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BLOOD INDICES AND HEMOPOIESIS IN THE INDIVIDUAL DEVELOPMENT OF FISH

I. N. Ostroumova

Changes in blood and hemopoiesis in fish in relation to age and certain environmental factors are examined in this paper.

Considerable attention is given to blood as one of the indices of the physiological state of fish.

Data are presented on the determination of blood indices in various stages of illness of rainbow trout which have been reared on synthetic food.

The paper is intended for physiologists and pisciculturists.

BLOOD INDICES AND HEMOPOIESIS IN THE INDIVIDUAL DEVELOPMENT OF FISH

/3

I.N. Ostroumova

INTRODUCTION

The study of the blood and the hematopoietic organs of lower vertebrates, which include fish, is of interest in understanding the development of individual physiological systems in the course of evolution in various representative of the animal world.

Man's practical activity necessitates an understanding of the blood and blood-formation in fish.

In piscicultural practice the fish which are being reared are usually assessed in terms of size and weight; however, in recent years the question of the necessity of assessing fish in terms of their physiological indices has been raised. Thus, the need for information on blood has increased since the blood may serve as one of the indices of the physiological state of an organism.

Familiarization with the published literature shows that in spite of the rather long list of articles on the blood of fish many questions still remain unexplained. Contradictory data on the blood-forming organs in fish are noticeable; it becomes evident that the information on the blood of fish in the early period of ontogenesis is scant and fragmentary and that little has been done in the study of the changes in the blood and, particularly, in blood-formation in fish

which are exposed to various environmental factors.

As a rule, the main emphasis in the study of the blood of fish is put on the quantity of hemoglobin, erythrocytes and leucocytes and on the leucocytic formula, whereas the morphology of red blood remains obscure. Meanwhile, the composition of red blood cells is of considerable interest with respect to the peculiarities of blood-formation in fish.

Because of intravascular blood-formation, we can determine by /4
means of peripheral blood smears the rate of the performance of blood-forming organs and the response of the blood-forming system upon exposure to a particular aspect of the environment.

Quantitative determinations of hemoglobin, erythrocytes and leucocytes in immature fish, using generally accepted methods, are only possible several months after they have emerged from the eggs. On the other hand, observations on the change in the qualitative composition of cells in many fish may be conducted by means of blood smears as early as the embryonic and larval stage of development.

The present paper defines more accurately the place of basic blood-formation in the fish under consideration, presents data on the changes in the blood of fish according to age in the early stages of development and establishes the reaction of blood and blood-forming organs to certain factors in the environment. Particular attention is given to the composition and morphology of red blood cells and the advisability of determining the qualitative composition of red blood cells during a physiological assessment of fish along side of quantitative determinations of hemoglobin and erythrocytes is demonstrated.

REVIEW OF LITERATURE

Blood-formation in fish has many features in common with blood-formation in higher vertebrates, however it also has a number of features associated with the overall low organization in fish and with their living conditions. As in mammals, the formation of the first blood cells in fish in the embryonic period occurs long before the appearance of fully developed blood-forming organs. However, whereas in higher vertebrates the formation of blood vessels and the formation of plasma and primary blood cells occur simultaneously (Freifel'd, 1947) and always in the early stage of development, in some fish, whose eggs are in water rich in oxygen, the formation of the erythrocytes considerably lags behind the formation of the blood vessels and blood plasma (Marcus, 1905; John, 1932; Pestova, 1955; Privol'nev, 1947).

John (1932) showed that the initial blood cells in herring appear in the lumen of the vessels only 30 days after emergence from the egg, 26-27 days after resorption of the yolk sac. Prior to this, a colourless fluid circulates in the vessel of the embryos and larvae. In fish whose eggs develop in conditions of less oxygen, the beginning of the formation of the vessels, blood plasma and blood cells occurs simultaneously and in the early stages of development. A similar phenomenon was noted by Marcus (1905) and Pestova (1955) in salmon which buries its eggs in the soil.

At the beginning of the embryonic period of all vertebrates, including mammals, the formation of erythrocytes occurs intravascularly, i.e. inside

the vessels. In fish, amphibians, reptiles and birds intravascular blood-formation is retained throughout life. This explains the large number of immature forms of erythropoiesis in their peripheral blood during the intensive functioning of the blood-forming organs. /5

In mammals, still in the embryonic period, when the liver becomes the place where erythrocytes are formed, blood-formation occurs outside of the vessels, i.e. it occurs extravascularly. With the appearance of bone marrow the myeloid tissue of the latter is the only place where the erythrocytes, granulocytes and trombocytes are formed in mammals. The lymphocytes are produced by the lymphoid tissue of the spleen and lymph glands. In fish the bone marrow and lymph glands are absent and the function of blood-formation in post-embryonic life is fulfilled by other organs.

In all lower vertebrates, including fish, strict localization of blood cell formation is lacking. The formation of lymphocytes and leucocytes occurs in the most diverse places - mainly in the connective tissue of the kidney, but also in the intestine, gonads, oesophagus (in Selachii), spleen and pancreas (Drzewina, 1905). The places where erythropoiesis occurs are less dispersed; however, as is pointed out by Zavarzin (1945), during increased activity in blood-formation in lower vertebrates the formation of erythrocytes may spread throughout the entire vascular lumen.

There is no single opinion concerning the focus of erythrocyte formation in bony fish. Some authors regard the spleen to be the main organ of erythropoiesis (Khlopin, 1946; Zavarzin, 1945; Pestova, 1954).

Others are of the opinion that in the majority of fish the bulk of erythrocytes are formed in the connective tissue of the kidneys (Jordan and Speidel, 1924; Rumyantsev, 1939; Duthie, 1939; Catton, 1951; Topf, 1953, 1955). The distribution of active zones of blood-formation in the kidneys differs in different species of fish. In chub the blood cells are formed mainly in the cephalic region (Rumyantsev), in trout and roach - throughout the length of the kidney, in Ctenolabrus rupestris - in the cephalic and caudal regions and in Cottus bubalis, similar to chub - only in the cephalic region (Catton, 1951).

Having weighed the intercanalicular tissue of the kidney and the tissue of the renal canalicules, Topf (1953) detected a considerable difference in the ratio of their weights in carp and frogs. In carp the weight of the intercanalicular connective tissue of the kidney, where intensive blood-formation takes place, was 70.2% of the weight of the tissue of the renal canalicules. In frogs, however, active blood-formation in the kidney was lacking and the weight of the intercanalicular tissue was only 12% as compared to that of the tissue of the renal canalicules.

Due to intravascular blood formation in the blood of lower vertebrates immature forms of erythrocytes as well as mature forms are often present. In the spring and summer, the number of immature erythrocytes in the blood of cold-blooded animals increases considerably (Werzberg, 1911). While /6 determining the rate of consumption of oxygen by blood cells, Korzhuev (1949) detected that this consumption increases in the spring and summer for amphibians and reptiles, thereby explaining the presence in the blood

of immature forms of erythrocytes which have hypermetabolism.

A rise in erythropoiesis in the spring in Atlantic salmon is noted by Nysenbaym (1951), who emphasized that intensive erythropoiesis is connected not only with seasonal changes but also with features in the biology of fish reproduction.

Immature forms of erythrocytes are present in the blood and in blood-forming organs at different stages of maturity. Topf (1953) singles out the following stages for erythropoiesis in carp: the parent cell, lymphoid haemoblast, erythroblasts from (a) the kidney and (b) the blood), the intermediate degree between the erythroblast and proerythrocyte, proerythrocyte and mature erythrocyte.

Using accepted medical terminology and taking into consideration the characteristic features of fish, Nysenbaym (1951) designates the following stages in erythropoiesis for Atlantic salmon: erythroblast, normoblast, basophil erythrocyte, polychromatic erythrocyte and mature orthochromatic erythrocyte. These designations are also used in the present paper.

The difficulty in studying the white blood of fish arises from its extremely diverse composition. In structure the white blood cells in fish differ substantially from the blood cells in mammals, however the basic nomenclature for them is generally taken from the classification adopted in medicine. However, detailed descriptions of cells and micro-photography are often lacking. Thus, one and the same cells may be named differently by different authors and, on the other hand, different cells may be grouped together.

A single classification for white blood does not exist.

Werzberg (1911) uses the classification of Pappengein* in his study of the white blood of fish: lymphocyte, mononuclear leucocyte (lympholeucocyte) with a round or pod-like nucleus, agranular leucocyte with a two-segment nucleus, neutrophil and eosinophil.

In the composition of white blood of the sterlet, Zwetkow (1925) finds polymorphonuclear leucocytes, eosinophil cells, myelocytes and trombocytes. Rubashev (1936) adheres to the following classification: small and large lymphocytes, monocytes, cells of the lymphomyeloblast type, neutrophilic granulocytes and eosinophilic granulocytes.

In carp, Topf (1953), Wunder and Dombrowski (1953) isolate lymphocytes, granulocytes (without specifying any particular form - I.O.*) and so called tromboblasts about which more detail will be given below.

In recent years, in the publications of the USSR, the classification /7
of the white blood of fish developed at the laboratory of the Moscow Technical Institute of the Fish Industry and Fisheries under the direction of Professor N.V. Puchkov has usually been used: lymphocyte, monocyte, polymorphonuclear leucocyte, neutrophil and eosinophil (Lyaiman and Shpolyanskaya, 1951).

Despite its diversity in cellular composition, the white blood of fish also have general features which are characteristic of all species of fish. Firstly, in contrast to higher vertebrates, the white blood of fish is lymphoid in nature, i.e. lymphocytes dominate among the white formed elements in the norm (Rawitz, 1900; Meinertz, 1902). Only a small percentage is accounted for by the remaining agranular and granular forms of white blood and it is mainly because of these forms that diversity is

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*Transliteration. Translator.

observed in the leucocytic composition of fish.

Only agranular forms exist in the blood of some fish, for example, in gobies (Zavarzin, 1945) and in perch (Meinertz, 1902). In salmon, the granularity of the leucocytes is very indistinct and insignificant and is detected frequently only during a peroxidase reaction (Rubashev, 1936). While staining blood smears according to the Pappengein method, Nysenbaym (1951) detected only agranular forms - lymphocytes, monocytes, polymorphonuclear and segmented nuclear leucocytes. In carp, authors usually note, in addition to agranular forms, leucocytes with well-defined granularity, assigning them to neutrophils or eosinophils, or they find that both neutrophil and eosinophil are present (Drzewina, 1911, 1912; Jordan and Speidel, 1924; Rumyantsev, 1939; Golodets, 1939; Catton, 1951).

Very little is known about the function of leucocytes in fish. Puchkov (1954) ascertained, that in contrast to mammals, agranular leucocytes - polymorphonuclear leucocytes and monocytes exist in fish with true Mechnikov phagocytes. Data exist on the participation of leucocytes in digestion and ovulation in fish (Zwetkow, 1956).

Besides red and white blood cells, in the blood plasma of both higher and lower vertebrates there are formed elements - trombocytes which take part in blood coagulation. In mammals the trombocytes are a blood platelets without nuclei. In lower vertebrates, including fish, it is believed that the function of the trombocytes is fulfilled by other forms which comprise cells containing an oblong nucleus and a narrow layer of cytoplasm

- spindle-shaped cells.

However, in recent years, Dombrowski (1953) and Topf (1953, 1955) established the presence in the blood of carp of a large quantity of the minutest formations (d 0.7 μ) which, in the opinion of the authors are trombocytes and take part in blood coagulation. The authors found /8 cells in the blood of carp which they named tromboblasts which resemble, in function, the megakaryocytes in mammals. According to Topf and Dombrowski the minutest trombocytes are formed in the tromboblasts and they fill the cytoplasm of the tromboblasts. Thus, in external appearance the tromboblasts resemble granular leucocytes. Unfortunately neither Dombrowski nor Topf give microphotographs of the tromboblast and trombocyte, restricting themselves to diagrams alone.

The blood is the most important physiological system which participates directly in the metabolic processes of an organism. Changes in metabolism due to inner and external conditions are reflected on the composition of the blood.

We know that blood does not remain invariable during the growth and development of fish. Pavolv and Krolik (1936) found two maximums in hemoglobin and proerythrocyte content in carp which are functions of age and development. The first maximum is observed in fingerlings and the second at age 2 + (in the third year of life - translator) when the fish are sexually mature. The authors found that each maximum coincides with the periods at which the rate of metabolism in the organism of the fish is highest. Antipova (1954) noted a general tendency towards an increase in the quantity of hemoglobin and erythrocytes in carp right up to age 2+. These indices became more or less stable in carp older than three years.

Antipova observed that the composition of white blood changes with age. Thus, in carp fingerlings the white blood is only represented by agranular forms. Neutrophils appeared for the first time at age 1 and the oesinophils at age 1+. Golodets (1954) noted that the quantity of monocytes and polymorphonuclear leucocytes increase with age in immature sturgeon, bream and zander.

Many authors observed a reduction in the quantity of lymphocytes and an increase in the quantity of monocytes in fish in the spawning period (Drabkina, 1951; Drabkina and Telkova, 1949; Puchkov and Drabkina, 1951; Antipova, 1954; etc.).

During the study of changes in the blood of fish most consideration is given to the late period of ontogenesis, with the changes in the blood of embryos and early postembryos being ignored. This is explained in part by the lack of a method for determining the indices of the blood in immature fish which possess an insignificant quantity of blood.

On the basis of a study of the blood smears of the embryos and larvae of Atlantic salmon and salmon in general, Nusenbaym (1951, 1954) was /9 able to trace the morphological change in red blood in the early ontogenesis of the fish. It was found that in the early stages of embryonic development red blood is mainly represented by primary immature forms - erythroblast and in larvae, following hatching, by primary erythrocytes which are subsequently replaced by the cell of normoblastic blood-formation.

The indices of the blood of fish are related not only to growth but also to the size of the fingerling. Privol'nev (1953) points out that salmon fingerlings of the same age had different hemoglobin content

depending on their weight. A large quantity of hemoglobin was found in larger fingerlings.

External conditions have a considerable effect on the blood of fish. However, the nature of the effect has not yet been adequately studied. Let us examine the data published on the effect of temperature, hunger and poor quality food on the blood.

From the study by Schlicher (1927) we know that a rise in the temperature of water increases the number of leucocytes in the blood of fish and on the other hand, a drop in temperature causes a decrease in number. According to the data of Puchkov and Federova (1951) the short term effect of low temperature on carp (in the course of 1 hr) is a decrease in the phagocytic force of the leucocytes, without any change in the number of blood cells and leucocytic formula. In respect to the affect of the temperature of water on red blood the data are contradictory.

Schlicher indicates that there is an increase in hemoglobin and erythrocytes in fish with a drop in the temperature of the water, and Schaeffer (1925) shows that there is a decrease. Ivlev (1955) cites various data on the quantity of hemoglobin and erythrocytes in various species of fish in relation to their behaviour during the winter.

Poor quality food, i.e. the lack of necessary vitamins and minerals, causes a sharp drop in the quantity of hemoglobin and erythrocytes in fish (Hoffmeyer, 1907; Phillips, 1940; Drabkina, 1951). In this connection it is interesting that complete starvation in experimental conditions did not lead to visible changes in the quantity of hemoglobin and erythrocytes (Schlicher, 1927; Puchkov and Federova, 1951). According to

the data of Puchkov and Federova only the phagocytic index decreased in carp which were subjected to starvation for 20 days, otherwise the red and white blood did not change. Drzewina (1911-1912) noted a decrease in cells with acidophilous granularity in some marine fish after lengthy starvation.

Besides being affected by temperature, lack of vitamins and /10
minerals, and complete starvation, the blood is affected by such factors as oxygen, rH of water, and salinity as well as various invasion and infectious illnesses in fish. The mobility in the composition of the blood of fish with respect to the effects of various environmental conditions has permitted a number of authors to use the blood indices as an estimate of the physiological state of fish (Drabkina, 1953; Privol'nev, 1953; Golodets, 1954). To extend this type of method of assessment it is necessary to complete our knowledge of the changes in the blood and in blood-formation with age and of the effects of various environmental factors on them.

SUBJECT MATTER AND METHOD

In our studies we used Baltic salmon, rainbow trout and carp (hybrid of carp and "eastern carp"). The work with the Baltic salmon was conducted at the Narva fish hatchery. The carp and rainbow trout were taken from the VNIORKh (All-Union Scientific Research Institute of Lake and River Fisheries - translator) experimental commercial station, "Ropsha".

The blood was taken from the fish by various methods depending on the size and age of the fish. It was difficult to obtain blood from embryos and larvae because of their small size. However, by using a specific technique it was possible to obtain a small amount of blood from which one could become acquainted with the morphology of the cells in the early period of development. To take blood it was necessary to cut off the head or caudal peduncle of the embryo or larva. A capillary tube (a thin drawn out glass tubule) was brought to the wound to catch the blood. The contents of the capillary tube were blown onto a microscopic slide and from a drop of blood a smear was prepared which did not exceed 1-2 cm^2 in area. In older fish, blood was taken directly from the caudal artery using the N.V. Puchkov method. In fish which were 1† in age and older blood was obtained from the gill vessels by means of capillary tubes.

Imprints of the blood-forming organs were prepared in the following manner. Part of the kidney, spleen or liver was taken from a dissected fish; a cross-section was brought to a microscopic slide, using a scalpel, and the tissue was pressed onto the slide several times.

Soon after the blood smears and impressions were dry they were stained with Mai-Gryunwal'd* dye fixative with subsequent dye finishing using Romanovskii azure-eosin (Pappengein method). It is very important that the dyeing be correctly timed. When the cytoplasm of mature /11
erythrocytes are stained on smears and impressions which have been kept for several days, it assumes a greyish azure or dark blue colour which is not peculiar to it, thus during an examination of the smears the

*Transliteration. Translator.

degree of maturity and the functional importance of the cell may be incorrectly judged. In order to obtain vivid smears and imprints it is necessary to stain them within a few hours after they have been prepared.

The hemoglobin was measured by means of a Sahli hemometer and the number of erythrocytes and leucocytes were counted in a Goryaev chamber, with solutions suggested by Golodets (1939) being used to dilute the blood in mixers.

The leucocytic formula was determined by the usual method given a count of 200 white blood cells. In order to determine the composition in percent of immature cells of the erythropoietic series the field of view of the microscope was decreased by placing a paper disc with an aperture 0.5-0.8 cm in diameter in the eyepiece. From 200 to 400 red blood cells were counted on each glass; subsequently, the percentage of all immature forms and the percentage of each stage taken separately was calculated. The count was taken while using a 7 eyepiece and a 90 lense (under immersion).

The diameters of the cells and nuclei were measured, given a 10 eyepiece and a 90 lense with an eyepiece micrometer, the graduation of which was established beforehand by means of an object micrometer. All measurements are converted into microns.

The present study was conducted under the direction of Professor T.I. Privol'nev. I take this opportunity to express my sincere gratitude for his daily assistance and guidance.

