Changes in Depth of Anesthesia of the Carp Anesthetized with a Constant Level of CO₂*¹

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Changes of respiration, behavior, and depth of anesthesia were examined in carp that were individually kept in a chamber supplied with water bubbled with an equi-mixture of CO₂ and O₂. CO₂ had an anesthetic effect on all the carp at Pco₂ = 125, 150, and 175 mmHg, but these partial pressures in the ambient water were fatal in 10, 20, and 70% of the carp, respectively. Although all the carp survived in the experiment using Pco₂ = 100 mmHg, only 50% of them became anesthetized. The depth of anesthesia progressed with time and the Pco₂. Recovery time from anesthetic state was 13-130 min, the mean recovery time being about 40 min in all experiments. The optimum anesthetic concentration for adult carp seemed to be between Pco₂ = 100 mmHg and 125 mmHg.

Anesthetics are often used for handling fish in the field and laboratory, and are also used for the transportation of live fish.¹⁾ However, most anesthetics are expensive and are not always safe for humans and fish. High concentrations of CO₂ are known to have an anesthetic effect on many animals including fish.²⁻⁷⁾ Many workers have examined its mechanism of action and the effects of CO₂ on various physiological factors so that it can be used as an anesthetic for humans and laboratory animals.⁸⁻¹¹⁾ However, few studies have been done in fish. In early studies,¹²⁻¹⁴⁾ CO₂ was examined only as an anesthetic for short-term anesthesia. CO₂ seems to be a suitable anesthetic for the transportation of live fish for human consumption because it is safe for humans.

Independently of previous workers, one of the present authors, H. Mitsuda, and his colleagues noted the anesthetic effect of CO₂ in early studies on the relationship between the partial pressure of CO₂ and catalase activity and on food preservation by CO₂.¹⁵⁻¹⁷⁾ They developed a technique to anesthetize fish with CO₂ by continuous bubbling of an equi-mixture of CO₂ and O₂ into the ambient water.^{2,4)} They confirmed that CO₂ was a safe anesthetic for long-term anesthesia as well as short-term anesthesia, and proposed that this anesthesia is useful for the transportation of live fish because of its effectiveness, low cost, and

safety.

Takeda and Itazawa,¹⁸⁾ using young carp and porgy *Pagrus major*, evaluated the efficacy of CO₂ for a long-term anesthesia and reported that the optimum concentration of CO₂ for carp is Pco₂ = 95-115 mmHg, and that CO₂ could not be used for porgies because they were highly sensitive to CO₂. However, in general, the optimum concentration of any drug changes with age, sex, and physiological conditions of the animals and also with acclimating conditions of fish.

In the present study, to apply this anesthesia for adult fish which is a main object for live fish transportation, we anesthetized adult fish with a constant level of CO₂ for 10 h, then examined the changes in their behavior, respiration, and depth of anesthesia. To determine the optimum concentration of CO₂, we examined its anesthetic effect with regard to the changes in the depth of anesthesia.

Materials and Methods

Eighty adult carp *Cyprinus carpio*, each weighing about 500 g (497 ± 49 g, mean ± SD), were used. The carp were obtained from a fish farmer in Kyoto Prefecture, and then transported to our laboratory. Each group of ten carp was kept in a 1,000 l plastic tank placed indoors and acclimated at 23 ± 1°C under a 14-h light/10-h dark

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Fig. 1. A schematic diagram of the experimental apparatus. T: Tank, F: Filter, V: Valve, H: Heater, P: Pump, TR: Thermoregulator, FM: Flow meter, MFC: Mass flow controller, BC: Bubbling chamber, EC: Experimental chamber.

cycle for at least one month. They were daily fed a commercial diet for carp, but fasted for at least 24 h prior to the experiment. The water in a tank was artificially aerated and filtrated, and partially exchanged with fresh water every week.

Figure 1 shows a schematic diagram of the experimental apparatus. At first, the carp were lightly anesthetized with MS222 (tricaine methanesulfonate) to measure their body weight. They were transferred into the experimental chamber (EC in Fig. 1, 10 × 40 × 13 cm), and then acclimated for at least 10 h before the beginning of the experiment. They were continuously irrigated with fully-aerated fresh water kept at 23°C at a constant flow rate of 1.5 l/min during acclimation. The light condition was maintained at 14L-10D throughout the experiment.

