NRCP Research Journal
Vol. 3, No. 2, July-September, 1993

DEVELOPMENT OF A PHOTOMETRIC DUAL-SLIT SYSTEM FOR THE MEASUREMENT OF RBC VELOCITIES IN SINGLE MICROVESSELS, USING COMMERCIALLY AVAILABLE COMPUTER COMPONENTS

XENIA T. TIGNO, PhD and EDGAR GENIO
Department of Physiology,
U.P. College of Medicine
Pedro Gil, Manila
Instrumentation Laboratory, National Institute of Physics,
University of the Philippines
Diliman, Quezon City

INTRODUCTION

Adequacy of tissue perfusion is the major function of the cardiovascular system. As such, an accurate method should be made available to evaluate the efficiency by which this function is performed, be it during normal physiologic reactions, or in pathologic states such as shock, cardiac failure, diabetes, hypertension and other conditions where perfusion is suspected to be compromised. Oftentimes, the functional state of the peripheral circulation is assessed by using conventional gross methods such as cardiac output, cardiac index, systemic blood pressure, or volume flow rates measured from large distributing arteries. However, it has become increasingly evident that changes in the distribution of blood within a particular regional circulation may occur, with little or no overt changes in these systemic parameters. It becomes imperative therefore to make measurements at sites where the actual exchanges between blood and tissue take place, namely in the microcirculation. Furthermore, variations in blood vessel reactivity and vascular impedence to flow are also best observed in the microcirculation, where the resistance vessels (small arteries, arterioles and post-capillary venules) are found.

Hemodynamic assessment of the microcirculation is usually performed by an intravital microscopic technique, which allows direct observation of the peripheral vasculature, and which uses the red blood cells themselves as tags for measurement of flow velocities.

This technique requires the use of high-precision, computer-assisted equipment, which may cost around a quarter of a million pesos, a kingly sum way beyond the moderate budgets usually allocated for locally funded research projects. In response to this, the project was undertaken to study the feasibility of constructing a computer-assisted RBC velocimeter, employing local manpower and technology, and improvising with commercially available computer components, thereby reducing equipment cost to around a twentieth of the cost of pre-fabricated models.

The project was a joint endeavour of the Department of Physiology and the National Institute of Physics, University of the Philippines System.

BASIC THEORETICAL PRINCIPLES

The dual-slit or two-window method of measuring RBC velocity was introduced by Wayland and Johnson in 1967 and has been employed by laboratories worldwide for the past twenty years. It consists of two photodiodes positioned along the axis of the vessel under observation. As blood flows past the first, or upstream diode, the stream of RBCs produces a signal pattern. As the RBCs traverse the second, or downstream, diode, a similar pattern is produced after a signal delay or time interval, T. Since the interdiodal distance is fixed, the velocity of RBC flow can be calculated from the formula

$v = d/T$, where d is the inter-diodal distance and T is the time interval between the appearance of the signal at the first and at the second diodes.

The time interval, T, is mathematically computed from the cross-correlation function defined as:

$$\varnothing(t) = \int_{t_o}^{t_o + T} f_u(t)\ f_d\ (t + T)\ dt$$

where f_u (t) is the amplitude of signal from first diode

f_d (t + T) is the amplitude of signal from 2nd diode

$\varnothing$ (t) is a function relating to the covariance or similarity of the 2 patterns

Knowing the velocity of the RBCs, the bulk flow velocity of whole blood can be estimated as velocity of RBC/1.6.

The volume flow rate in single vessels can then be calculated as:

$$Q = \text{volume/time} = \pi r^2 \times \text{velocity}$$

EXPERIMENTAL SET-UP

Laboratory white rats were anesthesized with ketamine hydrochloride (Ketalar, 100 mg/kg B.W.) and their tracheas cannulated to allow spontaneous breathing. The mesentery was then exteriorized through a mid-line incision on the abdomen, and then draped on a plexiglass stage for microscopic observation. The mesentery was immersed in a warm saline solution and covered with saran plastic wrap to prevent condensations on the objective, and to inhibit any constrictive effects of hyperbaric oxygen. The plexiglass cradle was then attached to a Zeiss Universal Research microscope and viewed with a Zeiss Neofluar 16/0.40 objective. An 8 inch tube, where two photodiodes had been mounted on one end, was attached to the ocular end of the microscope. The image of the microvessels are thereby projected to the plane of the photodiodes, with an effective image magnification of 240x. The two photodiodes, upstream and downstream, were always oriented perpendicular to the direction of flow. The distance between them was 2.00 mm, which corresponds to an effective distance of 8.33 microns, referred to the plane of the preparation. The distance corresponds closely to the diameter of an RBC, and is essential to ensure reliability and accuracy of measurement. Two identical pre-