BLOOD-FORMATION IN RAINBOW TROUT, SALMON AND CARP

To define more accurately the main organ of blood-formation in the fish under consideration impressions of the spleen and kidney were made for 31 carp, 33 rainbow trout and 24 salmon. The fish varied in age - from age 1 to sexually mature individuals. In addition, several impressions of the liver were prepared for all three species of fish. However, the first examination showed that blood-formation is lacking in this organ in all three species. Subsequently, only the impression of the kidney and spleen were studied.

In the examination of the blood-forming organs, we first had to deal with the different rates of erythropoiesis and leucopoiesis in fish in relation to their physiological state and environmental conditions. /12 In some cases a vigorous regeneration of red blood cells was observed and then the formation of white blood cells was considerably reduced and inversely, with reduced erythropoiesis an increase in leucopoiesis was frequently observed. However, sometimes a reduction occurred in both erythropoiesis and leucopoiesis.

For purposes of studying the cells of the erythropoietic series in blood-forming organs only those fish were used, in the blood of which a considerable quantity of immature erythrocytes (10-20% and greater) was observed (figure 1a), i.e. a certain degree of vigorous erythropiesis could already detected from the blood. In the impressions of the kidneys /13 of all of these fish a considerable predominance of erythropoietic cells

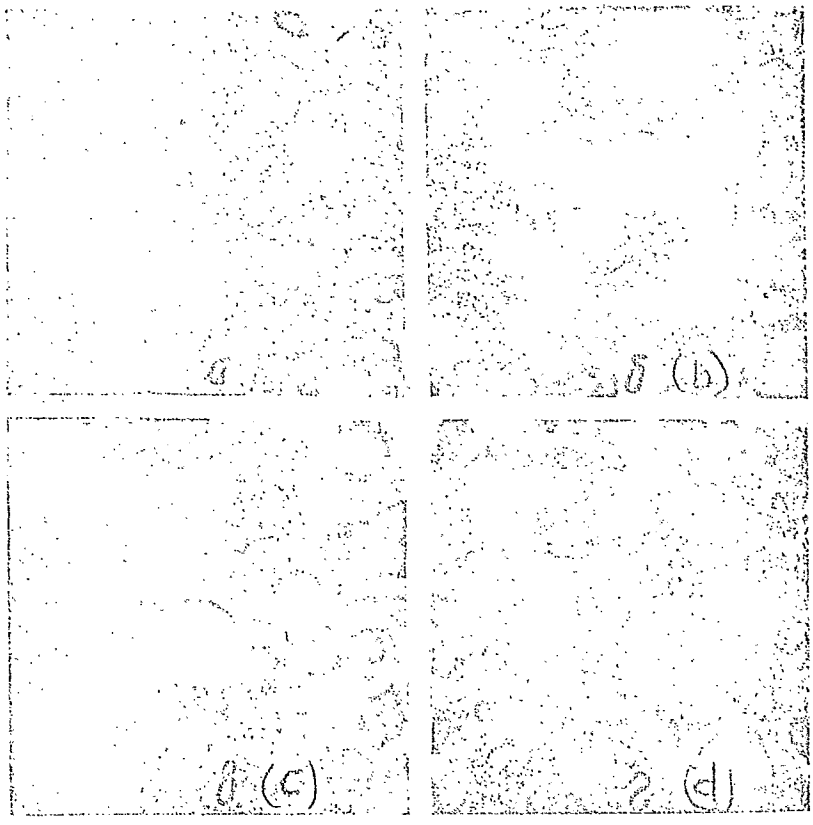

Fig. 1. Blood smears and impressions of the kidney and spleen of rainbow trout during the period of vigorous erythropoiesis (7 x eyepiece, 90x lense); a - blood smears. Presence of immature forms of erythrocytes; normoblast (round cells, basophilic and polychromatophilic erythrocytes; b - impression of kidney. Large cell, hemocytoblast, in the center. Many erythroblasts; c - impression of the kidney. Mitosis of the hemocytoblast; d - impression of the spleen. Early forms of erythropoiesis (hemocytoblasts and erythroblasts are lacking).

over white blood cells was noted.

In figure 1 b and c microphotograph of the impressions of the kidney of a rainbow trout are given. From the blood smear (figure 1a) one may already say that in this trout there is rather intensive erythropoiesis, but the impressions of the kidney provides still further evidence of the presence of the active formation of erythrocytes. All stages of maturity of erythrocytes, from the earliest to the latest, are revealed in the kidney.

The hemocytoblasts are parent cells from which all formed elements in the blood are formed, including erythrocytes. These are large round cells whose diameter averages 14 x 14 μ. Their characteristic features are a very narrow layer of cytoplasm which is deep dark blue in colour and a large round nucleus with a delicate, scarcely discernible reticular structure, which occupies almost the entire cell. The nucleus is rosy-lilac in colour. The mitotic division of these cells is observed (figure 1c).

The erythroblasts are the early stages of the erythropoietic series. They are round cells which are smaller than the hemocytoblasts (d 11 x 11 μ). In their shape, structure and colour the traits of erythropoietic cells already become apparent, due to which these cells are well distinguished from white blood. The nucleus of the erythroblasts is always in the center of the cells (figure 1b) and it is somewhat coarser in structure than the nucleus of the hemocytoblasts. The chromasemae stain a reddish-violet colour and stand-out sharply against a background of light-coloured intermediate sections. The layer of cytoplasm is wider

and is stained a lighter blue than the cytoplasm of the hemocytoblasts. Such cells are encountered very often in the impressions of the liver and the configurations of their mitotic divisions may be observed. Erythroblasts rarely appear in the peripheral blood. They only appear in moments of heightened erythropoiesis and in very small quantities.

Furthermore, the later stages of erythropoiesis which are observed in the blood are abundantly revealed in the impressions of the kidney. However, their true numbers in the kidney cannot be determined from the impressions since a certain amount of peripheral blood is present on the slide when the specimens are being prepared. Normoblasts, basophilic and polychromatophilic erythrocytes belong to these cells.

Normoblasts are round cells (d 10 x 10 μ) which have a wider layer of cytoplasm (figure 1a) staining in a lighter dark blue tone than the cytoplasm of the erythroblasts. The nucleus is round, rather large, in the center, stains reddish-violet and is darker than the nucleus of the erythroblasts.

In contrast to all of the previous stages the characteristic /14
feature in the basophilic erythrocytes stage is a somewhat elongated, rather than round, cell (figure 1a). Usually, the nucleus is also elongated but frequently it has round outlines. Lumps of chromatin in the nucleus begin to draw closer together, however intermediate light-coloured sections can still be well seen between them. The cytoplasm becomes considerably lighter in colour but it stains in a blue tone.

The polychromatophilic erythrocytes are cells in the last stage of maturation, already resembling very much the mature erythrocytes in shape and size (figure 1a). Its cytoplasm already has a definite quantity

of hemoglobin which is reflected in the colour of the cytoplasm, giving it a light greyish-violet hue. The nucleus is somewhat larger than in the mature erythrocyte. Lumps of chromatin, between which lighter-coloured sections can be seen, stand-out sharply.

The mature eyrthrocytes (d 14 x 8 μ) are ellipsoidal cells with an elongated nucleus. The cytoplasm stains in rosy-yellowish tones which indicates that it is filled with hemoglobin. The nucleus is narrow and is in the center; the lumps of chromatin are close together and the nucleus is dark violet in colour.

All of these stages represent the gradual maturation of red blood cells, therefore there are no abrupt transitions from one stage to another. Some stages such as the erythroblast and polychromatophilic eyrthrocytes are more pronounced whereas others such as normoblasts are less pronounced. Very often, one can already observe a certain amount of hemoglobin in the cytoplasm. The colour of the cytoplasm acquires a slight polychromtophilia but the cell is still round in shape.

Hence, it follows that when one isolates certain stages of erythropoiesis it is necessary to bear in mind the somewhat arbitrary nature of these designations and also the absence of sharp boundaries between the stages which are being singled out.

From the description of the successive stages in the erythropoietic theories it can be seen that the nucleus becomes smaller and more dense and the colour darker in proportion to the maturity of the erythrocytes. On the other hand, the layer of cytoplasm becomes larger, being initially deep dark blue in colour and gradually becoming lighter and turning a rosy-yellow.

The round cell and nucleus become elongated and take on an ellipsoidal shape. The mature erythrocyte is formed.

From an examination of the impressions of the spleen of trout it was ascertained that the same cells are observed in the spleen as in the blood. An absence of immature forms of erythrocytes in the blood leads to the complete absence of these forms in the spleen. Given a large of immature elements in the blood, a considerable quantity also accumulates in the spleen, however such early stages as hemocytoblasts and erythroblasts (figure 1d) are characteristically very rarely detected in the spleen. That is, essentially only the cells which are also observed in the peripheral blood are encountered in the spleen. Hence, it follows that only a very slight erythropoiesis is possible in the spleen of trout which is indicated by the very rare encounter of hemocytoblasts /16 and erythroblasts. In trout, the kidney assumes the leading role in erythropoiesis.

A similar picture is revealed during an examination of the imprints of the kidney and spleen of salmon and carp.

In fish whose blood did not reveal immature forms of erythrocytes (figure 2a) and hence lacked active erythropoiesis immature cells of the erythropoietic series were rarely encountered in the impressions of the kidney. The bulk of the cell consisted of white blood cells among which one could observe both mature forms and all of the transitional stages of maturation (figure 2b).

In the impressions of the kidney of trout and salmon one observed a large quantity of mature lymphocytes, polymorphonuclear leucocytes and

/15

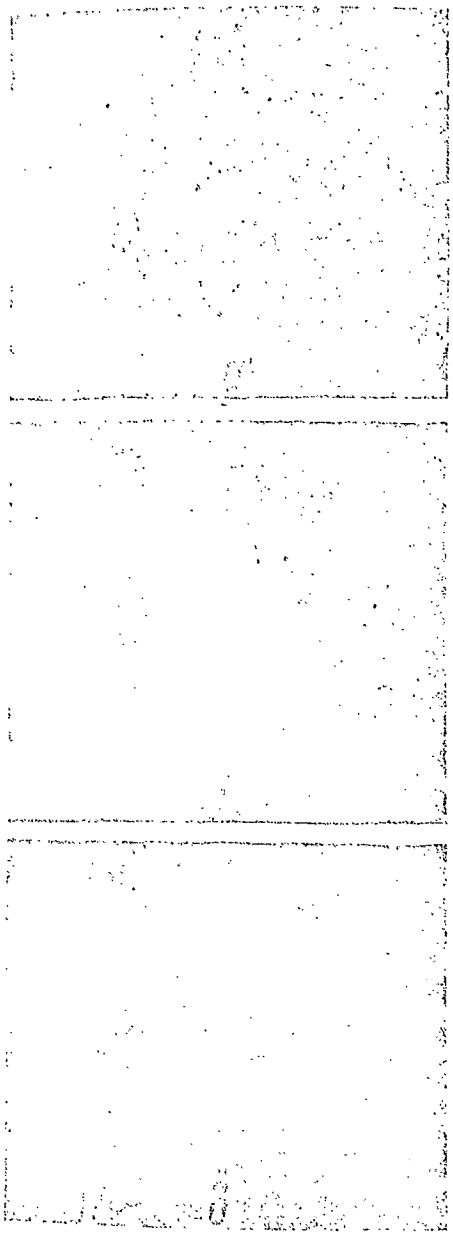

Fig. 2. Blood smear and impressions of the kidney and spleen of rainbow trout during the period of vigorous leucopoiesis (eyepiece 7 x, lense 90 x); a - blood smear. Immature forms of erythrocytes are lacking; b - impression of the kidney. Leucocytes are present in various stages of maturation. Immature cell of the erythropoietic series are not detected; c - impression of the spleen. Accumulation of lymphocytes. Immature forms of red and white blood are lacking.

monocytes which are also observed in the peripheral blood.

The lymphocytes are small round cells (d 8 x 8 μ) which very much resemble the lymphocytes of mammals. The nucleus is round, dense and occupies a large part of the cells; it stains dark violet in colour whereas the cytoplasm stains light azure. The edges of the cytoplasm are uneven; frequently it does not envelop the entire nucleus but only 1/3 of it, and sometimes it is only represented by one or two wide pseudopod-like protuberances (figure 3) or it is not seen at all and the lymphocytes seems to be holonuclear. Similar completely nuclear lymphocytes in carp were described by Topf (1953) and Dombrowskii (1953).

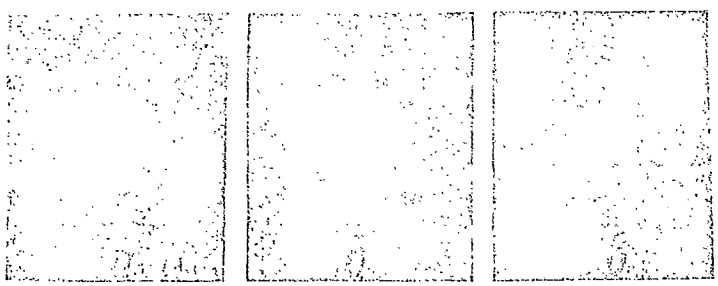

Fig. 3. Lymphocytes of rainbow trout; a - cytoplasm envelops nucleus on all sides; b - lymphocyte with a section of cytoplasm; c - completely nuclear lymphocyte.

As the lymphocytes mature the tendency is towards a decrease in the size of the cell, increased density and darkening of the nucleus, brightening of the cytoplasm and reduction of its total area. Thus, in the early forms the cytoplasm envelops the entire nucleus but later in proportion to the development, it is only preserved in the form of

individual sections.

The polymorphonuclear leucocytes are larger cells than the lymphocytes (d 13 x 11 μ). The nucleus is divided into several lobes or segments between which wide or narrow bridges are observed (figure 4). The nucleus stains a reddish-violet. The cytoplasm is usually a light azure grey; it is sometimes homogeneous but more often it has a light foamy structure. The more pronounced the segmentation of the nucleus the lighter in colour and the more transparent the cytoplasm. /17

Fig. 4. Polymorphonuclear leucocytes of rainbow trout

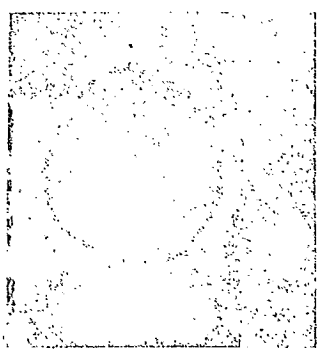

Fig. 5. Monocytes of rainbow trout

In terms of shape and structure of nucleus these cells resemble very much the neutrophils of mammals. Using a supravital dye, Catton (1951) detected granularity in the cytoplasm of similar cells in trout and on the basis of this assigned them to neutrophils. With Pappengeim* and Romanovskii staining, granularity is not observed in the cytoplasm of the polymorphonuclear cells.

*Proper name transliterated. Translator.

The monocytes are the largest cells in the blood (d 14 x 11 μ). A large bean-shaped nucleus lies at the edge of the cell (figure 5). The structure of the cytoplasm, in contrast to that of the polymorphonuclear leucocytes, is lumpy and uneven; the cytoplasm stains dark blue - sometimes quite dark. A lucid interval is frequently observed in the center where the nucleus sags.

On the impressions of the kidney and on blood smears of carp, lymphocytes and monocytes are also detected and in addition there are cells with fine rose-coloured granularity in the cytoplasm. The nucleus of such cells is at the edge and it is either round or divided into two lobes between which there is always a narrow or wide constriction (figure 6). Division of nuclei into more than two lobes is not encountered. Sometimes the cell is placed on a microscopic slide so that the nucleus turns out to be in the middle, however such cases are rarely observed. The cytoplasm of the cell is filled with a fine rose-coloured granularity, the granules being arranged in such a way that lucid intervals are detected between them. /18 Such cells are observed in the kidney of carp in large numbers. Similar cells in the kidney attracted the attention of many authors who were working with the blood and blood-forming organs of cyprinoid and certain other fish (Drzewina, 1905; Jordan and Speidel, 1924; Duthie, 1939; Rumyantsev, 1939; Catton, 1951). Some of the authors called the cells eosinophils however the majority assigned them to neutrophils allowing for, of course, a certain uniqueness in these cells as compared to the neutrophils of higher vertebrates. Duthie (1939) and

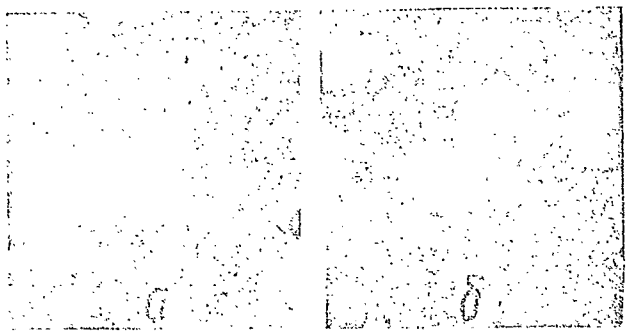

Fig. 6. Neutrophilic leucocytes of carp;
a - undivided nucleus; b - nucleus divided into two lobes.

Catton (1951) noted that the granularity of these cells does not stain on blood smears as a result of Gimz* staining (Romanovskii azure-eosin), but at the same time it is well revealed in the impressions of the kidney.

In our study we essentially used a combined stain of Mai-Gryunval'd + Romanovskii azure-eosin (Pappengeim method). Given this method of staining, the granularity of these cells is well revealed not only in the kidney but also on the blood smears of carp. Lack of knowledge of the function of these cells prevents us from determining their exact name. We shall arbitrarily designate them as neutrophils similar to the designation adopted by many authors. In terms of the nature and arrangement of the granularity, these cells most resemble the neutrophils of higher vertebrates.

In the kidneys of the fish under consideration we observe a large number of not only mature white blood cells but also transitional forms. One can already quite easily decide in the early stages whether to assign the cells to the erythropoietic series or to the developing white blood cells. Both in the series of polymorphonuclear cells and in the series

*Transliteration. Translator.

of monocytes and neutrophils the nucleus moves to the edge of the cell (figure 2) very early. This is never detected in the erythropoietic series. /19
Having moved to the edge of the cell, the nucleus begins to sag and adopt a bean-like shape. In the structure of the nucleus of the white blood cells one never sees the pronounced lumps of chromatin against an overall light-coloured background as can be observed in erythroblasts and normoblasts, etc. In the nuclei of the white blood cells the chromasemae are always quite densely arranged.

When the polymorphonuclear leucocytes of trout and salmon mature, one observes a gradual brightening of the cytoplasm and a branching of the nucleus. Sometimes the maturation of the cytoplasm lags behind the changes in the form of the nucleus and the nucleus begins to branch when the cytoplasm is still dark blue. Usually, however, with greater segmentation of the nucleus the cytoplasm stains in a lighter azure and even light-grey tone.

