A METHOD FOR CHRONIC INTRAVENOUS INFUSION IN FREELY MOVING RATS¹

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This report describes a system for infusing fluids either on a self administered or programmed basis into the blood stream of freely moving, unanesthetized rats. The method has proved to be successful for studying barbiturate and morphine addiction in rats (Davis and Miller, 1963; Weeks, 1960), and can be used whether drugs are to be given periodically or chronically 24 hr a day for weeks at a time. The system has been in use off and on for about a year with rats being infused on a 24 hr basis. The only major difficulty has been that occasionally rats will roll over on the lead-in tube. This may bend the tube or bring the swivel joint inside the animal's cage where it can be chewed. This can be prevented by lengthening the lead-in tube or lowering the ceiling of the enclosure.

The system consists of three basic parts: a chronic intravenous cannula; a lead-in tube of stainless steel connected to a water-tight swivel joint; and a subcutaneously implanted harness which supports the lead-in tube.

Cannula Construction

The cannula is an improved version of Model I described earlier by Weeks and Davis (1964). This one is easier to make and implant than the earlier model and incorporates a one-way valve on the tip which prevents blood from entering and clogging the cannula. It is made by joining a 20 cm length of polyethylene tubing (No. PE 60) to a 55 mm length of .012" inner diameter silicone rubber tubing (No. 7002-012)² and cementing to the latter,

two size 4-0 surgical silk anchor threads, one on the silicone rubber tubing, and the other at the polyethylene silicone tubing junction.

In making the junction between the two tubes, one end of the polyethylene tubing is very slightly flared by holding the end close to the tip of a hot soldering iron. One end of the silicone rubber tubing is inserted about 3 mm into the flared end of the polyethylene, and a half hitch of the 4-0 surgical silk is slipped over the polyethylene and pulled tight as close to the flare as possible. The thread is tied tightly enough to hold the polyethylene snugly against the silicone tubing, but not enough to constrict the tubing. When properly done, the silicone tubing can be moved back and forth inside the polyethylene, with little felt resistance. A square knot is then completed in the surgical silk, and the thread is trimmed to about 12 cm.

A second anchor is then attached to the silicone tubing 18 mm from the first, toward the tip of the cannula. The cannula is then trimmed so that it measures 30 mm from tip to anchor thread. Both the junction and the two anchor threads are then cemented to the cannula with RTV-11.³ This adhesive bonds to the silicone tubing, but not to the polyethylene. It is, therefore, applied to the junction so that it is in contact with the silicone tubing, covers the flare, and is in contact with the silk suture material. The flare on the polyethylene prevents the threads slipping off and provides a foundation to keep the adhesive in place.

This step in the procedure is facilitated by the use of a Plexiglas mold to hold the adhesive until it sets. Figure 1 illustrates the essential features of a mold that can be used to cement both the junction and the second an-

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²"Vivosil", available from Becton Dickinson Co., Rutherford, New Jersey.

³Available from General Electric Co., Silicone Products Dept., 1285 Boston Avenue, Bridgeport, Connecticut.

Fig. 1. Cannula mold. Sections B and B' are cemented to C, a support holding the molds the proper distance apart. A and A' are detachable sections screwed or clamped to B and B' while adhesive sets. They are removed to take the cannulas from the molds. Lower mold forms the RTV-11 around the polyethylene-Vivosil junction. Upper mold forms adhesive around the anchor thread nearest the tip of the cannula.

chor thread at the same time. When the adhesive has set (24 hr), the cannulas can be easily removed from the mold since RTV-11 does not bond to Plexiglas.

When the cannulas are to be used for infusion only and not for withdrawal, a one-way valve should be made by occluding the tip of the cannula with a drop of RTV-11. When this has set, a series of small holes are made in the end of the cannula with a sharp needle. These holes remain closed until fluid pressure in the lumen of the cannula expands the silicone tubing during infusion. The contents of the cannula are thus forced out. When internal pressure is reduced, the holes close and blood cannot enter. This modification has prevented any failures due to blood clotting in the lumen of the tube.

Surgical Procedure

To implant the cannula, the procedure described by Weeks and Davis (1964), is followed with few modifications. The silk threads cemented nearest the tip of the cannula are used to anchor it to the muscles adjacent to the

point where the cannula enters the vein. The silicone rubber between this point and the polyethylene-silicone rubber junction form the "U", which in the earlier model was formed in the polyethylene tubing. The threads cemented to the junction are used to anchor the cannula to midline neck muscles.

Subcutaneous Harness

When infusion is to be carried out over a long period of time (1 hr or more) active rats are likely to kink the connecting tube by turning more often in one direction than the other. This problem can be eliminated by connecting the tubing to a swivel joint fastened to a harness which is then strapped to the animal. Since harnesses strapped tightly enough to prevent the animal's slipping out tend to restrict movements and to promote lesions if kept on for appreciable lengths of time, a permanently implanted subcutaneous harness was developed, with a swivel joint attached.

The subcutaneous harness is made as illustrated in Fig. 2, consisting of a stainless steel base; fiberglass window screening;⁴ and two 1/2" 2-56 nylon binding head machine screws.⁵ For implantation, a 3 cm incision is made on the dorsal midline of the animal beginning about 3 cm caudad to the scapula. The skin is then separated from the underlying tissue by

⁴Available from any hardware store.

⁵Product Components Corporation, 15 Washington Avenue, Hastings-on-Hudson, New York 10706.

Fig. 2. Side and top view of the subcutaneous harness.

Fig. 3. Cross section of swivel joint. Body of joint is made of $\frac{1}{4}$ " diameter Plexiglas tubing machined to the dimensions indicated. When screwed in place, the left screw keeps the 18 gauge needle tubing from slipping out of the neoprene "O-ring". This screw must not be screwed down tight forcing the retainer ring against the "O-ring." If there is not a small amount of lateral play at this point, excessive friction will prevent the swivel from operating easily. The needle tubing on the right, soldered to the brass screw, is the input tube. It must be screwed firmly into the joint to prevent leaking. It may be necessary to use a washer filled into the bottom of its screw hole if fluid is passed through the system under pressure.

blunt dissection about 4 cm away from each margin of the incision. Puncture wounds are made through the skin, one on each side of the incision, to accommodate the screws. The stainless steel plate and screen is then laid under the skin, the screws are run through the puncture wounds, and the incision is closed.

The result is two nylon screws protruding through the skin supported subcutaneously by the stainless steel plate and the fiber glass screen. A swivel joint or supporting structure for the infusion tube can be easily connected to these screws.

Swivel Joint and Connection Tube

To prevent the rat from destroying the tube by chewing, the portion entering the animal's cage is made of 18 gauge needle tubing. A bracket made to fit the protruding screws from the rat's harness is soldered to this tube. A water-tight swivel joint permitting the animal to turn without producing kinks in the tube is mounted on the end of the needle tubing which protrudes above the top of the experimental chamber.

The details of construction of the swivel joint are illustrated in Fig. 3. The needle tubing that enters the joint through the screw at the left is connected to the animal harness at its other end and is free to revolve within the neoprene "O-ring". The neoprene "O-ring" provides a water-tight seal around this tubing. Fluid from the syringe driver enters the joint through the needle tubing on the right through plastic tubing. The plastic tubing is force fitted over the 18 gauge needle tubing protruding to the right from the 6-32 screw. This plastic tubing must be rigid enough to hold the right portion of the joint while the needle tubing on the left rotates within the "O-ring".

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