COMPARATIVE STUDIES ON TWO HEMOGLOBINS OF SALMON—V.
CHANGE IN PROPORTION OF TWO HEMOGLOBINS WITH GROWTH*

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It has been reported^{1,2)} preliminarily that in the blood of chum salmon, *Oncorhynchus keta*, there are two hemoglobins (Hb's), components F and S, whose quantitative proportion is fairly constant, component F:S being 55%:45% on an average. Among the individuals analyzed, however, there were some ones of which the proportion deviated notably from the average, as shown typically in the case of the fish collected on Jan., 1959, at Yamagata Pref.³⁾ It seemed to be interesting to the authors what is at the bottom of this phenomenon, and whether it is of simple physiological nature or of complicated hereditary one, for example which relates to the existence of plural varieties of this species. In order to reinvestigate this problem, the percentage proportion of both components was determined on 151 individuals of this fish, which were caught mainly at several places of this country and were examined relationships between the proportion and some factors, the place and season of catching, sex and body length of the fish, and water temperature. According to the results obtained, it was found that there is a positive correlation between the proportion and body length (or the stage of growth). This phenomenon seems to be noteworthy enough to report, though its true character is not yet be revealed.

Experimental

Materials The blood of chum salmon was collected individually at Hokkaido, Japan Proper, and the North Pacific Ocean, as shown in Figs. 2 and 3 in detail. The fish were matured sexually, which were caught at rivers and a few miles off the coasts of Mashike and Abashiri. All the fish caught at the North Pacific were about 50 cm. long and immature sexually. The blood collected was stored immediately in ice to bring back to our laboratory as quickly as possible, mostly in one or two days after collecting. Hemolyzates prepared as usual from these blood samples were analyzed as described below. The North Pacific blood samples were however partly hemolyzing due to the prolonged storage of more than two weeks and they were analyzed soon after the removal of plasma layer.

Method of estimation of the percentage proportion of both components
The percentage proportion of components F and S in the hemolyzate was estimated

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by the heat coagulation method⁴⁾. This method is based on the fact that there is a temperature zone in which component F is coagulated quantitatively while component S not at all when a mixed solution of both components is heated under a fixed condition. The concentration of the pigment in solution was determined spectrophotometrically with a BECKMAN DU spectrophotometer.

One of the difficulties on the estimation of the proportion was how blood samples should be brought to the Laboratory without any secondary changes affecting the proportion. To check whether the percentage proportion changes before analysis, some experiments were carried out using fresh blood samples which were ice-stored for only 6 hours after collecting at the Nitta River, Fukushima Pref. According to the results the proportion of both components determined after the ice-storage for more than 2 weeks was not different from the proportion at 6 hours after collection. Indeed there is no guarantee at present that no change of the proportion takes place during 6-hours storage in ice. Even if any change occurs, however, it will be very slight. Because it is reasonable to consider such a change to proceed gradually rather than drastically, and as mentioned above, there were no symptoms favorable to such a gradual change.

Results and Discussion

Results are represented conveniently in the quantitative ratio of component S to

Fig. 1. Distribution of the proportion of components F and S in all the blood samples. The proportion is represented conveniently as percentage of component S to the total amount of Hb. This note is common in all the following figures.

the total amount of both components (abscissa) versus the frequency expressed in the number or percentage of individuals (ordinate). The distribution curve of all the fish hitherto analyzed is an almost normal curve, the ratio of component S varying from 37 to 51% and the axis of symmetry being located at 44 to 45% (Fig. 1).

The distribution as classified by the place of sampling is shown in Figs. 2 and 3. It appears that the distribution is different more or less from one place to another, especially among the places of Hokkaido. Then the results of Japan Proper were examined from various points of view such as the ocean of catching, sex and body length of the fish, the water temperature of river, and the season of catching.

The distribution as divided into the ocean

Fig. 2. Distribution of the proportion as classified by the place of catching.

Mashike (Hokkaido, offshore), water temp. 16~17°C., Oct. 21, 1958.

Abashiri (Hokkaido, offshore) water temp. 12~13°C., Oct. 22, 1958.

Kitami (the Tokoro River, Hokkaido) water temp. 8.0°C., Oct. 23, 1958.

Shibetsu (the Shibetsu River, Hokkaido) water temp. 2.9°C., Nov. 29, 1958.

Chitose (the Chitose River, Hokkaido) water temp. 6.5°C., Dec. 4, 1958.

Tsugaruishi (the Tsugaruishi River, Iwate Pref.), water temp. 8.5°C., Jan. 8, 1960.

Haranomachi (the Nitta River, Fukushima Pref.), water temp. 12~13°C., Nov. 20~22, 1959.

Ishizuka (the Tone River, Ibaragi Pref.), water temp. 11°C., Nov. 25~26, 1959.

Fukura (the Takibuchi River, Yamagata Pref.), water temp. 7.2°C., Dec. 18, 1959.

Teradomari (the Shinano River, Niigata Pref.), water temp. 10°C., Dec. 6, 1959.

Toyama (the Jintsuji and the Joganji Rivers, Toyama Pref.), water temp. 9~13°C., Nov. 28, 1959.

of catching, is shown in Fig. 3, from which it seems that the distribution shifts to the left, though slightly, in the order of the Pacific Ocean, the Japan Sea, and the North Pacific.

