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Ichthyopathology

THE MORPHOLOGICAL AND HISTOLOGICAL STRUCTURE OF TUMOURS IN THE
„CAULIFLOWER DISEASE” (PAPILLOMA) OF EELS

MORFOLOGICZNA I HISTOLOGICZNA BUDOWA NAROŚLI
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Tumours appearing on eels with the „cauliflower disease” are morphologically different from each other. The present paper was aimed at the investigation of a possible effect of these differences on a histological structure. The author’s attention was also paid to the initial stages in the papillae formation; the role of particular corium elements in this process was also taken into consideration.

INTRODUCTION

The cases of papilloma (*Papillomatosis anguillarum*) called also the „cauliflower disease” of eels has been more and more frequently noted in recent years and they urge a detailed investigation of this disease.

Eels with characteristic growths were first noted in 1910 in the Jasmund Bay (Schäperclaus, 1953). Till 1944 the range of the „cauliflower disease” had been limited to a few parts of the Baltic coast, being noted in the Southern Sweden, Bornholm and the eastern coasts of Zealand and Lolland. Then its range expanded to the Baltic coast of Jutland and Germany covering also the whole Polish coast (Fig. 1). In the early fifties, according to the Prof. Grabda’s data, papilloma occurred commonly along the Polish coast being found also in the seashore lakes (Jamno, Łebsko) and in the Gdańsk Bay. At present it is more frequent in the western coast, mainly in the Szczecin Firth area and the

