

USEPA Pathology Report for PTU

MATERIALS AND METHODS

Slides were prepared by Experimental Pathology Laboratories, Inc. (EPL[®]). Head samples that were trimmed at EPA-MED were placed in cassettes with the cut side down so that the heads were sectioned in a transverse plane from caudal to rostral. Initially, five step sections (30 microns apart) were taken from each block, and two serial sections of each step were placed on each of five slides. These slides were examined by a technician to assure that a sufficient amount of thyroid tissue was present bilaterally in at least two of the step sections. If necessary to meet these criteria, additional step sections were cut from the block. The slides were stained with hematoxylin and eosin and the sections were covered with glass cover slips. Two of the step sections from each frog were selected for evaluation of the status of the follicular cells, the relative size of the follicle and associated colloid, and the size of the entire thyroid gland section on the slide. The changes are graded from 1 to 5 depending upon severity (1=minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high). Non-gradable changes are designated as present (P).

RESULTS

Stage 51 *Xenopus laevis* exposed to PTU for 21 Days

There were exposure-related changes in the thyroid gland, which included follicular cell hyperplasia, distension of thyroid follicles, and diffuse enlargement of the thyroid glands. At the highest exposure concentration (20 mg/L PTU), the colloid in nine of 10 thyroids had a light eosinophilic foamy appearance in contrast to the dark eosinophilic homogeneous appearance of the colloid in frog thyroids at all other exposure concentrations. Two frogs at the lowest exposure concentration (1.25 mg/L PTU) and one frog at the 2.50 mg/L exposure concentration had minimal distension of thyroid follicles. Overall, follicular distension accompanied by diffuse enlargement of the thyroid glands increased in prevalence and severity as the exposure concentration of PTU increased from 2.50 mg/L to 20 mg/L. Hyperplasia of follicular cells occurred at the two highest concentrations of PTU. The severity of follicular cell hyperplasia varied from minimal to slight/mild at 10 mg/L PTU, and from mild to moderately severe at 20 mg/L PTU. See Table 1 for a tabulation of the prevalence of the changes described above. One frog from each of the six exposure concentrations is illustrated by photographs taken at low magnification (1.25x) and high magnification (10x). The photographs are annotated to illustrate the various thyroid changes.

Table 1. Prevalence (as percentage of total) of selected changes in the thyroid gland of stage 51 *Xenopus laevis* exposed to PTU at various concentrations for 21 days.

PTU Exposure Concentration	0 mg/L	1.25 mg/L	2.50 mg/L	5.0 mg/L	10 mg/L	20 mg/L
Number of organisms examined	(9)	(9)	(10)	(10)	(7)	(10)
Colloid, Light Eosinophilic Foamy	0	0	0	0	0	90
Colloid, Light Eosinophilic Homogeneous	0	0	0	0	0	10
Follicular distension (enlargement)	0	22	10	80	100	100
Glandular hypertrophy	0	0	20	50	100	100
Follicular cell hyperplasia,	0	0	0	0	100	100

Stage 54 *Xenopus laevis* exposed to PTU for 14 days

There were exposure-related changes in the thyroid gland which included follicular cell hyperplasia, distension of thyroid follicles and diffuse enlargement of the thyroid glands. At the highest exposure concentration (20 mg/L), the colloid in all 10 frog thyroids had a light eosinophilic foamy appearance in contrast to the light eosinophilic homogeneous appearance of the colloid in six of ten frog thyroids at the 10 mg/L PTU exposure concentration. In all other frogs, colloid was homogeneous dark eosinophilic. The prevalence of thyroid follicle distension was exposure concentration related at 10 and 20 mg/L PTU, but was not exposure concentration related at concentrations lower than 10 mg/L. Diffuse enlargement of the thyroid glands increased in prevalence from the 5.0 to 20 mg/L exposure concentrations and increased in severity from slight/mild at 5.0 and 10 mg/L to moderately severe at 20 mg/L. Follicular cell hyperplasia occurred at only the highest exposure concentration and was moderate in severity. See Table 2 for a tabulation of the prevalence of the changes described above. One frog from each of the six exposure concentrations is illustrated by photographs taken at a low magnification (1.25x) and a high magnification (10x). The photographs are annotated to illustrate the various thyroid changes.

Table 2. Prevalence (as percentage of total) of selected changes in the thyroid gland of stage 54 *Xenopus laevis* exposed to PTU at various concentrations for 14 days.

PTU Exposure Concentration	0 mg/L	1.25 mg/L	2.50 mg/L	5.0 mg/L	10 mg/L	20 mg/L
Number of organisms examined	(10)	(10)	(10)	(10)	(10)	(10)
Colloid, Light Eosinophilic Foamy	0	0	0	0	0	100
Colloid, Light Eosinophilic Homogeneous	0	0	0	0	60	0
Follicular distension (enlargement)	10	0	30	10	40	80
Glandular hypertrophy	0	0	0	30	80	100
Follicular cell hyperplasia,	0	0	0	0	0	100

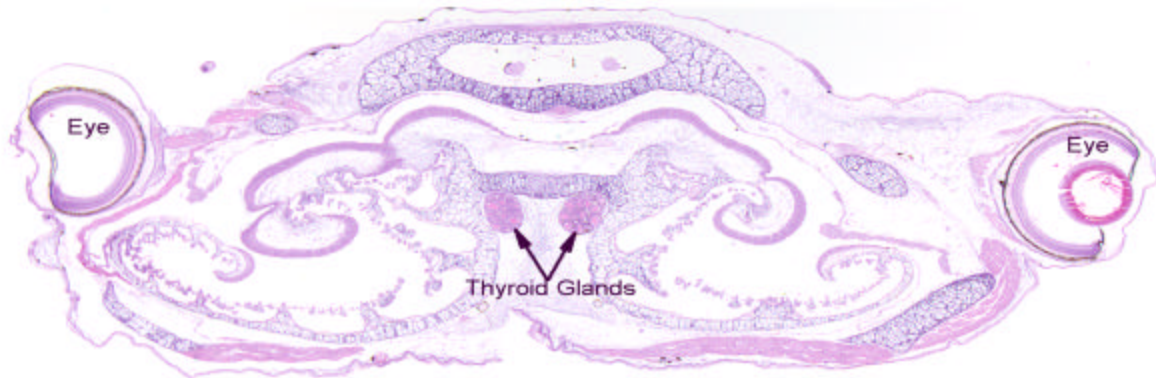
DISCUSSION

Effects of PTU on the thyroid glands of the African clawed frogs were apparent both in frogs that were exposed at Stage 51 for 21 days and Stage 54 for 14 days. At the highest exposure concentration of PTU, all the frogs were affected with thyroid follicular cell hyperplasia and diffuse thyroid gland enlargement despite the developmental stage of the frog at the start of the test or the duration of the test. However, the average severity of these changes was slightly greater in frogs exposed for 21 days than in frogs exposed for 14 days. In the 21-day exposure, the enlargement of the thyroid glands was observed minimally at the 2.50 and 5.0 mg/L exposure concentrations, whereas in the 14-day exposure, thyroid gland enlargement was observed first at the 5.0mg/L exposure concentration. Thyroid follicular cell hyperplasia occurred at both the 10 and 20 mg/L exposure concentrations in frogs exposed for 21 days, but it was diagnosed only at the 20 mg/L exposure concentration in frogs exposed for 14 days. It is not possible to determine

