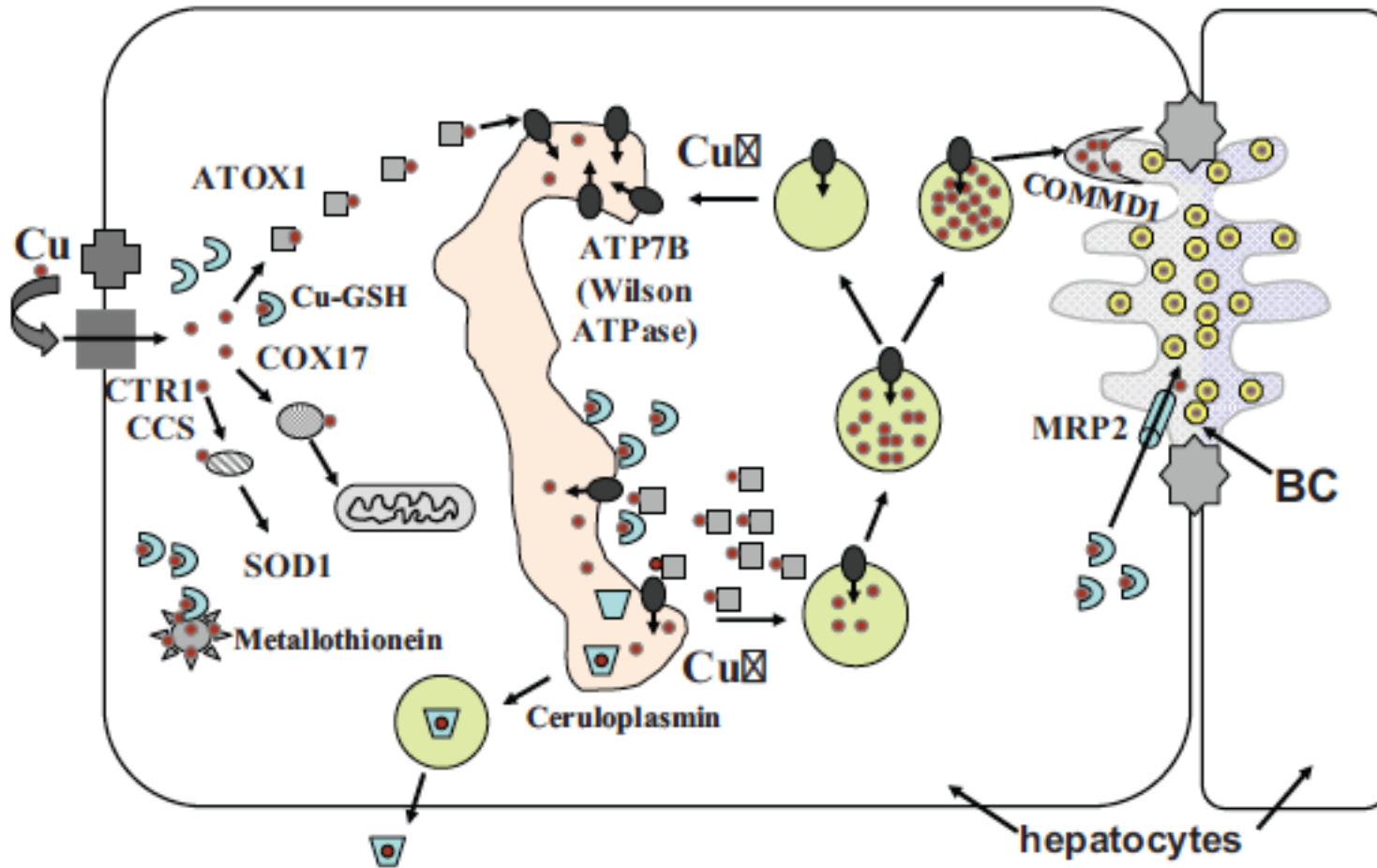


Copper , orcein, and keratin 7 ...

Alberto Quaglia
Institute of Liver Studies
King's College Hospital

Copper in hepatocytes



Roberts and Sarkar

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

Orcein

- Elastic fibres, HBV “ground glass hepatocytes”, and copper binding protein
- Copper-binding protein:
 - Wilson disease
 - Cholestatic disorders
 - Advanced stage chronic liver disease (cirrhosis) of any aetiology (patchy distribution)
 - Physiological (infant liver up to about 2-3 months)

Procedure

This procedure has been validated for use in this laboratory using formalin fixed, paraffin embedded liver tissue sections cut at a thickness of 4 microns. See NEQAS CPT Run 99 (November 2015)

Orcein staining is performed on multiple slides each run, and tissue sections will contain varying amounts of elastic fibres around blood vessels, which can be considered an internal control for this method. A control slide containing copper associated protein is stained as part of each run.

Verification of staining runs is performed by checking and scoring each run with BMS and pathologist sign-off using the form **KCH-LP-FM-HIS-Slide check and acceptance**.

EQA is confirmed by submission of slides to the UK NEQAS CPT General Pathology scheme.

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

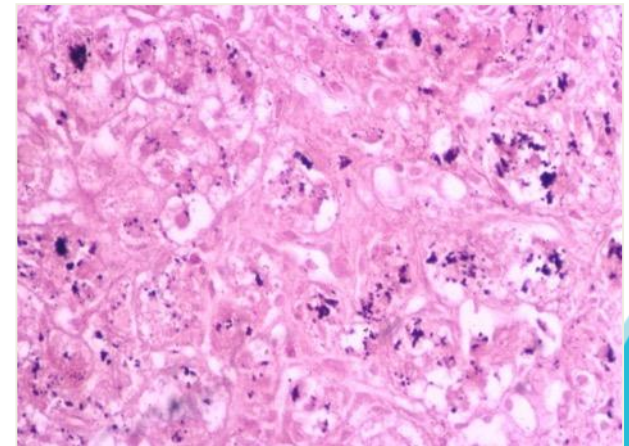
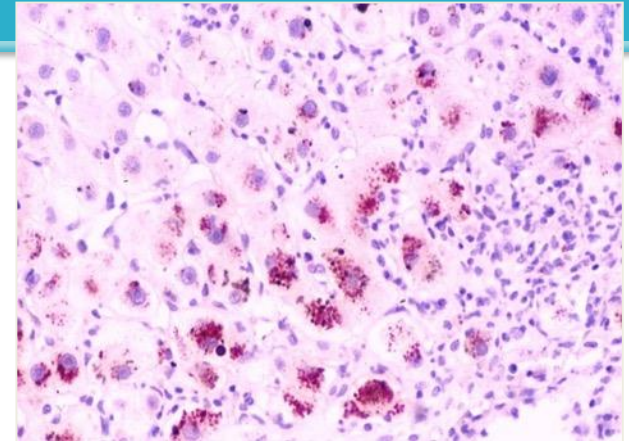
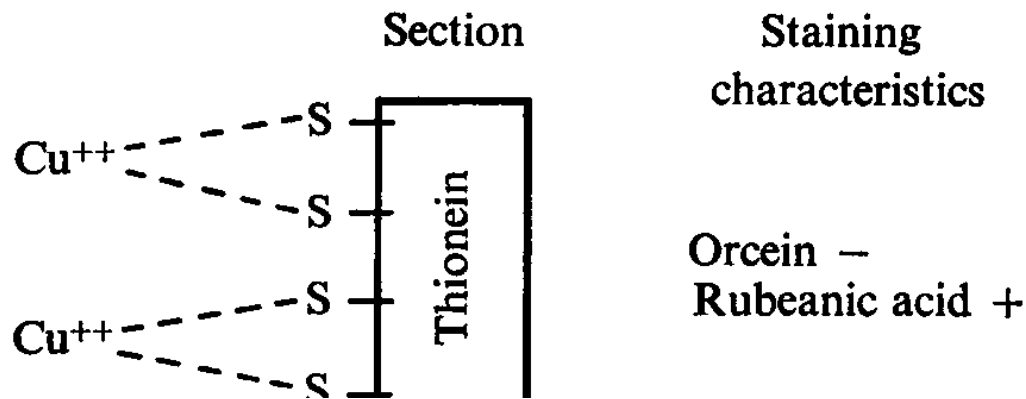
Concentration of dye needed varies between batches - check for each new batch of dye.

Make fresh solution every 7- 10 days, or earlier if the background staining is markedly increased.

Copper and copper associated protein

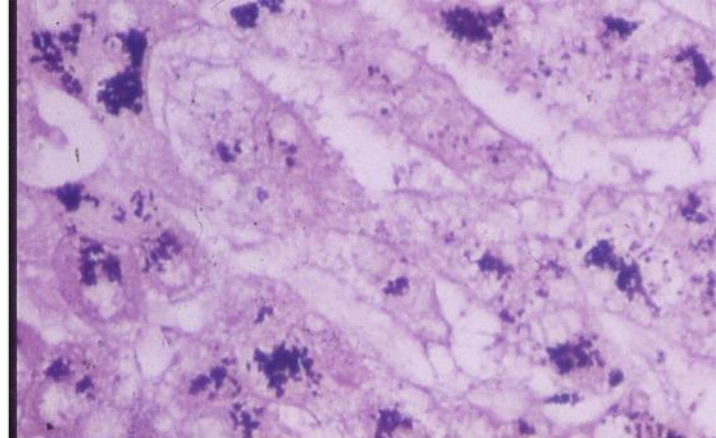
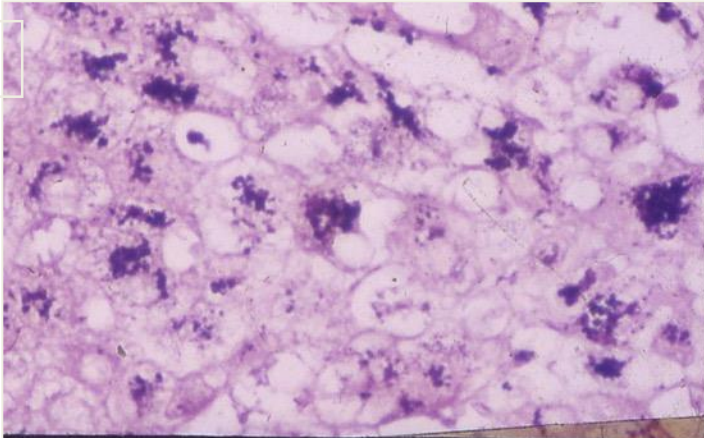
Metallothionein is known to contain sulphhydryl groups in large quantities and it binds copper to the apoprotein, thionein, through these residues by the formation of mercaptides (Morell *et al.*, 1961; Evans, 1973). These observations correspond with the observed characteristics of orcein-positive accumulations supporting the hypothesis that thionein and orcein-positive aggregations are related.

Schematically the results can be presented as follows:

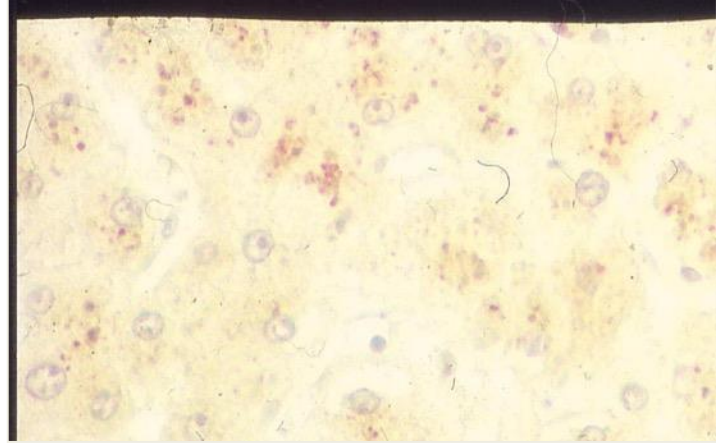
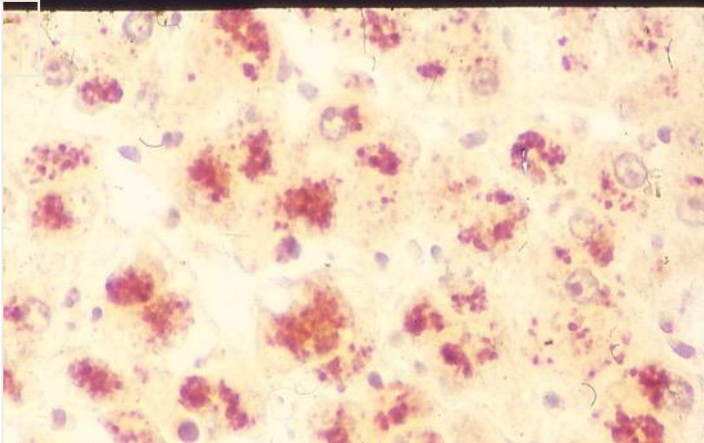


