"Histochemical observations on the adrenal tissue of the teleost fish, *Mandua*".

*(Folia Morphologica, Prague, 1974, --- In Press)*
The normal pattern of the teleostean interrenal tissue is thus fairly well known. Still much remains to be done primarily due to the innate variation in the general morphological setting of the adrenal tissue. To find out any difference from the normal pattern, if any, the genus *Nandus* was selected. Even preliminary observations of the fish pointed out the absence of the Corpuscles of Stannius hitherto known to be a common constituent of the adrenal apparatus of the teleostean fishes. Apart from the histological details, the usual histochemical procedures have also been applied to evaluate the exact functional position of the adrenal constituents of this interesting fish, as its the only genus of the family *Nandidae* (Order - Percoidel) found in India.

**MATERIAL AND METHOD**

A number of fresh specimens of *Nandus nandus* were collected locally from the Sagar lake. They were immediately dissected and the anterior kidney was fixed in the various fixatives. The sections were cut at 6 μ except for the mitochondrial preparations which were prepared at 4 μ. The outline details of the different procedures used are as follows:

1. **Hillarp and Hökfelt's chromaffin reaction (Hillarp and Hökfelt, 1955)**: The material was kept in a solution containing 10v of 5% potassium dichromate and 1v of 5% potassium chromate for 16 hours.
2. Wiesel's stain (Gray, 1954): The material was kept in Wiesel's fixative for four days and then transferred to 2% potassium dichromate solution for two days. Sections were stained in toluidine blue (1%) for 48 hours and counterstained by Safranin (1%).

3. Giemsa stain (Pearse, 1960): After fixation in the Zenker's fluid for 24 hours, freshly prepared Giemsa stain was applied to the sections for 72 hours.

4. Periodic Acid Schiff test for polysaccharides (Hotchkiss, 1943): The tissue was fixed in Aqueous Bouin, Zenker's fluid, Carnoy and Wiesel's fixative and sections were stained by PAS.

5. Iodate test for noradrenalin (Hillarp and Hökfelt, 1955).


8. Acid fuchsin and methyl green method for mitochondria (Gurr, 1965).

9. General stains like Haematoxylin - Eosin, Iron Haematoxylin - Eosin, Mallory's triple stain and Azan were used after suitable fixation for the study of histological details.

RESULTS

The adrenocortical tissue is located inside the anterior kidney along the course of the posterior cardinal vein. The anterior kidney on each side lies immediately
behind the pericardium. There is no apparent difference between the right and the left units of the anterior kidney. The cell masses form columns and many of them are grouped together into bunches of one to ten cells in sections.

The chromaffin cells are comparatively fewer in number than the adrenocortical cells. They lie intermingled with the adrenocortical cells (fig. 3).

**HISTOLOGY**

**ADRENOCORICAL CELLS**

The adrenocortical cells are roughly polygonal in shape with a large round nucleus. Average size of these cells is from 14 μ to 15 μ while the nucleus runs from 3 μ to 4 μ. They are grouped together into bunches of one to ten cells covered by collagenous fibres (fig. 1). The nucleus has a thick nuclear membrane and one large nucleous. In some nuclei a few chromatin granules are visible which are best seen in Azan stain. The adrenocortical nuclei are less basophilic than the nuclei of the kidney tissue.

Two varieties of the adrenocortical cells can be differentiated on the basis of the reaction of the cytoplasm to general stains like Haematoxylin-Eosin, Iron Haematoxylin-Eosin, Mallory's triple stain and Azan stain. In one variety the cytoplasm is dense, deep staining and strongly acidophilic.
In the other variety the cytoplasm is clear and scarcely reactive to stains. Further the cytoplasm of the second variety shows a number of granules which are coloured red by Azan stain. These granules are scarcely visible in the cytoplasm of the first variety perhaps due to its dense nature (fig. 2).

