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TECHNICAL NOTE

Chronic vascular constrictions and measurements of renal function in conscious rats

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Although a tremendous amount of physiologic information has been gained from experiments on anesthetized animals, there is often the question to what extent anesthesia itself, as well as the losses and replacement of fluid that accompany surgical preparations, might have influenced the function being studied. This possible pitfall may apply especially to studies on the kidney because the renal circulation is so exquisitely sensitive to anesthetic agents and changes in fluid balance. Chronic preparations in dogs have been [1] and continue to be used [2] successfully. But the far lesser expense of rats, as well as their common use in physiologic experiments, points up the desirability of a successful chronic preparation in this species. Renal clearances have been performed previously on unanesthetized rats [3, 4], but the methods have not been generally adopted.

We here describe a method that can measure renal clearances and extraction of PAH accurately and repeatedly in the same conscious rat over a period of weeks. For our own purposes [5] we also needed a method for constricting blood vessels. The design and construction of a suitable cuff for this purpose, likewise usable in unanesthetized rats, also form part of this report.

Methods

Construction of material. (1) Catheters for vessels. Medical grade tygon tubing (S-54-HL, Norton Co., Akron, Ohio) is used for this purpose: size 0.025 inch I.D., 0.040 inch O.D. for implants effected via the carotid artery or jugular vein, and size 0.015 inch I.D., 0.030 inch O.D., when the catheter is placed in the abdominal aorta through the femoral artery. Small dumbbells made of PE-60 or PE-100 tubing, flared at both ends by flaming (Fig. 1A), are used for tying the catheters to the vessels and adjacent fascia (Fig. 3A), and to prevent kinking.

(2) Cannula for bladder. A three-flanged cannula (Fig. 1B) is constructed from silicone rubber material (Silastic, Dow Corning). Three small pieces are cut from 0.020-inch thick silastic sheeting, and a small hole is bored in each with a sharpened steel tube. A piece of silastic tubing (0.078 inch I.D., 0.125 inch O.D.) is then threaded through each sheet, and the first and third sheet are glued to the tube, approximately 1 cm apart, with no. 891 medical adhesive (Dow Corning Co., Midland, Michigan). The middle sheet is left free to slide along the tubing. After trimming the flanges and the silastic tube to the desired shape and length, we inserted a stainless steel tube (14-G, 1.5-cm long) into the silastic tube (Fig. 1B). A tight fit is achieved by soaking the silastic tube in xylene (which causes the tube to dilate), inserting the steel tube, and then allowing the silastic to contract during vaporization of the xylene.

(3) Cuff for constricting vessels. We have developed a cuff that requires at most 1 mm of space and minimal dissection of the vessel, one that does not exert pressure on surrounding structures when inflated, and one that can sustain stable, partial occlusion of a vessel for hours. Construction of this cuff is shown in Fig. 2.

Whereas most cuffs are made from a combination of materials [6, 7], we use only silastic. No. 891 medical adhesive mixed in a 1:1 ratio with naphtha, is applied as glue with a syringe. A small, square piece is cut from 0.020-inch thick silastic sheeting, and a hole is bored through this square with a 16-G steel tube. A silastic tube (0.030 inch I.D., 0.065

