The salmon (*Salmo salar* L.)'s Ultimobranchial body Histophysiological study at various stages of the salmon's life cycle in fresh water streams

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The Salmon (Salmo salar L.)'s Ultimobranchial Body

Histophysiological study at various stages of the salmon's life cycle in soft-water streams

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In 1889, Van Bemmelen makes reference to glandular growths at the level of the pericardial wall of sharks, chimaera and sturgeons, which he calls "suprapericardial bodies". Supino (1907) and Giacomini (1908-1912) look into these "suprapericardial bodies", in number a variety of fishes. Watzka (1933) proceeds with a comparative study of the gland in various classes of Vertebrates and shows it is inexistent in Clyclostomata. Finally, Krawarick (1936), unaware of any work done previously in this respect, believes in the discovery of a new type of gland which he identifies in 31 Teleostean species. The modern expression "ultimobranchial body" apparently was introduced by Greil (1905) with reference to this organ situated at the level of the last branchial slit of the m periesophageal
muscular wall, underneath the heart.

The hypocalcemia-inducing role of the gland was shown by means of tests made on a chicken (Tauber, 1967), a selachian (Copp et al., 1967), a batrachian (Robertson, 1968), and a teleostean (Chan, 1968). We did observe a hypertrophy of the ultimobranchial body (Lopez et al., 1968) on the eel (Anguilla anguilla L.), following hypocalcemias experimentally induced by injections of Carp hypophysis or by removal of Stannius corpuscles.

Calculated with respect to the calcemia of the Salmon (Salmo salar L.) since a number of years at the Museum's physiology laboratory, calcium concentration reveals variations of the calcemia during the life cycle of this particular fish (Fontaine et al., 1969). It was therefore deemed interesting to undertake a histophysiological study of the Salmon's ultimobranchial body at various stages of its life cycle.

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The Salmon's ultimobranchial body.

Fig. 1 to 5, 9 to 12
Material and methods

Salmons were caught at two different times in the year. In spring, more precisely in April, juveniles were caught in the Oloron stream:
- male parrs, herein designated as spring salmon (10 individuals);
- parr-smolts, or salmons beginning to show their silvery garb (10 females and 10 males);
- smolts in the course of catadromous migration (6 females and 6 males).

Adult fish were caught at Peyrehorade, where the Oloron and Pau streams come to a confluent:
- Anadromous salmon running upstream towards the spawning-grounds (3 females and 4 males);
- a mended salmon on its way downstream towards the sea, after winter spawning (1 male).

In January of the following year, were caught in the Oloron stream spawning-grounds:
- adult salmon in the course of spawning (10 females and 8 males);
- male parrs, herein designated as winter salmon (9 individuals), a number of which are taken to the physiology laboratory of the
Museum in Paris. At the end of a few weeks, they turn into silver. They are known as "pseudo-smolts" because their livery, although definitely silver in colour, is not exactly that of the smolt (the fins especially are much darker than those of smolts fishè naturally). They were taken in March to the Laboratory (6 individuals).

The ultimobranchial body area is immediately fixed in an aqueous fixative (Bouin) and processed by means of regular histology methods (alcohol bath, dehydration to butylic alcohol, paraffin inclusion, 5 µ sections). Stains used are:

- hemalum-eosin, as a topographic stain which serves to identify the ultimobranchial body in the sections; Groats periodic-Schiff-(PAS)-stain
- hematoxylin acid and Cleveland Wolfe Cytoplasmic granulations

PAS were submitted to a histo-chemical analysis:

1.- digestion test by maltase in a PAS association for the purpose of detecting glycogen.

2.- PAS stain subjected to various controls as follows:

- reversible acetylation: blocking prior to oxydation of 1-2 glycol groups; PAS is then negative.
- liberation by saponification of acetylated 1-2 glycols: if the PAS that follows is positive, the presence of mucopolysaccharids is confirmed.
Signs & Abbreviations used in Figures:

- gr. - granulations
- blood vessels
- pycnotic nuclei colloid
- piriform cells

Fig. 1 - A parr's ultimobranchial body. Groat's PAS-hematoxylin stain (X360.). Cells presenting a clear cytoplasm and a few masses of granulations PAS+ at the lumen's edge (gr.), and elongated blood vessels, against the basal membrane.

Fig. 2 - A smolt's ultimobranchial body. Groat's PAS-hematoxylin stain (X 360.)

Fig. 3 - A parr-smolt's ultimobranchial body. Cleveland-Wolfe stain (X 350.)

Fig. 4 - A mended salmon's ultimobranchial body. Cleveland-Wolfe stain (X 360.) Cell masses intermingled with colloid organized patches to form a disorganized tissue, degeneration in the course of degeneration.
RESULTS

A histological examination shows the Salmon's ultimobranchial body as a compact organ whose pseudo-stratified epithelium presents vesicles partly blocking the gland's lumen. Robertson (1965) explains the Rana pipiens's epithelium's pseudostratification by the various shapes taken by the cell while it matures. In a primary stage, near the basal membrane, it is ovoid with a round nucleus, then it becomes elongated, its nucleus takes an oblong shape, granulations are formed. When the cell is degenerating, the nucleus is close to the lumen.

With the Salmon at the parr stage, the gland's epithelium is at a low level. Cells present a clear cytoplasm with few granules, a markedly basophilous nucleus, with the nucleolus hardly visible located at the base of the cells. Their apex contains a few masses of granulations PAS+, towards the lumen. Against the basal membrane, in the connective tissue, small blood vessels may be observed. Such is the picture of a gland having little activity. (Fig. 1).

