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THYROID GLAND IN *CLARIAS BATRACHUS* (LINNAEUS): A MORPHO-HISTOLOGICAL AND HISTOCHEMICAL STUDY

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RESUMO: Relatamos as mudanças morfo-histológicas e histoquímicas que ocorrem na glândula tireóide do peixe teleosteo, *Clarias batrachus* (Lin.) Com base nos conteúdos coloidais e atividades secretorias, os folículos da tireóide do peixe sob estudo podem ser dividido em 5 estágios (quiescente, não-secretório, secretório, secretório ativo e atrofiado) Foram feitas tentativas para correlacionar as mudanças morfo-histológicas e histoquimicas de vários folicolos da tireóide com sua atividade secretória.

ABSTRACT: The morpho-histological and histochemical changes occurring in the thyroid gland of the teleost fish, *Clarias batrachus* (Lin) are reported. On the basis of colloidal contents and secretory activities, the thyroid follicles of the fish under study may be divide into 5 stages (quiescent, non-secretory, secretory, active secretory and atrophied) Attempts were made to correlate the morpho-histological and histochemical changes of the various thyroid follicles with their secretory activity.

INTRODUCTION

Thyroid being an important endocrine gland in teleosts has drawn the attention of many investigators (Honma, 1956; Gorbman, 1969; Bose *et al.*, 1977; Leatherland, 1982) owing to its variable nature from scattered follicles to encapsulated form. Though some literature are available concerning the histological aspects of teleostean thyroid, the degree of functional state of thyroid follicles and the studies pertaining to their histochemical nature is rare and far from comprehension.

An attempt has, therefore, been made in the present study, to correlate the different stages of activities of thyroid follicles of *Clarias batrachus* as reflected by their histological changes along with the concomitant changes in their histochemical nature.

MATERIAL AND METHODS

The commonly available freshwater adult catfish, *C. batrachus* measuring from 21 to 32 cm in length and weighing about 69 to 195 g were collected from the local fish ponds during the spawning season (June to August) The ambient water temperatures during the period of study were $30 \pm 2°C$.

Fishes were regularly autopsied and the lower jaws were fixed in aqueous Bouin s fluid and neutral formalin for histological and histochemical studies respectively. Decalcification was made in a mixture of 5% formic acid and formaldehyde (1:1 by volume) for six days. Sections were cut at 5 um and stained in haematoxylin eosin for histological study. For histochemical study 10 um sections were prepared and subjected to the following histochemical tests:

1 Mercuric bromphenol blue (MBPB) for detection of protein (Mazia *et al* , 1953)
2. Periodic Acid Schiff s (PAS) for detection of carbohydrate (McManus, 1946)
3. Best carmine for detection of glycogen (Best, 1906)
4. Sudan Black B for detection of lipid (Mc Manus, 1946, cited by Pearse, 1975)
5. Millon reaction for detection of tyrosine (Baker, 1956)

OBSERVATIONS

In the present study, the thyroid follicles have been found to be unevenly distributed surrounding the ventral aorta and its afferent branchial branches. Their occurrence at the base of the gill arches has also been encountered (Fig. 1) The follicles are mostly round or oval in shape and are highly variable in their diameters ranging from 20 to 60.5 um (Fig. 2) They are encircled by a single layer of epithelial cells whose heights vary from 2.4 to 4 um. However, some follicles encircled by multiple layer of cells have also been found (Fig. 3) The lumina of the follicles are either fully or partially occupied by agranular eosinophilic colloid materials and in many occasions they are provided with resorption vacuoles, the number of which are variable. The affinity of the colloid materials towards the basic dyes has also been noticed Like those of thyroid follicles the diameters of the colloid also vary from 10 to 54.5 um. Budding of relatively smaller follicles from the larger ones has occasionally been encountered (Fig 4)

On the basis of the amount of colloid contained in the lumen, presence or absence of colloidal resorption vacuoles and the height of the columnar epithelial cells, altogether five stages representing the different state of activities of the thyroid follicles, duly supported by the histochemical analysis have been categorised in the present investigation.

