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The Acidophilic Granules (AG) in the Thyrotrophs and their Relation to Thyroid Hyperplasia among Laboratory-reared *Xenopus*[1)]

By

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(With 1 Text-figure, 2 Plates and *2 Tables)*

In the course of a cytological study of the pars distalis of *Xenopus laevis*, variable amount of acidophilic granules (AG) were occasionally observed in the cytoplasm of the thyrotrophs. Such granules have also been reported in the animals of the same species which have been kept in the laboratory (Guardabassi and Blanchi, 1962). These authors noted that the animals with the pituitary thyrotrophs bearing AG possessed the hypertrophied thyroid glands, and that the AG became conspicuous after thiouracil treatment. In rats similar granules have been known to occur in the thyrotrophs of the animal in long term thyroid deficiency (Catchpole, 1949; Purves and Griesbach, 1951; Halmi, 1952).

These facts may suggest that occurrence of the AG in this type of cell is related with a disorder of the pituitary-thyroid axis.

The present paper deals with histological variation of the thyroid and the morphology of AG with special reference to the relation between the AG and thyroid hyperplasia.

Materials and Methods

All the animals used in this investigation were obtained from the Institute of Endocrinology, Gunma University, where the larvae were procured by artificial ovulation through injection of chorionic gonadotropin. They were reared in an aerated large pond and fed with alfalfa powder and dry milk. The adults were maintained in aquaria and fed on chopped pork liver. After arrival to this laboratory they were kept in a large aquaria for one to ten months. During this period the water temperature was 10° to 25°C and all the animals appeared healthy and grew normally. Twenty-four animals, including eleven males weighing about 45 gm and thirteen females weighing about 65 gm, were chosen at random and were sacrificed for inspection. Of these, both the thyroid and the pars distalis of twenty-one animals were examined histologically. On occasions, the pars distalis was processed for electron microscopy.

Pars Distalis. The glands were fixed in Helly's fixative for 3 hr and embedded in

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paraffin. Serial sections were cut at 5μ. Most sections were stained with modified Azan stain, in which 1% acid fuchsin was applied prior to the phosphotungstic acid treatment of Heidenhain's Azan. In order to investigate the staining properties of AG, some sections were stained with the following methods:

1) Heidenhain's Azan
2) Modified Azan
3) Luxol Fast Blue (Shanklin *et al.*, 1959) followed by PAS-orange G
4) Gabe's (1953) or Gomori's (1950) aldehyde fuchsin

Cell count was performed on every tenth serial section by counting the thyrotrophs that contain AG and those devoid of AG. The count was made on 20 sections of different cut planes in each gland.

For electron microscopy, the tissues were fixed in 1% OsO_4 containing sucrose and buffered at pH 7.2 with veronal acetate for 2 hr. Occasionally, glutaraldehyde was applied before OsO_4 fixation. They were then dehydrated through a graded series of cold ethanol and embedded in Epon 812 (Luft, 1961), or in styrene-methacrylate (Kushida, 1961). Thin sections were cut on a Porter-Blum microtome and stained with uranyl acetate only or followed by lead hydroxide (Millonig, 1961). The sections were examined in a Hitachi HS-7 electron microscope.

Thyroid Gland. The thyroid glands were fixed in Helly's or Bouin's fluid. After fixation, the glands were cut transversely into two halves with a fine razor blade. Their paraffin sections were cut along the same plane, and the measurement of the size of the thyroid gland was made on these transverse sections as follows: The longest diameter of the gland and the one at the right angle to it in the same section were measured with an ocular micrometer, then their averaged value was multiplied twice by itself. The height of the follicular epithelium in each gland was measured in eighty places and their mode was taken.

Results

With the staining methods employed, the thyrotrophs of the pars distalis were occasionally observed to contain acidophilic granules (AG) that were ordinarily rather rare in this type of cells. Before describing the correlation of their occurrence and thyroid histology, it is necessary to depict their morphology.