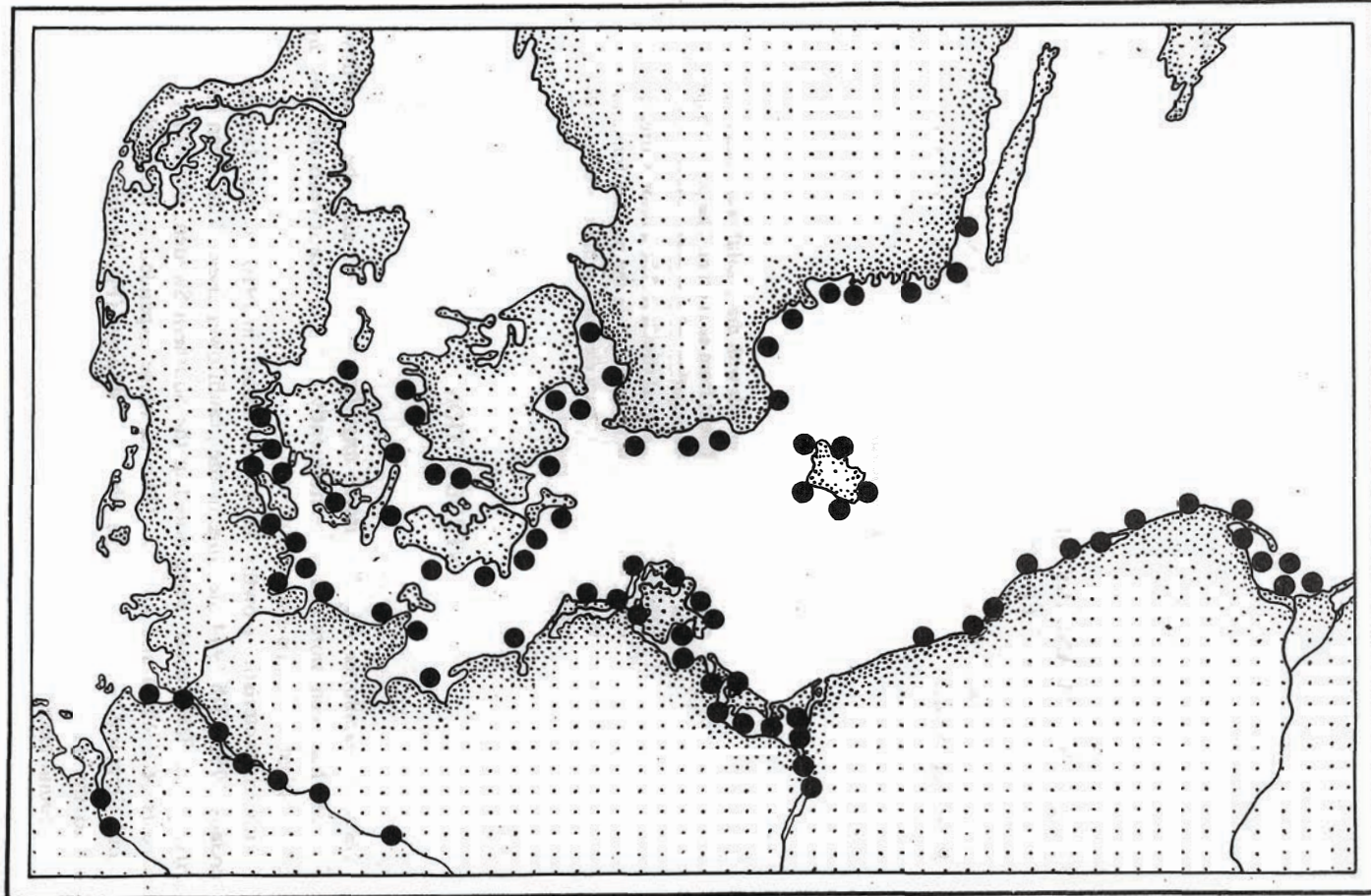


Fig. 1. Regions where eels with papilloma were found

lower part of the River Odra. In The North Sea coast this disease, rare before 1953, has been often encountered of Cuxhaven, in Elbe and more westwards in Weser, Emse (Lühmann and Mann, 1957) and Mosel (Koops and Mann, 1969). Is noted also in Black Sea (Radulescu, 1972). In 1950 the percentage of diseased eels found to be 5% in Szczecin Firth, (Schäperclaus, 1953) which is comparably high from that % Baltic coast (0,1–2%) by (Luhmann and Mann, 1957). To the west from Jutland the continuous increase in number of cases has been observed: for example in the mouth of Elbe in 1965 eels with tumours amounted to 11,4% (Koops and Mann, 1969), a few years later their number increasing even up to 40% (Peters, Peters and Bresching, 1972).

The „cauliflower disease” does not occur only in eels. In 1950 Schäperclaus (1953) found similar growths on snouts of cods, their structure, however, being different from that of eel papillae. Amlacher (1957) described papilloma of bleaks, the morphological structure of their growths clearly indicated a similarity to those of eels.

Christiansen and Jensen in 1947 and Schäperclaus in 1953 described the morphological and microscopic structure of the papillomatous growths. They found out that papillae consisted of more or less delicate stroma of the connective tissue and the proliferating epithelial elements. They attempted also to disclose the cause of the disease. Lühmann and Mann (1957) and Amlacher (1957, 1961) studied the tumours in details. They confirmed the previous observations in respect of the histological structure. Lühmann and Mann considered growing of tumours and their effect upon the whole organism. Amlacher compared the structural similarities of the growths in bleaks and investigated the role which the particular elements played in the eel papillae formation (Amlacher, 1961).

The latest studies resulted in many new informations regarding to the disease etiology (Koops and Mann, 1969; Pfitzner, 1969; Schmid, 1969; Peters and Peters, 1970), the microscopic appearance of tumours (Schmid, 1969; Schubert, 1969; Peters, Peters and Bresching, 1972) and the energetic changes occurring in the papillae cells (Peters and Peters, 1970; Peters, Peters and Bresching, 1972).

It is theoretically accepted that a halophile virus is a direct cause of the tumour formation (Schäperclaus, 1953; Koops and Mann, 1969) although a number of experiments with the artificial infection, which were carried out with the injection of tissue homogenate or implantation of tumours to the healthy eels, gave no positive results (Christiansen and Jensen, 1947; Schäperclaus, 1953; Koops and Mann, 1969; Pfitzner, 1969). The other authors tend to consider the cell disturbance during the transformation of energy (Peters and Peters, 1970) or the mutational changes (Peters, Peters and Bresching, 1972) as the cause of papilloma.

The present paper is aimed at completing the informations of the kinds of tumours, their structure, changes in papillae and investigating their youngest stages.

MATERIAL AND METHOD

The investigated material resulted from the catches taken in the Szczecin Firth (Zalew Szczeciński) off Trzebież. Four samples of diseased eels were taken from August till

October 1971. As a whole 77 eels with the body length and weight ranges from 29 to 68 cm (*longitudo totalis*) and from 30 to 320 g, respectively, were examined. Small individuals were represented in greatest quantities, the eels with the length not exceeding 40 cm amounted to approximately 60%. Only exceptionally the individuals with the minimum and maximum lengths 29 and 68 cm, respectively, were found in Table 1.

Table 1

Size and weight of the eels examined

Length class (l.t. — cm)	below 30	30–34	35–39	40–45	46–52	53–60	over 60
Number of fish	1	15	24	12	12	5	1
Percentages	1,4	21,4	34,3	17,1	17,1	7,1	1,4
Mean length (cm)	29,0	32,9	37,2	42,1	47,4	59,2	68,0
Mean weight (g)	30,0	49,3	68,4	95,8	162,0	285,0	320,0

All the eels were examined in details. The macroscopic changes in the newly formed growths were observed as well as the anatomopathologic changes of the visceral organs. Samples for the histological studies were taken from 31 eels; in most cases they originated in the head part of body where the youngest stages of papillae were found. The material investigated was treated with 10% formalin or other fixators, viz. Zenker, AFA, Suza, Bouin, Romeis and sublimate with formalin. The microscopic mounts were stained with haematoxyline and eozyne or with the Van Gieson's method (See — A. Dzidziul)

RESULTS

The newly formed papillae 1–2 mm in size were most often observed on the snout or near the nostrils. These parts appear to be the most susceptible to the papillomatous changes.

