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BRIEF COMMUNICATION

A Flexible Technique for Long Term Infusions in Unrestrained Rats

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NICOLAIDIS, S., N. ROWLAND, M.–J. MEILE, P. MARFAING AND A. PESEZ. *A flexible technique for long term infusions in unrestrained rats.* PHARMAC. BIOCHEM. BEHAV. 2(1) 131–136, 1974.– A technique is described which allows long term intravenous and intragastric infusions in rats; the advantages of these methods is discussed. A new double lumen watertight swivel is described for double infusions or infusion and sample experiments. Using the ensemble, rats have been infused for up to five months while at complete liberty of movement.

Infusion technique Long term Intravenous Intragastric Catheterisation Double lumen swivel Surgical procedure

INTRAGASTRIC (IG) [8, 11, 12, 13, 14, 15, 19] and intravenous (IV) [1, 3, 4, 5, 6, 7, 10, 20, 21, 22, 24] catheterisation procedures in the rat have been described, and most are suitable for acute infusion or sampling experiments. However, not all of these are adaptable to chronic, long term studies, the principal difficulty being to allow freedom to the animal even during its most active periods. The respective shortcomings and disadvantages of the literature methods may be briefly summarised. Painful tail vein catheters (e.g. [10]) are clearly inadequate, as are jugular techniques which use semirigid polyethylene catheters (PE) [1,3]. The commercially available harness device is severely restricting [12, 21, 24]. Fixations in the neck region or subcutaneously [6, 7, 11, 13, 15, 22] pull on the rat during attempted activity. The IG nasopharyngeal catheter developed by Epstein [8] and reviewed by Kissileff [14] has been used effectively [8, 14, 19], but since it does not eliminate sensations in the nasopharynx [13,18], it is not suitable for the study of purely gastric factors.

However, by using the best features from the above methods – for example all but one [5] advocate a watertight swivel joint – we have synthesised a cheap and simple system, which is minimally restraining for the rat. As is well known, many physiological and behavioral parameters are particularly susceptible to modification by minor stresses; our system, as an ensemble, is most suitable for chronic, long term infusion or sampling studies in these fields of research.

METHOD

Surgery

Semisterile conditions, and sterile instruments and catheters are used. Details are minimised where suitable references are available.

IV catheterisation. The catheters are made entirely of silicone rubber (Silastic®) as used by Corbit [4] and Steffens [20]; this is better tolerated than part PE catheters. Our basic design is shown in Fig. 1. We find a double mitered tip (E) effectively prevents coagulation and allows sampling, although other designs are possible [6,20]. The catheter is completed with a 20 mm curved length of 1 mm syringe needle (all metallic ends are polished smooth), and a tightly fitting double thickness of Tygon tubing (Technicon tubes) with pin stopper. Prior to surgery, the catheter is filled with saline.

Surgical technique is well documented [4, 6, 20, 24]; our catheter is designed for entry into the right external

[1] N.R. held a French government scholarship, and is now at University of Pittsburgh. The cages were constructed by Serge Massé.

CATHETER			(mm) DIMENSION id	od
	PE	n°8	1.19	1.70
I.G.	DC	602 175	.750	1.65
	DC	602 201	1.00	2.15
I.V.	DC	602 151	.635	1.19
	DC	602 175	.750	1.65

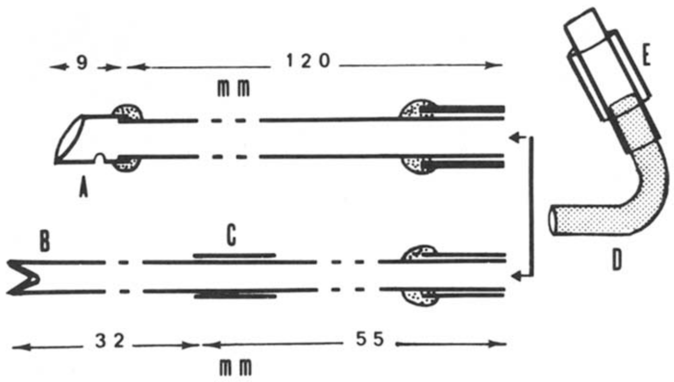

FIG. 1. Design and dimensions of IG and IV catheters. They are made of silicone rubber (Silastic®–Dow Corning, Catalogue number cited). Tip A of the IG catheter is PE. The tip for the IV catheter is cut in a double mitre B, and collar C fitted. Stippled portions are Silastic® glue. D is a 1 mm bore cut syringe needle; E is the flexible headpiece made of thick walled Tygon tubing (Technicon® tubing).

