THE MICROSCOPIC INNERVATION OF THE GILL-APPARATE IN SCORPAENA PORCUS

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The anatomical and structural difference of the gill-apparate in fishes was examined by several authors as it is proved by RAUTHER's monographical work (1937). Especially many data were given by BEVELANDER (1935) about the structure and function of their epithelial cells. Well-known examinations threw light upon the physiology of the gills (LUTZ, WYMAN 1932, INVING, SOLANDT 1935, COPELAND 1948) However, the problem of gill-innervation, mainly in microscopical respect, except DE BOYD's work (1936) may be considered uncleared. Concerning the innervation of the gills was determined in general that the first branchial arches are supplied with glossopharyngeal nerves and from 2—4 with vagal ones (BÜTSCHLI, ROMER, WIEDERSHEIM). There are only suppositions about the participation of the trigeminal, facial nerves and of the sympathetic system in the innervation of the gill-apparate.

Material and method

We studied the innervation of the gills in the *Scorpaena porcus*. This animal was preferred because we could get it in great number due to the kind help of the scientifical workers in the Research Institute of Split. On the other hand the function of their gills is usual and very surprising. They swim quickly in the bottom of the aquarium and stop many times, open the opercular apparate and move the gills. The moving became stronger lifting the animals. Under dry circumstances the skin of the head and the palatinal membrane became pinky, likely owing to the change of the blood circulation and breathing, and about 5 minutes the gills move regularly.

The gill apparate were fixed in 10 p. c. formaldehyde. The anatomical section was prepared with the *truncus arteriosus*. The microscopical slides were made with frozen microtome separating first the gill-lamellae, and the impregnation with BIELSCHOWSKY—ÁBRAHÁM's method. The thickness of the slides was 20—25 μ.

Different parts in the gill apparate and their innervation

The gill apparate has 4 well developed double branchial arches with two lamellae in *Scorpaena porcus*. The anterior ends of the arches came together in the *entoglossum* whereon the lingual part is formed. The posterior ends are connected with the *pharynx*. The whole apparate forms a unity with the hyoi-

dean hemibranch, with the mucous membrane of the oral cave and with the musculature belonging to the branchial, hyoideal and pharyngeal region.

The two lamellae are placed to outward side of the osseous branchial arch. They are situated on the concave pharyngeal margin of the branchial arch merged into one another at the basis. Opposite the gill-lamellae at the inward side, the branchial arch is fringed with bony processes, so-called gill-rakers. They are covered with the mucous membrane. This is continuation of that in the oral cave and spreads to the branchial arch too, forming the connection between the gill-lamellae and gill-rakers as well as the loose connective tissue around the branchial arch.

So, the above mentioned parts including their special and rich blood circulation and musculature, show difference of each other, not only structurally but also in respect of innervation.

Gill – lamellae

The structure of the gill-lamellae as common in the osseous fishes, consists of filaments of different size. They are longest at the middle and they gradually decrease towards the ends. In the central part of the filaments, in the loose connective tissue cartilagineous trabecle may be seen in right angles to the osseous branchial arch. Here are the arteries and veins, originating from the branchial vessels, running parallel to the supporting cartilage of filaments. Sometimes at the basis of filaments striated muscle fibres appear too. The entire surface of each of the filaments is covered with respiratory epithelial cells. Many delicate indentments to fine little filaments grow their respiratoric surface.

