Continuous measurement of tissue blood flow by laser-Doppler spectroscopy

MICHAEL D. STERN, DONALD L. LAPPE, PATRICK D. BOWEN, JOHN E. CHIMOSKY, G. ALLEN HOLLOWAY, Jr., HARRY R. KEISER, AND ROBERT L. BOWMAN
Laboratory of Technical Development and Laboratory of Experimental Therapeutics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20014; and Division of Bioengineering, University of Washington School of Medicine, Seattle, Washington 98195

Stern, Michael D., Donald L. Lappe, Patrick D. Bowen, John E. Chimosky, G. Allen Holloway, Jr., Harry R. Keiser, and Robert L. Bowman. Continuous measurement of tissue blood flow by laser-Doppler spectroscopy. Am. J. Physiol. 232(4): H441-H448, 1977 or Am. J. Physiol.: Heart Circ. Physiol. 1(4): H441-H448, 1977. — Laser light scattered from tissue in vivo is broadened in line width as a result of the Doppler shift produced by moving red cells in the microcirculation. A feasibility study was carried out to demonstrate use of this effect to measure and monitor tissue blood flow. Light from a helium-neon laser illuminated a 1-mm area of the skin of volunteers subjected to UV-induced erythema and was found to vary in an approximately linear manner with skin blood flow. The laser instrument provided continuous monitoring of blood flow fluctuations, including the pulsatile component. The instrument was used to monitor flow in the outer cortex of the rat kidney during administration of norepinephrine, angiotensin, hydralazine, dextran, dopamine, nitroprusside, and angiotensin blocked by saralasin. Dynamic and steady-state responses were consistent with known pharmacology and renal physiology, and with the assumption that vasoconstrictor angiotensin II receptors in the kidney are accessible to blood-borne inhibitors. The laser Doppler method is a promising tool for rapid monitoring of dynamic changes in tissue perfusion.

Microcirculation; capillary blood flow; coherent light; Doppler effect; xenon-133; renal blood flow; angiotensin; norepinephrine; hydralazine; renal cortex; blood flow velocity

The need to measure local blood flow in small volumes of tissue arises in many contexts in physiology, pharmacology, and clinical medicine. Available approaches include, depending on the tissue involved, washout of radioactive indicators (11, 15, 16), thermal uptake with heated probes (13), various forms of plethysmography (22), implanted hydrogen electrodes or radiation detectors (1, 10), and the trapping of labeled microspheres. An ideal method would provide a continuous reading of the blood flow in a small, localized region of tissue; it would not disturb the state of the circulation, and would be indefinitely repeatable for the same preparation; it would also provide absolute values independent of tissue characteristics, and respond instantaneously to changes. At present, no method approaches this ideal.

Laser-Doppler velocimetry (6) has been used to measure the motion of particulates in many applications. The possibility of using this method to estimate the velocity of flow in macroscopic vessels (via fiberoptic catheter) (19) and in retinal arteries and veins (20) has been demonstrated. Following up on the observation that laser light that has passed through a fingertip does not manifest the speckle phenomenon, we reported previously that coherent light scattered from skin shows Doppler broadening, which is a sensitive index of flow in the microvascular bed. Here we report the results of feasibility studies showing that this phenomenon can provide a useful method for measuring the local blood flow in tissues. Its principal advantages are that it provides virtually instantaneous and continuous monitoring, good localization, and appears to be essentially non-invasive (beyond the requirement of exposure of the tissue). Its drawbacks are that it is limited to the penetration depth of light (unless an invasive fiberoptic probe is used) and that it requires calibration against another method of flow measurement, since it is sensitive, in principle, to changes in the vascular bed geometry and optical properties of the tissues. We have shown that this method can be calibrated against the 133Xe uptake technique in human skin, and that it can be used to monitor flow in the outer cortex of the kidney in the rat during a variety of pharmacologic interventions. Promising preliminary results indicate that similar flow signals can be obtained from brain, liver, and other tissues.

