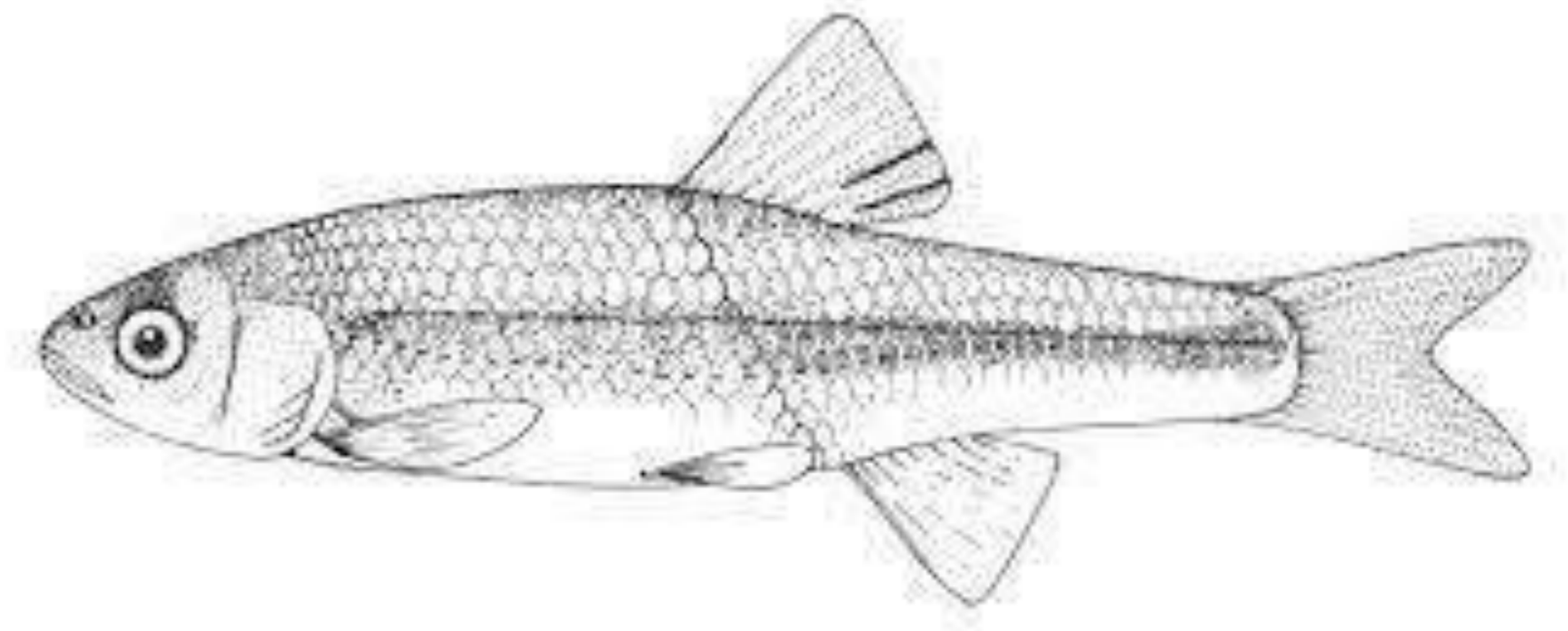
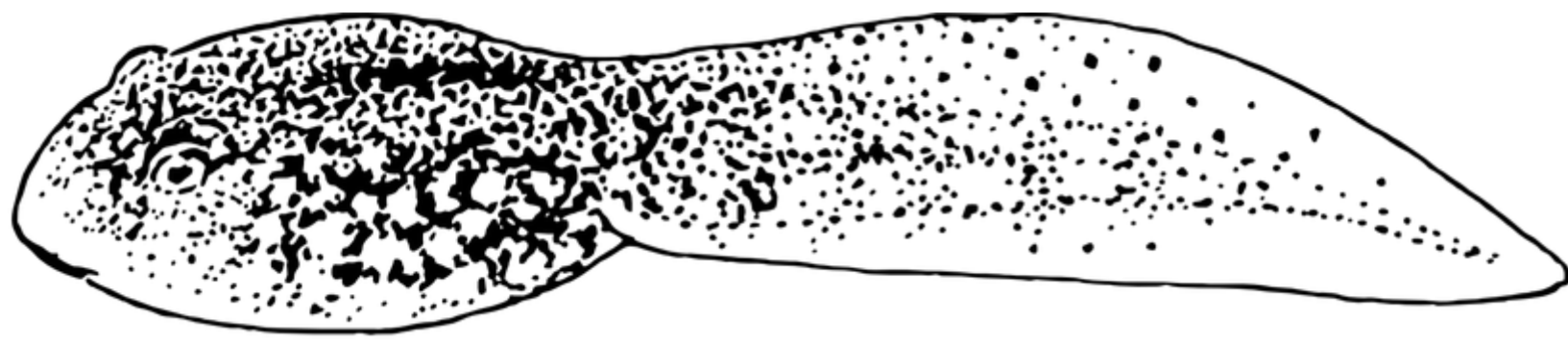


Pathological Alterations in Non-Target Tissues in AMA or FSTRA Studies Might Impact the Study Outcome.



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Examples

Summary

Standardized screening tests for potential endocrine-active substances such as the Amphibian Metamorphosis Assay (AMA) and the Fish Short Term Reproduction Assay (FSTRA) rely heavily on the histopathology endpoint. Tissue examinations in these assays are typically limited to only a few target organs such as the thyroid gland or reproductive tract, although liver and/or kidney are occasionally added. However, it is possible for studies to be impacted by pathologic changes that occur in non-target organs, many of which may be present in the same histological sections as the target tissues. Examples of such changes include cellular disturbances resulting from systemic toxicity, off-target toxicity, or stress, and immune responses to background infectious diseases. Such changes may help to explain histopathologic findings identified in target organs, and these pathologic processes may also affect other common study endpoints such as development, growth, and fecundity.

Although guidance documents for these studies either obliquely suggest or directly recommend the recording of study-relevant findings observed in non-target tissues, there may be resistance to this out of concern that this practice might add unneeded complexity to the study results. Given that a major purpose of these particular screening assays is to determine (if possible) whether effects of the test substances are endocrine-mediated, it is imperative that the pathologist obtain as much information as possible from the examined specimens. Furthermore, the pathologist has an ethical obligation to record any and all observed findings that may be study-relevant.

Guidelines/Guidances

OECD Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads

- Describe: histopathology of fish gonads
- No specific mention of what is to evaluate except some comments on kidney and liver

Histopathology Guidance Document for the Larval Amphibian Growth and Development Assay (LAGDA)

- Pathologists should specifically evaluate the target tissues identified in the guidelines; however, changes observed in other tissue types may also be recorded. This especially pertains to findings suspected to be treatment-related, or findings that might otherwise impact the study results (e.g., systemic inflammation or neoplasia).

