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special communication

A self-powered constant infusion device for use in unrestrained rats

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Brand, Paul H., Nianning Qi, Patricia J. Metting, and Steven L. Britton. A self-powered constant infusion device for use in unrestrained rats. *Am J Physiol Heart Circ Physiol* 278: H2157–H2162, 2000.—We developed a device that delivers fluid through a catheter at a constant rate and can be used in conscious animals to solve a variety of problems. For example, this device can be used for delivering drugs and maintaining intravascular catheter patency. The device provides infusions at low flows (1.0–1.5 ml/day), so that experimental agents may be administered with minimal volume loading of the rat. Arterial and venous catheter patency is maintained by infusion of heparinized saline through indwelling catheters attached to the device. The catheters exit from the rat in the intrascapular area and are routed through a protective spring to the device, which is suspended above the cage. The catheters may be attached to pressure transducers, blood may be sampled, and injections or infusions may be made without disturbing the rat. Because the device is self-contained, it can be suspended by a fluid-free swivel that rotates through 360°, providing minimal restraint. The device has been used successfully to measure arterial and central venous blood pressures in two studies using rats.

chronic instrumentation; blood pressure measurement; vascular access; catheterization

DIRECT MEASUREMENT of arterial blood pressure and infusion of experimental agents over prolonged periods is often required in studies in which small animals, such as rats, have unrestrained movement and free access to food and water. Direct pressure measurement and infusions require indwelling vascular catheters. The primary difficulty that arises in maintaining functional indwelling vascular catheters is thrombus formation at the catheter tip (1, 10). Thrombus formation may be prevented or delayed by infusion of heparinized saline, along with infusion of any required experimental agents. One approach to providing prolonged infusions in unrestrained animals is to use 360° swivels with fluid channels (5, 6) to compensate for animal movement. This approach can be technically challenging and expensive. Many studies have used commercially available swivel systems that allow free movement of the rat during infusion via a pump located external to the animal's cage (3, 7). Despite the wide use of commercially available swivel devices, our experience with these products has proven to be less than satisfactory under many circumstances. The major problem is stabilizing the interface between the animal and the catheter system. Because of variable rotational friction of the swivel systems, complicated tethering and harness systems are required to keep the skin from getting distorted.

Another approach to compensate for animal movement is to construct a rotating cage. Rotating cages are complicated to construct (8) and result in the animal being studied in an unusual environment, a circumstance that can influence the results by itself.

In two recent studies, we evaluated the development of hypertension in Dahl salt-sensitive rats (4, 9). For these studies, a simple and reliable approach was devised to measure arterial and central venous blood pressures directly over prolonged periods in unrestrained, chronically instrumented rats. With the device we created, catheter patency is maintained by slow infusion of heparinized saline. Because the device is self-powered and self-contained, it can be suspended above the rat's cage by a fluid-free swivel, which allows trouble-free rotation through 360°. This device has operated reliably in over 100 rats to maintain patency of arterial and venous catheters.

Because this device is capable of infusing fluid at a constant rate, it can also be used to provide infusion of experimental agents. Because of the low cost, reliability, ease of construction and use, and lack of skin distortion with this device, and its potential for providing constant infusion of experimental agents, we thought it would be of service to provide a detailed description of its design, characteristics, and use.

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METHODS

Self-Powered Infusion Device

The self-powered infusion device illustrated in Fig. 1*A* consists of a latex rubber bulb that acts as a pressure and fluid reservoir, a syringe filter to remove bacteria and particulate matter, and a hydraulic resistor to set the level of flow. The rubber bulb of the type commonly used for pipetting (2 ml; New Jersey Rubber) is connected in series with a 0.2-μm syringe filter (product no. 4192 sterile Acrodisc syringe filter, Gelman Sciences, Ann Arbor, MI) and a hydraulic resistor through a nylon three-way stopcock (K75, Baxter, Valencia, CA). The resistor is manufactured in our laboratory from fused silica capillary tubing (25 μm ID; Scientific Instrument Services, Ringoes, NJ). The tubing is flamed in a Bunsen burner to remove its outer coating, and a section ~4 cm in length is embedded within a 21-gauge blunt-tip hypodermic needle (305167, Becton-Dickinson, Franklin Lanes, NJ) using Dow-Corning Silastic glue.