In the kidney of carp one may trace all stages in the gradual filling up of cells with neutrophilic granularity. Cells are detected with azure cytoplasm in the center of which there is a small area which is filled with the finest rose-coloured granularity. Subsequently, one may encounter cells in which a large part of the cytoplasm is filled with granules and a narrow transparent azure layer of cytoplasm is observed only along the edge; finally, cells are observed in which the entire cytoplasm is filled with granularity. These are already mature neutrophils.

With the absence of heightened erythropoiesis, in the spleen, unlike the kidney, of trout, salmon and carp, one usually observes a certain number of mature forms of white blood cells. Immature forms are extremely rare. Sometimes, one can only detect lympocytes here, in quite large numbers (figure 2c).

Thus, in the spleen of all the fish which are being considered one observes only a slight erythropoiesis and, probably, a small formation of white blood cells, particularly lymphocytes. In trout, salmon and carp red and white blood cells are mainly formed in the kidney.

Approximately the same picture is revealed in the peripheral blood of trout and salmon. Besides erythrocytes, we encounter lymphocytes (predominantly), monocytes and polymorphonuclear agranulocytes on blood smears (figure 3, 4, 5). All of these cells are present in the blood in a mature state or in the late stages of maturation. On blood smears one frequently encounters so called spindle cells which have a dark elongated nucleus and a narrow layer of azure cytoplasm. In addition, in some blood smears of salmon, formations are detected which, in terms of shape and arrangement, very much resemble the trombocytes of mammals but which exceed the latter in size. These formations attained a diameter of 3 μ; there was no signs of nucleus or cytoplasm in them; they were irregular in shape; there were stained in a reddish-violet colour and were arranged very densely, similar to trombocytes in mammals (figure 7). These formations are also frequently detected in decomposed form in blood smears which points to their instability. We know that trombocytes /20 in mammals are also very unstable and decompose on contact with glass.

Similar formations were encountered in large numbers in the kidney of trout as well. The presence of such formations in fish is not indicated in the literature. The trombocytes in carp described by Topf and Dombrowskii differ by virtue of their very small size (less than 1 μ) and by the fact that they are revealed only with the aid of a special stain. It is possible that the formations which we detected in salmon and trout are analogous to the blood platelets in mammals.

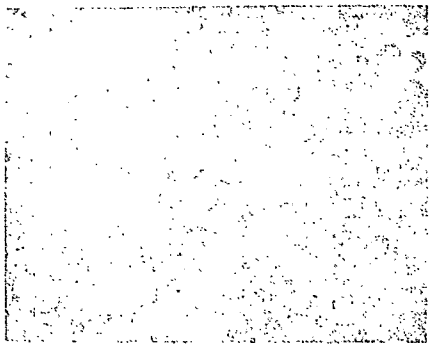

Fig. 7. Formations in the blood of salmon which resemble the trombocytes in mammals (eyepiece 7x, lense 90x)

In the blood of carp we detected the following white blood cells: lymphocytes, monocytes and neutrophils. Besides these we observed quite often cells which are apparently old deforming neutrophils. Jordan and Speidel (1924) wrote about such cells in their time. The nucleus of these cells was usually at the edge and stained in very dark violet tones. Its structure was not examined. The cytoplasm was absolutely transparent and colourless. Sometimes it was possible to detect dark violet inclusions of different sizes in it. More often there were no inclusions. The cells

resemble those which Topf and Dombrowskii designated as tromboblasts, being similar in shape and size, structure and location of nucleus. However, it is difficult to form an opinion about their function from the smear. Their appearance likely indicates decomposition of cells rather than a capacity for some activity. However, it is possible that a deterioration in the cells occurs during the fixation and staining of the specimen.

Thus, as a result of the examination of the blood smears of fish only immature cells were detected in the blood of salmon and trout, at least granulation was not revealed as a result of staining by the Pappengeim and Romanovskii methods. But in the blood of carp there were distinct leucocytes with rose-coloured granularity, tentatively designated as neutrophils. The kidney was the main blood-formation organ in all of the fish being considered.

CHANGES DUE TO AGE IN THE COMPOSITION OF THE BLOOD CELLS OF SOME FISH

/21

During an examination of changes in the blood due to age most attention was given to the early stages in the development of fish since they have been studied least. The blood smears of trout and salmon, beginning with the embryonic period, were examined.

The eggs of rainbow trout were transported from Ropsha in Narva to the fish farm on the day that they were fertilized and were incubated to begin with in the Vil'yamson* apparatus and subsequently in a floating river nursery. A mass hatching of larvae was noted 42 days after

*Transliteration. Translator.

fertilization. Larvae began to take food on the 10th day after hatching. In the early days they were fed on plankton and subsequently, on spleen and oligochartes.

The Blood Picture of Embryos of Rainbow Trout

It was possible to prepare the first blood smears on the 30th day after fertilization. By this time the eyes of the embryo in the egg are already being pigmented. A large number of round cells (d 10 x 10 μ) which have a large nucleus located in the centre (figure 8) are detected in the blood smears. The layer of cytoplasm is rather wide; it is stained in a dark blue colour; chromasemae which stand-out distinctly against an overall rose-coloured background are well seen in the nucleus. These cells resemble the erythroblasts of mature fish; however, in this period definitive blood-forming organs do not yet function and the cells are a product of primary erythropoiesis, i.e. they may be called primary erythroblasts. Similar cells were observed by Nusembaum (1954) in the blood of the embryos of salmon. In addition to these cells a certain number of earlier forms were detected in the embryos on the 30th day. These may be designated as primary proerythroblasts. They have a narrower layer of cytoplasm which is stained in a darker blue colour. The structure of the nucleus is less distinct than in erythroblasts; it is stained in rosy-lilac tones. Mature cells of the erythropoietic series are not detected in this period. Frequent mitosis is observed.

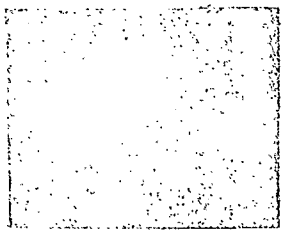

Fig. 8. Primary erythroblasts in the embryo of rainbow trout. Mitosis (eyepiece 7x, lense 90x)

Individuals cells are encountered which apparently may be assigned to early forms of white blood. These are large round or elongated cells with a nucleus which is round or bean-shaped and occupies most of the cell. The nucleus of such cells is at the edge so that the cytoplasm /22 on that side is seen in the form of a very narrow, scarcely noticeable, ring or is not seen at all. On the other hand, the cytoplasm is a wide layer which is stained in a deep dark blue colour. A lucid interval is observed where the nucleus sags in the cytoplasm. The nucleus is stained in rose tones and has a delicate reticular structure.

Thus, in the blood of the embryos of trout on the 30th day of development there are only very early forms of primary erythropoiesis and individual early forms of white blood cells. The dark blue colour of the cytoplasm of the proerythroblasts and erythroblasts indicates that they do not have traces of hemoglobin.

Blood smears which were obtained on the 34th day after fertilization

showed that the blood had undergone considerable changes in four days. The bulk of the red blood was no longer represented by erythroblasts but by the next stages of development - primary basophilic and polychromatophilic erythrocytes. These cells are distinguished from basophilic and polychromatophilic erythrocytes of mature fish by a round nucleus and cell. Polychromatophilic erythrocytes predominate in this period. A considerable amount of hemoglobin now accumulates in the cytoplasm of these cells as a result of which the cytoplasm becomes a greyish-violet colour. The nucleus of these cells becomes smaller and somewhat denser as compared to the earlier stages; however, lumps of chromatin and lucid intervals between them are well seen in the nucleus. Primary proerythroblasts and erythroblasts are very rarely encountered. In this period mitosis is characteristically observed very rarely and is evidently a division of proerythroblasts and erythroblasts.

In the white blood the same cells are encountered as were encountered on the 30th day of development but, in addition, cells appear which are similar in structure but smaller.

Thus, in four days, the composition of the blood, particularly the red blood, changed considerably. Most of the proerythroblasts and erythroblasts changed into basophilic and polychromatophilic primary erythrocytes, already having a certain amount of hemoglobin. However, the blood still completely lacks mature oxyphylic cells.

Blood Picture of Rainbow Trout in the Postembryonic Period of Development

Larvae began to hatch in mass on the 42nd day of embryonic development. Blood smears which were obtained on that day showed the following. The red blood was entirely represented by large round and elongated cells which had a wide layer of cytoplasm, stained in yellowish-rose tones which indicated that the cells were mature and that a considerable amount of hemoglobin was present. Similar cells had a small dense nucleus /23 with connivent lumps of chromatin. The nucleus was stained in a dark violet colour. These are primary mature erythrocytes (figure 9). They are distinguished from the erythrocytes of mature fish by a large, round nucleus and cell. Immature forms of primary erythropoiesis were absolutely lacking in the days immediately after hatching. Consequently, there was no intensive formation of primary erythrocytes in this period.

The contrary is seen in the white blood picture. Various stages in the maturation of lymphocytes and polymorphonuclear cells are detected. The polymorphonuclear cells of the larvae are distinguished from those of mature fish by the blueish-azure colour of the always homogeneous cytoplasm and by a light reddish nucleus. The nucleus of these cells is most varied in shape, ranging from a round or bean-like shape to an oddly bent form.

The lymphocytes in the larvae are somewhat larger than those of mature fish and they are considerably darker. This applies both to the nucleus and cytoplasm. Cells of the monocytoid type are very rarely encountered. Lymphocytes predominate in this period.

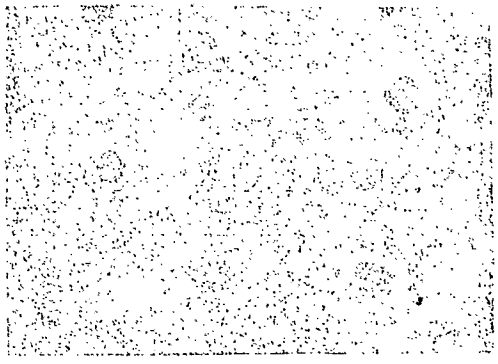

Fig. 9. Primary erythrocytes in a larva of rainbow trout on the day when it hatched (eyepiece 7x, lense 90x)

Thus, by the time the larvae hatches the entire mass of red blood is represented by mature primary erythrocytes. The white blood consists of lymphocytes, polymorphonuclear leucocytes and their immature forms. These cells differ somewhat from the cells of the white blood of mature fish.

The blood of trout larvae, 18-19 mm long, on the 7th day after hatching. The larvae become more mobile but as yet they do not eat; the yolk sac is approximately 40-50% of the primary sac. By this time, the first immature cells of secondary erythropoiesis already occur in the blood in large numbers. These are basophilic and polychromatophilic erythrocytes which already very much resemble the mature erythrocytes of fish in the shape and size of nucleus and cell. However, the azure and grey-violet colour of their cytoplasm as well as the structure of the nucleus indicate the immaturity of these forms. The immature cells /24 of secondary erythropoiesis comprised an average of approximately 26% on the 7th day after hatching. The remaining mass of red blood was represented by primary mature round erythrocytes. Lymphocytes, polymorphonuclear

leucocytes and monocytes are observed among the white blood cells. All of them are basically represented by mature forms.

The blood of trout larvae, 20-21 mm long, on the 9th day after hatching. The larvae swim in the water mass. Some of them attempt to seize plankton, however the bulk of them as yet do not feed. A single cyclops was found in only two of 20 dissected intestines. An examination of the blood shows that considerable changes occur in the blood during these days. The number of immature forms of secondary erythropoiesis increases to 44%, i.e. it is almost double that of the blood smears prepared two days previously. Earlier stages - erythroblasts (1%) and normoblasts (6%) - appear among the immature forms. Having matured, some of the polychromatophilic erythrocytes produce oxyphylic secondary erythrocytes - small oval cells with an oval nucleus and rosy-yellow cytoplasm. Compared to the erythrocytes of mature fish they are smaller and less elongated. Such cells already comprise approximately 16% of all the red blood cells. The number of primary erythrocytes drops to 40%. Thus, the cells of secondary erythropoiesis are replaced by primary erythrocytes (figure 10). At this time there are absolutely no immature forms of primary erythropoiesis, which are encountered in large numbers in the embryonic period. Only old primary erythrocytes are present in the blood. They are easily deformed, lie very unevenly on glass and spread, whereas the secondary erythrocytes are excellently preserved.

White blood cells are encountered in smears considerably more rarely than on the 7th day after hatching and an absolute reduction is observed. Their immature forms are lacking.

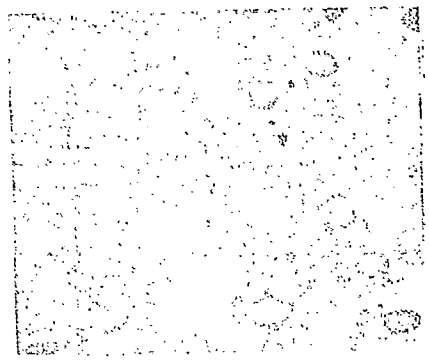

Fig. 10. Replacement of primary erythrocytes by secondary erythrocytes in rainbow trout larvae 9 days after hatching (eyepiece 7x, lense 90x)

Thus, considerable changes are observed in the blood of larvae by the time the transition is made to taking food. These changes are in the direction of the replacement of primary erythrocytes by secondary erythrocytes. The total number of leucocytes drops noticeably. /25

The blood of trout larvae, 22-23 mm long, on the 12th day after hatching. All of the larvae take nourishment; plankton were found in all of the dissected intestines (20 specimens). The yolk sac forms a thin layer along the central part of the trunk. The red blood presents the following picture. The primary erythrocytes only comprise 1/5 of all the cells (21%). The bulk of the erythrocytes are represented by cells of secondary erythropoiesis - immature cells (43%) and mature cells (36%). Among the immature cells polychromatophilic erythrocytes are observed in large numbers (27%), basophilic erythrocytes in smaller numbers (9%) and normoblasts and erythroblasts in still smaller numbers (2% and 1%, respectively).

Against the background of an overall reduction in the quantity of white blood one can notice a relative increase in polymorphonuclear leucocytes and a sharp relative and absolute decrease in lymphocytes.

Thus, on the 12th day after hatching the replacement of primary cells by secondary cells in the blood continues, with three forms of red blood elements being present - primary erythrocytes, secondary erythrocytes and their immature forms.

Blood of trout juveniles, 25-26 mm long, on the 29th day after hatching. The juvenile feeds actively and the yolk sac has been completely resorbed. The primary erythrocytes have disappeared completely from the blood and they have been replaced by secondary erythrocytes. However, a rather intensive formation of new red blood cells still continues which is indicated by the detection of approximately 19% of immature elements in the smears. The secondary erythrocytes are similar to the erythrocytes of mature fish but are smaller and frequently are less elongated (figure 11, figure 12).

The number of white blood cells has increased considerably, the blood having acquired a lymphoid character. Whereas in the previous period the leucocytes did not even comprise 0.5% of the erythrocytes, they now constituted an average of 4% and in individual specimens, 8% and more. The newly emerged cells differ somewhat from the white blood in the larval period of development. Now, the lymphocytes, polymorphonuclear leucocytes and monocytes are similar to the white blood cells of mature fish. The colour of the nucleus and cytoplasm of the lymphocytes is /26 considerably lighter than in similar cells in the larva. In the polymorpho-

nuclear cells changes are noted in the structure and colour of the cytoplasm and nucleus. The nuclei became denser and stain violet. The nuclei of the polymorphonuclear cells in larvae were more porous in structure and stained in lighter reddish-violet tones. In this period cells are detected which may be assigned to the immature stages of monocytes and polymorphonuclear leucocytes - large cells with a round nucleus at the edge and with cytoplasm stained dark blue. Apparently, a certain animation in leucopoiesis takes place.

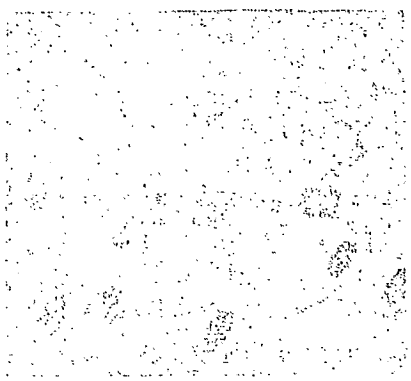

Fig. 11. Secondary erythrocytes and their immature forms in the blood of a rainbow trout juvenile 26 days after hatching (eyepiece 7x, lense 90x)

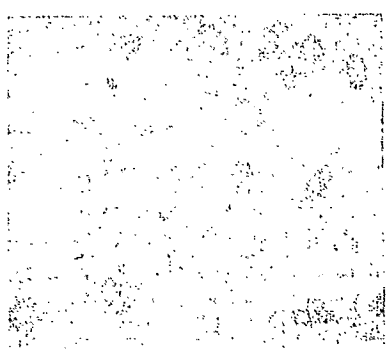

Fig. 12. Erythrocytes of a mature rainbow trout (eyepiece 7x, lense 90x)

Consequently, by the 29th day of postembryonic life, when the yolk sac had been completely resorbed and the larva had changed into a fingerling, changes also occurred in the blood. The embryonic and larval primary erythrocytes disappeared completely and were replaced by secondary erythrocytes. A certain number of immature forms indicates a continuing increase in the overall number of red blood cells. The overall quantity of white blood increased due to the appearance of new cells, which are similar to the cells of mature fish, and the blood adopted a lymphoid character. Thus, by this time the blood has generally adopted the appearance of the blood of normal mature fish, with the only difference being that quite a large number of immature forms are still observed both among the red and white blood cells.

Subsequently, by the 40th day after hatching the intensive formation /27
of blood elements decreases; 11-12% immature erythrocytes are detected in the blood. Early stages of white blood are not observed at all.

The change in blood cells is related to the development of the entire organism. Immature proerythroblasts and erythroblasts are characteristic of the early embryonic period. Having matured gradually and having become filled with hemoglobin they change into mature erythrocytes by the time of hatching. The period of transition to active feeding when the yolk sac has not been resorbed is also a transitional period for the blood. Primary erythrocytes are replaced by secondary erythrocytes. By the time the larva changes into a fingerling and the yolk sac is completely resorbed, only cells of secondary erythropoiesis are detected in the blood. By that time the white blood is also represented by forms which are similar

to those of the white blood of mature fish.

An examination of blood smears of salmon showed a very similar picture. However, because of a more extended embryonic and larval period the change in the blood cells of salmon larvae occurred over a longer period of time than in trout (figure 13, figure 14), but also parallel to the resorption of the yolk sac.

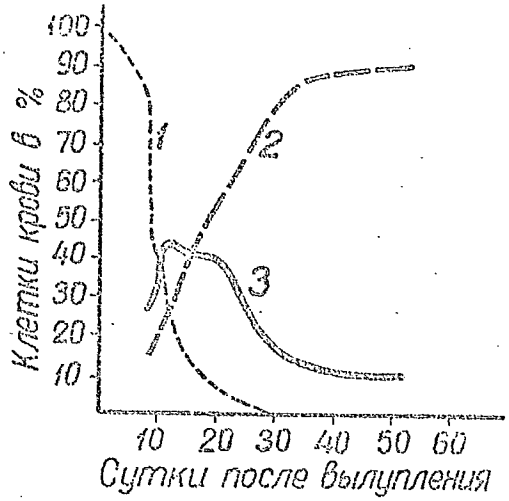

Fig. 13. Changes in the red blood of rainbow trout juveniles; 1 - primary erythrocytes; 2 - secondary erythrocytes; 3 - immature forms of secondary erythrocytes.

