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Measurement of Blood Pressure of Carp

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Abstract Cannulae combined with electrodes were devised for simultaneous recordings of intracardiac blood pressures and electrocardiograms from the carp ranging from 700 to 1,000 g in body weight. Cannulae were implanted at any two of three sites: dorsal aorta, bulbus arteriosus and ventricle. Size of cannula, site of cannula implantation and type of transducer adequate for the blood pressure measurements were investigated. In the confined but not anesthetized, systolic blood pressures in the bulbus arteriosus were either equal to or slightly less (about 1.0 mmHg) than those recorded in the ventricle. Pulsating pressures were much less in the bulbus arteriosus than in the ventricle. As in other fish, carp also showed a less of pressure across the gill vasculatre, and the loss amounts to about one-third of that generated by the heart. In an undisturbed state, heart rate became low and nonrhythmical. Ventricular contractions were performed in small group with short pauses between series.

Introduction

Blood pressures of fish have been studied as early as the later part of the nineteenth century (Schoenlein and Willem, 1894; Brünings, 1899) and thereafter have drawn the attention of many investigators as shown in various reviews (Mott, 1957; Johansen and Martin, 1965; Ozaki, 1968; Satchell, 1971). It was pointed out (Satchell, 1971), however, that there are few records for teleost fishes and most of the measurements are from the family Salmonidae. Moreover, a large majority of recordings have been taken during an anesthetized state which has been found to alter blood pressures to various degrees (Ngan, unpublished data).

In the present study blood pressures of confined but unaesthetized carp were measured with the aid of cannulae implanted at several sites in the circulatory system. At the same time, respiratory movements and electrocardiogrms of the fish were also recorded.

Materials

Common carp, *Cyprinus carpio* Linnaeus, ranging from 700 to 1,000 g in body weight, were used. Prior to the experiment the fish were acclimated to laboratory conditions in the manner previously described (Ngan et al., 1973). Blood pressures were measured through cannulae implanted at any two of three sites, namely dorsal aorta, bulbus arteriosus, and ventricle.

Polyethylene tubing, 50 cm in length, was used for making the cannula. One end of the tubing was fitted over the cut end of a disposable needle from which the plastic hub had been removed.

Since proper employment of the cannula required its immobilization to the body of the fish by sutures, the needle was bent almost at a right angle once it was implanted in the ventricle or the bulbus arteriosus. In order to fascilitate the bending, the needle had been annealed beforehand with a gasburner.

Procedure for cannulation

The method for implantation of cannula into the dorsal aorta has already been described (Ngan et al., 1973). In order to implant the cannula into the bulbus arteriosus or the ventricle, the fish was anesthetized deeply with MS 222, (1/10,000) then placed on its back in a V-shaped plastic holder. Three sutures were prepared and readied on the ventral side of the fish. The first was positioned about halfway between the base of the pectoral fins, the second slightly behind the first, and the third just anterior to the pelvic fins.

The cannula was filled with saline-heparin solution, and the bulbus arteriosus or the ventricle was approached through the thoracic