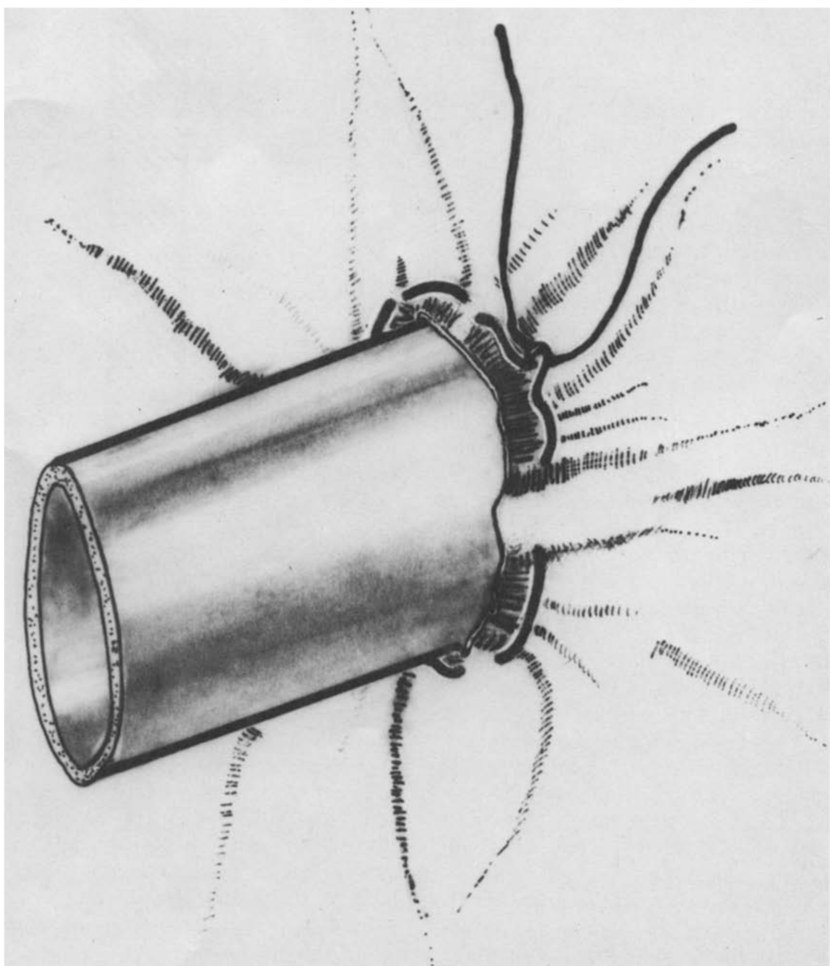

FIG. 2. Surgical implantation of IG catheter, showing how the purse string suture respects the vasculature of the stomach wall and upon tying around the catheter the blood flow is uninterrupted.

jugular vein some 5 mm before it dives under the clavicle. When pushed in as far as collar C, tip B is now at the auricular cavity. The vein is tied around the catheter, and held in place by further sutures around the vein as in [20]; other workers find hernia gauze satisfactory. When not in use, catheters are filled with viscous polyvinylpyrollidone solution as in [20].

IG catheterisation. Discounting nasopharyngeal methods, ours is a considerable methodological advance over that of Kohn [15]. The catheter is again of Silastic® except for a small PE tip A glued on (Fig. 1). The stomach is accessed by gently pulling it through an incision in the abdominal wall to the left of the midline and some 1 cm below the rib cage. An untied purse-string suture (about 5 mm in diameter) is made in the membranous portion (darker) of the stomach wall as shown in Fig. 2. The purse-string suture at the level of the adventice respects the vasculature, as shown in Fig. 2, and hence avoids necrosis. The rigid tip A' of the catheter is then forced through a small needle puncture made in the middle of the suture loop, and advanced so that all of the glue bulge is inside. The suture is tied firmly, but not so tightly as to collapse the catheter; the trauma to the stomach is minimal. The catheter cannot be pulled out, and remains functional and leakfree for months. The stomach is repositioned and the catheter led through the abdominal wall which is closed by sutures of each of the three muscular layers. It is advisable to feed these rats a finely ground chow; standard chow has larger particles which can clog these catheters.