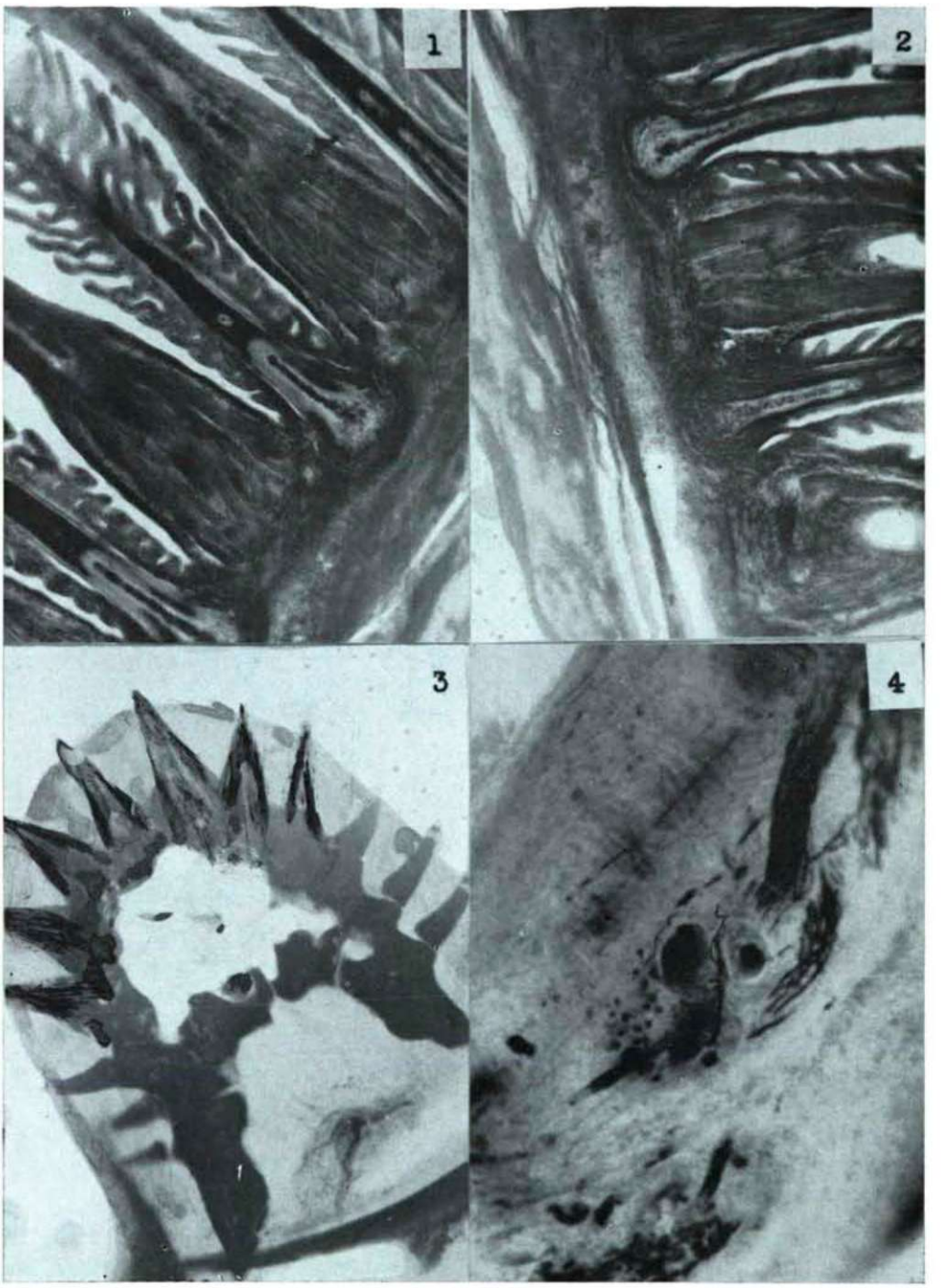
The gill-filaments of *Scorpaena porcus* have always broad base and show two types. One type of the filaments possesses supporting cartilage the wide diameter of their base originates from the large devided bulb. The other type shows surprising many striated muscle fibres, very large blood vessels and abundant connective tissue at the basal part. Their decrease caused the thinning of the filaments close to the top. The two kinds of filaments is regularly alternating (Table I. Fig. 1). At the endings of the lamellae the filaments show an absolute lack or very small cartilage and in most of the cases the muscle fibres do not occur either. These pictures are in full agreement with the description of the gill filaments of fresh water fishes by other authors (KRAUSE 1921, RAUTHER 1925, SCHÖTTLE 1910).

The nerves of the filaments reach the base in one trunk. They come from the connective tissue around the osseous arch. Here the main trunk is found in a close connection with the branchial artery and vein (Table I. Fig. 2). The nerves have many thick fibres forming coils in their way and quite thin fibres running

Table I.

Fig. 1. Alternating structure of gill filaments.
Fig. 2. Blood vessels and nerve trunks connections to the filaments.
Fig. 3. The thorns of a gill-tooth.
Fig. 4. Nerve cells in the arterial *plexus*.

TABLE I



always straight. Both, however more frequently the thicker ones have varicosities. The lateral branch of this trunk, the so-called filamentary nerve, runs obliquely to the filaments and forms a very rich *plexus* in the basal part.