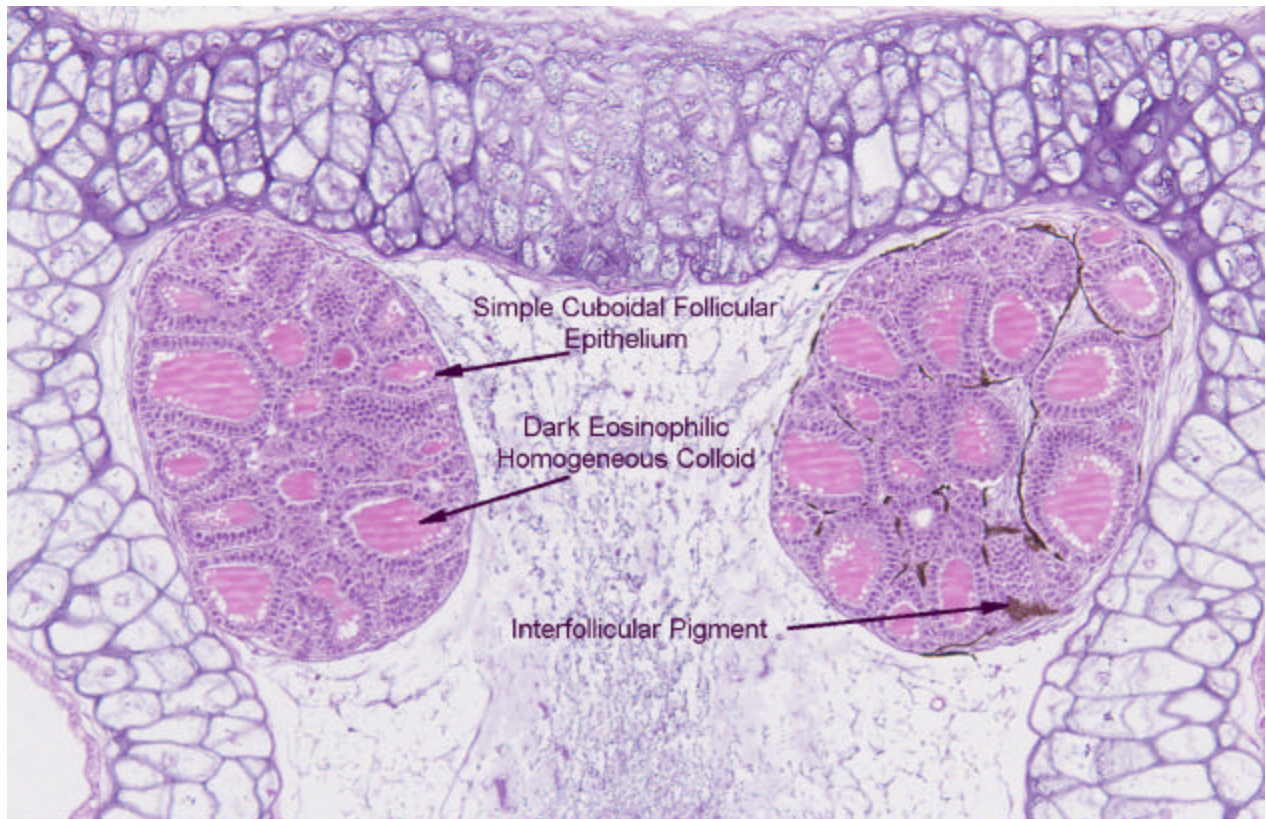
if the differences in response relative to exposure concentration in these two tests were due to the stage of frog development at the initiation of the exposure or to the length of the exposure.

There was a marked difference in the appearance of the colloid in frogs exposed to the highest concentration of PTU as compared to the colloid in frogs exposed to PTU concentrations lower than 20 mg/L. The colloid at the highest concentration was light eosinophilic and foamy. Most of the frogs at the other exposure concentrations had colloid that was dark eosinophilic and homogeneous. Colloid contains thyroglobulin, a high molecular weight glycoprotein. Upon proteolysis, active thyroid hormone is released from thyroglobulin. The foamy appearance of the colloid in thyroid glands that are inhibited in hormone production by the highest concentration of PTU may reflect the loss of thyroid hormone as it is being utilized but not replaced.

APPENDIX A: LIGHT MICROSCOPY PHOTOGRAPHS
STAGE 51 TOADS EXPOSED TO VARIOUS
CONCENTRATIONS FOR 21 DAYS

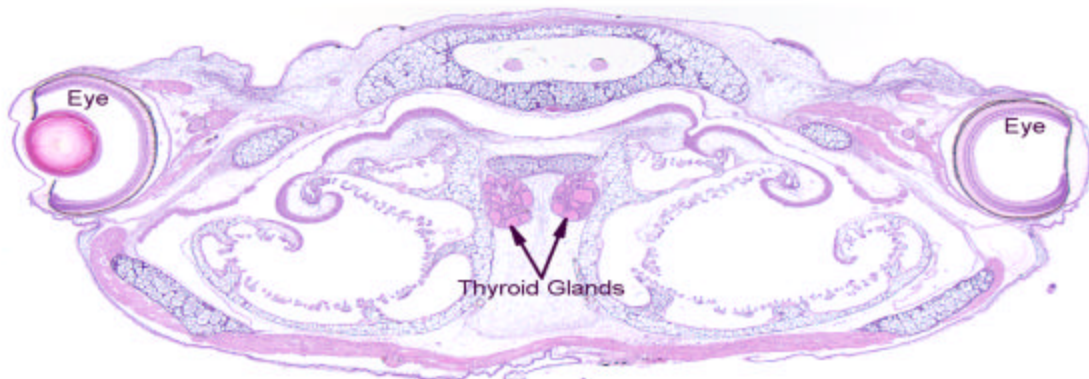


Z10391 Toad No. 51-1102 H&E
WA 04-02 X Section Head of *Xenopus laevis* 1.25x

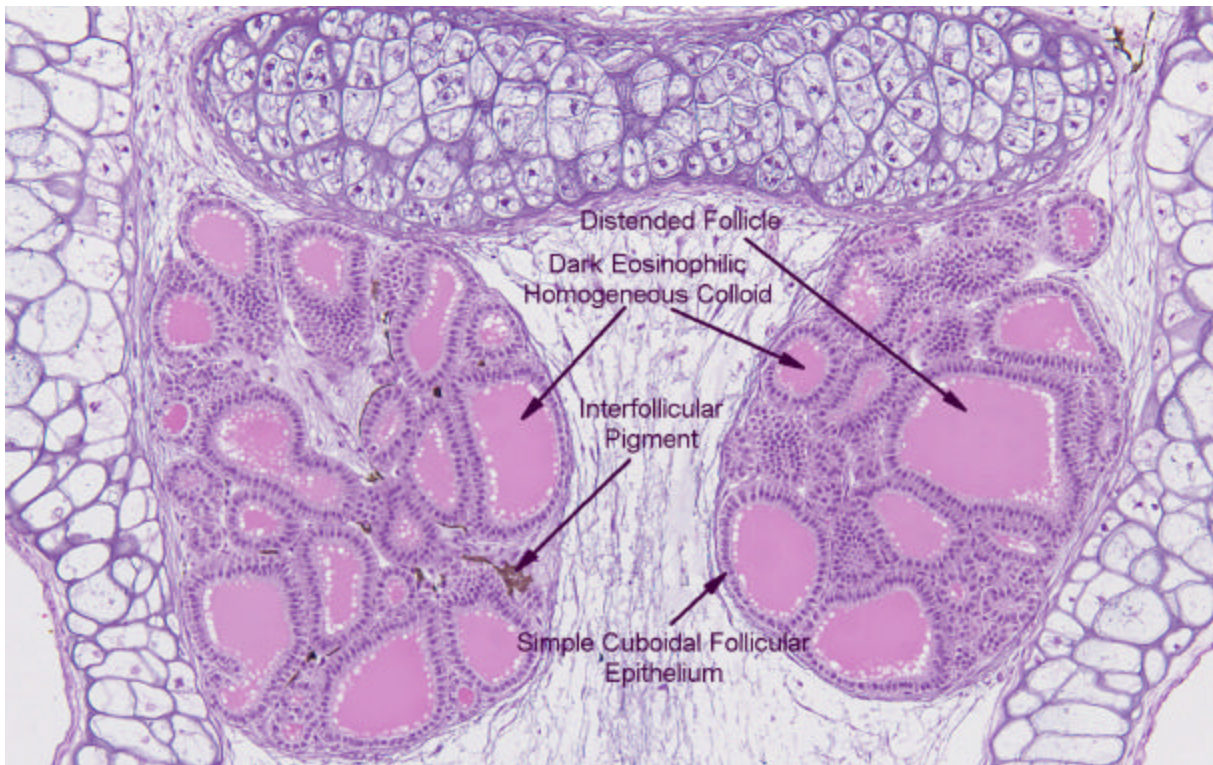


Z10400 Toad No. 51-1102 H&E
WA 04-02 Thyroid Glands of *Xenopus laevis* 10x

Z10391 and Z10400. Low (1.25x) and high (10x) magnification photomicrographs of thyroid glands from Stage 51 *Xenopus laevis* control. The colloid was dark eosinophilic and homogeneous. The thyroid follicles were lined by simple cuboidal epithelium.