Head fixation. Both types of catheter described, as well as others such as portal (Steffens' or Davis' unpublished techniques), left jugular, are pulled subcutaneously to a slit in the skin on the top of the skull. The use of screws and dental cement provides a reliable, non stressing mode of attachment [1, 4, 8, 14, 19, 20]. We use, in addition to small screws, a stainless steel bolt worked to the dimensions shown in Fig. 3. The T head is then slid into an elongated hole in the skull (around the lambda region), turned through 90° and the nut fastened. This is facilitated if the tip of the bolt is flattened as in the figure and held in clamps. The head of the bolt is thin and does not harm the dura, yet is strong enough to provide a 100% reliable anchor. Stereotaxic manipulations are most conveniently carried out immediately prior to fixture of the screws, and the embedding of metal part D of the catheter (Fig. 1) in the cement. This head fixture is particularly suitable for multiple catheter preparations – we are currently using cardiac + portal, cardiac + gastric, and bilateral jugular preparations. With practice, such operations take about 1 hr overall, with a simple catheterisation correspondingly more rapid.

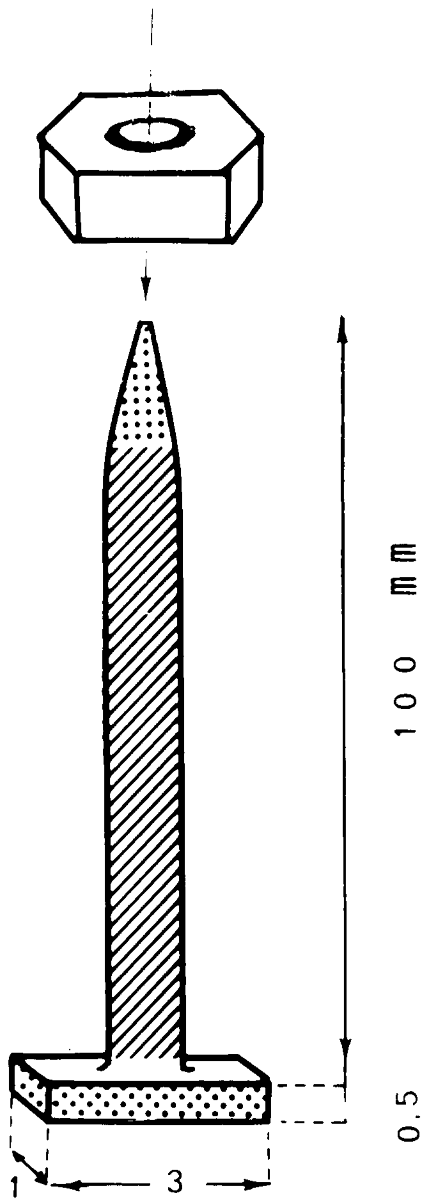

FIG. 3. Stainless steel head bolt lathed to dimensions shown.

APPARATUS

The Infusion Ensemble

Our experimental cages are essentially similar to those described by Blass [2], but built for long term infusions. Figure 4 shows that vertical bar (V) attached to the frame of the cage carries a Plexiglas beam which moves freely in all directions. The animals are permanently connected to a syringe and drive motor, maintained at the level of the resting rat. If the liquid reservoir is not at this level the resultant feeble hydrostatic gradients can cause air leaks at watertight swivels (which appear leakfree at high pressures). An adequate length of PE tubing leads from the syringe, through holes in the beam to a watertight swivel. An accurate length (see Fig. 4) of PE leads from swivel to rat, and is protected by metal sheath (Meccano® spring cord is excellent, but needs to be made slipfree on the PE catheter by a collar of larger diameter PE). The beam is counterbalanced with elastic thread, which means that the system has minimal inertia and responds to the rat's every movement. With the torsion adjusted so that the pull on the rat is of the order of 2 g wt. – or just enough to prevent the protected catheter from sagging. When the rat rears, the PE stays fairly taut. The PE catheter is connected to the rat's Tygon headpiece (E, Fig. 1) by a 1 cm length of 1 mm bore steel tube. The piece E is strong and flexible, and the

