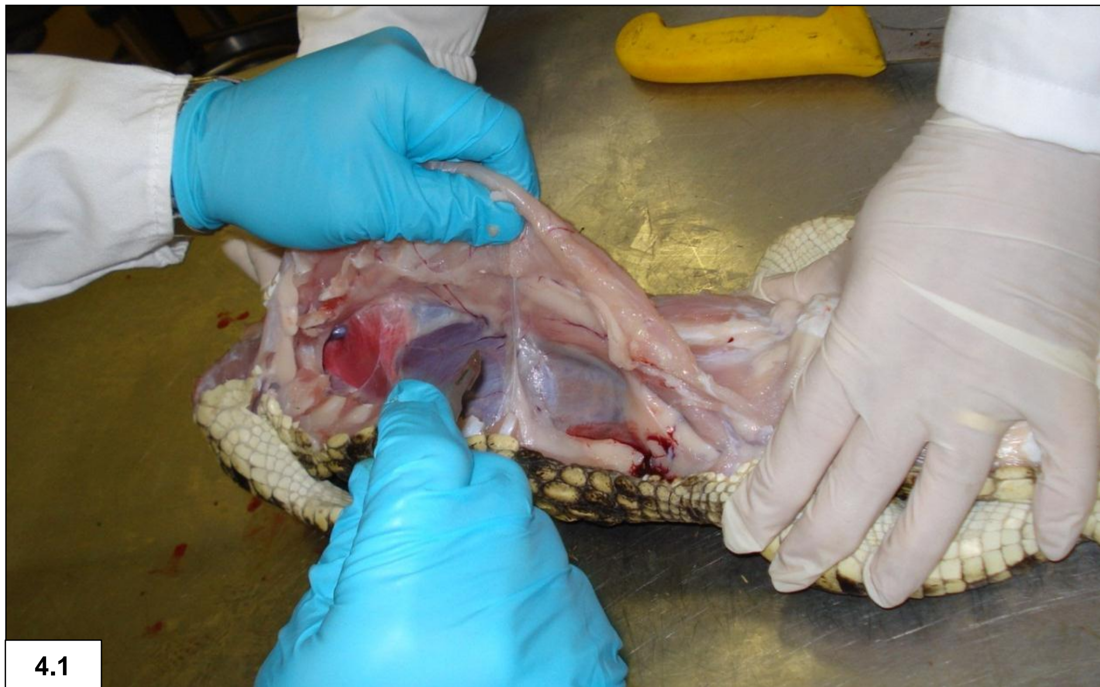


Figure 4.1: Lateral incision facilitating removal of the skin, ventral body wall and ribs.

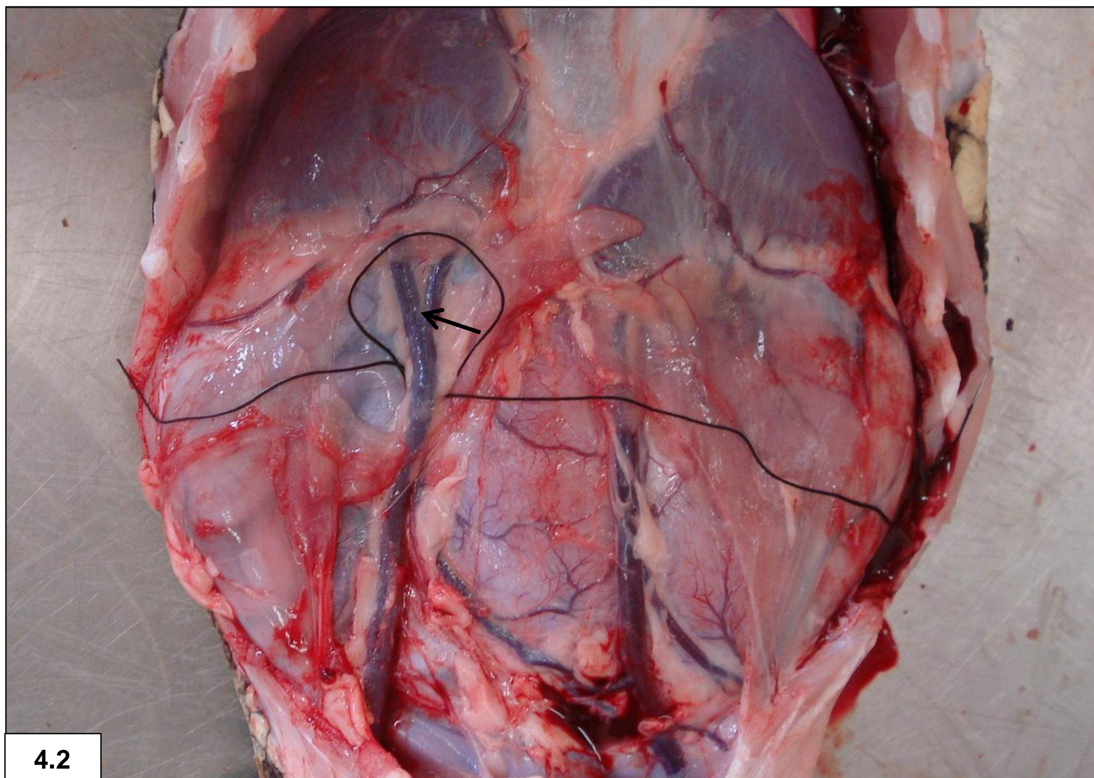


Figure 4.2: The portal vein to the liver was tied off *in situ.* Note the branching of the blood vessel (arrow).

Figure 4.3: The liver was removed from the body cavity and connected to a peristaltic pump.

Figure 4.4: A pale discoloration of the liver tissue indicated successful perfusion.

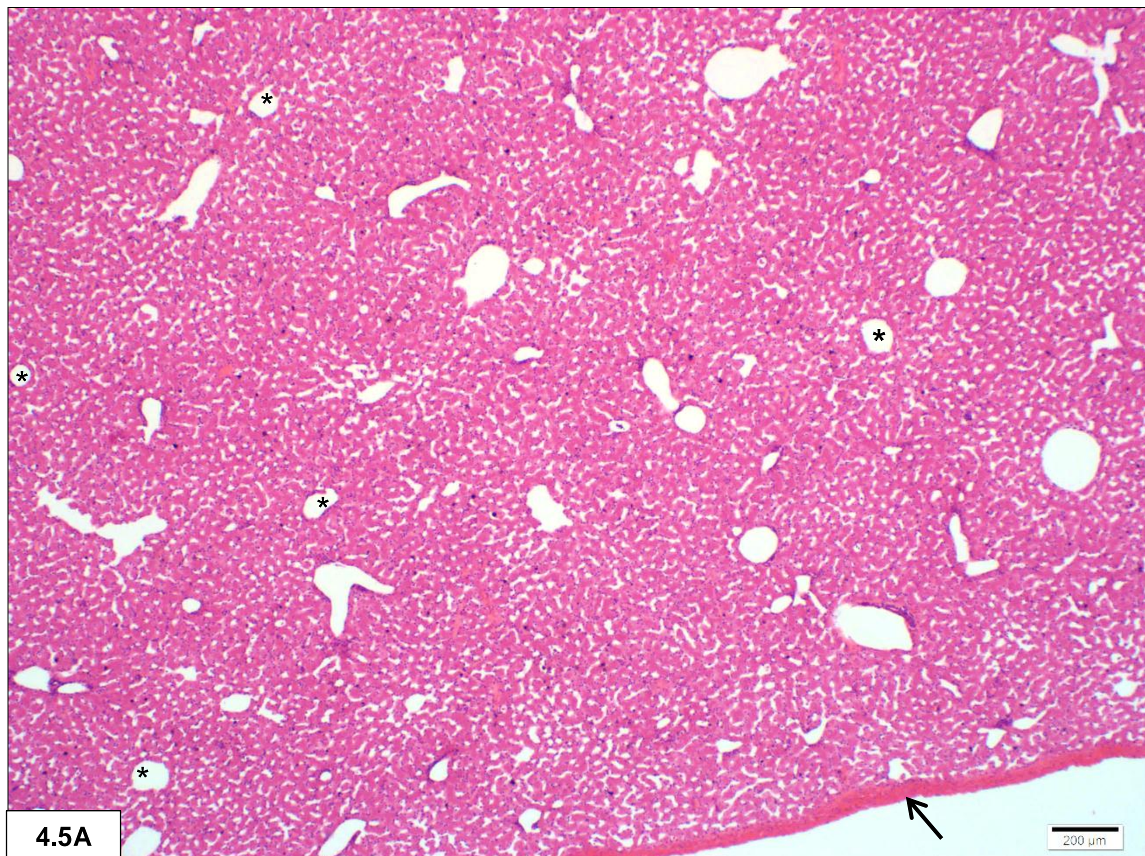
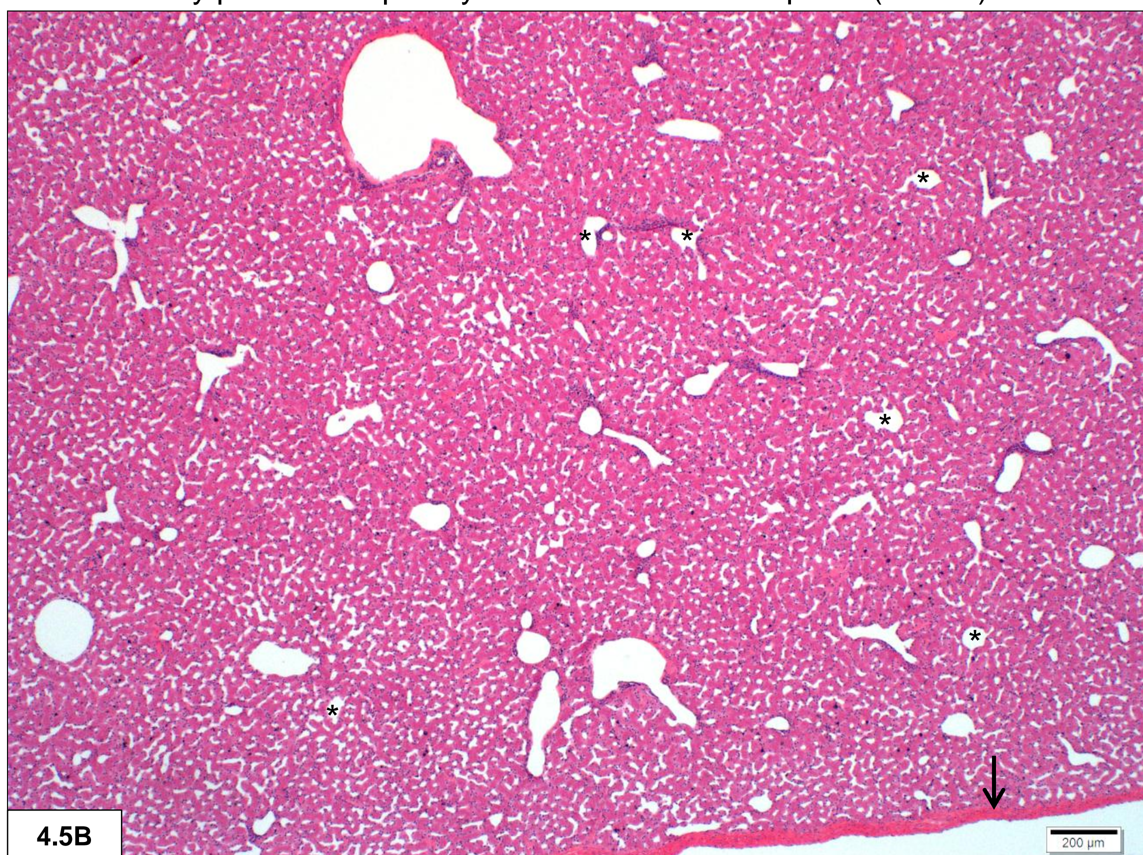


Figure 4.5 A & B: Haphazard arrangement of central veins (stars) and portal tracts surrounded by plates of hepatocytes. Note Glisson’s capsule (arrows).



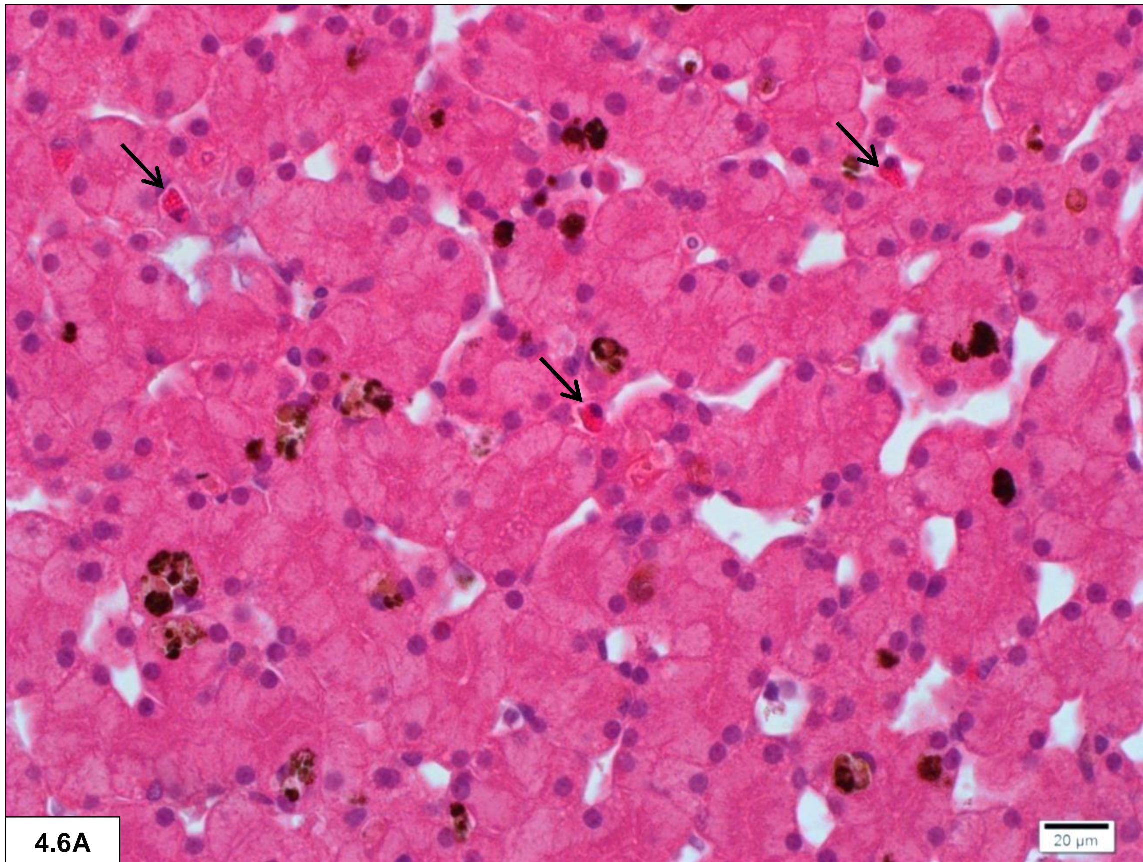
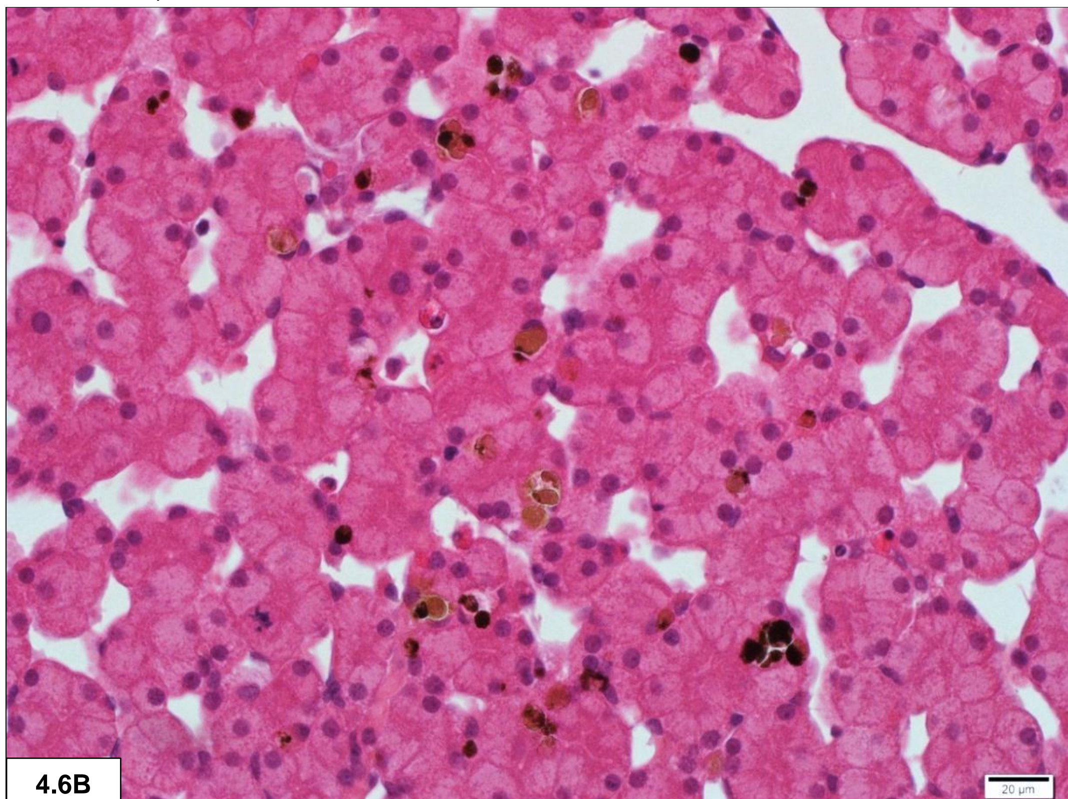


Figure 4.6 A & B: Eosinophilic hepatocyte cytoplasm with blue-staining basal nuclei next to the sinusoids. Distinctive pink-staining apical cytoplasmic inclusions are seen next to the bile canaliculi. Note brownish cytoplasmic inclusions in Kupffer cells. Note eosinophils (arrows in **A**).



