CHAPTER-2
HISTORICAL RESUME
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A. Adrenal Homolog of Fishes.

Some of the earliest studies on the adrenal homolog of fishes were reported in cyclostomes and elasmobranch by several workers (Balfour, 1878; Grynfelt, 1903; J. F. Gaskell, 1912; W. H. Gasekel, 1920 and Goodrich, 1930) where it was established that the interrenal tissue and the chromaaffin cells were homologues of the mammalian cortex and the medulla. In these fishes, the two tissues remained anatomically separate throughout life with the more dispersed chromaaffin cells being present in the great vessels, heart and in abdominal wall in close relation to sympathetic neurons along the vertebral column. Among the non–teleost fishes, Giacomini (1904) examined the adrenal homolog of the Baltic sturgeon Acipenser sturio and later in A. rutenus, Scaphirhynchus platyrhynchus and Polyodon spathula (Giacomini 1933; 1934) and found chromaaffin cells in the walls of the posterior cardinal veins and the renal veins.

As early as 1902, Giacomini first delineated the interrenal and chromaaffin cells in teleosts and in 1934 he first described the adrenal homolog as an organ located within the head kidney (also referred to as the cephalic or anterior kidney) and in contact with the posterior cardinal vein or with their tributaries, in both the adult and developing specimens. In 1960, Oguri studied the distribution of chromaaffin cells in the carp where they were found to be restricted to within the kidney and resembled more closely the distribution pattern of some amphibians than of other fish species, in which the chromaaffin cells were distributed from the kidney to the wall of the heart. Nandi (1961) gave a comprehensive account of the different distributional patterns of the interrenal and
chromaffin tissues as well as their relation with the blood vessels in about 125 species belonging to 55 families of teleosts. The interrenal morphology was found to be extremely variable in these fishes. It was reported that the surrounding tissues could be lymphoid or myeloid or both, and might or might not contain renal elements. The relationship of the interrenal to these tissues was also found to be variable. Usually the interrenal tissues was associated with the postcardinal veins or their branches or both, which ramified throughout the anterior kidney tissue.

Chromaffin cells were always found to be located within the walls of these veins. Usually the interrenal and chromaffin tissues were separate, although in most fish species there was some type of association between them. Nandi (1961) postulated that in fishes when interrenal and chromaffin cells occurred together around the veins within the anterior kidney, one of several possible arrangements might be found: (1) The chromaffin tissue may be interspersed among the interrenal cells, as in the Cyprinidae and Cyprinodontidae. (2) In the Gasterosteidae, clumps of interrenal alternate with clumps of chromaffin cells, the entire complex forming a cuff around the lumen of the postcardinal vein. More often only the chromaffin cells are seen within the walls of these veins, either adjacent to the endothelium or embedded within the connective tissue of the vein wall, whereas the interrenal is found external to both the vein wall and is associated with the chromaffin tissues. Even in the truly interspersed glands mentioned above, the chromaffin cells tend to be located closest to the vein lumen. An unusual arrangement of the interrenal and chromaffin tissues, not described previously, was observed only in fish from two closely related families: the Labridae (wrasses) and the Scaridae (parrot fishes). In all the examined species of Scaridae family and some members of the Labridae family, a reverse arrangement was seen where the interrenal cells were found immediately adjacent to
the endothelium of the vein, as a single layer of columnar cells, and the chromaffin cells were present between the interrenal and the connective tissue of the vein wall. However in other members of Labridae family, other arrangements of interrenal and chromaffin cells were seen, but it was particularly noted that the chromaffin cells maintained a relatively constant position in the vein wall despite marked variations in the distribution of the interrenal tissues.

Studies done in goldfish (Van overbreeke, 1960; Nandi, 1962; Mahon at al, 1962) revealed that in this fish the major proportion of the interrenal and chromaffin cells are randomly mixed in collars of cells between two and twelve cell thick, encircling the postcardinal veins. Isolated groups of these cells were also found in the lymphatic tissue extending as far back as the mesonephros. Usually the interrenal cells were found to be more numerous than the chromaffin cells.