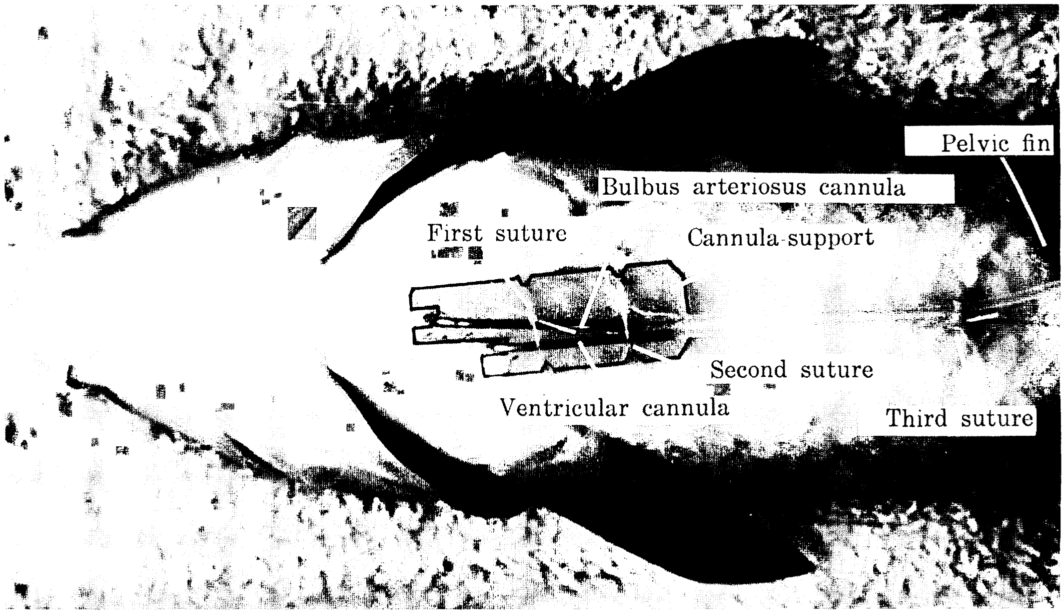

Fig. 1. Ventral view of ventricular and bulbus arteriosus cannulae fixed to the body of a fish by sutures and cannula support.

region of the fish. The bulbus arteriosus lies beneath the midline on the ventral side and just behind the coracoid. On the other hand, the ventricle is located slightly to the right and beneath the line which connects the two foremost points of the bases of the pectoral fins (Fig. 1). Once the tip of the needle was implanted into the bulbus arteriosus or the ventricle, the blood flowed into the cannula to replace the saline-heparin solution. A strong pincette was used to hold the needle at a point as near the fish body as possible, and the needle was bent backward with the upper edge of the pincette acting as a fulcrum. Since the needle had been annealed, the bend could be made easily and did not shift the position of the needle. Blood in the cannula was returned to the bulbus arteriosus or the ventricle by saline-heparin solution, and the free end of the cannula was pinched tightly with forceps and then sealed with clay. The cannula was then secured to the body of the fish with the three sutures prepared earlier. In order to prevent the needle from further entering the heart or from swinging to either side a piece of polyethylene tubing (about 0.3 cm in thickness) with notches fitted to the needle and the sutures was employed as a cannula-support. The fish was than allowed to recover and rest in an aquarium supplied with running water for two days prior to experimentation (Ngan et al., 1973).

Fish implanted with a cannula in this way could be used in the present study for about 10 days. A moment after sealing, a small amount of blood was usually seen to rise in the cannula. This is not believed to be due to the sealing but may be due of the diffusion of blood into the saline-heparin solution. When the cannula was not in use for a few days, it was usually found plugged with blood. It was, threfore, necessary to flush the cannula with saline-heparin solution everyday, even on those days when recordings were not taken.

Recordings of respiratory movements, electrocardiograms, and blood pressures

Respiratory movements were recorded with the aid of a pair of slender enamel-insulated copper wires functioning as electrodes, one attached to the operculum and the other to the claviculm of the fish. Insulation was stripped off from a short portion of the ends of the wires and respiratory movements were registered as changes in impedance between the electrodes as a result of operculum movements.

For electrograpy, two types of electrodes combined with the above described cannula were devised.

In the first type, enamel-insulated copper wire was used as a conductor between the needle of the cannula and the recorder. One end of the wire was soldered to the needle just in front of the polyethylene tubing. A shield, made of a piece of larger tubing (Igarashi's No. 35), was used to cover this portion. The needle, the shield and adjacent portion were then coated with a thin layer of plastic for insulation except for the tip of the needle, which served as an electrode (Fig. 2–above).

The second type was made of an enamel wire simply hooked to the tip of the needle (Fig. 2–below). The wire was then wound spirally along the polyethylene tubing or attached to it by collars. A portion of insulation on the upper side of the wire near the tip of the needle was stripped off to serve as an electrode. The length of enamel wire hooked inside the needle should be minimized in order to prevent the blood from clotting. In the present experiment, a length of 0.3 cm was found to adequately secure the wire in an effective position for days, provided that the needle was bent to the side on which the wire was not placed.

