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著者	KASHIWAGI Masaaki, YOSHIDA Fumikazu, SATO Ryuhei
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Blood Cell Constituents of Chum Salmon, *Oncorhynchus keta* (Walbaum), with Special Refererence to the Erythrocyte Series according to Growth of the Fish from Hatching

Masaaki KASHIWAGI, Fumikazu YOSHIDA and Ryuhei SATO

Department of Fisheries, Faculty of Agriculture,
Tohoku University, Sendai, Japan
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Summary

1. The alevins of chum salmon just after hatching had three kinds of erythrocytes: primary, polychromatic and basophilic. Their constitutents were 96.92 per cent, 1.60 per cent and 1.48 per cent respectively.
2. The primary erythrocytes, which were round in shape and showed a light red cytoplasmic stain with May-Giemsa's, decreased as the fish grew and disappeared at about 60 days when the yolk sac was absorbed completely.
3. Mature erythrocytes of oval shape and orthochromasia replaced the primary erythrocytes. They appeared at about 8 days then increased. The percentage reached 73.47 per cent at 40 days when the fish rose to the water surface and began feeding, 78.33 per cent at 60 days and 78.27 per cent at 130 days.
4. The percentage of polychromatic and basophilic erythrocytes showed a increasing at 21 days, i.e. 49.75 per cent and 22.10 per cent of erythrocyte population respectively. But those percentage were decreased to 10.07 per cent and 7.13 per cent at 40 days and became stable at 60 days.
5. The changes of the erythrocyte constitutents were correlated with the degree of yolk sac absorption with growth of the fish. The constitutents showed a tendency to be stabilized at the stage when yolk sac absorption was completed.
6. The average size of the erythrocytes became longer on the major axis, but shorter on the minor axis with the growth of the fish.

It is necessary to investigate physiologically chum salmon, *Oncorhynchus keta* (Walbaum), for maintaining their production naturally and artificially. The authors have been studying the chum salmon physiologically throughout their life for the purpose of propagation as already reported by Kashiwagi and Sato (1) and Sato and Kashiwagi (2). In the present paper, the morphology of the erythrocytes and their constitutents were observed on the alveins and fry with growth of the fish.

Materials and Methods

The chum slamon used in this study were carried to the laboratory at the stage of eyed period egg from the Tsugaruishi River Salmon Hatchery, Iwate Prefecture, Japan, and reared in the fresh water aquaria from January to May in 1966. The water temperature was 5.5 to 14.5°C during the rearing. The alevins rose to the water surface and began feeding at about forty days after hatching, so they were fed on a dry mixed ration.

The stage of the fish examined were just, 8 days, 21 days, 30 days, 40 days, 60 days and 130 days after hatching. The body size at each stage are shown in Table 1.

On the methods of blood collection, the just hatched and 8 day alevins were cut on the heads because they had small and limp bodies. The small amount of blood that flow out was taken with a capillary tube and placed on a slide glass immediately. The freshly filmed blood was stained with the May-Giemsa's technique (3). The routine caudal procedure was used on the other alevins and fry, and the blood was prepared in the same way.

The observations of the blood cells were made under oil immersion at a magnification of 1,500. One thousand erythrocytes in total were counted from each of five fish at one stage.

Results

The alevins of chum salwon just after hatching have a round yolk sac and transparent body. The erythrocyte series consisted of three kinds of cells. The first was a round shaped cell, irregularly oval, with an average size of 14.4 by 12.7μ and the cytoplasm was stained light red with May-Giemsa's. The chromatin network of the nucleus was minute. The authors call it "primary erythrocyte" to follow Ostromova's expression (4). The second was oval shaped and stained polychromatically. The third was round or oval shaped, stained basophilically and contained materials in the outline of the cell. These two cells were smaller than the primary reticulum cells. The percentage was 96.92 per cent in the first, 1.60 per cent in the second and 1.48 per cent in the thrid of the erythrocyte population (Table 1).

In the 8 day alevins, the yolk sac was elongated along the body, melanin appeared on a part of the back of the body and guanin on the operculum. In addition to the noted three cells, there were mature erythrocytes which had an oval shape and the cytoplasms stained orthochromatically. This mature erythrocyte was not different from that found in adult fish, except that the size of 14.5 by 9.0μ was smaller than that of 17.0 by 10.0μ for the 2 years old fish reared in a salt water pond (unpublished data). The chromatin of the nucleus was more coarse than those of the primary erythrocyte. The percentage of mature erythrocytes was 0.19 per cent.

