NECROSIS, REGENERATION AND REPAIR OF THE LIVER

NECROSIS OF HEPATOCYTES

- the high metabolic rate and some of the normal functions of hepatocytes make them prone to cell death (**necrosis**)
- e.g. hepatocytes are rich in membranous organelles and are therefore highly vulnerable to oxidant damage by reactive oxygen species (free radicals), especially if anti-oxidants such as vitamin E and reduced glutathione are depleted
- e.g. metabolism of toxins may generate more toxic intermediate metabolites and reactive oxygen species → death of cells performing the biotransformation steps +/- their neighbours

- causes of hepatocyte necrosis include hypoxia, toxins, severe metabolic disturbances, anti-oxidant deficiency, or the action of infectious agents or leukocytes

- **the distribution pattern of necrosis in the liver (and of the subsequent fibrosis in a surviving animal) provides important clues to the likely cause**

FOCAL NECROSIS

- **focal necrosis** refers to a solitary focus of necrosis of hepatocytes
- e.g. **focal acute hepatic trauma** - blunt trans-abdominal trauma to one part of the liver, penetration of a sharp foreign body from the reticulum in a cow
- e.g. **black disease** - local activation of a dormant intra-hepatic bacterial spore of a *Clostridium* species, with subsequent bacterial replication and release of necrotising exotoxins (see Lecture 14)
- in surviving animals (NOT those with black disease!), repair of the lesion will result in **focal hepatic fibrosis**

MULTIFOCAL NECROSIS

- multiple small focal aggregates of necrotic hepatocytes are randomly distributed throughout the liver
- the foci are usually numerous and appear grossly as tiny cream to white foci
- over time, the necrotic foci evolve into foci of inflammation (**multifocal hepatitis**)

- a **very common pattern**
- typical of **many viral**, **bacterial** or **protozoal infections** in which the infectious agent enters the liver via the blood stream (especially via the portal vein from the gastrointestinal tract)
- hence, multifocal necrosis is often referred to as an **embolic pattern** because the cause has entered the organ as a blood-borne embolus from somewhere else

- **trans-hepatic migration of larvae of metazoan parasites** can also result in multifocal necrosis

- multifocal necrosis is usually of little functional significance
- may lead to minor **multifocal fibrosis** but can sometimes completely resolve without any obvious scarring
- e.g. **telangiectasis** - very common in livers of cattle
  - multiple, randomly scattered, pinpoint to a few mm diameter red spots that ooze a tiny

volume of blood when incised
- correspond microscopically to distended blood-filled sinusoids
- thought to represent residual sites of previous embolism and hepatocellular necrosis that healed without significant fibrosis

ZONAL NECROSIS

= necrosis of hepatocytes restricted to distinct acinar zones

- often grossly visible as a **zonal pattern**
- necrotic zones appear as slightly depressed red areas due to dilation of sinusoids and haemorrhage
- zones of degenerate cells undergoing hydropic or fatty degeneration appear as slightly raised pale areas

- the zone(s) affected provide diagnostic clues as to the possible cause
- usually need microscopic examination to accurately determine which zone (1, 2 or 3) is affected

Periacinar (Centrilobular) Necrosis

- the **most common type of zonal pattern**

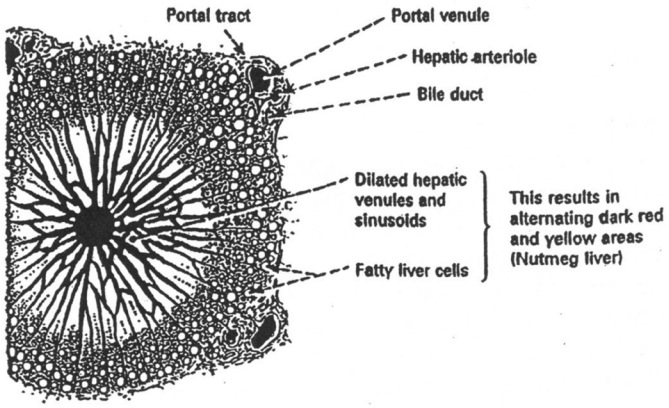
- hepatocytes in the periacinar zone (zone 3) are most distant from the afferent blood supply and are therefore most susceptible to hypoxia
- they also contain the greatest concentration of mixed function oxidase (cytochrome P450) enzymes that can transform exogenous drugs and toxins into more potent metabolites