Method

A small area of tissue is illuminated by the beam of a helium-neon laser (632.8 nm), and one coherence area of the backscattered radiation is sampled by a pinhole and allowed to fall on a photomultiplier tube. The backscattered light consists of Doppler-shifted light which has interacted with moving red cells and unshifted light
which has been scattered by fixed cells and stroma. At the photomultiplier cathode the Doppler-shifted components interfere with the unshifted light and with each other, producing spectrum of beat notes at audio frequencies related to the velocity distribution of the red cells. Since beat frequencies depend on the relative velocities of the red cells and fixed tissues, overall motion of the entire tissue has little effect on this spectrum. Because of multiple scattering, the incident light may have its direction partly randomized before interacting with red cells, and frequency multiples can be produced by scattering from multiple red cells. These complex effects preclude exact calculation of the shape of the Doppler spectrum. However, a dimensional argument shows that the bandwidth of the Doppler spectrum should scale in proportion to the red cell velocities if the latter are changed without altering the flow geometry. For viscous flow in a fine network, this bandwidth should scale in proportion to red cell flow, given fixed geometry, and this has in fact been demonstrated experimentally for blood in glass capillary tubes (20). On the other hand, the magnitude of the Doppler signal is expected to increase with the number of red cells in the flow field (not necessarily linearly because of multiple scattering). These arguments support the heuristic definition of the Doppler flow parameter \( F \) as the unnormalized root-mean-square (rms) bandwidth of the Doppler signal:

\[
F = \sqrt{\int_0^\infty \omega^2 P(\omega) d\omega}
\]  

where \( P(\omega) \) is the power spectrum of the Doppler signal as a function of Doppler frequency. In reality, changes in tissue perfusion and red cell content are generally accompanied by some change in the vascular geometry, red cell flow is not laminar, and red cells have a spinning motion as they flow. Therefore, the belief that \( F \) is proportional to flow must be based on empirical evidence. In practice, we find this to be so under a surprisingly wide range of circumstances (for example, the skin of different volunteers with or without ultraviolet (UV)-induced erythema). However, there is no a priori reason to expect that the calibration coefficient will be the same in different tissues.

The definition of the flow parameter (equation 1) is somewhat arbitrary; other weighted estimates of bandwidth might do as well. It was chosen because it can be evaluated in real time by means of relatively simple analog circuitry, obviating the need for a spectrum analyzer. This is done by electronically differentiating the photocurrent after passing it through a low-pass filter wide enough to pass the entire Doppler spectrum. The power of the resulting signal contains two terms, one due to the Doppler signal, which can be shown to be equal to \( F^2 \), and the other due to the shot noise of the photomultiplier tube, which is proportional to the mean photocurrent and independent of flow. Therefore, if \( R \) is the output of an rms detector that receives the differentiated signal, the flow parameter \( F \) can be computed as:

\[
F = \frac{\sqrt{(R^2 - s^2)I}}{I}
\]  

where \( I \) is the mean photocurrent and \( s \) is a constant which depends on the gain of the photomultiplier. Equation 2 includes a division by \( I \) which serves to normalize the flow parameter so that it is independent of the intensity of the laser and the total reflectivity of the tissue. In practice, \( s \) is most conveniently fixed by zeroing the instrument on a stationary object; the zero of flow does not, therefore, depend on a biological calibration. The arguments leading to equation 2 are detailed in the Appendix.

The configuration of the instrument, as used for studies of the kidney, is shown in Fig. 1. A 15-mW laser with a beam diameter of about 1 nm (Jodon Engineering Associates, HN-15) is used. The returning light passes through a 2-mm aperture at the surface of the tissue, which limits stray, scattered light, and a .5 mm pinhole 1 m away, which selects one coherence area. An interference filter (632.8 nm, 3 nm bandwidth) rejects ambient light; the light is detected by an EM 9658-R photomultiplier with high quantum efficiency in the red. The use of this wavelength was dictated by availability of the helium-neon laser. In theory, other wavelengths, such as the 800-nm isosbestic point of hemoglobin and oxyhemoglobin, might be preferable, although the easily available He-Ne line has proved entirely satisfactory in practice. The mean photocurrent and the rms detector signal are recorded on a strip chart (Beckman type-R Dynograph). The flow parameter may be computed by an on-line calculator, using equation 2, or by analog circuitry. Simultaneously, the Doppler spectrum is computed by a real-time spectrum analyzer (Saicor, 51-B) and recorded on an X-Y plotter. The spectra shown in the illustrations have been corrected for the shot noise (which is perfectly white) by a computation analogous to equation 2.

