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DIFFERENCES IN THE EEL PERIPHERAL BLOOD HEMATOLOGIC PARAMETERS
ASSOCIATED WITH PAPILLOMA (*PAPILLOMATOSIS ANGUILLARUM*)*

RÓŻNICE W PARAMETRACH HEMATOLOGICZNYCH KRWI OBWODOWEJ WĘGORZ
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As a result of studies on the eel (*Anguilla anguilla* L.) hematologic parameters, certain characteristic symptoms typical of the „cauliflower disease“ (*Papillomatosis anguillarum*) have been established, both the different stages in tumours' growth and anatomo-pathological lesions being taken into account.

INTRODUCTION

Owing to a constant expansion of papilloma (*Papillomatosis anguillarum*) within eel populations, various studies on the disease have been undertaken in recent years. Positive results of cell cultures (Pfitzner, 1969) as well as some alien elements detected in cells incubated with the diseased eels' blood (Schubert, 1969) indicated to a virus nature of papilloma. Also Schäperclaus (1953), Amlacher (1961), Koops and Mann (1969), and Schmid (1969) were of similar opinions despite partly negative results of eel artificial infections obtained either through tissue homogenate injections or transplantations of tumour fragments (Schäperclaus, 1953; Koops and Mann, 1969; Pfitzner, 1969).

As far as other problems are concerned, the morphologic and histologic structures of the papilloma tumours have been studied in detail (Christiansen and Jensen, 1950; Schäperclaus, 1953; Lüthmann and Mann, 1956; Amlacher, 1961; Schmid, 1969; Pilarczyk, 1973). Among the other things, the tumour growth nature, differences in the connective tissue stroma and epithelium patterns as well as a role played by skin elements in growth processes have been disclosed (Schäperclaus, 1953; Amlacher, 1961; Schmid,

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1969; **Pilarczyk**, 1973). Simultaneously to proliferation processes occurring in the papillae cells, particularly in those of the epithelium (**Pilarczyk**, 1973), regressive lesions leading to necrosis in outgrowth tissue fragments are in progress. An extent of the degenerative lesions intensity depends on the tumour growth stage and partly affects its macroscopic appearance. **Peters et al.**, (1972) obtained under the experimental conditions a growth retardation and gradual necrosis of the tumours resulting from a quinine sulphate treatment, while **Pilarczyk** (1973) ascribed the degeneration and disintegration of papillae under natural conditions to the influence of chemical compounds contained in polluted waters.

The papillomatous growths cause some apparent disturbances not only locally in the changed skin. Strong emaciation have been found to occur in fish with larger papillae, particularly with those growing on jaws around the snout (**Pilarczyk**, 1973). Those eels exhibited also an increased muscle hydration, lowered protein and fat contents as well as a drop in liver and gut weights (**Lühmann and Mann**, 1956).

Besides, a strong influence of a toxic agent, presumably viruses being involved here, causes definite pathological lesions in visceral organs (**Dzidziul**, 1973). The inflammatory and degenerative changes revealed by her in liver and kidney are related to eels with tumours appearing as flat and diffuse deposits or as large and stalk-like outgrowths.

In view of the above results it seems purposeful to supplement them with the analysis of the peripheral blood hematologic indices for eels of various tumour growth stages and anatomo-pathological changes.

MATERIAL AND METHODS

The material investigated originated from the Szczecin Firth waters near Trzebież, collected as a few samples from August through October 1971. As a whole, 77 fishes with papillomatous growths and 30 control ones were examined. The length and weight ranges amounted to be 29–69 cm (l.t.) and 30–600 g, respectively. In the material investigated small individual below 40 cm prevailed (**Pilarczyk**, 1973; Table 1 – the observations were carried out on the same specimens). The control fishes were selected out of a larger number (ca 150 individuals), the autopsy changes and hematologic parameters having been checked previously.

Before passing them under observation, the eels caught were kept from 17–20 hrs to 3 days in a tank filled with aerated current water. The blood to be examined was taken from the caudal vein with a Pasteur's pipette rinsed with a small amount of heparin. Always in the same way the morphologic blood elements were counted in first few drops. The quantitative examination of 50 diseased and 30 control fish individuals comprised:

1. Erythrocyte, leukocyte and thrombocyte counts per 1 mm³ peripheral blood, made in the Bürker chamber following the generally accepted procedure. The blood had been previously 200-fold diluted with violet staining solution and physiological fluid in the Potain mixer.
2. Photocolorimetric determinations of the hemoglobin level according to Green and Teal-modified Drabkin's method.