Medaka Extended One Generation Reproduction Test (MEOGRT)

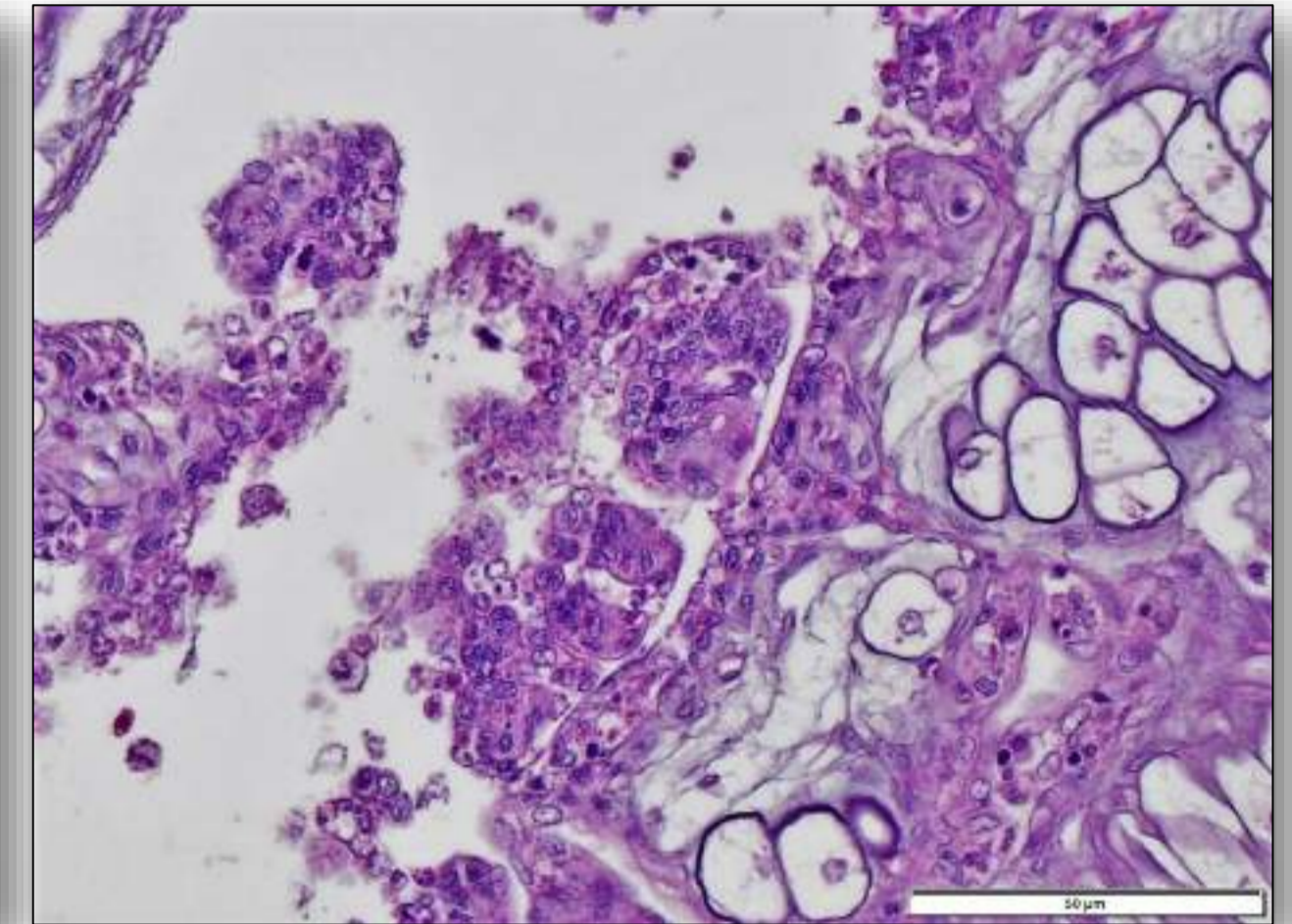
- '...Consequently, liver and kidney histopathology may also be assessed in detail to help better understand any responses in mechanistic endpoints. However, if these detailed evaluations are not performed, gross abnormalities observed incidentally during the histopathological evaluation should still be noted and reported...'

Guidance Document on Medaka Histopathology Techniques and Evaluation for the Medaka Extended One-Generation Reproduction Test (MEOGRT) - Part 1

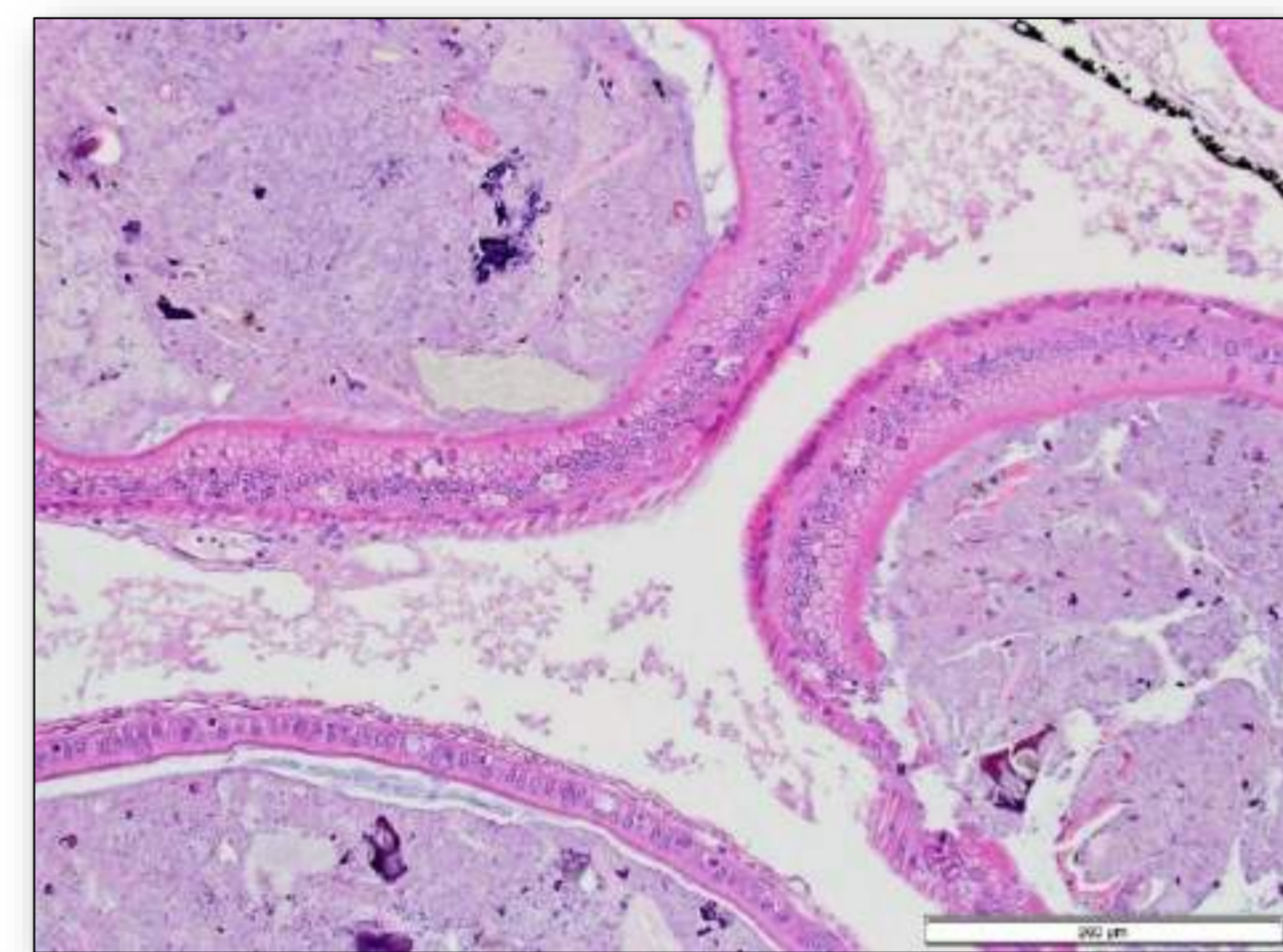
- '...Pathologists should specifically evaluate the target tissues identified in the guidelines; however, changes observed in other tissue types may also be recorded. This especially pertains to findings suspected to be treatment-related, or findings that might otherwise impact the study results (e.g., systemic inflammation or neoplasia)...'



