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Observations on Skin Structure and Sloughing in the Stone Fish *Synanceja verrucosa* and Related Fish Species as a Functional Adaptation to Their Mode of Life

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Summary. *Synanceja verrucosa*, *Inimicus filamentosus*, *Pterois volitans* and *Dendrochirus brachypterus* slough permanently their outer keratinised layer of epidermis. In *I. filamentosus* and *S. verrucosa* this layer is formed by the distal segments of the epithelial cells, where in *P. volitans* and *D. brachypterus* the whole superficial cells are keratinised.

During the process of ripening the outer cells become loaded with fibrillar material and detached from the skin. In *S. verrucosa* several keratinised layers accumulate on the fish body and become invaded by algae and other fouling organisms. This produces an envelope of cryptic and protective value over the fish. As this ballast becomes too thick, the fish slough the cover, remaining protected only by the youngest sheet of keratin, attached to the superficial epithelial cells. The structure of the slough and outer epithelial layer resemble the "Oberhäutchen" described for squamate reptiles.

Key words: Teleosts — Skin — Sloughing — Oberhäutchen — Light and electron-microscopy.

Introduction

The skin of animals proves to be one of the main parts by which the mode of adaptive evolution can be continuously traced. Especially in the vertebrate skin the pathway of morphological sophistication is evident: This extends from a simple single-layer epithelium that rests on a narrow spongeous connective tissue, to a well specialised and stratified epidermis, rich in derivates and supported by strong collagenous connective tissue. Along this line, as the animal adapts to a terrestrial mode of life, a prominent turning point is marked: the development of sloughing phenomenon. In advanced reptiles this sloughing occurs at once, synchronised all over the animal's body (Maderson, 1968). In lower aquatic vertebrates, especially in fishes, such a phenomenon of total and synchronised sloughing has as far as I know, never been described. Only Gilchrist (1920) and Heldt (1927) mentioned some type of partial ecdysis in fishes.

The aim of this work is to describe the skin structure and sloughing observed in the stone-fish *Synanceja verrucosa* and *Inimicus filamentosus* (Synanceiidae), and partly in *Pterois volitans* and *Dendrochirus brachypterus* (Scorpaenidae). All four species are poisonous fishes found in the shallow water of the Gulf of Aqaba (Fishelson, 1971). Part of the observations were performed in nature, but most were done in large aquaria of the section Marine Biology, Department of Zoology, Tel-Aviv University.

Materials and Methods

For histological observations pieces of skin from fishes were fixed in Bouin's fluid or in 10% neutral formol. Serial sections 8 μ and 10 μ thick were stained in Mallory azan. Safranin fast green hematoxylin-eosin; Mayer's-hematoxylin with light green and gentian violet; and toluidin blue. For electron microscopy pieces of skin were fixed for 2 hours in glutaraldehyde, then washed, postfixed in 2% osmium-tetraoxide and after dehydration, embedded in Epon 812. The sections were prepared with an LKB-ultramicrotome and examined in a Jeolco EM-7 electron microscope.

Observations

General Remarks on the Fishes

Having a wide circumtropical distribution along the coral reefs and being among the most poisonous fishes, the stone-fish and scorpion-fish served as a subject for numerous investigations, most of them dealing with their poisonous dermal glands (see summary in Halstead, 1970) and only few provide some information on their habitat (Endean, 1961).

Along the shallow waters of the Sinai Peninsula the stone-fish are usually found in a depth of 5 to 10 m, although here and there specimens were observed in the most shallow subtidal. They are always found singly dispersed, usually half buried in sediment, head and trunk dorsum protruding.

During the years of investigation performed in the Red Sea, those fishes were never observed to swim and rarely observed to escape from touch. For days and weeks they could be observed in the same place, and if disturbed, they "walk" slowly for a very short distance and then bury again, using the ventral and pectoral fins. Even in aquaria they rarely swim (Fig. 1).

This hiding and cryptic mode of life, is well supported by the general shape of the animal, especially by skin ornamentation: in the scaleless fish all the body is covered by numerous outgrowhts, and wart-like papillas, forming an irregular stonelike structure. All of this is covered by a thick brown-coloured sheet of algae, hydrozoans, diatomeans and other organisms, forms, that also cover the hard substrata around the places where the fish hide. In such a way, the stone-fish blends with the surroundings.

