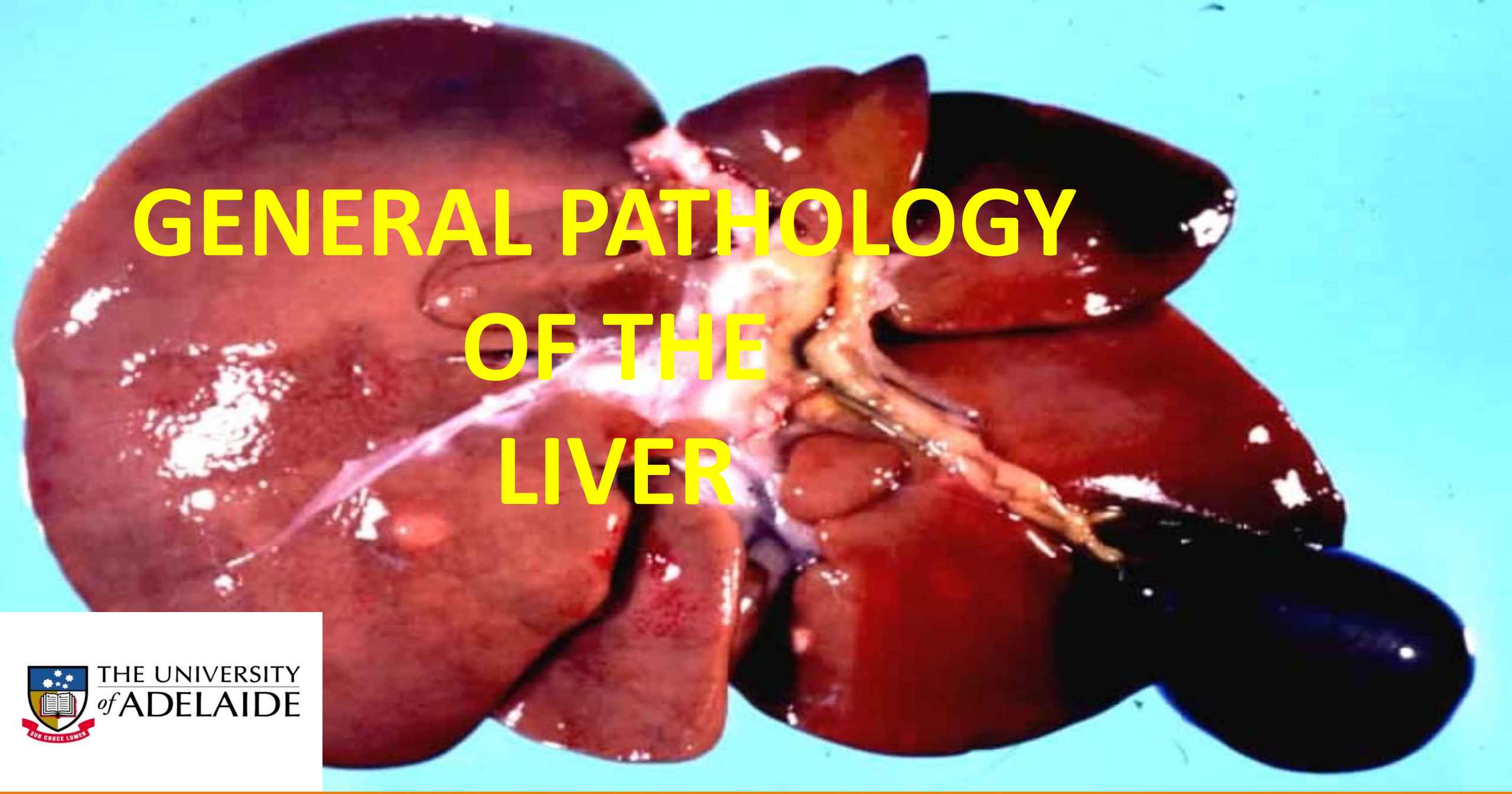


GENERAL PATHOLOGY OF THE LIVER

THE UNIVERSITY of ADELAIDE

A/Professor John Finnie

University Veterinarian, Office of the Deputy Vice-Chancellor (Research)

University of Adelaide

Myriad functions of the liver

Carbohydrate metabolism

Lipid metabolism

Protein synthesis

Bilirubin and bile acid metabolism

Xenobiotic metabolism

Immune function

Thus, hepatic dysfunction has far-reaching consequences

The liver is the largest internal organ and a marvel of biology

Guardian of homeostasis

Epicentre of the body's metabolic capability

A massive filter, detoxifying portal blood and releasing cleansed blood to the systemic circulation

An important part of the innate immune system, integrated into the complex system of defence against infection

The liver was perceived by the ancient Greeks to be immortal, not only because of its prodigious recuperative powers, but also because it was considered to be the seat of the soul and intelligence

The myth of Prometheus indicates that the ancient Greeks knew in some measure the liver's potential for repair (we know that 70% can be removed and it returns to normal size within a few weeks)

In Greek mythology, Prometheus was punished by the gods for his theft of fire for human use.

He was chained to a rock and each day an eagle was sent to feed on his liver. After overnight recovery of his “immortal liver”, the eagle’s meal was repeated.

Functional anatomy of the liver

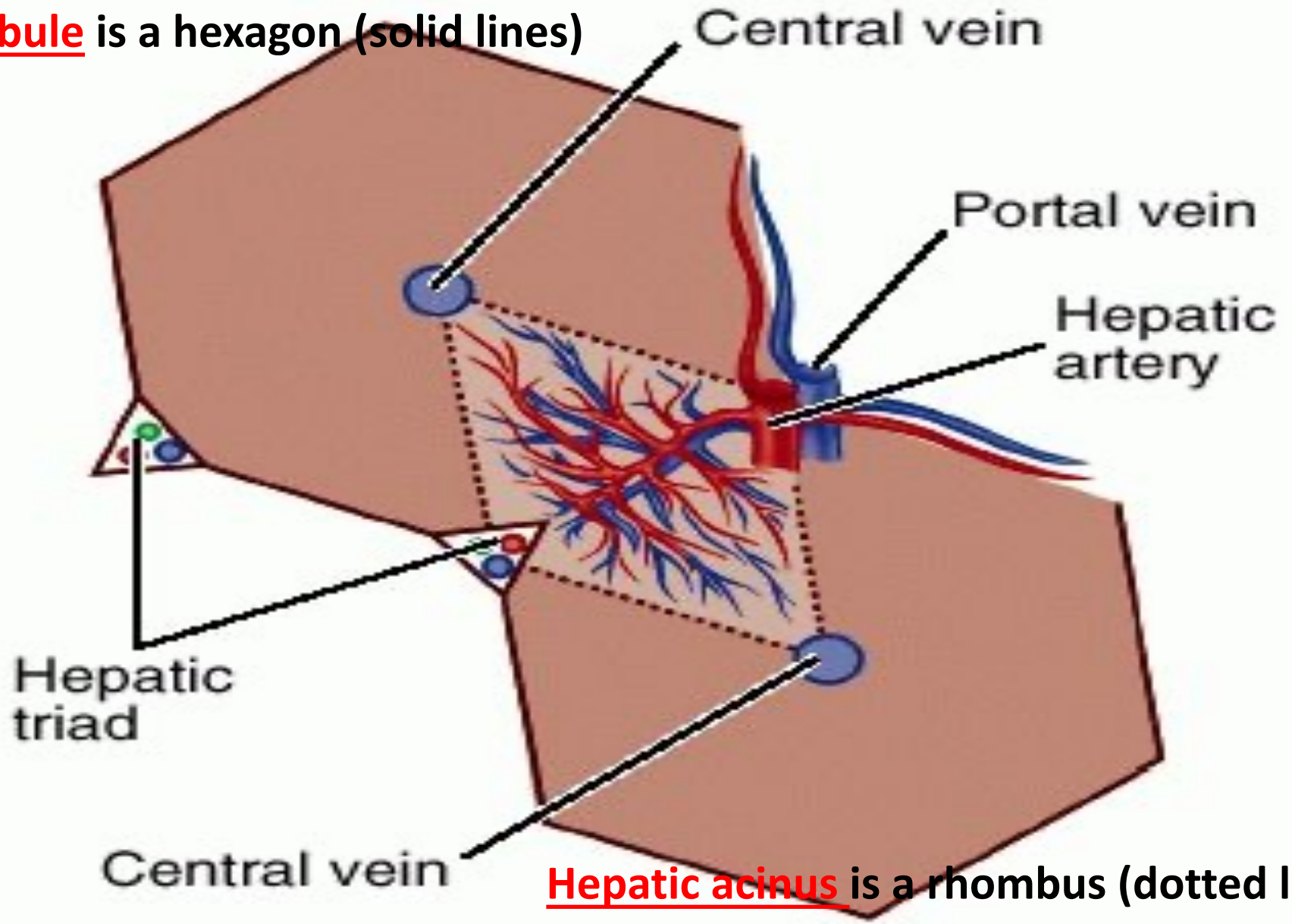
The vasculature of the liver defines its functional microanatomy, but debate continues as to what best represents the hepatic structural-functional unit

The 2 principal models of functional liver anatomy

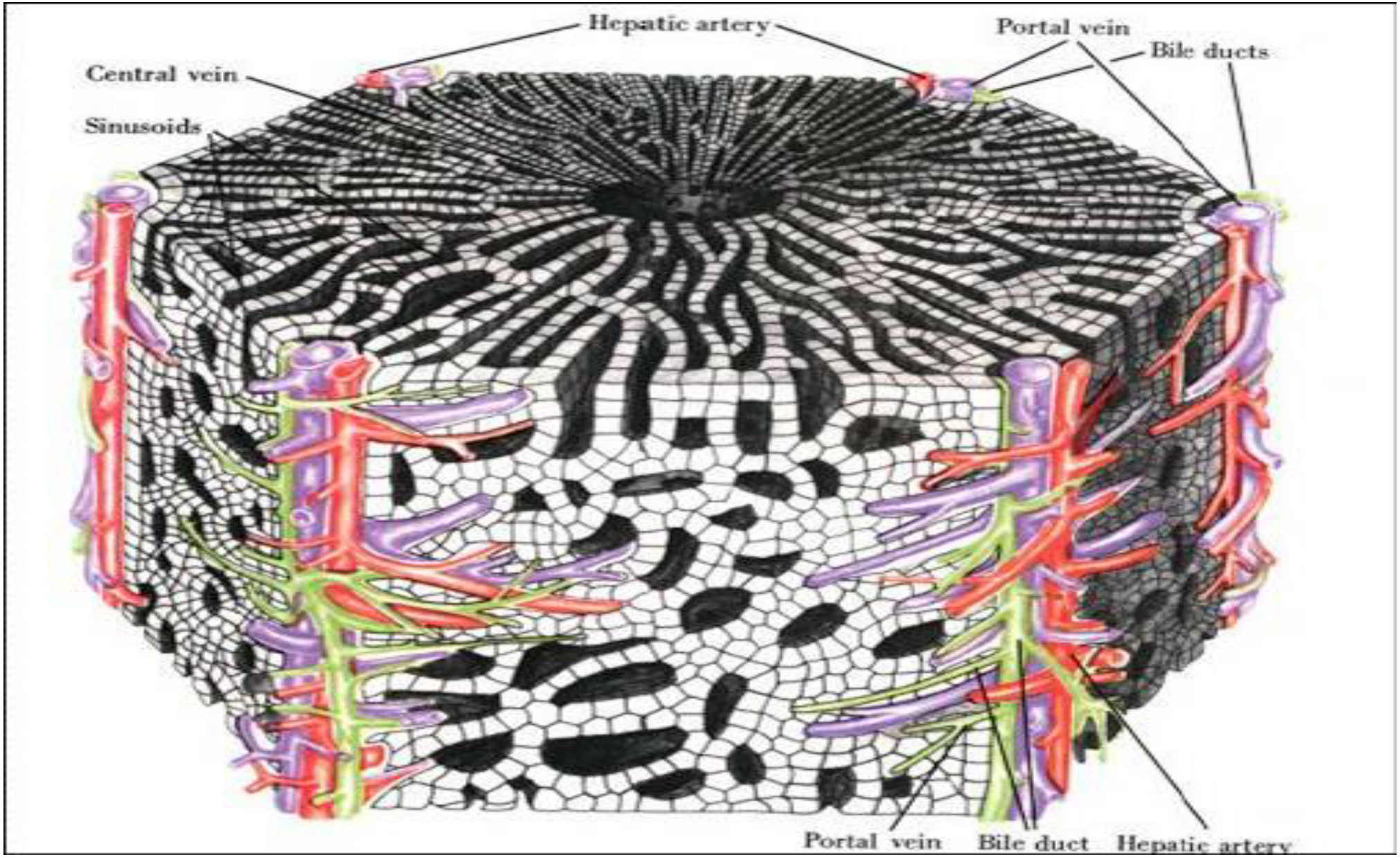
Classical hepatic lobule – a hexagonal anatomical arrangement of hepatocytes centred on the terminal hepatic venule

Hepatic acinus of Rappaport – terminal branches of portal vein and hepatic artery (at the centre of the acinus) project into the liver parenchyma, forming the long axis of a diamond-shaped acinus, with the hepatic venule (central vein) at the periphery

Hepatic lobule is a hexagon (solid lines)



Hepatic acinus is a rhombus (dotted lines)



HEXAGONAL HEPATIC LOBULE

Hepatic lobule

Arrangement with the hepatic venule (“central vein”) at its centre

Hepatocyte damage is then defined as being periportal (around the portal triad) or centrilobular (around the central venule)

Hepatic acinus

Divided into zones in relation to the blood supply:

1. **Zone 1 (periportal) – hepatocytes here are closest to oxygen and nutrient rich arterial and portal inflow**
2. **Zone 2 (transitional mid-zone)**
3. **Zone 3 (periacinar) – hepatocytes around the central venule**

Zone 1 - Periportal

Hepatocytes here are the main site of gluconeogenesis (formation of glucose from non-carbohydrate sources), protein synthesis, aerobic metabolism, urea cycle, lipid and cholesterol metabolism

These hepatocytes are susceptible to direct-acting toxicants due to their proximity to the vascular inflow

Zone 3 - Periacinar

These hepatocytes are more susceptible to hypoxic damage as they are furthest from the vascular inflow and are

Prone to injury from metabolically activated toxicants produced by cytochrome P450 and other detoxifying enzymes

Hepatic vascular supply

The liver receives ~25% of the cardiac output, plus that from

Portal vein supplies 70-80% of afferent blood, draining stomach, intestine, spleen and pancreas, and ~50% of oxygen (rest supplied by hepatic artery)

Portal flow is not regulated, but that in the hepatic artery is and responds to changes in portal flow

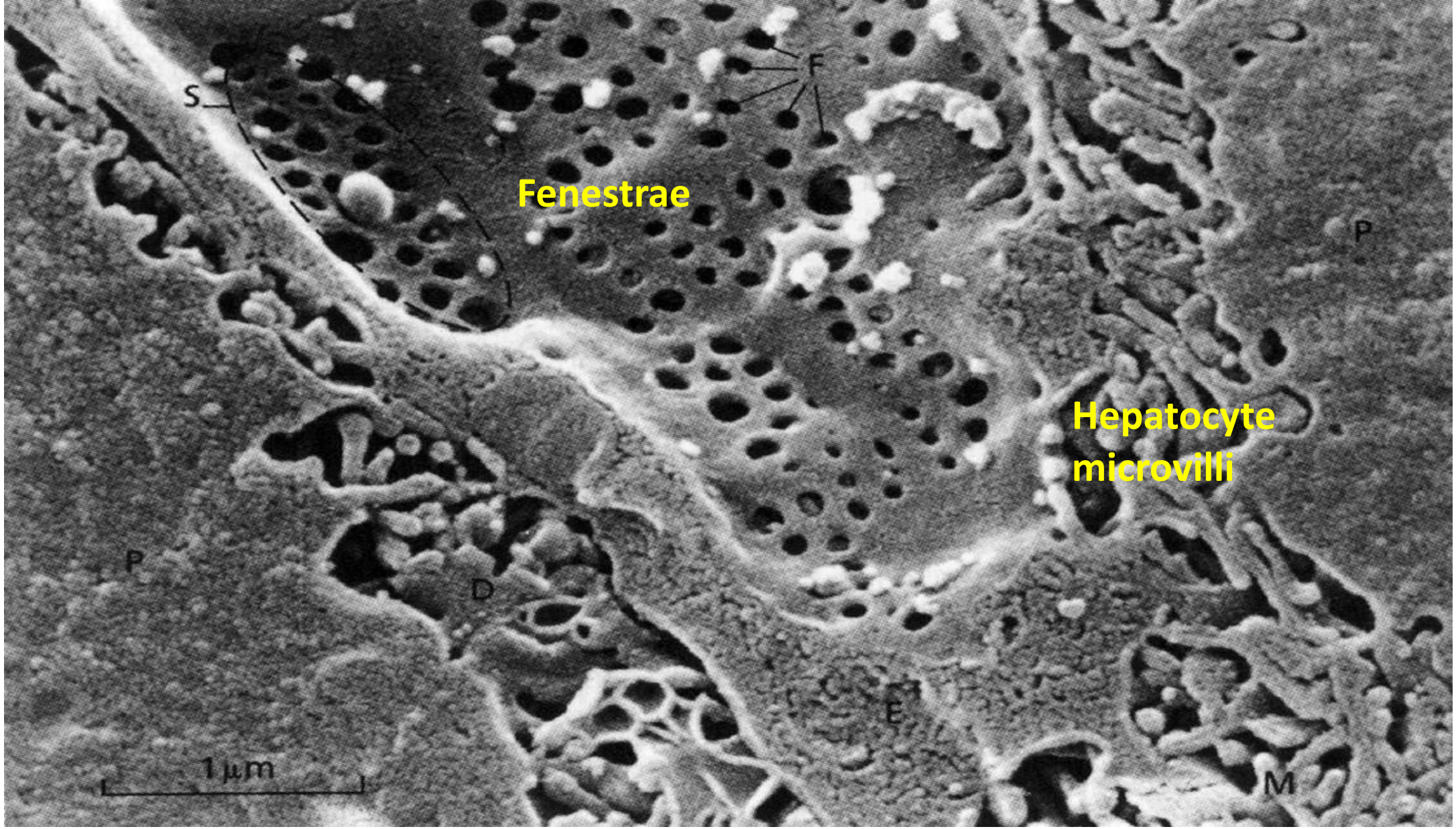
Portal and arterial blood eventually mix in low pressure sinusoids, leaving via hepatic veins into caudal vena cava