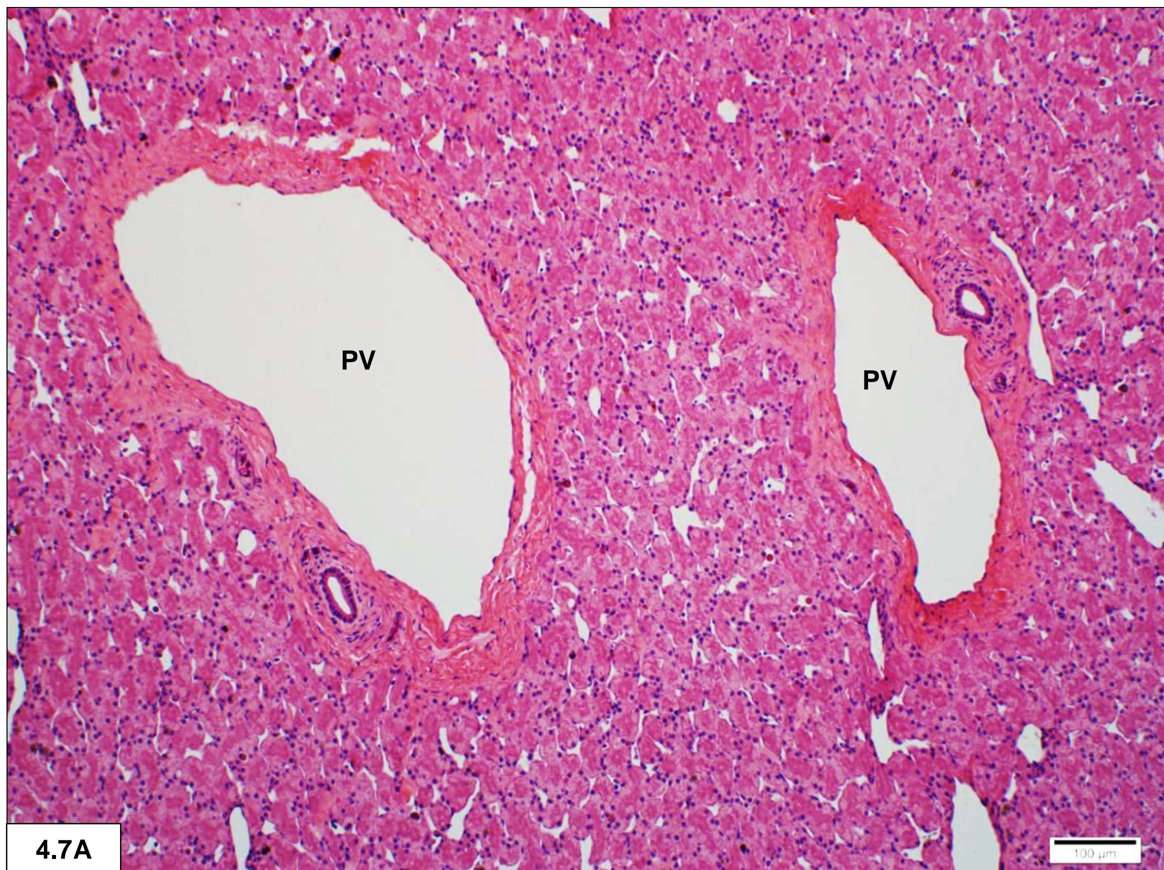
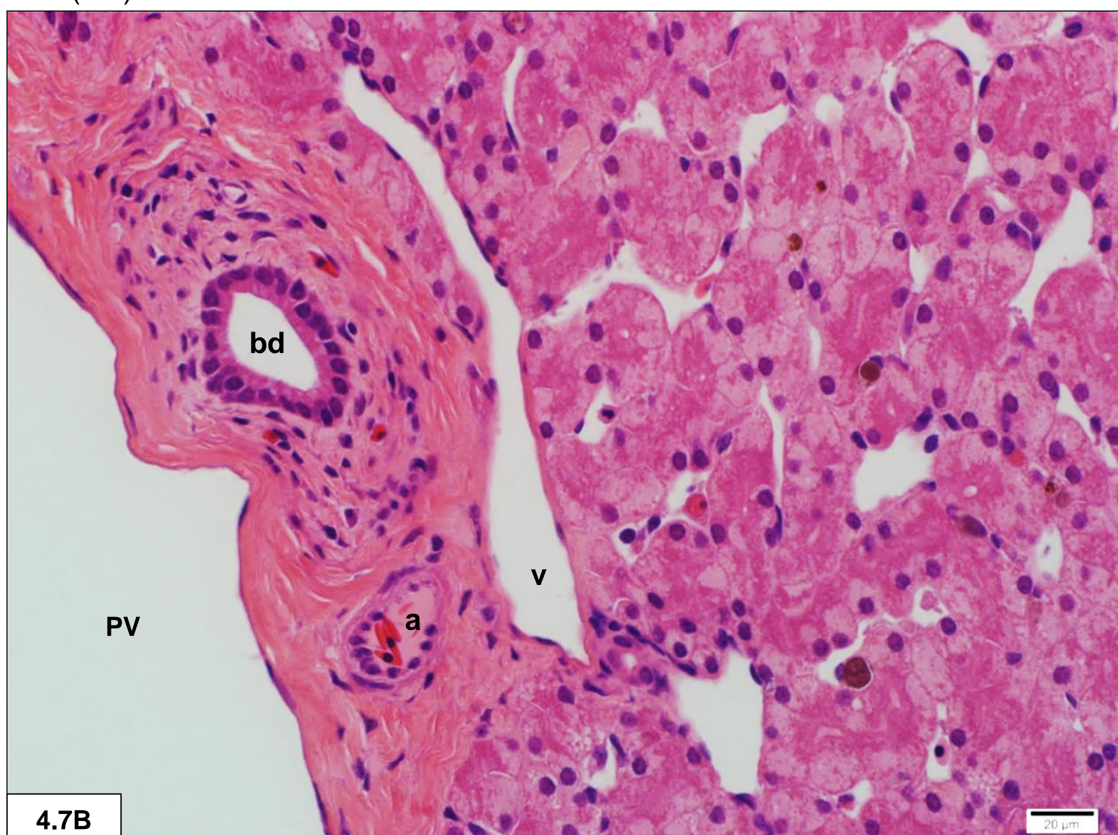


Figure 4.7 A: Two large branches of the portal vein (PV).
B: Portal triad consisting of a bile duct (bd), artery (a) and venule (v) next to a portal vein (PV).



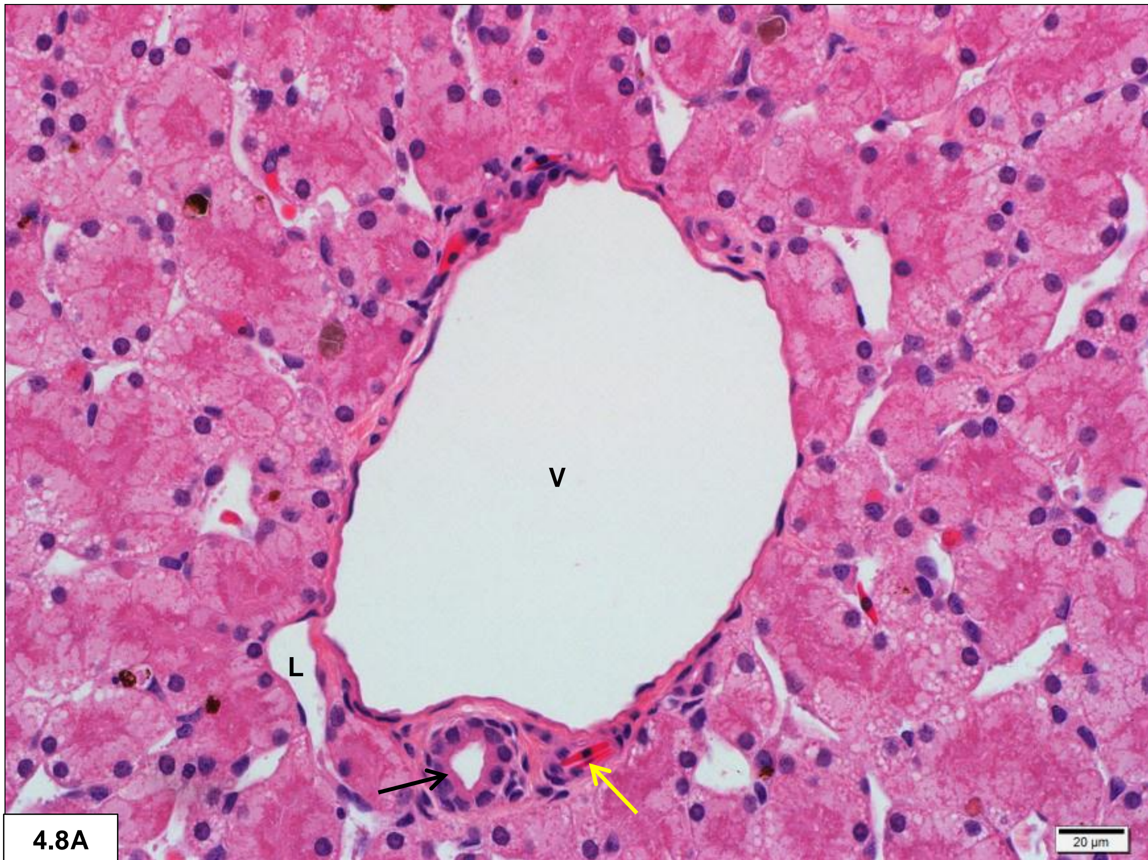
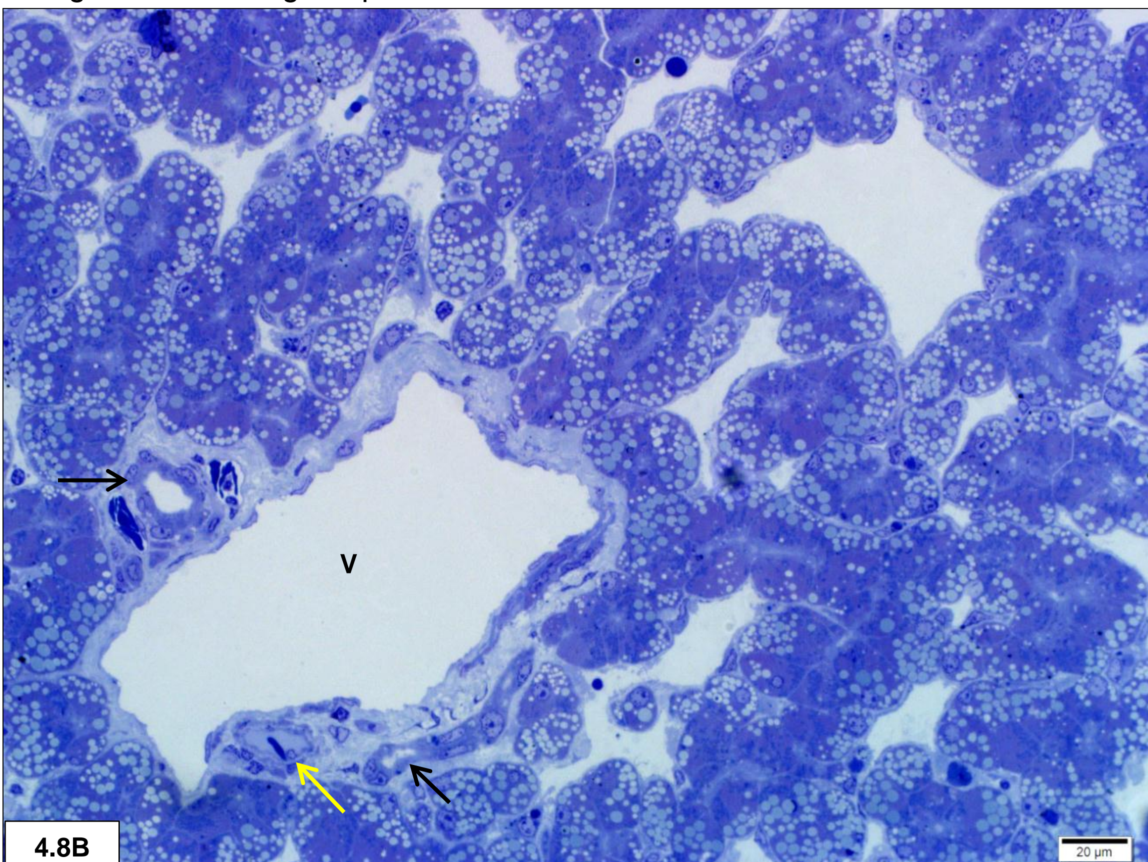


Figure 4.8 A & B: Portal triad – vein (V), artery (yellow arrow), bile duct (black arrow). Note lymphatic vessel (L in **A**) and frothy hepatocytes. H/E. **B:** Note pale vacuoles and collagen surrounding the portal triad. TB.