3. A hematocrit value determination using Wintrobe's micropipettes and heparinated capillaries.

Bases on the above parameters the typical erythrocyte indices, i.e., average hemoglobin content and concentration per one blood cell, and mean erythrocyte volume were calculated. Besides, all the eels were examined in detail, parasites as well as anatomico-pathological changes in the visceral organs being taken into account.

RESULTS

In most cases, the diseased eels exhibit increased numbers of morphotic elements per the peripheral blood volume unit (1 mm^3) (Tab. 1). In the first place, a average erythrocyte number/ mm^3 tends to increase; the deviations from the control level are connected partly with the papillae appearance and extent of development. Considerably increased erythrocyte counts (in average by $400,000/\text{mm}^3$) were found in eels with large, dark-stained tumours sometimes excessively overgrowing the fish snouts (Tab. 1, group 2). A further increase in the erythrocyte count (in average to $2,000,000/\text{mm}^3$ Tab. 1, group 3) is detected in tumours with more abundant blood supply, partially injured, with tissue fragments detached and bleeding wounds found. Similar symptoms become more pronounced with an additional fish head congestion, particularly so around jaws, fins and visceral organs, mainly abdominal membrane, liver and kidney.

The highest erythrocyte numbers (about $2,400,000/\text{mm}^3$) were found in the peripheral blood of eels with flat tumours covering vast areas of skin (Pilarczyk, 1973; tumour type C) showing strong vascularization and blood vessels distinctly dilated (Tab. 1, group 3a).

The erythrocyte number increase (Tab. 1, groups 1, 2, 3) does not necessarily cause any significant differences in the remaining parameters like hematocrit and hemoglobin content. Only in a few cases an evident hematocrit value increase has been noted in the group 1 showing relatively young, moderately developed tumours. Groups 2 and 3 revealed wider fluctuations and greater deviations from the mean values. The differences as well as the hemoglobin content changes are connected with intensified changes in skin and visceral organs.

A decline in the mean erythrocyte volume is seen from the hematocrit indices (Tab. 2, column 2), the differences being most clearly evident in the fish group 3 with and excessive blood supply in papillomatous growths; the average erythrocyte volume is $183.8\mu^3$ instead of $252.5\mu^3$. Furthermore, relatively strong deviations of both the minimum and maximum values in particular samples indicate to a considerable size differentiation in the erythrocyte populations.

Regarding the other indices, only the absolute hemoglobin index alters significantly (Tab. 2, column 3). The calculations made indicate to a clear drop of the mean hemoglobin content per 1 erythrocyte, the phenomenon being pronounced in tumours excessively grown or improperly supplied with blood.

The other anatomico-pathological changes related to papilloma (Tab. 1, fish groups 4, 5, 6) cause a different pattern of quantitative changes in erythrocyte and other

Table 1

The peripheral blood hematologic data in the papilloma-diseased eels

Group of fishes examined	Kind of changes	Number of females	Erythrocytes million/mm ³	Hematocrit %	Hemoglobin g%	Leukocytes per mm ³	Thrombocytes per mm ³
Control individuals		30	1.490 1.38–1.68	37 34–42	10.05 8.38–11.81	20.120 10.4–28.0	37.600 30.8–46.8
Individuals with papillomatous outgrowths 1	well-developed and newly formed	19	1.628 1.37–1.78	37 32–47	9.20 5.30–10.21	46.550 26.0–94.4	44.760 30.4–82.4
2	large, excessively overgrown	7	1.890 1.72–2.28	41 29–45	11.68 7.63–14.41	15.560 10.8–24.4	39.800 28.4–58.0
3	strongly congested or bleeding	13	2.091 1.87–2.44	39 29–47	10.17 7.63–13.14	52.290 40.0–80.8	55.000 39.6–70.8
3a	flat and diffuse include	2	2.405	46	12.04	61.400	39.600
4	papillae and other congestions, for instance in swim bladder	5	1.280 1.22–1.33	26 14–34	7.64 7.42–7.88	62.560 33.2–110.1	53.920 38.8–77.6
5	papillae and hyper-splenism of spleen	3	1.100 0.84–1.27	26 26–28	7.76 6.99–8.88	17.400 7.6–29.2	26.400 14.2–28.0
6	papillae and "red disease"	3	0.330 *0.17–0.81	14 *4–23	3.53 *0.57–5.15	35.700 *25.2–55.2	17.000 *12.0–19.0

