

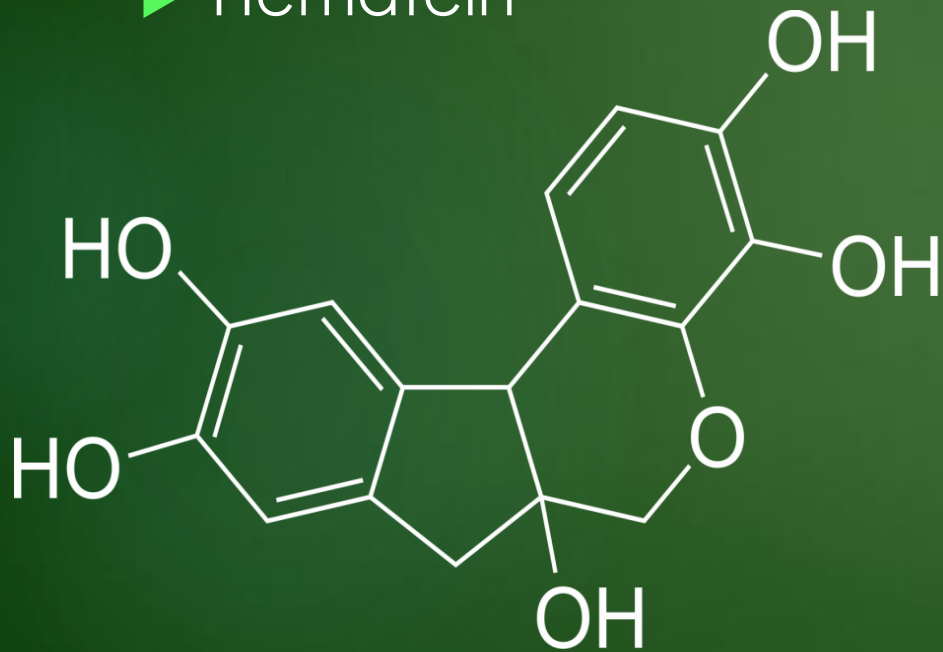
Eriochrome cyanine R: a substitute for hematoxylin

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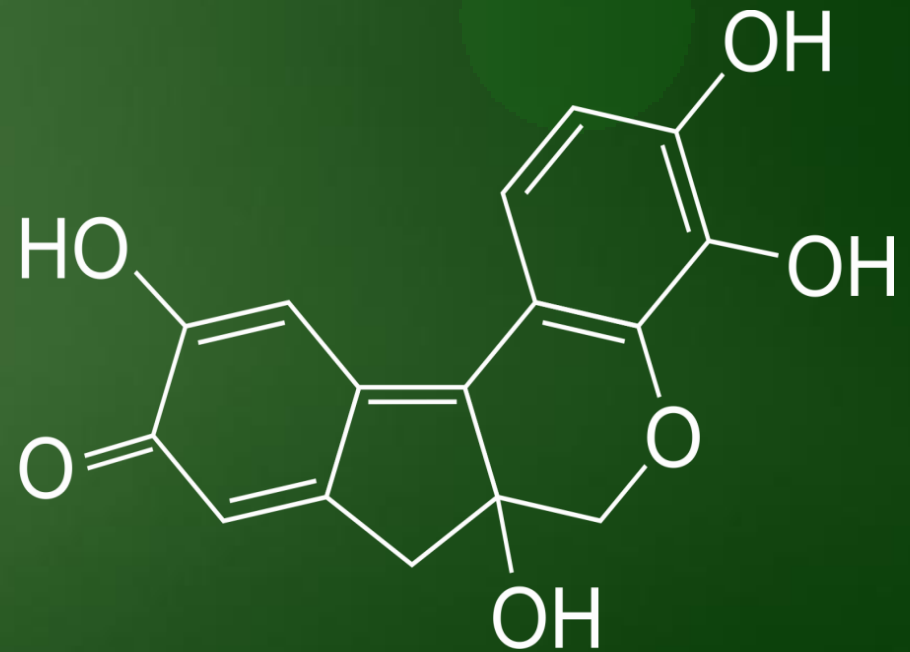
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Introduction: Hematoxylin & Hematein

- ▶ Hematoxylin, Natural Black 1, C.I. 75290
- ▶ Logwood tree, *Hematoxylon campechianum*
- ▶ Textile dyeing
- ▶ Hematein



