Japanese Journal of Ichthyology
Vol. 20, No. 2. 1973

魚類学雑誌
20巻2号 1973年

Implantation of Cannula into Dorsal Aorta of the Carp

Phan Van Ngan, Isao Hanyu, and Takashi Hibiya

(Received January 12, 1973)

Abstract Technique for implantation of cannula into dorsal aorta of fish was applied successfully to the carp, *Cyprinus carpio*, with body weight ranging from 500 to 1,000 g. Effects of anesthesia and surgical operation were also investigated with hematocrit value as a parameter. In the carp, it is unnecessary to secure the cannula with any kind of fixation. Implanted cannulae can be used for 10 days or more, and as far as hematocrit value is concerned, the fish should be allowed to rest for at least 2 days after being cannulated. A few hours after the operation, hematocrit value decreases drastically against the value measured at the time of operation and then gradually takes a stable level 2 days after the operation. Hematocrit value increases when the fish is anesthetized by immersion in a standing solution as well as by irrigation with an anesthetic solution.

Various techniques have been developed for cannulation of fishes at different sites in the circulatory system such as dorsal aorta (Conte et al., 1963; Smith and Bell, 1964; Houston, 1971), ventral aorta (Holeton and Randall, 1967), bulbus arteriosus (Chester Jones et al., 1969), and caudal vein (Hoar and Hickman, 1967).

Cannulation has several advantages over other available techniques and has been used in various studies relating to cardiovascular function. Materials used in these studies were mostly trout, salmon, or eel. In the present study, however, we tried to apply, with some modification, technique of cannulation of dorsal aorta described by Smith and Bell (1964) to carp, *Cyprinus carpio* Linnaeus, a fresh water fish which is commonly used as an experimental animal and is an important subject of fish culture in Japan. Carp has been implanted with catheters in both aortae by Garey (1970). The technique of implantation, however, was not described. We also investigated effects of surgical operation and anesthesia with hematocrit value as a parameter.

Material

Common carp ranging from 500 to 1000 g in body weight were obtained from a fish dealer and reared in an aquarium with commercial food. Before being cannulated, the fish were placed individually in small plastic compartments (15×15×50 cm) supplied with running water at the rate of 0.5 liter per min. In one or two days of acclimation, the fish assumed a quiet state. The fish were not fed during the experiment.

The cannulae were of polyethylene tubing about 50 cm long slipped over the cut end of a 21G disposable hypodermic needle (Terumo Ltd.) from which the plastic hub had been removed. Various kinds of tubing including polyethylene tubing No. 3 (Hibiki) and polyethylene tubing No. 20 (Igarashi Ika Kogyo Co., Ltd, Tokyo) were tried. Igarashi's polyethylene tubing No. 20 proved to be most suitable although the tube is somewhat less flexible. When Hibiki's tubing is used, it is advisable to have a short section of larger polyethylene tubing as a collar fitted closely over the overlapping portion of the cannula and the needle. This is to prevent the cannula from being cut by the end of the needle.

Procedure for cannulation

The procedure for implanting the cannula is basically similar to that decribed by Smith and Bell (1964). In carp, however, we found that the heat-flared plastic tubing used by them as a cannula support in the case of the salmon is unnecessary and since there is only a short portion of the cannula left unsupported inside the mouth cavity, it is also unnecessary to secure it further by any suture.

The fish was anesthetized by MS. 222, tricaine methanesulfonate, (1/10,000) for 10 min and then placed on its back in a V-shaped plastic holder. During the operation the fish was not

irrigated further by anesthetic solution. One end of a polyethylene tubing was inserted into the mouth cavity through a hole made on the midline of the head and slightly anterior to the nasal openings. The end was led out of the mouth, then fitted to the needle to make the cannula. The cannula was then filled with saline-heparin solution and the dorsal aorta was approached through the pharyngeal muscle on the roof of the mouth. As no gill arches are seen in the palate of the carp, the needle must be oriented along the skinfolds which run along the midline of the pharyngeal muscle. In order to facilitate the orientation of the needle, we used a specially made pincette, which could be adjusted to hold the needle tightly during the insertion and to release it when its tip was implanted in the aorta. Once the needle was in the aorta, as indicated by the blood flow into the cannula to replace the saline-heparin solution, another pincette was used to hold the needle in position and the cannula tubing was pulled from outside through the above-mentioned hole until the tubing formed a well fitted curve to the palate. While doing so, a great care must be taken to prevent the cannula from being twisted. With practice the needle could be implanted as deep as 1 cm in the aorta and the operation could be completed in about 20 min (Fig. 1). Saline-heparin solution was used to push blood in the cannula back into the aorta and the free end of the cannula was pinched tightly with a forceps and then sealed by clay. Without the help of the forceps it was difficult to prevent the blood from rising up again into the cannula and clots to plug it up. After the sealing, the fish was allowed to return to its compartment.