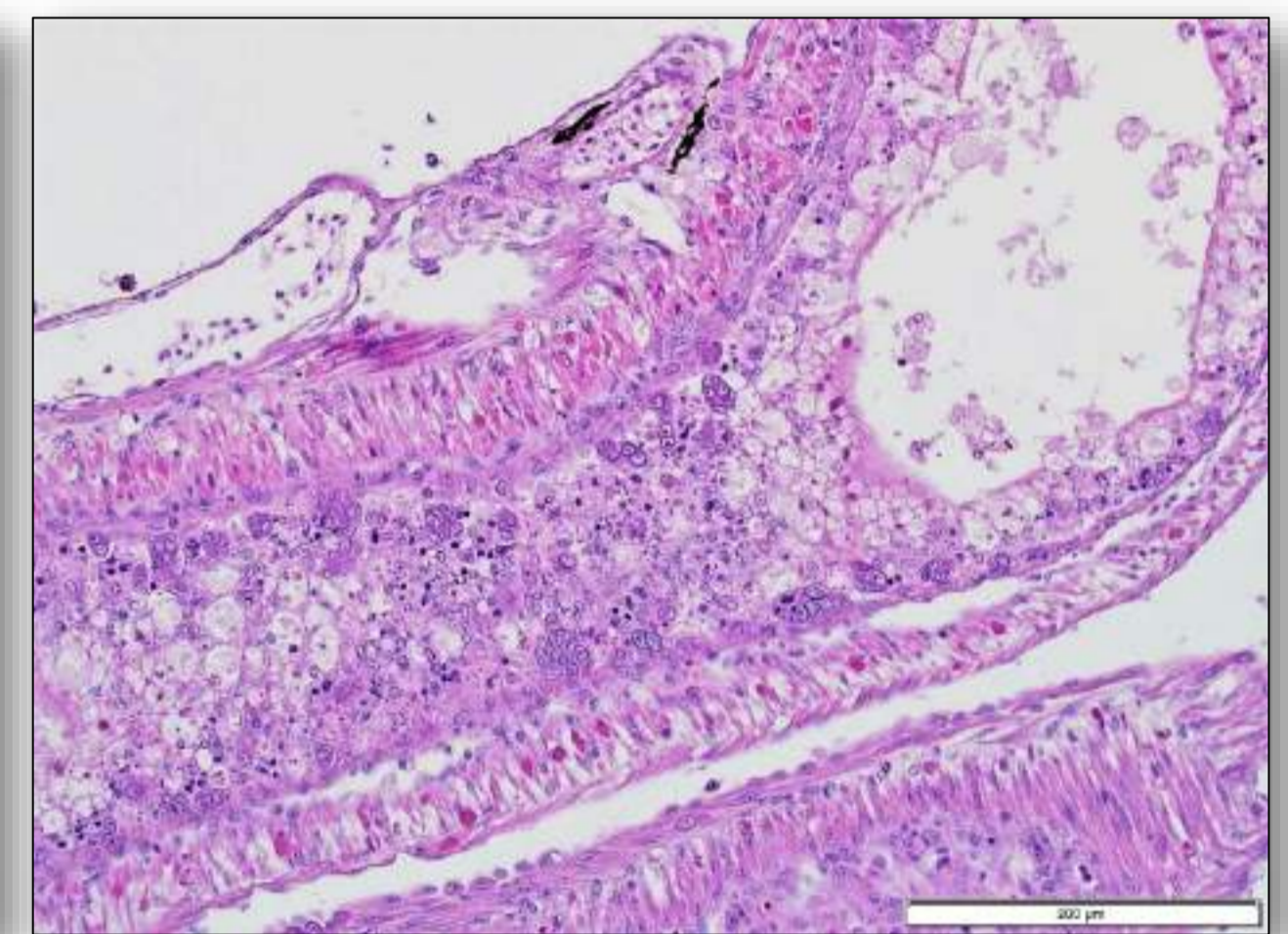
AMA. Tadpole. Normal Gills



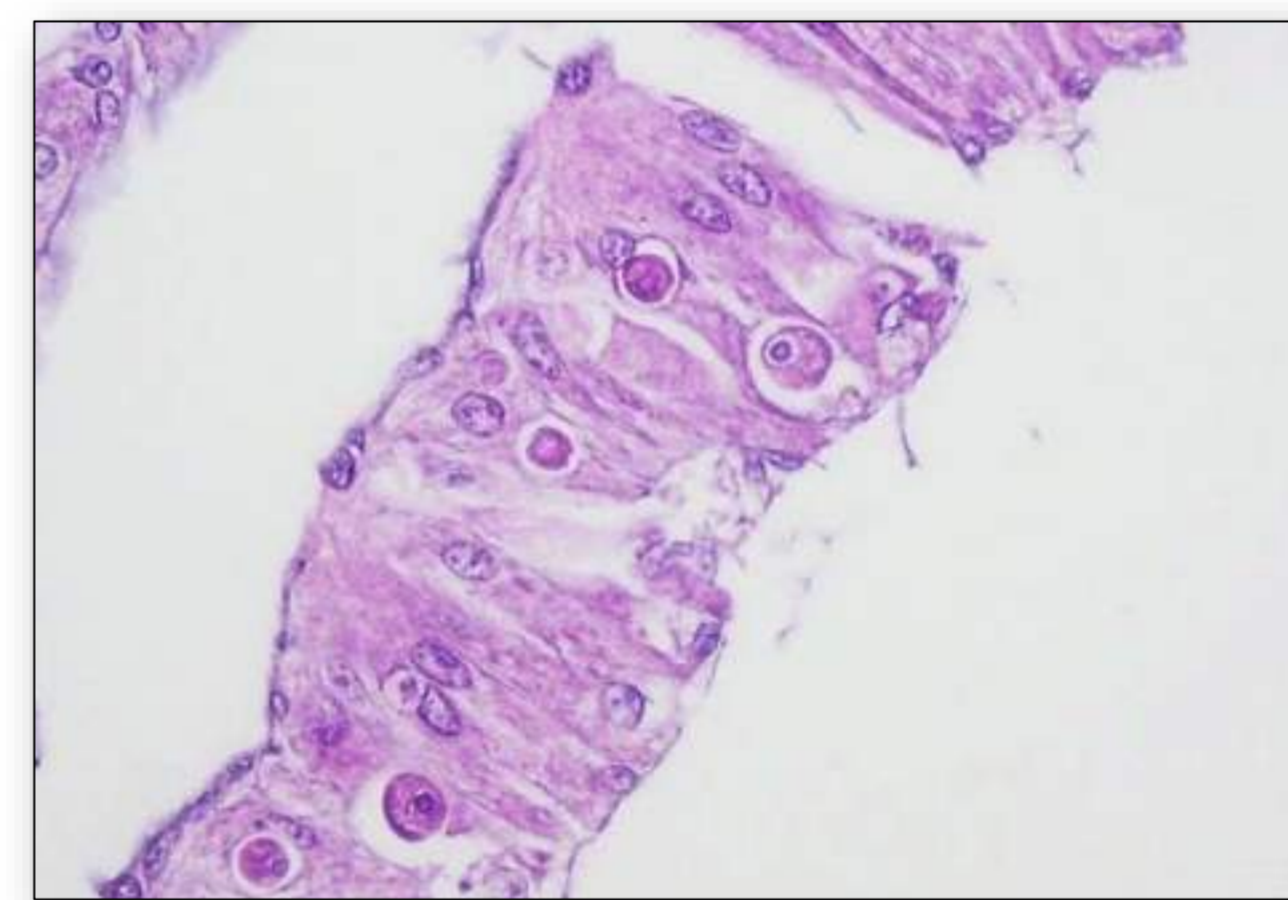
AMA. Tadpole. Epithelial necrosis and apoptosis in internal gill cavity induced by test item.



