IMPACT OF SEA LAMPREY PARASITISM ON THE BLOOD FEATURES AND HEMOPOIETIC TISSUES OF RAINBOW TROUT

Great Lakes Fishery Commission

TECHNICAL REPORT No. 46

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IMPACT OF SEA LAMPREY PARASITISM ON THE BLOOD FEATURES AND HEMOPOIETIC TISSUES OF RAINBOW TROUT

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ABSTRACT

Rainbow trout *(Salmo gairdneri)* held in the laboratory were subjected to sea lamprey *(Petromyzon marinus)* attack for prescribed time periods. Blood and hemopoietic tissue samples taken during wound development and wound healing provided information on the hosts' ability to recover after a lamprey attack. The blood features that were monitored included the hematocrit, hemoglobin, red blood cell precursors, leucocrit, and leucocyte differential. The spleen was the only hemopoietic organ which exhibited pathological change. During lamprey attachment the hematocrit and hemoglobin values for host fish were higher than for control fish, which was attributed in part to a stress response and the inability of the smaller sized lamprey (19.1 cm and 13.5g) to induce anemia. There was no significant change in the red blood cell precursors during this wound development period, and host fish demonstrated lymphopenia with concomitant neutrophilia. The hematocrit and hemoglobin values for fish in the wound healing group dropped significantly at 2 days following lamprey detachment, which is believed to be attributed to hemodilution via the wound area. The number of red blood cell precursors rose significantly during wound healing and reached a peak value at 1 month. Lymphopenia with concomitant neutrophilia was evident 2 days following lamprey detachment.

INTRODUCTION

The feeding mechanism of sea lamprey *(Petromyzon marinus)* is adapted for obtaining liquid food, principally blood sucked from the host fishes (Lennon 1954). Body fluids enter the lampreys' diet to a lesser degree and a considerable amount of reduced flesh, particularly muscle, is also ingested. Within the confines of the wound the capillaries are destroyed and blood and lymph are ingested by the lamprey. Lennon also found that the buccal gland secretions from sea lamprey prevented fish blood coagulation.

The daily blood consumption by sea lamprey at 10 ± 1°C feeding on rainbow trout *(Salmo gairdneri)* and lake trout *(Salvelinus namaycush)* ranged from 2.9 to 29.8% (average, $11.6 \pm 7.5\%$) of the lamprey's wet body weight per day (Farmer et al. 1975). They found the hematocrits of dying fish were greatly reduced to $1.9 \pm 1.7\%$ from control values of $34.4 \pm 1.8\%$ and the percentage moisture of the blood of dying fish increased from 84.5 ± 1.0 to $96.4 \pm 1.5\%$.

Because most of the sea lamprey's diet consists of blood, and teleosts have a relatively small blood volume, blood loss can result in marked changes in various blood features. By monitoring various blood features during the attack period and during wound healing one can determine what changes take place during lamprey attachment and how the host recovers during wound healing. Because the hemopoietic centers of the kidney and spleen will be taxed during lamprey feeding, the pathology of these organs was studied to determine what effects, if any, occur.

METHODS AND MATERIALS

EXPERIMENTAL ANIMALS

Rainbow trout used in the study were obtained from the Midwest Trout Farm, Harrison, Michigan. Before experimentation the fish were held in 1,000 L circular tanks that received flowing 12°C well water of pH 7.1, hardness 330 mg $CaCO_3$/L and alkalinity 325 mg $CaCO_3$/L. Dissolved oxygen was 9.0 mg/L or higher. The fish were fed Trout Chow (Ralston Purina, Checkerboard Square, St. Louis, MO) *ad libitum.* The averages for total length and weight of the trout used in the wound development study were 30.6 cm and 309.1 g; the averages for those in the wound healing group were 31.1 cm and 328.4 g, respectively.

Sea lamprey, obtained from the U.S. Fish and Wildlife Service Laboratory at Hammond Bay, Michigan, were juveniles that had recently completed metamorphosis. Lamprey were maintained in 150 L tanks receiving flowing well water from the same source as that used for the trout. The lamprey were allowed to feed on *carp (Cyprinus carpio)* before experimentation. All lamprey were actively growing, therefore those used in the later wound healing study were larger on the average (22.9 cm and 25.3 g) than those of the wound development study (19.1 cm and 13.5 g).

EXPERIMENTAL PROCEDURE FOR WOUND DEVELOPMENT

Individual trout were weighed, measured (total length), and placed singly in a 150 L fiberglass tank containing sea lamprey. When one of the lampreys attached itself to the trout, the time and position of attachment were noted and the remaining lampreys were removed to a holding tank. The lamprey was allowed to feed on the trout for a prescribed period after which the lamprey and trout were anesthetized in 80 mg/L tricaine methane sulfonate (MS-222), separated, weighed, and measured. Blood samples and the hemopoietic tissues of the kidney and spleen were collected from wounded fish at 4 h, 12 h, 2 days, and 10 days after the initial lamprey attachment. Five fish were used for each sampling period.

HEMATOLOGY PROCEDURE

A 0.5 cc blood sample was collected from the caudal blood vessel of the trout with a 22 gauge needle and 3.0 cc syringe. The blood was then placed in a 3.0 cc vacutainer tube treated with EDTA to prevent clotting.

