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Many investigators have reported that the the properties of blood change sensitively according to the changes of environment. The same can be said about the blood of fish. To make the mechanism of change of fish blood properties clear, we began by determining the total carbon dioxide and the hydrogen-ion concentration in the blood of carp.

Materials and Methods

The species of the fish used in the present investigation was a carp *Cyprinus carpio* LINNE of 76.8–233 g in body weight, 160–262 mm in body length. These carps were obtained in December 1966 from a pond of a private fish farm in the suburbs of Snedai City, Miyagi Prefecture. All the carps were tamed in the aquarium in our laboratory for at least a week before the experiment. Blood was drawn from the heart with a hypodermic syringe or a 0.2 ml measuring pipette and 0.1 ml of it was used to determine the carbon dioxide present. The total CO_2 was determined by a Conway's micro-diffusion method. Hydrogen-ion concentration of the blood was determined by a syringe type glass electrode.

The suffocation of the carp was tried in the air-tight vinyl bag filled with 500 ml water or solution. In other experiments, the carps were allowed to stand in the four litre solutions.

Results and Discussion

All the results are shown in Fig. 1.

In the normal condition, total CO_2 in the blood of the carp was in the range 19.6–29.8 meq/l blood, average value 23.1 meq/l blood when the water temperature was in the range 14–19°C. Hydrogen-ion concentration was in the range pH 7.62–7.70, average value pH 7.67.

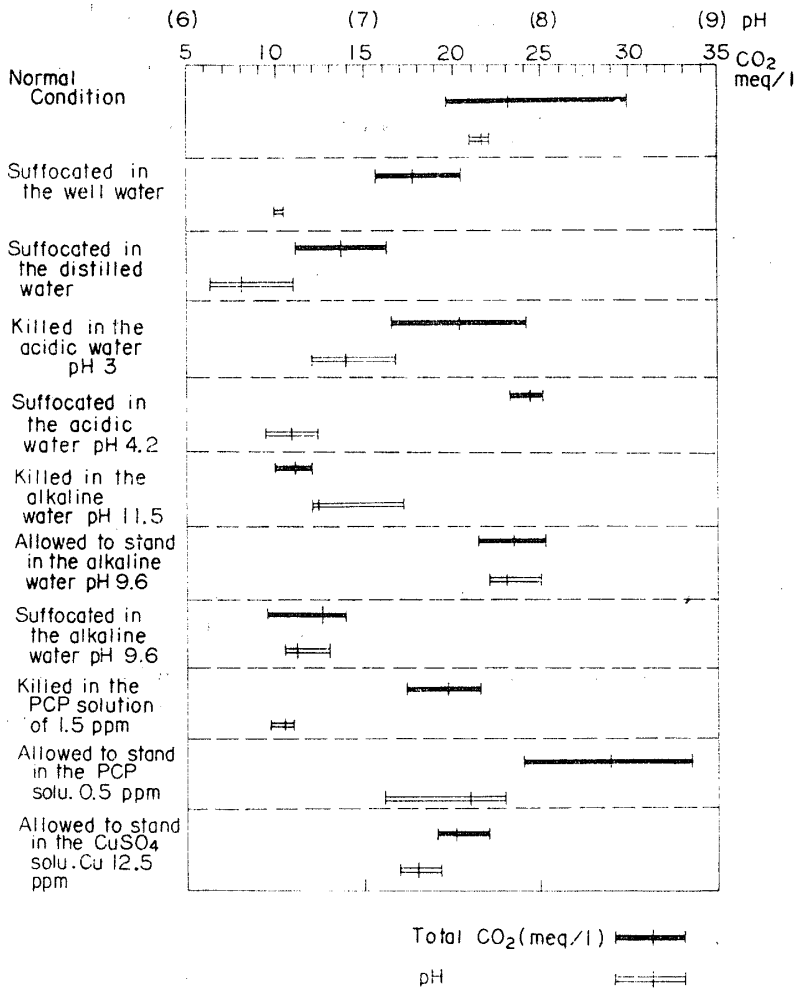

Fig. 1. The total CO_2 and the Hydrogen-ion concentration in the blood of carp in the different environments.

After death by the suffocation, there was a little difference between the one which died in the distilled water and the other in the well water showing high alkalinity.

In the blood of the carp suffocated in the distilled water, the total CO_2 was in the range 11.1–16.4 meq/l blood, average value 13.7 meq/l blood and the hydrogen-ion concentration was in the range pH 6.12–6.60, average value pH 6.31.

In the blood of the carp suffocated in the well water, however, the total CO_2 was in the range 15.7–20.5 meq/l blood, average value 17.7 meq/l blood and the hydrogen-ion concentration was in the range pH 6.47–6.57, average value pH 6.55. These value were a little higher than those involving the distilled water. Both the total CO_2 and the hydrogen-ion concentration in the blood of the carp suffocated in the distilled or well water were lower than those in the normal condition. The result that under the suffocative state carbon dioxide value in the blood of fishes decreased is in accord with those of the findings of Kokubo (1), Powers (2) and Itazawa (3).