No evident relation seems to be present between the ratio and sex, though the distribution curve of female only is lacking in individuals containing component S less than 42% (Fig. 4).

The distribution was analyzed from the point of body length, although most of the fish were adult and fairly large in body size because they were caught in spawn-

Fig. 3. Distribution of the proportion as classified by the ocean of catching.

Fig. 4. Distribution of the proportion as classified by sex.

Fig. 5. Distribution of the proportion as classified by body length.

ing season. As seen in Fig. 5, it seems that the longer the body length, the more the distribution shifts to the right. However it may be inadequate to point out conclusively this correlation only from these data, because of scarcity of the analysis on the fish of shorter body lengths.

Next the relation was studied between the ratio of component S and the water temperature of river where the fish were caught. The result is shown in Fig. 6. The border-line of temperature of 9°C. was selected on trial, considering the numbers of the fish caught and the water temperatures at all the places. It seems that the distribution is different to a certain degree between the two groups divided thus.

In Fig. 7, the results at the Takibuchi, Yamagata Pref., and the Tsugaruishi, Iwate Pref. are presented respectively over two seasons of catching. It is seen easily that the distribution varies appreciably with the season (or time) of catching at the same place.

Thus it seems that the percentage proportion of both components is more or less related to these factors except sex of the fish. Above all, the correlation with body length of fish is most probable: There is a tendency that the longer the body length, the more the quantitative ratio of component S increases. In fact, the majority of the fish caught at the North Pacific all of which were small (about 50 cm. long) contained component S less than 42% (refer to Fig. 3). So is the case with the situation of the Takibuchi in Jan., 1959 (refer to Fig. 7), in which all the fish were

Fig. 6. Distribution of the proportion as classified by water temperature. The border-line of 9°C. was selected arbitrarily.

Fig. 7. Distribution of the proportion as classified by the season of catching at the Takibuchi, Yamagata Pref. and at the Tsugaruishi, Iwate Pref..

of comparatively small size. These two findings seem to support the correlation between the percentage proportion of both components and body length. Thus, the correlations between the ratio and the several factors above mentioned may be regarded as different phases of one phenomenon.

In order to confirm further the correlation between the ratio of component S and body length, the same analysis was made on the blood of larval chum salmon (6~8 cm. long and 1.5 g. weighed on an average) which were caught on May 23, 1960, at the Tsugaruishi Salmon Hatchery, Iwate Pref. Blood from 73 individuals was combined to analyze, as only one to two drops of blood were obtained per fish by cutting tail. According to the result, the quantitative ratio of component S was only 23.6% in this sample. It is very clear that this supports strongly the correlation in question.

In this connection, it has been reported recently⁵⁾ that the percentage proportion of two components of Hb is different remarkably between red salmon and its land-locked form, Hime-masu, the quantitative ratio of component S being about 50% in the former and about 30% in the latter. This difference might be, however, of apparent nature based on the difference in growth stage, because the red salmon were adult and about 50 cm. long whereas the Hime-masus immature and only about 20 cm. long. The authors wish to throw more lights on this problem in the future.

Thus it was found that in the blood of chum salmon there are always two types of Hb whose percentage proportion correlates to body length or the degree of growth of the fish. Then either of both seems not to be such abnormal Hb as in the case of anemic diseases of human. It has been reported on sheep^{6,7)} or cattle⁸⁾ that some breeds possess two types of adult Hb and the other breeds only either of the two, and the difference of the occurrence of the Hb's seems to be determined genetically. The present phenomenon should be however regarded as a different one from it. ADINOLFI *et al.*⁹⁾ has reported that in the blood of lamprey there is present one type of Hb, which is likely to correspond to fetal Hb in mammalia, in addition to ordinary adult type of Hb, both types of Hb occurring in a nearly same concentration at larval stage while most of the fetal-like Hb disappearing at adult stage. The present case seems to be compared rather with the case with lamprey, although they should be distinguished in the point that at adult stage chum salmon possess both Hb's in an almost same concentration, while lampreys mainly adult Hb.

Next problem is what physiological significances the present phenomenon implies. As reported previously¹⁰⁾ respiratory properties of both components of chum salmon Hb are different remarkably from each other: Component F is one of the Hb's of the lowest oxygen affinity among fishes. The oxygen affinity is affected strongly by pH. In a great contrast component S shows a fairly high oxygen affinity and is absent completely in the pH effect. On the other hand salmon are very specific ecologically in moving down from fresh water (river) to sea at larval stage and returning to fresh water again to spawn. These two facts, i.e., the difference in respiratory function of both

components and the ecological specificity of this fish, may be related directly to this phenomenon, although no reasonable explanation is yet given on this matter. Further studies will be continued to clarify this phenomenon.

Summary

The estimation of the percentage proportion of two Hb's was made by the method of heat coagulation on many individual samples of adult salmon and on a combined sample from larval salmons.

The frequency curve of all the fish analyzed showed an almost normal curve, the proportion of component F:S being 63~49%:37~51% and about 55%:45% on an average. It seems that the proportion correlates to several factors such as the place and season of catching, water temperature, and body length except for sex. Most probable is the correlation with body length, which is supported by the proportion (F:S=24%:76%) of larval blood. Correlations with the other factors might be different phases of the correlation with body length.

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