HAYWOOD, JOSEPH R., RICHARD A. SHAFFER, CRAIG FASTENOW, GREGORY D. FINK, AND MICHAEL J. BRODY. Regional blood flow measurement with pulsed Doppler flowmeter in conscious rat. Am. J. Physiol. 241 (Heart Circ. Physiol. 10): H273–H278, 1981.—Development of techniques for the continuous measurement of regional blood flow and vascular resistance in intact small animals has been impeded primarily by the bulkiness of flow probes. The availability of an ultrasonic pulsed Doppler flowmeter system enabled us to construct miniaturized probes using 1-mm-diameter piezoelectric crystals that emit a 20-MHz signal and receive the reflected sound waves from passing blood cells. The finished flow probe is approximately 2.5–4 mm long and 2 mm in cross-sectional diameter with lumen diameters appropriate for the rat, ranging from 0.7 to 1.2 mm. This report describes the materials and methods involved in constructing and implanting the probes in rats to monitor renal, mesenteric, and hindquarter blood flow velocity. The accuracy of the pulsed Doppler method in detecting changes in regional blood flow and vascular resistance was established by the demonstration of a highly significant correlation between velocity signals recorded from the Doppler unit and volume flow recorded simultaneously. These data indicate that the ultrasonic pulsed Doppler flowmeter provides the opportunity to measure changes in regional blood flow and vascular resistance in a conscious freely moving rat.

Flow Probe Construction

The ultrasonic pulsed Doppler flowmeter used in this study was constructed by the Bioengineering Resource Facility of the University of Iowa from the plans generously supplied by C. J. Hartley of Baylor College of Medicine. The system is basically the same as that described by Hartley and Cole (5); however, several modifications have been made to increase the sampling frequency of the echo signal to improve the accurate detection of high-velocity signals. Modifications have also been made in the preparation of the crystal and the construction of the flow probe.

Flow Probe Construction

The pulsed Doppler flow probe consists of a Silastic cuff around a piezoelectric crystal with insulated copper wire leads. The four stages of construction are detailed by Hartley and Cole (5), in which several probes are driven by a common oscillator. Like the continuous-wave Doppler, the pulsed Doppler flowmeter offers the advantage that zero flow can be determined electronically. However, in contrast to the continuous-wave flowmeter, the detection system of the pulsed Doppler consists of a single lightweight piezoelectric crystal that emits a 20-MHz ultrasonic signal. The same crystal receives the reflected signal from the passing blood cells in the intervals between ultrasonic pulses. Although the system measures Doppler shift, a relative index of blood flow, the reduced size of the crystals permits the chronic implantation of multiple flow probes in small animals, such as the rat.

This report describes detailed methods for the preparation of the crystal and construction of the flow probe for implantation. Although Hartley et al. (6) have presented preliminary observations on the use of this flowmeter system in the conscious rat, we report refined procedures for the acute or chronic placement of these probes in small- and large-diameter arteries in rats for application of the pulsed Doppler flowmeter in chronically instrumented rats. Finally, evidence is presented that changes in the velocity signals recorded from the flow probes are directly and reliably proportional to changes in true volume flow.
in Fig. 1. A dissecting microscope should be used for each stage of construction.

Stage A. The 36-AWG insulated silver-plated copper wire leads (CZ1174/SPC, Cooner Sales, Chatsworth, CA) are prepared for soldering by first removing 1 mm of insulation from each end. Care must be exercised to prevent the multistranded wire from being nicked or cut. Noncorrosive 65/35 resin core solder is used to tin both ends of the wire. A miniature soldering iron (6 V, 6 W, pencil type) is used for all soldering steps. A very small bead of solder is placed on one end of the wire after tinning. The PZT-5A piezoelectric crystal (20 mHz, 1 mm diam x 0.004 mil, compress mode, fine-ground, chrome-gold plate; Valpey-Fisher, Hopkinton, MA) is placed on a firm flat surface. A thin film of TIX solder flux is applied to a small area near the edge with a 27-gauge needle. The flux will allow the solder to flow smoothly onto the chrome-gold plating. The beaded end of the wire is placed on the fluxed area, with the edge of the insulation flush with the edge of the crystal (do not apply pressure to the surface of the crystal). The solder bead is gently touched with the soldering iron until the solder flows onto the crystal surface, and the solder is kept to a small area of the crystal. If the solder is allowed to cover too much surface, the vibrating characteristics of the crystal will be diminished. The wire is then rotated carefully until the solder joint is face down. The other lead wire is soldered to the remaining surface (Fig. 1A). The wire leads are taped down and very gently bent up to elevate the crystal into a horizontal position above the work surface.

