# METHODS

# Ultrasound as a Tool in Experimental Research of Macro- and Microcirculation

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Peculiarities of application of two modes of ultrasonic measurements of blood flow in experimental research are considered, which are based on Doppler effect and on differential transit-time of upstream and downstream sound propagation. The efficiency of high-frequency ultrasound flowmeter equipped with 26.8 MHz transducers was demonstrated in measurements of blood flow in rat midbrain and coronary arteries. This approach can be used for evaluation of the dynamics of cardiac output with an intravascular catheter 0.6 mm in diameter working at 33 MHz. The probe and electronic scheme of the devise for measuring blood flow in microvessels are described. Blood flow rate measured in mesentery and *m. cremaster* arterioles under normal conditions was 2-12 mm/sec. One-element probe working at 38.5 MHz provided stable recording of blood microflows in 30-40-μ vessels.

**Key Words:** *ultrasound; biomicroscopy; microcirculation; blood flow transducers*

Clinical and experimental measurements of blood flow in major vessels are routinely performed with devices based on electromagnetic or acoustic methods. These devices provide sufficient precision and high reproducibility of the measured pulse flow curve. By contrast, ultrasonic technique is more frequently used for measurements in small vessels (<1 mm in diameter). This technique is characterized by high sensitivity and small size of transducers. Ultrasonic devices for measuring blood flow are based on two phenomena: Doppler effect [14] and differential transit-time of upstream and downstream sound propagation, implemented in phasometric and pulse mode of operation [13,15]. The devices based on the latter principle are characterized by high precision, because ultrasonic beams passing through the vessel integrate the velocity profile of the blood flow [13]. This feature was corroborated in tests of pulse-operated ultrasonic device with pulsing flows in Pitot tubes conducted in Department of Biological Engineering of Institute of General Pathology and Pathological Physiology [10]. The Doppler-operated device based on reflection from blood cells recorders a spectrum of ultrasonic signals reflecting distribution of blood elements within the flow profile. Mathematical processing of this Doppler spectrum yields mean velocity over the cross-section of the vessel. This parameter is used for calculation of the blood flow rate, which is measured with cuff-type transducers.

The above guidelines were used at the Department of Biological Engineering to develop specialized ultrasonic devices, which were tested with blood flows [4,5]. The cuff-type transducers of Doppler- and pulse-operated devices were placed together on the carotid and femoral arteries and on the abdominal aorta in dogs. The probes of pulse-mode device were preliminary calibrated in units of blood flow rate, and they served as the control. Experiments showed that readings of Doppler-type device depend on various factors and mainly on the geometrical proportions between blood vessel and piezoceramic ultrasonic trans-

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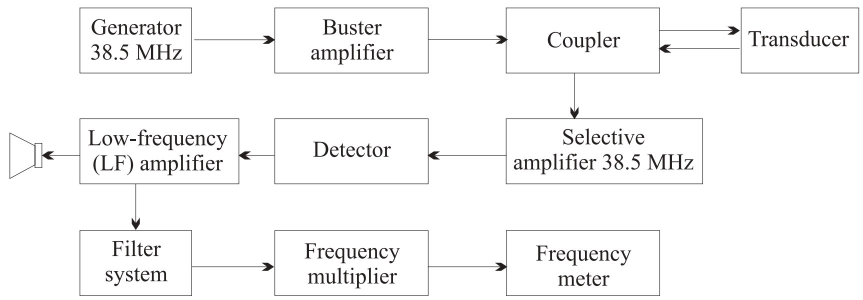


**Fig. 1.** Block diagram of flowmeter designed to measure blood flow in microvessels.

ducer. This relationship is used for calculation of blood flow rate measured with cuff probe of Doppler-type device.

One of the main advantages of Doppler technique is possibility to attain high sensitivity by increasing ultrasonic frequency. In the developmental devices we successfully used frequencies of 5, 6.5, 8, and 13 MHz in two-element probes, in which radiation and receiving of ultrasound were performed by individual piezoceramic crystals [2,9]. To measure blood flow rate in small vessels with diameter of 150-200 μ, we constructed a device working at 26.8 MHz and supplied it with miniature one-element probes of contact- and cuff-types, which radiated and received ultrasound [8]. This device made it possible to measure blood flow in coronary arteries and in branches of the midbrain artery in rats, which was principal in the studies of cerebral and coronary circulation [8,11,12].

Further development of high-frequency ultrasonic technique in experimental studies was performed with an ultrasonic catheter [7]. A transducer with a diameter of 0.6 mm was introduced into the aorta via the carotid or femoral artery and used for evaluation of the dynamics of cardiac output, stroke volume, and other hemodynamic parameters during acute or chronic experiment. High sensitivity and small size of intravascular transducer resulted from the use of 33-MHz ultrasound.

