Routine Direct Measurement of Arterial Pressure in Unanesthetized Rats. (25937)

JAMES R. WEEKS AND JULIE A. JONES (Introduced by M. H. Kuizenga)
Dept. of Pharmacology, The Upjohn Co., Kalamazoo, Mich.

Difficulties and possible inaccuracies associated with existing indirect methods for determining blood pressure routinely in rats (lack of agreement with direct pressure measurements, requirements that animals be heated or anesthetized, errors due to size and placement of occluding cuffs) have been summarized(1,2,3). To circumvent these difficulties, we have adapted for routine use the method of permanent aortic intubation first described by Still *et al.*(2,4). Still's original method involved inserting a small polyethylene tube into the lumen of the aorta and leading it beneath the skin to an exit on the back of the neck. The free tip was then heat sealed and cut off for each measurement. We have made 3 modifications: (1) employing hypodermic needle tubing to bring the tube through the skin, (2) using a wire to plug the free end of polyethylene tubing, (3) using small size polyethylene tubing only for insertion into the aorta, the remainder being a larger size. The needle tubing allowed easy replacement of the end piece of polyethylene as it stretched and frayed with use; while larger tubing allowed systolic and diastolic pressures to be measured.

Methods and materials. Construction of the tube to be implanted involves fabrication of 3 items: (1) a "J"-shaped piece of polyethylene tubing (passing from aorta to back of the neck), (2) a piece of hypodermic needle tubing (which exits through the skin), and (3) an end piece of polyethylene stiffened by a jacket to resist mechanical trauma (Fig. 1). The tube is implanted as follows. Make a transverse skin incision about 15 mm behind the ears in anesthetized rats at least 200 g in weight. Open the abdomen on the midline, remove viscera and cover with gauze. Using a dry gauze sponge, wipe the aorta clean in the region of the bifurcation. Pass a 14 cm hubless trocar (made from 16 ga needle tubing with blunt end enlarged to about 2.5 mm o.d. by a drop of solder) through the psoas muscles lateral to left genito-femoral nerve and medial to left ureter, then on to the incision in neck. Using a few mm of PE 50, couple aortic tube and attached needle tubing to a 16 cm length of 22 ga needle tubing, insert into the trocar as far as possible, and pull through the tissues to neck incision. Thrust a needle through the skin in the midline about 6 mm in front of incision. Using the needle as a guide, pass needle tubing through the skin. Slip on the reinforced PE 50 "end piece" and attach to a tube leading to a syringe filled with isotonic saline. Using silk sewing thread (size A), make a single stitch through neck muscles, tie a square knot thus forming a loose loop anchored to the neck, then tie securely to the polyethylene tubing. Close neck incision. Anchor polyethylene tubing alongside the aorta just above the bifurcation by one stitch of the attached silk thread through psoas muscles. Cut the tip of the PE 10 on a bevel at a point slightly below renal arteries and fill tubing with saline. Occlude the aorta with the tip of a finger and puncture with a 26 ga needle about even with the anchor stitch. Withdraw needle and insert polyethylene tubing. Although seldom necessary, bleeding may be controlled by a wisp of cotton-type oxidized cellulose and gentle pressure. Replace viscera and close abdomen. No anticoagulant is necessary in the tube. About 80% of intubations were successful. Since troublesome infections occurred occasionally, we sterilized instruments and materials in a 1:1000 solution of benzalkonium chloride. Procaine penicillin G 40,000 units and dihydrostreptomycin 50 mg (as 0.2 ml, i.m., of Dihydrocillin, Upjohn) were given postoperatively. Rats must be caged individually. Blood pressure was measured using a Statham P23G pressure transducer (Statham Instruments, Hato Rey, Puerto Rico) and a Grass Model 5 polygraph