Hepatic vascular supply

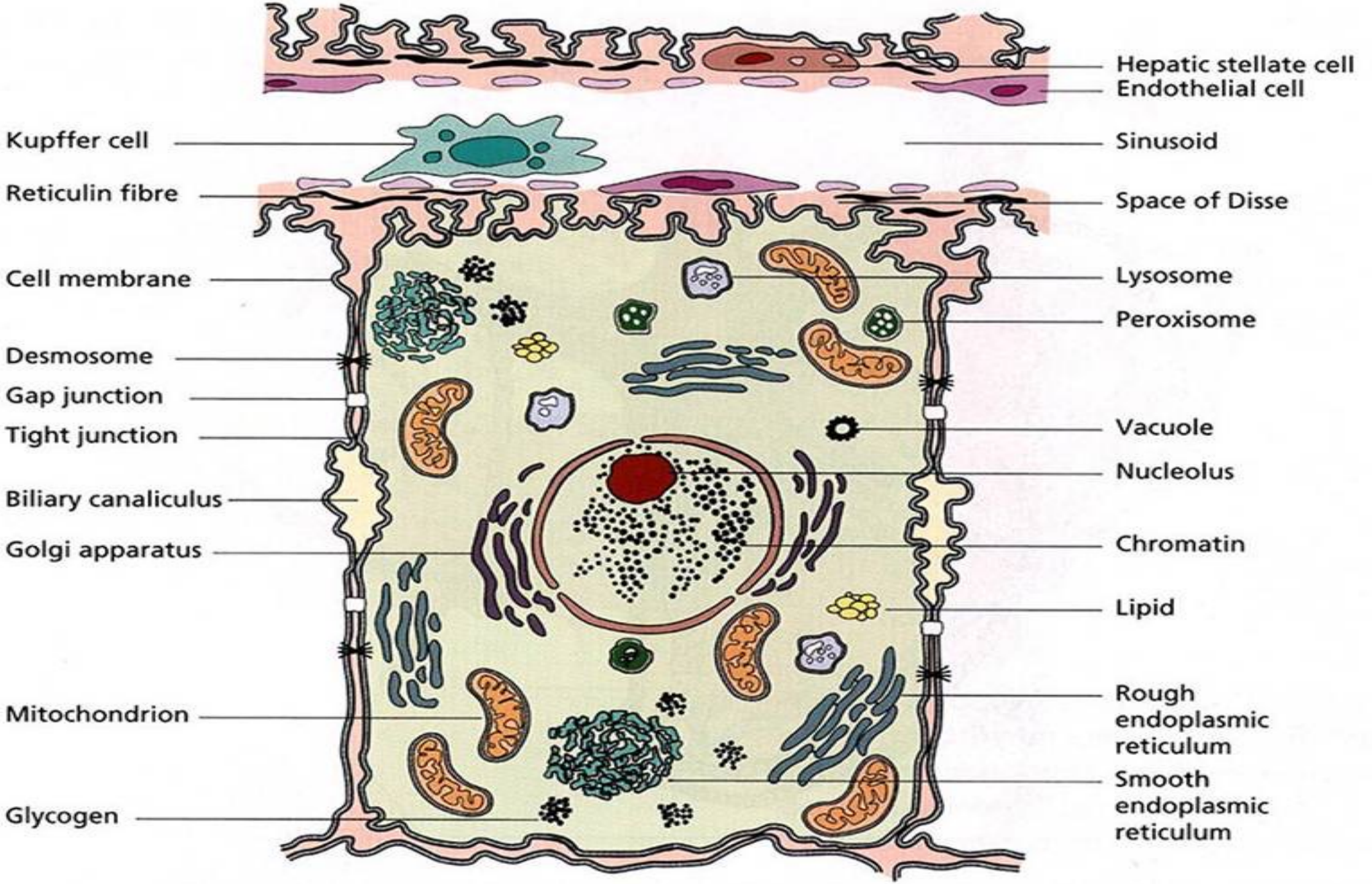
Sinusoids are lined by specialised endothelial cells, which are fenestrated and lack a basement membrane (being supported instead by loose extracellular matrix), providing free exchange of various macromolecules in the peri-sinusoidal space of Disse

Fluids from peri-sinusoidal space drain into lymphatics, then into hepatic lymph nodes and eventually thoracic duct

Liver is largest lymph producer in body – lymph is high in protein, containing 85-95% of the protein of plasma and a high lymphocyte and macrophage content



FENESTRATED SINUSOID


Hepatic stellate cell
Endothelial cell
Kupffer cell
Sinusoid
Reticulin fibre
Space of Disse
Cell membrane
Lysosome
Peroxisome
Desmosome
Gap junction
Tight junction
Vacuole
Nucleolus
Biliary canaliculus
Golgi apparatus
Chromatin
Lipid
Mitochondrion
Rough endoplasmic reticulum
Smooth endoplasmic reticulum
Glycogen

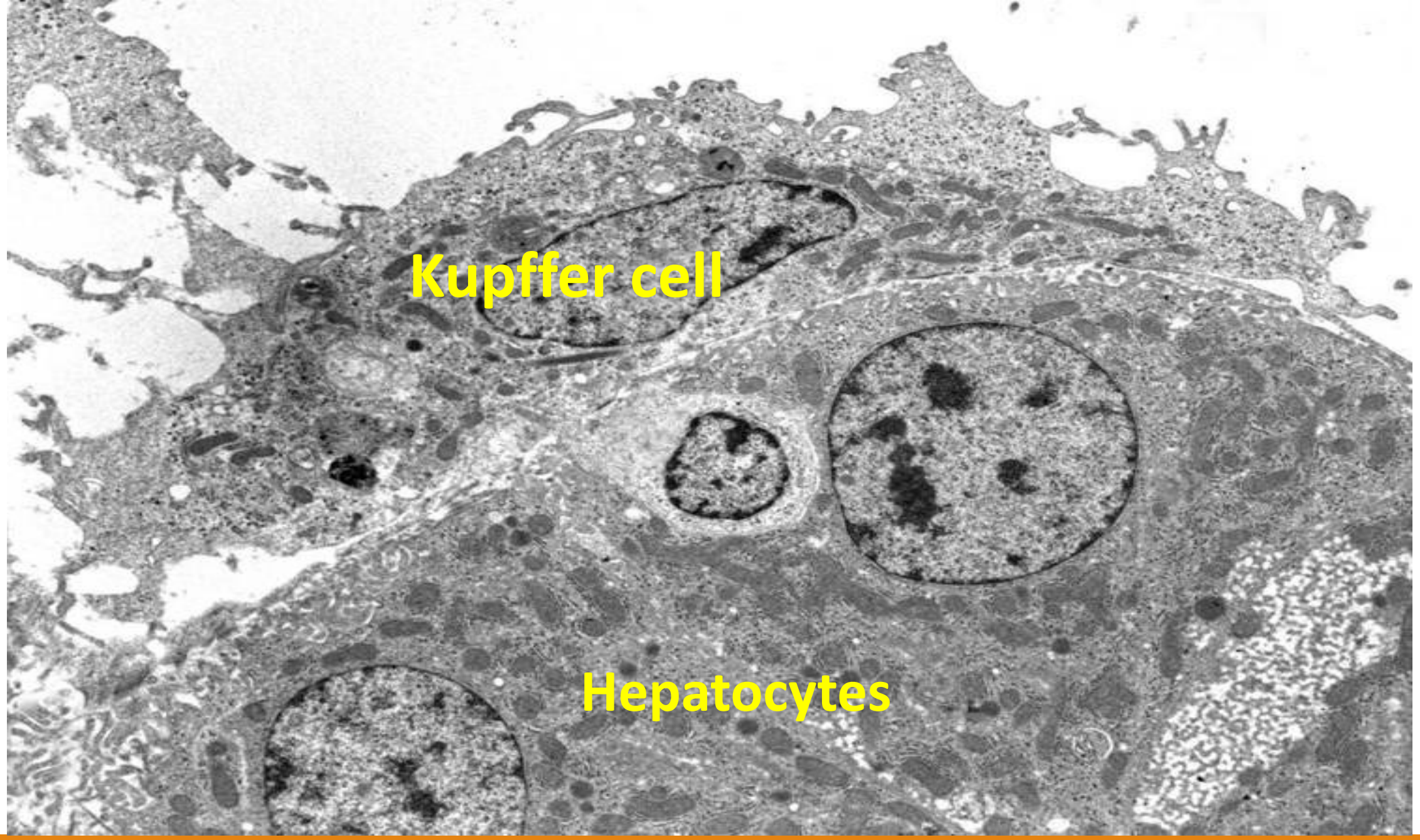
Kupffer cells

Specialised macrophages located in sinusoidal lumens and can migrate along the sinusoid to areas of tissue injury

May have a dual origin from blood-borne monocytes and indigenous cells, which are locally proliferative, especially in inflammation

Not efficient antigen-presenting cells, but are proficient phagocytes of effete cells, particulate matter, and microorganisms

Can secrete cytokines and other inflammatory mediators


Kupffer cell
Hepatocytes

Portal tract

Portal tract contains branches of the hepatic artery, portal vein, lymphatics and bile ducts

with a bile storage diverticulum (gallbladder)

Gallbladder is absent in horses and rats

Hepatocytes

These parenchymal cells constitute ~70-80% of the liver mass, but a large percentage is also composed of non-parenchymal cells (bile duct epithelium, sinusoidal endothelium, Kupffer cells) and itinerant cells (leucocytes)

Hepatocytes are arranged in single-cell thick plates, separated by sinusoids and each hepatocyte is exposed to blood on 2 sides

Highly metabolically active and organelles support a variety of functions, including synthesis and secretion of plasma proteins, coagulation factors, and acute-phase proteins

Hepatocytes

Store nutrients in times of adequate energy and release glucose when needed

Key modulators of lipid metabolism and synthesis and secretion of lipoproteins

Synthesise bile acids and secrete them into bile

Detoxify most xenobiotics and secrete them into bile

Hepatocytes

The liver is exposed to a variety of nutritionally-based insults and toxin-related damage

Normal hepatocytes contain abundant glycogen + stored triglycerides and proteins

3 morphologically and functionally distinct hepatocyte surfaces

Sinusoidal domain faces the space of Disse and has numerous microvilli, increasing the hepatocyte surface area to facilitate exchange with blood

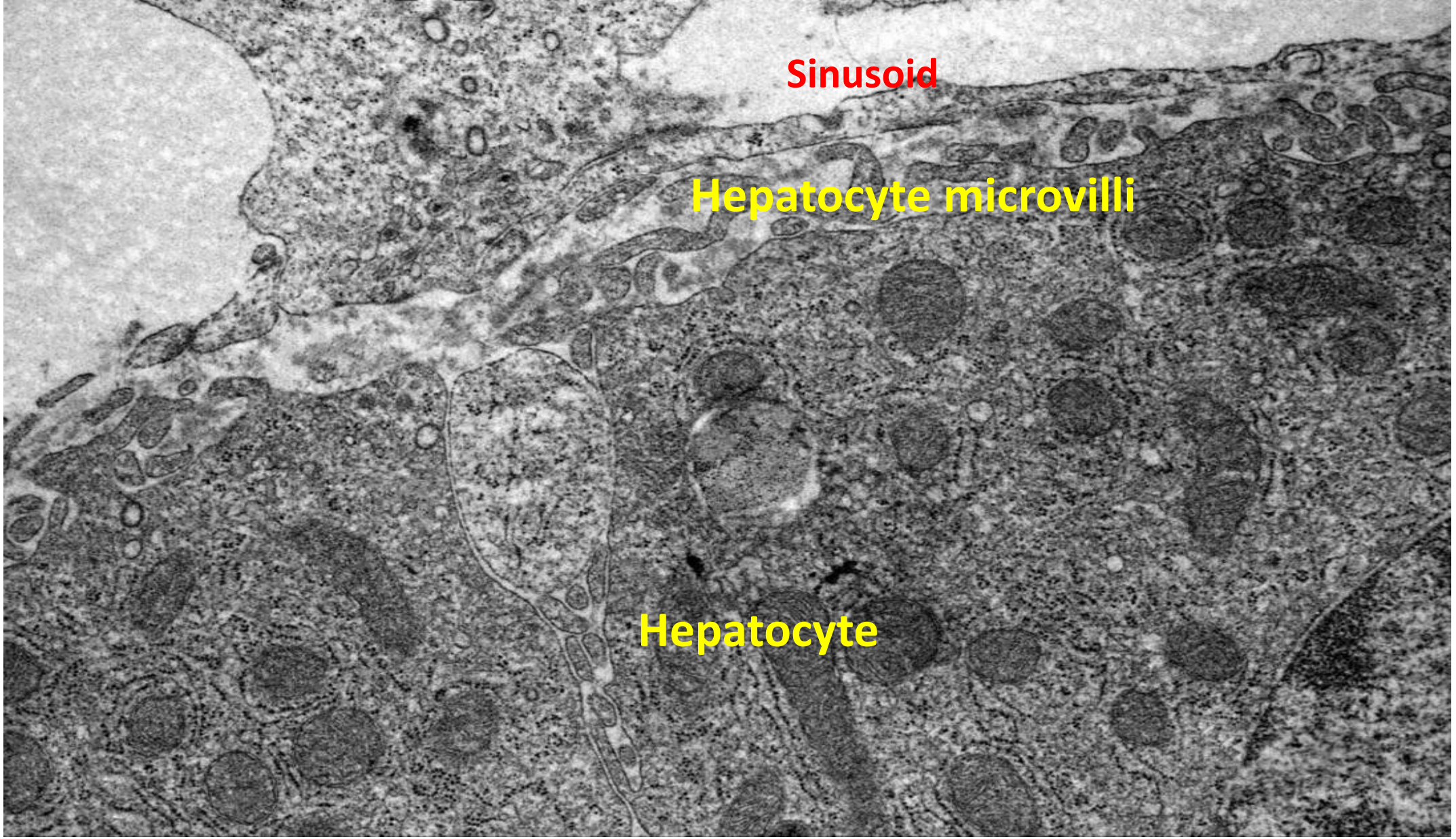
Lateral domain extends from the sinusoidal surface to the edge of the canaliculus and is specialised for adhesion via junctional complexes and intercellular communication via gap junctions

Canalicular domain is the beginning of the bile drainage system (intercellular space between 2 adjacent hepatocytes, isolated by junctional complexes). Canalicular surface is covered by microvilli and bile is propelled along canaliculi by contractile microfilaments

Canals of Hering

Partly lined by biliary epithelium and partly by hepatocytes and connects bile canaliculi to cholangioles and eventually interlobular bile ducts

Epithelial cells of this transition zone between hepatocytes and bile ductules have substantial regenerative potential – contains undifferentiated, pluripotential stem (oval) cells, which can generate new hepatocytes or bile ductules, the latter proliferating in many forms of liver injury



HEPATOCYTE SINUSOIDAL DOMAIN

Haematopoiesis

Occurs in perisinudoidal space of Disse in fetal life, which then declines, but can return (extramedullary haemopoiesis) with increased demand

The normal mouse liver at birth still contains a substantial amount of haemopoietic tissue, these foci disappearing 1-2 weeks post-partum

Other hepatic cells

Liver contains large numbers of lymphocytes (~5% of the entire liver cell population) involved in innate immune responses to maintain homeostasis by responding to immunological challenges, suggesting that the liver should be considered to be a lymphoid organ

Hepatic dendritic cells are found in portal tract and, with other antigen-presenting cells in liver (sinusoidal endothelium and Kupffer cells) play an important role in the induction and regulation of immune responses

Hepatic stellate cells (also termed lipocytes, Ito cells and fat-containing cells) reside in the space of Disse

Hepatic stellate cells

Store retinoids, including vitamin A

Maintain and remodel the sinusoidal extracellular matrix

Produce growth factors

Regulate sinusoidal diameter by contraction of cellular processes

They are important in hepatic fibrosis – when activated, they lose their lipid (possibly catabolising lipid to support an activated state) and develop a myofibroblast phenotype that can restrict sinusoidal flow. After hepatic injury, they can secrete a dense, less permeable matrix resembling a basement membrane and sinusoidal endothelial cells lose their fenestrae (termed "capillarisation" of sinusoids)

Hepatic progenitor (oval) cells

Hepatic progenitor (oval) cells are bipotential and can mature into biliary epithelium or hepatocytes

They can also proliferate and constitute a ductular reaction

When hepatocytes are damaged, oval cells can proliferate dramatically

Cholangiocytes/pit cells

Cholangiocytes (bile duct or biliary epithelium) differ from hepatocytes in phenotype and function, although derived from common embryonic progenitor cells, and actively modify the composition of bile

Natural killer (pit) cells adhere to sinusoidal endothelium and participate in immune defences

