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A Preliminary Study on Gas Exchange in Carp

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Water can be regarded as an environment good for CO_2 output and unprofitable for O_2 uptake. Fish show a sharp sensitivity against increased CO_2 concentration in water.[1)2)3)] Shift in Hb-HbO_2 dissociation curve of blood caused through changes in P_{CO_2} associates with the ecological speciality of fish species.[4)5)] Many studies of this sort suggested that characteristics in gas exchange in fish resulted from the qualities of the water for gases. Recently ITAZAWA[6)] published an extensive study on the gas concentration in venous and arterial blood, which had scarcely been carried out beforehand, as the latter is hardly obtainable in fish.

The gas exchange is carried out only by the concentration gradient due to the difference in partial pressure of the gas. The investigations ever achieved have mainly been forcused on the Hb-HbO_2 dissociation curve in vitro or gas concentration alone in the blood. The partial pressures of O_2 and CO_2 in venus and arteral blood (P_{vO_2}, P_{aO_2}, P_{vCO_2}, P_{aCO_2} respectively) participating in the gas exchange remain still univestigated.

In the present paper a trial investigation on the gas exchange in carp is represented, in which curves showing the relationship between partial pressure and gas vol-% in blood were obtained, and partial pressures of O_2 and CO_2 in blood in vivo were determined by the use of the curves.

Procedure and Materials

The fish (*Cyprinus carpio*) respirated the water lead by a vinyl tube connecting the mouth of the fish and a Marriot's flash after the device made by WINTERSTEIN.[7)] The openning of the tube leading the expired water situated 3 mm higher than the effective niveau of the water in the flash. The water could flow over the gillsurface through the sucking force by the movement of the respiratory apparatus except the mouth opening.

The former half of the whole body of the carp was covered with gum membrane of an ice bag. The whole body was settled in a wooden box to prevent the bag from unnecessary expansion. The box was freed from the side wall at the portion

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where the access both to Aorta dorsalis and Vena dorsalis was achieved after the mode described by ITAZAWA. The ventra-lateral side of the vertebrates was exposed through dissecting the lateral muscle layer as shown in the figure 1. A 1/4 injection

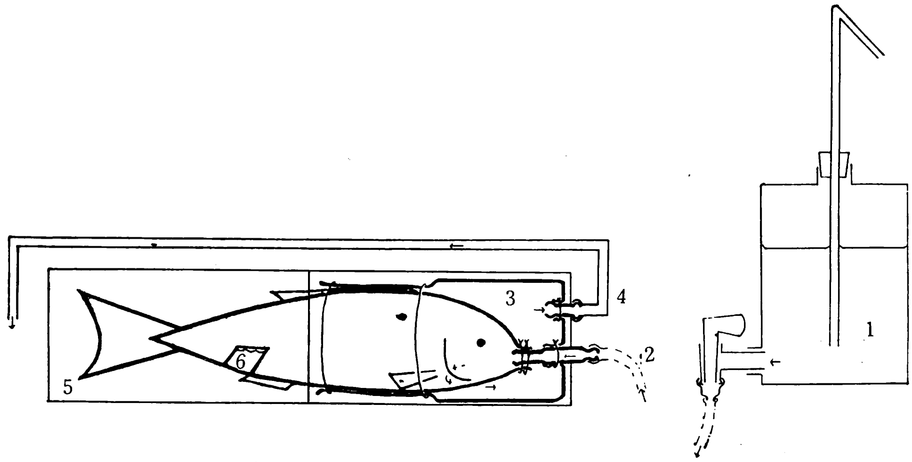

Fig. 1. Scheme of the experiment arrangement.

1. Marriot's flash.
2. tube transporting inspired water.
3. ice bag membrane.
4. tube transporting expired water.
5. holder.
6. portion of the vessel exposure.

↑ shows the direction of the respirated water stream.

needle of which inside was wet with 8% heparin and 4% NaF dissolved in physical saline water was inserted separately into Aorta and Vena running just along the ventral side of the vertebrates. From the vessels the arterial and the vonous blood were anaerobically sucked into the syringe connected with the needle and analysed by the use of van Slyke's monometer in the volume percent of O_2 and CO_2 contained.

To obtain the partial pressure in the blood in vivo one must employ the Hb-HbO_2 dissociation curve or the curve representing the relationship between the partial pressure and the volume percent of the gas contained in the blood (P_{CO_2}-CO_2 vol % curve or P_{O_2}-O_2-vol % curve). When a standard curve is drawn for the blood of a species and reference measurement, corresponding to alkalireserve determination, was conducted in each individum, the partial pressure of a gas in the blood of individums under almost uniform condition can be determined in the way analogous to the one commonly employed in human physiology.