Due to the small eggs and hatched larvae of carp it was not possible to trace the changes in the blood by periods as was the case for trout and salmon. However, a certain number of smears were obtained in the larval period in the days immediately following hatching. In the smears it was possible to detect individual round cells with yellow cytoplasm and a small round nucleus stained violet. Apparently, round primary /28 erythrocytes are characteristic of carp larvae. After some time, on the 10th

day after hatching, when the carp juvenile already feeds actively, we observed in the blood only ellipsoidal cells with an elongated nucleus. Immature forms were observed among them. Thus, apparently in carp there is a similar change in blood cells which, in this fish, takes place in a very short period, together with rapid resorption of the yolk sac and the tranformation from larva into fingerling.

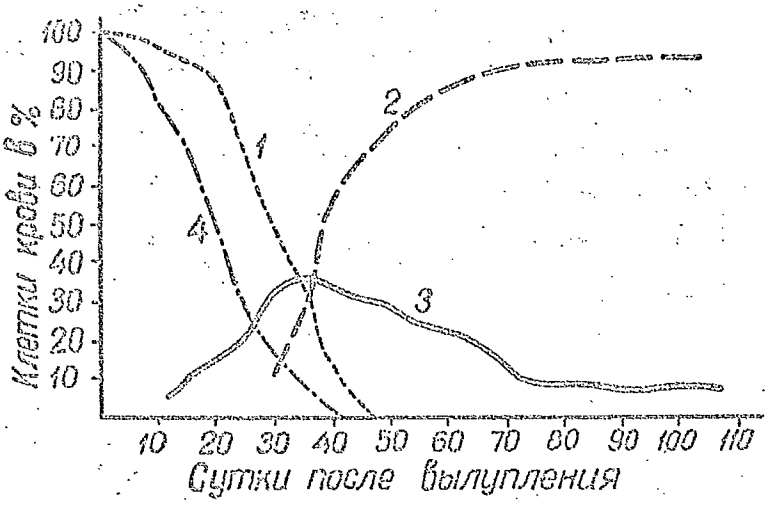

Fig. 14. Changes in the red blood of Baltic salmon juveniles by age; 1 - primary erythrocytes; 2 - secondary erythrocytes; 3 - immature forms of secondary erythrocytes; 4 - percent of yolk sac

EFFECT OF ENVIRONMENTAL FACTORS ON THE BLOOD AND BLOOD-FORMATION OF SOME FISH

Effect of Water Temperature on the Blood and Blood-Formation of Some Fish

The vital functions of fish as cold-blooded animals is closely related to the thermal conditions of the environment.

A decrease and increase in the temperature of water causes a change in the rate of metabolism in fish which cannot but reflect on the state of the blood which takes an active part in the metabolic processes of an organism. This holds true both for the early stages of development and for the late period of ontogenesis in fish.

Whereas there is some published data on the effect of water temperature on the blood of mature fish, information is lacking on the effect of temperature on the formation of blood in the larval period.

Effect of Temperature on the Rate of Development of Blood in Baltic Salmon Larvae /29

To set up the experiments, in April of 1954, 2,000 Baltic salmon eggs were obtained from the Narva fish hatchery. The eggs were placed in aquariums in a cooler at a water temperature of 3-4°. Eggs which were incubated at the Narva fish hatchery served as the control group. The mass hatching of larvae had already begun in transit. During the same days the hatching of larvae also occurred at the fish hatchery.

During rearing, the juveniles of the control group lived in the following temperature conditions: in April, 2-4°; in the first half of May, 3.3-7°; in the second half of May, 6-13°; in the first half of June 13-17°; in the second half of June, in July and August, 3-4°.

During all of this time the juveniles of the experimental group lived in water at a temperature of 3-4°.

In the days immediately after hatching the red blood of larvae from the experimental group in no way differed from the blood of larvae reared at the fish hatchery. The blood consisted entirely of primary erythrocytes. No other cells either of primary or secondary erythropoiesis were observed.

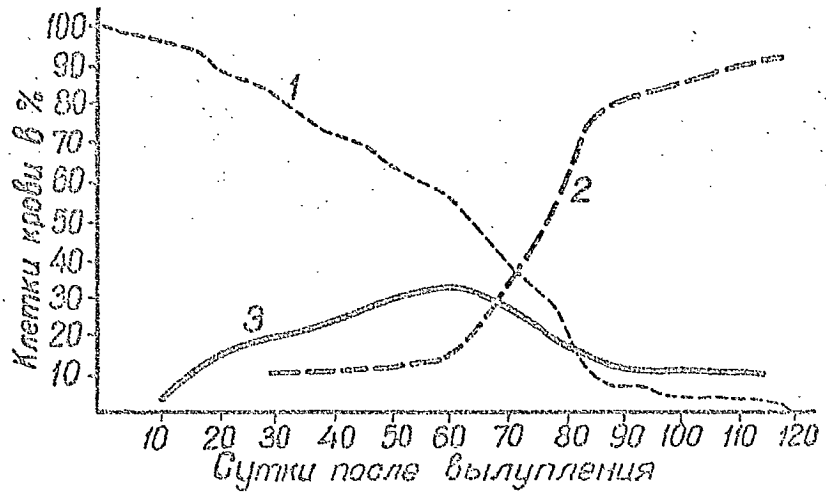

Fig. 15. Changes by age in the blood of salmon juveniles reared in conditions of low temperature: 1 - primary erythrocytes; 2 - secondary erythrocytes; 3 - immature forms of secondary erythrocytes.

The first immature forms of secondary erythropoiesis - basophilic and polychromatophilic erythrocytes - appeared on the 10-13th day after hatching in the blood of larvae from the experimental group and the control group. Subsequently, the development of the blood of larvae reared in the cooler gradually began to lag behind that of larvae in the control group (figure 15; compare with figure 14). This delay occurred along /30 with a delay in the development and growth of the entire organism. Thus, on the 60th day after hatching the average length of larvae from the

control group was 33 mm and of larvae from the experimental group, only 26.6 mm. In juveniles reared in normal conditions, the yolk sac had already been resorbed completely by the 60th day and only the forms of secondary erythropoiesis were present in the blood. A different picture was observed in larvae reared at a reduced temperature. The yolk sac in them was still rather large, and up to 57% primary erythrocytes and only 47% secondary erythrocytes and their immature forms were detected in the blood. Thus, in these larvae a change in the blood cells was taking place whereas in the juveniles of the control group it had already ended. In the juveniles of the experimental group, the yolk sac was completely resorbed and the primary erythrocytes gave way entirely to the cells of secondary erythropoiesis only by the 120th day of postembryonic development.

Consequently, in the first 10-13 days, when the water temperature for the larvae of the experimental group and control group was approximately the same (2-4^{o}), no difference was observed in the blood of the larvae. Subsequently, the reduced temperature into which the larvae of the experimental group were forced sharply inhibited their growth and development which also caused a delay in the replacement of primary erythrocytes by secondary erythrocytes. The process of change in the red blood cells was not only delayed but was also considerably slower and smoother than in the control larvae, however similar to the latter it was parallel with the resorption of the yolk sac.

Effect of Change in Water Temperature on the Indices of the Blood of Some Fish

Carp and rainbow trout fingerlings and catfish of age 1+ were used in the experiment. The study was conducted in the winter of 1954-1955 at the Central Experimental Commercial Station of the All-Union Scientific Research Institute of Lake and River Fisheries, "Ropsha".

Some of the carp and all of the rainbow trout were studied as soon as they were taken out of the ponds in which the water temperature had not exceeded 3° for 1-2 months. The other carp and the catfish (the latter were obtained from the Zhivorybnyi* base in Leningrad) were held from 10 to 30 days in large aquariums (vats) in which the water temperature was 2-4°. After the state of the blood was determined for fish which lived in conditions of low temperature, the fish which had not been used were transferred to aquariums in which the water temperature reached 14-16° in the course of 3-4 hours. The fish were kept in these aquariums from 1 to 13 days, the indices of their blood being systematically determined. The samples of blood were taken directly from the caudal artery of the fish according to the method of N.V. Puchkov.

The data obtained on the blood are presented in Table 1. The composition of the leucocytes is given in relative (in % %) and absolute (in 1 mm^3 of blood) quantities. The latter were calculated on the basis of /31 the total number of leucocytes counted in a Goryaev chamber and on the basis of the leucocytic formula determined from blood smears.

Firstly, attention is drawn to the striking low number of leucocytes

*Transliteration - meaning live fish. Translator.

in carp which are in conditions of reduced temperature (2.2 thousands per 1 mm^3 of blood). However, the leucocytic composition does not deviate greatly from the norm and bears a lymphoid character.

When carp are transferred to aquariums with water at 16° the picture changes sharply after one day. The total content of leucocytes in 1 mm^3 of blood increases several-fold, in considerable degree due to the sharply increased absolute and relative quantity of neutrophils. In conditions of low water temperature these cells constituted an average of 13% (0.3 thousand per 1 mm^3 of blood), and in the course of 1 day at a higher temperature the average quantity of these cells increased to 66% (9.1 thousand per 1 mm^3 of blood), i.e. more than 30-fold. In individual cases neutrophils reached 80-90% with respect to other blood cells.

On the third day after the carp had been transferred to water with a temperature of 16° both an absolute and relative decrease in neutrophils and a certain increase in lymphocytes was observed. Subsequently, the number of neutrophils continued to drop alongside with a relative and absolute increase in the number of lymphocytes. On the 9th day, in conjunction with the overall increase in the number of white elements (up to 40.9 thousand per 1 mm^3 of blood), the usual lymphoid character of the blood was established. The lymphocytes constituted 96% (39.3 thousand per 1 mm^3) and the bulk of the neutrophils disappeared from the blood stream. These cells now accounted for only 2% (0.8 thousand per 1 mm^3 of blood). Among the lymphocytes cells were observed which are somewhat larger than ordinary lymphocytes with a wide layer of cytoplasm enveloping the nucleus on all sides. The appearance of such

young lymphocytes indicates heightened lymphocytosis in the organism.

In spite of the small number of catfish at our disposal, it could be seen that the changes undergone in the blood as a result of temperature change were very similar to that of carp. At a low temperature (4°) the number of leucocytes was greatly decreased (3.3 thousand per 1 mm^3 of blood). With increase in water temperature ranging from 4 to 6° the number of cells with neutrophilic granularity (smaller than in carp) increased sharply initially. The same thing occurred when catfish were transferred to water which had a temperature of 16°. After the fish had stayed in conditions of increased temperature (16°) for 10 days, the total number of white blood cells greatly increased (up to 52.5 thousand per 1 mm^3 of blood) and the leucocytic formula adopted a normal lymphoid character.

We were not able to see such changes in trout. At a low temperature /32-33 the content of leucocytes in the blood of the fish was quite considerable (16.6 thousand per mm^3) and of these 92% were lymphocytes, 4% were polymorphonuclear immature cells and 4% were monocytes. After the trout had remained in water temperature of 14° for 1 day, the number of leucocytes on the average remained unchanged and the leucocytic formula, as before, bore a lymphoid character with no pronounced changes. On the whole, cells with granularity were not detected in the blood of rainbow trout nor were they observed in the kidney.

It was not possible to keep trout in the aquariums more than one day. However in the autumn of 1955, we succeeded in collecting data on the blood of rainbow trout which was brought from Ropsha and was being

Table I

1 Изменение показателей крови рыб под влиянием температуры воды

2 Рыбы	3 t^0 воды	4 Продолжит. повышен. t^0	5 Гемоглобин %	6 Гемоглобин г%	7 Эритроциты млн/мм³	8 Лейкоциты тыс/мм³	9 Состав лейкоцитов: 10 Лимфоциты %	15 Лимфоциты тыс/мм³	11 Моноциты %	Моноциты тыс/мм³	12 Полиморфноядерные %	Полиморфноядерные тыс/мм³	13 Нейтрофильные %	Нейтрофильные тыс/мм³	14 Количество исследованных рыб
16 Радужная форель	1		43	6,8	0,98	16,6	92	15,3	4	0,7	4	0,7	—	—	20
	14	1	41	6,5	1,10	14,3	90	12,9	4	0,6	6	0,8	—	—	17
	13—16	≈15	45	7,2	1,10	26,7	97	25,9	1	0,3	2	0,5	—	—	32
17 Карп	1—4		50	8	1,30	2,2	80	1,8	7	0,2	—	—	13	0,3	26
	16	1	46	7,3	1,51	13,9	33	4,6	1	0,1	—	—	66	9,1	25
	16	3	49	7,9	1,38	16,0	62	9,9	2	0,3	—	—	36	5,8	13
	16	5	43	6,8	1,19	13,7	85	11,6	1	0,1	—	—	14	1,9	8
	16	9	52	8,2	1,62	40,9	96	39,3	2	0,8	—	—	2	0,8	25
	16	13	47	7,5	1,25	35,6	98,5	35,2	0,5	0,2	—	—	1	0,3	11
18 Сом	4		—	—	—	3,3	90	3,0	5	0,2	—	—	5	0,2	4
	6	1	—	—	—	24,0	45	10,8	3	0,7	—	—	52	12,5	1
	16	1	—	—	—	27,3	37	10,1	2	0,5	—	—	61	16,7	4
	16	10	—	—	—	52,5	95	49,9	2	1,0	—	—	3	1,6	4

Key to Table I:

1. Change in the indices of the blood of fish with change in water temperature
2. Fish
3. Water temperature, t^{o}
4. Duration of increased temperature, days
5. Hemoglobin
6. G % (transliteration - translator)
7. Erythrocytes, millions per mm^3
8. Leucocytes, thousands per mm^3
9. Composition of leucocytes
10. Lymphocytes
11. Monocytes
12. Polymorphonuclear elements
13. Neutrophils
14. Number of fish studied
15. Thousands per mm^3
16. Rainbow trout
17. Carp
18. Catfish

reared in floating river-hatcheries in the Narva River. For a period of 15 days the temperature of the water in the river fluctuated between 13-16°. The number of leucocytes in trout averaged 26.7 thousand per 1 mm^3 of blood.

Thus, at an increased water temperature quite a large number of leucocytes was detected in the blood whereas reduced temperature caused a drop in the number of white blood cells for all of the fish under consideration. However, the decrease in the number of leucocytes in carp and catfish when the temperature was 1-4° was very significant (to 1/10 - 1/20); with an increase in temperature there was an increase in the number of leucocytes in the first days of this period, being generally at the expense of the neutrophils, initially.

But in the winter, the number of leucocytes in trout was only 2/3 - 1/2 of that in trout living at a temperature of 13-16°. When trout remained for one day at a temperature of 14°, this did not cause a rapid reaction in the blood.

The cause of the non-uniform reaction of the white blood must be sought in the various behaviors of fish towards low water temperatures. In the winter, when the temperature drops to 4° and lower, carp and catfish stop taking food and their metabolism drops sharply.

Trout, on the other hand, belong to fish which, because of a high rate of metabolism (Strel'tsova, 1953), carry on an active mode of life, swim freely and search for food during the entire winter, i.e. their physiological state is qualitatively different from that of carp and catfish in the same conditions.

The white blood cells are direct participants in the inner metabolic processes of the organism (Kassirskii, 1955). Accordingly, the number of white blood cells in the blood depends on the rate of general metabolism. This, apparently, explains the significant drop in the number of leucocytes in carp and catfish as compared with trout, given low temperature.

An increase in temperature helps carp and catfish to make the transition from a passive to an active state. This transition requires a /34 certain length of time during which the fish again acquires all of its vital functions.

In the blood this period is marked by a sharp increase in the number of neutrophils and, later, the gradual disappearance of them and an increase in the number of leucocytes to a normal state. Apparently, the appearance of a large number of cells with neutrophilic granularity reflects the changes which have begun in the metabolic processes of the organism relative to the transition to an active mode of life. It is interesting to note that a sharp increase in the number of neutrophils in the blood is even detected during an increase in water temperature from 4 to 6° (in catfish).

In trout, an increase in the temperature of water does not cause an abrupt change in leucocytic composition.

As may be seen from Table 1, change in water temperature does not cause definite deviations in the amount of hemoglobin and number of erythrocytes either in trout or carp. While determining these blood indices, considerable individual fluctuations were observed. In individual carp living at a temperature of $1\text{-}4^{\circ}$ the quantity of hemoglobin rose to 65% whereas in others this was not observed. The average figures did not show a pronounced increase.

On the basis of the experiments conducted on the effect of water temperature on the blood of fish, it may be concluded that temperature has a definite effect both on the development of blood in the larval period and in the cellular composition of the blood of older fish, the reaction of the blood depending in many respects on the behavior of the entire organism towards certain temperatures.

Effect of Starvation and Heightened Feeding on the Blood and Blood-Formation in Salmon and Carp

The admission of food matter into an organism is one of the basic conditions which ensure normal vital activity in an animal. The absence and inadequacy of feeding leads to disturbance in the balance between the two sides of metabolism, to a predominance of disintegration over synthesis, which leads to the exhaustion of the organism. Naturally, such shifts in the organism must be reflected on the peripheral blood and the functional state of blood-forming organs.

The study of the effect of starvation on the blood and blood-formation in fish has been inadequate.

To explain the reaction of the organs of blood-formation and peripheral blood on starvation we attempted to answer two questions. On the one hand, to determine the effect of starvation on the development and formation of blood in the early stages of ontogenesis, i.e. in the period of transition in the larvae from yolk feeding to active feeding.

And, on the other hand, to explain the changes in the blood and blood-formation, affected by starvation, in fully formed fish.

Effect of Starvation on the Development of the Blood in Baltic Salmon Larvae /35

To explain the effects of starvation on the formation of the blood in early ontogenesis we chose Baltic salmon larvae as our subject. The larval period of salmon is very extended and this facilitated the observation of deviations in the process of the formation of the cellular composition of blood.

The study was conducted at the Navra fish hatchery in 1954-55. Experiments which were set up both in 1954 and 1955 produced the same results. We shall present the data for 1955.

At the Navra fish hatchery the juveniles were reared in floating river nurseries. Five hundred larvae were isolated for the experiment. They were placed in a separate nursery which was situated alongside of the commercial nurseries. Larvae from the commercial nurseries served as controls.

The formation of the cellular composition of the blood of salmon larvae during starvation is presented in Figure 16 and in larvae which feed normally, in Figure 14.