amplifiers were also mounted at the tube close to the photodiodes, so that the signals were amplified and buffered before going to the correlator via long cables. Because a video-camera is attached to the microscope, simultaneous observation on a video monitor is possible, with total magnification of ca. 500x in the video-monitor. The image can also be recorded for future reference on a video-cassette recorder (Sony Superbetamax). The video camera has an image-inserter input, so that markers generated by a microcomputer-based video-angiometer can be fed into the monitor to allow on-line and precise measurement of vessel inner and outer diameters. The video-angiometer software was also developed during the period of the project as an additional accessory.

ACTUAL MEASUREMENTS

Blood flowing through successive bifurcations are known to undergo successive reductions in velocities. The above system was tested by observing the characteristics of flow along several microvessel networks and the measuring velocities at different generations of microvessels. The system proved to be reliable and versatile, capable of measuring velocities from below 0.5 mm/s to around 7 mm/sec. Values obtained were within the range and closely similar to previously published data on the microcirculation.

The following example serves to illustrate this:

		diameter	velocity
Inflow vessel:	A1		
Branches	A12	14.3 μ	2.6 mm/s
	A13	11 μ	1.7 mm/s
	A14	6.7 μ	1.4 mm/s
	A15	10.1 μ	.4 mm/s

This figure shows that with our present velocimeter, we are able to make continuous, on-line measurements of the velocity, so that temporal variations in flow are detectable. The precision of the device is such that velocities from around .4 mm/sec to around 7 or 8 mm/sec are detectable. From this and other recordings, variations in flow due to cardiac contraction and respiration are also detectable, in contrast to previously espoused notions that flow in the microvessels are "steady" and non-pulsatile.

Another example from the same network (Fig. 4) compares perfusion in a true capillary, and in an AVA or shunt vessel. In capillaries, RBC flow is "single-file", so that periods of RBC flow may alternate with periods of pure plasma flow only. Notice that our velocimeter is sensitive enough to record such fluctuations, such that velocity values go to zero if only plasma is present. Velocity values for the capillary here averaged 0.6 mm/s, as compared to the AVA with a velocity of 1.8 mm/s, or thrice that of a capillary.

Eventually, all measurements both of blood pressure and velocity, will be stored and analyzed by the microcomputer, which will also average and process the data. To be added to the system is an RBC counter which will count each RBC flowing through one diode at a given time interval. This measurement will be used in calculating for the dynamic capillary hematocrit, which measures the efficiency of oxygen delivery to the periphery. The project is 95% complete, and the additional accessories hopefully finished within the next few months, depending on the state of affairs of the electrical wiring of the college and the frequency of brown-outs.

However, suggestions for possible applications of the system to clinical problems and evaluation of therapeutic regimens are most welcome.

ADDENDUM: CIRCUIT DESCRIPTION

Miniature photodiodes (HP5205) about 1.5 mm diameter were mounted close to each other (2.0 mm center to center) on a printed circuit board. The photodiodes were operated in the photocurrent mode using a current to voltage converter. Low noise and low input current Op-Amps (AD545) were used or this. Circuit lay-out considerations associated with such op-amps were obeyed. The photodiodes were mounted as close as possible to the op-amp inputs which were surrounded by a guard connected to ground. The output of the current to voltage converter was amplified by another op-amp whose offset can be adjusted. The signals coming from this circuit board mounted on the tube connected to the eyepiece was fed into the correlator via long shielded cables.

To evaluate the cross-correlation, the upstream signal was delayed, multiplied by the downstream signal and time-averaged. The

time delay is achieved by using an analog-shift register, composed of sample and hold circuits arranged in a bucket brigade period Tc derived from a voltage controlled oscillator (VCO). There are 12 stages with taps at 4th, 8th and 12th stages corresponding to time delays of 4Tc, 8Tc and 12Tc producing u(t-4 Tc), u(t-8 Tc) and u(t-12 Tc). These are multiplied by the downstream signal (using an analog multiplier) and averaged by an integrator to produce c(4 Tc), c(8 Tc), and c(12 Tc). By adjusting the clock frequency until the three point correlogram is symmetric, i.e. c(8 Tc) > c(4 Tc) = c(12 Tc), the time delay can be made to coincide with 8 Tc. Hence the velocity is d/8 Tc, d being the effective distance between the two photodiodes. By using the difference between c(4 Tc) and c(12 Tc) to control the oscillator via a feedback loop, the symmetry condition can be maintained so that the clock frequency is always proportional to the velocity even as it changes with time. This difference voltage is considered as an "error" signal. The feedback signal is composed of two terms: a proportional signal, which is just the error signal amplified, and an integral term, the time integral of the error signal. This feedback signal drives the voltage controlled oscillator (Fig. 5).

