Studies of the Conditioned Reflex in the Lower Vertebrates
V. Respiratory Defensive Conditioned Reflex in Carp[1)2)3)]

By

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It has been noted in our previous papers dealing with the food conditioned reflex, that the number of reinforcements requried for the formation of conditioned reflex in lower vertebrates such as fish is not as accurately determined as in higher animals (Tuge, Ii, Kanayama, and Ochiai, '55; Tuge, '56). In this connection the defensive conditioned reflex may be of much more use.

On the other hand, fish living in water are always under the influence of the surrounding media, through which their activities are greatly modified. Among such media which may be considered harmful to fish, CO_2, acids, sulfides and others may be counted. There are possibilities in the natural conditions showing that such a harmful chemical substance, as an unconditioned stimulus, will cause a defensive conditioned reflex in combination of any indifferent stimuli.

Chernova ('53) succeeded to obtain in carps a defensive conditioned reflex very quickly, by combining CO_2 with light. It is well-known that the CO_2 solution would have influence not only upon the chemical sense organs of the mouth or the body surface of the fish, but also directly upon the respiratory center through the blood circulation. There arises a question as to whether or not such a direct stimulation would be able to establish such a quick and stable temporary connection. If so, CO_2 or H_2CO_3 introduced into the water in excessive quantity may be supposed to exercise a profound influence upon the behavior of fish.

In the pressent experiment, we dealt with the formation of respiratory conditioned reflex under application of CO_2 and HCl as an unconditioned stimulus. The latter will be considered only as a stimulant of the chemical sense organs.

MATERIAL AND METHOD

For the present experiment 17 carps (*Cyprinus carpio*) of one to two years of

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age were used. They ranged from 17 to 23 cm in body length, including the tail. The experiments were undertaken from July to September. Water temperature was kept at 22-26°C.

For the experimentation, the fish was tied with bands as illustrated in Fig. 1.

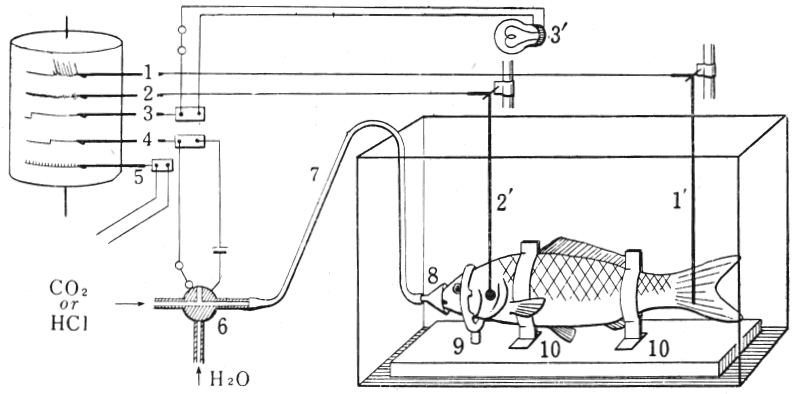


Fig. 1 Arrangement of the set-up for the experimentation. Explanation in the text. 1 - 1′, movement of tail; 2 - 2′, movement of operculum; 3-3′, light; 4, CO_2 or HCl; 5, time; 6, a three-way cock; 7, a tube leading to the small funnel; 8, a small funnel; 9, ring; 10, bands.

The method to tie the fish was according to that of Kogan and Shitov ('54, p. 18). The fish tied as above was placed into an experimental aquarium containing water of about 20 *l*. Both the movements of the operculum and tail were registered on a kimographion by means of the levers, the ends of which were gently placed onto the operculum and the tail respectively.

During the experimentation, water of about 0.8 *l* per minute was constantly poured onto the mouth from a small funnel, to which the tank, filled with water, was connected by a tube. Either CO_2 or HCl solution, used as an unconditioned stimulus, was poured from the respective tanks through the same funnel by means of a three-way cock. The CO_2 saturated solution contained 1.1 g per litre of CO_2, for which titration was made, according to R. B. Warder's method. As HCl solution, the solutions of 1/10,000, 1/1,000 and 1/100 mol were used. However, in the present experiment 1/100 mol solution was used exclusively, since it was most adequate as an unconditioned stimulus.

As conditioned stimuli were applied light, a buzzer and the water current given onto the lateral line. The electric light was suspended far above the aquarium, and its intensity was about 75 luxes at the surface of the aquarium. The buzzer was sounded at about 2 m above the aquarium. Stimulation of the water current was given slantwisely onto the lateral line of the abdominal region, through a small glass tube.