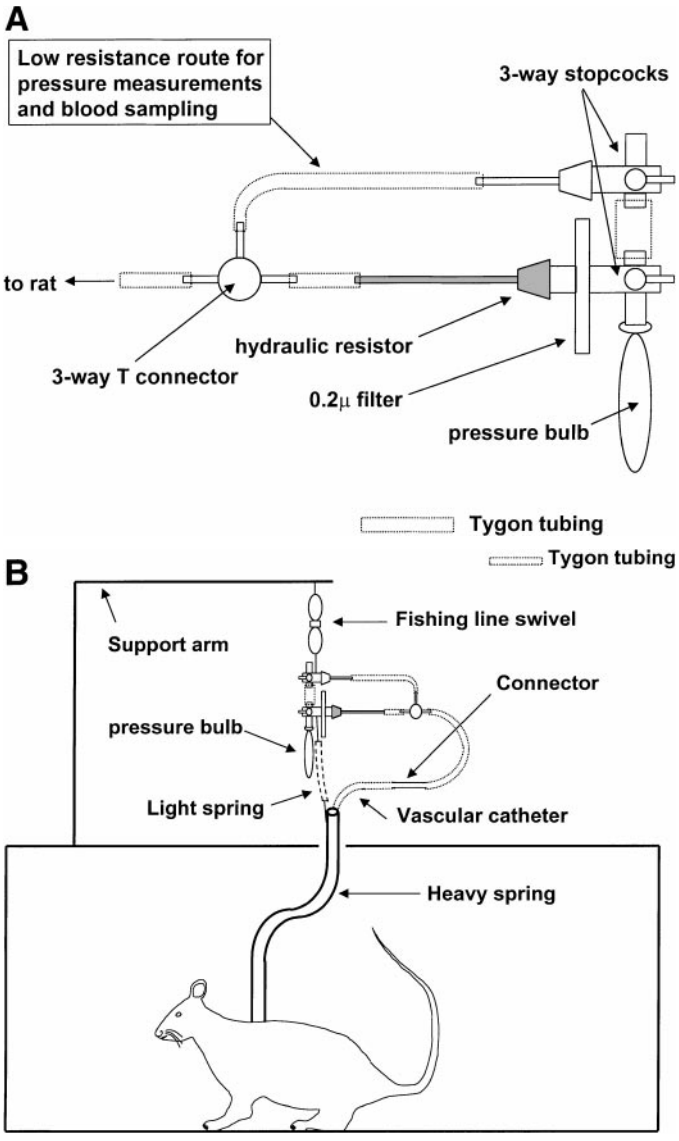

Fig. 1. *A*: self-powered constant infusion device with low-resistance pathway for blood pressure measurements and blood sampling. *B*: setup for chronic intravascular infusion in unrestrained rats. Rat shown in home cage.

During use, each vascular catheter is connected to its own infusion device. To provide a low-resistance route for blood sampling and blood pressure recording through the vascular catheters, a second three-way stopcock is connected in parallel to the stopcock holding the resistor (Fig. 1*A*) by a short length of Tygon tubing to provide mechanical stability and a three-way T connector to direct fluid movement (20 gauge/3 way, Small Parts, Logansport, IN). During infusions, the stopcock for the low-resistance route is closed to the animal, whereas during pressure measurements or blood sampling, the stopcock to the hydraulic resistor is closed to the animal. The device is suspended from a support arm above the rat's cage through a ball-bearing fishing line-type swivel (obtained from a local sporting goods store) that rotates freely in either direction through 360°(Fig. 1*B*).

In Vitro Tests of Flow and Pressure Characteristics

Constancy of fluid delivery. To determine the flow versus time characteristics of the fluid delivery device, six similar devices with 45 mm × 25 μm ID capillary tube resistors were prepared, the bulb was filled with ~10 ml of water, tared with an analytic balance sensitive to 0.00001 g, and allowed to deliver water freely. Weight was monitored once or twice daily for 67 h to determine the decrease in weight with time due to delivery of water from the devices.

