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BLOOD CELL CONSTITUENTS IN FISH

II. Peroxidase Staining of the Leucocytes in the Kidney and Some Other Organs

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In the previous paper (Yûki, 1957), the author reported that the leucocytes of the blood stream in the teleost fishes clearly show the positive reaction by the peroxidase staining, that these stained cells are easily discernible from the other blood elements owing to their characteristic colouration, and that fluctuation of the ratio measured between the positive and negative cells is utilizable in fish physiology as a biological indicator.

In course of the present study, it was found that the positive leucocytes in the blood stream fluctuate in number markedly corresponding to some unknown physiological changes in the fish. For example, sometimes these cells increase to several times the usual number, while in other cases they decrease to as few as almost none, though those in the blood stream are usually small in number. It is presumable that such a fluctuation of the positive cells is a response of the hematopoietic organ to physiological changes of the fish. To make clear the causes of such a fluctuation in the number of these cells, examination was first made in which organ they are generated and stored abundantly.

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MATERIALS AND METHODS

In the present experiment, the rainbow trout, *Salmo irideus*, 2 – 4 years old was mainly used, which had been reared at Nanae, near Hakodate City. Further, the following forms and species of fishes were also examined: adult marine and young freshwater forms of Masu, *Oncorhynchus masou*, anadromous form of chum salmon, *O. keta*, Atka mackerel, *Pleurogrammus azonus*, 2 species of rockfish, *Sebastes taczanowskii* and *S. thompsoni*, 2 species of puffer, *Fugu vermicularis porphyreus* and *F. vermicularis paradalis*, starry flounder, *Platichthys stellatus*, and wingfish, *Lepidotrigla microptera*. All these fishes were collected in the natural field, river and sea.

The peroxidase-positive leucocytes in the following organs were examined: liver, kidney (mesonephros), spleen, gill, pyloric caeca and brain. Before dissection of the

fish is made, enough blood was drained out cutting the caudal part. From the blood thus obtained, the blood serum was prepared for use in the following mince- and smear-preparation of the organs. A piece of each organ taken from the fish is set on a slide glass, and a few drops of the serum are poured on it. Then, the piece is minced with scissors, and the smear preparation of the organ is made. Finally, the residues of the piece are removed from the slide glass. The smear preparations thus made were stained by the two methods, Wright-Giemsa's and peroxidase stainings the same as used in the previous observation (Yûki, 1957).

OBSERVATIONS

The peroxidase-positive leucocytes were found in most of the organs examined, and they were identical with those which were observed in the blood stream. Among the organs examined, the positive cells were found most abundantly in the kidney. Further, as shown in Fig. 2, a, the other elements of the blood cells belonging to the various stages were also observed abundantly in this organ.

In the case of the mince- and smear-preparations employed in the present observation, it is difficult to indicate the absolute number of the positive cells occurring in each organ as they are not smeared in most cases so as to distribute uniformly on a slide glass. However, it seems reasonable that an approximate tendency of the occurrence of these cells in the organ may be judged by the following method. That is, in place of a micrometer, a glass plate with a notched area of 4×6mm is inserted into the ocular of the microscope; sliding the preparation from side to side, sums of the respective numbers of the erythrocytes and positive cells observed successively within the notched area are counted under the microscope; then the ratio of these two kinds of blood cells is calculated. The following table shows the number of the positive cells per 10,000 erythrocytes in each organ and that of the blood stream in the rainbow trout. Each number was calculated from the average of 3 measurements on each preparation, using the method described above.

