Hemoglobin Content, Erythrocyte Sedimentation Rate and Hematocrit of the Blood in the Young of the Carp (*Cyprinus carpio*)

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(Text-figs. 1-3; Tables 1-5)

(I) INTRODUCTION

Several works have been reported on the hemoglobin content (Hb), erythrocyte sedimentation rate (E.S.R.) and hematocrit (Ht) of fish blood. As to the hemoglobin content or the hematocrit of the carp (*Cyprinus carpio*) blood, there are recent studies by SAITO (1954)[1] and BLACK (1955)[2]. It appears, however, that the carp used as the material in all of the experiments hitherto reported were adult or nearly so and that not more than twenty of them were examined as a single lot.

It is very probable that hematological tests will find practical value in fish culture in diagnosing certain physiological abnormities of the fish. This subject has already been reviewed by DAVIS (1956)[3].

The present work has been undertaken to obtain basic data for carrying out hematological tests on fishes. Young carp under one year of age have been selected as the material and the normal values of hemoglobin content, erythrocyte sedimentation rate and hematocrit have been determined. Carp of this age represent the stage called the "seed carp" and are regarded with much importance in carp culture.

Prior to the determination of the aforementioned normal values, the methods of determination were critically studied. And the methods which give satisfactory results were developed.

(II) MATERIALS

All the carp used as the material arose from the eggs laid on a single occasion in June 1957, and were raised under the same suitable conditions.

They were fed on the artificial diet composed of fresh fish meat, ground dry silkworm pupa, wheat middlings and small quantity of silkworm feces. The food was given in such manner that fresh food was always, even in midwinter, present in the rearing tank; so that, the fish presented the appearance of satiety.

Those fish which showed any sign of illness were discarded in the course of the raising.

(III) METHODS AND THEIR CRITICAL STUDIES

Since carp blood has large clotting ability[4] and its erythrocytes are susceptible to hemolysis, and since the carp used as the materials were small in size, the apparatus

and procedures which are usually applied to the examination of human blood were somewhat modified and employed in the present work.

(A) *The region from which the blood was collected*

The blood was drawn from bulbus arteriosus[5] into the heparinized glass capillary.

In order to expose bulbus arteriosus, a carp was wrapped in wet gauze as soon as it was captured, then the ventral end of right clavicle and coracoid were cut with scissors, and the muscle was cut open toward the heart. By this method, the heart was already exposed with little bleeding in only 20 to 30 seconds after the fish was captured.

(B) *Heparinized glass capillary*

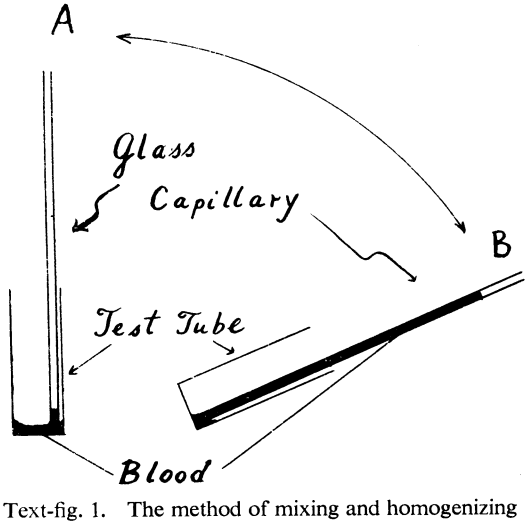
Heparinized glass capillaries were used to draw blood from bulbus arteriosus, since employment of a syringe and a metal needle often caused coagulation or hemolysis in carp blood.

The glass capillaries were 2 mm in diameter and 150 mm in length. They were sharpened at one end just as the injection needles, and were lined with the film of heparin.

When the heparin film with which a capillary was lined dissolved into a capillary-full of blood, the concentration of the heparin was estimated at 0.1–0.2 mg per ml of the blood. This concentration of heparin is regarded as sufficient to lengthen the coagulation time of the blood of man[6]. Indeed, the heparinized blood sample of the carp did not coagulate for more than three hours at 30°C.