EXPERIMENTAL RESULTS

Experiments with CO_2

Light and CO_2 5 fishes were used for the experiment in order to work out the temporary connection between light and CO_2. The orientation reactions to light, if existing, are easily extinguished, except in only a few fishes. The combination of light with CO_2 was made as follows: 15 to 30 seconds after the light

Table 1

Speed of formation of the temporary connection between the chemical substances (CO_2 and HCl) and the various conditioned stimuli.

Kinds of combination	Cases	Number of reinforcement required for the weak temporary connection	Number of reinforcement required for the stable temporary connection
CO_2 and light	*C. no. 152*	1	2
	C. no. 153	4	—
	C. no. 155	1	2
	C. no. 187	3	11
	C, no. 188	4	9
CO_2 and buzzer	*C. no. 160*	2	4
	C. no. 161	—	2
CO_2 and water current	*C. no. 157*	2	—
	C. no. 158	2	—
	C. no. 164	3-6	—
	C. no. 165	4	5
HCl and light	*C. no. 176*	5	7
	C. no. 181	3	7
	C. no. 182	3	6
	C. no. 184	2	9
	C. no. 185	2	9
	C. no. 186	2	9

was switched on, CO_2 solution was poured onto the mouth for about 10 to 30 seconds. Usually, the temporary connection is formed very quickly. The speed of formation of the temporary connection is shown in table 1. Figure 2 illustrates the kimographic records of the formation of the temporary connection.

Buzzer and CO_2 In two carps used for combining buzzer with CO_2, the temporary connection was formed after 2 times of reinforcement of CO_2, as shown in Table 1.

Water current and CO_2 Four carps were subjected in order to make the temporary connection. As seen in table 1, the temporary connection was established after 2 to 4 times of reinforcement with CO_2. In comparison with the other cases, such as with light and buzzer, however, the stable conditioned respiratory reflex was hardly obtainable. At least, it required more than 5 times of reinforcement to obtain the stable temporary connection (Fig. 3).

Experiments with HCl

Six carps were used to make the temporary connection between HCl and light. In this case, only light was used as the conditioned stimulus. As seen in table 1,

Fig. 2.

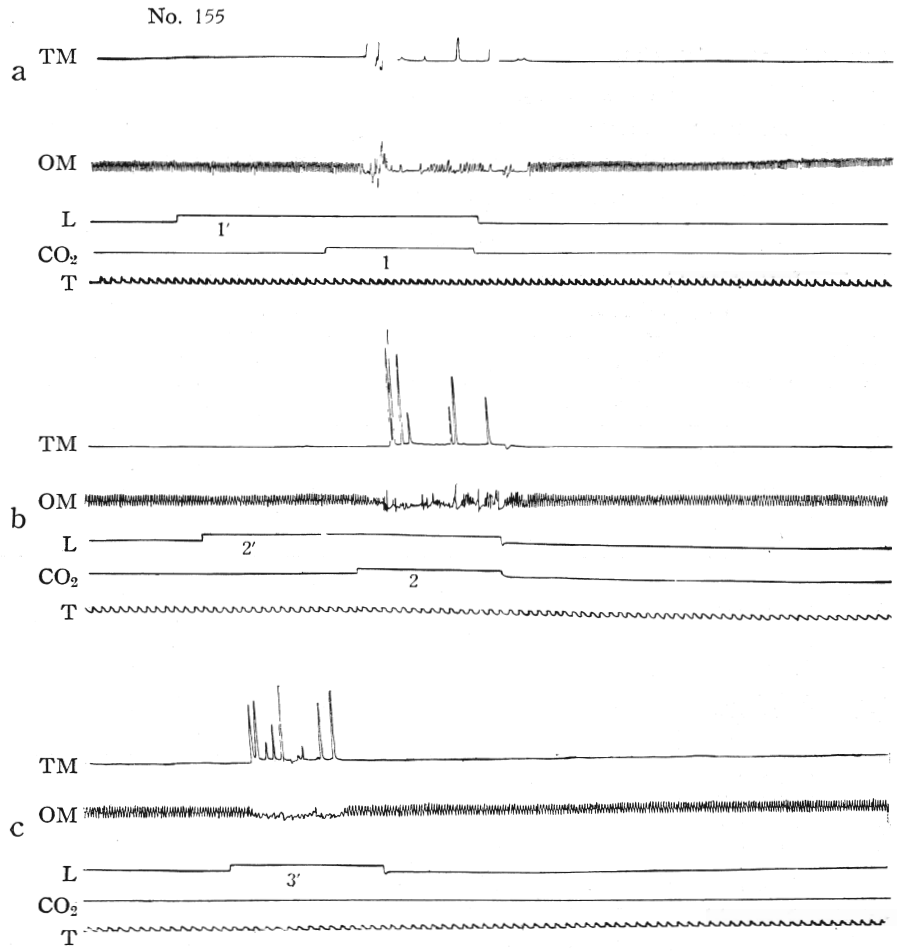
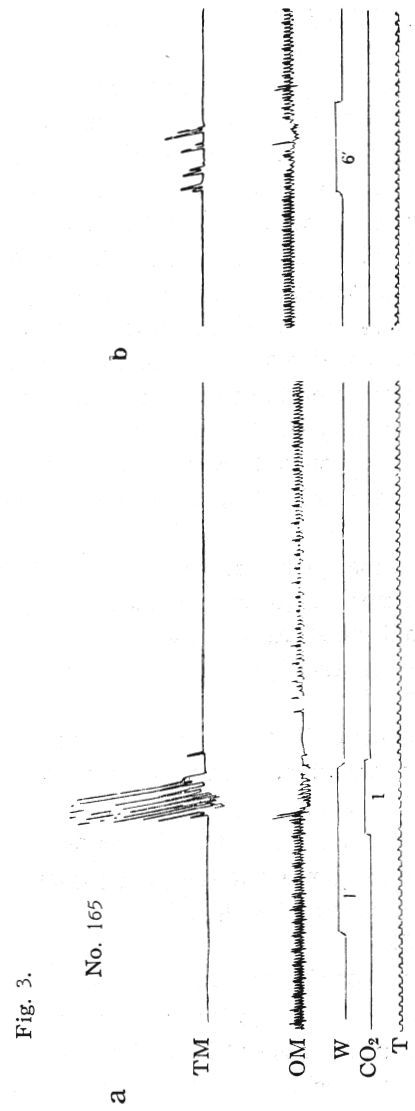