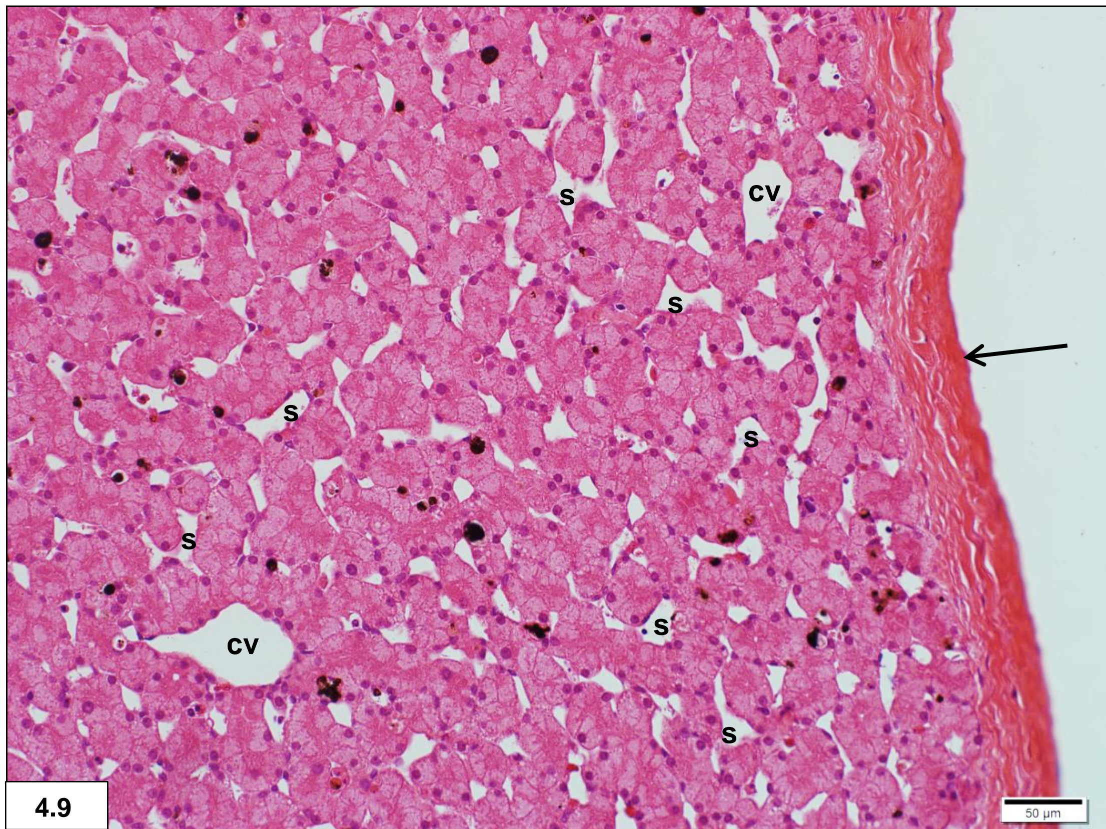


Figure 4.9: Glisson's capsule (arrow), central veins (**cv**) and sinusoids (**s**).

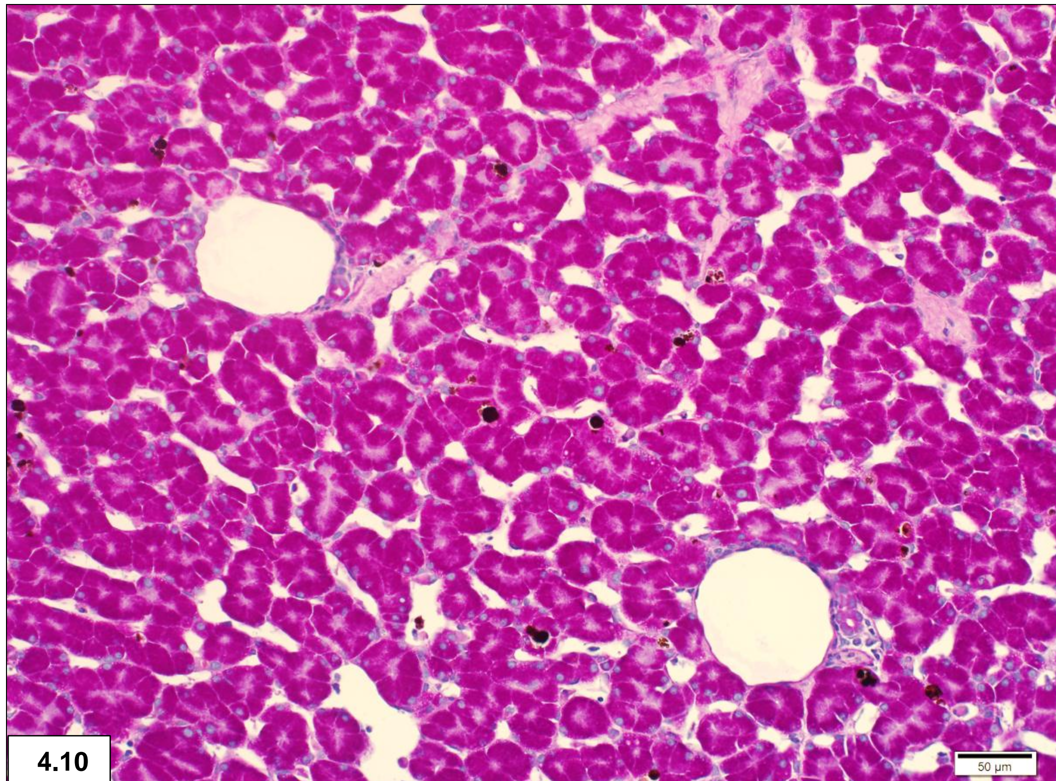


Figure 4.10: PAS **-** magenta cytoplasmic positivity indicating the presence of carbodydrates.

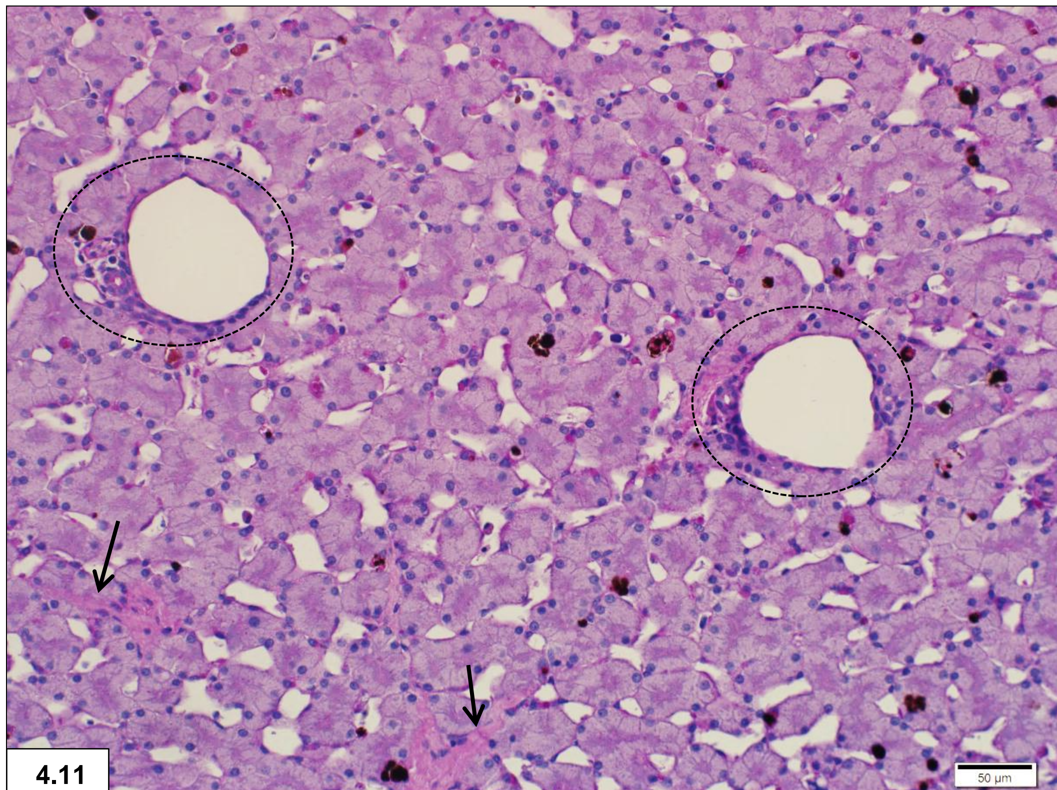


Figure 4.11: PAS-D - elimination of the magenta positivity by diastase digestion indicating the presence of glycogen. Note portal triads (dashed lines) and collagen trabeculae (arrows).

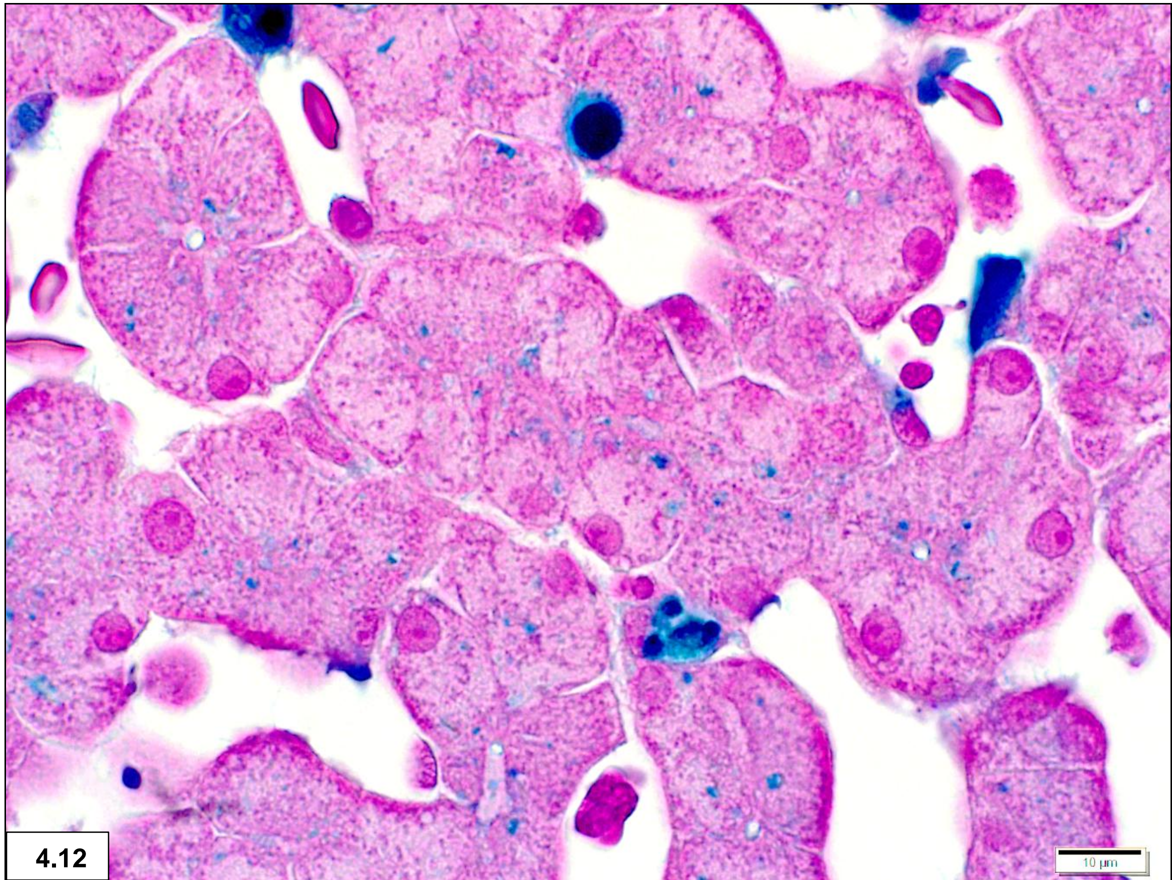


Figure 4.12: Perls' Prussian blue stain demonstrating fine blue peribiliary hemosiderin granules.

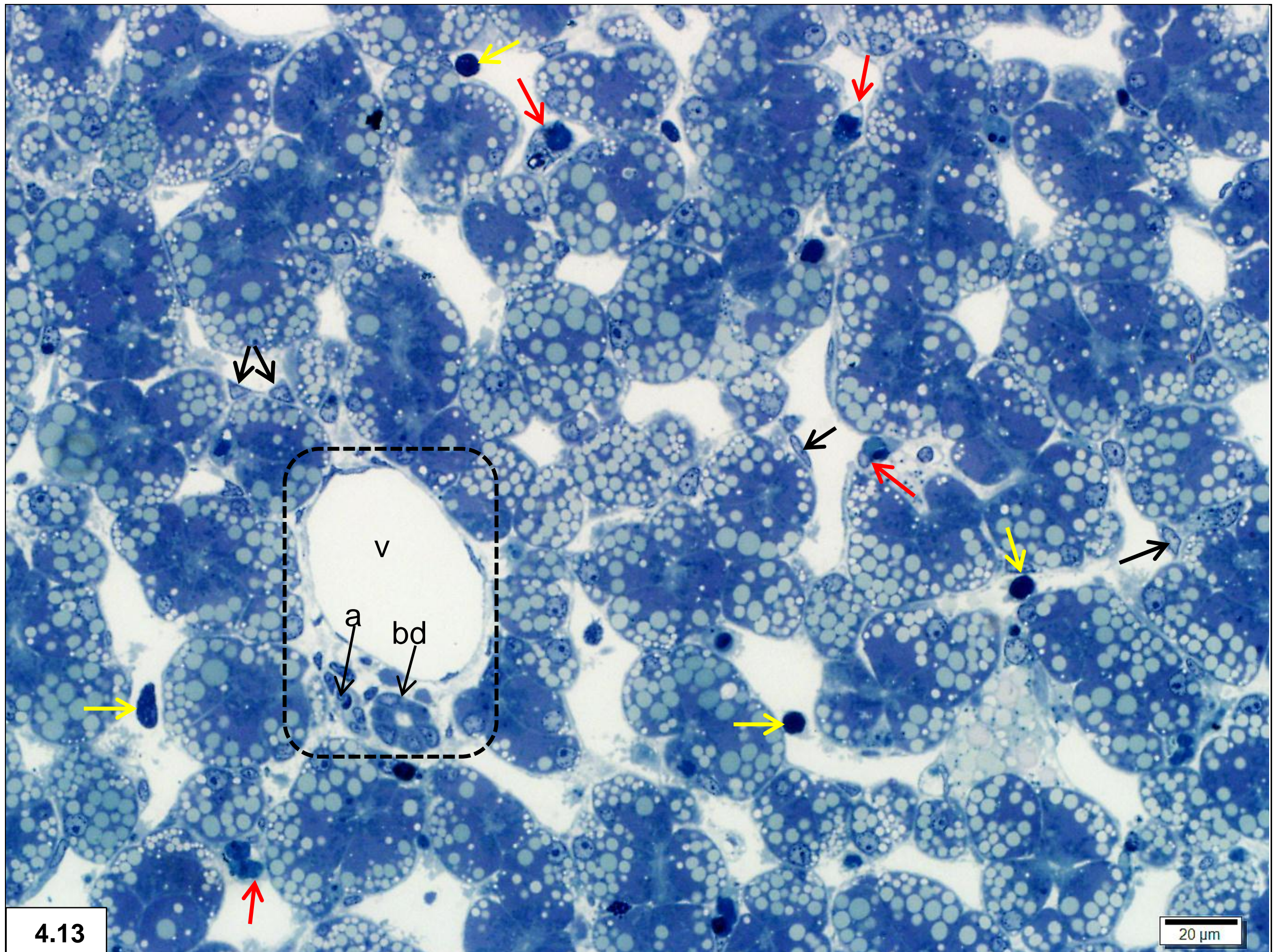


Figure 4.13: Toluidine Blue – endothelial cells (black arrows), Kupffer cells (red arrows), blood cells (yellow arrows). Note portal triad (dashed line) consisting of a vein (v), artery (a) and bile duct (bd) and variably sized pale vacuoles in the hepatocytes indicating lipid droplets. The basal nuclei of the hepatocytes have a pale blue colour with distinctive dark blue nucleoli.