Copper / Copper-associated protein

Orcein



Rhodanine

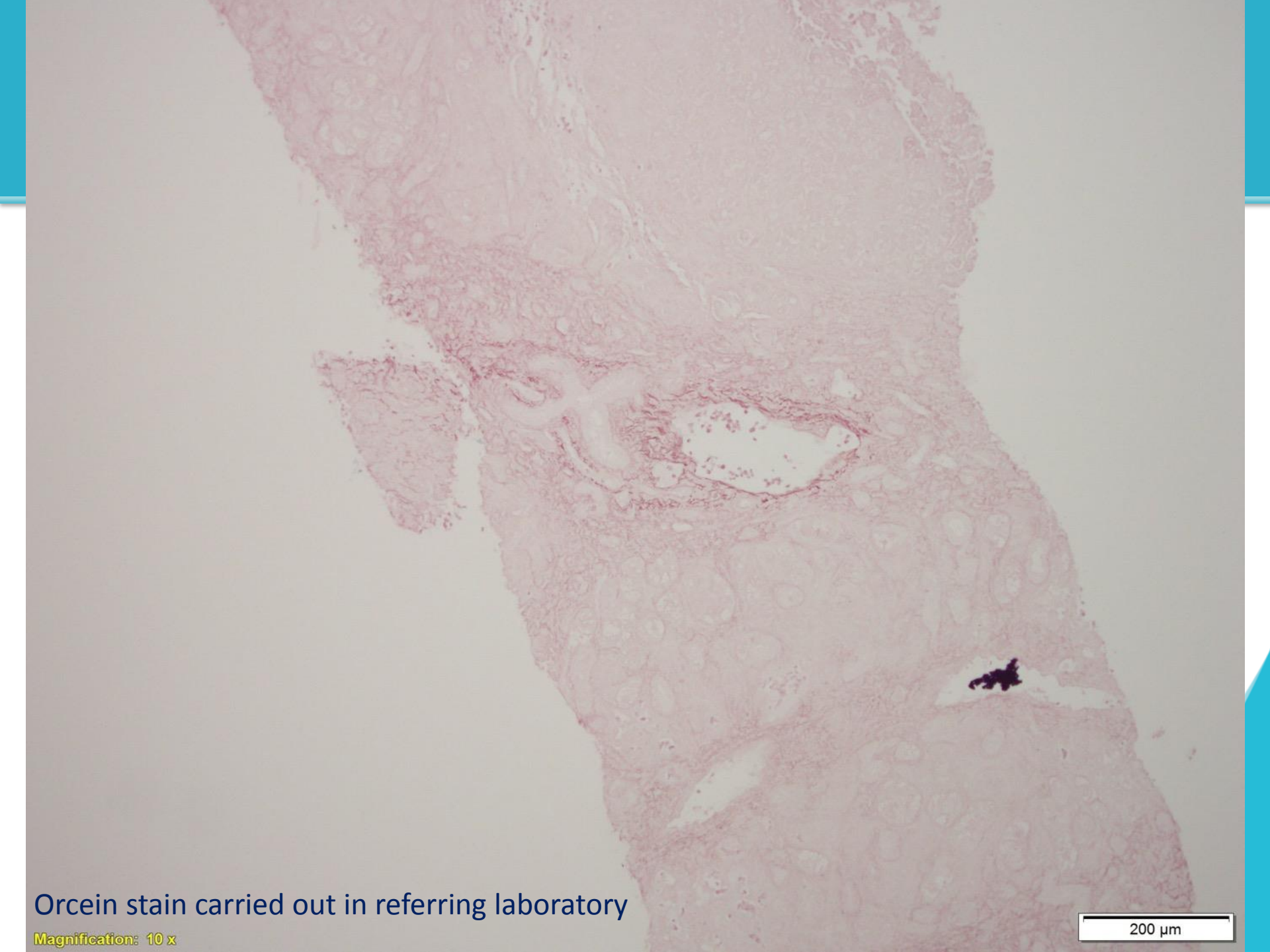


Immediate processing

After 3 wks in formol 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

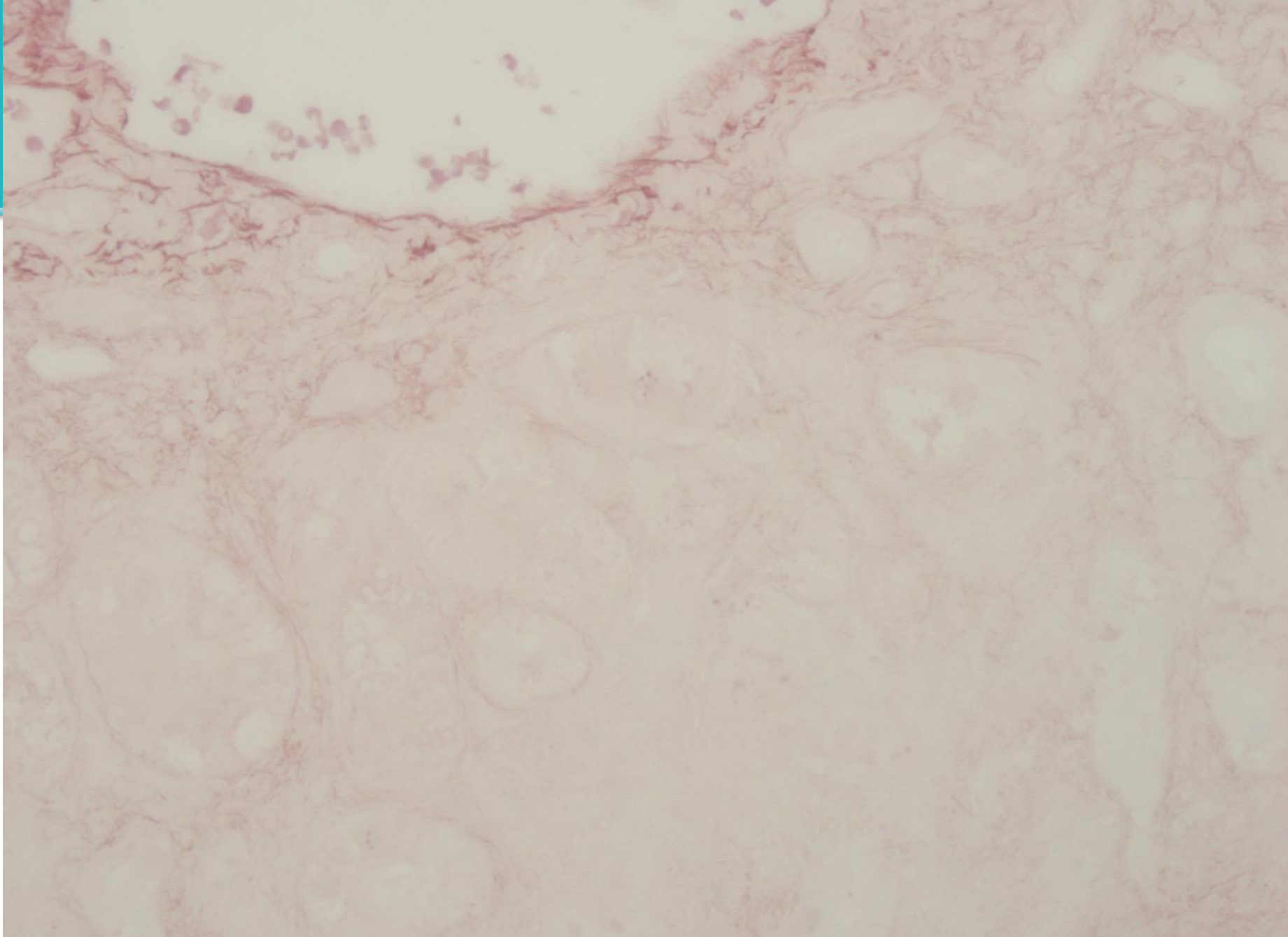
Magnification: 10 x

200 μ m

Orcein stain carried out in referring laboratory

Magnification: 40 x

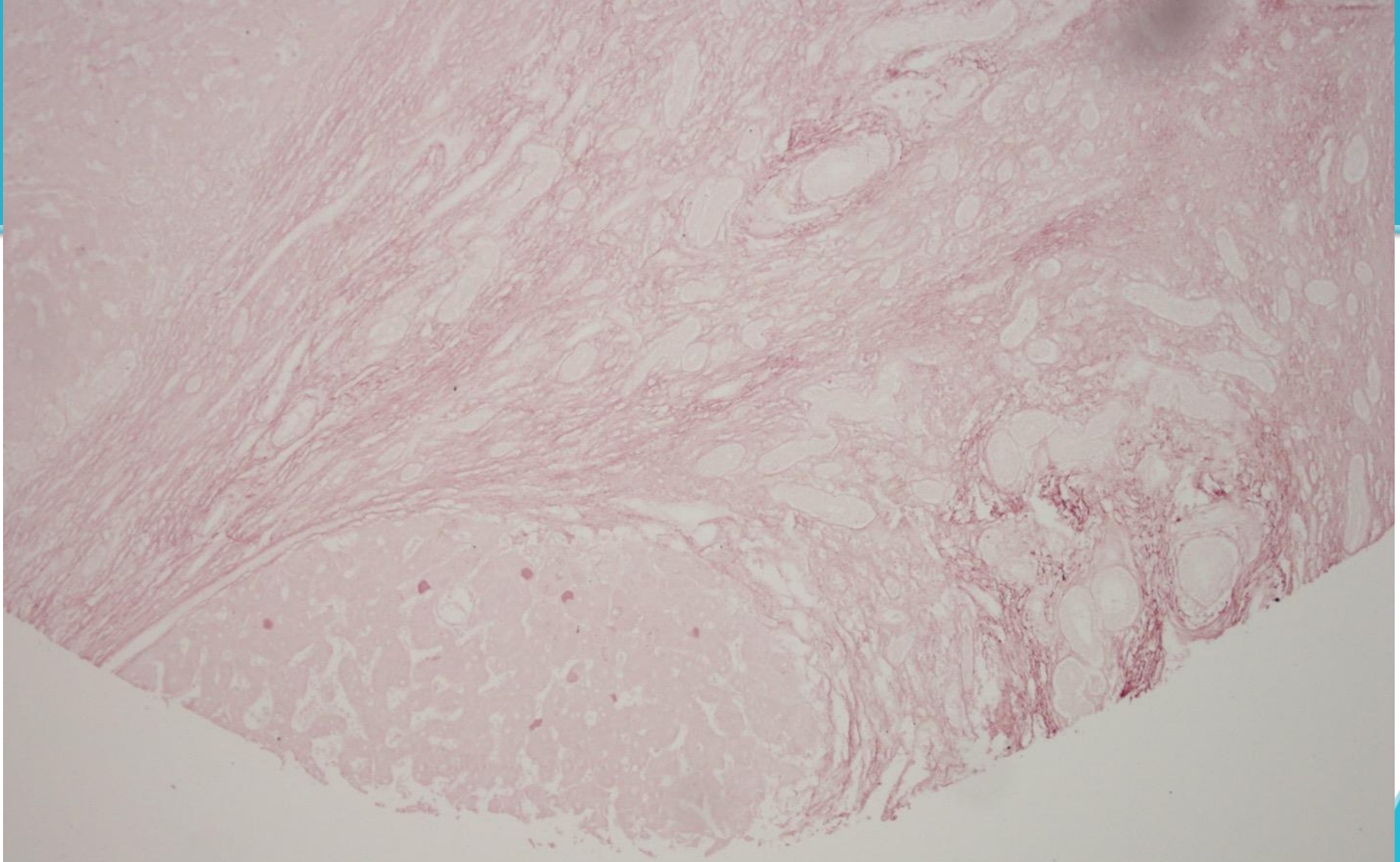
50 μ m



Orcein stain carried out in referring laboratory

Magnification: 40 x

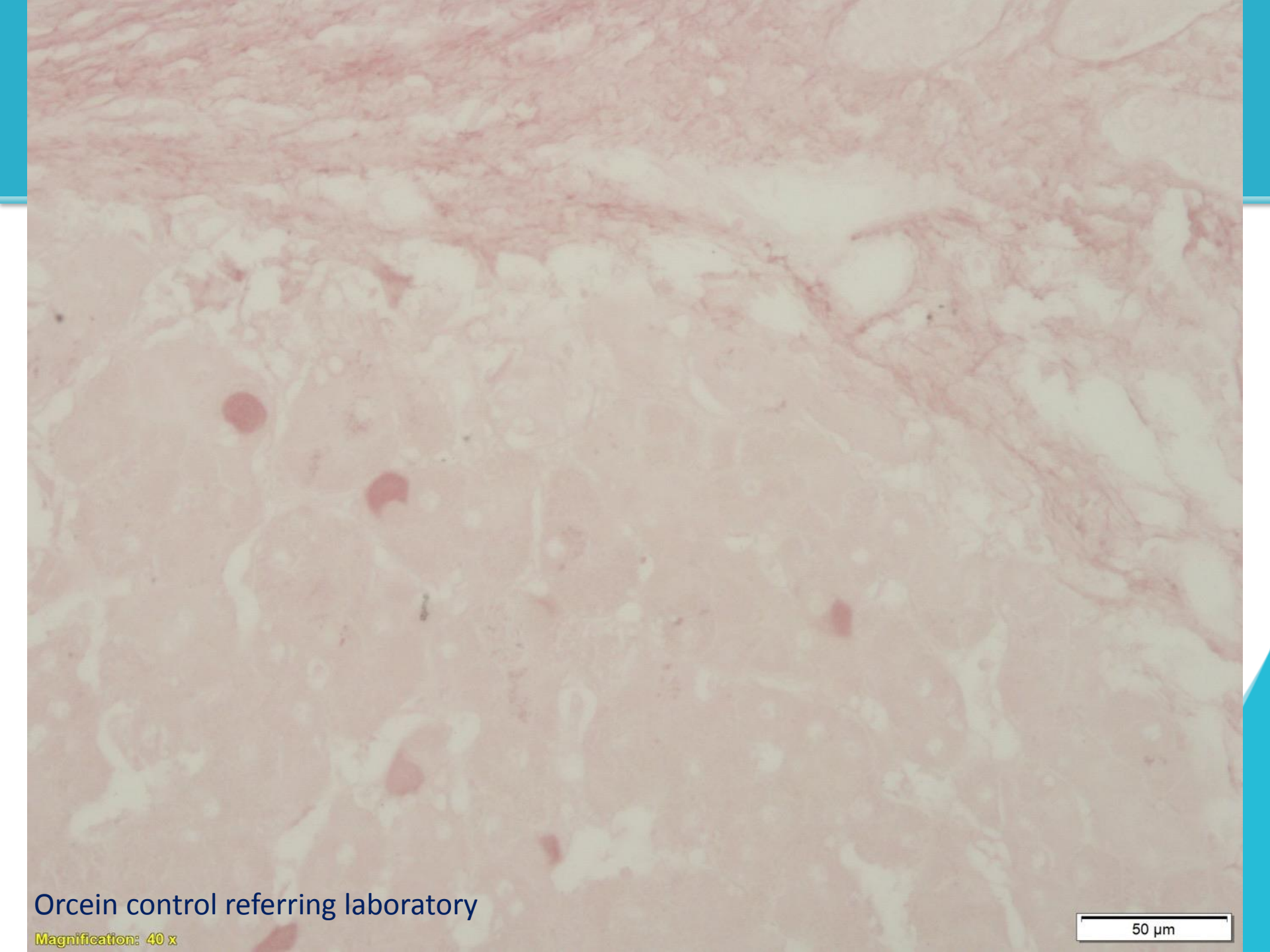
50 μ m



