炭酸ガスの鎮静効果による長時間麻酔

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The Use of Sedating Action of CO_2 for Long-term Anesthesia in Carp*1

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As an anesthetic for long-term anesthesia, two concentrations of CO_2 were used: a high concentration (Pco_2≒200, 225, or 250 mmHg) for inducing anesthesia quickly and a low concentration (Pco_2≒100 mmHg or 125 mmHg) for maintaining anesthesia. Adult carp were kept in water containing the high or low concentration of CO_2 for a total of 10 h; the duration of each treatment was 15 min or 30 min at the high concentration and 1 h or 9.5 h at the low concentration. The time needed for the induction of anesthesia was shortened by the use of the high partial pressure of CO_2 (Pco_2≒250 mmHg). The carp could be anesthetized or sedated for 10 h, using Pco_2=250 mmHg as the high concentration and Pco_2=100 mmHg as the low concentration of CO_2.

In the preceding study, we examined the changes in the anesthetic stage of the adult carp exposed to a constant level of CO_2 (Pco_2=25–175 mmHg) and reported that CO_2 has two drawbacks as an anesthetic for long-term anesthesia. One is that its adequate range is extremely narrow, and the other is that the time needed for the induction of anesthesia is relatively long. In general, the time needed for the induction of anesthesia is shortened by raising the concentration of an anesthetic. Thus, the time needed for the induction of anesthesia can be shortened by the use of higher concentrations of CO_2 provided that the concentration does not surpass a certain permissible limit. We previously reported that more than 1 h was required to anesthetize the carp at the concentration of Pco_2≒175 mmHg, which was the highest concentration examined in the preceding study, and that this Pco_2 was fatal in 70% of the carp when they were anesthetized for 10 h.

In the present study, to overcome the above-mentioned drawbacks of CO_2, we adopted two concentrations of CO_2: a high concentration for inducing anesthesia quickly and a low concentration for maintaining anesthesia. At first, we tried to find the appropriate concentration of CO_2 for short-term anesthesia, and then we tried to maintain the adult carp in a favorable anesthetic stage for 10 h by using high and low concentrations of CO_2 in various combinations.

Materials and Methods

The conditions for rearing the carp and experimental procedures were the same as those previously described.[1] In this study, seventy adult carp (524±52 g, mean±SD) were used.

First, to determine the adequate concentration of CO_2 to safely shorten the time needed for the induction of anesthesia, the carp were kept in water containing a high Pco_2 for a short time. The partial pressures applied in this study were Pco_2=200 mmHg (Exp. 1), 225 mmHg (Exp. 2), and 250 mmHg (Exp. 3), because more than 1 h was required to anesthetize the carp adequately at Pco_2≒175 mmHg.[1] Each Pco_2 corresponds to a bubbling volume of an equi-mixture of CO_2 and O_2 at a constant rate of 1.17, 1.45, and 1.78 *l*/min, respectively (Table 1). Treatment with the high concentration of CO_2 was carried out for 30 min.

In Exps. 4–7, we used two concentrations of CO_2: a high concentration for inducing anesthesia quickly and a low concentration for maintaining anesthesia. As the high concentration of CO_2, we chose Pco_2=250 mmHg from the satisfactory results obtained in Exps. 1–3. As the low concentration of CO_2, we chose Pco_2=100 mmHg and 125 mmHg from the results obtained in the preceding study.[1] The carp were treated with

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the high concentration for 30 min or 15 min and with the low concentration for 9.5 h or 1 h. The treatment with the low and high concentrations of CO_2 was carried out for a total of 10 h.

Each treatment (Exps. 1–7) was carried out on ten carp. After the introduction of the mixed gas, the changes in the behavior, respiration, and anesthetic stage in the carp were examined and at the same time the changes in the pH, Pco_2, and Po_2 of the water running through the experimental chamber, in which a carp was kept, were also monitored. The anesthetic stage (Stage 0-V) was originally defined depending upon the opercular movement, swimming motion, and the sense of equilibrium as the criteria.[1] After the 30 min or 10 h bubbling of the mixed gas, the chamber was reperfused with fresh water for the recovery of the carp.

