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THE EFFECT OF EXPERIMENTALLY INDUCED INFLAMMATION ON THE BLOOD PATTERN AND HAEMOPOIETIC ORGANS OF THE TELEOST, CLARIAS LAZERA.

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ABSTRACT

The peripheral blood picture and the histological appearnce of haemopoietic organs, were examined after intramuscular injection of turpentine oil in the catfish, **Clarias lazera**. Ranges and means of all types of blood cells were determined. Leucocytosis, mainly due to neutrophilia and to a lesser extent to increase in other granulocytes, occurred. Both lymphopaenia and thrombocytopaenia took place. Consideration is given to the probable role of turpentine oil in the activation of haemopoietic sites of the fish.

INTRODUCTION

Leucocytes of lower vertebrates have been studied by experimentally promoting inflammatory reactions using irritating chemicals, such as turpentine or thorotrast. Various workers employed such chemicals in non-mamalian vertebrates to detect the specific cell type that responds to the used agent. It is known that in mammals, turpentine oil injections provoke an enormous increase in the relative and absolute numbers of neutrophilic granulocytes i.e. (neutrophilia). "Sterile abscess" was also recorded to occur due to muscular injection with turpentine (Yokoyama, 1960).

Earlier accounts of haematological reactions in poikilothermic animals have involved such experimental conditions as injected foreign matter, (Easton, 1952 and Weinreb and Weinreb, 1969), acute inflammation (Weinreb, 1958 and Yokoyama, 1960), viral infection, (Watson et al., 1956), bacterial infection, (Katz, 1950) and x-irradiation (Watson, 1961 and Schechmeister et al., 1962). The response revealed from these studies, particularly notable in circulating blood cells, is probably universal among animal cells. However, the specific responding cell types in teleosts remain to be defined more clearly and require further elaboration.

The purpose of this study is to determine the specific responses of **Clarias lazera** to experimentally induced inflammation using turpentine oil, as manifested by the circualting blood cells and haemopoietic tissues.

MATERIAL AND METHODS

to February 1986, from Bab El-Abid zone which is an unpolluted area of Lake Mariut, near to Alexandria. They were maintained in continually aerated aquaria at room temperature (17°C). The fish was kept for 48 hours for acclimatization. Blood samples were made by severing the caudal peduncle, for haematological studies and various haemopoietic organs i.e. liver, kidney, spleen, heart and intestine were fixed in 10% neutral formalin, and stained with eosin-haematoxylin (Pearse, 1972).

The most satisfactory diluting solution for erythrocyte, leucocyte and thrombocyte counts was found to be Yokoyama mixture (Yokoyama, 1960). The classical May-Grunwald blood stain, Giemsa-Romanowsky, Leishman, Wright's stain and the Panoptic method were used as differential stains, but Giemsa and Panoptic methods gave valuable results in staining both blood smears and tissue imprints.

Fish were divided into four groups, each of 12 healthy fish. The first group was given a dose 0.1 ml of commercial turpentine oil per 100 gm body body weight. This was made by injecting each fish intramuscularly just posterior to the pectoral fin. The second group was given a dose of 0.2 ml of turpentine oil per 100 gm weight. The third and fourth groups were used as controls and were injected with 0.1 ml and 0.2 ml piscine saline per 100 gm body weight.

After 24 hours of injection (Yokoyama, 1960), blood samples were collected from the four groups. Smears and imprints were air dried and fixed in absolute ethyl alcohol. Haematological methods proceeded as mentioned above, haemopoietic organs were dissected, removed and fixed in 10% neutral formalin, dehydrated and embedded in wax, sections were cut 4 µm thick and stained.

Statistical methods used for the analysis of the present results were according to Arkin and Colton (1963), using the t-test analysis. Significant differences were established at 0.05 level.

RESULTS

Table 1 shows that there is a slight decrease in erythrocyte count of the group of animals injected with 0.1 ml of the oil. However, a slight increase occurred in the second group of animals injected with 0.2 ml of the oil. Examination of blood smears taken from the second group revealed that erythrocytes exhibit a case of poikilocytosis i.e. cells of different shapes (Fig. 1) . There were also a large number of degenerating and deformed red blood cells and some normoblasts.