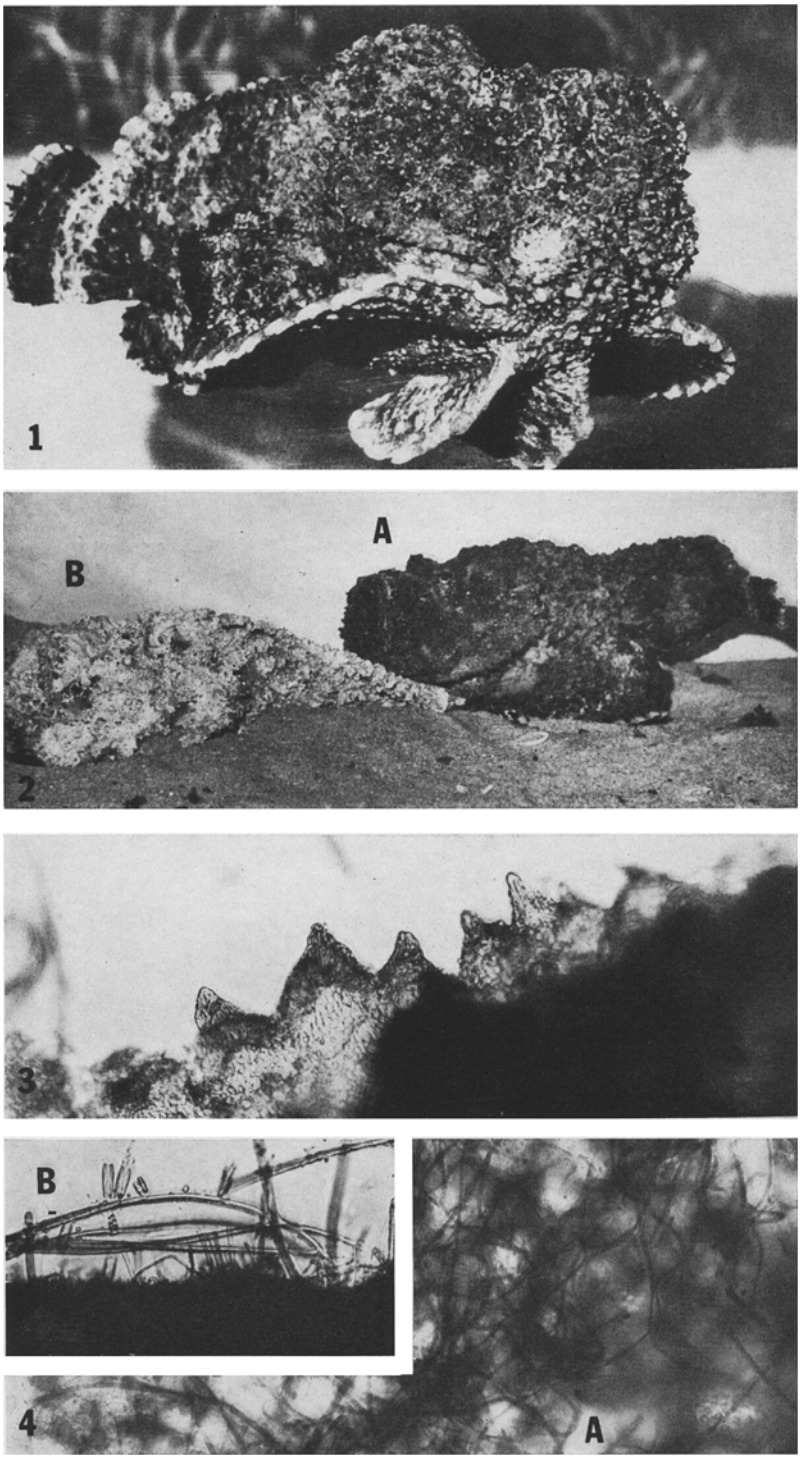
Inimicus filamentosus, the other representative of the same family, has the same skin structure. Also its behaviour resembles that of *Synanceja*, being a strictly sessile and stationary one. Being predators, that feed on fishes and other animals, these fishes use the camouflage to snatch the approaching prey. Its swallowing ability is very high and in aquaria it was observed that a taken prey may have the length of the predator. Being cannibalistic, they were found to be able to feed on slightly smaller specimens of their kind as well as on other poisonous

Fig. 1. A stone-fish swimming in aquarium (1/3 nat. length)