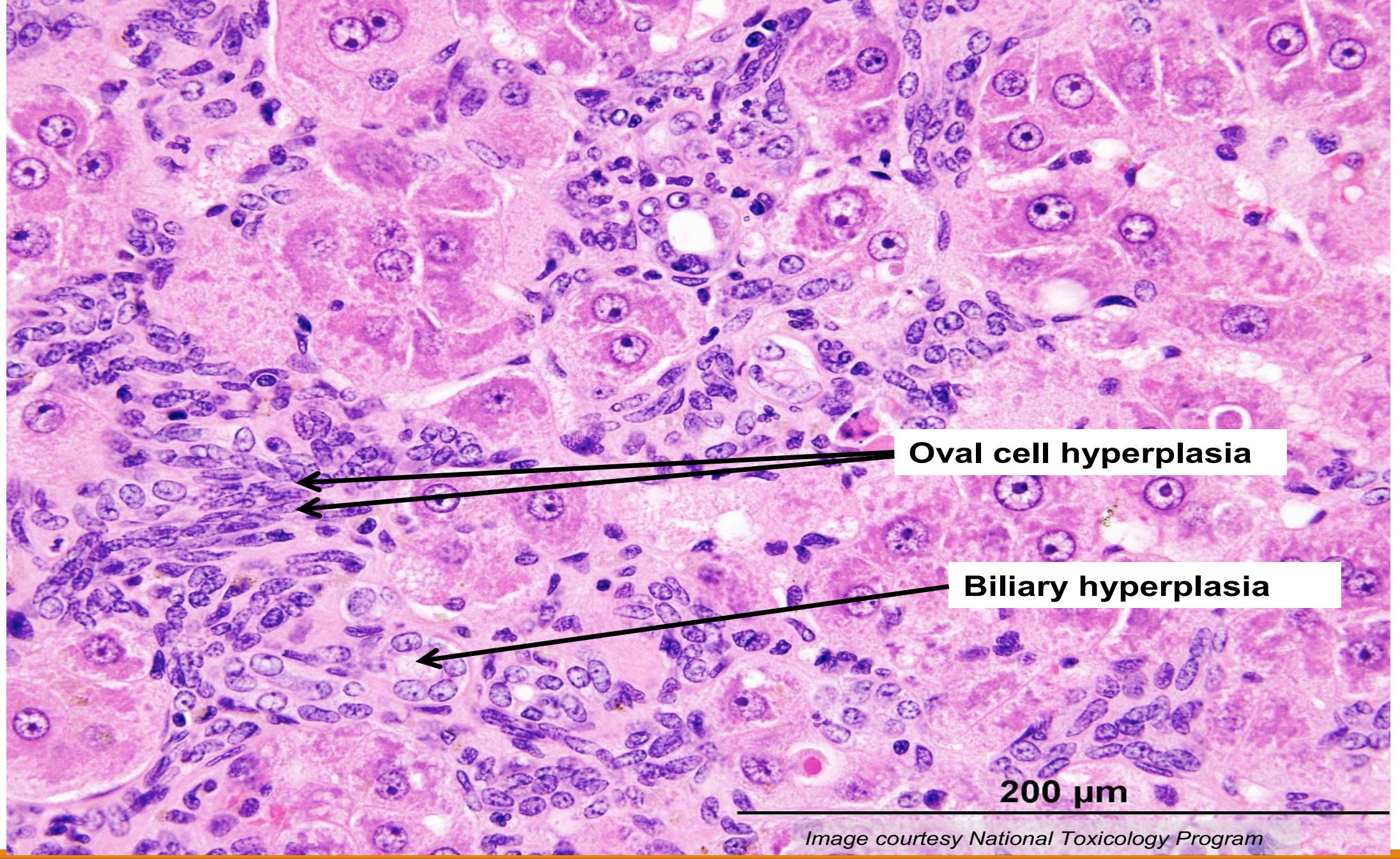
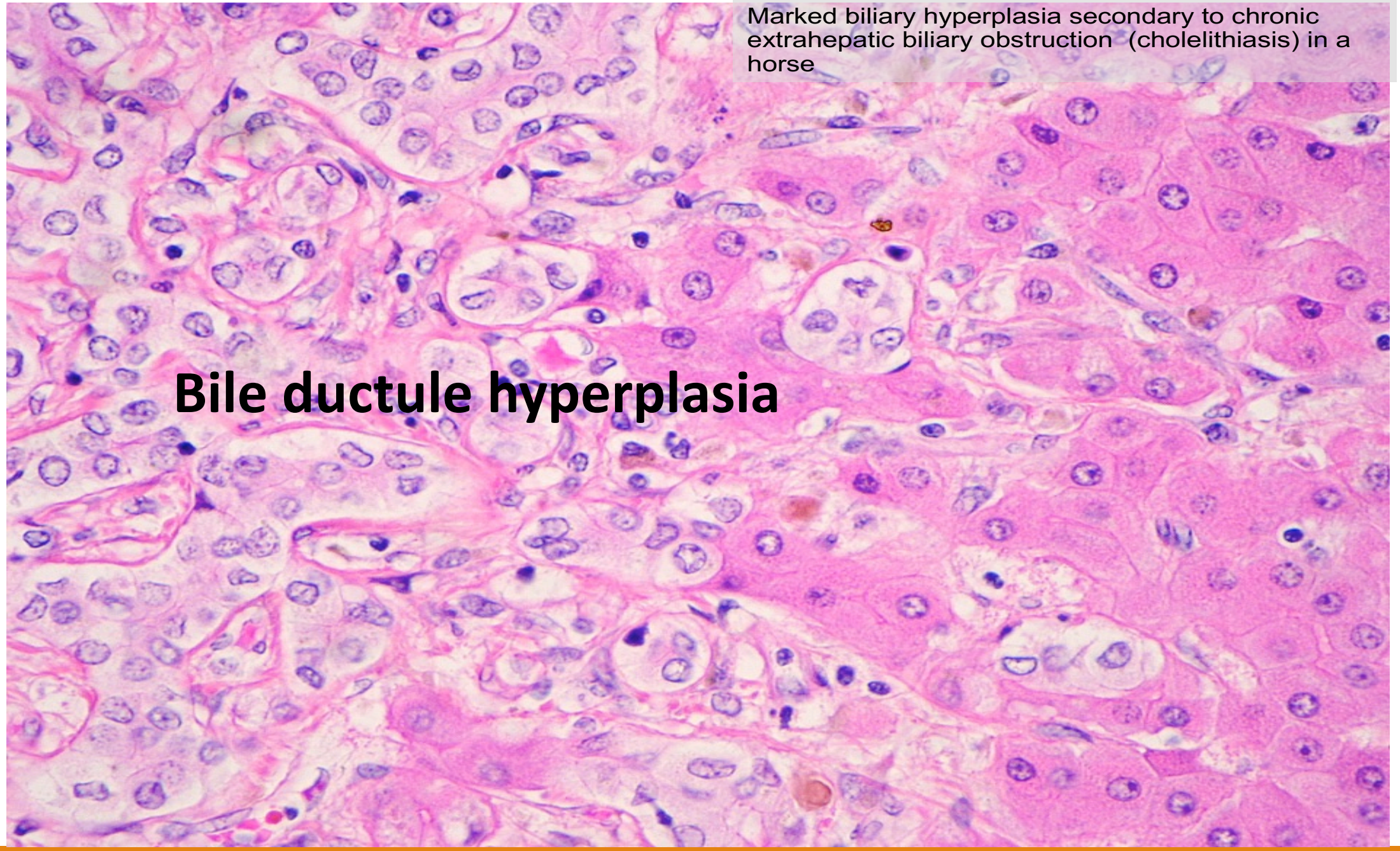


Image courtesy National Toxicology Program



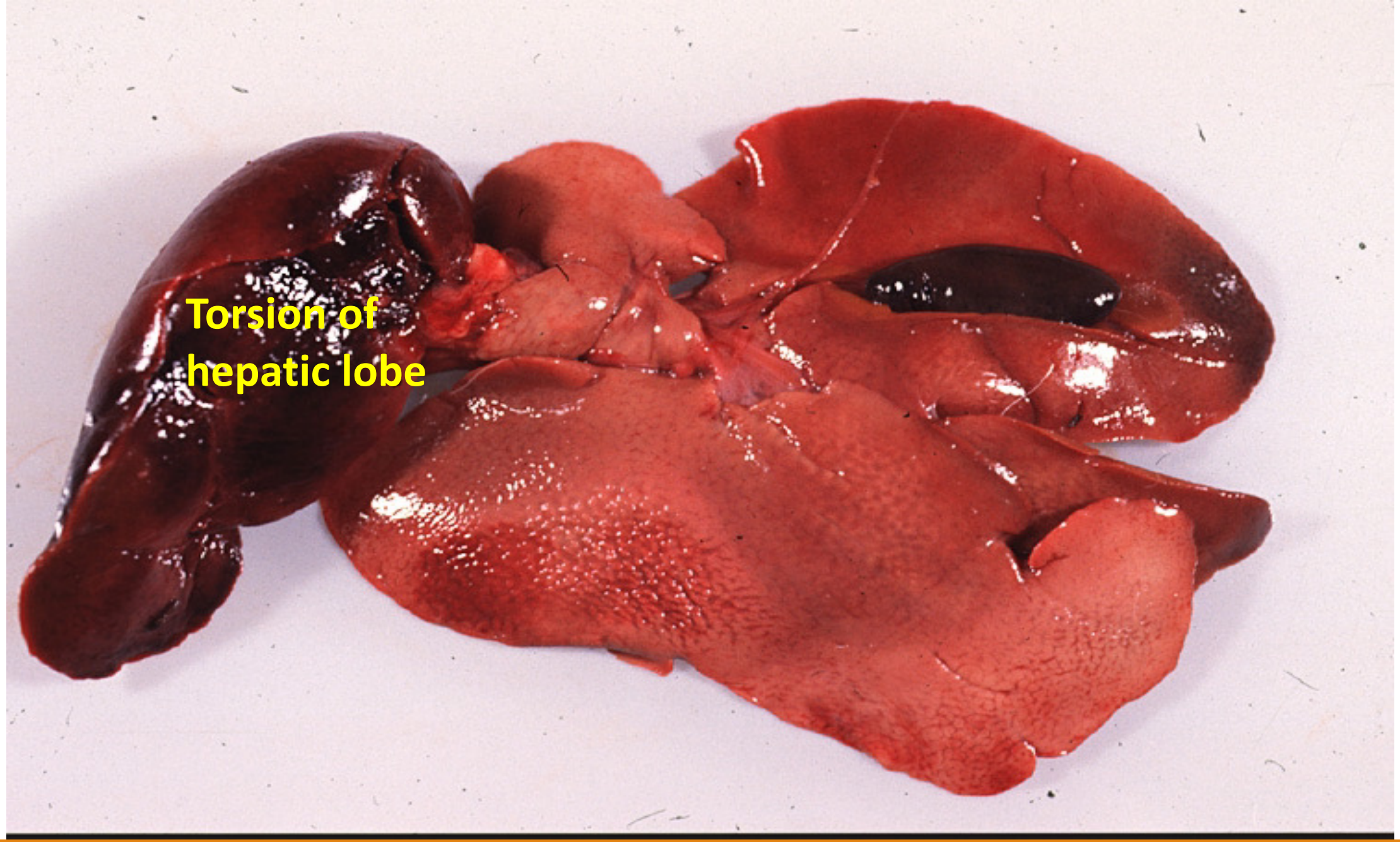
Marked biliary hyperplasia secondary to chronic extrahepatic biliary obstruction (cholelithiasis) in a horse

Liver displacement, torsion and rupture

Displacement usually occurs caudally

Lobe torsion results in vascular occlusion and infarction - can cause death from shock/haemorrhage

Rupture usually occurs from trauma. The liver is relatively fragile and rupture may be clinically silent until substantial blood loss has occurred


Torsion of
hepatic lobe

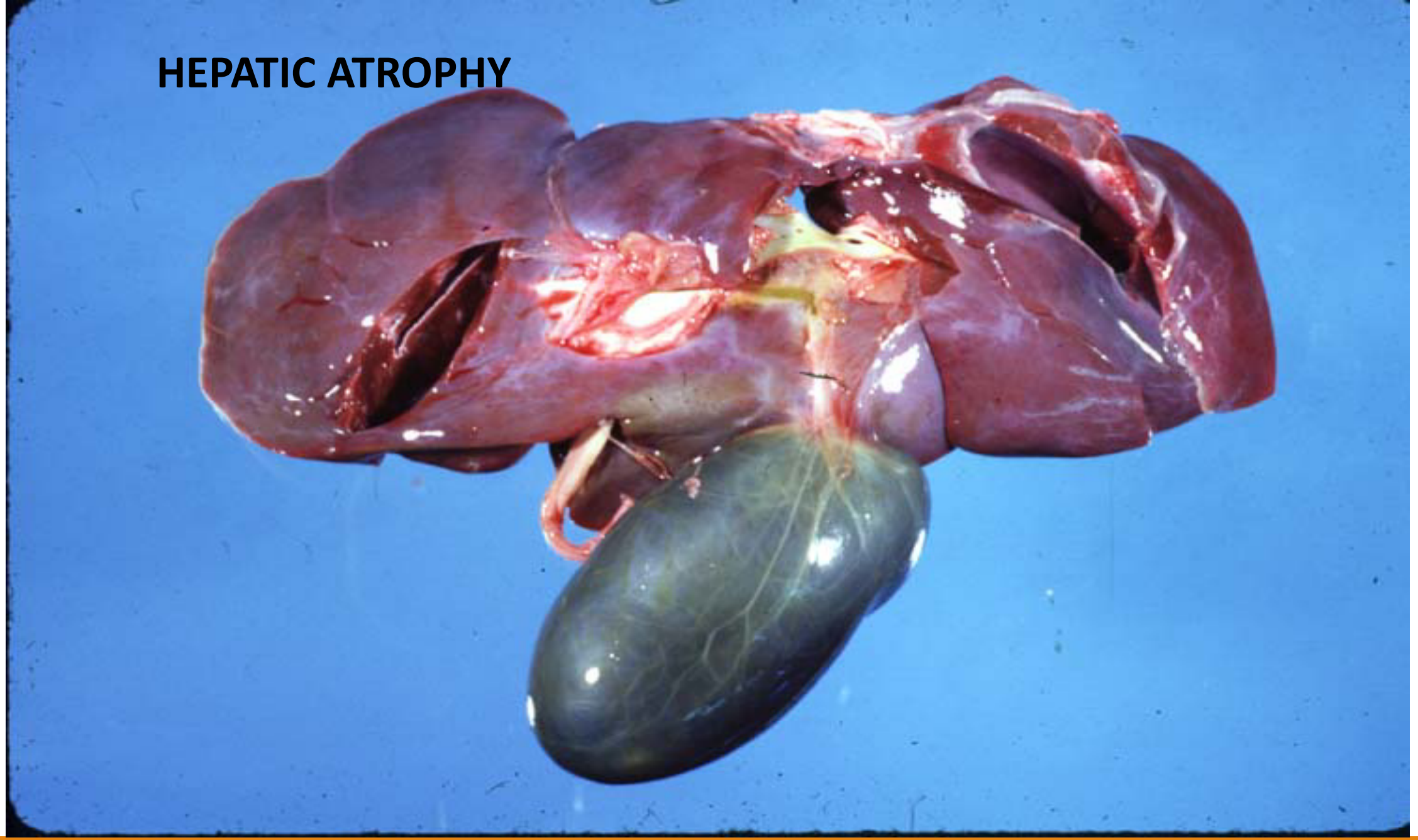
Hepatic response to functional demand

The liver must be highly adaptable to match metabolic function to changing demand

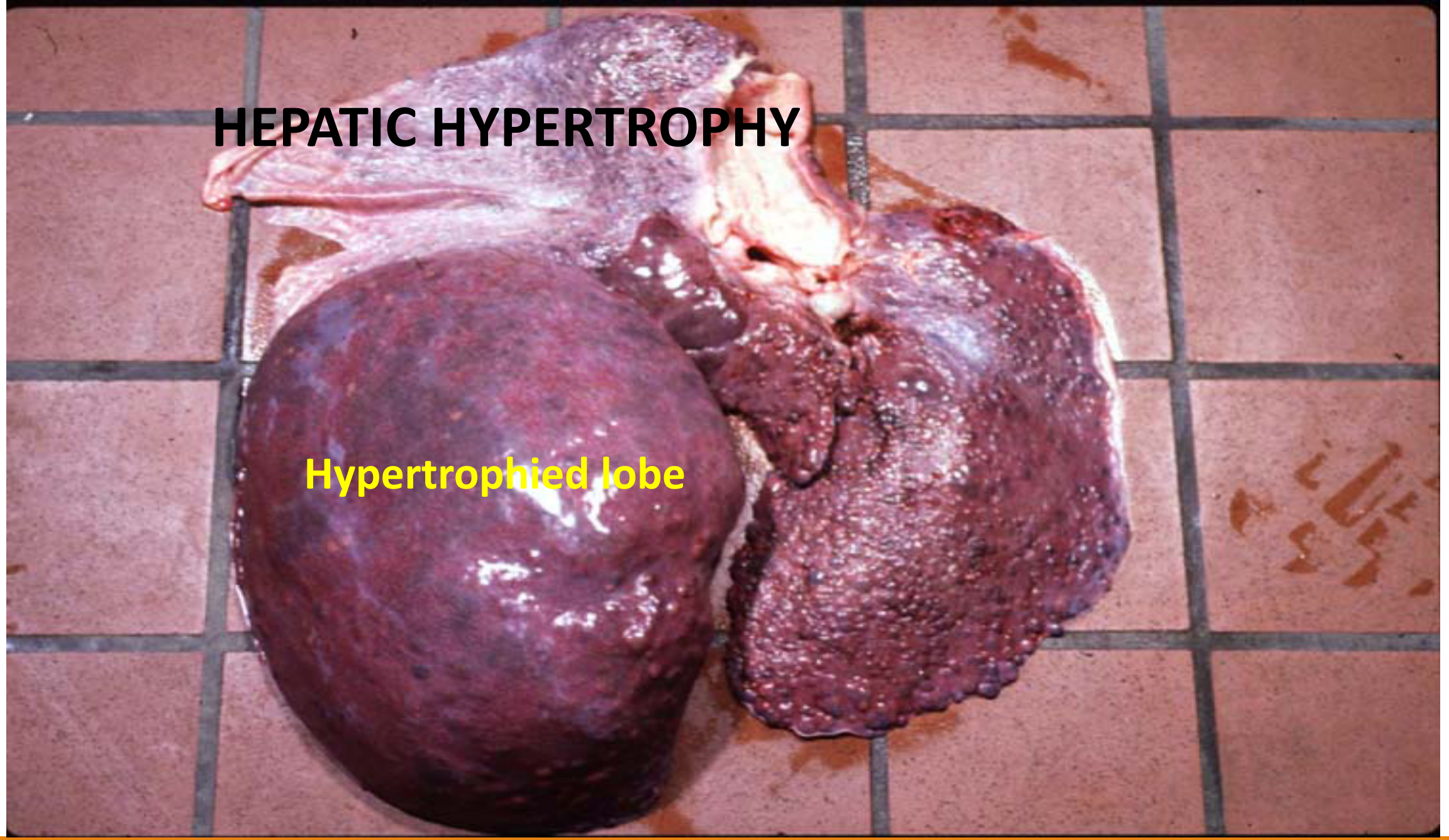
Increased demand leads to hypertrophy/hyperplasia of hepatocytes