Table 1 shows that total leucocyte count increases considerably in group 1. Table 2 indicates that the increase in the total leucocyte count is accompanied by an increase in the total granulocytes especially neutrophils, the most abundant granulocytic type of the peripheral blood. Total leucocyte

count elevated greatly in the second group. The leucocyte differential patterns of injected fish (Table 2) shows that the marked increase is due to an elevation of the granulocytic type of cells. However, eosinophils and Basophils showed a considerable increase above the control.

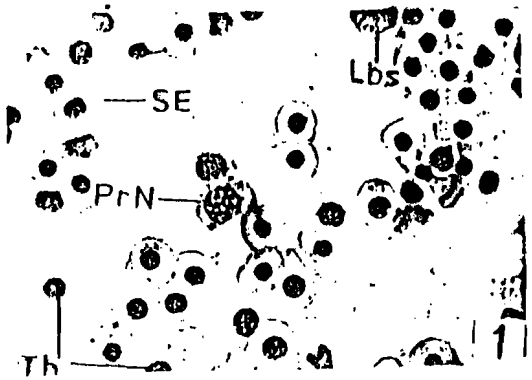

Fig. (1)
Blood smear from fish injected with 0.1 ml turpentine oil / 100 gm of body weight, Panoptic method, shows poikilocytosis, many degenerating erythrocytes or senile erythrocytes (SE), erythrocytes developmental stages: pronormoblast (PrN), degenerating thrombocytes (Th) and lymphoblast (Lbs). X 1250.

TABLE 1
Erythrocyte, total leucocyte and thrombocyte counts of Clarias lazera after 24 hours from injection with Turpentine oil.

Item	Control I		Group I		Control II		Group II	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Erythrocyte count ($\times 10^6$/ mm)	1.500 - 3.345	2.752	2.180 - 2.845	2.374 NS	1.670 - 3.450	2.613	2.[illegible] - 3.252	2.872 NS
Total leucocyte count: ($\times 10^3/mm^3$)	40.000 - 70.000	50.[illegible]	56.000 - 86.000	69.333 P< 0.05	42.125 - 74.220	53.204	65.000 - 98.[illegible]	[illegible] P<0.01
Thrombocyte count ($\times 10^3/mm^3$)	19.150 - 32.307	24.093	14.649 - 25.275	18.819 P<0.05	17.236 - 34.663	22.903	9.[illegible] - 19.[illegible]	[illegible] P<0.001

Control I : Fish injected with 0.1 ml saline; Control II: fish injected with 0.2 saline.
Group I : Fish injected with 0.1 ml Turpentine oil; Group II: Fish injected with 0.2 ml Turpentine oil.
The number of fish employed in each experiment is 12.
P< 0.05: significant; P<0.001-0.01: hihgly significant, NS: insignificant.

TABLE 2

Different leucocyte counts of Clarias lazera after 24 hours from injected with Turpentine oil.

Cell Type (%)	Control I		Group I		Control II		Group II	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Neutrophils	27.0 - 54.4	36.0	27.2 - 56.4 P<0.05	42.3	29.7 - 57.3	38.5	31.4 - 52.6	44.9 P<0.05
Eosinophils	2.3 - 7.6	4.6	5.0 - 8.8 P<0.05	6.9	1.9 - 8.7	5.2	8.8 - 15.0	10.7 P<0.05
Basophils	1.3 - 2.7	1.8	1.3 - 2.5 NS	2.0	0.9 - 3.0	1.6	3.9 - 6.7	5.2 P<0.05
Total Granulocytes	30.6 - 64.7	42.4	33.5 - 67.7 P<0.01	51.2	32.5 - 69.0	43.7	44.1 - 74.3	60.8 P<0.001
Small Lymphocytes	29.1 - 50.4	43.3	12.3 - 29.4 P<0.001	21.3	27.4 - 51.0	44.0	10.5 - 21.7	14.3 P<0.001
Large lymphocytes	6.2 - 19.0	14.3	20.0 - 37.1 P<0.001	27.5	5.8 - 17.6	12.4	15.2 - 34.2	24.9 P<0.001
Total Agranulocytes	35.3 - 69.4	57.6	32.3 - 66.5 P<0.01	48.8	33.2 - 68.6	56.4	25.7 - 55.9	39.2 P<0.01

Control I and Control II: Fish injected with 0.1 and 0.2 ml saline respectively

Group I and Group II: Fish injected with 0.1 ml and 0.2 ml Turpentine oil respectively.