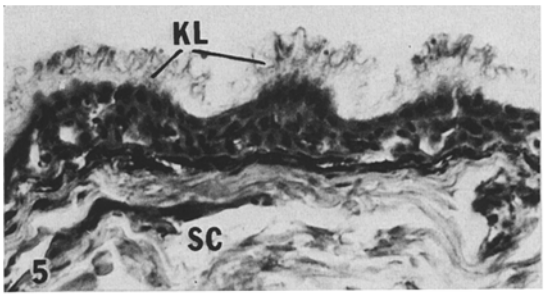
Fig. 2A and B. Two specimens of stone-fish. A before sloughing; B after (1/5 nat. length)

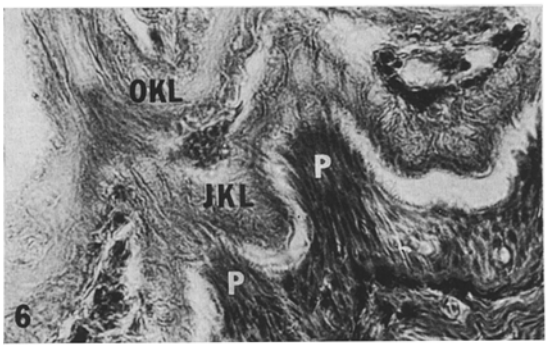
Fig. 3. Protuberances on the skin of stone-fish (×40)

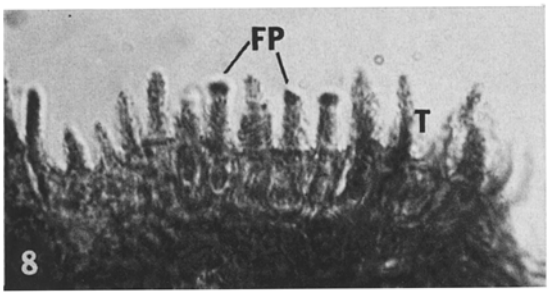
Fig. 4A and B. Fouling organisms of stone-fish. A filamentous algae; B Diatomacea (1×60)

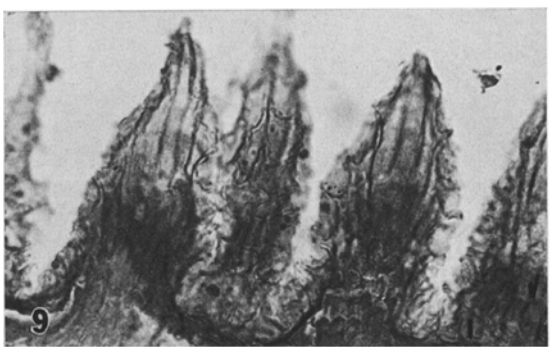


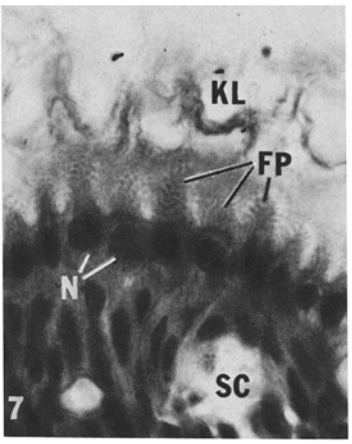
Figs. 1–4

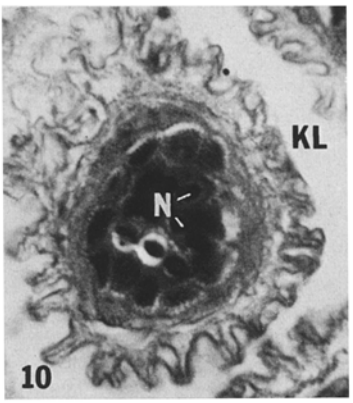












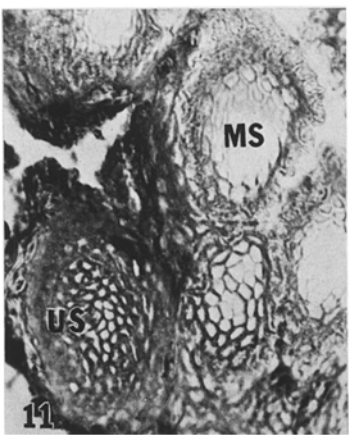


Fig. 5. Vertical skin section. *SC* stratum compactum; *KL* keratinised layer (Azan, ×60)

Fig. 6. Vertical skin section. *P* papillous outgrowths; *JKL* juvenile keratine layer; *OKL* older keratine layer (Azan, ×80)