* values not included into the mean

Table 2

The peripheral blood hematocrit indices of the papilloma-diseased eels

Group of fishes examined	Kind of changes	Mean erythrocyte volume in μ^3	Mean hemoglobin content per 1 erythrocyte	Mean hemoglobin concentration (%)
	1	2	3	4
Control individuals		252.5 231.7–269.2	67.10 58.19–74.47	26.63 24.64–28.68
Individuals with papillomatous cutgrowths:				
1	well-developed and newly formed	230.5 154.7–306.5	54.31 31.54–65.70	24.26 15.78–31.90
2	large, excessively overgrown	192.0 127.7–254.2	59.17 33.46–73.89	25.72 24.02–26.91
3	strongly congested or bleeding	183.8 127.1–244.7	48.76 41.75–53.85	26.50 23.80–30.74
4	papillae and other congestions, for instance in swim bladder	202.5 106.0–278.6	59.93 59.80–62.54	31.77 22.44–52.28
5	papillae and hypersplenism of spleen	283.2 204.7–333.3	67.40 55.03–105.7	28.05 25.58–31.70
6	papillae and "red disease"	289.4 *23.5–295.0	71.60 *3.33–79.83	24.70 *0.14–27.05

*values not included into the mean

parameters. Thus an additional hyperemia in the connective duct or swim bladder results in a decrease in erythrocyte counts, hematocrit, and hemoglobin content in average by $210,000/\text{mm}^3$, 11% and 2.4 g%, respectively. A similar drop is seen in the functional spleen insufficiency (Tab. 1, group 5); particular hematocrit indices do not decrease there, but in contrary, the average erythrocyte volume becomes typically increased (Tab. 1, column 2).

The acute form *Erysipelosis anguillarum*, enhancing the pathological lesions (appearing through extensive hyperemia in abdomen skin, fins, particularly the anal one, losses and depigmentation of skin as well as a strong hyperemia in the visceral organs) results in the greatest drop in the parameters discussed. The erythrocyte numbers, hematocrit and hemoglobin content are maintained there on the level of $300,000/\text{mm}^3$, 14% and 3.53 g%, respectively (Tab. 1, group 6). In the effusion form of this disease, comprising sanguineous fluid within pericardial and peritoneal cavities as well effusions and clots among the visceral organs, the peripheral blood erythrocyte number drops to about $100,000/\text{mm}^3$. Trace hemoglobin amounts reaching 0.5 g% occur, too.

The eel papilloma proceeds usually accompanied by a clear leukocytosis reaction. Average leukocyte amounts in the peripheral blood reach ca $50,000/\text{mm}^3$, the maximum ones reaching to $100,000/\text{mm}^3$, while the control level is ca $20,000/\text{mm}^3$ (Tabl. 1). The leukocyte level tends to rise in various stages of the diseases with fine, newly formed tumours and well-developed, strongly vascularized ones. It is also increased following acute internal symptoms (in swim bladder congestion, among the other) as well as in the typical form of the „red disease”. No leukocytosis has been found in the blood of eels with older, well-shaped tumours stained brown with a distinct superficial layer of necrotic tissue (Tab. 1, eel group 2). Also the leukocyte number shows the control level range or drops below the normal value in the spleen insufficiency.

One of other parameters, the differences in the peripheral blood thrombocyte counts for particular groups of the fishes examined are no less characteristic. The thrombocyte number shows the highest increase (average and maximum values up to 55,000 and $70,000/\text{mm}^3$, respectively) when the tumours are excessively supplied with blood, the smaller increase being noted when the papillae are old and partly dead. With regard to the spleen hypersplenism a decline of these cells amount is recorded (in average by $26,400/\text{mm}^3$, while in its minimum to $14,200/\text{mm}^3$) as well as similar symptoms associated with the „red disease” (in average to $17,000/\text{mm}^3$; Tab. 1).

DISCUSSION

The chronic form of pseudopolycythaemia appears to be the most frequently encountered symptom associated with the papilloma, characterized by and increase in the relative erythrocyte number (per volume unit) whereas the hematocrit and hemoglobin levels fluctuations are less pronounced. Moreover, the peripheral blood erythrocyte are of a smaller average volume and hemoglobin content per one cell. The decrease in the two parameters allows to suspect an anisocytosis symptom and a compensative erythroblastic reaction taking place.