The number of fish employed in each experiment is 12.

P<0.05: Significant; P<0.001-0.01: Highly significant; NS: insignificant.

Examination of blood smears taken from samples of both groups of injected fish showed an abundant appearance of developmental stages as well as mature granulocytes, a case which is not met with in normal blood smears (Figs. 2 and 3). Agranulocytes, on the other hand, seem to behave in a reciprocal manner. Nevertheless, the number of large lymphocytes, increased considerably to reach about twice their original level. It is noteworthy that, this increase was accompained by the presence of certain type of leucocytic cells similar to the plasma cell of other vertebrates (Figs. 2 and 4). Plasma cells are not known to exist in normal blood smears of vertebrate animals. Furthermore, in the second group of injected fish, although the total number of agranulocytes decreased yet large lymphocytes still represent the highest ratio of agranulocytes.

Table 1 shows that thrombocyte count of first group of injected fish decreased considerably, a clear case of thrombocytopaenia. This case of thrombocytopaenia becomes an acute state in the second group of injected fish, since thrombocyte level reachs less than half its original value. Blood smear examinations showed noticeable scarcity of all types of thrombocytes, some of which were degenerated, (Fig. 1).

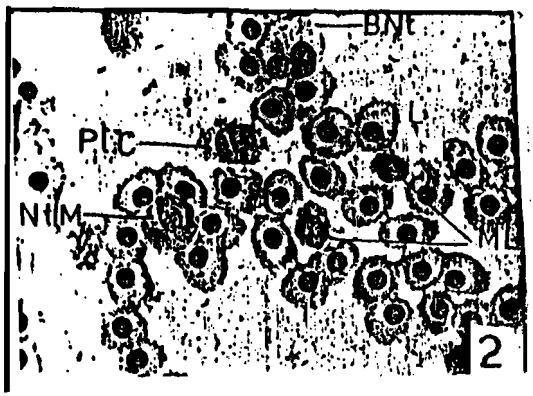

Fig. (2)
Blood smear from a fish injected with 0.1 ml turpentine oil / 100 gm of body weight, Panoptic method, shows a mature bilobed neutrophil (BNt), neutrophilic myelocyte (Nt M), plasma cell (Plc) and two medium-sized lymphocytes (ML). Notice also poikilocytosis of eruthrocytes. X 1250.

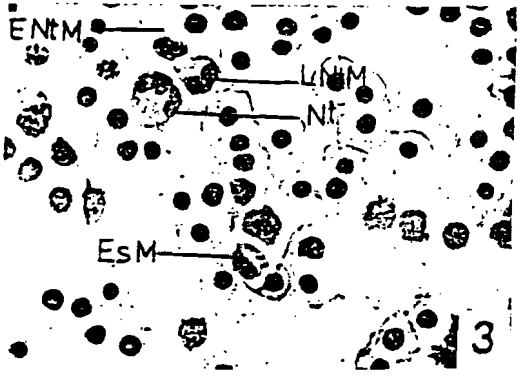

Fig. (3)
Blood smear from a fish injected with 0.2 ml of turpentine oil/100 gm of body weight, Panoptic method, shows various developmental stages of granulocytes: an early (ENt M) and two late neutrophilic myelocytes (L Nt M), eosinophilic myelocyte (Es M) and a mature neutrophil (Nt). X 1250.