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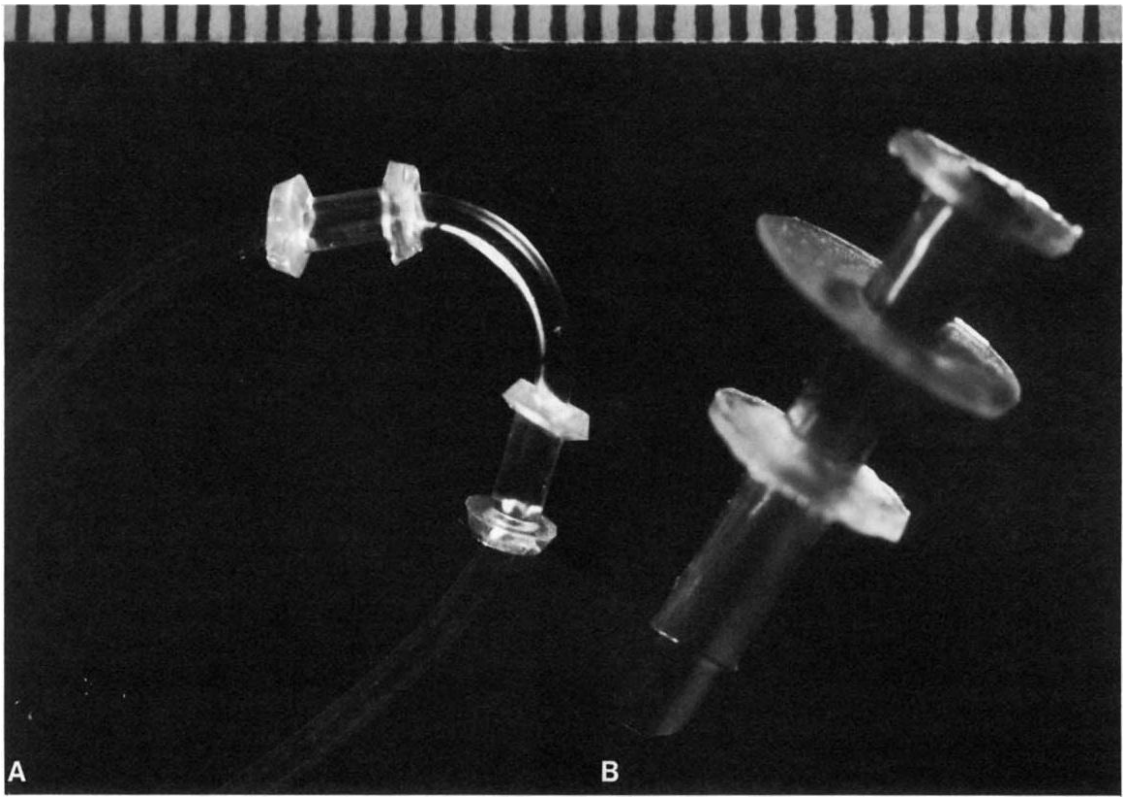


Fig. 1. **A** *Typical catheter for implantation in a vessel, showing dumbbells that are used for securing the catheter to the vessel and fascia, and to prevent kinking.* **B** *Bladder cannula, showing the three flanges and the stainless steel tube.* See text for detailed descriptions. (Scale = 1 mm divisions.)

inch O.D.) is fed through the hole of the sheet, and this sheet is glued to the proximal end of the tube (Fig. 2a). When the glue has set, the end of the tube is trimmed flush with the square sheet by means of a razor blade (Fig. 2a). A slightly larger second square is then cut from 0.005-inch thick silastic sheeting, and glue is applied to the periphery of one side of this square (Fig. 2b). The parts shown in Fig. 2, a and b, are then apposed (Fig. 2c), taking care not to occlude the lumen of the tube with adhesive. Next, three short silastic tubes (0.025 inch I.D., 0.047 inch O.D.), which will accommodate sutures, are glued to the assemblage: two tubes to the 0.020-inch thick square and the proximal side of the tube, and one to one edge of both squares (Fig. 2d). The cuff is then built up, with additional glue, to the contour that is shown in Fig. 2d; further glue is applied between the sheets and the tubing, to lend strength. Finally, the silastic tubes are trimmed (Fig. 2g).

The cuff is secured to a vessel as follows (Fig. 2, g and h). Two sutures are tied together at their center and one of the resulting four limbs is cut off at the knot. One of the remaining three limbs is threaded through the vertical tube until the knot lies within that tube (Fig. 2h), and each of the other two limbs of suture are passed through the horizontal tubes and tied together. One of the two ends from this tie is cut at the knot (Fig. 2g), so that finally there remain two sutures that, when tied, secure the cuff to the vessel (Fig. 2g). The vertical tube with its contained suture is passed beneath the vessel. Figure 2, e and f, show the cuff as it looks when it has been implanted on a vessel, uninflated (Fig. 2e) and inflated with water, with the 0.005-inch thick sheeting bulging into the vessel lumen (Fig. 2f).

The blowout pressure of the cuff varies with the amount of adhesive used; for most of the cuffs that we have tested, this pressure is approximately 25 psi.

Surgical preparation. All materials are sterilized in a 1:1,000 solution of benzalkonium chloride (Zephiran), and rinsed in sterile saline immediately before implantation. The rats are anesthetized with ketamine (60 mg/kg body weight i.m.) and pentobarbital (20 mg/kg body weight i.p.). Catheters are implanted in either the descending aorta via the left carotid artery or in the abdominal aorta via the femoral artery, as well as in the left superior vena cava by way of the left external jugular vein. The use of the dumbbells is shown in Fig. 3A. The catheters are passed under the skin with the help of a 16-G trochar, and they are then exteriorized at the back of

