

Liver Biopsy in the Assessment of Medical Liver Disease

Thursday 4th April 2019



The Royal College of Pathologists

Pathology: the science behind the cure

How to handle liver biopsy specimens

Fixation, processing and staining methods

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Royal Free London NHS Foundation Trust

UCL Cancer Institute, Research Department of Pathology

Proper handling, processing and staining is essential to:

- minimise inherent **sampling** error
- contain effects of **fragmentation**
- avoid **artefacts** affecting interpretation
- provide **sufficient** tissue for full work-up
- provide sufficient **spare** tissue for future need

Specimen collection / fixation

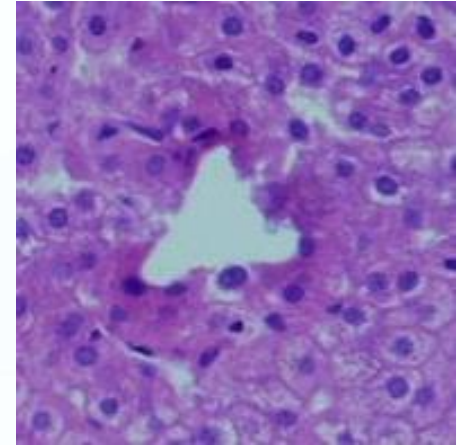
- **Conventional histology**
 - Immediately in buffered formalin or formol saline
 - Free floating, no blotting paper
 - at least 2-4 hours fixation
- **Copper / iron estimation**
 - Wet blotting paper
- **Snap frozen tissue**
 - OCT
 - enzymology - metabolic disorder (specialised centres)
 - Molecular (RNA later)
- **EM**
 - 1mm³ in glutaraldehyde:
 - Limited use
 - Byler (PFIC1), mitochondriopathy, Wilson, Storage disorders, other

Handling the biopsy specimen

- **Minimise crushing artefact**
 - Use disposable pipettes for transfer into cassette (small fragmented specimens)
- **Avoid packing between foam sponge**
- **Use fine mesh cassettes**
- **Processing:**
 - Overnight
 - Fast track urgent
 - Sufficient fixation time between biopsy and processing
 - 13:30 deadline → H&E ~16:30
- **Embedding**
 - flat, all core(s) on same cut plane

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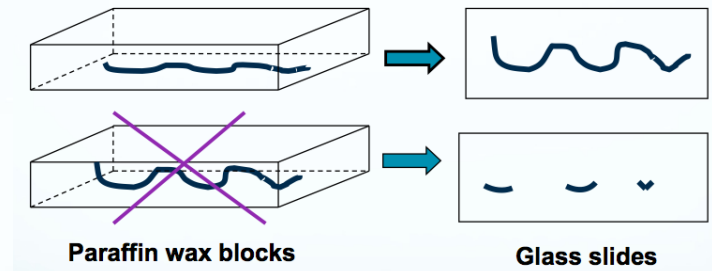


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Routine set of stainings

- 14 sequential pair-mounted numbered sections

□ 1, 8 = H & E

□ 3 = Silver for reticulin

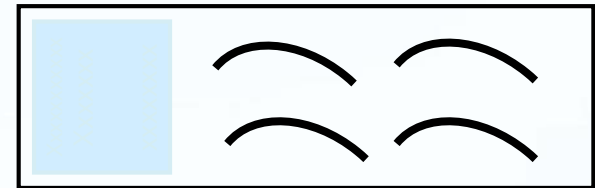
□ 4 = Perls' for iron

□ 5 = Periodic acid Schiff after diastase

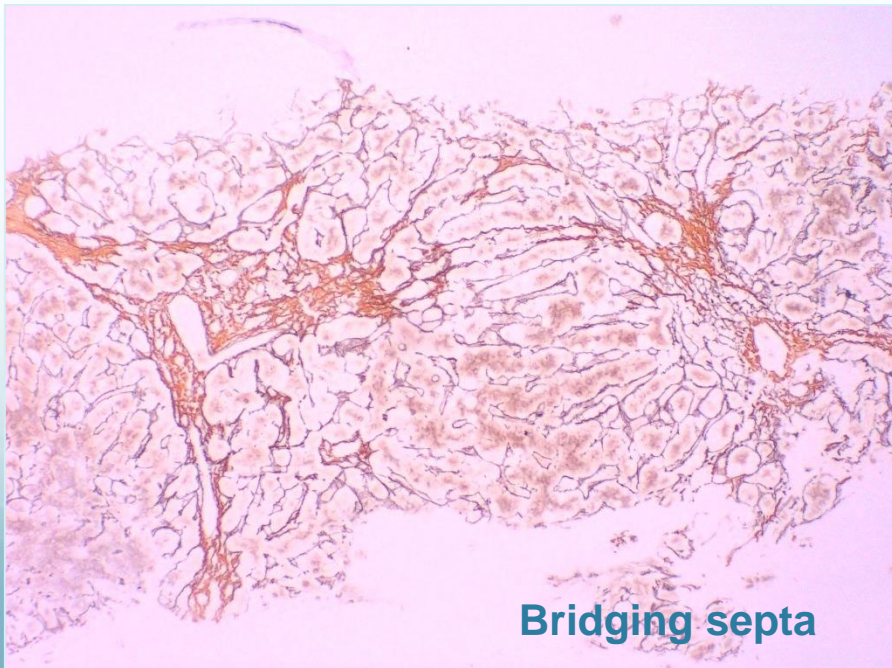
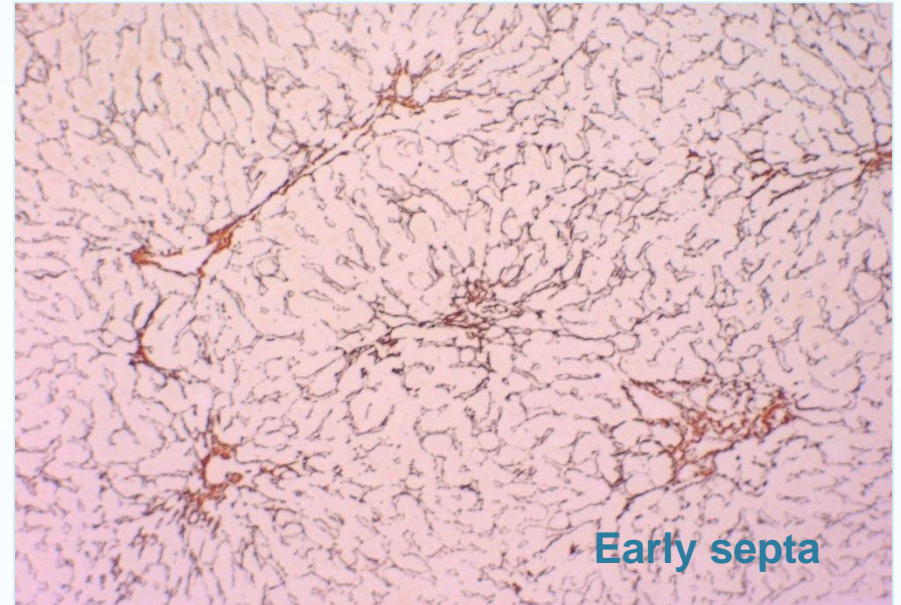
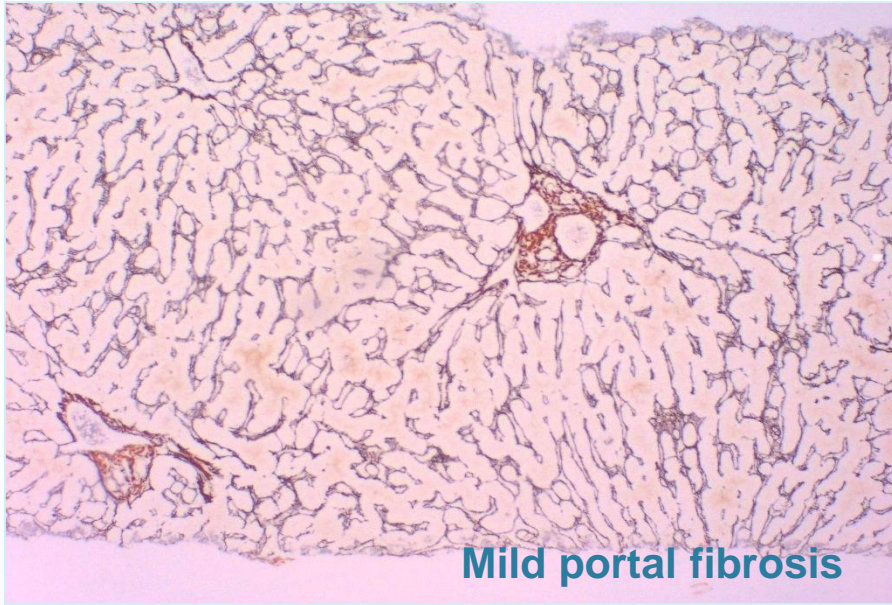
□ 6 = Orcein (modified Shikata' s)

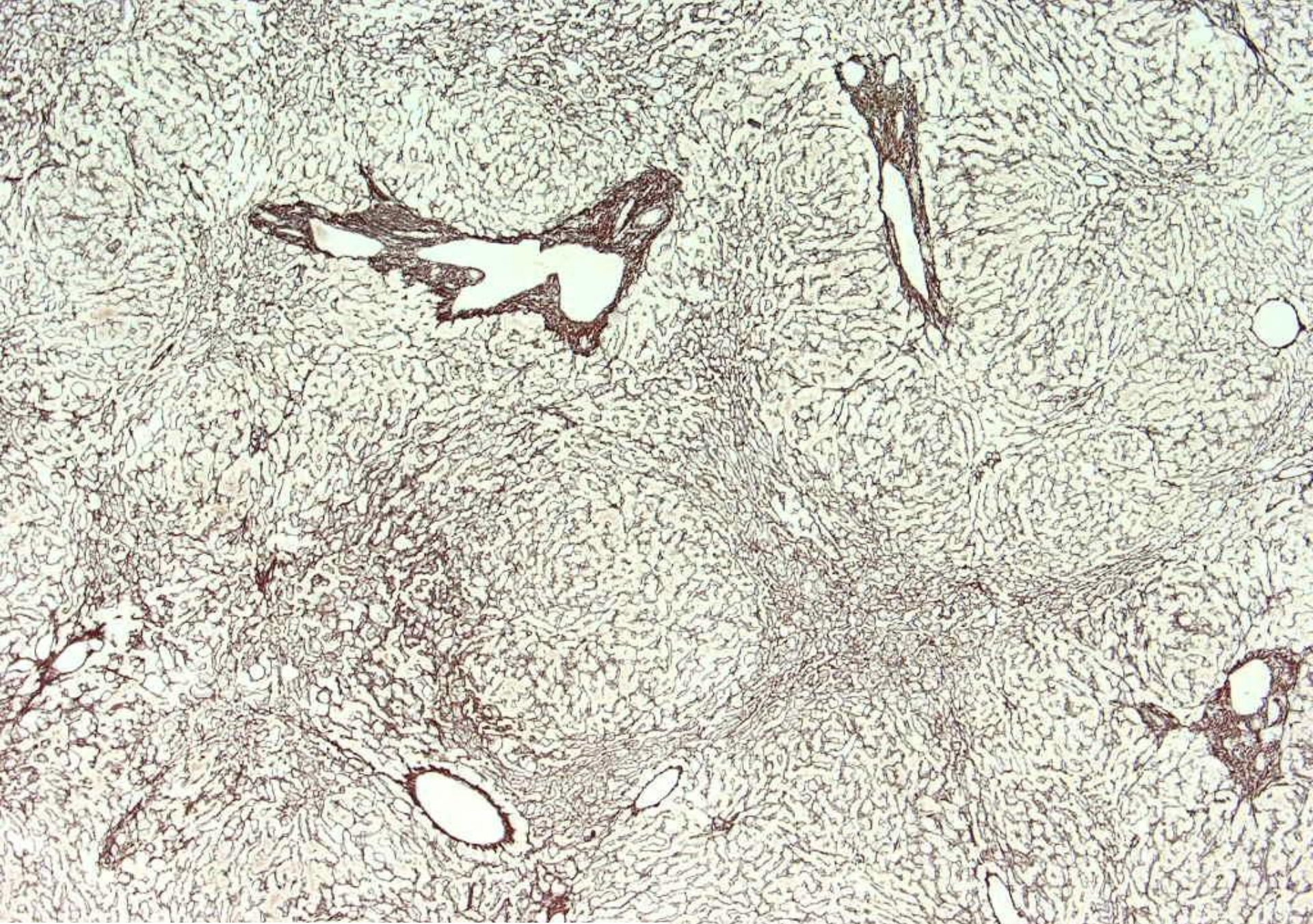
□ 2 = Held unstained

- e.g. HVG, EVG (acute vs chronic injury)

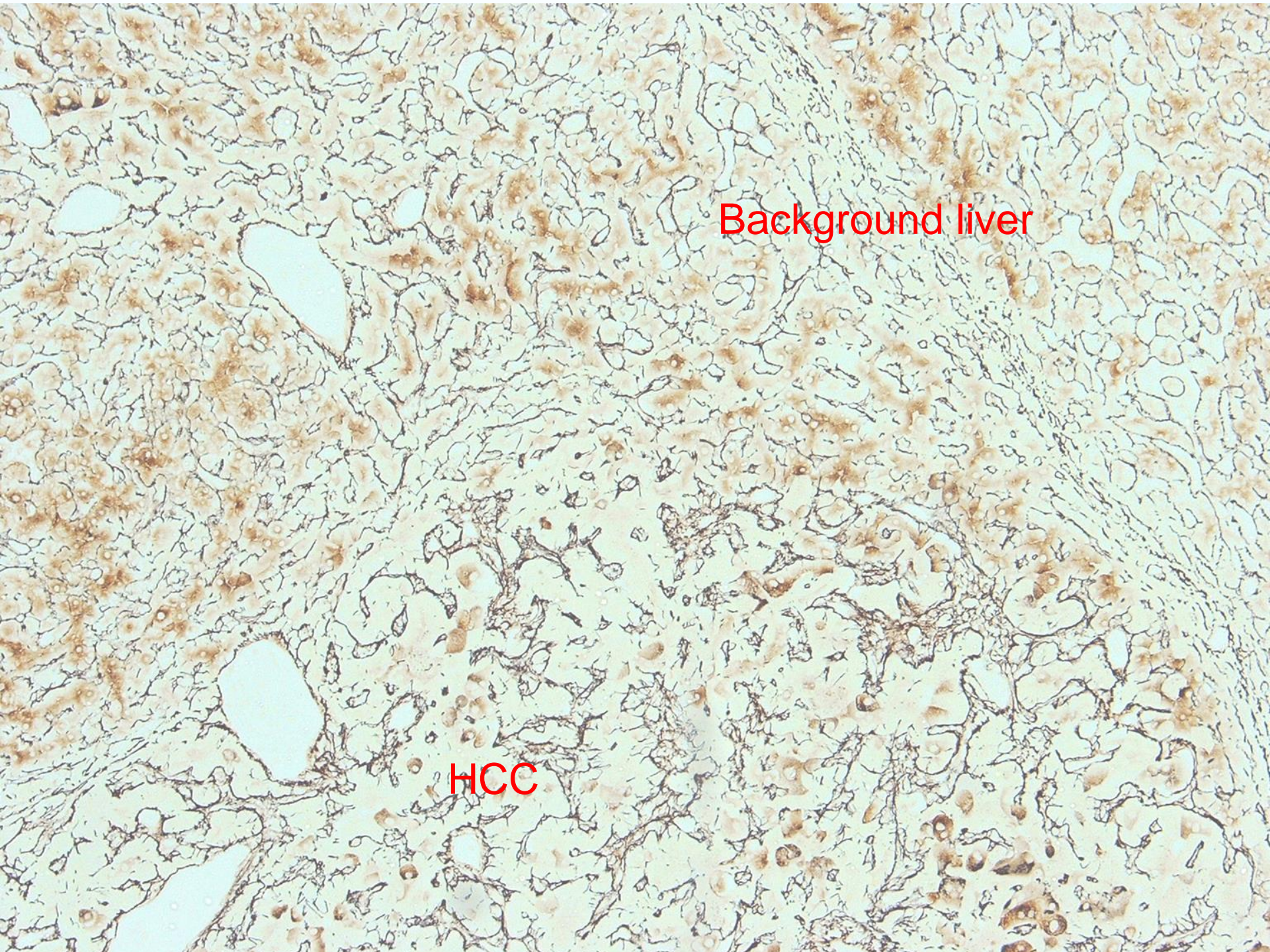


Gordon-Sweets' silver method for reticulin (collagen III)





Nodular regenerative

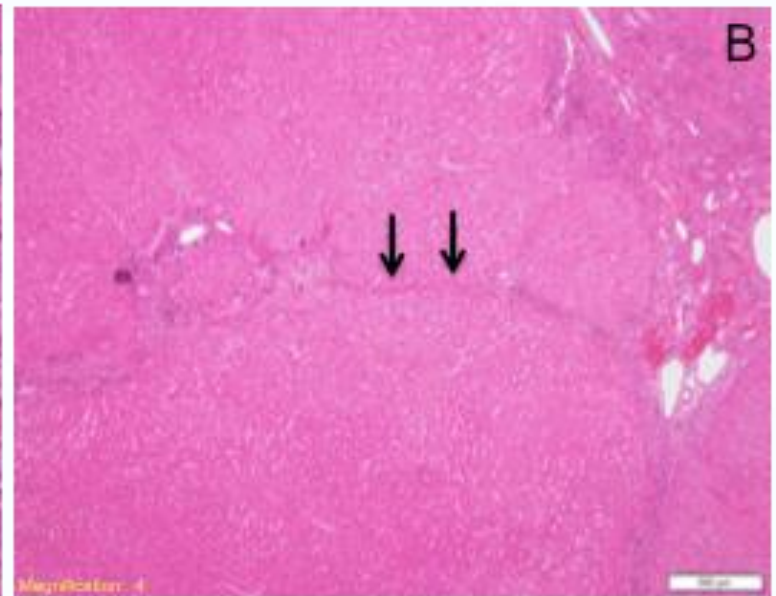
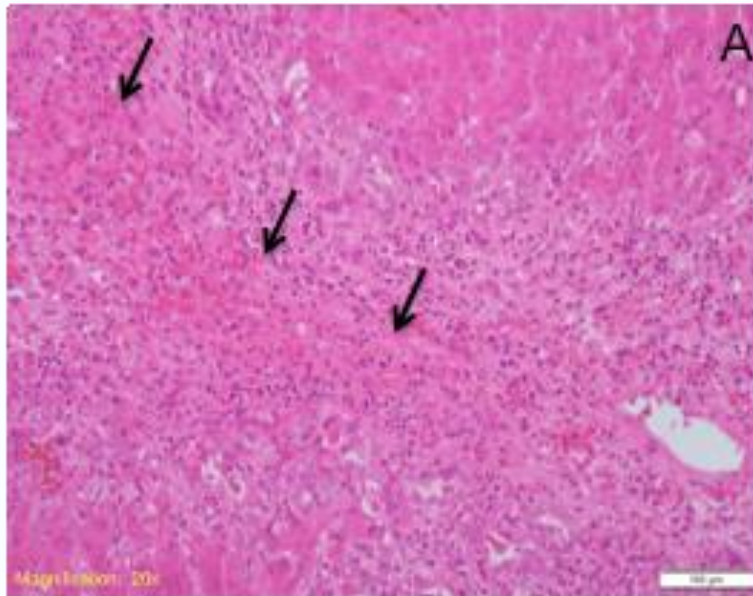


Background liver

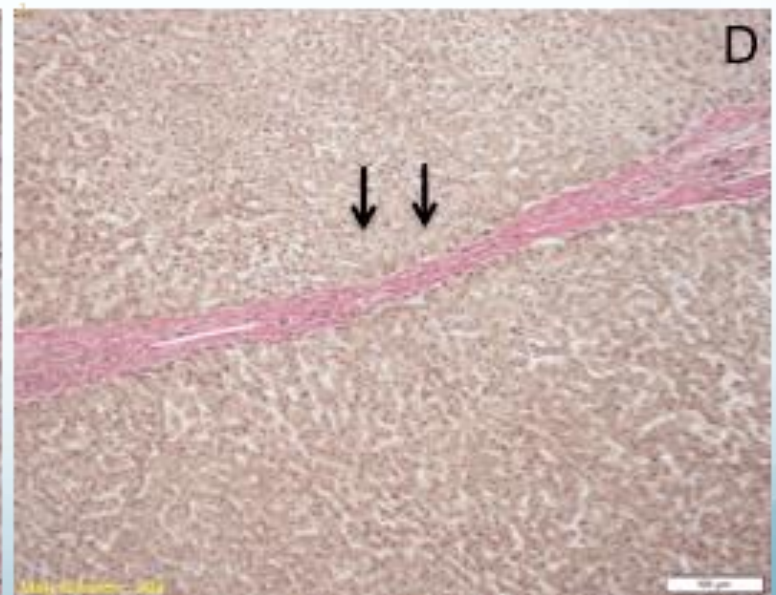
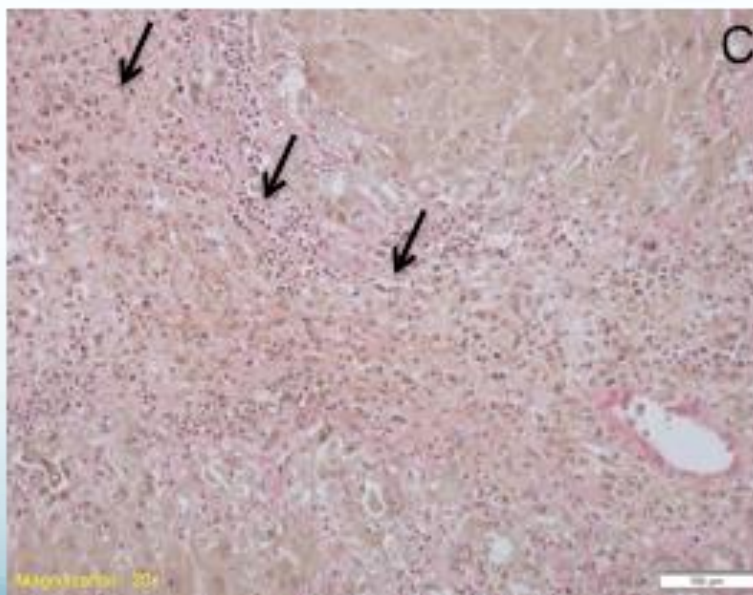
HCC