Alternative change can be seen not only in the structure of filaments but also in the innervation. The supporting filaments are always poor of nerves but the others are so richly supplied that is a rarity (Fig. 1). Three kinds of arrange-

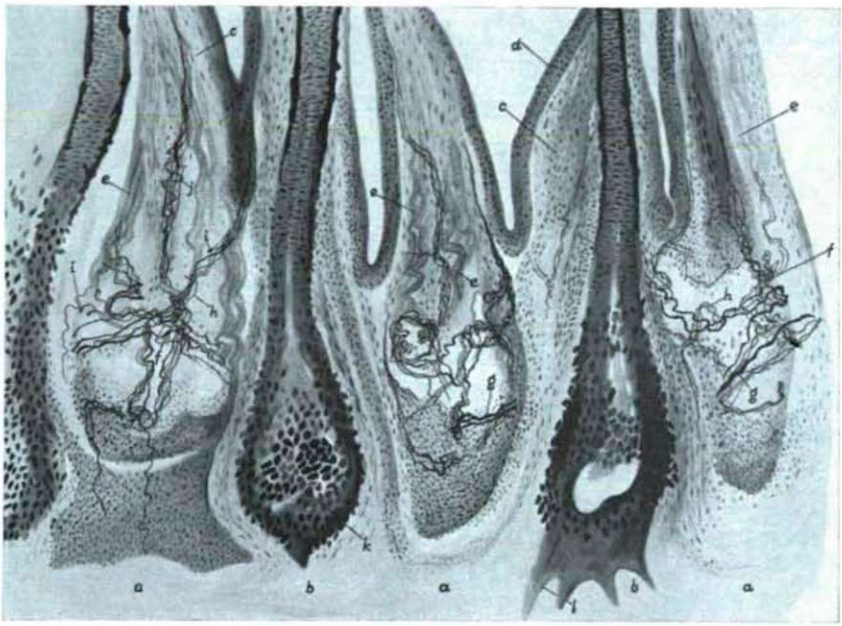


Fig. 1. *Scorpaena porcus:* Alternating structure and innervation in the basal parts of the gill filaments in the middle of branchial lamellae. a — bases of the moving and vascularised filaments, b — bases of supporting filaments, c— connective tissue, d — respiratory epithel, e — striated muscle fibre, f — ring *plexus*, g — thick nerve fibre, h — thin nerve fibre, i — nerve ending, j — spiral fibre, k — cartilage, l — thorns of the gill raker. BIELSCHOWSKY—ÁBRAHÁM's method. Magn. 120x.

ments could be separated in the dense *plexus* at the base of the filament. One is quite superficial and shows ring form. A part of the fibres runs to the striated muscle fibres and an other one to the filamentary artery. We suppose most of the thick fibres are connected with the musculature. The filamentary muscle fibres get very rich nerve supply. Their special innervation will be described in the chapter of „The muscles of the gill apparate".

Some of the fibres originating from the ring *plexus* run to the filamentary arteries and form very rich and dense plexuses in the arterial wall together with the fibres coming here in the *adventitia* of the vessels and in all probability belong

to the cranial sympathetic system. Sometimes little black end-spots or rings could be seen at the endings of the nerve fibres, especially in the *media* and larger end-plates with neurofibrillar structure in the *adventitia*. The former must be the endings of the *effector* fibres and the latter of the receptors.

May be that some of the free end-fibres of the plexuses run quite close to the epithelial cells accumulated between the two filaments but intimate connection was never visible. Some of the fibres of the plexuses run upwards along the filamentary arteries. Many spiral fibres appeared among them (Fig. 1, j). However, we would emphasise that the top fibres originating from the plexuses reach only the middle of the filaments. No fibres could be found in the upper part of the filaments where the respiratory epithelial cells and the capillary system were dominant. So the function of the respiratory cells is similar to that in the lungs of higher vertebrates free of close nerve connection.