The size of AG was strikingly variable from cell to cell, with the largest one measuring up to about 2μ in diameter (Figs. 1, 2, 3 and 4). In most cases they were round in shape. In some cells, they were found to aggregate with each other so that they could not be visible separately.

In Table 1 the staining properties of the AG are compared with those of ordinary thyrotrophs and acidophils. It is apparent that the AG do not correspond to the latter in the staining characteristics and, therefore, in chemical nature.

In electron microscopy, two different structures were observed which may probably correspond to the AG at the light microscopic level. One is the spherical bodies of 250–700 mμ in diameter which were found intermingled with ordinary secretory granules (Fig. 12). These bodies were less electron-dense than the secretory granules. They were surrounded by a loose membrane. Within

Table 1. Comparison of the staining properties between the ordinary thyrotrophs, AG and alpha cells

Stain	Ordinary thyrotrophs	AG	Alpha cells
Heidenhain's Azan	blue	red	orange
Modified Azan	blue	red	red
PAS	+	−	−
Luxol Fast Blue-PAS-orange G	red purple	orange	green
Gabe's AF	+	+	−
Gomori's AF	+	−	−

Table 2. The relation between thyroid histology and the frequency of AG-bearing thyrotrophs of laboratory-raised *Xenopus*

Animal number	Sex	Thyroid Gland			Pars Distalis	
		Maximal diameter of whole gland (mm)	Cube of diameter	Epithelial height (mode values in μ)	Ratio of AG-bearing thyrotrophs/ AG-free thyrotrophs	Per cent of AG -bearing thyrotrophs
1	♀	5.95	210.6	4.7	634/1610	28.3
2	♀	4.05	66.4	3.3	135/1568	7.9
3	♂	4.00	64.0	5.1	83/1284	6.1
4	♂	2.85	23.2	3.8	43/1348	3.1
5	♂	2.75	20.8	4.2	55/1397	3.9
6	♀	2.75	20.8	4.9	27/1536	1.7
7	♀	2.60	17.6	4.0	177/2149	7.7
8	♀	2.40	13.8	5.9	5/2244	0.3
9	♀	2.00	8.0	6.1	2/1246	0.2
10	♀	1.95	7.2	6.4	14/1651	0.8
11	♂	1.90	6.9	4.7	5/1583	0.3
12	♂	1.85	6.3	5.3	21/1481	1.4
13	♂	1.80	5.8	6.2	13/1308	1.0
14	♂	1.75	5.4	4.0	2/1192	0.2
15	♂	1.70	4.9	4.8	34/1543	2.2
16	♂	1.70	4.9	5.9	8/1447	0.5
17	♀	1.65	4.5	6.2	0/1697	0.0
18	♂	1.60	4.1	4.9	5/1469	0.3
19	♀	1.30	2.2	6.1	3/1316	0.2
20	♀	1.10	1.3	5.5&7.1	43/1102	3.8
21	♂	1.00	1.0	6.0	7/1602	0.4
22	♀	—	—	—	(processed for EM)	
23	♀	—	—	—	(〃)	
24	♀	—	—	—	(processed for various staining methods)	

the membrane, three to four bodies were frequently seen coalescing to form a beadlike structure (Fig. 12, arrows). The other structure is electron-dense bodies within cytoplasmic vesicles (Figs. 13 and 14). These bodies were also spherical in shape. Their size varied strikingly, ranging from 50 mμ to more than 1.5μ in diameter. When they occurred in small size, their number per vesicle was larger and vice versa. The cytoplasmic vesicles containing dense bodies bear close resemblance to those depicted in the rat pituitary after thyroidectomy (Farquhar and Rinehart, 1954).