Acute vs chronic liver injury

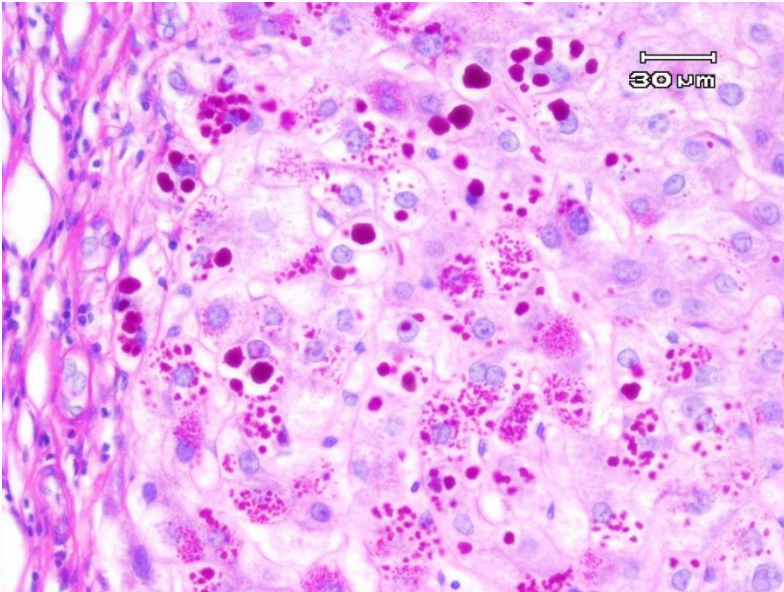
H&E



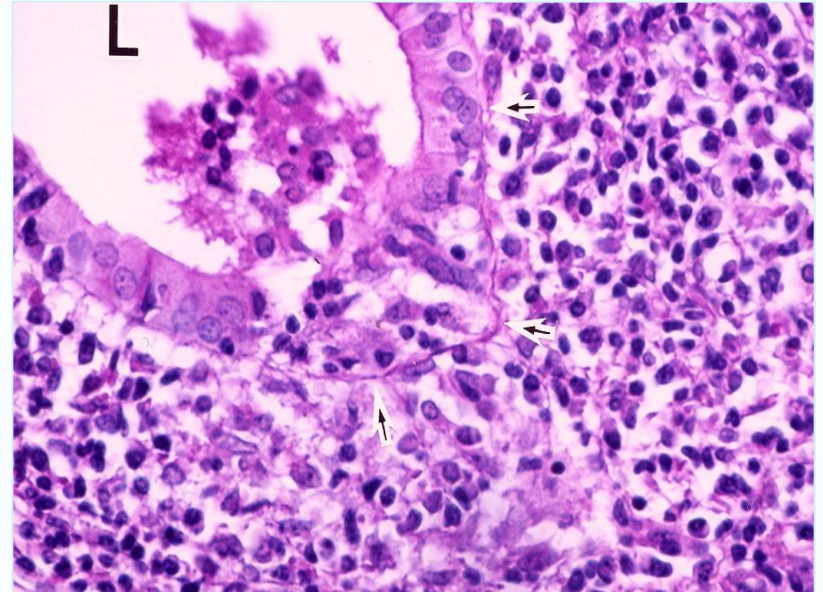
HVG



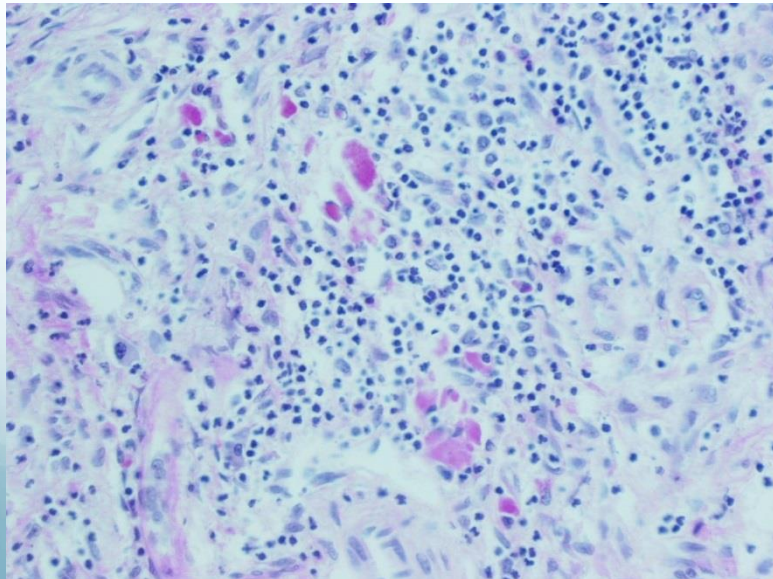
PAS after diastase



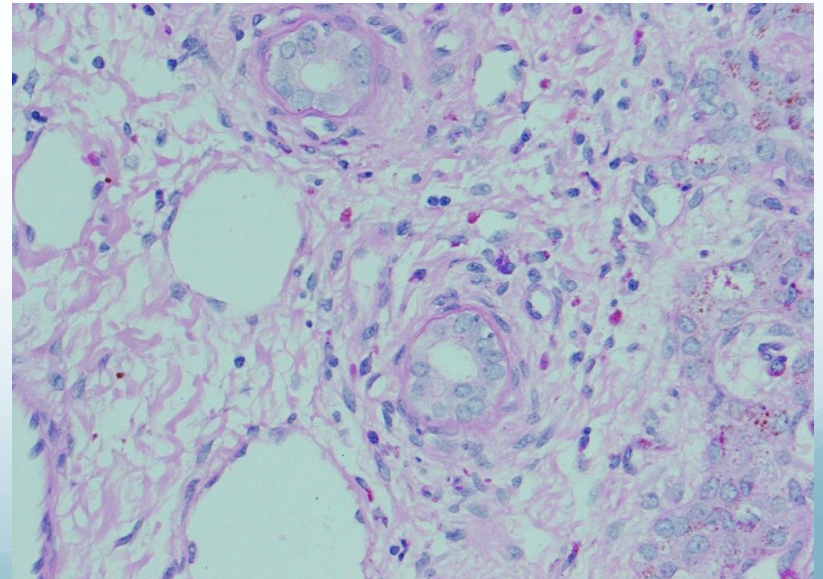
α 1-antitrypsin globules



Bile duct basement membranes



Scavenger macrophages



Perls' for iron

Ferritin + haemosiderin

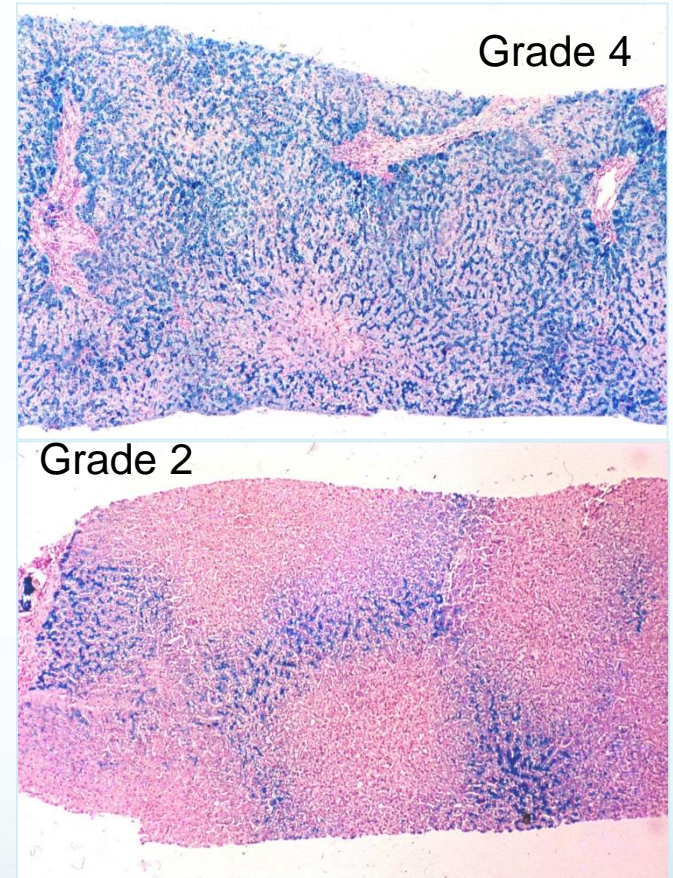
Ferritin

diffuse blue blush (DD artefact)

• Haemosiderin

intense blue granules

- Hepatocytes
 - Haemochromatosis
 - Secondary (e.g. ALD)
- Sinusoidal (endothelium/Kupffer cells)
 - Haemolysis, blood transfusions, systemic chronic disorders, acute hep
 - Ferroportin disease

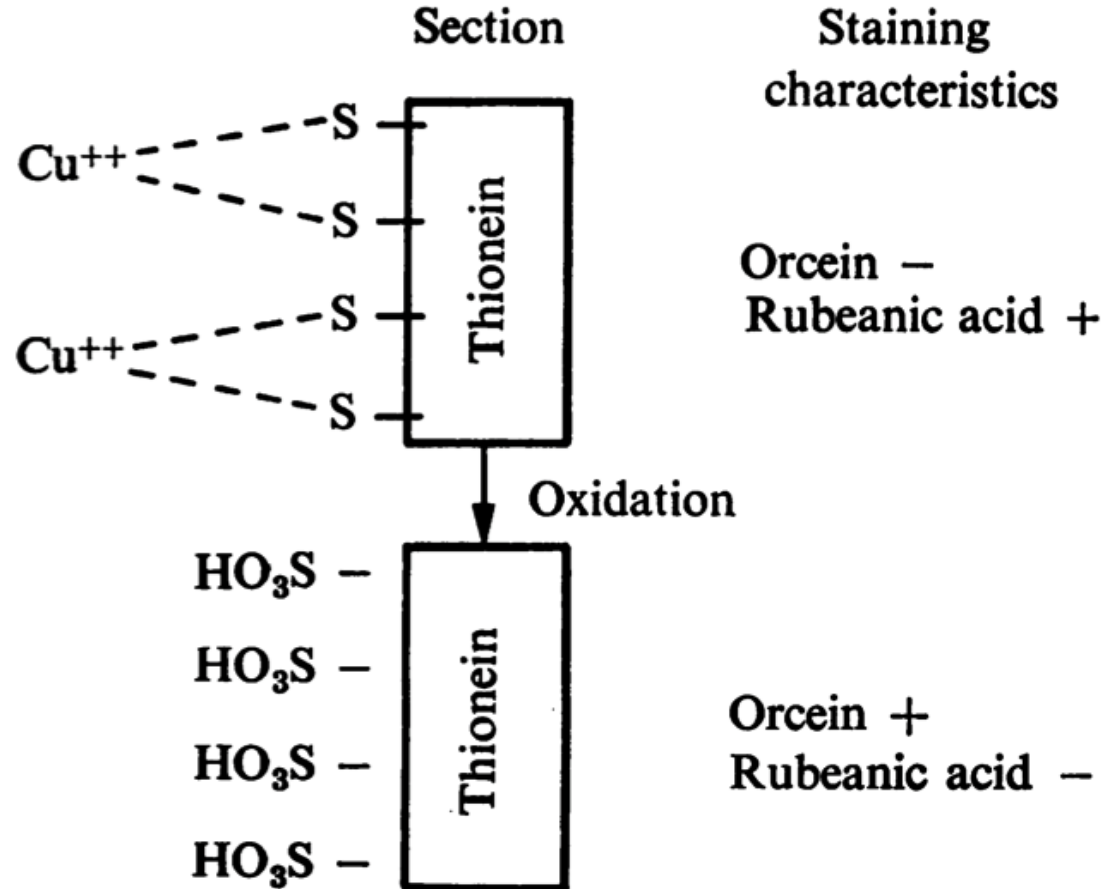


Parenchymal iron
graded on a 0-4+
scale

Orcein

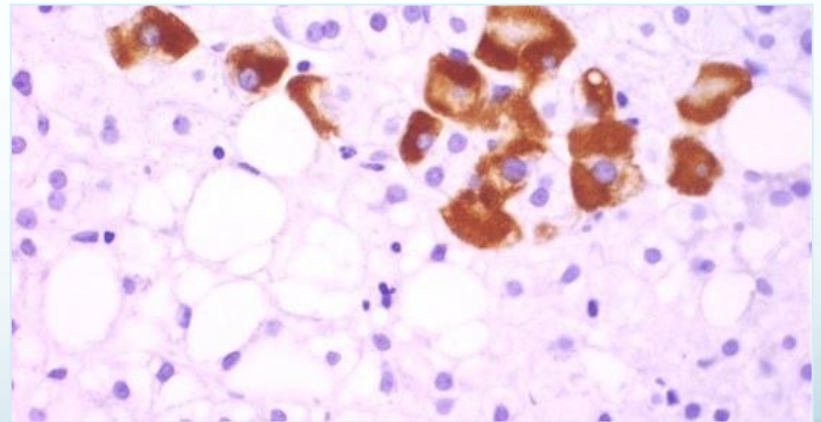
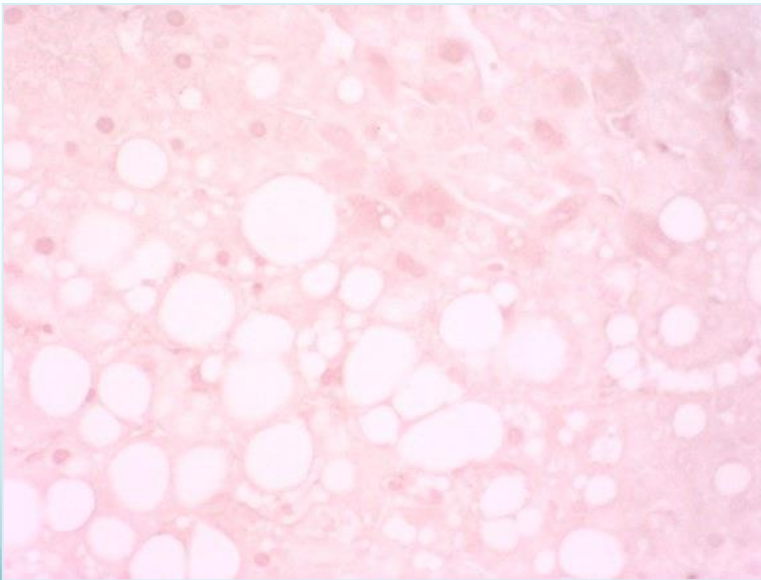
Disulphide bonds

- Hepatitis B surface antigen
- Elastic fibers
- Copper-binding protein

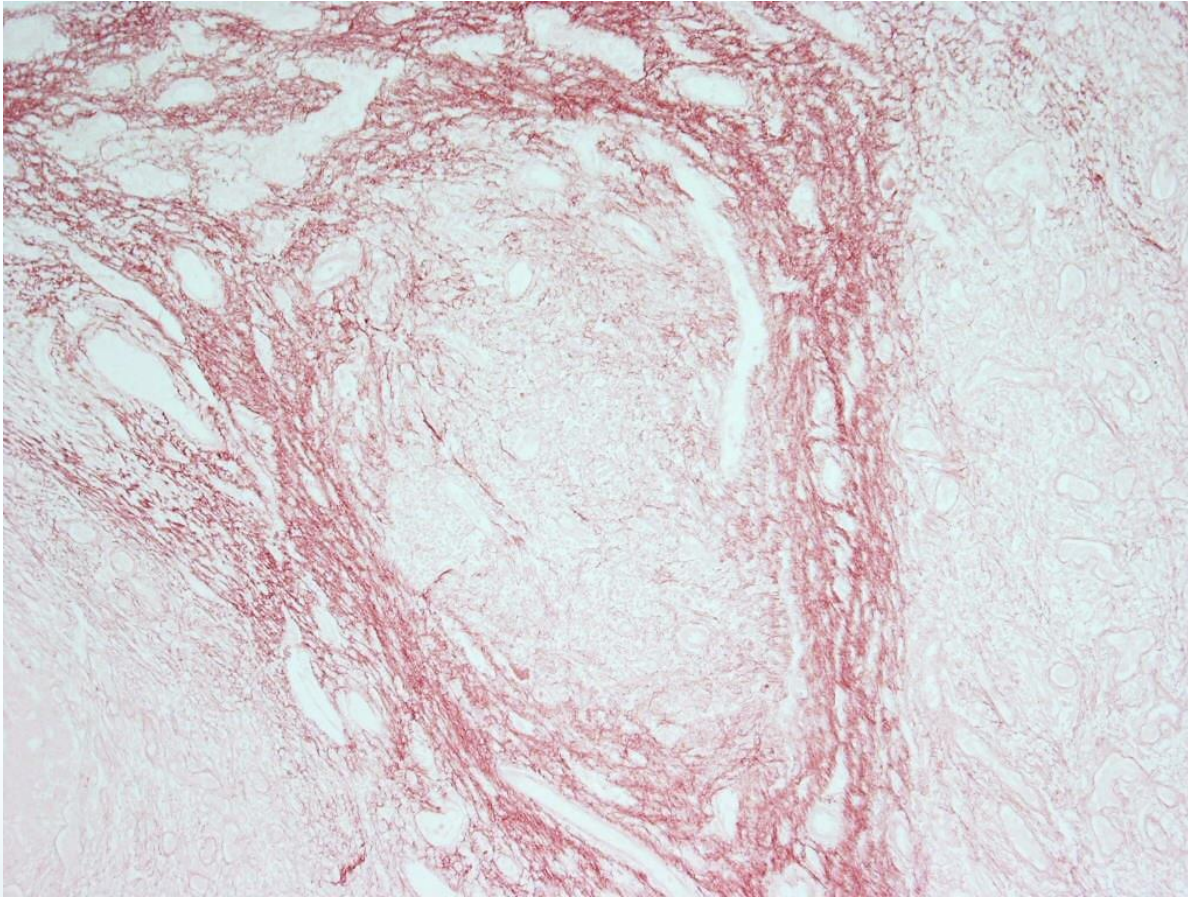


Orcein

- Hepatitis B surface antigen
 - ⇒ **Homogeneous cytoplasmic inclusions**
 - Low sensitivity (↓ in buffered formalin)
 - Does not detect membranous deposition



Orcein



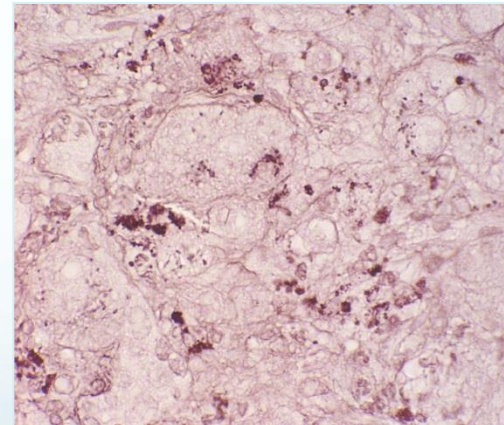
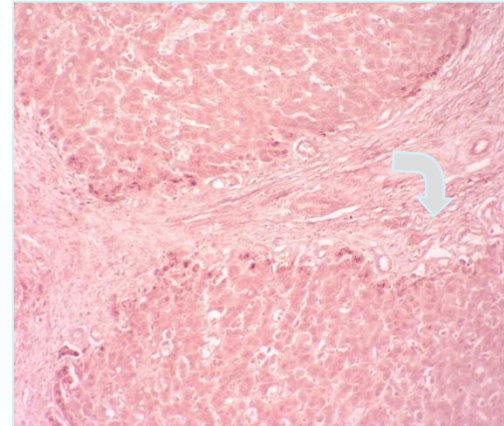
Elastic tissue

- Age of areas of parenchymal collapse
- Vascular thrombi

Orcein

Copper-associated protein \Rightarrow Dark brown granules

- Chronic cholestasis
 - periportal / periseptal
- Wilson's disease
 - random \pm Kupffer cells