TABLE 1. *Erythrocytic Constituent of Chum Salmon at Different Stages*

Days after hatching	Body length (cm)	Body weight (gm)	Water temp. (°C)	Yolk sac absorp. (%)	erythrocyte series				
					prim. (%)	poly. (%)	Baso. (%)	Mature (%)	Senile (%)
Just	2.2	0.3	9.0	0	96.92	1.60	1.48	—	—
8	2.6	0.3	8.0		90.99	4.50	4.32	0.19	—
21	2.8	0.3	7.0	50	22.15	49.75	22.10	6.00	—
30	2.8	0.3	8.0		11.12	27.38	9.22	52.28	—
40	2.9	0.3	8.5	80	9.33	10.07	7.13	73.47	—
60	3.3	0.4	12.8	100	0.07	14.13	7.47	78.33	—
130	6.2	2.7	14.5		—	15.60	6.13	78.27	—

In the 21 day alevins, both melanin and guanin content increased, and the yolk sac was about fifty percent absorbed. The percentage of primary erythrocytes decreased and showed 22.15 per cent of the erythrocyte population. Those of the polychromatic, basophilic, and mature erythrocytes increased to 49.75 per cent, 22.10 per cent and 6.00 per cent respectively.

In the 30 day alevins, the polychromatic and bsophilic erythrocytes decreased to 27.38 per cent and 9.22 per cent, though the mature erythrocytes increased to 52.28 per cent. The primary erythrocyte count was 11.12 per cent as shown in Table 1.

In the 40 day alevins, the side of the body was covered with guanin and remarkable so-called parr mark appeared. The yolk sac was about eighty per cent absorbed. Most of the fish rose to the water surface and began feeding, so they were fed on a dry mixed ration as mentioned previously. The percentage of primary erythrocyte decreased to 9.33 per cent, while the mature erythrocytes increased to 73.47 per cent. The polychromatic and basophilic erythrocytes were 10.07 per cent and 7.13 per cent.

In the 60 day fry in which the yolk sac was completely absorbed, the primary erythrocytes were few. The percentage of polychromatic, basophilic and mature erythrocytes were 14.13 per cent, 7.47 per cent and 78.33 per cent respectively.

In the 130 day fry in which the parr marks disappeared, no primary erythrocyte was observed and senile erythrocytes on the course of collapse were seen. But the erythrocytic constituents resembled those of 60 day fry as shown in Table 1 and Fig. 1.

The changes of the erythrocytic constitutents of the chum salmon according to their growth from hatching were correlated with the degree of yolk sac absorption. And the constituents became stable at the stage of complete absorption of the yolk sac. On the other hand, there are some changes in the size of erythrocytes, too. The major and minor axis of the 100 cells of the erythrocyte series were measured in random and shown in Fig. 2 as the Price-Jones curve. The Price-Jones curves were divided into three types. In the first, there is a mode of 14.0μ of the major axis and of 12.5 to 13.0μ of the minor axis. This