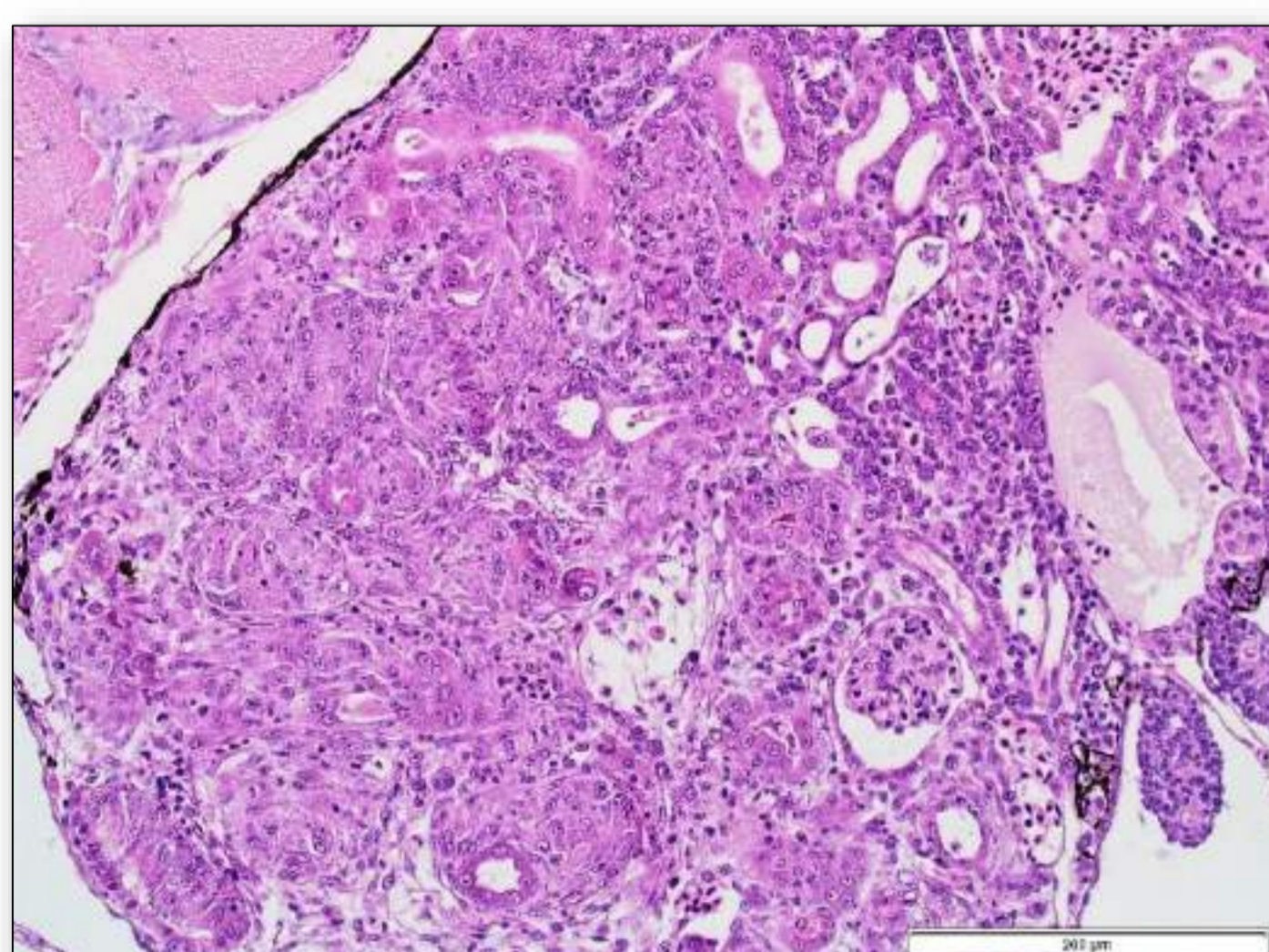
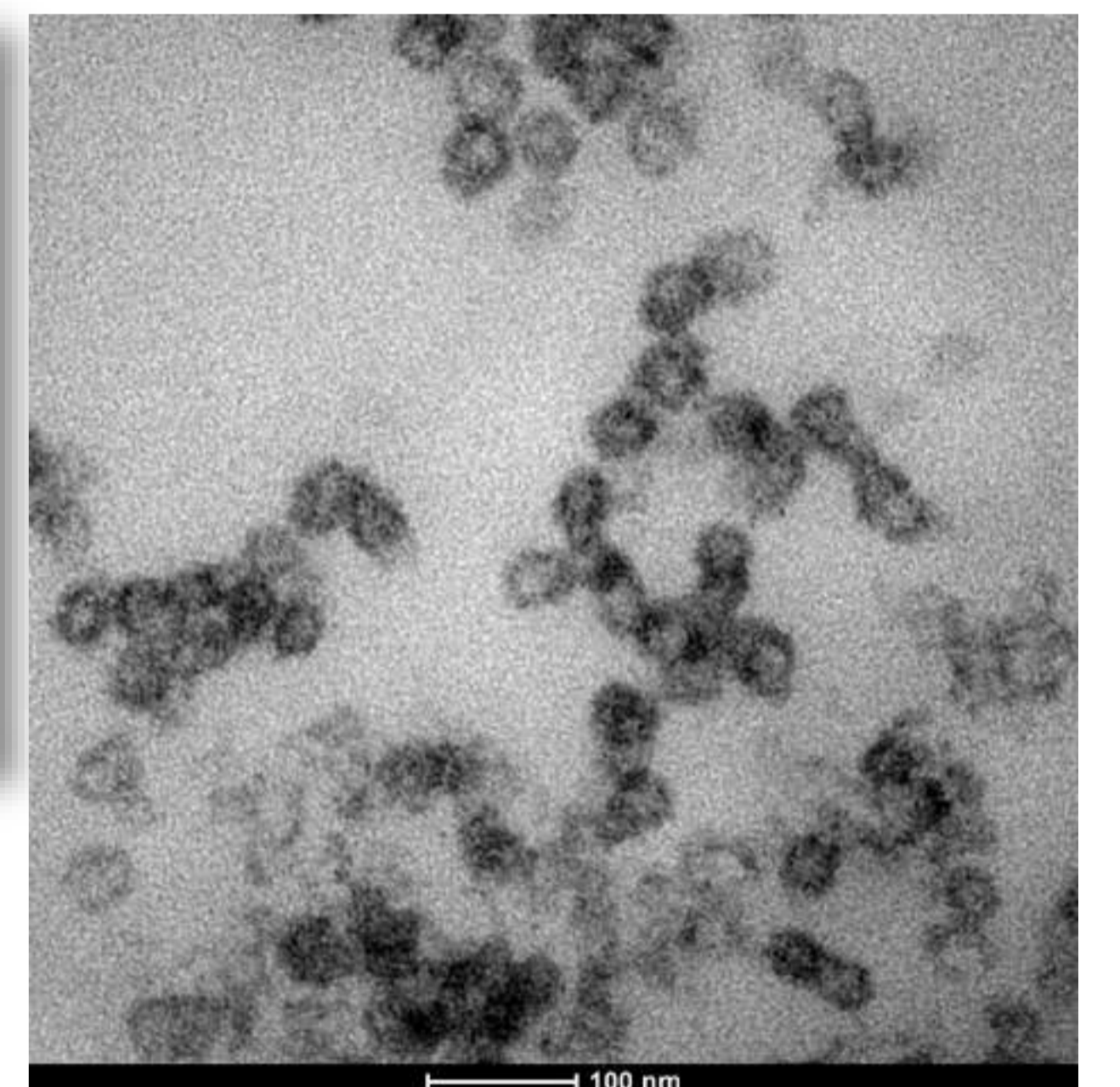
AMA. Tadpole. Normal intestinal mucosa