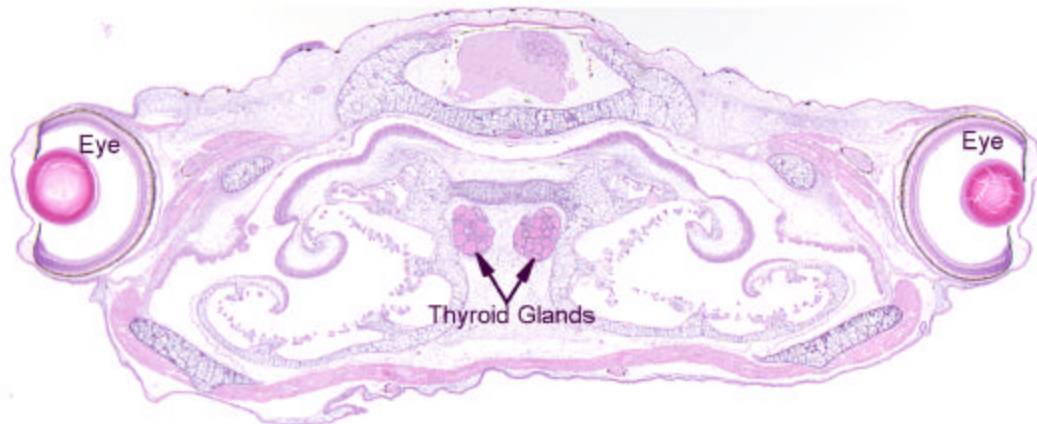


Z10396 Toad No. 51-2101 H&E
 WA 04-02 X Section Head of *Xenopus laevis* 1.25x

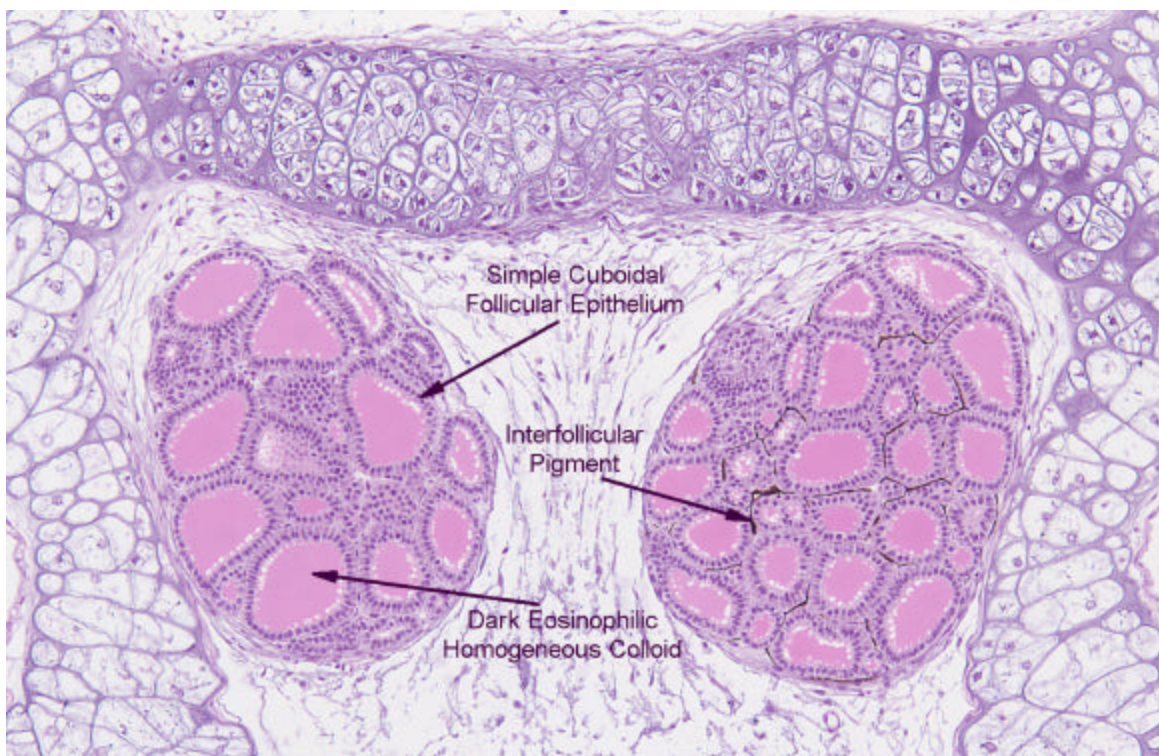


Z10397 Toad No. 51-2101 H&E
 WA 04-02 Thyroid Glands of *Xenopus laevis* 10x

Z10396 and Z10397. Low (1.25x) and high (10x) magnification photographs of thyroid glands from Stage 51 *Xenopus laevis* exposed to 1.25 mg/L PTU for 21 days. A few follicles were distended minimally in relation to other follicles in the glands.

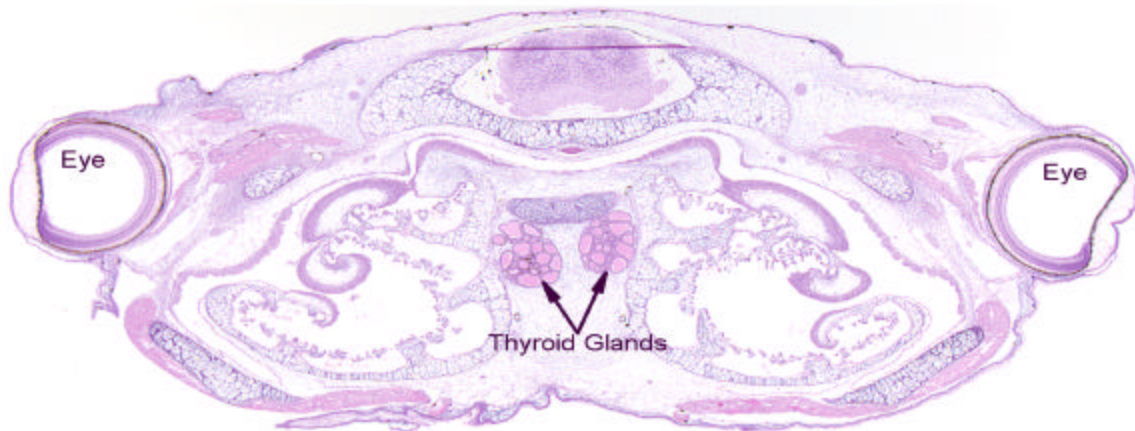


Z10395 Toad No. 51-3101 H&E
 WA 04-02 X Section Head of *Xenopus laevis* 1.25x

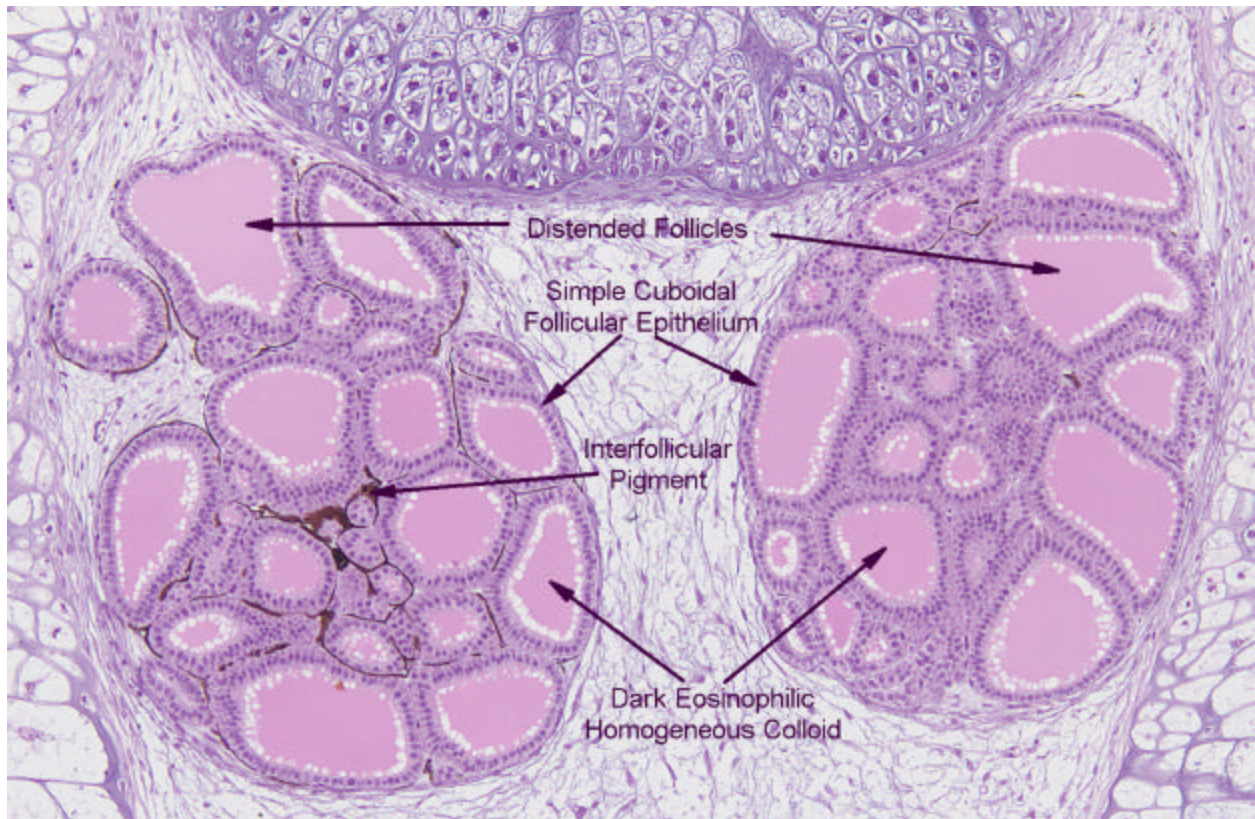


Z10399 Toad No. 51-3101 H&E
 WA 04-02 Thyroid Glands of *Xenopus laevis* 10x

Z10395 and Z10399. Low (1.25x) and high (10x) magnification photomicrographs of thyroid glands from Stage 51 *Xenopus laevis* exposed to 2.50 mg/L PTU for 21 days. This toad (No. 51-3101) had minimal diffuse enlargement of the thyroid glands.