Stage B. The piece is encapsulated with epoxy to strengthen and protect the crystal and solder joints (Fig. 1B). After being mixed, the epoxy (Plast no. 86/87, Fibre-Glast Developments, Dayton, OH) is degassed under vacuum for 15–20 min to remove all trapped air. If not removed, this trapped air will greatly attenuate the signal emitted and received by the crystal. The pot life of the epoxy is about 1 h. The epoxy is applied with a 27-gauge needle to both surfaces and edges of the crystal, which should be covered by a very thin film of epoxy when finished. The epoxy is allowed to cure for 24 h, and the work is not moved during this time.

Stage C. After 24 h, the crystal is placed in a Silastic mold designed to hold the crystal at an approximately 45° angle to the vessel, the optimum angle for detection of flow velocity. The mold is a 3-mm-length of Silastic tubing (1.0 x 1.0 mm; Dow Corning, Midland, MI). One end is cut perpendicular to the lumen, the other end is cut at a 45° angle to the lumen. A squared-off 22-gauge needle with sharpened edges is used to punch a hole in the side of the mold. The paired probe wires are pulled through this hole until the crystal is inside the mold. The crystal should now be perpendicular to the lumen, facing the 45° angle cut (Fig. 1B). The beveled end of the Silastic mold is filled with sticky dental wax (Caulk Red Sticky Wax; R. D. Caulk, Milford, DE). A 27-gauge needle is used to fill the unbeveled end of the mold with Silastic medical adhesive, which is allowed to cure for 24 h. This material stabilizes the crystal in the mold. A thin film of polyurethane foam (Plast 24/25A, Fibre-Glast Developments) is spread with a 27-gauge needle over the surface of the cured Silastic. The polyurethane foam, which acoustically baffles the back side of the crystal preventing the detection of flow from any surrounding areas (Fig. 1C), also is allowed to cure 24 h.

Stage D. The final stage of probe construction (Figs. 1, C and D) is the formation of the Silastic cuff that is sutured around the vessel. The crystal assembly is then attached to a stainless steel hypodermic needle, appropriate in size to the diameter of the vessel, by warming the wax slightly and pressing the mold flush with the needle (Fig. 1C). When the wax cools, it will hold the mold firmly to the needle. A small amount of adhesive (Silastic medical adhesive type A 891, Dow Corning) is placed on the end of a 27-gauge needle and painted on

![Diagram](http://apjheart.physiology.org/10.22033.5/on August 28, 2017)
the crystal mold complex (Fig. 1D) by rotating the needle to cover all sides. A large cork may be used to hold the needle to facilitate rotation of the mold during encapsulation. Care should be taken to prevent a bulky buildup of Silastic on the mold. The adhesive should be rather thin on the upper part of the mold but thicker on the underside opposite the probe to add strength for suturing. After 24-h curing, the encapsulated probe is removed from the needle by cutting parallel to the needle on the side opposite the crystal. The cut should be straight to ensure even flaps for suturing. A warm soldering iron is placed on the needle to melt the dental wax, and the probe is very gently peeled off the needle. The remaining wax is removed from the probe cavity with small forceps. The pencil soldering iron is then inserted into the cavity to melt away any remaining wax from the surface of the crystal.