Orcein control referring laboratory

Magnification: 10 x

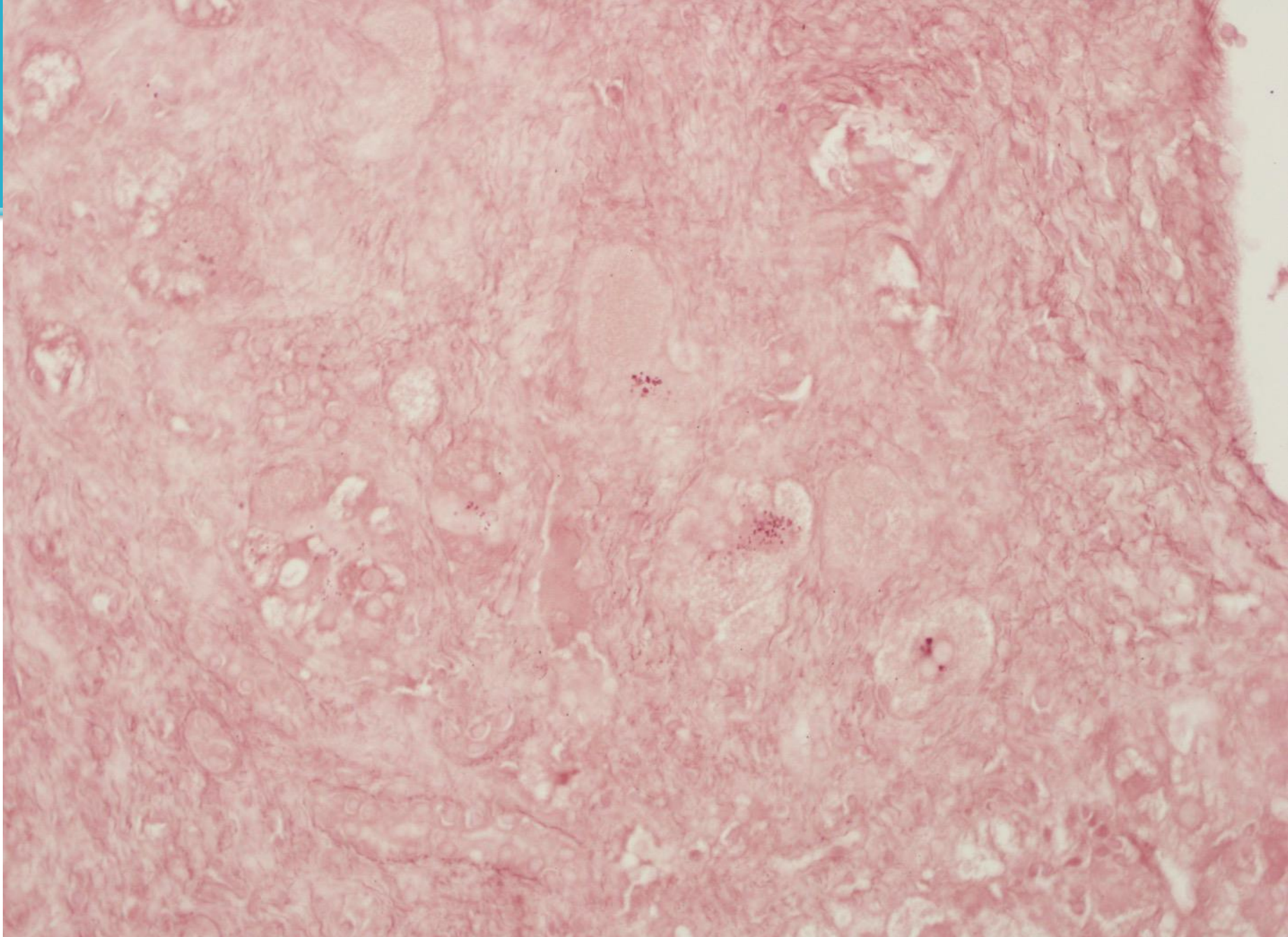
200 μ m



Orcein control referring laboratory

Magnification: 40 x

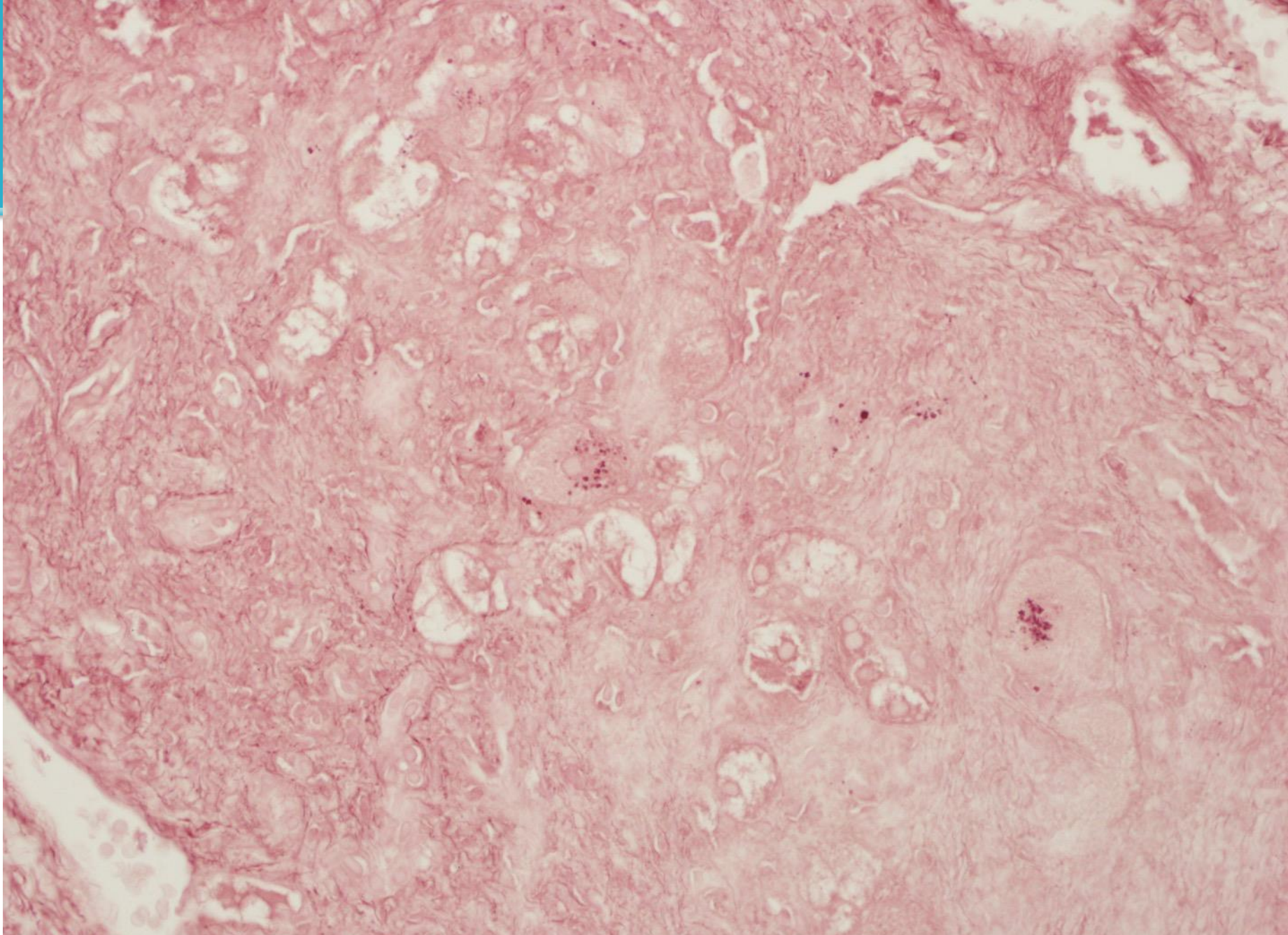
50 μ m



Orcein stain carried out at King's College Hospital

Magnification: 40 x

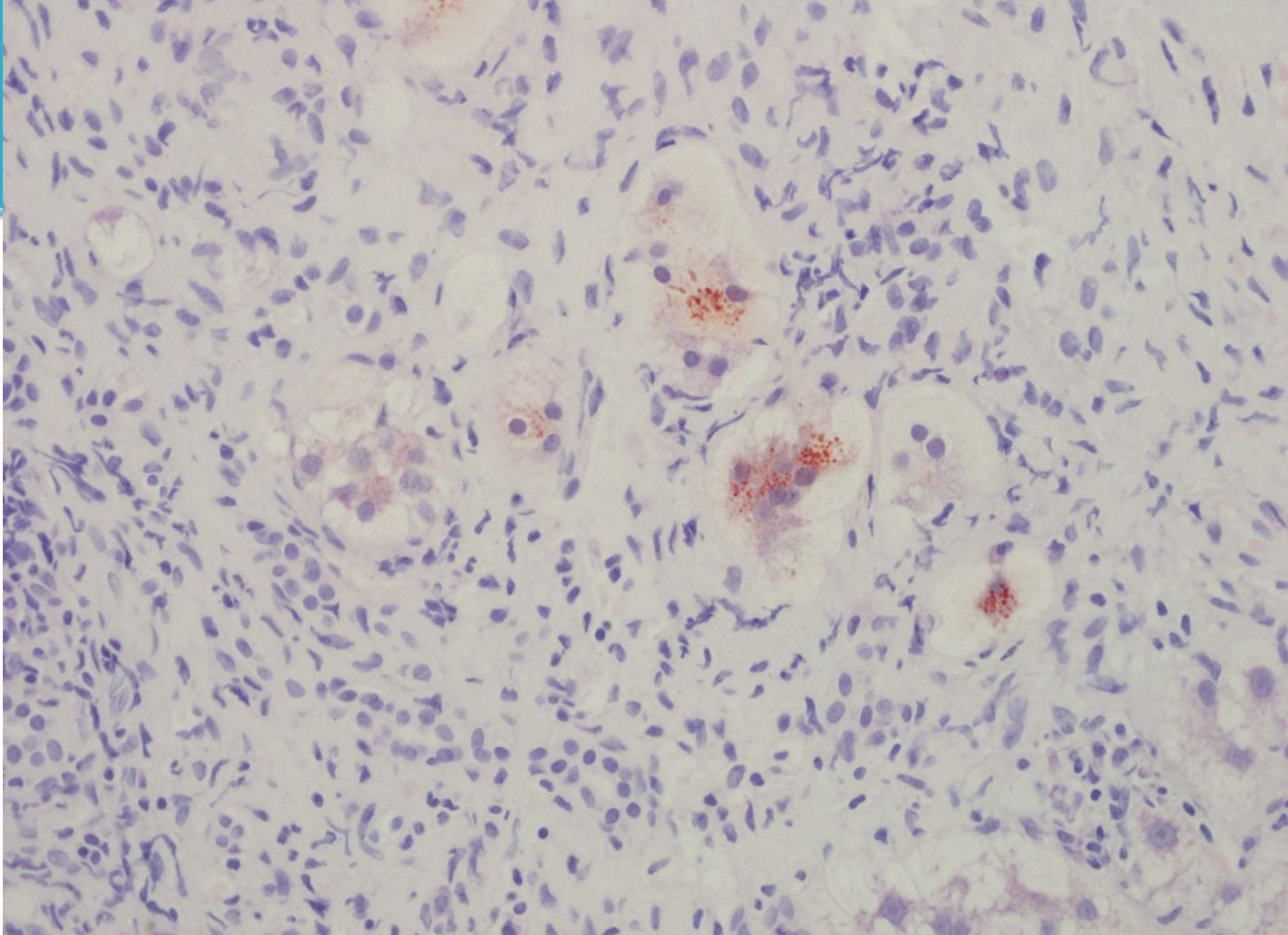
50 μ m



Orcein stain carried out at King's College Hospital

Magnification: 40 x

50 μ m



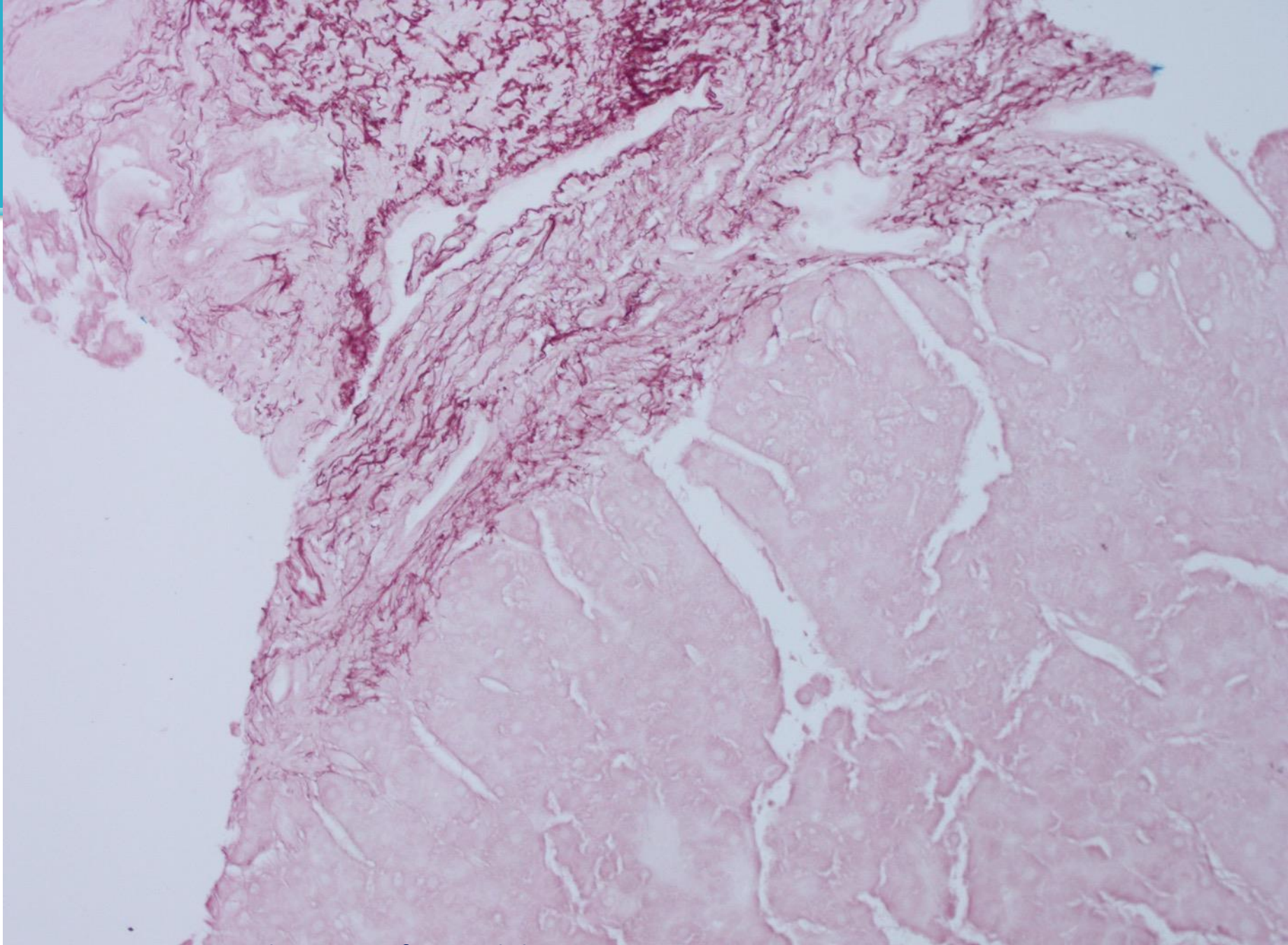
Rhodanine stain carried out at King's College Hospital