Two blood smears were prepared immediately. One smear was stained with Wright's stain and the other was fixed in absolute methanol as a spare. The stained smear was examined under oil immersion (1,000x) for immature red and white blood cell differential counts. The immature red blood cells (RBCs) were determined as a percentage of the first 500 red blood cells counted. The white blood cell count included the differentiation of the first 100 white blood cells into lymphocytes, thrombocytes, and granulocytic, metagranulocytic, immature, or segmented neutrophils (Lehmann and Sturenberg 1975).

Hematocrit and hemoglobin were determined by the microhematocrit and cyanmethemoglobin methods, respectively. Two heparinized microhematocrit capillary tubes were used in each hematocrit test and the two readings were averaged.

Leucocrit was determined by measuring the buffy coat at the surface of the packed red blood cells using an ocular micrometer and the determination was made by techniques described by McLeay and Gordon (1977).

HISTOPATHOLOGIC TECHNIQUE

Tissue samples of the spleen and anterior kidney were removed and fixed immediately in 10% buffered neutral formalin, then imbedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (Luna 1960).

EXPERIMENTAL PROCEDURE FOR WOUND HEALING

Lamprey were allowed to attach on trout for 8 days. The fish were then separated from the lamprey by anesthesia in MS-222 and returned to holding tanks for a prescribed time period. To monitor the hosts' recovery, blood samples for hematocrit, hemoglobin, leucocrit, and blood smears were taken immediately after lamprey detachment and at 1 week intervals thereafter. The trout were weighed and measured at the time of each blood collection. Five wounded fish were sacrificed to obtain tissue samples of the spleen and anterior kidney at 2 days, 2 weeks, 1 month, and 3 months after lamprey detachment.

RESULTS AND DISCUSSION

BLOOD FEATURES OF WOUND DEVELOPING AND WOUND HEALING FISH

During lamprey attachment the hemotocrit progressively increased from a control value of 24.1± 1.8% to a significantly higher value of 30.7± 1.8% after 12 h of attachment (Fig. 1A). This significant rise in the hematocrit could be attributed in part to stress response (Casillas and Smith 1977). Wedemeyer (1970), Nilsson and Grove (1974), and Schreck et al. (1976) found the stress response in fish resulted in additional erythrocytes entering the circulatory system. The hematocrit was 27.9±1.4% at 10 days of lamprey attachment indicating the inability of the smaller lamprey (19.1 cm and 13.5 g) to induce anemia even after 10 days of attack.

Changes in the hemoglobin concentration closely parallelled the changes in the hematocrit (Fig. 1C). The initial hemoglobin value was 6.6rt0.7 g/d1 but after 12 h of lamprey attachment it was 8.6±0.4 g/dl. This significant rise was consistent with the rise in the hematocrit. The hemoglobin then decreased to 7.8±0.4 g/d1 after 10 days of lamprey attachment, following in line with the hematocrit. There was no significant change in the red blood cell (RBC) precursors during this wound development period (Fig. 1E).