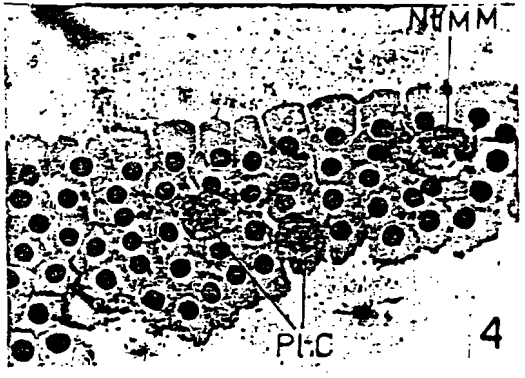

Fig. (4)
Blood smear from a fish injected with 0.1 ml turpentine oil/100 gm of body weight, Panoptic method, shows two plasma cells (Plc) and neutrophilic metamyelocyte (Nt MM). Notice poikilocytosis caused by turpentine oil. X 1250.

After injection with 0.1 ml of turpentine oil, the haemopoietic tissue (HT) of the kidney showed a noticeable activity (Fig. 5) i.e. large numbers of developing and mature blood cells were observed in the tissue. After 0.2 ml injection (Fig. 6), more leucopoietic activity was observed.

Fig. (5)
Formalin-eosin haematoxylin. T.S. of head kidney from a fish injected with 0.1 ml of turpentine oil / 100 gm of body weight. Notice intertubular space containing haemopoietic tissue (HT). In the latter, blood cells are seen, they are also on the uriniferous tubules (UT). Venous sinusoid (VS) appears in continuity with the stroma (S). Notice early myelocytes (EIM). X 500.

Fig. (6)
Formalin-eosin haematoxylin. T.S. of head kidney from a fish injected with 0.2 ml of turpentine oil/100 gm of body weight. Notice increasing activity, more blood cells than those seen after 0.1 ml of turpentine oil injection (compare with fig. 5). Venous sinusoids (VS), Malpighian bodies (MB), uriniferous tubules (HT) and stroma (S). X 500.

The spleen showed apparent activity of haemopoiesis after the injection of 0.1 ml of turpentine oil (Fig. 7). On increasing the dose of turpentine oil to 0.2 ml, further activity was noticed, (Fig. 8).

Fig. (7)
Formalin-eosin haematoxylin. T.S. of spleen from a fish injected with 0.1 ml of turpentine oil/100 gm of body weight, showing developing erythrocytes (DE), thrombocytes (Th), and lymphocytes (L). X 1250.

Fig. (8)
Formalin-eosin haematoxylin. T.S. of spleen from a fish injected with 0.2 ml of turpentine oil/100 gm of body weight. Note neutrophil (Nt), reticular cell (RC), polychromatophil (PN), blood forming tissues (Notice arrows) and haemocytoblast (Hc b). X 500.

The liver cells showed shrinkage after injection with 0.1 or 0.2 ml turpentine oil (Figs. 9 and 10). Increase of haemopoietic activity of the liver was obvious in case of the higher dose of turpentine (Fig. 10).

Fig. (9)
Formalin-eosin haematoxylin. Section of liver from a fish injected with 0.1 ml of turpentine oil/100 gm of body weight. Notice neutrophil (Nt), Thrombocyte (Th), bile canaliculi (bc) and reticular cells (RC). X 1250.

Fig. (10)
Formalin-eosin haematoxylin. Section of liver from a fish injected with 0.2 ml of turpentine oil/100 gm of body weight. Increased number of red and white blood cells as compared with fig. 9. X 500.

The ileum, also showed increased haemopoietic activity (Fig. 11). Large numbers of red blood cells, eosinophils were observed beside scattered lymphocytes in the lamina propria of the ileum.

Fig. (11)
Formalin-eosin haematoxylin. T.S. of ileum from a fish injected with 0.1 ml of turpentine oil/100 gm of body weight. See eosinophils (ES) and lymphocytes (L). X 1250.

DISCUSSION

The blood response of **Clarias lazera** elucidated by turpentine oil injection is more or less similar to that reported in other animals; including the dog fish (Reznikoff and Reznikoff, 1934); the turtle (Ryerson, 1943); the chiken (Bradley, 1937); the perch (Yokoyama, 1960) and the goldfish (Weinreb and Weinreb, 1969). However, certain points of interest were revealed from the present work.