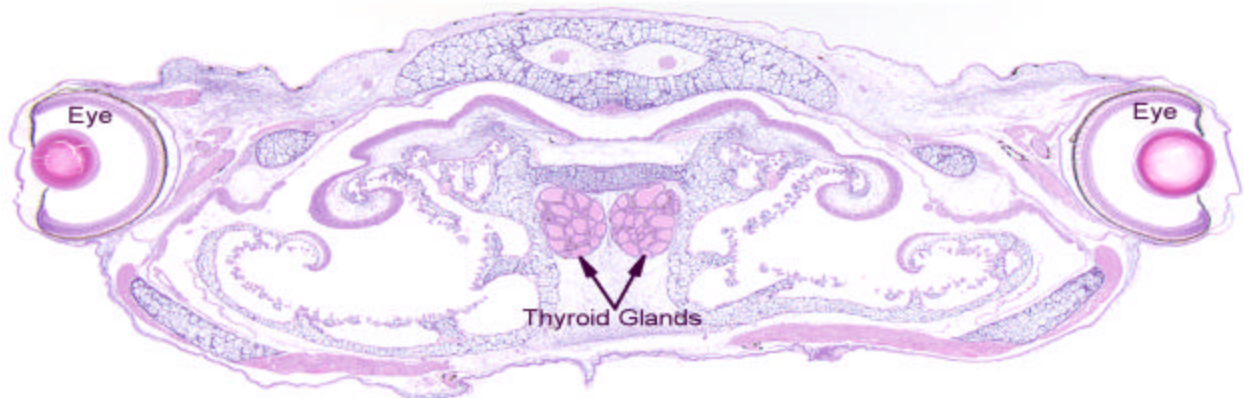


Z10394 Toad No. 51-4105 H&E
 WA 04-02 X Section Head of *Xenopus laevis* 1.25x

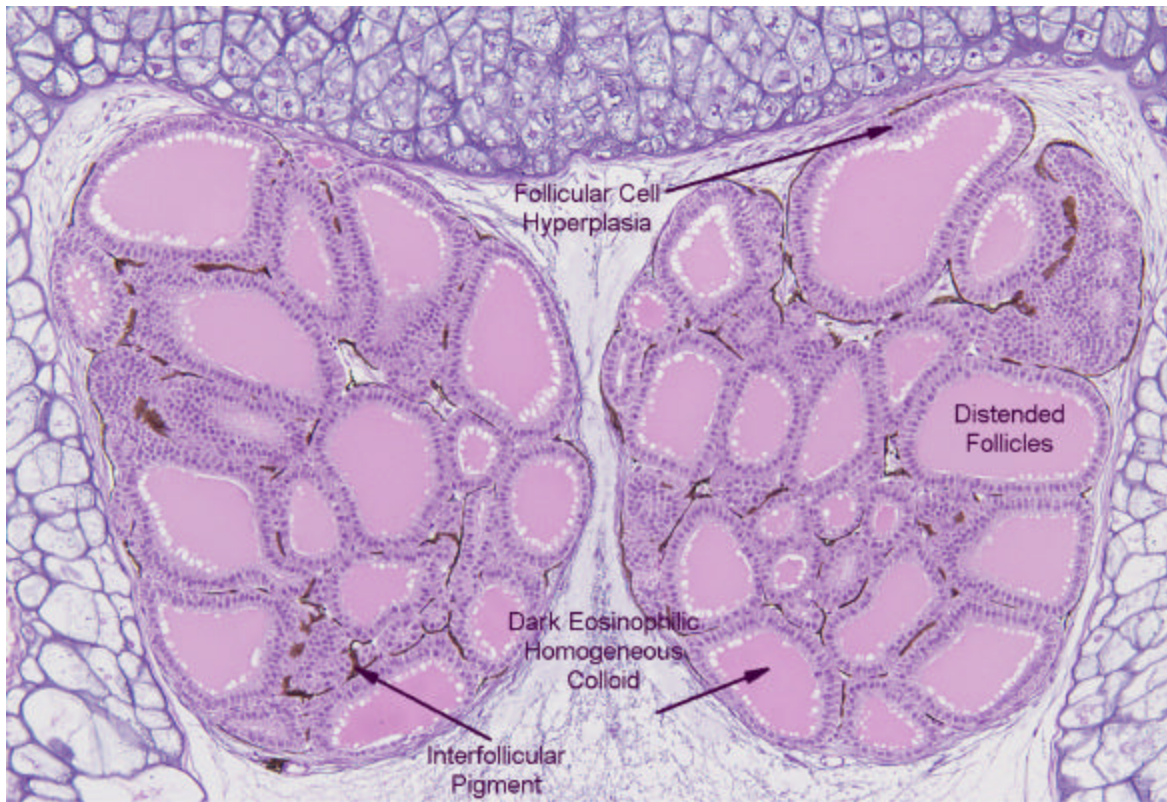


Z10398 Toad No. 51-4105 H&E
 WA 04-02 Thyroid Glands of *Xenopus laevis* 10x

Z10394 and Z10398. Low (1.25x) and high (10x) magnification photomicrographs of thyroid glands from Stage 51 *Xenopus laevis* exposed to 5.0 mg/L PTU for 21 days. This toad (No. 51-4105) had minimal diffuse enlargement of the thyroid glands. A few follicles were distended minimally in relation to other follicles.