- **Physiological (infant liver up to 2 months of age)**
- **Cirrhosis of any aetiology (patchy)**

Cu and Cu-associated protein in WD

- Early Wilson :
 - Cytosolic Cu highly soluble + too small to be seen histologically (rhodanine stain +/-)
 - Lysosomal Cu binding protein may not be significant yet
- ⇒ Negative staining does not exclude WD
- ⇒ Fresh tissue for Cu estimation

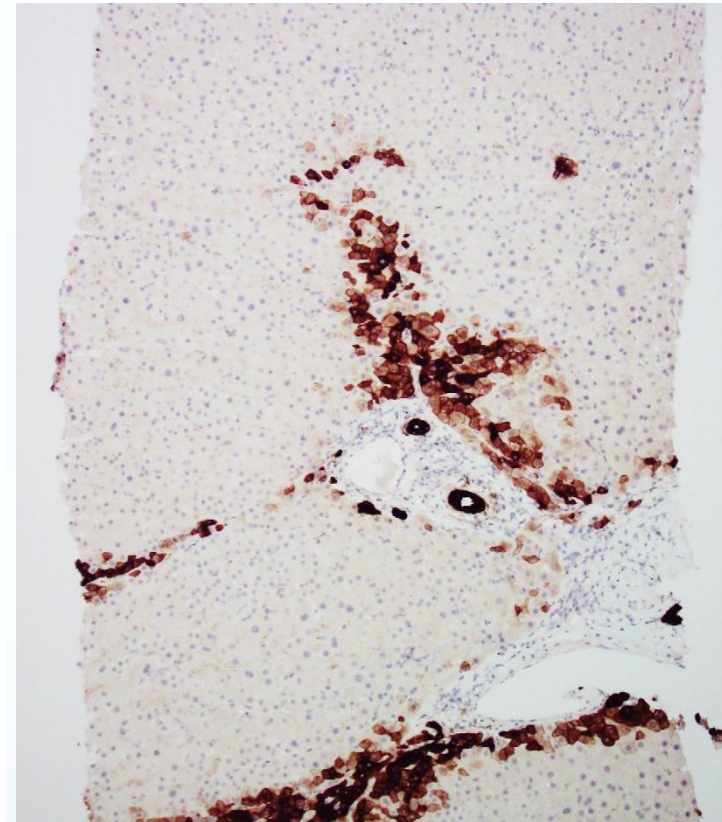
Table 2. Summary of results

Diagnosis (no. of cases)	Hepatocytes positive with polyclonal rabbit anti-human keratin*			Hepatocytes positive with monoclonal anti-cytokeratin no. 7†			Hepatocytes positive with monoclonal anti-cytokeratin no. 19†		
	PV	PP/PS	Both	PV	PP/PS	Both	PV	PP/PS	Both
Primary biliary cirrhosis									
stage I (3)	0	1	0	0	0	0	0	0	0
stage II (2)	0	0	1	0	0	2	0	0	0
stage III (4)	0	0	4	0	1	3	1	0	0
stage IV (4)	0	0	4	0	0	4	0	1	0
Primary sclerosing cholangitis (3)	0	0	3	0	0	3	0	3	0
Extrahepatic biliary obstruction (8)	1	1	4	0	2	3	0	0	0
Toxic/drug-induced liver disease									
'simple cholestasis' (10)	1	2	2	1	0	2	0	0	0
hepatitis (4)	0	0	3	0	0	2	0	0	0

* On paraffin sections.

† On cryostat sections.

PV = perivenular (acinar zone 3); PP = periportal (acinar zone 1); PS = periseptal; Both = both in perivenular and periportal or periseptal location.



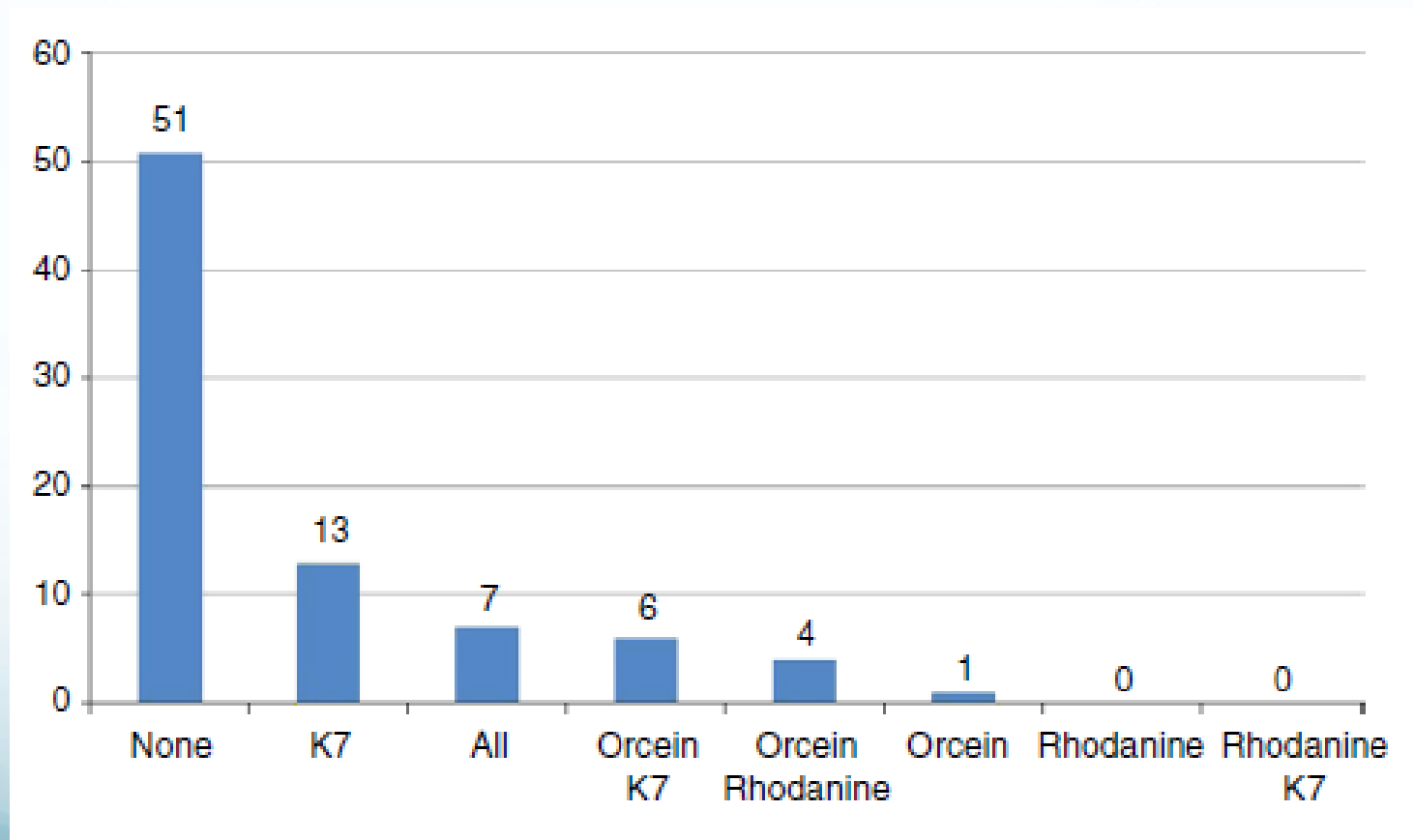
A cytokeratin immunohistochemical study of cholestatic liver disease: evidence that hepatocytes can express 'bile duct-type' cytokeratins.

Van Eyken P1, Sciort R, Desmet VJ.

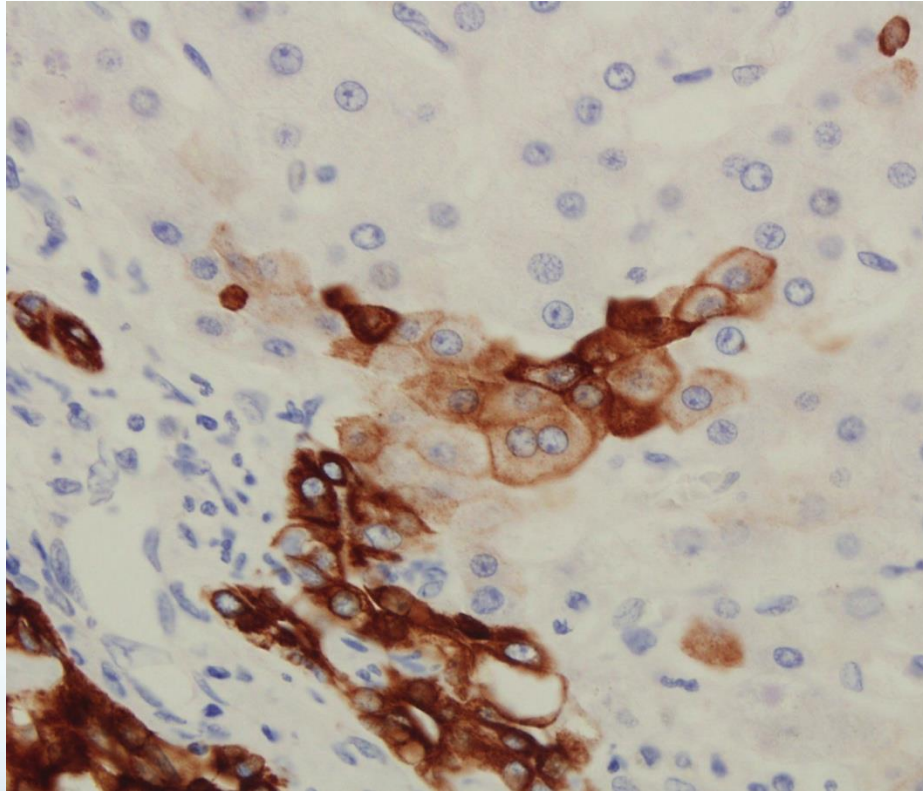
Histopathology. 1989 Aug;15(2):125-35.

Orcein vs Rhodanine vs Keratin 7 in biliary disorders

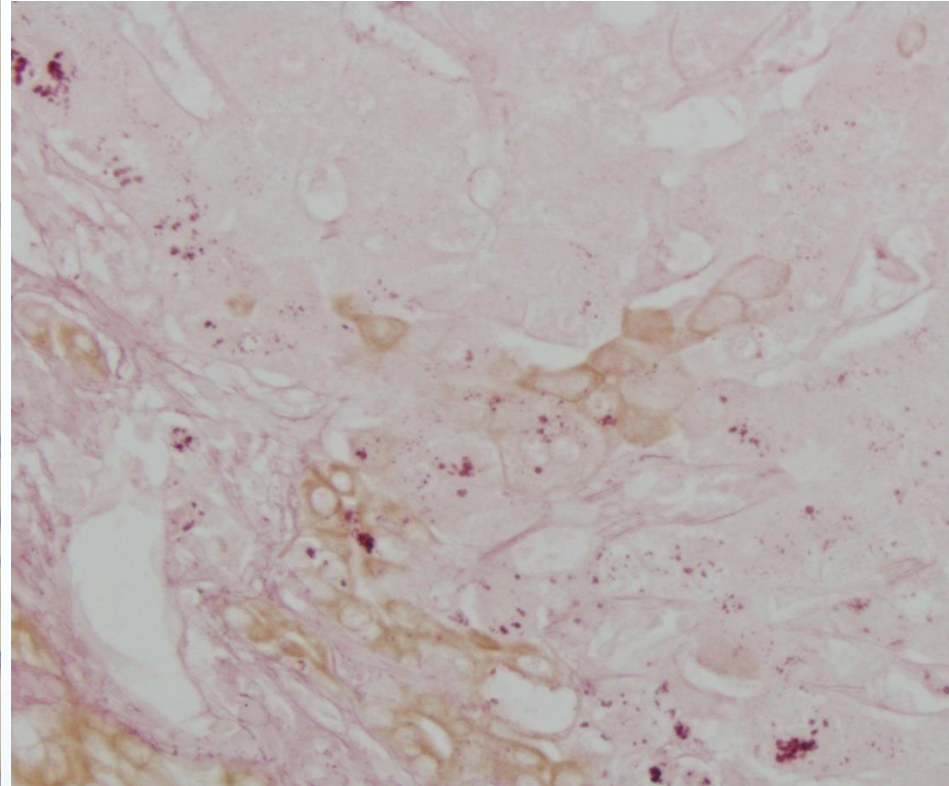
82 portal tracts. 12 biopsies. Portal tract number range 1-14, median 6



Sequential cytokeratin 7 and orcein stain in advanced stage chronic biliary disorders



Cytokeratin
7



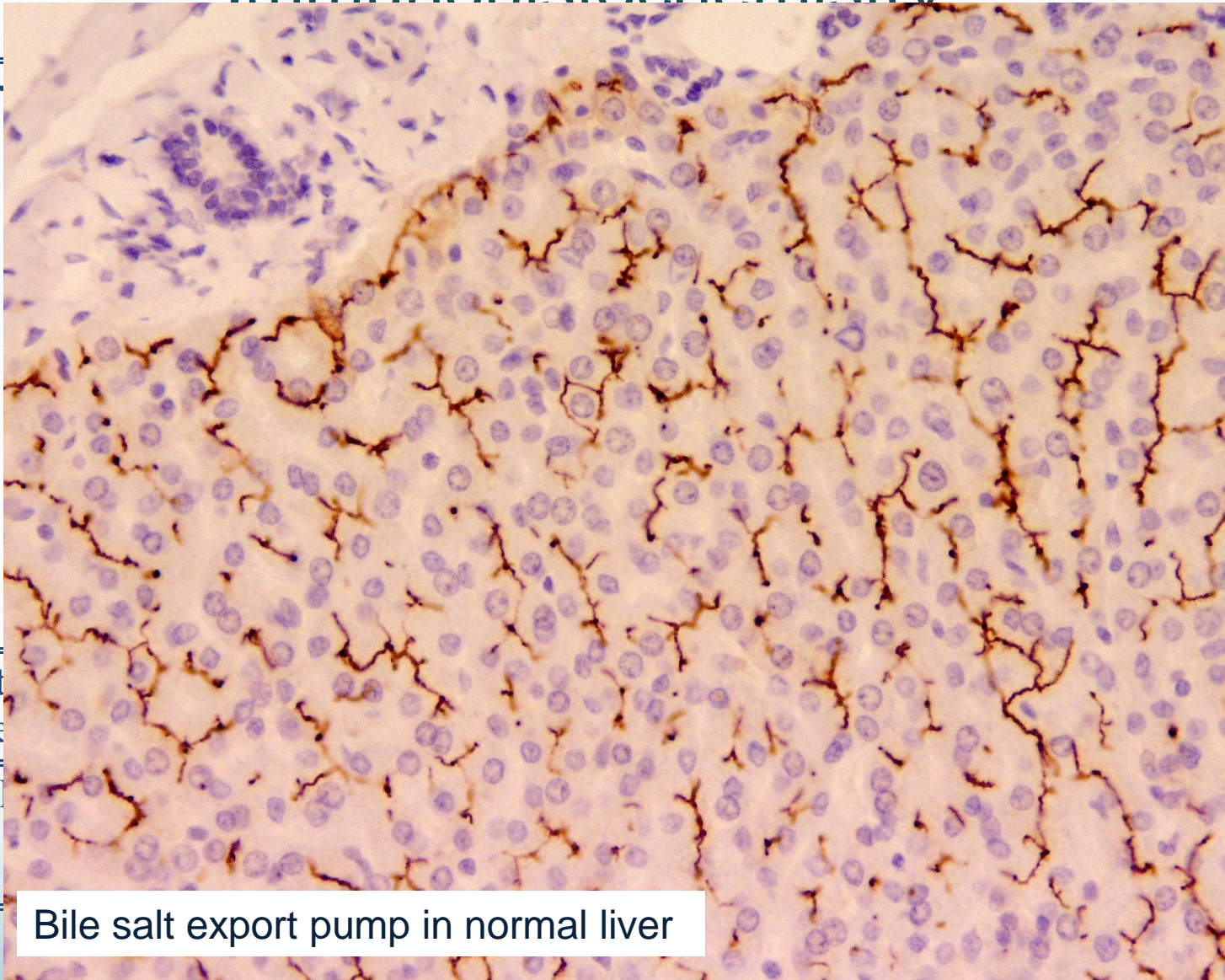
Orcein

Immunohistochemistry

<p>General repertoire</p>	<ul style="list-style-type: none"> • Cytokeratins Pan, KER 7,19,20 • Hep Par 1 • Canalicular markers pCEA, CD10 • Sinusoidal cells (CD34, CD68) • Tumour typing • LCA, CD3, CD20, CDX2, PSA, TTF-1, ER/PR, HMB45, Desmin, Synaptophysin, chromogranin, Ki67) • MUC1,2,5,6, CA19.9
<p>Additional canalicular markers</p>	<p>CD13, BSEP, MDR3, GGT, cMOAT, Claudin-1, TJP2, DCD2</p>
<p>HCC/HCA work-up</p>	<p>SAA, Glutamine Synthetase, beta-Catenin, Glypican-3, FABP, HSP70, Arginase, Sall 4, CD34, others</p>
<p>Virus</p>	<p>HSV, Adenovirus, HBsAg, HBcAg, EBV (ISH), CMV</p>
<p>Other</p>	<p>CD1a, C4d, CD133, Epcam, Perforin , Tubulin Beta 3, Catalase</p>

Immunohistochemistry

Gener



Addit
marke

HCC/

Virus

Other

, TTF-

(Ki67)

, DCD2

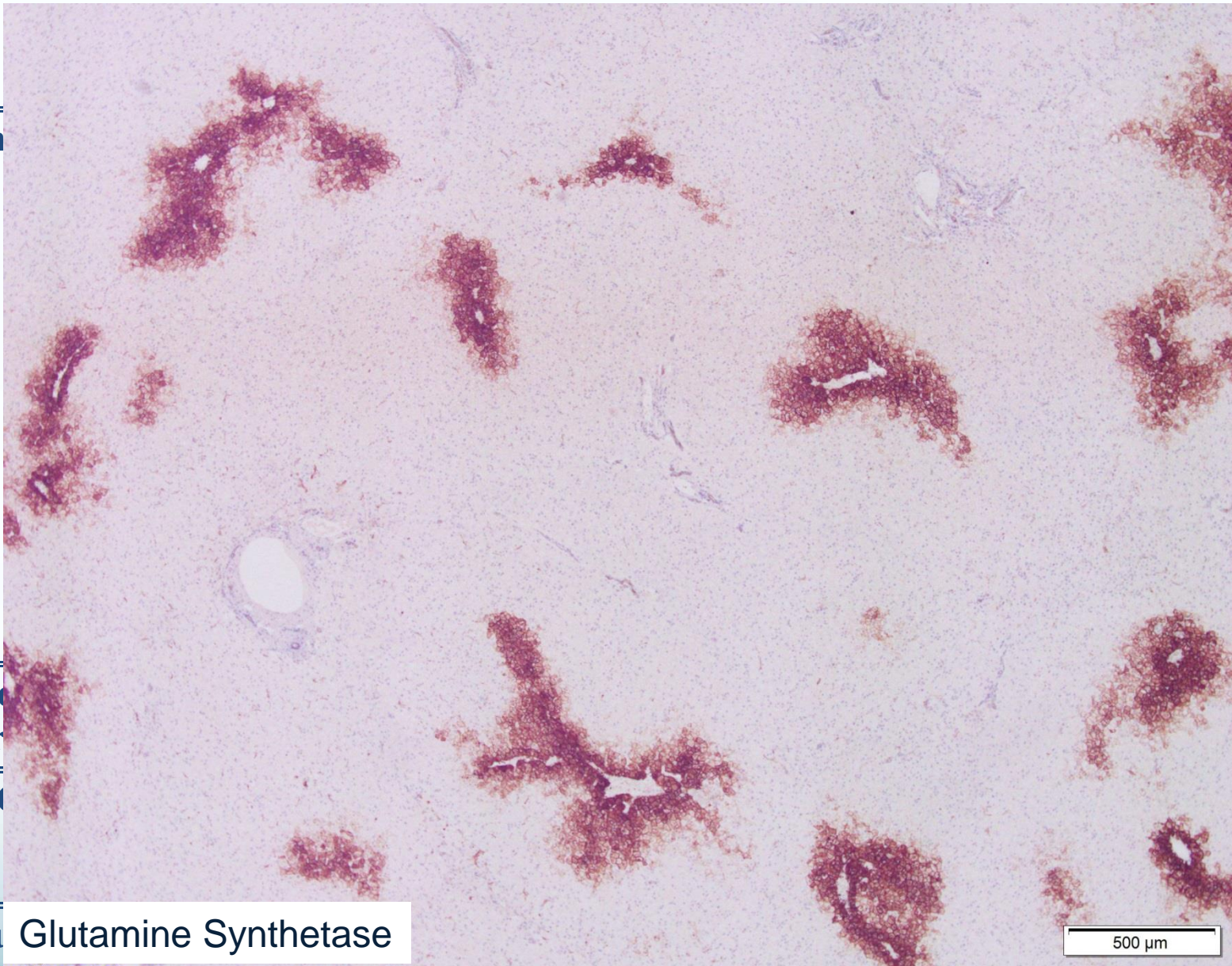
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Gen