An equi-mixture of CO₂ and O₂ was introduced into the bubbling chamber (BC in Fig. 1) according to Mitsuda *et al.*^{3,4)} The partial pressure of CO₂ (Pco₂) in the water running through the experimental chamber rose rapidly and reached a

Table 1. Criteria of anesthetic stage for CO₂ anesthesia in carp

Anesthetic stage	Opercular movement	Swimming motion	The sense of equilibrium
0	Normal	Normal	Normal
I	Normal	Normal	Partial loss
II	Normal	Normal	Total loss
III	Weak	Partial loss	Total loss
IV	Very weak	Total loss	Total loss
V	Stop	Total loss	Total loss

stationary level in about 30 min. The bubbling volume of the mixed gas was calculated in advance by examining the relationship between the Pco₂ in the running water and the volume of the mixed gas. The carp were supplied with water containing a constant partial pressure of CO₂ (Pco₂ = 25, 50, 75, 100, 125, 150, and 175 mmHg) at a constant flow rate of 1.5 l/min, and with fresh water as a control. After 10 h of bubbling of the mixed gas, the carp were reperused with fresh water. Each experiment was carried out on ten carp.

Fig. 2. Changes in the P_{CO_2} of the water after the introduction of an equi-mixture of CO₂ and O₂; the P_{CO_2} before the introduction of the mixed gas was 9 ± 2 mmHg (mean $\pm$ SD, $n=80$). ■: Exp. 1, ●: Exp. 2, ▲: Exp. 3, ▼: Exp. 4, □: Exp. 5, ○: Exp. 6, △: Exp. 7, ▽: Exp. 8.

The changes in the behavior, respiratory rate, and depth of anesthesia in the carp and the changes in pH, P_{CO_2} , and P_{O_2} in the water running through the experimental chamber were examined at 0.5, 1, 2, 3, 5, and 10 h after the onset of the introduction of the mixed gas. The P_{CO_2} and P_{O_2} were measured with a blood gas analyzer (Instrumentation Laboratory, IL-213-05) partially modified to measure high partial pressures of CO₂ and O₂ at 23°C. The depth of anesthesia was measured in anesthetic stages (Table 1), which were defined for anesthesia with CO₂ according to McFarland's criteria.¹⁹⁾

Results

General Features of Anesthesia with CO₂

When high concentrations of CO₂ were introduced into the ambient water, the carp progressed through an anesthetic state from Stage 0 to V (Table 1) and showed the following behavioral changes. The carp became excited and struggled violently at first. Consequently, their respiratory rates increased rapidly, but later gradually decreased with time. After a while, they partially lost their sense of equilibrium and repeatedly woke up and fell down (Stage I). Next, they lost their equilibrium, and they no longer woke up; however,

Fig. 3. Changes in the P_{O_2} of the water after the introduction of an equi-mixture of CO₂ and O₂; the P_{O_2} before the introduction of the mixed gas was 157 ± 18 mmHg (mean $\pm$ SD, $n=80$). ■: Exp. 1, ●: Exp. 2, ▲: Exp. 3, ▼: Exp. 4, □: Exp. 5, ○: Exp. 6, △: Exp. 7, ▽: Exp. 8.

their swimming motion still remained normal (Stage II). They swam slowly against the stream, turning over. Then, they almost stopped swimming completely and kept quiet, showing a reduction in opercular movement (Stage III). Next, they did not move at all except for slight opercular movement (Stage IV). Finally, they stopped breathing (Stage V). When fresh water was reperfused, the carp recovered from the anesthetic state.

Results of 10 h Anesthesia with a Constant Concentration of CO₂

Using the present experimental apparatus (Fig. 1), we could maintain the constant level of pH, P_{CO_2} , and P_{O_2} in the water running through the experimental chamber except for the first 30 min (Figs. 2–4). The P_{CO_2} and P_{O_2} before the introduction of the mixed gas was 9 ± 2 mmHg and 157 ± 18 mmHg, respectively, and the values of the P_{CO_2} after the introduction of the mixed gas were well in accordance with expected values (Fig. 2 and Table 2). The average pH before the introduction of the mixed gas was 7.0.