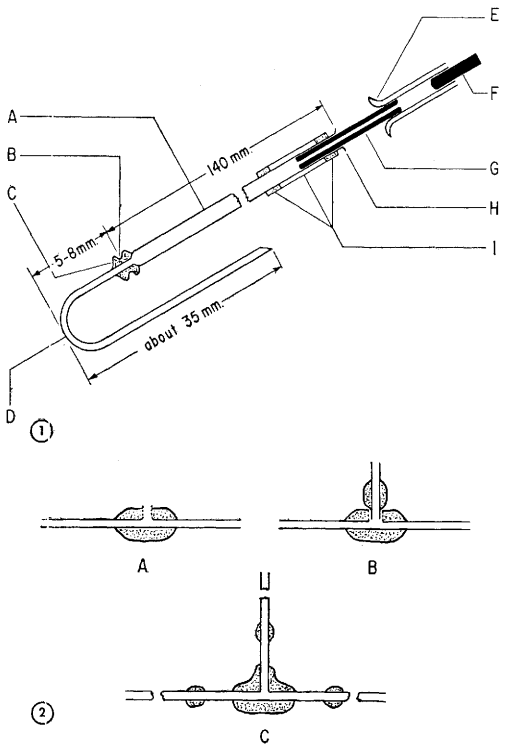

FIG. 1. Polyethylene tubing and accessories for aortic intubation. A. PE 50 tubing, 140 mm length for rats to 350 g (Clay-Adams, N. Y.). B. 4-O silk suture tied between ridges for anchoring tubing alongside aorta. C. PE 50 sealed to PE 10 [Insert pipette probe wire (Fisher 6-214, ground smaller at tip if necessary) into lumens, melt together by rotating slowly against pointed tip of moderately hot soldering iron (Ungar 4035, No. 332 tiplet, operated at about 70 volts). Melt again on PE 50 to form second ridge.] D. PE 10 fixed into "J"-shape (form loosely around glass rod and dip into boiling water). E. PE 50 with PE 160 protective jacket (hold near flame to seal together). F. 7 mm wire plug made from 18 ga needle stylet, inner end bluntly tapered (cut and grind this wire and needle tubing using dental separating disc with stream of water playing continuously on both sides). G. 20 mm long 22 ga stainless steel needle tubing with smooth rounded ends. H. PE 50 projecting beyond PE 50-160 seal flared by heating near a small flame. Tubing anchored to neck muscles by tying in the groove between flare and seal. I. PE 160 jacket 20 mm long sealed at both ends over PE 50 as in C above using a 21 ga needle stylet to maintain lumen.

FIG. 2. Preparation of polyethylene "T"-tubes. A. Insert wire (21 ga needle stylet) into lumen of PE 50, thicken wall by rotating against chisel tip (Ungar No. 333) of moderately hot soldering iron. Drill hole (No. 60) for side arm. B. Make similar thickening, cut one end very short, insert stub into hole, insert wire into side arm, and seal by rotating thickened joint against pointed tip, taking great care not to touch the thin walls of the tubing. C. Attach additional lengths of PE 50 to the arms of the "T" by melting together over a wire.

(Grass Instrument Co., Quincy, Mass.). The transducer was arranged with side outlet connected to controlled air pressure for calibration. A 3-way stopcock on the recording outlet allowed saline to be injected into the rat. Connection between rat and transducer was by a length of PE50 tubing with a piece of 22 ga needle tubing on the end. The rat was immobilized by wrapping in a towel with head exposed, the end piece of polyethylene was occluded by grasping with a shod hemostatic forceps, the wire plug removed, the tube connected to transducer and flushed with about 0.2 ml of isotonic saline, then B.P. recorded continuously for at least 1 minute. Average pressure could easily be estimated. If the tube seemed obstructed, saline was forcibly injected using a 0.25 ml syringe. At other times, it was necessary to massage the aorta gently through abdomen. Presumably, the tip of the tubing was lying against the wall of the aorta or partially occluded by a small piece of fibrin. Aortic thrombi and aneurisms have been rare since the tube was anchored alongside the aorta as described. Rats so prepared have been used for as long as 6 months. We have used this method routinely in B.P. studies for over a year and have prepared at least 300 rats. Drugs may be injected into the aorta with only a few seconds interruption of pressure recording. A "T"-joint was fabricated in the PE 50 tubing leading from transducer to rat (Fig. 2) and the side-arm of "T" connected through a small stopcock to a syringe. After closing the connection to the transducer, drugs were injected, then flushed into the animal by about 0.1 ml of saline from the syringe on the transducer.