The fish in the wound healing group all experienced an 8 day sea lamprey

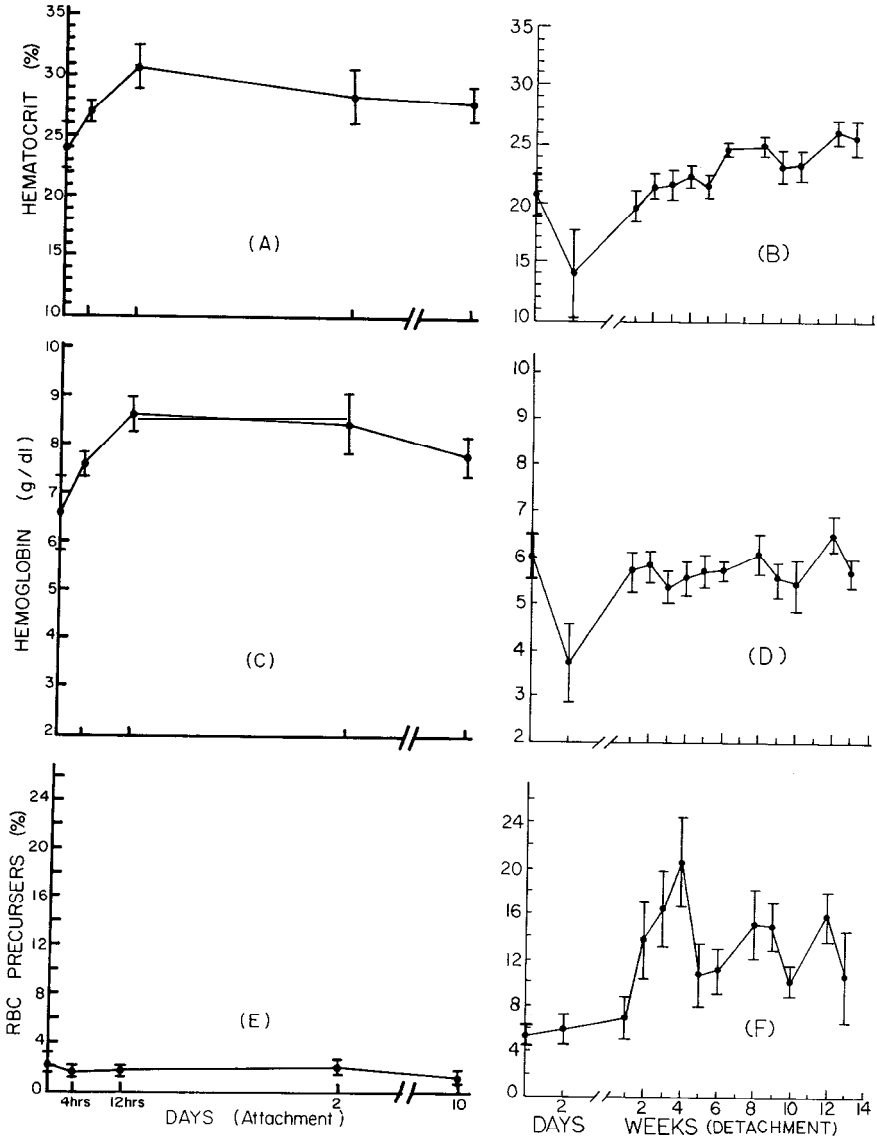

FIG. 1. Hematocrit, hemoglobin, and red blood cell precursor values during wound development and wound healing in lamprey-attacked fish.

(A) Hematocrit (%) in wound development fish.
(B) Hematocrit (%) in wound healing fish.
(C) Hemoglobin (g/dl) in wound development fish.
(D) Hemoglobin (g/dl) in wound healing fish.
(E) Red blood cell precursors (%) in wound development fish.
(F) Red blood cell precursors (%) in wound healing fish.

attachment. The hematocrit curve was similar to that of the hemoglobin during the wound healing period (Fig. 1B and D). Hematocrit and hemoglobin at lamprey detachment were 20.7± 1.9% and 6.0±0.5 g/dl, respectively, and both dropped significantly to 14.0±3.5% and 3.7±0.9 g/d1 at 2 days post detachment. The decline was attributed to a dilution effect on the blood as the wound was open and the fish was no doubt subjected to a flux of incoming water in the wound area. Kirk (1974) reported that the osmoregulatory status of fish may cause the hematocrit to change. The hematocrit and hemoglobin increased to 19.8± 1.3% and 5.7±0.4 g/dl, respectively, at 1 week. The hematocrit continued to rise slightly over time, with some slight fluctuations, reaching a value of 25.7± 1.4% at 3 months; the hemoglobin continued at a fluctuating plateau to 3 months when it had a value of 5.7±0.2 g/dl.

The increased hematocrit during wound healing was attributed to the formation of new epidermis over the wound area which would prevent hemodilution due to the influx of water. An accompanying red blood cell precursor response could also account for the rising hematocrit at 1 week and beyond (Fig. 1F). The red blood cell precursor value at detachment was 5.3±0.9% of the total red blood cells and was significantly higher than the control value of 2.2±0.7%. The number of RBC precursors continued to rise significantly after lamprey detachment. By week 2 the value was 13.9±3.1% and by week 4 the value reached a peak of 20.9±3.5%. By week 5 the number of RBC precursors had dropped to 10.6±3.0% and remained at a highly fluctuating level to 3 months when the value was 10.4±4.2%. One would expect that the increased number of RBC precursors during wound healing would result in a lower hemoglobin concentration because of the reduced amount of hemoglobin carried by these immature cells (Walker 1975). Although the hemoglobin concentration fluctuated around 5.7 g/d1 from 1 week through 3 months of wound healing, it was lower than the hemoglobin concentration of 6.6 g/d1 in fish that did not experience a lamprey attack (Fig. 1C and D).

The RBC precursor response tends to agree with results reported by Walker (1972). After withdrawing 20 to 30% of the whole blood volume from rainbow trout he found the reticulocytes appeared 5 days later and a high count of 40.4% remained at 20 days. He concluded the reticulocyte increase in the peripheral circulation is long-term since the response was still in progress on day 20.

Walker (1975) removed 40% of the blood volume from rainbow trout within a 7 day period and reported significant effects on the hematocrit, hemoglobin, and reticulocyte count. He found significant hematocrit recovery after 16 days and the hemoglobin concentration had recovered 30 days after bleeding. This difference in hematocrit and hemoglobin recovery indicated a replacement of the lost red blood cells by immature cells which did not contain complete hemoglobin molecules.

McLeay and Gordon (1977) introduced a test called the leucocrit which is the volume of packed leucocytes and thrombocytes expressed as a percentage of the whole blood. They found that the number of circulating leucocytes and thrombocytes was a more accurate reflection of a fish's reaction to stress than the number of erythrocytes. Using coho salmon *(Oncorhynchus kisutch)* and rainbow trout, they observed that the leucocrit and leucocyte-thrombocyte counts for

both species were depressed from control values after 96 h of exposure to stressful conditions.