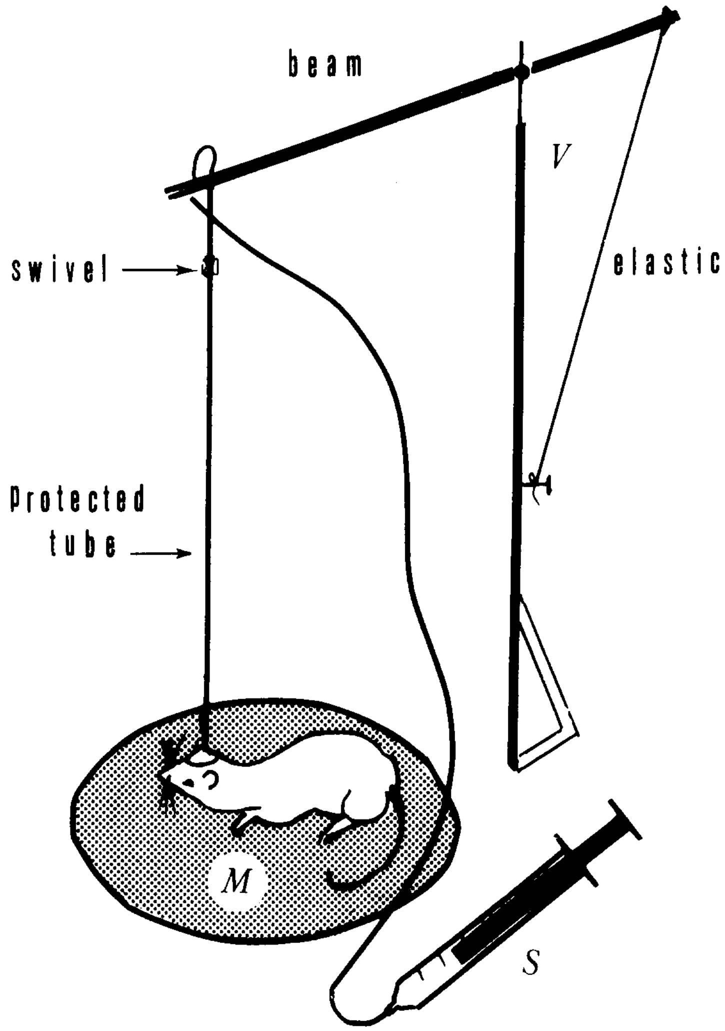

FIG. 4. The infusion ensemble. The cage, of which mesh floor M and vertical bar V attached to frame are shown, is a modified metabolism cage with cylindrical Plexiglas walls. The syringe S containing the infusion liquid is maintained at the level of the resting rat. The light beam pivots freely and is counterbalanced with thin elastic thread.

system allows the rat to sleep in a natural posture with the head curled under the body, while allowing free passage of fluids. This piece, in some rats, may need infrequent changing. All the dimensions of the system are so chosen to give tight fittings – it is not easy for man to pull the ensemble apart and virtually impossible for the rat.

Watertight Swivel

In addition to the excessively heavy commercial swivels, several lightweight and reliable home-made efforts are available in the literature (e.g. [7, 9, 23]). Our own version is a Plexiglas cylinder which is divided into two minute fluid chambers by an O ring. The casing exerts an even radial force on the O ring, which consequently forms a watertight seal of minimum surface area with the rotating metal tube at its inner circumference. This has been developed into a double channel model, shown in Fig. 5. The principle is the same, but two O rings form two tiny, independent fluid compartments. The main trick is the accurate machining of the Plexiglas casing, so that the radial pressure on the O rings is sufficient to maintain the joints leakfree, but not so great as to impose a high torque for turning the inner tubes. Well made, the torque is little greater than for a simple swivel.