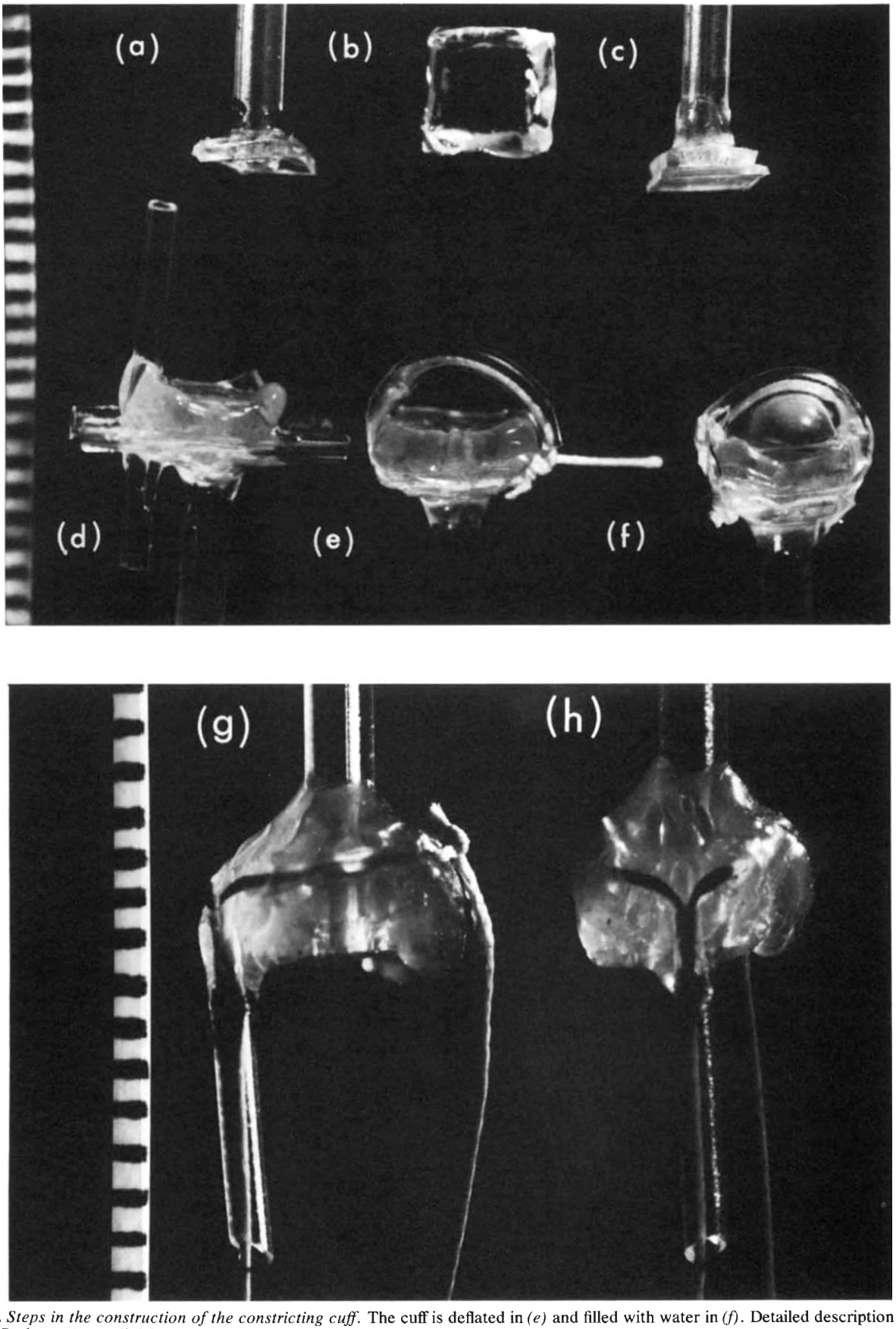
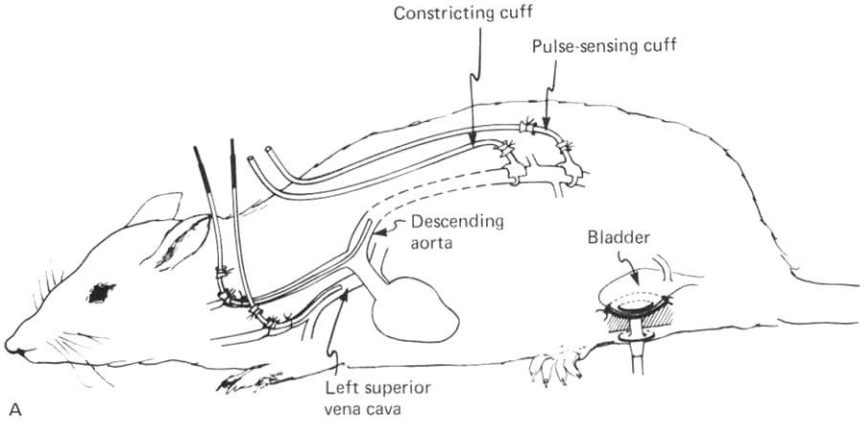


Fig. 2. *Steps in the construction of the constricting cuff.* The cuff is deflated in *(e)* and filled with water in *(f)*. Detailed description is in text. (Scales = 1 mm divisions.)