Magnification: 40 x

50 μ m

Case 2

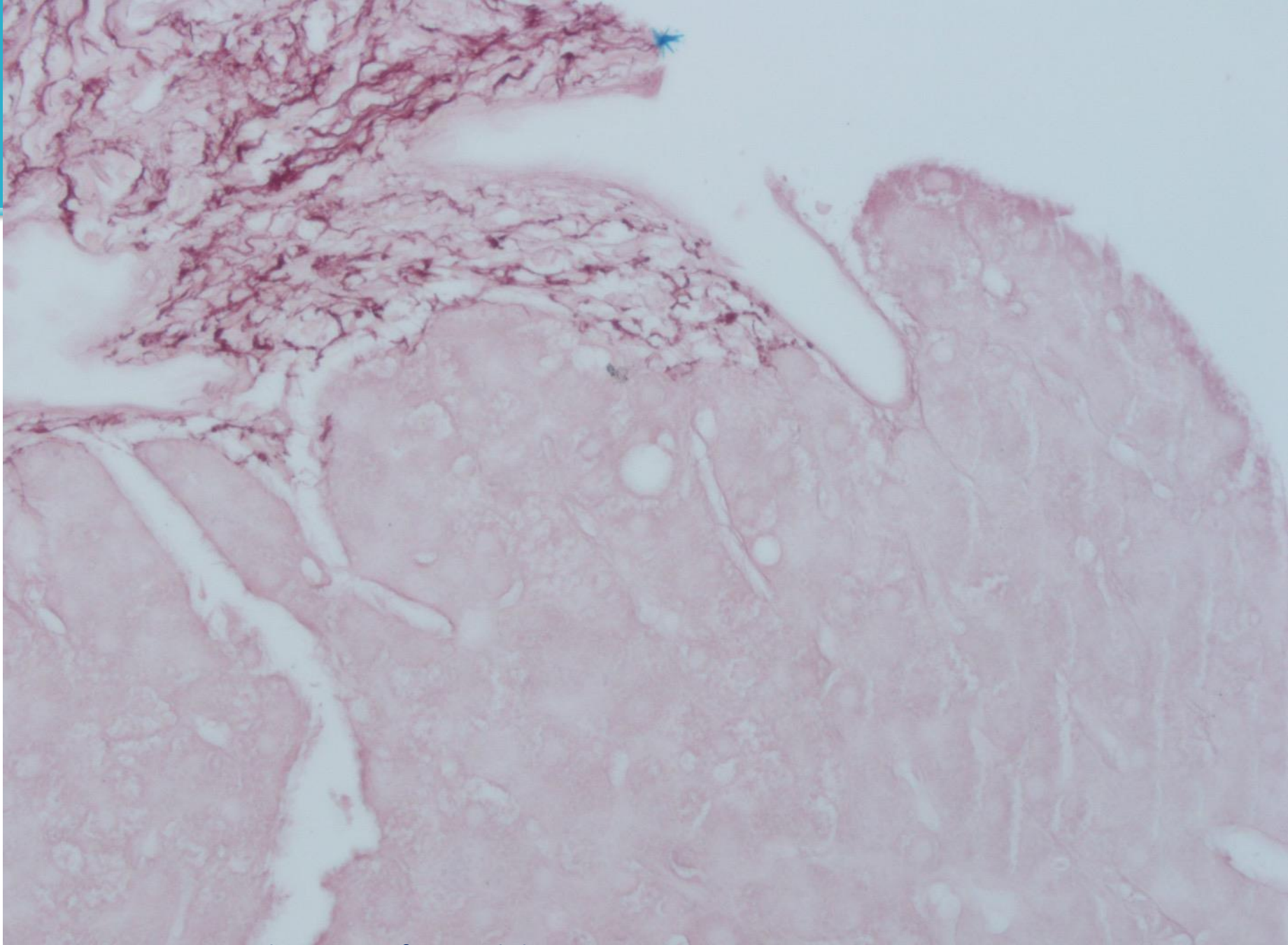
- 47 year old female, referred to King's College Hospital from another hospital
- - Crohn disease since 1995
- - Azathioprine, mecaptopurine and thioguanine
- - Increase in spleen size on MRI, and grade 1 varices on endoscopy indicating portal hypertensive gastropathy
- - Liver biopsy ? nodular regenerative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