Performance

The rats do lead unhampered experimental lives of months. The use of sterile liquids for infusion, and addition of antibiotic, postpones the infections which finally account for the rats. The double swivel, together with the

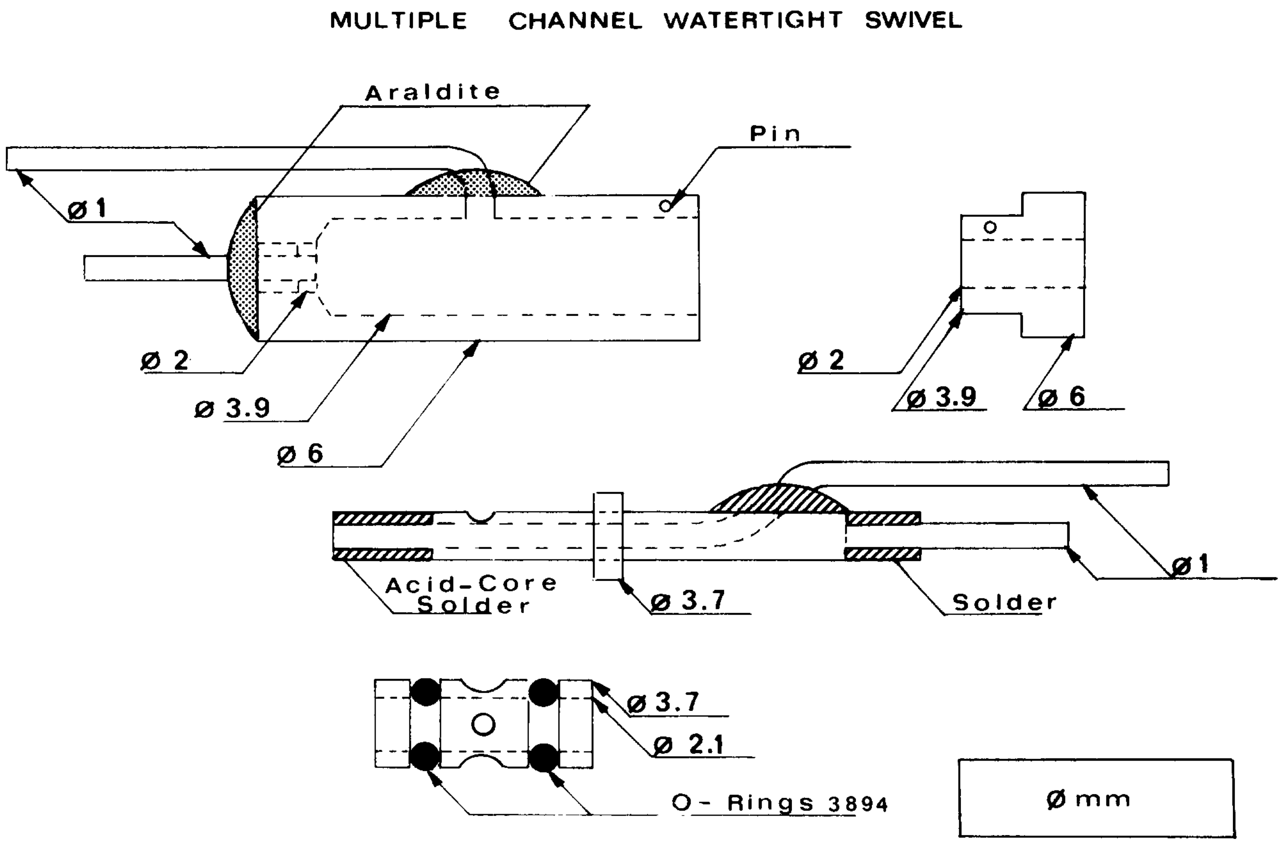

FIG. 5. Double channel watertight swivel. The outer casing is accurately machined Plexiglass of circular cross section. All solder is acid cored, and tubes of stainless steel. The O rings are made by STEFA.

described possible multiple catheterisations allows a whole range of permutations of infusions and sampling in undisturbed rats. One limitation seems to be the size of rat: below about 100 g bodyweight, the skull is too soft to take screws; but using a suitably small jugular catheter rats have received continuous nutritive infusions during growth from 100 through 180 g (Rowland, unpublished data). The system has a low dead space, and is sufficiently light and compact to have been used in a closed metabolic chamber for study of respiratory exchanges [16].

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