PERFORMANCE

The system was tested using a sinusoidal signal and a phase shifted conversion of it achieved by passing the sinusoidal signal through an RC filter. With these kind of signals, the circuit operates as a phase locked loop with the error signal acting as a phase detector. d (t) is the reference signal and u(t) is the signal whose phase is to be adjusted in order to lock onto the reference signal. This is understandable since for complex signals, the phases of the dominant frequency components are in phases at maximum crosscorrelation.

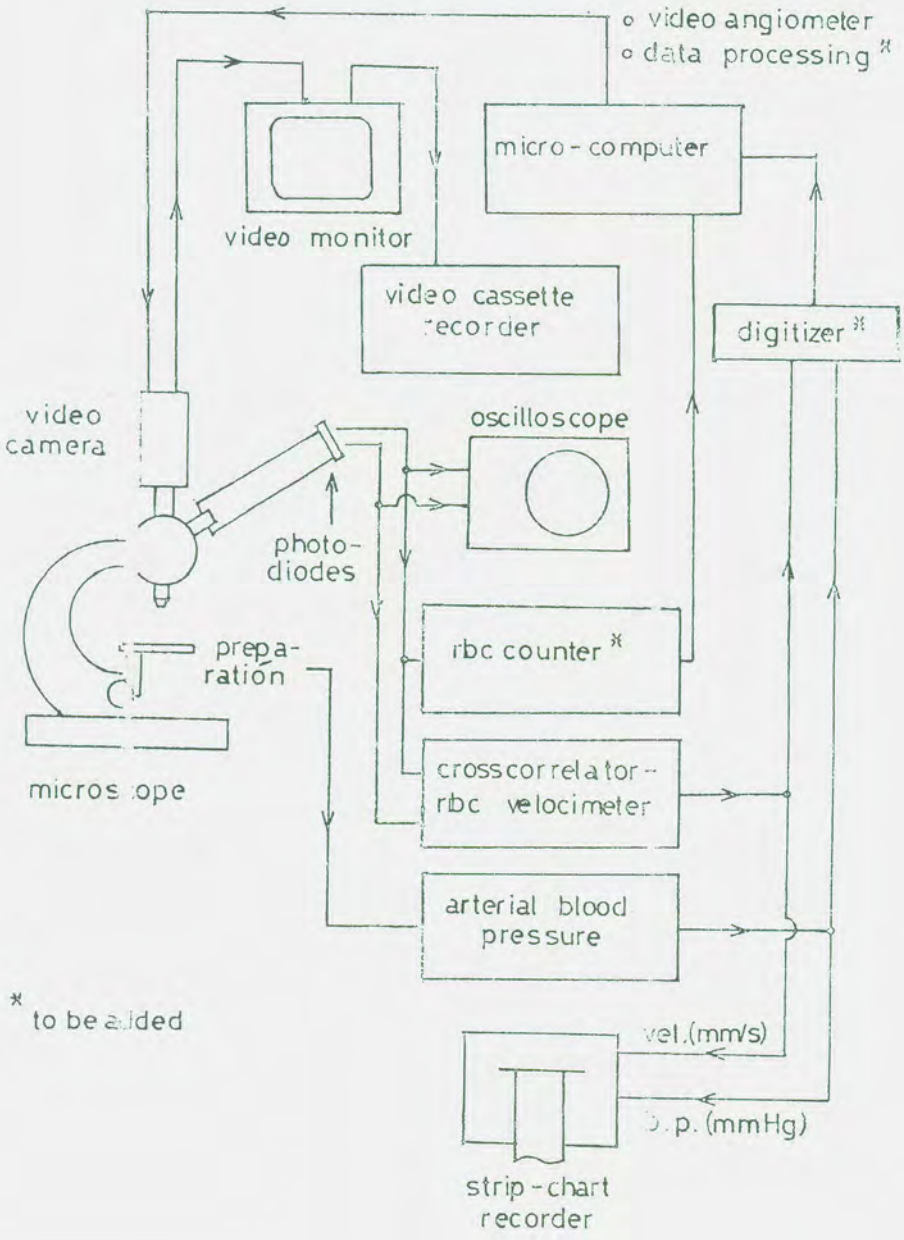


Figure 1. Set-up for microcirculatory studies.