Application of high-frequency ultrasonic technique in biological experiments showed that it can be applied to study microcirculation under conditions of biomicroscopy. To this end, we tested some variants of electronic devices. A device based on one-element probe working in continuous-wave mode was the best. In this probe (width 0.7 mm, height 1 mm) the sensitive element was a 0.3-mm$^2$ piezo-crystal plate made of titanium-zirconium lead salt. The piezo-element is placed in the probe at the angle of 50$^o$ to the vessel plane. It beamed ultrasound at 38.5 MHz, which propagated via an acoustic lens with focal distance of about 1 mm. Transversal resolution of ultrasonic beam in the focal plane was 200-250 μ. To determine this parameter, we used an ultrasound scanner. The targets were made of materials with high reflection power. Specifically, we used fine wires with diameter of 30-45 μ and aramid fibers with total diameter of 20-60 μ.

The device was constructed according to routine scheme and consisted of two parts: a high-frequency unit and Doppler-signal processing unit (Fig. 1). The major element of the high-frequency unit was a clock generator tuned at 39.5 MHz. Its high-frequency output was fed to buster amplifier and then (via a limiting

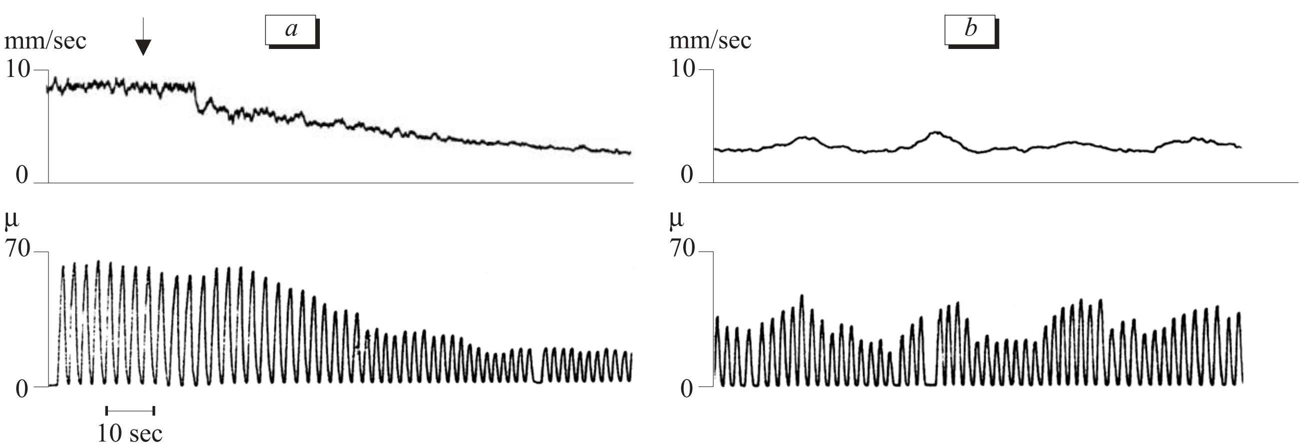


**Fig. 2.** Simultaneous measurements of blood microflow and arteriole diameter in rat mesentery. *a*) effect of epinephrine (0.8 μg); b) vasomotion at low blood flow rate in microvessel. The upper and lower curves show flow rate and vessel diameter. Here and in Fig. 3: the arrow marks the moment of stimulation.