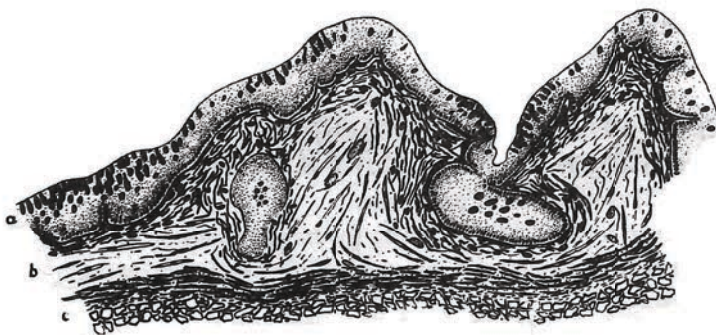


Fig. 2. A Fragment of skin from the terminal part of snout; the initial changes in the connective tissue, leading to the epithelium swelling and divisions, are visible: a) epithelium, b) connective tissue of corium, c) hypodermal layer

The initial deformations are noticed in the connective tissue of skin, the outgrowth of the connective tissue becomes visible and its fibres form characteristic arrangements. Then slight folds and swelling of the epithelium form (Fig. 2). At the same time the connective tissue outgrows. Proliferate towards the surface (Fig. 3, 4, 5). The collagenous fibres penetrate among the epithelial cells giving rise to the connective tissue wedge-like appendixes. Simultaneously, a clearly visible reproductive layer appears on the connective tissue border. The epithelial cells proliferate significantly. The connective tissue penetrates towards the surface in two ways. The first one is the formation of digital appendixes which are then surrounded by the epithelial cells (Fig. 3, 4). Their marrow built of the

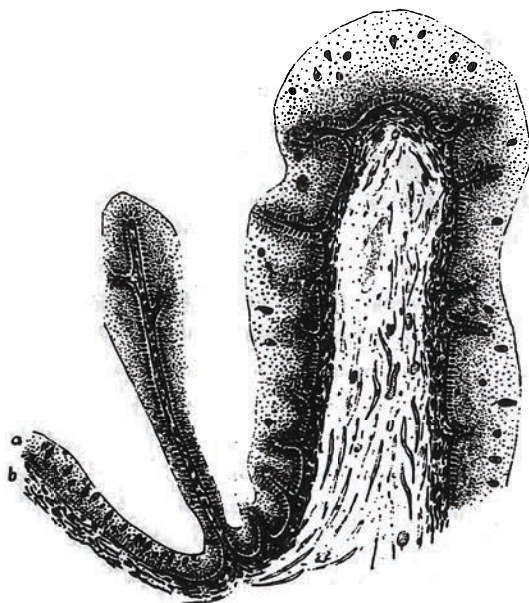


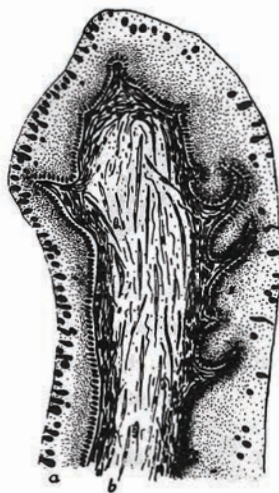
Fig. 3. The snout side cross-section; in its base a branched connective tissue process is formed, surrounded by the epithelial cells; changes in the „labium”, i. e. epithelium proliferation and connective tissue penetration towards the surface, also visible

connective tissue forms manifold branches. The further intense epithelial proliferation results in the uniform tumour mass interspersed by the connective tissue strongly vascularized. In the second case, the narrow streaks of connective tissue fibres penetrate into the epithelium (Fig. 5) branching off, interlacing one with another and cutting the fragments of an outgrowing tissue off. It results in the formation of an arrangement appearing on the sections as an irregular reticulum (Fig. 6). In the meshes of this reticulum the lobules typical for papilloma are formed from the epithelial and the connective tissue cells. In the initial growth stage the cylindric layer produces cells typically epithelial; they change their shape from elongated to cuboid as the distance from the layer increases. The mucus-containing cells and the spadicose ones, rather rare in young



Fig. 4. The connective tissue penetration towards the surface is visible on the snout side cross-section; the epithelial cells proliferate around the connective tissue appendixes: a) epithelium, b) connective tissue

Fig. 5. A cross-section of the terminal part of snout with conspicuous appendixes of the connective tissue, penetrating between the epithelial cells: a) epithelium, b) connective tissue



papillae, are disposed in various densities within the epithelial tissue. The layer arrangement is subject to changes during the tumour growth. The newly formed cells shift towards the centre and the glandular cells laying centrally lose their specific excretional properties.