In both cases the reference electrode was either a small piece of metal placed in the aquarium or a enamel wire with its exposed end wound to the cannula (Fig. 2–above).

By using these two types of electrode-cannulae, blood pressures and electrocardiograms from inside of the heart could be recorded simultaneously. The fish was subjected to only one operation, i. e. implantation of the cannula.

The recorder was a 4-channel polygraph (RM-45, Nihon Koden Co. Ltd.) two channels of which were used to record blood pressures while the remaining two were used to record respiratory movement and electrocardiogram.

For measurement of blood pressures, free ends of the cannulae were connected to transducers (MPU–0.5, Nihon Koden Co. Ktd.) which in turn were attached to the recorder.

Technical considerations and results

1. Cannula size: In an attempt to find a suitable cannula size for recording blood pressures, two experiments were performed. In the first experiment, fish were opened ventrally and polyethylene tubings of three dif-

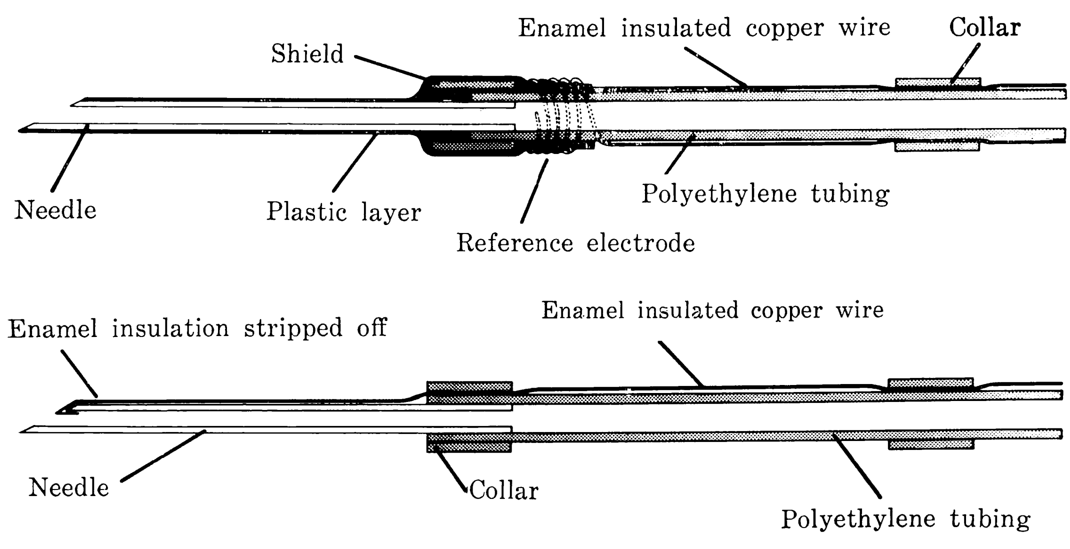

Fig. 2. Two types of electrode-cannulae for the simultaneous recording intra-cardiac blood pressure and ECG of fish.

ferent sizes (Igarashi's No. 20, No. 25, and No. 30 whose outer diameters were approximately 1.3, 1.6 and 1.9 mm, respectively) were inserted into the bulbus arteriosus one after another. Blood pressure was then recorded with the aid of a standard pressure transducer. It was found that the pressures conveyed by the No. 25 and No. 30 tubings were identical, while that conveyed by the No. 20 tubing was significantly modified. The modifications consisted of both reduction in peak sharpness and decrease in pulsating pressures, which were apparently caused by the smaller diameter of the tubing.

The same modifications were also confirmed in the second experiment in which two cannulae of different sizes were implanted in unopened fish (Fig. 3). Based on these results, it was decided to use the No. 25 tubing as the cannula in our experiment.