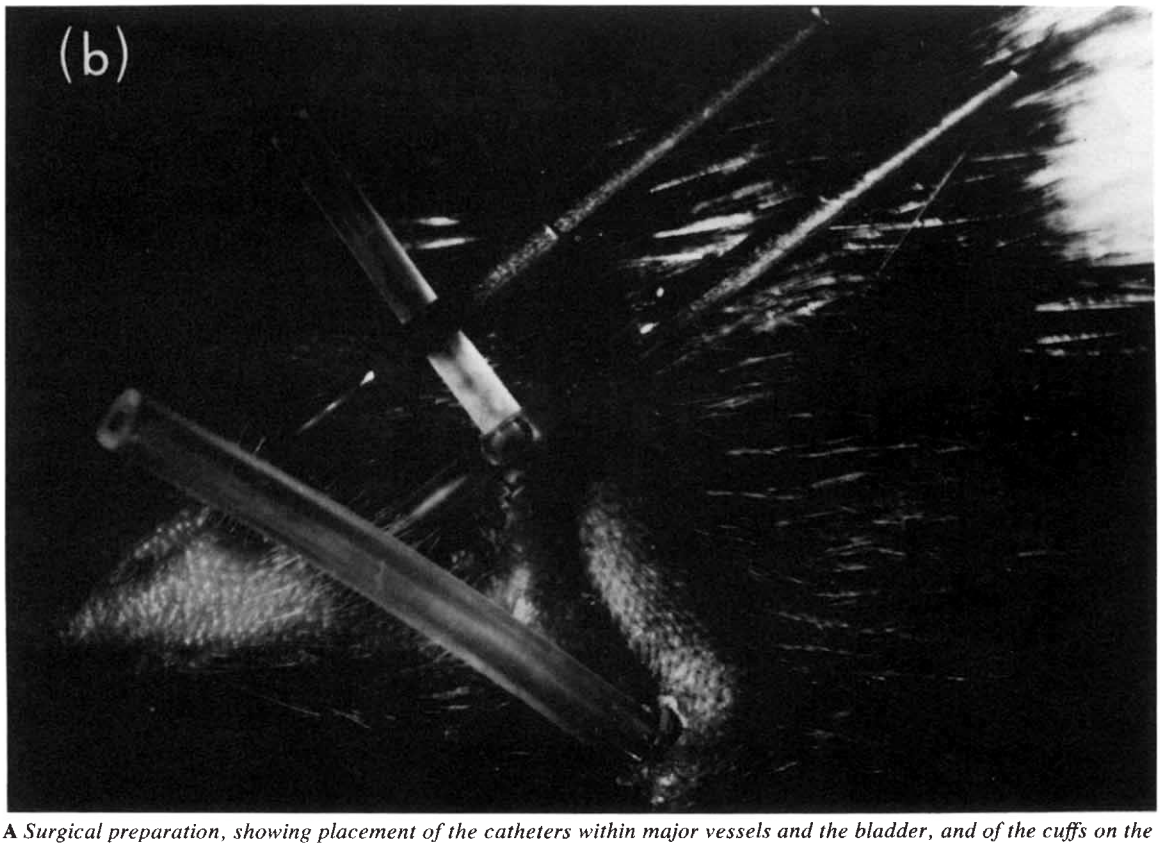


Fig. 3. A *Surgical preparation, showing placement of the catheters within major vessels and the bladder, and of the cuffs on the aorta.* The pulse-sensing cuff is constructed like the constricting cuff but inflated minimally. **B** *Neck of a rat, showing how catheters protrude without any formal holding device.* The vessel catheters are sealed with stainless steel rods.

the neck. We have the impression that healing occurs faster and handling is made easier if the ends of the catheters are left free (Fig. 3B) and if the use of some holding device, such as a button sewn to the back of the neck, is avoided. The catheters are filled with heparinized saline and sealed through insertion of stainless steel rods.

To implant the bladder cannula, the bladder is exposed through a midline incision. The first flange of the cannula (Fig. 1B) is inserted through a small incision in the bladder, and the cannula is secured with a 4-0 silk purse-string suture. The second, unglued flange is then pushed onto the bladder wall, and it is tied to the wall with interrupted sutures (Fig. 3A). The abdominal muscle and fascia are closed between the second and third flange (Fig. 3A), and finally the skin is closed around the cannula and on top of the third flange. Thus, only the

stainless steel tube and part of the silastic tube protrude through the skin.