Gill – rakers

The gill-rakers are seemingly very simple and insignificant formation of the gill apparate but according to their structure and innervation they must play an important role in the life-function, especially in the feeding of the *Scorpaena porcus*. Taking into consideration their structure they consist of separated hillocks at the opposite ends of the supporting gill-filaments. Structurally they are similar to the teeth of the higher vertebrates and probably have the function like that of the teeth, as the real maxillar and mandibular teeth are good enough to seize the food but not so strong to chew it. So it is right to call them gill-teeth. The main substance of the gill-teeth is compact bone forming 8—16 thorns towards the oral cave and *septa* of different sized to the *pulpa* cave. Outside they are covered with the mucous membrane but the ends of the thorns run through most of the cases the membrane (Table I. Fig. 3).

Inside of the gill teeth the connective tissue full of blood vessels and nerve fibres is found. The blood vessel system, mostly capillaries, forms rich *reticulum*. The rich nerve *plexus* coming from several smaller nerves runs at the base of the rakers series in the connective tissue of the branchial arch and gives lateral branches to the gill-teeth. The nerves consist of thicker and thinner fibres but structurally they differ from that in the main gill trunk mentioned before. Despite the near position, both of the trunks situated in the connective tissue of the branchial arch no connection could be noted between them.

The nerves of the gill-rakers possess always fewer thick fibres and very many thin ones. They never showed varicosities or coils in their pathways. Very richly branching in the connective tissue they form a dense *plexus* closely connected with the connective tissue cells (Fig. 2). The thick fibres as a rule showed dendronlike ramification or free coils at the end, the thin fibres follow the processes of the connective tissue cells and end freely or with little knobs on the cell bodies or quite next to that.

The small arteries or veins of the connective tissue in the gill teeth seem to have own nerve supply. Small nerve trunks with fine thin nerve fibres or quite

Fig. 2. *Scorpaena porcus:* Structure and innervation of the gill tooth. a — *epithelium* of the mucous membrane, b — *lamina propria,* c —bone rounding the *pulpa,* d — connective tissue of *pulpa,* e — capillaries, f — nerve *plexus,* g — thick nerve fibre, h — thin nerve fibre, i — goblet cell. BIELSCHOWSKY—ÁBRAHÁM's method. Magn. 200x.

separated thin fibres may be connected with the vessel wall. The capillaries are mostly poor of nerve fibres.

The pictures obtained were very similar to the teeth-pulpa innervation of mammals and human. Relatively as many fibres were found in the gill-teeth *pulpa* of *Scorpaena porcus* as by other authors (BERKEBACH VAN DER SPRENKEL 1935, BERNICK 1948, CHRISTENSEN 1938, GORDON-JÖRG 1953, HATTASSY 1958, 1960, HELD- BAUD 1953, LOEWENSTEIN-RATHKAMP 1955, MARTINO 1941) in the teeth *pulpa* of higher vertebrates. So we consider the connective tissue of the gill-teeth as a very sensory area of the gills.

The mucous membrane

In the field of the gill-apparate the whole branchial arches are covered with mucous membrane. The mucous membrane structurally and in respect to its innervation is in full agreement with that of the oral cave. We stated their identity in the *Scorpaena porcus*. The mucous membrane consists of the outer epithelial and the inner connective tissue layers. The *epithelium* is represented by the stratisfied squamous, not keratinized type full of goblet cells. The *lamina propria* shows special structure and forms in places double layer. The higher layer is in close connection with the basal membrane of the *epithelium* and the deeper one is connected with the osseous or muscular elements. Both can be considered as loose connective tissue but in the higher layer the connective tissue cells, in the deeper layer the fibrous elements are dominant. The cell bodies with the processes form *reticulum* in the higher layer, appearing here a very distinct form of the *reticulum* of the connective tissue cells due to the strinking strong affinity to the silver.

The cell *nuclei* have more *nucleoli* and could be observed very frequently their amitotic propagative forms. Numerous small arteries and capillaries were found here and particularly dense nerve *plexus* is characteristic of this layer (Fig. 3). Three kinds of fibres are distinguished in the *plexus*. The three kinds of fibres are located in one trunk in most of the cases. According to their location, structure and end-connections we think the thick fibres (Fig. 3, d) are receptoric fibres of the epithelial cells. The thin ungranulated fibres (Fig. 3, f) must be effectors to the vessels and the glandular (goblet) cells. The fine granulated fibres (Fig. 3, e) which form the greatest part of the fibres, seem to be very sensitive receptoric fibres belonging to the connective tissue cells.

The deeper layer of the lamina propria which may be considered as a *lamina submucosa*, is especially thick in the branchial branch and at the lingual part of the gill-apparate. In this layer, the number of the connective tissue cells are relatively few and the number of the interstitial substance especially that of the collagenous fibres is increased.