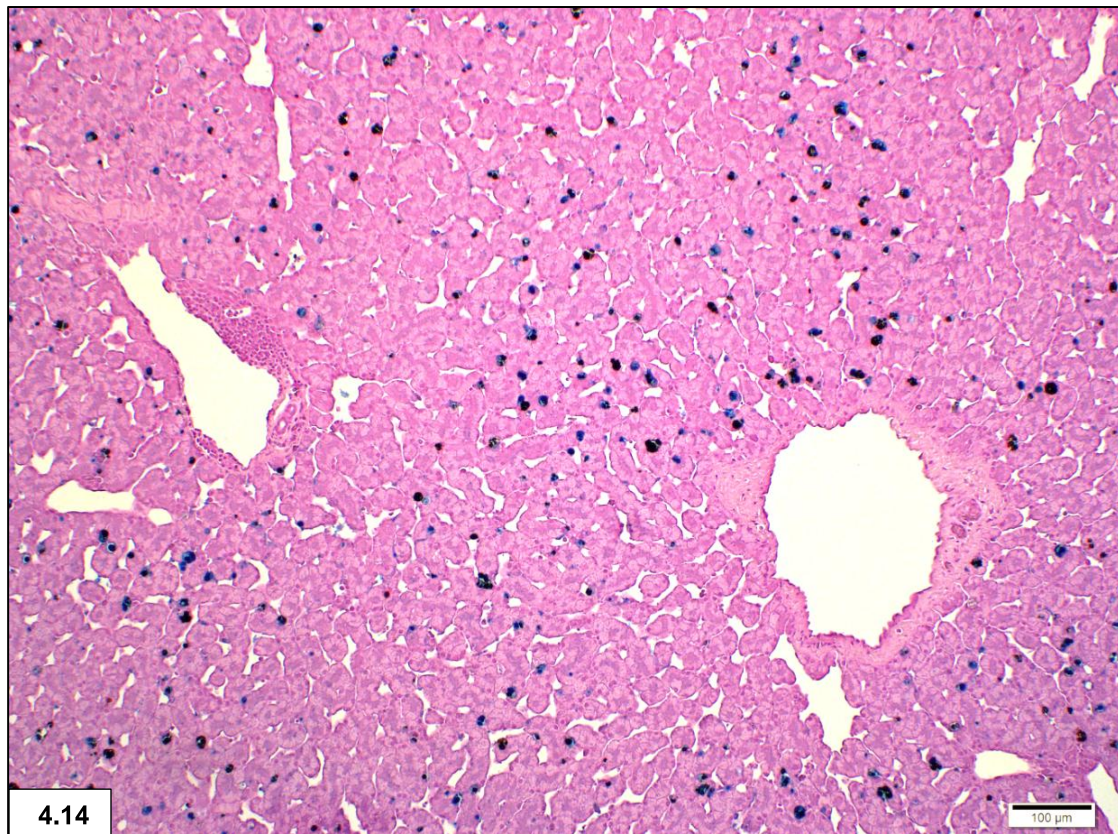


Figure 4.14: Perls' Prussian blue – blue positivity for iron deposits in Kupffer cells.

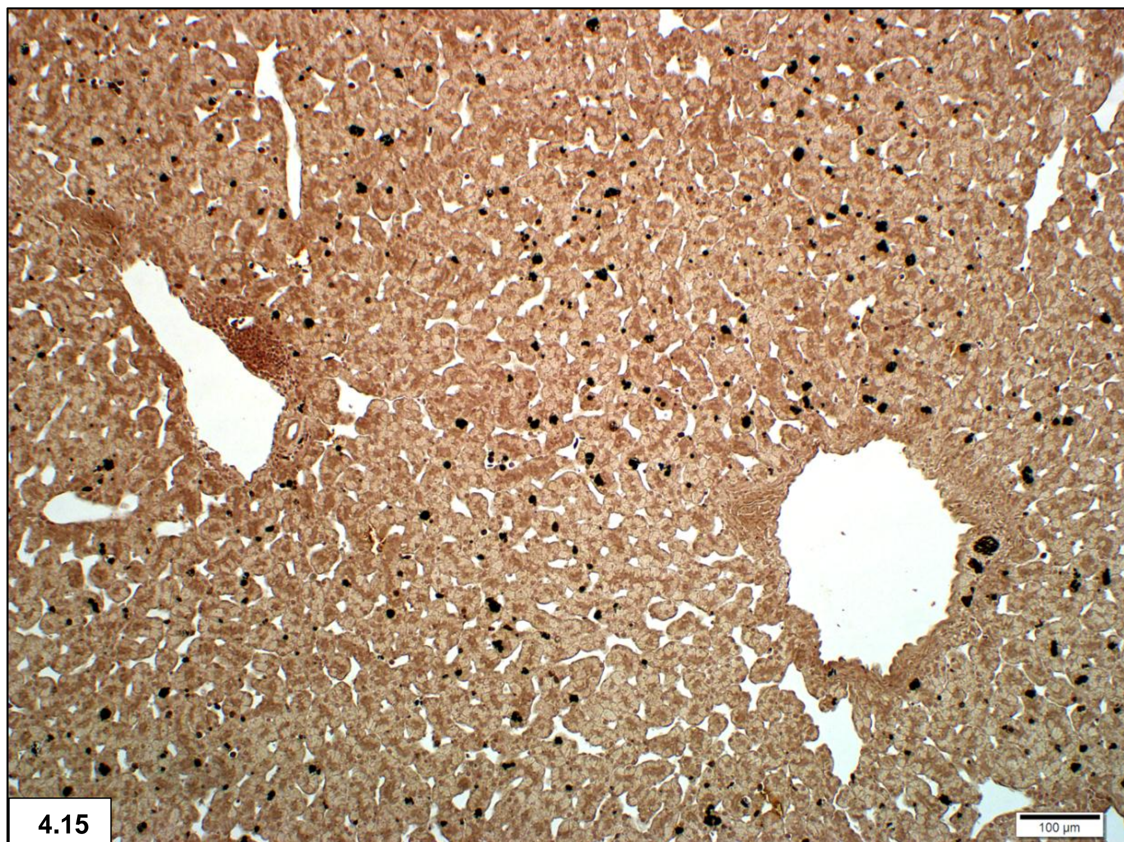


Figure 4.15: Masson-Fontana – black positivity for melanin in Kupffer cells .

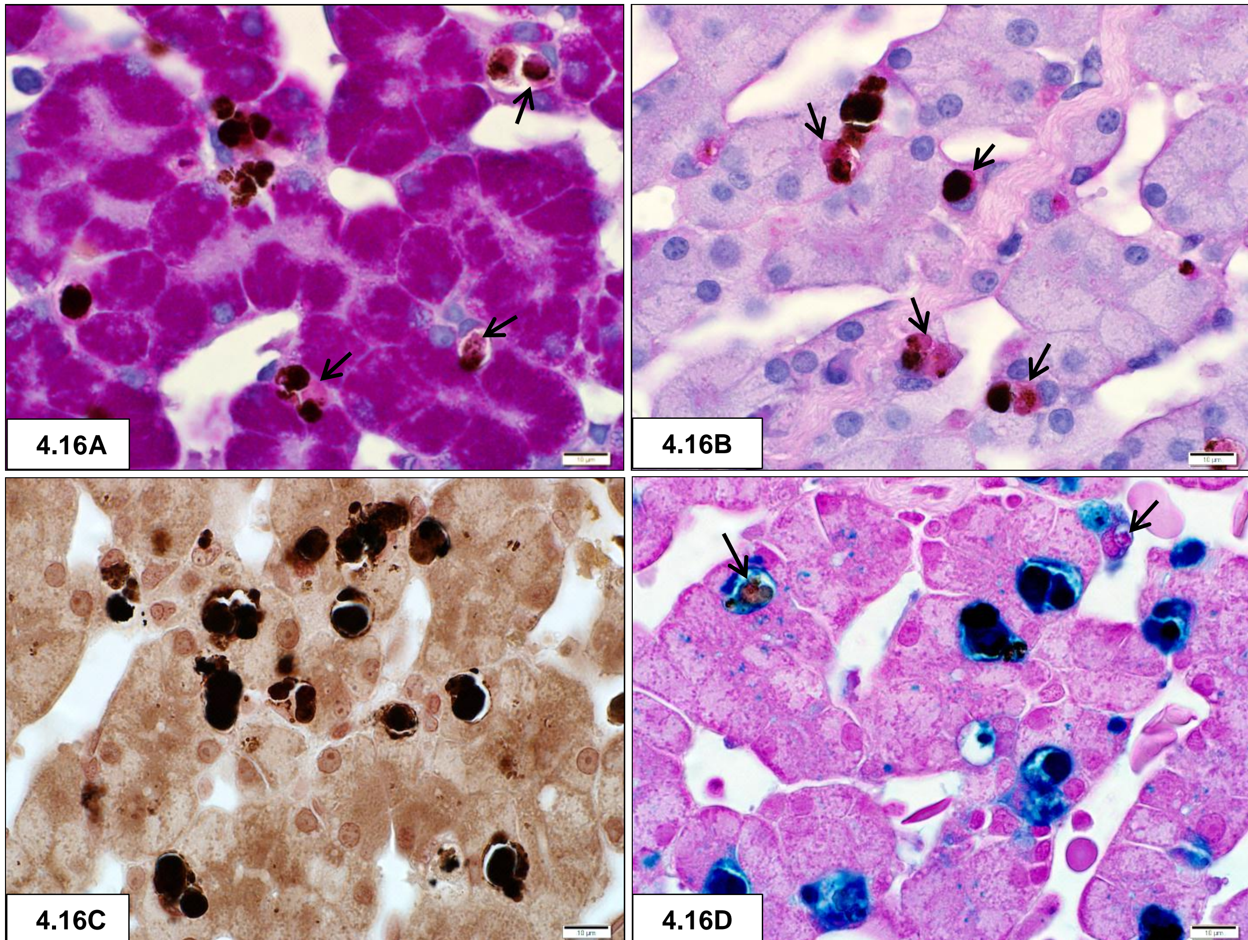


Figure 4.16: A-D – Granule comparison with different staining methods to show variable contents of the granules in Kupffer cells. **A** – PAS; **B** – PAS-D; **C** – Masson-Fontana; **D** - Perls' Prussian blue.

In **A** & **B** the granules are brownish-black with a pinkish component (ceroid or lipofuscin - arrows) in some granules. Note pink outline of some of the hepatocyte groups in **B** indicating a putative basal lamina. In **C** there are distinctive black granules (melanin) mixed with a brownish component. **D** shows partially blue granules (hemosiderin) mixed with a black (melanin) and in some a pink (ceroid or lipofuscin - arrows) component. Note the fine blue peribiliary granules indicating the presence of hemosiderin in the hepatocytes.

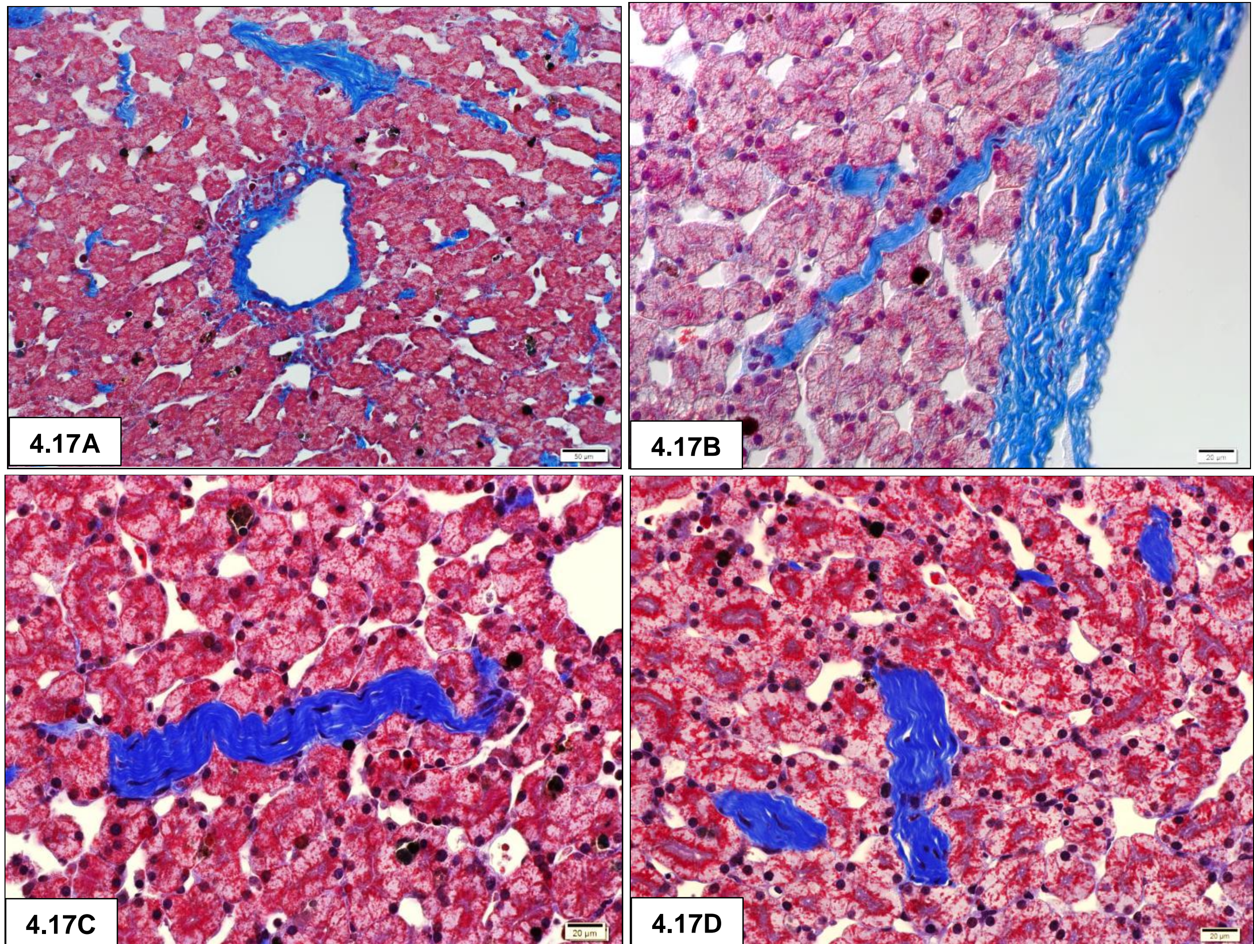


Figure 4.17 A – D: Masson trichrome stain for collagen.
B – Differential interference contrast.

Note collagen network around portal triad in **A,** collagen extending from Glisson's capsule in **B** and prominent collagen fibres between hepatocytes in **C** & **D.**

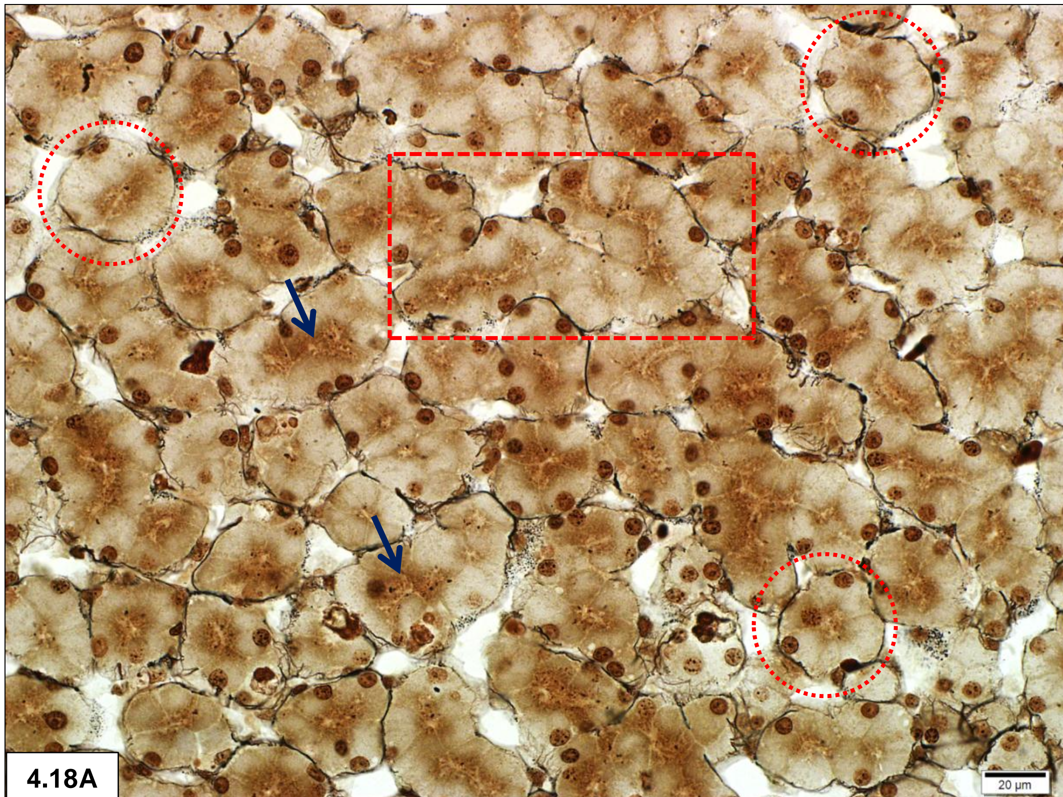
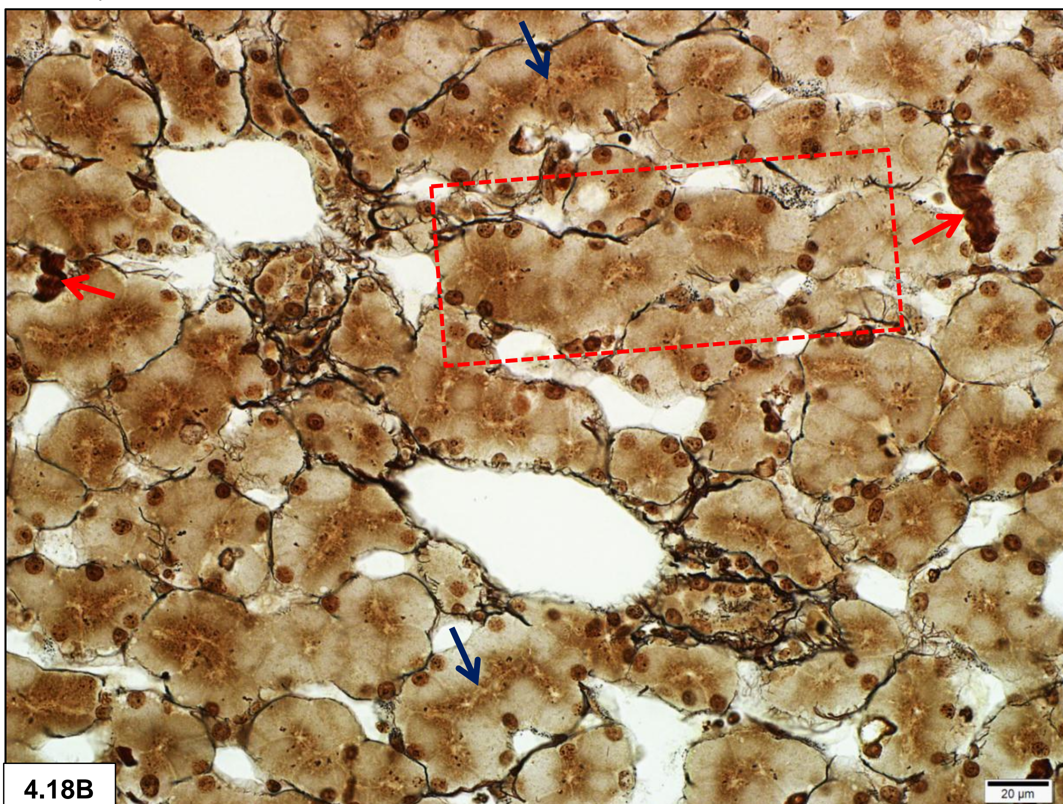