- e.g. **most toxic insults**
- e.g. **severe hypoxic injury** - severe anaemia, right-sided congestive heart failure and other causes of passive venous congestion of the liver, shock, a prolonged agonal period in which circulatory function is declining etc
- e.g. **some viral infections** - canine adenovirus-1 (infectious canine hepatitis virus), Rift Valley Fever virus (an exotic disease of small ruminants)
- e.g. **equine serum sickness** (Theiler's disease)

- particularly in the context of passive venous congestion of the liver caused by right-sided congestive heart failure, the gross zonal pattern is often referred to as **"nutmeg liver"** (because it resembles the cut surface of a nutmeg) (Figure 1)

- **periacinar** (centrilobular) **fibrosis** may be identifiable in survivors (this is sometimes referred to as **cardiac sclerosis** in animals with chronic right-sided congestive heart failure)

Figure 1



Reference: "Pathology Illustrated" – A.D.T. Govan, P.S. Macfarlane, R. Callander, 4th edition, Churchill Livingstone, Edinburgh, 1995

Midzonal Necrosis

- see Figure 2
- in midzonal necrosis, hepatocytes in zone 2 (mid zone) are chiefly affected by the necrosis
- **uncommon**
- seen in **some intoxications**, e.g. ngaione (*Myoporum* species) in sheep, some cases of aflatoxicosis in pigs and horses, and rarely blue-green algal poisoning

Figure 2

Reference: "Pathology Illustrated" – A.D.T. Govan, P.S. Macfarlane, R. Callander, 4th edition, Churchill Livingstone, Edinburgh, 1995

Periportal Necrosis

- in periportal necrosis, hepatocytes in zone 1 (periportal zone) are mainly affected
- **uncommon**
- seen in **some intoxications** (e.g. acute bovine liver disease, phosphorus poisoning, some cases of blue-green algal poisoning)
- periportal necrosis is also typical of **rabbit calicivirus** infection

- surviving animals may have permanent **portal/periportal fibrosis**

- **portal/periportal fibrosis** (**biliary fibrosis**) is also expected in animals that survive an episode of chronic cholangiohepatitis (Lecture 14) or chronic extra-hepatic bile duct obstruction, with the scar tissue centred on bile duct branches in the portal areas

MASSIVE NECROSIS

- **massive necrosis = necrosis of entire hepatic acini**
- **NOT necrosis of the entire liver** but large areas of the organ may be involved

- as no cells survive in the affected acini, local regeneration of hepatocytes is impossible
- the connective tissue scaffold of the liver is also destroyed in the areas of massive necrosis → collapse of the necrotic areas → broad bands of scar tissue (so-called **post-necrotic scarring)** in survivors + regenerative nodular hyperplasia of hepatocytes in adjacent unaffected parenchyma

- the affected liver may be slightly enlarged, small or of normal size
- **acute phase** – grossly, appears as a mosaic of red, grey and yellow tissue (red = collapsed zones of necrosis and haemorrhage; grey-yellow = areas of surviving but degenerate tissue)
- **chronic phase** – grossly see bands of scar tissue crisscrossing the liver, with intervening zones of nodular hyperplasia of hepatocytes

- **causes of massive hepatic necrosis** are:

  - **severe toxic injury**
  - **acute vascular accidents** - e.g. sudden thrombosis of an intra-hepatic branch of the portal vein, torsion of a liver lobe, strangulation of a liver lobe in a diaphragmatic hernia
  - **severe damage by reactive oxygen species** - e.g. due to deficiency of anti-oxidants or sudden increased demand for anti-oxidants (e.g. following iron injection)

Hepatosis Dietetica in Pigs

- hepatosis dietetica is a syndrome of **acute massive hepatic necrosis** seen in young, rapidly growing pigs
- often responsible for sudden death
- due to **anti-oxidant deficiency** caused by concurrent deficiencies of sulphur-containing amino acids, vitamin E and/or selenium → hepatocellular membrane peroxidation by reactive oxygen species → massive hepatic necrosis

HEPATIC FIBROSIS AND STELLATE CELLS

- animals that survive an episode of hepatocellular necrosis are likely to develop some degree of **hepatic fibrosis** (**scarring**)
- so too are animals with recurrent or persistent inflammation of the hepatic parenchyma

Hepatic Stellate Cells and Progressive Fibrosis

- the major source of new collagen deposition in the liver are the **stellate cells** (also known as the **Ito cells**) of the liver
- these are specialised lipocytes (fat-storing cells) located in the perisinusoidal space immediately external to the sinusoidal endothelial cells
- in health, they contribute (with hepatocytes and endothelial cells) to the production and maintenance of the delicate extracellular matrix that supports the sinusoidal endothelium and maintains the spatial relationship of the endothelial cells to the hepatocytes