Reproducibility of flow. Five similar devices were prepared, again with 45 mm × 25 μm ID resistors, filled with 10 ml of water, and tared as above. Flow from each device was measured as the decrease in its weight during three consecutive ~24-h periods.

Flow versus length of resistor. The utility of this device as a constant flow delivery system may be improved if different flows can be readily achieved. One way in which variable flows can be obtained is by using devices with resistors of different lengths. To test the effect of the length of the resistor on flow, seven devices were prepared with 25-μm ID capillary tube resistors of lengths varying from 30 to 47 mm. Flow was determined over 24 h from each of these devices as described above.

Constancy of pressure head. Given that the devices are prepared with fixed resistors, constancy of flow implies that there must be a constant pressure source. The pressure source is the rubber-nipple bulb fluid reservoir. We hypothesized that a constant pressure head may be provided by the bulb following the law of Laplace (2): pressure = [(wall stress)(wall thickness)]/radius.

Preliminary observations suggested that pressure declines very little as the bulb delivers fluid through the resistor, suggesting that the product of wall stress and thickness declines in proportion to the decline in radius as the fluid leaves the bulb. To characterize simultaneous changes in pressure and volume in the bulb, a bulb was attached via a four-way stopcock to a syringe mounted in a constant infusion-withdrawal pump (Harvard Apparatus, South Natick, MA) and to a Statham 23 Db pressure transducer. The bulb was filled with ~12 ml of water. A 12-ml syringe was tared and placed in the syringe pump, and water was withdrawn from the bulb into the syringe by the pump at a nominal rate of 0.12 ml/min. After ~10 ml had been withdrawn from the bulb, the experiment was stopped, and the syringe was reweighed to determine the actual volume withdrawn from the bulb. While water was withdrawn from the bulb, the pressure in the bulb was determined from the voltage signal from the transducer. The signal was amplified on a Sensormedics R-611 polygraph, sampled at 300 Hz, and then digitized by a Data Translation analog-to-digital board (DT 2801, Data Translation, Marlboro, MA) The digital signals were sampled

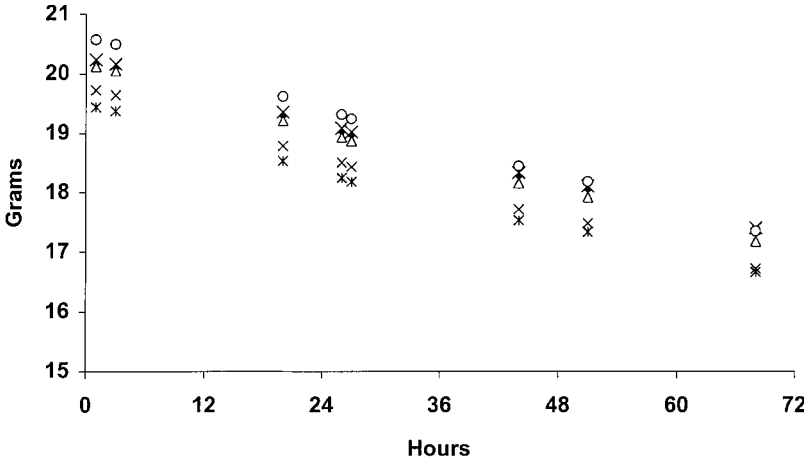

Fig. 2. Six constant infusion devices showing decrease in weight due to delivery of fluid from devices over 67 h. Each symbol represents a single device.

at 0.1 Hz by a PC (Dell Dimension P75t, Dell Computer, Round Rock, TX) using Labtech Notebook V8.0 (Andover, MA) software and stored on the hard drive for off-line analysis. One to four repeat simultaneous measures of pressure and volume were made on each of four bulbs. To determine absolute pressures, the transducer was calibrated with a mercury manometer.