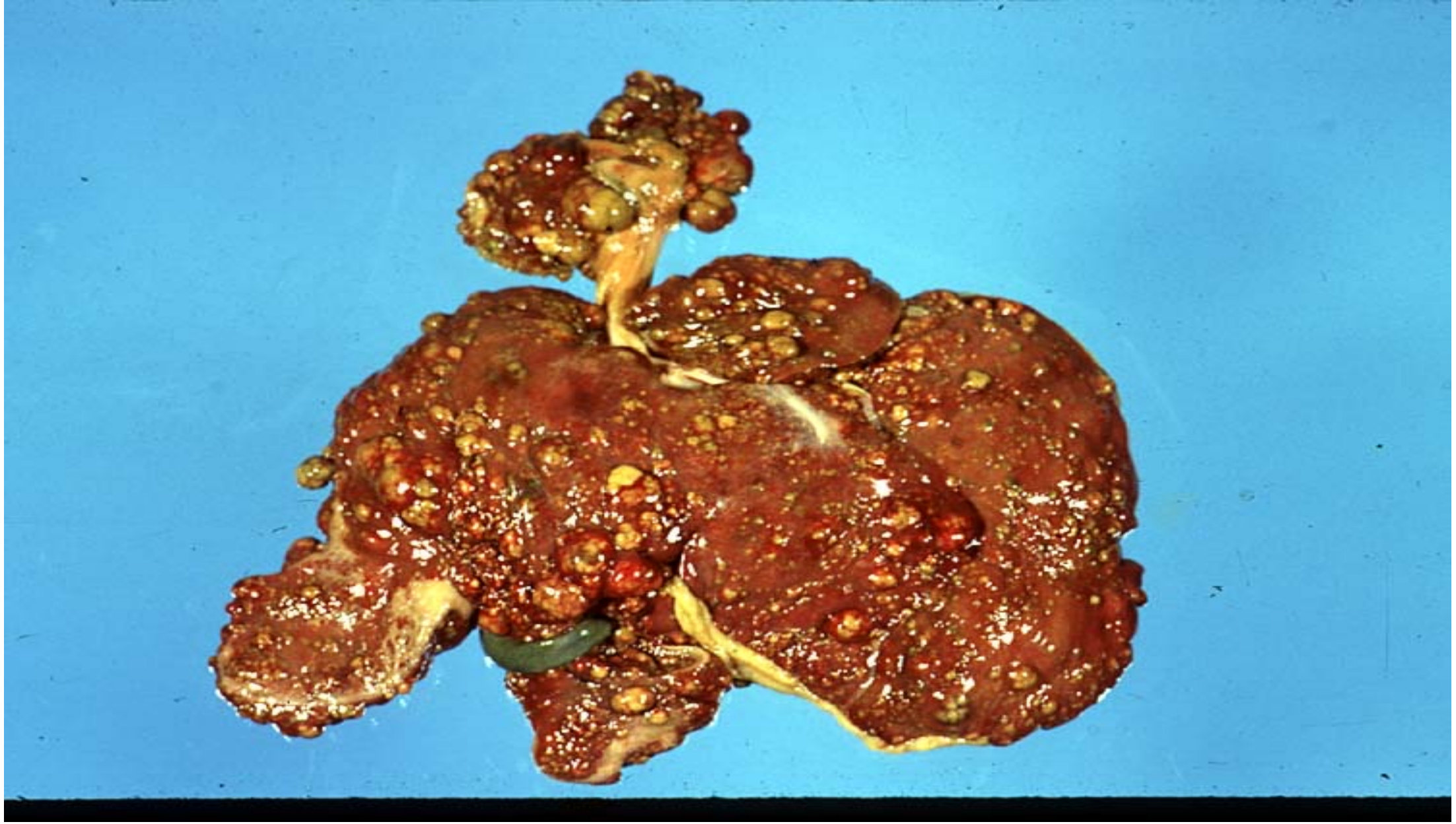
Decreased demand (prolonged illness, starvation) causes atrophy, apoptosis or autophagocytosis (cytoplasmic organelles aggregate, then are degraded by lysosomes) of hepatocytes

HEPATIC ATROPHY



HEPATIC HYPERTROPHY





NODULAR REGENERATIVE HYPERPLASIA

Hepatocellular responsiveness

Hepatocytes have a relatively long lifespan of several months

Trophic factors supplied by the portal circulation regulate hepatocyte replication and liver mass

Exposure to xenobiotics (foreign compounds) can reversibly increase hepatocyte volume, particularly the smooth endoplasmic reticulum (SER)

Hepatic pigmentation

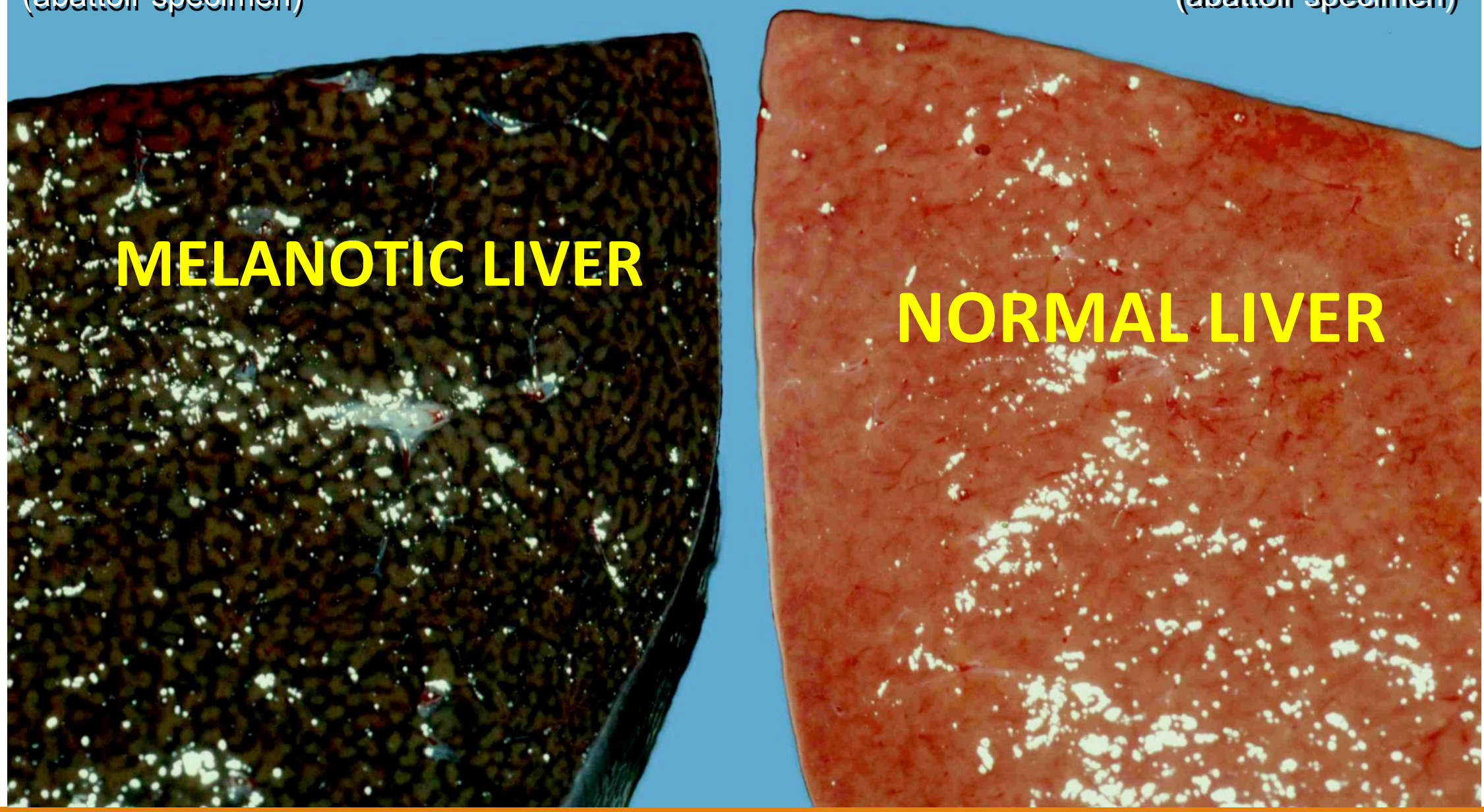
Melanosis: congenital

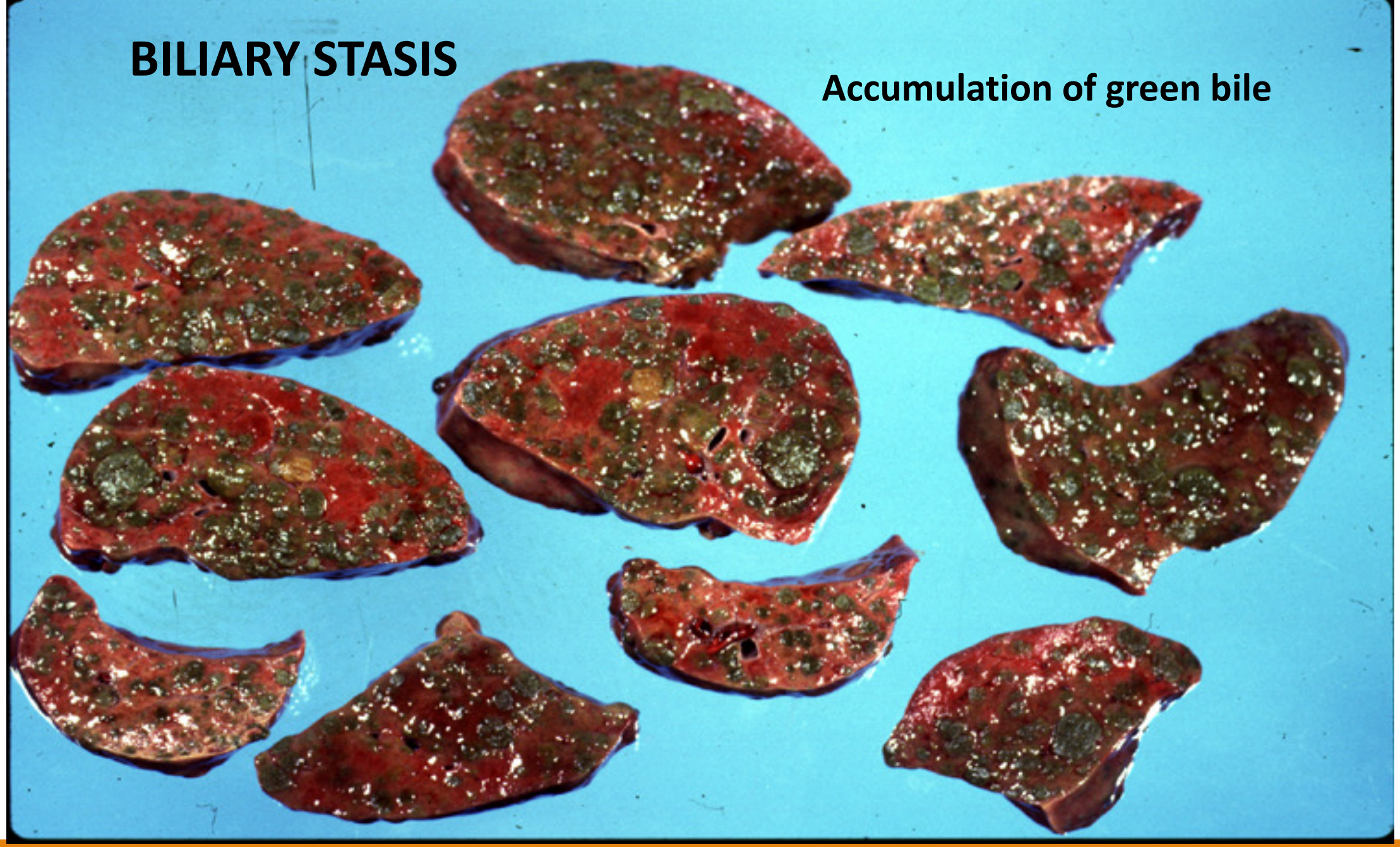
Lipofuscin - periacinar hepatocytes contain more golden-brown lipofuscin pigment with increasing age (and atrophy, increased turnover of membrane lipids) – derived from lipid component of cell membranes

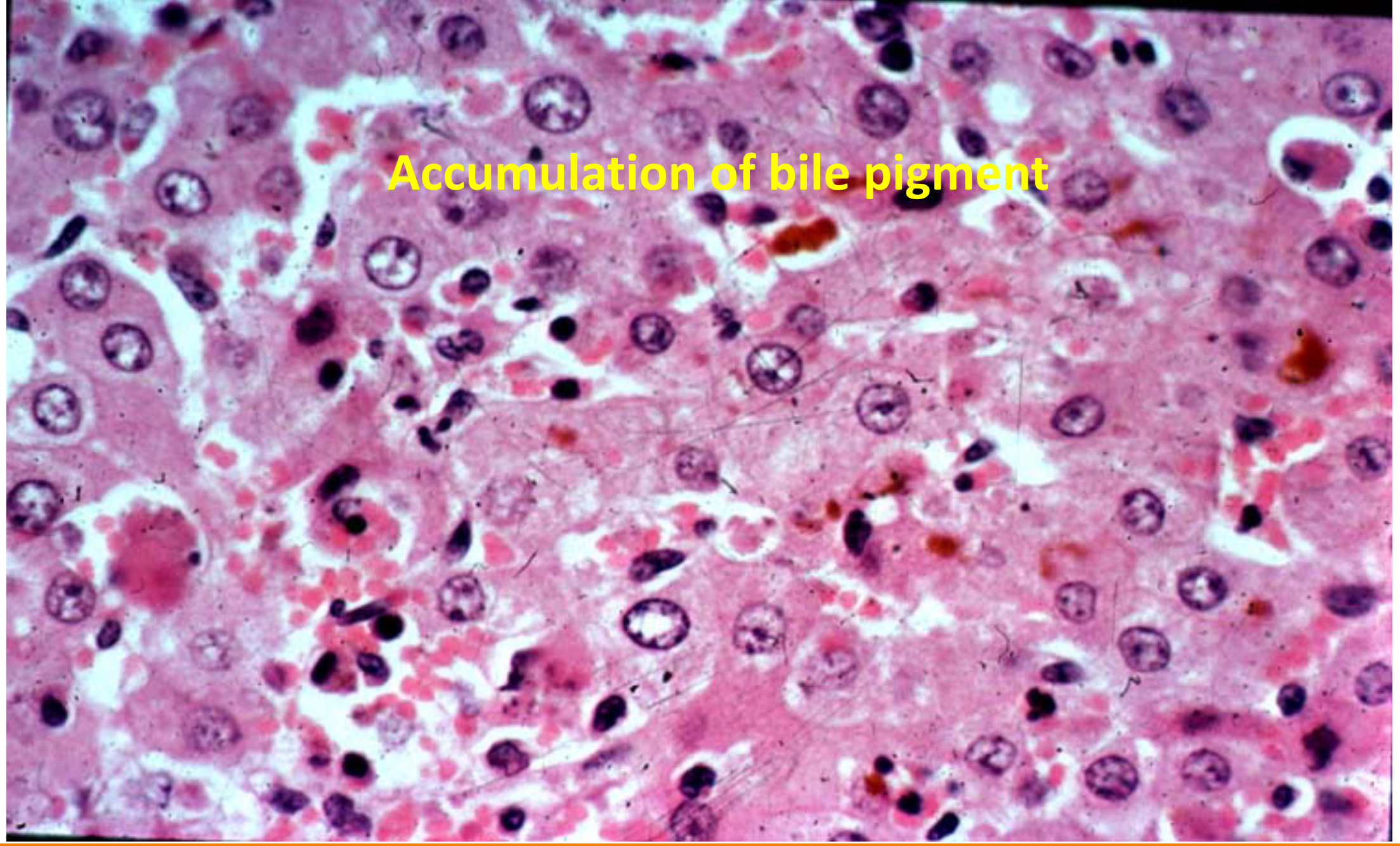
Bile - obstruction to biliary outflow - olive green colour grossly and, histologically, golden-brown

Environmental melanosis, bovine liver
(abattoir specimen)

Normal bovine liver
(abattoir specimen)




BILIARY STASIS
Accumulation of green bile


Accumulation of bile pigment

Iron overload

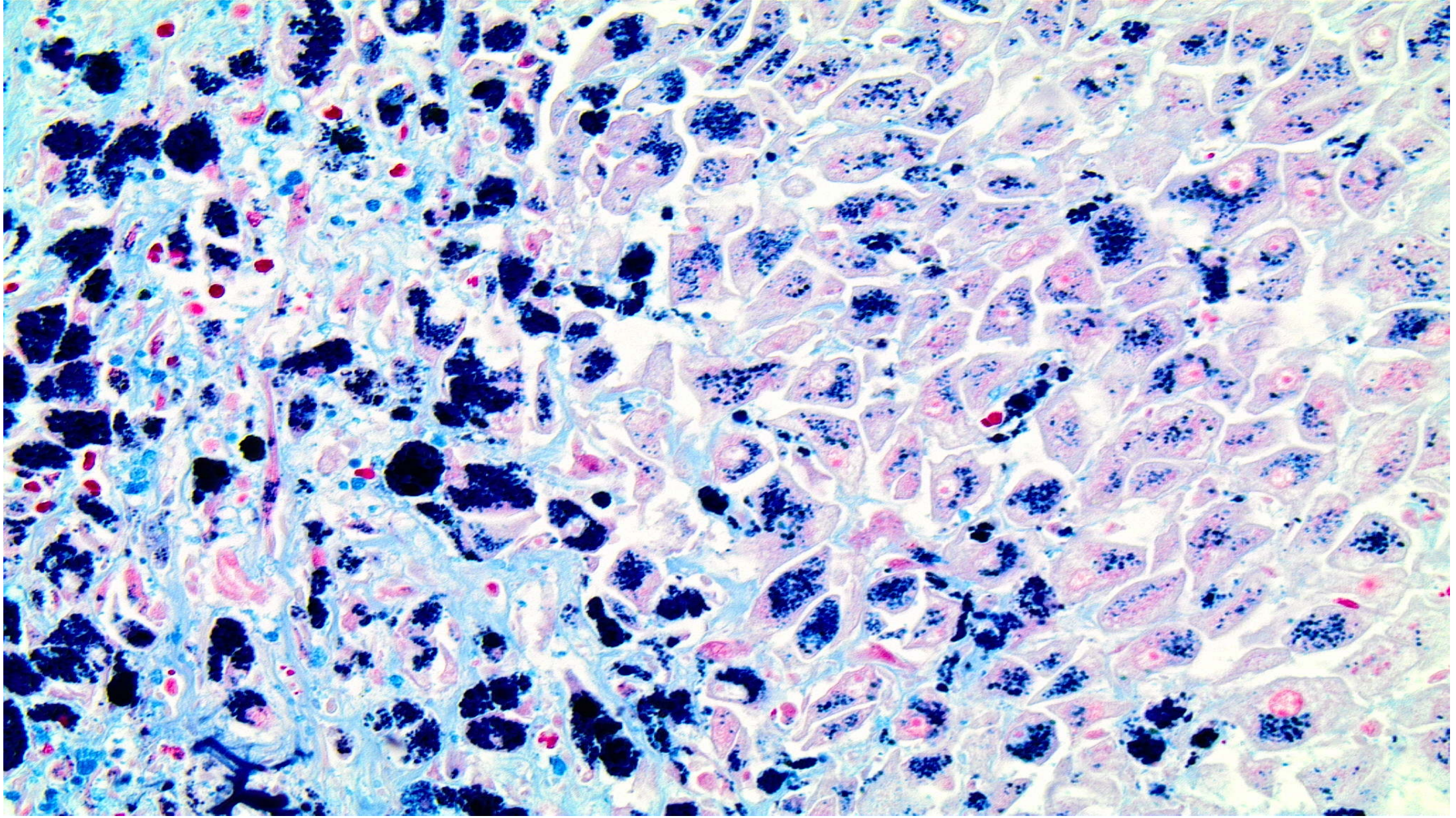
Haemosiderosis when there is an excess of hepatocellullar Fe and

Haemochromatosis when excess Fe storage produces fibrosis and inflammation with hepatic injury