**Chronic Implantation of Flow Probes**

Measurement of mesenteric, renal, and hindlimb blood flow velocity in the conscious animal requires chronic placement of the Doppler flow probes on the major arteries. To accomplish this, rats are anesthetized with 50 mg/kg pentobarbital sodium, and a midline abdominal incision is made, exposing the superior mesenteric and left renal arteries and the abdominal aorta. After the lumen of the probe is filled with coupling gel (Ultrasound Gel, Tucker Laboratories, Orange, NJ), the probe is placed around the blood vessel and loosely sutured closed with 6-O ophthalmic silk (Fig. 2). The insulated wire leads are anchored to the abdominal wall to prevent a change in the orientation of the probe to the vessel. The wires are then tunneled out the back of the abdominal cavity and led subcutaneously to the back of the neck. The wire leads are soldered to an ultraminiature receptacle (GF-6, Microtech, Boothwyn, PA). Three holes are then drilled in the skull (no. 68 drill bit) and small stainless steel screws are inserted (no. 52-10, Lomat Watch, Montreal, Canada). The receptacle is placed over the screws, and the entire unit is attached to the skull with cranioplastc cement (Plastic Products). An ultraminiature plug (GM-6, Microtech) with shielded miniature twisted pair cables (NMFU-2/30-4046SJ, Cooner Sales) with a flexible spring covering connects the Doppler flowmeter to the receptacle containing the flow-probe wires. The signals are then recorded either as pulsatile or mean flow velocity of a Doppler shift (kHz) on a Beckman Dynograph. In addition, polyethylene catheters (PE-50) are placed in the femoral artery and jugular vein and exteriorized through the back of the neck. Femoral artery pressure is monitored with a Century CP-01 pressure transducer, permitting calculation of regional vascular resistances from pressure and mean flow recordings. Drug injections are made via the venous catheter.

**Comparison of Pulsed Doppler and Electromagnetic Flowmeters**

To ascertain the validity of the pulsed Doppler flowmeter for use in determining acute changes in regional blood flow, a simultaneous comparison was made of responses measured with Doppler and electromagnetic flowmeters. Blood flow to the left kidney was measured with an electromagnetic flowmeter using the autoperfusion method developed by Fink and Brody (2). This technique involves the diversion of blood from the left carotid artery through an extracorporeal flow probe retrograde into the lower aorta. Blood flow is supplied only to the left kidney by occluding the aorta between the superior mesenteric artery and the left renal artery, thus creating a cul-de-sac in the aorta and leaving only the kidney through which blood may pass. By cannulating the lower aorta, a section of the vessel was free for placement of the Doppler flow probe with an 18-gauge lumen while leaving the kidney and renal artery untouched. All other blood vessels between the lower aorta and left renal artery were ligated. Alterations in renal blood flow were induced by injection of graded doses of norepinephrine (10, 20, and 40 ng) and acetylcholine (3, 10, and 30 ng) intra-arterially into the extracorporeal tubing.
Measurement of Doppler Shift

Velocity signals recorded from flow probes around vessels are made optimal for each probe separately. The range adjustment of the flowmeter is altered until the peak voltage displacement (analogous to the Doppler shift) is achieved. This corresponds to focusing the ultrasound on the midstream of the vessel. Zero flow is established by turning the ultrasound signal off. (In all experimental trials electronic zero was equal to zero flow obtained by arterial occlusion distal to the probe.)

Data Analysis

Changes in renal blood flow (ml/min) and flow velocity in Doppler shift (kHz) were compared by least-squares regression analysis. A similar correlation was made for percent changes in vascular resistance calculated for the two methods. Significance of the correlation was determined by testing the significance of the Pearson product-moment correlation. Significance was assumed at the \( P < 0.05 \) level.

RESULTS

The flow probes constructed in our laboratory and the pulsed Doppler flowmeter have been used repeatedly in both acute and chronic rat preparations. As shown in Fig. 2, excellent pulsatile velocity profiles that coincide with arterial pressure pulses were recorded from conscious rats. During short-term chronic experiments (2–6 days), rats prepared with Doppler flow probes have remained healthy. No experiments are performed the day after surgery. In chronic experiments, the incidence of probe failure has been very low.

A typical response to an intravenous bolus of norepinephrine administered to a conscious rat 3 days after implantation is shown in Fig. 3. The predictable decreases in flow to the gut and kidney occurred. Little change in hindquarter flow, presumably resulting from nearly simultaneous direct vasoconstriction and reflex vasodilation, was seen. All three vascular beds were calculated to exhibit an increase in vascular resistance. Ganglionic blockade with hexamethonium caused a large decrease in arterial pressure, leading to a passive decrease in mesenteric and hindquarter blood flows. Renal flow remained relatively constant despite the large decrease in arterial pressure, presumably due to autoregulation. Following hexamethonium, the same dose of norepinephrine again caused decreases in mesenteric and renal blood flow and a slight increase in hindquarter flow. In this example, the increase in hindquarter blood flow is presumably due to the larger increase in arterial pressure redistributing cardiac output to the muscle bed. The calculated change in vascular resistance to all three vascular beds during norepinephrine injection after ganglionic blockade was potentiated.