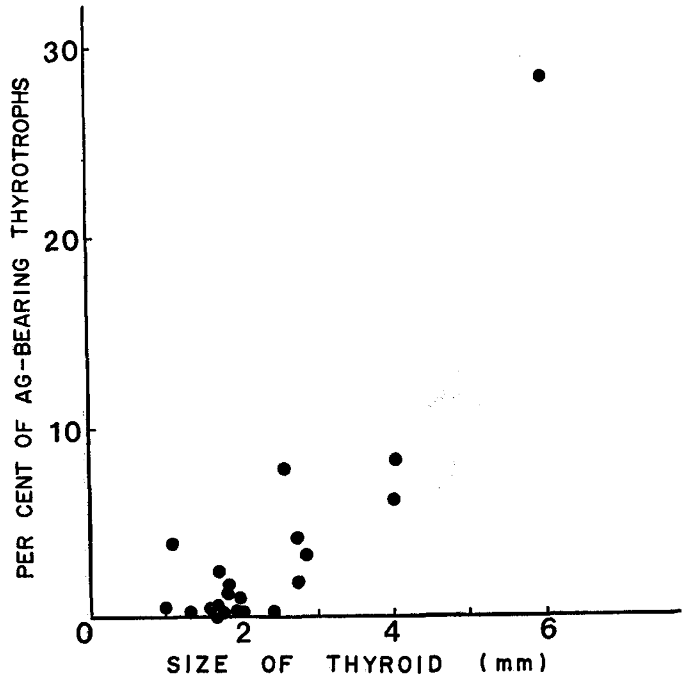

Fig. 1. The percentage of AG-bearing thyrotrophs plotted against the size of the thyroid gland.

Table 2 summarises the histological features of the thyroid glands and the number of the thyrotrophs that contain AG. The relation between the size of the thyroid and the percentage of the AG-bearing thyrotrophs is given in Text-figure 1. It was a general trend that the AG-bearing thyrotrophs occur more frequently in the animals that possess relatively large thyroid. In addition to their large size, these goiter-like thyroids were found to have the following characteristics: (1) the presence of many "micro-follicles" (Fig. 5), (2) the epithelium was relatively low and frequently folded to make irregular masses (Fig. 6), and (3) nuclei of the epithelium were irregular in shape and pycnotic in some places.

Discussion

It is well established that thyroidectomy results in the formation of the so-called "thyroidectomy cells" in the pars distalis of various species (Severinghaus *et al.*, 1934; Severinghaus, 1937; Zeckwer *et al.*, 1935; Elftman, 1958; Dent, 1961; Cardell, 1964). In rat thyroidectomy cells, some authors have recognized coarse granules of an unknown nature (Halmi, 1952; Purves and Griesbach, 1951; Catchpole, 1949). According to Catchpole (1949), they are insoluble in the reagents which remove glycoproteinaceous hormone. In the present study, too, considerable difference in the staining properties was observed between the AG and ordinary thyrotrophs. It may be reasonable to conclude, therefore, that the AG are not thyrotrophin itself.

In electron microscopy, dense bodies have been observed within the cytoplasmic vesicles of foamy appearance. These structures are similar to those described in rat thyroidectomy cells (Farquhar and Rinehart, 1954). These authors are of the opinion that they correspond to the coarse granules, or presumably the AG, which have been revealed by light microscopy. In addition, in *Xenopus*, somewhat different structures were found in the cytoplasmic matrix intermingled with ordinary secretory granules. These structures may also correspond to the AG as the afore-mentioned dense bodies do. As to the relation of these two different types of bodies and their respective functional significance, the present electron microscopic observations do not permit any definite conclusions.