Haemosiderin in excess is usually suggestive of exaggerated haemolytic activity

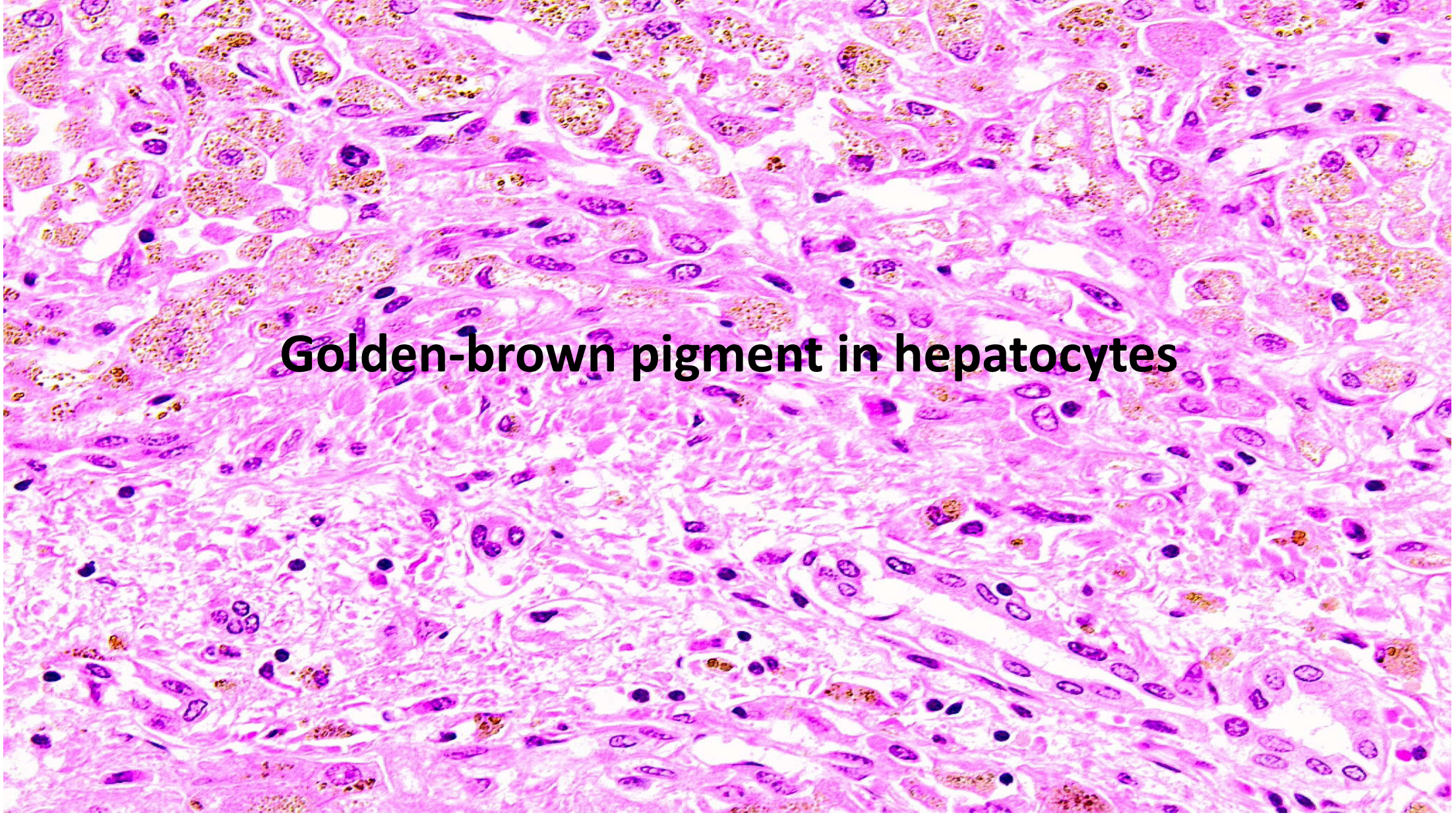
Fe can be stained with **Perl's Prussian blue**

Hematin as formalin pigment – artefact produced in sections when tissues rich in blood contact formalin fixative – action of formic acid on Fe (must be distinguished from haemosiderin)



Fe-containing haemosiderin stains dark blue with Perl's Prussian blue stain

HAEMOCHROMATOSIS



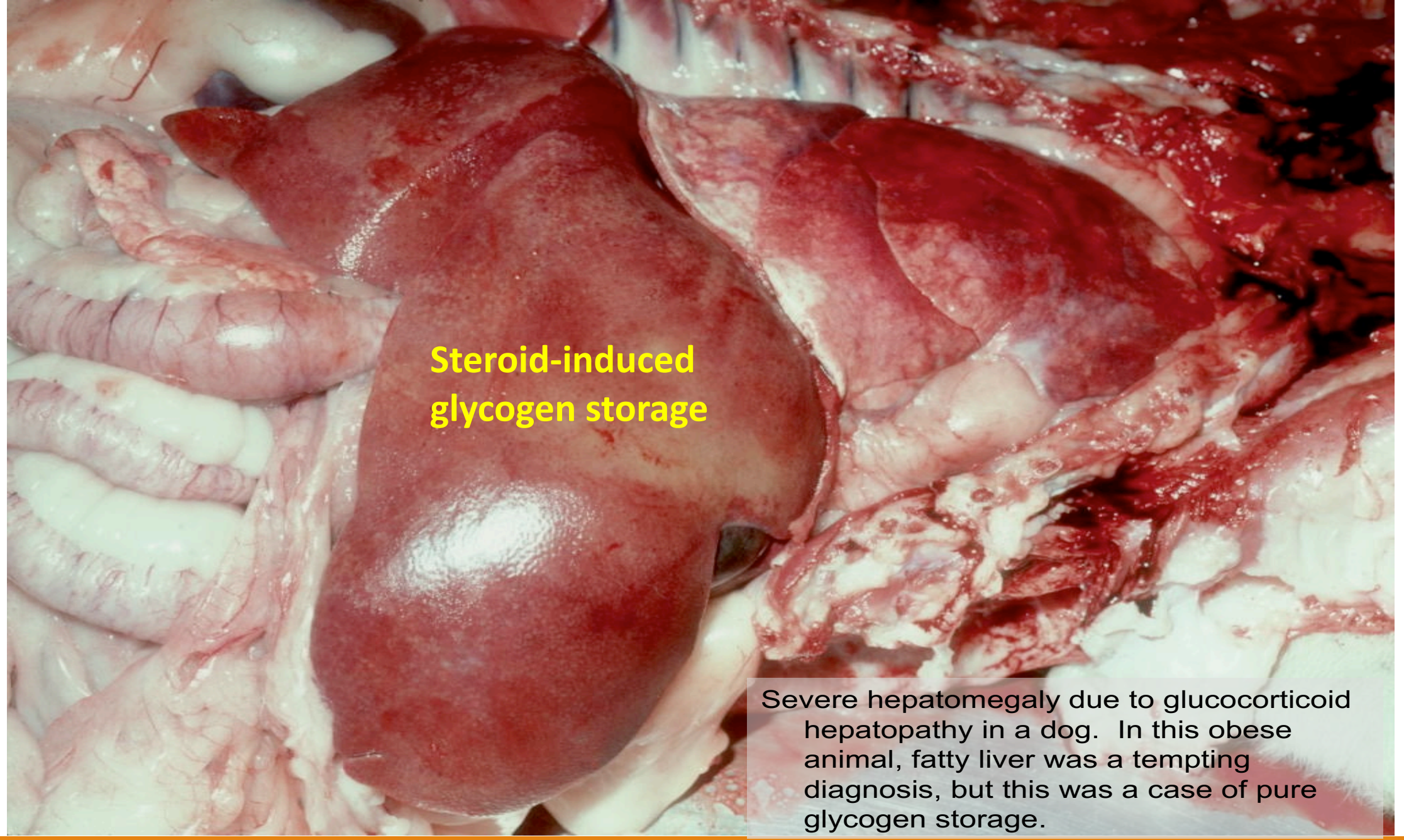
HAEMOCHROMATOSIS

Hepatocyte vacuolation

Hydropic degeneration – cytoplasmic ballooning due to excess water – nucleus tends to remain centrally located (versus lipid, where nucleus tends to be displaced to periphery of cell) – potentially reversible

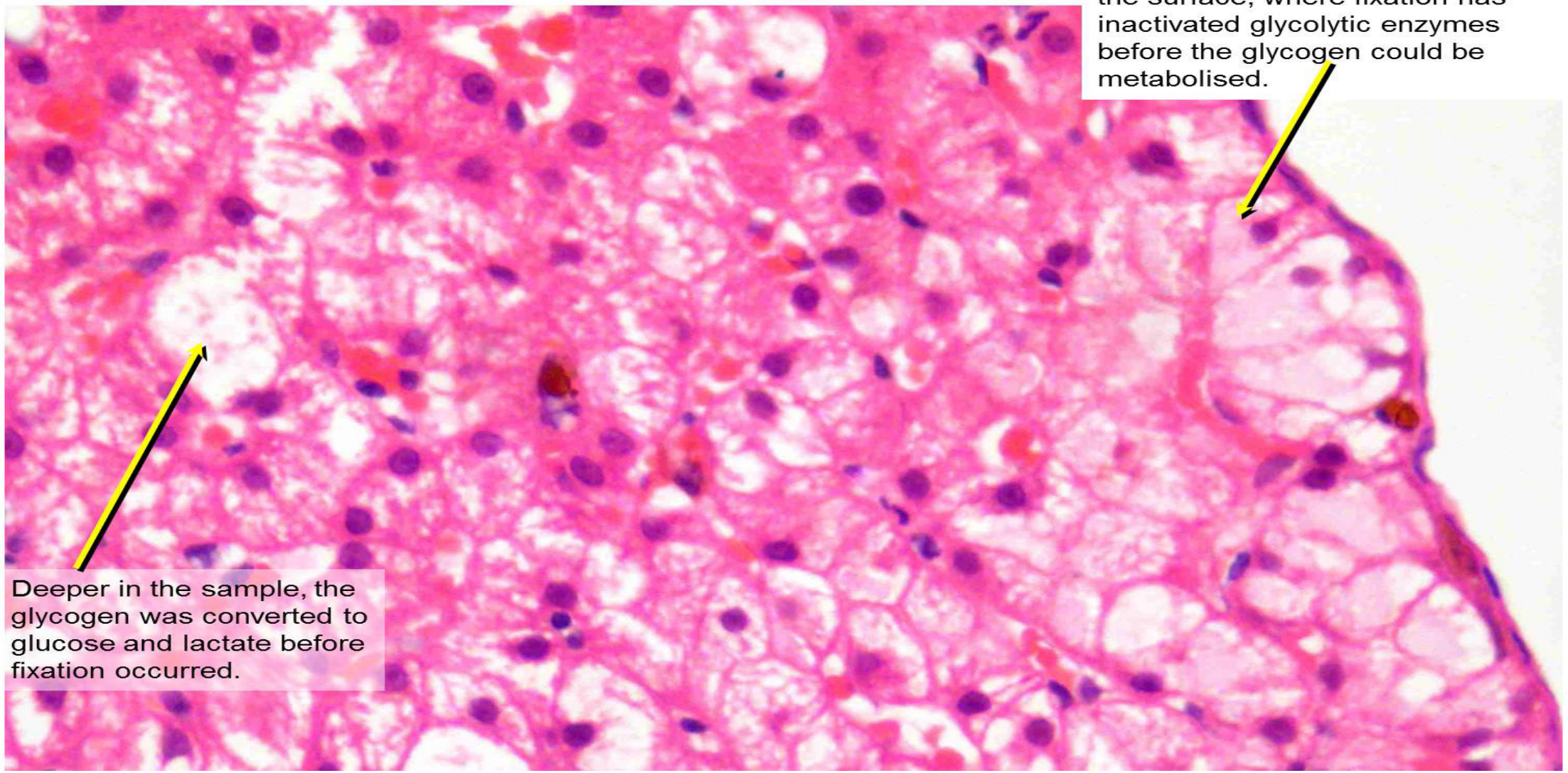
Steroid-induced– PAS-positive glycogen accumulation causes swelling of hepatocytes by an unknown mechanism (especially in dogs)

Fatty change (lipidosis, steatosis) – either small triglyceride globules (microvesicular) or larger coalesced droplets (macrovesicular)

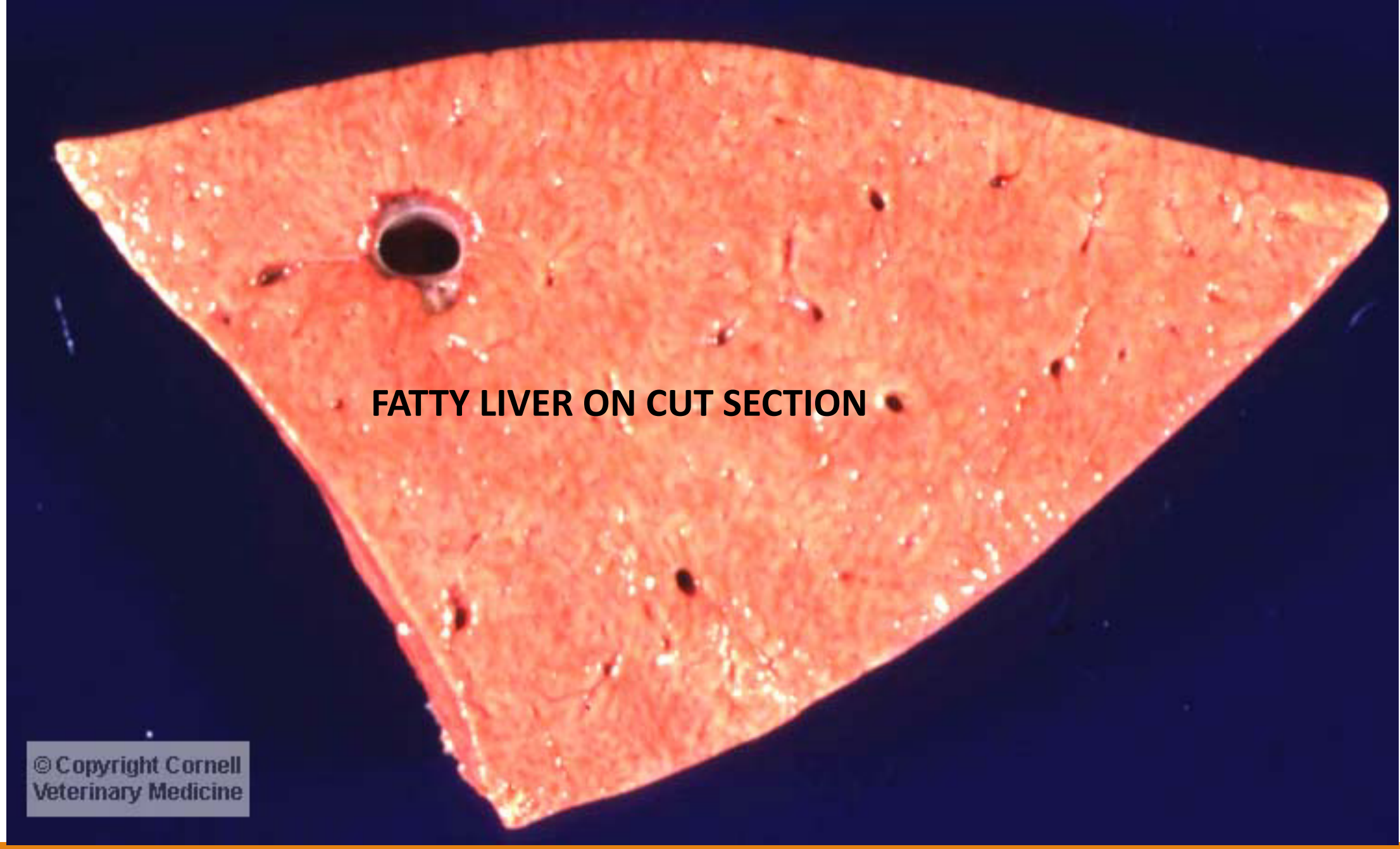


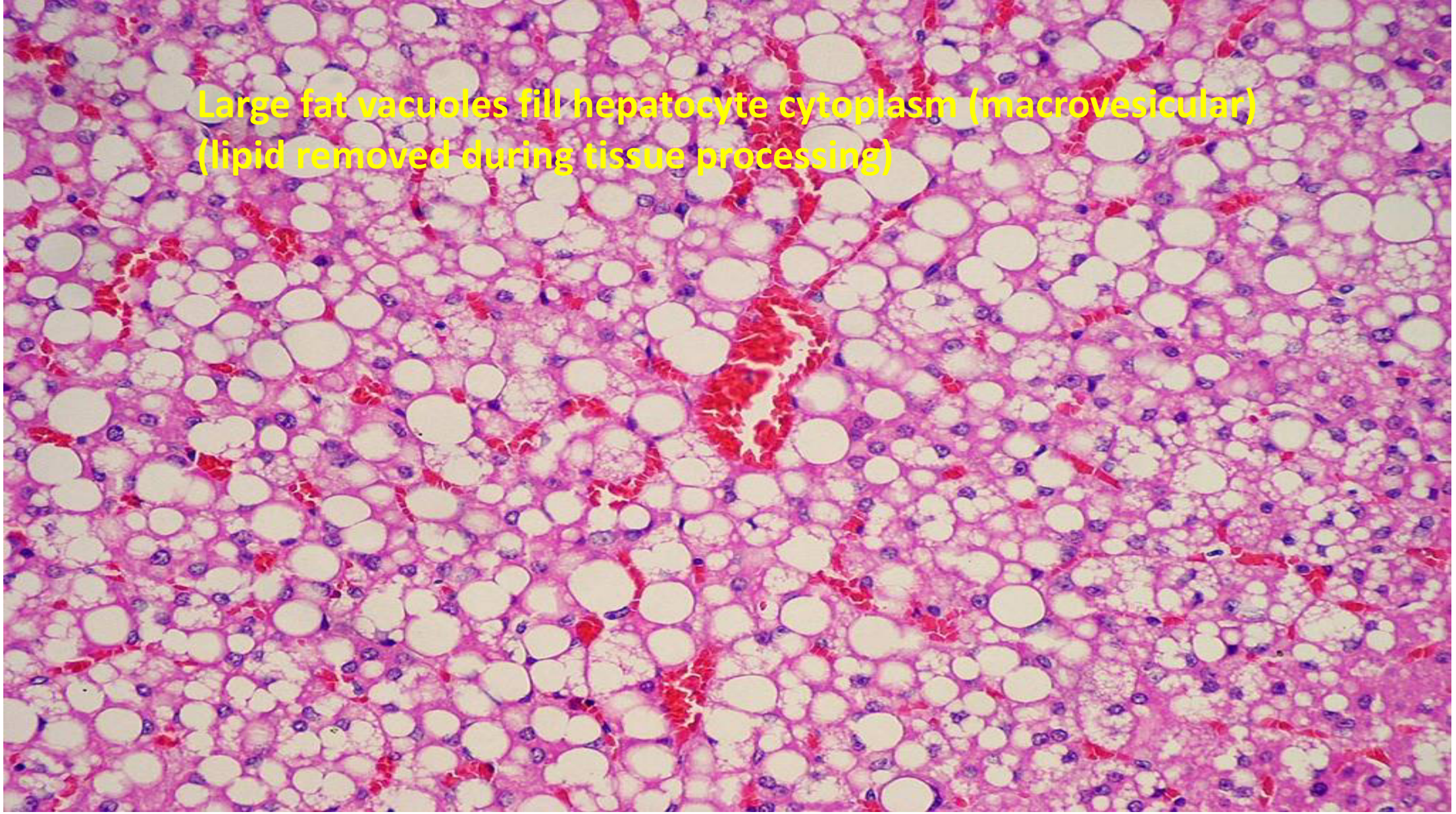
Severe hepatomegaly due to glucocorticoid hepatopathy in a dog. In this obese animal, fatty liver was a tempting diagnosis, but this was a case of pure glycogen storage.