Fig. 2. The figure illustrates the formation of the temporary connection between CO_2 and light. (a) First application of CO_2. (b) Second application of CO_2. Very slight disturbance in the movement of operculum was observed. (c) Application of conditioned stimulus (light) after two times of reinforcement of CO_2. Abbreviations; TM, movement of tail; OM, movement of operculum; L, light; T, time (2 seconds).

the weak temporary connection was formed after 2 to 5 times of reinforcement and, the stable connection after 6 to 9 times of reinforcement (Fig. 4). Accordingly, the formation of respiratory conditioned reflex by application of HCl as unconditioned stimulus is not considered so slow, when compared with the case of CO_2.

Extinction

We could not follow the process of extinction to a complete result, because of certain circumstances in our laboratory. The temporary connection established by CO_2 appeared to be extinguished 9 to 16 times of application of extinctive stimulus without reinforcement of CO_2. It is very interesting in the case of HCl, where it required a large number of extinctive stimuli to extinguish the respiratory conditioned reflex. In one example, it required more than 40 timesof extinctive



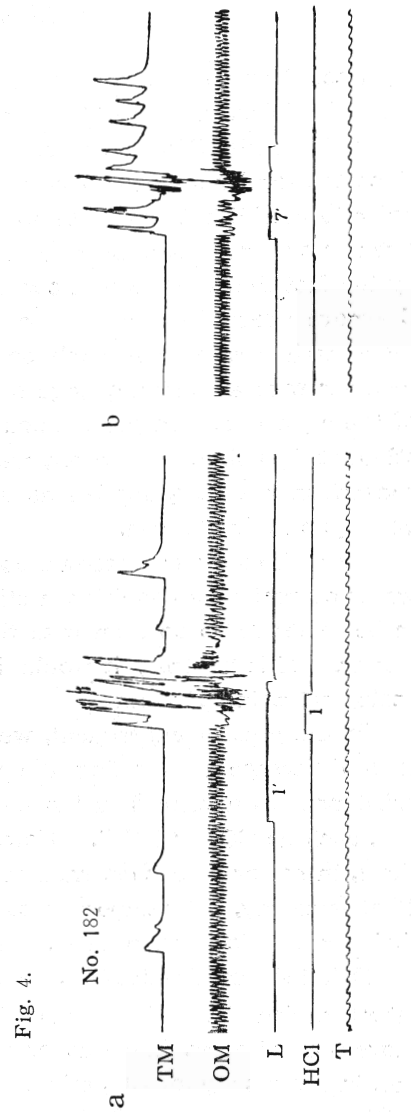


Fig. 3. The figure illustrates the formation of the temporary connection between CO_2 and water current onto the lateral line. (a) First application of CO_2. (b) Conditioned respiratory reflex elicited after 5 times of reinforcement of CO_2. Abbreviations are the same in Fig. 2 except W (water current).