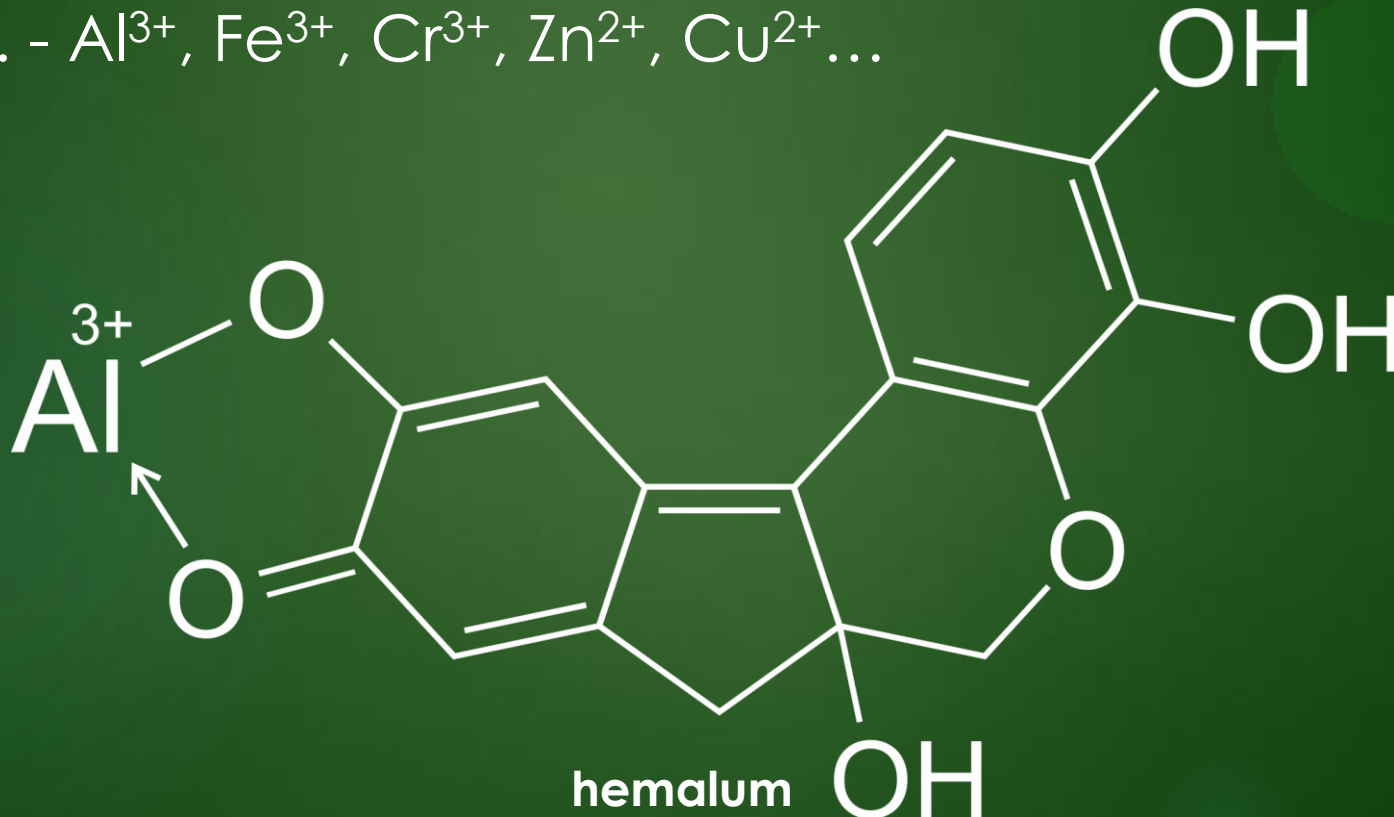
hematoxylin



hematein

Introduction: Mordants & Mordant Dyes

- ▶ A transitional metal ion with valency of at least two
- ▶ Forms chelates with certain dyes (crab's claws)
- ▶ E.g. - Al^{3+} , Fe^{3+} , Cr^{3+} , Zn^{2+} , Cu^{2+} ...