Fig. 7. As Fig. 6: *KL* keratine layer; *FP* fingerlike protrusions of outer epithelial cells; *N* their nuclei; *SC* secretory cells (Hematoxylin-Eosin, ×600)

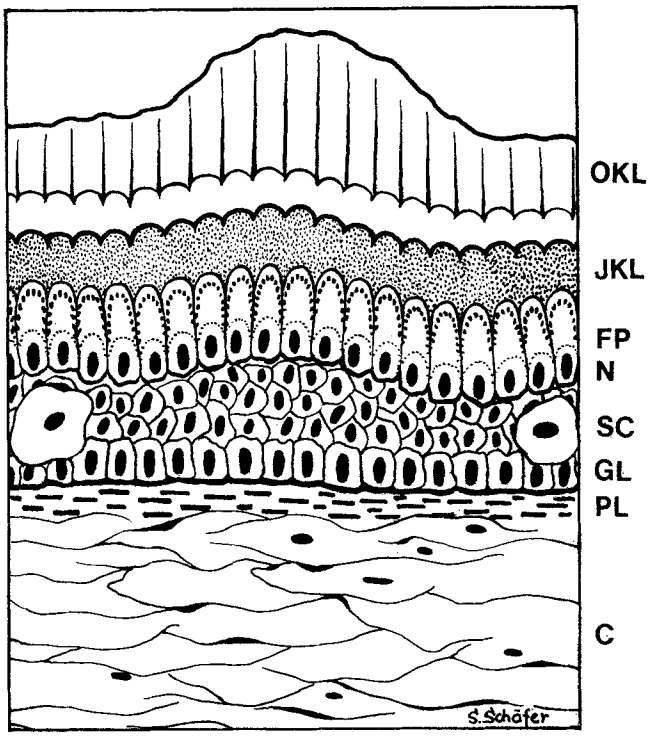


Fig. 5a. *C* corium, *PL* pigment layer, *GL* germinative layer, *SC* secreting cells, *N* nuclei of external epithelial layer, *FP* fingerlike protrusions (villi punctated), *JKL* juvenile keratinised layer, *OKL* old keratinised layer during slough

species. *Pterois volitans* and *Dendrochirus brachypterus* are usually found around stones and coral heads. In contrast to the first two species, they behave less cryptic and when feeding they actively search for their prey.

Occasionally instead of being cryptic, specimens of stone-fish observed in nature had a bright, red-whitish colouration and so could be easily located (Fig. 2). Also in aquaria on several occasions the fish were observed to have lost their dark brown colour, demonstrating their bright skin. It was later found that the first sign of such an act to occur is the "milky" colour that developed over the fish eyes. Around this time the fish also stop to feed.

Usually with time such specimens crawl over the bottom and with strong tail hits "jerk" in the water. After several jerks, the real skin becomes exposed and the old cover, broken in large thick parts, floats around. On several specimens this phenomenon was observed to occur two or three times a year. Such after-shed specimens remain motionless buried in sand for several days, not

Fig. 8. As Fig. 7, but without the keratine layer, villi visible (Azan, ×800)

Fig. 9. Columnar keratine over epithelial outgrowths (Hemalaun, ×60)

Fig. 10. Cross sections of epithelial outgrowths. *N* nuclei (Hematoxylin-Eosin, ×200)

Fig. 11. As Fig. 10. but sectioned higher. *MS* the middle part; *US* close to the upper surface of papillae (as Fig. 10)

feeding. Gradually, the body becomes again brown dark and the fish renews its activity. The examination of the shed skin showed that its main material was composed of some macroscopical irregular structures of warts and tubercules (Fig. 3), almost entirely covered by fouling organisms (Fig. 4). The thickness of this layer ranged between 150 μ to 400 μ.

Jerks were also observed in the *Pterois* and *Dendrochirus* species, in them also accompanied with sloughing. In these fishes the shedded skin forms a delicate, transparent membrane 10 μ to 20 μ thick, that disappears in the aquarium at once.

Skin Histology of the Stone-Fish and Other Species

In the stone-fish the basic layer of skin is formed by the corium 180 μ to 820 μ thick, mainly consisting of stratum compactum of collagenous fibers and elastic filaments (Fig. 5). Toward the basal membrane of the epidermis the corium ends with a narrow sheet of spongeous connective tissue, mixed with numerous pigment cells. This last layer, rich in blood capillaries, forms various protuberances towards the exterior and so modulates the outer structure of the skin (Fig. 5a).