This layer surrounds the osseous branchial arch, gives tissue to the *pulpa* of the gill teeth and carries the vessels and nerves to the different parts of the gill-apparate. It is poor of nerves but sometimes free or capsulated coils can be found in this layer (Fig. 4). It is especially interesting because the incapsulated nerve endings are unknown in the fishes, amphibians and reptiles. We could see in the coils thicker and thinner fibres with many longish varicoses in both kinds of fibres. The coils are round or longish and their origin is in all probability from the sensory trigeminal nerve.

The branchial blood vessels

The branchial vessels giving the filamentary branches are lateral branches of the *truncus arteriosus*. They are located in the connective tissue of the branchial arch close to each other the artery, vein and the main nerve trunk of the gill-lamellae. In respect to the innervation of the vessels the artery has always very rich

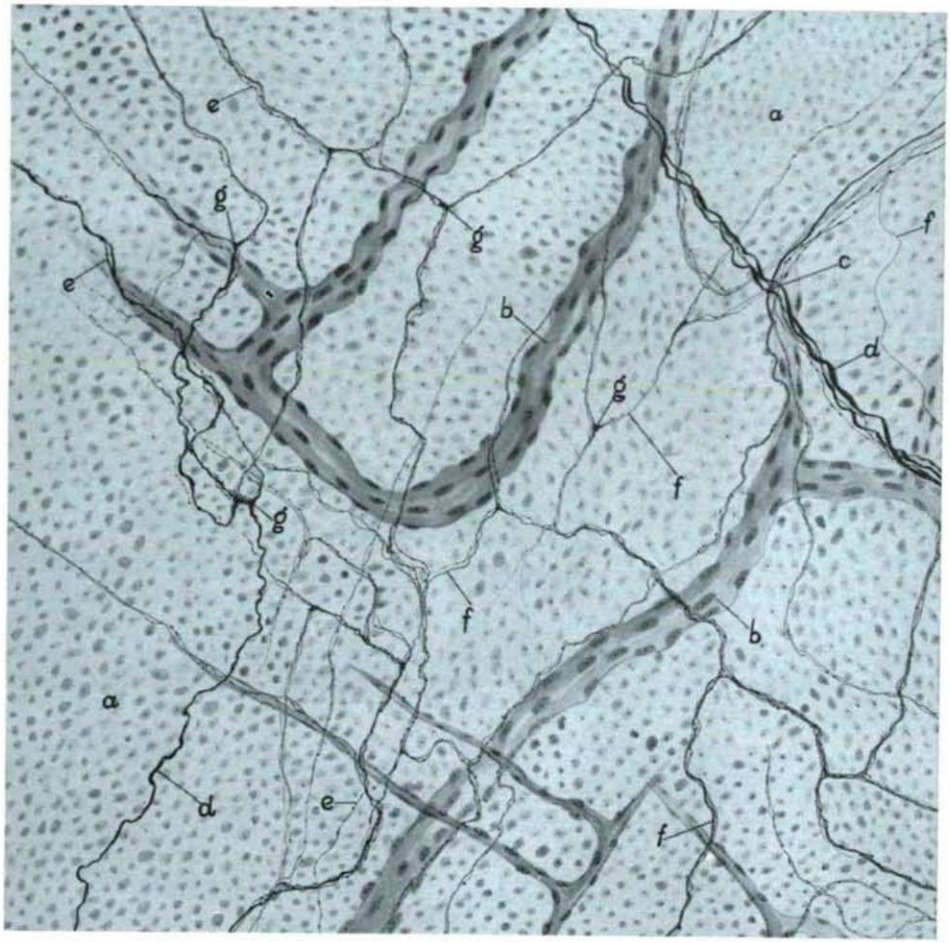


Fig. 3. *Scropaena porcus:* Innervation in the outher layer *of lamina propria* in branchial arch II, a — connective tissue cells, b — capillaries, c — nerve *plexus*, d — thick nerve fibre, e — granulated thin nerve fibre, f — not granulated thin nerve fibre, g — dendron-like branching, BIELSCHOWSKY—ÁBRAHÁM' s method. Magn. 400x.

adventitial *plexus* with very many thick and fewer thinner fibres. The vein is very poor of nerves. In the *plexus* of the branchial artery little groups of nerve cells occurred (Table I. Fig. 4). The cells are multipolar, as a rule 3—6 cells in one group.