CTF-

57)

Ad
mar

CD2

HCC

ABP,

Virus Glutamine Synthetase

500 μm

Other

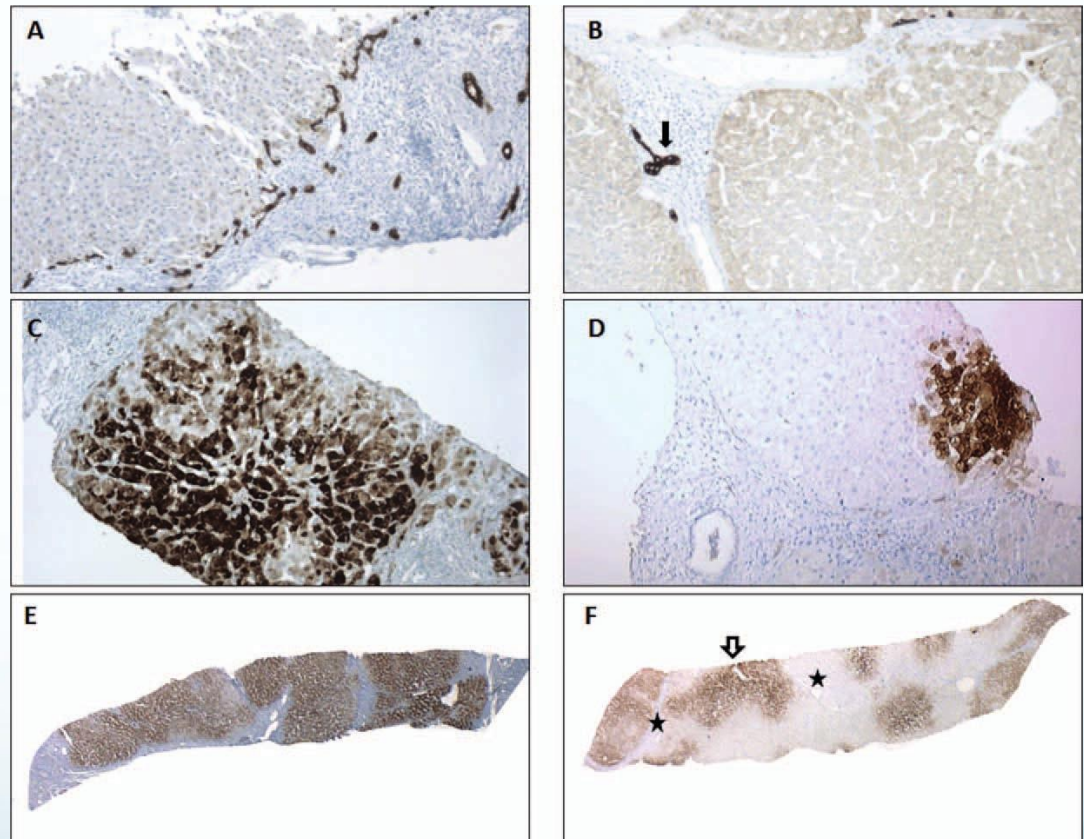
CD1a, C4d, CD133, Epcam, Perforin , Tubulin Beta 3, Catalase

- 6 December 2019
- Royal College of Pathologists
- One day conference dedicated to diagnostic histopathology of liver tumours
 - Course organisers: TuVinh Luong, Jennifer Watkins and Alberto Quaglia
- CPD in progress
- Registration begins on 1 May 2019
 - Please send email to Mr Andrew Hall
email: livertumourconference@outlook.com



Chronic liver disease: progression, regression and remodelling *(Histopathology. 2016 Jun;68(7):953-67)*

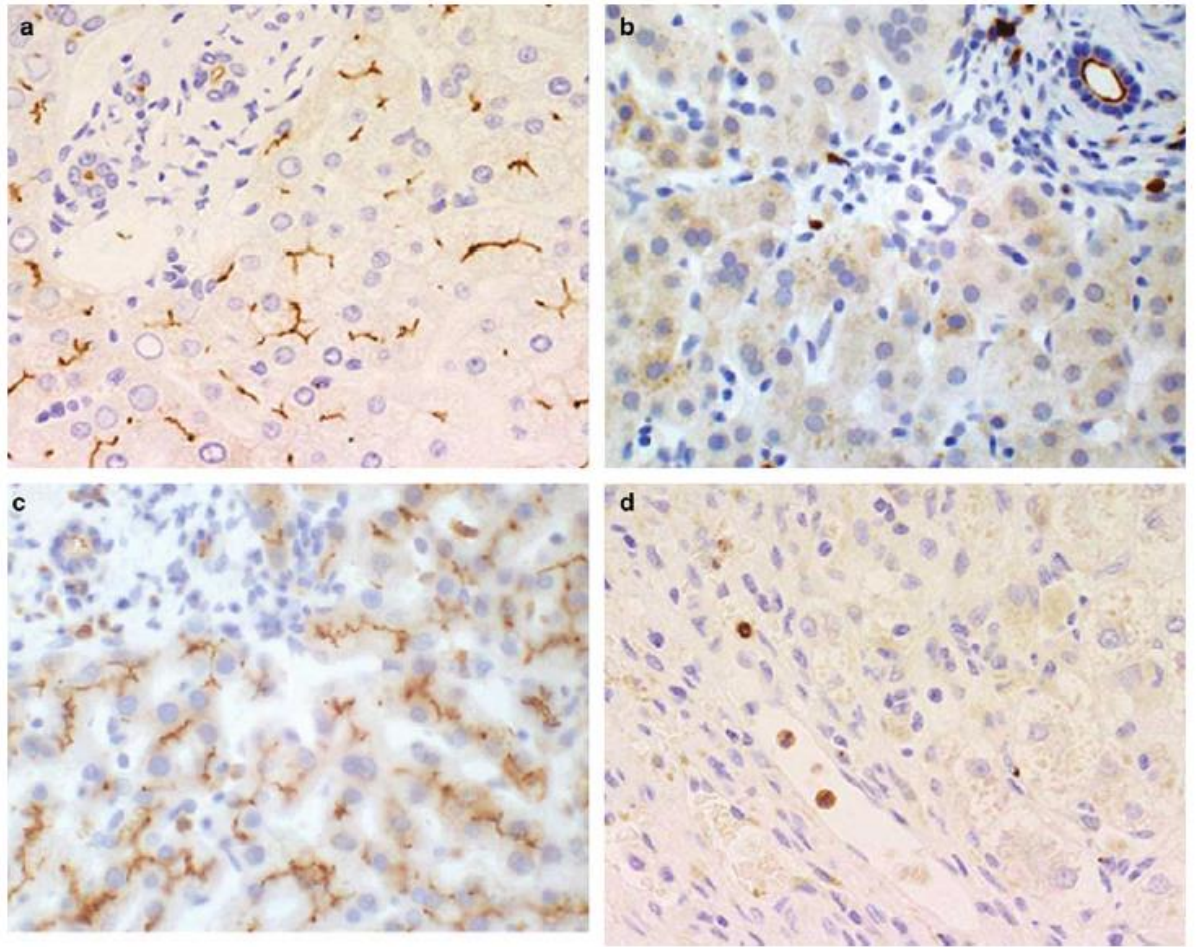
- Not just collagen
- Other histological signs of regression:
 - Reduced ductular reaction
 - Restored metabolic zonation (glutamine synthetase/CYP2E1)



D'Ambrosio et al. A morphometric and immunohistochemical study to assess the benefit of a sustained virological response in hepatitis C virus patients with cirrhosis. *Hepatology* 2012;56:532-43

Canalicular markers

- *CEA (polyclonal)*
- *CD10*
- *CD13*
- *GGT*
- *BSEP*
- *MRP2*
- *MDR3*
- *Others*

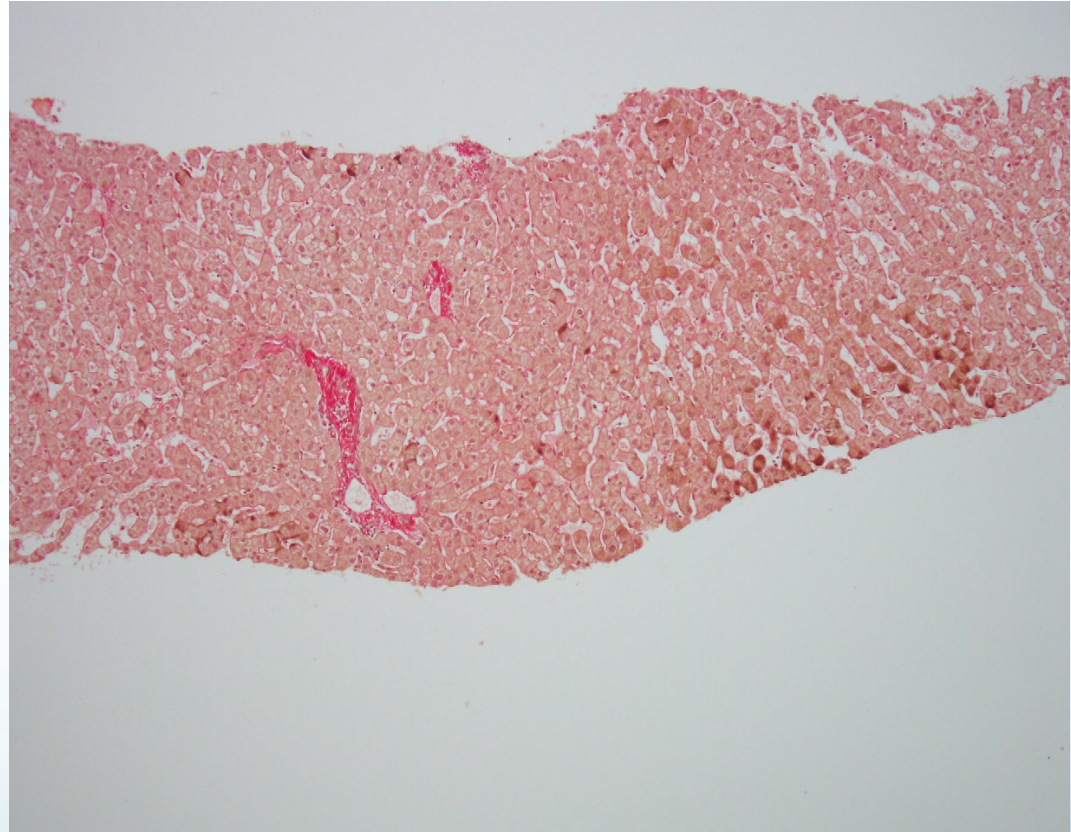


Lack of hepatocellular CD10 along bile canaliculi is physiologic in early childhood and persistent in Alagille syndrome.

Byrne JA, Meara NJ, Rayner AC, Thompson RJ, Knisely AS.
Lab Invest. 2007 Nov;87(11):1138-48

Picrosirius Red

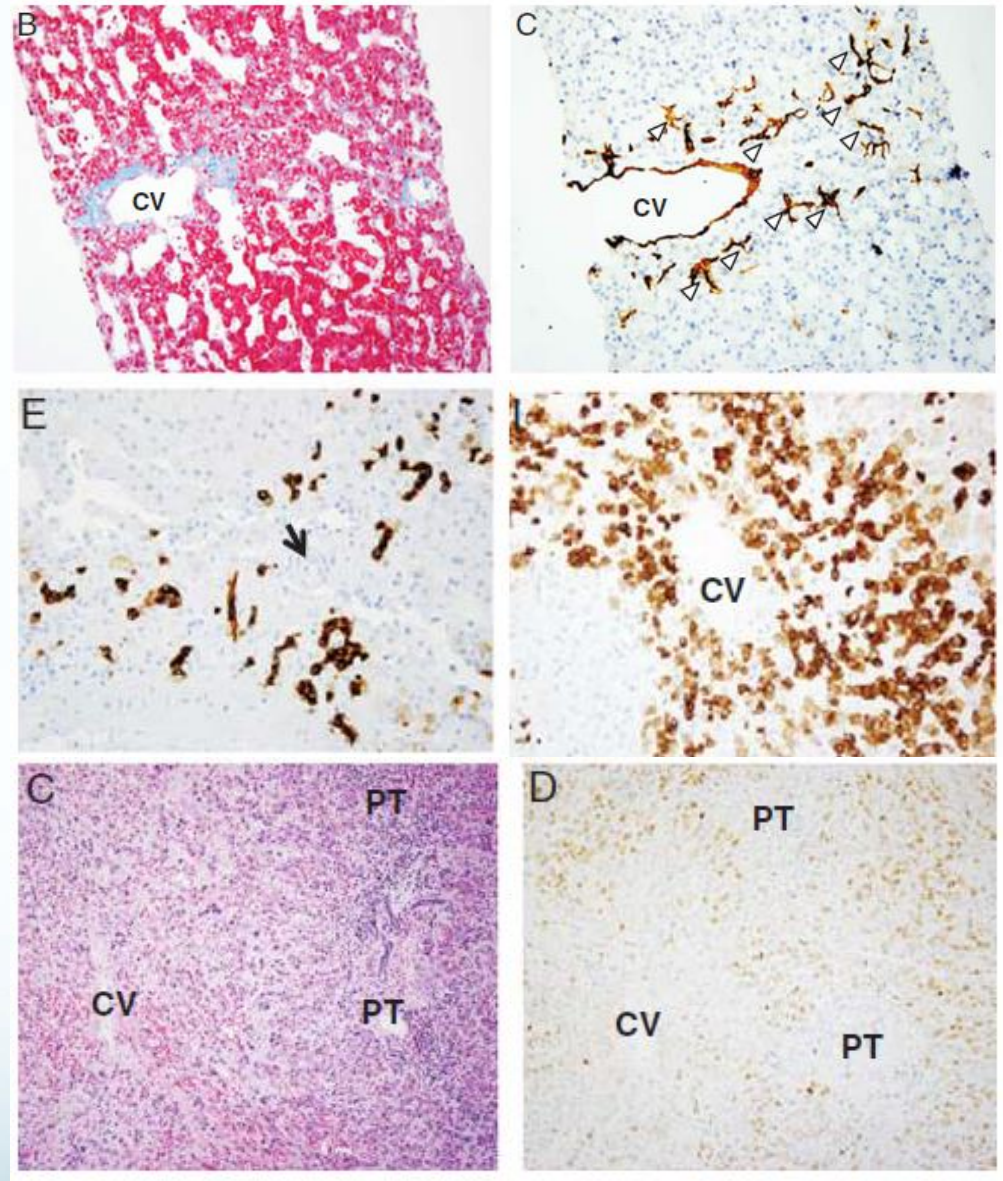
- Binds specifically to collagen
- Fibrosis assessed by image analysis
 - Good correlation with clinical parameters



- 61 cases from 58 patients with chronic VOD
- 48 needle biopsies, 8 autopsies, 2 explants, 2 wedge biopsies, 1 partial hepatectomy

Centrilobular changes:

- Arterioles in 82%
 - Centrizonal CD34 microvessels in 92.6% (90% of cases without fibrosis)
 - Centrizonal sinusoidal capillarization on 96.3% (80% of cases without fibrosis)
 - Ductules in 72.1%
 - Centrizonal CK7+ve hepatocytes in 92.5% of cases
 - Loss of metabolic zonation (glutamine synthetase 91.7%)
-
- Fibrosis stage:
 - 1= 36.1%
 - 2=24.6%
 - 3=32.8%
 - 4=6.6%



Aberrant centrizonal features in chronic hepatic venous outflow obstruction: centrilobular mimicry of portal-based disease. Krings G1, Can B, Ferrell L. *Am J Surg Pathol*. 2014 Feb;38(2):205-14

Biopsies to assess focal lesions

- Best to evaluate firstly on H&E sections
- About 6 spare sections on PLL coated slides held for immunohistochemistry.
More if specimen particularly small and thin
- Due to scarcity of tissue more judicious selection recommended based on histological appearances + clinical guidance

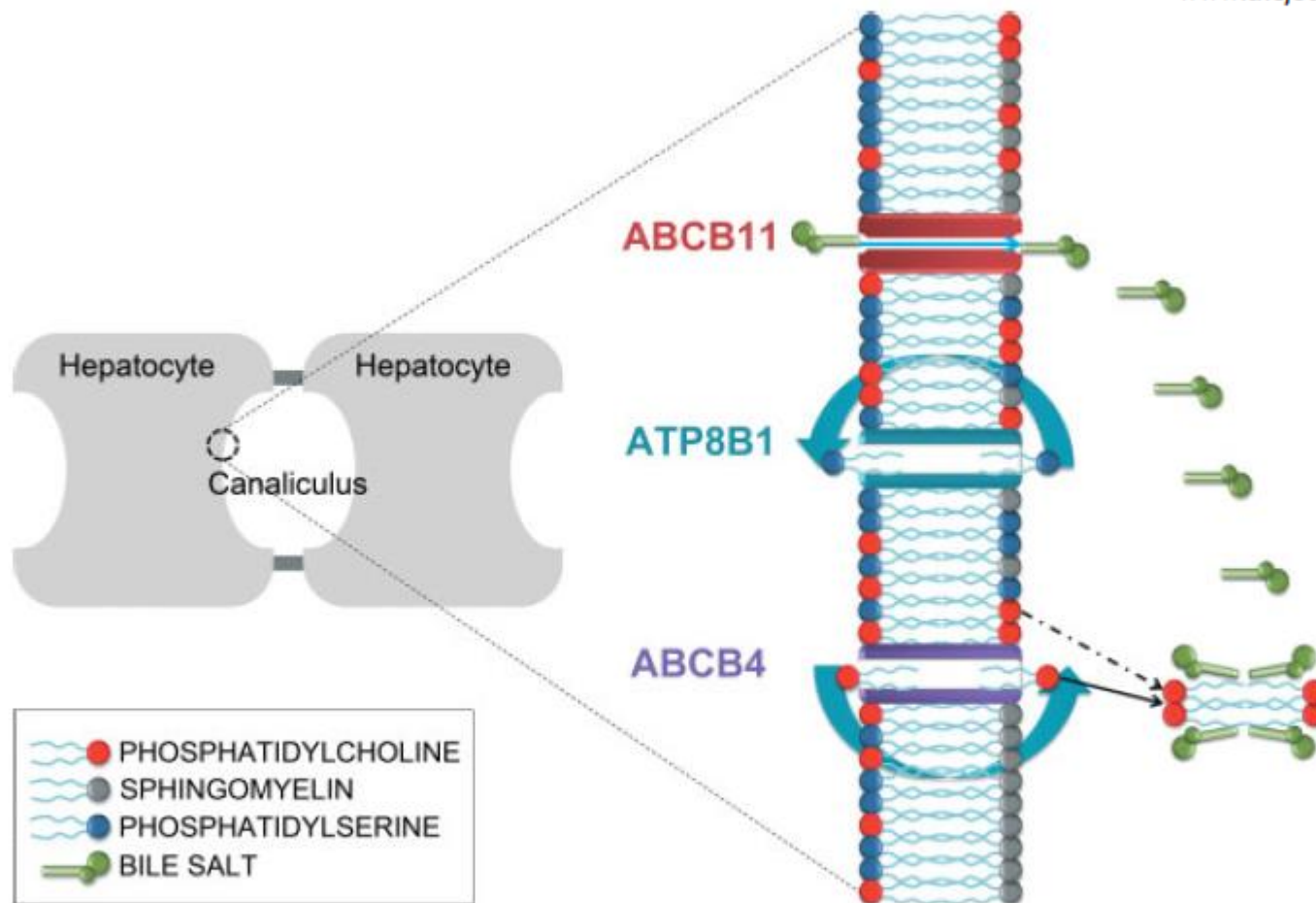


Figure 3. Maintenance and role of lipid asymmetry at the canalicular membrane. Lipid asymmetry at the plasma membrane is known to be important for barrier function. Bile salts are exported into the bile canalculus by ABCB11. The PC floppase ABCB4 flops PC into the outer leaflet of the canalicular membrane, where it is extracted into the bile by bile salts. Flopping of excess PC destabilizes the canalicular membrane, causing PS to flop spontaneously into the outer leaflet (alternatively, ABCB4 may flop PS directly). The dilution of sphingomyelin in the outer leaflet induces a liquid disordered phase and renders the canalicular membrane more sensitive to detergent solubilization. The PS flippase ATP8B1 is therefore required to flip PS back into the inner leaflet of the canalicular membrane to maintain its liquid crystalline order.

Keratin 7 in hepatocytes

The presence of cytokeratin no. 7 and/or no. 19 in these hepatocytes implies that, in a variety of cholestatic diseases, hepatocytes can express cytokeratin polypeptides which in the normal liver are restricted to bile duct cells and this lends further support to the concept of 'ductular metaplasia of hepatocytes'. This phenomenon is thought to contribute to the increased number of ductules observed in various liver diseases (Buysens 1962, Jorgensen 1973, Uchida & Peters 1983, Vanstapel *et al.* 1984, Desmet 1985, 1987b, Nakanuma *et al.* 1986, Sciot *et al.* 1986, Van Eyken *et al.* 1987a, Yamada, Howe & Scheuer 1987).