**CHROMAFFIN CELLS:**

Chromaffin cells are also grouped together into bunches and surrounded by a thin collagenous covering. They are found scattered among the adrenocortical cells. There is no uniformity in their distribution. Sometimes they are nearer to the internal wall of the posterior cardinal vein and at other times on the very outer periphery of the adrenocortical masses of cells. Minute capillaries are seen interspread among the entire adrenocortical cells and the chromaffin cells. The capillaries are specially prominent in relation to the bunches of chromaffin cells. The cytoplasm is clear, without granulation and nonreactive in all the general stains used. Faint markings are seen in the cytoplasm of some chromaffin cells. The nuclei are irregular, round or oblong and eccentric. They are less basophilic than the adrenocortical nuclei. Fine chromatin granules are seen in the nucleoplasm. The nucleolus is visible in some of them (figs. 1 and 2).
ADRENOMELANOCYTIC TISSUE

A curious feature of the structure of the anterior as well as the posterior kidney of Nandus is the presence of round encapsulated bodies containing masses of ill-defined cells. The histochemical evidence and discussion on the adrenomelanogenic nature of these encapsulated bodies are given in Chapter III.

HISTOCHEMICAL REACTION

CHROMAFFIN REACTION: In Nandus, the chromaffin cells give the typical reaction of mammalian adrenal medullary tissue. They are coloured yellow with potassium dichromate containing fixatives. This is the reaction on which the name chromaffin is actually based. Wiesel's stain and Giemsa stain give typical green colour to these structures thus confirming their chromaffin nature (fig. 3).

A test devised by Hillarp and Hökfelt (1955) to differentiate between two types of chromaffin cells produces uniformly yellow colour both in the chromaffin as well as in the adrenomelanogenic capsules suggesting the presence of noradrenaline. There is neither dark brown coloration given by adrenaline nor any indication of two types of chromaffin cells.
The Iodine test for noradrenaline (Hillarp and Nöckfelt, 1955) was tried but the results were not uniform.

SUDAN BLACK B: The adrenocortical cells as well as the chromaffin cells were all positive to Sudan black B staining. All these structures took an intense dark stain with the method of Mc Manus (Pearse, 1960).

The lipid reaction in the chromaffin cells is clearly shown in figure 4 and 5. Figure 4 is a microphotograph of the chromaffin reaction before applying Sudan black B. Figure 5 shows the same section after Sudan black B reaction for comparison.

ASCORBIC ACID: The adrenocortical cells react positively to the ascorbic acid test of Chinoy (1969). The granules are coarse but mostly of uniform size (fig. 6). Many areas are without any indication of ascorbic acid. Stray granules are occasionally seen in the kidney tissue as well. Chavin (1966) studied a number of fresh water and marine fishes and found the adrenocortical tissue positive to ascorbic acid.

The chromaffin cells are negative to ascorbic acid test.

PAS REACTION (Hothkiss, 1948): Both the adrenocortical cells and chromaffin cells are PAS positive. There is slight variation in the appearance of the cytoplasm according to the fixative used. With potassium dichromate and potassium
chromate containing fixatives fine granulation is seen in the cytoplasm.

MITOCHONDRIA (Regaud's Iron Haematoxylin, Carleton, 1946; and Acid fuchsine-Methyl green, Carr, 1965) : The cytoplasm of the adrenocortical cells is full of scattered fuchsinophilic particles. Some of them are larger in size and appear to be mitochondria. Similar structures are seen with Iron Haematoxylin staining (fig. 7). Thus there is no distinction between fuchsinophilic and nonfuchsinophilic particles in the cytoplasm.

DISCUSSION

The situation of the anterior kidney is quite normal and there is no asymmetry as found in Betta splendens (Van Overbeeke, 1960), Acanthobrama (Yaron, 1970) and Xenentodon canalis (Gupta, 1969; Gupta and Shrivastava, 1971 a,b.).

The adrenocortical as well as the chromaffin cells are typically teleostean in position. There are, however, two types of adrenocortical cells - one with dense acidophilic cytoplasm and the other with clear nonreactive cytoplasm in Nandus. Mahon, et al. (1962) found the cytoplasm varying from intensely acidophilic to basophilic in goldfish. The two cell types in Nandus do not show any structural difference and hence the varieties observed seem to be mainly due to their physiological status at a particular time. The
cells with clear cytoplasm have many granules and thus appear to be more active physiologically.