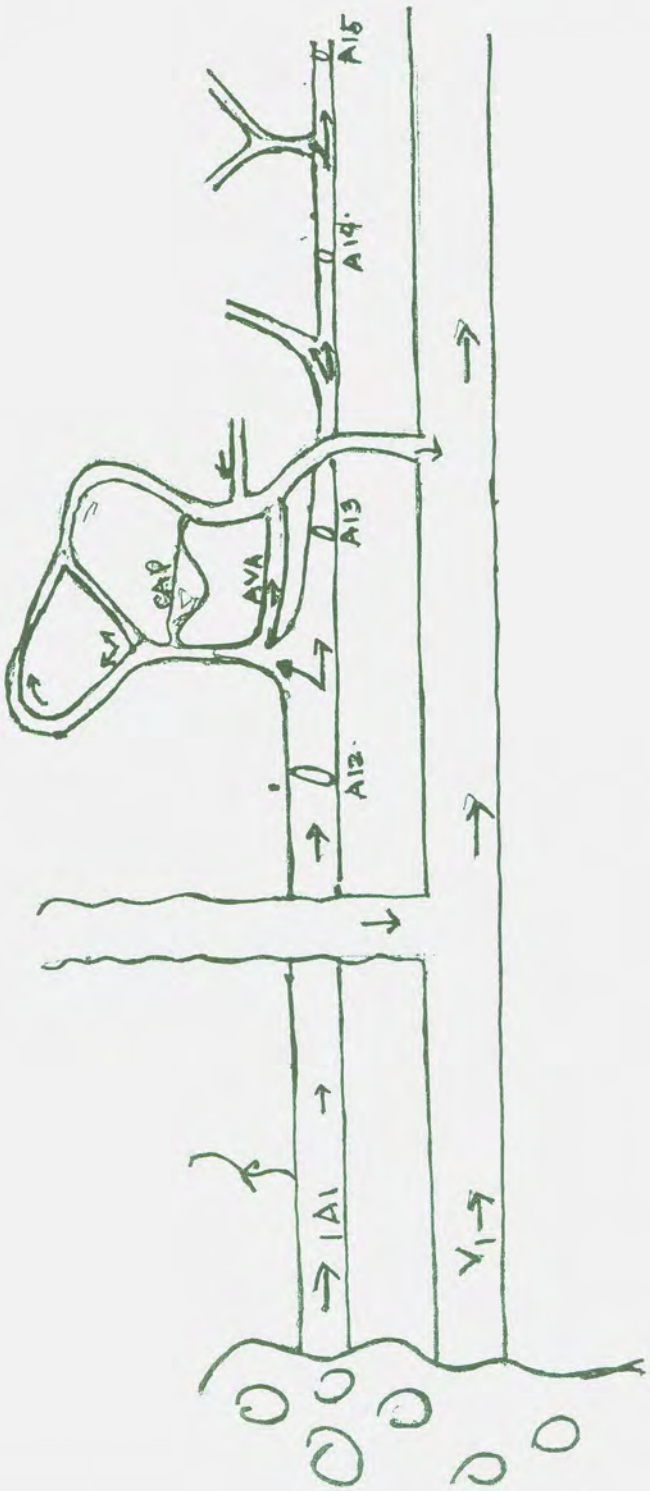