2. Site of implantation: Although it is not theoretically true that the pressure can vary within each chamber of the heart, it may be possible that cannulae implanted at different sites within heart chamber are obstructed to different degrees, thus conveying different values. In order to check this possibility, blood pressure in the bulbus arteriosus (Fig. 6, left) or in the ventricle was recorded with two cidentical cannulae implanted at different sites of the chamber. No difference could be detected in pattern of blood pressure recorded in both cases except for an unusual height in ventricular pressure. Systolic pressure in the ventricle of carp of the above-mentioned size ranged from 25 to 56 mmHg. However, when two cannulae were implanted into the ventricle, the pressure was usually below 20 mmHg. It might perhaps be that the existence of the two needles had hindered its normal functions.

3. Transducers: Trials were conducted with two selected types of transducers (Nihon Koden, Co. Ltd.), a standard pressure transducer (MPU-0.5; 10∼360 mmHg) and a low pressure transducer (MPU-0.1; 20-1,000 mm H_2O). Blood pressure from the bulbus arteriosus and the ventricle of the same fish were first recorded with a pair of low pressure transducers and then a pair of standard pressure transducers. The pressure records thus obtained revealed that the standard pressure transducer was sensitive enough to follow minor changes corresponding to operations of valves, whereas the low pressure transducer did not detect such changes and even caused serious deformations of the pressure pattern.

4. Blood-pressure changes: Intracardiac pressures as measured by the method described above displayed regular cyclic changes, the sequence of which can be interpreted in the

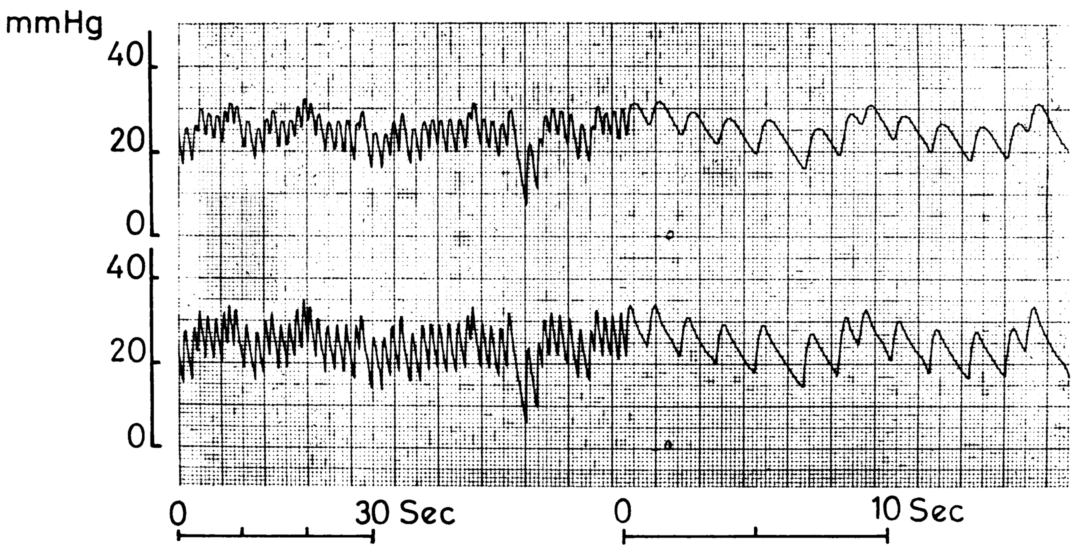

Fig. 3. Blood pressure in the bulbus arteriosus of the same fish recorded simultaneously by two cannulae of different sizes. Upper: Igarashi tubing, No. 20. Lower: Igarashi tubing, No. 25.