Follicles at the first stage, the stage of quiescent, the follicular lumen is not completely filled up with the colloid and thus a peripheral empty zone has been encountered around the centrally places colloid materials (Fig. 5) In this stage, the colloid material is relatively dense and devoid of any colloidal resorption vacuoles. The epithelial cell height appears to be minimal Follicles at the second stage, the non-secretory stage, the follicular lumen is completely filled up with dense colloid and thereby making the latter to be in contact with the epithelial cell layer. Hence, no empty peripheral zone is detectable in the lumen (Fig. 6) The epithelial cell height is also at its minimum and the colloidal resorption vacuoles are yet to be appeared Follicles of third stage, the secretory stage, show the appearance of few small colloidal resorption vacuoles along the outer margin of the colloid which is in a state of liquefaction (Fig. 7) In the follicles at the fourth stage, the active secretory stage, the number of colloidal resorption vacuoles increases considerably and they are found to be around the entire periphery of the liquefied colloid (Fig. 8) The height of the epithelial cell layer attains maximum during this stage. Follicles of the next stage, the stage of collapse or atrophied, are much smaller in comparison to those of other stages. They appear to be distorted with scanty colloid and sometimes become acolloidal and finally get collapsed (Fig. 8) The follicles of fourth and fifth stages have mostly been detected to be adjacent to the wall of the blood vessels (Fig. 8)

Various histochemical techniques employed in the present study indicate that the different categories of thyroid follicles as mentioned above exhibit variable intensity of reactions towards protein (Figs. 9,10), lipid (Fig. 11), periodate reactive carbohydrates (Fig 12), glycogen (Fig. 13) and tyrosine (Fig. 14) However, the follicles of first and second stages always display an intense reactions towards the different histochemical tests adopted while the intensity of the same has been found to be reduced gradually as the follicles pass through the subsequent stages, i.e. the third, fourth and fifth stages.

DISCUSSION

With few exceptions like *Sarda sarda* (Gudernatsch, 1911), *Gymnarchus* (Thomopoulos, 1950), *Channa punctatus* and *Heteropneustes fossilis* (Belsare, 1959) and *Latimaria chalumnae* (Chavin, 1972) in which the thyroid glands are in encapsulated forms, the structure of teleostean thyroids have been a continuing interest due to the scattered nature and migratory behavior of their follicles. The present investigation on the morpho-histological structure of thyroid follicles in *C. batrachus* corroborates the findings of Srivastava and Sathyanesan (1971) and Joy and Sathyanesan (1981) However, the histological observations duly supported by the histochemical studies indicate that the degree of functional state of thyroid follicles in *C. batrachus* could conveniently be divided into five stages on the basis of the amount of colloid material, epithelial cell height and presence or absence of colloidal resorption vacuoles. In the present study, the chromophilic nature of the colloid has not been taken into consideration due to the fact that the colloid substances of the same follicle differs tinctorially The tinctorial differences of colloid in thyroid follicles were considered by Hewer (1927) for determining their state of activities. Payne (1957) also stated that the colloid materials stain basophilically depending upon the degree of secretory activity of the epithelial cells. On the other hand, Bose and Firoz (1974) reiterated the opinion that the chromophilic nature of the colloid may not be the correct criteria to determine the functional states of thyroid follicles. Nevertheless, Mukherjee (1975) recognised three stages of functional state of thyroid follicles in *Heteropneustes fossilis* by taking into consideration of epithelial cell height, amount of colloid and its resorption vacuoles along with the chromophilic nature of the colloid materials. While working on the cyclical activity of the thyroid gland in *Heteropneustes fossilis*, Pandey *et al* , (1975) considered the epithelial cell height and the follicular diameters to express the different functional states of thyroid follicles. In *Phoxinus phoxinus* (Barrington and Matty, 1954), *Fundulus heteroclitus* (Gorbman and Berg, 1955), *Carassius auratus* (Fortune, 1955, 1956; Hoar and Eales,

1963) and *Salmo gairdneri* (Fontaine and Fontaine. 1957; Eales, 1965) the epithelial cell height was taken into account as main criterion for determining the diverse activities of thyroid follicles.