During wound development in our study the leucocrit value decreased slightly, but not significantly, from the control value of 0.97±0. 10% to 0.89±0.04% and 0.84±0.13% at 12 h and 10 day attachments, respectively (Fig. 2A). The differential leucocyte count indicated that the lymphocytes decreased during lamprey attachment. The lymphocyte control value of 95.6±1.3% dropped significantly to 91.4±1.0% and 79.4±6.1% at the 4 and 12 h attachment periods, respectively, before rising to 82.2±7.5% at the 2 day lamprey attachment period (Fig. 2C). There were some relative increases in the neutrophilic series and thromboyte percentages during wound development which may have balanced out the decreased percentage of lymphocytes, thus explaining why the leucocrit did not drop significantly during this time. Many researchers have shown that stress in teleosts plays an important role in lymphopenia accompanied by neutrophilia (Weinreb 1958; Slicher 1961; Belova 1965; McLeay 1973a, 1973c; Bennett and Gaudio Neville 1975).

Wistar and Hildermann (1960) found that ACTH and adrenocorticoids cause a depression of lymphoid cells and that chronic stress results in leucopenia and loss of immunological responsiveness in mammals. There is also evidence that stress causes increases in circulating adrenal corticosteroid levels in teleostean fishes (Hane et al. 1966; Fagerlund 1967; Wedemeyer 1969; Singley and Chavin 1975a, 1975b; Mazeaud et al. 1977). Non-specific stress in fish results in lymphopenia (Weinreb 1958; Ball and Slicher 1962; Pickford et al. 1971; McLeay 1973a, 1973b, 1973c, 1975; Bennett and Gaudio Neville 1975).

In the wound healing group of our experiment, the leucocrit at detachment was 0.59±0.04% which was significantly lower than the control leucrocrit of 0.97±0.10% (Fig. 2B). After detachment the leucocrit increased significantly to a value of 1.16±0.09% at 1 week and remained at a fluctuating plateau for nearly 3 months. The decreased leucocrit at detachment would tend to be caused by a decreased lymphocyte number. The lymphocytes at detachment had a value of 92.8±2.2% (Fig. 2D). Although a relatively high lymphocyte percentage was present, this only represents a relative percentage of the total white blood cells. In fact, there probably was an absolute drop in both lymphocytes and the neutrophilic series as evidenced by the decreased leucocrit. The relative lymphocyte percentage then dropped significantly to 62.7± 8.1% at 2 days before increasing again to a sustained higher level at 1 week. The lower leucocrit and lymphocyte percentages at 2 days could be attributed to the stress caused by the possible influx of water through the opened wound area. The lymphopenia at 2 days was also accompanied by an increase in the percentage of the neutrophilic series.

During wound development the thrombocyte concentration increased significantly from a control value of 0.8±0.5% to 10.4±2.2% at the 12 h attachment (Fig. 2E). This value then fell somewhat at 2 days attachment. This conforms with the findings of Casillas and Smith (1977) who found that thrombocyte counts increase after stress. The thrombocyte concentration during wound healing remained at a slightly elevated level throughout the 3 month period (Fig. 2F).