The epidermis is formed by stratified epithelium of 4 to 6 layers of cells. Occuring in form of papillas and tubercles. This layer in stone-fish is 25 μ to 45 μ thick in the narrowest places and 120 μ to 200 μ thick in the widest places (Fig. 5, 6, 14). Over the fine membranes this layer is only 18 μ to 40 μ thick.

Among the cell layers found in the epithelium, the basal layer which borders the corium, and the outermost one, differ from those found in the intermediate layers.

The basal, so called germinative stratum, (see also Mittal and Munsi, 1970) is formed by slightly columnar cells 5 μ to 6.5 μ in height. They are very chromophilic, showing various stages of mitotic cycles in their nuclei.

The cells found above them are polygonal or rounded; their nuclei slightly elongeted, perpendicular to the epithelial surface. Among those cells gland cells are sparsely scattered, one type round and small, and the other elongeted and large. These large glandular cells usually extend from the basal layer to the outer surface of the skin (Fig. 5a).

The epithelial cells of the most external stratum have the most peculiar structures: Those are elongeted structures 9.0 μ to 12.0 μ long, perpendicular to the skin surface. In these cells two parts could be observed (Fig. 5a):

I. The basal part of the cell with the nucleus on the upper border. Those basal parts form a well organised chromophilic layer all along the skin (Fig. 7 and 8). The nuclei of those cells are oval-shaped, sometimes perpendicular, often parallel to the skin surface.

II. The distal part of the outer cells are fingerlike (1.5 μ to 3.0 μ high and 0.3 μ to 0.7 μ thick), rising above the skin's surface. Those protuberances are less chromophilic than the basal parts of the cell and are very numerous on the dermal papillae and warts. High magnification of those cell-endings show that they are covered by villi that form brush-like structures (Figs. 8, 16).

The epithelium is covered by a thick sheet of cuticle-like layers that extends all over the warts and protuberances (Figs. 5, 9). The innermost part of those layers envelops the finger-like extensions and villi of the epithelial cells. In such a manner, cuticular columns are formed over protuberances and in them the original, longitudinal striation produced by the adjacent cells, could be traced (Fig. 9).

Before sloughing in the stone-fish this outer sheet of keratinised material is 150 μ to 400 μ thick, of which the innermost part is the juvenile one. This cell-attached sheet is in such intimate contact with the protrusions and microvilli of the outer epidermal cells, that in well prepared sections the border between them cannot be identified (Fig. 6). These two parts are also equally negative to most stains, reacting positively to light green and gentian violet, typical stains for keratinised structures.

It seems that the outermost parts of the epithelial cells produce the keratinised layer by transformation. In cross sections it is well visible that the higher the cells are, the less stain they absorb, and more amorphic structures are observed (Figs. 10, 11).

Structures like these were also observed on *Inimicus* (Fig. 12) but not found in the scorpion-fish species *P. volitans* and *D. brachypterus*. In these last two species as in other fishes, the epithelium extends over the scale, forming a more or less narrow, smooth layer. A delicate, membranous layer of keratinised cells extends over their body (Fig. 13).

Sloughing in the Stone-Fish

During the time of sloughing, the separation of the exuvium occurs along a line that divides the upper layers of the keratinised sheet from a delicate layer of 10 μ to 20 μ wide, that remains attached to the epithelium (Fig. 14). The sloughed part is formed by two or three layers invaded by rhisomes of algae and hydrozoans (Fig. 15). The innermost surface of the moult lowest layer demonstrates a polygonal pattern which is a replica of the epithelial cells. This part is also slightly eosinophilic, demonstrating the presence of remnant cytoplasm. Older parts, found above this sheet, are less chromophilic being inert to cytoplasmatic staining, but react well to light green and gentian violet. The most superficial part of the exuvium which is densely invaded by various fouling organisms, again becomes highly reactive to various tissue stains. A similar development was also observed in *I. filamentosus*.

In the other fishes observed (*Pterois* and *Dendrochirus*) the exuvium is composed only of the lowest layer that shows the polygonal structure, which is a replica of the epithelium layer.

Electron Microscopy

This part of the investigation was performed only on the external cell layer of the stone-fish for two reasons: Firstly, these are the cells that participate in the sloughing and secondly, all the other parts of the skin seem not to differ too much from those described for other fish species.