The constricting cuff is placed on the aorta, usually just proximal to the renal arteries (Fig. 3A), through an incision in the left flank. The cuff is secured to surrounding muscle with a dumbbell made of PE-190 tubing, and the catheter leading from the cuff is exteriorized at the back of the neck (Fig. 3B). In most animals, a second cuff is implanted on the aorta distal to the renal arteries, as a means of gauging the degree of constriction effected by the first cuff (explained further below).

We have also developed a technique for determining renal plasma flow (RPF) by measuring extraction of paraaminohippurate (PAH) in conscious rats. For this purpose, a specially prepared tygon catheter (0.025 inch I.D., 0.040 O.D.) is threaded into the left renal vein. Prior to insertion, a flexible right-angle "hook" is fashioned at the end of the catheter by bending the terminal 7 to 8 mm under heated oil. One or two holes are punched close to the bent, tapered end. The left renal vein is exposed through a short midline, ventral incision; the flexible hooked end of the catheter is then threaded, from a cut-down at the femoral vein, and guided into the left renal vein under direct visualization. The catheter is tied to the femoral vein and surrounding fascia with polyethylene dumbbells, and it is exteriorized at the back of the neck, as described above. Maintenance of this catheter is identical to that of the aortic and other venous catheters.

During the operation, great care is taken to monitor the level of anesthesia and to minimize loss of blood and fluid, as well as trauma to tissues. As a result, we achieve a successful preparation in roughly two out of three tries.

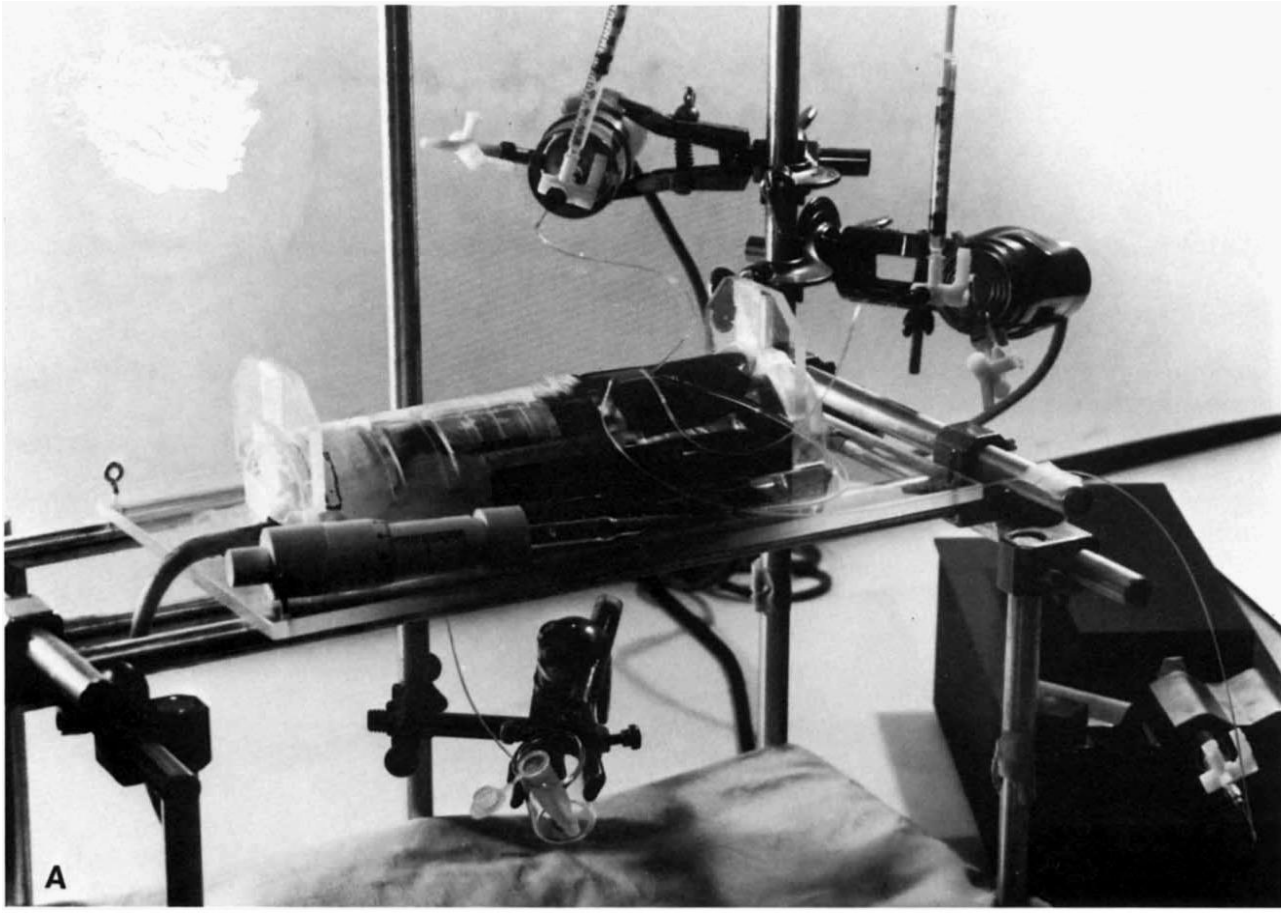
After the operation, each animal is placed in a separate cage on top of a wire mesh that is covered with absorbent pads (Healthco. Inc., Boston, Massachusetts). The animal is watched carefully during the immediate 4 hours after completion of the surgery. By that time, it is fully awake and moving about the cage; by the next morning, the rat walks freely, feeds, can be picked up with ease, and appears to be without pain. Penicillin (100,000 U i.m. daily) is given on the day of operation and for 4 consecutive days thereafter. The urinary bladder is flushed daily with distilled water, and the catheters are rinsed with heparinized saline two or three times a week. Wounds are kept clean with cotton swabs. These procedures are continued for the duration of the preparation.