From the foregoing literature it is apparent that proper attention has not been given to determine the exact nature of various functional state of thyroid follicles in teleosts. In the present study, however, besides epithelial cell height, other histological features have been considered to denote five distinct stages representing the different degree of activities of thyroid follicles. These various functional stages of thyroid follicles are well in correlation with the different degree of intensity towards the different histochemical tests adopted. Freinkel (1964) observed histochemically that the lipid contents of mammalian thyroid fluctuate in parallel to the thyroid activity Similarly, Pandey (1974) also reported the fluctuations of thyroid phospholipid in accordance with the physiological activity of the gland in a freshwater teleost, *Rasbora daniconius*.

In the present study, the intense reactions towards the different histochemical tests at the first and second stages of activity of thyroid follicles and weak reactions at the fourth and fifth stages may be probably be interpreted owing to the fact that the follicles of the two former stages being inactive, retain their full content of hormones thereby giving an intense reactions. On the other hand, the follicles of two latter stages are partially evacuated by way of releasing their hormones into the blood stream and thereby making the follicles to react weakly to the respective histochemical tests employed in the present study

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Figs. 1 to 14 Photomicrographs of transverse sections of thyroid follicles in *Clarias batrachus.*

Fig. 1 Showing the occurrence of thyroid follicles (arrow heads) at the base of gill arch (GA) Haematoxylin-Eosin (HE), × 100

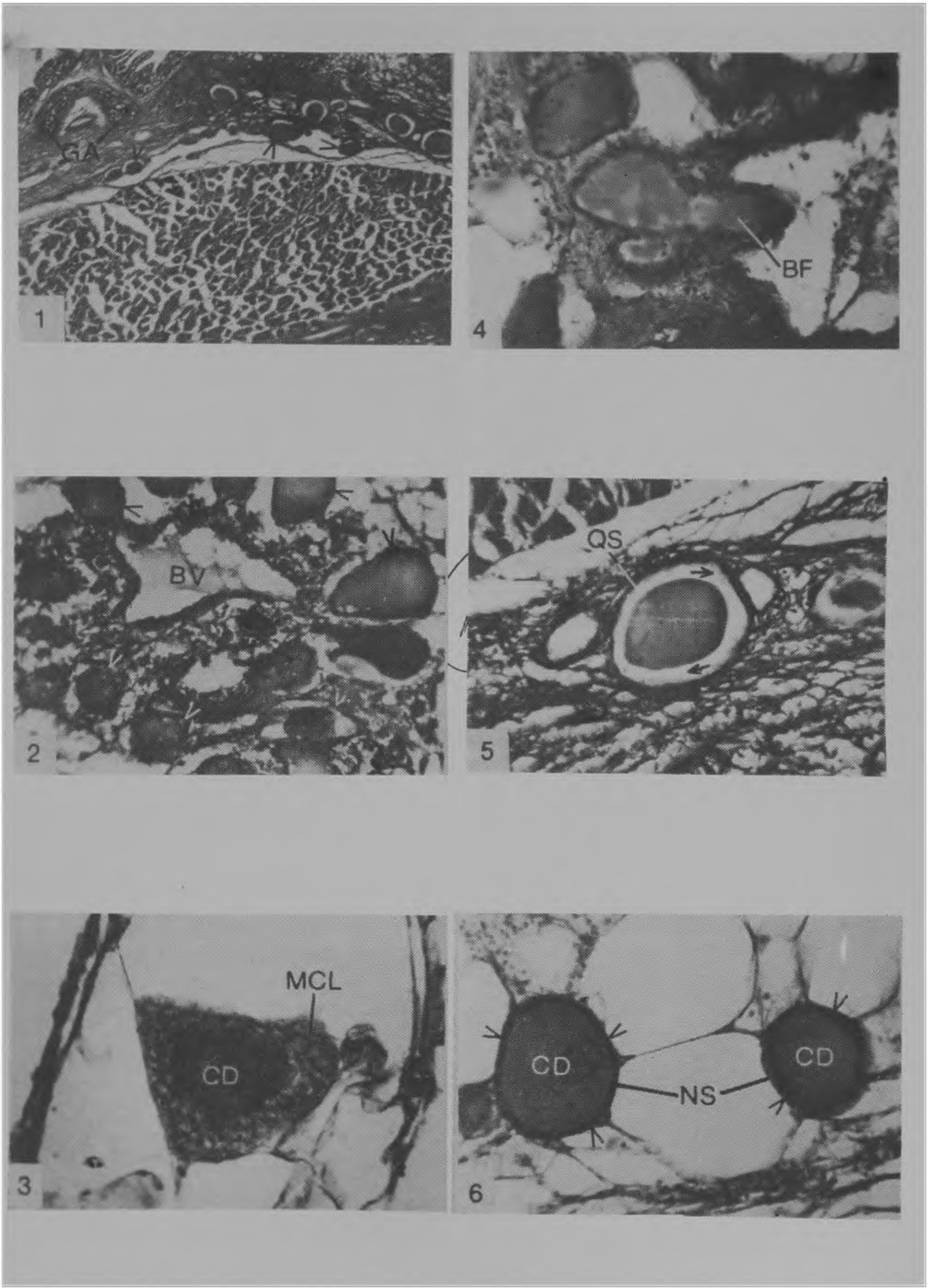
Fig. 2. Thyroid follicles (arrow heads) of various shapes and sizes occurring at the close proximity of the blood vessel (BV) HE, × 400.