Results

Results of Short-term Anesthesia with High Concentrations of CO_2

On the introduction of an equi-mixture of CO_2 and O_2 into the ambient water, the Pco_2 and Po_2 in the water running through the experimental chamber rose rapidly and reached a maximum level in about 30 min, as described in the preceding report.[1] The partial pressures of CO_2 and O_2 examined at 30 min after the introduction of the mixed gas were $Pco_2 \fallingdotseq 200$ mmHg and $Po_2 \fallingdotseq 325$ mmHg in Exp. 1, $Pco_2 \fallingdotseq 225$ mmHg and $Po_2 \fallingdotseq 330$ mmHg in Exp. 2, and $Pco_2 \fallingdotseq 250$ mmHg and $Po_2 \fallingdotseq 350$ mmHg in Exp. 3. As expected, the Pco_2 values were nearly the same as the estimated values. The pH in the running water decreased from 7.2–7.4 to 4.9–5.1 due to the dissolution of CO_2.

Table 1 summarizes the results of Exps. 1–3. In the experiments using $Pco_2 = 200$ mmHg and 250 mmHg, all the carp became anesthetized to Stage III or higher stage. The time needed for inducing all the carp into Stage III was 17 ± 4 min and 15 ± 4 min in Exps. 1 and 3, respectively. However, in the experiment using $Pco_2 = 225$ mmHg, only 80% of the carp achieved Stage III, and the others remained in Stage II even at 30 min after the introduction of the mixed gas. When fresh water was reperfused to them after bubbling of the mixed gas for 30 min, all the carp came out of the anesthetic stage, the mean recovery time being 15–25 min. From these results, we adopted $Pco_2 = 250$ mmHg as the high concentration for the following long-term anesthesia.

Results of Two-step Anesthesia with High and Low Concentrations of CO_2

After a 30-min treatment with the high concentration ($Pco_2 \fallingdotseq 250$ mmHg), water containing the low partial pressure of CO_2 ($Pco_2 \fallingdotseq 100$ mmHg in Exp. 4 and 125 mmHg in Exp. 5) was supplied to the carp for the following 9.5 h. Table 2 summarizes the results of Exps. 4 and 5. As was expected, all the carp became anesthetized to Stage III or higher stage within 30 min. The

Table 1. Results of short-term CO_2 anesthesia

Exp. No.	n	Flow rate of an equi-mixture of CO_2 and O_2 (*l*/min)	Expected value* of Pco_2 in water (mmHg)	Time needed for Stage III (min)		Percent of carp in Stage III or more at 30 min (%)	Recovery time (min)	Survival rate (%)
				mean±SD	range		mean±SD	
1	10	1.17	200	17±4	12–23	100	15± 3	100
2	10	1.45	225	17±2 (n=8)	—	80	25±22	100
3	10	1.78	250	15±4	9–20	100	19± 4	100

* Each value is Pco_2 which reaches a constant level in about 30 min after the introduction of an equi-mixture of CO_2 and O_2.

Table 2. Results of two step anesthesia with high and low concentrations of CO_2

Exp. No.	n	Pco_2* in water (mmHg)	Time needed for Stage III (min) mean±SD	Percent of carp in anesthetic state at 10 h (%)	Recovery time (min) mean±SD	Survival rate (%)
4	10	250→100	13±2	60	29±23 (n=6)	100
5	10	250→125	15±3	90	45±59 (n=8)	90

* The carp were treated with the high concentration of CO_2 ($Pco_2 \fallingdotseq 250$ mmHg) for 30 min and with the low concentration ($Pco_2 \fallingdotseq 100$ mmHg for Exp. 4, $Pco_2 \fallingdotseq 125$ mmHg for Exp. 5) for the following 9.5 h.