- this matrix is largely made up of type IV collagen

- in health, stellate cells store lipid droplets that are rich in retinyl esters, including **vitamin A**
- they can become greatly distended by lipid, especially in cats
- they also function in the control of microvascular tone and regulate sinusoidal blood flow

- hepatic injury (e.g. inflammation and/or necrosis) can lead to local release of cytokines (e.g. transforming growth factor-β, interleukin-1, tumour necrosis factor, reactive oxygen species) from leukocytes, activated Kupffer cells or injured hepatocytes → stimulation of stellate cells → mitotic division and transformation from fat- and vitamin A-storing cells into collagen-producing myofibroblasts → fibroplasia and often permanent fibrosis
- the new collagen (especially types I and III) and other matrix components (e.g. laminin, fibronectin and chrondroitin sulfate proteoglycans) is largely deposited by the stellate cells in the perisinusoidal space

- if the hepatic insult is mild and transient, the immature collagen may be degraded
- severe, persistent or repetitive hepatic insults → progressive fibrosis that is virtually irreversible → **capillarisation of sinusoids** (i.e. sealing of the normal endothelial fenestrae, filling in of the perisinusoidal space by collagen, and loss of microvilli from the hepatocyte surface) → impaired perfusion of hepatocytes and compromised secretion of hepatocellular products into sinusoidal blood
- capillarisation also increases the resistance to blood flow through the sinusoids

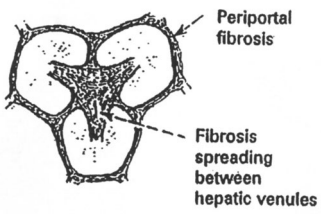
- in a heavily scarred liver, abnormal connections (**anastomoses**) often develop between afferent hepatic arterial and portal venous branches and between afferent blood vessels and efferent hepatic veins → impaired perfusion of hepatocytes
- capillarisation of the sinusoids and new vascular anastomoses within the liver both contribute to ongoing hepatocellular injury and hence to cirrhosis

- the two types of hepatic fibrosis that are commonly associated with progression of hepatic disease to cirrhosis are:

- **bridging fibrosis** = **fibrosis that links adjacent portal areas or links portal areas to central veins** (Figure 3)

- **diffuse** (or **dissecting**) **hepatic fibrosis** = fibrosis extending irregularly throughout the hepatic parenchyma, within and across acini
  - usually results from a diffuse, chronic or repetitive insult to the hepatic parenchyma

Figure 3



Reference: "Pathology Illustrated" – A.D.T. Govan, P.S. Macfarlane, R. Callander, 4th edition, Churchill Livingstone, Edinburgh, 1995

REGENERATION OF THE LIVER

- the liver has **considerable regenerative capacity**

- hepatocytes have a long lifespan (approximately 30% of animal's lifespan)
- hepatocytes are **stable cells** – normally low replication rate in adulthood but retain capacity to rapidly divide
- mature hepatocytes are usually in the post-mitotic or G_o resting stage of the cell cycle but, under the influence of growth factors (e.g. interleukin-6 and transforming growth factor-α from activated Kupffer cells and hepatocyte growth factor from stellate cells), can enter the cell cycle and undergo mitotic division

- in young animals, most hepatocytes have the capacity to replicate
- this capacity declines with age
- in mature animals, periportal (zone 1) hepatocytes have the greatest capacity to replicate

- can surgically remove up to 70% of liver → complete regeneration within a few weeks to normal size (but not shape)
- such regeneration involves mitotic division of surviving hepatocytes on the existing connective tissue scaffold rather than formation of new lobes or lobules (some new lobules may form from subdivision of existing lobules)

- restoration of normal hepatic mass therefore requires retention of the scaffold
- if the scaffold is destroyed, parenchymal collapse and permanent post-necrotic scarring result
- complete regeneration also requires an adequate (if not optimal) blood supply and biliary drainage

- once normal hepatic mass has been restored, release of transforming growth factor β by macrophages → cessation of mitotic division of hepatocytes

- **other potential sources of new hepatocytes** following hepatic parenchymal necrosis include:

  - **oval cells** (also known as **ductal precursor cells**)

    - small bipolar epithelial stem cells located in the lining of the terminal bile ductules (canals or ducts of Hering or cholangioles) located in the periportal (zone 1) parenchyma adjacent to the portal areas
    - these can divide and then differentiate into either cholangiolar epithelial cells or hepatocytes