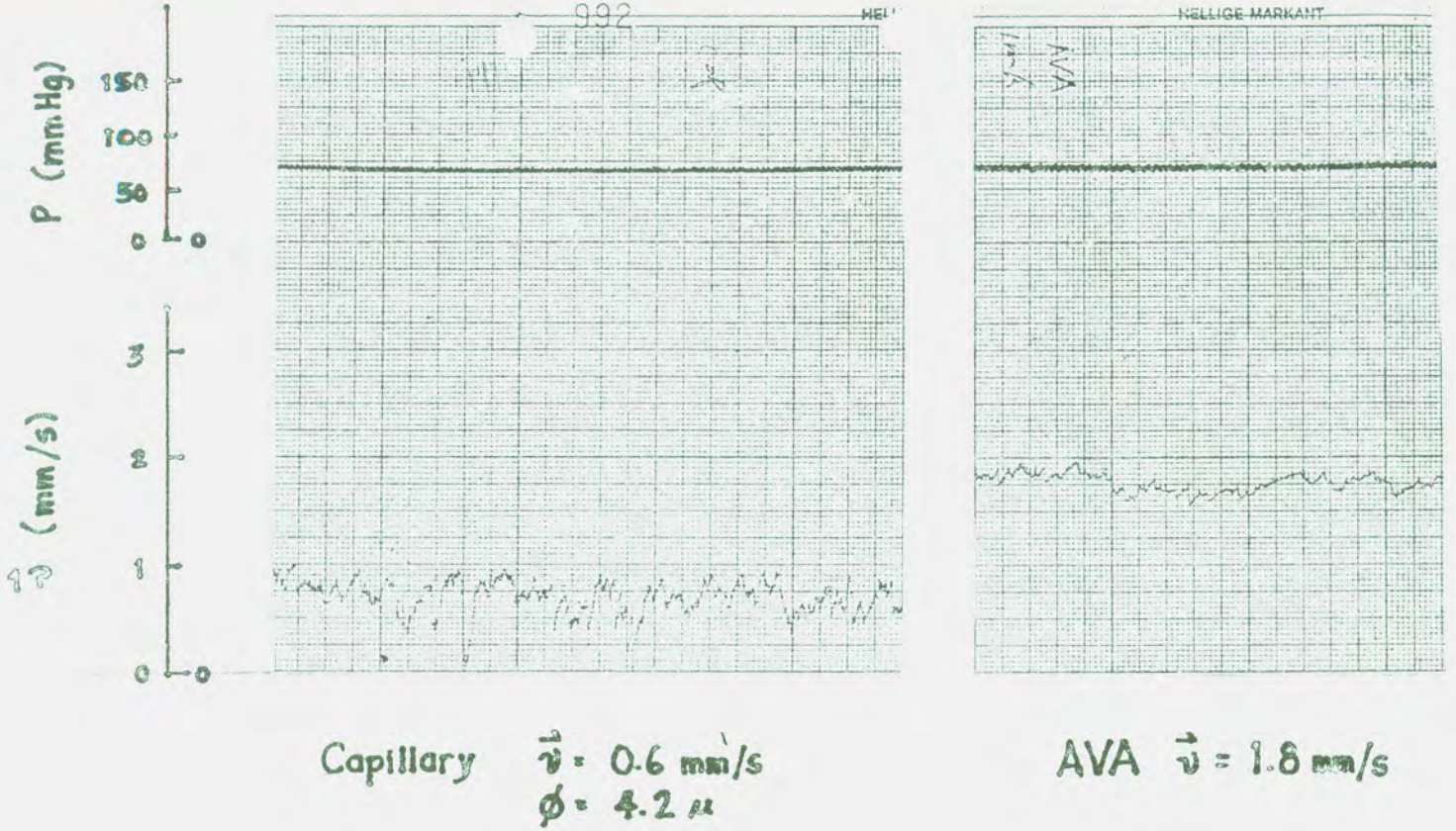