In the present investigation the P_{CO_2}-CO_2 vol % curve was first determined, by means of which the P_{aCO_2} was obtained, corresponding to the CO_2-volume percent in the blood. The association of O_2 with Hb is much shifted through the change of P_{CO_2}. The P_{O_2}-O_2 volume % curve must be drawn under the influence of the P_{CO_2} same to that in the blood in vivo. Blood was equilibrated with gas mixtures of various P_{O_2} and of P_{CO_2} equal to that found in blood in vivo and and analysed with van Slyke's apparatus.

To equilibrate blood with gas mixtures 2 cc of blood was taken, put into tonometer ball and shaken for 15 minutes, being bubbled with the gas mixtures. 1 cc of the blood was taken and analysed with van Slyke's apparatus. The gas mixtures were arranged beforehand by the use of Scholander's micro-gas-analyser in fraction of O_2 and CO_2; to those gas mixtures of which fractions were found to be unsuitable either in O_2 or CO_2, gases were added till adequate composition was obtained.

The box in which the fish was settled, was laid on a side, therefore, the fish also. This position was different from the normal for fish but otherwise it was impossible to take blood from vessels along the vertebrates. In this abnormal, experimental position carp remained still even for a long time so far as water was supplied from the experiment arrangement.

The P_{O_2} in the inspired and expired water was determined by the use of Oxy-

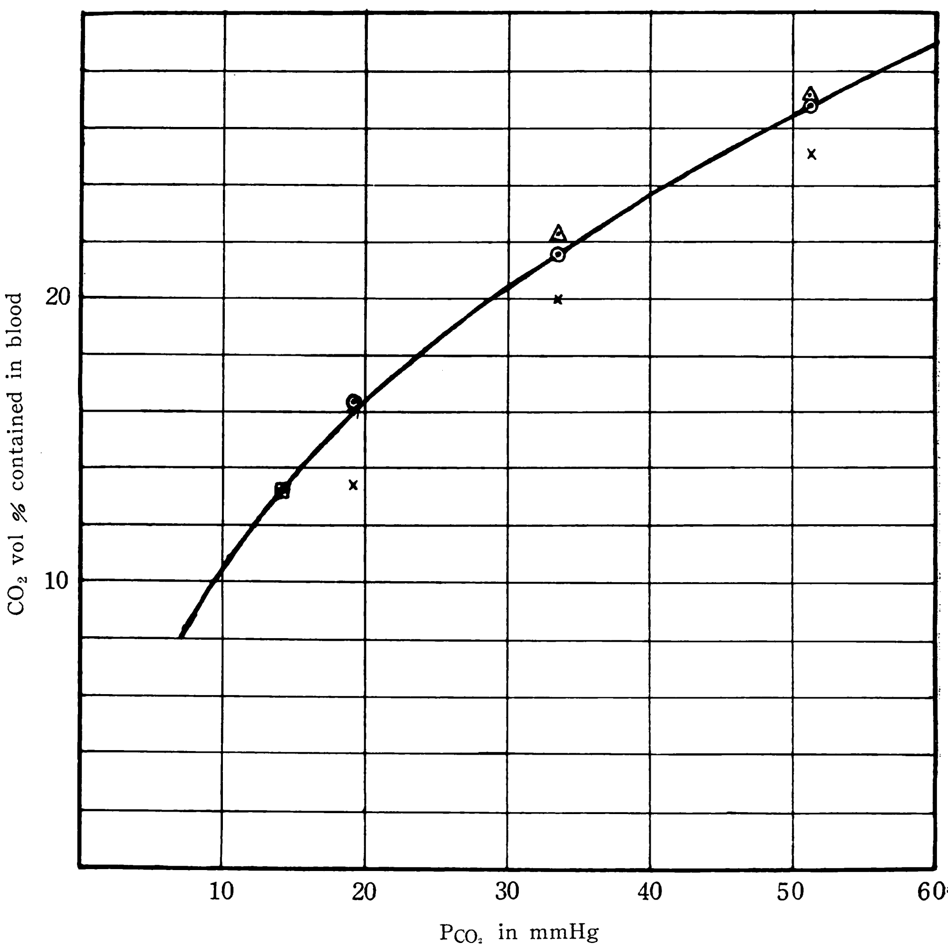

Fig. 2. Curve representing the relationship between P_{CO_2} and CO_2 vol %.
(P_{CO_2}-CO_2 vol % curve)

graph.[9] The respirated volume was measured throgh the difference in the scale on the wall of the Marriot's flash. The volume of the consumed O_2 was calculated through multiplying the P_{O_2} difference between in the expired and inspired water with O_2 dissolving coefficient and the volume of respirated water.