Prior to feeding, i.e. on the 25th day after hatching, the blood pictures of larvae from the experimental and control groups were

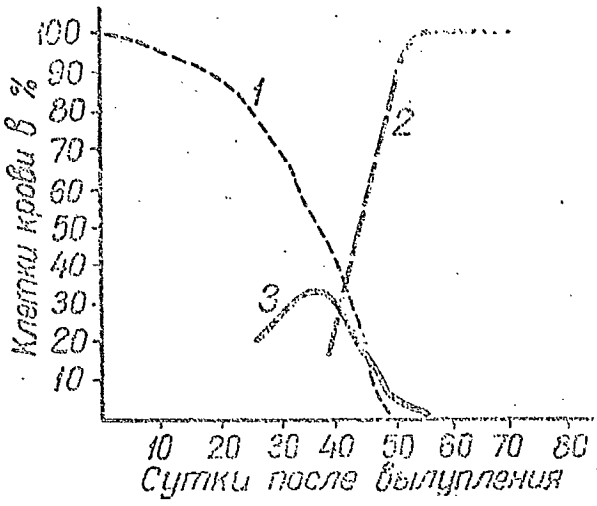

Figure 16. Changes according to age in the red blood of young salmon during starvation: 1 - primary erythrocytes; 2 - secondary erythrocytes; 3 - immature forms of secondary erythrocytes

the same. Primary erythrocytes comprised the bulk of the red blood cells. Approximately an average of 20% was accounted for by the cells of secondary erythropoiesis which had appeared but were immature. The greater part of these consisted of polychromatophilic erythrocytes and the smaller part, of basophylic erythrocytes and normoblasts.

When the transition to seizing food was made, the control larvae /36 were given the eggs of sprats, small oligochaetes, spleen and dry Daphnia. No food was placed in the experimental nursery.

Seven days after the beginning of feeding (on the 37th day after hatching) a difference could already be detected between the blood of the larvae which were eating and that of the starving larvae. By that

time the yolk sac in both groups of larvae had still not been completely resorbed. It was detected in the form of a narrow strand along the ventral part of the body. A large number of the blood cells of the feeding larvae was already represented by secondary erythrocytes (46%), and primary erythrocytes totalled no more than 20-26%. That is, the replacement of the cells of primary erythropoiesis was approaching the end. Immature cells of secondary erythropoiesis comprised 34%.

A delay in the development of the blood was observed in the starving larvae. The majority of the mature blood cells were still primary erythrocytes (50%). Secondary mature erythrocytes comprised only 17% and their immature forms, 33%. Thus, the absence of food in the period of mixed nutrition caused a delay in the development of the blood of the larvae.

On the 19th day after the beginning of the period of mixed nutrition (49 days after hatching) no trace of the yolk sac was observed either in the controls or in the experimental larvae. The control larvae ate actively. No food was placed in the experimental nursery.

The starving larvae noticeably lagged behind in growth. Their average length was 28.7mm whereas the lengthh of the feeding larvae was 31.7mm.

In the blood of the control larvae primary erythrocytes are no longer seen, however intensive secondary erythropoiesis continues (until the immature cells comprise 20-25% of the blood). Hence, the number of erythrocytes is insufficient in order to achieve breathing and

all the new lots of maturing secondary erythrocytes enter the blood in proportion to growth and development.

The blood picture of starving larvae is different. The replacement of primary erythrocytes by secondary erythrocytes has also been completed. However, at the same time, secondary erythropoiesis has also been sharply reduced. In blood smears 1-4% polychromatophilic erythrocytes, at the threshold of maturation, are encountered. No other immature elements of red blood were detected. After another five days neither were polychromatophilic erythrocytes observed. The entire mass of blood consisted of mature secondary erythrocytes. In feeding larvae an intensive replenishment of the erythrocytes of the blood continues; immature forms in smears comprise approximately 20%.

Thus, the replacement of primary erythrocytes by secondary erythrocytes occurs in starving larvae solely on the basis of yolk nutrition, but is somewhat retarded in comparison with normally feeding larvae. After the residues of the yolk sac have been completely resorbed there is a halt in the blood-forming process in the starving larvae, /37 whereas in the feeding larvae intensive blood-formation is observed which continues with a certain degree of deceleration during the entire first summer.

Effect of Starvation on Carp Fingerlings

Keeping tench, bream and goldfish in conditions of prolonged starvation, Schlicher (1927) did not detect sharp changes in the quantitative indices of the blood of these fish as compared to normally feeding fish.

Puchkov and Federova (1951), having subjected carp to 20-day starvation, also did not find changes in the quantity of hemoglobin, erythrocytes, leucocytes and the composition of the leucocytic formula. Only the phagocytic index changed.

Hence, according to the data provided by these authors, starvation for a period of several weeks did not reflect on the quantitative indices of the red blood of fish.

By means of a study of blood smears and impressions of the blood-forming organs of starving fish we succeeded in eestablishing, as will be shown below, that starvation has an effect on blood-formation, reflecting in this case on the qualitative composition of the peripheral blood cells.

The effect of starvation on the blood of juveniles which were fully formed was determined for small carp. The juvenile carp were reared in ponds at the Central Experimental and Commercial Station of VNIORKh - "Ropsha".

Prior to the setting up of the experiments blood was taken from 30 one-month-old juveniles and smears were prepared. It is impossible to determine the content of hemoglobin and erythrocytes in such carp due to their small size (22-25mm).

Thirty days after hatching the red blood of carp consisted mainly of secondary erythrocytes and, their immature forms comprised only 10%. In the majority of cases these were polychromatophilic erythrocytes and individual basophilic erythrocytes and normoblasts. Few leucocytes were detected - approximately 1% with reference to the erythrocytes. Lymphocytes

comprised the main part (93%), leucocytes with very fine neutrophylic granularity were calculated at approximately 3% and monocytes, approximately 4%.

In the experiment 50 fingerlings were placed in two aquariums, 25 in each.

Aquarium No. 1 (control group) was given plenty of small chironomid larvae and oligochaetes. Aquarium No. 2 (experimental group) did not receive food.

Eight days after the beginning of the experiment blood smears and kidney impressions were examined from 10 fish from aquarium No. 1 and 10 from aquarium No. 2.

Aquarium No. 1. Heightened feeding of the fingerlings caused intensive erythropoiesis. Immature erythrocytes in the smears comprised approximately 40%. Among these one can observe all stages of maturation /38 of erythrocytes - from erythroblasts to polychromatophilic erythrocytes. No marked changes are observed in the white blood. With reference to erythrocytes, leucocytes as before comprise 1-2%.

An enormous number of cells of the erythropoietic series were detected in the kidneys. The late stages of maturation are observed most frequently - basophilic and polychromatophylic erythrocytes - that is, cells which are encountered in the peripheral blood in large numbers. However, earlier stages of erythropoiesis are also quite often detected - erythroblasts of various sizes (from 8.5X8.5 μ to 14X14 μ in diameter). Mitotic divisions of parental cells and erythroblasts are observed quite frequently.

Cells of the leukopoietic series are considerably fewer in number. Among them both mature and immature forms are encountered.

There are a large number of immature forms of erythrocytes in the blood and kidneys, and the frequent occurrence of mitotic division of cells indicates a vigorous regeneration of red blood cells.

Aquarium No. 2. The picture of the red blood and blood-forming organs is completely different for the starving fingerlings. Immature forms of erythropoiesis are lacking completely. All of the blood consists of mature erythrocytes filled with hemoglobin. At first glance one gets the impression that the blood was taken from mature fish and doubt arises only because of the small size of erythrocytes which is a peculiarity of the fingerlings.

There are no definite changes in the composition of white blood as compared to that of the feeding fingerlings. The relative quantity of lymphocytes increases somewhat at the expense of other white blood cells.

When examining the impressions of the kidney, attention is first drawn to the large number of cells with very fine rosy-yellowish granularity. These cells which are called neutrophils in mature carp are smaller, with smaller granules. Sometimes, the granularity is so fine that it is impossible to examine it. Only the slight rosy-yellowish color of the cytoplasm is detected. The nucleus of these cells is usually oval and is located at the edge, at times having two lobes. Cells with larger yellowishreddish granularity is exceptional. Cells of the erythropoietic series are considerably fewer in number than those of the leucopoietic

series, some of the erythroblasts and basophilic erythrocytes, apparently, being destroyed. They become smaller and the color of their nucleus and cytoplasm becomes darker. Large erythroblasts are lacking and polychromatophilic erythrocytes are encountered rarely.

Thus, starvation for a period of eight days led to a sharp reduction in erythropoiesis and to an increase in the quality of neutrophils in the kidney.

After the blood was analyzed food was placed in larger quantities not only in aquarium No. 1 but also in aquarium No. 2. After eight days /39 one could detect considerable changes in the blood of the experimental and control carp fingerlings. The feeding of the fingerlings was intensified for a period of 16 days. Whereas in the previous analysis a large number of immature erythrocytes was detected in the blood of these fingerlings, this time the young forms comprised no more than 5-6%. Blood-formation was reduced considerably.

Aquarium No. 2. The fingerlings starved for a period of eight days and in the following eight days were fed intensively.

In all blood smears a large number of immature forms of the erythropoietic series was detected, comprising on the average one half of all the red blood cells. Vigorous blood-formation occurred. No changes were noted in the leucocytic formula.

After 10 more days, during which the fingerlings in both aquariums continued to feed, no definite deviations were observed in the blood of

fingerlings in aquarium No. 1. The intensity of blood formation in aquarium No. 2 decreased; however, up to 15% immature erythrocytes were observed in the blood.

The experiments show an undisputed relationship of blood formation in fingerlings to feeding and starvation. Intensified feeding for several days leads to a considerable rise in blood formation whereas starvation causes a temporary reduction in the activity of the blood-forming organs. All of these phenomena are very clearly-reflected in the peripheral blood picture.

The considerable relationship between blood formation and feeding in fish is supported in the results of experiments in which older fingerling carp (8-9 months) were used.

After the carp had been kept in water at a temperature of 1-2° for one month during which conditions they did not take food and were in an inactive state, the fish were transferred to aquariums in which the water temperature was 14°. Chironomid larvae* were given in abundance every day to aquarium No. 3, with the carp beginning to take this nourishment only 4 days after being transferred to warm water. In aquarium No. 4 the fish did not eat.

An analysis of the blood on the 15th day after the carp had been transferred to higher temperature conditions showed the following: A vigorous regeneration of the blood began in the feeding fish (up to 40%

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*Read bloodworm. Translator.

immature erythrocytes were detected in the peripheral blood). On the other hand, in starving fish no signs of heightened blood formation were observed. The number of leucocytes increased both in the control and experimental fish in relation to an increase in water temperature.

Thus, despite the increased temperature and, consequently, a certain increase in the rate of metabolism, intensified blood-forming activity did not occur in the starving fish whereas a considerable intensification in the work of the blood-forming organs was observed in the feeding fish.

A month later, the vigorous blood-formation in the feeding carp /40 dropped considerably and immature forms of erythrocytes in the peripheral blood constituted about 13%. After another month the number of immature forms in the blood of feeding fingerlings was reduced to a minimum. Single polychromatophilic erythrocytes were encountered in the smears.

During all of this time no signs of blood formation in the blood were detected in the starving fingerlings, however neither were signs of anemia observed. The quantity of hemoglobin and erythrocytes was maintained at a constant level. Only after four months did the content of hemoglobin and erythrocytes fall in starving fingerlings, however intensification in blood formation was absent as before.

On the basis of the experiments conducted on the effect of starvation and heightened feeding on the blood and blood formation in fish the following may be stated. The absence of timely fattening of larvae during the change over to active feeding causes a delay in blood

formation and a parallel delay in the development of the entire organism. However, the substitution of embryonic and larval primary erythrocytes by normoblastic secondary erythrocytes occurs, though with a certain time lag, on the basis of a yolk diet alone. After the yolk sac has been resorbed, secondary erythropoiesis in the starving larvae ceases.

Starvation in month old immature carp suspends the formation of erythrocytes in several days. On the other hand, heightened feeding causes a vigorous regeneration of red blood.

Erythropoiesis is connected with the feeding of fish and less with the change in water temperature. When carp are transferred from low temperature to higher temperature a considerable increase in blood formation is only observed in the feeding fish. The change in temperature did not bring about an intensification in erythropoiesis in starving fish.

The number of leucocytes in the blood is related to a greater degree to the temperature of the water in which the fish is found than to the amount of consumed food. When the water temperature for both the starving and feeding fish is the same, a constant number of leucocytes was observed. However, a change in water temperature caused a change in the number of leucocytes, as was seen in the experiments on the effect of water temperature on the blood of fish.

Effect of the Nature of Food on the Blood and Blood Formation of Rainbow Trout

The rearing of fish on synthetic food frequently leads to illnesses

connected with the absence or inadequacy of certain components (vitamins and mineral salts) in the food. This leads to a disturbance in the activity of various organs and, in particular, to a disturbance in the function of blood formation which entails the development of anemia.

Having studied the blood of immature sturgeon living in ponds and feeding on plankton and benthos, and the blood of fish reared in basins on oligochaetes, Drabkina (1951) showed a pathological deviation in the blood of immature fish which fed on oligochaetes alone. The absence /41 in oligochaetes of a number of vitamins and mineral salts (especially iron salts) led to a sharp reduction in hemoglobin (less than 10%) and erythrocytes in the blood of immature sturgeon. Hemolysis of the red blood cells was detected in the smears.

Anemia in rainbow trout, reared on poor quality synthetic foods, has been described by a number of authors (Hoffmeyer, 1907; Phillips, 1940). However, in examining the blood, these authors only employed data on the content of hemoglobin and erythrocytes and did not deal with the qualitative nature of red and white blood.

In order to control phenomenona of avitaminosis accurate indices are required for the physiological state of the organism of fish and, in particular, of blood. Accordingly, in addition to quantitative determinations of hemoglobin and erythrocytes it is also necessary to pay attention to the qualitative composition of blood cells which reflects the intensity of activity of the blood-forming organs of fish. As will be shown below, the composition of red and white blood cells is a more

sensitive index of the degree of illness in an organism than is the quantitative data on the hemoglobin and erythrocyte content.

A rainbow trout was kindly submitted to us by a colleague at VNIORKh, K.A. Faktorovich. This rainbow trout was used in experiments conducted at Ropsha to explain changes in fat exchange in the liver under the influence of different foods (Faktorovich, 1956).

We had trout which was reared on natural foods and trout fed on synthetic mixtures (spleen, horse meat, fish, barley meal and meat-and-bone meal, oilcake and bran).

There were various age groups among the fish being studied (from age 1 to age 2+). The hematological picture of the studied trout is given in Table 2 in which the data is arranged in accordance to the degree of disturbance in the blood, i.e. fish with the least deviation in the blood from the norm are in the upper part of the table and fish with the greatest deviation, in the lower part. No fewer than three smears were prepared from the blood of each fish. Thus, the data on the morphology of red and white blood represent the average of three figures.

From Table 2 it is seen that trout which was fed on natural foods possesses a normal amount of hemoglobin (58-61%) and erythrocytes (1.25-1.33 millions per $1mm^3$). Immature erythrocytes are encountered very rarely; they constitute less than 1%. Among the white blood cells lymphocytes predominate (90-95%) and polymorphonuclear leucocytes and monocytes only account for 5-10%. This state meets the norm (Figure 17a).

The blood picture of fish fed on synthetic mixtures indicates the presence of anemia which is more or less defined. Doubt is raised only /42-43 in the case of trout which possess quite a high content of hemoglobin (53%) and have a very small quantity of immature elements in the blood (1.0%). A shift in the leucocytic formula in the direction of a relative decrease in the number of lymphocytes and increase in the number of polymorphonuclear cells and monocytes may be due to the spawning state.

There is no doubt of the presence of anemia in the remaining fish./44 From the data on fish presented in the Table it is seen that anemic fish are found in various states. In some, anemia is quite weak and in others it is more acute. The following three stages of anemia in the examined fish may be isolated, based on data on the blood and the blood-forming organs.

Stage I. The trout has red gills and red blood which does not differ in external appearance from the blood of healthy fish. Quantity of hemoglobin and erythrocytes is somewhat reduced, however it is close to the norm. Quite a large percent of immature erythrocytes (up to 46%) is detected in the blood smears. Most of the immature erythrocytes consist of polychromatophilic erythrocytes which are on the threshold of maturation and conversion to mature forms (Figure 17b). A small relative decrease in the number of lymphocytes (75-79%) is detected in the leucocytic formula.