The epithelial cells in young, growing papillae, in which the stroma of connective tissue is being formed, do not change their character. The adult form is exposed to the degeneration process as well as to the proliferation changes. The disintegrational changes degree of the stroma and epithelial tissue varies depending on the size, structure and kind



Fig. 6. A horizontal section of the growth with noticeable characteristic „lobules”:

a) epithelium, b) connective tissue

of a papilla. In certain papillae the creative process exceeds the disintegration remaining in equilibrium or being even less marked in the other ones. The epithelial cells hydropic degeneration is the most common form of changes in all epithelial growths; in the significantly intensified degenerative processes the changes occur also in the reproductive cylindric layer (Fig. 7).

The disintegration of a necrotic character is related, among the others, to changes in the blood vessels. They appear as the considerable vasodilatation, disturbance in blood flow as well as extravasation. The embolisms, often connected with the blood cells extravasation (Fig. 7), as well as clots with noticeable precipitates and the blood cells disintegration (Fig. 8, 9, 10) are most frequently formed in the blood vessels or in their centres of degeneration. The circulation retarding, formation of a stasis leads to the changes being noticed as degeneration, including the necrosis. It is one of causes of the tumour atrophy. The degenerative changes in epithelial cells, apart from the vacuolization, may be formed in various ways. Cells from the great part of a papilla decay with the characteristic nucleus decomposition leading to its obliteration in the way of the rearrangement and decomposition of chromatin (Kariorrhexis). Besides, most commonly the epithelial cells decaying proceeds through the deformation, condensation and pycnosis of the cell nucleus. In another case the swelling followed by the cell plasmolysis and dissolving the chromatic matter, nucleus membrane or the whole nucleus occur during cariolytic (Fig. 8). Sometimes this phenomenon is accompanied by the cellular infiltration gathering usually near the palisade tissue (Fig. 12-a). The similar changes occur also in the glandular cells: mucous and spadicose ones (Fig. 12-b), where swelling of an excretory part of a cell and the nucleus deformation leading to its disintegration is observed. Depending upon a tumour development degree, the reversal processes occur in the terminal parts (in younger tumours) and in the greater part of papillae (in older ones). In the extremal cases no degenerative changes are observed in the reproductive cylindric layer only.

These changes, leading to many directions, give in result the degenerated tumour fragments. The dead tissue forms a compact mass, being only to a small extent superficially exfoliated.

