

FIG. 2. Drop recording of blood flow in an anesthetized cat. Effects of intra-arterial test doses of acetylcholine (injection volume 0.1 ml).

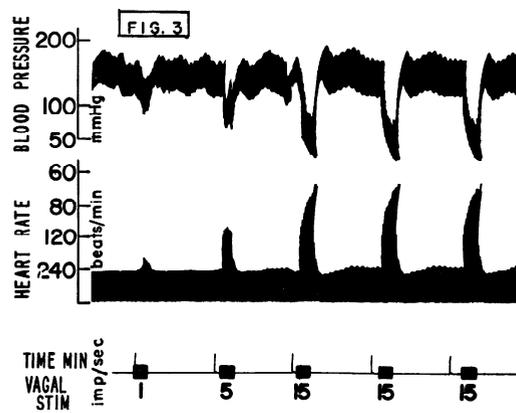


FIG. 3. Recording of blood pressure and heart rate in an anesthetized cat. Effects of vagal stimulation (2 v, 5 msec, various frequencies, stimulation periods 10 sec/min).

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## A watertight swivel joint permitting chronic injection into moving animals<sup>1</sup>

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SEVERAL MODERN EXPERIMENTAL TECHNIQUES involve the delivery of fluids from fixed reservoirs through flexible tubes into the brain (1, 2), stomach (3, 4), or blood vessels (5) of freely moving rats. The twisting and kinking of the flexible tubes produced by the animal's circular movements limit the duration of these experiments.<sup>2</sup> This difficulty has most often been avoided by physically restraining the animal or by restricting the fluid delivery to short daily periods. In order to study direct, intragastric self-injection of food and water by rats bearing nasopharyngeal gastric tubes (4) without artifacts introduced by

restraint of movement or short-term testing, we have devised a light, watertight swivel joint for plastic tubing that has been used successfully even with active rats that move in only one direction around their cages.

The principle is simple. The flexible tube is interrupted just above the animal's head by the swivel joint, consisting essentially of a hollow cylinder that fits snugly within a housing. The flexible tube is connected to the cylinder, and the housing, fixed firmly to the animal's skull, rotates around the cylinder. The animal's circular movements, therefore, are not transmitted to the tube and it does not twist or kink.

The joint and its component parts are shown assembled in Fig. 1A and partially disassembled in 1B. All parts, except for the stainless steel outlet tube, are of virgin Lucite. The flange in the midportion of the cylinder (*part B*) is engaged by the lower surface of the cap (*part C*) when it is screwed into the housing (*part A*), thus preventing the withdrawal of the cylinder from the housing. All moving surfaces and the threads are

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<sup>2</sup>This problem has been dealt with successfully by C. M. Rhode et al. (6) with larger animals such as the dog. They used a very flexible form of polyvinyl chloride tubing that does not easily kink when held under tension. Very active rats, however, defy this arrangement.

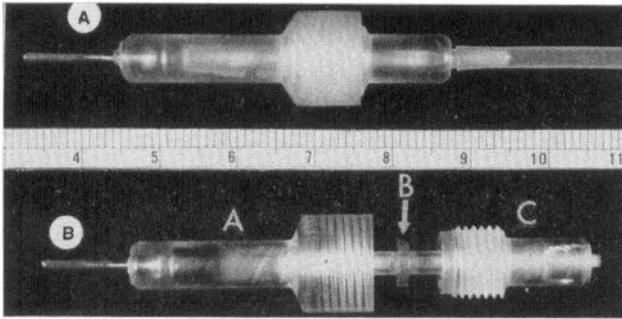


FIG. 1. Watertight swivel joint for plastic tubing shown assembled in *A* and partially disassembled in *B*. Part *A* is housing; part *B* is flange in midportion of cylinder that fits snugly within housing; part *C* is cap that engages flange when joint is assembled. Scale in centimeters.

sealed against leaks with ordinary stopcock grease which also lubricates the moving parts. The outlet tube is 0.5 in. of 15-gauge stainless steel needle tubing fixed in place at the end of the housing with epoxy resin cement (Eastman 910). When assembled, the joint is 5.0 cm long (excluding the outlet tube) and weighs 2.5 g.