In **Clarias lazera** the erythrocyte counts of the first group of injected animals showed a slight decrease, whereas, the second group showed an slight increase. This result is in agreement with those reported by Weinreb (1958) on the rainbow trout and Yokoyama (1960) on the perch. Weinreb and Weinreb (1969) stated that the total peripheral blood picture of **Carassius auratus** appeared to be normalized after 12 hours of a single intraperitoneal injection of thorotrast. Dick and Dixon (1985) recorded insignificant reduction in erythrocyte concentration in rainbow trout after exposure to acute dose of copper.

Poikilocytosis was found by most of erythrocytes in the second group of injected **Clarias lazera.** It seems that turpentine oil affects, in some way, the process of erythropoiesis in this teleost, a phenomenon that has not been recorded in the available literature. However, during the present experiments, large numbers of degenerating and deformed red blood cells along with some normoblasts were frequently noticed on blood smears of turpentine injected **Clarias lazera.**

Investigation of leucocyte differential counts of injected fish showed a marked increase in the granulocytic types of cells; especially neutrophils. Also, both eosinophils and basophils showed a considerable elevation with increase of the dose. This observation is in harmony with that of Weinreb (1958) who reported significant increase in heterophils (neutrophils) of the injected rainbow trout. Menkin (1940) reported a case of leucocytosis in dogs, intrapleurally injected with turpentine, that continued for 24 hours. However, he found that leucocytosis was not due to the direct action of turpentine, but due to the presence of a leucocytosis-promoting factor in the inflammatory exudate. However, Yokoyama (1960) reported a similar case of neutrophilia in the perch injected with turpentine oil, but she showed that this neutrophilia was not too high to cause leucocytosis, since the total leucocyte counts were not altered. The same author, also, stated that eosinophils which occur in very small number in the perch, showed no change with turpentine oil injections.

During these experiments a case of lymphopenia is observed. Lymphopenia was reported for other stressed fish like the perch (Yokoyama, 1960), the gold fish (Weinreb and Weinreb, 1969) and the rainbow trout (Dick and Dixon, 1985).

It is of interest to notice that elevation of large lymphocytes in injected fish was accompanied by the presence, in the peripheral blood, of plasma cells; which is not a normal case in the vertebrates. The presence of plasma cells in the blood of experimental animals indicates the transformation of lymphocytes to immunoglobulin manufacturing cells due to the presence of an anthigen like material in the blood. Evans (1968) repoted that some toxic substances can induce chemical alterations or compination of the hapten type. Therefore, it is propable that turpentine oil injection, might have induced this type of immune reaction which would account for the presence of plasma cells in the peripheral blood of the experimental fish. In accordance to this, Chiler et al. (1969) reported the response of antibody of immunocomponent cells in the spleen and anterior kidney of the rainbow trout, **Salmo gairdneri.** Ellis (1977) discussed the effect of antigen and inflammation on the developmental stages of lymphocytes in fish.

In the above experiments, thrombocytopaenia was noticed in the blood of injected **Clarias lazera,** as indicated by the acute drop in thrombocyte counts in the fish. This observation is in disagreement with that reported on rainbow trout by weinreb (1958) who showed that no significant change

occurred in thrombocyte numbers in the first 24 hrs. After injection with turpentine. Dick and Dixon (1985), on the other hand, recorded an increase in thrombocyte count of rainbow trout exposed to copper poisoning.

In the present work, changes in the peripheral blood picture may be attributed to change in production and output of cells from haemopoietic sites and changes in cell populations in the circulation. All haemopoietic organs investigated showed hyperactivity to produce more blood cells of different types. Certain haemopoietic organs which are normally incative during the time of experiment, become active after the injection of turpentine oil. The large numbers of both mature and immature blood cells noticed in blood smears or between the haemopoietic tissues are a further proof. In agreement with this result is the work of Zinkle (1981) who reported the presence of immature forms of various cell types in the blood of animals suffering from inflammatory diseases, Weinreb and Weinreb (1969) reported similar observations but attributed induced endocytosis transformation and differentiation of various cell population in the circulattion of the thorotrast-injected fish.

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