In Vivo Experiments: Use of Flow Device to Maintain Catheter Patency

Animal preparation and experimental setup. The animals used in this study were John Rapp inbred salt-sensitive (SS/Jr) and salt-resistant (SR/Jr) rats. Results of studies of the mechanisms leading to hypertension using these rats have been published previously (8, 9). All animals were anesthetized with a solution of ketamine (80 mg/kg), xylazine (12 mg/kg), and atropine (0.032 mg/kg) via an intraperitoneal injection. Two micro-Renathane catheters (0.040 in. OD × 0.025 in. ID; Braintree Scientific, Braintree, MA) extended with Tygon plastic tubing (0.60 in. OD × 0.020 in. ID; Norton Performance Plastic, Akron, OH) were inserted into the right carotid artery for ~2.0 cm and into the right jugular vein for ~3.2 cm, respectively, for the direct measurement of arterial pressure, central venous pressure, collection of blood samples, and injection of experimental agents. The free ends of the tubing were tunneled subcutaneously to exit the skin at the intrascapular region through a flanged entry portal that was fixed beneath the skin. The entry portal was made in our laboratory from stainless steel tubing (4 mm OD × 3 mm ID; Small Parts, Logansport, IN). The exteriorized tubing was routed out of the animal cage through a helical stainless steel wire spring (0.40-mm wire, 5.0-mm diameter spring, Walter Gogel, Toledo, OH) to protect the tubing from manipulation by the animal. This spring was fixed on the tubing entry portal at the bottom end and attached to the fluid delivery device at the top end through a lighter wire extension spring (0.20-mm wire, 4.0-mm diameter spring, Walter Gogel) that was flexible enough to allow maximum movement of the rat in the cage (a standard polyvinyl rat cage, 26.5 × 20.5 × 16.0 cm^3) with minimum tension on the tether. The catheters exiting from the heavier spring were connected to the fluid-delivery device by a 21-gauge, 1.5–2 cm length of stainless steel tubing. The device provided a flow of saline containing 50 U/ml of heparin at 1–1.5 ml/day to maintain catheter patency. The fluid delivery device was suspended from the support arm (Fig. 1*B*) by a ball-bearing fishing line swivel, so that the device rotated freely, without twisting the tethering spring or catheters. As a result, the rats were allowed essentially unrestrained movement. The only required management of the animal and the device is to refill the reservoir bulb to a volume of 10 ml about every other day. All animals appeared to tolerate the tethering device without any discomfort or restriction of motion.

RESULTS

In Vitro Tests of Flow and Pressure Characteristics

Constancy of fluid delivery. Constancy of fluid delivery was measured as a decrease in weight, using six similar infusion devices. Figure 2 shows the decline in weight for each device sampled over 67 h. The linear regression of weight versus time was calculated for each device; data are shown in Table 1. For six devices manufactured with resistors of similar length and diameter, the range of flows was from 0.99 to 1.147 ml/24 h. The square of the Pearson correlation coefficient for flow versus time was >0.99 for all six devices, indicating that almost all of the variability of flow with time fit closely to a linear model during 67 h of measurement.

Reproducibility of flow. Flow was determined from five devices as the decline in weight during three consecutive 24-h periods. Reproducibility was estimated from the percent coefficient of variation (standard deviation/mean × 100) and ranged from 1.86 to 8.78% for the five devices (Fig. 3).

Flow versus length of resistor. Flow, measured in seven resistors varying in length from 30 to 47 mm, declined by 0.05 ml/24 h per millimeter length. For the linear correlation between flow and length, the square

Table 1. *Linear regression of flow during 67 h for six infusion devices*

Device No.	Flow, ml/24 h	R^2	F	P
5	1.036	0.99777	2237.01	8×10^{-8}
7	1.068	0.99778	2702.06	3.4×10^{-9}
9	1.048	0.99791	2862.44	2.9×10^{-9}
10	1.010	0.99789	2842.96	2.9×10^{-9}
11	0.990	0.99399	991.662	6.8×10^{-8}
12	1.147	0.99926	8112.53	1.3×10

R^2, Pearson correlation coefficient; F, F ratio; P, probability.