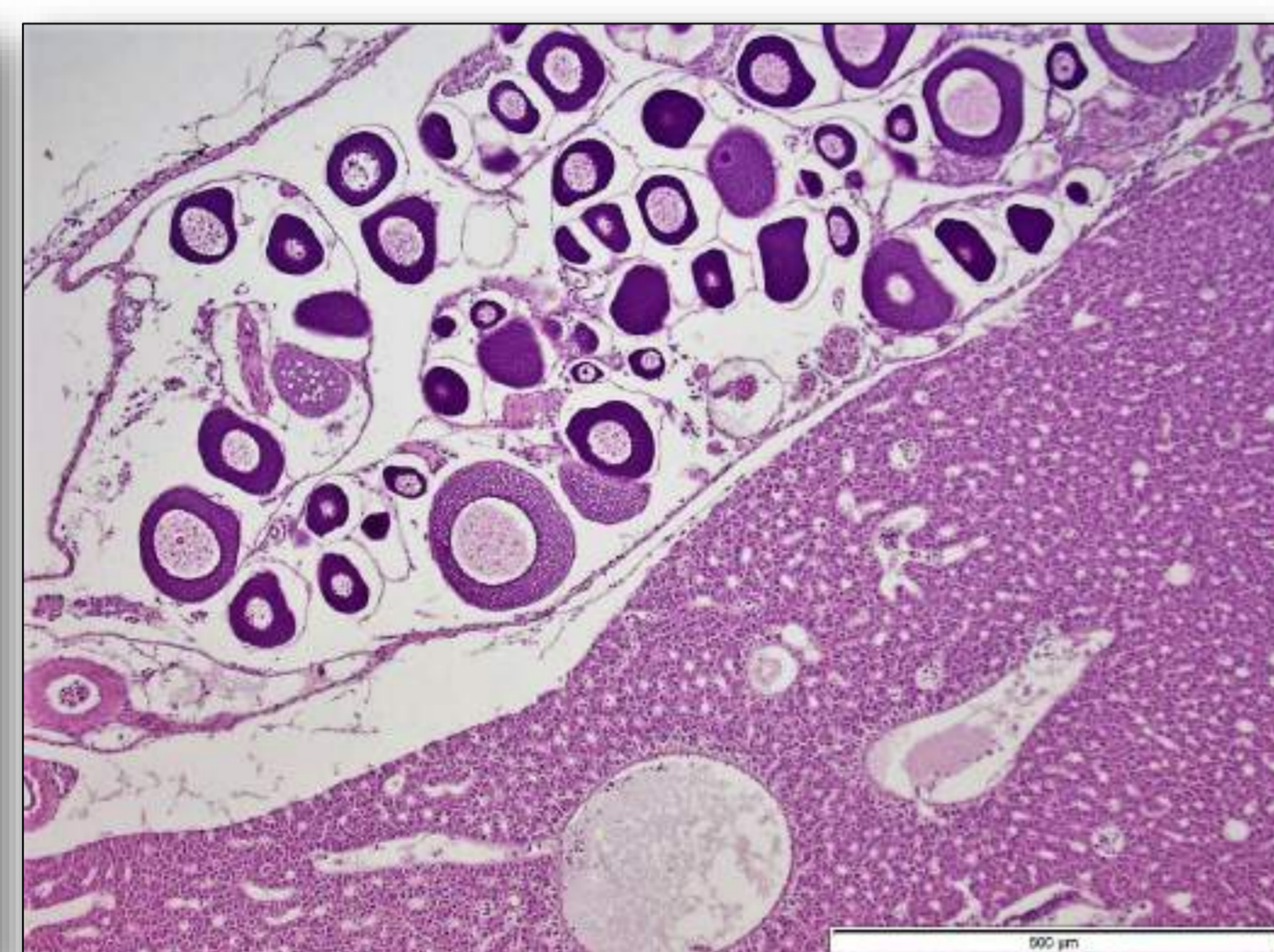
AMA. Tadpole. Intestine. Necrosis and apoptosis of mucosal epithelia induced by test item.