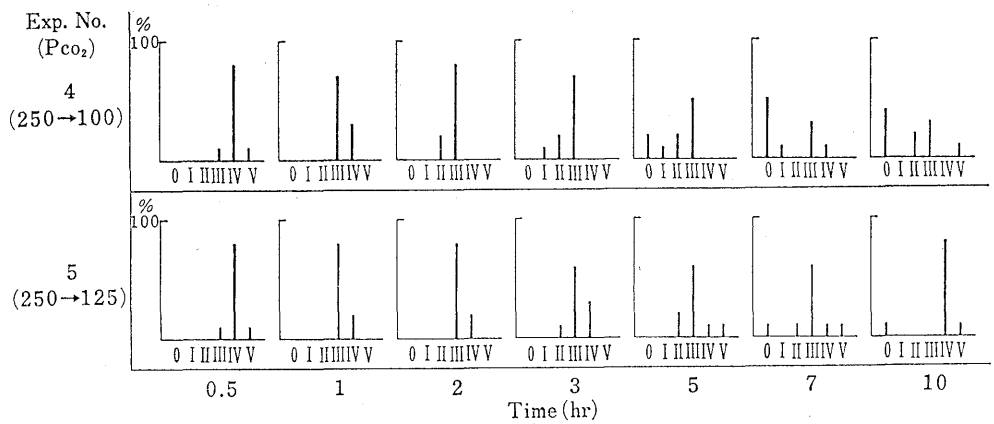

Fig. 1. Changes of anesthetic stage in the carp treated with the high concentration of CO_2 ($Pco_2 \fallingdotseq$ 250 mmHg) for 30 min and with the low concentration ($Pco_2 \fallingdotseq 100$ mmHg for Exp. 4, $Pco_2 \fallingdotseq$ 125 mmHg for Exp. 5) for the following 9.5 h. Anesthetic stage was examined seven times (at 0.5, 1, 2, 3, 5, 7, and 10 h) in both experiments. Each result is shown in the seven bar charts. The abscissa of the bar chart shows the anesthetic stage (0–V) and the ordinate indicates the percentage of the carp in each stage.

time needed for inducing all the carp into Stage III was 13±2 min in Exp. 4 and 15±3 min in Exp. 5.

When the carp were continuously irrigated with water containing the low partial pressure of CO_2, their anesthetic stage gradually became shallower. Fig. 1 shows the changes in the anesthetic stage in the carp. In Exp. 4 using $Pco_2=100$ mmHg as the low concentration, the carp in Stage II were observed at 2 h after the treatment with the high concentration of CO_2 and some carp came out of the anesthetic state within 5 h. Also in Exp. 5 using $Pco_2=125$ mmHg as the low concentration, the carp in Stage 0 appeared during the treatment with the low concentration (Fig. 1). In the experiment using $Pco_2=125$ mmHg as the low concentration, the survival rate was 90% although all the carp survived in the experiment using $Pco_2=100$ mmHg as the low concentration.

We previously reported that the optimum partial pressure for adult carp seemed to be between $Pco_2=100$ mmHg and 125 mmHg.[1] However, we can conclude that an optimum partial pressure of CO_2 does not evidently exist because some carp recovered from the anesthetic state during the treatment with the concentration of $Pco_2 \fallingdotseq 125$ mmHg and this concentration had been fatal in 10% of the carp. Accordingly, we used the high ($Pco_2=250$ mmHg) and low ($Pco_2=$ 100 mmHg) concentrations of CO_2 repeatedly.

Results of Anesthesia with High and Low Concentrations of CO_2 used Repeatedly

At first, a 30-min treatment with the high concentration and a 1-h treatment with the low concentration were repeated, because some carp recovered from the anesthetic state at 1.5 h after the introduction of the low concentration of CO_2 ($Pco_2=100$ mmHg) in Exp. 4. Table 3 summarizes the experimental results and Fig. 2 shows the changes of anesthetic stage in the carp. The carp became anesthetized in Stage III or higher stage at the first treatment with the high concentration, and at the subsequent first treatment with the low concentration their anesthetic stage became a little shallow but still Stage III or higher

Table 3. Results of anesthesia with high and low concentrations of CO_2 used repeatedly

Exp. No.	n	Pco_2* in water (mmHg)	Time needed for Stage III (min) mean±SD	Percent of carp in anesthetic state at 10 h (%)	Percent of carp in sedate state at 10 h (%)	Recovery time (min) mean±SD	Survival rate (%)
6	10	$250\rightarrow100/250_{30}$	13±2	70	30	14± 2 (n=4)	70
7	10	$250\rightarrow100/250_{15}$	13±1	60	40	31±17 (n=6)	100

* The carp were anesthetized first with the high concentration of CO_2 ($Pco_2 \fallingdotseq 250$ mmHg) for 30 min, and after that they were treated repeatedly with the low concentration of CO_2 ($Pco_2 \fallingdotseq 100$ mmHg, 1 h) and with the high concentration of CO_2 ($Pco_2 \fallingdotseq 250$ mmHg, 30 min for Exp. 6 or 15 min for Exp. 7).