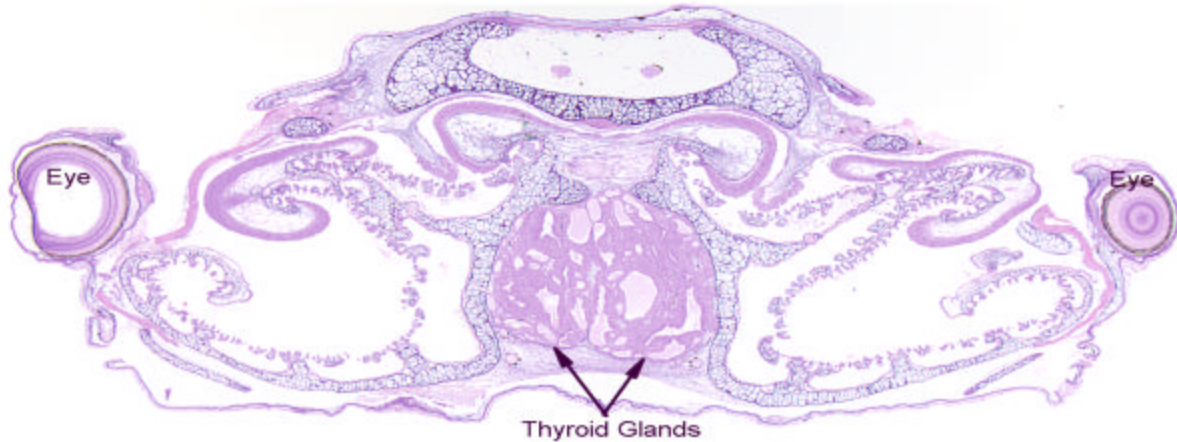


Z10393 Toad No. 51-5101 H&E
 WA 04-02 X Section Head of *Xenopus laevis* 1.25x

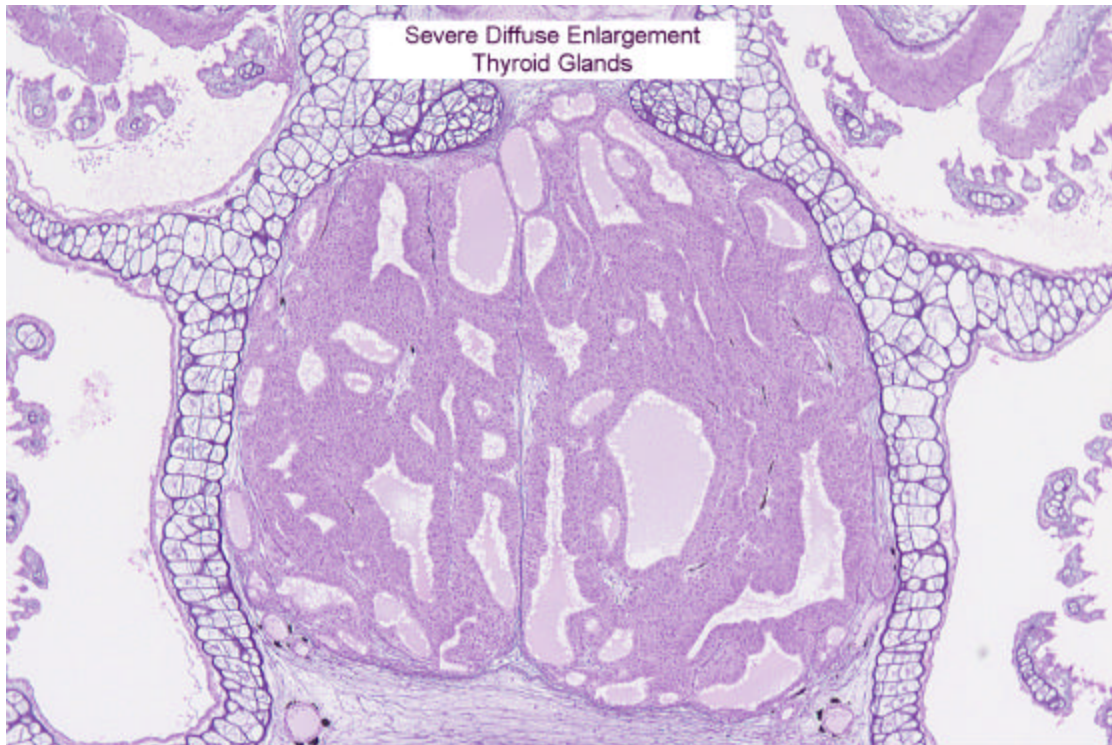


Z10401 Toad No. 51-5101 H&E
 WA 04-02 Thyroid Glands of *Xenopus laevis* 10x

Z10393 and Z10401. Low (1.25x) and high (10x) magnification photomicrographs of thyroid glands from Stage 51 *Xenopus laevis* exposed to 10 mg/L PTU for 21 days. This toad (No 51-5101) had moderate diffuse enlargement of the thyroid glands, slight/mild distended follicles, and minimal follicular cell hyperplasia.

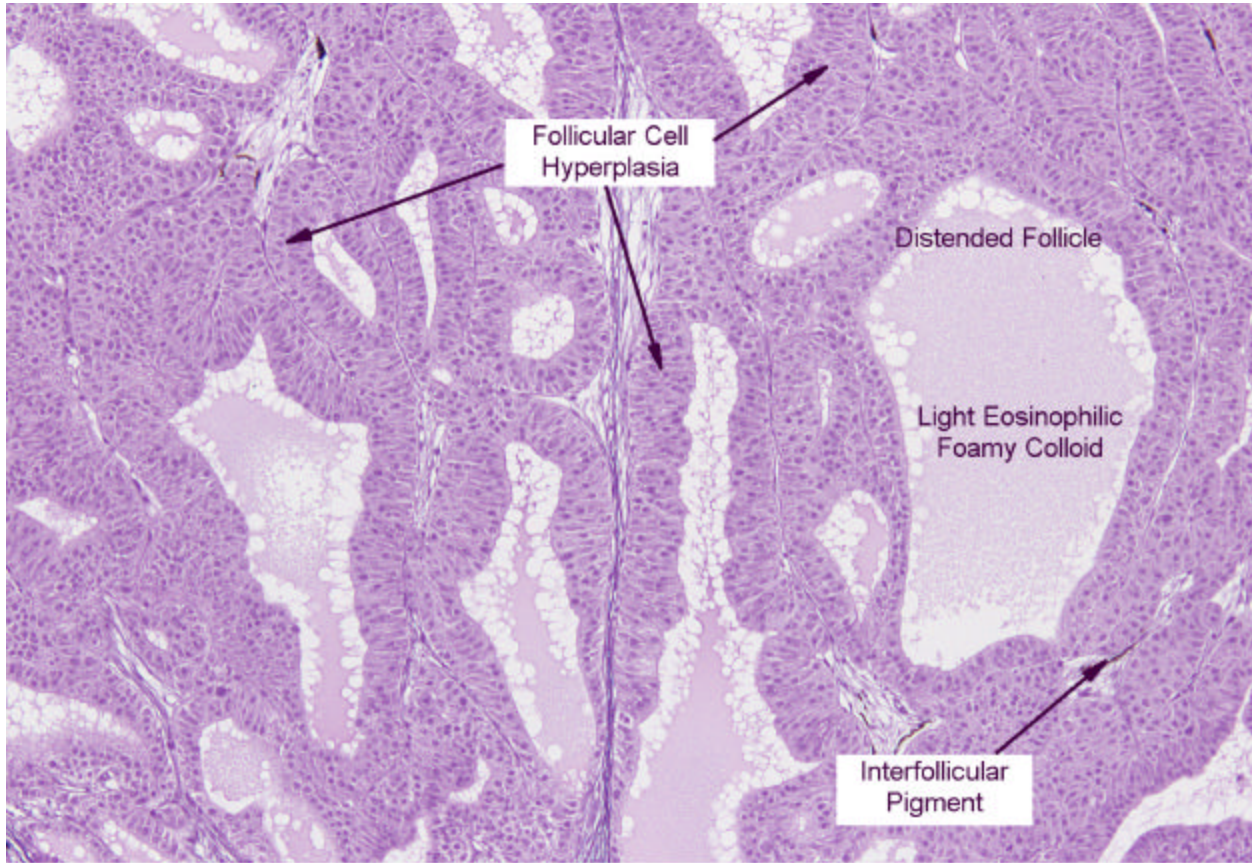


Z10392 Toad No. 51-6101 H&E
 WA 04-02 X Section Head of *Xenopus laevis* 1.25x



Z10403 Toad No. 51-6101 H&E
 WA 04-02 Thyroid Glands of *Xenopus laevis* 4x

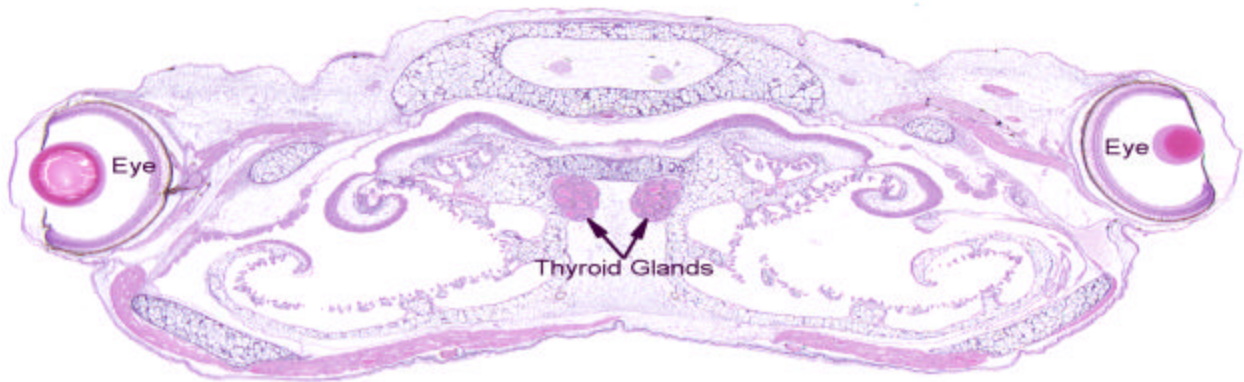
Z10392 and Z10403. Two relatively low magnification (1.25x and 4x) photomicrographs of thyroid glands from Stage 51 *Xenopus laevis* exposed to 20 mg/L PTU for 21 days. This toad (No. 51-6101) had diagnosed severe diffuse enlargement of the thyroid glands, moderate distended follicles and moderately severe hyperplasia of the thyroid follicular cells. In contrast to the homogeneous dark eosinophilic colloid in the follicles of toads exposed to concentrations of PTU lower than 20 mg/L, this toad had a light eosinophilic foamy colloid. See Z10402 for an annotated photomicrograph at 10x magnification of thyroid glands from this same toad.



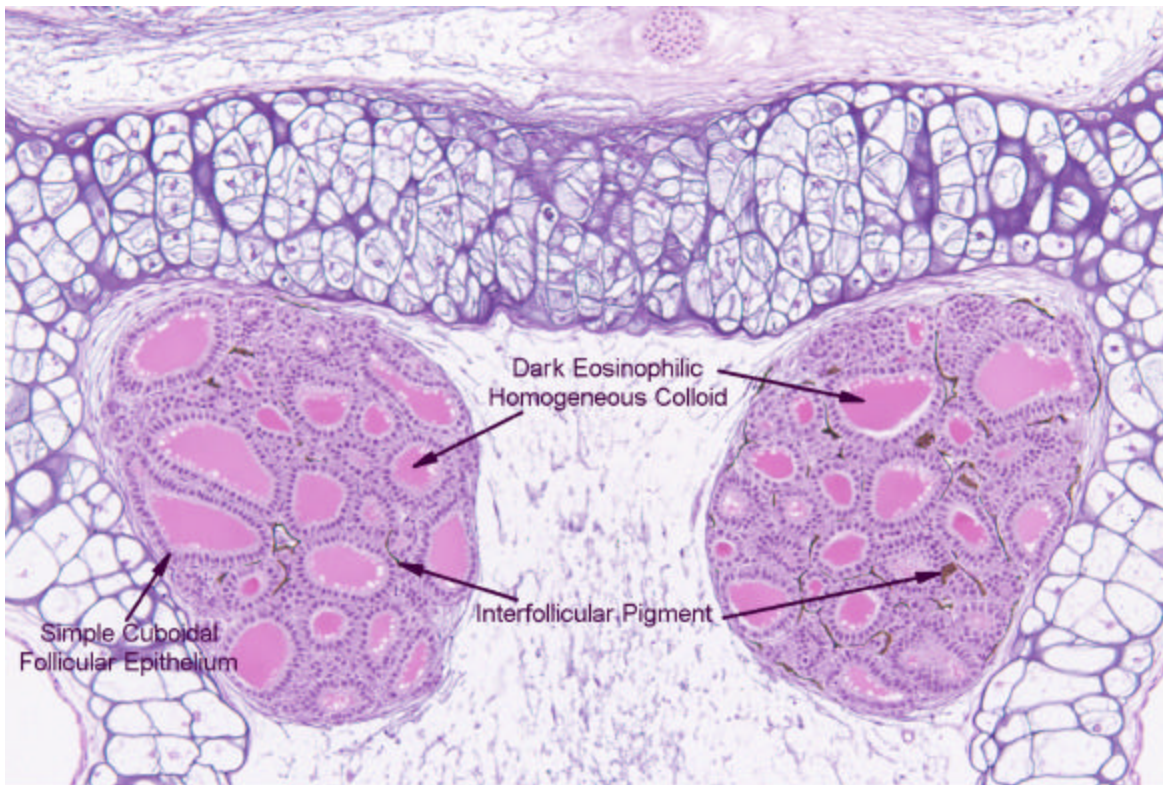
Z10402 Toad No. 51-6101 H&E
WA 04-02 Thyroid Glands of *Xenopus laevis* 10x