**Measurements on Human Skin**

Spectra from human skin and the time course of occlusion and release of the brachial artery by a blood-pressure cuff have been reported previously (18). To determine if the flow parameter \( F \) could serve as a quantitative measure of flow, we compared the method with washout of \(^{133}\)Xe injected intradermally in saline. Studies were conducted on seven healthy caucasian volunteers of ages 21-32 yr. Erythema was produced on both forearms 5 cm below the elbow by exposure to a UV lamp. The methods of producing and grading the erythema, and the \(^{133}\)Xe uptake technique have been previously described (4, 5). On the day following exposure to UV, blood flow was measured by both techniques in the erythema region, and in neighboring normal skin on either or both forearms. If the subject’s time permitted, measurements were made again later in the day when the erythema had begun to fade. Five points within a 2.2-cm circle (arranged as the 5 spots on a die) were measured with the Doppler instrument, averaging for 20 s at each point. The flow was then measured by uptake of \(^{133}\)Xe injected at the center of the circle, after which the Doppler measurements were repeated. All 10 Doppler measurements were averaged to minimize the effects of point-to-point fluctuations (mottling) and time
variations in local skin blood flow due to sympathetic tone. Throughout the measurements the subject remained supine at an ambient temperature of 22 ± 1°C; 30 min equilibration time was allowed prior to starting the experiment.

Table 1 shows the results of these measurements for each of the subjects. Plotting the Doppler values (arbitrary units) against the xenon values reveals a positive bias in the Doppler values. The likely cause of this is the effect of motion of the forearm, which was controlled only by an ad hoc arrangement of sandbags. Motion of the tissue introduces a "motion noise" into the flow signal which is uncorrelated with the signal due to red cell flow. It therefore adds in quadrature to the flow parameter:

\[
F_{ \text{Doppler}}^2 = F_{ \text{True}}^2 + N^2
\]

where \( N \) is the flow equivalent of the motion noise. In order to obtain an unbiased estimate of the calibration coefficient of the instrument, a linear regression was performed between the squares of the xenon and Doppler flow measurements. The data were least-squares fit to the regression:

\[
F_{ \text{Doppler}}^2 = A^2 F_{ \text{Xenon}}^2 + B
\]

and the value of \( A \) was used as the calibration coefficient to convert the arbitrary Doppler units to milliliters per minute per 100 g. Figure 2 shows a plot of the calibrated Doppler values against the corresponding xenon values. Since scaling of only the Doppler axis was done, the shape of this plot and the first-order correlation coefficient of 88% do not depend on the normalizing procedure just described.

The Doppler instrument is capable of recording the pulsatile components of microvascular flow; however, these are partially obscured by the presence of fluctuations of similar frequency, presumably due to variation of the tone of the microvessels in the small region samples. To extract an accurate phasic wave form, we averaged the Doppler flow synchronously over a number of cardiac cycles using the QRS complex of the electrocardiogram (ECG) to gate a digital correlator-probability analyzer (Saicor, 42-A). In this mode the correlator is
used as a computer of average transients, calculating the average of the flow parameter from (typically) 150 consecutive cardiac cycles superimposed, so that the QRS-complex of each cycle (as detected by a Hewlett-Packard 780-7A ECG monitor) appears as time zero. The wave form of the phasic component as obtained from a fingertip is shown in Fig. 3. It represents about 10% of the steady flow. The pulse-wave propagation time and dicrotic notch are visible. The appearance of the dicrotic notch is of interest, although it is not possible to determine whether or not it is present in true capillaries since arterioles and venules are also present in the region of tissue sampled.