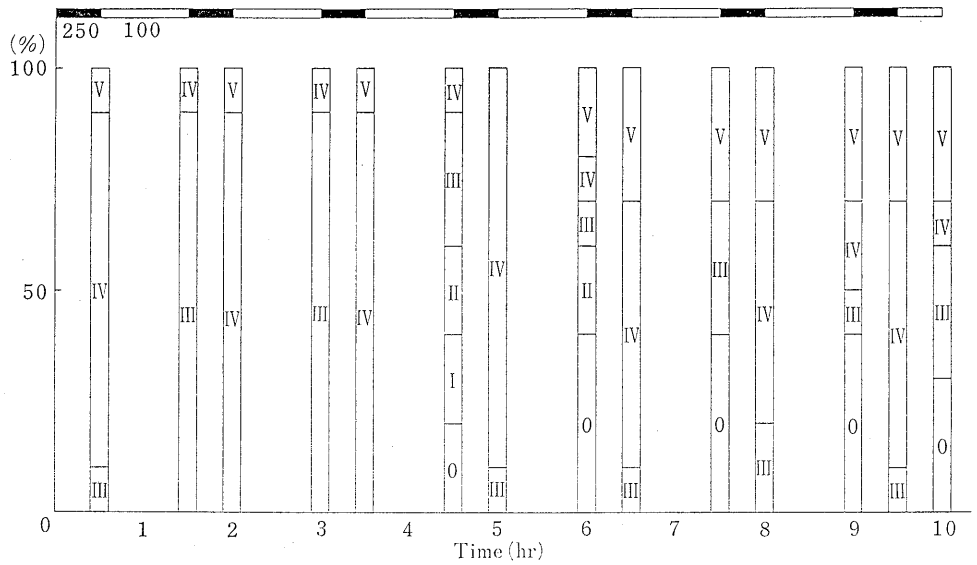

Fig. 2. Changes of anesthetic stage in the carp treated with CO_2 in the condition of Exp. 6. ▓: treatment period with the high concentration of CO_2, □: treatment period with the low concentration of CO_2.

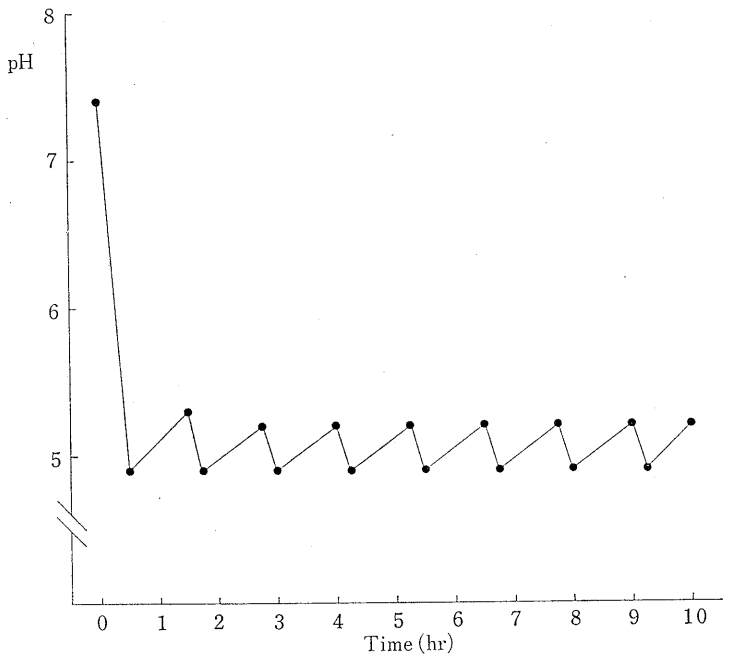

Fig. 3. Changes in the average pH of the water running through the experimental chamber in Exp. 7.

stage. The anesthetic stage, in general, progresses with time, so it is naturally expected that the anesthetic stage becomes deeper at a later treatment than at an early treatment, compared with the treatments with the same concentration. However, surprisingly, some carp recovered from the anesthetic state after the third treatment with the low concentration (Fig. 2). In this experiment, although the treatment with the low concentration was repeated seven times, the anesthetic stage tended to become shallower with every treatment with the low concentration and the