A considerable shift in the leukocytes towards the peripheral leukocytosis occurs in the pseudopolycythaemia. Another characteristic feature of this stage of the disease is variously intensified thrombocythaemia indicating, among other things, to an intensified differentiation of cells in the system as well as to a possibility of haemorrhage and clots likely to appear. In general, the polycythaemia is associated with certain forms of a circulation system insufficiency as well as quantitative changes within the plasma accompanied by a simultaneous translocation of intracellular fluids. As far as the pseudopolycythaemia is concerned, mammals affected show variable amounts of blood in their organisms, sometimes smaller than those under the normal conditions owing to the plasma volume decrease. The symptoms described are most often associated with a considerable epidermal vascularization, vaso-motor distempers, vein stasis, and damages in the blood vessels, particularly the capillary ones.

The symptoms as described above have not been hitherto studied in detail in fish, also as far as eel papilloma is concerned. The macro- and microscopic observations reveal a strong superficial vascularization of the tumour tissue. Pilarczyk (1973) found changes within the connective tissue stroma blood vessels involving vasodilatation, formation of embolism, and exovascular effusions. Following the blood vessels degenerative lesions, tumour fragments or larger parts tend to atrophy. Frequently associated with the above-described changes, the epidermal symptoms have been recorded as strong congestion and spotty ecchymoses usually observed on head or abdomen skin. Various damages of the skin, even the mechanical ones, causing a local epidermal inflammation, when combined with the other lesions make the differences between the peripheral blood parameters to be more profound.

Apart from the above stated facts, in 18 cases out of 39 fish individuals analyzed, liver parenchyma was found to be injured due to an even, strong congestion or a congestion of the terminal part of lobes in the organ. Dealing with large and stalk-like tumours, Dzidziul (1973) found the blood vessels' disintegration within the liver parenchyma in an acute inflammation accompanied by the connective tissue proliferation. Stronger are the changes in eels exhibiting flat and diffuse tumours, the fishes' livers showing inflammatory changes combined with the colliquative necrosis. The kidney glomerulae and canaliculi are destroyed to various degrees in both the eel groups studied by the author, thus partly or completely eliminating the active excreting role of the organ.

Therefore both the skin symptoms and histopathologic changes, combined with those dissection-revealed occurring in this stage of papilloma indicate to the acute insufficiency of the blood system and osmo-regulation mechanism distempers; as a result, definite changes in the peripheral blood appearing as pseudopolycythaemia, leukocytosis, and thrombocythaemia take place.

The other stage in the „cauliflower disease” (with their defined anatomic-pathological changes occurring) comprise hypochromatous anemia usually proceeding with a leukocytosis and thrombocythaemia or thrombocytopenia reactions. A moderate anemia with a large amounts of leukocytes and thrombocytes was noted in additional swim-bladder congestion cases showing a strong erythrocyte extravasation, clots and sanguineous effusions being formed at the same time.

A typical form of the „red disease” combined with papilloma displays the intensifying anemia symptoms in the course of the disease progress and emaciation of the organism. The anemia is caused by high blood losses resulting from haemorrhages and intraperitoneal clots as well as from a sanguineous effusion within the peritoneal and pericardial cavities. Not always this stage of the disease is accompanied by acute leukocytosis since it depends on the extent of damages in kidney hemopoietic centres. Thrombocytopenia, however, is typical as a result of the general vessels' distempers in excessive haemorrhages of the organ. **Kreutzmann** (1973) pointed out to a fact that eels suffering from the „red disease” showed the blood parameters decrease too, although to a somewhat smaller extent, i.e., down to ca 50%; the erythrocyte number, hematocrit and hemoglobin content dropped in average to $779,000/\text{mm}^3$, 26.7% and 4.92g%, respectively.

In the other group of eels examined by the author, the ones showing hypersplenism (possibly on the background of a chronic form of papilloma), an intensifying anemia, leuko- and thrombocytenia were observed. The differences should be ascribed to an excessive blood elements destructions in spleen as well as to distempers in the maternal cells formation and differentiation in kidney. A detailed analysis of changes taking place within the blood system, attempting to explain their mechanism, will be a further step in the studies on papilloma.

The results of the peripheral blood parameters analysis in the control eels from various waters tend, in general, to converge. The numerical data obtained from the eels of the Szczecin Firth are close to the results given by **Zajceva** (1967) for the fishes of the Kurski Firth, Baltic Sea. Small differences found are concerned with the hemoglobin values which are somewhat lower, in average by 2 g%, in the Szczecin Firth eels, taking the seasonal variations into account. Also the hematocrit indices within the average ranges of hemoglobin content and concentration in a blood cell are similarly smaller. **Kreutzmann** (1973) found very close values in eels caught from Born/Darz Sounds; only somewhat higher hematocrit values having been obtained throughout the whole period investigated (six months) should be stressed here.