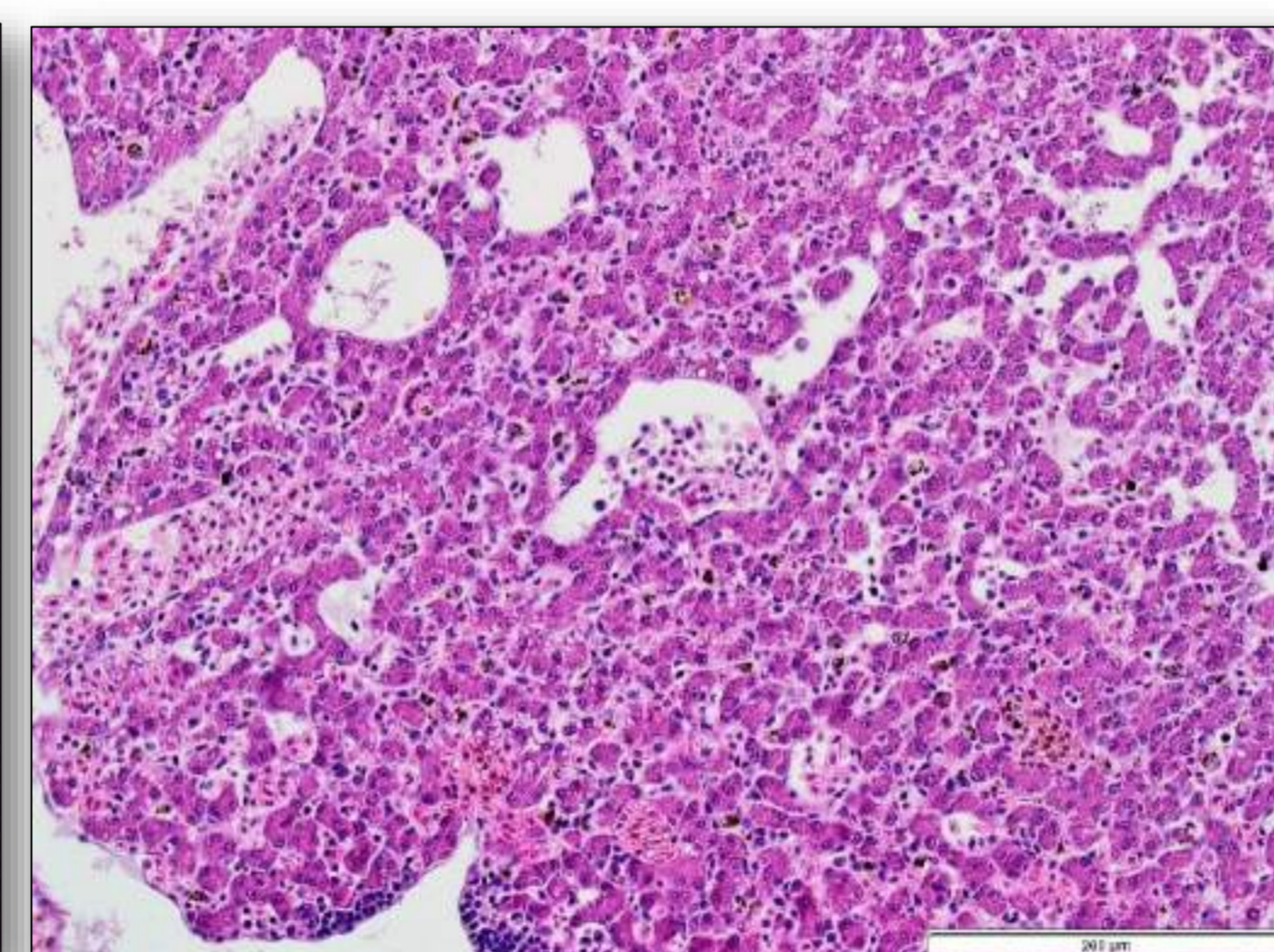
AMA. Tadpole. Oral mucosa with single cell necrosis and inclusion bodies. Inclusion bodies related to virus particels by TEM.



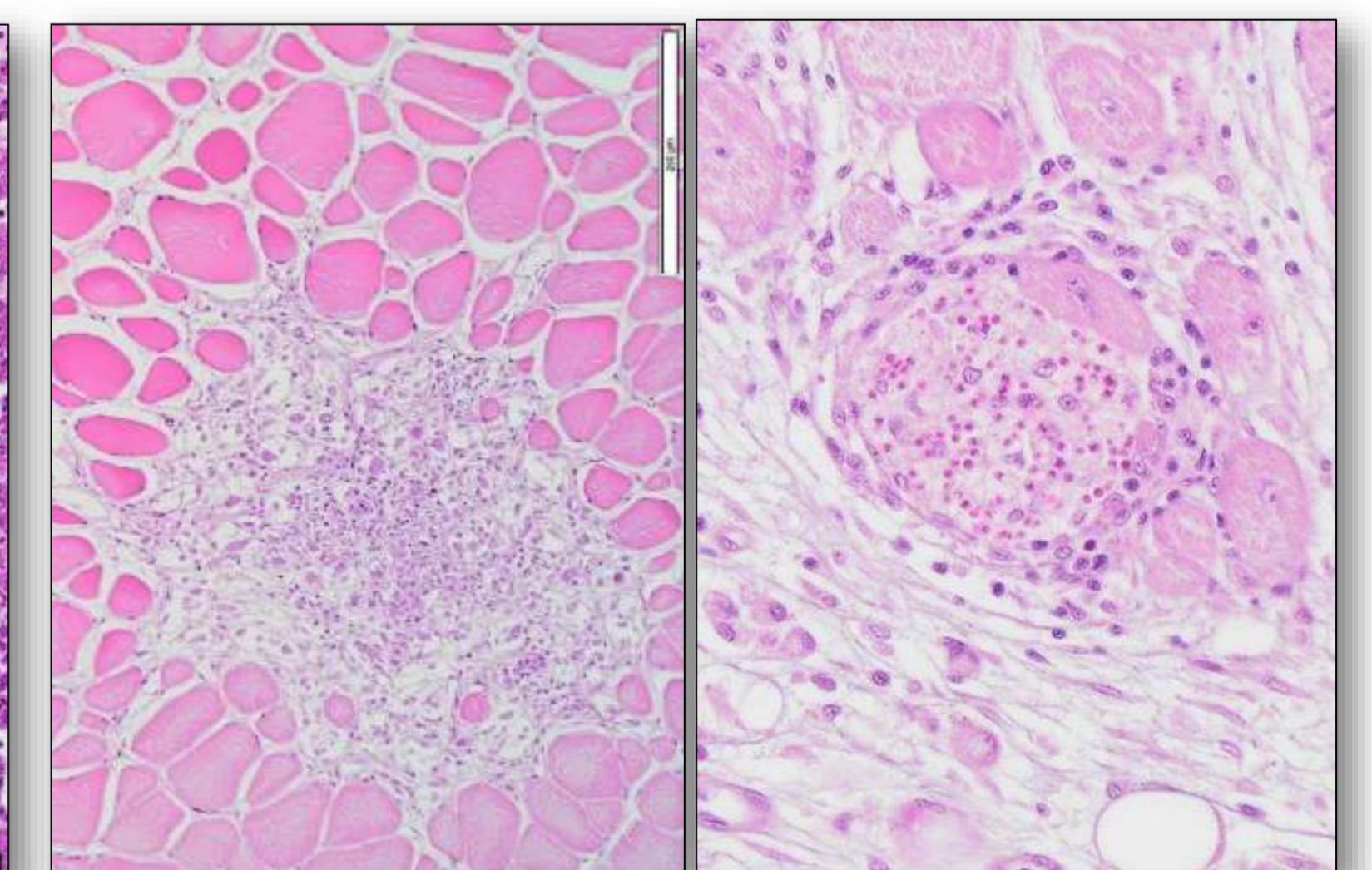
FSTRA. Fathead minnow. Nephritis induced by test item.