Liver biopsy, glucocorticoid hepatopathy



Steroid-induced glycogen storage


FATTY LIVER ON CUT SECTION
© Copyright Cornell
Veterinary Medicine



FATTY CHANGE (LIPIDOSIS, STEATOSIS) IN HEPATOCYTES

Hepatic lipidosis

Common in injured hepatocytes because there is normally a high throughput of fatty acids, which can be impeded at various stages in the complex pathway of lipid metabolism and eventual secretion of very low density lipoproteins (VLDL)

Common in conditions that cause increased mobilisation of body fat stores when there is increased demand for energy over a short time period (e.g. pregnancy and lactation)

Fatty change of hepatocytes

Also seen with nutritional disorders, including obesity (increased transport of dietary lipids or mobilisation from adipose tissue), protein-calorie malnutrition (impaired lipoprotein synthesis) and starvation (increased mobilisation of triglycerides)

Hypoxia (commonly anaemia and passive venous congestion) leads to triglyceride accumulation as lipoprotein synthesis/transport depend on oxidative metabolism

Hepatic steatosis

Intoxications often cause fatty liver + hepatocellular necrosis

In **diabetes mellitus**, insulin deficiency or inactivity accelerates lipolysis from adipose tissues

Fatty change of hepatocytes

In acute injury, small, discrete microvesicles of lipid

With more protracted injury, lipid globules tend to coalesce into macrovesicles that displace the nucleus to the periphery of hepatocyte and compress sinusoids

Liver yellowish, enlarged, and friable with rounded edges and tends to float in fixative

Since agents used during preparation of paraffin-embedded sections dissolve fat, need formalin-fixed frozen sections stained with oil red O or Sudan stains to demonstrate lipid

Hepatic lipidosis

Lipid accumulation is a sensitive response to hepatocellular injury, but triglyceride globules *per se* are not harmful to hepatocytes and the process is potentially reversible

However, chronic fatty livers tend to show fibrosis and nodular regeneration

and are more susceptible to a wide range of insults

Hepatic lipidosis

Sometimes fat-laden hepatocytes rupture or fuse to form fatty cysts and may undergo further metabolism to form lysosomal residues of ceroid

Fatty change also occurs in neonates of species in which the milk is rich in fat

Physiological fatty change

Physiological fatty change occurs in late pregnancy and heavy lactation, especially in ruminants

In response to increased energy demand, triglycerides are mobilised from adipose tissue and the liver is presented with a large load of fatty acids

In addition, the liver depends mainly on fatty acid metabolism for its own large energy needs and the export of protein as lipoprotein at a time when dietary energy intake is insufficient for production demands

Hepatic amyloidosis

Hepatic manifestation is usually part of a generalised amyloidosis

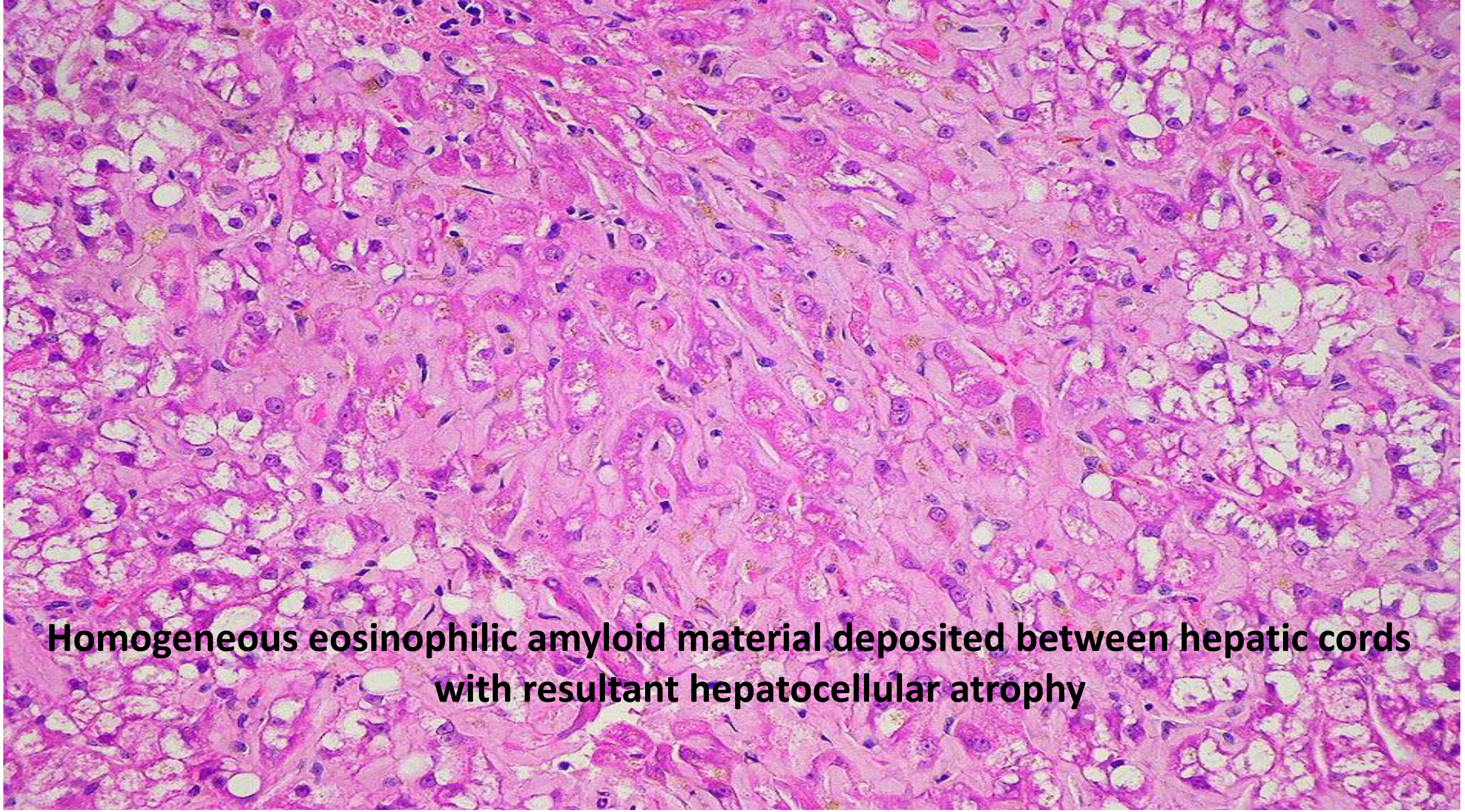
Caused by overproduction of amyloid A, a highly inducible acute phase protein, in response to chronic inflammation

Affected livers are prone to rupture and bleeding

Amyloidosis

Amyloid is deposited in the perisinusoidal space, between the sinusoidal lining and hepatocytes, leading to atrophy of hepatocyte cords

In H&E sections, amyloid appears as an eosinophilic homogeneous material and stains positively with Congo red



Homogeneous eosinophilic amyloid material deposited between hepatic cords with resultant hepatocellular atrophy

AMYLOIDOSIS

Hepatocyte death and loss

2 main forms are recognised – necrosis and apoptosis

But these are opposite extremes of the spectrum of cell death and there is a continuum between these extremes

Many insults incite necrosis and apoptosis

Necrosis

Characterised by cell swelling, loss of plasma membrane integrity, cell lysis, release of cell contents (including enzymes) and inflammation

Often caused by mitochondrial dysfunction, leading to ATP depletion, loss of ion and membrane homeostasis, influx of water (swelling or oncosis), and Ca influx, activating enzymatic degradation

Sometimes cells die individually (single cell necrosis)

Apoptosis (programmed cell death)

Permits active removal of cells, usually without leakage or inflammation

Nucleus fragments and cytoplasm condenses to form apoptotic bodies (plasma membrane intact)

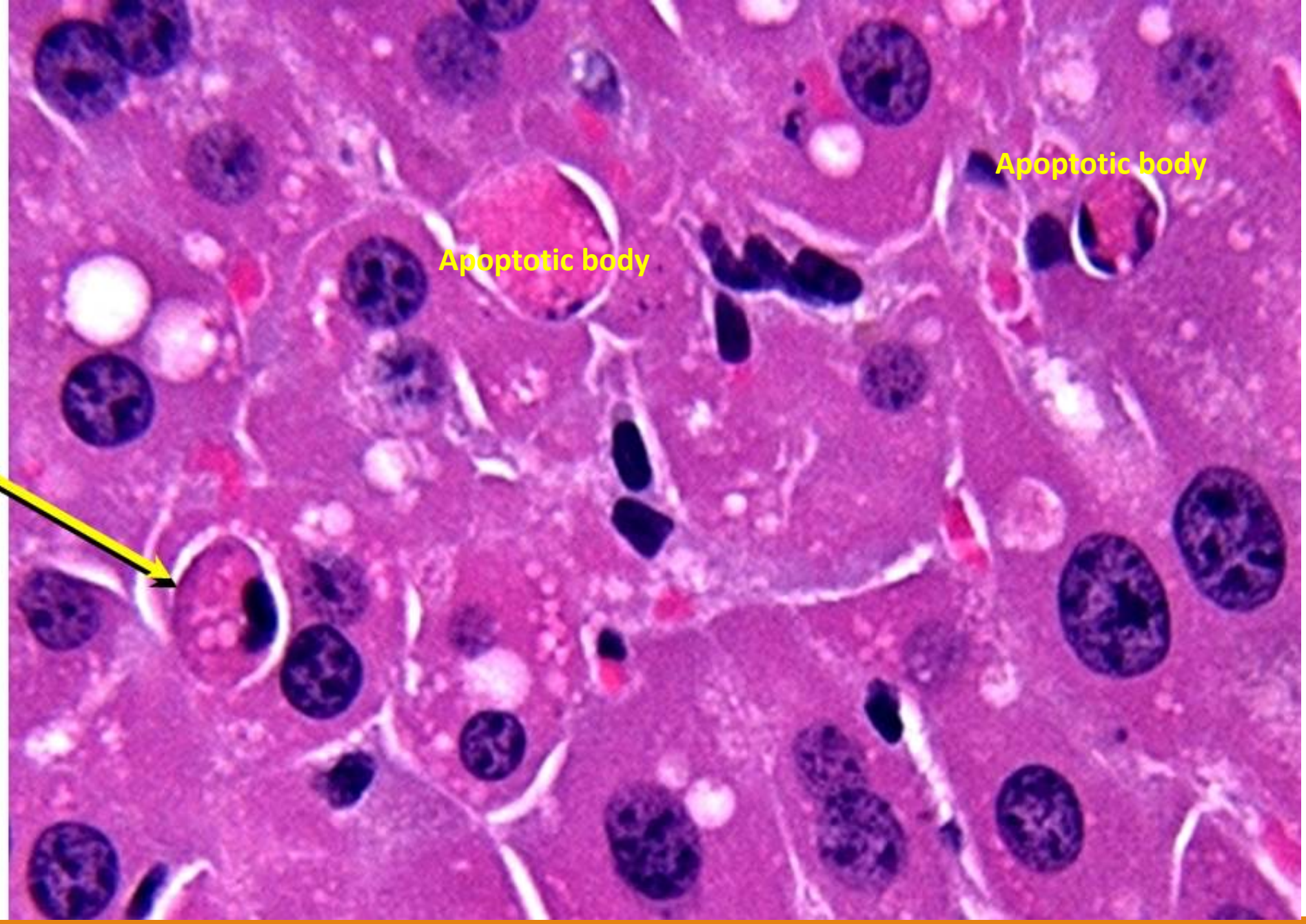
ATP needed to initiate the execution phase – energy-dependent, active process (versus necrosis)

Apoptosis

Apoptotic bodies are rapidly phagocytosed by neighbouring hepatocytes and Kupffer cells, which minimises secondary inflammation

Apoptosis can be initiated by intrinsic or extrinsic factors, often mediated by caspases

Apoptosis can be detected immunohistochemically with antibodies to e.g. caspases or biochemically (TUNEL reaction)


Apoptotic body
Apoptotic body
Apoptotic body, taken up by hepatocyte

Patterns of hepatocyte cell death

1. **Focal** (or more commonly **multifocal**) necrosis

2. **Zonal necrosis** (periportal, mid-zonal and periacinar)

3. **Massive necrosis**

Focal hepatic necrosis

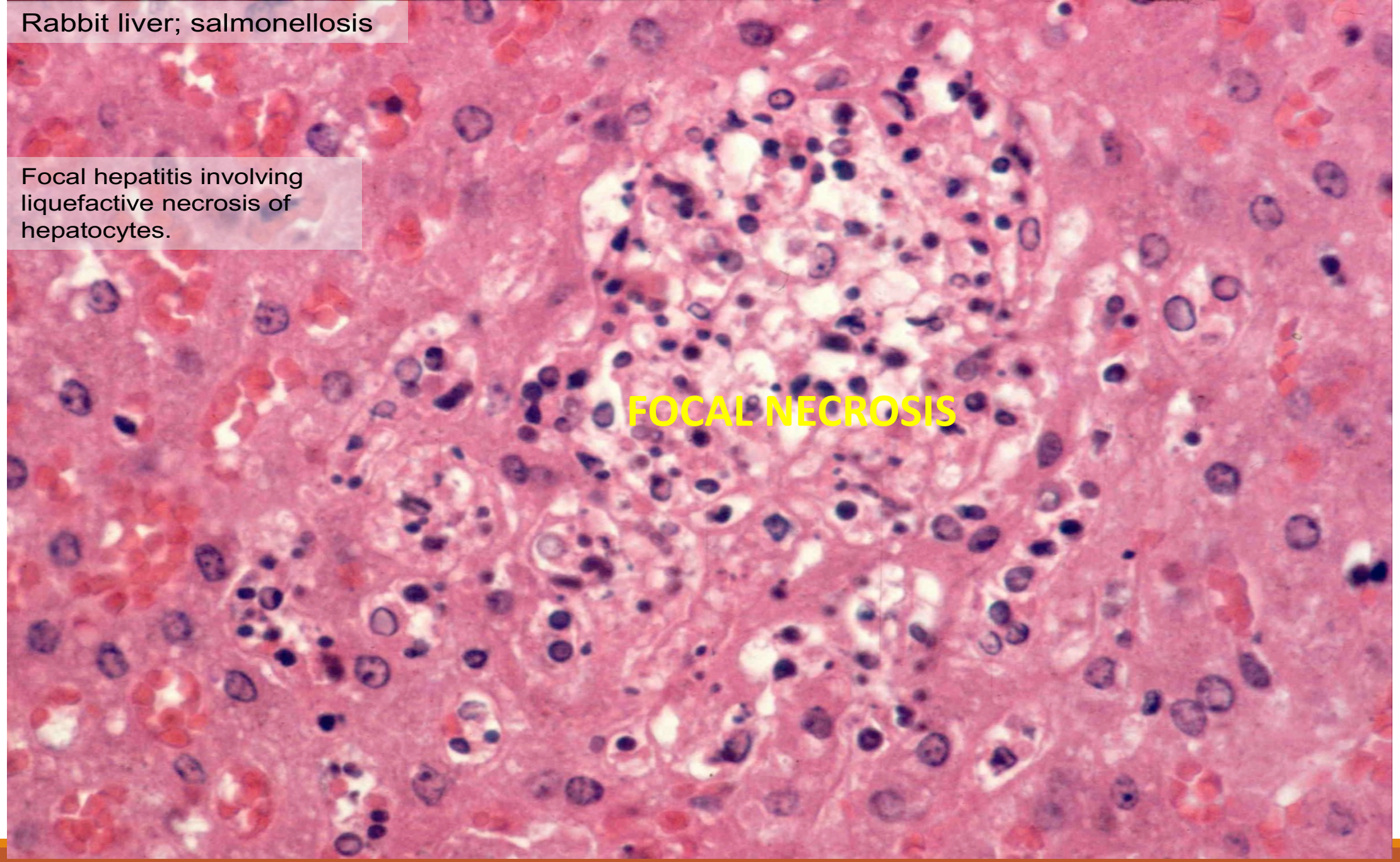
Commonly in infections (viral and bacterial) and parasitic migrations, usually attended by inflammation (focal hepatitis)