Orcein stain carried out in referring laboratory

Magnification: 20 x

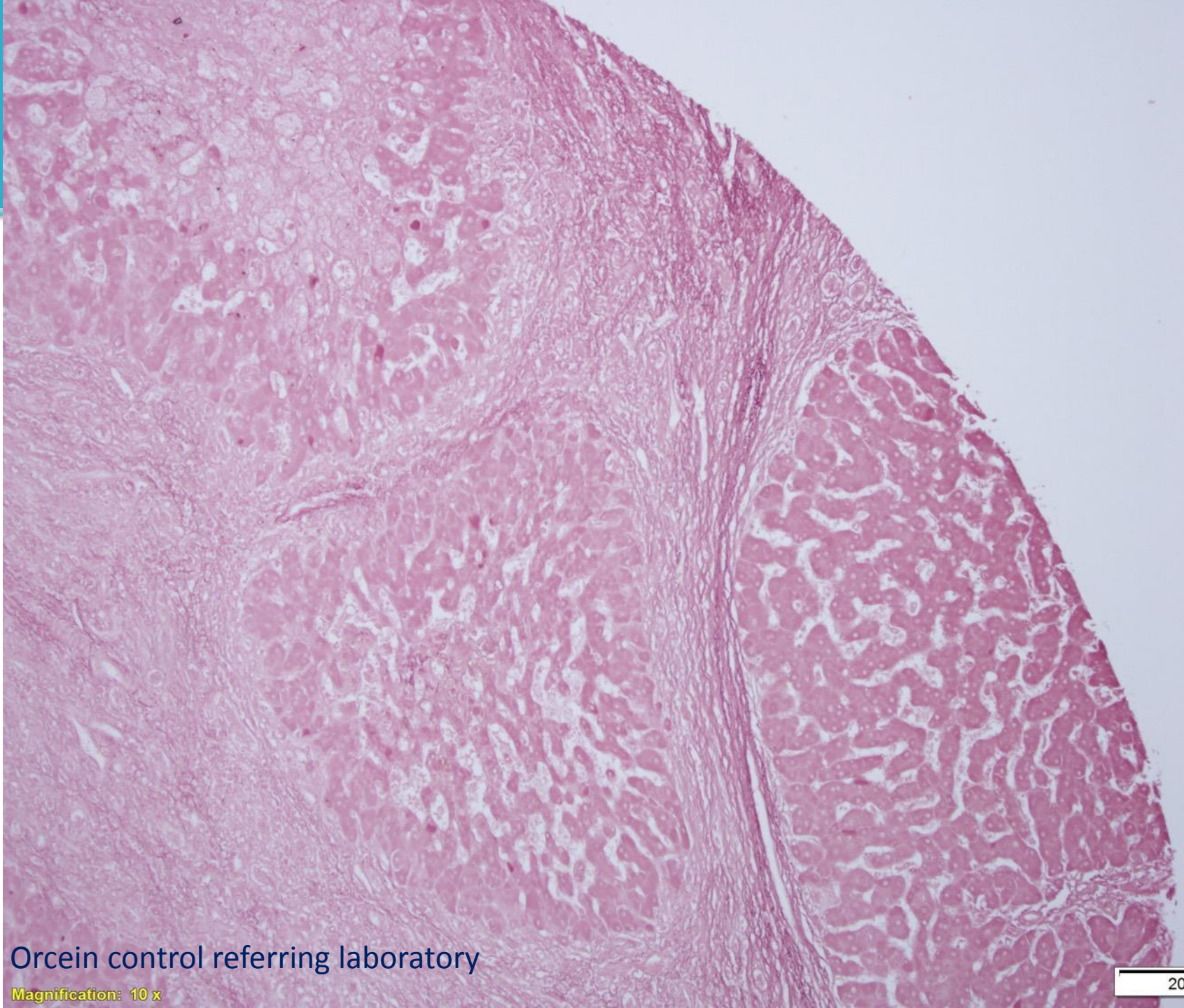
100 μ m



Orcein stain carried out in referring laboratory

Magnification: 40 x

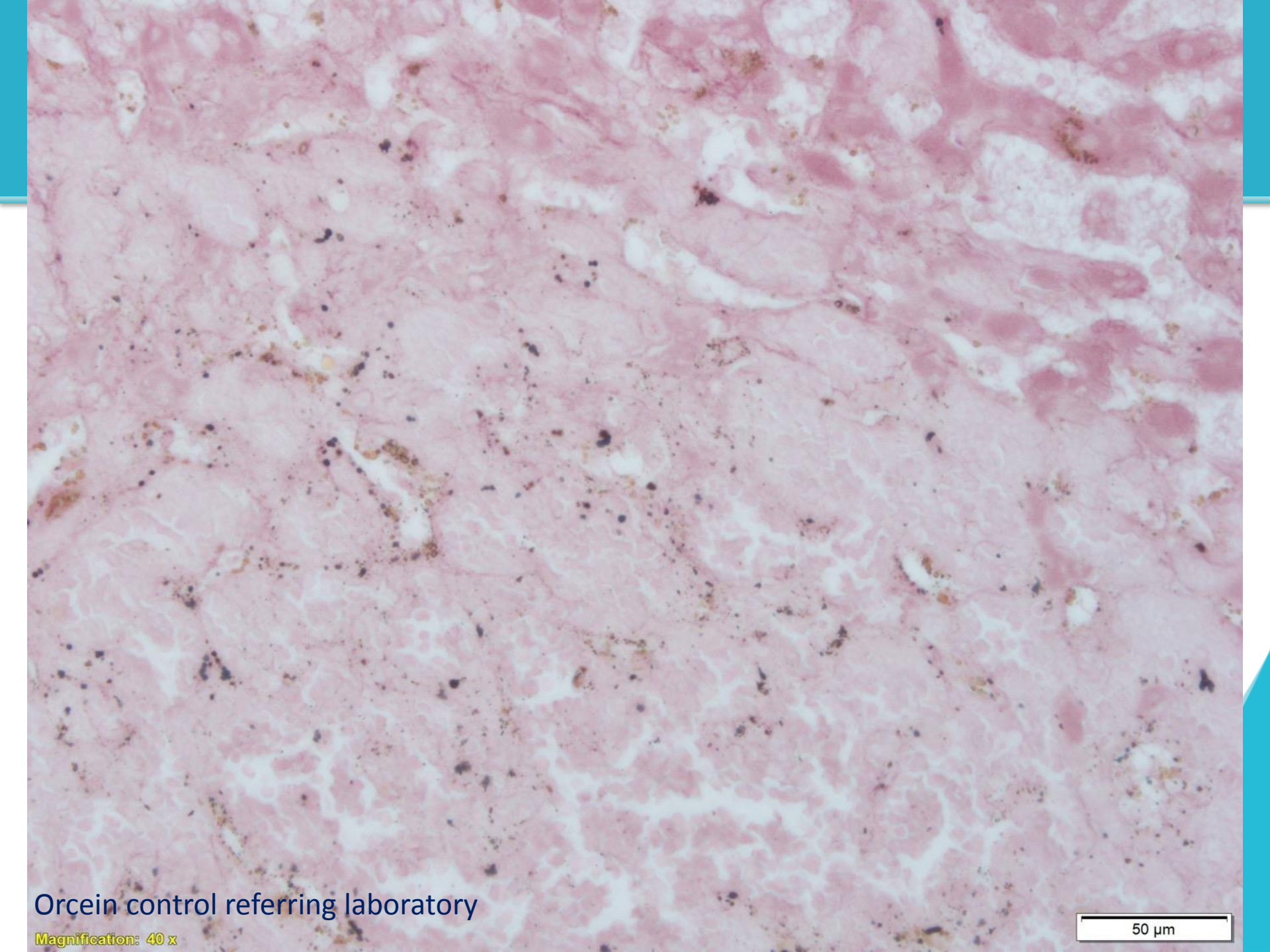
50 μ m



Orcein control referring laboratory

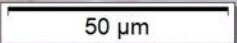
Magnification: 10 x

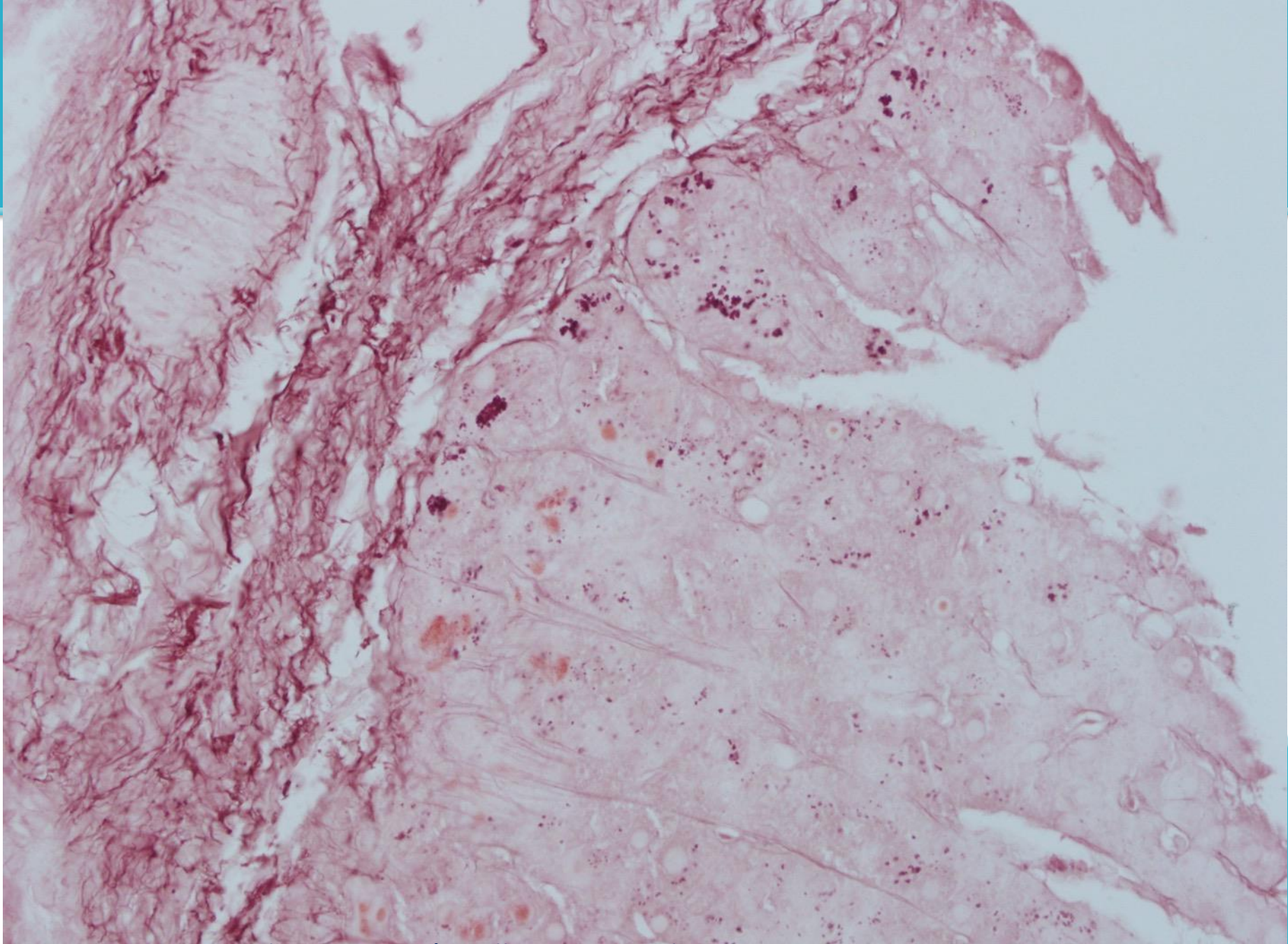
200 μ m



Orcein control referring laboratory

Magnification: 40 x

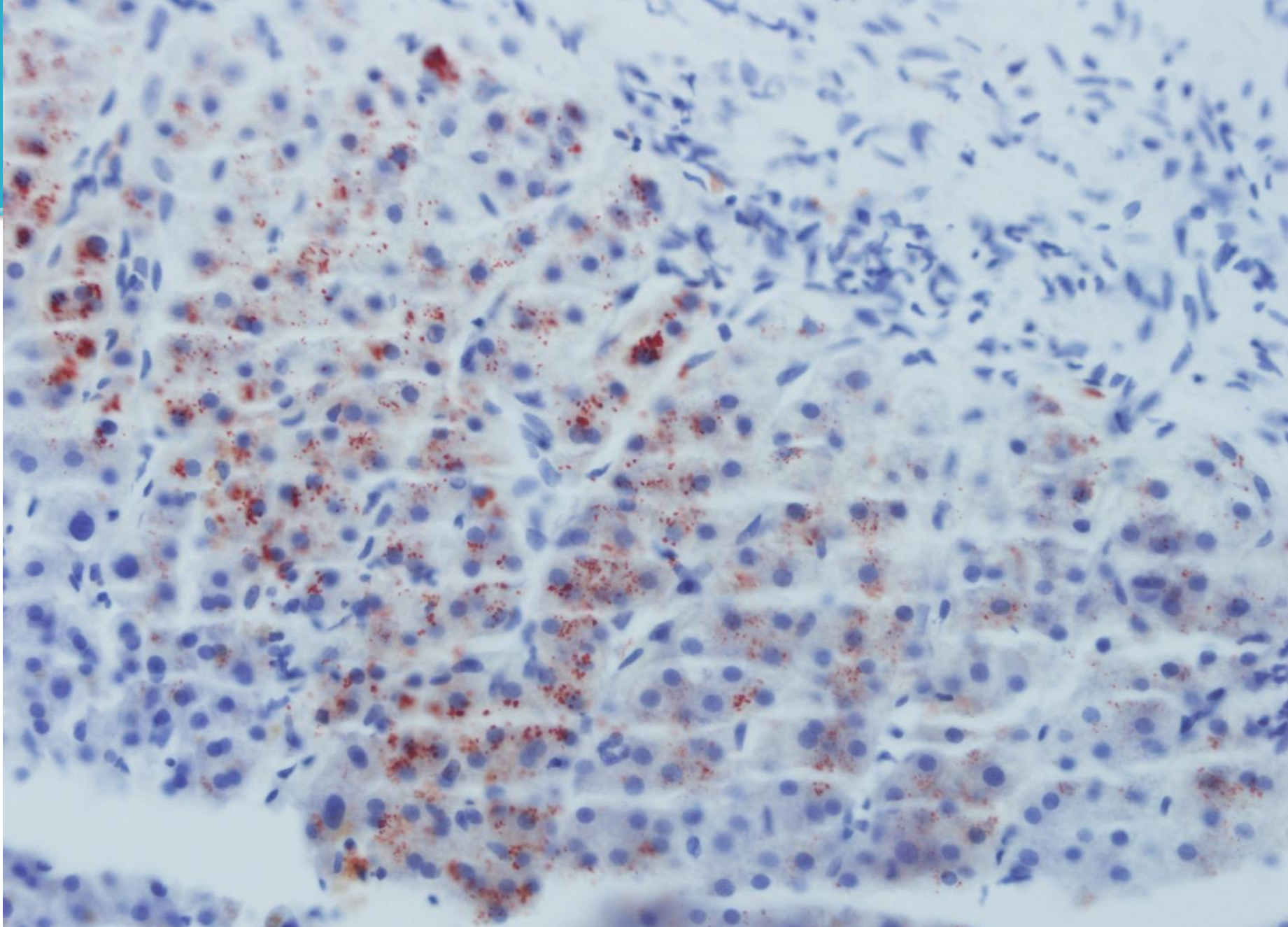




Orcein stain carried out at King's College Hospital

Magnification: 40 x

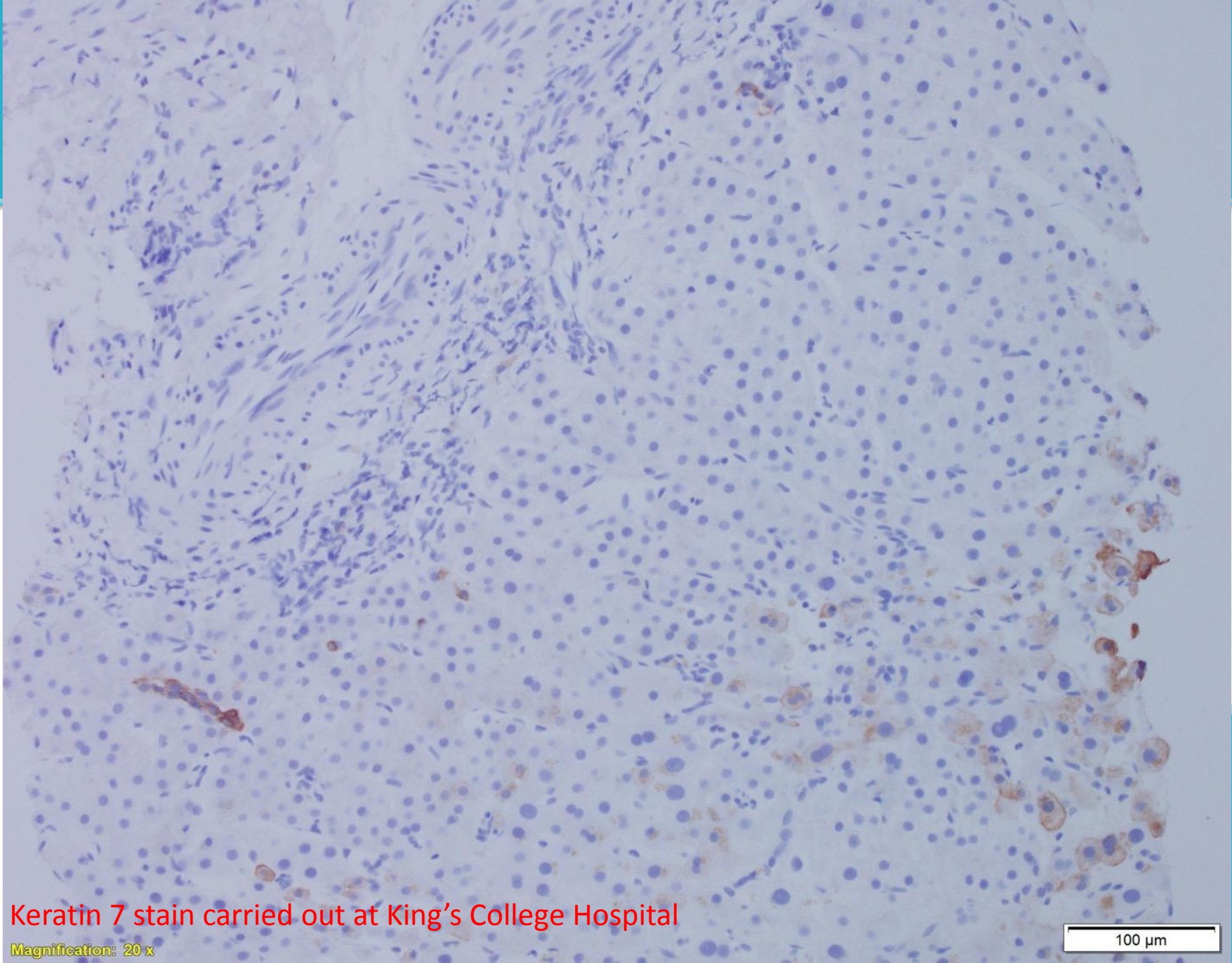
50 μ m



Rhodanine stain carried out at King's College Hospital

Magnification: 40 x

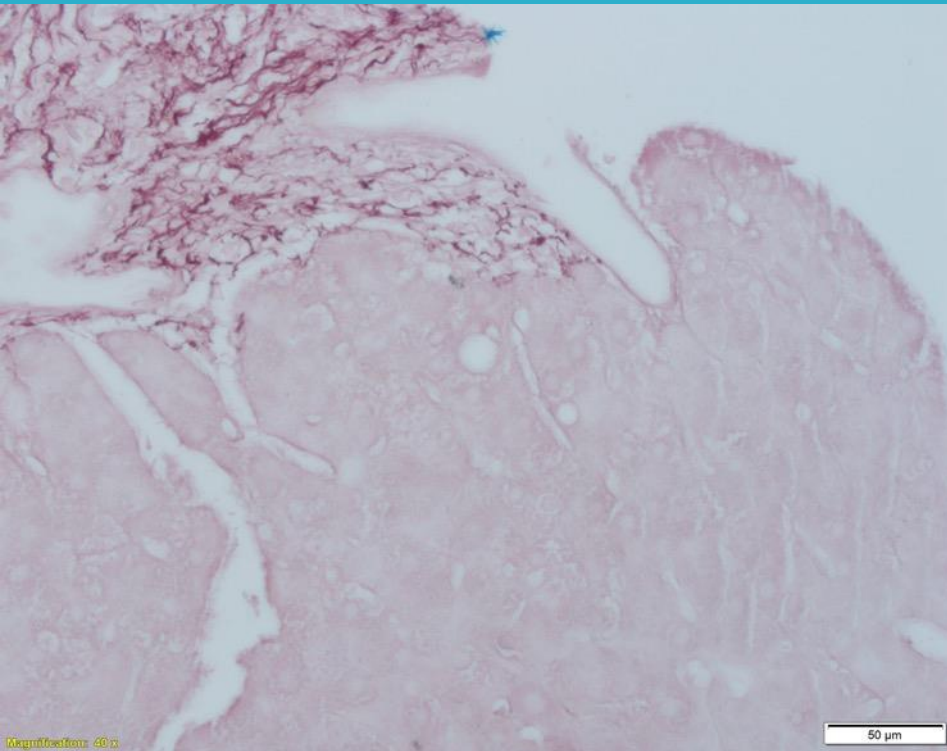
50 μ m



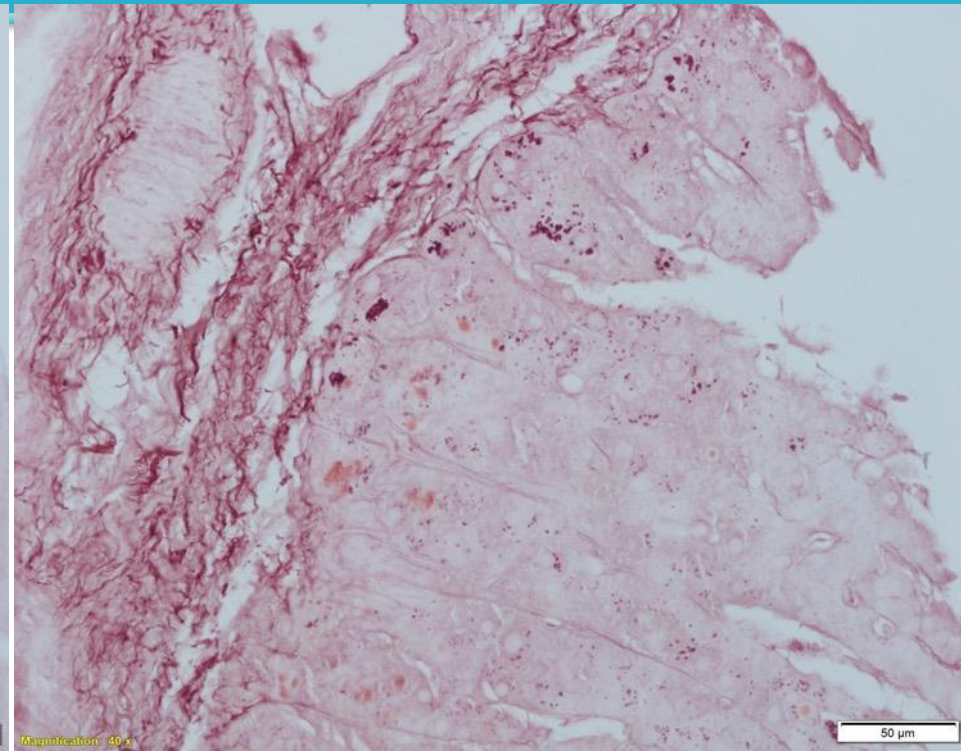
Keratin 7 stain carried out at King's College Hospital