Figure 4.18 A & B: Gordon & Sweet's stain demonstrating the fine black reticular fibre network around groups of hepatocytes, sinusoids and portal tracts. The transverse (dashed circles) and longitudinal hepatocyte groups (dashed squares), basal nuclei and central bile channels (blue arrows) are well illustrated. Note brown collagen fibers (red arrows) in **B.**



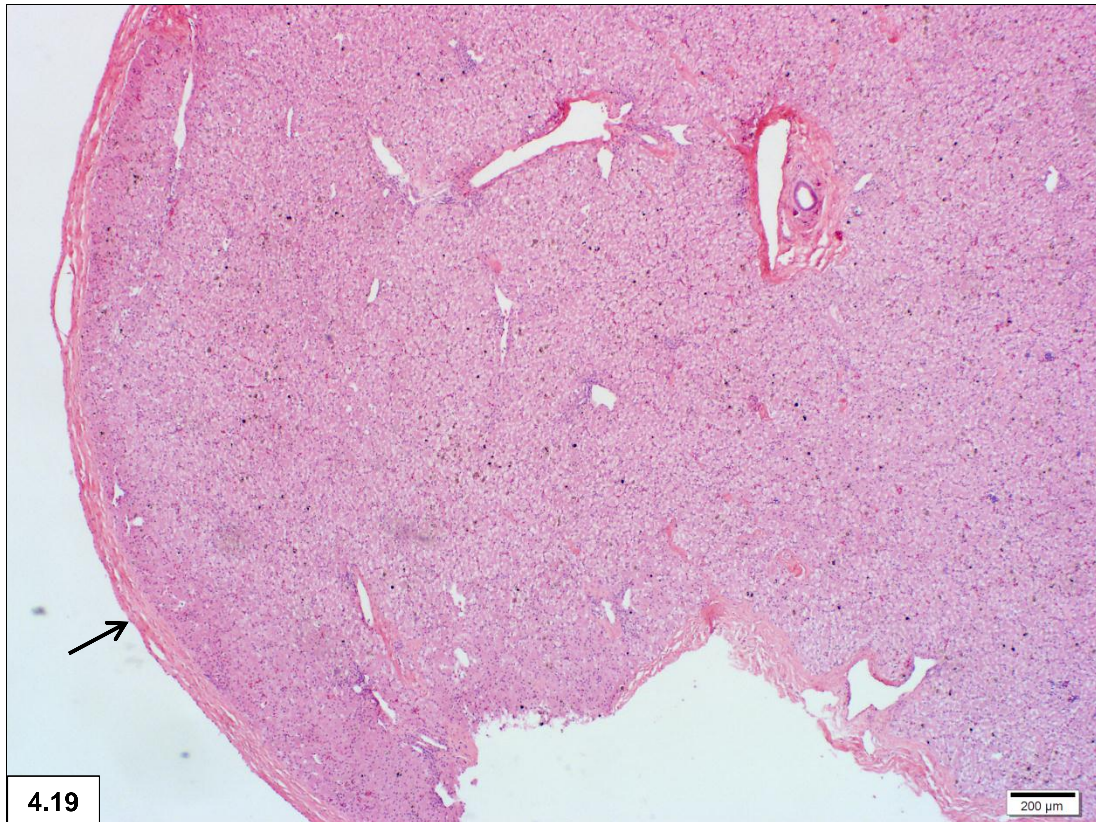


Figure 4.19: Low power view of the general architecture of the **isthmus** enclosed by Glisson's capsule (arrow). H/E.

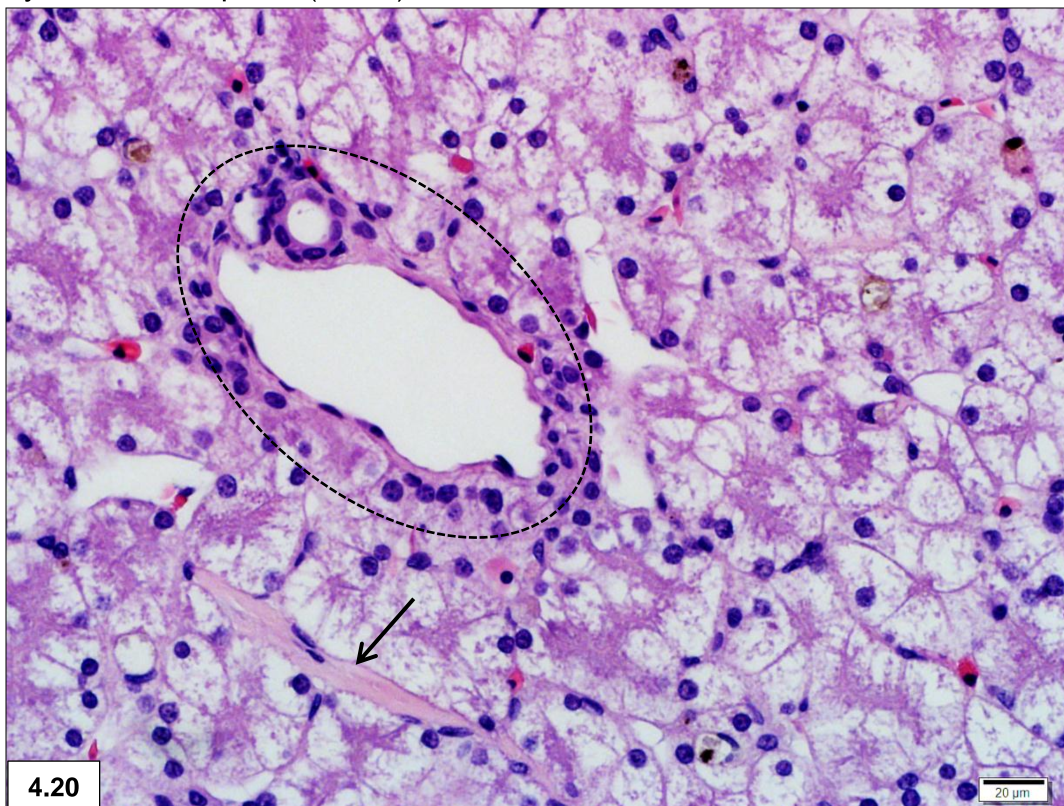


Figure 4.20: Isthmus - Portal triad (dashed lines) and hepatocytes exhibiting a clear cytoplasm. Hepatocyte tubular structures are evident. Note collagen trabecula (arrow). H/E.

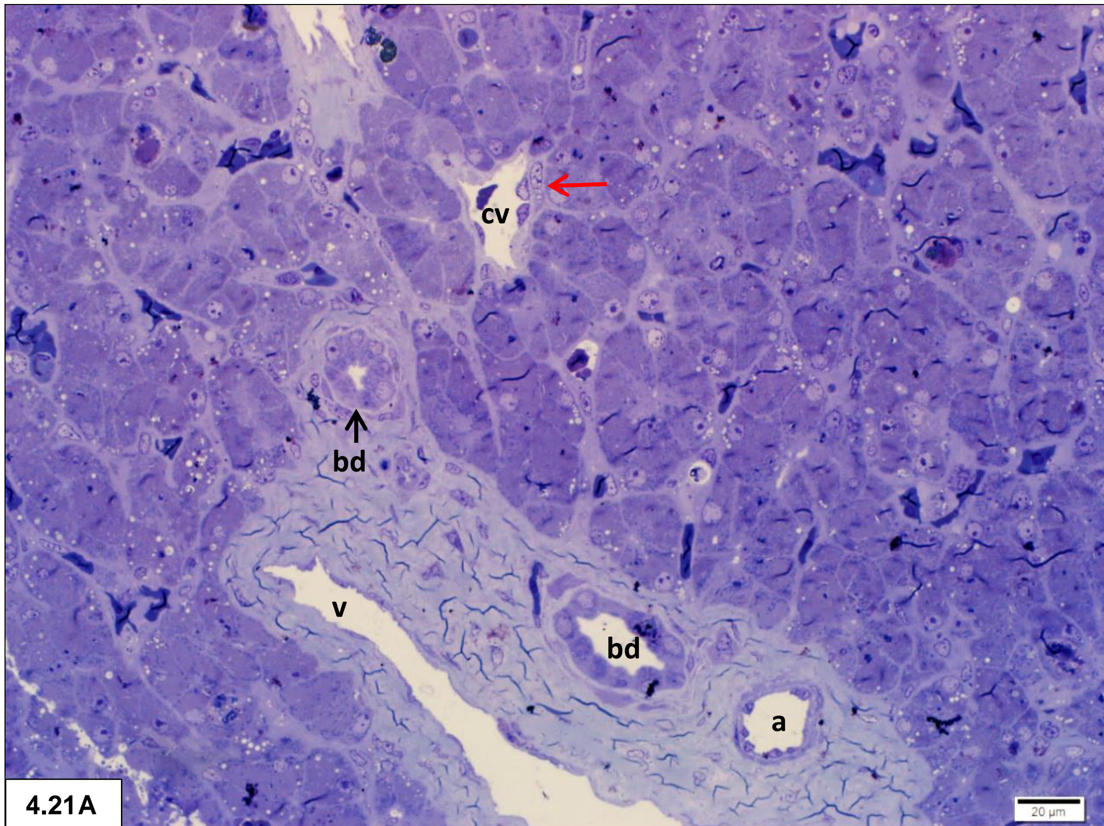


Figure 4.21: A – Isthmus - Portal triad enmeshed in collagen. Note central vein and stellate cell (red arrow) with underlying endothelial cells. Vein (**v**); bile duct **(bd)**; artery **(a)**

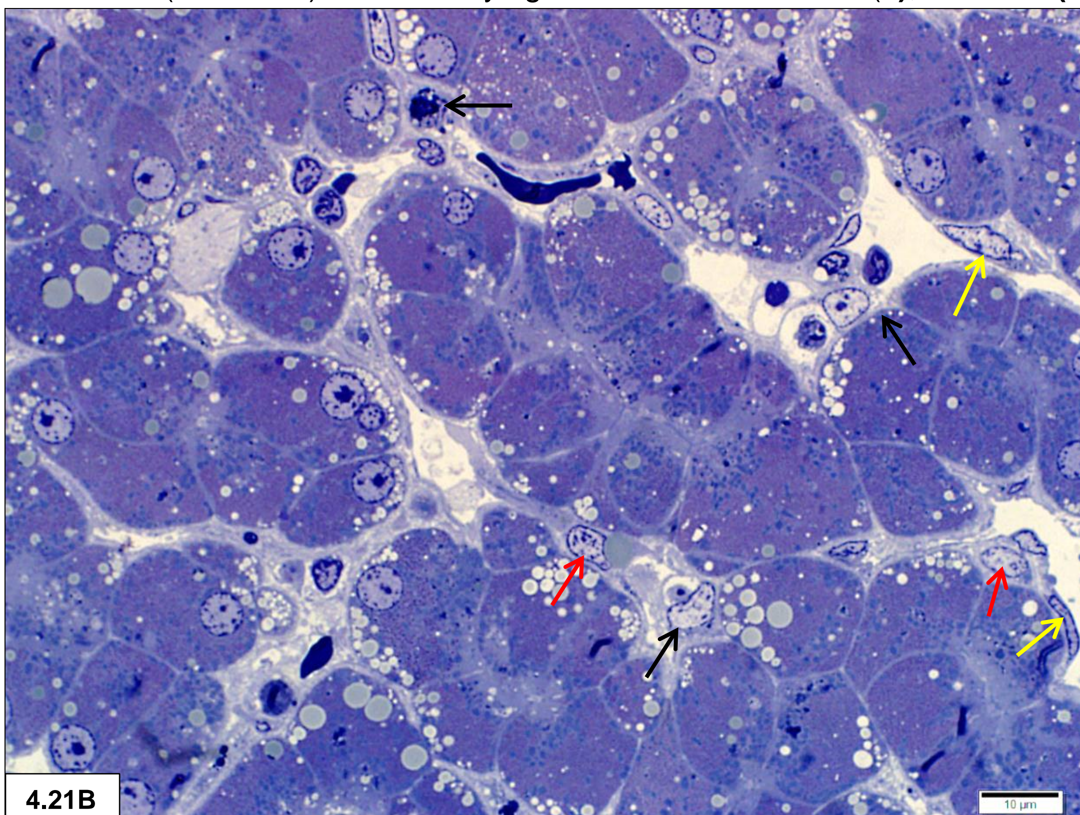


Figure 4.21: B – Note stellate (red arrows), endothelial (yellow arrows) and Kupffer cells (black arrows). A few blood cells are evident. Note pinkish metachromasia indicating glycogen. TB.