The ultimobranchial body of smolts, parr-smolts and smolts is much the same as the gland we have just described. No great difference may be found (Figures 2 and 3.).
With adult Salmon in anadromous migration, the epithelium is S-shaped, which, as a result, increases the distribution surface between the cells and the blood. The blood vessels are in large number and dilated. Nuclei are located at the base of the cells and abundantly often present outlines of mitosis. The cytoplasm is filled with granulations PAS/ with the optical microscope being adjusted to optimal power (figures 5, 7 and 10). The PAS stain followed by a positive reversible acetylation enables us to identify the granulations as comprising mucopolysaccharides; digestion by maltase being negative excludes all glycogens. In this case the gland appears to be storing up material.

In the case of salmon in the spawning stage, the epithelium's aspect is altogether different. It is very high, is made up of pyriform cells, the cytoplasm of which is clear and apparently devoid of granulations. The cell apex, swollen up, is vacuolated. The nuclei, very elongated, are hardly basophilous and contain two or three large nucleoli taking highly to stain. Towards the lumen, may be found pycnotic nuclei affixed to the membrane of the cell apex and giving a scalloped aspect to the epithelium.
The ultimobranchial body of a salmon migrating upstream

Fig. 5 - A spawning salmon's ultimobranchial body. Groat's PAS-hematoxylin stain (X 80.) Scalloped-shaped epithelium. Large vascular network filled to the brim with red blood corpuscles around the organ. (See details figures 7 and 10.)

Fig. 5 - A spawning salmon's ultimobranchial body. Groat's PAS-hematoxylin stain. (X 80.) High epithelium. Clear cytoplasm and nuclei. (See details figures 8, 9, 10, 11 and 12.)

Fig. 7 - Closer view of figure 5 (X 360.) Vague aspect of cytoplasm is due to abundant very fine granulations PAS.}

Fig. 8 - Closer view of figure 6 (X 360.) Note triangular blood vessels, one angle of which pushes its way between epithelial cells.
These degenerating nuclei are often associated with colloid patches
PAS (figures 6, 8, 9, 11 and 12.) The blood vessels then assume an angle through the basal membrane, triangular shape, and make their way through the epithelium, angleways. This particular aspect was already noticed in the eel, the ultimobranchial body being stimulated (Lopez et al., 1968), and would appear as a sure sign of the gland's activity. Measurements were made of nuclei diameters but the fact that they frequently take on another shape makes it difficult to interpret results.

At the mended phase, the ultimobranchial body has undergone complete degeneration. Masses of active cells still remain in close association with cell wastes and pycnotic nuclei although in a disorderly fashion. (Fig. 4).

DISCUSSION

Blood calcium, applied to these same individuals revealed differences (not always significant) between parrs and smolts, while a drop of calcemia is clearly seen between the adult salmon going upstream and the spawning salmon (Fontaine et al., 1969). This particular stage of the life cycle, the gland shows hyperactivity.
In the case of parrs, smolts, parr-smolts and pseudo-smolts, the gland appears as being in an inactive stage. The epithelium is made up of cells similar to the "storage cells" noted by Robertson (1969) in the frog. Apex of these cells is filled with masses of granulations. It would appear this material becomes especially abundant in the glands of salmon going upstream. Here, the epithelium takes a scalloped aspect, the cells, assembled together at the base, push the storage cells towards the center. On the other hand, the number and size of the blood vessels may lead to understand that the gland has reached the stage for extensive activity. But, between this phase and the spawning stage, calcemia drops between 25 to 35% (highly significant), which may be due partly to an increased activity of the ultimobranchial body, which secretes calcitonine.

We know for a fact that (O'Dor, 1969) calcitonine may be extracted in important quantities from the ultimobranchial body of the Pacific Salmon (Onchorynchus) and that this hormone is 25 to 50 times more active in the rat in a mammal. With fish, injections of the hormone apparently give very different results, according to the species. Logan (1967) obtained a hypocalcemia with
a phosphatemia, by injecting thyrocalcitonine to a Telostean (Ictalurus melas); similarly, Chan (1968) injected calcitonine to the European eel (Anguilla anguilla), and immediately observed a lowering of the calcemia rate. On the other hand, Pang (1967), while attempting the same experiment with Fundulus heteroclitus in sea water, did not produce any reduction in the calcemia. This may be explained by the fact that the two first fish stated have a cellular line

while Fundulus's which is non-cellular, does not appear to present any normal bony reabsorption (Opp, 1969).

The ultimobranchial body of a spawning salmon is actually a gland presenting sure signs of activity and "depletion": disappearance of secretory granules, glandular hypertrophy and modification of the nuclear and nucleolar outlines. The three types of cells described by Robertson (1968) are found in the case of a hypercalcemia produced experimentally with Rana pipiens: basal cells, secretory cells and degenerating cells. Complete degeneration areas found in a mended salmon's gland have already been observed in the case of the Stannius corpuscles of the mended salmon (Lopez, 1969), and would be associated with regulation of the calcium metabolism (Fontaine, 1964-67; Lopez, 1969).
For its going upstream, important calcium transfers are the production of essential for genital material; surprisingly enough, salmon's only do not seem to present any excessive brittleness. This is probably due to the fact that the ultimobranchial body, stimulated as we have shown, secretes calcitonine which slows down the outset of an osseous reabsorption described by Tchernavin (1938-1940).

At any rate, the histophysiological study of the salmon's gland in normal physiological conditions seems to confirm the gland's role in regulating calcium metabolism with Teleostean fish.

SUMMARY

REFERENCES