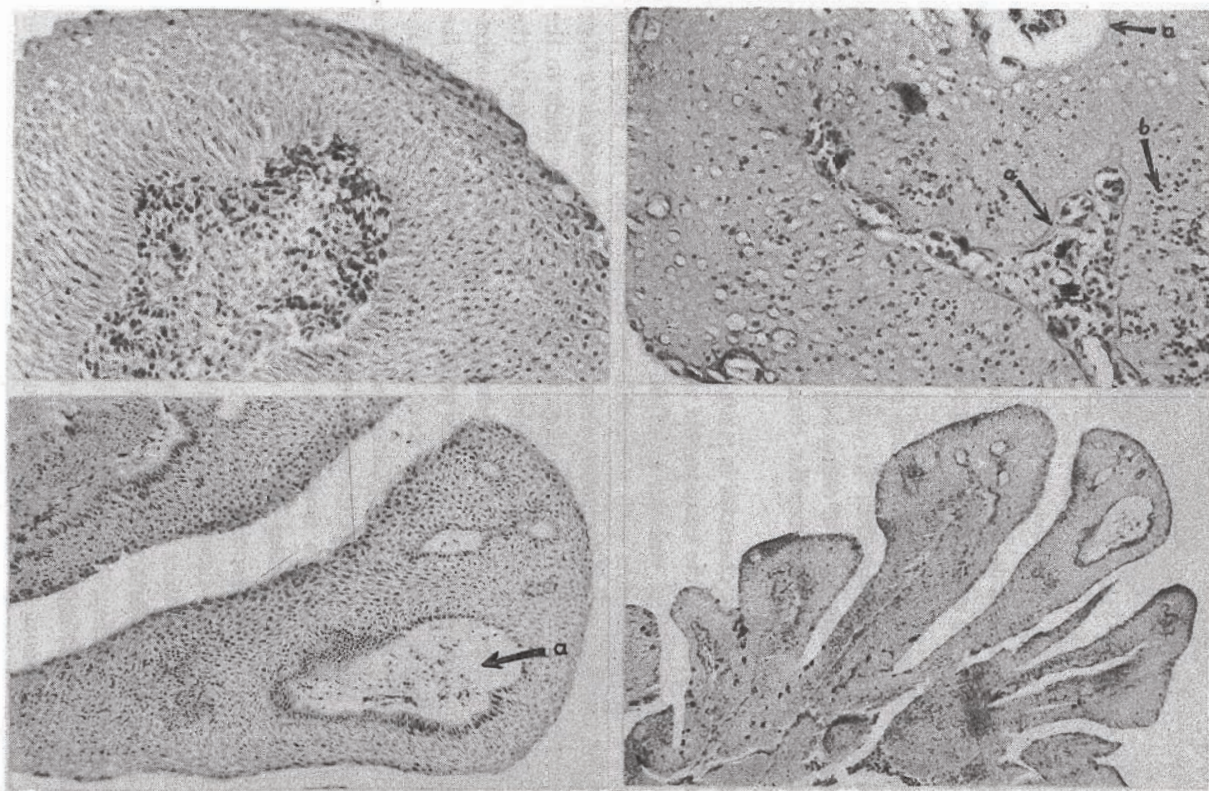


Fig. 7. A fragment of a papilla with cells strongly vacuolized, the blood elements extravasation visible within the connective tissue (Fot. by Waluga) Fig. 8. A cross-section through the superficial part of a tumour. The cario- and plasmolysis variously developed in the epithelial cells, the cylindric layer disintegration, the cells borders, especially those lying externally, become obliterated. Vasodilatation and the blood cells disintegration in observed (a) a fine-grained infiltration between the tumour cells (b) (Fot. by Waluga) Fig. 9. The top fragment of a tumour with the vasodilatation, clot and the blood cells disintegration. The epithelial cells vacuolized and the nucleus pycnosis in various stages (Fot. by Waluga) Fig. 10. A vertical section through a rugged papilla with clear reversal changes (Fot. by Waluga)

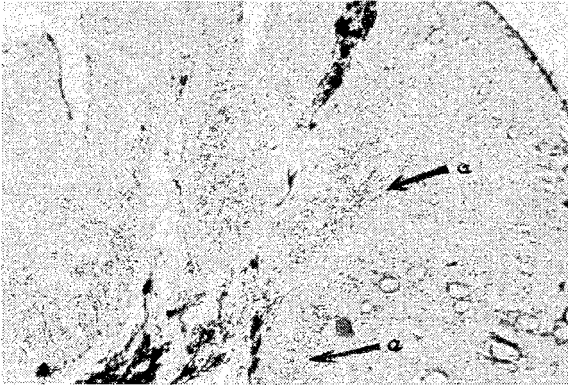


Fig. 11. The papilla disintegration visible on the cross-section, resulting from the vacuolization of epithelial and glandular cells forming a growth; the cylindric layer; the degeneration of stroma connective tissue. A dark pigment gathered in the connective tissue; a finegrained infiltration in the central part of a tumour (a). (Fot. by Waluga)

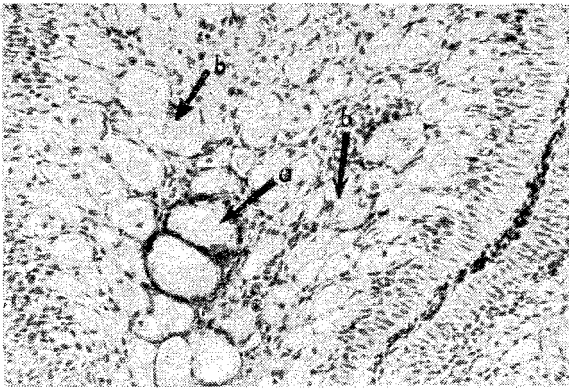


Fig. 12. A cross-section through a compact tumour with a fragment of the connective tissue stroma. In the mucuous (a) and spadicose (b) cells clear degenerative changes are observed. (Fot. by Waluga)

The tumours from various eels are considerably different from one another, the differences exhibited in shapes, sizes, colour, hardness and the connection with skin. Basing on a similarity of these features, papillae were divided conventionally into three distinct types: A, B and C.

A. Tumours with a single stalk-like connection with the skin (Fig. 13, 14) are observed most commonly. This kind of a growth occurs on „labiae“, in the lower part of snout, in

the upper part of head, between the eyes and in the fin bases. They have a compact structure with a rough surface; they are relatively soft, of a jelly-like consistence. These papillae are strongly vascularized showing a considerable amount of a dark pigment discolouring them brown – greenish. Their growth proceeds through the papillae volume increase, no expansion to the adjacent part of skin being observed.

B. The second type of papillae is characterized by a similar way of formation. They are relatively rare in the investigated material. The difference between them and those described earlier lies in their external surface, strongly rugged and with many blisterous appendices of various shapes (Fig. 15). When observed *in vivo*, their appearance is, however, somewhat different, they are covered with a great amount of mucus that agglutinate the appendices giving a papilla the tumorous homogenous form. The rugged papillae, in contrary to the previously described compact forms, are hard, bright, with the single blood vessels disposed in spots.