Z10402. At 10x magnification only a portion of the enlarged thyroid glands was captured in the field. These are the thyroid glands from Stage 51 toad No. 51-6101 seen in the previous two photomicrographs. Note the severe hyperplasia of the follicular cells and the light eosinophilic foamy cytoplasm.

APPENDIX B: LIGHT MICROSCOPY PHOTOGRAPHS
STAGE 54 TOADS EXPOSED TO VARIOUS
CONCENTRATIONS FOR 14 DAYS

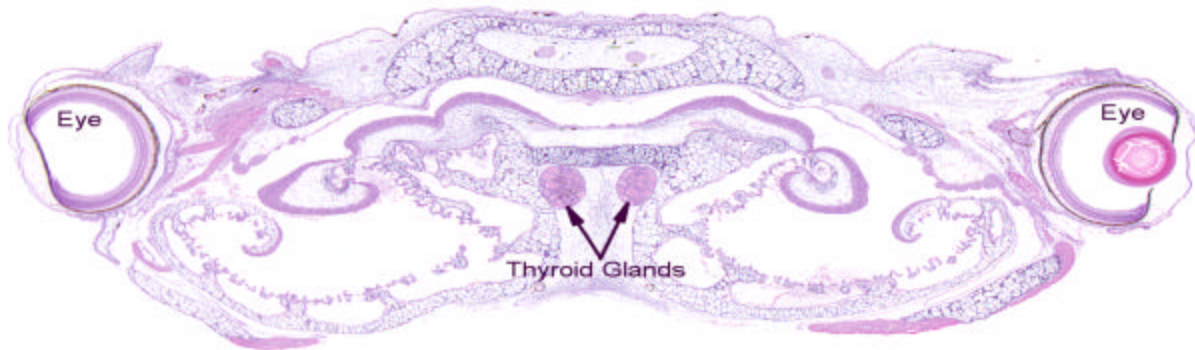


Z10373 Toad No. 54-1205 H&E
 WA 04-02 X Section Head of *Xenopus laevis* 1.25x

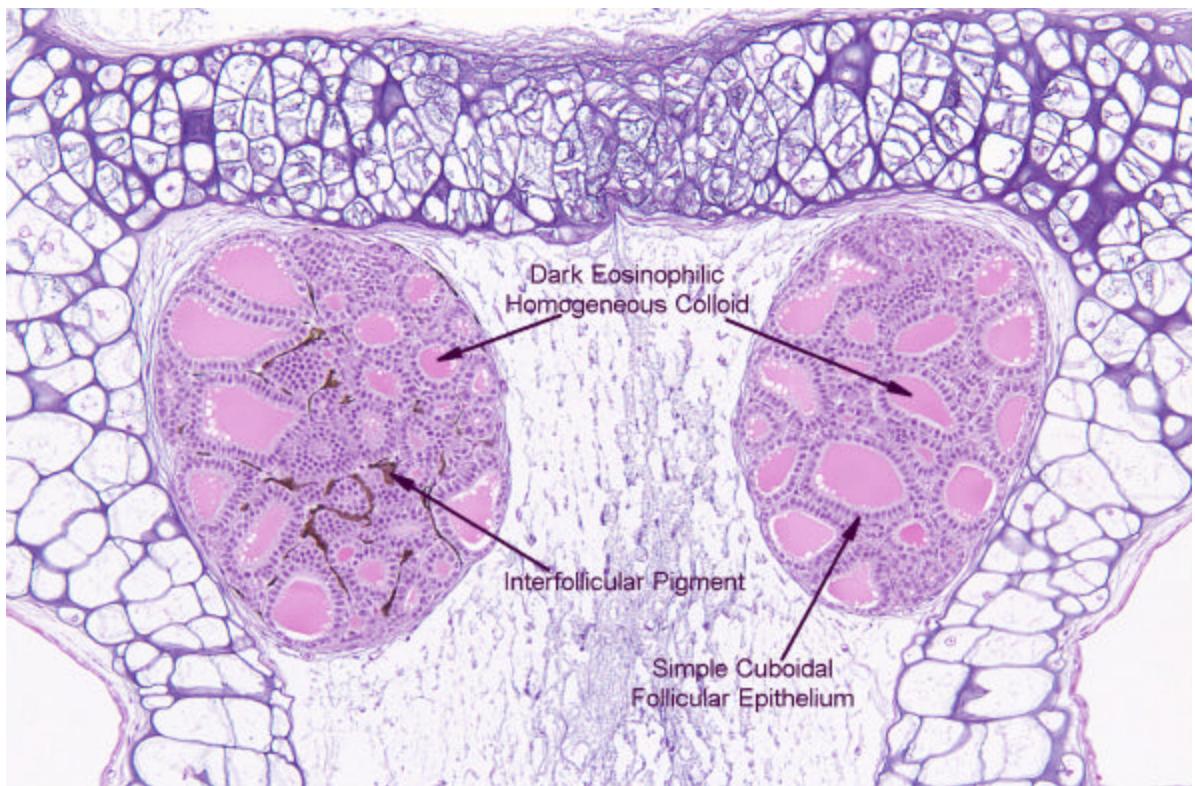


Z10386 Toad No. 54-1205 H&E
 WA 04-02 Thyroid Glands of *Xenopus laevis* 10x

Z10373 and Z10386. Low (1.25x) and high (10x) magnification photomicrographs from a Stage 54 control *Xenopus laevis* (No. 54-1205). The colloid was dark eosinophilic and homogeneous. The follicles were lined by simple cuboidal epithelium.