Conduct of experiment. Experiments are run beginning 4 to 7 days after the surgical preparation. For about 3 days prior to the surgical preparation and for approximately 5 days after the operation, animals are accustomed to the restraining cage (Fig. 4, A and B) by placing them in the cage once daily for approximately 60 min. This cage is made of a hinged plastic cylinder having openings at the bottom for the legs and the bladder cannula, and at the top for the various catheters; the openings are in the form of slits with dimensions of approximately 6 × 1.5 cm. Rats are usually more quiet if the head-end of the cylinder is covered with some sort of opaque tape. After several days of training, the animals will remain in the cage, quietly, for up to 5 hours. Satisfactory quietness can be assessed by a stable pulse rate and blood pressure. These two variables are monitored continuously beginning 20 to 30 min after the rat is placed in the restraining cage; heart rate stabilizes at 360 to 420 beats/min and mean arterial blood pressure at 95 to 110 mm Hg. If the animal is uncomfortable or restless, this fact is immediately apparent in erratic pulse rate and blood pressure. When that occurs and cannot be quickly corrected, the experiment is terminated.

Renal clearances are measured by standard procedures. When serial measurements over a 24-hour period are required [8], the animal is periodically put in the restraining cage for 1 hour while renal clearances are performed. Between the clearance periods, the rat is returned to its usual, larger cage; during this time, urine is not collected, but the other catheters can remain connected to the infusion pump and transducer, provided that the rat is supervised continually.

In those experiments where a major vessel is constricted [5], the cuff is first filled with water by inserting PE-10 tubing to the bottom of the cuff and then injecting water while the tubing is being withdrawn; this procedure prevents air from being trapped in the cuff. The diaphragm of the cuff is then blown up with water by means of a micrometer syringe that leads, via polyethylene tubing, to a 16-G steel tube that fits snugly into the catheter (Fig. 4, A and B).

In normal rats, the degree of constriction is assessed by arterial blood pressure recorded from the aorta distal to the constriction (Fig. 5). A different method must be resorted to in Brattleboro homozygotes [5] in which, for reasons as yet unknown, the back leg becomes lame or gangrenous when the femoral artery to that leg is catheterized. In these animals, the degree of constriction can be gauged by the aortic pulse (Fig. 6A) sensed with a cuff placed on the aorta just distal to the constricting cuff (Fig. 3A); the pulse-sensing cuff can be cali-