Beside the adventitial *plexus* there are some places where special thick nerve fibres were found staining dark black with silver. They are wavy and end with dendron-like forms. (Table II. Fig. 1). Though they have no neurofibrillar endplates at the ends, yet they may be considered as the simpler forms of the *pressoreceptor* system in the arterial trunks of the higher vertebrates. Structurally

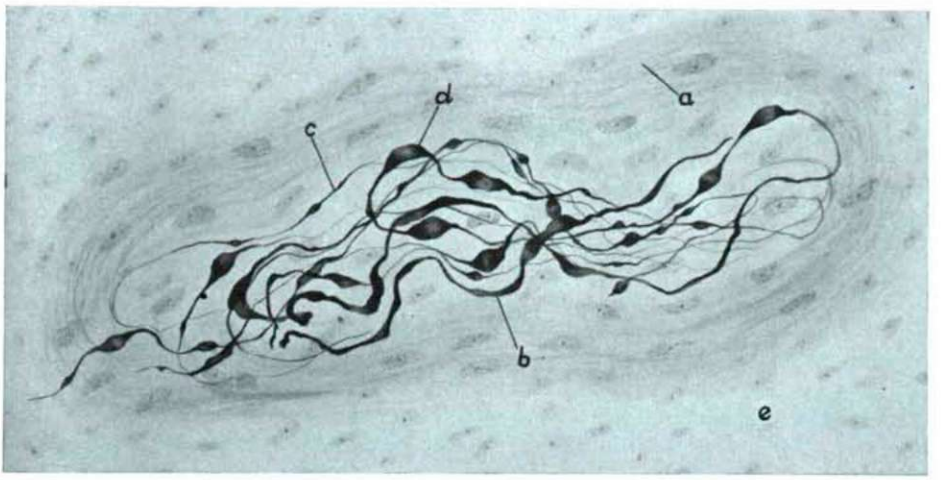


Fig. 4. *Scorpaena porcus:* Capsulated nerve ending in the inner layer of *lamina propria* of branchial arch I, a — capsule, b — thick nerve fibre, c — thin nerve fibre, d — varix, e — interstitial connective tissue. BIELSCHOWSKY—ÁBRAHÁM's method. Magn. 600x.

they are in full agreement with our findings in the swim-bladder (ÁBRAHÁM, STAMMER 1954). Single thick nerve fibres with dendron-like endings appear sometimes on the filamentary arteries, too. They may have similar function, their forms, however, are simpler. We tried to count the number of the pressoreceptors in one gill-apparate and could be stated only 2—4 larger ones in the branchial arteries and 4—6 simpler types in the filamentary arteries. It is true that the I. arch couple has always the largest pressoreceptoric area where 4—6 thick fibres end near each other. Often they were not found in the II. or IV. arches. Where the branchial receptors were absent 3—4 filamentary arteries had receptoric endings.

Quite near the branchial arteries in the connective tissue of the branchial arch some cell groups appeared with intimate nerve connection. Probably they posses similar but simpler structure as the chemoreceptors in higher vertebrates. The nerve trunks coming to these cells contain only thick nerve fibres and show wavy pathway. Their fine end branches are closely connected with the cells (Table II. Fig. 2).

The receptoric end fibres of the branchial vessels found in the *Scorpaena porcus* show the same regulation of the blood circulation in fishes as demonstrated by HEYMANS and NEIL (1960) in the highest vertebrates. Our morphological findings are supported by the physiological data of LUTH-WYMANN, IVNING, SOLANDT, MC. WILLIAM who experienced pressoreceptoric function in *Squalus, Mustelus* and *Anguilla.*

Finally it may be mentioned that the vessels in the gill-teeth and in the mucous membrane were never connected with the branchial vessels and never had any receptoric endings. They are the continuation of the main vessels of the lingual part and the oral cave.

The muscles of the gill apparate

The active movement of the gills in the *Scorpaena porcus* is connected with the well developed branchial-, hyoideal-, and pharyngeal musculature. However, the muscles at the basal part of the filaments, representing the filamentary musculature, seem to be different from the other muscles of the gill apparate. We suppose the differences are in the structure, origin and nerve supply between the two types of the muscles.