In the kidney of fish which are in the first stage of anemia a mass of cells of the erythropoietic series is observed in various stages of maturation. These are mainly polychromatophilic and, to a lesser degree,

Table 2

1 Изменения показателей крови радужной форели в зависимости от характера пищи

2 Корм	3 Пол	4 Стадия зрелости	5 Гемоглобин %	5 Гемоглобин г %	6 Эритроциты млн/мм³	7 Лейкоциты тыс/мм³	8 Незрелые эритроциты	9 Незрелые эритроциты по группам, в % 12 эритробласты	13 нормобласты	14 базоф. эритроциты	15 полихромат. эритроциты	10 Лейкоцитарная формула в % 16 Лимфоциты	17 Полиморфноядерные лейкоциты	18 Моноциты	19 Стадия анемии
20 Естественный	22 Самка	II	61	9,7	1,33	21,0	35 един.	0	0	0	един.	95	5	2	
		—	58	9,2	1,25	25,0	"	0	0	0	"	90	9	1	
21 Искусственный	23 Самец	V	53	8,4	1,02	28,0	10	0	0	0	10	76	17	7	
	"	III	—	—	—	—	12	0	0	1	11	77	10	13	I
	22 Самка	III	42	6,7	0,81	30,5	35	0	2	10	23	75	18	7	
	23 Самец	II	38	6,1	0,8	23,2	46	0	6	19	21	79	16	5	
21 Искусственный	22 Самка	II	24 меньше 10	1,5	0,23	16,0	60	2	10	38	10	77	22	1	II
	"		меньше 10	1,5	—	—	70	2	10	46	12	70	23	7	
	23 Самец		меньше 10	1,5	0,25	10,5	85	1	7	48	29	70	25	5	
21 Искусственный	22 Самка	III	меньше 10	1,5	—	—	10	1	3	4	2	32	50	18	
	"	III	меньше 10	1,5	0,29	54,0	3	3	0	0	0	48	14	38	III
			меньше 10	1,5	0,15	23,2	0	0	0	0	0	26	50	24	
	23 Самец	II	меньше 10	1,5	—	—	0	0	0	0	0	18	20	62	

Key to Table 2:

1. Changes in the indices of the blood of rainbow trout in relationship to the nature of food.
2. Food
3. Sex
4. Stage of maturity
5. Hemoglobin
6. Erythrocytes, millions per mm^3
7. Leucocytes, thousands per mm^3
8. Immature erythrocytes
9. Immature erythrocytes by groups, in %
10. Leucocytic formula in %
11. g% (transliterated - translator)
12. Erythroblasts
13. Normoblasts
14. Basophilic erythrocytes
15. Polychromatophilic erythrocytes
16. Lymphocytes
17. Polymorphonuclear leucocytes
18. Monocytes
19. Stage of anemia
20. Natural
21. Synthetic
22. Female
23. Male
24. Less than
25. One

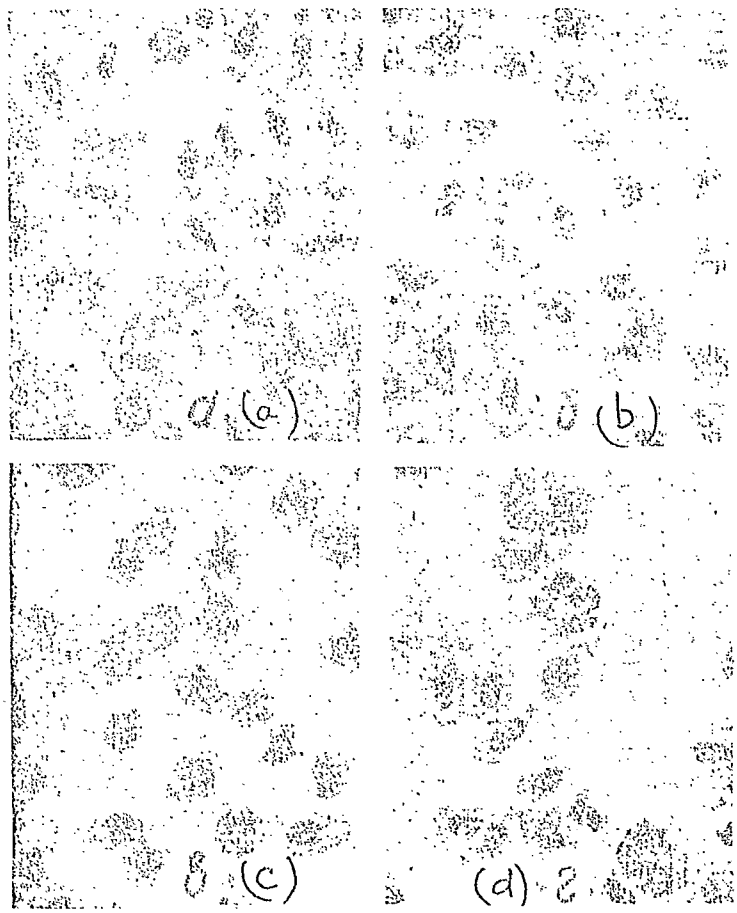

Fig. 17. Blood of a healthy and a sick rainbow trout (Eyepiece 7x, lens 90x): a - blood of healthy rainbow trout; b - blood of sick rainbow trout. Stage I of anemia. Immature forms of erythrocytes are present; c - blood of sick rainbow trout. Stage II of anemia. Large number of immature forms of erythrocytes in the early stages of maturation; d - blood of sick rainbow trout. Stage III of anemia. Immature forms of erythrocytes are absent. At the bottom to the right and at the top, vacuolized monocytoid forms. Polymorphonuclear leucocyte with a segmented nucleus.

basophilic erythrocytes. Erythroblasts, hemocytoblasts and their mitotic divisions are quite often encountered. The white blood cells are represented by mature polymorphonuclear leucocytes, however they are very few in comparison with the cells of erythropoiesis. Heightening of leucopoiesis is not observed.

A large quantity of polychromatophilic and basophilic erythrocytes are detected in the spleen. However, early forms of erythropoiesis, i.e. erythroblasts, are very rarely encountered. Thus, essentially the same cells which are present in the peripheral blood are present in the spleen. Single erythroblasts only point to the possibility of very slight erythropoiesis.

The state of peripheral blood and blood-forming organs indicate an active resistance of the organism against the onsetting anemia. Heightened blood formation by the organs maintains the quantity of erythrocytes and hemoglobin at quite a high level, with the major formation of blood cells occurring in the kidney.

Stage II. The blood is pale rose in color and the gills are tinged pale rose. The total quantity of blood in fish in the second stage of anemia is distinctly less than in fish in the first stage of anemia. It is very difficult to get enough blood from the gill vessels in order to determine all of the indices.

The quantity of hemoglobin and erythrocytes is sharply reduced. The hemoglobin content is so small that it cannot be determined by a Sahli hemometer, i.e. it is less than 10%. The number of erythrocytes does not

exceed 0.25 million per $1mm^3$ of blood. Attention is drawn to the extremely small number of mature erythrocytes and the sharp increase in immature forms (up to 85%) in the smears, with basophilic erythrocytes predominating, being somewhat smaller in size than usual. Among the basophilic and /45 polychromatophilic cells one quite often detects large elongated cells with a constriction in the nucleus or cells with two nuclei (Figure 17c) and, finally, cells with a constriction in the cytoplasm. Apparently, this is amitotic division which is possible during vigorous regeneration of blood cells.

In the impressions of the kidney in this stage a significant predominance of red blood cells over white blood cells, as in stage I, is also observed. However, they consist mainly of basophilic erythrocytes. Mature erythrocytes are encountered still more rarely than in the blood. There are many erythroblasts and hemocytoblasts. Both mitotic and amitotic divisions are detected.

The composition of the cells of the spleen reflect the composition of the cells of the peripheral blood. There are many basophilic erythrocytes, fewer polychromatophilic erythrocytes and very few mature erythrocytes. Erythroblasts are lacking and, therefore, blood formation is also lacking.

In stage II, more profound disturbances are detected than in stage I. However, the large number of immature forms in the blood and kidney still indicate an active state of the blood-forming organs.

Stage III. The color of the blood and gills of the fish in this stage in no way differs from that of stage II. There is no difference in the quantity of hemoglobin and erythrocytes, being just as small as in stage II. However, a completely different picture is observed in the blood smears and impressions of the kidney and spleen. Immature forms of erythrocytes are almost completely lacking in the blood (Figure 17d). Only rarely are very early forms of erythropoiesis encountered in some fish. The majority of the cells consist of mature erythrocytes with cytoplasm which stains a yellowish-rose color. The erythrocytes frequently become deformed in a smear. Among the white blood cells both the relative and absolute number of lymphocytes decreases sharply. The latter comprise only 18-48%. On the other hand, the number of polymorphonuclear cells and monocytes increases considerably, with cells with a very segmented nuclei predominating among the polymorphonuclear cells. In addition, monocytoid cells, which normally are not encountered, appear in the blood. These are large, frequently vacuolized and deformed, cells with irregular cytoplasm which stains dark blue and with a branched nucleus which is generally not detected in monocytes. Their nuclei are considerably larger in size than those of polymorphonuclear cells. The cytoplasm stains in a darker color.

In contrast to the first two stages of anemia, the connective tissue of the kidney is generally not rich in blood elements. Late stages of erythropoiesis - basophilic and polychromatophilic erythrocytes - are almost lacking. Erythroblasts are encountered here and there, however the majority of them are vacuolized or deformed. Their cytoplasm which /46

is usually a deep dark blue is slightly decolorized. On the other hand, a large number of polymorphonuclear cells (as compared to erythrocytes), having a considerably or slightly branched nucleus, are observed. There are many monocytoid forms which are similar to those which we detected in the peripheral blood.

Immature forms of erythropoiesis are absolutely lacking in the spleen. Only mature erythrocytes are detected in the spleen, i.e. as before. The picture corresponds to the peripheral blood in which the main part of the red blood is represented by mature forms.

From the foregoing it follows that fish which were fed on natural food did not have deviations from the norm in the blood, whereas those fed on synthetic mixtures in which important food nutrients were obviously lacking developed anemia.

We traced three successive stages of anemia in rainbow trout and the reaction of the blood-forming organs in each stage.

Stage I of anemia is characterized by a certain reduction in the quantity of hemoglobin and erythrocytes and, correspondingly, an increase in the intensity of blood formation.

Stage II is the highest point in the activity of the blood-forming organs. Stage III indicates the exhaustion of the blood-forming organs and the depression of erythropoiesis. This stage is the most serious since in the first two stages the organism actively struggles against the deficiency in hemoglobin and erythrocytes whereas in the third stage it is passive.

Whereas stage I differs from stage II both visually and in the quantity of hemoglobin and erythrocytes, stages II and III cannot be distinguished, given the existing method of determining hemoglobin and erythrocytes. However, the quality of the composition of blood cells determined in the smears indicates a difference in the physiological state of fish in stage II and III despite the same quantitative data on the blood. In stage II there is an intensive regeneration of blood whereas in stage III depression in the process of erythropoiesis is observed.

An examination of the impressions of the kidney and spleen of rainbow trout in all three stages of anemia showed that the composition of the blood cells of spleen always resembles the composition of the peripheral blood. Early forms of blood formation - hemocytoblasts and erythroblasts - are only encountered in stage I and, even so, very rarely. The kidney plays the leading role in the formation of erythrocytes as well as leucocytes in both sick and healthy rainbow trout.

DISCUSSION OF THE DATA /47

In the textbooks and scientific literature published in the USSR the opinion has been established that the main and even only organ of erythrocyte formation in bony fish is the spleen (Zavarzin, 1945; Zavarzin and Rumyantsev, 1946; Zavarzin and Shchelkunov, 1954; Khlopin, 1946; Pestova, 1954; Puchkov, 1954). However, in the works of a number of foreign authors in the past, in a study by Rumyantsev (1939) and,

recently, in the published studies of Catton (1951) and Topf (1953, 1955), it was demonstrated that the formation of erythrocytes in the majority of bony fish takes place in the connective tissue of the kidney. Thus, the opinion that the spleen is the predominant organ in the formation of erythrocytes in all bony fish must be reexamined.

In Table 3 we give the results of authors who have been directly involved with the study of the blood-forming organs of bony fish. In this Table we also include our own data.

As may be seen from the Table, the majority of the authors conclude that the main organ of blood formation in bony fish is the kidney. According to Jordan and Speidel (1924) as well as Catton (1951) blood formation in various fish occurs either in the kidney or in the spleen. However, in the conclusions of these studies it is stated that the kidney must be regarded as the main organ of erythropoiesis in bony fish. The spleen is of secondary importance. This conclusion is again asserted by Jordan in his review on comparative hematology (1938).

The Table shows that the dominant role of the kidney in the formation of erythrocytes in carp is indisputable. We know about the blood formation in Salmonidae from the work done by Catton who studied the formation of blood cells in trout and who concluded that both the kidney and the spleen are equally active in blood formation in these fish. In the study by Catton it is pointed out that immature forms of erythrocytes are not observed in the blood of the mature trout which were examined. Hence, it may be concluded that active erythropoiesis

was lacking in these fish at the moment of examination. Determination of the location of the formation of red blood cells during the absence of active erythropoiesis may result in unreliable conclusions since the number of early forms of erythrocytes in the blood-formation organs during such periods is sharply reduced.

In the kidney of salmon and trout during the active period of /48-49 erythropoiesis we always observed a certain number of immature early forms of the erythropoietic series - erythroblasts - whereas these forms were very rarely encountered in the spleen. Only late stages of erythropoiesis, which are also observed in the peripheral blood, were detected in large numbers in the impressions of the spleen. On the basis of this one may conclude that the main organ of erythropoiesis in salmon and trout as well as in carp is the kidney.

It should be noted that the majority of authors who are engaged in the study of the blood-forming organs of fish usually do not take into account the state of erythropoiesis; at least, in the majority of studies there is no indication of the intensity of the process of blood formation. Meanwhile, the state of erythropoiesis or leucopoiesis can be of decisive importance when determining the site of red or white blood cell formation in fish. Usually, heightened erythropoiesis rarely reduces leucopoiesis and, on the other hand, during heightened leucopoiesis one does not detect a large number of early forms of erythropoiesis in the blood-forming organs. The alternation of heightened erythropoiesis and leucopoiesis is also observed in the embryonic and larval period.

Table 3

/48

/ Органы кроветворения у костистых рыб по данным различных авторов

2 Название рыб	3 Место основного кроветворения	4 Автор
Leuciscus alburnus 5	Почка + селезенка	Bizzozero u. Torre, 1884
Carassius auratus (золотой карась) goldfish 6	Почка + селезенка	" "
Tautoga onitis	Селезенка	Jordan a. Speidel, 1924
Stenotomus chrysops (скап) scup 7	Селезенка	" " "
Fundulus heteroclitus	Селезенка	" " "
Carassius auratus (золотой карась)	Почка	" " "
Ictiobus bubalis (из чукучановых) 8	Почка	" " "
Paralichthys dentatus (из камбаловых) 9	Почка	" " "
Leuciscus cephalus (голавль) 10	Почка	Румянцев, (Rumyantsev) 1939
Trigla hirundo	Почка	Duthie, 1939
Trigla cuculus	Почка	" " "
Labrus bergulta	Почка	" " "
Labrus mixtus	Почка	" " "
Щука 11	Селезенка	(Khlopin) Хлопин, 1940
Ctenolabrus rupestris	Почка	Catton, 1951
Trigla cuculus	Почка	" " "
Spinachia vulgaris	Почка	" " "
Cottus bubalis	Почка	" " "
Salmo trutta (форель) 12	Почка + селезенка	" " "
Perca fluviatilis (окунь) 13	Селезенка	" " "
Rutilus rutilus (плотва) 14	Почка	" " "
Cyprinus carpio (карп)	Почка	Topf, 1953
Cyprinus carpio (карп) 15	Почка	Topf, 1955
Perca fluviatilis (окунь)	Селезенка	(Pestova) Пестова, 1954
Salmo irideus (радужная форель) 16	Почка	Остроумова, (Ostroumova) 1957
Salmo salar (балтийский лосось) 17	Почка	" " "
Cyprinus carpio (карп)	Почка	Остроумова, 1957

Key to Table 3:

1. Blood-forming organs in bony fish according to the data provided by various authors.
2. Name of fish.
3. Main blood formation site.
4. Author.
5. Kidney + spleen.
6. Goldfish.
7. Scup.
8. From Catostomidae.
9. From flounders.
10. Chub.
11. Pike.
12. Trout.
13. Perch.
14. Roach.
15. Carp.
16. Rainbow trout.
17. Baltic salmon.

A study of the morphology of the blood of salmon and trout in early ontogenesis showed that each period has its characteristic forms. In the early embryonic period immature primary elements - proerythroblasts and erythroblasts - were observed. Later, they changed into primary basophilic and polychromatophilic erythrocytes and, finally, by the time of hatching the entire mass of red blood was represented by primary mature erythrocytes. There were no immature forms.

What is the origin of primary erythrocytes? Special studies have not been conducted on this question however certain assumptions can be made on the basis of published data.

Maksimov (1918) wrote that the first red blood corpuscles of the embryos of all animals, having a yolk sac, are formed in the cavities of the vessels of the latter. They are spherical and disc-like cells with a wide layer of cytoplasm and a small nucleus. During a considerable part of the life of an embryo they enter the circulation system and function as carriers of oxygen. Later, they are replaced by cells which emerge in the special blood-forming organs which have appeared (spleen, liver and, finally, marrow - in mammals). /50

The primary erythrocytes of fish are, apparently, analogous to the primary functioning of blood cells in other animals and are generally formed in the vessels of the yolk sac.

Apart from morphological features, embryonic blood in mammals also have their physiological features. In 1910, Vakulenko, using physical-chemical methods, established a difference between the embryonic

and mature form of hemoglobin in man (Likhnetskaya, 1950).

The works of Likhnetskaya (1950) and a number of other researchers have demonstrated that the erythrocytes in the blood of the embryos of mammals contain a special embryonic hemoglobin which has a great affinity to oxygen. Given low partial pressure, the percentage of saturation of the embryonic hemoglobin will be greater than the percentage of saturation of hemoglobin in mature man in the same conditions.

A comparison of our data on blood with the data of Privol'nev (1947, 1949) on the breathing of larvae and immature salmon would lead one to think that the primary erythrocytes in the larvae of fish also contain hemoglobin which has a considerable affinity to oxygen. The larval period in salmon - when larvae feed on the yolk sac - is characterized by a low critical tension of oxygen even during an increased rate in breathing. Later, the critical tension of oxygen for the immature fish increases in proportion to development. Since the blood of larvae consists of primary erythrocytes during the period of yolk feeding, then, apparently, these cells are also carriers of hemoglobin with high affinity for oxygen which can bind the necessary quantity of oxygen, given a low partial pressure of it, in water.

Thus, during the period of yolk feeding the red blood of the larvae is represented by primary erythrocytes which have morphological and physiological peculiarities and which are mainly formed in the walls of the vessels of the yolk sac.

In the period of transition to external feeding, while the yolk sac has not yet been completely resorbed, a transitional state is also

observed in the blood of the larvae. The primary erythrocytes gradually disappear giving way to secondary erythrocytes, and by the time that the transition to active feeding is complete and the larvae have become fingerlings only second erythrocytes and their immature forms are detected in the blood. In structure, the secondary erythrocytes are similar to /51 the cells of mature fish (differing only by being smaller) and, apparently, are already a product of a definite blood-forming organ. In all probability the kidney is the main site of blood cell formation in the majority of teleosts.

By the time the conversion to active feeding is complete the white blood also already consists of forms similar to the cells of mature fish. Thus, by the time of the transition to active feeding and transformation of larvae into fingerlings, the basic transformations in the blood are already taking place and, apparently, a definitive blood-forming organ is already functioning.

The transformtion in the composition of the blood cells of fish is parallel to the development of the entire organism. In salmon, the period of yolk feeding is extended and the blood develops slowly. In trout, together with a more rapid resorption of the yolk sac, there is a rapid change in blood cells. In carp, all of these processes are apparently still more rapid.

The rate of blood formation is not constant in each period of development. In the hatching period, there are no immature red blood cells in the smears. In the period of replacement of primary erythrocytes by secondary erythrocytes the rate of blood formation rises sharply (immature forms in the blood constitute up to 40%). A high rate of blood formation

is observed in the first 10-15 days of active feeding, however, later the activity of the blood-forming organs drops and during the entire first summer the immature forms of erythrocytes detected in the blood of the immature fish comprise 7-10%.

The sharp increase in the number of immature forms during this period indicates that the immature fish are in a poor state which may be caused by a deficiency or absence of necessary vitamins and mineral substances in the food, unfavorable temperature and oxygen conditions, contamination by parasites and a number of other external environmental factors.

The process of blood formation in the larval period as well as the composition of the blood and the rate of blood formation in fish which are completely formed depend to a great extent on the effect of various environmental factors on the organism.

The prolonged effect of low temperature (2-4^{o}) on the larvae of salmon considerably delays the development of the entire organism. The replacement of embryonic and larval blood cells by secondary erythrocytes and the resorption of the yolk sac take place at a considerably slower rate than in the control group. This process lasts more than 100 days whereas in the control group it only lasts 50 days.