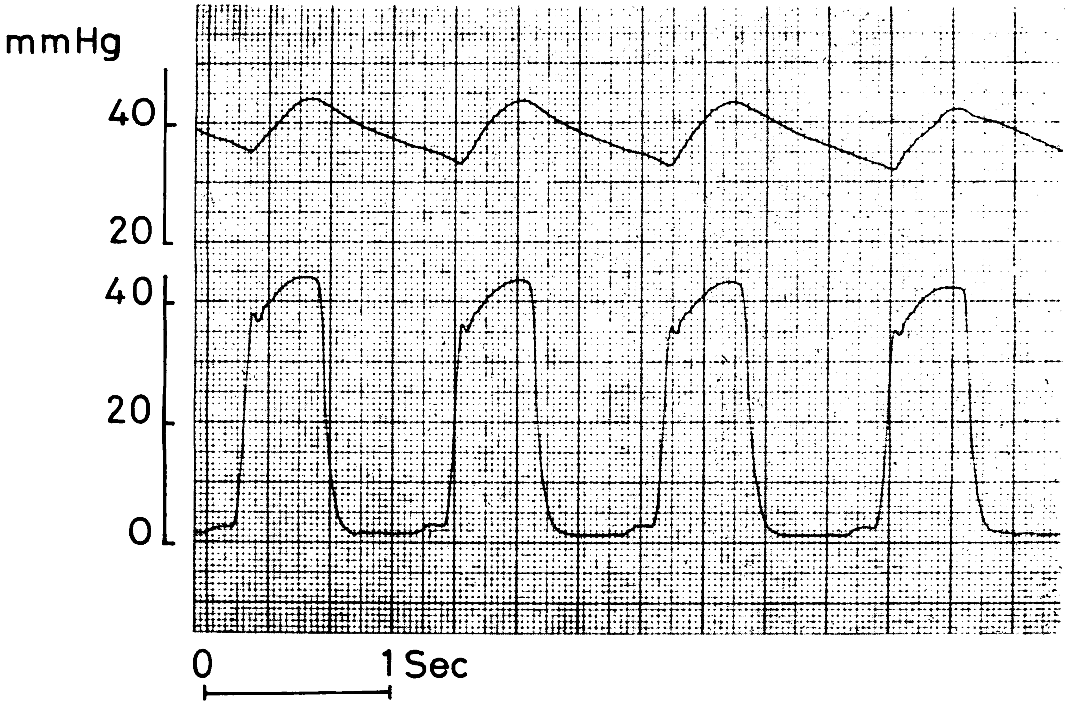

Fig. 4. Details of ventricular (lower) and bulbus arteriosus (upper) pressures recorded simultaneously by standard pressure transducers.

following way:

As a result of atrial systole the blood is forced into the ventricle, causing an appreciable rise in ventricular pressure (Fig. 4, lower). The atrioventricular valve closes when the subsequent contraction of the ventricle causes a rapid increase in pressure. The ventricle contracts isovolumetrically until the pressure exceeds that in the bulbus arteriosus. At this instant, the valve between the ventrice and the bulbus arteriosus opens and the blood is forced into the latter. This brings about a small decrease in the intraventricular pressure, which is exhibited as a sharp notch in the pressure record. The pressure then starts to rise again, but less rapidly, and the rate of rise is almost the same as that of the pressure in the bulbus arteriosus (Fig. 4, upper). Intraventricluar pressure falls rapidly as the ventricle relaxes. The relaxation is isovolumetric until the atrial systole reopens the atrioventricular valve. Concomittant to ventricular relaxation, pressure in the bulbus also begins to decline. This decline is, however, far less rapid and continues until the ventricular contraction reopens the valve between the ventricle and the bulbus arteriosus in the next cycle.

As is clearly demonstrated in Fig. 4, the amplitude of the pressure change is much less in the bulbus (32～44 mmHg) than in the ventricle (2–44 mmHg). Thus, the bulbus acts as an organ which reduces the rapid rise and fall of ventricular pressure before the blood enters the delicate network of blood vessels in the gill.

Obviously the systolic pressure of the bulbus is almost equal to that of the ventricle, whereas its diastolic pressure displays a close relationship with the interval of the ventricular contractions, i. e. the longer the interval the lower the pressure.