Introduction: Applications

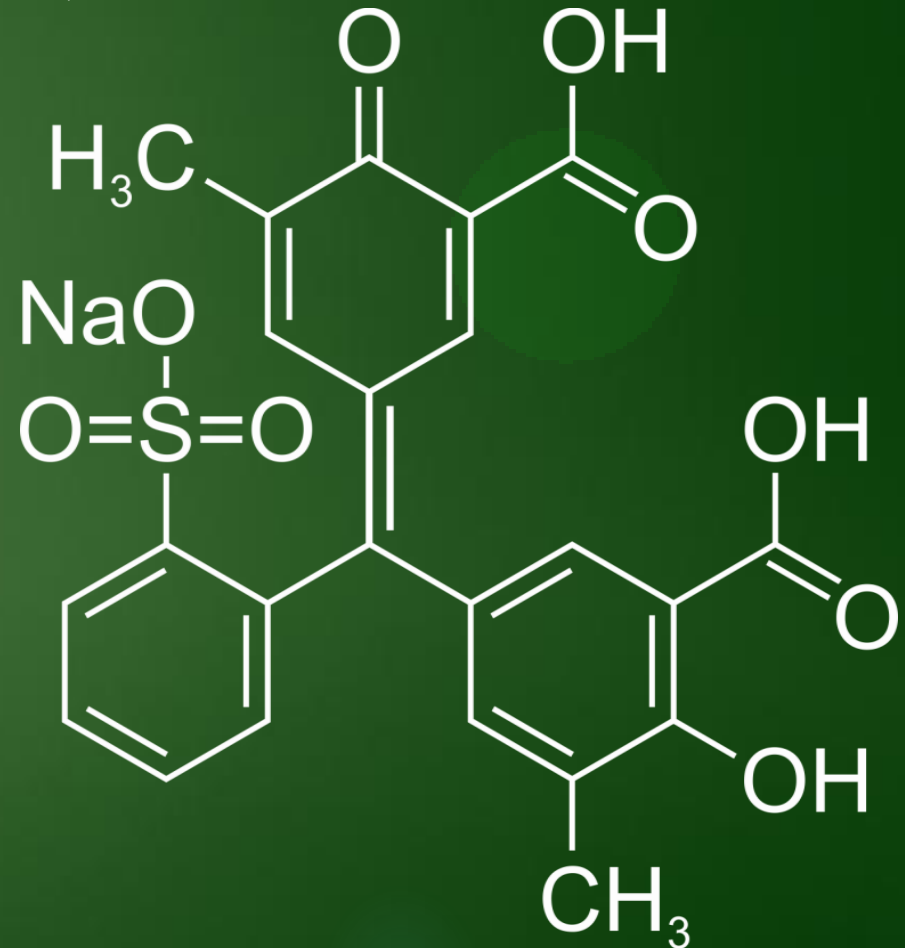
- ▶ “Hematoxylin & eosin” (H&E) = hemalum: Böhmer, 1865
- ▶ Progressive vs. Regressive
- ▶ Iron-hematein
- ▶ PTAH
- ▶ Other metal ions + hematein complexes (used seldom)
- ▶ Detection of metal deposits within a tissue

Introduction: Shortages of Hematoxylin

- ▶ In 1920s, 1970s, 2008...
- ▶ Due to technical & economical complexities of the supply chain
- ▶ Ecological responsibility?
- ▶ Potential substitutes:
 - Cationic thiazine dyes: Methylene blue, Azure B and Toluidine blue
 - Various synthetic metal-complexing dyes: **Celestine blue**
Eriochrome cyanine R

Introduction: Eriochrome Cyanine R

- ▶ Solochrome or Chromoxane CR, Mordant blue 3, C.I. 43820
- ▶ Synthetic sulfonphthalein anionic dye (red)
- ▶ pH indicator
- ▶ Forms anionic complexes with transitional metal ions



Introduction: Iron + ECR

- ▶ Kiernan (1984) describes 4 complexes, red & blue in color
- ▶ A few published methods:
 - Progressive & regressive
 - Selective nuclear staining
 - Selective myelin staining
 - Dichromatic method
- Fairly acid-resistant, lesser to alkaline sol.
- Long shelf-life of the working sol – up to 10 years!

Our Goal:

- ▶ Is Llewellyn's progressive Fe-ECR method (1978) a fair substitute for hemalum?
- ▶ How does it work in combination with eosin Y?
- ▶ How does it work on tissues fixed in:
 - Neutral buffered formaldehyde?
 - Buffered zinc-formaldehyde?
 - Neutral buffered glyoxal?