In taking blood, the pinched and sealed portion of the cannula was cut off and consequently

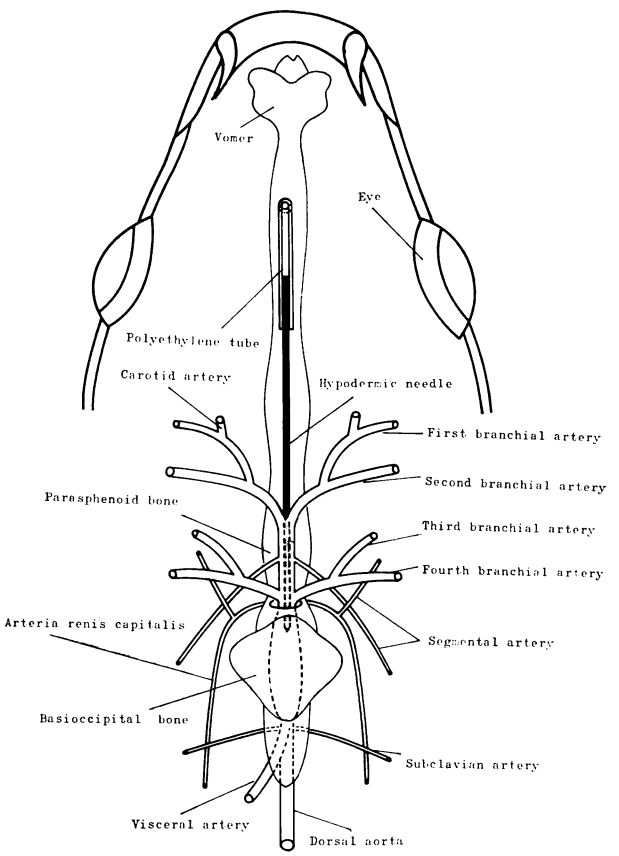

Fig. 1. Diagram showing position of dorsal aortic cannula. (Adapted from Okamura, 1941).

the cannula became shorter after each sampling. If the length of the cannula is to be kept constant, an accessory tubing of the same size may be connected to the cannula by a connector, which is a piece of a needle or of polyethylene tubing. Sealing is made at the free end of this portion. In the subsequent samplings the connector is removed so that sample can be taken from the free end of the main cannula.

Recovery period

Smith and Bell (1964) suggested that a minimum period of 4 to 6 hrs is required for a pink salmon to recover after cannulation. Later, the need for a much longer recovery period was indicated by Houston et al. (1969). In order to estimate the minimum period needed for a cannulated carp to recover, we followed the condition of the fish for a long period of time and with various intervals using hematocrit value as a parameter.

To investigate the variations of hematocrit value in the first few days after the cannulation, five fish ranging from 780 to 950 g were cannulated and hematocrit values were measured immediately after the operation and at 1, 3, 6, 12, 24, 48 and 96 hrs afterward. Hematocrit value was measured in capillaries after centrifugation at 12,000 rpm for 5 min. The measurement was performed in duplication and amount of blood drawn each time was estimated to be about 0.05~0.07 ml including the amount discarded at the beginning of each bleeding.

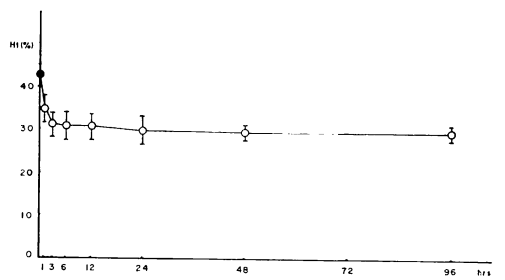

Fig. 2-1. Variations of hematocrit value after implantation of cannula.

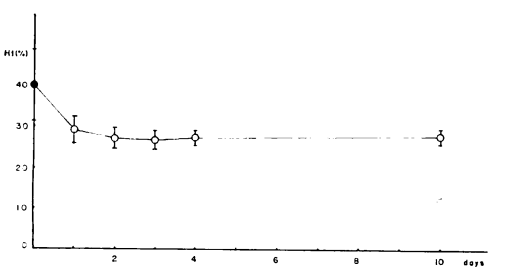

Fig. 2-2. Variations of hematocrit value after implantation of cannula.
● Values taken at the time of operation.
○ Values taken after transferring into fresh water.
Vertical bars indicate standard deviations.