When the fish was recovering from anesthesia or resting after disturbances, the heart rate became high and rhythmical. Such a high heart rate was always associated with slightly augmented systolic pressure in the ventricle and definitely elevated diastolic pressures in the bulbus. When the fish was undisturbed for a long while, however, the heart rate became low and nonrhythmical. The ventricular contractions were displayed in small groups, with short pauses in between. The number of contractions in a group usually ranged from 2 to 5. Accordingly, both the pressures in the ventricle and the bulbus showed wider but rather regular fluctuations (Fig. 5).

This performance is tentatively called 'grouping' and believed to be a 'normal' performace of the heart when the fish is at rest and free from disturbances.

As in other fishes (Robertson et al., 1966; Stevens and Randall, 1967) carp also showed a loss of pressure across the gill vasculature and the loss amounts to about one third of that generated by the heart. For example, in Fig. 5, ventricular pressure fluctuates from 2~56 mmHg, whereas that in the dorsal aorta varies from 22~32 mmHg. The pressure in the dorsal aorta also displays a 'grouping' as those in the ventricle and bulbus arteriosus.

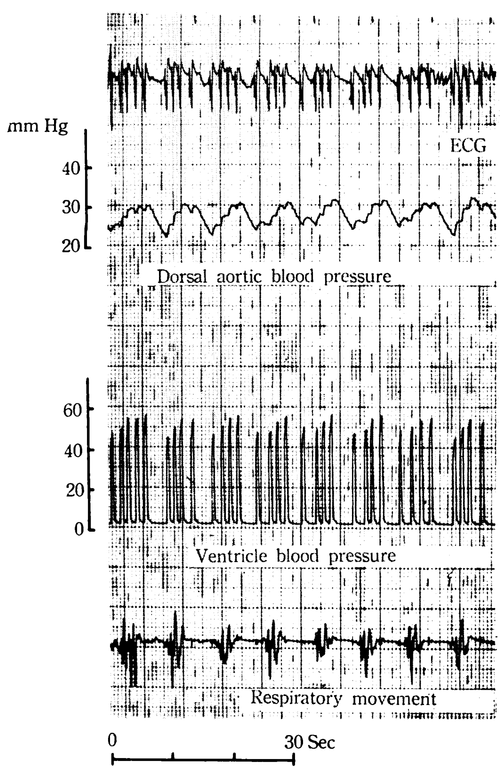

Fig. 5. Patterns showing a close relationship among ventricular pressure, dorsal aortic pressure, ECG, and respiratory movement.

However, there is a lapse of time between the peak of a ventricular systolic pressure and its corresponding peak in dorsal-aorta pressure.

'Grouping' in fact, occurred not only in cardiac contractions but also in respiratory movements. In this respect we colud record a close relationship between respiratory movements, blood pressure in the ventricle, blood pressure in dorsal aorta and ECG (Fig. 5).

Discussion

For a correct measurement of blood pressure, transducers now available require the cannula which is larger than a certain degree. It may, therefore, be difficult to record blood pressures, especially in the dorsal aorta of small fish such as goldfish, although the implantation of cannula into its dorsal aorta has been successfully conducted (Ngan, unpublished data).

There have been no studies on effects of implantation of cannula into the ventricle, but it seems that, among others, the needle of the cannula may hinder its normal functions in spite of the fact that a cannula implanted in it could be used for days and almost all fish survived after their release. Implantation of cannula into the ventricle, therefore, should be avoided as much as possible. Effects of cannula on bulbus arteriosus may be much less judging from its structure and function. Systolic pressures in the bulbus arteriosus are either equal to or slightly less (about 1.0 mmHg) than those in the ventricle. The former, therefore, can be used to estimate the latter in the case of cannulation of the ventricle is not desirable.

Several attempts have been made to implant cannula into the auricle, but little success has yet been achieved. Fish with cannula implanted in the auricle usually died several hours after the implantation. This may be due to the fact that the wall of the auricle is thin and less elastic compared to that of the bulbus arteriosus or the ventricle. Attachment between the wall and the cannula is not tight enough to prevent the wound from bleeding.