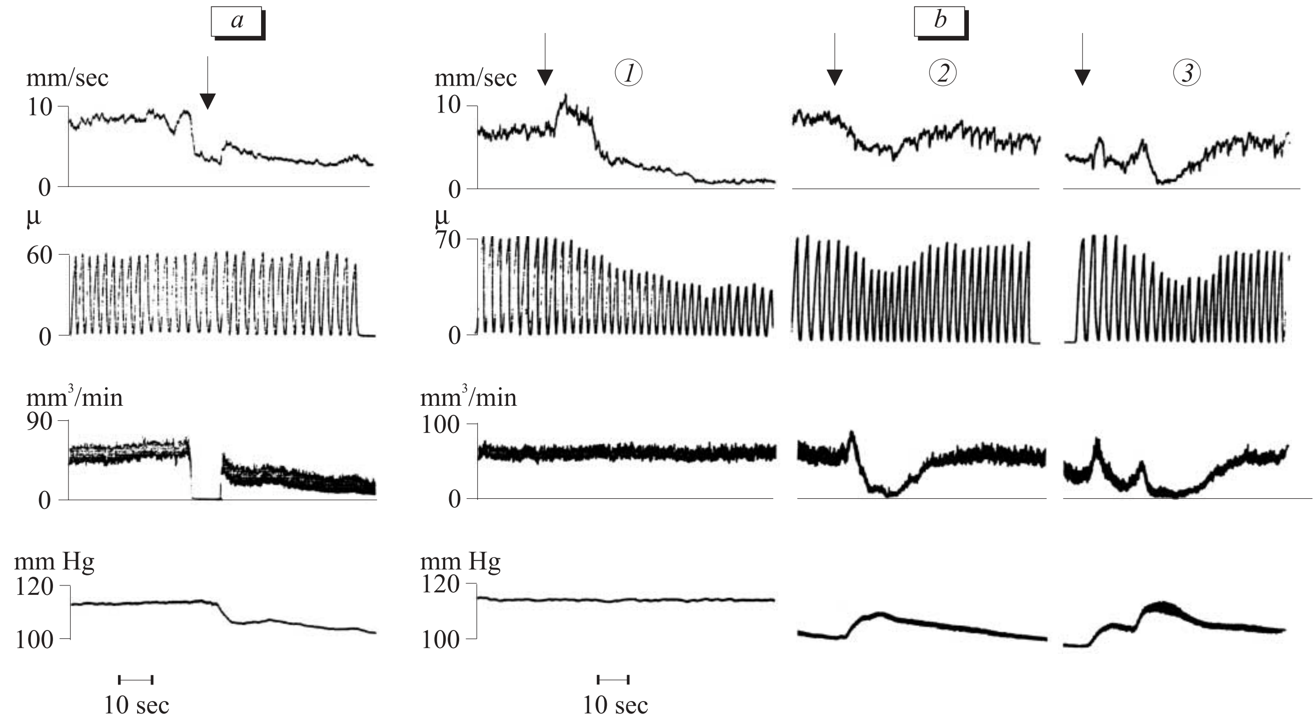


**Fig. 3.** Effect of epinephrine and occlusion of arterial macrovessel on macro- and microflows. *a*) occlusion of mesentery arterioles (diameter 200 μ). The upper and lower curves show flow rate and vessel diameter, blood flaw in microvessels, blood pressure; *b*) effect of epinephrine. *1*) application of epinephrine (0.8 mg) to arteriole with diameter of 70 μ; *2*) application of the same dose of epinephrine to artery with diameter of 200 μ; *3*) intravenous injection of epinephrine (0.5-1.0 mg).

resistor) to piezo-element of the transducer. A peculiar feature of this unit was low level of modulation noise. During continuous radiation of ultrasound focused on blood microvessel, the piezo-element of the probe was affected by the wave reflected from the blood formed elements. Superposition of incident and reflected waves on the piezo-element resulted in ultrasound beating. Via a phase-shifting chain, this signal traveled to low-noise high-frequency amplifier, and then it was rectified to yield the Doppler frequencies. These frequencies were amplified by a low-frequency amplifier for sound control. They were also used in Doppler signal processing unit to measure the flow rate.

The Doppler-signal processing unit consisted of a selective amplifier, a filter system of low and high frequencies, frequency multiplier, and a zero-crossing counter (frequency meter). The non-linearity of frequency meter was 7-10 % in the range of 20 Hz to 6 kHz.

The described device was tested together with Dr. M. I. Timkina. The vessels were chosen in rat mesentery and *m. cremaster*. The ultrasonic transducer was focused on microvessel under MBI-15 microscope with the help of micromanipulator, the ultrasonic beam being directed along a microvessel. The optimal position of the transducer was chosen according to sound signal. There were no problems in using 1-mm-height transducer with short-focus objectives widely used in biomicroscopy.

Measurements of microvessel diameter were performed by image splitting method modified by P. N. Aleksandrov [1]. The tests of the first specimens of ultrasonic transducers demonstrated their stable work on the vessels with diameter of 30-40 μ. The value of linear velocity recorded in mesenteric and *m. cremaster* arterioles was 2-12 mm/sec under the normal conditions (Fig. 2). Since the angle between the piezo-element and vessel plane was 50º, the calculated value of Doppler frequency corresponded to 40 Hz per 1 mm/sec, which was within the range of sensitivity of the device.

To demonstrate interrelation between macro- and microcirculation, in some experiments we simultaneously recorded blood flow in an arteriole and in the corresponding feeding artery (Fig. 3).

Measurements of blood flow in proximal subdivisions of resistance macrovessels were performed with ultrasonic cuff-type transducers (diameter 200 μ, frequency 26.8 MHz). These transducers were calibrated in units of blood flow.

Reactions of the vascular system were induced by physiologically active substances affecting various parts of vascular bed. In addition, occlusion of macrovessels was performed.

The reported studies were carried out to demonstrate the possibility of combined precise study of regional blood macroflow and microcirculation based on common methodical approach.

Thus, use of high-frequency Doppler technique during regional biomicroscopy promotes the study of complex hemodynamic relationships between various levels of integrative circulatory system and reveals changes of these relationships during normal and pathological conditions.

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