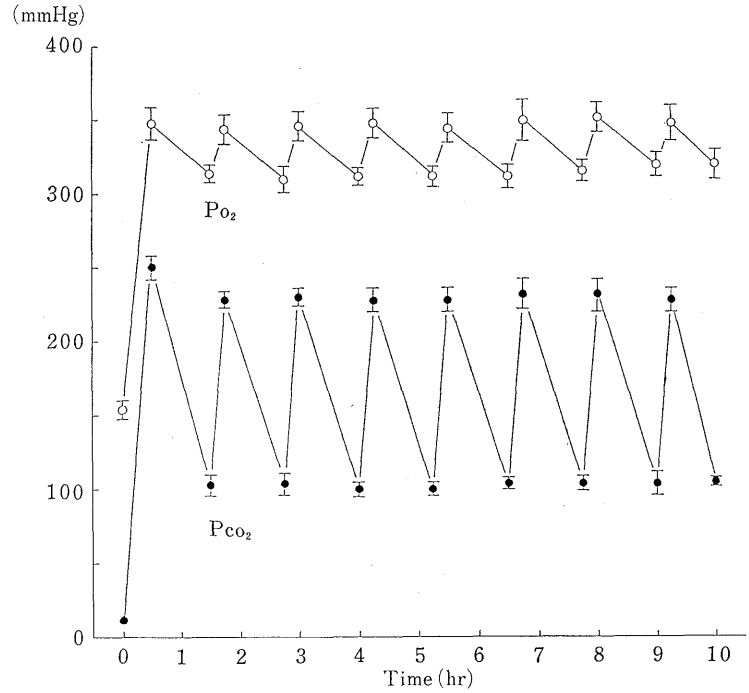

Fig. 4. Changes in the Pco_2 and Po_2 of the water running through the experimental chamber in Exp. 7.

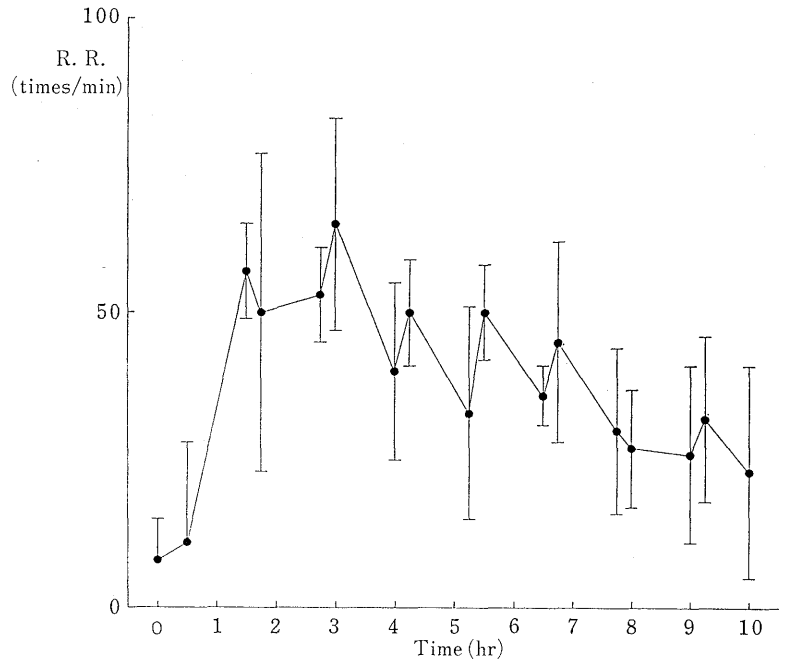

Fig. 5. Changes of the respiratory rate in the carp anesthetized repeatedly with the high concentration of CO_2 ($Pco_2 \fallingdotseq 250$ mmHg, 30 or 15 min) and with the low concentration of CO_2 ($Pco_2 \fallingdotseq 100$ mmHg, 1 h).