    - **biliary hyperplasia** (**proliferation of new biliary channels in the portal areas** and in periportal parenchyma) is a common non-specific finding in recently damaged livers
    - biliary hyperplasia is thought to reflect mitotic division of the oval cells after necrosis of hepatocytes → formation of new cholangioles and replacement of lost hepatocytes

  - a putative multipotent **periductular liver progenitor cell** – derived from circulating bone marrow stem cells

- in chronic or repetitive hepatic insults, the parenchymal regeneration tends to be nodular (**nodular hyperplasia**) → architectural distortion
- although the regenerative nodules increase the hepatic parenchymal mass, the component hepatocytes may not function optimally
- blood flow into the nodules and biliary drainage from them may be abnormal → hydropic or fatty degeneration of the hepatocytes +/- cholestasis within the nodules
- abnormal communications between portal veins and central veins may also develop as a consequence of fibrosis → vascular bypass of some hepatocytes in the nodules

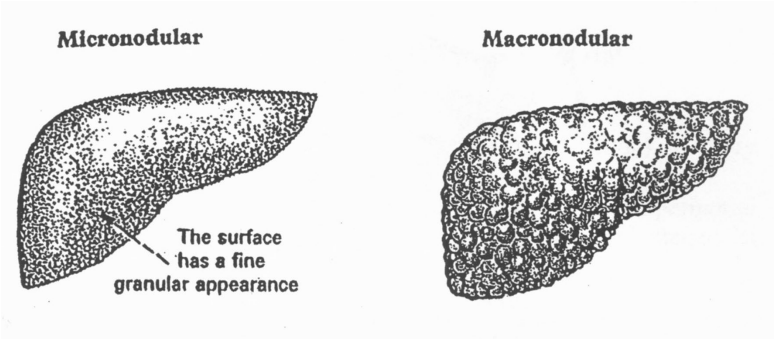
CIRRHOSIS

- **cirrhosis = an end-stage liver**

- a cirrhotic liver is characterised by:

  - **involvement of the entire liver** (not necessarily uniformly)
  - **bridging** or **diffuse fibrosis**
  - **regenerative hyperplastic parenchymal nodules** (either macronodular or micronodular) (Figure 4)
  - **permanent distortion of the architecture of the liver**, with development of new vascular anastomoses within the liver

Figure 4



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- once established, it may be impossible to accurately establish the original cause of cirrhosis

- **common causes of cirrhosis** in domestic animals are:

  - chronic poisoning of herbivores with plant toxins or mycotoxins (Lectures 15 and 16)
  - chronic cholangitis/cholangiohepatitis (Lecture 14)
  - chronic hepatitis in dogs, including chronic administration of anticonvulsant drugs and inherited disorders of copper metabolism (Lecture 14)

Consequences of Cirrhosis

- cirrhosis leads to **portal hypertension** (increased pressure in the portal vein and its upstream venous tributaries) due to increased resistance to portal venous blood flow into the sinusoids

- in cirrhosis, abnormal anastomoses develop between hepatic arterial and portal venous branches in areas of hepatic scarring; these may also contribute to portal hypertension by feeding higher pressure arterial blood into low pressure venous channels

- **portal hypertension** leads to:

  - **congestion of the viscera normally drained by the portal vein** - stomach, intestines, spleen, pancreas and their mesenteries

  - **ascites** - typically a transudate (low protein, low nucleated cell count)
    - due to increased plasma hydrostatic pressure within congested capillary beds of the splanchnic viscera and their mesenteries

  - **acquired portosystemic shunting**

Acquired Portosystemic Shunting

- **results from persistent portal hypertension**, usually due to cirrhosis
- occasionally results from gradual obstruction of portal venous inflow to the liver (e.g. by external compression of the portal vein by an expansively growing intra-abdominal tumour or abscess) or, rarely, from a congenital anastomosis between a branch of the hepatic artery and a branch of the portal vein

- unlike congenital portosystemic shunts, acquired portosystemic shunts appear as **MULTIPLE, tortuous venous channels** connecting the portal vein and the systemic venous circulation
- the shunts most often develop between the mesenteric veins and the caudal vena cava, right renal vein or gonadal vein
- the shunting vessels may be difficult to identify post mortem

- portal venous blood is shunted into the systemic circulation, bypassing the liver → similar consequences to a congenital portosystemic shunt (e.g. **hepatic encephalopathy** and deprivation of the liver of trophic factors)