Magnification: 20 x

100 μ m

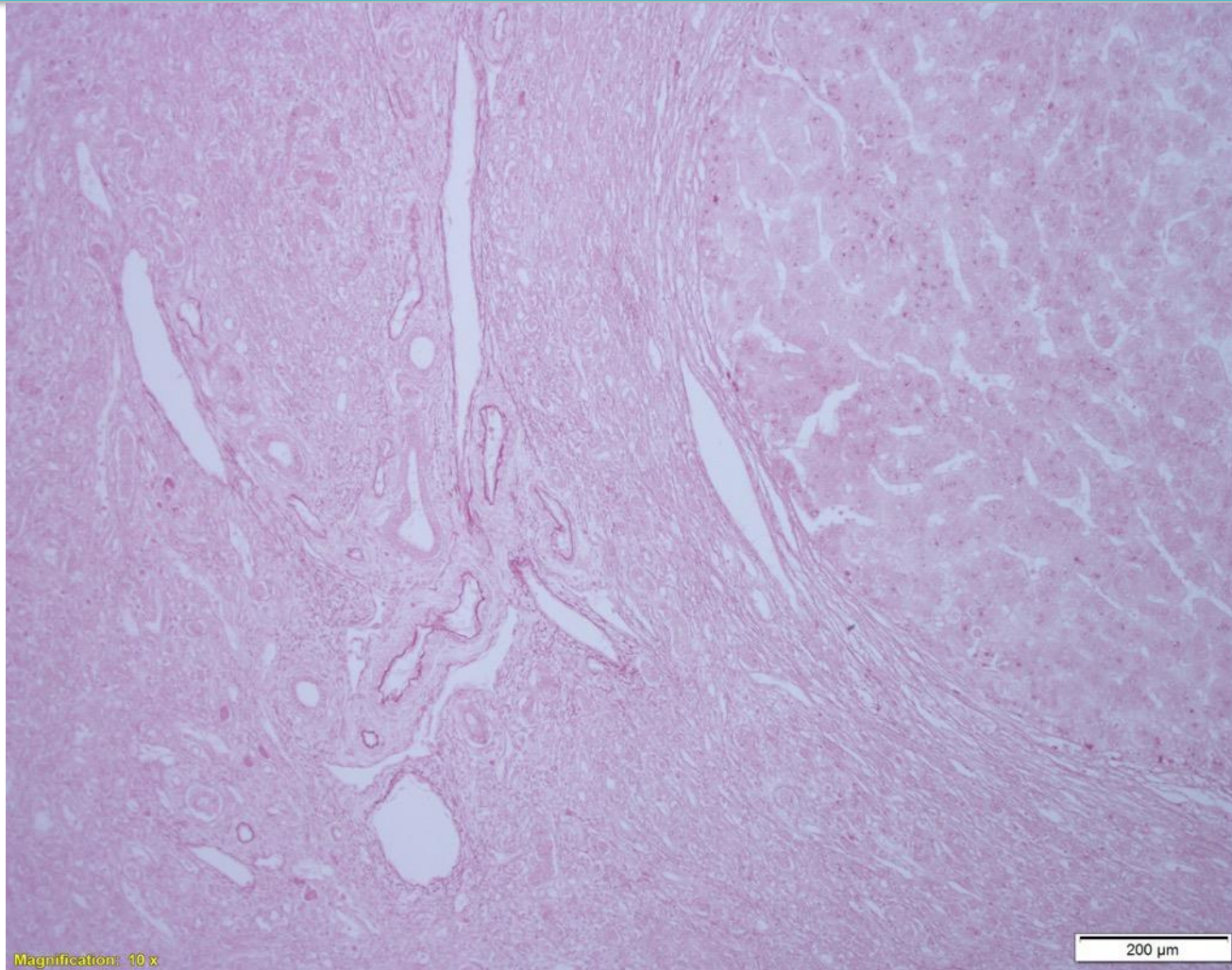


Orcein stain carried out in referring laboratory

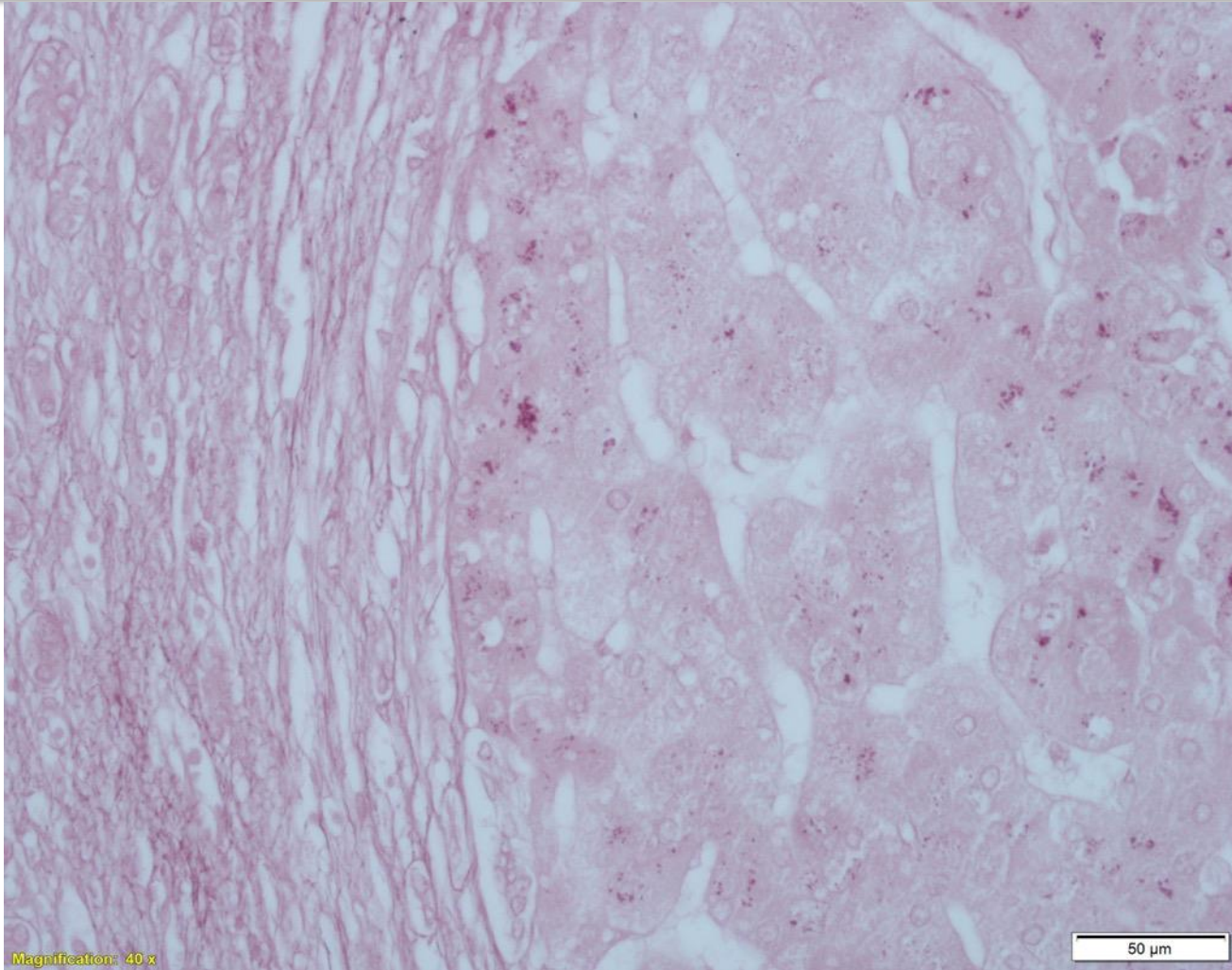


Orcein stain carried out at King's College Hospital

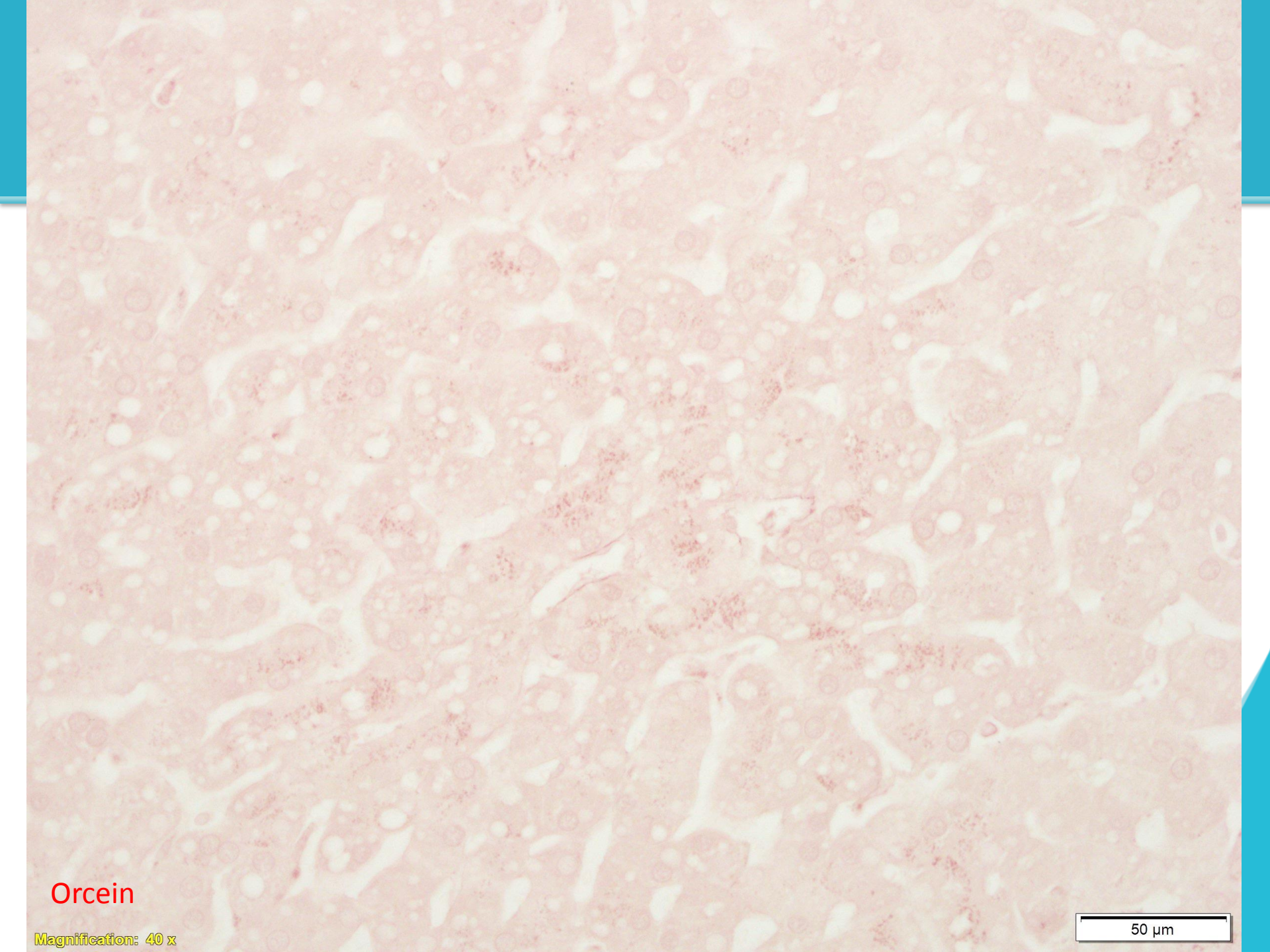
KCH orcein control



KCH orcein control



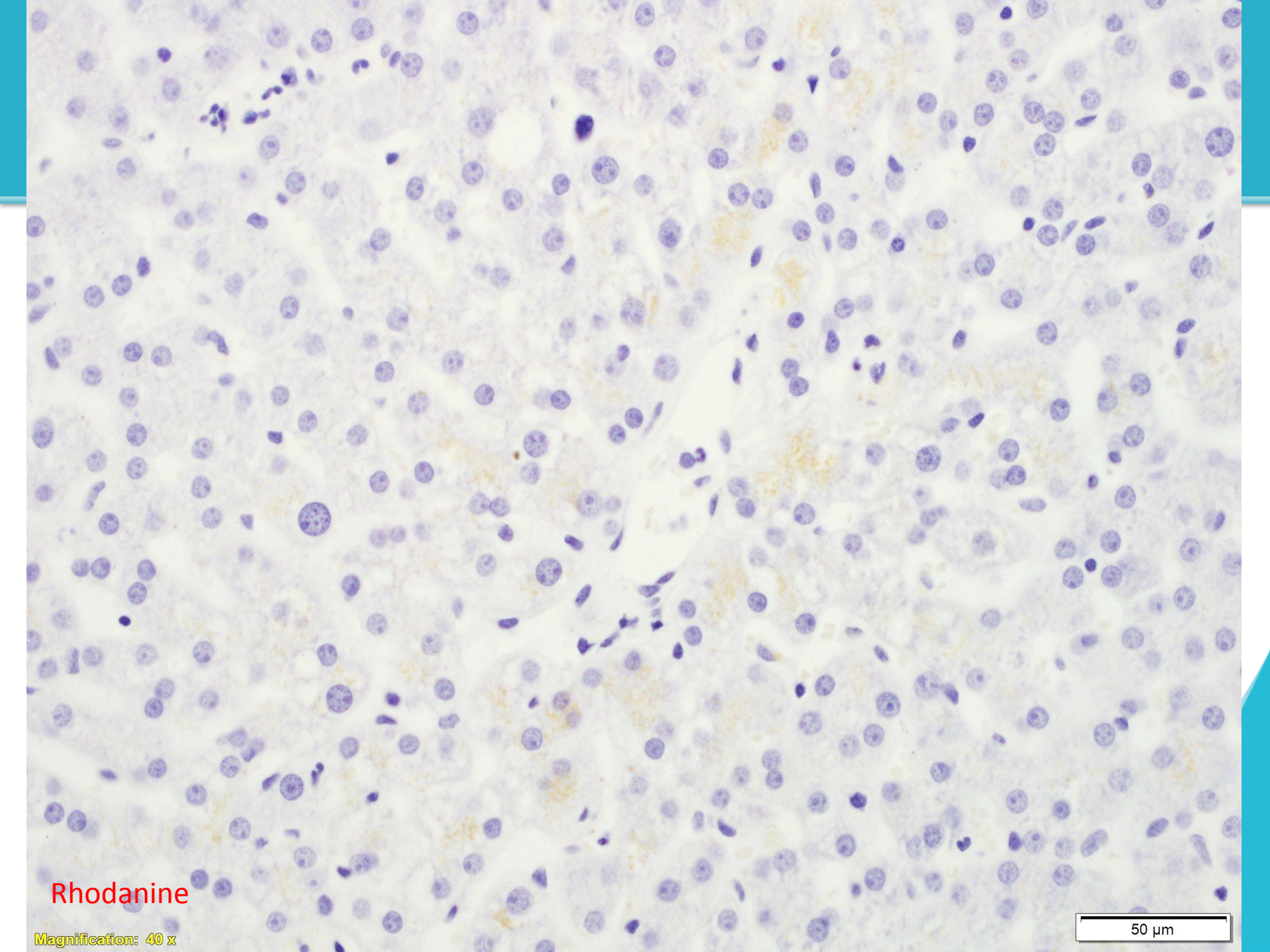