In the present study it was revealed that the frequency of occurrence of AG-bearing thyrotrophs is related to the degree of thyroid hyperplasia. That is, the higher the percentage of AG-bearing thyrotrophs contained in the pituitary, the larger the thyroid gland possessed by the animal. Then a question arises: What is the reason of the hypothyroidism in these "unoperated" and "untreated" animals? Morpurgo and Zacchei (1954) have ascribed the cause for the malformations to a special "thyroid instability" from their comparative studies on the questioned glands of the animals in natural habitat and those in laboratory environment. It must be taken into consideration that the feeding of a high protein diet such as liver causes a hyperplasia of the thyroid glands in brook trout (Marine, 1914) and in rats (Burget, 1917; Hou, 1940). The toads after metamorphosis have been fed exclusively on pork liver. This high protein diet may lead the animals to hypothyroidism with enlarged thyroid gland. Of course, besides diet there are many other factors which must be taken into consideration, such as water temperature and environmental stresses. Apart from the cause of the hypothyrodism, special care must be paid by laboratory investigators who intend to study the thyroid-pituitary axis using this animal.

Summary

Histological features of the thyroid gland and the pars distalis have been investigated of twenty-four specimens of laboratory-raised *Xenopus*. The thyroid

glands were variable to a great extent in size, ranging from 1.00 to 5.95 mm in diameter. Some thyrotrophs in the pars distalis of these animals contained acidophilic granules (AG). It was found that the larger number of AG-bearing thyrotrophs are observed in the animals that possess larger thyroid gland.

Both the light and the electron microscopic observations confirmed that the AG are similar to those reported in the thyrotrophs of athyroid animals or the animals with insufficient supply of thyroid hormone. The cause for hypothyroidism in laboratory-raised *Xenopus* is discussed.

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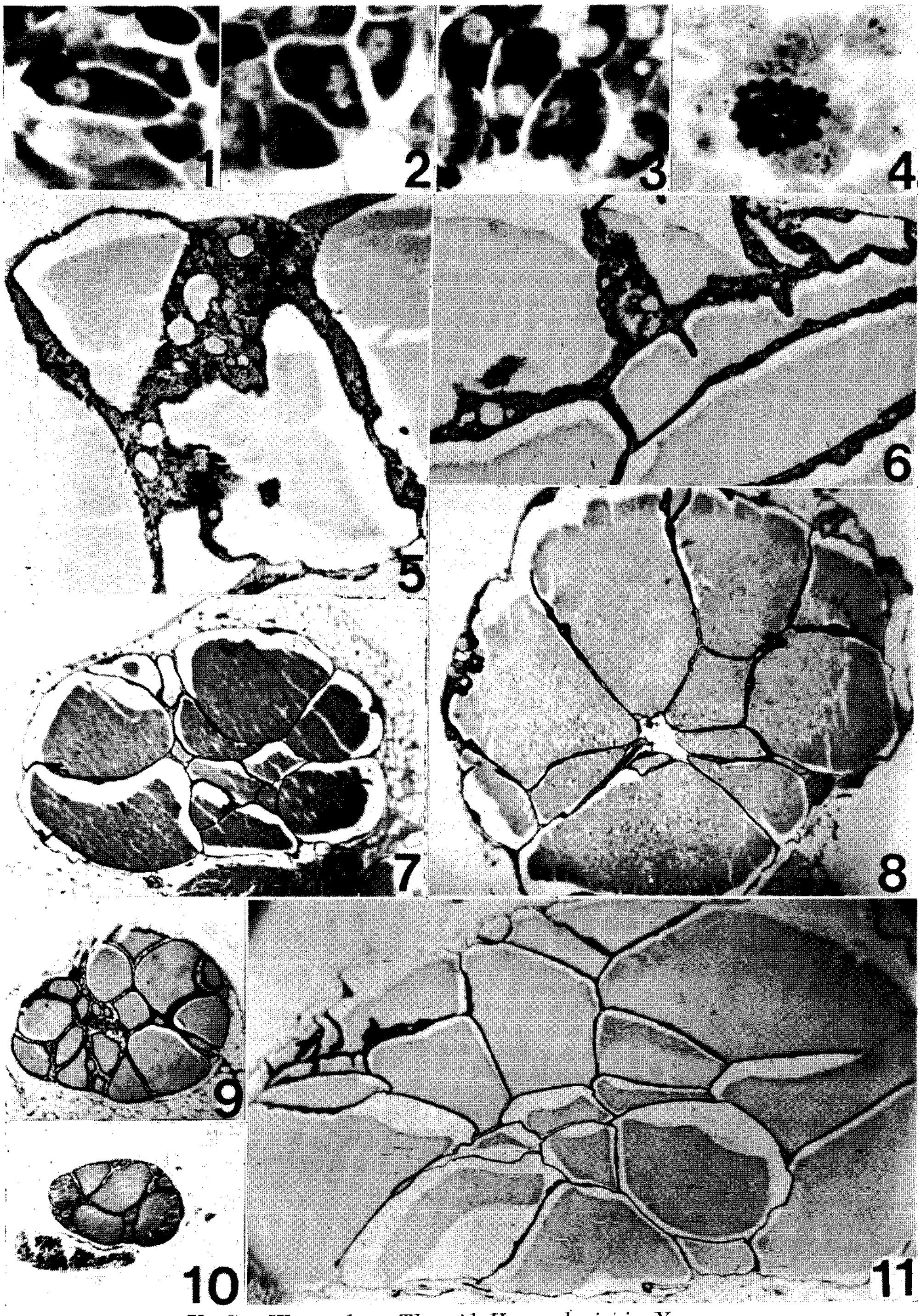