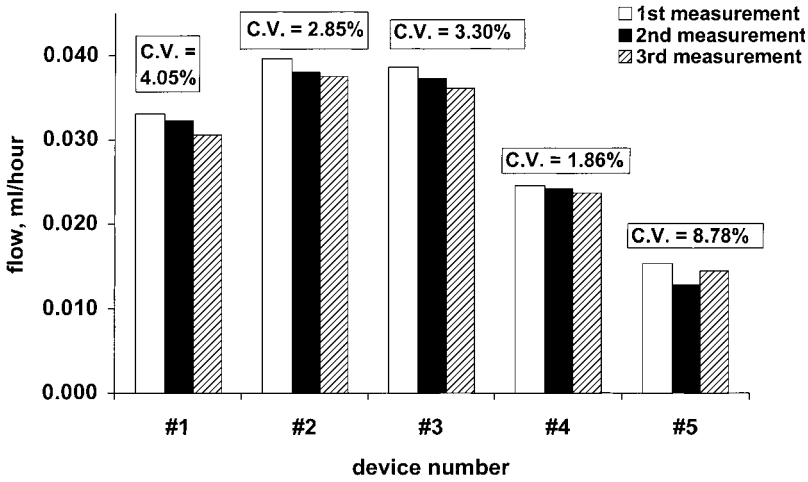

Fig. 3. Three consecutive measurements of flow from each of five devices, showing reproducibility of flow measurement. CV, coefficient of variation.

of the Pearson correlation coefficient was 0.78, suggesting that variations in length are closely associated with variations in flow.

Constancy of pressure head. Simultaneous measurements of pressure and volume were made during withdrawal of water in four bulbs. Two patterns were seen. In two bulbs, pressure declined slowly with volume until ~6 ml had been withdrawn from the bulb. Further removal of water from the bulb resulted in a more rapid decline in pressure (Fig. 4, *A* and *B*). In the other two bulbs, pressure increased slightly as volume decreased, until about 6 ml had been withdrawn, and then pressure declined rapidly (Fig. 5, *A* and *B*). Similar patterns were seen for all of the trials for the bulbs that showed an initial increase in pressure and for those that showed a decrease. Considering all 17