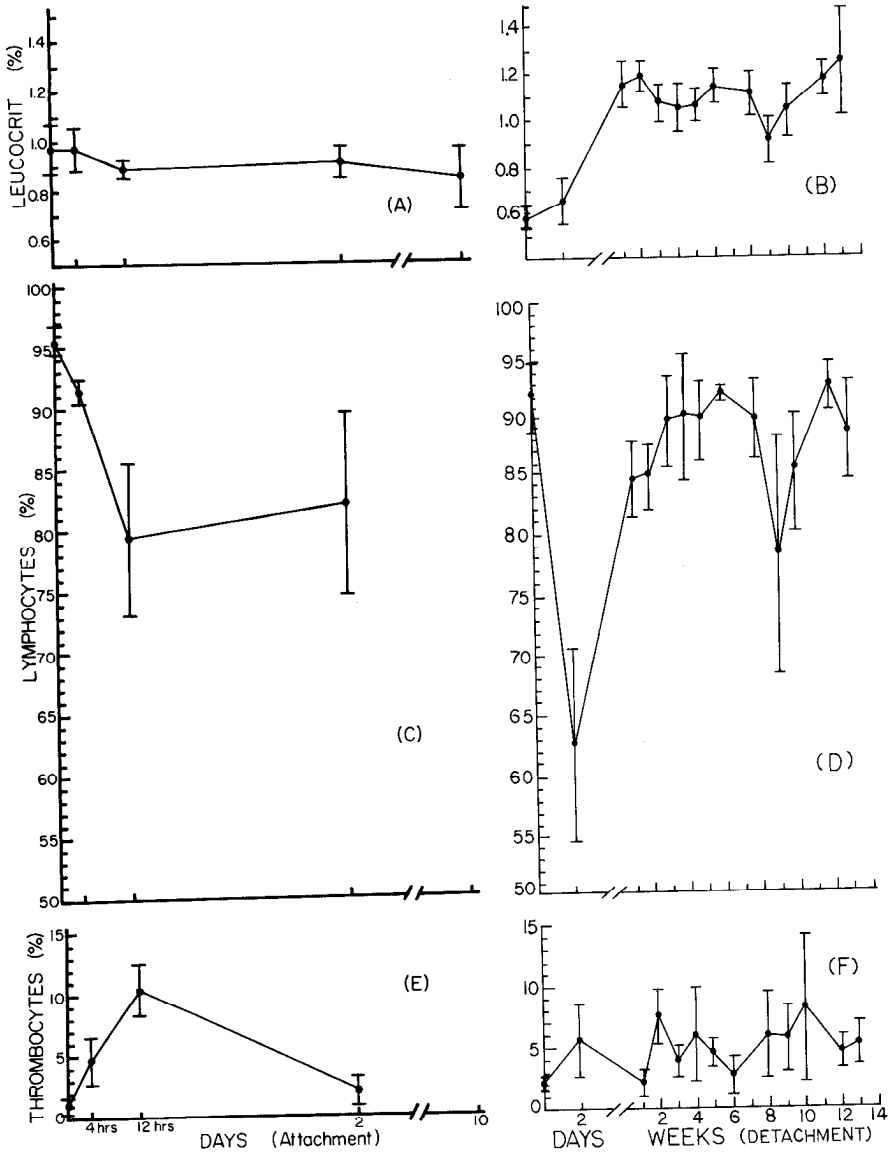

FIG. 2. Leucocrit, lymphocyte, and thrombocyte values during wound development and wound healing in lamprey-attacked fish. The blood smears obtained at 10 day lamprey attachment were of poor quality, therefore a white blood cell differential was not obtained.

(A) Leucocrit (%) in wound development fish.
(B) Leucocrit (%) in wound healing fish.
(C) Lymphocyte percent of white blood cell differential in wound development fish.
(D) Lymphocyte percent of white blood cell differential in wound healing fish.
(E) Thrombocyte percent of white blood cell differential in wound development fish
(F) Thrombocyte percent of white blood cell differential in wound healing fish.

During wound development the neutrophilic series increased from a control value of 3.6± 1.8% to 15.8±6.3% after 2 days of attachment (Fig.3A). This indicates the start of the inflammatory response of the fish toward the wound.

In wound healing, the neutrophilic series at detachment was 4.7± 1.9% and then rose sharply to 31.5±7.2% at 2 days (Fig. 3B). This also happened to be the time when the highest cellular response was noted in histological sections of the wound area. The increased percentage of the neurophilic series in the blood at this time was most likely a protective mechanism as the wound was open to the environment and was an ideal entry area for pathogens. The neutrophilic series remained elevated at week 1 and had a value of 13.4±2.3%.

During wound development the granulocytes and immature and segmented neutrophils increased through the attachment periods; the immature and segmented neutrophils had the largest increases at 2 days attachment (Fig. 3 C, E and G). The metagranulocytes were present in insignificant numbers.

During wound healing the granulocytes and immature and segmented neutrophils were significantly elevated at 2 days; the segmented neutrophils attained the highest percentage of 20.3±4.7% (Fig. 3 D, F, and H). This demonstrates the ability of the fish to respond to adverse conditions by increasing the relative percentage of mature neutrophils which would no doubt be the most capable of handling incoming pathogens. Metagranulocytes were again insignificant during wound healing.

No cells were identified as monocytes, basophils, or eosinophils in our study. Ellis (1977) reported that the literature is extremely confused on the designation of monocytes in fishes and some workers even deny the existence of monocytes in teleost fish. McCarthy et al. (1973) were unable to find any cells in the blood of rainbow trout that resembled the mammalian monocytes. Catton (195 1) had similar difficulty with the blood cells of trout and roach. Blaxhall and Daisley (1973) could not identify monocytes in the blood of brown trout *(Salmo* trutta) though they reported that neutrophils and metamyelocytes could easily be mistaken unless cytochemical staining methods were used. Basophils and eosinophils have not been observed from the blood of rainbow trout (Klontz 1972) and brown trout (Blaxhall and Daisley 1973). Ellis (1977) claimed that the entire literature concerning eosinophils in fish is contradictory in that there have been reports of their presence and absence in many fish species. Ellis (1976) reported the absence of eosinophils and basophils from the circulation in plaice *(Pleuronectes platessa).*

Blood Features of Mortally Wounded Fish

The hemoglobin, hematocrit and leucocrit values of mortally wounded fish at detachment were as low as 0.2 g/dl, 2.0% and 0.30%) respectively (Table 1). The red blood cell precursors were as high as 15% in one fish.

White blood cell differential counts from a fish near death 10 days after lamprey detachment had a neutrophilic series of 0% at detachment, 4.0% at 1 week, and 25.0% at 10 days after detachment. The immature and segmented neutrophils made up the greatest percentage of the neutrophilis series. The lymphocytes were 100% at detachment, 96.0% at 1 week, and 74.0% at 10 days