Kidney	*Spleen*	*Liver*	*Brain*	*Pyloric caeca*	*Gill*	*Blood*
4500–6100	250–400	>50	0?	0?	+?	20–30

Although as stated in the above the numbers of positive cells shown in the table do not indicate the absolute value of each organ, it is clear that the cells are much more abundant in the kidney than in the blood stream (Figs. 1 & 2). Possibly, the number of the cells in the former organ may run up to a few hundred times as many as that of the latter. In comparison with the kidney, the positive cells are much less in both spleen and liver in the rainbow trout (Figs. 3 & 4). In both brain and pyloric caeca, not a single cell which showed the positive reaction was observed, at least in the present experiment. The positive cells were found in the gill rarely, but they might possibly be

derived from the circulating blood in this organ.

Except the starry flounder and wingfish, the positive leucocytes were found to be stored abundantly in the kidney of all the forms and species of the fishes examined, similarly as in the rainbow trout. In the exceptional two species, the positive cells were found to be rather more abundant in the liver in the starry flounder and in the spleen in the wingfish than in the kidney of the respective species.

DISCUSSION

Jordan and Speidel (1924) have reported from their detailed observations concerning hematopoiesis of the fish that in teleost the intertubular connective tissue of kidney (mesonephros) is a predominant hematopoietic site and spleen is only of a secondary importance in this function. Dawson (1935) has also reported that in the teleost fishes the mesonephros is an important hematopoietic locus for both erythrocyte and granulocyte. Walter (1955) has pointed out from his recent study that the kidney is the most important organ for the hematopoiesis in carp, too. Although the method employed in the present observation differs from those of the previous workers who used either the anatomical smear or the section of tissues, the author's result also agrees with those of them.

As stated in the previous paper (Yûki, 1957), the peroxidase-positive leucocytes are composed of both groups of the monocytic and granulocytic cells. It is clearly seen in the present mince- and smear-preparations that the positive cells found in the kidney are formed also of both the monocyte and granulocyte or either of them as they included elements of various stages from the undifferentiated to the mature cells. However, it is apparently curious that these positive cells are few in the blood stream, while they are found to be abundant in the kidney. Regarding this phenomenon, the author has held the opinion that it is resulted from the following two causes. It is conceivable as the first cause that the positive cells are short-lived in the blood stream. If that is the case, abundant cells must be generated and stored in some organ to supply them continuously into the blood stream, owing to their short longevity. As the second cause, it may be considered that the abundant existence or the storage of the positive cells in the fish kidney is essential for the performance of their physiological function, besides the phagocytosis of them (Irie, 1932). Further, various kinds of enzymes which are contained within the stored positive cells are considered to have some important roles to accomplish the function of the kidney. The fact that these cells show the positive reaction to the peroxidase staining suggest that they contain the peroxidase and some other enzymes, even if the staining is only a secondary reaction as Lison (1953) has stated. In such a meaning, the present finding might throw light upon an analysis of the physiological function of the fish kidney, though it has not yet been clarified in many respects.

SUMMARY

1. To find in which organ the peroxidase-positive leucocytes are generated and stored, the mince- and smear-preparations of the liver, kidney, spleen, brain, gill and pyloric caeca were examined using both stainings of the Wright-Giemsa and the peroxidase. Although the observation was made mainly on the organs of the rainbow trout, the following forms and species of fishes were also used: adult marine and young freshwater forms of Masu, anadromous form of chum salmon, Atka mackerel, 2 species of rockfish, 2 species of puffer, and wingfish.

2. The positive cells were found most abundantly in the kidney. In the rainbow trout, the approximate number of these cells in the kidney reaches about 200 times as many as those found in the blood stream. Probable causes concerning such a marked difference of the occurrence of the positive cells between the kidney and blood stream were discussed briefly.

3. In the rainbow trout, the positive cells of the spleen are relatively small in number being only about 1/20 that of the kidney; those of the liver are about 1/100 of the same organ. None of the positive cells were found in either brain or pyloric caeca. In the gill, they were found rarely.

4. Among the fishes examined, the occurrence of the positive cells was somewhat characteristic in the starry flounder and wingfish. That is, they are relatively abundant in the liver in the former species and in the spleen in the latter one. The results in the other forms and species of the fishes examined are almost identical with those of the rainbow trout.