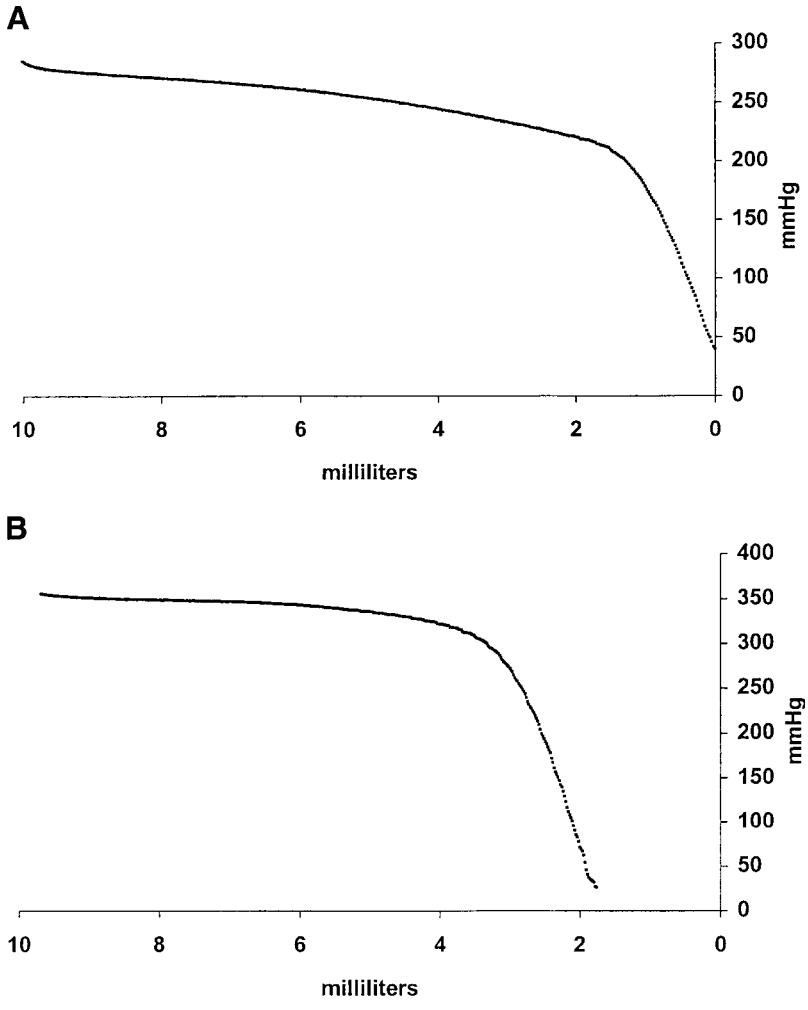

Fig. 4. Simultaneous measurement of volume and pressure in bulb, showing two bulbs (*A*: *bulb 1*; *B*: *bulb 2*) in which pressure decreased with decreasing volume.

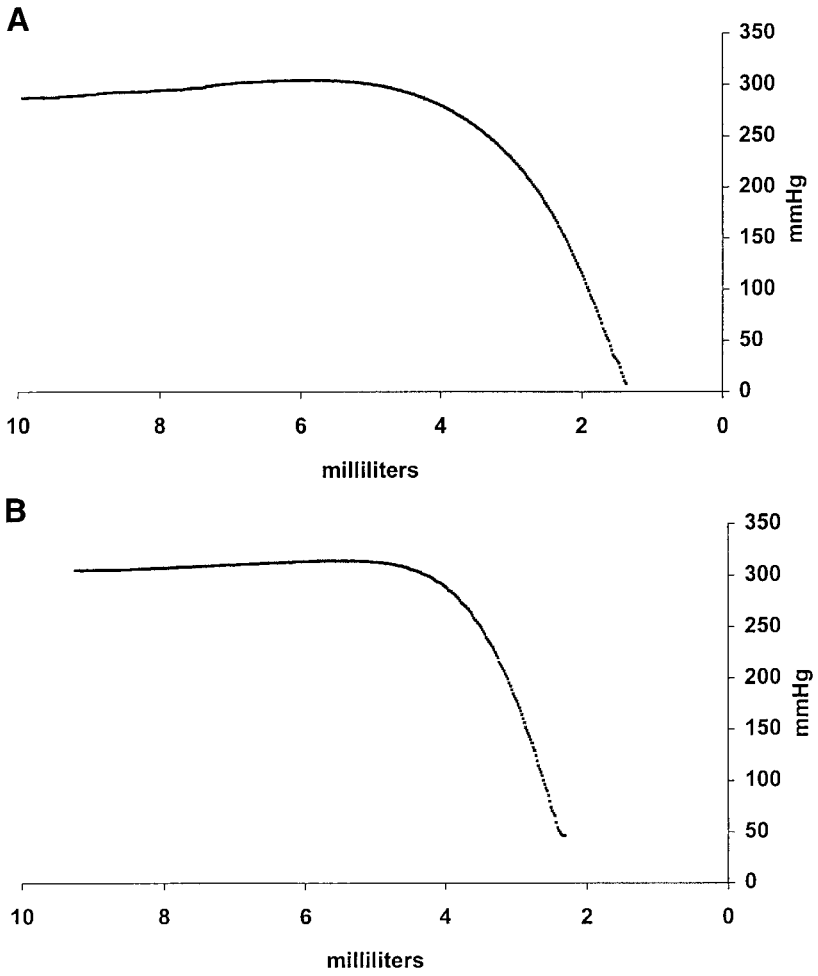

Fig. 5. Simultaneous measurement of volume and pressure in bulb, showing two bulbs (*A*: *bulb 3*; *B*: *bulb 4*) in which pressure initially increased with decreasing volume.

trials in the four bulbs, during the withdrawal of the first milliliter of water from the bulb the range of change in pressure was +1.73 to −6.65 mmHg.

