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## Preface

The liver consists of different cell types including hepatocytes, endothelial cells, stellate cells, Kupffer cells, pit cells, and bile duct cells. Hepatocytes, the parenchymal cells, account for approximately 80% of the liver mass. Although other hepatic cells play a significant role in various aspects of liver physiopathology, hepatocytes exhibit unrivaled complexity and diversity of functions. They produce the majority of circulating plasma proteins including transporters (such as albumin, ceruloplasmin, transferrin, and lipoproteins), protease inhibitors ( $\alpha_1$ -antitrypsin, antithrombin, and  $\alpha_2$ -macroglobulin), blood coagulation factors (fibrinogen, prothrombin, factors V, VII, IX, X, etc.), and modulators of immune complexes and inflammation (complement C3, C-reactive protein). Hepatocytes control the homeostasis of fuel molecules such as glucose/glycogen and fatty acids including triglycerides as well as other essential compounds such as cholesterol, bile acids, and vitamins A and D. They metabolize amino acids, metals such as copper and iron, and endogenous compounds such as heme and bilirubin. In addition, hepatocytes play a critical role in detoxifying xenobiotics such as diet and environmental pollutants (plant, fungal, and animal toxins, pesticides, herbicides, derivatives of domestic and industrial combustions, organic solvents, dyes, preservatives, etc.) and, more importantly, drugs. Hence, hepatocyte function strongly impacts on the pharmacokinetics, side effects, and toxicity of drugs (1, 2).

As highly differentiated cells, hepatocytes rarely divide in the adult individual under normal (healthy) conditions. However, it is known since antiquity that the liver possesses a remarkable ability to regenerate after partial hepatectomy. This process of regeneration is primarily dependent on the proliferation of hepatocytes and other hepatic cell types, as documented by numerous studies in rodent models (3). Although partial hepatectomy aimed at treating some liver pathologies may be the source of serious failure (4), it is certainly not the primary cause of liver injury in mankind. Indeed, the major etiologic agents of liver diseases are xenobiotics (such as amatoxins, carbon tetrachloride, and cyanides), drugs (acetaminophen, isoniazide, halothane, estrogens, etc.) (5, 6), alcohol (7–9), hepatitis A, B, C, D, and E viruses (10–14), and immune and genetic disorders (15–17). In a variety of human liver diseases, notably in the cirrhotic stage, proliferation of senescent hepatocytes is inhibited. This results either from telomere shortening, chronic inflammation, presence of growth factors, and presence of DNA-damaging agents (reactive oxygen species and nitrogen species) or from combinations of these different agents (18). Under these conditions, the liver regeneration relies on the emergence of a heterogeneous population of small poorly differentiated bipotent progenitor cells, named oval cells in rodents (19) and liver progenitor cells (LPC) in man (20). The recruitment of LPC in the diseased liver is marked by the ductular reaction and increases with the extent of liver injury and inflammation (21–23). These progenitors, the origin of which is still a matter of debate, accumulate in the portal or periportal zones of the liver acinus (canal of Hering), invade the parenchyma generally in the form of neoductules and differentiate into mature hepatocytes and cholangiocytes.

It is not surprising that these exceptional functional, metabolic, and proliferative properties of hepatocytes have been the object of a tremendous interest from the scientific community. Hence, numerous studies have been carried out in animal hepatocytes (mostly rodents). However, it is now evident that species specificity is an important factor (even within the rodent species), so that direct investigations on *human* hepatocytes are mandatory to avoid risky extrapolations from animal studies (24). In addition, the possibility of using human hepatocytes for the biotherapy of liver diseases has generated a huge interest within the last decade. The emphasis in this volume has therefore been placed on *human* hepatocyte models (although data on animal hepatocytes are presented in some chapters), but I believe that the information provided will be useful for those working on hepatocytes from other species.

The aim of this volume is to provide the reader with methods, technical protocols, and review chapters focusing on selected areas of hepatocyte biology including isolation, culture, differentiation and stem cells, and hepatocyte use in clinical, basic, and applied research. Here is a brief survey of the content of this volume: A number of hepatocyte culture models have been designed, developed, and improved in order to maintain these cells in a high level of differentiation (*see Chapters 1–3, 6, 7, and 23*), while intense efforts have also been placed on the cryopreservation of these precious cells (*see Chapters 4 and 5*). Hence, the primary culture of adult human hepatocyte has become the gold standard model in different fields such as endogenous compound metabolism (*see Chapters 19 and 22*), drug/xenobiotic metabolism and transport (*see Chapters 1 and 15–18*), drug side effects (*see Chapters 15 and 16*), and drug toxicity (*see Chapter 21*) (25, 26). Interestingly, recent developments have led to the discovery of a human hepatoma cell line named HepaRG that, in contrast to any other existing cell lines, does differentiate *in vitro* to hepatocyte-like cells that exhibit a series of phenotypic markers close to those observed in normal human hepatocytes, notably in terms of detoxication (*see Chapters 1, 13, and 20*) (27). Further investigations point to other applications of primary hepatocytes in the field of virology (*see Chapters 24 and 25*) and liver biotherapy including hepatocyte transplantation and bioartificial liver devices (*see Chapters 2, 10, 28, and 29*) (28–30). Although isolation of hepatocytes from the human liver does not represent a challenge any more, the dramatic shortage of human liver of adequate quality for this purpose is now a real problem. It has therefore become mandatory to develop new alternative sources of human hepatocytes. The possibility to generate a wide diversity of tissue-specific cells from the differentiation of adult and embryonic stem cells, including hepatocytes, represents promising opportunities (31). Indeed, recent publications reveal that hepatocyte-like cells can be generated from the differentiation of intrahepatic progenitor cells, embryonic stem cells, adult multipotent progenitor cells, hematopoietic stem cells, mesenchymal stem cells, and induced pluripotent stem cells (*see Chapters 8–12 and 14*) (32). Moreover, animal studies suggest that progenitor cells could be the biotherapeutic agents for the treatment of liver disease in the near future (*see Chapters 10, 26, and 27*) (33). In conclusion, this volume will be useful to those who are currently using or envisaging to use human (or animal) hepatocytes to investigate any aspect of liver physiopathology or who are interested in the liver development and/or liver stem cells and liver biotherapy.

Unfortunately, it has not been possible to cover all the contributions of hepatocytes to liver physiopathology nor to avoid some overlaps between chapters. I would like to express my sincere apologies to those readers who may regret such omissions or redundancies. Yet, it is also important at this stage to emphasize what has been *voluntarily omitted* in this volume, i.e., hepatoma cell lines such as HepG2, Huh-7, and Hep3B and derived

cellular clones. Such cell lines are being used routinely in basic research for investigating different aspects of liver physiopathology including gene regulation, virus replication, endogenous metabolism, and cell cycle control. Although these cell lines represent very useful research tools and allow the gathering of valuable and important information, they are still too often improperly referred to as *human hepatocytes*. Such a confusing statement must be avoided. Indeed, these cells *are not hepatocytes*. They are dedifferentiated and exhibit abnormal hepatic phenotype with deregulated proliferation, defects in gene expression, perturbed signaling pathways and host anti-viral responses, deficient endogenous and xenobiotic metabolism, impaired responses to cytokines, growth factors, infectious agents, etc.

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*Patrick Maurel*

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