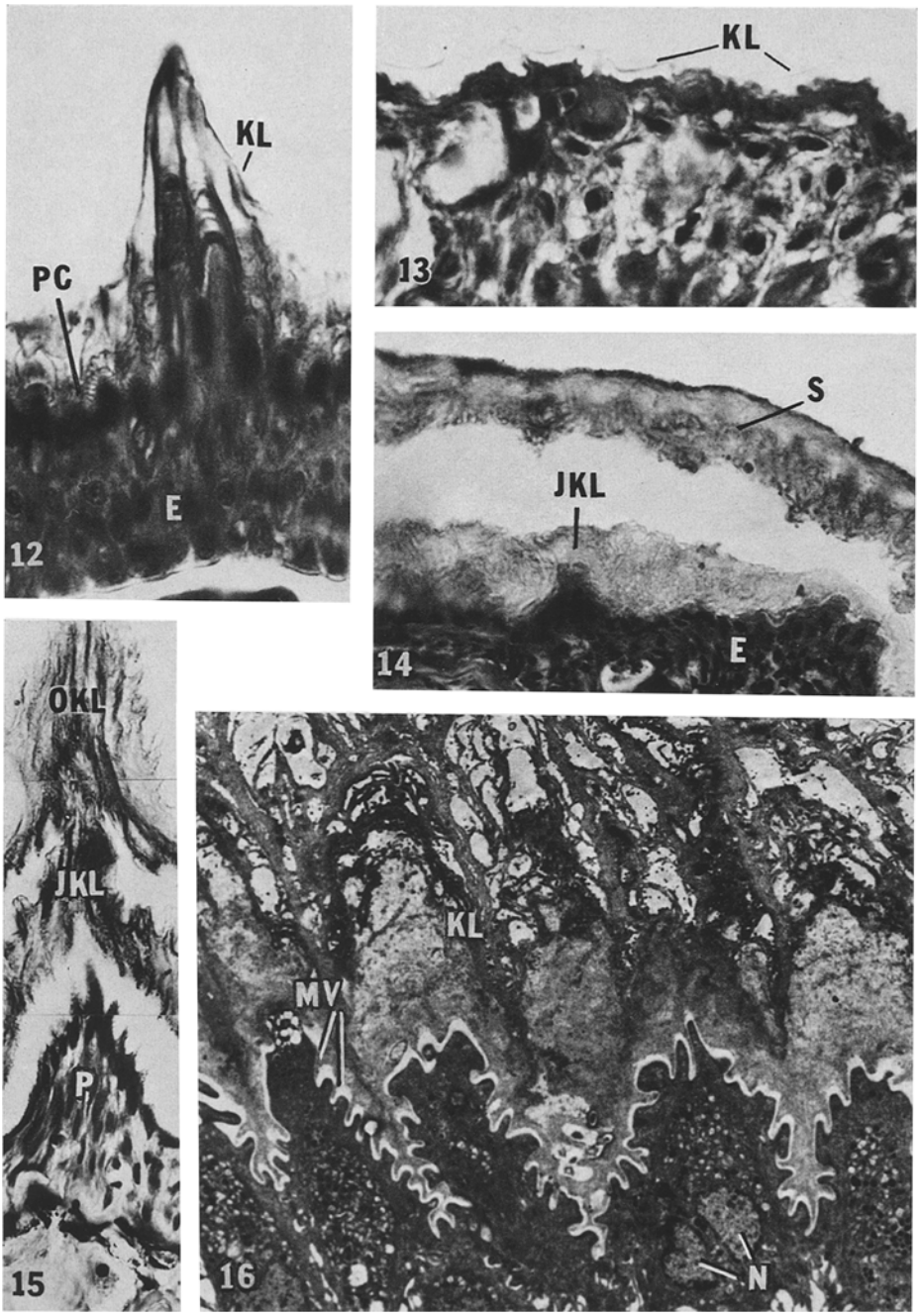


Fig. 12. Vertical skin section of *Inimicus filamentosus*. *E* ectodermis; *KL* keratinised layer; *PC* outer cell layer (Azan, ×120)

Fig. 13. The same in *Dendrochirus brachypterus*. *KL* keratinised membrane (Azan, ×120)

In low magnification, vertical sections, each villiferous outer growth of the epithelium seems to be formed by a single cell in which two strata are visible (Figs. 16, 17): The central part formed by one, but sometimes two large irregular nuclei and highly cisternised ergastoplasm. As in other fishes (Henrikson and Matolsky, 1968) close to the nuclei are extensive granulated reticula, Golgi apparatus, lysosoms and mitochondria. The outer part, composed of cytoplasma close to the cell membrane, has some fibrillar structure. Such division of cell content was also described by Wellings *et al.* (1967) from the sole *Hippoglossiides elassodon*.