Carp employed had been kept by a keeper in the water of temperature 8 to 10°C from the end of autumn, were brought into the water of 15°C in the laboratorium and operated after a few hours.

The experiment was carried out during December of 1956 and January of 1957.

Results

1) P_{CO_2}-CO_2 vol % curve. Blood of an individium was seperated two to four samples, equilibrated with gas mixtures containing CO_2 at various partial pressures.

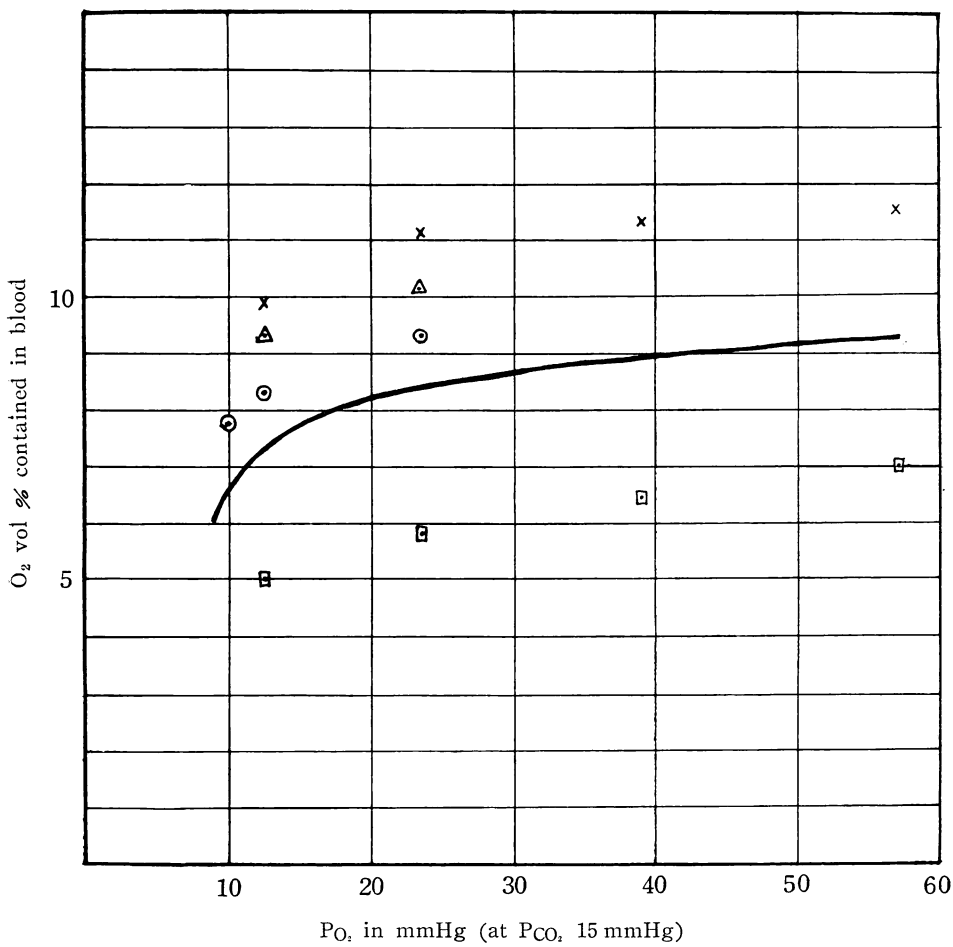

Fig. 3. Curve representing the relationship between P_{O_2} and O_2 vol %. (P_{O_2}-O_2 vol % curve)