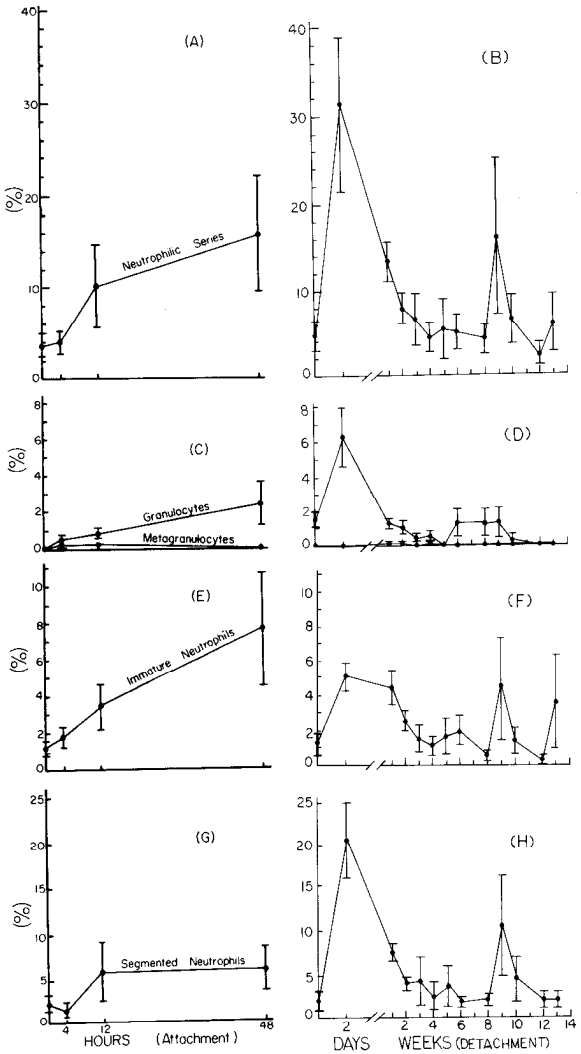

FIG. 3. Neutrophilic series and constituents during wound development and wound healing in lamprey-attacked fish expressed as a percentage of the white blood cell differential. The blood smears obtained at 10 day lamprey attachment were of poor quality therefore a white blood cell differential was not obtained.

(A) Neutrophilic series (%) in wound development fish.
(B) Neutrophilic series (%) in wound healing fish.
(C) Granulocyte (%) and metagranulocyte (%) in wound development fish.
(D) Granulocyte (%) and metagranulocyte (%) in wound healing fish.
(E) Immature neutrophil (%) in wound development fish.
(F) Immature neutrophil (%) in wound healing fish.
(G) Segmented neutrophil (%) in wound development fish.
(H) Segmented neutrophil (%) in wound healing fish.

after detachment. The thrombocytes attained a value of 1.0% 10 days following detachment.

It is obvious that the large blood loss from the fish plays a role in its death. Using the formula log y = 3.3 11 - 1.533 log x which was developed by Farmer et al. (1975), it is possible to calculate x which is an estimate of the amount of blood lost per day, expressed as a percentage of the host fishes blood volume, since one knows y, the time to its death in days. For instance, if the fish near death after 5 days of lamprey attachment (Table 1) was used to find the daily blood volume loss, a value of about 50% would be obtained. This is indicative of the great demands on the hemopoietic organs of the fish, because an amount equivalent to its blood volume would have to be replaced every 2 days. Because the RBC precursors were only 15% of the red blood cells (Table 1), it is obvious that once the blood reserves from the spleen and kidney are depleted the fish would die.

One fish near death 10 days following lamprey detachment had progressively lost weight from 3 11.5 g at detachment to 279.9 g 10 days later. The wound area became infected and fungal infection was seen at 4 days. The hematocrit dropped from 15% at lamprey detachment to 10% at 7 and 10 days following detachment (Table 1). This drop could be attributed to extensive hemorrhaging that occurred in the wound area during this time. The number of RBC precursors had risen during this period to 9.6% at 10 days. The leucocrit was depressed to a level of 0.44% at detachment indicating its immunological response was somewhat depressed; the white blood cell differential showed 0% neutrophilic series and 100% lymphocytes. Ten days following lamprey detachment, the neutrophilic series increased to 25% of the total white blood cells of which most were immature and segmented neurophils, and the lymphocytes dropped to 74%. The leucocrit at this time also rose to 1.12% indicating an absolute rise in the neutrophils with the lymphocytes staying depressed possibly because of the increased stress condition at this time. This increase in neutrophils could be attributed to the infected wound. Similar results were reported by Hines and Spira (1973) who found that mirror carp infected with Ichthyophthirius

TABLE 1. Blood features of mortally wounded fish

Length of lamprey attachment	Hematocrit at detachment (%)	Hemoglobin at detachment (g/dl)	Leucocrit at detachment (%)	RBC Precursors at detachment (%)
5 days	8.0	1.7	0.44	15.0
7 days	2.0	0.2	0.30	not obtained
8 days	2.0	0.4	0.59	not obtained
8 days[a/]	15.0	6.0	0.44	0.4
1 week after detachment	10.0	3.4	0.59	1.5
10 days after detachment	10.0	5.0	1.12	9.6

a/ This fish was not near death until 10 days following detachment and thus blood samples were taken at 1 week and 10 days.

multifiliis had a sharp lymphocyte drop with a concurrent rise in neutrophil percentages.

Lennon (1954) observed that rainbow trout mortally wounded by sea lamprey had erythrocyte counts which were 14.9% of control fish and their blood hemoglobin was reduced by at least 90%. There was a 49.2% drop in white cells in lamprey wounded trout compared with healthy rainbow trout. This is consistent with our research results.

HISTOPATHOLOGY OF THE KIDNEY AND SPLEEN

The most striking changes occurred in the spleen (Fig. 4). Some fish exhibited blood congestion in the red pulp regions of the spleen especially those of the 10 day wound development and 2 day wound healing groups (Fig. 5). The lymphoid (white pulp) region may have released excess numbers of red blood cells into the red pulp region to meet the demand of lamprey feeding. The most striking change in the spleen was the depletion of the white pulp region in the anemic fish with a subsequent loss of the once discernible red and white pulp regions (Fig. 6). This is evidence of a degeneration of hemopoietic tissue due to the excess burden of blood loss. No significant changes in the hemopoietic tissues in the kidneys of any of the fish were evident.