In the basal parts where those external cells touch one another, they have strongly interdigitating membranes rich in desmosomal bridges (Fig. 17). In the distal parts of the cells, the adjacent cell membranes become more and more divided by prolonged lacunae, only here and there sealed by desmosomes.

The peripheral fibrillar cytoplasm becomes more pronounced, enveloping all the central organelle-rich cell content. In those regions, the endoplasmic reticulum is less regular, the lysosomes large, and the dark zymogenic granules numerous (Fig. 17).

The outer, free membranes of the cells are with protuberances that are almost entirely packed with the fibrillar cytoplasm. This fibrillar structure can easily be observed to form the closely attached layer of the produced outer sheet and could also be traced to the sloughed exuvium. In some sections it looks as if there is not a membrane between the villous cell surface and the adjacent keratinised sheet and that the transition from one to the other is a gradual one, forming a continuity.

Discussion

The modification observed in the skin structure of *Synanceja verrucosa*. *Inimicus filamentosus*, and also partly in *Pterois volitans* and *Dendrochirus brachypterus*, differs essentially from those occuring in other teleost fishes (Bertin, 1958; Oosten, 1964). In *S. verrucosa* and *I. filamentosus*, the epidermal derivates and sloughing generally at large resemble structures and processes described for reptiles (Maderson, 1965, 1968) than for fishes. As in squamate reptiles, also in the stone-fish, some kind of epidermal generation occurs, that enables the animal to produce the enveloping layers of keratinised material and also to slough them at once all over the body.

This producing layer consists of the outer stratum of epithelial cells, and attached to it the keratin sheet (Fig. 5a). In stone-fish as in reptiles, those layers are chromophilic in their innermost parts, and chromophobic on their external surfaces. Such selective affinity to stains, especially prominent in the presloughing stage, was also described for Tuatara (Maderson, 1968). This preferential staining

Fig. 14. Sloughing in stone-fish. *S* slough; *JKL* juvenile keratinised layer; *E* ectoderm (Hematoxylin-Eosin Safranine, ×40)

Fig. 15. As in Fig. 14, over an epidermal outgrowth (×120)

Fig. 16. Outer epithelial cells in stone-fish. *KL* keratinised layer; *MV* villi; *N* nuclei (×7000)