Blood pressures recorded in the present study are those of fish confined in small compartments. Under this condition, blood pressures and heart rates can be easily affected by external disturbances as well as by the activities of the fish itself. What pattern of cardiac pressure a fish displays in its natural environment is yet a problem to be studied.

The above described 'grouping' occurred whenever the cannulated fish were kept undisturbed. The occurence was so regular that it can be taken as a sign of tranquility of the experimental fish. Although the physiological significance of this phenomenon is not yet understood at present, it is worthwhile mentioning that simple rhythmical beats reported in fish heart is not the case with the confined, unanesthetized carp.

There have been several reports dealing with the relationship between blood pressure and size of fish (Burger and Bradley, 1951; Greene, 1904). Within the range of body weight used in our study, however, on such relation could be established.

During an extended period of measurement, a cannula may become plugged by blood which has diffused into it. In this case, record of blood pressure will be affected and the cannula must be flushed with a saline-heparin solution. Comparisons of patterns of blood pressure in the bulbus arteriosus recorded with two cannulae, where one is plugged and the other normal, are given in Fig. 6.

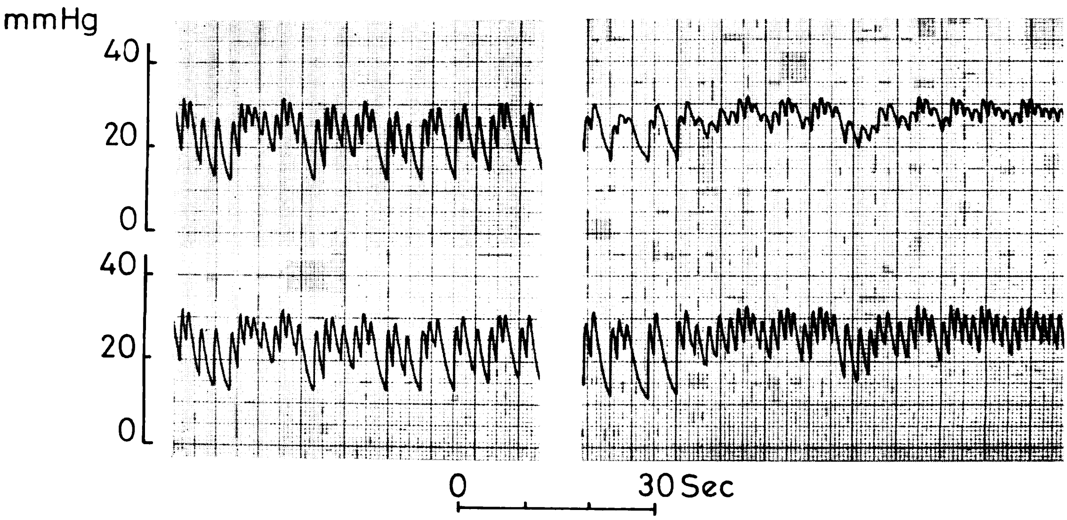

Fig. 6. Pattern of bulbus arteriosus pressure recorded by two identical cannulae, one of which is plugged while the other is normal. Left: Both are normal. Right upper: plugged Right lower: normal.

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コイの血圧測定

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コイ（体重 700～1000 g）の心臓内血圧と心電図とを同時測定するために電極兼用カニューレを考案した．カニューレの挿入は，心室，動脈球ならびに背大動脈のいずれか二つに対して行ない，血圧測定に適するカニューレの太さ，挿入部位，トランスデューサーの型なども検討した．狭い水槽内に拘束された無麻酔のコイでは，動脈球における収縮期圧は心室内のそれとほとんど変らないが，前者の脈圧は，後者の場合より遙かに小さい．また，他の魚種におけると同様，鰓毛細管による血圧の低下が見られ，心臓内圧の約 1/3 に達する．安静時には，心拍数が減じ，かつ，律動性を失なっており，心拍動は数回続いてはしばらく休むという経過を繰り返す．

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