Stress may also play a role in the white pulp depletion in the spleen. Rasquin (195 1) reported the spleen was completely depleted of lymphoid tissue in *Astyanax mexicanus* in response to an injection of ACTH, or when the fish were held under adverse conditions.

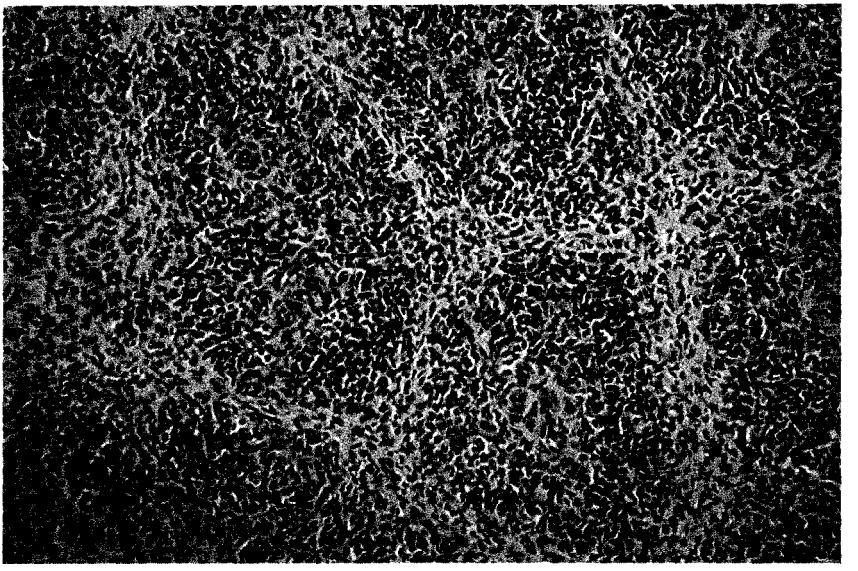

FIG. 4. A normal rainbow trout spleen showing the red and white pulp regions H & E X100.

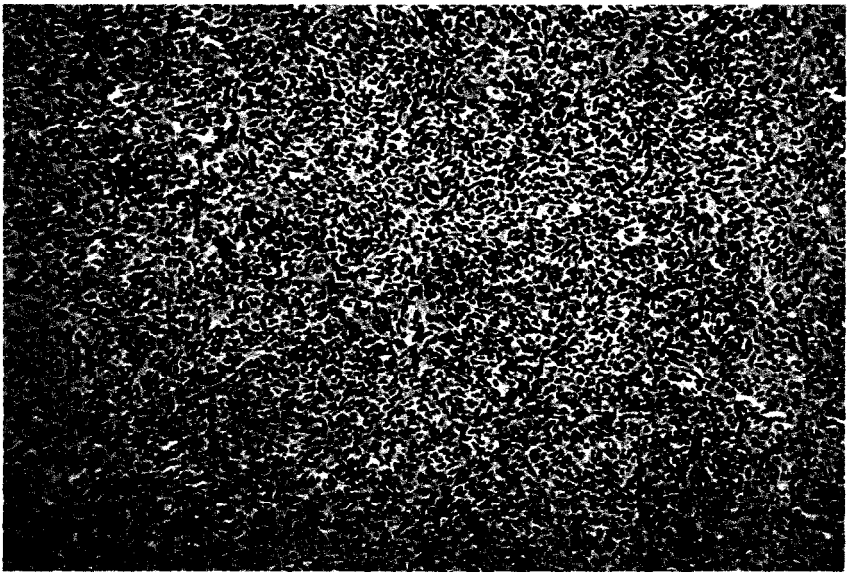

FIG. 5. Red blood cell congestion in the red pulp region of the spleen. H & E. X100.

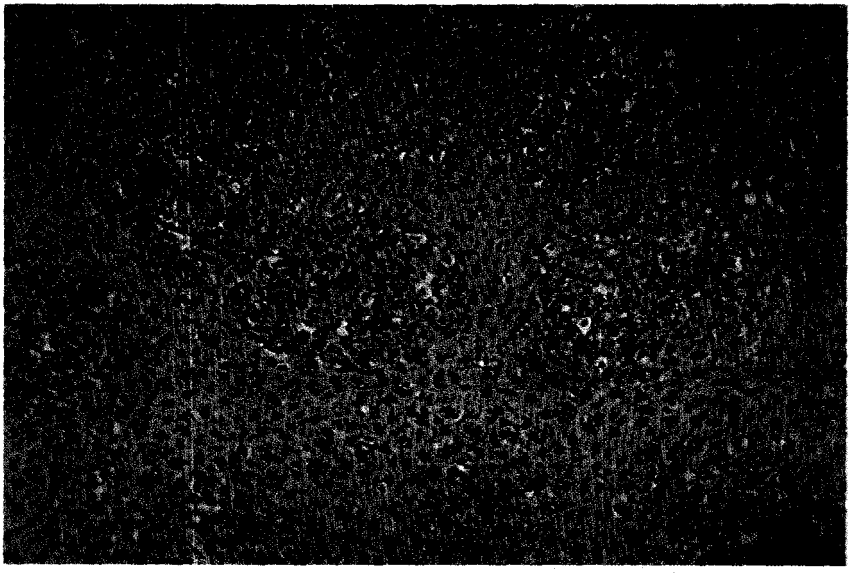

FIG. 6. Spleen from a rainbow trout that was near death after five days of sea lamprey attack-note the diminished white pulp regions. H & E. X100.