The muscle fibres in the filaments are short and thin without any close connection. The muscle bundles run in many directions in the filaments. Their *nuclei* are rounded and rarely visible. Very dense and clear cross striations characterise their myofibrils. The nerve supply of the muscle fibres is very rich. In our opinion on the filamentary muscle fibres get not only one but more nerve end connections. The endings are dendron-like with little end-plates or knobs (Fig. 1, i). The muscle *nuclei*, however, are never collected round the nerve endings. These end-forms are very similar to our findings in the intrinsic musculature of the reptiles and bird's eyes (STAMMER 1962). So we suppose a special innervation of these muscles. It may be that they get not voluntary but autonom motoric innervation.

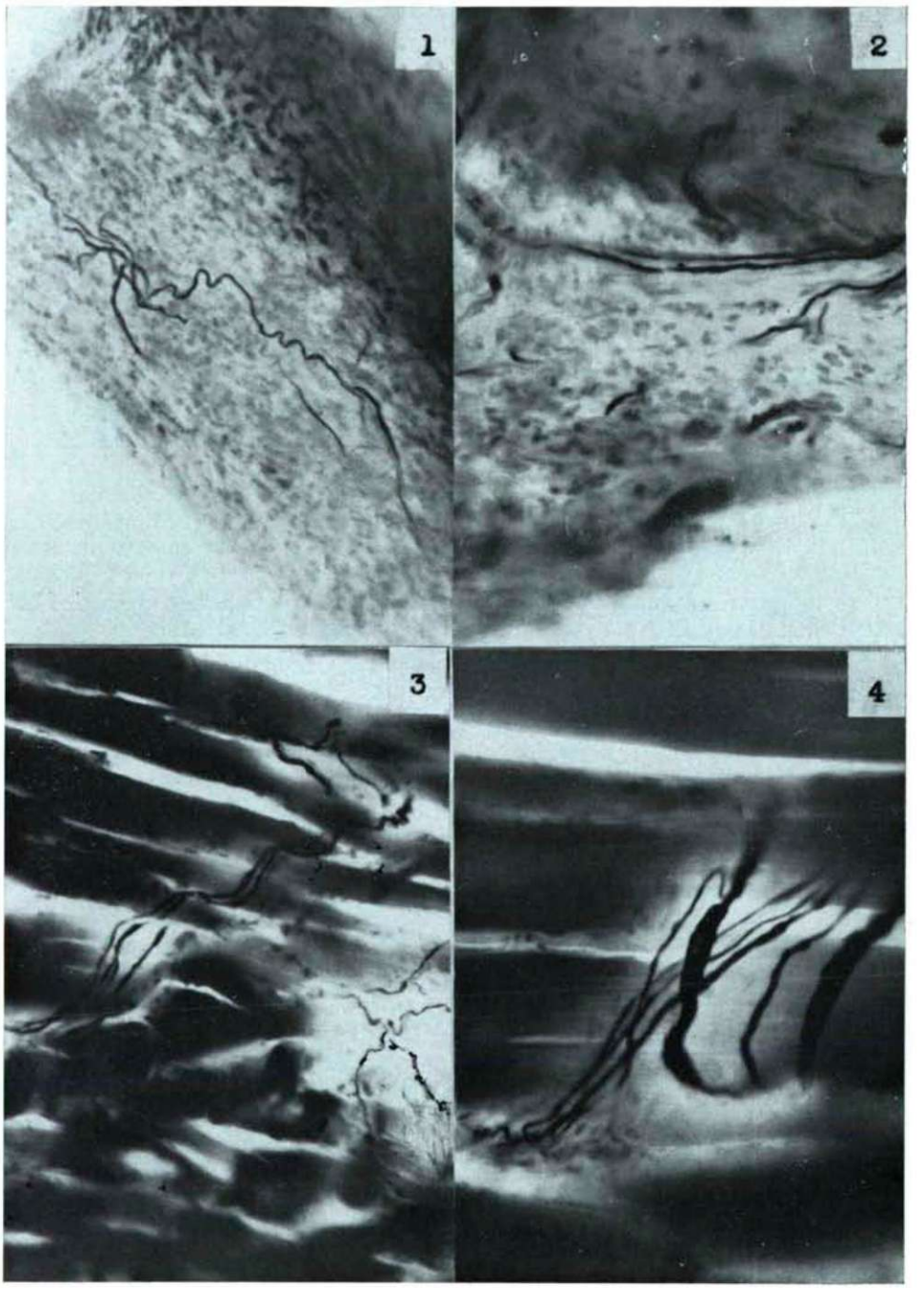
The musculature found at the lingual and pharyngeal ends as well as the groups of the branchial arches, represent the branchial, hyoideal and pharyngeal musculature agree with each other in every respect. These muscles *(Musculus branchialis, hypobranchialis, coracobranchialis, coracohyoideus, pharyngobranchialis* and *mm. interarcuales)* are differentiated to new function from the lateral musculature of the trunk, however, their skeletal characteristics remained. Their fibres showed not so dense and strong cross striation as those mentioned above. The forms of their *nuclei* are always longish and well visible in near position to the *sarcolemma*. Their nerves as everywhere in the skeletal muscles of the fishes consist of very thick and very thin nerve fibres. It is remarkable that the thin fibres appear as lateral side-branches of the thick fibres (Table II. Fig. 3), and the thick fibres become quite thin and after certain distance they are quite thick again. Both appearences were experienced in the skeletal and eye muscles of fresh water fishes (STAMMER 1960). Beside the rapid caliber changes, small or large varices could be noted in the nerve fibres. The nerve trunks of the muscles run obliquely to the parallel muscle fibres, closely connected to each other and give lateral end-branches to both sides (Table II. Fig. 4). The end-forms remind of the motoric end-plates of higher vertebrates but they are simpler and possess poor collection of the muscle *nuclei*.