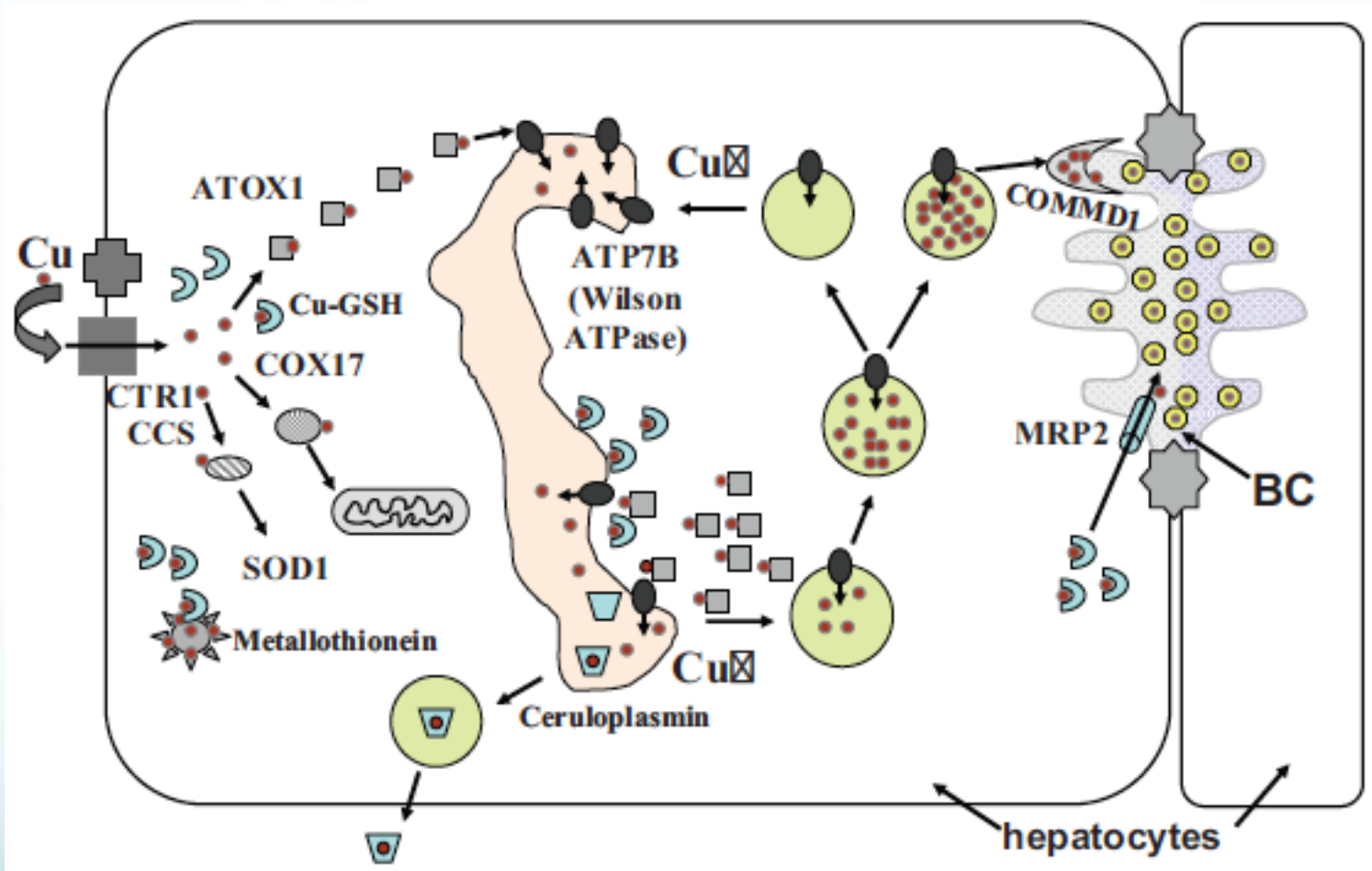
Hepatocytes in acinar zone 1 or in the periphery of cirrhotic nodules which are immunoreactive for cytokeratin no. 7 (and/or no. 19) might indeed represent cells of an intermediate phenotype which are transforming into bile duct-type cells. This is supported by continuity between proliferating ductules and hepatocytes reactive

A cytokeratin immunohistochemical study of cholestatic liver disease: evidence that hepatocytes can express 'bile duct-type' cytokeratins.

Van Eyken P1, Sciot R, Desmet VJ.

Histopathology. 1989 Aug;15(2):125-35.

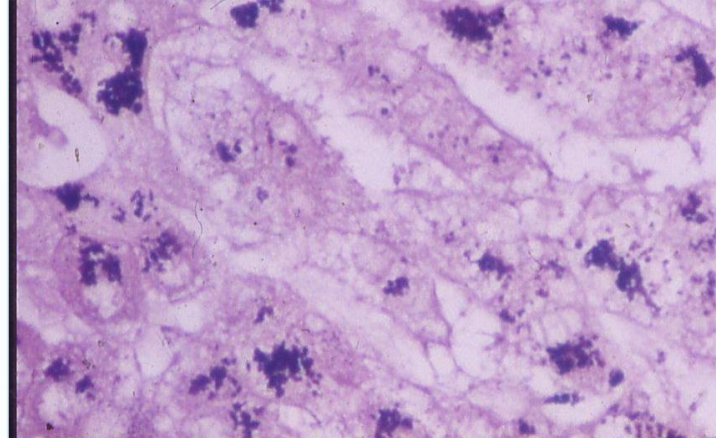
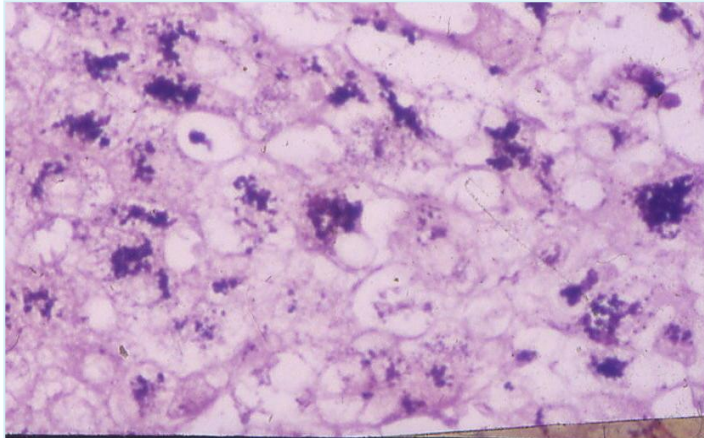
Copper in hepatocytes



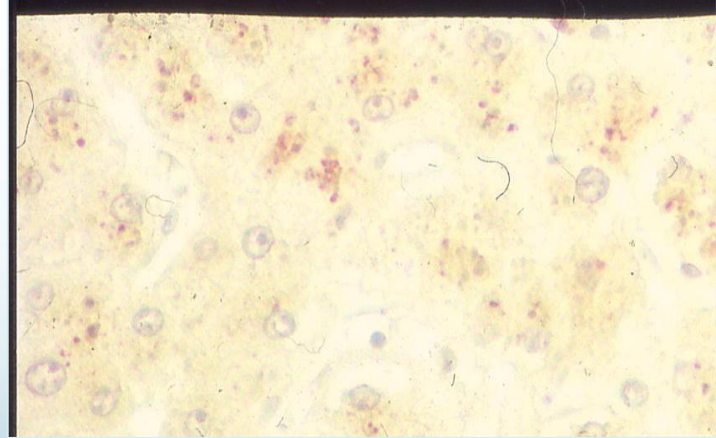
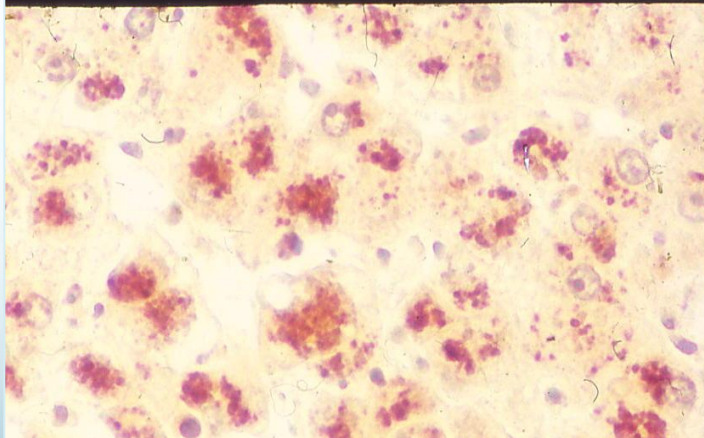
Roberts and Sarkar
Am J Clin Nutr 2008; 88 (suppl): 851S-4S

Copper / Copper-associated protein

Orcein



Rhodanine



Immediate processing

After 3 wks in formal saline

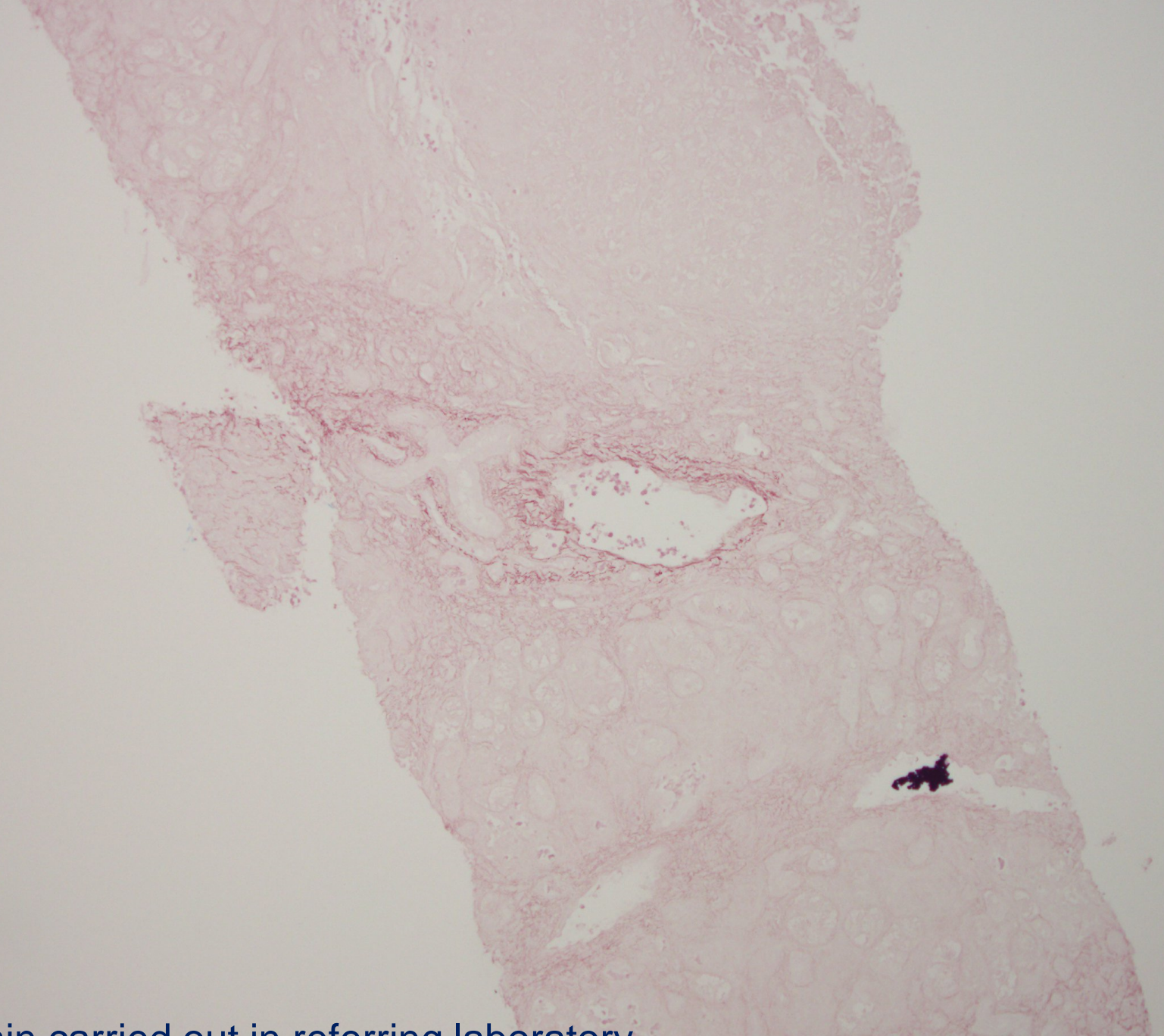
Case 1

- 18 year old male referred to King's College Hospital from another hospital
- Right upper quadrant pain
- Raised IgG levels
- ? Autoimmune hepatitis
- A full set of stained sections received including orcein, plus paraffin block