Average of hematocrit value decreased drastically in the first 1 hr and continued to decrease gradually until it assumed a constant level after about 48 hrs. Individually, there were fish whose hematocrit value was maintained at a constant level 1 hr after the operation (Fig. 2-1). Once the hematocrit value attained its constant level after operation, the level remained stable for a long period of time as seen in the following experiment.

Five fish ranging from 700 to 940 g were cannulated and hematocrit values were determined immediately after the operation and every day thereafter for 4 days. The fish were then allowed to rest for 6 days before the final measurement. Average hematocrit value, as expected, fell markedly during the first day and, after a further fall of a minor degree during the next day, reached a constant level on the third day (Fig. 2-2).

To investigate the cause of the drastic decrease of hematocrit value in the first few hours after operation, another group of five fish ranging from 800 to 900 g were used. The fish were cannulated and allowed to rest for 2 days. Hematocrit value was taken at the time of operation and after 2 days of recovery. The fish were then subjected to 10 min of anesthetization in a 1/10,000 solution of MS. 222 followed by 20 min of exposure to air and then allowed to recover in fresh water for 1 hr. These treatments simulated the conditions the fish encountered during the operation for cannulation except for surgery.

After 10 min in anesthetic solution and 20 min of exposure to air, the hematocrit value was found to increase by 9.9% and 32.8% respectively over the initial level, that is. the level before the treatments (Fig. 3). The initial level was restored after 1 hr of recovery. It was also noticed that individually after the

treatments, hematocrit value might take a similar level attained at the time of actual operation or even exceeded it. These results suggested that the high hematocrit value obtained just after the operation could be ascribed to the anesthetization and perhaps handling as well as surgical operation itself. The fall in hematocrit value therefore can be considered as its restoration to 'normal' after a temporary elevation caused by surgery, anesthetization, and handling during the operation.

In another experiment five cannulated fish ranging from 750 to 940 g were subjected to 2 days of successive experiments. On the first day, the fish had been anesthetized by simply being immersed into a 1/10,000 solution of MS. 222 for 40 min and then allowed to recover in fresh water. On the second day, the fish were anesthetized by solution of MS. 222 of the same concentration irrigated through the mouth by means of a syphon for also 40 min and then allowed to recover in fresh water with running water supplied through the mouth. Flow rate of anesthetic solution was about 2 liters per min. In both experiments hematocrit values were measured before the experiment, during the anesthetization and during recovery with interval of 10 min for 40 min. The two methods of anesthetization did not unexpectedly show much difference (Fig. 4).

Experiments with a less concentrated solution of MS. 222 (1/20,000 and 1/40,000) showed that hematocrit value increased in the first few ten minutes and then tended to decrease to initial level even during anesthetization (Fig. 5).

It was noticed that in high concentration of anesthetic solution (1/10,000) the fish laid quietly on its side after a short period of time and its respiration movements were almonst unnoticeable. In lower concentrations, how-

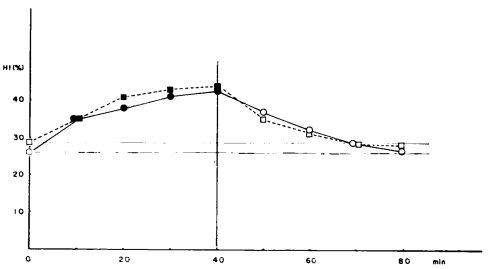

Fig. 4. Variations of hematocrit value in response to the same anesthesia under two different methods of anesthetizations and recoveries.

● ■ Values taken when the fish were under anesthesia.
○ □ Values taken when the fish were in fresh water.
●—● Anesthetization by immersion into standing solution.
■---■ Anesthetization by irrigating with solution.
○—○ Recovery by transferring into running fresh water.
□---□ Recovery by irrigating with fresh water.
— - — Initial level.

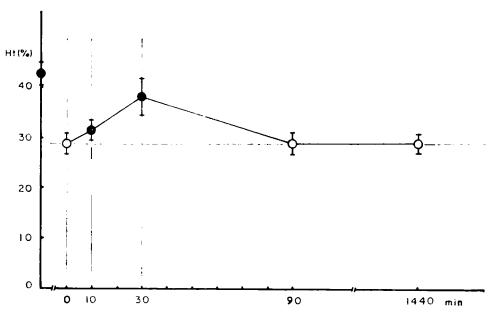

Fig. 3. Variations of hematocrit value under anesthetization, air exposure and recovery in fresh water.