Material & Methods

- ▶ Tissues fixed in neutral buffered formaldehyde 4% (**NBF**)
 - Various ovine and porcine organs
 - Surgical biopsies of human organs
- ▶ Murine tissues fixed in:
 - Buffered zinc formaldehyde 1%+4% (**BZnF**)
 - Neutral buffered glyoxal (ethanedial) 4% (**NBG**)
- ▶ All tissues: processed in an ethanol-aliphatic xylene substitute-paraffin sequence, embedded in paraffin

Material & Methods

- ▶ Staining solution:
 - Modified Llewellyn's progressive Fe-ECR
 - pH should be between 0,9 and 1,0
 - Fe³⁺:ECR molar ratio around 1:1

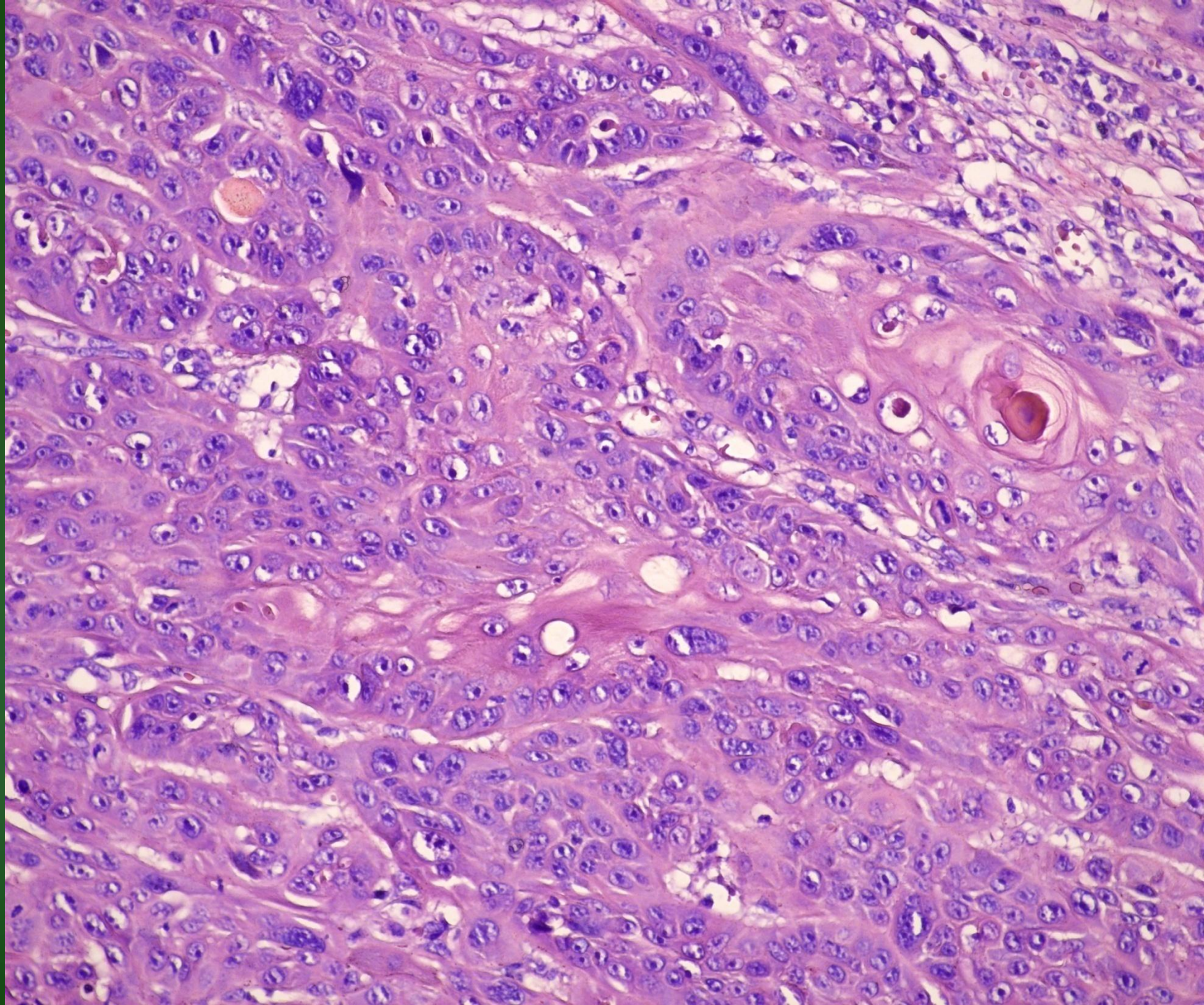
Material & Methods

► Staining procedure

1. Bring sections to water
2. Stain in the working sol – 5 min
3. Wash under running tap water – 3-5 min
4. “Blue” in 0,5% Na-acetate – 3 min
5. Wash well in dH₂O – 30 sec with agitation
6. Counterstain with aq 0.1% eosin Y, pH 5,0 – 1 min
7. Wash & differentiate in running tap water – 30 sec
8. Dehydrate in 95% & 3x100% EtOH rapidly with agitation
9. Clear & mount