The Hillarp and Hökfelt's dichromate based test for differentiation between noradrenalin and adrenalin may be said to be quite reliable when both yellow and brown colorations appear among the chromaffin cells. The iodate reaction (Hillarp and Hökfelt, 1955) although considered specific was not uniform in its results in Nandus. According to Chavin (1966) there is "Inter specific variation in degree of intensity to the iodate reaction in several species of fresh water and marine species". In most of the teleosts worked out so far (Van Overbeke, 1960; Chavin, 1966; Yaron, 1970) doubts have been expressed towards the presence or absence of one of them. However use of Reserpine by Olivereau (1963) and Yaron (1970) indicates that noradrenalin is the common secretory product of the chromaffin cells among the teleost fishes. In Nandus also noradrenalin appears to be the normal secretion of the chromaffin cells which is further supported by Reserpine injections (Chapter V). Further there is no evidence to support the presence of two kinds of chromaffin cells producing noradrenalin and adrenalin.

The Sudan black B test for lipids is positive in most of the teleost fishes and appears to be characteristic of the adrenocortical cells. Oguri (1960) however found the interrenal cells negative to lipids test after staining frozen sections with Sudan III. According to Mabon and his
associates (1962) the Sudan black B stains interrenal sudanophilic granules from light grey to black. In Nandus (figs. 4 and 5) the adrenocortical cells are strongly positive to Sudan black B. In addition the chromaffin cells take dark stain with Sudan black B. Chavin (1966) found chromaffin cells negative to Sudan black B staining by the method of Chavin and Kovacevic (1961). Figures 4 and 5 bring out the conditions clearly in the case of Nandus. Thus the possibility of the presence of lipids or lipoprotein inside the chromaffin cells can not be ruled out among the teleost fishes.

Mahon et al. (1962) found that only adrenocortical tissue reacts positively to ascorbic acid test. Chavin and Kovacevic (1961), as referred to by Mahon et al. (1962) show the presence of ascorbic acid in the kidney tissue as well. In Nandus conditions are similar to those of Chavin and Kovacevic (1961).

Mahon et al. (1962) have demonstrated fuchsinophilic particles in the adrenocortical cells in gold fish Carassius auratus. According to him the granules appear as small aggregations near the nucleus. Sometimes they are in the form of large granules, rods or clumps. In Nandus apart from fine granules large sized round bodies are uniformly visible and randomly distributed in the cytoplasm more or less similar to the control tissue of Carassius plumbeus (Van Overbeke, 1966).
REFERENCES


Balfour, F.M. 1882 : On the nature of excretory organ in adult teleosteans and ganoids which is usually regarded as the head kidney or pronephros. Quart. Jour. Micros. Sci., 30 : 11-16.


Chinoy, N.J. 1969: On the specificity of the alcoholic, acidic silver nitrate reagent for the histochemical localization of ascorbic acid.
Histochemie 20: 105-107.

Callinge, W.E. and Vincent, S. 1897: The suprarenal bodies of fishes.

Comolli, A. 1913: Ricerche istologiche sull'interrenal dei Teleostei.

Fontaine, M. and Hasey, J. 1953: Recherches sur le contrôle hypothalamiqne de l'interrenal d'un poisson téléostéen, l'anguille (A. Anguilla L.) variations pondérales de l'interrenal antérieur.

Fontaine, M. 1954: Recherches sur l'interrenal antérieur des téléostéens.

Giacomini, E. 1902: Sulla existenza della sostanza midollare nelle capsule surrenali dei Teleostei.
Monitore Zoologico Ital., Ano XIII, n. 0.

1908: II. sistema interrenale e il sistema cromaffine (sistema feocromico) nelle anguille adulte, nelle cieche e nei Leptocelti.
Giacomini, E. 1910 : II sistema interrenale e il sistema cromaffine dei Ciprinidi.
Rend. R. Acad. Di. Sc. Dell'Inst. di Bologna, VI, XVI.

--------- 1911 : II sistema interrenale e il sistema cromaffine dei Labiobranchi.
R. Acad. Di Sci. dell'Inst. di Bologna, VI, XVI.

Blakiston Company, New York.

Gupta, O.P. 1969 : The blood vascular system of Xenentodon (Ham.).
Morph. Jb., 114(2) : 177-203.

Gupta, O.P. and Shrivastava, R.K. 1971a : Studies on the location, anatomy and histology of the endocrine glands of Xenentodon cancila (Ham.).

-------------------------- 1971b : Morphological studies of the interrenal bodies, mesonephric kidneys and the urinary bladder of the Indian Gar-pike, Xenentodon cancila (Ham.).

Currie, E. 1965 : The rational use of Dyes in Biology and General staining methods.
Leonard Hills : London.

