GENERAL CONCLUSIONS

The micro-morphology of the juvenile Nile crocodile liver and gallbladder has not previously been described. Selected information is available on reptiles in general and more specifically on the Saltwater crocodile, the West African crocodile and the caiman. It is essential to have a thorough knowledge of the normal histology and ultrastructure of the liver and gallbladder in order to properly evaluate the pathology of disease in these organs of the Nile crocodile. Due to the shortage of information on the subject matter, this study had to rely on findings in other vertebrates for assessment and comparison. The study illustrated the topography, gross anatomy, histology and ultrastructure of the liver and gallbladder of *Crocodylus niloticus.* The emphasis was on distinguishing the different cell populations by using light microscopy and transmission electron microscopy.

The two liver lobes were connected dorso-medially by an isthmus, which also consisted of liver tissue, and were located in the coelomic cavity demarcated by the post-pulmonary and post-hepatic membranes. The right lobe was larger than the left lobe, with both lobes having a triangular shape. The isthmus in this species may be a developmental adaptation to allow for the inclusion of other organs in the body cavity.

The **light microscopical features** of the liver in this study confirmed the findings in most reptiles and in the few crocodilians investigated, namely, that the classic lobular growth pattern could not be identified and was indeed absent from the juvenile Nile crocodile. Instead, a haphazard sinusoidal and portal tract arrangement determined the parenchymal growth pattern. The cross-sectional parenchymal tubular structures were similar to the descriptions in four non-mammalian vertebrate classes and in the two crocodilian species, *Caiman latirostris* and *Osteoleamus*. The tubules consisted of four to five pyramidal hepatocytes surrounding a central lumen. In the longitudinal sectioning plane the tubules consisted of two-cell-thick plates with an elongated lumen. The liver cords were seen to

branch and anastomose. The nuclei of the hepatocytes were eccentrically located nearest to the sinusoidal lumen as in some reptiles and in the West African crocodile. Peribiliary cytoplasmic granules were positive for hemosiderin and variably sized lipid droplets and glycogen were present.

The non-parenchymal component of the liver consisted of sinusoids lined by flat endothelial cells, accompanied by Kupffer cells and extrasinusoidal stellate cells. The shape of the sinusoids were intermediary between sacculosinusoidal and tubulosinusoidal, i.e. irregular and angular, and therefore the same as that of young alligators. Many single Kupffer cells were found in different locations in the liver, namely, in the sinusoid, in the space of Disse and forming part of groups of hepatocytes. This differs from other reptiles where the Kupffer cell was either bound to the sinusoidal wall or prominent Kupffer cell collections were noted or the cell was a rare finding. The cells contained large inclusions consisting of melanin, hemosiderin and a third component that stained pink with the PAS reaction.

The liver was enclosed by a noticeable fibrous covering, Glisson's capsule, which extended fibrous trabeculae into the parenchyma with the trabeculae traversing the parenchyma in a haphazard manner. The fibrous trabeculae are suggested to prevent damage to the liver during trashing movements of the crocodile. A prominent collagenous sheath containing fibroblasts, plasma cells, lymphocytes and phagocytes surrounded the portal tract areas. A delicate network of reticular fibers existed around the parenchymal tubules.

The architectural components of the juvenile *Crocodylus niloticus* liver were further clarified by **transmission electron microscopical examination**. The large polygonal hepatocytes contained eccentric nuclei displaying compact nucleoli consisting of variably arranged electron-dense material. The cytoplasm was filled with alpha or beta glycogen particles and contained variable numbers and sizes of lipid droplets. Many mitochondria with matrix granules, peroxisomes with an amorphous content, peribiliary lysosomes containing hemosiderin granules and cholesterol slits, were present. Occasional centrioles, melanin granules and prominent bile pigments were exhibited. Granular and smooth endoplasmic reticulum and pericanalicular Golgi complexes were identified. The apical surfaces extended microvilli into bile canalicular lumina that were sealed off by junctional complexes. Unlike in mammals, bile canaliculi were absent between the lateral borders of

the hepatocytes in the juvenile Nile crocodile. Desmosomes and microvilli were present between lateral cell membranes. Certain reptiles display a basal lamina around hepatocyte groups, but in the Nile crocodile a basal lamina was only present in support of hepatocyte groups next to Glisson's capsule. Most groups of hepatocytes were surrounded by reticular fibres and scanty collagen fibres instead.