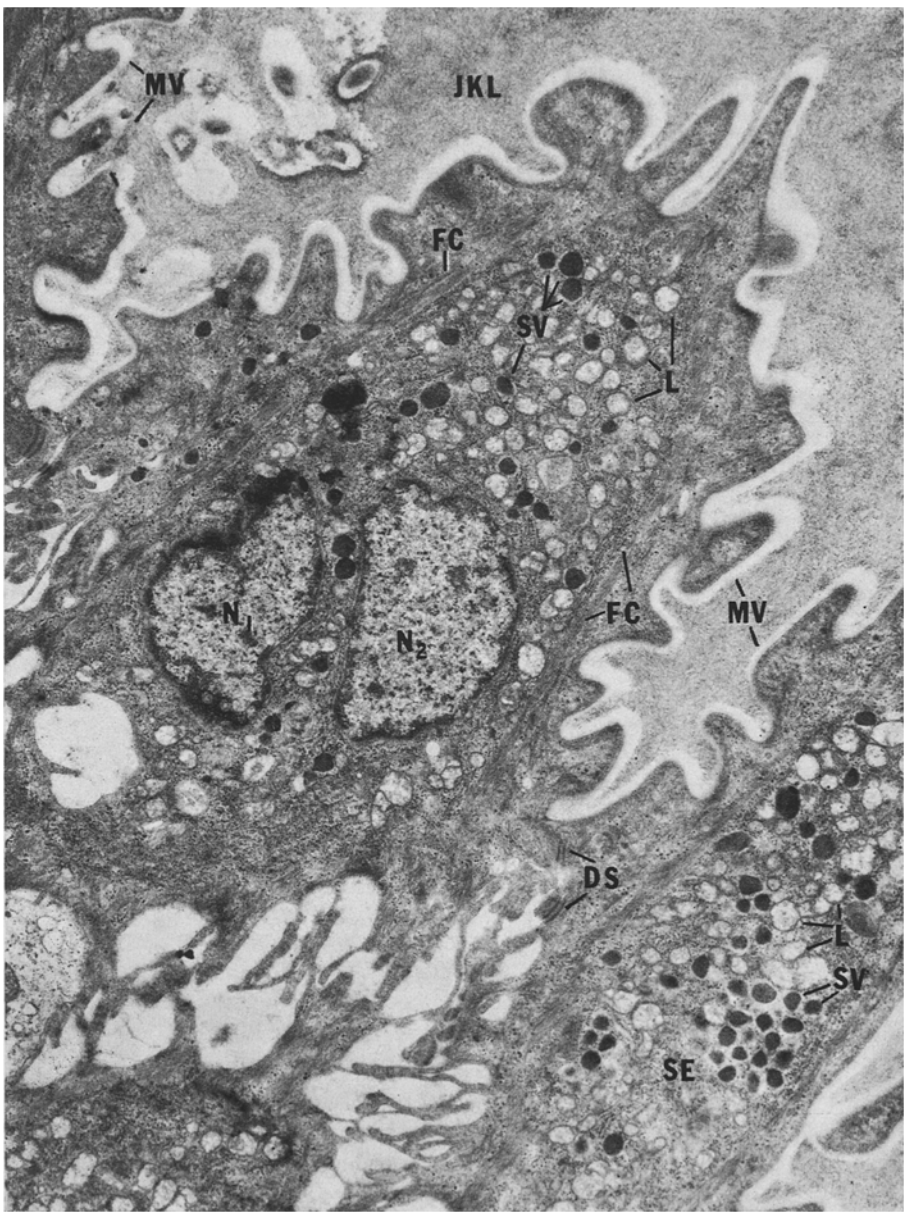


Fig. 17. A single outer epithelial cell. *FC* filamentous cytoplasm; *SV* secretion vesicles; *DS* desmosomes; *L* lysosomes; *SE* secretory ergastoplasm; *JKL* juvenile keratinised layer; *MV* villi (×22000)

seems to indicate some ripening processes occuring in the produced keratin, during which it loses the affinity to some stains, and begins to strongly absorb other ones (Light Green and Gentian Violet) typical for keratin or cuticle.

It seems that the keratinous layers of the stone-fish, are analogous to the „Oberhäutchen" of reptiles (Wurmbach, 1964; Maderson, 1968). During sloughing, the superficial epithelial cells remain covered by a narrow sheet of keratin (Fig. 14). functionally analogous to the α-layer of the outer epidermal generation in *Gecko*.

The profound differences between the keratinisation of the stone-fish and of the reptiles, are in the nature of slough-production: In Reptilia this layer is actually formed by outer, entirely keratinised epidermal cells. Characteristic for this process is the gradual disappearance of the cell-nuclei and the deposition of keratinous material within the cell-boundaries.

In the stone-fish the keratinised sheets are not formed by entire cells but only by their outermost segments. Those parts become loaded with fibrillar material (Fig. 7) and after this they become detached. The remaining part of the cell, containing the nucleus, continues to be active and organised. This is evident from their uninterrupted affinity to cytological stains during the various steps of development and keratine production. Only the distal portions of these cells become more and more chromophobic, indicating their keratinisation. The question of how long the outer layer of epithelial cells are active remains open.

As mentioned, sloughing in stone-fishes occurs only after three or four keratinised sheets have been accumulated over the fish body. From those sheets, the youngest one remains attached to the producing cells, whereas the older ones, invaded by fouling, break apart. It seems that this fouling enforces the sloughing, enabling the animal to free itself from the accumulated ballast.

In *Pterois volitans* and *Dendrochirus brachypterus*, the produced keratin forms only a delicate membrane, transparent, with recognizable cell borders. This membrane often detaches from the animal.

In reptiles the total sloughing process lasts from 10 to 12 hours; in the stone-fishes it takes from 3 to 4 hours, whereas in the observed scorpion fishes from only 20 to 40 minutes. Although exact information is not on hand, it seems that the higher the temperature the shorter the intersloughing periods, and the faster the keratin detachment.

The adaptive value of sloughing in the stone-fishes seems to be well connected with the digging, cryptic behaviour of those animals: They are scaleless fishes and instead of developing protective mucous layers as other fishes (Oosten, 1958) they discovered the third way of protection: sloughing and thus detaching the most external cover. Such fishes expose their true color only for a short time.

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