When freshly greased, the joints will tolerate at least 100 psi of water pressure without leaking. This was determined by connecting a commercial water pressure gauge to a length of plastic tubing interposed between the joint and a 10-cc syringe with the entire system filled with water. The outlet tube of the joint was occluded and transient force was applied to the water by pushing on the plunger of the syringe. This was done three times for each of three different joints. There were no leaks up to 100 psi. This was the maximum pressure tested. As the grease seal deteriorates over time small leaks will appear at lower pressures. This, however, is unpredictable. It may occur within a few days or the joint may not leak for weeks after it has been greased. By routinely disassembling and regreasing the joints every 3 days leaks can be entirely prevented.

In our application the animal is anesthetized with hexobarbital (150 mg/kg, i.p.) and a small plastic tube (PE 50) is passed into its stomach through the nasopharynx. The roof of the nostril and the overlying skin of the snout are slit on the side of the insertion and a second small incision is made in the scalp between the ears. A subcutaneous tunnel is created by undermining the skin between the two incisions. The free end of the plastic tube is then brought through the incision in the scalp by bending it backward sharply on itself where it emerges from the nostril and passing it through the tunnel under the skin of the snout and scalp. In order to avoid kinking the plastic tube, the sharp angle at the nostril is formed by a short length of 22-gauge stainless steel tubing bent into a U-shape to fit the snout. The intragastric portion of the tube and its free end are therefore separate lengths of PE 50 tubing (5.5 and 2.5 in. long, respectively) that are forced onto the arms of the metal

U-tube before the animal is anesthetized. With the free end of the tube emerging from the incision in the scalp, the defect in the nostril is sutured closed over the metal U-tube thus concealing under the animal's skin all parts of the gastric tube except for its free end. The free end of the tube is then fitted onto a short piece of 20-gauge stainless steel needle tubing bent to project upward between the rat's ears. This technique for gastric intubation has been described in detail elsewhere (4).

After the animal has fully recovered from the insertion of the gastric tube, it is again anesthetized and its head is fixed in the head-holder of a stereotaxic machine (7). A thick-walled, hollow, soft-rubber connector (made by paring down and boring through a 0-0 stopper) about 1 in. long is fitted onto the steel tube projecting upward between the animal's ears, and both the connector and the tube are fixed rigidly to the rat's skull with methylmethacrylate cement and stainless steel screws. Three screws (0-80,  $\frac{1}{8}$  in.) are placed in a triangular arrangement in the thicker portions of the skull (anterior portion of frontal bone, posterior-lateral portion of parietal bone, and cerebellar bone) after the skull has been cleared of periosteum. They are screwed into holes drilled down as far as the lower table of bone with a no. 57 cylindrical dental burr. The rubber connector joined to the free end of the gastric tube is then placed vertically within the triangle formed by the screws, and the field is filled with the cement to form a single rigid block including the screws and the connector.

A length of large-gauge polyethylene tubing (PE 260) is then forced onto the tapered end of the hollow cylinder (see Fig. 1*A*). This tube conducts the fluid from the reservoir to the joint. It is flexible to lateral displacements but resists torque movements and will not twist easily. Since more force is required to twist the PE 260 tube than to turn the cylinder within the housing and since the housing is fixed to the animal's skull, only the housing rotates with the animal.

The moving surfaces of the cylinder and cap are greased and the joint is assembled. The outlet tube of the swivel-joint housing is then plugged into the top of the hollow rubber connector. A few drops of Eastman cement are used here to assure a firm union. Finally, when the animal has recovered from the anesthesia, the PE 260 tube is counterweighted over a pulley suspended over the box in which the animal is housed.

In our current work, rats fitted with such gastric tubes and swivel joints are trained to press a lever that activates an automatic pump thus delivering small volumes of fluid from the reservoir directly into their own stomachs. By keeping the swivel joint well greased and firmly screwed together and by using automatic programming and recording equipment, we have worked successfully for as long as 62 consecutive days with active rats that feed themselves day and night by pumping a liquid diet into their stomachs.

The joints were made by Mr. Otto K. Hebel, Martin Biological Laboratory, Swarthmore College, Swarthmore, Pa. Mr. Hebel cooperated with the authors in their design.

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