Endothelial cells with flattened nuclei and long, thin fenestrated cytoplasmic extensions lined the sinusoids. When active their nuclei and cytoplasm bulged into the sinusoidal spaces. Numerous pinocytotic vesicles and lysosomes attested to the transmembranous activity of the endothelial cells. The basement membrane normally surrounding blood vessels was absent around the sinusoids – instead the space of Disse consisting of a clear area displaying microvilli extending from the base of the hepatocyte, and cytoplasmic projections of stellate and myofibroblastic cells, was present.

The Kupffer cells of *Crocodylus niloticus* were highly mobile cells found in different locations in and around the sinusoids and as part of hepatocyte groups. This differs from other vertebrates where these cells were described as fixed cells or resident in the sinusoidal lumen. Large phagosomes contained three different elements, notably melanin, hemosiderin and ceroid. Melanin is synthesised by the Kupffer cells and traps the potentially harmful superoxides formed by iron as a defensive reaction. Ceroid was the third 'pink' component seen with the PAS reaction as these crocodiles were too young to have accumulated the 'wear and tear' pigment lipofuscin. Lipid droplets were a normal constituent of most Kupffer cells in contrast to mammalian Kupffer cells where they were absent. The vermiform processes found in other vertebrates were absent. The most conspicuous structure, namely the 'tubulosomes', found in the Nile crocodile Kupffer cells, have not been described in other reptiles. These organelles may have the tripartite function of breaking down phagosome contents, melanin synthesis and promoting cell mobility. The melanomacrophages seen forming part of hepatocyte groups were also designated Kupffer cells due to their analogous cytoplasmic content.

Stellate cells, regarded as the storage site for vitamin A, were located in the space of Disse and contained a few large non-membrane bound lipid droplets sometimes displacing and indenting the nucleus. This differs from the numerous lipid droplets found in the stellate cells of the West African crocodile. The cells extended long subendothelial cytoplasmic processes and were in close contact with the endothelial cells, as well as

Kupffer cells and myofibroblastic cells in the space of Disse. Some stellate cells exhibited a solitary cilium protruding into the space of Disse and a chemoreceptor or sensory function was ascribed to these immotile cilia. Sparse multivesicular bodies were found in contrast to the West African crocodile that showed a consistent presence of these bodies. Stellate cell cytoplasm contained filaments, microtubules, coated and pinocytotic vesicles.

Another type of cell was found in the same location in the space of Disse as the stellate cells – the two cells sometimes occurred simultaneously. This cell displayed features of both fibroblasts and smooth muscle cells and was called a myofibroblastic cell. Lipid droplets were absent and filaments forming densities and subplasmalemmal plaques as well as dilated granular endoplasmic reticulum were noted. The myofroblastic cells may be responsible for regulating the sinusoidal blood flow and for maintaining the extracellular connective tissue component. Previous reports stated that hepatic stellate cells lose their vitamin A content and transform into myofibroblasts during detrimental circumstances. However, both the stellate cell and the myofibroblastic cell were present simultaneously in the same location – perhaps these are two different cell populations with different functions. Another explanation is that differentiation from stellate into myofibroblastic cell occurs in the same region where stellate cells already reside.