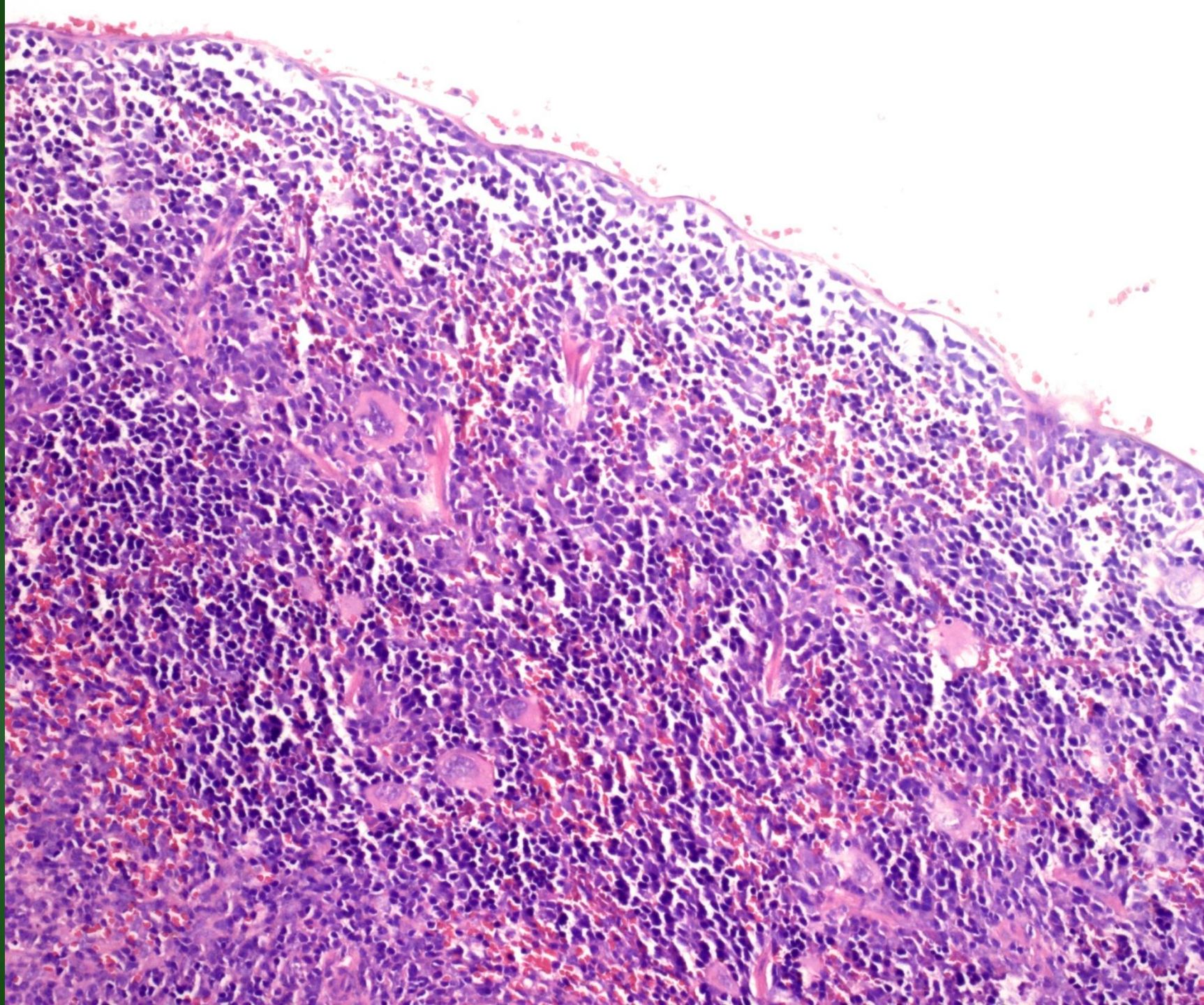
Results:

NBF fixed tissue
200x mag



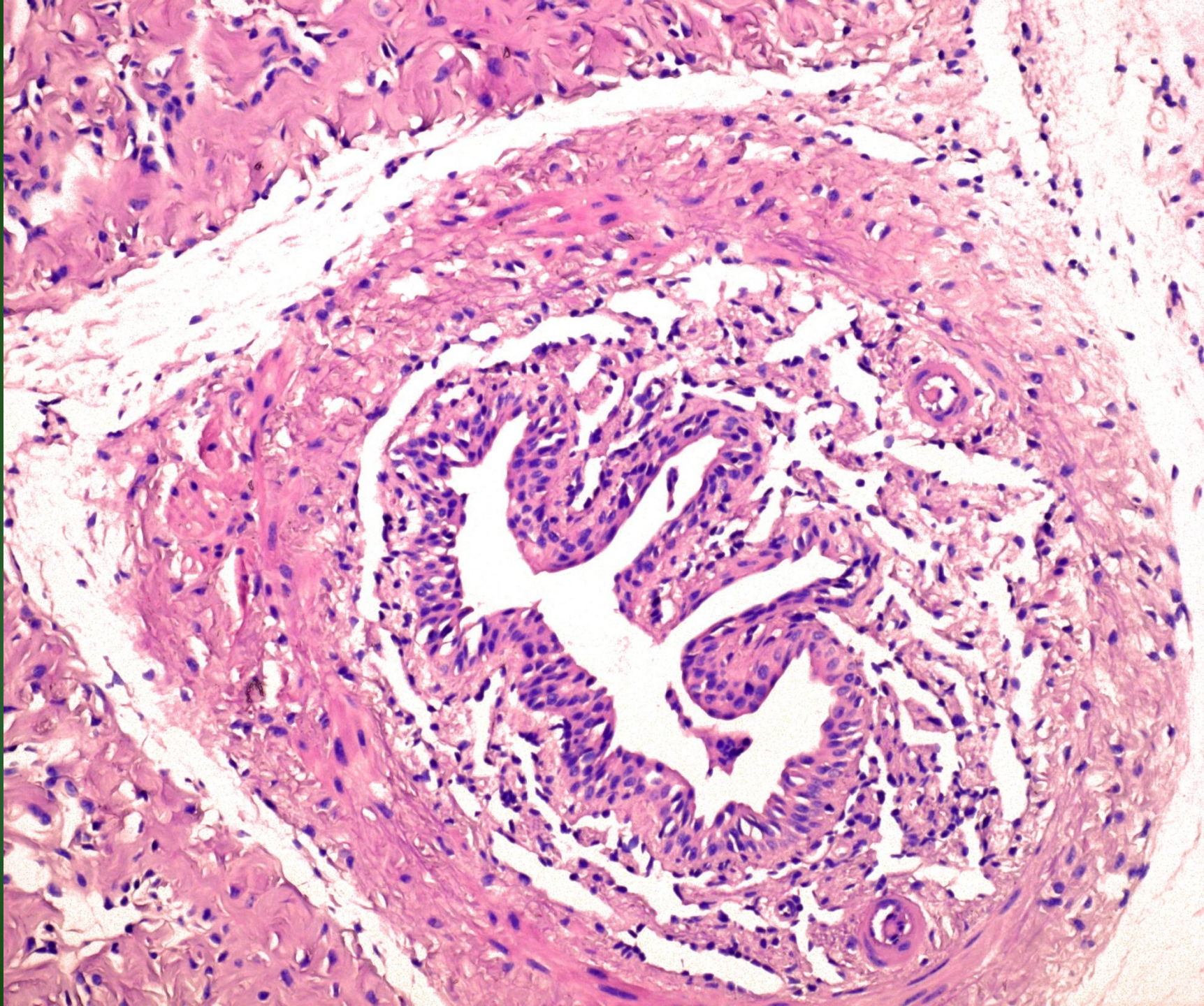
Results:

BZnF fixed tissue
200x mag



Results:

NBG fixed tissue
200x mag



Conclusions

- ▶ (Modified) Llewellyn's progressive Fe-ECR method – 5+
- ▶ Goes well with eosin Y as a H&E substitute
- ▶ Works well on NBF, BZnF and NBG fixed tissues
- ▶ Btw, NBG fixes pleasantly better compared to NBF.
- ▶ **Further comparative studies on human pathological material are needed...**

Thank you for your attention!

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