Given normal temperature and an absence of timely fattening of salmon larvae, the supply of yolk sac is sufficient to transform the larva into a fingerling in external and internal features, particularly the blood. Thus, primary erythrocytes are replaced by secondary normoblastic erythrocytes on the basis of yolk feeding alone. However, /52

in the absence of fattening the growth of larvae in length is drastically slowed down and the nutritive substances in the yolk sac are basically used for development, i.e. the transformation of the larva into a fingerling. Not having received food on time, the immature fish are extremely small and look starved, however the red blood, following resorption of the yolk sac, already consists entirely of secondary erythrocytes. When the yolk sac has been resorbed blood formation in the starving immature fish ceases and immature forms of the erythropoietic series are not detected in the smears, whereas during the same period in the feeding of immature fish basophilic and polychromatophilic erythrocytes are observed comprising up to 25% and erythropoiesis is intensive.

The effect of different environmental factors is also reflected on the composition of the blood and the rate of blood formation in fully formed fish.

From the experiments conducted by Schlecher (1927), we know that the number of leucocytes in fish increases with increase in water temperature and decreases with decrease in water temperature.

On the whole, our data coincide with the data provided by Schlecher; however, in addition, we were able to ascertain that the effect of different temperatures results in different reactions on the part of the white blood of fish which behave differently towards low temperatures. Fish are cold-blooded animals and, correspondingly, low water temperatures cause a reduction in the metabolic rate and along with this a drop in the number of leucocytes in carp and catfish as well as in trout. However, in trout, which lead an active mode of life in the

winter, the number of leucocytes is only reduced to 2/3-1/2. In thermophilic fish - carp and catfish - which are in low-temperature water, the number of leucocytes decreases to 1/15-1/20, dropping to an extremely small number for fish (2-3 thousand per $1mm^3$ of blood). This may be explained by the sharp drop in the rate of metabolism and the transition of these fish to a qualitatively different passive state in conditions of low temperature.

When carp and catfish are transferred to conditions of high temperature there is a sharp increase in the number of neutrophyls in the days immediately following the transfer (from 0.3 thousand at a low temperature to 9.2 thousands when transferred to a higher temperature), but after several days the blood again acquires its usual lymphoid character. This phenomenon is not detected in trout.

Due to the lack of knowledge about the functional nature of cells with neutrophylic granularity in fish, it is impossible to ascertain the physiological importance of this phenomenon for the organism. On the basis that these cells correspond, in their nature, to the neutrophyls in mammals, as many authors will acknowledge, it is worth considering the following circumstance. In his book Hematology of Farm Animals (1940), Vasil'ev shows that an increase or decrease in the overall acidity in an organism causes considerable morphological changes in the blood. /53 During acidosis the appearance of a large number of neutrophyls is observed and during alkalosis a large number of lymphocytes is observed.

Possibly, with the transition of carp and catfish from a passive state, which lasted for several weeks, to a qualitatively different physiological state, changes in the pH factor in the organism take place in the first few days, causing acute neutrophilia which is later replaced

by heightened lymphocytosis.

It is interesting to note that the total number of leucocytes increases in carp transferred to warm water irrespective of the feeding of the fish. There was a parallel increase in the number of leucocytes in both the feeding fish and starving fish. Schlicher (1927) detected a similar phenomenon in perch and goldfish.

Puchkov and Federova (1951) noted that, in the case of carp, starvation for a period of 20 days did not change the quantitative and qualitative composition of leucocytes. According to our data there was no change in the composition of leucocytes of carp living at a constant temperature of 15-16^{o} even as a result of 4 months of starvation.

Thus, if starvation - be it quite prolonged - occurs at a constant temperature, it does not cause specific changes in the white blood of fish. On the other hand, changes in water temperature, irrespective of feeding and starvation, have an effect on the qualitative and quantitative composition of the leucocytes in the blood of fish.

How does temperature and starvation affect the red blood of fish?

Various authors have obtained different data on the quantity of hemoglobin and erythrocytes in fish. Schlicher states that these indices of the blood increase during the winter, whereas Schaeffer (1925) shows that they decrease. Ivlev (1955) concluded that the quantity of hemoglobin and erythrocytes during the winter depends on the behavior of the fish.

In our experiments no clear-cut deviations in these indices of the blood in either direction were observed as a result of change in temperature although there were cases of a considerable increase in the

quantity of hemoglobin and erythrocytes in carp in conditions of low temperature. The increase in these indices in carp during the winter cannot, of course, be explained by heightened blood formation since the passive state in which the fish live can hardly create conditions for the intensive functioning of the blood-forming organs. Schlicher explained this phenomenon by the thickening of the blood, by the depletion of water in it, or by the expulsion of erythrocytes from the blood supply. However, apparently, an increase in the quantity of hemoglobin and erythrocytes in fish as the result of low temperatures is not a regular phenomenon. The contradictory data of different authors are indicative of this.

Starvation in goldfish and perch for a period of 30 days /54 (Schlicher, 1927) and in carp for a period of 20 days (Puchkov and Federova, 1951) did not result in distinct changes in the blood. Schlicher noted that starvation reduces the consumption of oxygen, but it is not known whether this reflects on the red blood corpuscles.

We obtained the same picture in the initial months in our experiments. Starvation which lasted three months did not reflect on the hemoglobin and the number of erythrocytes and only with the passage of the fourth month did these indices drop considerably.

However, the determination of hemoglobin by means of a Sahli hemometer and the count of erythrocytes do not always reveal such an important phenomenon as the process of blood formation and the formation of new erythrocytes. A certain increase and decrease in the activity of the blood-forming organs usually goes unnoticed when red blood cells are counted in a Goryaev chamber whereas during an examination of blood

blood smears this is detected distinctly.

As the results of experiments have shown blood formation is closely related to starvation and feeding of fish. An increase in food causes vigorous regeneration or red blood cells and the cessation of feeding suspends erythropoiesis.

These data coincide with the results of experiments on the change in blood formation in rabbits which were starved (Machavariani, 1952; Zaitseva, 1956 and Poberii, 1956). The starvation of young rabbits led to the reduction and complete cessation of erythropoiesis in the very first days whereas leucopoiesis continued, though with some slowing down. The parenteral introduction of protein led to heightened erythropoiesis in the marrow. On the strength of this the authors conclude that cessation in erythropoiesis during starvation is explained by the disturbance in this process in view of the absence of the admission of protein from without.

The cessation of blood formation due to starvation in fish and rabbits apparently has a common cause. However, we cannot agree that the abrupt inhibition of blood formation in the very first days of starvation is caused by the absence of supplies of protein or any other nutrients to the organism.

An additional experiment was conducted to demonstrate this. For a period of several days food was not given to five carp fingerlings. An examination of the blood smears showed that immature forms of erythropoiesis in the blood were lacking. Following this a large quantity of blood was taken from the caudal artery of all five fish. Thus, artificial anemia was caused. In the days that followed the carp continued to fast, and

in spite of this vigorous regeneration of blood began (immature forms in the blood comprised up to 50-60%).

Hence, it may be concluded that the rapid reduction in erythropoiesis in the early days of starvation is explained not by the absence of a particular product but by a sudden decrease in the requirement by the organism of a supply of new erythrocytes in the blood. Thus, the result is not a /55 disturbance in blood formation but a suspension of it. There is no doubt that the activity in the formation of erythrocytes - carriers of oxygen - depends primarily on the requirements of the organism for oxygen. The absence of food reduces considerably the need for oxygen. Thus, the rate of breathing in mammals in the early days of fasting drops up to 43%, i.e. almost in half. Vinberg (1956) cites data of various authors showing that even in the early days starvation in fish is accompanied by a considerable decrease in the need for oxygen. It is obvious that starvation is accompanied by a reduction in the need for oxygen in all living beings since the initial stage and basic condition of normal metabolism is the admission of food into the organism and its subsequent conversion with the indispensible participation of oxygen.

Thus, fish which have a normal oxygen regime but which are not receiving food should not experience oxygen deficiency, especially in the early period of starvation, when the organism has not arrived at a state of dystrophy. The number of erythrocytes in these fish ensures completely a reduction in oxygen consumption. The absence of a need for an increase in the number of erythrocytes suspends their formation. But the intake of new portions of food after starvation naturally will cause heightened blood-

formation. This may explain the rapidity of the reaction of the blood-forming organs to the cessation of feeding and to an increase in food intake by fish.

In experiments with salmon larvae it was established that in spite of the absence of timely fattening in the period of transition from yolk feeding to active feeding there is rather intensive secondary erythropoiesis though with some time lag.

Apparently, this is caused by the gradual aging and disappearance of embryonic and larval blood cells - primary erythrocytes - from the blood which unavoidably must lead to an intensification in secondary erythropoiesis.

It is interesting to note that the replacement of blood cells in the development of the larva, the appearance of normoblastic blood formation, is directly linked to the beginning of active feeding - an important factor in heightening the metabolism.

Thus, there is a very close relationship between the requirement of food and oxygen and blood formation in the organism of fish. Intensification in feeding produces a large need for oxygen which stimulates an increase in the rate of blood formation; on the other hand, reduced feeding leads to a reduction in the need for oxygen and to the suspension of erythropoiesis.

In speaking about the stimulating effects of oxygen deficiency on blood formation it is essential to remember that the formation of blood cells can only occur in the presence of specific substances in the organism. Experience with artificial anemia in carp showed that a certain /56 supply of these substances does exist in the organism of fish. However

we do know that given a systematic absence or deficiency of these substances in food animals develop anemia.

From the medical literature we know the following about the nature of these substances. Normal blood formation can only take place given the internal factor (hemogenesis which develops in the stomach) and the external factor (the hemogene and vitamin B_{12}) which is taken in with food (Alekseev, 1953). The interaction of these factors forms a hemopoietic substance ("hemopoietin") which is deposited in the liver. Hepatic hemopoietin is identical with folic acid which is a component of the vitamin B complex. Another essential substance in blood formation is iron which takes part in the formation of the hemoglobin molecule and causes an overall stimulation of the process of blood formation (Tur, 1950). The absence of essential substances in food leads to anemia. We observed such anemia in rainbow trout which was reared on synthetic foods. Three stages in anemia were isolated as a basis for determining the qualitative and quantitative cellular composition of blood. Each stage was characterized by specific indices, the second and third stages being distinguished only by means of blood smears.

It should be emphasized that the picture of blood resulting from absolute starvation and the picture of blood resulting from an absence of certain vitamins or mineral salts in food are completely different. During starvation no immature erythrocytes are detected in the smears whereas during avitaminosis they are usually observed in abundance. An exception is an advanced stage of anemia when the blood-forming organs are exhausted and when immature forms of erythrocytes are completely

absent or are detected singly in the blood.

The difference in the blood pictures is explained by the fact that with the absence of individual food components the need for oxygen cannot be sharply reduced. The intake of the usual quantity of food by an organism requires that the quantity of erythrocytes in hemoglobin in the blood be maintained at a specific level. However, disruption in the maturation of erythrocytes caused by the absence of essential elements leads to a decrease in the number of red blood cells contrary to the norm. This leads to an intensification in the process of blood formation and to the utilization of all reserves of substances necessary in the formation of erythrocytes. This process continues until erythropoiesis is completely inhibited (stage III of anemia in trout).

In medicine and veterinary science such inhibition in blood formation is called aplastic anemia (cessation of the formation of new blood cells).

In conclusion it may be said that up to this time in the studies /57
of the blood of fish insufficient attention has been given to the composition of red blood cells in fish and the morphological peculiarities of erythrocytes. Korzhuev (1949) wrote that the characteristic feature of previous works on the blood of various animals was the neglect of the morphological features of blood. Meanwhile, the morphological attributes which characterize carriers of hemoglobin - erythrocytes - are very important for an understanding of the features of the blood of any animal.

The question of the necessity of detailed study of the morphology of the blood of fish was also raised at the first conference on the

physiology of fish which was held in Moscow in 1956.

The present paper shows that the morphology of red blood in fish undergoes important changes relative to the effect of various environmental factors and is clearly reflected in the work of the blood-forming organs. These facts provide the basis for assuming that in assessing the physiological state of fish according to the blood it is necessary to use such a sensitive index of the state of an organism as the qualitative composition of the red blood cells.

In so doing one must remember that heightened blood formation and the appearance of a large number of immature forms in the blood of fish may be normal and is related to the poor state of an organism.

In some cases the appearance of a large number of immature erythrocytes in the blood is a normal phenomenon of age or season (in the period when primary erythrocytes are replaced by secondary erythrocytes immature cells comprise up to 40%; a large number of immature forms in the spring). However, in the majority of cases a sharp increase in the number of immature forms of erythrocytes in the blood of fish is a reaction of the blood-forming organs to the unfavorable effect of external conditions on the organism of fish which may be manifested in a reduction of oxygen in the water, deficiency in essential vitamins and mineral substances in food, infection of fish by various parasites and a number of other environmental factors which have an effect on blood formation but which have not as yet been established. Determination of the qualitative composition of blood cells and the quantitative indices enable changes which have begun in an organism to be observed at an early stage and makes it

possible to eliminate the hazard through an appropriate reexamination of the living conditions of the fish. /58

CONCLUSIONS

1. The main organ in the formation of both erythrocytes and white blood cells in trout, salmon and carp is the kidney. Only an insignificant degree of blood formation occurs in the spleen. On the basis of published data and the data obtained as a result of the present study it is necessary to reexamine the opinion that the spleen is the main organ in bony fish in the formation of erythrocytes.

2. During active erythropoiesis the impressions of the kidney of fish consist mainly of cells of the erythropoietic series. With a slackening in erythropoiesis in the kidney a predominance of white blood cells is observed. Thus, it follows that in defining the sites of erythrocyte and leucocyte formation in various fish it is necessary to take into account the state of the process of erythropoiesis or leucopoiesis at the moment of examination.

3. Each period in the development of fish has its specific blood cell composition. In the embryonic period in the blood of salmon and trout there are immature forms of primary erythropoiesis and leucopoiesis. By the time hatching takes place the red blood consists entirely of mature primary erythrocytes. Many immature forms are encountered among the white blood cells. In the transitional period of mixed feeding the replacement of primary round erythrocytes by secondary

ellipsoid erythrocytes is observed in the blood of larvae. By the time the yolk sac is resorbed the primary erythrocytes have disappeared completely from the blood. The number of white blood cells greatly increases. In structure and color the lymphocytes, polymorphonuclear leucocytes and monocytes of this period already coincide with the cells of mature fish.

4. The greatest activity in blood formation (when immature forms in the blood comprise up to 40%) in the post embryonic period is observed when the cells of primary erythropoiesis are replaced by those of secondary erythropoiesis. Henceforth, the rate of blood formation decreases. In the summer immature erythrocytes in the blood are observed to comprise 7-10%.

5. The pronounced disturbance in the ratios of blood cells characteristic for each specific period is indicative of the poor state of the immature fish, the cause of which must be sought in poor maintenance conditions.

6. Decrease and increase in water temperature caused different reactions on the part of the blood in different fish. Given decreased temperature (2-4^{o}) carp and catfish change over to a passive state which results in a sharp drop in the number of leucocytes (1/15-1/20) in their blood whereas in trout which lead an active mode of life throughout the winter the number of leucocytes decreases only to 2/3-1/2 at this temperature.

7. The transition of carp and catfish from a passive state to an active state, given an increase in temperature, coincides with the

ejection into the blood of a large number of neutrophyls which gradually disappear in the course of several days, being replaced by lymphocytes. /59 In trout, such a change in the leucocytic composition, given an increase in water temperature, is not observed.

8. The absence of additional food for fish larvae during the period of yolk sac resorption has little effect on the replacement of primary erythrocytes by secondary erythrocytes. This replacement of blood cells can occur on the basis of yolk feeding alone with only a small time lag. By the time all traces of the yolk sac have disappeared in the blood there are absolutely no priamry erythrocytes. Later, however, active blood formation ceases in the starving fingerlings in contrast to the feeding fingerlings. There are no immature forms of erythrocytes in the blood.

9. Starvation shortens the process of blood formation in fish. In this case, the curtailment of erythropoiesis is caused not by the disruption of it or by the absence of some kind of substances which are consumed with food, it is only a temporary suspension which is connected with a decrease in the need for oxygen. Loss of blood, despite the absence of food, leads to a heightened regeneration of blood in starving fish. Feeding the fish after starvation causes a vigorous regeneration of red blood cells.

10. Starvation and intensified feeding does not cause noticeable changes in the absolute and relative quantity of white corpuscles in fish. However, when anemia is caused by avitaminosis, there is a gradual reduction in the number of lymphocytes and increase in the number of

polymorphonuclear leucocytes and monocytes.

11. Anemia was detected in trout which was reared on synthetic foods. A determination of the quantitative and qualitative indices of blood made it possible to isolate three stages in anemia which are distinguished by the quantity of hemoglobin and erythrocytes, the leucocytic formula and the red blood picture. Blood smears aided in establishing the reaction of the blood-forming organs in trout in each stage and in distinguishing stage II from stage III.

12. The red blood picture distinctly reflects the reaction of the blood-forming organs of fish to changes in the organism which are influenced by various environmental factors. Thus, in making a physiological assessment of fish which are being reared it is necessary to make use of the data on the morphological composition of blood cells as well as the quantitative data on blood.

BIBLIOGRAPHY

1. Alekseev G.A. Anemia (pathogenesis, symptoms and treatment). Ministry of public health of the USSR. Central institute for the advanced training of doctors. Moscow. 1953.
2. Antipova P.S. Changes in the morphological composition of the blood of carp due to season and age. Problems in ichthyology, issue 2, 1954.
3. Vasil'ev A.V. Hemotology of farm animals. State agricultural, cooperative and kolkhoz publishing house, 1948.

4. Vinberg G.G. Rate of metabolism and food requirements of fish. Belorussian state university im. V.I. Lenin. Minsk. 1956.

5. Golodets G.G. The morphological picture of the blood of some fish. Transactions of the Moscow technical institute of the fish industry and fisheries, issue 2, 1939.

6. Golodets G.G. Changes in the composition of blood of young fish with respect to the conditions of rearing. Problems in ichthyology, issue 2, 1954.

7. Drabkina B.M., Telkova L.P. Maturity of the sexual products in the females of Kuban sturgeon and the leucocytic formula of the blood. Transactions of the laboratory of pisciculture principles, volume II, publishing house of the Academy of Sciences of the USSR, 1949.

8. Drabkina B.M. Change in the morphological composition of the blood of bream and zander with respect to the spawning period. Transactions of the all-union hydrobiological society, volume 3.

9. Drabkina B.M. Study of the blood of immature sturgeon and sevriuga with respect to differences in feeding. Doklady of the Academy of Sciences of the USSR. New series, volume 76, No. 6, 1951.

10. Drabkina B.M. Effect of food ration on the physiology of sturgeon fingerlings which are being reared. Fisheries, No. 2, 1951.

11. Drabkina B.M. Physiological assessment (by blood indices) of sturgeon fingerling reared in artificial conditions. Transactions of the Saratov division of the Caspian branch of the All-union scientific research institute of sea fisheries and oceanography, vol. 2, oblast' state publishing house, 1953.

12. Zavarzin A.A. Essays on the evolutionary histology of blood and connective tissue. State publishing house of medical literature, issue I, 1945.