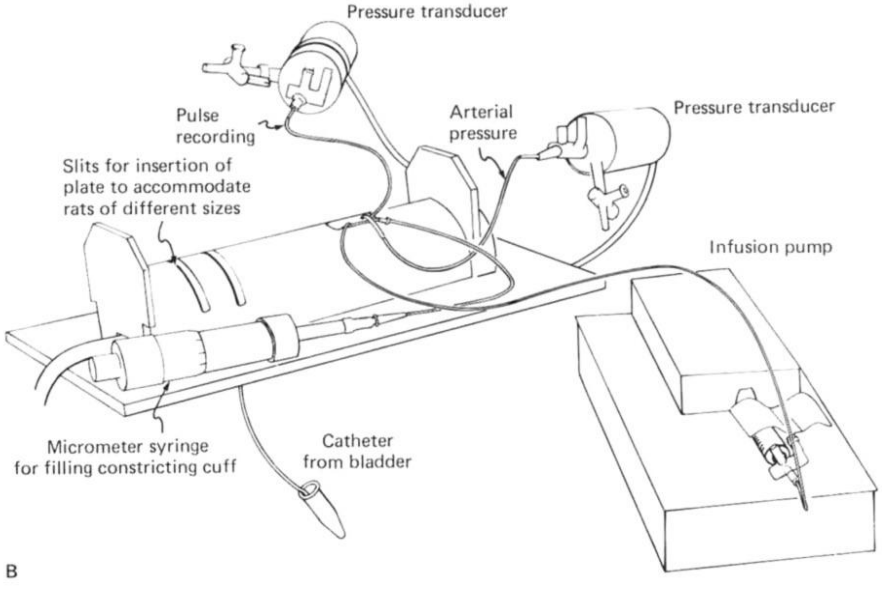


Fig. 4. A *Preparation as it looks when an experiment is being conducted on an unanesthetized rat.* **B** *Diagram of the above.*

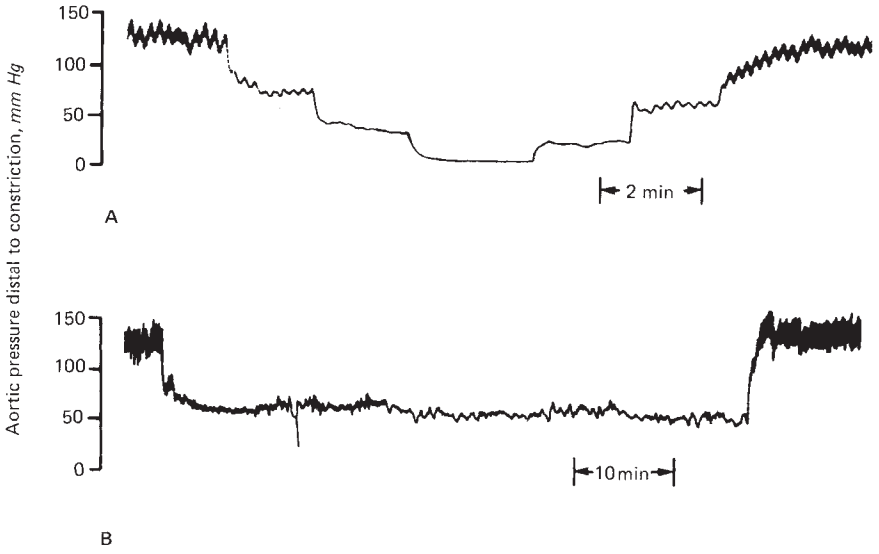


Fig. 5. *Aortic pressure distal to the constriction in an unanesthetized rat.* Decreases in the distal pressure in response to increments of constriction are shown in **A**, and **B** illustrates the stability of the constriction.

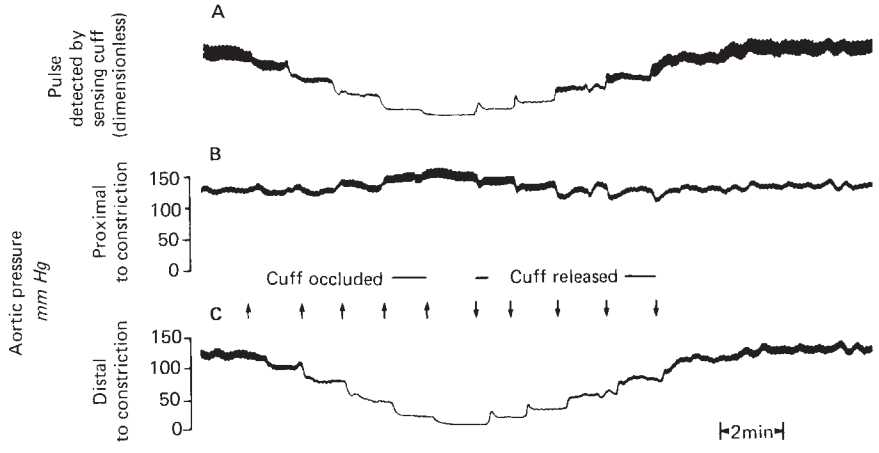


Fig. 6. *Pressure measurements during progressive aortic constriction in an unanesthetized Brattleboro homozygous rat.* The tracing of the pulse pressure in **A** is dimensionless in the sense that the gain in the recording system is so great that the excursions shown amount to only 1 to 4 cm of H_2O. The pulse tracing can be calibrated by recording the simultaneous aortic pressure distal to the constriction **C** at the end of the experiment.

brated by recording the simultaneous aortic pressure via a femoral artery (Fig. 6C) in the same homozygous animal at the end of the experiment.