Measurements on Rat Renal Cortex

The action of pharmacologic agents on the regional blood flow of internal organs is a matter of some importance physiologically and for drug screening. The non-uniform distribution of blood flow to the anatomic regions of the kidney (12) and the unique sensitivity of the outer cortex to vasoconstrictors (2) are of special interest, because the outer cortex of the kidney may be the target organ for vasoactive interventions, both deliberate and inadvertent. We have demonstrated the use of the Doppler instrument for rapid monitoring of the perfusion of the outer cortex of the rat kidney.

Preparation. Male Sprague-Dawley rats weighing 350-450 g were anesthetized with sodium pentobarbital (Veterinary Laboratories), 6 mg/kg, intramuscularly. The trachea was cannulated with 3 cm of no. 12 thin-walled polyethylene tubing. The carotid artery on one side was cannulated with .023-inch ID polyethylene tubing and the internal jugular vein was cannulated with .011-inch ID polyethylene tubing. When two drugs were to be infused simultaneously, the femoral vein was also cannulated. The left kidney was exposed by left abdominal approach, freed from underlying connective tissue by blunt dissection, supported on a glass half-ring, and bathed in mineral oil at 37°C as for micropuncture. Arterial pressure was monitored with a strain-gauge manometer (Statham, P23db). The electrocardiogram and heart rate were monitored by a standard ECG monitor (Hewlett-Packard, 780-7A) connected to a frequency meter (Hewlett-Packard, 500C). Drugs were infused intravenously with a syringe infusion pump (Harvard Apparatus, 933) at flow rates not exceeding .05 mg/min. Comparable infusions of physiologic saline produced no effect. Preparations in which blood pressure fell below 90 mmHg or heart rate exceeded 350 beats/min under control conditions were discarded. These animals were usually found to be bleeding, and were clearly distinguishable from the experimental group. The following drugs were used in the experiments: norepinephrine (Levophed, Winthrop), hydralazine HCl (Apresoline, CIBA), angiotensin II amide (Hypertensin-CIBA), saralasin acetate (P-113, Norwich), dopamine HCl (Intropin, Armar-Stone), isoproterenol (Isuprel, Winthrop), Dextran 70 (Macrodex, Pharmacia), epinephrine (Parke-Davis), and sodium nitroprusside (Nipride, Roche). Steady-state dose-response curves were obtained for the short-acting drugs, each drug being infused at constant rate until all monitored parameters became steady, at which time a reading was taken. The animals were allowed to recover to a steady baseline between points, which were taken in random order. Doses were chosen to cover a full range from threshold effects to levels at which intolerable side effects (e.g., cardiac arrhythmia, severe hypotension) occurred.

For long acting hydralazine, the animal was permitted to achieve a steady baseline (variation in flow parameter of less than 5% over 30 min), after which single intravenous doses of .1 mg/kg were given at 5-min intervals until either a maximum increase in flow parameter was obtained or mean blood pressure fell below 50 mmHg. The mean blood pressure was then

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**Fig. 2.** Laser-Doppler flow parameter plotted against corresponding $^{133}$Xe washout flow measurement for subjects in Table 1. Laser axis has been scaled to give absolute units by procedure described in text. Solid line is line of equality, not of best fit.

**Fig. 3.** Pulsatile component of skin flow measured by laser Doppler method, obtained by averaging many pulse cycles synchronously with ECG. Units are arbitrary and only pulsatile component is shown. Steady state flow is ca. 10 times larger.
restored to 100 mmHg by infusion of 6% Dextran 70 (maximum 3 ml), and a further reading of the flow parameter was obtained.

RESULTS

Kidneys in the control state showed values of flow parameter (arbitrary units) in the range of 1.12-1.4 for all animals, usually falling within the range 1.25-1.35. These values were quite stable, varying by about 5-8% over the surface of the kidney, and less than 5% over 30 min at a single point. If the renal pedicle was tied, or when the animal was sacrificed by an overdose of anesthesia, the flow parameter immediately fell to less than .05 U, and the Doppler spectrum became flat. This uncertainty in the zero value, which also exists for stationary objects, is due to instrument error (aggravated by the steep slope of the correction equation (equation 2) near zero flow), and is not a fundamental limit on the ability to measure low flow states (which can be done by reducing the processing bandwidth).