Orcein stain carried out in referring laboratory

Magnification: 10 x

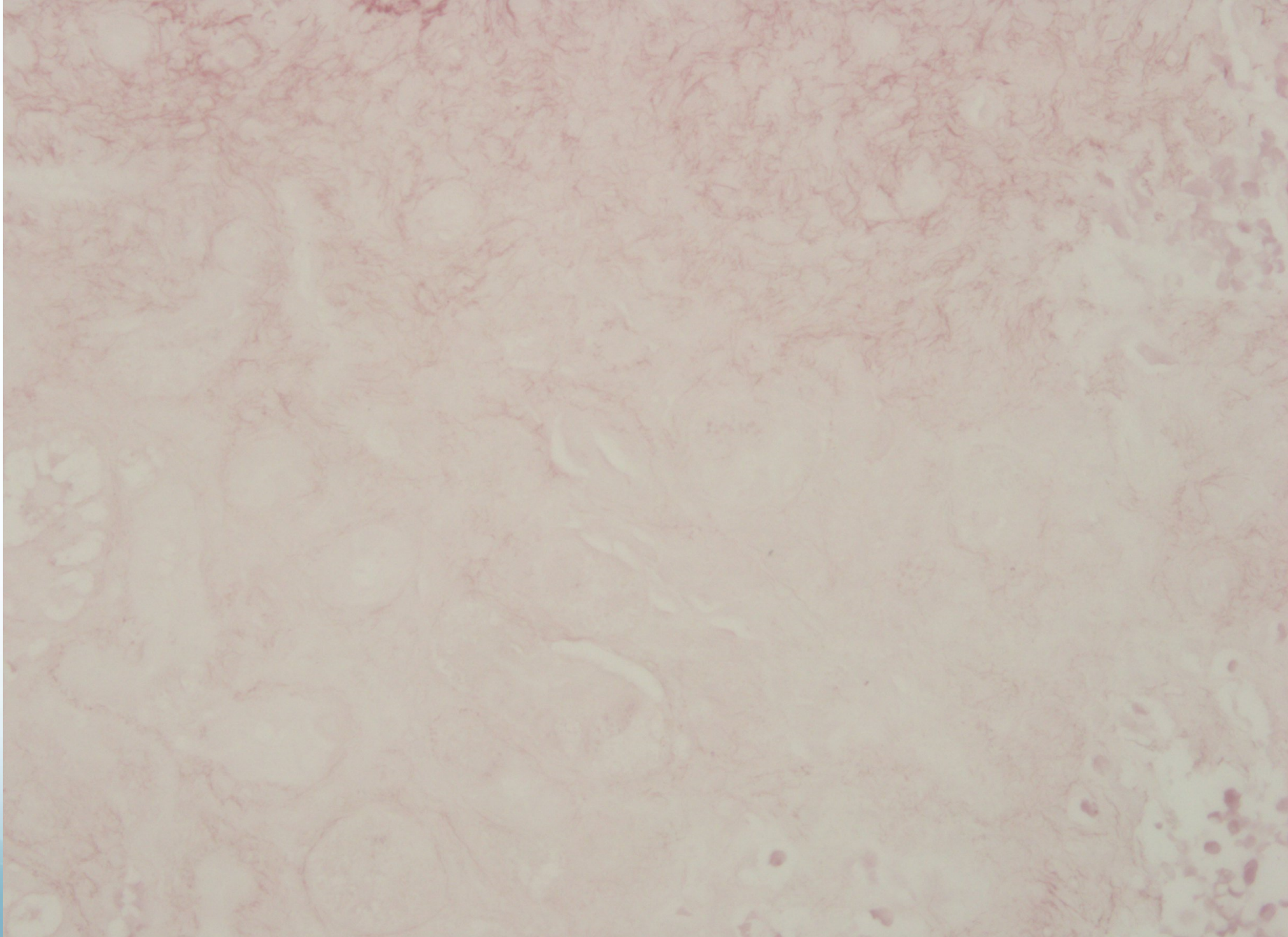
200 μ m

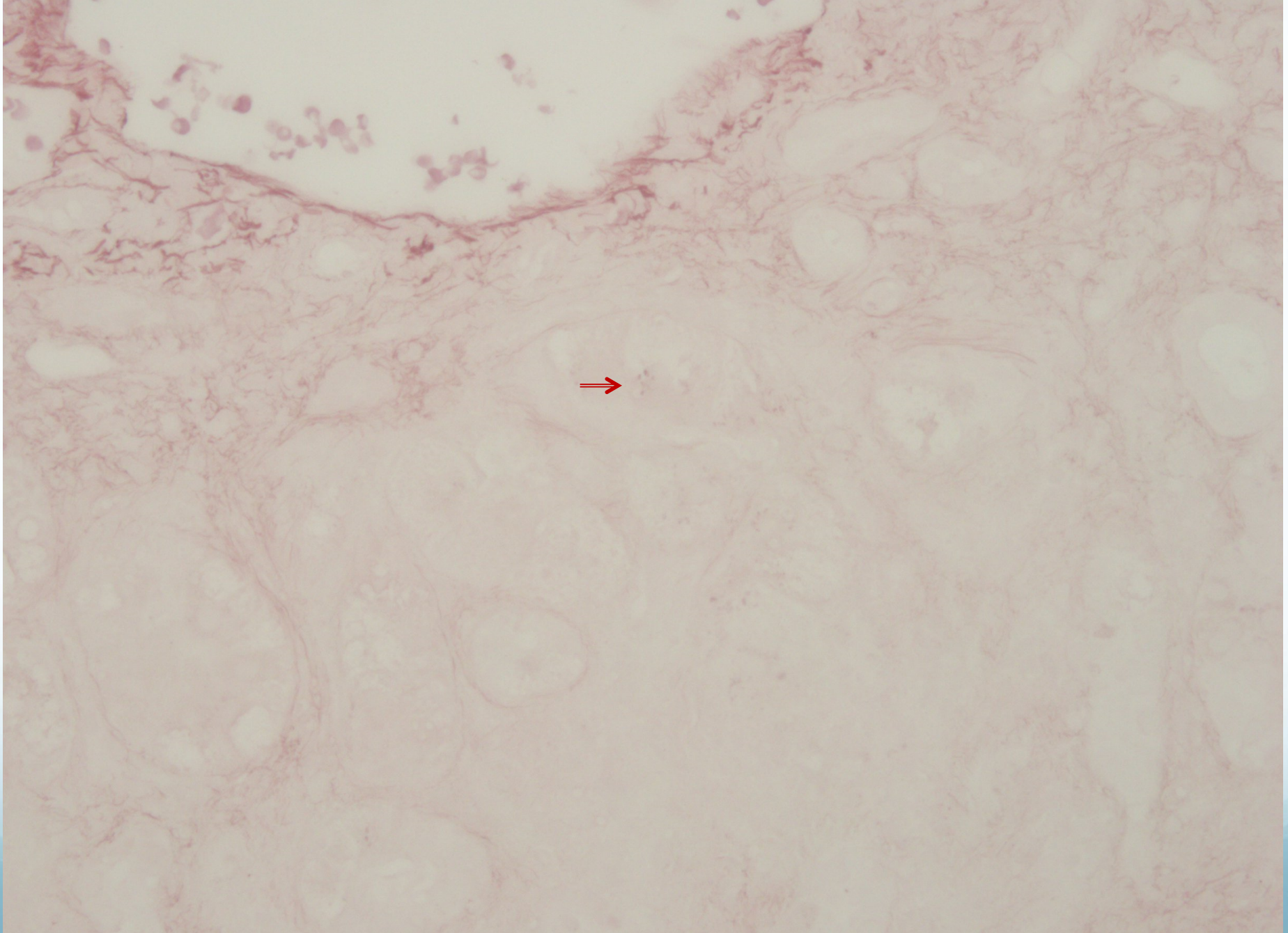


Orcein stain carried out in referring laboratory

Magnification: 40 x

50 μ m

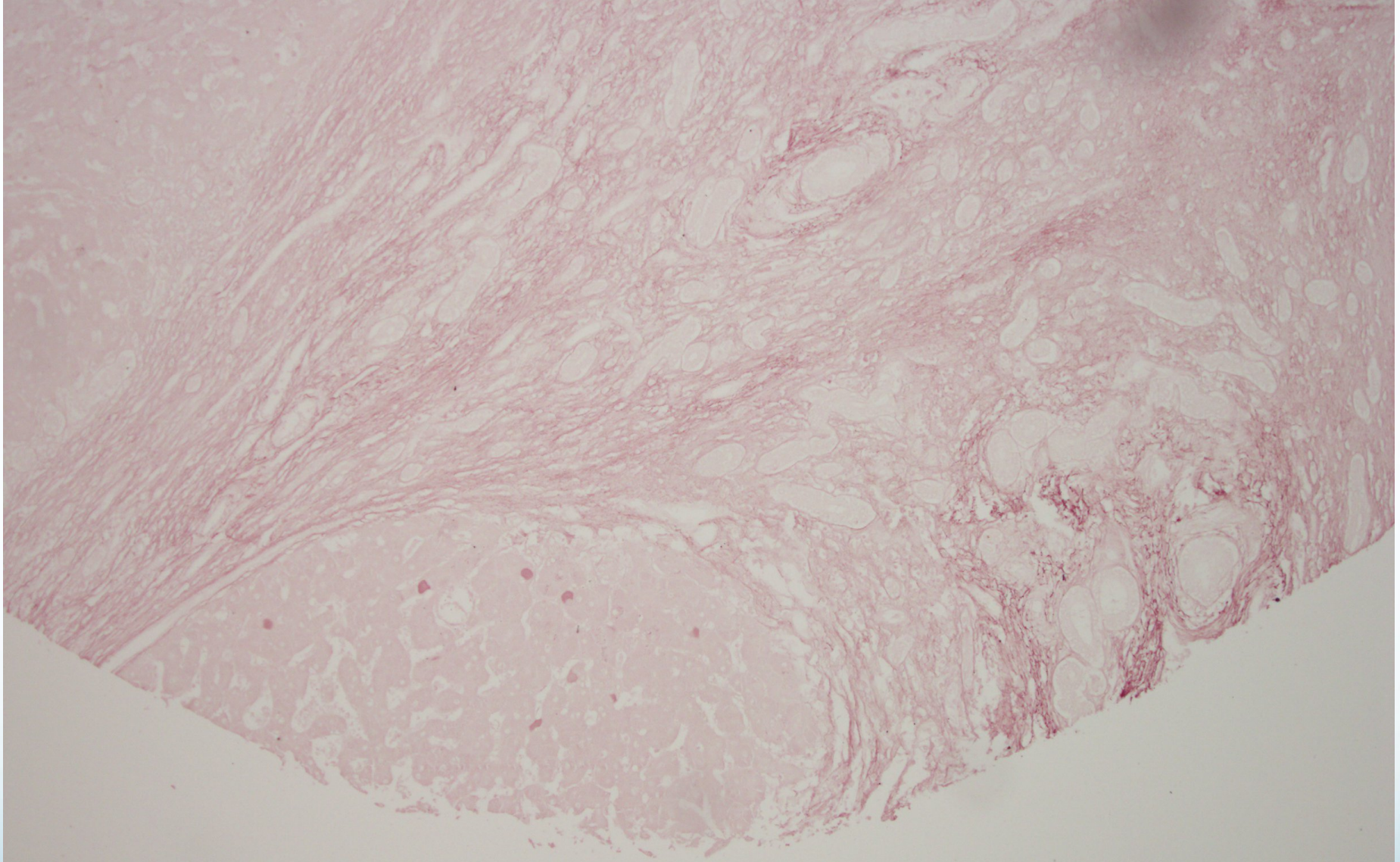




Orcein stain carried out in referring laboratory

Magnification: 40 x

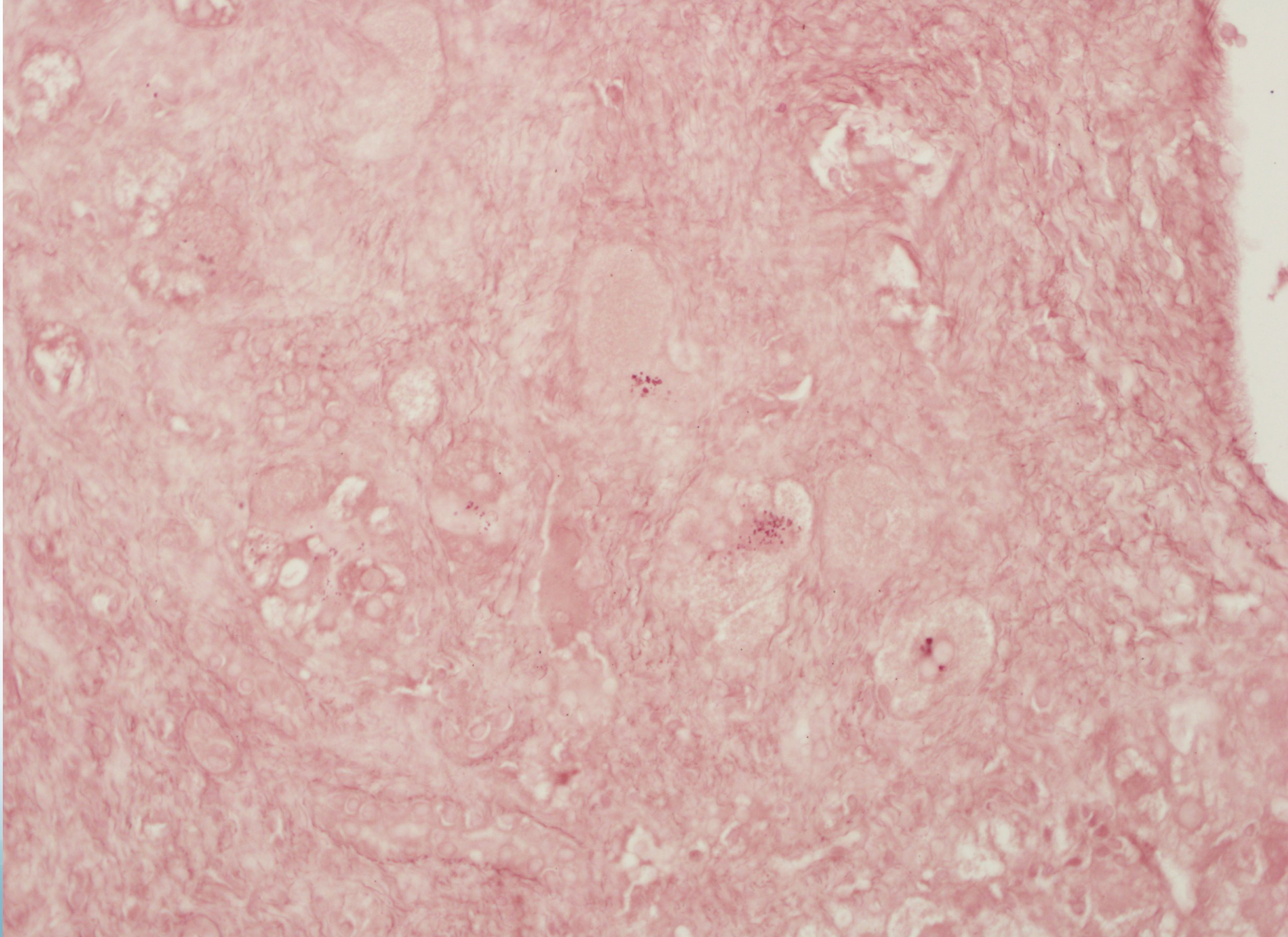
50 μ m



Orcein control referring laboratory

Magnification: 10 x

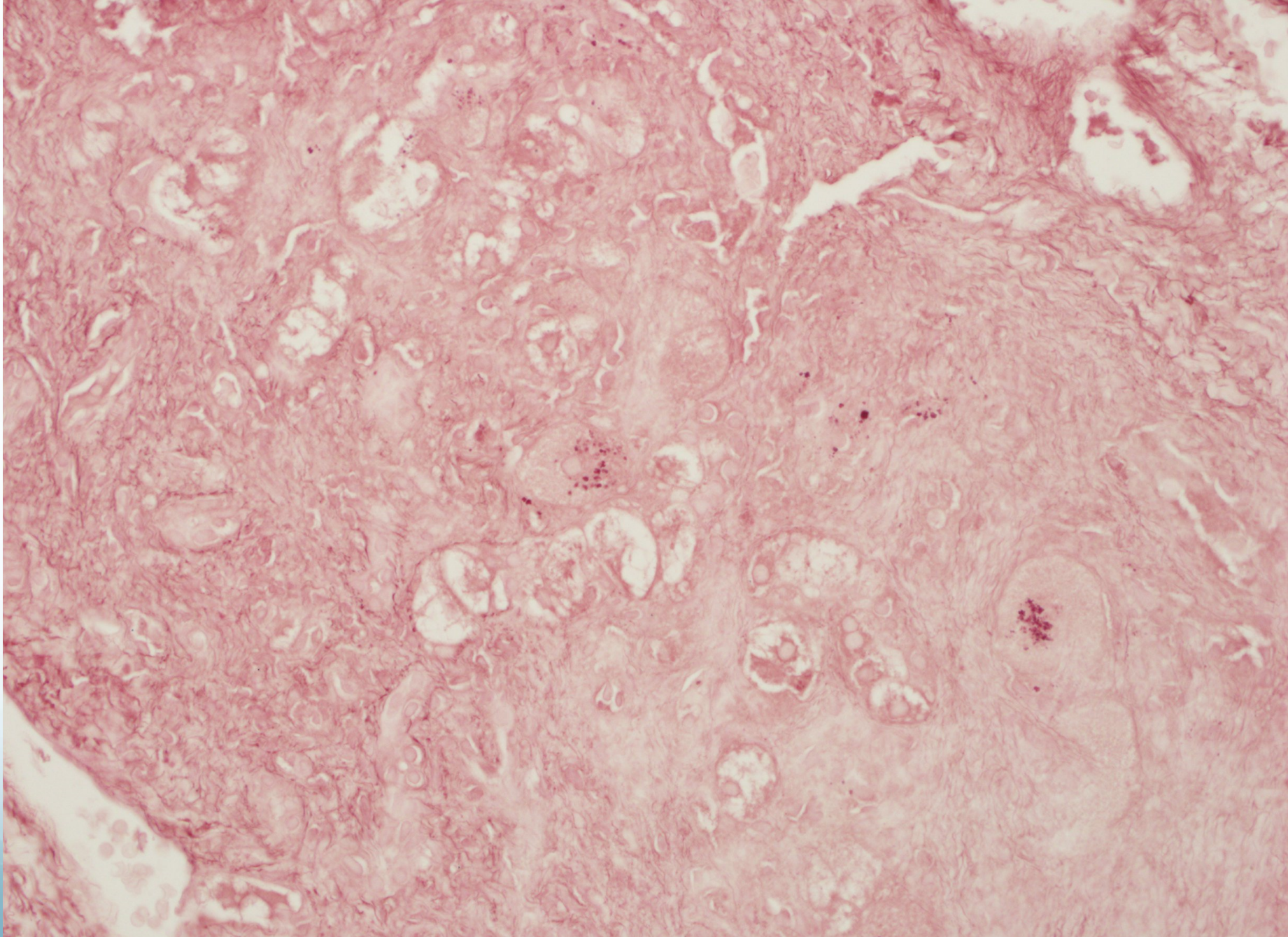
200 μ m



Orcein stain carried out at King's College Hospital

Magnification: 40 x

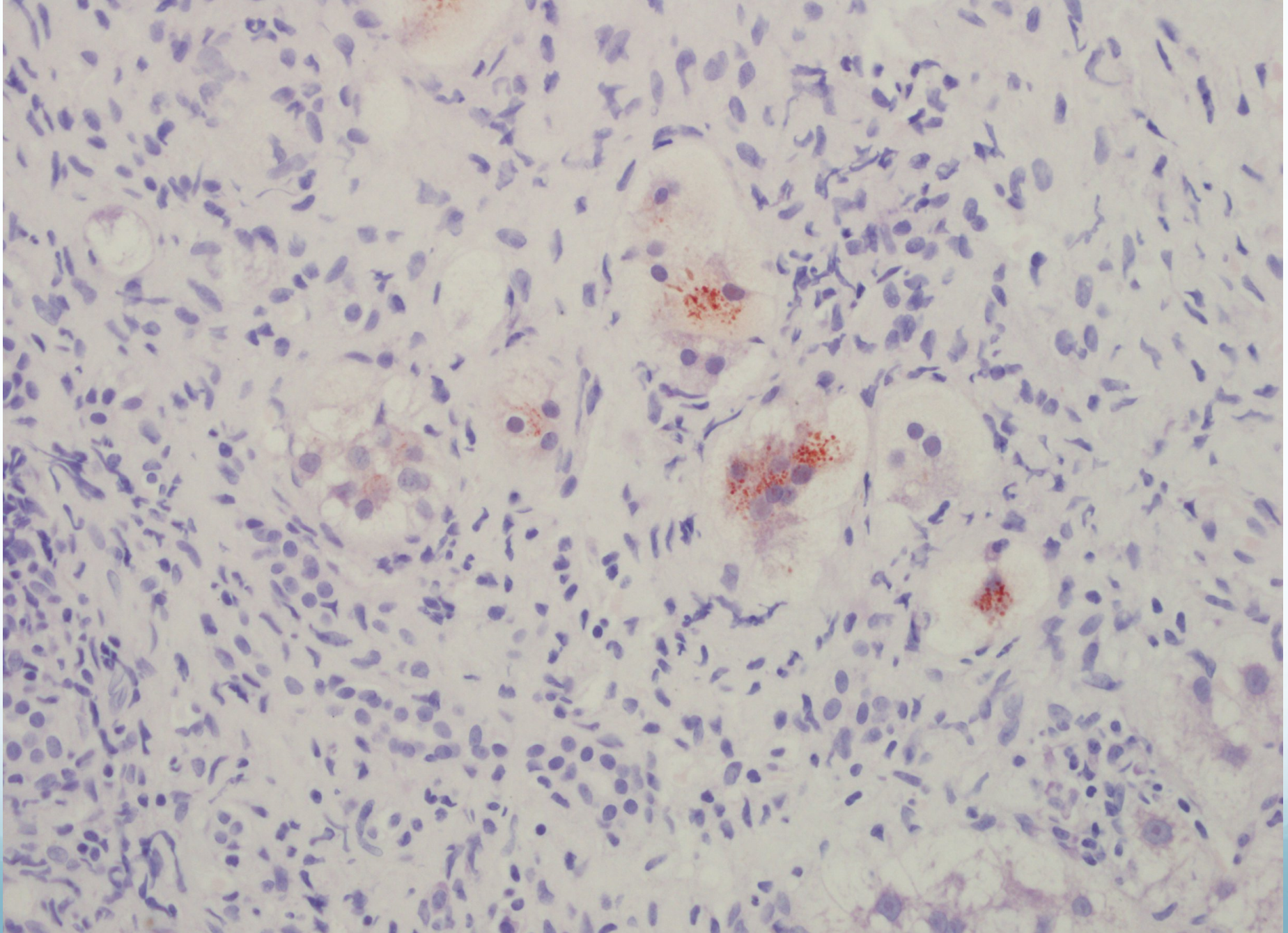
50 μ m



Orcein stain carried out at King's College Hospital

Magnification: 40 x

50 μ m



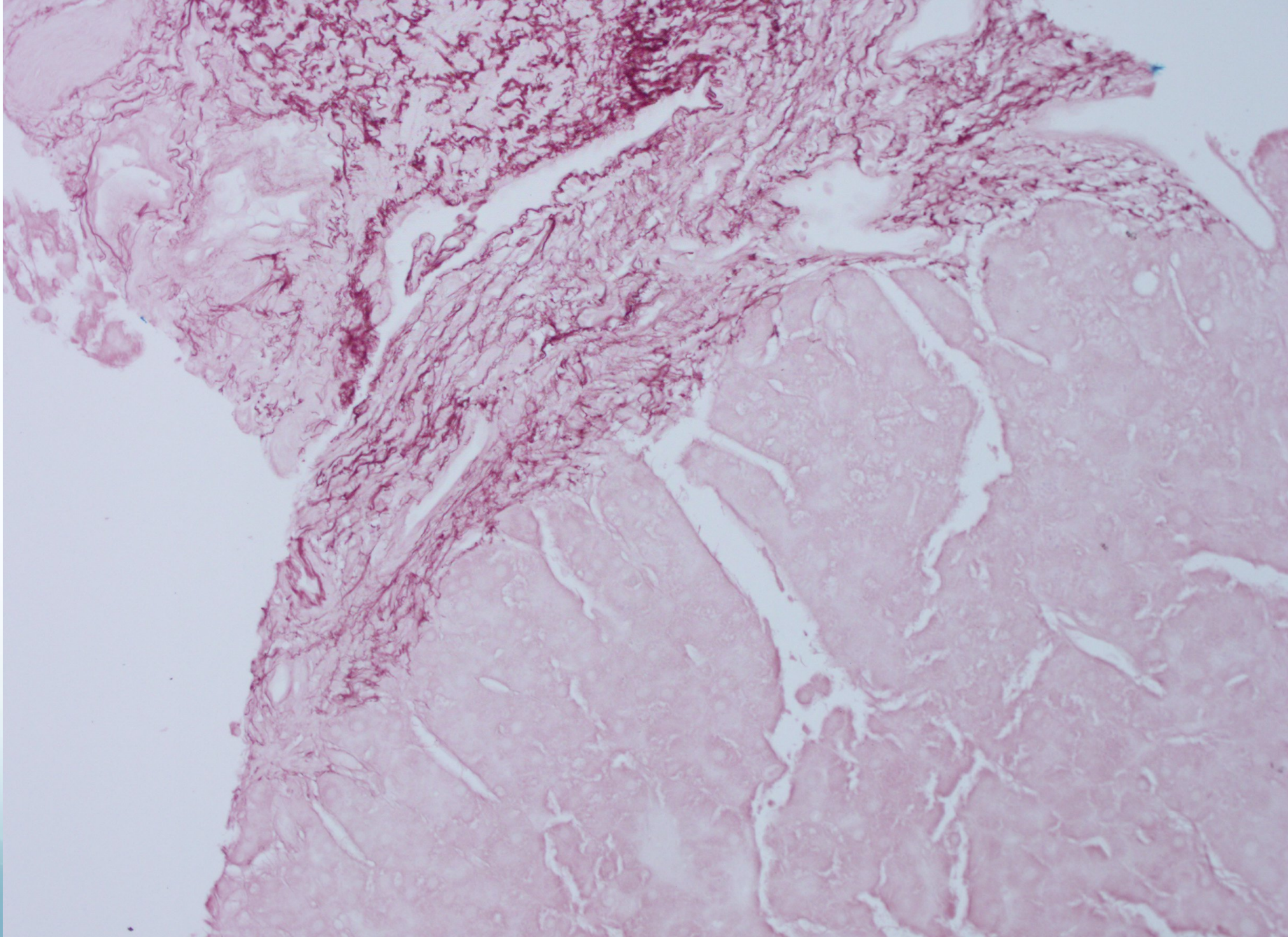
Rhodanine stain carried out at King's College Hospital

Magnification: 40 x

50 μ m

Case 2

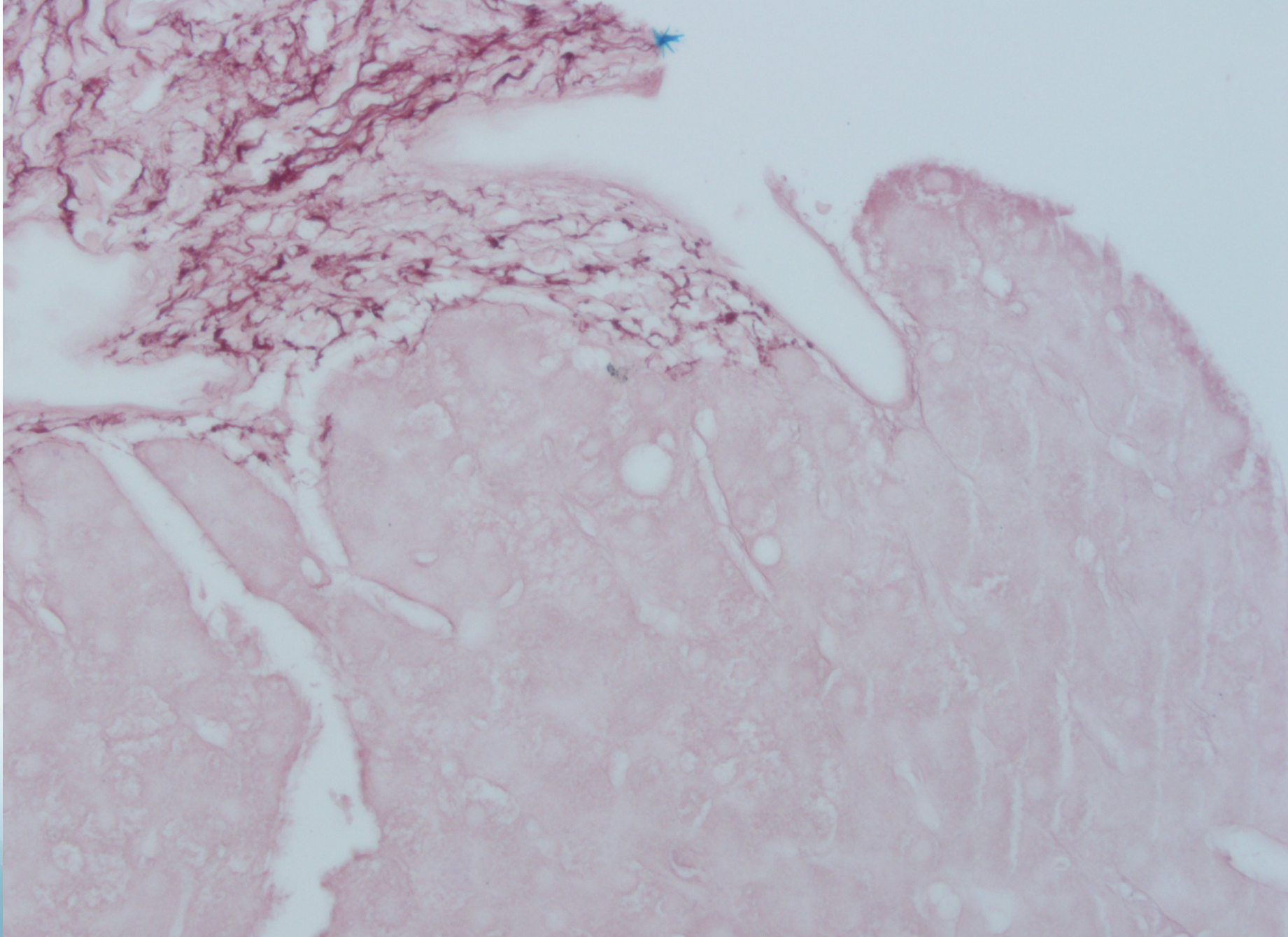
- 47 year old female, referred to King's College Hospital from another hospital
- - Crohn disease since 1995
- - Azathioprine, mercaptopurine and thioguanine
- - Increase in spleen size on MRI, and grade 1 varices on endoscopy indicating portal hypertensive gastropathy
- - Liver biopsy ? nodular regenerative hyperplasia ? PSC
- -A full set of stained sections received including orcein, plus paraffin block