The decrease in total CO_2 in the blood seemed to be caused by the fishe's hyperpnea for the lack of dissolved oxygen in the water.

Decreased dissolved oxygen in the water, the total CO_2 in blood decreased. In this case, acidosis which is well-known as the property under the suffocative stage was not caused by the accumulation of carbon dioxide but it seemed to be caused, as Cordier and Chanel (4) reported, by the decrease of alkali-reserve according to the increase in organic acid such as lactic acid. It could be assumed that the possibility of an increase in the total CO_2 might be caused when the gas exchange between the water and the blood was obstructed by the degeneration of gill lamellas. And when the carp was killed in the acidic water prepared pH 3 with H_2SO_4, color tone of its gill changed noticeably to grey by errosion. The total CO_2, however, was in the range 16.5–24.2 meq/l blood, average value 20.3 meq/l blood. It was a little lower than that in normal condition. The hydrogen-ion concentration was in the range pH 6.70–7.18, average value pH 6.87 and its blood showed acidosis. This might have been due to the effect of the environmental water.

These results suggested that the gas exhcange between the blood and the water was not obstructed and the cause of death was not the suffocation by the degeneration of gill lamellas.

When the carp was suffocated in the water prepared pH 4.2 with H_2SO_4, the hydrogen-ion concentration was in the range pH 6.45–6.71, average value pH 6.57 and was nearly the same value as that of the carp suffocated in the distilled or well water. Colour change of its gill could not be recognized.

In this case, in spite of hyperpnea, the total CO_2 did not decrease because of the delay of exchanging carbon dioxide. The total CO_2, however, was not so much as that in the normal condition so that the cause of death was supposed not to be the decline of organic function by the carbon dioxide but by the decline of oxidation due to the lack of oxygen.

In the case of the carp killed in the water prepared pH 11.1–11.6 with NaOH, its total CO_2 was in the range 10.0–12.0meq/l blood, average value 11.2 meq/l blood, a remarkably low value owing to the carbon dioxide intake of alkaline water. However, the hydrogen-ion concentration in the blood was in the range pH 6.70–7.22, average value pH 6.73 and it showed acidosis. It seemed to be due to the fluctuation of $H_2CO_3/NaHCO_3$ by the absorption of carbon dioxide into the alkaline water.

In the case of the blood of the carp suffocated in the water pH 9.6 prepared with NaOH, its total CO_2 was in the range 9.6–13.9meq/l blood, average value 12.6 meq/l blood and its hydrogen-ion concentration was in the range pH 6.55–6.80, average value pH 6.61. Both showed a noticeably low value. But in the case of the living carp under the same conditions, its total CO_2 was in the range 21.6–25.2 meq/l blood, average value 23.6 meq/l blood and was nearly the same value as the one in the normal condition. It showed that in pH 9.6 there was no degeneration

of gill lamellas such as to cause suffocation.

In order to examine whether there was the change of the total CO_2 after death, the carp was clubbed to death and was allowed to be in the air. At 7 hours after the death its total CO_2 was in the range 21.3–26.0meq/l blood, average value 24.0 meq/l blood, which was nearly the same value as in normal condition. However, after 12 hours, the value increased and to the range 29.4–30.8 meq/l blood, average value 30.1 meq/l blood.

In the case where the carp's body was stuck on the viniyl bag without water to take away the contact with the air and was kept as it was, the total CO_2 in its blood did not change either after 12 hours or after 25 hours.

From the above results, it may be possible to predict the condition of environments in which the fish died.

In the case where the carp was kept in the $CuSO_4$ (Cu^{++} 12.5 ppm) solution for 7 hours, its total CO_2 was in the range 18.6–28.3 meq/l blood, average value 21.8 meq/l blood and the hydrogen-ion concentration was in the range pH 7.2–7.42, average value pH 7.30. These were almost the same values as for the normal condition. It is suggested that for these reasons gas exhange might not be obstructed even though the gill lamellas were degenerated and changed in colour.

In the case of the carp killed in the 1.5 ppm PCP (Pentachlorophenol) solution, the total CO_2 was in the range 17.3–21.5 meq/l blood, average value 19.7 meq/l blood and hydrogen-ion concentration was in the range pH 6.47–6.61, average value pH 6.55. As these were nearly the same value in the case of suffocation in the well water, the difficulty in respriation which was known as one of the toxic symptoms by PCP in man might be caused.

But in the case of the 0.1–0.5ppm PCP solution, the total CO_2 was in the range 24.0–33.5 meq/l blood, average value 28.8 meq/l blood and the hydrogen-ion concentration was in the range pH 7.12–7.80, average value pH 7.60.

The total CO_2 was a little higher than the normal value. It is known that PCP is extremely virulent to fishes and its 48 hours T.L.M. to the carp is about 0.15 ppm. Dilute PCP solution have a narcotic effect on the carp.

On the basis of the findings, it will be possible to elucidate mechanism which change the fishe's blood properties in different environmental water and to prove the cause fish suffocation and also to determine other factors related to the changes of fish blood properties.

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