No.	Sex	Body Weight gr	Condition	Blood Source	Actual measurement				Reference meas.		ΔP_{CO_2} mmHg	Ventilation L/hr.	O_2-uptake cc/kg. hr.	P_{O_2} mmHg in inspired Water	P_{O_2} mmHg in expired Water	Cardiac output cc/min kg	recal-curated Cardiac output cc/min kg
					CO_2-vol %	O_2-vol %	P_{CO_2}-mmHg	P_{O_2}-mmHg	CO_2-vol %	O_2-vol %							
1	♀	538	30 min. exposed to air	Vein	24.5		48		(33.5)* 20.1	(19.6)* 11.3							
2	♂	345	50 min. exposed to air	Vein	28.9	1.5	56		(33.5) 22.3	(19.6) 11.6							
3	♂	606	respirating from M's flash	Art. Vein	12.2 20.6	11.4 2.0	13 27		(33.5) 22.2	(19.6)	14	3.2	23.3	149	56	4.1	4.5
4	♀	602	respirating from M's flash	Art. Vein	5.9 12.0	8.4 2.6	7 16		(17.5) 12.1	(23.7)	9	5.0	42.0	156	43	12.0	13.2
5	♂	564	respirating from M's flash	Art. Vein	9.9 20.8	8.4 1.4	13 36	10 2	(17.5) 12.5	(23.7) 9.9	23	4.9	50.2	149	45	12.0	13.2
6	♂	431	respirating from M's flash	Art. Vein	16.5 27.7	7.9 1.4	13 39	12 2	(14.1) 18.3	(57.2) 9.7	26	8.2	40.3	147	100	10.5	11.6
7	♂	487	respirating from M's flash	Art.	8.2	6.4	9		(14.1) 9.8	(57.2)		4.0		148			
8	♂	528	30 min. exposed to air	Art. Vein	17.5 18.8	2.3 1.0	31 34		(14.1) 9.8	(57.2) 9.0	3						
9	♂	416	50 min. exposed to air	Art. Vein	17.0 18.9	0.8 0.6	22 26		(14.1) 13.0	(57.2) 8.8	4						

* P_{CO_2} and P_{O_2} in the reference gas mixture.

The CO_2 vol % contained in the blood was determined. Figure 2 shows the curve obtained.

2) P_{CO_2} in the blood. In the way described above the P_{CO_2} in the blood was determined by the use of the P_{CO_2}-CO_2 vol % curve shown in figure 2 and of the reference measurement. The average values obtained were 30 and 13 mmHg in Vena dorsalis and in Aorta dorsalis respectively as shown in the table.

3) P_{O_2}-O_2 vol % curve. The P_{CO_2} was about 13 mmHg under the experiment condition. Blood was equilibrated with several gas mixtures which were 15 mmHg in P_{CO_2} but different in P_{O_2}, analysed in O_2 vol %. The data obtained were plotted in the figure 3.

4) P_{O_2} in the blood. The P_{O_2} was determined in Aorta and Vena dorsalis separately. For the determination of the latter the Hb-HbO_2 dissociation curve obtained by WASTL was employed. 10 and 12 mmHg were found in Aorta in two specimens. About 2 mmHg was obtained in average of 4 specimens for Vena dorsalis.

Discussion

For P_{CO_2}-CO_2 vol % curve the measurement was carried out in three individiums separately. The data obtained are similar in three cases as shown in figure 1. It is known from this that the curve would be always similar with a slight drift according to individiums.

P_{aCO_2} and P_{vCO_2} were determined in the way, as above mentioned analogous to the one employed in human physiology. The values were 13 mmHg and 30 mmHg respectively in average. These values are remarkably small in comparison with the higher vertebrates. For example in man these are 40 and 46 mmHg respectively.[11] The difference between P_{aCO_2} and P_{vCO_2} ($\Delta P_{CO_2} = P_{vCO_2} - P_{aCO_2}$), however, is bigger in fish than in man. This means an effective gas exchange in fish. Due to the great solubility of CO_2 in water CO_2 is put out effectively from the venous blood during the passage of the blood through gillcapillaries, causing a remarkable rise in the affinity of the blood for O_2. When the arterial blood rich in O_2, poor in CO_2 flows to capillaries in tissue, CO_2 diffuses rapidly into the blood due to the sharp gradient in P_{CO_2} between the tissue and the arterial blood, lowering the blood in the affinity to O_2. The blood liberates 80% of O_2 to the tissue. Such an effectiveness in gas exchange in fish may be ascribed to the qualities of water.

The CO_2 capacity of fishblood is smaller than that of mammals.[10] The CO_2-vol % obtained in the present investigation was about the half of those obtained by WASTL[10] and ITAZAWA.[6] Moreover it was reported that the CO_2-vol % was equal both in Aorta and Vena[6] (that is, R. Q. in blood is zero). In the present investigation a remarkable difference was obvious. R. Q. in blood was calculated from the O_2 vol %- and CO_2 vol %- difference in Vena and Aorta to be about 1 in three cases. Therefore, the CO_2 vol % difference changes according to the alternation in the O_2-vol % difference, i. d. to the alternation in the rate of O_2 consumption by the tissue. In the other three cases the value exceeded 1; in two cases ca 1.6, in one cases where the fish was kept out of water ca 9. Though the reason on the remarkable fluctuation in R. Q. remains unknown, it may take place occasionally in fish. KAWAMOTO[12] reported that goldfish put out CO_2 without any consumption of O_2 in a water poor in O_2.