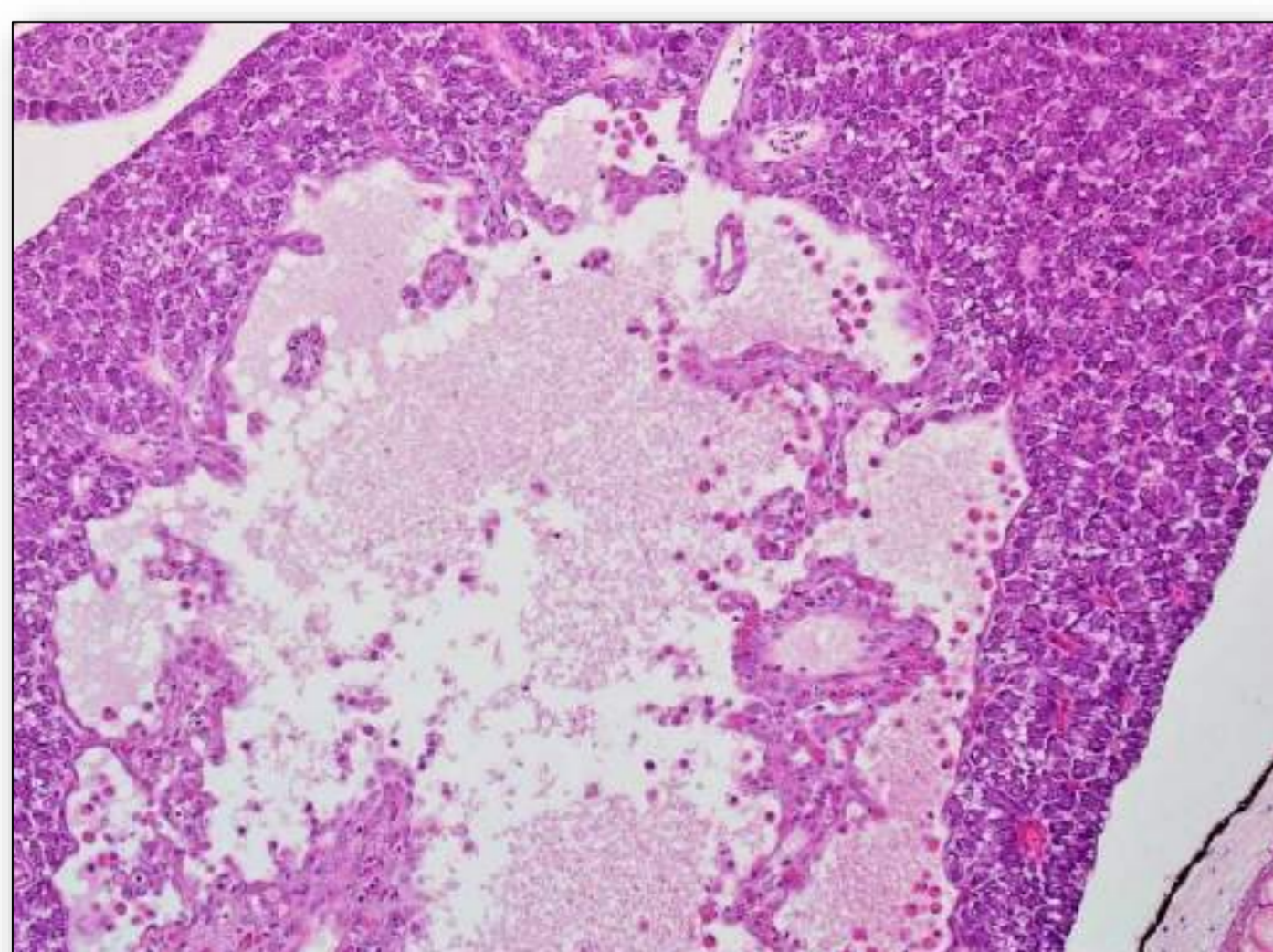
FSTRA. Fathead minnow. Low stages of ovary development related to nephritis.



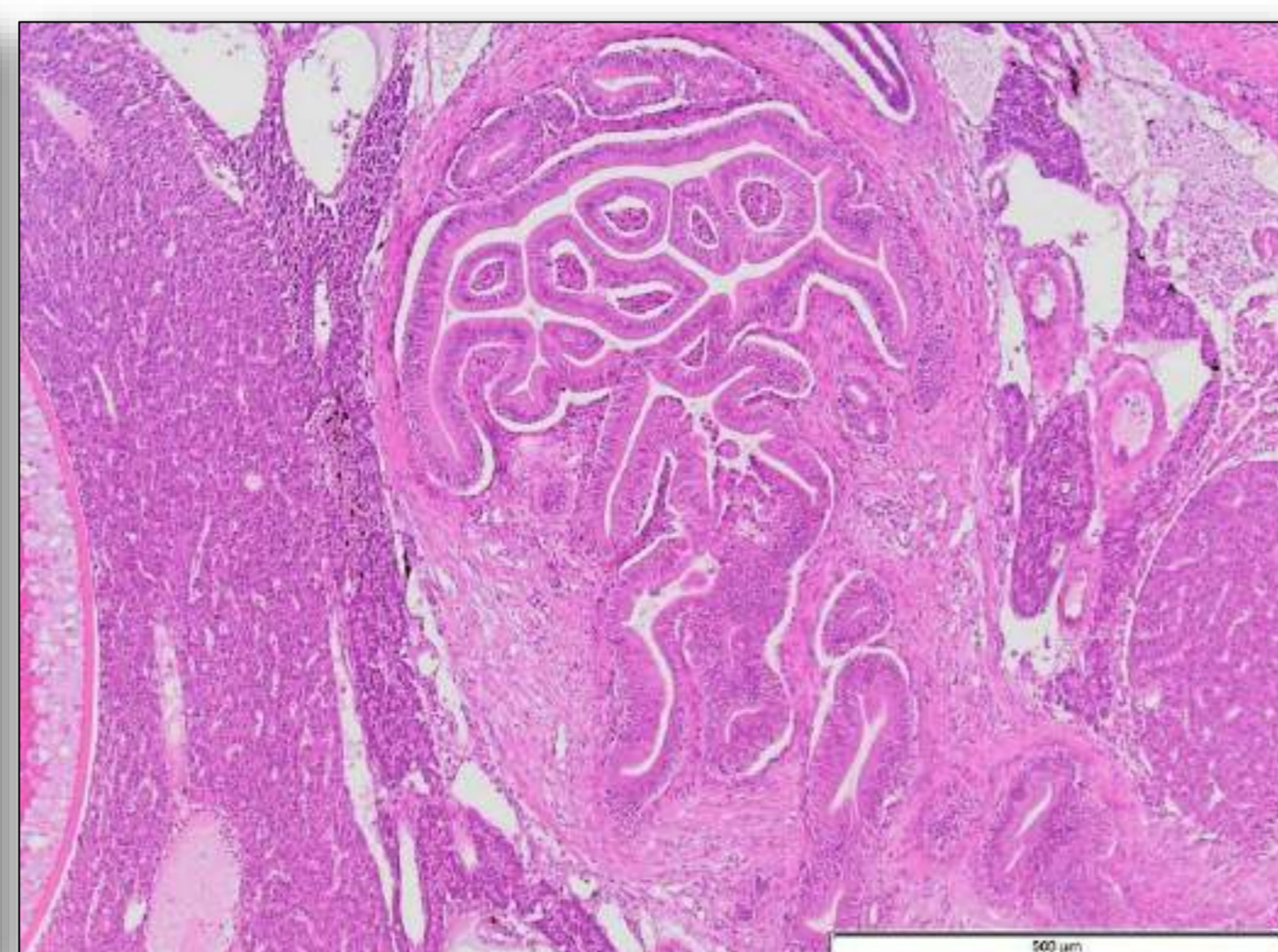
FSTRA. Fathead minnow. Reduced hepatocellular glycogen related to nephritis.