In Vivo Experiments: Use of Flow Device to Maintain Catheter Patency

The fluid delivery devices were used to maintain patent arterial or venous catheters in 100 chronically instrumented rats. In vivo, fluid delivery devices with resistors of 3.2 cm in length produced a flow of ~1 ml/day in the arterial catheters and ~1.5 ml/day in the venous catheters. Both arterial and venous catheters were kept patent on average for as long as 3 wk without any need for additional flushing. No experiments were terminated because of catheter failure.

All animals were maintained in good health with an average daily body weight gain of 4.6 g. The daily consumption of food and water was in the normal range for rats of this age (23.2 ± 0.7 g and 26.3 ± 0.7 ml).

DISCUSSION

Chronic intravascular infusion in small animals is a useful experimental technique in physiological and pharmacological studies. The infusion device described here provides an effective long-term access for administration of experimental agents with minimal volume loading, repeated hemodynamic measurements, and blood sampling in small, unrestrained animals. This device is unique in that the delivery of fluid is self-powered by the elasticity of the rubber bulb. Because the device is self-powered and thus self-contained, and because it weighs only ~20 g (including 10 g of saline), it can be suspended by a simple fishing line swivel. Consequently, the device rotates freely with the movement of the animal. Furthermore, the light extension spring attaching the device to the catheter-protecting spring allows lateral movements with minimal tension. As a result, movement of the animal within its home cage is essentially unrestricted. The device is easy to manufacture, and all the required materials are readily available. The approximate cost for the materials to create one flow device is less than $10. By creating continuous fluid infusion without interrupting normal activity of the animal, this device eliminates the problems that often occur in chronic cannulations with a pump mounted outside of the animal cage and a commercial swivel. By using this device to infuse heparinized saline at 1.0–1.5 ml/day in over 100 rats in our laboratory, we have observed that both arterial and venous catheters could be kept patent for a period of at least 3 wk without daily flushing. In one study from our laboratory (9) Dahl salt-sensitive and salt-resistant

inbred rats were successfully instrumented with carotid arterial and jugular venous catheters that remained patent for 15 days. The infusion device was also used to obtain blood samples for serial measurements of hematocrit and plasma volume (by dilution of Evan's blue). In a second study from our laboratory, DiPaola et al. (4) used the infusion device for measurement of arterial pressure and to observe the acute effects of captopril and saralasin on arterial blood pressure. These two studies illustrate the utility of the device in maintaining patent vascular catheters, measuring blood pressures, and administering experimental agents during various experimental maneuvers.

Infusion of only 1 to 1.5 ml/day allows maintenance of catheter patency or administration of experimental agents with minimal fluid volume expansion and therefore presumably minimal effects on cardiovascular and renal function.

Adjusting the length and inner diameter of the silica capillary tubing in the resistor achieves variable rates of fluid delivery to suit the needs of different studies. Given our observation that the rate of fluid delivery is linear and nearly constant over 67 h (Fig. 2 and Table 1), the device may be used to infuse experimental agents at known rates for several days.

Constancy of flow is a result of the use of a fixed resistor and the minimal changes in driving pressure observed during the simultaneous measurements of pressure and volume in the pressure bulbs. Although two patterns of change in pressure (increase or decrease) with declining volume were noted in different bulbs, the range of changes in pressure was small (+1.73 to −6.65 mmHg) during delivery of 1 ml of fluid. Delivery of 1 ml of fluid over 24 h is sufficient to maintain catheter patency.

We have no data to account for the small increases or decreases in pressure that occurred during delivery of fluid from the bulb but would speculate that these pressure patterns are a result of variations in the characteristics of different pressure bulbs. For example, from the law of Laplace (see *Constancy of pressure head*), if the radius of the bulb declined with decreasing volume more quickly than the product of wall stress and wall thickness, pressure would increase. These changes presumably account for the observation that in two bulbs pressure increased initially with fluid delivery.

In summary, the self-powered catheter infusion device we developed offers advantages of simplicity, dependability, capacity for quantitative adjustment of continuous flow over extended periods, ease of manufacture, minimal expense, and unrestrained activity of the animal.

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