Observations were also made on the composition of adrenal tissues that indicated that the tissues surrounding the interrenal and chromaffin cells could be lymphoid or myeloid or both and may or may not contain renal elements (Nandi, 1961). The head kidney of the cichlid *T. mossambica* (Sailendri and Muthukkaruppan, 1975) was found to be encapsulated by thin strands of collagen fibers and included lymphatic and blood vessels and sinuses. The head kidney of the plaice, *P. platella* had a haemopoietic parenchyma supported by a network of reticuloendothelial cells (Ellis at al., 1976), whereas in *Mystus gulio* (Deivasigamani, 2007) the head kidney was found to be composed of haemolymphoid and endocrine (interrenal) tissues whereas the trunk kidney was purely renal tissue.

In majority of catfish, the presentation is such that the head kidney is well separated from the trunk kidney. The head kidney represents the pronephros and trunk kidney the functional excretory organ in the adults,
and the two parts are ontogenetically and in the adult anatomically distinct. Studies on *Mystus vittatus* (Gurumani, 1983) and *Mystus gulio* (Deivasigamani, 2007) showed that in these fishes the head kidney is well separated from the trunk kidney by the airbladder and the connection between the two being the postcardinal veins.

Grassi Milano *et al.* (1995), Manelli *et al.* (1996) and Abelli *et al.* (1996) suggested that different patterns could be found in some teleost species showing their anatomical relationship with the renal system and venous vessels. Grassi Milano *et al.* (1997) studied adrenal tissue in the developing and adult teleost of several families:— Salmoniformes — *Oncorhynchus mykiss* (rainbow trout), *Salmo trutta fario* (brown trout), *Coregonus lavarecus* (white fish); Cyprinodontiformes — *Gambusia affinis* (mosquito fish). Perciformes — *Dicentrarchus labrax* (sea bass), and *Sparus aurata* (sea bream). They found that in the adults of all species, steroidogenic and catecholaminergic chromaffin cells were found in the head kidney which is pronephric in origin and subsequently transformed into a haematopoietic lymphatic organ. While the steroidogenic cells are always confined to the head kidney in all the families studied, the chromaffin cells were found to be variably distributed around the anterior and posterior cardinal veins and ducts of Cuvier in Perciformes around the posterior cardinal veins and in the haematopoietic tissue in Salmoniformes around the ducts of Cuvier and posterior cardinal veins in Cyprinodontiformes while a few were also visible around the sinus venosus. In Perciformes and Salmoniformes, numerous chromaffin cells are also present in the posterior kidney, derived from the opisthonephros, in contact with the caudal vein. The finding of Grassi Milano *et al.*, 1997 supported the concept that the structure of the adrenal gland in teleosts is intermediate between that of
the other actinopterygians and that of tetrapods. The development differs from that of tetrapods in that it occurs mainly in the pronephros and only later do chromaffin cells reach the opisthonephric kidney.

Thus on the basis of comprehensive studies by several workers over the years, it was clearly outlined that the most primitive arrangement occurs in fish where chromaffin and steroidogenic cells, which are considered as functional equivalent of adrenal gland of tetrapod vertebrates, are mostly separated and scattered inside the renal tissue forming the adrenal gland so called the adrenal homolog (see Chester-Jones and Mosley, 1980; Mastrolia et al., 1981; 1984; Gallo et al., 1993; Abeli et al., 1996). In some teleosts, the two tissues mingle to varying degrees and depending on species, take up different positions in relation to the renal vessels. The topographic position of the adrenal tissues within the kidney, their relation with blood vessels and the relations between chromaffin and steroidogenic cells were found to have important functional implications (Gallo et al., 1993, Hankil and Kloas, 1995; Reid et al., 1995).