● Values taken when the fish were under anesthesia.
○ Values taken when the fish were in fresh water.
— - — Initial level.
Vertical bars indicate standard deviations.

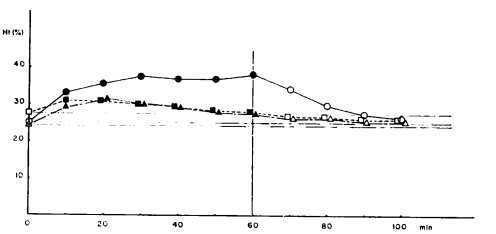

Fig. 5. Variations of hematocrit value in response to anesthesia with different concentrations.

○□△ Values taken when the fish were in fresh water.
●■▲ Values taken when the fish were under anesthesia.
●—● MS. 222 (1/10.000).
■---■ MS. 222 (1/20.000).
▲---▲ MS. 222 (1/40.000).
—·— Initial level.

ever, (1/20,000 and 1/40,000) the fish continued to perform its respiration and other body movements during the whole experiment and in many cases (1/40,000) was able to keep its 'normal' position.

Anesthetization with other anesthesia such as benzocaine, quinaldine and urethane also increased hematocrit value. For example, 40 min of anesthetization with 1% urethane increased average hematocrit values of three fish from 26% to 34%. Recovery from anesthetization with urethane seemed to be less smooth and take longer time than recovery from anesthetization with MS. 222 (Fig. 6).

In an attempt to investigate the effect of low oxygen concentration in water on the hematocrit value, water supply to compartments of a group of three fish was cut off for 6 hrs. Hematocrit value and oxygen concentration in water were measured at the beginning of the experiment, 2 and 6 hrs after water supply was stopped and 3 hrs after water supply was resumed. Oxygen concentration was measured with the aid of a portable D. O. meter (Riken, OX-200). Two hrs after water supply was stopped, oxygen concentration decreased from 88.7% to 17.0% and hematocrit value remained unchanged. After 6 hrs oxygen concentration decreased further to 11.0% and average hematocrit value increased by 35.3%. Hematocrit value was restored to the initial level at the next measurement after water supply was resumed (Fig. 7).

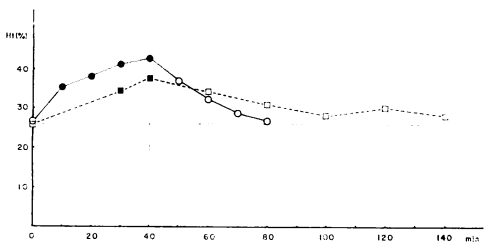

Fig. 6. Variations of hematocrit value of the same group of fish in response to different anesthesia.
● ■ Values taken when the fish were under anesthesia.
○ □ Values taken when the fish were in fresh water.
●—● M.S. 222 (1/10.000).
■---■ Urethane 1%.
—·— Initial level.

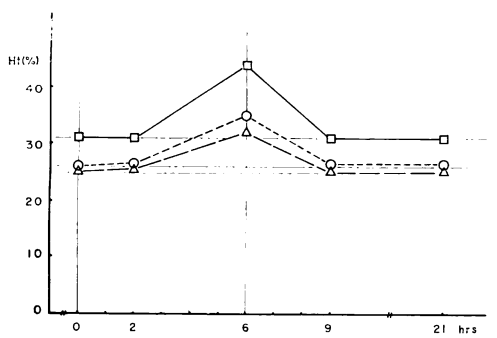

Fig. 7. Variations of hematocrit value caused by suffocation in unaerated aquarium (see text).
△ Fish No. 1.
○ Fish No. 2.
□ Fish No. 3.
—·— Initial level.

Discussion and conclusion

The present study has revealed that cannulation of dorsal aorta of carp is possible without cannula support and other fixations. No attempt has been made to investigate the duration in which an implanted cannula can be used, but, in practice, cannulated fish have been used successfully for 10 days or more. In this connection, it was reported that little success has yet been made in maintaining an aortic catheter in brook trout, *Salvelinus fontinalis*, for more than about 2 days (Houston et al., 1969).

After cannulation was completed, a recovery period of 4~6 hrs was earlier suggested (Smith and Bell, 1964). Our data and others (Houston et al., 1969; 1971a, b), however, indicated that the period may be much longer. As far as hematocrit value is concerned, after cannulation the fish should be allowed to rest for at least 2 days. A few hours after the operation, hematocrit value decreases drastically against the value obtained at the time of operation and then gradually takes a stable level 2 days after the operation.

