Communication

EFFECTS OF CARBON DIOXIDE ON CARP

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Fish is the most common animal protein source in Japan. The fish proteins are rich in essential amino acids and present a good amino acid profile for human requirements (1). The necessity for the transport and handling of living fish has increased, thus the development of safe techniques has been of great concern. One effective method has been the use of anesthetics to suppress swimming, which results in a decrease in oxygen consumption and the alleviation of pollution. The effects of anesthetics on fish have been extensively investigated under experimental conditions but have had little practical application.

Here, we examined the use of carbon dioxide instead of anesthetics.

Carp (Cyprinus carpio) were reared in a 1,200-liter acrylic container composed of three square compartments. The water was circulated and aerated. Carp were classified into three groups; those of small size weighing 20 to 80 g, those of medium size weighing 200 to 400 g and those of large size weighing 700 to 800 g. They were usually fed every Saturday, but were fasted for at least 24 hr prior to an experiment. Glass vessels used in the experiment were 30×45×30 (height) cm. Usually one carp was placed in 15 liters of fresh water after which carbon dioxide was bubbled into the glass container at room temperature. The flow rate (1 liter/min) was kept constant. Oxygen (1 liter/min flow rate) was mixed with the carbon dioxide to avoid loss of oxygen consumption by the carp. Nitrogen was also given and the results are compared with the effects of carbon dioxide.

The dissolved oxygen concentration was measured with a Dissolved Oxygen Meter (YSL Model 57). The dissolved carbon dioxide concentration was not measured directly, but estimated in terms of the hydrogen ion concentration with a pH meter (Horiba M-5).

Electrocardiograms (ECG) were monitored with a Polygraph RM-6200

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(Nihon Koden) to determine the effect of CO2 on the heart. Carp used for ECG measurements were those of small size ranging from 30 to 50g. A 3-liter polyethylene beaker with a diameter of 16 cm, that nearly fitted the size of the carp, was used. Three electrodes were inserted into the carp. One was inserted into the heart, one into the ventral muscle mass, and the third into the muscle mass approximately one inch from the second electrode location, and this one was used as a ground.

The behavioral changes in carp induced by carbon dioxide are shown in Fig. 1. The pH was also followed, since tracing the pH indicates changes in the dissolved carbon dioxide concentration which may be related to behavioral changes in the carp. The swimming pattern at the initial period of excitation may be equivalent to Stage I-2 of the McFarland’s criteria (2). This appearance is common to anesthesia in fish if they are exposed to high drug concentrations. A time around (C) in Fig. 1 should be the boundary between Stage I-2 and Stage II-1. At that time, the pH reached a constant value of 4.50. After the mixed gas was stopped, the pH change was very slow and slight. After the carp turned on its side, significant changes appeared in the opercular rate and in both the amplitude and the regularity of the

![Fig. 1. Change in the behavior of carp under CO2 gas treatment.](image)

- Excitation was induced.
- Swimming was too active to allow a count of the opercular rate, sometimes being accompanied by darting movements to the water surface.
- Excitation ended in slow, rolling movements together with a decline in the opercular rate and a decrease in the amplitude of opercular movements.
- The mixed gas was stopped.
- The carp lay on its side at the bottom; opercular movements became somewhat irregular together with a further decline in the opercular rate.
- Regular opercular movements returned.
- Respiratory movements deepened.
- The carp began to move.
- The normal position was resumed.

opercular movements until recovery. The former and the latter in this period may be equivalent to Stage II-2 and Stage III, respectively. Regardless of the above behavioral changes the carp still lay on its side at the bottom of the vessel (Fig. 2). When the pH reached 4.80 after 395 min the carp began to move, after which it recovered within 10 min; this was a total sedation period of 6 hr. Recovery appears to rely upon the pH, viz. the dissolved CO$_2$ concentration. In fact, when returned to fresh water carp usually recovered within 5 min. In addition, if the carp were not small they sometimes recovered even after cessation of opercular movements. In contrast, a technique by which the pH value was kept constant during sedation induced by CO$_2$ prolonged the period of sedation and enabled carp to survive for 30 hr. These observations suggest that the period of sedation can be controlled simply by the dissolved CO$_2$ concentration, and are thus of great interest.

It is also of interest to observe how much oxygen carp consume under CO$_2$ treatment. The mean oxygen consumption of carp during sedation at 15°C was 2.4 ml/kg·hr for large-sized carp and 5.4 ml/kg·hr for medium-sized ones. These values were much lower than those in normal swimming conditions (284 ml/kg·hr for large carp and 221 ml/kg·hr for medium ones), and were comparable to those of motionless marine animals such as the arkshell (Arca inflata) and the sea cucumber (Stichopus) (3).

Effects of MS222 and quinaldine on carp were tested and compared to CO$_2$. Frequent ventral fin movement and subsequent swimming with imbalanced equilibrium were the major differences. It should be noted that the overall behavioral features under CO$_2$ were similar to those under the anesthetics MS222 and quinaldine. Nitrogen had no significant effects on the carp in phosphate buffer solution (pH 4.5) without CO$_2$ and showed no significant changes in their
swimming patterns. These results add to the evidence that the series of behavioral patterns found here are based on CO₂. The patterns shown in Fig. 1 are common to those of other carp under identical conditions. For the small carp, individual variations were relatively great during the period of sedation.

There were significant changes in ECG determinations of carp under CO₂ treatment. Five minutes after the introduction of the mixed gas, the carp began to turn on its side. This appeared to cause a significant change in the QRS-complex peak, with gradual decline of cardiac rate. This fall in the pulse rate was also accompanied by a fall in the opercular rate. This shows that there may be a connection between the cardiac and respiratory (opercular) centers in the brain, as Randall has contended (4). Subsequent ECG profiles were not altered much while the carp was lying on its side.

The sedation of carp induced by simple handling of CO₂ caused day-long sedation. The mixed gas of carbon dioxide and oxygen, at varied concentrations of both gases, likewise induced the anesthesia-like sedation for the marine-fish, *Chrysoophys major* and *Seriola aurevittata*. Compared to anesthetics, carbon dioxide gas is much cheaper and is as an absolutely safe additive; thus, sedation with carbon dioxide might be of potential use for the transport of live fish.

Details of the sedative action of carbon dioxide are being studied at the physiological and neurological levels.

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