Handin, R.I., Nandi, J. and Bern, R.A., 1964 : Effect of hypophysectomy on survival and on thyroid and interrenal histology of the cichlid teleost Tilapia mossambica.
Hanke, W. and Chester-Jones, I., 1966: Histological and
Histochemical studies on the adrenal
cortex and the Corpuscles of Stannius
of the European eel (Anguilla anguilla L.).

Hillarp, N.A. and Hökfelt, B. 1955: Histochemical demonstration
of noradrenalin and adrenalin in the
adrenal medulla.
J. Histochem. Cytochem., 3 : 1-5.

Hotchkiss, R.D. 1948: A microchemical reaction resulting in the
staining of polysaccharide structures in
fixed tissue preparations.
Arch. Biochem., 16 : 131-141.

Leloup-Hatey, J. 1964: Fonctionnement de l'interrénal antérieur de
deux Télécétacéens: le Saumon atlantique et
l'Anguille européenne.

Mahon, E.F., Hoar, W.S. and Tabata, S. 1962: Histophysiological
studies of the adrenal tissues of the
goldfish.

Mc Bride, J.R. and Van Overbeeke, A.P. 1969: Hypertrophy of
the interrenal tissue in sexually maturing
sockeye salmon (Oncorhynchus nerka) and
the effect of gonadectomy.


Yaron, Z., 1970: The chromaffin and interrenal cells of *Acanthobrama terae-sanctae* (Cyprinidae Teleostei).
Fig. 1 - Section of anterior kidney of *Nandus*
showing collagenous walls over bunches
of adrenocortical cells and chromaffin
cells.

Aqueous Bouin : Haematoxylin-Eosin. X 450

Abbreviations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD.C</td>
<td>Adrenocortical cells</td>
</tr>
<tr>
<td>CH</td>
<td>Chromaffin cell</td>
</tr>
<tr>
<td>COLL</td>
<td>Collagen fibres</td>
</tr>
<tr>
<td>COLL.W</td>
<td>Collagenous wall</td>
</tr>
<tr>
<td>N</td>
<td>Nucleus</td>
</tr>
<tr>
<td>PCV</td>
<td>Posterior cardinal vein</td>
</tr>
</tbody>
</table>
Fig. 2 - Section of anterior kidney of Nandus showing two types of the adrenocortical cells in the interrenal tissue.

Aqueous Bouin : Haematoxylin-Eosin. X 450.

Abbreviations:

ADC. I - Adrenocortical cells with dense and acidophilic cytoplasm
ADC. II - Adrenocortical cells with granular cytoplasm
CH - Chromaffin cells
KID - Kidney tissue

Fig. 3 - Section of anterior kidney of Nandus showing typical arrangement of adrenal tissue.

Zenker’s fluid : Giemsa. X 100

Abbreviations:

AD.C - Adrenocortical cells
CH - Chromaffin cells
KID - Kidney tissue
PCV - Posterior cardinal vein
Fig. 4 - Section of anterior kidney of *Nandus* showing the chromaffin reaction. Potassium dichromate and potassium chromate. X 100.

Abbreviations:

AD.C  - Adrenocortical cells
CH    - Chromaffin cells
KID   - Kidney tissue
PCV   - Posterior cardinal vein

Fig. 5 - The same as in Figure 4 after Sudan Black B stain. Compare figure 4 and 5 for lipid reaction in the chromaffin cells apart from that in the adrenocortical cells. Potassium dichromate and potassium chromate. Sudan Black B. X 100.

Abbreviations:

AD.C  - Adrenocortical cells
CH    - Chromaffin cells
KID   - Kidney tissue
PCV   - Posterior cardinal vein
Fig. 6 - Section of anterior kidney of *Nandus* showing ascorbic acid granules in the adrenocortical tissue. Stray granules of ascorbic acid are seen in the kidney tissue as well.

Silver nitrate : X 450.

Abbreviations:

AD.C - Adrenocortical cells
KID - Kidney tissue
PCV - Posterior cardinal vein

Fig. 7 - Portion of adrenocortical tissue magnified about 1000 times to show the mitochondrial particles in the cytoplasm.

Regaud's fixative - Regaud's Iron Haematoxylin. Similar results are obtained with acid fuchsin and methyl green : X 1000.

Abbreviations:

AD.C - Adrenocortical cells
KID - Kidney tissue
MIT - Mitochondria
N - Nucleus