The nerve trunks run in the muscles segmentally and so there are large areas without any nerve connections. Bearing in mind that the limit of the muscle fibres is roughly at the same level so we consider the segmental innervation where one muscle fibre possesses only one nerve ending.

Table II.

Fig. 1. Pressoreceptoric fibres in the adventitional *plexus* of the branchial artery.
Fig. 2. Chemoreceptoric fibres in the adventitional *plexus* of the branchial artery.
Fig. 3. Segmental innervation of the pharyngeal musculature connected to the gill apparate.
Fig. 4. Motoric nerve ending of the branchial musculature.

TABLE II



The origin of the nerves in the gill apparate

Without experimental examinations is very difficult to speak about this question in fishes. However, to study the degeneration peripherally and centrally which means the only way to solve the problems, seems to be impossible at present. Cutting the nerves or any injury of the gill apparate causes high mortality in fishes.

Considering the obtained pictures of our microscopical examinations, the equalities and differences of the nerve supply as well as the better known nerve connections in highest vertebrates we think about the origin as follows:

1. The common participation of the trigeminal and facial nerves in the mucous membrane.

2. The common participation of the glossopharyngeal and vagal nerves in the branchial area.

3. Sensory fibres of the branchial vessels belonging to the glossopharyngeal and vagal complex.

4. Cranial sympathetic fibres connected always to the plexuses of the vessels, especially to the arteries.

5. The motoric fibres originate from cerebral nerve XII. to the lingual and branchial musculature and from cerebral nerves IX and X. to the pharyngeal musculature.

6. The filamentary muscles get their fibres from the nerve complex of IX. and X. but it may be from the parasympathetic part of these nerves.

7. The gill teeth are innervated only by sensory trigeminal fibres. Their endforms, the sensory coils appeared in the connective tissue of the branchial arch.

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Summary

Special structures and innervation was found in the different parts of the gill apparate in *Scorpaena porcus*.

Beside the structurally alternating gill filaments of the gill lamellae, strong gill raker, consist of separated teeth, and well developed musculature are characterised the gill apparate of this animal.

Very rich sensory system was noted in the field of the gill apparate in forms of incapsulated sensory coils and dendron-like end branchings.

The sensory coils are situated in the connective tissue round the branchial arches and inside the gill rakers. The dendron-like end-branchings occurred in the mucous membrane and connected to the branchial vessels.

On the basis of the very rich sensory innervation was stated very important receptoric areas in the gill apparate giving afferent foots to the feeding and breathing reflexes.

The motoric innervation belonged to the musculature and the vessels of the gill apparate.

The author believes the innervation of the gill apparate originates from cerebral nerves V, VII, IX and X and the cranial sympathetic system. They may be abundantly mixed up centrally and peripherally.

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