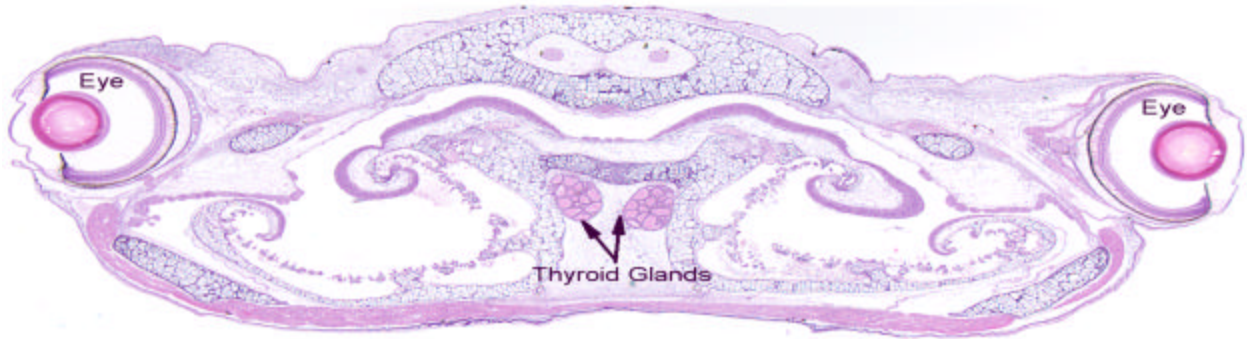


Z10381 Toad No. 54-2103 H&E
 WA 04-02 X Section Head of *Xenopus laevis* 1.25x

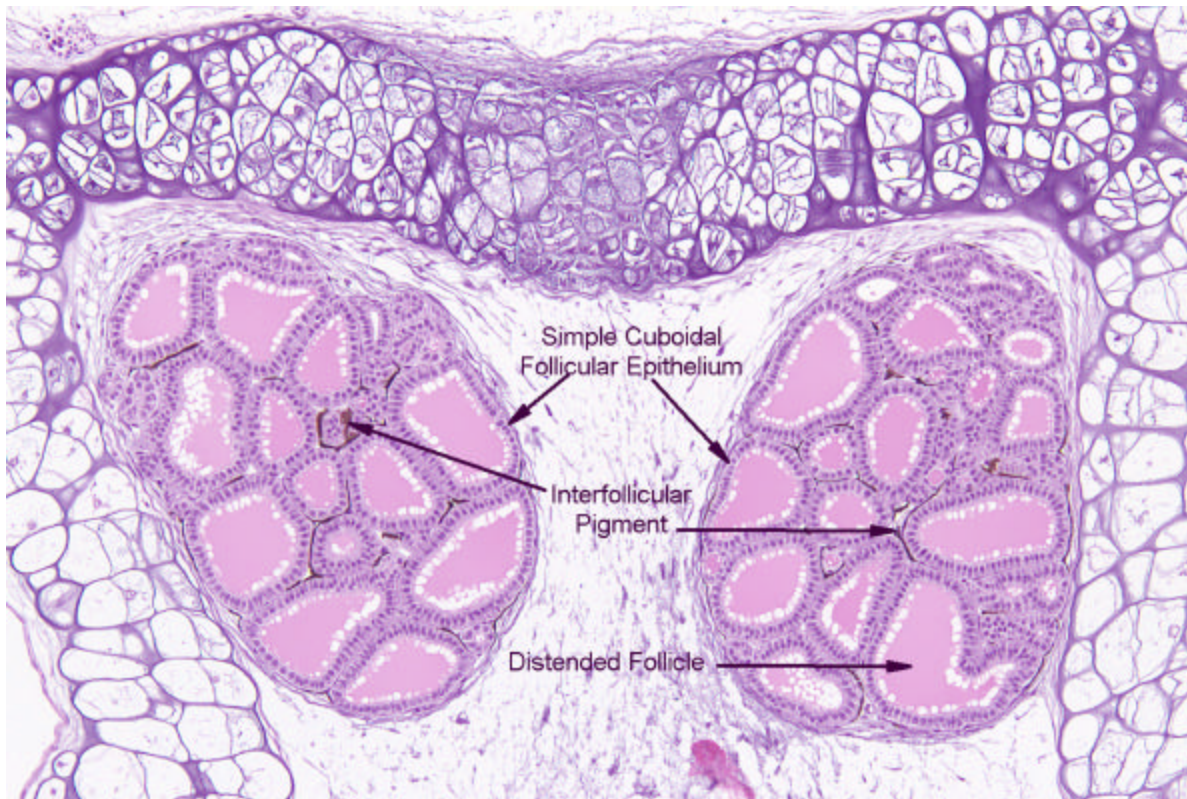


Z10390 Toad No. 54-2103 H&E
 WA 04-02 Thyroid Glands of *Xenopus laevis* 10x

Z10381 and Z10390. Low (1.25x) and high (10x) magnification photographs from a Stage 54 *Xenopus laevis* exposed to 1.25 mg/L PTU for 14 days. The features of the colloid and follicular cells were similar to those observed in the control toad (photographs Z10373 and Z10386).

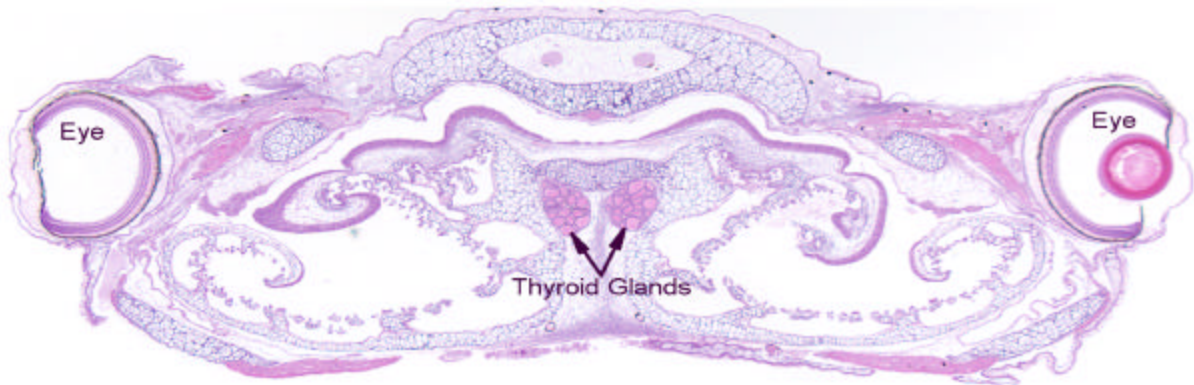


Z10383 Toad No. 54-3104 H&E
 WA 04-02 X Section Head of *Xenopus laevis* 1.25x

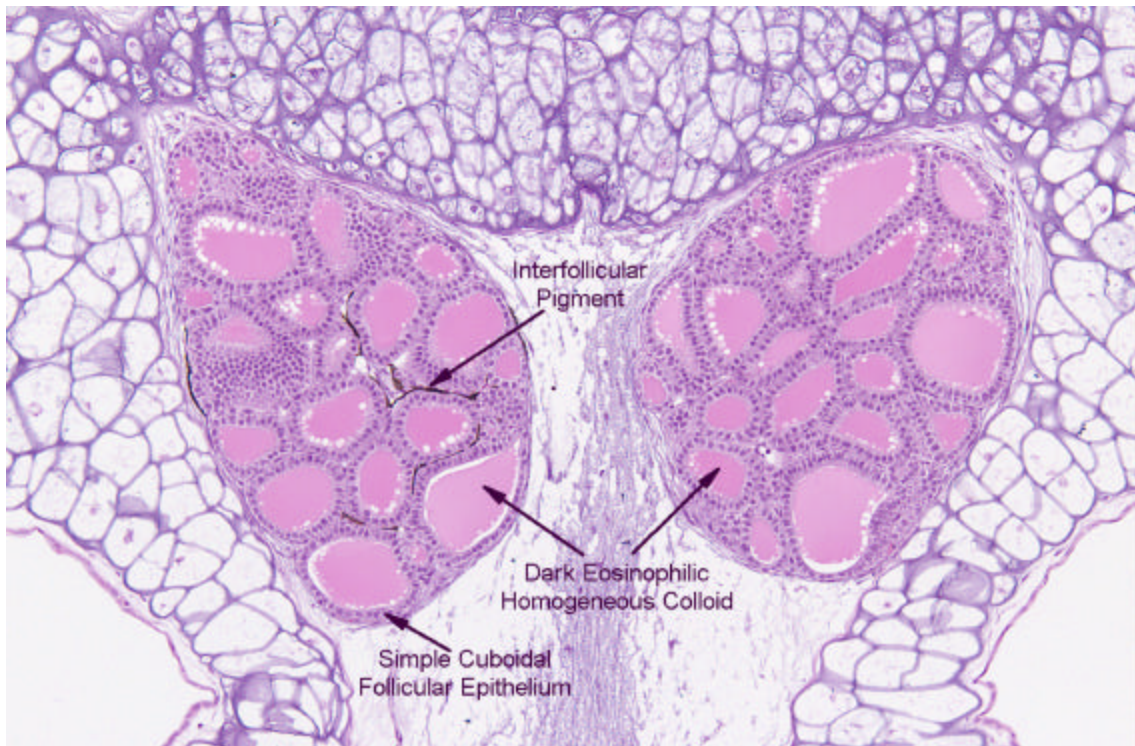


Z10389 Toad No. 54-3104 H&E
 WA 04-02 Thyroid Glands of *Xenopus laevis* 10x

Z10383 and Z10389. Low (1.25x) and high (10x) magnification photomicrographs from a Stage 54 *Xenopus laevis* exposed to 2.50 mg/L PTU for 14 days. This toad (No. 54-3104) had diagnosed minimal distension of thyroid follicles, but the thyroid glands otherwise resembled those from control toads.