Post-reperfusion (time 0) liver allograft biopsy



Orcein

Magnification: 40 x

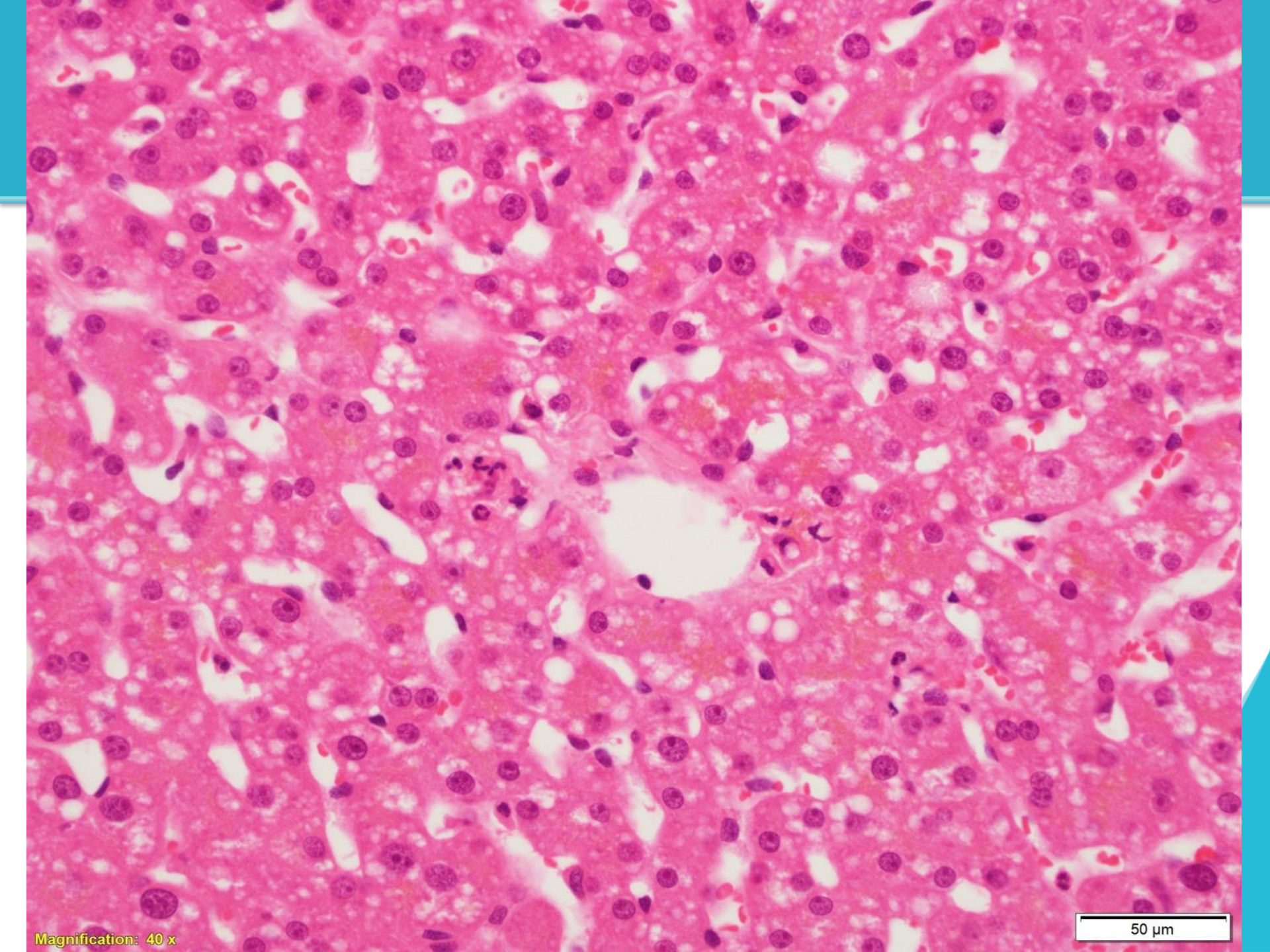
50 μ m



Rhodanine

Magnification: 40 x

50 μm



Magnification: 40 x

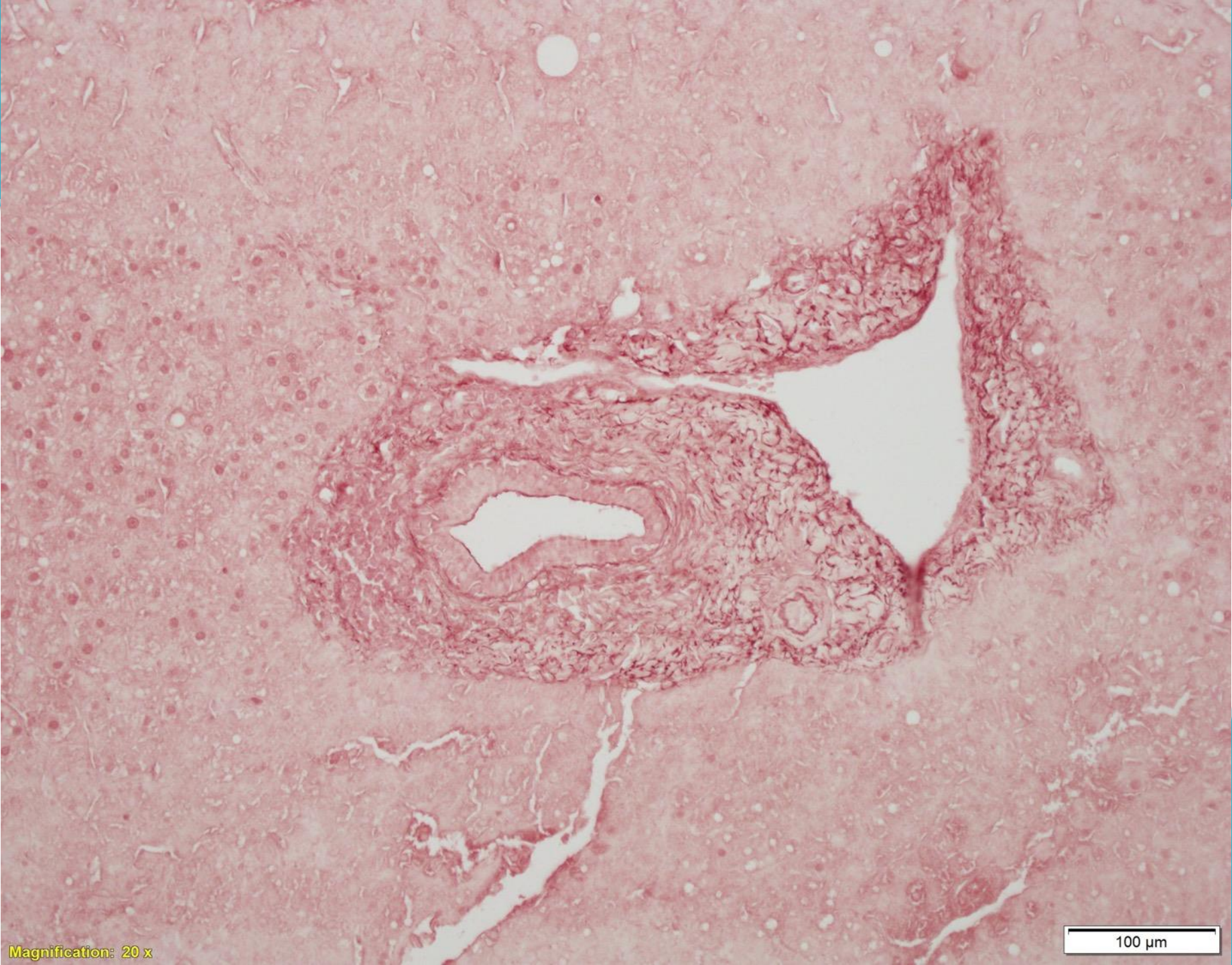
50 μm

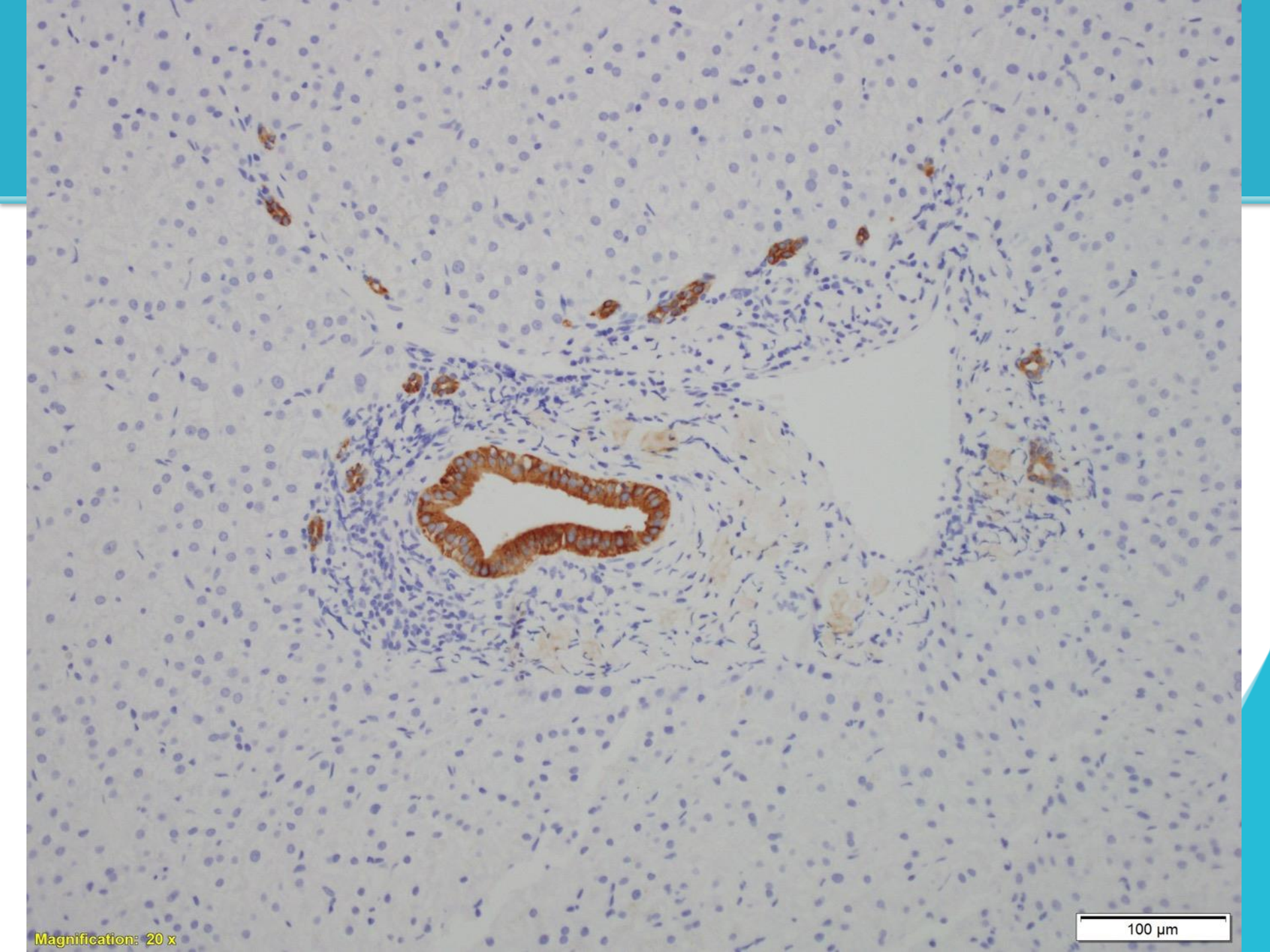
Liver EQA case

(AMA+ve)