(C) *Drawing the blood, mixing with heparin and homogenization*

When the needle-shaped end of the heparinized glass capillary was inserted in bulbus arteriosus, the blood flowed into the capillary to the accompaniment of the beat of the heart. In three or five seconds the capillary was filled with the blood. Then it was drawn out from the heart and was stood erect in the little test tube, allowing the blood flow onto the bottom of the test tube (Text-fig. 1, A).



Text-fig. 1. The method of mixing and homogenizing carp blood.

Next, both the capillary and the test tube were tipped; this caused the blood to flow back into the capillary by capillary attraction (Text-fig. 1, B). Then the capillary was put back in the state of erectness, the blood being transferred into the test tube again. This mixing operation was repeated ten times; the blood was now completely homogenized and mixed with heparin without disintegrating the erythrocytes.

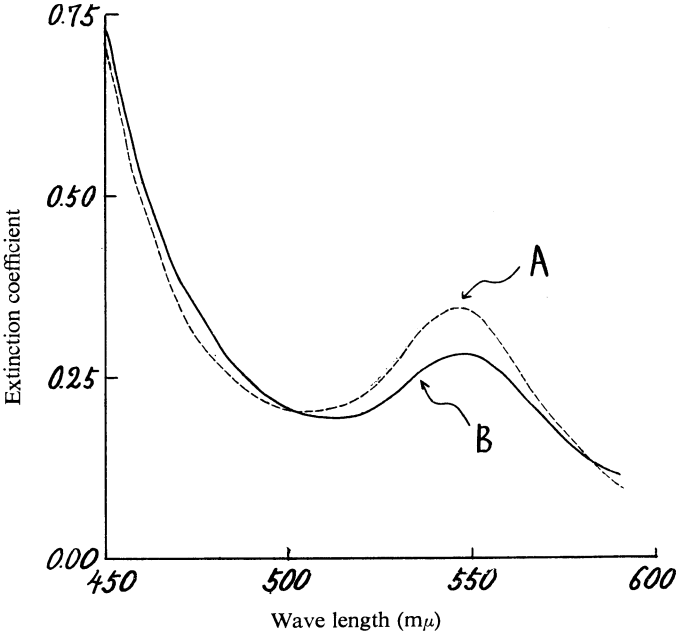
The blood sample thus homogenized was immediately subjected to the determination of hemoglobin content, erythrocyte sedimentation rate and hematocrit.

(D) *Hemoglobin content*

"Sahli's acid hematin method" is probably the simplest and easiest method to determine the hemoglobin content of blood, but many faults of this method have been pointed out in respect of accuracy[7]. Besides, the colour tone of the acid hematin derived from carp blood often fails to accord with that of the colour standard of the hemometer.

For these reasons, the "cyan-hematin method"[7][8] was used in the present study. This method has advantage over other methods in that pure hemin can be used as the colour standard.

The absorption curves of the cyan-hematins derived from hemin and from the hemoglobin of carp blood were determined spectrophotometrically (Text-fig. 2). The two



Text-fig. 2. Absorption curves of the cyan-hematin derived from hemin (A) and from the hemoglobin of carp blood (B).

preparations of cyan-hematin agreed in the wave length of maximum absorption band (545 mμ). So that the hemoglobin content of carp blood could be determined accurately by "cyan-hematin method".

(E) *Erythrocyte sedimentation rate and hematocrit*

"Kato's micro-hemopipette"[9] was used in determining erythrocyte sedimentation rate and hematocrit. Erythrocyte sedimentation rate was expressed in terms of "volume percent" in an hour at 25°C.

After the determination of the erythrocyte sedimentation rate, the hemopipette was centrifuged for 30 minutes at 3,700 r.p.m. in order to read the hematocrit. The centrifuge used in this experiment had a sufficient radius to produce a relative centrifugal force of about 2,260g at 3,700 r.p.m.[10].

(F) *Critical studies*

In order to check whether a blood sample was rendered really homogeneous by the method described in (C), the critical test was carried out as follows.