Orcein stain carried out in referring laboratory

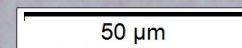
Magnification: 20 x

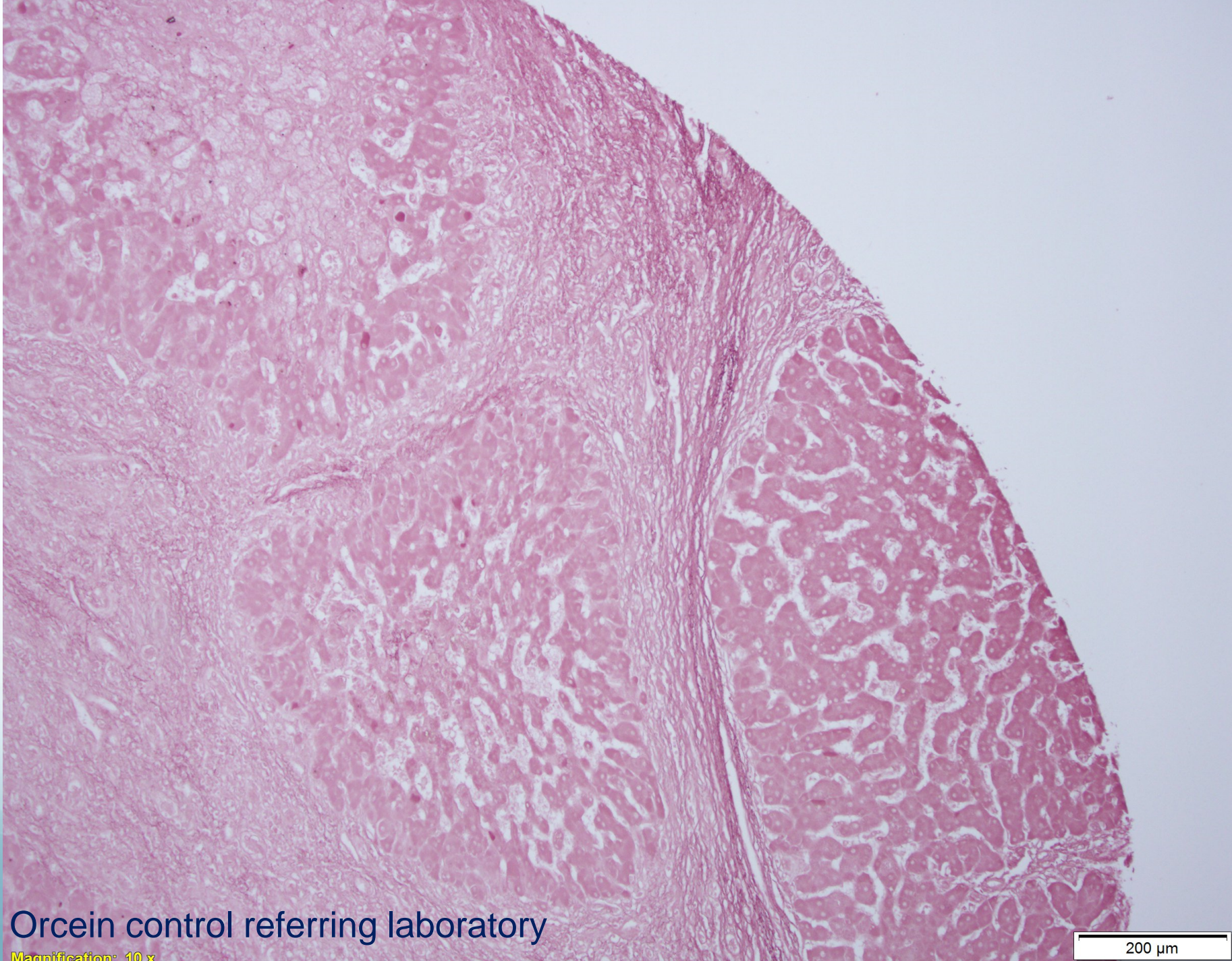
100 μ m



Orcein stain carried out in referring laboratory

Magnification: 40 x

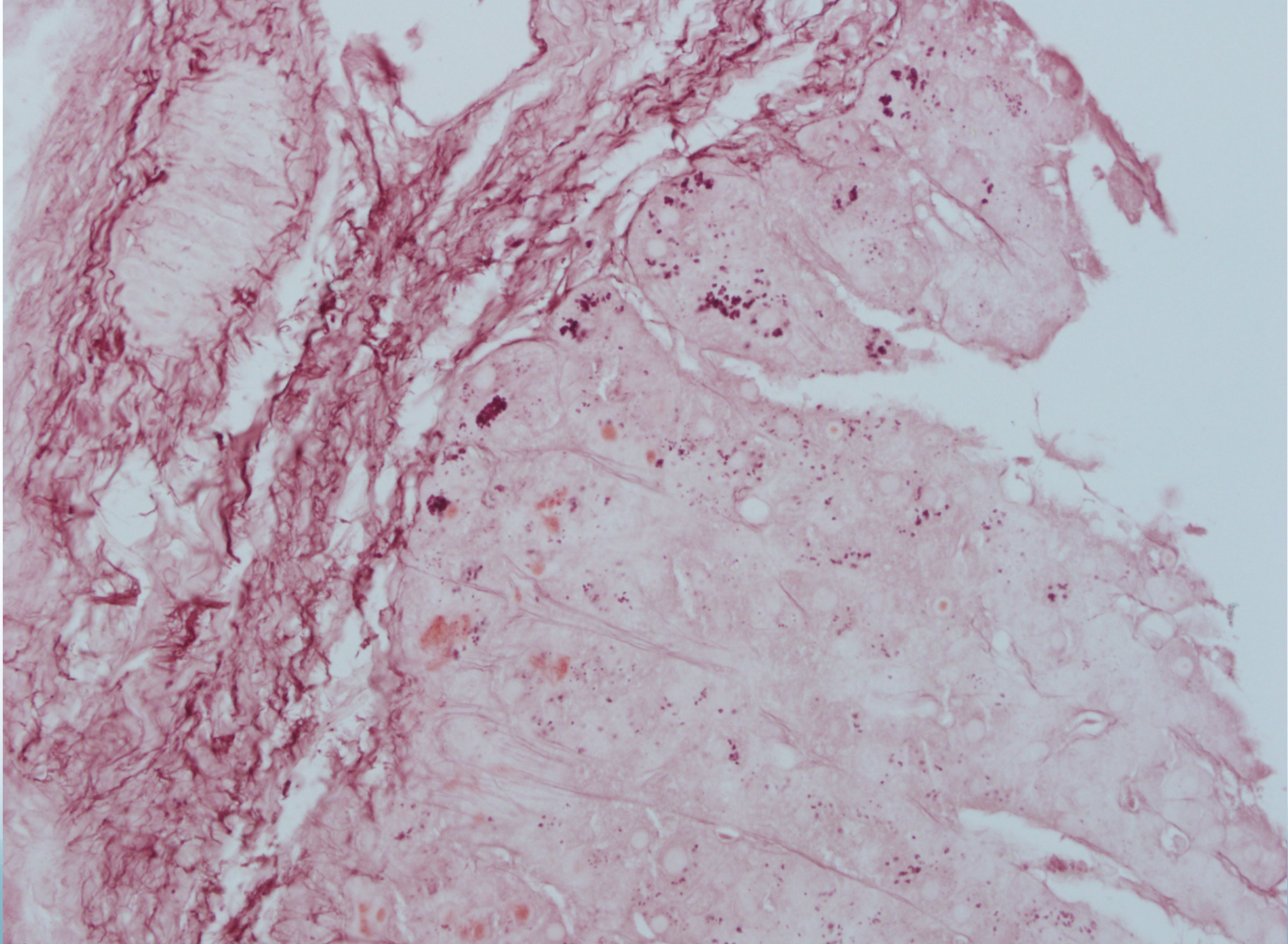




Orcein control referring laboratory

Magnification: 10 x

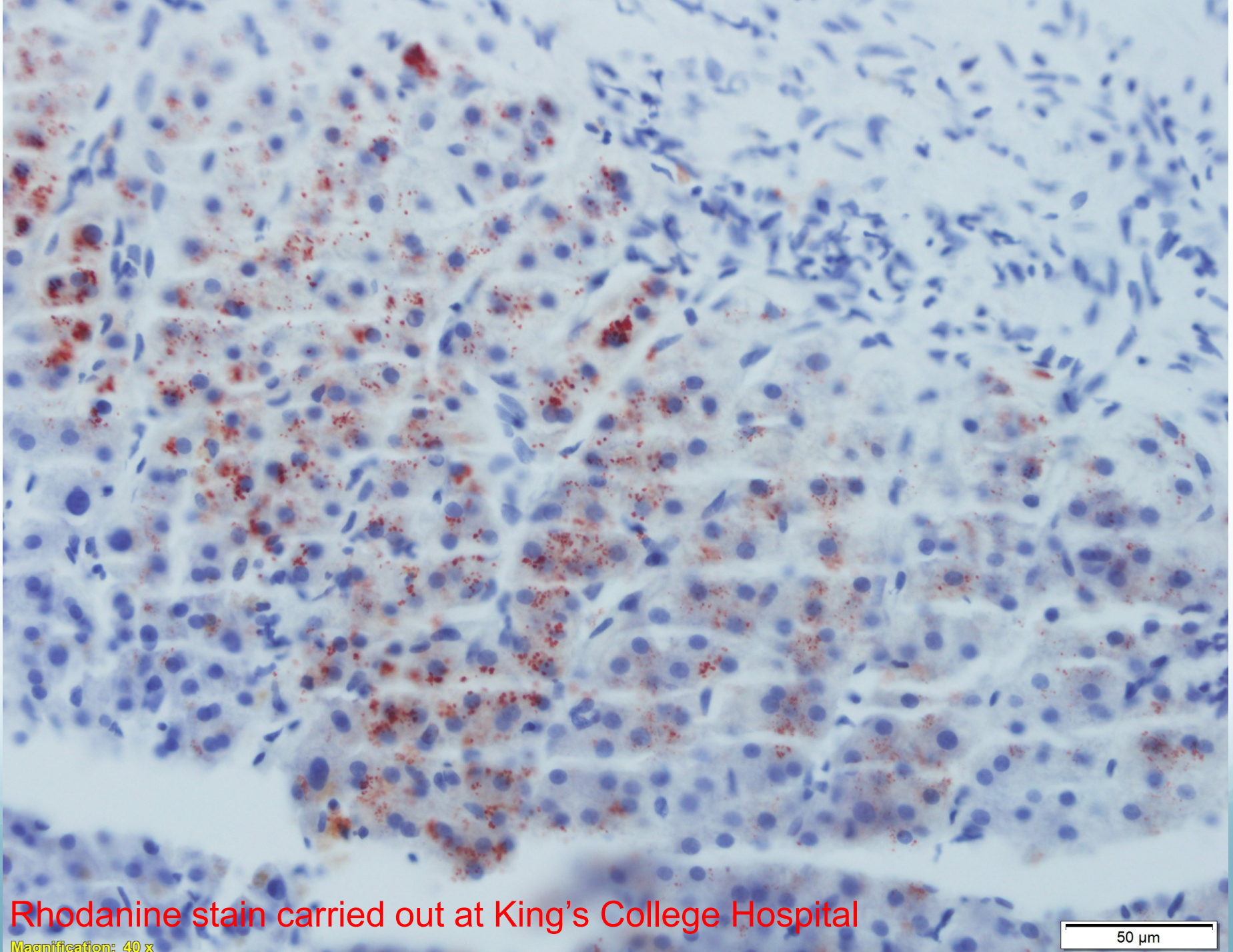
200 μ m



Orcein stain carried out at King's College Hospital

Magnification: 40 x

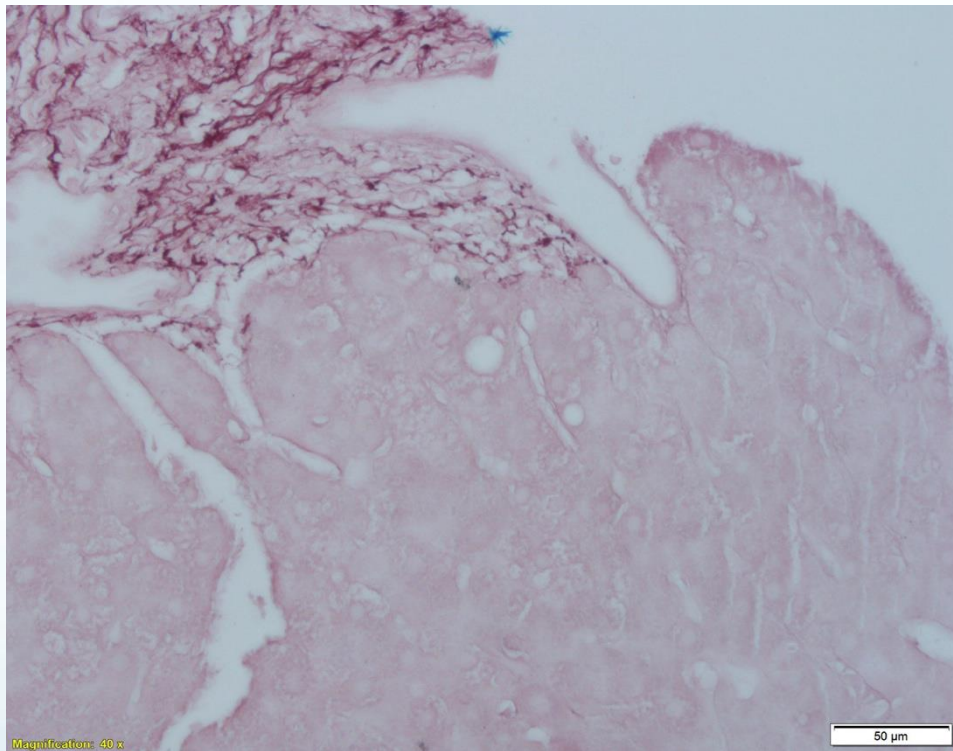
50 μ m



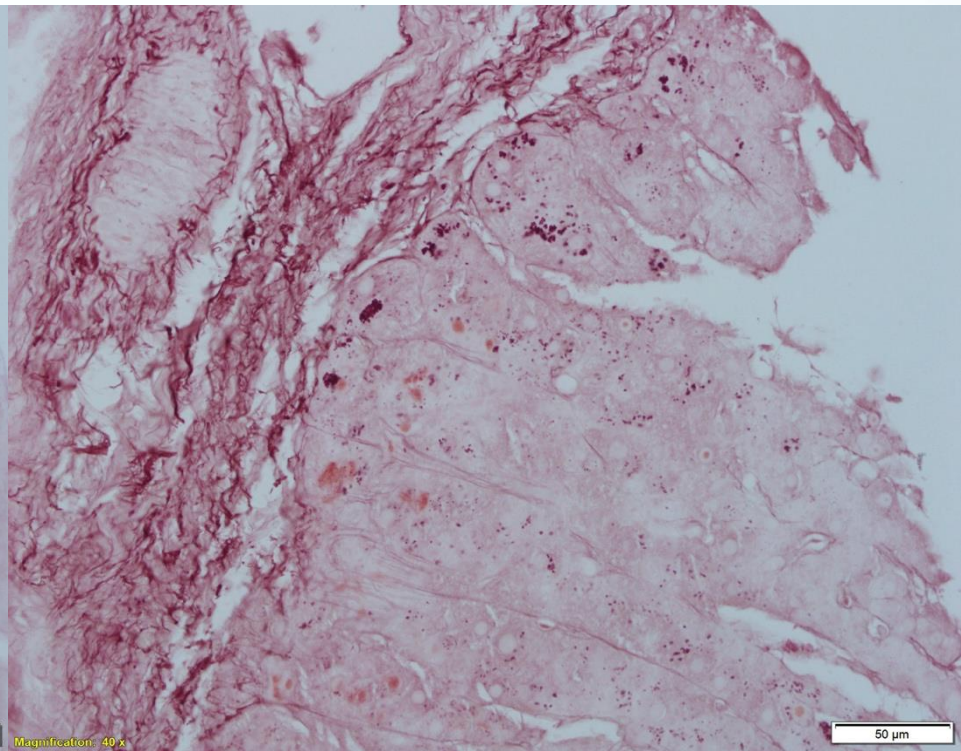
Rhodanine stain carried out at King's College Hospital