Magnification: 20 x

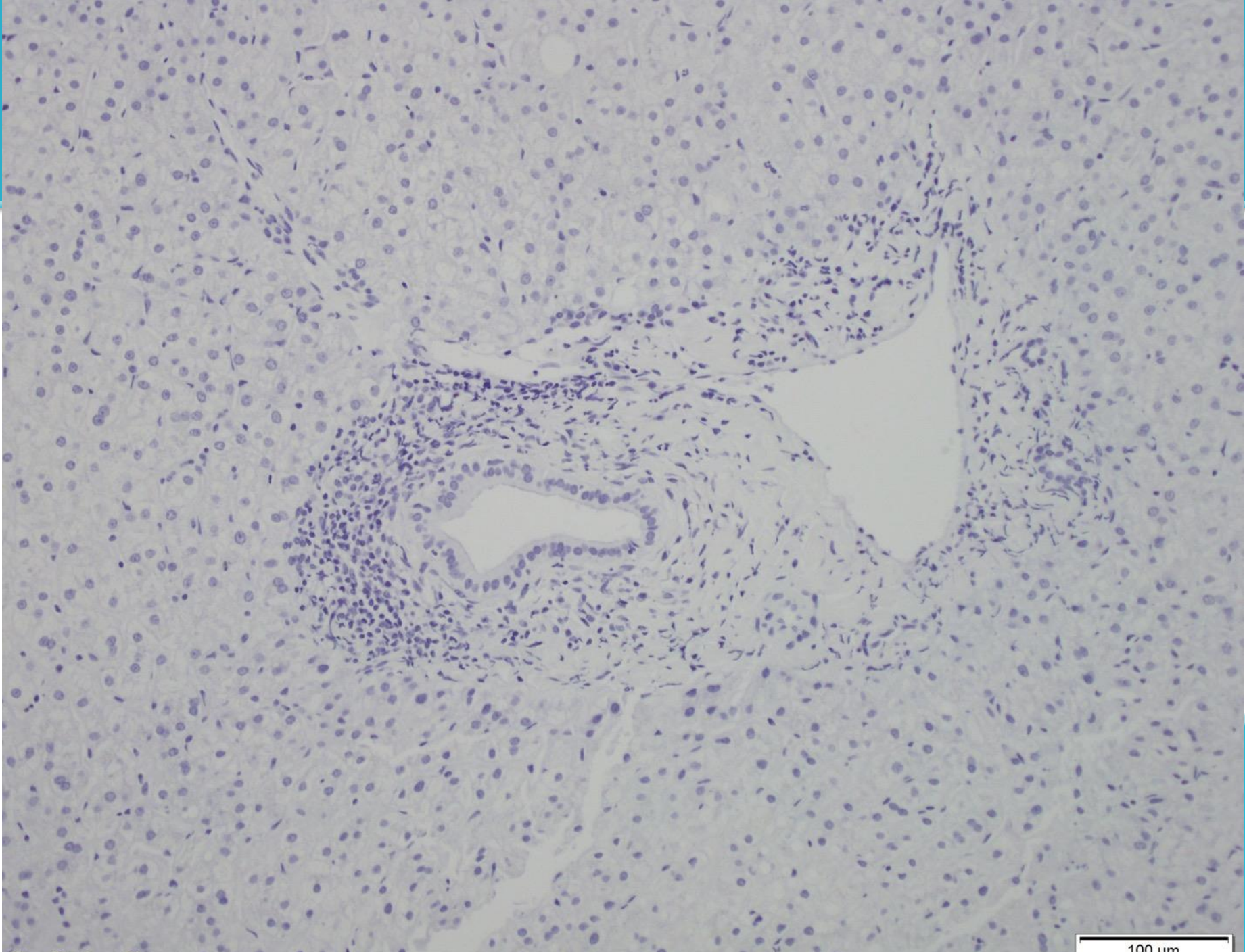
100 μ m





Magnification: 20 x

100 μ m



Magnification: 20 x

100 μ m

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 P, Sciot R, Desmet VJ.

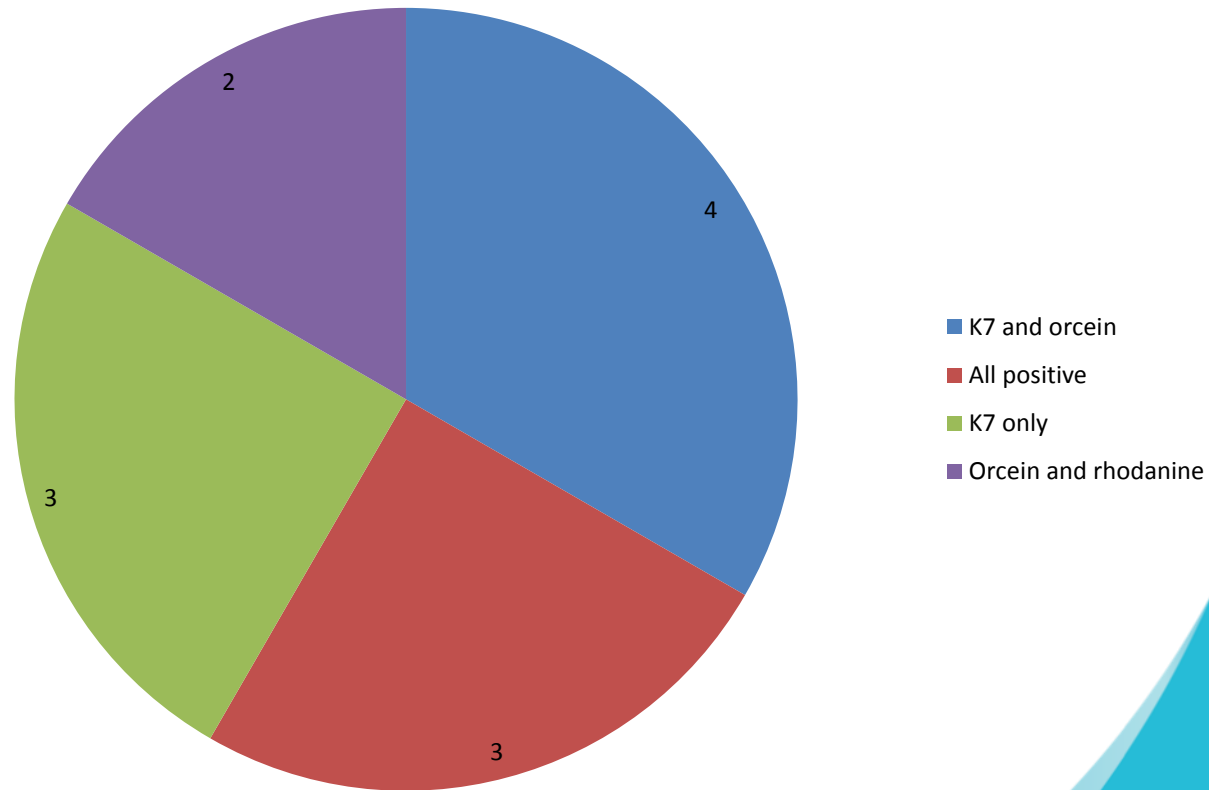
Histopathology. 1989 Aug;15(2):125-35.

Orcein vs Rhodanine vs Keratin 7 in biliary disorders

- 12 patients who underwent liver biopsy because of a clinical suspicion of a biliary disorder and/or who showed a biliary pattern of injury histologically (age range 4-67, 9 females)
- Each sample with three sections routinely stained for orcein, keratin 7 and rhodanine in the liver histopathology laboratory of the Institute of Liver Studies at King's College Hospital;
- Each sample needed to have one or more portal tracts that could be identified in all three stained sections
- Each portal tract visible in all three sections was marked, and each stain in the hepatocytes around it was assessed as follows:
 - negative—no visible granules with rhodanine or orcein stain, and no periportal K7+ve hepatocytes;
 - positive – the presence of rhodanine-positive or orcein-positive granules, ranging from a few granules identified after a careful search at high magnification (x400), or very occasional periportal K7+ve hepatocytes, to granules obvious at lower magnifications and abundant periportal K7+ve hepatocytes.

Orcein vs Rhodanine vs Keratin 7 in biliary disorders

Whole liver biopsy stains
(i.e. portal tracts
considered cumulatively)

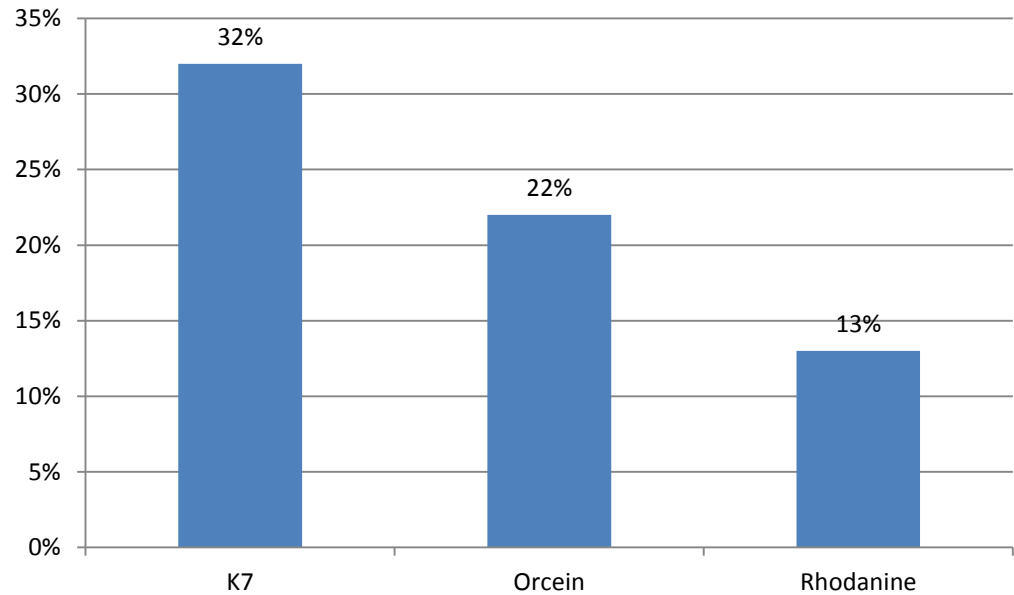


Quaglia and Bhathal Histopathology 2017;71:1003

Orcein vs Rhodanine vs Keratin 7 in biliary disorders

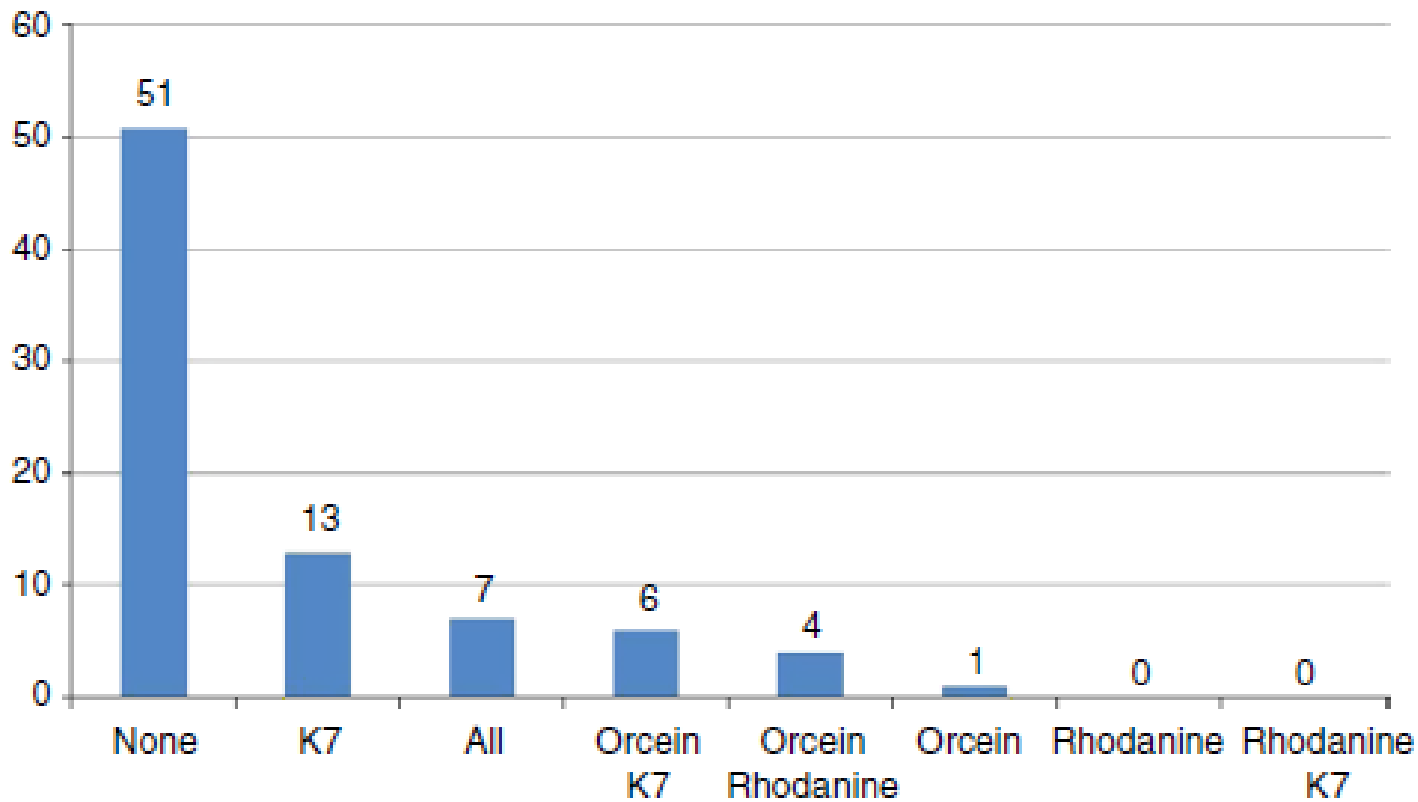
82 portal tracts. 12 biopsies. Portal tract number range 1-14, average 6.8

- K7+ve hepatocytes around 26 PT
- Orcein stain around 18 PT
- Rhodanine stain around 11 PT



Orcein vs Rhodanine vs Keratin 7 in biliary disorders

82 portal tracts. 12 biopsies. Portal tract number range 1-14, average 6.8



Summary

- Orcein stain false negative main issue
 - Histochemical technique
 - Control tissue critical
 - Interpretation
 - Coarse granules
 - 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 copper, copper-binding protein and K7 stain
 - Not reciprocally linked
 - Not clear whether one or the other becomes positive earlier
 - Orcein and K7 recommended , particularly in those cases when clinically and/or on H&E there is a suspicion of biliary pathology