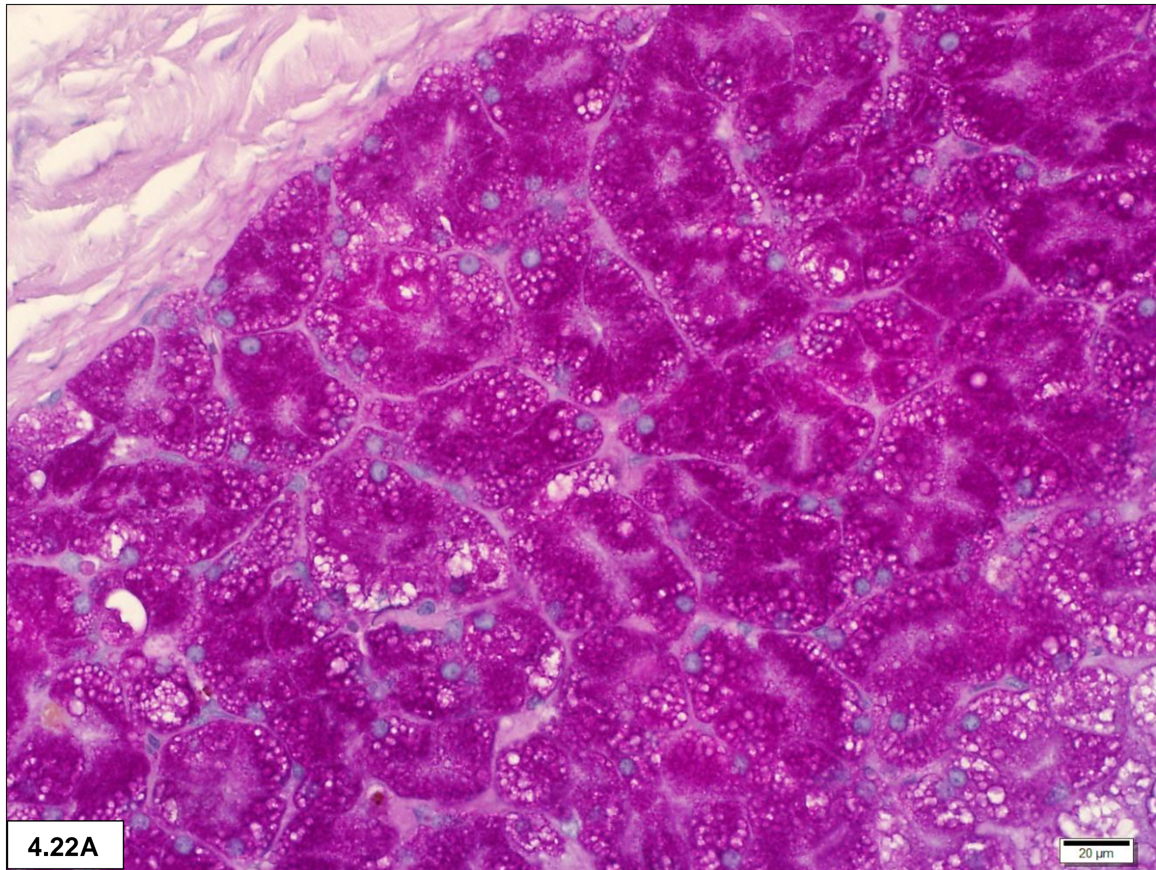


Figure 4.22 A: Magenta PAS positivity for glycogen.

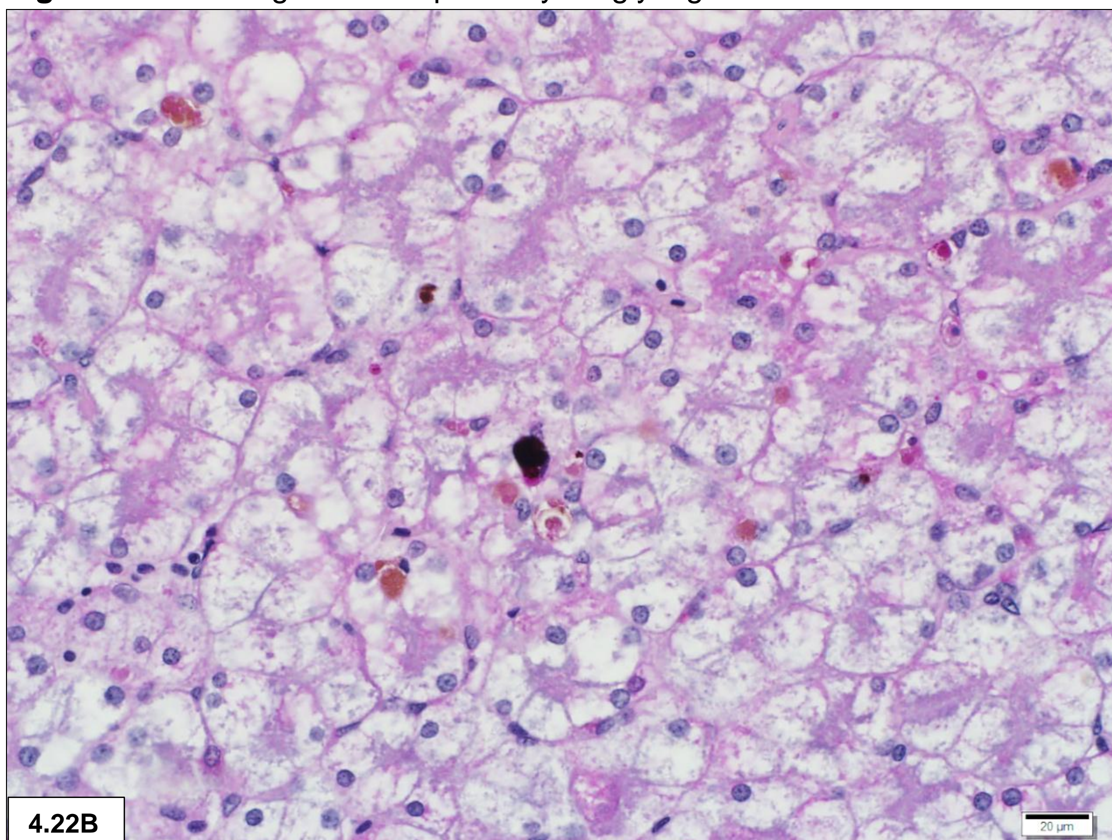


Figure 4.22 B: A similar region to that shown in Fig 4.22 A, demonstrating PAS diastase removal of glycogen.

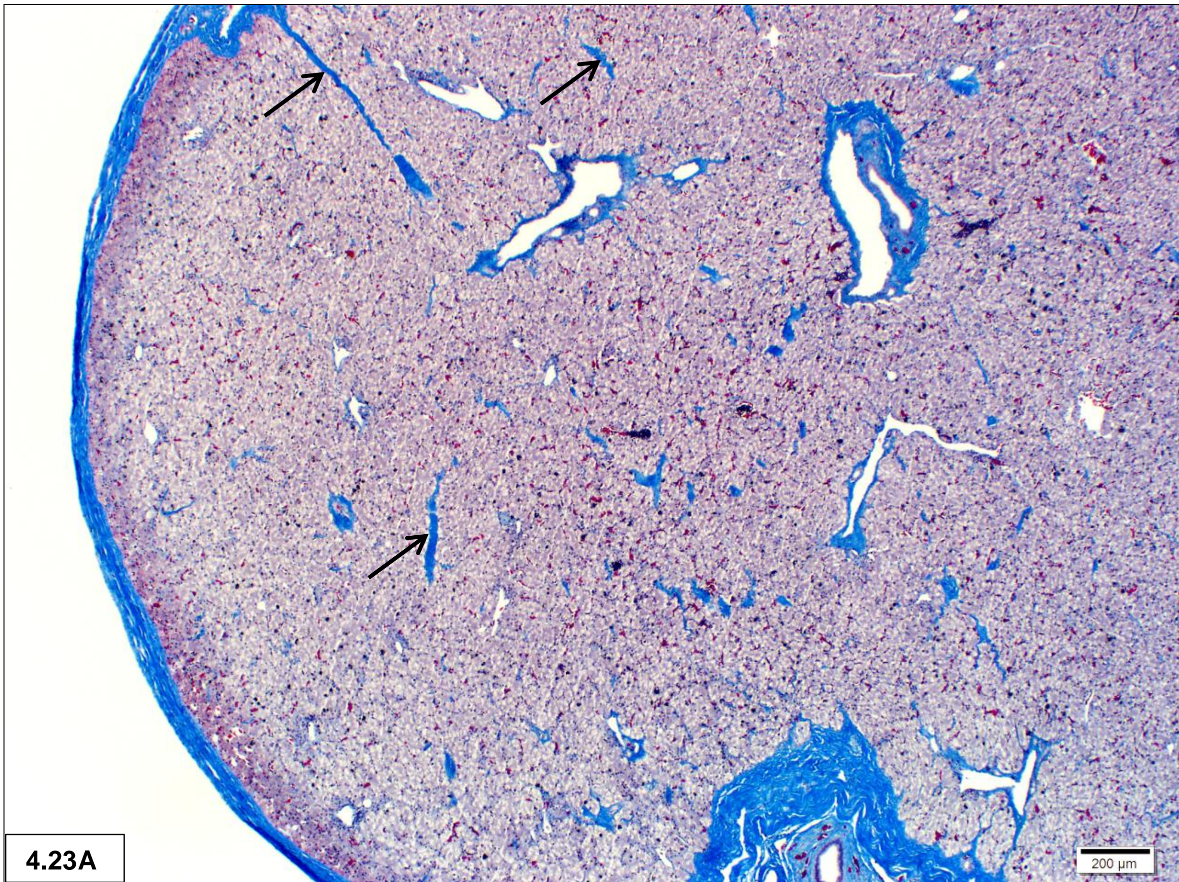
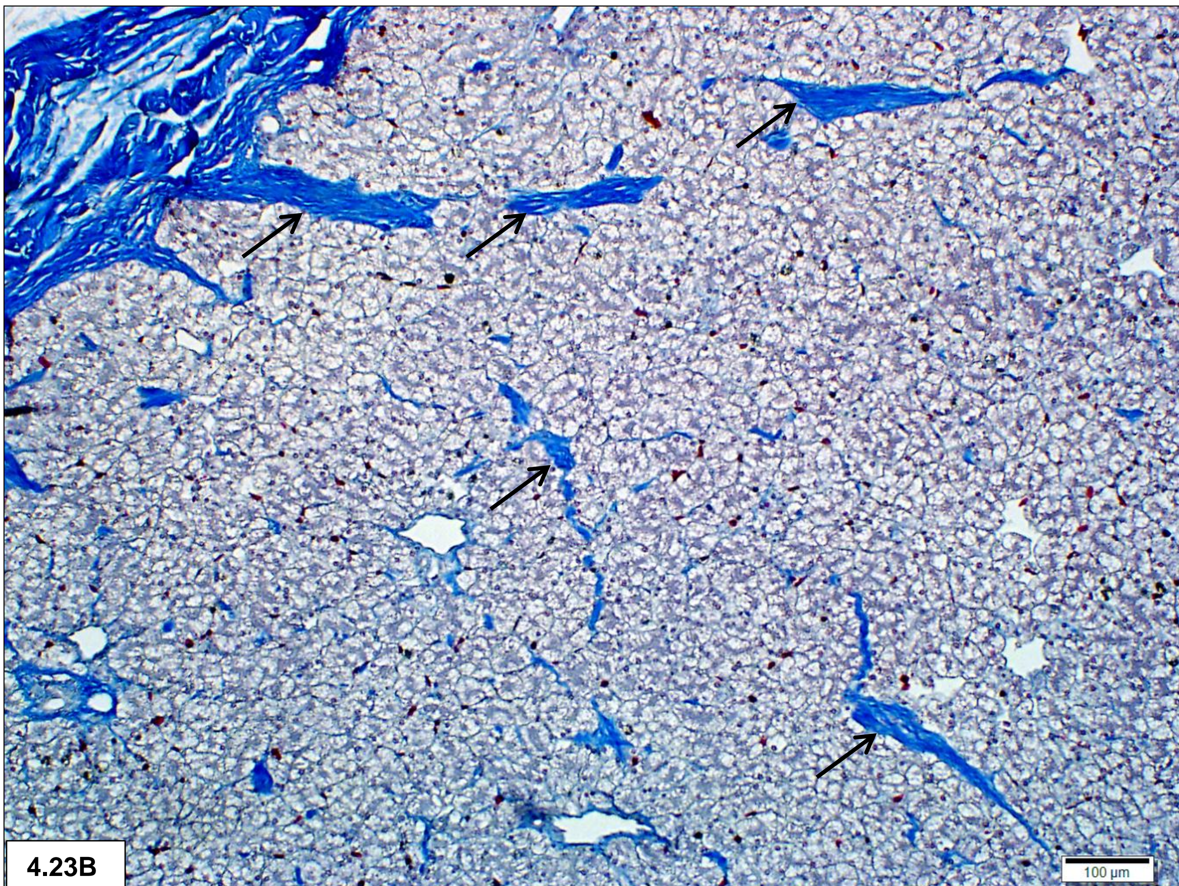


Figure 4.23 A & B: Blue collagen fibres (arrows), occasionally extending from the capsule, traversing the parenchyma haphazardly. Masson trichrome stain.



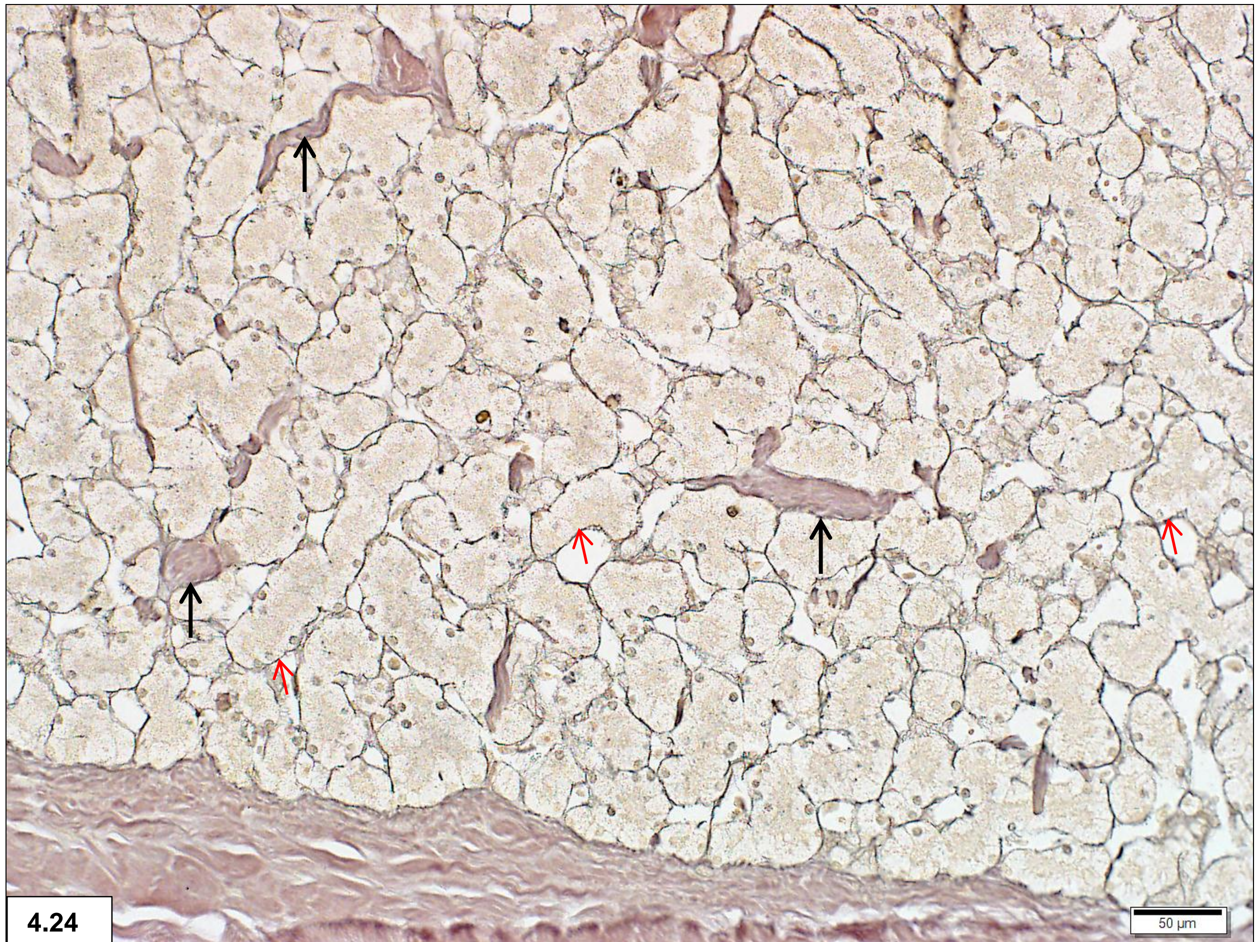


Figure 4.24: Black reticular fibres (red arrows) surrounding the hepatocyte tubules. Gordon & Sweet's stain. Note collagen fibres (black arrows).