APPENDIX

APPENDIX TABLE 1. Hematocrit, hemoglobin, red blood cell precursor, and leucocrit values for wound development and wound healing fish. $\bar{X} \pm S.E.$

Wound stage and time	Hematocrit (%)	Hemoglobin (g/dl)	RBC precursors (%)	Leucocrit (%)
Wound development				
Control	24.1±1.8	6.6±0.7	2.2±0.7	0.97±0.10
4 h	27.0±0.8	7.6±0.3	1.5±0.4	0.97±0.09
12 h	30.7±1.8	8.6±0.4	1.5±0.4	0.89±0.04
2 days	28.412.3	8.5±0.6	1.9±0.6	0.91±0.07
10 days	27.9±1.4	7.8±0.4	1.1±0.4	0.84±0.13
Wound healing				
Detachment	20.7±1.9	6.0i0.5	5.3io.9	0.59±0.04
2 days	14.0±3.5	3.7±0.9	6.0±1.1	0.66±0.09
1 week	19.8±1.3	5.7±0.4	6.9±1.9	1.16±0.09
2 weeks	21.5±1.1	5.8±0.4	13.9±3.1	1.19±0.06
3 weeks	21.6±1.3	5.4±0.3	16.3±3.6	1.07±0.08
4 weeks	22.4±0.9	5.6±0.3	20.9±3.5	1.05±0.10
5 weeks	21.6±0.9	5.7±0.4	10.6±3.0	1.06±0.07
6 weeks	24.7±0.4	5.7±0.2	10.9±2.3	1.14±0.07
8 weeks	25.0±0.7	6.1±0.4	15.4'2.9	1.11±0.09
9 weeks	23.3±1.3	5.5io.3	15.2±1.7	0.91 ±0.10
10 weeks	23.4±1.2	5.4±0.5	10.141.4	1.04±0.11
12 weeks	26.1±0.9	6.5±0.3	15.91-2.3	1.17±0.07
13 weeks	25.7± 1.4	5.7±0.2	10.4±4.2	1.25±0.22

APPENDIX TABLE 2. Components of the white blood cell differential by percent occurrence for wound development and wound healing fish. X'-S.E.

Wound stage and time	Neutrophilic Series	Granulocytes	Metagranulocytes	Immature Neutrophils	Segmented Neutrophils	Lymphocytes	Thrombocytes
Wound development							
Control	3.6±1.2	0	0	1.2±0.4	2.4±0.9	95.6±1.3	0.8±0.5
4 h	4.0' 1.4	0.4±0.4	0.2±0.2	1.8±0.6	1.6±0.9	91.4±1.0	4.6±2.0
12 h	10.2±4.5	0.8±0.4	0.2±0.2	3.4t1.2	5.8±3.3	79.416.2	10.4±2.2
2 clays	15.8±6.3	2.4± 1.3	0	7.6±3.1	5.8'12.6	82.217.5	2.0±1.2
Wound healing							
Detachment	4.7±1.9	1.5±0.5	0	1.2±0.7	2.0± 1.1	92.8±2.2	2.21t0.7
2 days	31.5±7.2	6.3± 1.7	0	5.0t0.9	20.3±4.7	62.7±8.1	5.7±2.8
I week	13.4±2.3	1.3t0.3	0.1±0.1	4.4±1.o	7.7±1.5	84.5±3.2	2.1±1.1
2 weeks	7.9± 1.4	1.0±0.4	0.1±0.1	2.6'0.5	4.1±0.8	84.7±2.8	7.5±2.2
3 weeks	6.5±3.3	0.4t0.2	0	1.6±0.7	4.5±2.6	89.6±4.1	3.9±1.3
4 weeks	4.3±1.9	0.5±0.3	0.1±0.1	1.1 i-o.5	2.5±1.4	89.9±5.6	5.9±3.9
5 weeks	5.5±3.2	0	0	1.7±0.9	3.8±2.5	89.7±3.5	4.7±1.2
6 weeks	5.3±1.9	1.3±0.8	0	2.0±0.8	2.0±0.8	92.0±0.4	2.7±1.6
8 weeks	4.3±1.6	1.3±0.8	0	0.5±0.3	2.5±0.9	89.8±3.5	6.0±3.5
9 weeks	16.0±9.1	1.3±0.9	0	4.5±2.9	10.3±5.6	78.3± 10.0	5.7±2.7
10 weeks	6.5±2.8	0.3±0.3	0	1.5±0.7	4.8±2.1	85.3±5.1	8.3±5.7
12 weeks	2.5±1.2	0	0	0.3t0.3	2.3±0.9	92.8±2.1	4.7±1.3
13 weeks	6.0±3.5	0	0	3.7±2.7	2.3±0.9	88.7±4.5	5.3±1.7

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