Figure 4 shows a family of Doppler spectra from the same kidney during various steady-state dose rates of intravenous norepinephrine. This drug is a potent, outer cortical vasoconstrictor (14) and produces the expected narrowing of the Doppler spectra. The shape of the spectra is characteristic of those that we obtained from translucent tissues. Its most conspicuous feature, a logarithmic singularity at low frequencies, can be accounted for by a heuristic theory of the multiple-scattering process (18).

We have obtained complete dose response curves for the flow parameter as a function of norepinephrine dose rate in eight animals. The flow parameter always decreased monotonically with increasing dose, and in all the animals but one, the curves had a convex, generally exponential shape. The points of three of these animals are plotted in Fig. 5, with the abscissa scaled for each animal so as to give a common rate of logarithmic decrement. The absolute sensitivities of different animals varied as much as threefold. For comparison, over the range of doses represented in Figs. 4 and 5, the heart rate varied from 280 to 420 beats/min (falling again at the highest doses) and the blood pressure from 125 to 250 mmHg. The dynamic response to a bolus of norepinephrine is shown in Fig. 6. With higher bolus doses it is possible to reduce flow temporarily to zero; this could not be done in the steady state because of cardiac arrhythmias. Hydralazine, in contrast, is a known renal vasodilator when given in doses that do not overly depress blood pressure (21). Cumulative doses of hydralazine produced the expected increase in flow parameter until blood pressure fell below 80 mmHg, at which point flow fell or plateaued. Restoration of blood pressure with dextran produced a variable further increase. These effects are shown for six rats in Fig. 7. The more modest effect of vasodilators than of vasoconstrictors is probably accounted for by the tendency of blood to redistribute away from the outer cortex during states of vasodilation (17), although a nonlinearity of the instrument cannot be ruled out at present.

Angiotensin II was infused into the jugular vein at rates of 0-5 µg/min, which raised mean blood pressure from 100 to 180 mmHg. A steady-state dose-response curve was obtained, with points in random order, each repeated 2 or 3 times (variation less than 3%). A steady-state infusion of saralasin (a competitive antagonist of angiotensin II) was begun in the femoral vein at 25 pg/kg per min, sufficient to block the hypertensive effect of...
FIG. 6. Transient response of laser renal flow parameter and other physiologic variables to a bolus of 2.5 μg of norepinephrine injected intravenously over 5 s at time shown by vertical arrow. Flow parameter is shown as percent of its control value prior to the injection. Mean photocurrent and output of root-mean-square (RMS) detector from which flow parameter is computed by equation 2 are shown as they were recorded during experiment.

FIG. 7. Percent change in renal cortical flow parameter produced by hydralazine given intravenously, and by hydralazine followed by volume expansion with Dextran 70 to restore mean arterial pressure to 100 mmHg in 6 rats. A: changes produced by interventions compared to control state immediately prior to injection. B: random variations occurring over a comparable period (30 min) without intervention, in the same animals, prior to 1st dose of hydralazine.

the highest dose of angiotensin, and the entire dose-response curve was then repeated in the same animal. The two curves, shown in Fig. 8, reveal total blockade of renal vasoconstrictor receptors (8). Of interest for drug-screening purposes, this entire experiment required only 5 h (including anesthesia, surgery, and preparation of the illustration). Saralasin alone produced no detectable effect.

With the exception of epinephrine, which produced effects similar to norepinephrine, the drugs (isoproterenol, nitroprusside, dopamine) did not produce major or consistent effects on the flow parameter in doses at which their other hemodynamic effects were marked. Isoproterenol and nitroprusside acted as vasodepressors in our preparation; the absence of marked changes in flow parameter presumably indicates either autoregulation or a balance between vasodilation and redistribution of flow away from the outer cortex (3). Dopamine was the only vasopressor agent that did not produce marked decrease in outer cortical flow, even at doses that raised blood pressure to 220 mmHg. This is consist-
ent with the fact that the kidney is believed to possess vasodilator dopamine receptors (9) in addition to adrenergic vasoconstrictor receptors which respond to dopamine.