P_{O_2}-O_2- vol % curve was obtained at P_{CO_2} of 15 mmHg. There was a remarkable difference in the O_2 vol % in blood. But the slope of the curve was apparently suggested and the determination of P_{O_2} was tried.

It was, however, only in 2 cases obtained; in NO. 9 10 mmHg, in NO. 13 12 mmHg. These values are too small in comparison with the P_{O_2} in expired water. Obviously a considerable amount of respirated water did not take parts in gas exchange, due to the particular experiment condition.

The blood of the carp which had been kept out of water was analysed and it was found that the P_{aCO_2} was high, the O_2- vol % contained in arterial blood was very small. The P_{vCO_2} was still higher than the P_{aCO_2}, though the latter was remarkable elevated in comparison with the carp respirating water. And the O_2- vol % in the arterial blood still exceeded that in the venous blood, though the former was found considerably decreased. This shows that uneffective gas exchange takes place at the gill capillaries of carp even apart from water, i. d., in direct contact with atmosphere. The wet and heavy gillfilaments lie on one another, adhering to each other by the surface tension of the little amount of water on the filaments. Ventilation of air over the filaments, therefore, is imcomplete. Moreover, gases diffuse from liquid phase to gas phase or vice versa, resulting possibly in a decrease in efficiency of gas exchange. CO_2 accumulation lowers the rate in oxygenation of blood passing through gillcapillaries.

Decrease in P_{CO_2} means that the P_{CO_2} gradient between arterial blood and tissue diminished and the drift of O_2 dissociation curve due to the change in P_{CO_2} does not occur at peripheries any more, therefore, O_2 contained in the arterial blood can not be liberated so effectively. This is true from the experimental result obtained; the value of O_2- vol % in venous blood did not decrease so much even in the carp exposed 30 minutes to the atmosphere from that found in the carp respirating water. Shortly speaking, the blood is not ready to take O_2 at gill capillaries and not inclined to liberate O_2 to the tissus at peripheries.

This is a phenomenon remarkably different from the anoxya observed in the O_2-poor water. KOKUBO[14] and ITAZAWA[6] reported that both the O_2-and the CO_2-vol % in blood diminished at the same time in fish kept in O_2 poor water. In this case the blood is ready to take O_2 in the gillcapillaries and to librate to the tissue.

Any way, judging from the small value of P_{vO_2} obtained in the present investigation, the tissue of carp seems to endure hypoconcentration of O_2 even in the normal condition. BRONK et al.[15] found that the rate of O_2 consumption does not decrease even at $P_{O_2}=2$ mmHg at 32°C in slice of tissue and concluded that tissue can be alive at such a low P_{O_2}, so far as P_{O_2} is uniform in the tissue.

Cardiac output was also calculated by means of Fick's law and listed in the table. The venous blood participating in the gas exchange is not the simple segmental venous but the mixed one. The O_2 vol % in the latter is about the half of the former, judging from the data reported by ITAZAWA.[6] Referring this, the cardiac output was recalculated to be 13.2 in two cases, 4.5, and 11.6 cc/min. kg. each in a case.

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Prof. Y. SUYEHIRO, Fisheries Department of Tokyo University, who stayed abroad, believed that the study on the gas exchange could be much convinienced in the pulmonary function research laboratory in the Applied Electricity Research Institutes of Hokkaido University and asked Prof. M. MOCHIZUKI, the chief of the laboratory, if the author could be allowed to study there.

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Summary

A trial study on the gas exchange was performed in carp. Curves showing the relationship between partial pressure and CO_2- or O_2- volume % contained in blood were obtained. In the carp respirating water from Marriot's flash the P_{CO_2}, P_{O_2} were determined both in Aorta and in Vena. O_2 consumption was measured. Cardiac output was calculated.

Results obtained under the experiment arrangement are as follows.

1. P_{aCO_2} was about 13 mmHg in 4 cases.

2. P_{vCO_2} was 16 to 39 mmHg (average 30 mmHg) in 4 cases.

3. P_{aO_2} was found to be 10 and 12 mmHg in 2 cases. The small value must have resulted from the experiment arrangement.

4. P_{vO_2} was found 2 mmHg. This suggests that the tissue of carp survives at a low P_{O_2}.

5. The difference in CO_2-vol % between Aorta and Vena changes according to that in O_2.

6. Both P_{aCO_2} and P_{vCO_2} are very small in comparison with those in man. On the contrary, ΔP_{CO_2} in carp exceeds that in man.

7. At periphery the arterial blood liberates 80% of O_2 contained.

8. It seems probable in carp exposed to the atmosphere that gas exchange proceeds to some extent.

9. Cardiac out put in carp was calculated to be about 13 cc/min. kg.

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