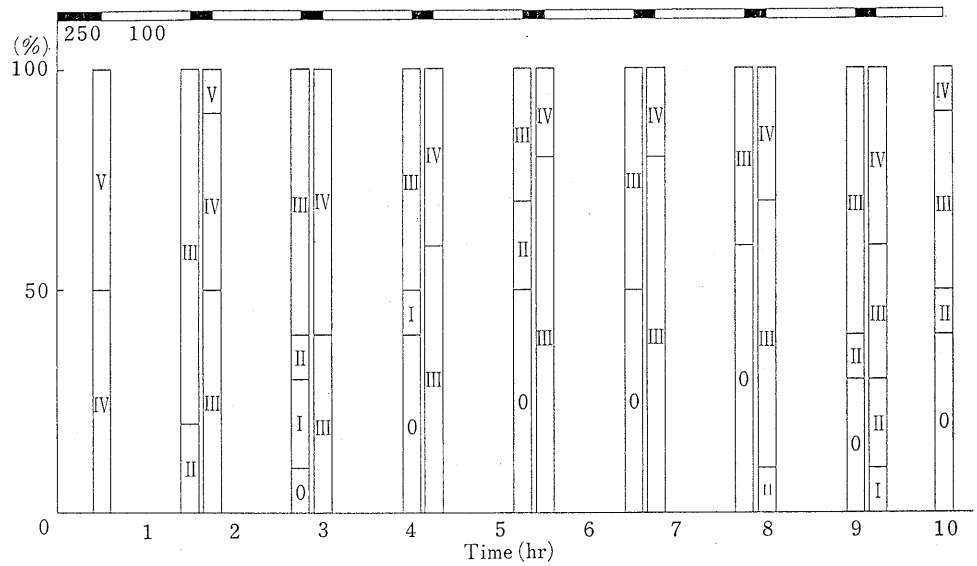

Fig. 6. Changes of anesthetic stage in the carp anesthetized with CO_2 in the condition of Exp. 7. ▓: treatment period with the high concentration of CO_2, □: treatment period with the low concentration of CO_2.

number of the carp in Stage 0 also increased with time. When we first found a carp in Stage 0, we thought we had lost control of the Pco_2 in the ambient water, but the pH, Pco_2, and Po_2 showed quite normal values.

Throughout the experiments, we constantly observed the behavior of the carp. When we encountered the carp in Stage 0 for the first time, we noticed by chance that the recovering carp almost stopped swimming and only kept equilibrium. Then, we applied mechanical stimulation to the carp through the experimental chamber (hitting the chamber light), but the carp did not respond at all. These carp seemed to remain in sedate conditions even after they had recovered from the anesthetic state during the treatment with the low concentration. We hit or vibrated the chamber when the carp recovered from the anesthetic state. All the carp remained in a sedate state. Lightly sedate carp did not respond to hitting or vibration and deeply sedate carp did not move at all even when touched directly.

We could not maintain the carp in a favorable anesthetic state for 10 h, and it seemed to be impossible to maintain anesthesia for 10 h. To accomplish 100% anesthesia, the time of the treatment with the high concentration must be longer or the Pco_2 used as the low concentration must be higher, but both procedures will undoubtedly increase mortality.

Therefore, we tried to shorten the time of each treatment with the high concentration of CO_2 from 30 min to 15 min except for the first treatment. As shown in Figs. 3 and 4, the pH, Pco_2, and Po_2 in the ambient water repeated fluctuation with a settled interval. Figs. 5 and 6 show the changes in the respiratory rate and anesthetic stage in the carp and Table 3 summarizes the results. Some carp in Stage 0, or the sedate state, appeared at about 3 h and its number gradually increased. At the end of the treatment, 40% of the carp were in a sedate state and 60% were at Stage II or higher stage. All the carp survived and the mean recovery time was 31 min.

Discussion

A high concentration of CO_2 has been proven to be safe as an anesthetic for short-term anesthesia, and CO_2 has been used for honey bee, abalone, and fish.[2-6] In the present study, we reaffirmed its safety. The carp became anesthetized within 30 min at Pco_2=200–250 mmHg and they all recovered from the anesthetic state. Its safety has also been demonstrated by electrocardiography (ECG). During anesthesia with CO_2, the ECG pattern of the carp slightly changed in its wave and electrical axis, but at the time of recovery from anesthesia the ECG showed an almost normal pattern (unpublished data).