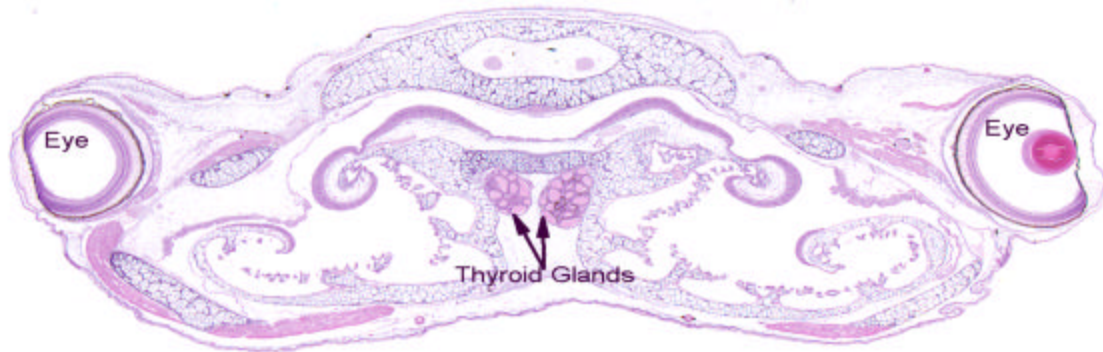


Z10377 Toad No. 54-4103 H&E
 WA 04-02 X Section Head of *Xenopus laevis* 1.25x

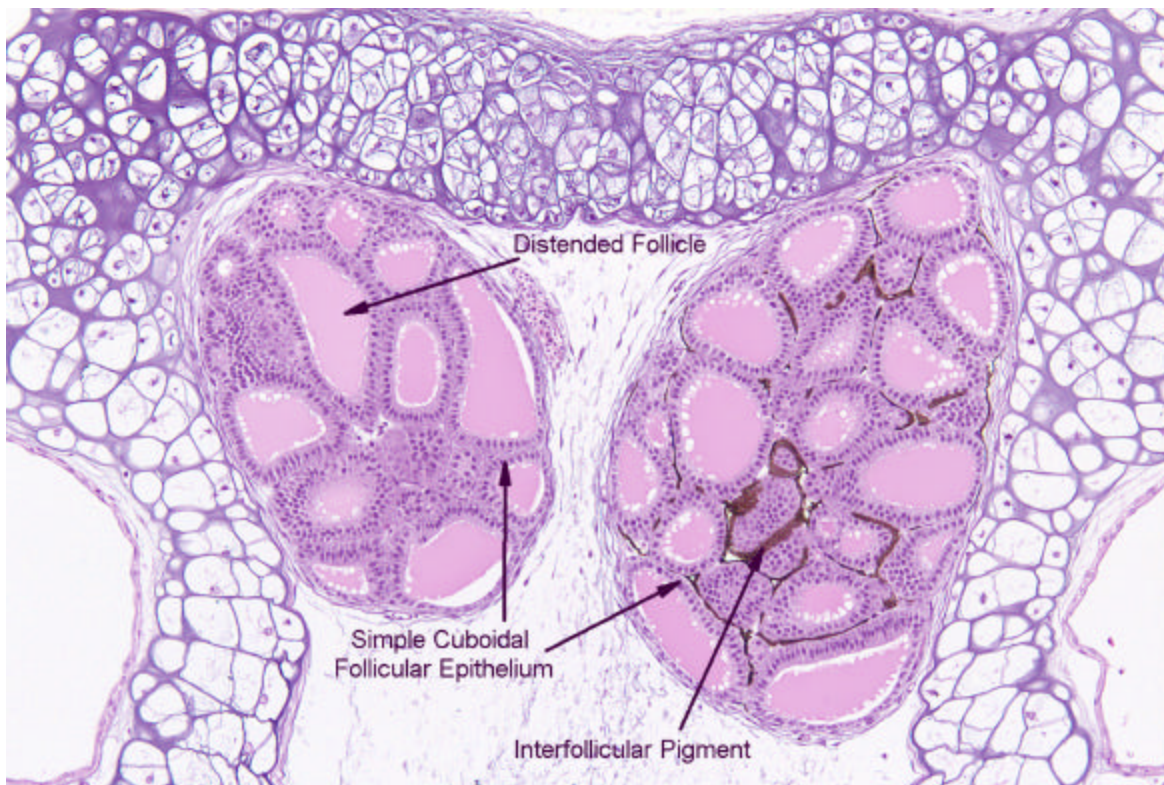


Z10388 Toad No. 54-4103 H&E
 WA 04-02 Thyroid Glands of *Xenopus laevis* 10x

Z10377 and Z10388. Low (1.25x) and high (10x) magnification photomicrographs from a Stage 54 *Xenopus laevis* exposed to 5.0 mg/L PTU for 14 days. There was mild diffuse enlargement of the thyroid glands in this toad (No. 54-4103). Compare the width of the interglandular interstitium in this photograph (10x magnification) with the width of this space in photomicrographs of toads exposed to doses of PTU lower than 5.0 mg/L.

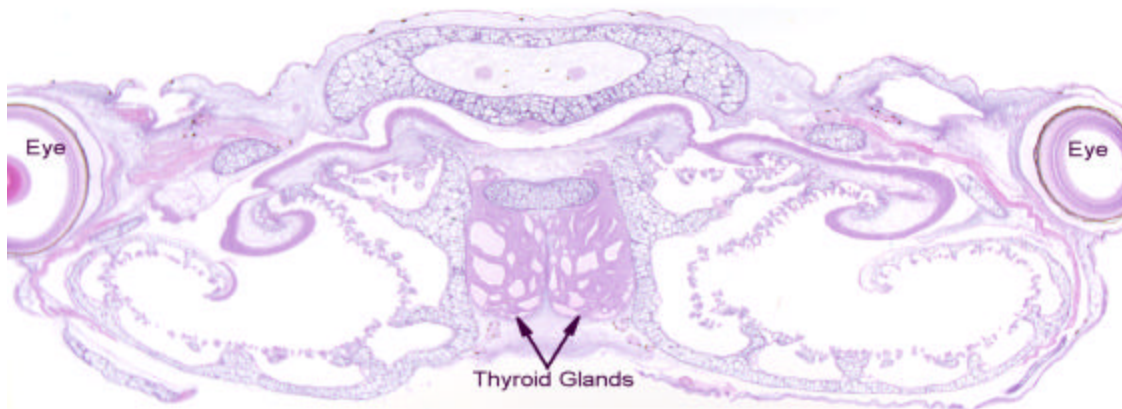


Z10380 Toad No. 54-5105 H&E
 WA 04-02 X Section Head of *Xenopus laevis* 1.25x

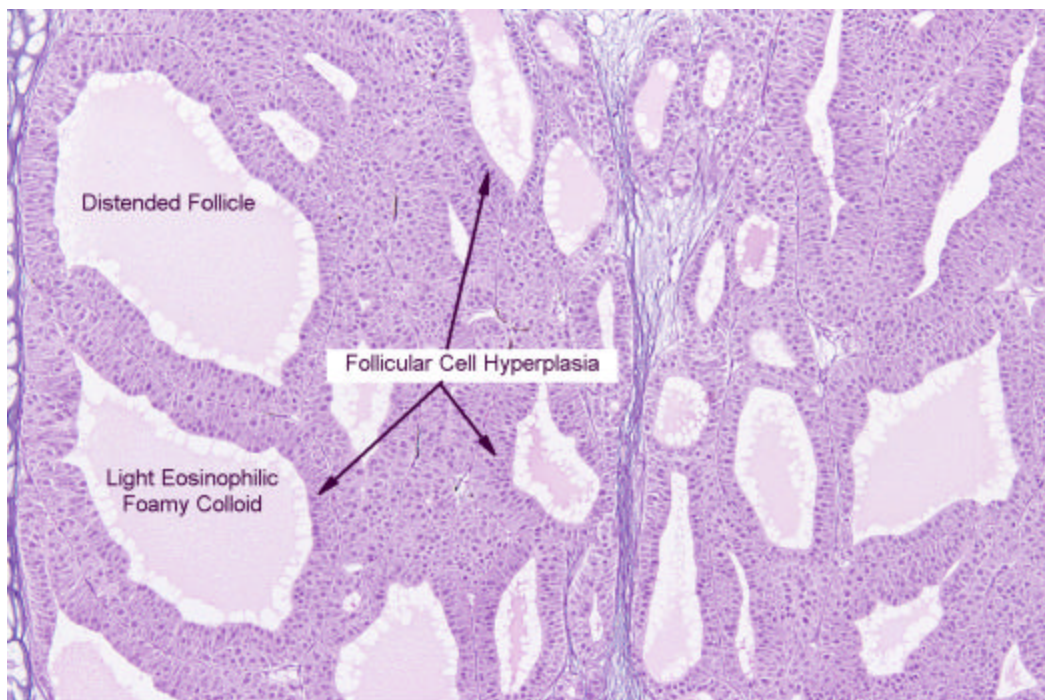


Z10387 Toad No. 54-5105 H&E
 WA 04-02 Thyroid Glands of *Xenopus laevis* 10x

Z10380 and Z10387. Low (1.25x) and high (10x) magnification photographs from a Stage 54 *Xenopus laevis* exposed to 10 mg/L PTU for 14 days. This toad (No. 54-5105) had mild diffuse enlargement of the thyroid glands and minimal distension of thyroid follicles.



Z10374 Toad No. 54-6103 H&E
WA 04-02 X Section Head of *Xenopus laevis* 1.25x



Z10385 Toad No. 54-6103 H&E
WA 04-02 Thyroid Glands of *Xenopus laevis* 10x

Z10374 and Z10385. Low (1.25x) and high (10x) magnification photographs from a Stage 54 *Xenopus laevis* exposed to 20 mg/L PTU for 14 days. This toad (No. 54-6103) had moderately severe diffuse enlargement of the thyroid glands, moderate follicular cell hyperplasia and mild distension of thyroid follicles. The colloid was light eosinophilic and foamy.