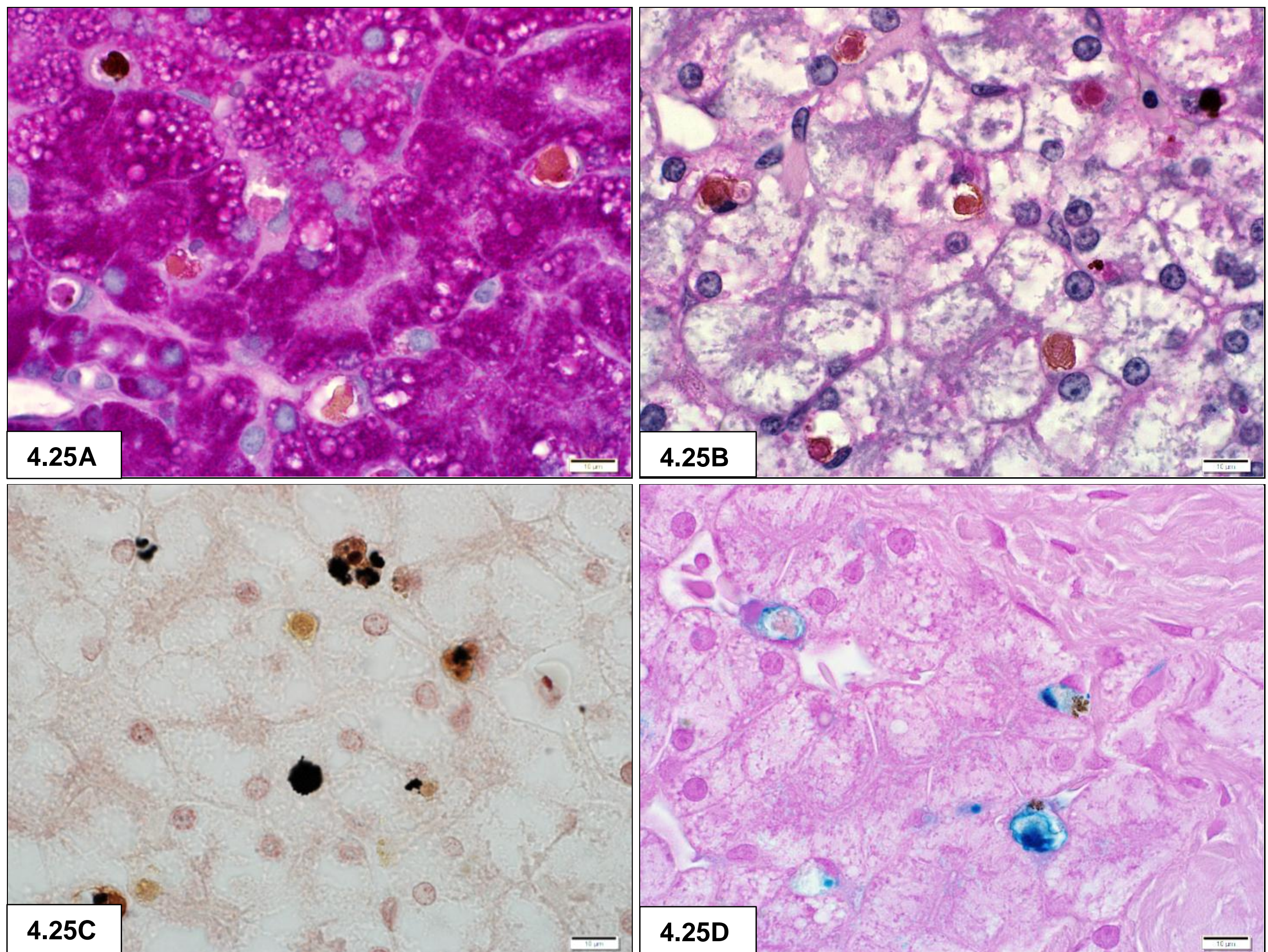
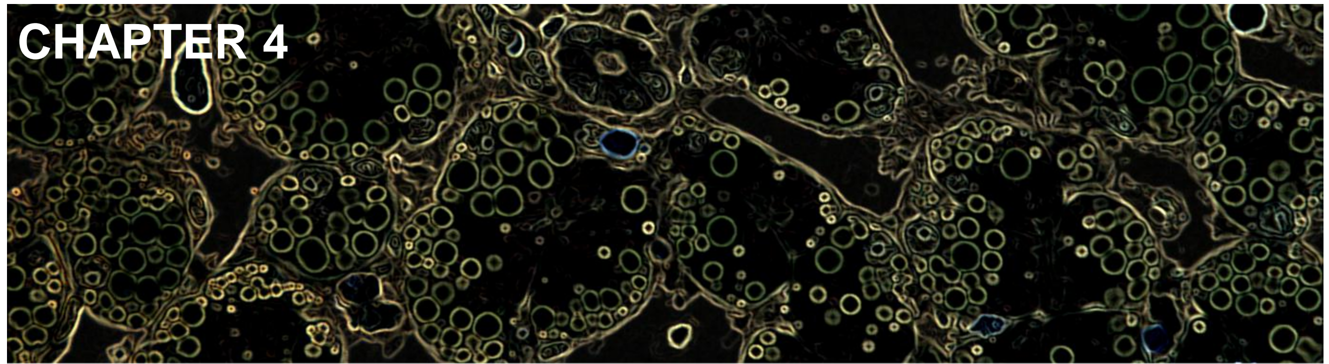


Figure 4.25: A – PAS; **B** – PAS-D; **C** – Masson-Fontana; **D –** Perls' Prussian blue

Large compound granular inclusions positive for melanin, hemosiderin and lipofuscin or ceroid. Note the diminished presence of peribiliary hemosiderin granules in **D**.



LIGHT MICROSCOPY OF THE LIVER

4.1 INTRODUCTION

The literature abounds with histological descriptions of the vertebrate liver. Hans Elias (1955) described the vertebrate liver as a muralium (system of walls) consisting of one- or two-cell-thick liver plates in which a sinusoidal network is suspended. Fried (2008) explained this muralium as a "three-dimensional lattice of interconnected plates made up of epithelial cells" tunnelled by lacunae containing sinusoids.

In 1986 Beresford and Henninger tabulated the variations in the microstructure of the liver of mammals, birds, fish and reptiles, but crocodiles were not mentioned. A brief overall view of reptilian liver histology was given by McClellan-Green *et al.* (2006) and Jacobson (2007). Schaffner (1998) wrote that the structure and cells of the reptilian liver were comparable to that of other vertebrates. Henninger (1982) and Moura *et al.* (2009) documented the light microscopy of the box turtle and the freshwater turtle respectively. Beresford (1987) briefly discussed the histology of the liver of *Caiman crocodilus* and Storch, Braunbeck and Waitkuwait (1989) included light microscopy in their ultrastructural study of the West African crocodile liver.

4.2 MATERIALS & METHODS

Liver samples were obtained from five 1-year old Nile crocodiles that were donated to the Faculty of Veterinary Science by Izintaba Crocodile Farm near Brits, North West Province, South Africa. They were euthanized by injecting sodium pentobarbital into the supra-vertebral vein. After muscular relaxation a lateral incision was made and the skin, ventral body wall and ribs were removed to expose the internal organs (Fig. 4.1).

Perfusion fixation was chosen as the method of fixation as the structural relationships in the liver are obscured by congestion when it is immersion-fixed. The portal vein to the right lobe was tied off *in situ* (Fig. 4.2), the liver removed from the body cavity and connected to a peristaltic pump (Fig. 4.3) (H.R. Flow Inducer, Watson Marlow Limited, England). The liver was perfused with a 0.5% heparin sodium (5000 IU/ml) saline solution, using the pump at 80 ml/min, to remove the blood from the sinusoids. The heparin solution was replaced with 2.5% glutaraldehyde in Millonig's buffer (pH 7.4, 0.13 M) that was pumped through the organ for approximately 20 minutes until a pale discoloration of the liver tissue indicated successful perfusion (Fig. 4.4).

With the completion of the perfusion procedure, tissue blocks were dissected from nine different areas in each of the five right liver lobes. The isthmus tissue failed to perfuse and a cross-sectional tissue block of this area was therefore immersion-fixed. All tissue samples were placed in 10% acqueous buffered formalin and fixed for a minimum of 24 hours before dehydrating through a graded ethanol series, clearing in xylene and infiltrating with paraffin wax in a Shandon Excelsior Thermo Electron Corporation tissue Processor. The samples were then embedded using a Thermolyne Histo Center 2 Embedding Unit and 3-5 micron thick sections were cut with a Reichert Jung rotary microtome.

Stains were performed to: (a) illustrate general liver architecture (haematoxylin & eosin, n=21); (b) identify glycogen (Periodic Acid-Schiff reaction & Periodic Acid-Schiff reaction with diastase treatment, both n=7), collagen (Masson Trichrome, n=14), reticular fibres (Gordon & Sweet's, n=9), iron deposits (Perls' Prussian blue reaction, n=9) and melanin (Masson Fontana, n=10) (Bancroft 2003). Toluidine blue of semi-thin resin sections was also performed (n=66).

4.3 IMAGE CAPTURING & PROCESSING

Slides of optimally perfused areas were examined by bright field & differential interference contrast illumination with an Olympus BX63 compound microscope and images were recorded with an Olympus DP72 digital camera. The Olympus CellSense software, version 1.5, was used to adjust the brightness and contrast, and a sharpening filter was applied where needed.

4.4 RESULTS

The liver consisted of parenchymal cells, i.e., hepatocytes, and diverse non-parenchymal cells, namely, endothelial cells, Kupffer cells and stellate cells. The parenchymal component occupied the largest part of the liver. The non-parenchymal cells were localized in and around the hepatic sinusoidal walls. Blood cells were occasionally found in the sinusoidal lumen and between the hepatocytes. A connective tissue stroma comprising blood and lymphatic vessels, blood cells, fibroblasts and bile ducts in a collagenous network made up the remainder of the liver. The isthmus contained liver tissue with a parenchymal and a non-parenchymal component.

4.4.1 Parenchymal organization

The principal cell in the juvenile Nile crocodile liver was the **hepatocyte**. A specific structural pattern such as the classic lobule, the portal lobule or the liver acinus was not distinguishable in the parenchyma. There was a haphazard arrangement of central veins and portal tracts surrounded by hepatocytes (Figs. 4.5 A & B, 4.9). The parenchyma was formed by anastomosing and branching cell cords consisting of two-cell-thick plates in the longitudinal sectional plane and at least 5 hepatocytes in the cross-sectional plane (Fig. 4.18 A & B). In longitudinal section long central bile channels could be discerned. The cross-sectional tubular configuration appeared gland-like with a common bile duct forming the ‘lumen’. The hepatocytes appeared pyramidal in shape in cross sections and cuboidal in longitudinal section.

Hepatocytes

- Hematoxylin and eosin (H/E) staining reaction:

  The cytoplasm was eosinophilic and contained blue-staining eccentric nuclei in the basal portion of the cell nearest to the sinusoids (Fig. 4.6 A & B). Distinctive pink cytoplasmic staining was present at the apical aspect of the cell abutting the bile canaliculi (peribiliary). Cytoplasmic vacuoles (Figs. 4.8 A & B) were present in the hepatocytes, most notably in the basal part of the cells, but could be present throughout the cytoplasm. The quantity of vacuoles present varied between the five livers and gave the hepatocytes a frothy

appearance. Other cytoplasmic inclusions in different shades of brown were also commonly observed (Figs. 4.6 A & B).

- Toluidine blue stain (TB) (semi-thin resin sections):

  The eccentric pale-blue nuclei contained prominent dark-blue nucleoli (Fig. 4.13). The cytoplasm showed pale round inclusions of varying sizes, seemingly lipid droplets, with the rest of the cytoplasm appearing a darker blue colour (Figs. 4.8 B & 4.13).

- Periodic acid-Schiff (PAS) staining reaction:

  There was a prominent magenta-staining cytoplasmic positivity indicating the presence of carbodydrates (Fig. 4.10).

- Periodic acid-Schiff staining reaction with diastase digestion (PAS-D):

  The elimination of the magenta positivity by diastase digestion revealed that the hepatocytes contained an abundance of the carbohydrate glycogen (Fig. 4.11).

- Perls' Prussian blue reaction:

  A fine blue granular positivity for hemosiderin was found in the peribiliary hepatocyte cytoplasm (Fig. 4.12).

- Masson-Fontana staining method:

  Melanin granules were present, but it was difficult to discern whether the granules were present in the hepatocytes (Fig. 4.15).

4.4.2 Non-parenchymal organization

The sinusoids in the juvenile Nile crocodile liver were asymmetrical. Depending on the plane of sectioning endothelial cells, Kupffer cells and stellate cells were seen in and around the irregular sinusoids with hematoxylin and eosin staining. Small numbers of blood cells also appeared in this area.

The **Endothelial cells** were flat cells that lined the sinusoids (Fig. 4.13).

Kupffer cells (Fig. 4.13) were large pleomorphic cells that appeared to be lining the sinusoids or protruding into the sinusoidal lumen. Large yellow to brown granular inclusions (H/E - Figs. 4.6 A & B) were positive for melanin with the Masson-Fontana method (Figs. 4.15 & 4.16 C) and positive for hemosiderin with the Perls' Prussian blue method (Figs. 4.14 & 4.16 D) The granules consequently contained both melanin and hemosiderin - there was an additional light-pink staining component revealed in certain granules with the periodic-acid Schiff and the PAS-D reaction (Fig. 4.16 A & B).

Stellate cells (Fig. 4.21B) were difficult to identify in light microscopy sections, but occupied a subendothelial position.

Occasional **blood cells** (Fig. 4.13) were present in the sinusoidal lumen. Prominent bright-pink cytoplasmic granules with hematoxylin and eosin staining showed some of these cells to be eosinophils (Fig. 4.6 A) that in certain instances were also found between the hepatocytes.

4.4.3 Connective tissue stroma

The liver was enveloped by a **connective tissue capsule** (Glisson's capsule) (Fig. 4.9) ranging in thickness between 40 µm and 60 µm. It comprised an outer single layer of mesothelial cells covering a layer of prominent pink-staining fibres that sometimes extended from the capsule into the parenchyma between the hepatocytes. These fibres, present in all five livers, were of varying width and length and traversed the parenchyma in a haphazard manner. The fibres stained a vibrant blue with the Masson trichrome stain indicating the presence of **collagen** (Fig. 4.17 A - D). The capsule was in direct contact with the parenchymal tubular structures.

The parenchymal cellular arrangement was interrupted by **blood vessels, lymphatic vessels** and **bile ducts**, enclosed by a connective tissue network which also showed blue positivity for collagen using the Masson's stain (Fig. 4.17 A). Randomly distributed **portal triads** (Figs. 4.7 A & B; 4.8 A & B) consisting of an artery, a vein and a bile duct, surrounded by a blue collagenous meshwork (Figs. 4.8 B & 4.17 A), were seen. Lymphatic vessels (Fig. 4.8 A) sometimes accompanied the triads. The bile ducts were lined by simple cuboidal epithelium. Several cells, probably **fibroblasts, plasma cells** and **lymphocytes** were present in the surrounding fibrous sheath in some areas. Black-staining **reticular fibres**

(Gordon & Sweet's; Fig. 4.18 A & B) formed a delicate framework around hepatocyte groups, sinusoids and portal areas. Distinctive pink borders, partially surrounding some of the hepatocyte tubules, were seen with the PAS-D reaction, indicating the presence of a basal lamina (Fig. 4.16 B).

4.4.4 Isthmus

The **isthmus** (Fig. 4.19) consisted of liver tissue comprising parenchymal and non-parenchymal cells enclosed by Glisson's capsule. Collagen fibres, occasionally extending from the capsule, traversed the parenchyma haphazardly (Fig.4.23 A & B). Portal triads (Fig. 4.20) enmeshed in collagen (Fig. 4.21 A) and central veins were evident, although no lobulation was seen. The hepatocytes exhibited a clear cytoplasm (Fig. 4.20) in areas and the tubular nature of the hepatocyte cords could be discerned despite the tissue being immersion-fixed. PAS and PAS-D staining reactions revealed the hepatocyte cytoplasm to be filled with glycogen (Figs. 4.22 A & B). The immersion fixation procedure made the recognition of the sinusoids, endothelial cells, stellate cells and blood cells (excluding red blood cells) difficult in H/E preparations, but Toluidine blue resin sections revealed these structures adequately (Fig. 4.21 B). Kupffer cells were identified by means of large granular inclusions that were again melanin, hemosiderin and PAS-D positive (Fig. 4.25 A-D). There was a diminished presence of peribiliary hemosiderin granules (Fig. 4.25 D). A fine network of black reticular fibres (Fig. 4.24) surrounded the groups of hepatocytes and clearly showed their tubular nature.