As mentioned above, the carp showed an increased respiratory rate after the introduction of the mixed gas (Fig. 5). Their respiratory rates

Fig. 4. Changes in the pH of the water after the introduction of an equi-mixture of CO_2 and O_2 ; the pH before the introduction of the mixed gas was 7.0 ± 0.2 (mean $\pm$ SD, $n=80$). ■: Exp. 1, ●: Exp. 2, ▲: Exp. 3, ▼: Exp. 4, □: Exp. 5, ○: Exp. 6, △: Exp. 7, ▽: Exp. 8.

reached a maximum within 5 min and then gradually decreased. At the high partial pressure of CO_2 ($\text{Pco}_2=50\text{--}175$ mmHg, $\text{Po}_2=250\text{--}350$ mmHg), their average respiratory rate rose from 9 strokes/min to about 60 strokes/min, but at the low partial pressure ($\text{Pco}_2 \approx 25$ mmHg, $\text{Po}_2 \approx 225$ mmHg) the carp showed only a slight increase in respiration compared with the control. The control carp maintained a constant respiratory rate, less than 10 strokes/min, throughout the experiment. Judging from their constant respiratory rates, we assumed that they were kept in a quiet state in the experimental chamber without any stress.

Fig. 5. Changes of the respiratory rate in carp anesthetized with CO_2 ; the respiratory rate before the introduction of the mixed gas was 9 ± 7 strokes/min (mean $\pm$ SD, $n=80$). ■: Exp. 1, ●: Exp. 2, ▲: Exp. 3, ▼: Exp. 4, □: Exp. 5, ○: Exp. 6, △: Exp. 7, ▽: Exp. 8.

Table 3 shows the changes in anesthetic stage in the carp during anesthesia. One carp became anesthetized (Stage I) for the first time at $\text{Pco}_2 \approx 50$ mmHg at 0.5 h, however, this carp and another carp (Stage I) anesthetized with $\text{Pco}_2 \approx 75$ mmHg soon recovered from the anesthetic state and did not become anesthetized again. This early anesthetic state observed in the experiment at $\text{Pco}_2 \approx 50$ mmHg and 75 mmHg seemed to be due to a transient increase in the CO_2 in the blood by a rapid increase in respiratory rate. When the mixed gas was bubbled to raise the Pco_2 over 100 mmHg, some carp fell into an anesthetic state without waking up during the bubbling of the mixed gas. However, only 50% of the carp were

Table 2. Results of CO_2 -anesthesia with carp kept in water bubbled with an equi-mixture of CO_2 and O_2 for 10 h

Exp. No.	n	Body weight of carp (g) mean $\pm$ SD	Flow rate (l/min) of an equi-mixture of CO_2 and O_2	Expected value of Pco_2 (mmHg)	Survival rate (%)	Recovery time (min)	
						mean	range
1	10	522 $\pm$ 50	0.00	—	100	—	—
2	10	491 $\pm$ 56	0.10	25	100	—	—
3	10	516 $\pm$ 48	0.20	50	100	—	—
4	10	484 $\pm$ 33	0.34	75	100	—	—
5	10	518 $\pm$ 49	0.48	100	100	46	13– 78
6	10	499 $\pm$ 43	0.64	125	90	40	21– 72
7	10	481 $\pm$ 38	0.80	150	80	42	14– 130
8	10	471 $\pm$ 42	0.98	175	30	48	32– 79

Table 3. Changes of anesthetic stage in carp after introducing of an equi-mixture of CO₂ and O₂

Exp.	Pco ₂	Percentage of carp in each anesthetic stage at (h)																	
		0.5						1						2					
No.	(mmHg)	0	I	II	III	IV	V	0	I	II	III	IV	V	0	I	II	III	IV	V
3	50	90	10					100						100					
4	75	90	10					90	10					100					
5	100	60	30	10				50	30	20				50		50			
6	125	10	20	70						70	30					60	40		
7	150			90	10					60	40					30	70		
8	175			70	30					20	60	20					60	40	

Exp.	Pco ₂	Percentage of carp in each anesthetic stage at (h)																	
		3						5						10					
No.	(mmHg)	0	I	II	III	IV	V	0	I	II	III	IV	V	0	I	II	III	IV	V
3	50	100						100						100					
4	75	100						100						100					
5	100	50		50				50		40	10			50		20	30		
6	125			30	70						90	10					60	40	
7	150			10	80	10				10	50	40				10	20	50	20
8	175				30	60	10					80	20					30	70

in anesthetic states even after 10 h in the experiment using Pco₂≐100 mmHg. All the carp became anesthetized in the experiment at Pco₂=125–175 mmHg.