On the other hand, the **Kawamoto's** calculations (1929) obtained from the hematologic data for *Anguilla japonica* show a well marked difference. The peripheral blood erythrocyte numbers for *A. japonica* and *A. anguilla* are above 2,000,000 and (in average after various authors) $1,400,000/\text{mm}^3$, respectively. When compared to these, the hematocrit shows a remarkably low values, ranging within 24.0–29.7% and 34–44% in *A. japonica* and *A. anguilla*, respectively, the latter range as quoted by various authors.

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RÓŻNICE W PARAMETRACH HEMATOLOGICZNYCH KRWI OBWODOWEJ WĘGORZY PRZY BRODAWCZYCY (*PAPILLOMATOSIS ANGUILLARUM*)

Streszczenie

Na podstawie oceny ilościowych parametrów krwi obwodowej węgorzy z brodawczycą (*Papillomatoses anguillarum*) wyznaczono różnice w przebiegu schorzenia w zależności od stadium wzrostu narośli i zmian anatomopatologicznych w narządach wewnętrznych. Najczęstszym objawem towarzyszącym brodawczycy jest nadkrwistość pozorna (*pseudopolycythaemia*) z narastającą leukocytozą i trombocytemią. Odczyny te występują, między innymi, w związku z ostrą niewydolnością układu krążenia oraz uszkodzeniem mechanizmów osmoregulujących.

Przy innych dodatkowych objawach, w przypadku przekrwień pęcherza pławnego z silnym wynaczynieniem erytrocytów z wytworzeniem skrzepów oraz krwistego wysięku, ustalono niedokrwistość niedobarwliwą z wysoką leukocytozą i trombocytemią. W połączeniu z rumieniścią (*Erysipelosis anguillarum*) zaznacza się ostra niedokrwistość przeważnie z nasilającą się leukocytozą obok trombocytopenii. Stany ostrej niedokrwistości spowodowane są znaczną utratą krwi w wyniku wylewów i skrzepów dootrzewnowych oraz krwistego wysięku w jamach osierdziowej i otrzewnowej.

W stadium nadczynności lub nieprawidłowej czynności śledziony (hypersplenizm) przy schorzeniu brodawczycy obserwowano niedokrwistość niedobarwliwą z leukocytopenią i trombocytopenią, wynikającą z nadmiernego niszczenia względnie zaburzeń w wytwarzaniu i różnicowaniu elementów krwi.

РАЗНИЦЫ В ГЕМАТОЛОГИЧЕСКИХ ПАРАМЕТРАХ ПЕРИФЕРИЧЕСКОЙ КРОВИ УГРЯ
ПРИ ПАПИЛЛОМЕ (*PAPILLOMATOSIS ANGUILLARUM*)

Р е з ю м е

На основе оценки количественных параметров крови угря, заражённого папилломой (*Papillomatosus anguillarum*), определили различия в течении болезни, зависящие от стадии развития опухолей и анатомо-патологических изменений во внутренних органах. Наиболее частым симптомом, сопровождающим папиллому, является ложное полнокровие (*pseudopolycythemia*) с возвращающим лейкоцитозом и тромбоцитемией. Эти реакции возникают и в связи с острой недостаточностью кровеносной системы, а также с нарушением осморегуляторных механизмов.

При других дополнительных симптомах, при гиперемии плавательного пузыря с острым экстравазатом эритроцитов с образованием стустков и кровяного экссудата, обнаружена гипохромная анемия с повышенным лейкоцитозом и тромбоцитемией. Наряду с эритемией (*Erysipelosis anguillarum*) отмечается острая анемия преимущественно с возрастающим лейкоцитозом и тромбоцитемией. Состояния острой анемии вызваны значительной потерей крови в результате внутрибрюшинных излияний и тромбов, а также кровяного экссудата в перикардиальной и брюшинной полостях.

В стадии гиперфункции или нарушения функции селезёнки (*hypersplenismus*) при заболевании папилломой наблюдали гипохромную анемию с лейкопенией и тромбоцитемией, возникающими в результате повышенного разрушения или же нарушения в образовании и дифференцировании элементов крови.

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