4.5 DISCUSSION

4.5.1 Parenchymal organization

The present study concurs with several authors that the classic lobular pattern seen in the livers of mammals does not apply to reptiles in general (Henninger 1982; Goldblatt *et al.* 1987; Schaffner 1998; McClellan-Green *et al.* 2006) or to the West African crocodile and the broad-nosed caiman (Storch *et al.* 1989; Starck *et al.* 2007). Hepatocyte tubules (McClellan-Green *et al.* 2006; Jacobson 2007) or plates (Richardson *et al.* 2002) may be organized radially around central veins or around portal veins (Henninger 1982). Parenchymal lobulation may be completely absent in some reptiles (McClellan-Green *et al.* 2006) as in the case of the juvenile Nile crocodile. 'Discrete hepatic lobules' were found by Richardson *et al.*

(2002) in the Saltwater and Australian freshwater crocodiles and Kassab *et al.* (2009) described classic hepatic lobules in the Desert tortoise.

Beresford & Henninger (1986) reviewed the various microscopical differences in the livers of mammals, birds, fish, reptiles & amphibians - crocodiles were however excluded. The authors' criteria for perceived parenchymal tubules, namely separate round cross-sectional profiles of pyramidal cells with central lumina, have been described in the four non-mammalian vertebrate classes. This interpretation is shared by the current findings of a tubular parenchymal structure in the juvenile Nile crocodile and was also described in *Caiman latirostris* (Starck *et al.* 2007) and in the freshwater turtle by Moura *et al.* (2009). The branching and anastomosing of hepatocyte tubules found in the juvenile Nile crocodile, as well as the two-cell-thick tubules, were also described in the crocodilian *Osteoleamus* by Storch *et al.* (1989) and in some reptiles (Elias and Bengelsdorf 1952, Schaffner 1998). The findings of Goldblatt *et al.* (1987) in the Newt liver and in *Phrynops geoffroanus* (Moura *et al.* 2009) that the hepatocyte chords were two to five cells thick corresponds to the current observations of the tubules in longitudinal and cross-sectional profile.

A limiting plate consisting of a single layer of hepatocytes beneath Glisson's capsule and also encircling the portal tracts is present in the human liver (Elias 1955, Fried 2008, www.pathologyoutlines.com). This continuous single cell layer was absent in the Nile crocodile where the parenchymal structures abutted the capsule and portal tracts directly.

The location of hepatocyte nuclei differs among the reptiles. Central nuclei were found in both the Newt (Goldblatt *et al.* 1987) and the freshwater turtle (Moura *et al.* 2009). Nuclei were described as being 'near to the vascular pole' in the West African crocodile (Storch *et al.* 1989) and basal nuclei were found by Henninger (1982) in the box turtle. The latter matches the eccentric nuclei seen in the juvenile Nile crocodile.

Henninger (1982) found hemosiderin positive granules in the apical hepatocytes of the Box turtle. Some of the pink (H/E) cytoplasmic granules seen peribiliary in the hepatocytes stained positive for hemosiderin (Perl's Prussian blue reaction) whereas the remaining pink granules may represent normal cytoplasmic organelles, for example mitochondria. The large brownish inclusions that seemed to be part of the parenchyma may be credited to the presence of bile pigments in hepatocytes (Kumar & Kiernan 2010). Electron microscopy (Chapter 5) will shed more light on these two observations.

The five livers in the present study contained varying quantities of vacuoles that in H/E preparations imparted a frothy look to the hepatocytes. The toluidine blue stain showed distinctive pale-coloured round vacuoles of differing sizes, mostly in the basal region, indicating lipid droplets. Starck *et al.* (2007) found lipid droplets in the apical part of the hepatocytes in the caiman. At the light microscopical level (H/E) vacuolation may be ascribed to the presence of water, glycogen, lipid or other material (Divers & Cooper 2000). Moura *et al.* (2009) attributed the vacuolated appearance of the hepatocytes in the freshwater turtle to an abundance of glycogen. Storch *et al.* (1989) described basal glycogen and the presence of lipid in glycogen areas. Ultrastructural examination (see Chapter 5) resolves this uncertainty.

4.5.2 Non-parenchymal organization

Elias and Bengelsdorf (1952) proposed that narrow, cylindrical **sinusoids** be called tubulosinusoidal and wide, irregularly shaped sinusoids be called sacculosinusoidal. They found the livers of lizards and tortoises to be of the sacculosinusoidal type and that of young alligators to be intermediate between the two sinusoidal types. The sinusoids of the broad-nosed caiman were described as 'very narrow' by Starck *et al.* (2007). The sinusoids in the present study were also of the intermediary type.

Some authors differentiate between **Kupffer cells** and melanomacrophages and some call these cells 'pigment cells' or 'specialized Kupffer cells' when containing melanin granules (Storch *et al.* 1989; Schaffner 1998; McClellan-Green *et al.* 2006; Jacobson 2007). Barni *et al.* (1999) deemed the pigment cells to derive from Kupffer cells. The Kupffer cells in this study were located in different areas and not only bound to the sinusoidal wall – it is difficult to type these cells as Kupffer cells or melanomacrophages at the light microscopical level and this matter will be considered in Chapter 5. Pigment cell clusters or collections of specialized Kupffer cells have been noted in other reptiles (Henninger 1982; Henninger & Beresford 1990; McClellan-Green *et al.* 2006; Jacobson 2007), but cell collections were not seen in the juvenile Nile crocodile liver. Instead, numerous but discretely scattered pigmented Kupffer cells were observed. Pigment cells were rare in the juvenile West African crocodile (Storch *et al.* 1989) and in some other reptile species (Hack & Helmy 1964; McClellan-Green *et al.* 2006). The hepatocytes of the lizard *Sceloporus* also contained pigment in addition to the pigment found in the Kupffer cells (Ells 1954). Moura (2009) did not mention Kupffer cells in the liver of the Freshwater turtle, but commented on the

presence of many melanomacrophages in the parenchyma. Perhaps the cells seen containing the identical yellow-brownish granules as Kupffer cells, and that were part of the hepatocyte groups in the Nile crocodile, are in fact melanomacrophages.

The large yellow-brownish cytoplasmic inclusions seen in the Nile crocodile Kupffer cells were positive for both **melanin** and **hemosiderin**, a feature also described by Jacobson (2007) in other reptiles, and also contained a third element. Hemosiderin is usually seen in cells responsible for the breakdown of effete red blood cells and consists, among others, of ferritin and glycoproteins (Kumar & Kiernan 2010). Glycoproteins are PAS positive and may account for the third pink element in the large yellow-brown granules. Other possible contenders for the third pink component may be either **ceroid** or **lipofuscin** as both these pigments are PAS positive (www.pathologyoutlines.com, Schaffner 1998). According to these two references ceroid pigment represents degraded cellular debris and lipofuscin denotes the accumulation of indigestible material mixed with lipid droplets. Kumar & Kiernan (2010) however describe ceroid as a type of lipofuscin. Ultrastructural features (see Chapter 5) distinguished between the two pigments.

4.5.3 Connective tissue stroma

The livers of the Saltwater and Australian freshwater crocodiles were covered by a ‘thick fibrous capsule’ and showed ‘relatively little interstitial connective tissue’ (Richardson *et al.* 2002). The connective tissue layers separating neighbouring liver lobules in some mammals were not evident in reptiles (Jacobson 2007). The juvenile Nile crocodile liver revealed prominent collagenous fibres of varying sizes criss-crossing the liver parenchyma from Glisson’s capsule to the portal areas. Beresford (1987) found connective tissue only in the liver capsule, portal tracts and large hepatic venules of the juvenile *Caiman crocodilus,* but a further study (Beresford 1993) found thin collagenous trabeculae in the parenchyma in three out of four Caiman livers examined. The liver of *Alligator mississippiensis* showed intermediate trabeculae of collagen linking the connective tissue of the liver capsule and portal tracts. Beresford (1993) hypothesized that the function of the collagen trabeculae in the alligator liver was to withstand thrashing of the body when subduing prey. Goldblatt *et al.* (1987) found connective tissue to be sparse in the Newt liver.

Schaffner (1998) desribed the portal tracts of reptiles to be randomly organized and Moura *et al.* (2009) noted an abundant connective tissue support for the portal tracts in the

Freshwater turtle. This is in agreement with the findings in the juvenile Nile crocodile. Beresford (1987) described the larger bile ducts of the Caiman to have a thick collagenous and cellular wall and speculated that this feature may have a supporting function.

The liver architecture in the present study was characterised by a delicate network of black reticular fibers (Gordon & Sweet's stain) around the hepatocytic tubules, sinusoids and portal areas. This was also found to be true of the Freshwater turtle (Moura *et al.* 2009). Kassab *et al.* (2009) described 'a fine meshwork of reticular fibres around the sinusoids and within the perisinusoidal spaces' of the Desert tortoise liver. Many studies do not mention the staining of reticular fibres.

Henninger (1982) and Storch *et al.* (1989) both noticed distinct PAS-positive boundaries, indicating the presence of basal lamina, around the hepatocyte tubules of the box turtle and the West African crocodile respectively. These boundaries, although incomplete and not present around all tubules, were demonstrated by the PAS-D reaction in the Nile crocodile. (See Chapter 5).

4.5.4 Isthmus

The light microscopical findings supported the macroscopical assumption (Chapter 3) that the flattened isthmus consisted of liver tissue and consequently contained parenchymal and non-parenchymal components. Marycz & Rogowska (2007) and Kassab (2009) described the isthmus of tortoises as consisting of connective tissue. Some authors mention the isthmus (Mushonga & Horowitz 1996), or dorsal bridge (Huchzermeyer 2003), or 'middle constricted portion' (Chiasson 1962), but do not elaborate further on its composition. Other authors do not refer to the existence of an isthmus in reptiles (Schaffner 1998, McClellan-Green *et al.* 2006).

The clear cytoplasm of the hepatocytes in this region may be due to the leaching of glycogen & lipid during histological processing. The light microscopical results of the isthmus indicate that this narrow tissue bridge is an extension of the two liver lobes with the same functional capabilities. Perhaps the existence of an isthmus can be ascribed to a developmental adaptation for the incorporation of other organs in the body cavity.

4.6 REFERENCES

BANCROFT, J.D. 2003. *Theory and Practice of Histological Techniques*. New York, Churchill Livingstone.

BARNI, S., BERTONI, V., CROCE, A.C., BOTTIROLLI, G., BERNINI, F. & GERZELI, G. 1999. Increase in liver pigmentation during natural hibernation in some amphibians. *Journal of Anatomy,* 195: 19-25.

BERESFORD, W.A. 1987. Some light microscopic histology of the liver in *Caiman crocodilus*. *The Anatomical Record,* 218: 16A.

BERESFORD, W.A. 1993. Fibrous trabeculae in the liver of alligator (*Alligator mississipiensis)*. *Annals of Anatomy,* 175: 357-359.

BERESFORD, W.A. & HENNINGER, J.M. 1986. A tabular comparative histology of the liver. *Archives Histology Japan,* 49: 267-281.

CHIASSON, R.B. 1962. Digestive system, in *Laboratory anatomy of the alligator.* USA: WM.C. Brown, pp. 36-37.

DIVERS, S.J. & COOPER, J.E. 2000. Reptile hepatic lipidosis. *Seminars in Avian and Exotic Pet Medicine,* 9: 153-164.

ELIAS, H. & BENGELSDORF, H. 1952. The structure of the liver of vertebrates. *Acta Anatomica,* 15: 297-337.

ELIAS, H. 1955. Liver morphology. *Biological Reviews,* 30: 263-310.

ELLS, H.A. 1954. The gross and microscopic anatomy of the liver and gall bladder of the lizard, *Sceloporus occidentalis biseriatus* (Hallowell). *The Anatomical Record,*119: 213-229.

FRIED, G.H. 2008. Liver, in *AccessScience.* © McGraw-Hill Companies.

http://www.accessscience.com

GOLDBLATT, P.J., HAMPTON, J.A., DIDIO, L.N., SKEEL, K.A. & KLAUNIG, J.E. 1987. Morphologic and histochemical analysis of the Newt (*Notophthalmus viridescens*) liver. *The Anatomical Record,* 217: 328-338.

HACK, M.H. & HELMY, F.M. 1964. A comparative chemical study of the liver of various vertebrates. *Acta Histochemistry,* 19: 316-328.

HENNINGER, J.M. 1982. Histology of the liver in the box turtle. *The Anatomical Record,* 202: 79A.

HENNINGER, J.M. & BERESFORD, W.A. 1990. Is it coincidence that iron and melanin coexist in hepatic and other melanomacrophages? *Histology and Histopathology*, 5: 457-459.

HUCHZERMEYER, F.W. 2003. Crocodiles and alligators, in *Crocodiles: biology, husbandry and diseases,* edited by F.W. Huchzermeyer. Cambridge: CABI Publishing, 1:1-52.

JACOBSON, E.R. 2007. Overview of reptile biology, anatomy, and histology, in *Infectious diseases and pathology of reptiles,* edited by E.R. Jacobson. CRC Press, Taylor & Francis Group, 6000 Broken Sound Parkway NW, Suite 300, Boca Raton, FL 33487-2742, pp.1-130.

KASSAB, A., SHOUSHA, S. & FARGANI, A. 2009. Morphology of blood cells, liver and spleen of the Desert Tortoise (*Testudo graeca*). *The Open Anatomy Journal,* 1*:* 1-10.

KUMAR, G.L. & KIERNAN, J.A. 2010. *Education guide: Special stains and H & E.* Dako North America, California. www.dako.com.

MARYCZ K., ROGOWSKA K. 2007. The liver morphology and topography of Horsfield's (*Testudo horsfieldi*) and Hermann's (*T. Hermanni*) terrestrial tortoises. *Electronic Journal of Polish Agricultural Universities,* 10: 1-9.

MCCLELLAN-GREEN, P., CELANDER, M. & OBERDÖRSTER, E. 2006. Hepatic, renal and adrenal toxicology, in *Toxicology of reptiles,* edited by S.C. Gardner & E. Oberdörster. CRC Press, Taylor & Francis Group, 6000 Broken Sound Parkway NW, Suite 300, Boca Raton, FL 33487-2742, pp.123-148.

MOURA, L.R., SANTOS, A.L.Q., BELLETI, M.E., VIEIRA, L.G., ORPINELLI, S.R.T. & DE SIMONE, S.B.S. 2009. Morphological aspects of the liver of the freshwater turtle *Phrynops geoffroanus* Schweigger, 1812 (Testudines, Chelidae). *Brazilian Journal of Morphological Science,* 26: 129-134.

MUSHONGA, B. & HOROWITZ, A. 1996. Serous cavities of the Nile crocodile (*Crocodylus niloticus). Journal of Zoo and Wildlife Medicine,* 27: 170-179.

RICHARDSON, K.C., WEBB, G.J.W. & MANOLIS, S.C. 2002. Digestive system, in C*rocodiles: inside out,* edited by K.C. Richardson. Australia: Surrey Beatty & Sons PTY LIMITED, pp. 89-94.

SCHAFFNER, F. 1998. The hepatic system, in *Biology of reptilia: volume 19, Morphology G: Visceral organs,* edited by C. Gans & A.B. Gaunt. Society for the Study of Amphibians and Reptiles, Missouri, pp. 485-531.

STARCK, M.J., CRUZ-NETO, A.P. & ABE, A.S. 2007. Physiological and morphological responses to feeding in broad-nosed caiman (*Caiman latirostris*). *The Journal of Experimental Biology,* 210: 2033-2045.

STORCH, V., BRAUNBECK, T. & WAITKUWAIT, W.E. 1989. The liver of the West African crocodile *Osteolaemus tetraspis.* An ultrastructural study. *Submicroscopical Cytology and Pathology,* 21: 317-327.

WWW Site reference:

www.pathologyoutlines.com