A part from the studies on topography, composition and distribution of the adrenal tissues in fishes, several ultrastructural studies of the interrenal and chromaffin cells were undertaken in several teleost species that revealed steroidogenic nature of interrenal cells. As early as 1980, Yoakim and Grizzle found that in the fat head minnow *Pimephales promelas*, one type of interrenal cell and two types of chromaffin cells were present. The interrenal cell showed features characteristic of steroid producing cells such as abundant endoplasmic reticulum and mitochondria with tubulo-vesicular cristae. The chromaffin cells were categorized into adrenaline producing cells and noradrenaline producing cells on the basis of electron density of cytoplasm and that of granules present in these cells. This was followed by studies on some more teleosts
Aphanius fasciatus (Mastrolia and Gallo, 1989), stickleback, Gasterosteus (Gallo et al., 1993), characids Brycon cephalus (Rocha et al., 2001), goldfish (Sampour, 2008) and grouper, Epinephilus tauvina (Abdel Aziz et al., 2010). The only ultrastructural study on a catfish adrenal tissue was conducted by Vermeulen et al., 1995 in which a large number of steroids including cortisol were localized in the interrenal cells by enzyme histochemistry. Apart from stickleback where two types of interrenal cells were observed — the light and dark cells, in most cases only one type of interrenal cells were seen. In 1993 Gallo et al., examined the catecholamine content of the two types of chromaffin cells using HPLC methods in the head kidney of the stickleback, Gasterosteus aculeatus and suggested that cells with electron dense granules contained noradrenaline and those with electron lucent vesicles contained adrenaline. Similar biochemical confirmation was done by other workers (see reviews by Chester Jones and Mosley, 1980) and later by Reid et al., 1998, and Gallo and Civinni 2003.

While most ultrastructural studies of the adrenal tissues have been performed on teleosts, few studies have dealt with non-teleost fishes. The available data is regarding the chromaffin cells of the holostean Amia calva (Youson, 1976) and the dipnoan, Protopterus and Lepidosiren (Scheuermann, 1993) and Neoceratodus (Chapin and Bennett, 1995). In all of these fish species, only one type of chromaffin cells containing granules with strongly electron dense content was described. However, in the chondrostean, Huso huso (Gallo et al., 2004) the chromaffin cells were found to have a wide distribution such as throughout the length of the kidney and showed sparse innervation which were considered primitive aspects. However, ultrastructural characteristics revealed two types of chromaffin cells.
Gallo and Civinni 2003 in his review on the adrenal homolog in teleosts concluded that the adrenal homolog of teleost is not a compact organ as the adrenal gland of most vertebrates but is composed of aminergic chromaffin and steroidogenic interrenal cells located mostly inside the head kidney which generally has a hematopoietic function.

B. Stress Responses of the Adrenal Tissues

By the mid twentieth century, it was clearly established that the interrenal gland in fishes was homologous to the mammalian adrenal cortex (see Chester Jones and Philip 1960; Mahon, et al., 1962) and was presumed to be the source of adrenocortical steroids (Butler 1965, Nandi and Bern 1965). This instigated several experimental studies which demonstrated histological changes in the interrenal cells in response to various stressors or stressful physiological activities. Oguri (1960) observed histological changes in the interrenal cells of goldfish after ACTH treatment whereas Mahon et al (1962) observed similar changes in response to cold stress. Crandall and Goodnight (1963) reported widespread histological change in Poecilia reticulata after long term exposure to several pollutants and they suggested that these were due to stress and/or poor metabolic utilization of food. Lang (1967) observed similar generalized change in a study on Carassius auratus.