13. Zavarzin A.A., Rumyantsev A.V. Course in histology. State publishing house of medical literature, 1946.

14. Zavarzin A.A., Shchelkunov S.I. Manual of histology. State publishing house of medical literature, 1954.

15. Zaitseva R.A. Regeneration processes in the marrow of a young organism under normal conditions, during starvation and parenteral feeding. Author's abstract of a dissertation submitted for the degree of Candidate of medical sciences, Moscow. 1956.

16. Ivlev V.S. Effect of winter conditions on the blood of some fresh-water fish. Bulletin of the Moscow society of naturalists. Biological division, volume LX, No. 4, 1955.

17. Kassirskii I.A., Alekseev G.A. Clinical haematology. State publishing house of medical literature, 1955.

18. Korzhyev P.A. Evolution of the respiratory function of blood. Publishing house of the Academy of sciences at the USSR, 1949.

19. Korzhyev P.A. Physiological methods of assessing the quality in mature commercial fish being reared. Thesis of reports presented at the conference on pisciculture. Moscow, 1954.

20. Lichnitskaya I.I. The change in the oxygen-binding properties of blood in the embryonic period. Publishing house of the Academy of medical sciences of the USSR, 1950.

21. Lyaiman E.M., Shpolyanskaya A. Yu. Change in the leucocytic formula of the blood of carp during a chronic form of erysipelas and the use of it in the diagnosis of the illness. Transactions of the Moscow

technical institute of the fish industry and fisheries, issue 4, 1951.

22. Maksimov A.A. Fundamentals of histology. Part II. Study of tissues. 1918.

23. Machavariani E.N. Morphological changes in the peripheral blood and the blood-forming apparatus during experimental alimentary distrophy. Author's abstract of a dissertation leading to a degree of Candidate of medical sciences. Tbilisi, Georgian state publishing house of medical literature, 1952.

24. Nusenbaum L.M. Study of the sex glands and blood of Atlantic salmon with respect to features of its reproduction. Author's abstract of a dissertation leading to the degree of Candidate of biological sciences. Leningrad. 1951.

25. Nusenbaum L.M. Blood cells of salmon embryos and larvae. Doklady of the Academy of sciences of the USSR, Volume XCVII, No. 3, 1954.

26. Pavlov V.A., Krolik V.G. Hemoglobin content and number of erythrocytes in the blood of some freshwater fish. Transactions of the Borodino biological station, Volume IX, issue I, 1936. /61

27. Pestova I.M. Blood-forming capacity of the vascular endothelium in the ontogenesis of bony fish. Archives of anatomy, histology and embryology, No. 4, 1954.

28. Pestova I.M. Formation of erythrocytes in the early ontogenesis of bony fish with respect to their development conditions. Doklady of the Academy of sciences of the USSR, Volume 101, No. 1, 1955.

29. Poberii I.A. Processes of regeneration in the spleen and lymph nodes

given parenteral introduction of protein, in conditions of starvation. Author's abstract of a dissertation leading to the degree of Candidate of medical sciences. Moscow, 1956.

30. Privol'nev T.I. Change in breathing in the ontogenesis of fish, given various partial oxygen pressure. Bulletin of the All-union scientific research institute of lake and river fisheries, Volume XXV, issue I, 1947.

31. Privol'nev T.I. Critical periods during the post embryonic development of fish. Bulletin of the All-union scientific research institute of lake and river fisheries, Volume XXIX, 1949.

32. Privol'nev T.I. Physiological indices for immature salmon from the Salets River and "Pelcha" fish hatchery. Bulletin of the All-union scientific research institute of lake and river fisheries, Volume XXXIII, 1953.

33. Puchkov N.V., Federova A.L. Study of the change in the composition of the blood of carp exposed to hunger and cooling. Transactions of the Moscow technical institute of the fish industry and fisheries, issue IV, State scientific and technical publishing house of the food industry, 1951.

34. Puchkov N.V., Drabkina B.M. Study of the physiology of ovulation in sturgeon. Transactions of the Moscow technical institute of the fish industry and fisheries, issue IV, State scientific and technical publishing house of the food industry, 1951.

35. Puckhov N.V. Physiology of fish. State scientific and technical publishing house of the food industry, 1954.

36. Puchkov N.V. Role of leucocytes in digestion and in the processes of ovulation in fish. Thesis of reports presented at the Conference on the study of the physiology of fish. Moscow, 1956.

37. Rubashev S.I. Study of the physiology of the blood of fish. White blood corpuscles of some freshwater fish. Transactions of the Borodino biological station, Volume IX, issue 1, Publishing house of the Academy of sciences of the USSR, 1936.

38. Rumyantsev N.N. The reticuloendothelicium and structure of blood-forming organs of some species of bony fish. Archives of anatomy, histology and embryology. Volume 21, issue 2, Publishing house of the Academy of sciences of the USSR, 1939.

39. Strel'tsova S.V. Methods of determining the heat tolerance and cold tolerance in adaptable species of fish. Transactions of the Conference on the problems of the acclimatization of fish and fodder invertebrates. Publishing house of the Academy of sciences of the USSR, 1954.

40. Tur A.F. Hematology of infancy. Publishing house of the Academy of medical sciences of the USSR, 1950.

41. Faktorovich K.A. Disease in rainbow trout, age 1 , when reared on synthetic foods. Problems in ichthyology, issue 6, 1956.

42. Freifel'd E.I. Hematology. State publishing house of medical literature, 1947.

43. Khlopin N.G. Tissue culture. State publishing house of medical literature, 1940.

44. Khlopin N.G. General biological and experimental bases of histology. Publishing house of the academy of sciences of the USSR, 1946.

German bibliographic items 003835 Return to: Mrs. C. Girouard, Slavonic Section

1. Genesis of red blood cells in the various vertebrate classes. . . . . 95(1), 1884
3. Hematological studies on carp and on several other fresh-water fishes.
4. Contribution to the study of lymphoid tissues of ichthyopsida (NB. Translator's note: the latter term is ancient classification!)
7. Studies on normal and pathological fish blood.
11. Contribution to the knowledge of blood genesis in teleosts.
12. Contributions to the comparative morphology of white blood cells.
14. Blood cells of several fishes.
16. Comparative physiological studies on blood cell counts in teleosts.
17. Hemopoietic sites in carp.
18. Hemopoiesis and hemopoietic sites in carp (Cyprinus carpio L.)
19. Studies on comparative hemocytology of several poikilotherm vertebrates.
20. Studies on infectious ascites in carp.
21. Blood morphology of the chondrostean Acipenser ruthenus.

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ЛИТЕРАТУРА

1. Алексеев Г. А. Анемии (патогенез, клиника, лечение). Министер. здравоохран. СССР. Цент. ин-т Усоверш. врачей. М. 1953.
2. Антипова П. С. Сезонные и возрастные изменения морфологического состава крови карпа. Вопросы ихтиологии, вып. 2, 1954.
3. Васильев А. В. Гематология с-х животных. Сельхозгиз, 1948.
4. Винберг Г. Г. Интенсивность обмена и пищевые потребности рыб. Изд. Белгосуниверситета им. Ленина. Минск. 1956.
5. Голодец Г. Г. О морфологической картине крови некоторых рыб. Тр. Мосрыбвтуза, вып. 2, 1939.
6. Голодец Г. Г. Изменения состава крови молодых рыб в связи с условиями выращивания. Вопросы ихтиологии, вып. 2, 1954.
7. Драбкина Б. М., Телкова Л. П. Зрелость половых продуктов у самок кубанской севрюги и лейкоцитарная формула крови. Тр. лабор. основ рыбоводства, т II, изд. АН СССР, 1949.
8. Драбкина Б. М. Изменение морфологического состава крови леща и судака в связи с нерестовым периодом. Тр. Всес. гидроб. общ., т. III.
9. Драбкина Б. М. Исследование крови молоди осетра и севрюги в связи с различием в питании. Доклады АН СССР. Нов. сер., т. 76, № 6, 1951.
10. Драбкина Б. М. Влияние пищевого рациона на физиологию выращиваемой молоди осетровых. Рыбное хоз., № 2, 1951.
11. Драбкина Б. М. Физиологическая оценка (по показателям крови) молоди осетра, выращиваемой в искусственных условиях. Тр. Саратов. отд. Касп. фил. ВНИРО, т. 2, Облгиз, 1953.
12. Заварзин А. А. Очерки эволюционной гистологии крови и соединительной ткани. «Медгиз», вып. I, 1945.
13. Заварзин А. А., Румянцев А. В. Курс гистологии. Медгиз, 1946.
14. Заварзин А. А., Щелкунов С. И. Руководство по гистологии. Медгиз, 1954.
15. Зайцева Р. А. Регенерационные процессы в костном мозгу молодого организма в норме, при голодании и парэнтеральном питании. Автореферат дисс. на соискание уч. степени канд. мед. наук, М. 1956.
16. Ивлев В. С. О влиянии зимних условий на кровь некоторых пресноводных рыб. Бюл. Мос. Общ. испыт. природы. Отд. биолог., т. LX, № 4, 1955.
17. Кассирский И. А., Алексеев Г. А. Клиническая гематология. Медгиз, 1955.
18. Коржуев П. А. Эволюция дыхательной функции крови. Изд. Акад. наук СССР, 1949.
19. Коржуев П. А. О физиологических методах оценки качества выращиваемой молоди промысловых рыб. Тезисы докладов на совещании по рыбоводству. Москва, 1954.
20. Лихницкая И. И. Изменение кислородосвязующих свойств крови в эмбриональный период. Изд. Акад. мед. наук СССР, 1950.
21. Ляйман Э. М., Шполянская А. Ю. Изменение лейкоцитарной формулы крови карпов при хронической форме краснухи и использование ее для диагностики заболевания. Тр. Мосрыбвтуза, вып. 4, 1951.
22. Максимов А. А. Основы гистологии. Часть 2. Учение о тканях. 1918.
23. Мачавариани Э. Н. Морфологические изменения периферической крови и кроветворного аппарата при экспериментальной алиментарной дистрофии. Автореферат дисс. на соискание уч. степени канд. мед. наук. Тбилиси, Грузмедгиз, 1952.
24. Нусенбаум Л. М. Исследование половых желез и крови семги в связи с особенностями ее размножения. Автореферат дисс. на соискание учен. степени канд. биолог. наук. Ленинград. 1951.
25. Нусенбаум Л. М. О клетках крови эмбрионов и личинок лосося. Доклады АН СССР, т. XCVII, № 3, 1954.
26. Павлов В. А., Кролик В. Г. Содержание гемоглобина и число эри-

троцитов в крови некоторых пресноводных рыб. Тр. Бородинск. Биолог. ст. т. IX, вып. I, 1936.

27 Пестова И. М. Кроветворная способность сосудистого эндотелия в онтогенезе костистых рыб. Архив анат., гист. и эмбр., № 4, 1954.

28 Пестова И. М. Образование эритроцитов в раннем онтогенезе костистых рыб в связи с условиями их развития. Доклады АН СССР, т. 101, № 1, 1955.

29 Поберий И. А. Процессы регенерации в селезенке и лимфатических узлах при парэнтеральном введении белка в условиях голодания. Автореферат дисс. на соискание уч. степени канд. мед. наук. М., 1956.

30 Привольнев Т. И. Изменение дыхания в онтогенезе рыб при различном парциальном давлении кислорода. Известия ВНИОРХ, т. XXV, вып. I, 1947.

31 Привольнев Т. И. Критические периоды при постэмбриональном развитии рыб. Известия ВНИОРХ, т. XXIX, 1949.

32 Привольнев Т. И. Физиологические показатели молоди лосося из реки Салда и прудов рыбоводного завода «Пелчи». Известия ВНИОРХ, т. XXXIII, 1953.

33 Пучков Н. В., Федорова А. Л. Исследование изменения состава крови карпа под влиянием голодания и охлаждения. Тр. Мосрыбвтуза, вып. IV, Пищепромиздат, 1951.

34 Пучков Н. В., Дрябкина Б. М. Исследование по физиологии овуляции у осетровых. Тр. Мосрыбвтуза, вып. 4, Пищепромиздат, 1951.

35 Пучков Н. В. Физиология рыб. Пищепромиздат, 1954.

36 Пучков Н. В. Роль лейкоцитов в пищеварении и процессах овуляции у рыб. Тезисы докладов на совещании по изучению физиологии рыб. Москва, 1956.

37 Рубашев С. Н. Исследование по физиологии крови рыб. О белых кровяных тельцах некоторых пресноводных рыб. Тр. Бородинск. биологич. станц., т. IX, вып. I, изд. АН СССР, 1936.

38 Румянцев Н. Н. О ретикуло-эндотелии и строении кроветворных органов некоторых видов костистых рыб. Архив анат., гист. и эмбр. т. 21, вып. 2, изд. АН СССР, 1939.

39 Стрельцова С. В. Методы определения теплостойкости и холодостойкости у акклиматизируемых видов рыб. Тр. Совещания по пробл. акклимат. рыб и кормовых беспозвочных. Изд. АН СССР, 1954.

40 Тур А. Ф. Гематология детского возраста. Изд. АМН СССР, 1950.

41 Факторович К. А. Заболевание двухлеток радужной форели при выращивании на искусственных кормах. Вопросы ихтиологии, вып. 6, 1956

42 Фрейфельд Е. И. Гематология. Медгиз, 1947.

43 Хлопин Н. Г. Культура тканей. Гос. изд. мед. литерат., 1940.

44 Хлопин Н. Г. Общебиологические и экспериментальные основы гистологии. Изд. АН СССР, 1946.

1 Bizzozero J. und Torre A. A. Ueber die Entstehung der rothen Blutkörperchen bei den verschiedenen Wirbelthierklassen. Archiv für path. Anat. und Physiol., Bd., XCV, H. 1, 1884.

2 Catton W. T. Blood Cell Formation in Certain Teleost Fishes. Blood. The Journal of Hematology. Vol. VI, № 1, 1951.

3 Dombrowski H. Untersuchungen über das Blut des Karpfens und einiger anderer Süsswasserfisch. Biol. Zentral., 72, 3/4, Leipzig, 1953.

4 Drzewina A. Contributions á l'étude du tissu lymphoïde des Ichthyopsidès. Arch. zool. exp., t. III, 1905.

5 Drzewina A. Contribution á l'étude des leucocytes granuleux du sang des poissons. Arch. d'anatomie microsc., v. 13, 1911—1912.

6 Duthie E. S. The origin, development and functions of the blood cells in certain marine teleosts. I. Morphology. J. Anat., v. 73 (3), 1939.
7 Hoffmeyer C. W. Untersuchungen über normales und abnormales Fischblut. Allg. Fischerei-Zeit., V. 32, № 3, 1907.
8 John C. C. The origin of erythrocytes in the hering (Clupea harengus). Royal society of London, Ser. «B», V. 110, 1932.
9 Jordan H. E. and Speidel C. C. Studies on lymphocytes. II. The origin, functions and fate of the lymphocytes in fishes. Journal of morphol., V. 38, № 4, 1924.
10 Jordan H. E. Comparative haematology. Downey's handbook of hematology, V. II, 1938.
11 Marcus H. Ein Beitrag zur Kenntnis der Blutbildung bei Knochenfischen. Arch. f. Mikrosk. Anat., Bd. 66, 1905.
12 Meinertz J. Beiträge zur vergleichenden Morphologie der farblosen Blutzellen. Archiv für path. Anat. und Physiol., Bd. 168, H. 3, 1902.
13 Phillips A. M. The development of anaemia in trout fed a synthetic diet and its cure by the feeding of fresh beef liver. Progr. Fish-Cult., № 48, 1940.
14 Rawitz B. Über die Blutkörperchen einiger Fische. Arch. für Mikrosk. Anat., Bd. 56, 1900.
15 Schaeffer A. A. The number of blood corpuscles in fishes in relation to starvation and seasonal cycles. Journal of Gener. Physiol., V. VII, 1925.
16 Schlicher J. Vergleichend-physiologische Untersuchungen der Blutkörperchenzahlen bei Knochenfischen. Zoolog. Jahrb. Allg. Zool. und Physiol., Bb. 43, H. 2, 1927.
17 Topf W. Über die Blutbildungsstätten beim Karpfen. Zoolog. Anzeiger, Bd. 150, H. 5—6, 1953.
17 Topf W. Die Blutbildung und die Blutbildungsstätten beim Karpfen (Cyprinus carpio L.). Zeitschrift für Fischerei und deren Hilfswis., Bd. IV, H. 3—4, 1955.
19 Werzberg A. Studien zur vergleichenden Hämozytologie einiger poikilothermer Vertebraten. Fol. Haemat. Arch., Bd. XI, 1911.
20 Wunder W. und Dombrowski H. Untersuchungen über die ansteckende Bauchwassersucht des Karpfens. Zeitschrift für Fischerei und deren Hilfswissensch., Bd. II, H. 5—6, 1953.
21 Zwetkov I. I. Zur Frage der Morphologie des Blutes der Knorpelganoiden (Acipenser ruthenus). Zeitschr. für Mikrosk-Anat. Forschung, Bd. IX, H. 3, 1935.

BLOOD INDICES AND HAEMOPOIESIS IN THE INDIVIDUAL DEVELOPMENT OF FISH

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Summary

As a result of an investigation of blood—forming organs in the rainbow trout (Salmo irideus), the Baltic salmon (Salmo salar) and the carp (Cyprinus carpio), the kidney has been found to be the main site of haemopoiesis in these fishes. The spleen plays but a minor part in the formation of blood cells.

The blood of fry is modified with its growth and development. Only immature forms of primary erythropoiesis are to be found in the early embryo. The blood larvae, living upon substances supplied by the yolk sack, consists of mature primary erythrocytes having circular shape of nuclei and cell.

In the period, when feeding activity of fry is developing, the primary erythrocytes are replaced by secondary elliptic red blood cells. At the time when resorption of the yolk sack is accomplished only secondary erythrocytes and their immature forms are to be found.

Environment influences the blood picture and haemopoiesis of the fish.

Alteration of water temperature affects the quantitative and qualitative changes in white blood cell contents. Starvation brings about a sharp decrease in the rate of blood formation, so that no more immature forms of erythrocytes are to be found in circulating blood. Feedings following a fast, as well as increasing the quantity of food, as compared to that administered over a preceeding period, cause enhanced blood regeneration, being accompanied by considerable morphological alterations.

When rainbow trout is raised upon artificial foods, deficient in vitamins and mineral salts, a disease accompanied by anaemia occurs. Examination of blood smears in affected trouts has revealed a sequence of three stages in the evolution of this anaemia with a clear cut reaction of blood forming organs corresponding to each of the stages.

The differential blood cell contents is thus shown to be a trite index of haemopoietic activity and to be a highly sensitive sign of the degree of pathological alterations taking place in the body of the fish. In raising fish, for assessing its physiological condition, determination of quantitative blood indices (haemoglobin level, red and white cell counts) should be supplemented by examination of cell morphology.

TABLE OF CONTENTS