Fig 3. Thyroid follicle with the colloid (CD) surrounded by multiple cell layers (MCL) HE, × 400.

Fig.4. A budding follicle (BF) originating from a larger one. HE × 400

Fig. 5. Thyroid follicle at the quiscent stage (QS) Note the peripheral empty zone (arrows) HE, × 400.

Fig. 6. Thyroid follicles at the non-secretory stages (NS) Note the intimate contact of dense collid (CD) located inside with the epithelial cell layer (arrow heads), HE, × 400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1
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4
BV
2
QS
5
MCL
CD
3
CD
CD
NS
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Fig. 7 Thyroid follicles at non-secretory (NS) and secretory stages (SS) Compare the staining intensity between the two stages. Note the appearance of colloidal resorption vacuoles (V) and the liquefaction of collloid at te secretory stage. HE, x 400.

Fig. 8. Thyroid follicles at the active secretory stage (AS) with increased number of colloidal resorption vacuoles (arrow heads) and at the collapse stages (CS) with distorded appearances. Note the presence of these follicles adjacent to the wall (arrow) of the blood vessel (BV) HE, x 400.

Fig 9. Thyroid follicle at the non-secretory stage (NS) showing intense reaction for protein. Mercuric bromphenol blue, x 400.

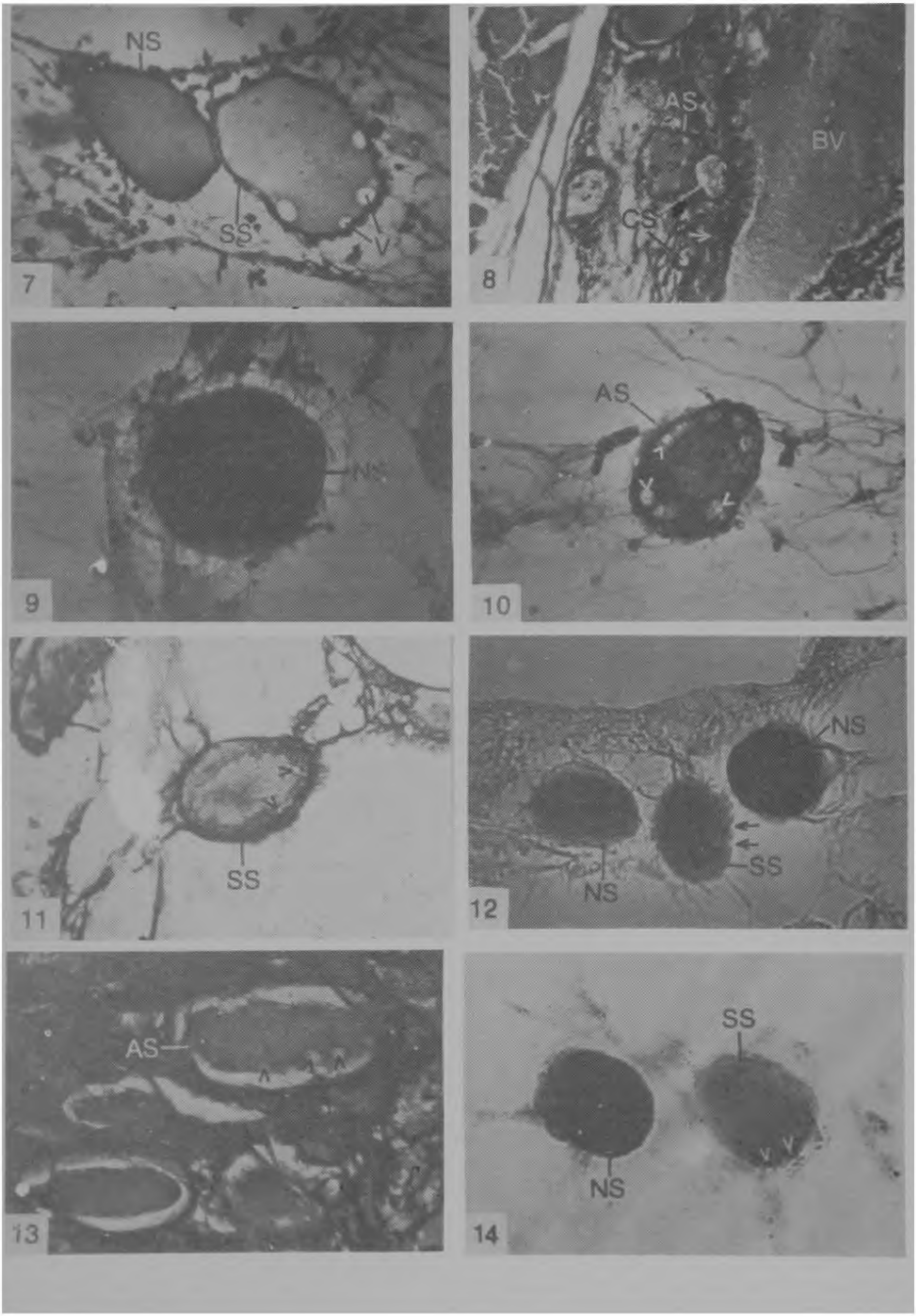
Fig. 10. Thyroid follicle at the active secretory stage (AS) showing weak reaction for protein. Note the presence of colloidal resorption vacuoles at the periphery (arrow heads) Mercuric bromphenol blue, x 400.

Fig. 11. Thyroid follicle at the secretory stage (SS) showing weak localization of lipid. Note the appearance of colloidal resorption vacuoles at the periphery (arrow heads) Sudan Black B, x 400.

Fig. 12. Thyroid follicles at non-secretory stages (NS) showing relatively higher localization of periodate reactive carbohydrates than that of the secretory stage (SS) with colloidal resorption vacuoles (arrows) PAS x 400.

Fig. 13. Thyroid follicle at the active secretory stage (AS) showing weak localization of glycogen. Note the presence of colloidal resorption vacuoles at the periphery (arrow heads) Best carmine. x 400.

Fig. 14. Thyroid follicle at the non-secretory stage (NS) showing intense localization and at secretory stage (SS) with colloidal resorption vacuoles (arrow heads) showing weak localization of tyrosine. Millon s reaction, x 400.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