Magnification: 40 x

50 μ m

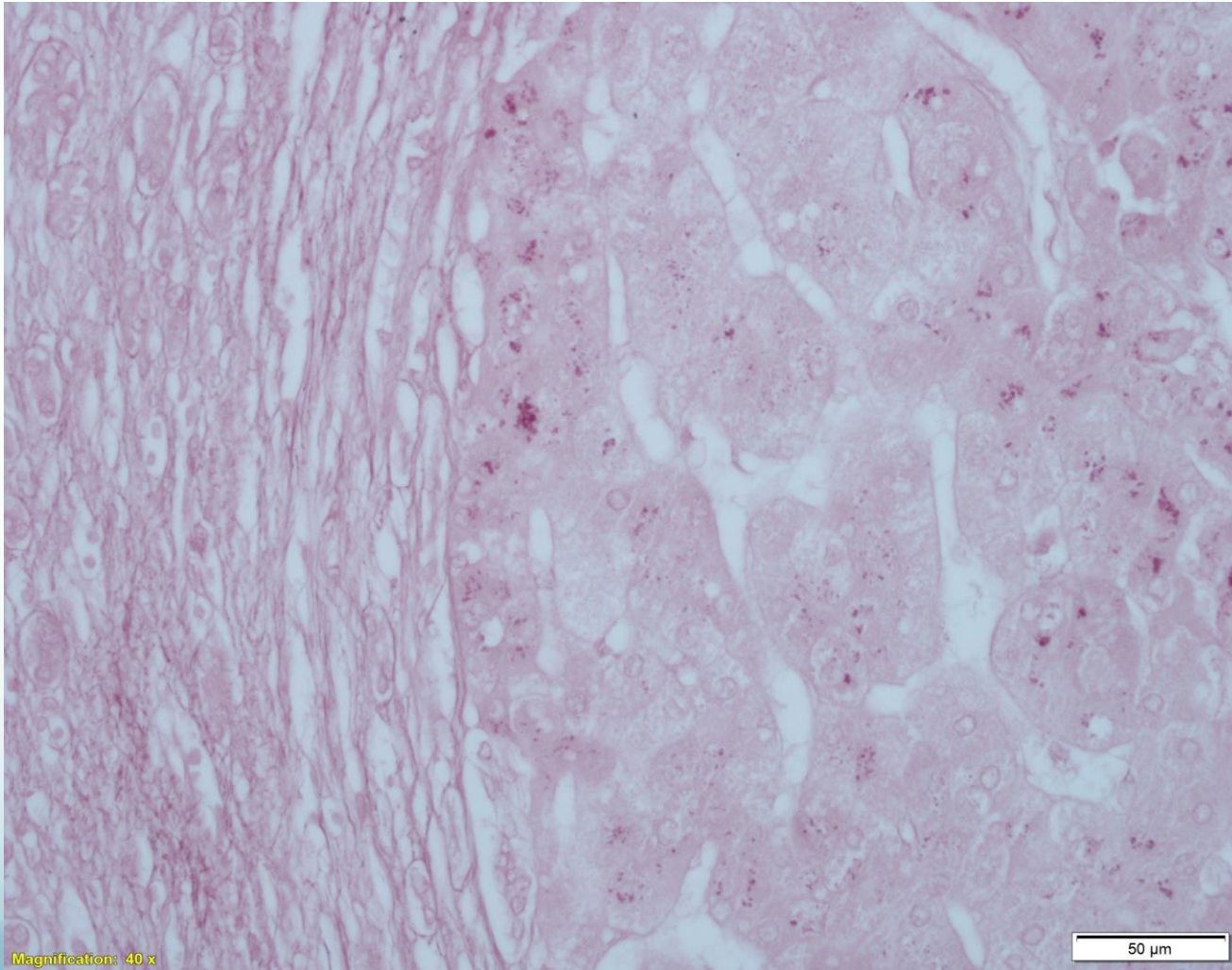


Orcein stain carried out in referring laboratory

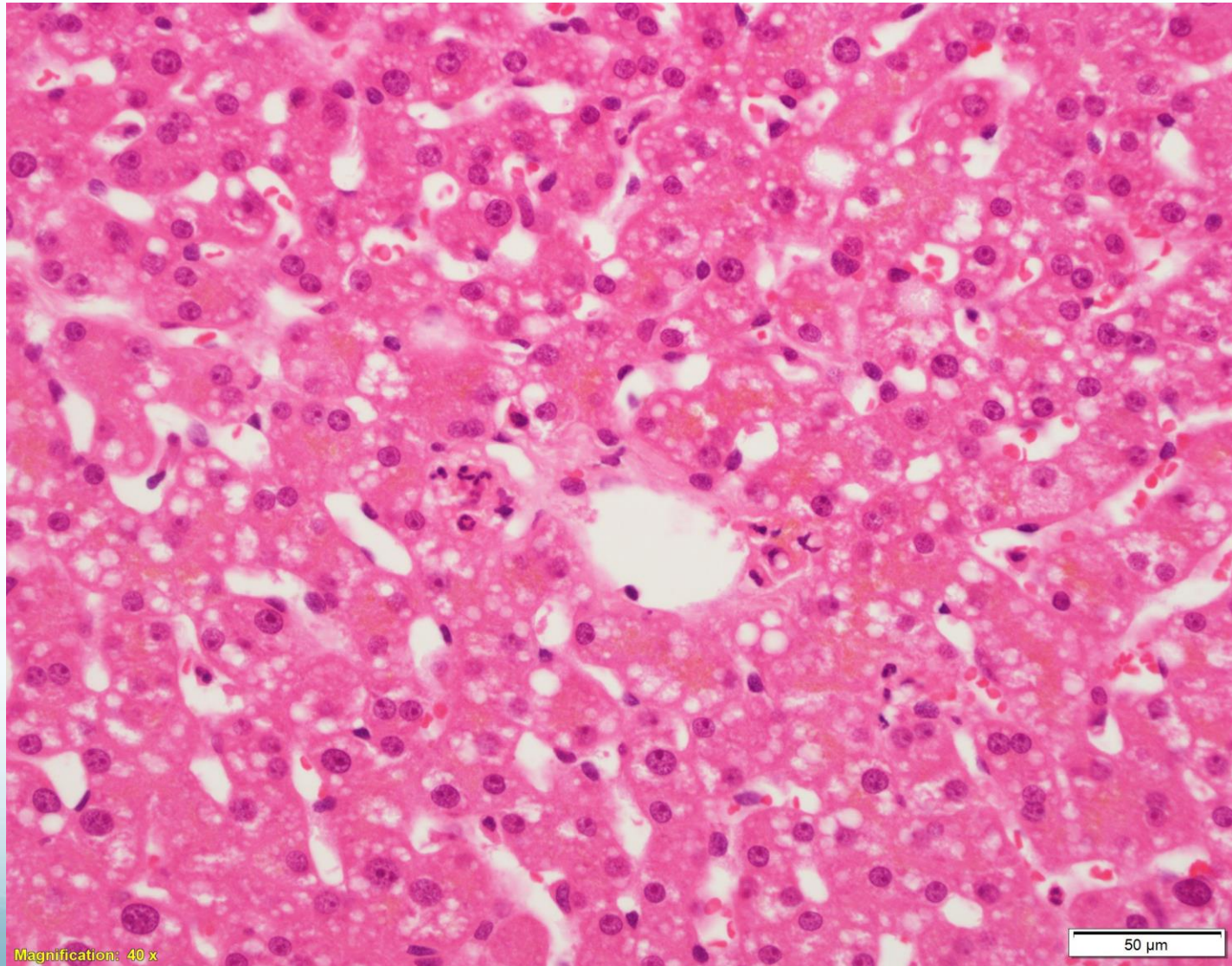


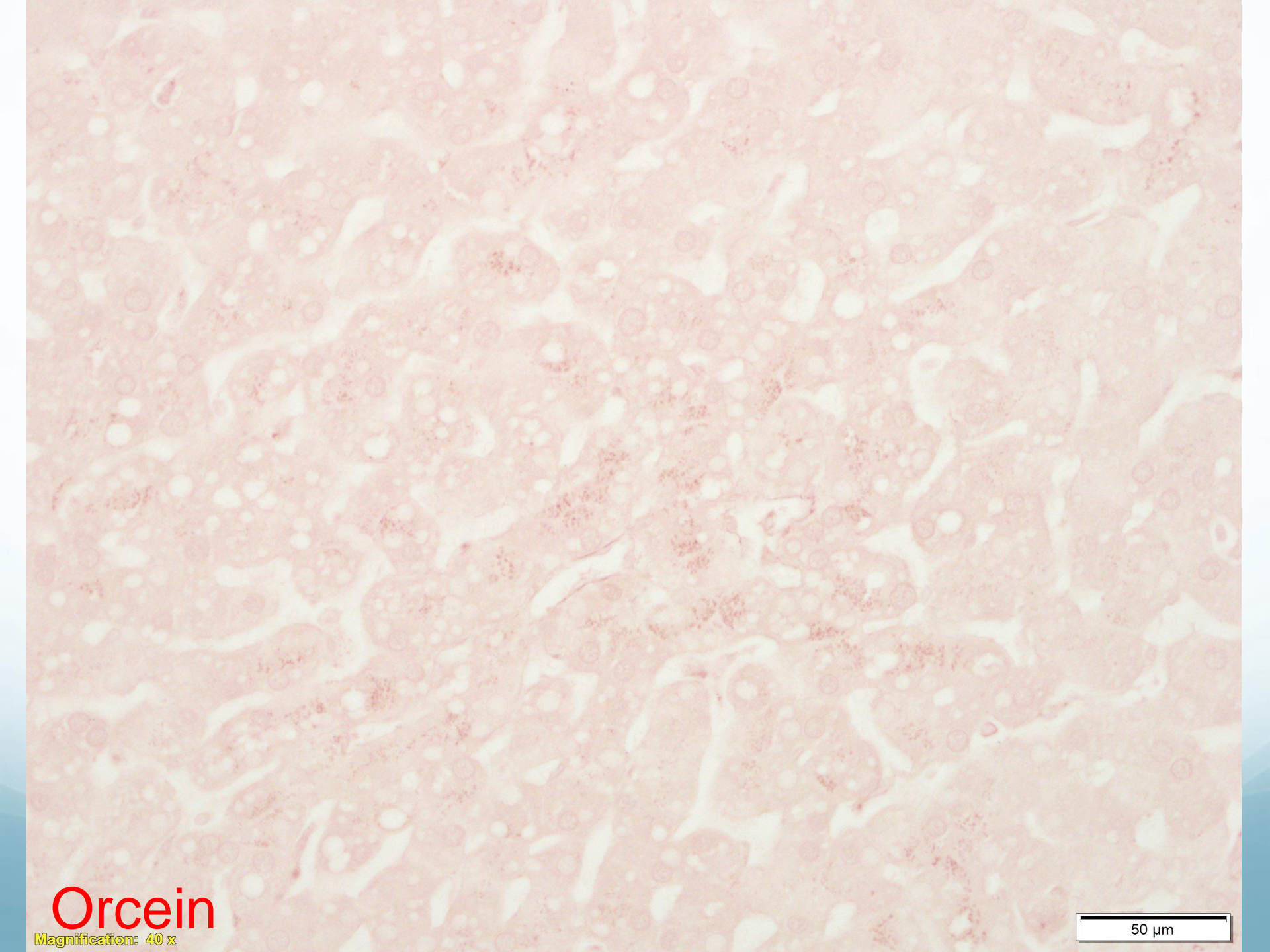
Orcein stain carried out at King's College Hospital

KCH orcein control



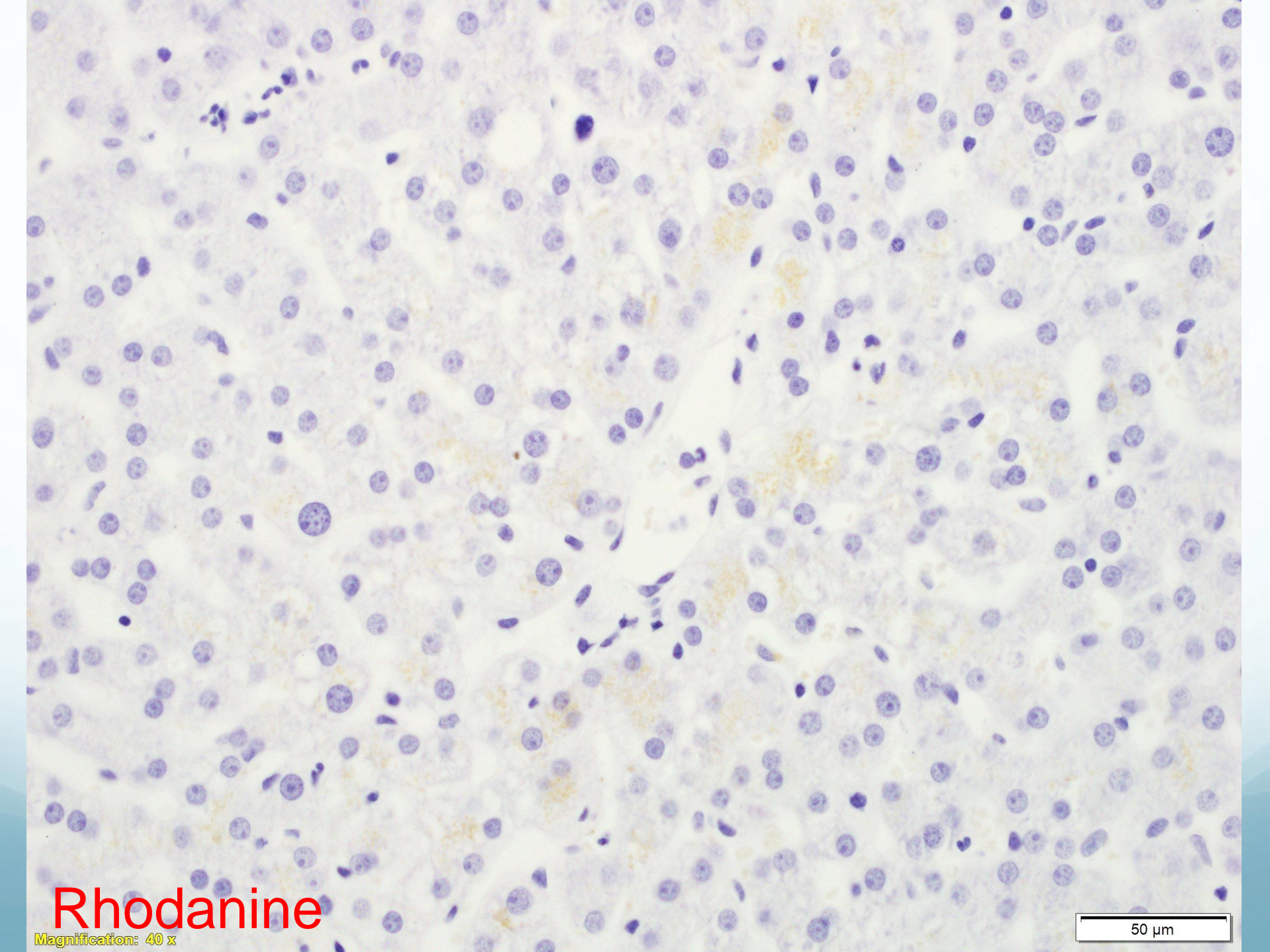
Post-reperfusion (time 0) liver allograft biopsy





Orcein
Magnification: 40 x

50 μ m



Rhodanine

Magnification: 40 x

50 μ m

ORCEIN

METHOD:

1. Dewax sections in xylene and take to distilled water
- 2. Oxidise in acidified potassium permanganate, 5 minutes
3. Wash in tap water, then rinse in distilled water
4. Bleach in 1% oxalic acid
5. Wash in several changes of distilled water
- 6. Immerse in orcein stain in jar for 1 hour at room temperature (This may need longer if the solution is new – check staining after 1 hour)
7. Rinse in 70% alcohol
8. Dehydrate rapidly through alcohols, clear in xylene and mount in DPX

RESULTS:

HBsAg	Grey-purple
Copper associated protein	Black-purple granules
Elastic fibres	black-purple

SOLUTIONS:

Acidified potassium permanganate

0.5% Potassium permanganate	47.5ml
3% Sulphuric acid	2.5ml

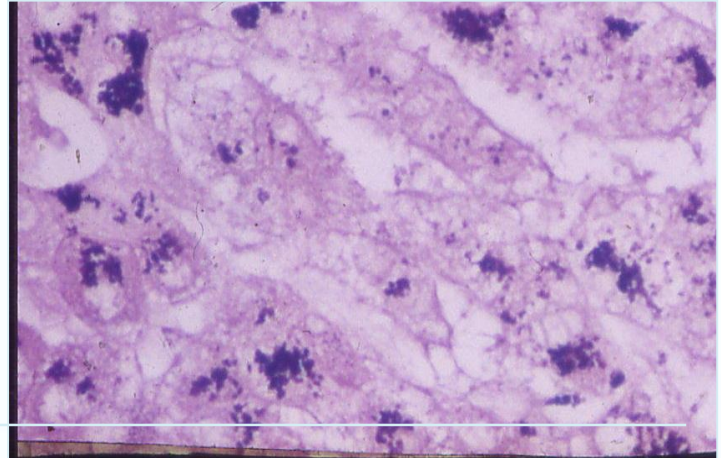
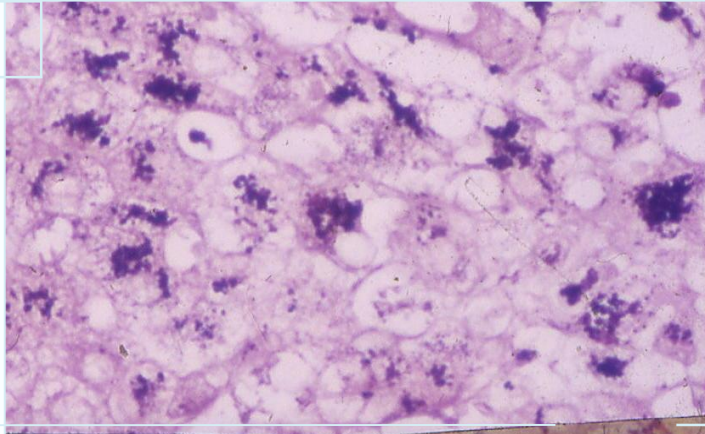
- Method works best with fresh solution - discard when it starts to precipitate and change every 2 weeks.

Orcein staining solution

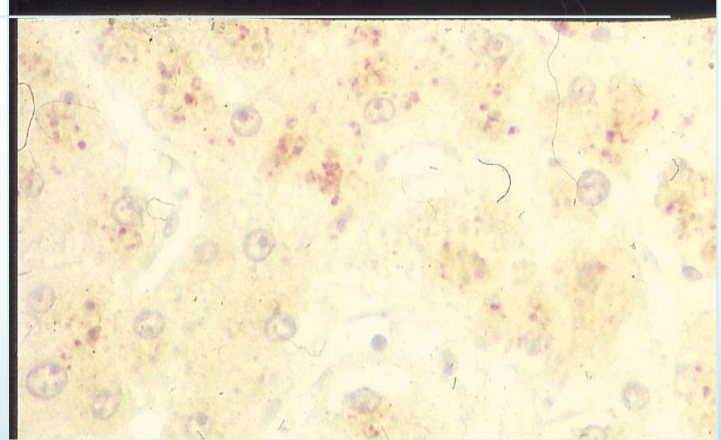
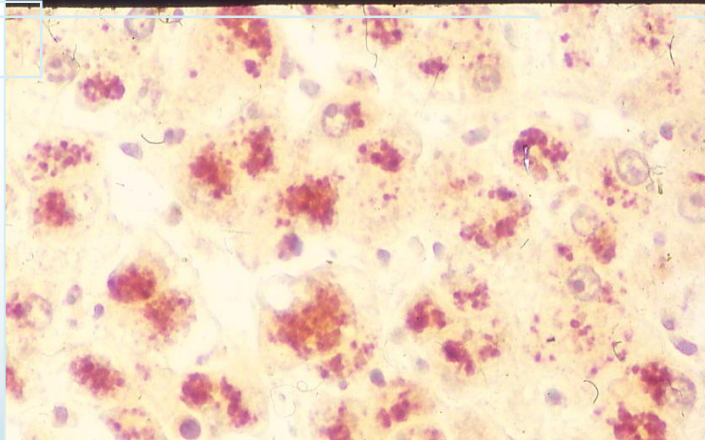
- Synthetic orcein (Merck) 0.5-1.0g
- 1% HCl in 70% alcohol 100ml
- Concentration of dye needed varies between batches - check for each new batch of dye.
- Make fresh solution every 7- 10 days, or earlier if the background staining is markedly increased.

Copper / Copper-associated protein

Orcein



Rhodanine



Immediate processing

After 3 wks in formol saline

Summary

- Orcein stain false negative main issue
 - Histochemical technique
 - Control tissue critical, must include copper-binding protein
 - Interpretation
 - Coarse granules, easy
 - Fine focal granules:
 - easily missed, needs to be looked at high magnification
 - ? Other pigment, add rhodanine, K7
 - Rhodanine, red stain easy to interpret
- Biliary disorders
 - Patchy distribution of rhodanine, orcein and K7 stain
 - Not reciprocally linked
 - Not clear whether one or the other becomes positive earlier
 - Orcein and K7 recommended in the first instance, particularly in those cases when clinically and/or on H&E there is a suspicion of biliary pathology

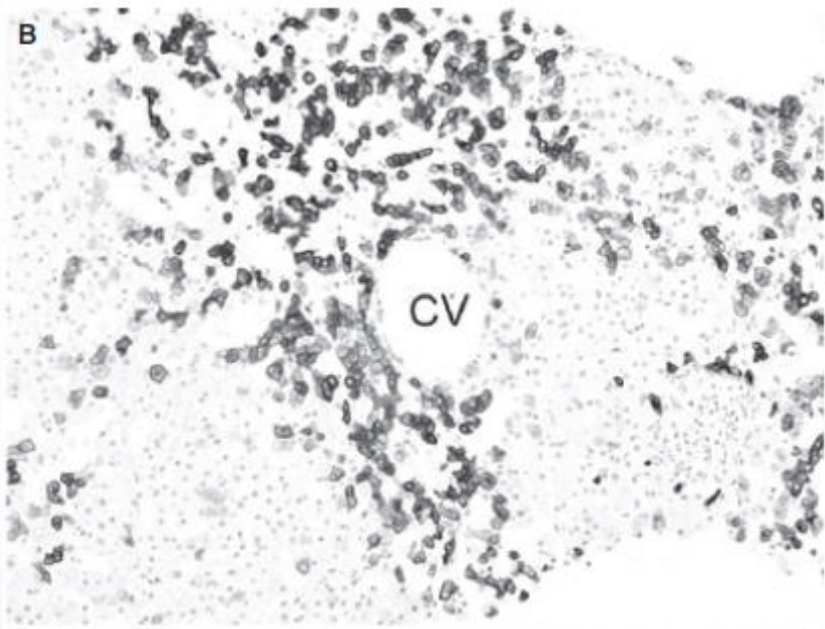


Table 1. Liver diseases and aberrant cyokeratin 7 expression of centrilobular hepatocytes of 113 examined specimens

Category of liver disease	Cases, <i>n</i>	Cases of CK7 + CHs, <i>n</i> (%)	CK7 + CH score 0/1/2/3, <i>n</i>
HCV infection only	34	17 (50.0)	17/4/10/3
HBV infection only	21	8 (38.1)	13/6/0/2
NASH only	14	9 (64.3)	5/5/3/1
ALD only	13	8 (61.5)	5/0/1/7
PBC	7	4 (57.1)	3/2/1/1
Autoimmune hepatitis only	5	2 (40.0)	3/1/1/0
Drug-induced liver injury only	4	0 (0)	4/0/0/0
Combined aetiology*	8	4 (50.0)	4/2/0/2
Other diseases†	7	4 (57.1)	3/2/0/2
Total	113	56 (49.6)	57/22/16/18

Aberrant cyokeratin 7 expression of centrilobular hepatocytes: a clinicopathological study
 Matsukuma S1, Takeo H, Kono T, Nagata Y, Sato K.
 Histopathology. 2012 Nov;61(5):857-62