Bilirubin is a main physiological product of heme degradation possessing important antioxidant and antiinflammatory properties. On the other hand, it could be neurotoxic during severe unconjugated hyperbilirubinemia combined with insufficiency of blood-brain barrier (neonatal jaundice). It is secreted from the body via bile and is further metabolized in the intestine. Part of the substance is reduced to urobilinoids, part is adsorbed to the intestinal content and some part could be reabsorbed back to the systemic circulation. This enterohepatically and enterosystemically circulating fraction varies in size depending on the rate of bilirubin secretion, solubility in the intestine and intensity of its intestinal metabolism. Under specific circumstances, EHC and ESC may significantly increase serum and bile bilirubin levels and influence physiological as well as pathological processes occuring in the body. Among the most important is the protective elevation of UCB levels in Gilbert syndrome subjects and dangerous increase in severity of neonatal jaundice.

In the presented thesis, the mechanisms affecting EHC and ESC of bilirubin and tools for further research in BP metabolism were investigated. The solubility of intestinal UCB is strongly decreased by addition of divalent cations. However, such approach to decrease EHC of bilirubin could be significantly harmful to metabolism of inorganic ions. The solution might be the use of insoluble matrices containing exposed divalent cations. Our study proved that feeding of hyperbilirubinemic Gunn rats with zinc methacrylate led to an important decrease in serum bilirubin levels without affection of zinc metabolism and pathologic changes in the intestine. Another important factor decreasing EHC is hydrogenation of intestinal UCB by intestinal bacteria belonging to the genus *Clostridium*. We proved that eradication of intestinal *Clostridia* of Gunn rats led to significant increase in hyperbilirubinemia while recolonization with a sole strain of *C. perfringens* capable of reducing bilirubin partially restored the bilirubin homeostasis. Bilirubin metabolism of this strain was further characterized using chromatographic methods. Urobilinogen was found to be a main product of hydrogenation of a number of substrates including mesobilirubin, bilirubin dimethylester, bilirubin ditaurate but not bilirubin bisglucuronoside. The investigation of cellular and tissue bilirubin metabolism is important for understanding of EHC and ESC of bilirubin as well as its antioxidant and toxic properties. However, due to its low cellular levels and high instability, valid analytical method for tissue bilirubin determination was unavailable. Therefore, we developed and validated a

highly sensitive and precise HPLC assay for quantification of bilirubin in cells and tissues under normobilirubinemic as well as hyperbilirubinemic conditions.

In conclusion, we found that regulation of EHC and ESC of bilirubin is a promising approach to control hyperbilirubinemia. Furthermore, we also partially characterized a metabolic pathway of intestinal bilirubin degradation. Finally, we developed a novel analytical method for further investigation of bilirubin metabolism.