Fig. 4. The figure illustrates the formation of the temporary connection between HCl and light. (a) First application of HCl. (b) Conditioned respiratory reflex elicited after 6 times of reinforcement of HCl. Abbreviations are the same in Fig. 2.

stimuli to attain complete extinction. It is also noted, that the conditioned reflex which had once been extinguished, was again vigorously elicited on the following day. In either case, CO_2 or HCl, the process of extinction appeared to take a wave-like course.

DISCUSSION OF THE RESULTS

According to the results of Chernova ('53), a strong temporary connection was established in almost all the fishes experimented upon, by merely one combination of light with CO_2. Our results, however, differ from those obtained by Chernova, since the former seemed to require over 2 times of reinforcement with CO_2 in order to establish a strong temporary connection in cases where light, buzzer, or a water current was applied as a conditioned stimulus. This may be explained by the fact that there was a considerable difference between the methods employed. Chernova adopted the method of pouring a CO_2 saturated solution at once into the aquarium where the whole body of the fish would receive the full effect of CO_2, together with a sudden change of the water pressure, due to the partial draining of the aquarium. In our method the mouth region of the fish would be locally stimulated at first. However, we can support the conclusion that the temporary connection with CO_2 applied as an unconditioned stimulus, tends to be formed very quickly in the fish.

Even though our materials used for the experiment in combining CO_2 with water current as a conditioned stimulus, were not adequate enough, it seemed not to be easier to do so than with the other conditioned stimuli used, either light or buzzer. If it be true, it would be worth to reinvestigate this matter from an ecological point of view.

In the present experiment, we intended to make it clear whether or not there is a difference in forming the temporary connection, using different chemical substances as unconditioned stimuli with their respective physiological effects on fish, such as CO_2 and HCl. However, we could find but little difference between them, in so far as it concerned the speed of formation of the temporary connection. That is to say, the temporary connection by means of CO_2 might be formed a little more quickly than that by means of HCl.

Detailed examination of the kimographic records obtained showed that the latent period from the stimulation of CO_2 to the reactionary change of the movement of operculum was longer than that in the case of HCl. Roughly speaking, in most cases of CO_2 stimulation, the latent period is from 2 to 8 seconds, while in the cases of HCl, it is within 2 seconds. Figures 2 and 4 may illustrate the differences between the respective latencies. In both cases, however, there are almost no marked differences in the respiratory mevements produced. In short, in both cases, by application of a stimulus, there occurred irregular respiratory movements with increased amplitudes, and then apnoea or abnormal respiration, accompanying the movement of the body. Such irregular respiration continued for a while after the pouring of those chemical substances onto the mouth had ceased. Afterwards, it recovered gradually to normal respiration. In most cases there occurred a disappearance of the cleaning reflex or a decrease of its frequency. As stated above, we could not determine in the present experiment that there are marked differences between the stimuli of CO_2 and HCl. Accordingly, we should hesitate to conclude from our experiment dealing with fish that CO_2 stimulates

exclusively the respiratory center.

Apart from the question as to what extent harmful chemicals, are dissolved at any given time in the water in which the fish live, it is beyond doubt that CO_2, or acids, would, to some extent, bear influence upon the behavior of fish. It is therefore, quite reasonable to take into consideration that the defensive conditioned reflex will be very quickly formed in fish while in their natural environment, where they would be free from the danger of such chemical substances. Our result, together with that of Chernova, seems to indicate that there is a possibility of providing a conditioned reflex between dangerous chemicals and various indifferent stimuli, in order to ensure the life of fishes in their variable circumstances. Consequently, it may be emphasized that this matter has a deep bearing upon the fishery industry, especially upon fish farming.

SUMMARY

1) In order to establish a respiratory defensive conditioned reflex in carp, both the solutions of CO_2 and HCl were used as unconditioned stimuli. Light, buzzer and water current onto the lateral line were applied as conditioned stimuli.

2) In combination of CO_2 with either light or buzzer, a respiratory conditioned reflex was stably formed after 2 times of reinforcement. In case of water current, a stable conditioned reflex was hardly obtainable, though a weak conditioned reflex was formed after 2 times of reinforcement as well.

3) In combination of HCl with light, a respiratory conditioned reflex was formed after 2 times of reinforcement, but it requires many more repetitions of reinforcement to establish a stable one.

4) Concerning the speed of formation of respiratory conditioned reflex, it may be said that the temporary connection by means of CO_2 is slightly quicker than that of HCl. But, no marked differences were found in the changes of respiratory movements between both solutions.

5) For the extinction of the respiratory conditioned reflex it required a large number of extinctive stimuli, and in case of HCl it seemed to require many more extinctive stimuli than in the case of CO_2.

6) It is emphasized, that the quick formation of a defensive conditioned reflex by application of harmful chemical substances, such as CO_2 and HCl, when in excessive quantities in the water, has proved to ensure the life of fishes in their variable circumstances.

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