C. The third tumour group is essentially different in its macroscopic appearance. Its connection with a fish skin is different. Papillae are flat, flaccid, occur in the form of a thin and soft deposit (Fig. 16). Initially their surfaces are rough, then in older tumours more porous. In a „jelly-like” translucent mass the dilatated blood vessels are visible. Such tumours are bright unregarding the substratum colour. Their outgrowth shows characteristic features. The tumours are produced from single, fine papillae. Their growth proceeds through the adjacent skin areas implantation without any vertical proliferation. Usually the small „cauliflower-like” papillae are formed in the terminal parts of these flaccid forms; during further development they unite into a homogenous mass. Such a form may be found on the whole fish body.

The histologic picture confirms the macroscopic differences in the tumours morphologic structure. The papillae consist of the connective tissue stroma and the epithelial cells reproducing on it, the proportions between the epithelial tissue and the connective stroma being variable. In the compact papillae (A type) attached stalk-like, the epithelial cells prevail (Fig. 6). The distribution of this tissue within the whole mass of a tumour is uniform, excluding the connective tissue condensation in the papilla marrow. The fibre streaks are regular, usually parallel giving rectangular elongated „lobules” which can be seen on sections. The flat flaccid tumours (C type) have another arrangement of the connective tissue. They are extensively connected to skin, the epithelium is interspaced with the connective tissue irregularly arranged, in the growth base. The main part of a tumour, however, is of a different character. The connective tissue reticulum is visible as very narrow streaks. In such a structure the reproductive layers almost touch each other with their basal membranes. The connective tissue – vascular stroma of these tumours is very irregular resulting in narrow „lobules”. The skin – epithelium border may be partially obliterated; the atrophy of the basal membrane occurs then. Almost the entire mass of a flat growth consists of fine untypical cells chaotically disposed, different from those of the epithelial tissue of stalk-like papillae. They have small nuclei of various shapes, intensively discoloured due to hyperchromasis. The glandular cells are not observed in such tumours, the arrangement of cells in layers not being found either.