DISCUSSION

The comparison between the Doppler and xenon methods in human skin indicates that the computed flow parameter varies in an approximately linear manner with flow. The scatter in this experiment is compounded of a number of factors, the most important of which are, probably, variation between individuals and point-to-point and time-to-time variations in skin blood flow. The latter fluctuations are quite pronounced in small areas of skin, and the impossibility of measuring flow simultaneously by xenon washout and Doppler spectroscopy probably introduced excessive apparent variance between the two methods. In contrast, the measurements obtained from the kidney of anesthetized animals showed great stability when the overall physiologic state was stable. The changes produced by pharmacologic agents are consistent with the known physiology of these drugs. In order to calibrate the method for the kidney, and determine whether or not it is truly point-to-point and time-to-time variations in skin blood flow and intrarenal flow distribution by the radioactive microsphere technique, which we are not equipped to do.

The penetration depth of the laser-Doppler method is difficult to estimate accurately. The theory of multiple scattering suggests that the average depth of penetration of light before emerging is of the same order as its lateral spread, giving an estimate of 1 mm for penetration depth. By covering the renal cortex with a slice of dead rat renal cortex, we have found that the signal is almost totally attenuated by a 1-mm layer. Presumably, the penetration depth in light-colored tissues (such as skin) is somewhat greater.

The ease of performing repeated or continuous measurements by the Doppler method suggests that it may be useful in a number of clinical applications, as well as in drug screening. Its use in quantitative physiologic experiments appears promising; however, more extensive calibration is required to determine if, in fact, this use is possible.

REFERENCES


APPENDIX

Derivation of Equation 2

The photocurrent produced by the backscattered light consists of two components: the Doppler signal, which is an audio signal the spectrum of which is the Doppler shift spectrum \( P(\omega) \), and the shot noise, which is a perfectly white noise of infinite bandwidth and spectral power density proportional to the mean photomultiplier current (DC). Thus, we have

\[ i = i_d + i_v \]  

where \( i_d \) is the Doppler signal and \( i_v \) is the shot noise. The latter contains theoretically infinite power (since it has infinite bandwidth). By passing the signal through a low-pass filter that is slightly wider than the Doppler bandwidth, we limited the noise to manageable levels without affecting the signal. The spectral power density of the combined signal is simply the sum of that for the Doppler signal and the noise, since the two are statistically independent. Therefore, the total spectrum is

\[ S(\omega) = P(\omega) + \alpha I, \omega < B \]  

where \( \alpha \) is a constant that depends on the gain of the phototube, \( I \) is the mean photocurrent (DC), and \( B \) is the bandwidth of the low-pass filter. If the entire signal is differentiated at this point, the power spectrum \( S(\omega) \) will simply be multiplied by \( \omega^2 \). The output of an rms detector applied to this signal will be the square root of the total integrated power in the spectrum.

\[ R = \sqrt{\int_{-B}^{B} \omega^2 S(\omega) \, d\omega} = \int_{-B}^{B} R'(\omega) \, d\omega + \int_{-B}^{B} \alpha I \, d\omega \]  

The first term under the second square root is the square of the flow parameter as defined by equation 1, while the second term can be integrated directly to give \( \alpha R''(\omega) \), or \( \alpha I \) if \( s \) is an appropriately defined constant which now depends on the phototube gain and the processing bandwidth. The result is

\[ R = \sqrt{F^2 + \alpha I} \]  

which rearranges to

\[ F = \sqrt{R^2 - \alpha I} \]  

If the flow parameter is redefined by dividing it by the mean photocurrent, so as to normalize it to unit intensity, equation 2 results.

APPENDIX

The authors thank Don N. Kennedy and Lee Morin for invaluable technical assistance.

Part of the work reported here was conducted under Public Health Service Grant HL-5678-02.

An Abstract of this study was presented at the Federation of American Societies for Experimental Biology Meeting, April 1976.

Received for publication 5 May 1976.