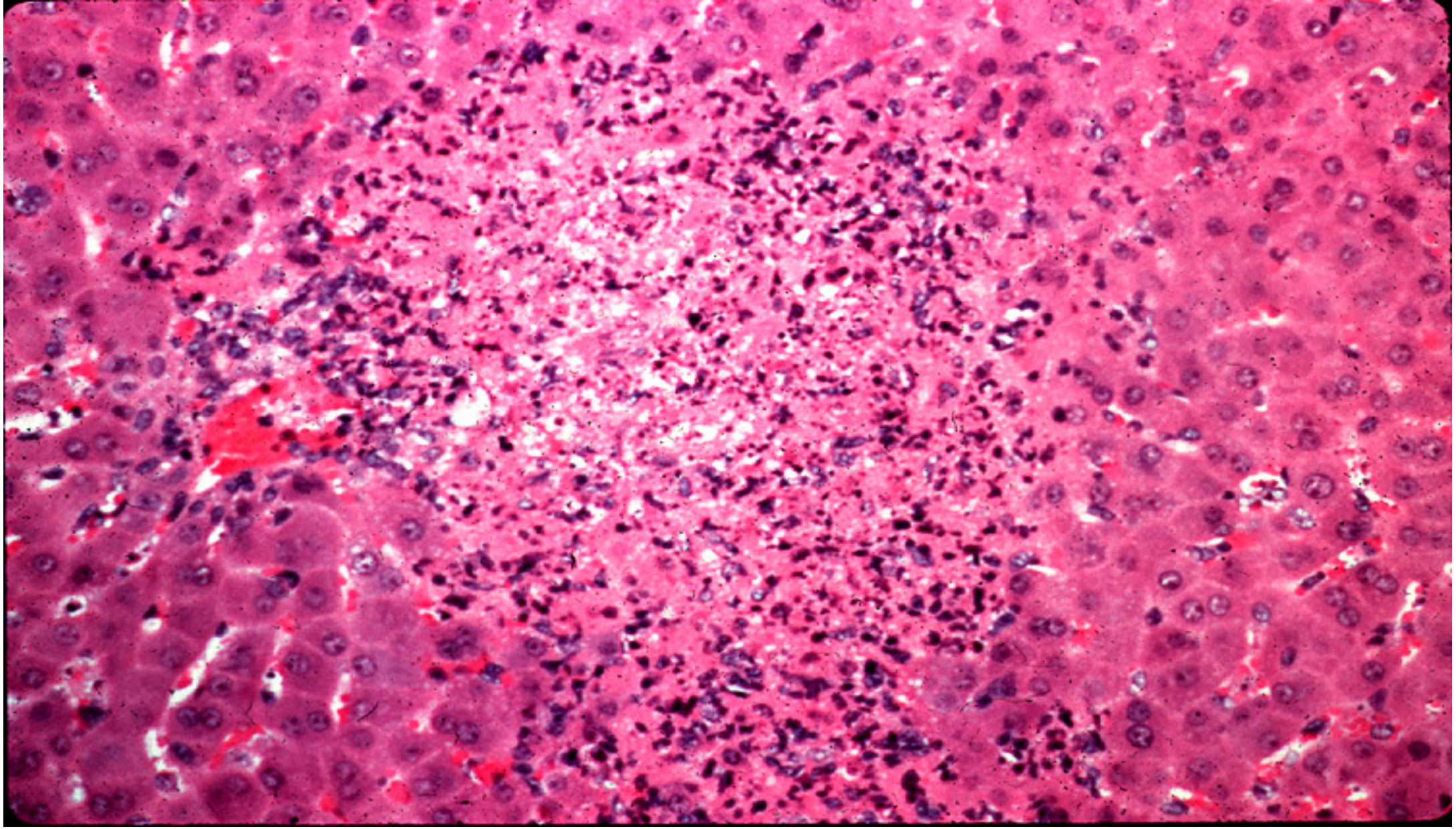
In septicaemias, bacteria produce focal necrosis from bacterial embolism or Kupffer cell reaction (latter in e.g. salmonellosis)

Focal necrosis has little functional significance, even when multifocal and widespread

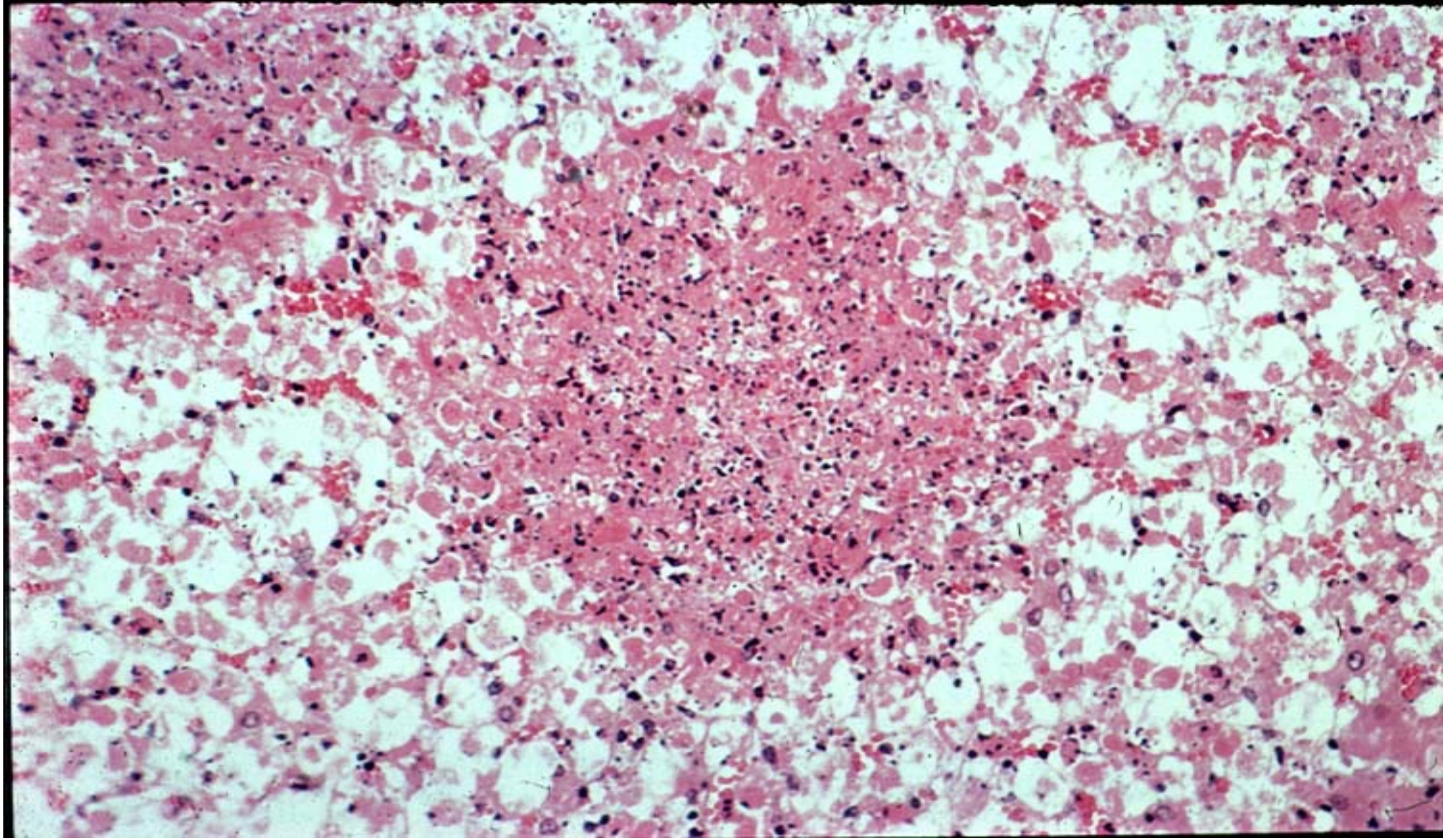
Rabbit liver; salmonellosis

Focal hepatitis involving liquefactive necrosis of hepatocytes.





FOCAL HEPATOCELLULAR NECROSIS



FOCAL HEPATIC NECROSIS WITH NUCLEAR PYKNOSIS AND KARYORRHEXIS

Periacinar necrosis

Most common because:

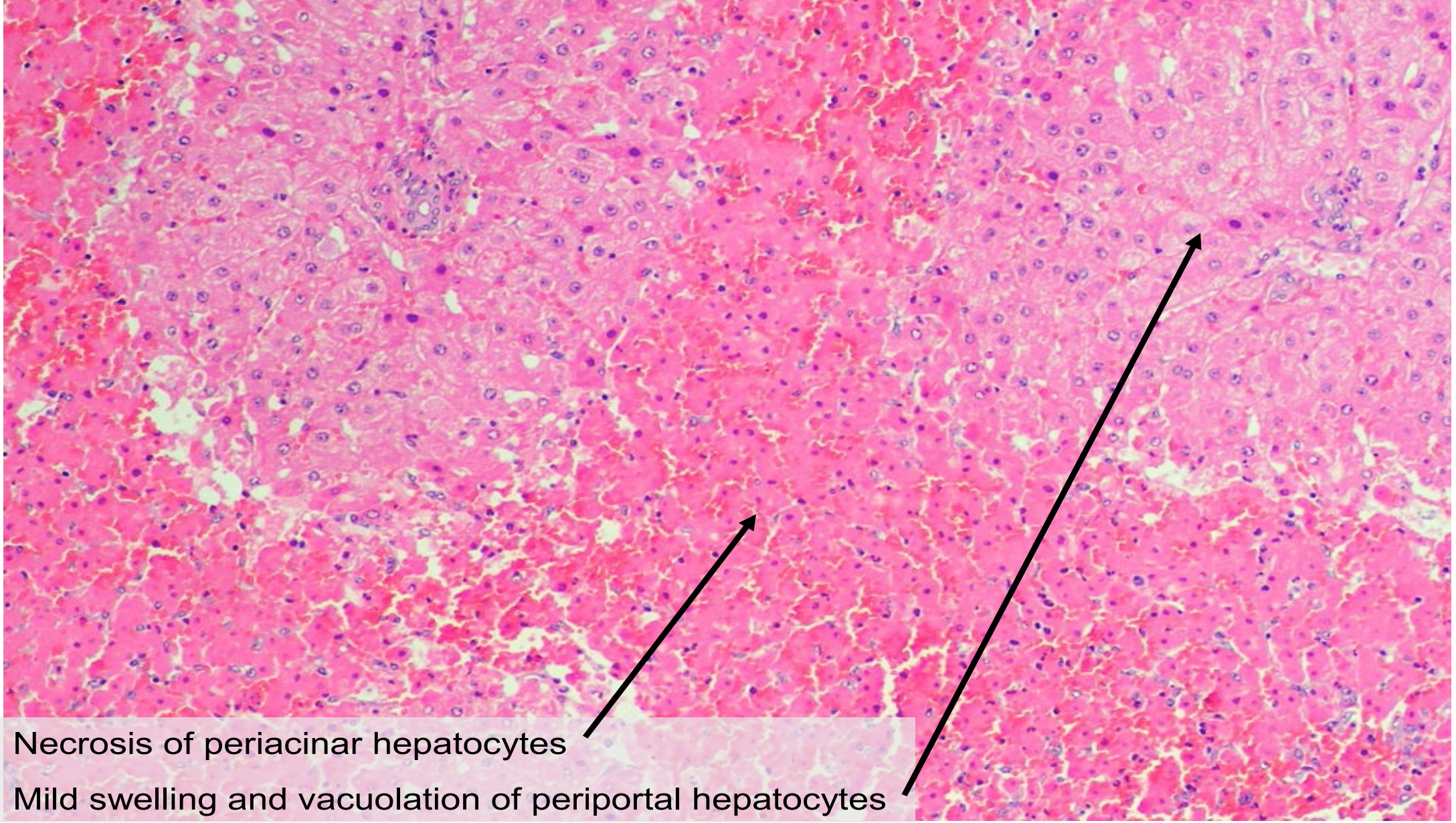
(1) these hepatocytes are furthest from incoming blood carrying oxygen and nutrients and

(2) have highest concentration of P450 cytochrome that converts some foreign substances to active metabolites that can injure or kill hepatocytes

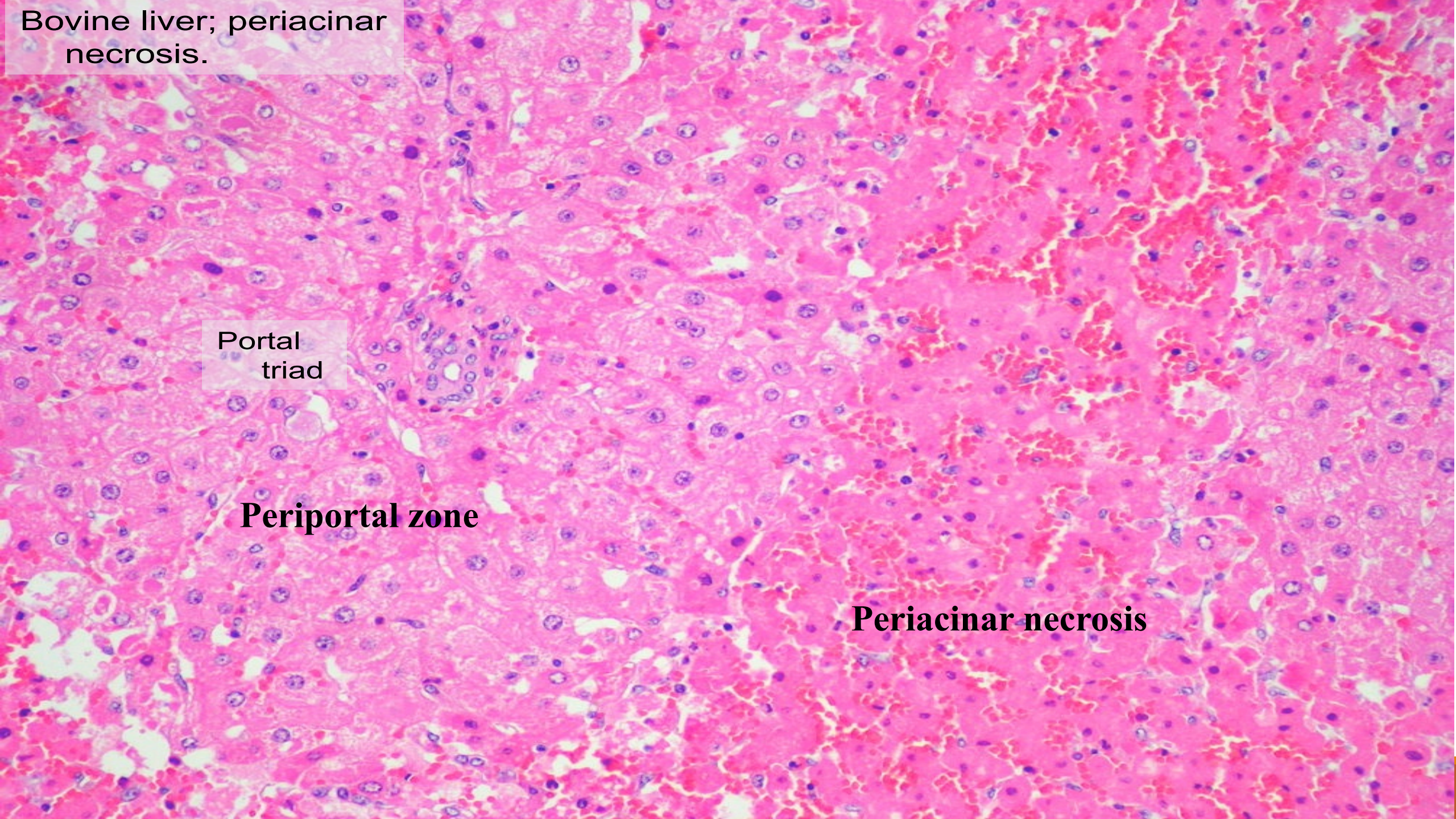
Periacinar necrosis

Often also damages sinusoids, permitting erythrocytes to enter the perisinusoidal space of Disse (haemorrhagic necrosis)

Seen in animals that die slowly – progressive hypoxia due to failing circulation



PERIACINAR NECROSIS

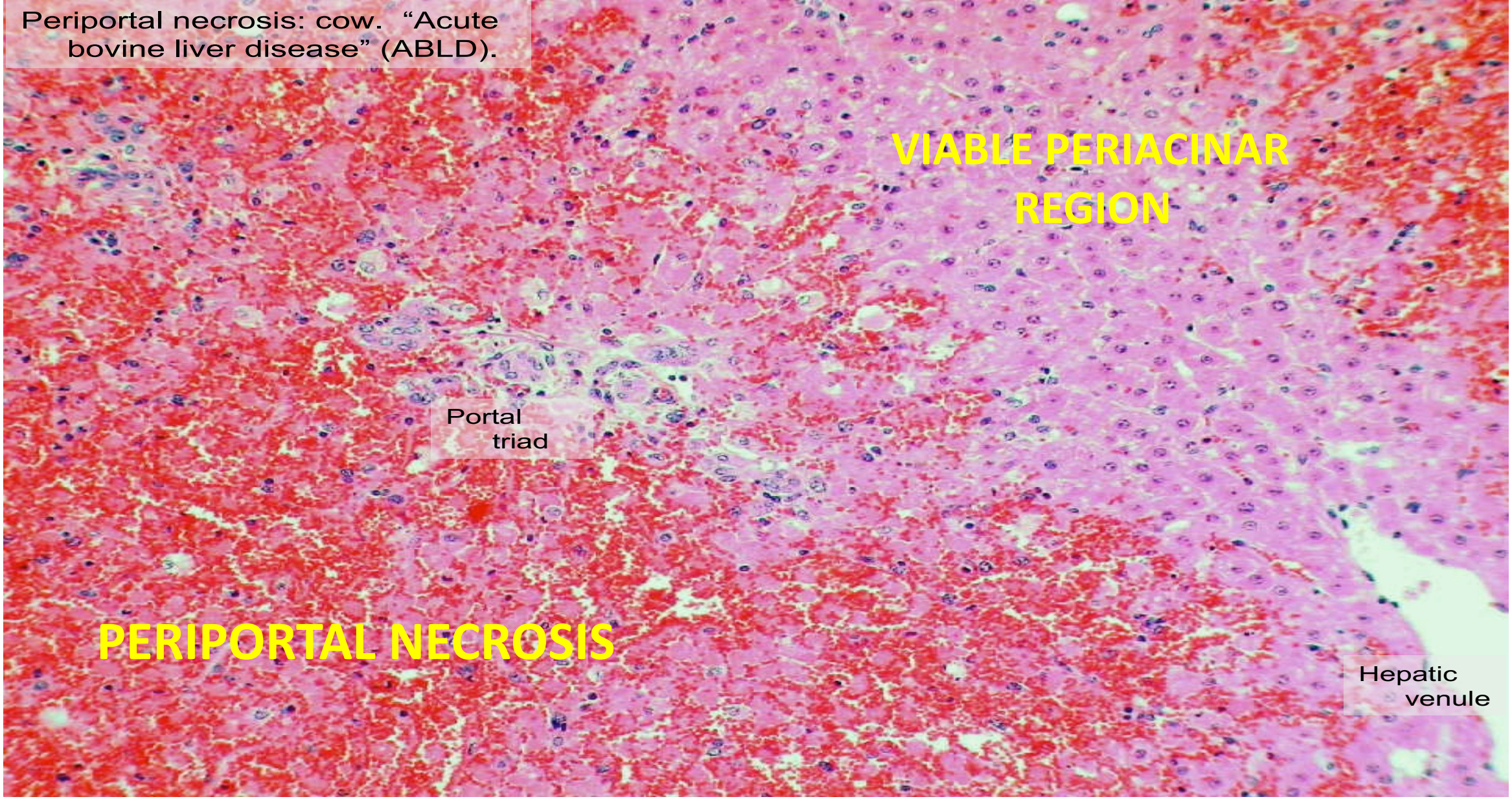

Bovine liver; periacinar necrosis.
Portal triad
Periportal zone
Periacinar necrosis