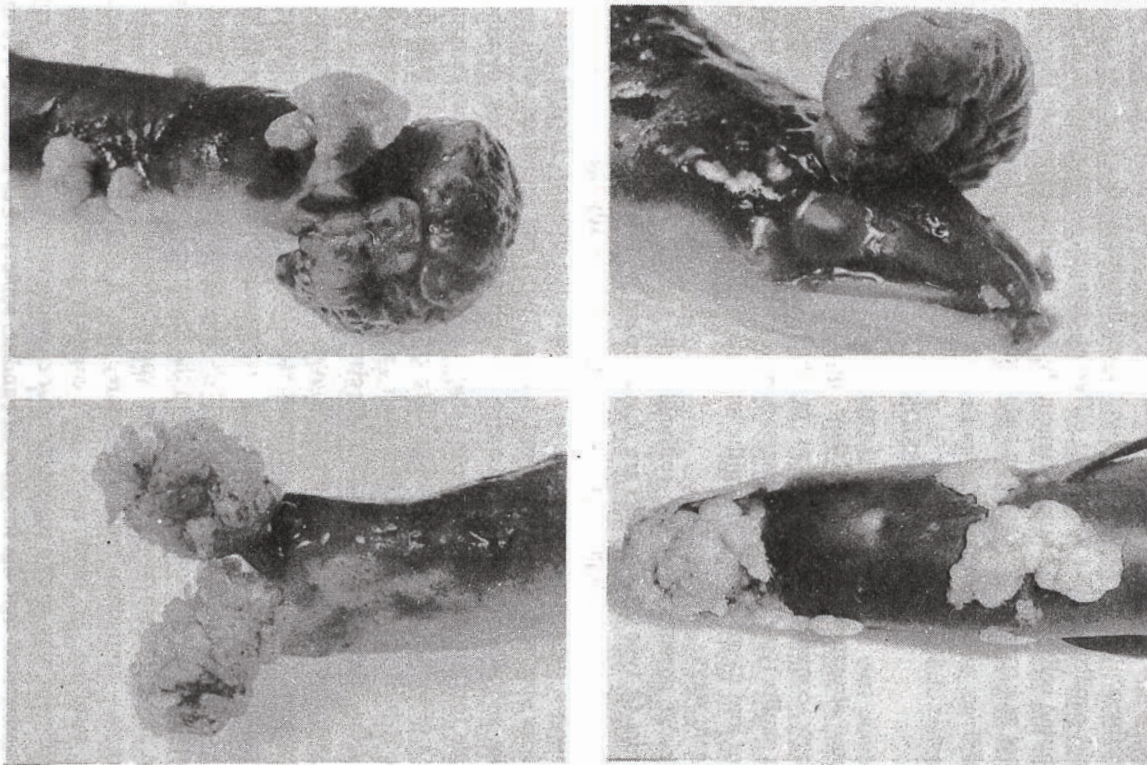


Fig. 13. A big stalk-like tumour of a compact structure, situated between the fish eyes, and fine blisterous papillae near the nostrils. The necrotic loss in the mandible with a noticeable proliferation of the epithelium Fig. 14. An eel with several tumours; a big compact pigmented one on the fish mandible and a brighter, spot-congested ones on the maxilla, near the eyes and gills Fig. 15. A strongly rugged tumour set on the maxilla and mandible terminal parts Fig. 16. An eel with flaccid strongly vascularized flat tumours on the upper side of his head

The disintegration processes result in specific changes in the macroscopic appearance of a papilla. Centrally situated cells, particularly the mucous and spadiceous ones decompose most quickly (Fig. 12). The dead tissue of the lobules becomes cracked and it crumbles away. The tumours are subjected to divisions into villi chaotically arranged. A papilla changed in this way in most cases dies out entirely, the cylindric layer stop functioning and the connective tissue stroma undergoes degeneration.

The flat flaccid papillae exhibit somewhat different changes. Great numbers of fine, irregularly distributed cells proliferate simultaneously with the disintegration; they shift towards the surface as a compact fold and run out on the tumour.

DISCUSSION

The microscopic structure of tumours, their appearance and the manner of their disintegration show considerable differences in their structure indicating a various character of each papilla type. Histologic observations reveal differences in the connective tissue arrangement and its relation to the epithelium in a papilla. They are also connected with skin in different ways.

In the flat flaccid growths (C type), epithelial cells are different from the typical stalk-like epithelium (A type). These cells are characterized by a hyperchromasis as well as an atypia, the nucleus – cytoplasm ratio moving over towards the nucleus. These features would indicate the different kind of flat and stalk-like growths. The flat papillae are relatively seldom observed. **Christiansen and Jensen** (1947) had found eels with such growths. **Lühmann and Mann** (1957), when analysing the tumour outgrowing, described the fusion of single foci into one and suggested that it might form big compact tumours. Since the author's observations indicate rather different way of growth of the stalk-like and flat tumours, it is to conclude that **Lühmann's** and **Mann's** description concerns the flat flaccid growths. The other authors (**Schäperclaus**, 1953; **Amlacher**, 1957, 1961) described an appearance and structure of a typical, mature growth. The structure presented by them corresponds with the compact stalk-like papilla (A type). This type has been most commonly observed in the material examined. Descriptions given by the authors mentioned above present a histologic picture of tumours composed of regular cells. The structural analysis of papillae in eels from the Szczecin Firth reveals the disintegration processes in most individuals. This fact contributes to the changes in their character, the stroma – epithelium ratio being altered too. A degenerated mass becomes stucked together resulting, in most cases, in a compact papilla. The disintegration proceeding in a different way gives a rugged tumour characteristically situated at the end of the snout. It is suggested that the location of papillae, subjecting to an active washing out, should result in the dead tissue removal and in the formation of digital tumour fimbriae. This type of a growth has been seldom encountered and it was not recorded by the other authors. The present investigations fail to find out whether the form described above is a last stage of a complete growth deterioration or only results from a temporary

inhibitor in the cells proliferation. **Peters, Peters and Bresching** (1972) found out that a chininsulfate treatment gave rise to a secondary differentiation of epithelial cells, the arrangement in layers, typical for a healthy epithelium, being formed followed by vanishing of the tumour.

Chemicals found in polluted waters are likely to belong to the factors contributing to the papillae degeneration and disintegration in the Szczecin Firth eels. **Peters et al.** (1970) reported a degeneration of cells which formed the papillae. They described disintegration changes in the glandular cells of the fin epithelium, but they did not indicate, however, whether it was also true for the remaining cells and whether it had an effect on the whole growth picture. The earlier papers had given no information on this subject. In the Prof. Grabda's mounts from eels taken in the fiftieths in Polish waters, there were no disintegrational changes in tumours either.

The author's opinion as to the papilla formation and the part played by the corium elements in this process is somewhat different. Most authors (**Schäperclaus**, 1953; **Amlacher**, 1961; **Schmid**, 1969) consider the corium connective tissue as functioning only as a support and nutrition for epithelial cells, and changes in this tissue being a result of transformations of a covering layer. In the material described in this paper, a stimulation of the connective tissue (its thickening, creasing of the stroma and the collagen fibres penetration between the epithelial cells) is clearly observed before the changes in the epithelial tissue occur. It might indicate an active participation of the corium in formation and growth of the „cauliflower disease“ tumours.