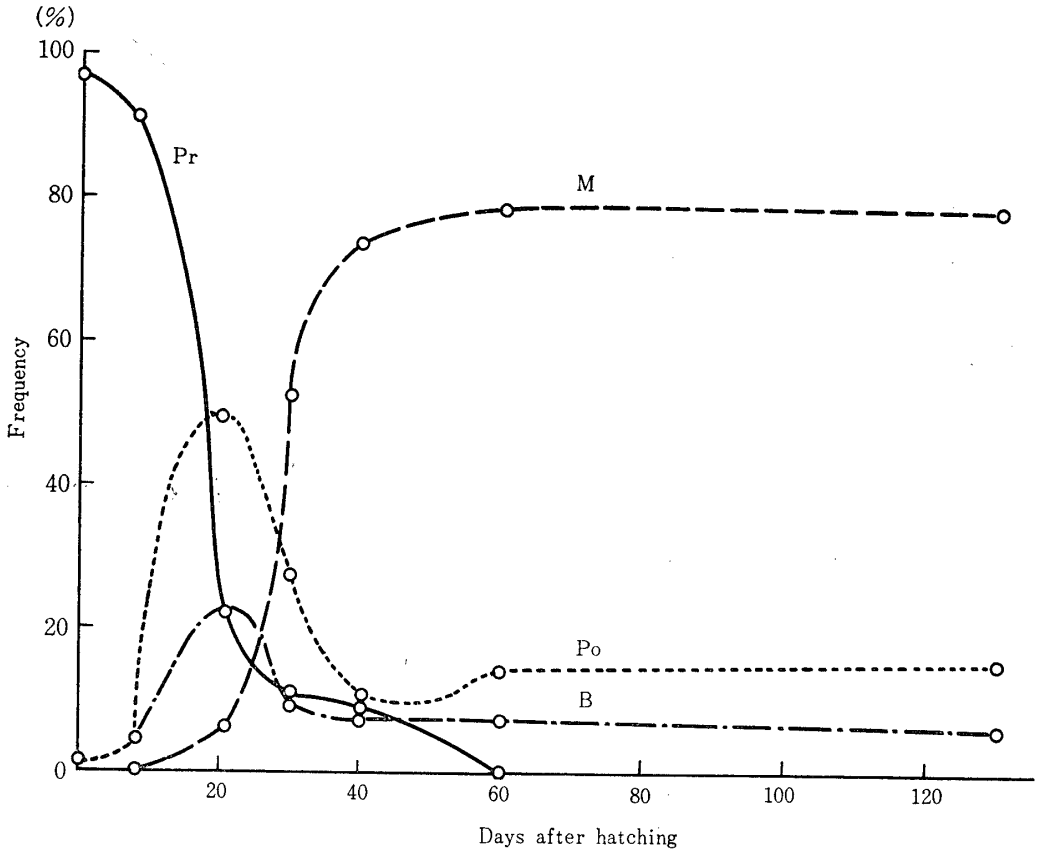

FIG. 1 Changes of the erythrocyte constituents of the chum salmon According to their growth after hatching.
Pr; Primary Erythrocyte Po; Polychromatic Erythrocyte
B; Basophilic Erythrocyte M; Mature Erythrocyte

TABLE 2. *Average Size of Erythrocytes of Chum Salmon at Different Stages.*

Days	Average Diameter		Type of the Price-Jones curve
	Major axis (μ)	Minor axis (μ)	
Just	14.12	12.65	I
8	13.93	12.21	
21	14.06	10.33	II
30	13.95	10.36	
40	14.49	9.40	III
60	14.52	9.32	
130	14.61	8.85	

* Price-Jones curve were divided into three types as shown in Fig. 2.

type showed a characteristic of round shaped cell for the just hatched and the 8 day alevins. In the second, there is no characteristic mode but wide distribution for the 21 day and the 30 day alevins. In the third, there is a 14.5 μ mode of