Results and Discussion

Results are presented here, not primarily for their scientific interest but rather to demonstrate the feasibility and reliability of our preparation. Some of the results have been presented in abstract form [5, 8], and more detailed reports will be published shortly.

We have performed more than 180 polyfructosan (Inutest, Laevosan-Gesellschaft, Linz-Donau, Austria) clearances [10] in 8 unanesthetized Long-Evans normal rats (4 females, 4 males) and 42 unanesthetized Brattleboro homozygotes (16 females, 26 males) [9]. The control GFR, recorded in several different projects, was 1288 ± (SEM) 66 μl/min · 100 g body weight in the Long-Evans rats and 979 ± 58 μl/min · 100 g body weight in the Brattleboro homozygotes. Although the difference between the means is statistically significant ($P < 0.05$), the biologic meaning of this difference, if any, is not known. What seems to us important is that the mean values and statistical variability of the measurements are in line with those that have been

measured in more conventional preparations; the results for Long-Evans rats are, in fact, at the upper limits of reported values (see Fig. 3 of Reference 11).

It is also possible to perform serial measurements over an extended period in the same animal. In one Long-Evans rat, for example, three consecutive, weekly measurements of GFR yielded values of 1315, 1190, and 1251 μl/min · 100 g body weight.

Renal extraction of PAH was 89 ± (SEM) 2.4% in 5 unanesthetized Brattleboro homozygotes, and the renal plasma flow in these animals was 3,752 ± (SEM) 378 μl/min · 100 g body weight. Filtration fraction in the same series was 0.25 ± (SEM) 0.02.

Finally, this preparation has been used to study the effects of aortic constriction. Mild aortic constriction (mean aortic pressure distal to the constriction, 70 to 80 mm Hg) was sustained for a period of 3 hours in 17 unanesthetized Brattleboro homozygotes. In 8 of these animals, there was autoregulation of the GFR [5], whereas in 9, the GFR decreased by an average of 21%. Any desired degree of constriction—including occlusion—can be effected by the use of this cuff.

We believe that these results demonstrate the feasibility of truly chronic, serial measurements of renal function in conscious rats. With just slight modifications, it is possible to apply the constricting cuff not only to the aorta but also to smaller vessels; we have, for example, applied such cuffs to the carotid artery and renal artery.

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References

1. VERNEY EB: The antidiuretic hormone and the factors which determine its release. *Proc R Soc Lond (Biol)* 135:25-106, 1947
2. UFFERMAN RC, SCHRIER RW: Importance of sodium intake and mineralocorticoid hormone in the impaired water excretion in adrenal insufficiency. *J Clin Invest* 51:1639-1646, 1972
3. KLEINMAN LI, RADFORD EP JR, TORELLI G: Urea and inulin clearances in undisturbed, unanesthetized rats. *Am J Physiol* 208:578-584, 1965
4. MALVIN RL, FUSCO MM: Renal function in unanesthetized normal and diabetes insipidus rats. *J Appl Physiol* 22:380-382, 1967
5. EDWARDS BR, GELLAI M, VALTIN H: Urinary concentration in the absence of ADH without a decrease in GFR. *Abst VII Int Congr Nephrol*, 1978, p. Q-3
6. KHOURI EM, GREGG DE: An inflatable cuff for zero determination in blood flow studies. *J Appl Physiol* 23:395-397, 1967
7. PELUSO FP, MELBIN J, NOORDERGRAAF A, PYLE L: A new method for fabrication of latex cuffs. *J Appl Physiol* 39:166-169, 1975
8. EDWARDS BR, GELLAI M, VALTIN H: Renal response to fluid deprivation in Brattleboro homozygous (DI) rats: Chronic clearances in conscious rats. *Abst X Annu Meeting Am Soc Nephrol*, p. 103A, 1977
9. VALTIN H: Animal model of human disease: Hereditary hypothalamic diabetes insipidus in the Brattleboro strain of rat. *Am J Pathol* 83:633-636, 1976
10. EDWARDS BR, STERN P: Renal clearances of ^{14}C-inulin and polyfructosan in the rat: Effect of increased ureteral pressure. *Pfluegers Arch* 369:281-283, 1977
11. SACHTJEN E, RABINOWITZ L, BINKERD PE: Renal concentrating ability in the uninephrectomized rat. *Am J Physiol* 233:F428-F437, 1977