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PLATE I

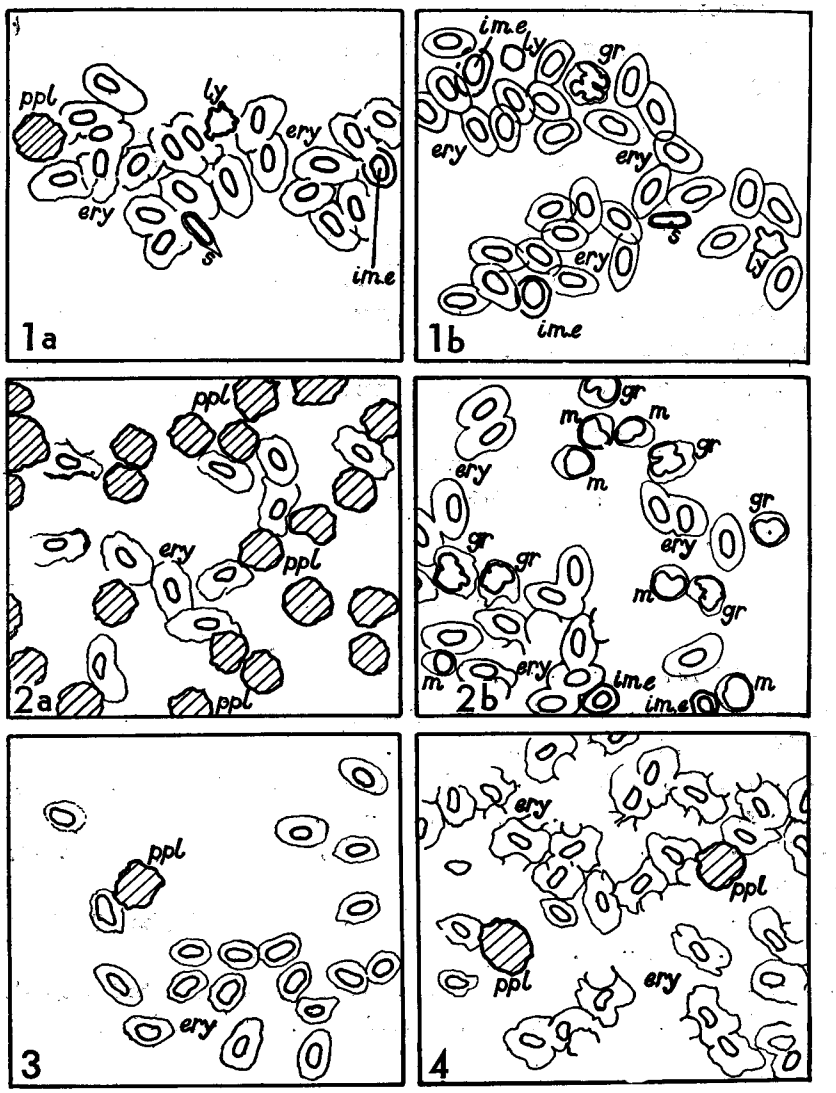
Explanation of figures

All figures were photomicrographed from the preparations of the circulating blood and certain organs in the rainbow trout. Magnification of the figures is about 600 diameters.