A blood sample drawn from a carp was divided into four subsamples, and the hemoglobin content and the hematocrit of each subsample were determined. It was found that the hemoglobin contents of the four subsamples were almost of the same level, and that their hematocrits also agreed with each other (Table 1). This test was repeated four times and the homogeneity of each blood sample was confirmed.

Table 1. Hematocrit and hemoglobin values for different portions of a blood sample. (Four measurements were made on a sample by dividing it into subsamples.)

Subsample No.	Hemoglobin Content	Hematocrit
I	13.7 g/dl	45.2%
II	13.7	45.0
III	13.8	45.2
IV	13.7	45.4

Another test was also carried out. Two full capillaries of blood were consecutively drawn from a carp. Then the hemoglobin content and the hematocrit of the blood in each capillary were determined. This test was repeated five times. An example of the results is shown in Table 2.

Table 2. Hematocrit and hemoglobin values for the two blood samples consecutively taken from the same fish.

	Hemoglobin Content	Hematocrit
1st Sample	14.1 g/dl	51.7%
2nd Sample	14.1	51.5

It is indicated by Table 2 that the character of the blood of a carp can be adequately represented by a single blood sample.

(IV) RESULTS AND DISCUSSION

In order to determine the normal values of various blood properties, blood samples were taken in late winter (February, 1958) and in late spring (May, 1958) from the carp reared under the aforementioned suitable conditions. The numbers of the fish sampled were 65 and 61, respectively. In addition, blood samples were taken in May, 1958 from the 46 carp which had been starved for 7 weeks.

The results of the analysis of the bloods of these three groups are shown in Tables 3, 4 and 5.

In February 1958 the water temperature of the rearing tank ranged from 1.6 to 7.8°C, and the carp, being eight-month-old, were inactive and showed poor appetite. On the contrary, in May 1958, the water temperature ranged from 16.0 to 22.3°C, and the carp, eleven-month-old, moved actively and had strong appetite.

Table 3. Normal blood values for the young of the carp in late winter (Measurements were made on 65 fish in February, 1958).

	Body Weight g.	E.S.R. %	Ht. %	Hb. g/dl	M. C. H. C. %
Maximum	36.4	4.6	65.7	16.0	31.0
Minimum	21.6	0.3	15.8	3.3	13.3
Mean	26.82	1.66	37.45	9.97	26.37
Fiducial limits at 95% probability	±0.80	±0.20	±1.94	±0.52	±1.02

Table 4. Normal blood values for the young of the carp in late spring (Measurements were made on 61 fish in May, 1958.)

	Body Weight g.	E. S. R. %	Ht. %	Hb. g/dl	M. C. H. C. %
Maximum	93.4	1.3	74.7	16.9	26.9
Minimum	21.7	0.1	26.4	5.5	16.1
Mean	40.38	0.46	50.08	11.03	22.17
Fiducial limits at 95% probability	±2.74	±0.06	±2.06	±0.48	±0.52

Table 5. Blood values for the starved young of the carp in late spring (Measurements were made on 46 fish in May, 1958.)

	Body Weight g.	E. S. R. %	Ht. %	Hb. g/dl	M. C. H. C. %
Maximum	37.5	7.3	52.3	12.4	27.8
Minimum	19.9	0.9	15.0	2.9	12.7
Mean	26.96	3.89	32.99	7.11	21.35
Fiducial limits at 95% probability	±1.01	±0.40	±2.13	±0.20	±0.94