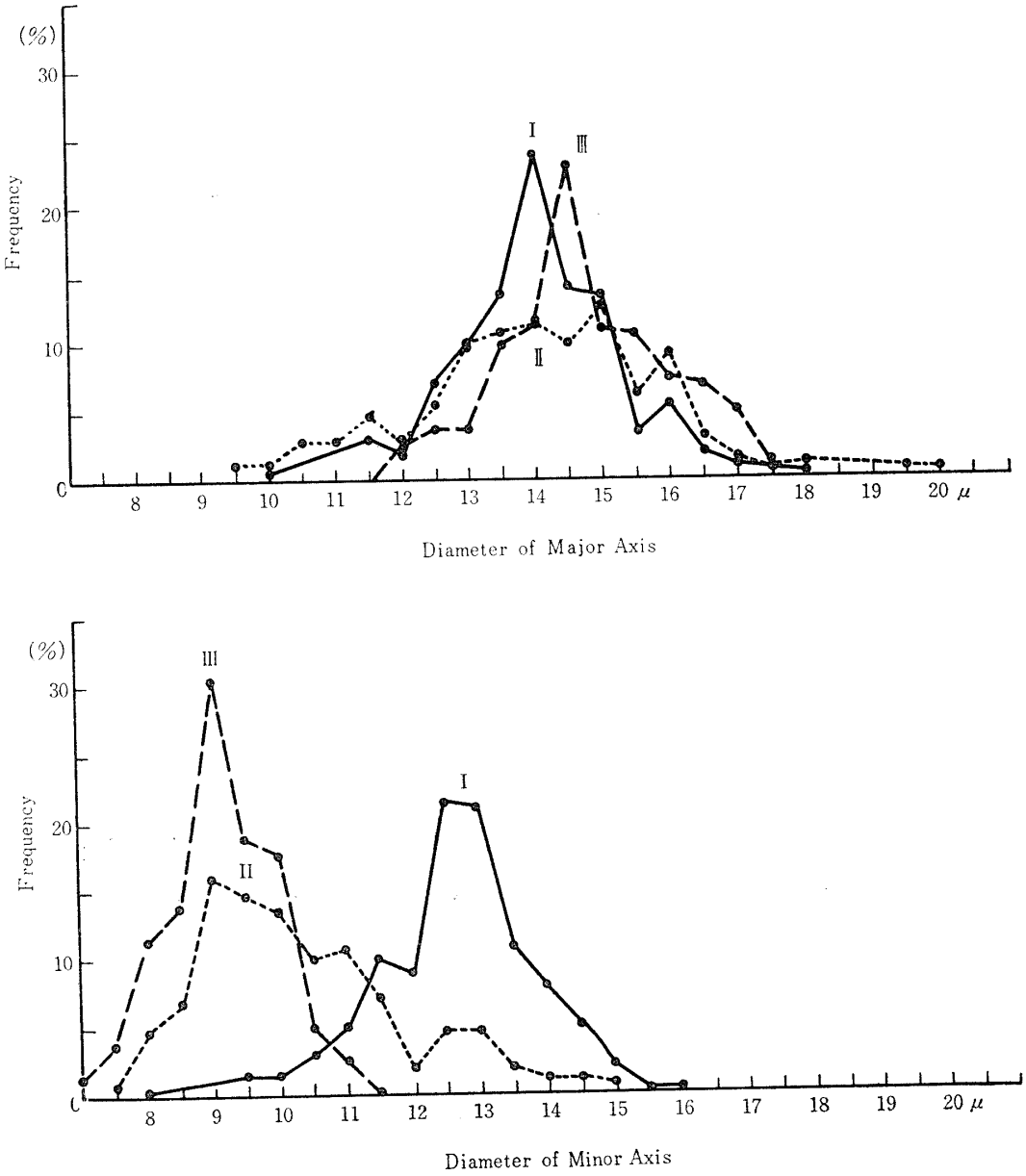

FIG. 2 Price-Jones curve of the erythrocytes of the chum salmon at different stages.
I; alevins just after hatching II; 21 day alevins III; 40 day alevins

the major axis and 9.0μ of the minor axis. This type showed a characteristic oval shaped cell for the 40 day alevins, the 60 and the 130 day fry. Therefore, the average erythrocytes grew in the major axis and shortened in the minor axis according to the growth of the fish as shown in Table 2. The morphological changes of the erythrocyte series above mentioned were shown in Fig. 3 to 6 of the just hatched, 21 day and 40 day alevins and 130 day fry.