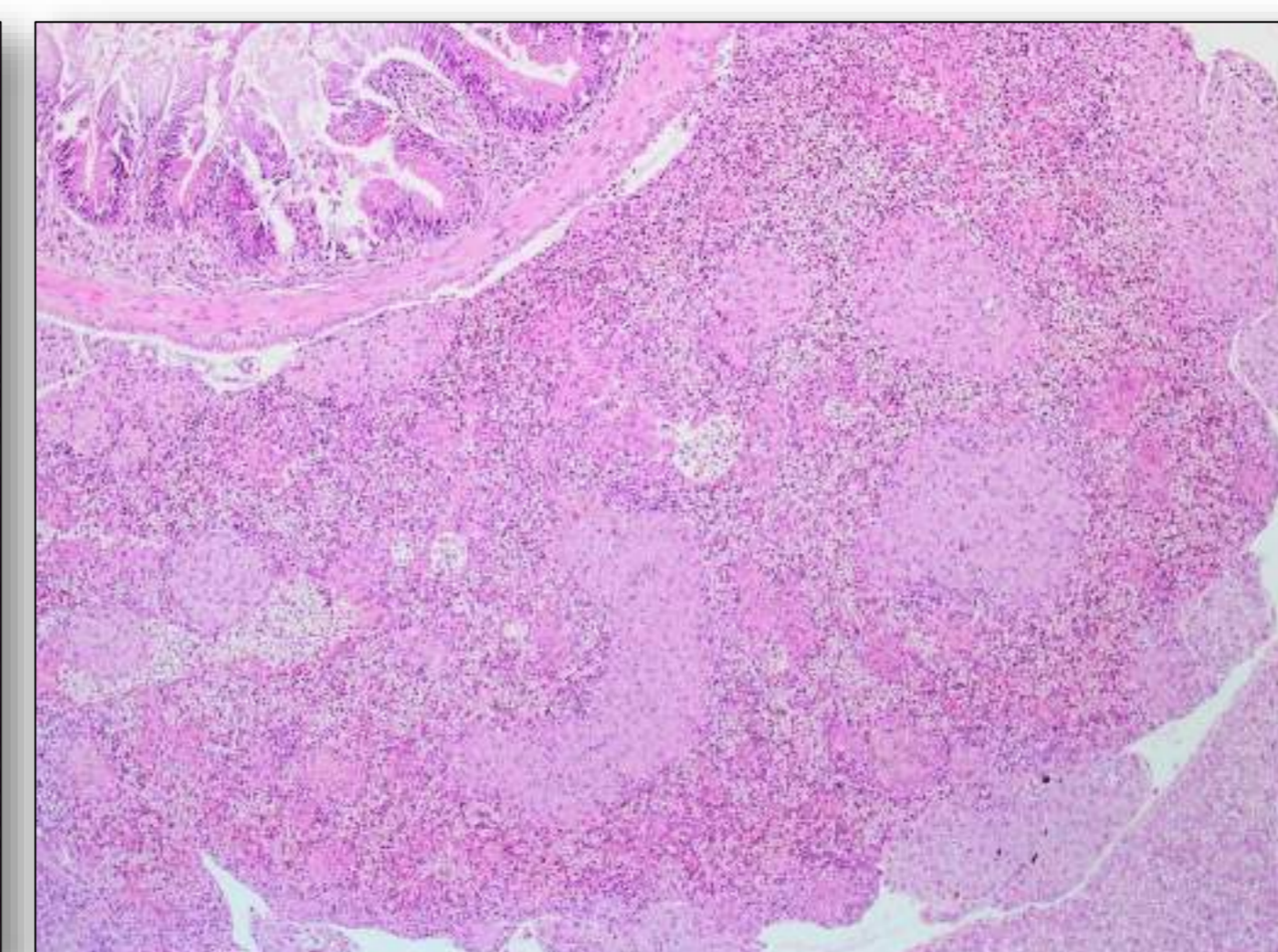
FSTRA. Fathead minnow. Necrosis of dorsal musculature. Infection by *Myxoplasma spec.*



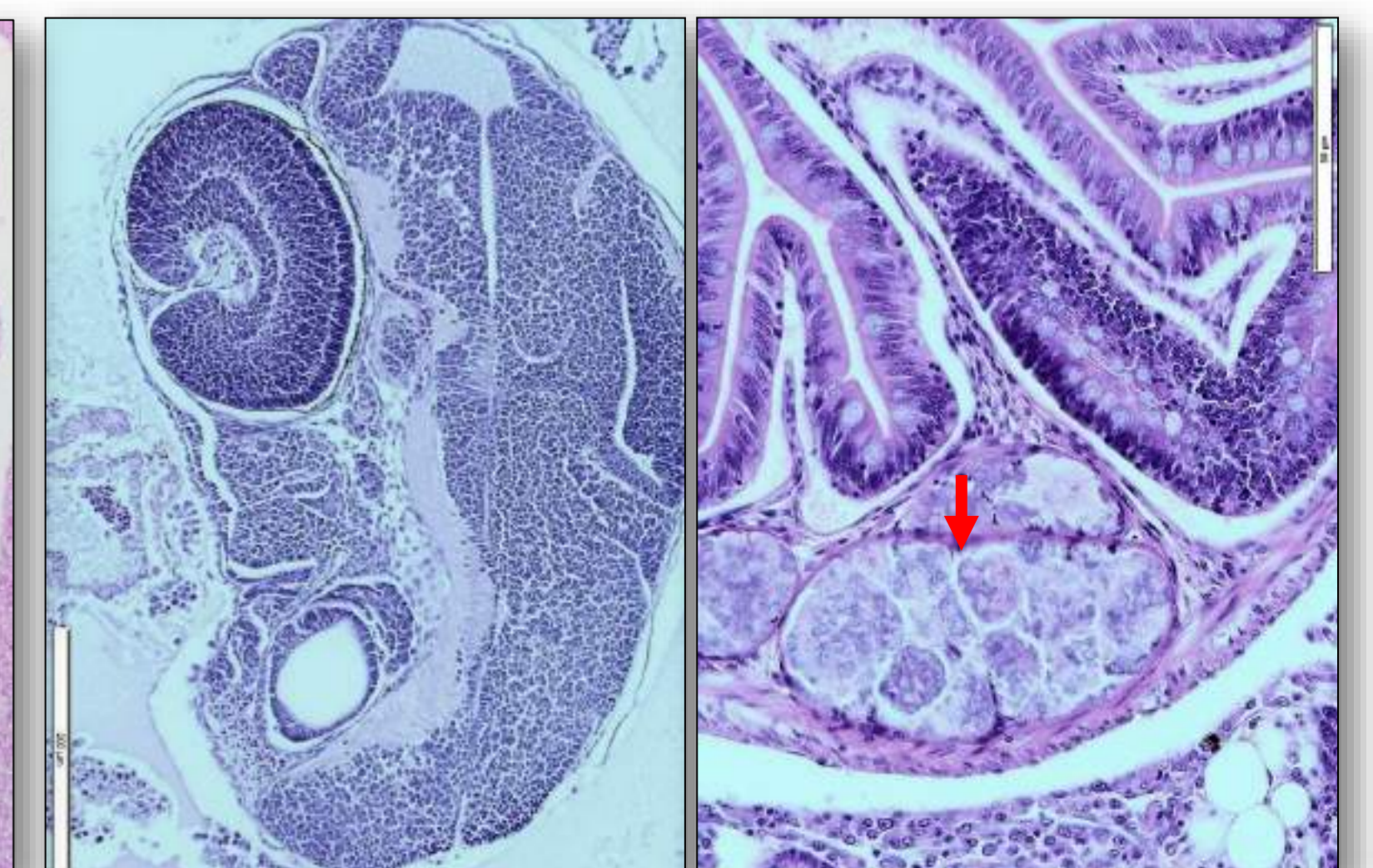
FSTRA. Fathead minnow. Angiomatous lesion in liver.



FSTRA. Fathead minnow. Periductal adenoma in abdominal cavity.



FSTRA. Fathead minnow. Granulomas in spleen deemed to be by infection.



FSTRA. Fathead minnow. Manifestation of cestode (helminth) in intestine, likely *Botrycephalus spec.* Right side: larval cysts,