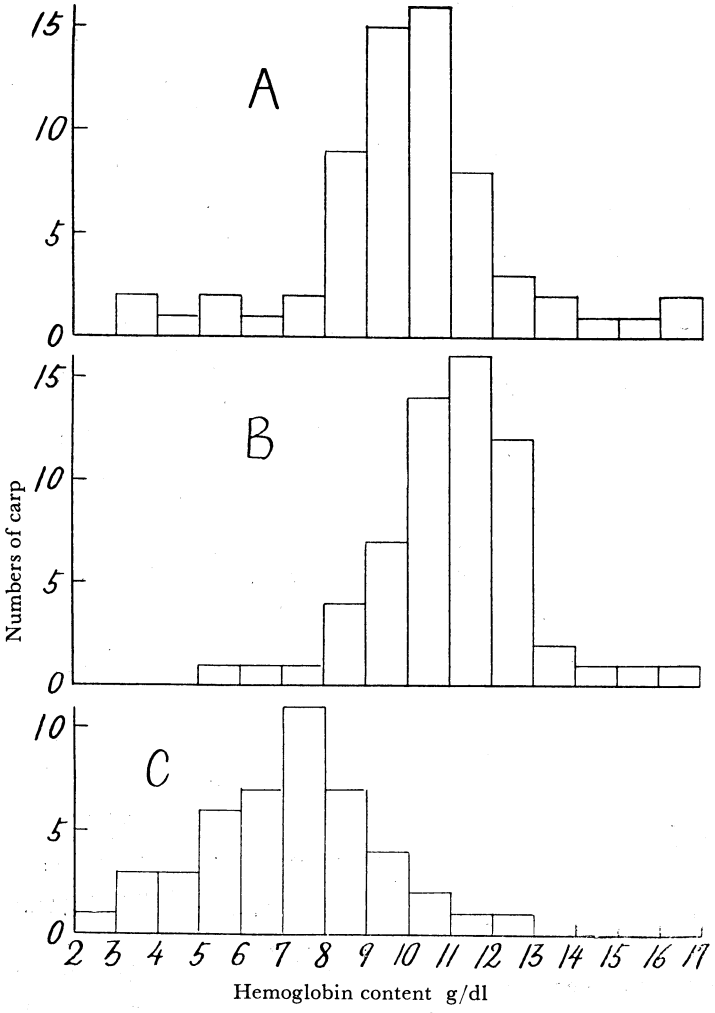
As is seen in Tables 3 and 4, the blood taken in late winter gave lower Hb and Ht but higher E.S.R. and M.C.H.C. (Mean Corpuscular Hemoglobin Concentration) as compared with the blood taken in late spring.

The mean hemoglobin content of 9.97 grams per cent for the blood taken in late winter is close to 9.4 grams per cent reported by BLACK (1940)[11], and the mean content of 11.03 grams per cent for the sample taken in late spring is also close to 11.2 grams per cent reported by BLACK (1955)[2].

The blood taken in late spring from the carp starved for seven weeks showed higher E.S.R. and lower Ht, Hb and M.C.H.C. compared with the bloods of the other two groups (Table 5). The fall of Hb and the rise of E.S.R. were especially remarkable.

After completion of the experiment in May 1958, 10 carp remained in the rearing tank. These were reared under suitable conditions until February, 1959, when their blood was analyzed. Although the result is not printed here because the samples were not numerous, the means of E.S.R., Ht, Hb and M.C.H.C. were nearly equal to those of the blood taken in February, 1958.

From these experiments it was concluded that such characteristics of the blood as Hb, E.S.R., Ht and M.C.H.C. vary both with season and with nutritive conditions.



Text-fig. 3. Frequency distributions of hemoglobin contents in the blood of the carp.
(A) Blood samples taken in late winter.
(B) Blood samples taken in late spring.
(C) Blood samples taken in late spring from starved fish.

Text-fig. 3 shows the histogram of the frequency distribution of Hb in each group of the blood samples. In each group the range of the hemoglobin content, from the minimum to the maximum, was considerably wide, but the distribution was approximately normal.

(V) SUMMARY

(1) The methods for determining hemoglobin content (Hb), erythrocyte sedimen-

tation rate (E.S.R.) and hematocrit (Ht) of the blood of young carp were critically tested and the proper methods were settled.

(2) Normal values of Hb, E.S.R., Ht and M.C.H.C. of the blood of young carp were determined in winter and in spring.

(3) The blood of the carp gave lower Hb and Ht, but higher E.S.R. and M.C.H.C. in winter than in spring.

(4) The blood of the carp starved for seven weeks gave abnormal values on various blood characteristics.

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