Discussion. Although we have routinely recorded mean arterial pressures (by electronic damping of the pulse pressure), systolic and diastolic pressures may also be measured. The same values were obtained when, in an anesthetized rat, pressures were recorded simultaneously from one carotid artery directly to a transducer using a 20 ga needle as a cannula, and from the other carotid artery using a complete aortic tube assembly connected to a second transducer. In the latter however, the contour of the pulse wave was rounded.

Normal mean arterial pressure, based upon 44 Wistar origin females, age 2 to 5 mo., 3 successive measurements per rat on different days, averaged 129 mm Hg. Analysis of variance showed the rat to rat standard deviation (square root of rat component of variance) to be 7.7 mm Hg, and day to day standard deviation on a given rat to be 6.2 mm Hg(5). Most of our experience has been with Grollman type(6) renal hypertensive rats, in which B.P. as high as 300/200 have been recorded. We have considered rats as hypertensive when their mean B.P. was over 150 mm Hg, most of them ranging between 160 and 200 mm Hg.

Reliability of readings from the photoelectric tensometer(7) were studied by simultaneous recording of direct aortic B.P. Our tensometer, built within The Upjohn Co., recorded cuff pressure and light transmission through the foot simultaneously on a paper chart. Personal bias was minimized by accepting all clear-cut readings. A cuff as designed by Rosett and Handler was used(2). Five successive readings were made, the cuff removed and replaced in apparently the identical manner, and another 5 readings made; 4 such series an each of 10 different rats. Actual B.P. from the aorta varied only a few mm of Hg. Tensometer B.P. agreed well within the 5 replicate readings when the cuff was undisturbed (average range 25 mm Hg), but there were often large variations between averages of replicate series on the same rat (up to 70 mm Hg, average range of series means 40 mm Hg), which could be due only to cuff placement. That the presence of the tube in the aorta might have altered blood flow to the legs, and so caused such variations, seems doubtful since a similar series using a polyethylene tube tied into a carotid artery showed the same discrepancies. (Carotid cannulated preparations remained functional only a few days.) Within the limits imposed by cuff placement variation, tensometer and direct aortic systolic pressures agreed.

Summary. Method is described whereby polyethylene tubes, permanently implanted in aorta, may be used routinely for repeated direct measurement of arterial pressure in unanesthetized rats.

1. Ablondi, F., SubbaRow, Y., Lipchuck, L., Perseneus, G., *J. Lab. Clin. Med.*, 1947, v32, 1099.

2. Still, J. W., Whitcomb, E. R., *ibid.*, 1956, v48, 152.

3. Rosett, T., Handler, P., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 391.

4. Still, J. W., Pradhan, S. N., Whitcomb, E. R., *J. Appl. Physiol.*, 1956, v8, 575.

5. Snedecor, G. W., *Statistical Methods,* (5th edit.) 1956, Iowa State College Press, Ames, 259.

6. Grollman, A., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 102.

7. Kersten, H., Brosene, W. G., Jr., Ablondi, F., SubbaRow, Y., *J. Lab. Clin. Med.*, 1947, v32, 1090.

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Influence of Certain Basic Compounds on Conversion of Mevalonic Acid To Cholesterol by Liver Homogenates.* (25938)

LEMUEL D. WRIGHT AND ESTHER ENGLERT
Graduate School of Nutrition, Cornell University, Ithaca, N. Y.

Evidence has been presented that a polyanion is essential for maintenance of ATP levels in whole rat liver homogenates incubating aerobically(1). The "natural" polyanion in this system appears to be RNA(2) but other polyanions such as DNA, polyethylene sulfonate, and heparin may substitute for it(3). ATP levels are not maintained in the presence of the *polycation* protamine presumably because of formation of an anion-cation complex that renders the essential polyanion unavailable(4). Additional data are presented here concerning the specificity of the polycation as an inhibitor of the system essential for

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