hepatic artery and portal vein and are distributed with branching vasculature.[374,375] The nerve fibres include preganglionic parasympathetic fibres derived from the anterior and posterior vagi and sympathetic fibres which are mostly postganglionic with cell bodies in the coeliac ganglia, and which receive their preganglionic sympathetic connections from thoracic spinal segments T7–T10. The hilar plexuses also include visceral afferent fibres and some phrenic nerve fibres, probably afferent in character.[375] Immunohistochemistry (IHC) studies of human liver, using antibodies to common neural proteins such as protein gene product (PGP) 9.5 and N-CAM, have shown that nerve fibres not only are present around vascular structures in portal tracts but extend into the parenchyma, running along the sinusoids. Fluorescence histochemistry[376] and IHC using antibodies to dopamine–β-hydroxylase and tyrosine hydroxylase[377] have shown that the majority of intrasinusoidal fibres are sympathetic; many contain neuropeptide tyrosine (NPY), a regulatory peptide commonly found in adrenergic nerves. Unmyelinated nerve fibres can be seen in the space of Disse using TEM.[307] They are frequently surrounded by Schwann cell processes, but a few bare nerve endings or varicosities are found in close apposition to hepatocytes or HSCs. Synaptic clefts have been identified at points of contact, suggesting direct innervation of these cells, but true synapses are not found.[254]

An extensive cholinergic (parasympathetic) network is found in rat liver.[378] In human liver, Amenta et al.[379] described parasympathetic cholinergic innervation of portal tract vessels with only limited innervation of the parenchyma. IHC studies have also identified intrahepatic fibres containing two neuropeptides—substance P and calcitonin gene-related peptide (CGRP)—which are commonly found in afferent nerves.[380,381] Such afferent fibres may be involved in chemo- and osmoreception as well as in vasomotor regulation.[375] It seems likely that the liver would have sensory receptors since it is exposed to the nutrient and solute load delivered via the portal circulation from the gut.[382,383]

Within the parenchyma, release of neurotransmitters from the intrasinusoidal sympathetic fibres may modulate hepatocyte, LSEC and HCS function.[384] Adrenergic nerves play a role in the control of hepatocyte carbohydrate and lipid metabolism and in intrahepatic haemodynamics and response to metabolic stress,[385] including helping to maintain hepatic glucose homeostasis even when insulin regulation of hepatic glucose uptake is disrupted.[386] Beyond that, stimulation of hepatic sympathetic nerves *in vivo* produces hyperglycaemia. This effect is caused by enhanced glycogenolysis in hepatocytes and appears to be under α-adrenergic control.[385] The regulation of hepatic carbohydrate metabolism by sympathetic nerve fibres may be enhanced further by gap junctional communication between hepatocytes.[387]

Bioulac-Sage et al.[254] have speculated that adrenergic nerves may induce contraction in HSCs, thereby regulating intrasinusoidal blood flow. This may have relevance to evidence from experimental animals and humans,[388] that the liver's normal response to hypovolaemic shock is impaired by denervation, and this may result in hepatic ischaemic injury. Loss of intrasinusoidal nerve fibres in the cirrhotic liver may contribute to impaired metabolic function.[389] Whether this loss accounts for some of the abnormalities of portal blood flow is not clear.[389–391]

Within portal tracts, the neurobiology of cholangiocytes also has garnered considerable interest.[301] Cholangiocytes express the M3 acetylcholine (ACh) receptor. Acetylcholine, acting on M3 receptor subtypes, potentiates secretin-induced choleresis and can stimulate cholangiocyte proliferation. Pharmacological or surgical denervation can induce cholangiocyte apoptosis, suggesting that adrenergic innervation has a key role in regulating cholangiocyte proliferation during regeneration.[392] Cholangiocytes may also express receptors for the neurotransmitter histamine (the G-protein-coupled H3R receptor), for serotonin, and for the α-type calcitonin gene-related peptide 1 (α-CGRP1), all of which may influence cholangiocyte proliferation.[297] Recognizing that the cholangiocyte compartment of bile ductules and canals of Hering contains putative stem cells with evidence for neuroresponsiveness.[293,294]

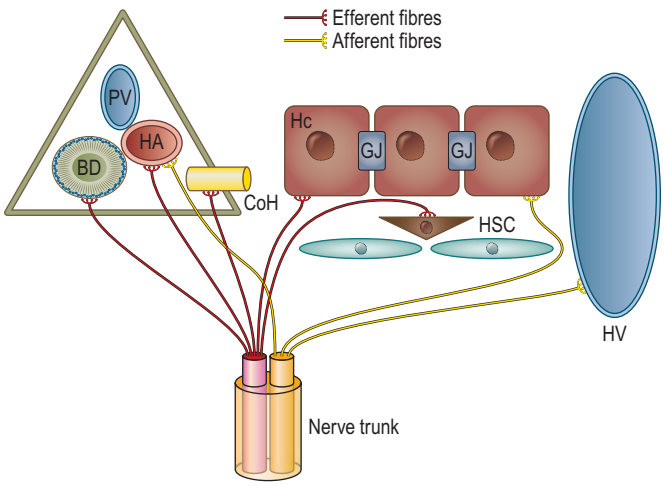

Figure 1.24 Schematic representation of hepatic afferent and efferent innervation. *BD*, Bile duct; *PV*, portal vein; *HA*, hepatic artery; *CoH*, canal of Hering; *Hc*, hepatocyte; *GJ*, gap junction; *HSC*, hepatic stellate cell; *HV*, hepatic vein.

General concepts of liver injury and repair

The morphology of liver disease reflects the convergent influences of liver damage and liver recovery. The liver is vulnerable to a wide variety of metabolic, toxic, microbial, circulatory and neoplastic insults. However, the liver has a remarkable capacity for self-repair, including complete restitution of liver mass after loss, from either necroinflammatory events or surgical resection. An understanding of liver pathology requires knowledge of the causes of liver damage and the mechanisms by which the liver responds. Table 1.3 presents the fundamental causes of liver injury, which fall into the general classes of infectious, immune mediated, drug- and toxin-induced hepatotoxicity, metabolic, mechanical and environmental. This table