Mitsuda *et al.*[7,8] Mishra *et al.*[9] and Takeda and Itazawa[10] attempted to apply this anesthesia for long-term anesthesia, but they did not establish a sufficient method of anesthesia for adult fish. We also failed to find adequate conditions to maintain the adult carp in a favorable anesthetic state for 10 h, possibly as a result of large individual differences. However, we showed that CO_2 has a strong sedating action. Mishra *et al.*[9] also pointed out the sedating action of CO_2. This action seems to be due to the lasting effects of high concentrations of CO_2, because the carp remained in a sedate state even in fresh water for a while after they were once exposed for 30 min to water containing a high partial pressure of CO_2 ($Pco_2 \fallingdotseq$ 250 mmHg). Moreover, they were not put into a sedate state when they were treated with a low concentration of CO_2 alone. The sedation ranged from a light state in which the carp did not respond to vibration, to a deep state in which they hardly moved even when touched directly.

The problem in transporting live fish is mainly the aggravation of its ambient water and injury of the fish by their excitement during transfer or transportation. Such excitement is reduced by the anesthetic action and strong sedating action of CO_2. We could maintain the carp in a sedate or anesthetized state for 10 h, using two concentrations of CO_2. Thus, this anesthesia is useful for transportation of live fish.

In the present study, we used carp *Cyprinus carpio* as an experimental fish, however, as a rule, the carp is a tough species of fish. As discussed in the preceding report,[1] the anesthetic action of CO_2 is due to the decrease in pH in the brain. Therefore, our method should be tested before use on species which are highly sensitive to a low pH.

We regarded Stages III-IV of anesthesia as being suitable for the transportation of live fish,[1] and tried to maintain the carp in Stage III or higher stage. On the other hand, Durve,[11] Taylor and Solomon,[12] and Ferreira *et al.*[13] stated that the sedate state is suitable for live-fish transport. Ferreira *et al.*[13] explained the reason as follows; the fish in Stage I showed active swimming movements to rectify imbalance, and the fish in Stage II or higher stage sank to the bottom, where there would be less oxygen. However, active movement is only transiently observed at the initial phase of anesthesia, and the problem of fish sinking to the bottom is solved by putting them into separate compartments. As previously reported,[1] the anesthetic stage progressed with time. Thus it is still impossible to maintain a certain anesthetic stage although, if possible, the most suitable stage would be Stage III-IV.

The previous and present studies were carried out using adult carp that were acclimated to the condition of 23°C. If the ambient temperature decreases, the concentration needed for the induction of anesthesia also decreases, and its effective range can be widened. Moreover, low temperature is known to have an anesthetic effect.[14,15] We are now studying a new method of anesthesia combining CO_2 with a low temperature.

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References

1) H. Yoshikawa, Y. Ishida, S. Ueno, and H. Mitsuda: *Nippon Suisan Gakkaishi*, **54**, 457–462 (1988).
2) O. Kaftanoglu and Y. S. Peng: *J. Apicult. Res.*, **21**, 3–6 (1982).
3) M. Sugiyama and Y. Tanaka: *Bull. Natl. Res. Aquaculture*, **3**, 37–44 (1982).
4) F. F. Fish: *Trans. Amer. Fish. Soc.*, **72**, 25–29 (1943).
5) H. E. Booke, B. Hollender, and G. Lutterbie: *Prog. Fish-Cult.*, **40**, 11–13 (1978).
6) G. Post: *Prog. Fish-Cult.*, **41**, 142–144 (1979).
7) H. Mitsuda, K. Nakajima, H. Mizuno, F. Kawai, and A. Yamamoto: *J. Nutr. Sci. Vitaminol.*, **26**, 99–102 (1980).
8) H. Mitsuda, S. Ueno, H. Mizuno, T. Ueda, H. Fujikawa, T. Nohara, and C. Fukada: *J. Nutr. Sci. Vitaminol.*, **28**, 35–39 (1982).
9) B. K. Mishra, D. Kumar, and R. Mishra: *Aquaculture*, **32**, 405–408 (1983).
10) T. Takeda and Y. Itazawa: *Nippon Suisan Gakkaishi*, **49**, 725–731 (1983).
11) V. S. Durve: *Aquaculture*, **5**, 53–63 (1975).
12) A. L. Taylor and D. J. Solomon: *Fish. Mgmt.*, **10**, 153–157 (1979).
13) J. T. Ferreira, H. J. Schonbee, G. L. Smith: *Aquaculture*, **42**, 169–174 (1984).
14) W. N. McFarland and G. W. Klontz: *Fed. Proc.*, **28**, 1535–1540 (1969).
15) K. S. Chung: *Nippon Suisan Gakkaishi*, **46**, 391 (1980).