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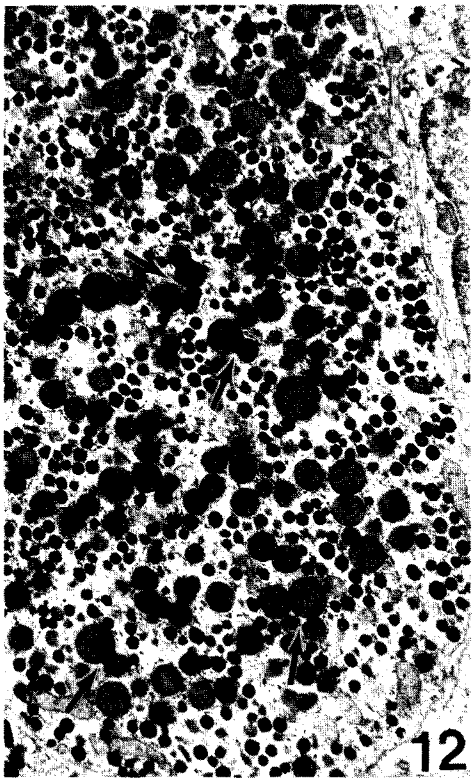

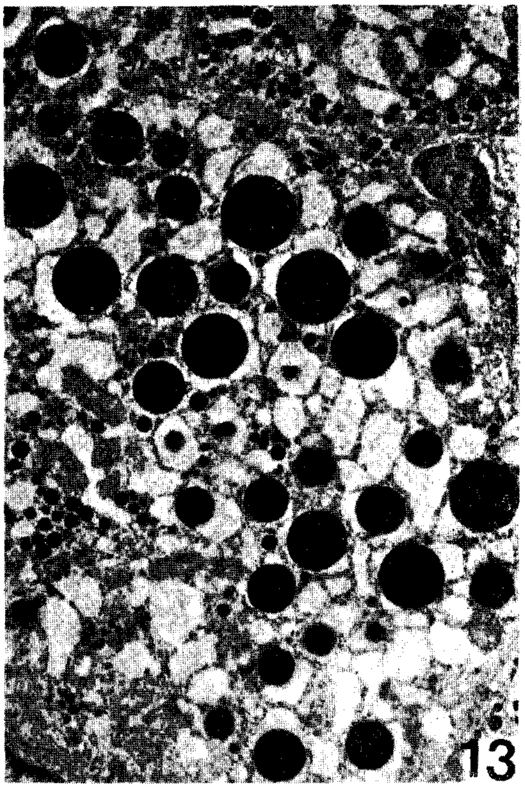

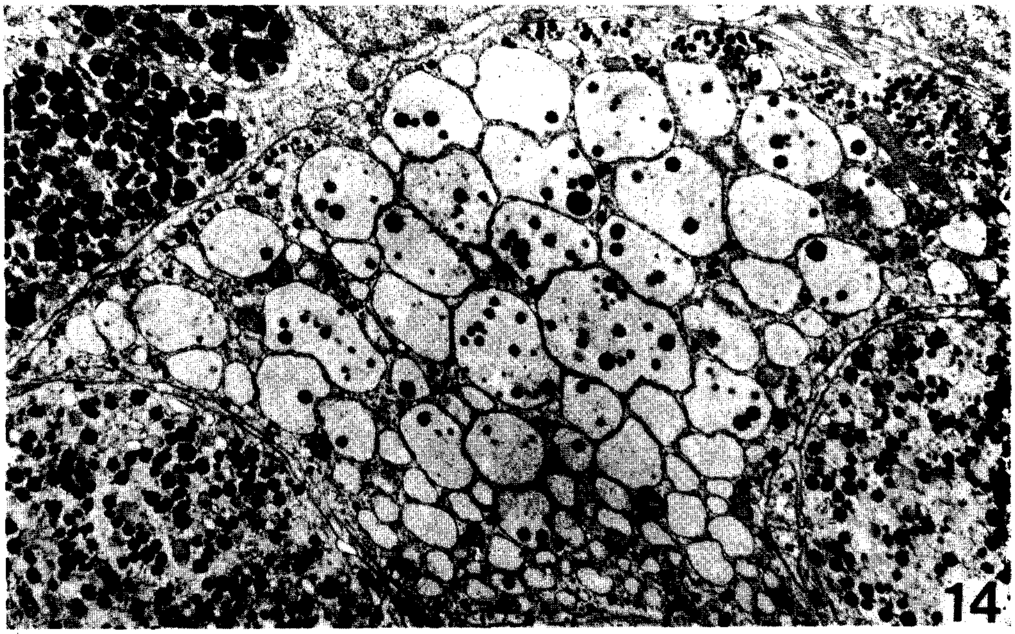

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Explanation of Plate IV

Figs. 1, 2, 3 and 4. Microphotographs of thyrotrophs with acidophilic granules (AG) of different size. × 800.

Fig. 5. The thyroid gland of animal no. 15. Abundant "micro-follicles" are seen between the ordinary large follicles. × 56.

Fig. 6. The thyroid gland of animal no. 1. Note irregularly folded epithelium. × 43.

Figs. 7–11. Transverse sections of the thyroids from animals no. 7 (Fig. 7), no. 3 (Fig. 8), no. 17 (Fig. 9), no. 21 (Fig. 10) and no. 1 (Fig. 11), respectively. Note striking variation in size of the gland. × 15.

Explanation of Plate V

Figs. 12–14 are electron micrographs of the thyrotrophs from animals no. 22 and no. 23.

Fig. 12. Electron micrograph showing spherical bodies occurring intermingled with ordinary secretory granules. Coalescing bodies are frequently seen (arrows). Styrene-methacrylate embedded, uranyl-lead stain. × 9,000.

Fig. 13. Large dense bodies found within cytoplasmic vesicles. The largest one measures about 1.5μ in diameter. In this cell, secretory granules are few in number. Epon embedded, uranyl stain. × 6,000.

Fig. 14. Thyrotroph filled with abundant cytoplasmic vesicles. The dense bodies contained in these vesicles are relatively small in size. These structures have close resemblance to those described in rat thyroidectomy cells (Farquhar and Rinehart, 1954). Styrene-methacrylate embedded, uranyl-lead stain. × 9,000.