Their anesthetic stages progressed with time and the partial pressure of CO₂, in general, in order from Stage I to V. However, some carp in Stage V sometimes showed a kind of convulsion in the initial period. The most advanced stages were Stages III, IV, V, and V at Pco₂≐100, 125, 150, and 175 mmHg, respectively. The time needed for the induction of anesthesia was shortened concomitantly with the increase in the Pco₂. For example, it took all the carp more than 3 h to fall into Stage III at Pco₂≐125 mmHg and just over 1 h at Pco₂≐175 mmHg.

When the anesthetized carp were reperfused with fresh water after 10 h bubbling of the mixed gas, they recovered from anesthesia within 13–130 min, but the vigorous swimming motion which had been noted at the initial phase of anesthesia, was not observed. The mean recovery time was about 40 min in all the experiments.

The survival rate was 100% in all the experiments at Pco₂=25–100 mmHg and the control; however, in the experiments at Pco₂=125–175 mmHg, the survival rate was 90, 80, and 30% at Pco₂≐125, 150, and 175 mmHg, respectively. Most of the dead carp were in Stage V at 10 h after the introduction of the mixed gas, but one carp was in Stage IV at Pco₂≐125 mmHg. This carp repeatedly woke up from Stage I for more

than 20 h, and finally died, possibly as a result of a high sensitivity to CO₂.

Discussion

From the present study, it is evident that CO₂ has two drawbacks as an anesthetic for long-term anesthesia. One is that its adequate range is extremely narrow. In the experiment at Pco₂≐100 mmHg, although all the carp survived, only 50% of the carp became anesthetized. While all the carp became anesthetized in the experiment at Pco₂≐125 mmHg, this Pco₂ was fatal in 10% of the carp. The optimum concentration for adult carp might be between Pco₂=100 mmHg and 125 mmHg. This value is well in accordance with that for young carp reported by Takeda and Itazawa,¹⁸⁾ Pco₂=95–115 mmHg. They pointed out that CO₂ was inappropriate for porgies *Pagrus major* because they are highly sensitive to high concentrations of CO₂.

The anesthetic or narcotic action of CO₂ is associated with a marked flatness of the electroencephalogram (EEG) in animals anesthetized with CO₂.²⁰⁾ The EEG pattern shows a characteristic change during anesthesia with CO₂. The potential of the EEG diminishes concomitantly with the progression of anesthesia, and finally becomes flat. In a preliminary experiment, we also demonstrated the same pattern in the carp anesthetized with CO₂ (unpublished data). This flat pattern in the EEG or narcotic condition is

caused by the decrease in the pH on the surface of the brain rather than the increase in the concentration of CO₂ itself.^{8,20)} It follows, therefore, that CO₂ can not be used in species which are sensitive to a low pH.

Another drawback is that the time needed for the induction of anesthesia is relatively long. We originally defined the anesthetic stages, using opercular movement, swimming motion, and the sense of equilibrium as the criteria, with reference to McFarland's criteria.¹⁹⁾ By these criteria, we could easily judge the depth of anesthesia from the appearance of the fish without giving any additional stress. Although the anesthetic stage III-IV, probably equivalent to the stages II-2-III of McFarland's criteria, is suitable for the transportation of live fish, it took all the carp more than 3 h to achieve Stage III in the experiment at Pco₂ = 125 mmHg. Even in the experiment at Pco₂ = 175 mmHg, when the mortality was 70%, it took more than 1 h. The time needed for the induction of anesthesia is shortened by raising the partial pressure of CO₂. Thus, it is possible to shorten the time by the use of higher concentrations although the mortality will increase.

The drawbacks of CO₂ as an anesthetic for long-term anesthesia are common to all the anesthetics. Unlike most anesthetics, CO₂ is absolutely safe for humans even if the anesthetized fish are used as food, as is coldnarcosis or electro-narcosis.²¹⁾ Any anesthetic used should be absolutely safe for humans especially when the fish are to be eaten. Therefore, we investigated the use of two concentrations of CO₂: a high concentration for inducing anesthesia quickly and a low concentration for maintaining anesthesia. These results will be described in the accompanying paper.

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