The liver-specific natural killer cells, pit cells, were found in the sinusoidal lumen and in close association with endothelial and Kupffer cells. Pit cells act as the first line of defence in the liver and destroy target cells by bringing about apoptosis and necrosis. They contained numerous small electron-dense membrane-bound granules and larger vesicles that were either electron-lucent or contained an amorphous electron-dense interior. The latter feature differed from other reports as they did not have the distinctive internal rod bridging the diameter of the vesicle. Another difference was the demonstration of single pinocytotic vesicles in the pit cells of the juvenile Nile crocodile giving them a pinocytotic function as well.

Intercalated cells with an electron-lucent cytoplasm, due to the lack of organelles, were present in groups of hepatocytes and in the space of Disse. They resembled lymphocytes, although with more abundant cytoplasm, and are reported to have an immune function.

The structure of the portal triad correlated with other descriptions and consisted of a portal vein, hepatic artery and bile duct, sometimes accompanied by a lymphatic vessel.

Additionally, concentrations of lymphoid tissue were found in this area, perhaps for increased immunity.

Plasma cells are antibody-producing cells and were regularly found in the portal tracts where antigens may enter the liver.

The isthmus consisted of the same components as the liver lobes. However, due to it being immersion-fixed and the sinusoidal contents not being removed, cytoplasmic remnants of hepatocytes were found in the sinusoids. One author explained the reason for the presence of cellular debris in the sinusoids as being a way of eliminating redundant cellular waste or to provide the body with essential substances.

The fully distended pouch-like **gallbladder** was attached caudally to the right liver lobe in the dorso-medial region by the hepatocystic ligament which is in accordance with the situation in other crocodilians. Three anatomical regions, the neck area closest to the hepatocystic ligament, the middle area constituting the body of the organ, and the blind end, were recognised. The layers of the gallbladder wall were separated into an epithelial layer, a *lamina propria,* a muscular and a serosal layer. The epithelial layer consisted of pseudostratified columnar epithelium and exhibited the normal features of absorptive cells, namely, surface microvilli, junctional complexes and basolateral interdigitations. Desmosomes were also present between the deeper lateral cell membranes. Microvilli were decreased in numbers in areas of apical bulging to allow for cell membrane permeability during exocytosis. The scanty long microvilli observed may have a possible mechano- or chemo-sensory function. Sparse cilia were also present in the epithelial cells and could have a sensory function. The presence of single cilium has not been described in other reptiles. The appearance of the epithelium depended on the current phase of the secretory cycle. Slender goblet cells represented the resting phase, the clustering of secretory granules in the subapical region signified the start of the secretory phase, followed by apical bulging, exocytosis of mucous granules and the stripping of the apical structures into the lumen being the final stages of the mucus secretory cycle. A combination of merocrine, apocrine and holocrine secretion was observed to take place. The juvenile Nile crocodile gallbladder thus has both absorptive and secretory functions. Proliferation of dark cells were present in areas of secretory activity and this is ascribed to their probable role as stem cells in balancing cell turnover. They may also represent dying cells. Glycogen granules were present. Lymphocytes were increased in number in the

basal epithelium and in the underlying *lamina propria* in areas where the epithelium was compromised and the basal lamina was thickened underneath the same regions pointing to a defense and strengthening function. The *lamina propria* consisted of a collagenous stroma containing fibroblasts, collagen fibers, nerve ganglia, blood vessels, lymphocytes and plasma cells. Smooth muscle cells with intervening collagen and fibroblasts constituted the *muscularis externa* that also contained nerve ganglia, lymphatic and blood vessels. The even nuclear profiles of these smooth muscle cells contrasted with the invaginated nuclear membranes described in other smooth muscle cells. Slivers of elastic fibers were present between the muscle-collagen layers in the *muscularis externa.* The serosal layer comprised mesothelial cells supported by a basal lamina with the cells containing prominent pinocytotic vesicles, lipid droplets, intermediate filaments and mitochondria. Cytoplasmic interdigitations and desmosomes existed between neighbouring cells. A collagenous stroma consisting of blood vessels and connective tissue supported the basal lamina and constituted the subserosa. The macroscopic and microscopic features of the juvenile Nile crocodile gallbladder are similar to that of mammals.