A comparison of instantaneous responses detected by the pulsed Doppler system and the electromagnetic flowmeter (Fig. 4) indicates that changes in Doppler shift elicited by renal nerve stimulation correlate very well, both qualitatively and quantitatively, with alterations in renal blood flow. When changes in renal blood flow produced by graded doses of acetylcholine and norepinephrine were compared by both methods of blood flow measurement, a linear relationship was observed (Fig. 5), with a highly significant correlation coefficient \( (r = 0.969, P < 0.001) \). Individual regression lines for the five animals were very consistent, as evidenced by minimal scatter of the data points (Fig. 5). When the responses to norepinephrine and acetylcholine were compared in terms of percent change in initial vascular resistance (changes in Doppler shift/initial Doppler shift), the two methods again showed a highly significant correlation \( (r = 0.973, P < 0.001) \).

DISCUSSION

The pulsed Doppler flowmeter affords an unique op-
Regional blood flow in conscious rat

Regression analysis yielded linear relationship with correlation coefficient of $r = 0.969$.

Wires tunneled subcutaneously do not impede normal abdominal aorta of the rat without interfering with the function of other organs. Furthermore, the fine-gauge wires around the renal and mesenteric arteries and the lower is approximately 2.5-4 mm diam. The probes easily fit blood vessels in the same animal. The advantages of the pulsed Doppler system, on the other hand, are numerous.

The use of a single piezoelectric crystal and 36-AWG wire leads permits miniaturization of the flow probe so that it is approximately 2.5-4 mm diam. The probes easily fit around the renal and mesenteric arteries and the lower abdominal aorta of the rat without interfering with the function of other organs. Furthermore, the fine-gauge wires tunneled subcutaneously do not impede normal body movements. Zero flow is determined without an occluding cuff. Finally, although volume flow is not derived quantitatively, percentage changes in flow, and thus vascular resistance, can be calculated accurately.

Detection of flow with the pulsed Doppler system is dependent on changes in the emitted ultrasonic frequency caused by the reflection of the signal off moving blood cells. The change in frequency, referred to as Doppler shift, is proportional to the velocity of the blood cells in the vessel, given that the angle of the crystal to the vessel and the velocity of sound through the fluids and surrounding tissues remain constant. Then, assuming the cross-sectional area of the vessel adjacent to the probe remains constant, the Doppler shift is also proportional to the actual blood flow to the organ. This linear relationship between volume flow and Doppler shift exists for both the continuous-wave Doppler flowmeter and the pulsed Doppler flowmeter.

The proportionality of the changes in blood flow detected by the ultrasonic pulsed Doppler and the electromagnetic flowmeter systems indicate the Doppler method is a valid technique for measuring alterations in regional blood flow or changes in vascular resistance in the awake rat. The observations described in this paper demonstrate the feasibility of simultaneous measurement in the rat of renal, mesenteric, and hindquarter blood flow and arterial pressure. The development of refined techniques for Doppler flow-probe construction and implantation in the rat permit the opportunity to gain further understanding of the regulation of the cardiovascular system during experimental or pharmacologic interventions in the conscious freely moving rat.

Although we have now had considerable experience with these techniques in rats (3, 7, 9, 10, 12), this report describing flow-probe construction and animal preparation make the pulsed Doppler flowmeter system available for extensive application. In fact, investigators at our institution are now using modifications of the basic technology described here to record cerebral blood flow velocity from stereotaxically placed probes mounted on needles (8), coronary flow velocity in human subjects (10A) and in rats (11) using probes attached to the cardiac surface with suction (10A), pial artery flow velocity recorded through a pial window (1), and regional hemodynamics in the conscious cat. It is anticipated that many other investigators with special needs for blood flow measurement will be attracted to the advantages of the pulsed Doppler approach.

Received 3 March 1980; accepted in final form 16 March 1981.

REFERENCES