Most of the studies during this period suggested that stress was associated with heightened interrenal activity in fish and the secretion of the interrenal gland might be responsible for the diverse changes that followed the increase in interrenal activity. Studies by Chavin (1956) which showed interrenal hypertrophy with saline treatment probably due to increased ACTH from pituitary and those by Van over Breeke and Ahsan (1966) which demonstrated interrenal hypertrophy after ACTH
injection and atrophy after hypophysectomy pointed out that the interrenal tissue in fishes is controlled by the pituitary. Basu *et al.*, (1965) indicated that measurements of interrenal nuclear diameter could be utilized as an index of interrenal function. They used this method to show the effects of hypophysectomy and of hormone treatments on the interrenal cells of *Tilapia mossambica* and suggested that a pituitary-interrenal axis resembling the pituitary adrenocortical axis of mammals existed in this fish.

A significant finding which was one step closer to interrenal-cortisol association was by Fuller (1974) who reported that increase in interrenal cell nuclear diameter closely paralleled increased in cortisol secretion as measured by a sensitive competitive protein binding assay. N. R. Bromage and A. Fuchs (1976) observed histological changes in the interrenal cells of the head kidney of goldfish in response to sublethal concentrations of detergent sodium lauryl sulphate and considered them to be indicative of interrenal cellular activation.

This was followed by several electron microscopic observations which indicated cytological modification of some organelles as nuclei, mitochondria, endoplasmic reticulum of the interrenal cells of several fish species in response to various stressors. In this respect, studies with osmotic stress and handling on *Aphanius* (Mastrolia and Gallo, 1989), in relation to annual reproductive cycle in male stickleback, *Gasterosteus aculeatus* (Civinni *et al.*, 2001) are significant. More recently ultrastructural evidence of piecemeal degranulation in response to osmotic stress in the chromaffin cells of *Aphanius fasciatus* (Crivellatto *et al.*, 2006) have been reported.

Along with the histological changes that were seen in the interrenal glands, increased secretion of adrenal steroids upon exposure to stressors were also reported in several studies leading to the belief that
they were responsible for the widespread and diverse changes that followed increase in the interrenal activity. McKimm (1966) found increased urinary excretion of adrenal steroids upon exposure to numerous sub lethal environmental stressors whereas circulating levels of cortisol were found to be elevated due to handling, physical exercise and disease (Fagerlund, 1967), forced swimming and chronic exposure to chromium (Hill and Fromm, 1968). A lot of research on the elevations of plasma corticosteroids mainly cortisol (Patrio at al, 1987) in response to various types of stressors has been well documented (see Pickering, 1987; Schreck, 1981; Barton, 1988 and Donaldson, 1981). These studies established cortisol elevation as an important hormonal or primary response to stress (Mazeaud at al., 1977) and activation of pituitary interrenal axis as an indicator of stress in fish (see Donaldson, 1981). It was postulated that cortisol is released primarily from the interrenal cells (adrenal cortex equivalent) of the head kidney tissue in fish following stimulation by adrenocorticotropic hormone (ACTH) (see Donaldson, 1981) and its release is controlled by negative feedback of cortisol at all levels of the hypothalamic-pituitary (HPI) axis (Fryer and Peter, 1977; Donaldson, 1981; Bradford at al, 1992 and Wendelaar Bonga, 1997).

Another major hormonal stress response to stress that emerged from studies (see Mazeaud and Mazeaud, 1981 and Nilsson, 1984, Boutilier at al, 1986) is the secretion of catecholamines, primarily epinephrine (adrenaline) into circulation following sympathetic stimulation of the secretory tissue. Catecholamines were found to be produced primarily by chromaffin cells in the head kidney tissue (adrenal medulla equivalent) and therefore their release is rapid elevating the circulation levels immediately after stress (Mazeaud at al, 1977; Randall and Perry, 1992 and Reid at al, 1998). The release of cortisol in teleostean and other bony fishes is delayed as compared to the
catecholamine release. A general review by Axelrod and Reisine (1984) indicates that regulation of these major hormone groups – corticosteroids and catecholamines is far more complex as the neuroendocrine control of both the hormonal axes is interrelated. Gallo and Civinni, (2003) in their review on adrenal homolog of teleosts suggested that other than the neuroendocrine control, local paracrine interaction may also play important role in modulating the stress responses.