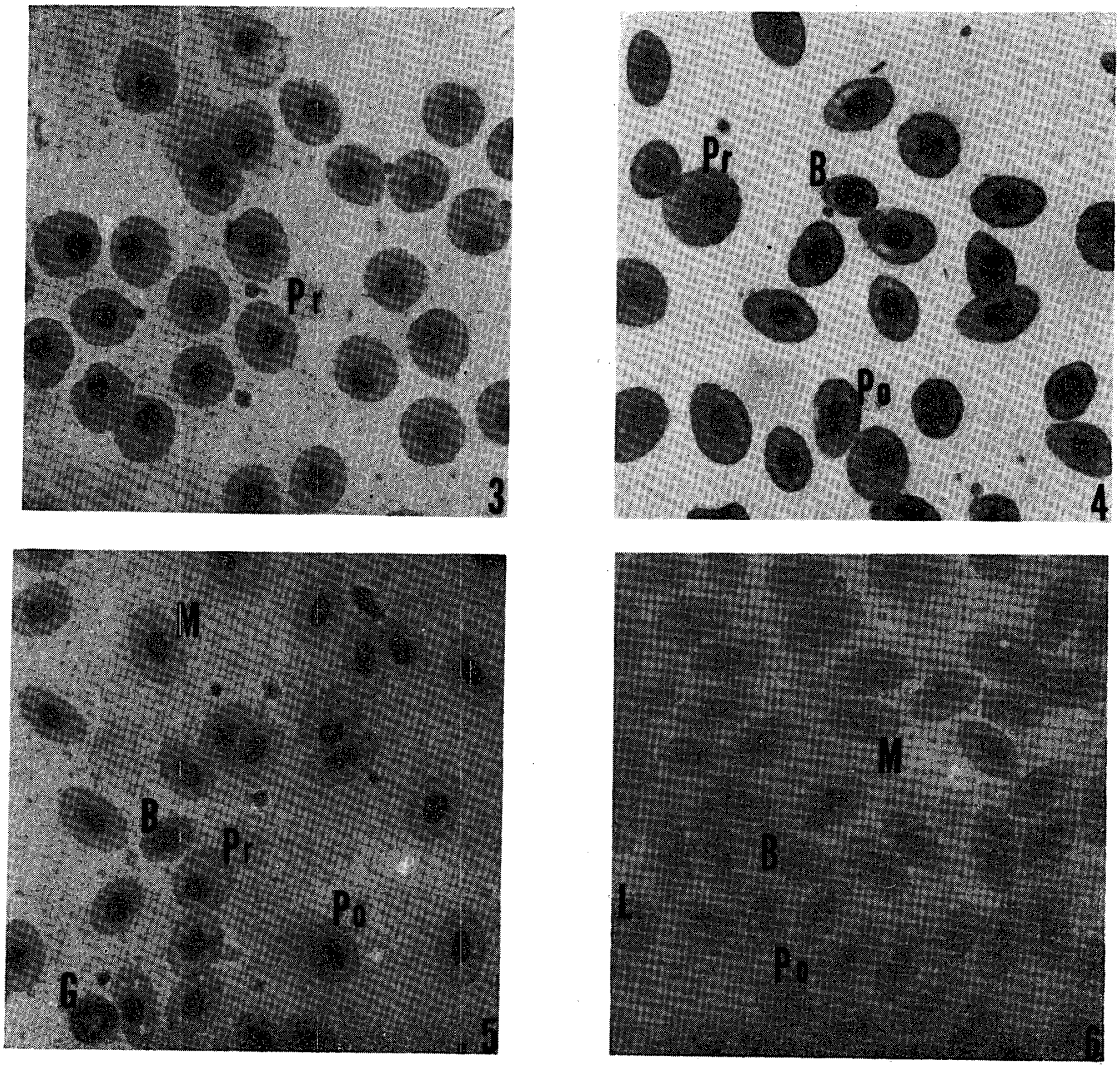

PLATE 1.

Explanation of Figures

FIG. 3 Blood cells of chum salmon alevins just after hatching. ×600
FIG. 4 Blood cells of the 21 day alevins. ×600
FIG. 5 Blood cells of the 40 day alevins. ×600
FIG. 6 Blood cells of the 130 day fry. ×600

Pr; Primary erythrocyte — Po; Polychromatic erythrocyte
B; Basophilic erythrocyte — M; Mature erythrocyte
G; Glanulocytic leucocyte — L; Lymphocytic leucocyte

Discussion

Of the many hemaological investigations of fish, a report by Ostromova (4) on chum salmon is one of absorbing interest. Ostromova studied the constitutents of the erythrocyte series according to the growth of the fish from hatching, and stated that the percentage of primary erythrocytes was 100 per cent for the one day alevins, 60 per cent for the 28 day when the yolk sac was 80 per cent absorbed, and non-existent at 50 days when the yolk sac was completely absorbed. On the contrary, mature erythrocytes made up 8.0 per cent at 28 days, 70 per cent at 50

days, 77 per cent at 60 days and 95 per cent at 130 days of life. The results of the present observations which show a decrease of primary erythrocytes and an increase of mature ones is similar to that of Ostromova's findings. However, the percentage of mature erythrocytes for the 130 day fry is higher in Ostromova's results than in the present study. It is caused by the fact that the polychromatic erythrocytes were not recognized by Ostromova though they were considerable in the present study. Ostrovova may have interpreted the polychromatic erythrocytes to be mature ones, because there is almost no differences between the two results in percentage of basophilic erythrocyte. According to Dawson (5) and catton (6), the polychromatic erythrocyte was classified into immature erythrocyte with the basophilic ones while Ostromova interpreted only the basophilic ones as immature. More detail studies including the hemopoietic organs are desirable on this development of erythrocyte series.

Basophilic erythrocytes in the present study were found in the alevins just after hatching, although they were not measured in the one day old alevins by Ostromva.

Anyhow, the changes of the erythrocytic constitutent which correlated with the degree of yolk sac absorption of the fish agreed with Ostromova. It may merit some attention that the erythrocytic constitutent had a tendency to stabilize at 40 days when the fish rose to the water surface and began feeding. Also, it is undoubted that the size variation of erythrocytes according to the growth of the fish reflected the change of erythrocytic constitutent, with special reference to the decreasing of round shaped primary erythrocytes and the increasing of oval shaped mature ones.

Besides, the internal organs including the hemopoietic ones seemed to be formed at the stage when feeding of the fish started. A histological observations of the hemopoietic organs according to the growth of the fish from hatching will be desirable in relation to the development of the erythrocyte series and its constitutents.

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