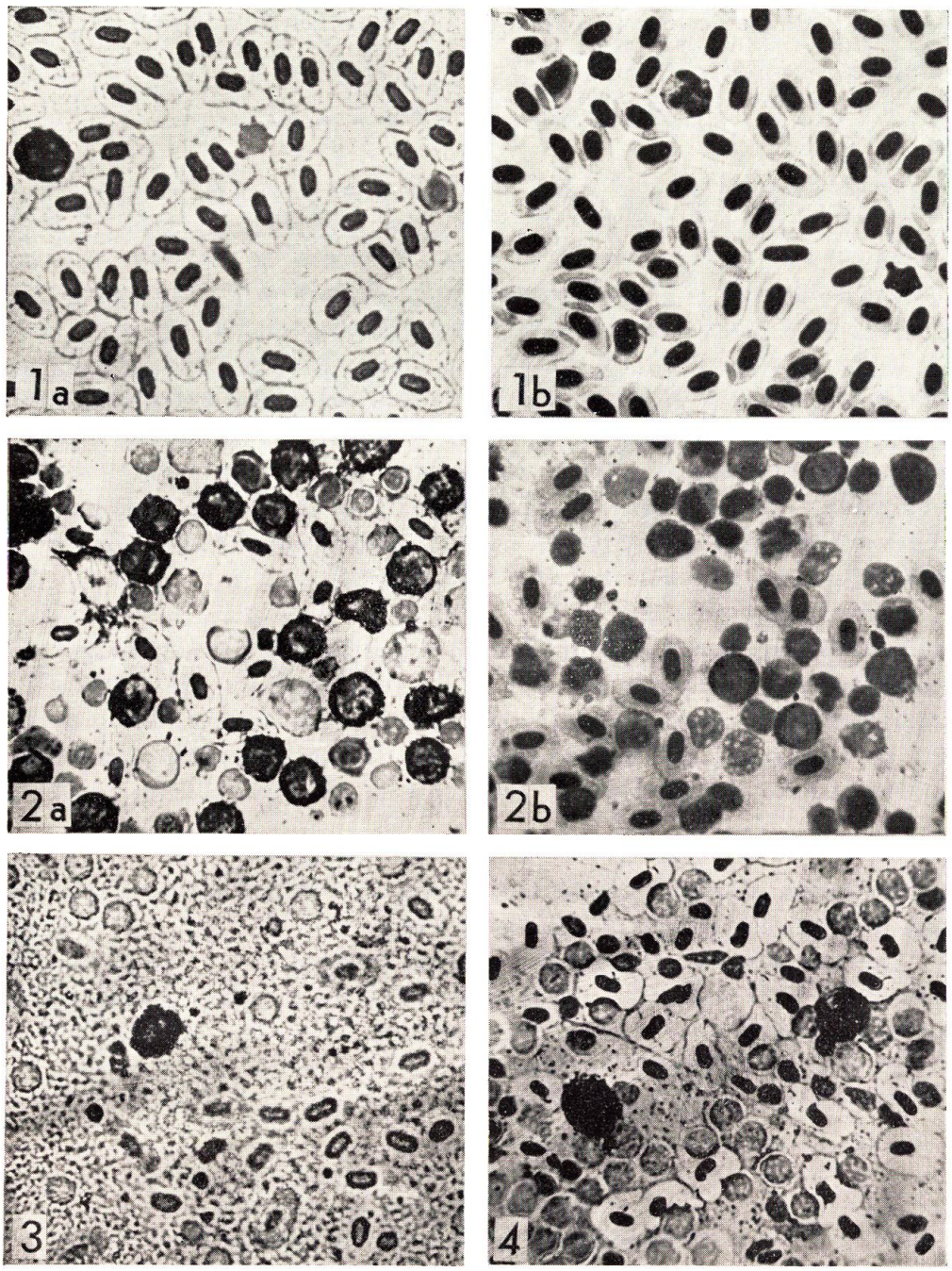
Fig. 1. Circulating-blood smears. a: peroxidase staining; b: Wright-Giemsa's staining.

Fig. 2. Mince- and smear-preparations of kidney. a: peroxidase staining; b: Wright-Giemsa's staining.

Figs. 3 and 4 show mince- and smear-preparations of liver and spleen obtained by peroxidase staining.



ery Erythrocyte *gr* Granulocyte *im.e* Immature erythrocyte *ly* Lymphocyte *m* Monocyte *ppl* Peroxidase-positive leucocyte *s* Spindle cell



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