Mid-zonal, periportal and massive necrosis

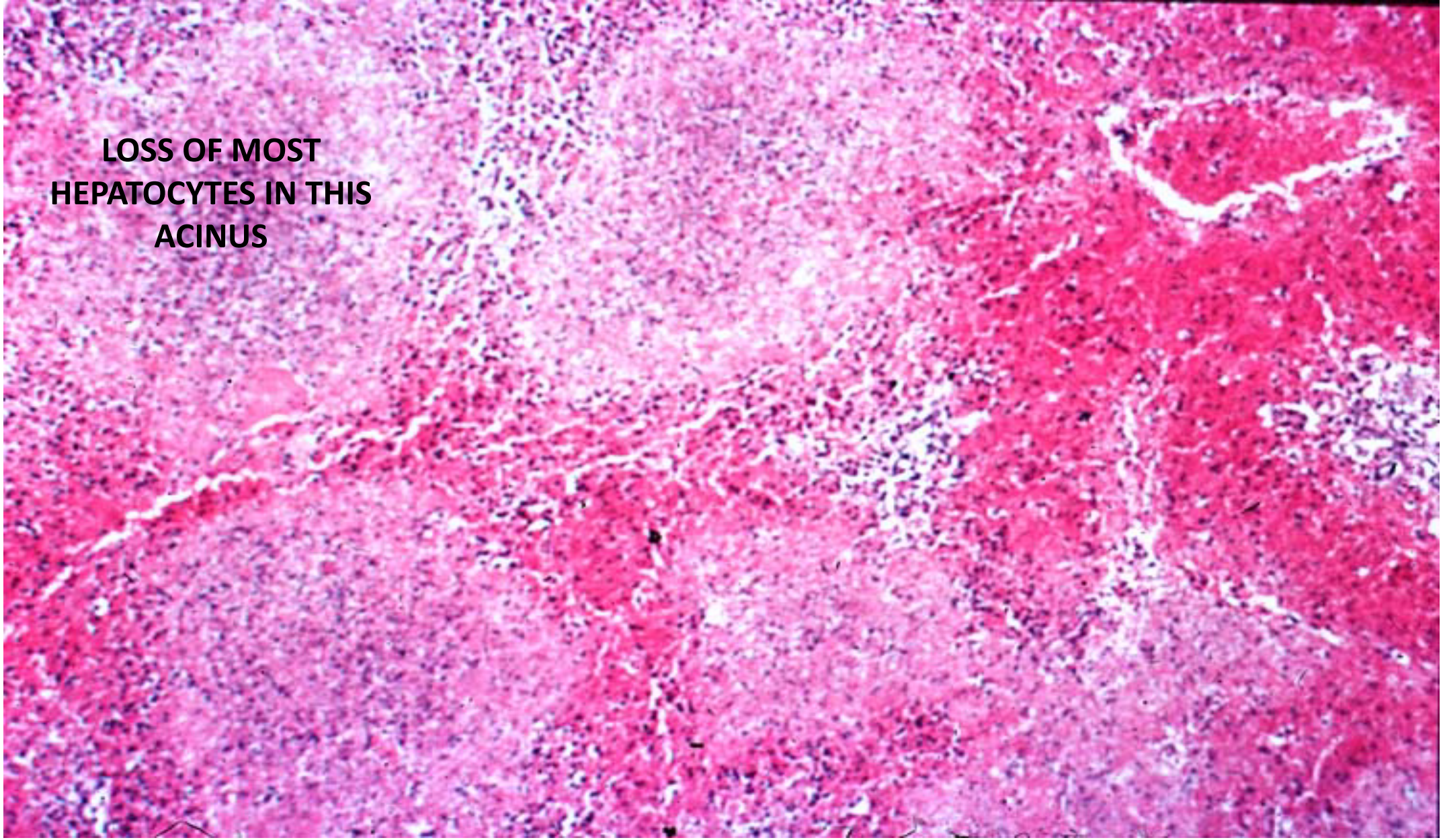
Mid-zonal necrosis: caused by some intoxications

Periportal necrosis: uncommon – usually direct-acting hepatotoxins

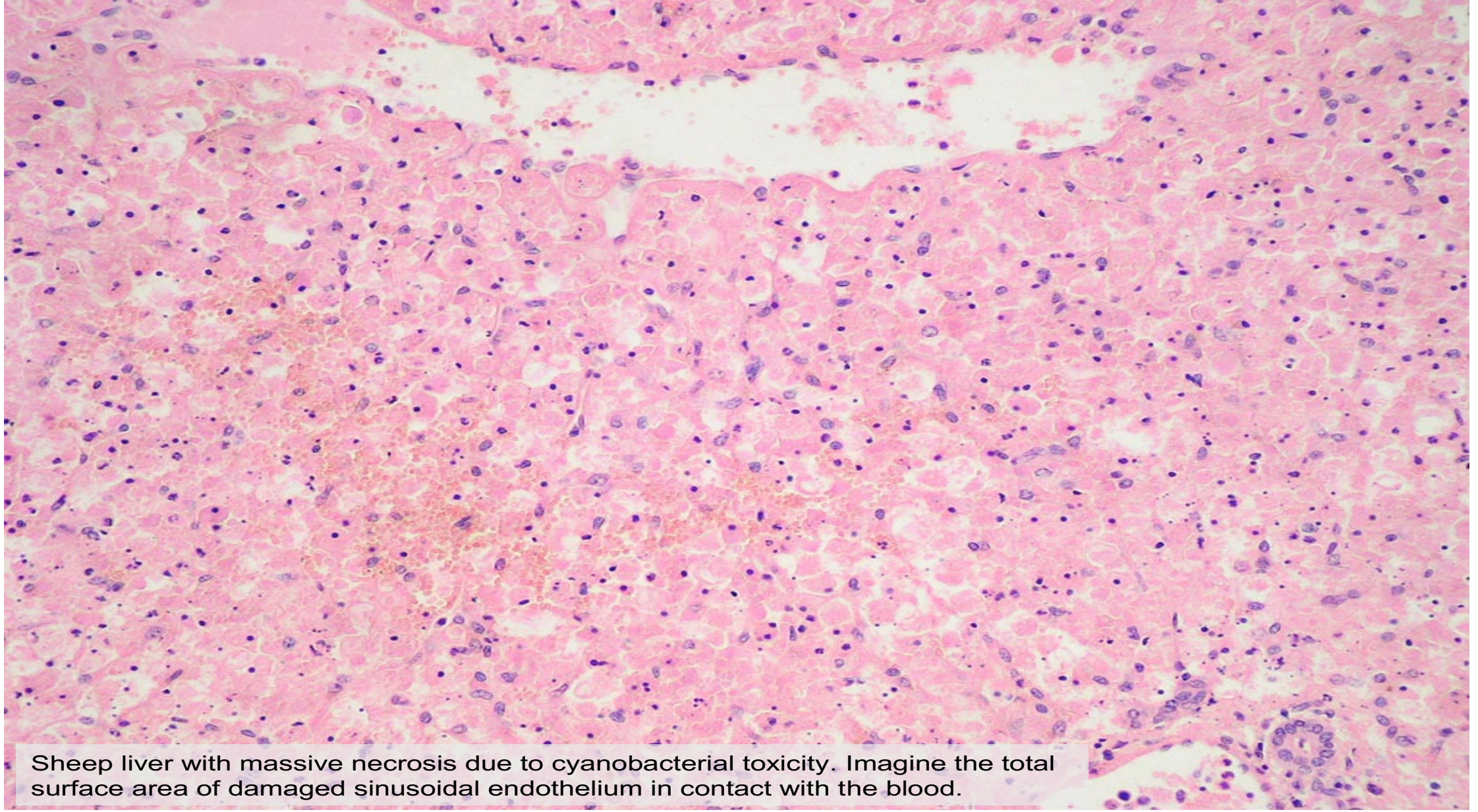
Massive necrosis: necrosis of an entire acinus, but some acini are spared. Since stem cells are also killed, no regeneration, and acinus collapses (and depressed below the capsular surface), with condensation of stroma and fibrosis (post-necrotic scarring)



PERIPORTAL NECROSIS



MASSIVE HEPATIC NECROSIS



Sheep liver with massive necrosis due to cyanobacterial toxicity. Imagine the total surface area of damaged sinusoidal endothelium in contact with the blood.

MASSIVE HEPATOCELLULAR NECROSIS INVOLVING NEARLY ALL HEPATOCYTES

Decreased hepatic functional mass (hepatic insufficiency)

70% or more of functional hepatocytes must be lost before alterations of hepatic function are detectable by serum biochemistry

Functions evaluated include protein synthesis (albumin, globulins, clotting factors), uptake and excretion of bilirubin and bile acids, uptake of ammonia and conversion to urea (BUN or blood urea nitrogen), glucose homeostasis and glycogen storage, and uptake and excretion of exogenous dyes (e.g. bromosulphalein)

Cholestasis

Can be intrahepatic (in bile canaliculi and ductules) or extrahepatic (in gallbladder or common bile duct)

May cause induction and release of certain membrane-bound hepatic enzymes, including alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT), elevating their serum concentration

Also results in retention and reflux of bile, increasing serum concentrations of substances normally excreted in bile (bilirubin, bile acids)

Bile acids can also cause hepatocyte injury by damaging their cell membranes

Bile duct hyperplasia

Common reaction to many types of injury, especially bile duct obstruction and portal inflammation/fibrosis

Can also occur independently of parenchymal changes, particularly when the injurious agent is concentrated in portal triads

May be reversible when the stimulus ceases

Bile ductules probably arise from pluripotential stem (oval) cells in canals of Hering, which can differentiate into either hepatocytes or cholangioles, and proliferate with hepatic injury

Hepatic fibrosis

Potentially reversible form of healing in which there is accumulation of extracellular matrix (ECM) components

May be resorbed completely but, when injury is extensive, progressive deposition of ECM occurs, leading to the “final common pathway” and ending in fibrosis and cirrhosis

Cirrhosis

Diffuse process characterised by fibrosis + conversion of normal liver architecture into structurally abnormal lobules

Not merely an end-stage of ECM accumulation, but a multifaceted distortion of hepatic parenchymal architecture and hepatic vascular anatomy

Portosystemic shunts (between portal veins and vena cava, by-passing the liver) and venous occlusion can result, interfering with hepatic function and driving portal hypertension

Results in hepatic insufficiency in the form of ascites (retention of excess low-protein peritoneal fluid, also often associated with systemic venous congestion of right-sided heart failure) and hypoproteinaemia

Hallmarks of cirrhosis

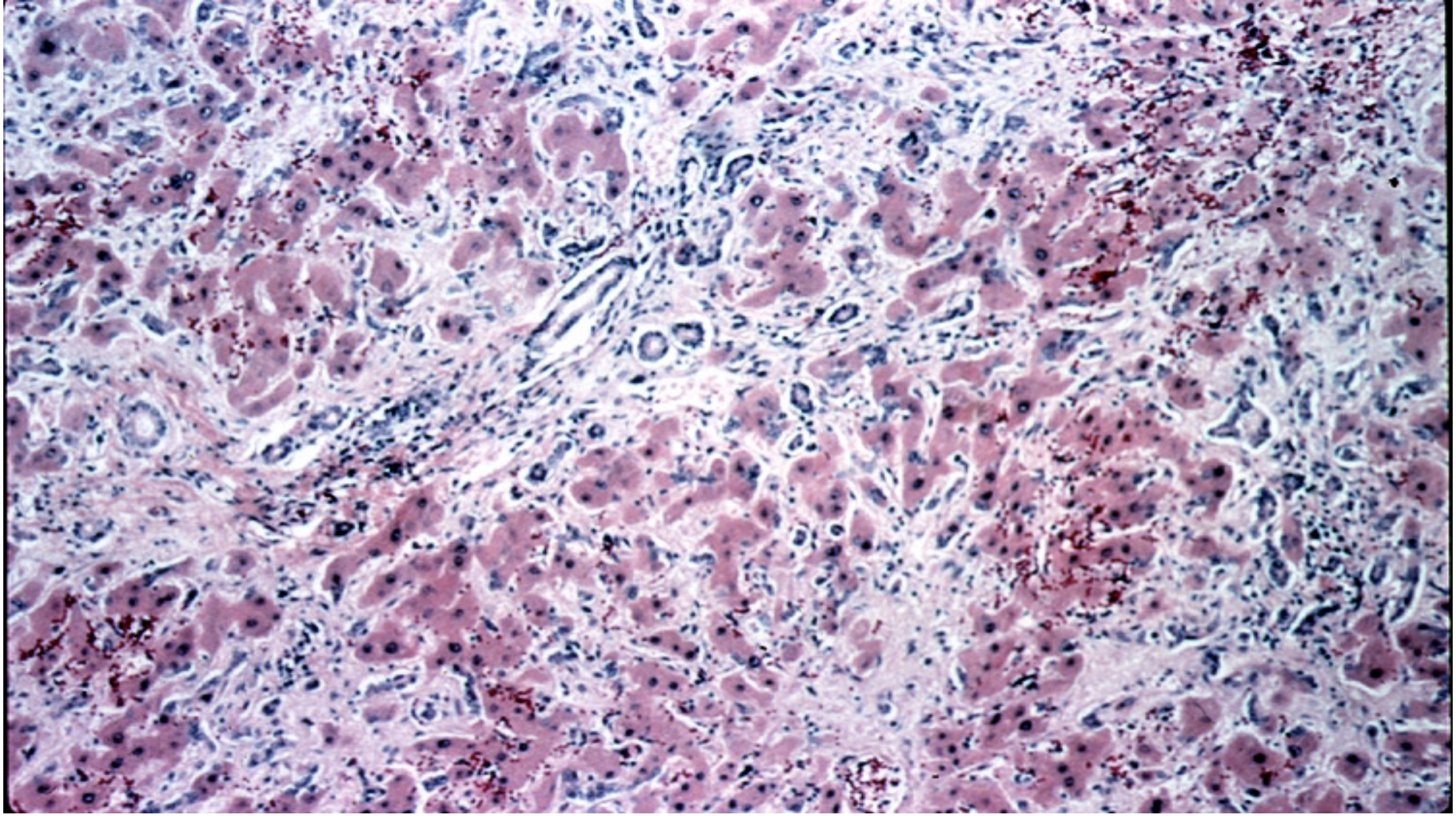
Bridging fibrous septa

Impaired exchange between sinusoids and hepatocytes due to increased perisinusoidal ECM

Parenchymal nodules created by hepatocyte regeneration

Ongoing damage and reorganisation of hepatic connective tissue

Cirrhosis usually results from several pathogenic processes – cell death and active inflammation with chronic fibrosis



DISSECTING FIBROSIS WITH PRESSURE ATROPHY OF HEPATOCYTES

Hepatic regeneration

Up to 70% of the liver can be removed and it will return to normal size (but not shape) within several weeks

Hepatic mass is tightly controlled by regulation of mitosis and apoptosis

Post-mitotic hepatocytes under the influence of growth factors (including from stellate cells can re-enter the cell cycle and replicate

Under protracted stimulus, progenitor (oval, stem) cells can differentiate into hepatocytes

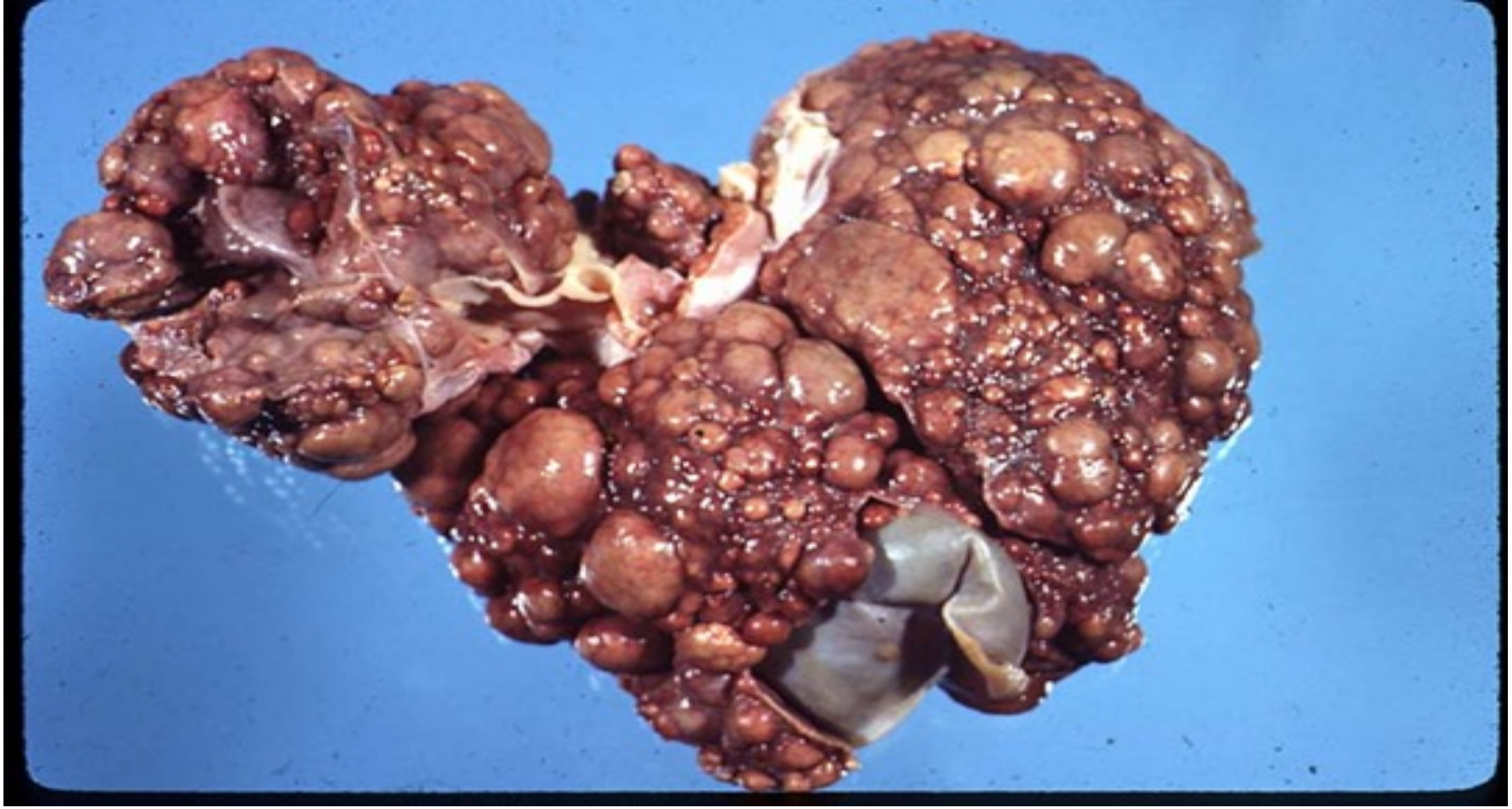
Hepatic regeneration

Regeneration tends to be nodular rather than diffuse and only occurs successfully if the underlying reticular scaffolding is intact

Success also depends on the access of hepatocytes to a vascular supply and bile drainage

If necrosis and inflammation are prolonged, substantial fibrosis ensues with hepatocyte atrophy

Some hepatotoxins (e.g. aflatoxin, pyrrolizidine alkaloids) inhibit hepatocyte proliferation (anti-mitotic effect)



NODULAR REGENERATIVE HYPERPLASIA