Schäperclaus (1953) found the growths only on eel heads, but **Christiansen and Jensen** (1947) had earlier described an eel with growths all over its body (presumably it had been a flaccid form of papilloma). More recent observations (**Lühmann and Mann**, 1957; **Amlacher**, 1961) showed that papillae were found most commonly on the skin of head and less frequently on the other parts of a fish body. Apart from the head, papillae may appear on the fin bases or on the fins themselves, on the tail and the dorsal side of fish; they are, however, rather rare there. The observations carried out on eels from the Szczecin Firth confirm the previous conclusions.

In the first description of the „cauliflower disease“ of eels **Christiansen and Jensen** (1947) pointed out that the diseased individuals were most commonly found among big fishes, especially the silver ones. **Schäperclaus** (1953), however, found this disease both in big and small specimens, the silver and green ones. According to him the disease is most often found in the small eels (30–35 cm of length). **Lühmann and Mann** (1957) confirmed these observations only partially assuming, however, that papillae were evenly distributed among the big and small individuals. They found cauliflower-like growths on eels of 9 cm of length. The present investigations show that eels of various ages are affected by the disease symptoms, most commonly the small ones (30–40 cm, Table I). The big fishes exhibit the growths less frequently.

The analysis of the material collected (Table I) indicates that the mean weight in particular groups differs only slightly from the weights of healthy fishes of the same length. In each length class, however, there existed 2–3 individuals with a 30–40% re-

duction of weight as compared with an average. The degree of the disease development (showed as an obstruction of mouth) indicated the prolonged starvation of these fishes. The similar observation were made as to fishes with growths widely distributed over a considerable length of a body with leaning of these fishes noticed as well.

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MORFOLOGICZNA I HISTOLOGICZNA BUDOWA NAROŚLI PRZY BRODAWCZYCY WĘGORZY

Streszczenie

Obserwacje makroskopowe węgorzy z brodawczą, pochodzących z Zalewu Szczecińskiego, zebranych latem 1971 roku, wykazały różnice w wyglądzie zewnętrznym narośli.

Najczęściej w badanym materiale spotykało się narośla o zwartej budowie, osadzone szypułkowato, z reguły w części głowowej. Rzadziej natomiast występowały twarde, silnie postrzępione brodawki, osadzone na końcu pyska. Trzecim typem były płaskie narośla, szeroko rozlane na znacznej powierzchni ciała ryby.

Na preparatach histologicznych, poza normalnymi szczegółami budowy, w obrębie komórek tworzących narośl, w większości przypadków, stwierdzono zmiany rozpadowe, które mogą być następstwem destruktywnego działania na brodawki związków chemicznych dostających się do wody wraz z zanieczyszczeniami. W przypadku brodawek postrzępionych zmiany zwyrodnieniowe były daleko posunięte, a umiejscowienie na końcu pyska sprzyjało usuwaniu martwej tkanki i rozpadowi narośli. Odmiennie zbudowane są brodawki rozlane, płaskie. Zaciera się u nich granica skórno-nabłonkowa, całą masę stanowią drobne komórki, inne niż w poprzednich brodawkach.

Prócz tego prześledzono początkowe stadia tworzenia brodawek. Na uwagę zasługuje fakt, że tworzenie narośli oraz ich wzrost odbywa się przy czynnym udziale tkanki łącznej skóry.

МОРФОЛОГИЧЕСКОЕ И ГИСТОЛОГИЧЕСКОЕ СТРОЕНИЕ ОПУХОЛЕЙ ПРИ ПАПИЛОМЕ УГРЕЙ

Р е з ю м е

Макроскопические наблюдения над большими папилломой угрями, выловленными в Щецинском заливе летом 1971 года, выявили различия во внешнем виде опухолей.

Чаще всего в исследуемом материале наблюдались опухоли плотного строения, имеющие вид сосочков, размещающиеся, как правило, в головной части. Реже встречались твёрдые, очень шероховатые опухоли, размещённые в носовой области головы. Третьим типом были плоские опухоли, широко разлитые по всей поверхности тела рыб.

На гистологических препаратах кроме обычных особенностей строения в области клеток, образующих опухоль, в большинстве случаев обнаружены дегенерационные изменения, которые могут быть результатом деструктивного действия на опухоли химических веществ, попадающих в воду вместе с загрязнениями. Что касается шероховатых опухолей, то в них дегенерационные изменения были довольно глубокими, а размещение их в носовой области головы благоприятствовало удалению мёртвой ткани и распаду опухолей. Иначе построены разлитые, плоские опухоли. Стирается у них кожно-эпителиальная граница, всю массу их составляют мелкие клетки, однако иные, чем в предыдущих опухолях.

Кроме того, были прослежены начальные стадии образования опухолей. Заслуживает внимания тот факт, что образование опухолей, а также их рост происходят при активном участии соединительной ткани кожи.

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