Hematocrit value increases when the fish is anesthetized with MS. 222, urethane, quinaldine and benzocaine. The increase of hematocrit value takes place when the fish is anesthetized by immersion in a standing solution as well as by irrigation with an anesthetic solution. Degree of increase depends on the concentration of the solution and on the duration of anesthetization.

Hematocrit value was used as the only parameter in this study because its measurement is simple and amount of blood used in the measurement is small. The measurement, therefore, can be made punctually and repeatedly with little effect on physiological conditions of the fish. However, with hematocrit value only it is difficult to determine whether the changes which take place after implantation of cannula are physiological or pharmacological.

Hematocrit value and other blood characters, at the same time, fluctuate depending on various factors such as handling, anesthetization, surgical operation, asphyxia, etc., consequently, data taken by conventional bleeding methods, may be doubted whether they are normal or not. To some extent, cannulation can provide more accurate data. At present, however, this technique can be used only in the laboratory. It is, therefore, necessary to improve the technique or to develop a new method so that field works also can be benefitted.

Acknowledgment

We are indebted to Dr. K. Yamamori of for his continued interest and technical assistance in carrying out this study. This study was supported in part by a research grant from the Ministry of Education.

Literature cited

Chester Jones, I., D.K.O. Chan, and J.C. Rankin. 1969. Renal function in the European eel (*Anguilla anguilla* L.): Changes in blood pressure and renal function of the freshwater eel transferred to sea water. J. Endocr., 43: 9～19, figs. 1～3.

Conte, F. P., H. H. Wagner, and T. O. Harris. 1963. Measurement of blood volume in the fish (*Salmo gairdneri gairdneri*). Am. J. Physiol., 205: 533～540, figs. 1～6.

Garey, W. 1970. Cardiac output of the carp (*Cyprinus carpio*). Comp. Biochem. Physiol., **33**: 181～189, figs. 1～3.

Hoar, W. S. and C. P. Hickman, Jr. 1967. A laboratory companion for general and comparative physiology. Appendix V. Prentice Hall, Inc. pp. 276～277, fig. 1.

Holeton, G. F. and D. J. Randall. 1967. Changes in blood pressure in the rainbow trout during hypoxia. J. Exp. Biol., 46: 297～305, figs. 1～7.

Houston, A. H., M. A. DeWilde and J. A. Madden. 1969. Some physiological consequences of aortic catheterization in the trout, *Salvelinus fontinalis*. J. Fish. Res. Bd. Canada, 26: 1847～1856, figs. 1～5.

Houston, A. H. 1971. A simple improvement in the vascular catheterization procedure for salmonid and other teleost fish. J. Fish. Res. Bd. Canada, 28: 781～783, fig. 1.

Houston, A. H., J. A. Madden, R. J. Woods, and H. M. Miles. 1971a. Some physiological effects of handling and tricaine methanesulfonate anesthetization upon the brook trout, *Salvelinus fontinalis*. J. Fish. Res. Bd. Canada, 28: 625～633, figs. 1～3.

Houston, A. H., J. A. Madder R. J. Woods, and H. M. Miles. 1971b. Variations in the blood and tissue chemistry of brook trout, *Salvelinus fontinalis*. Subsequent to handling, anesthesia and surgery. J. Fish. Res. Bd. Canada, 28: 635～642, figs. 1～5.

Okamura, S. 1941. Dobutsu Jikken no Shishin Daikando, Tokyo. In Japanese. pp. 429～446.

Smith, L. S., and G. R. Bell. 1964. A technique for prolonged blood sampling in free-swimming salmon. J. Fish. Res. Bd. Canada, 21: 711～717, figs. 1～3.

(Laboratory of Fish Physiology, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, 113, Tokyo).

コイの背大動脈内へのカニューレ挿入法

Phan Van Ngan·羽生　功·日比谷　京

魚の背大動脈に対するカニューレ挿入技法を体重500～1.000 g のコイに応用するとともに，その際の麻酔ならびに手術の影響をヘマトクリット (Ht) 値を指標にして調べ，次の結果を得た。コイではカニューレを固定する必要が無く，Ht 値に関する限り，挿入後10 日間以上にわたって使用することができる．Ht 値は手術直後急激に減少し，約 2 日後に安定レベルに達するので，2 日間の安静期間を要する．Ht 値の上昇は，魚を止水中で麻酔しても，あるいは麻酔液を口中に流し込んでも，同じように起こる．

(113, 東京都文京区弥生　東京大学農学部魚類生理学研究室)