Figure 2.



COMPARISON BETWEEN FLOW IN CAPILLARY AND FLOW IN AN AVA

Figure 3.

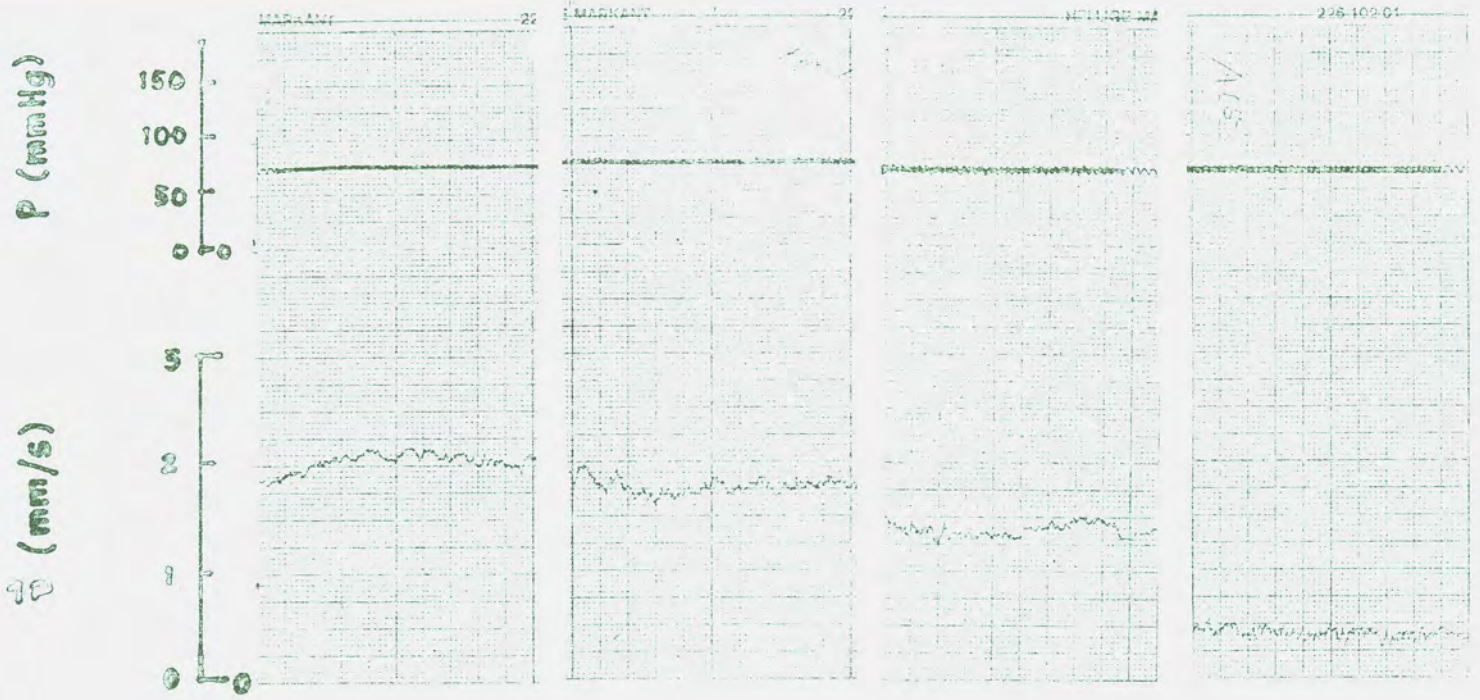
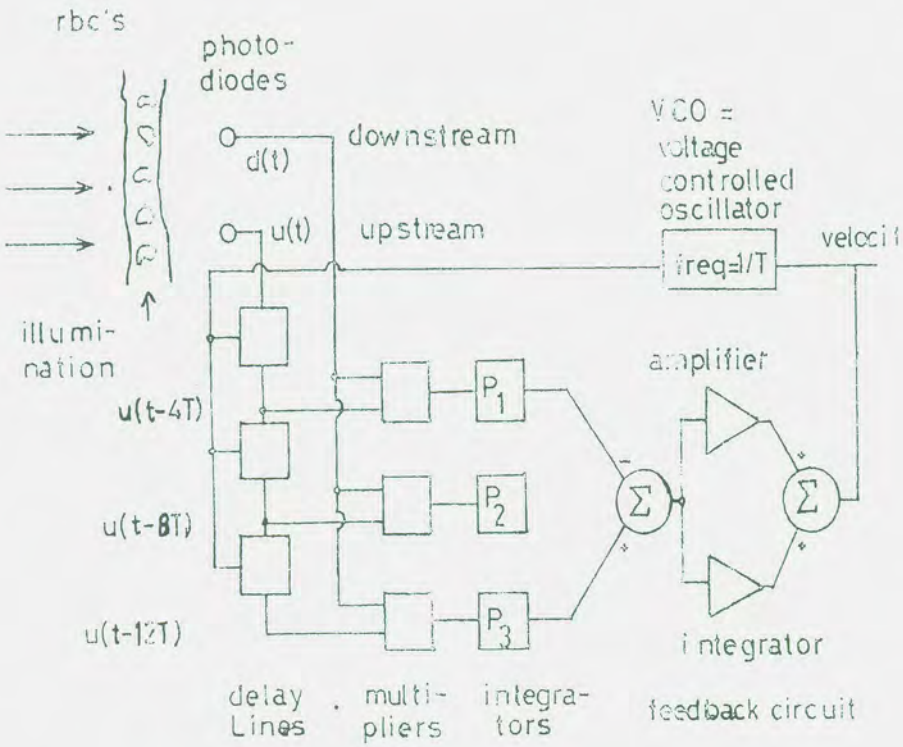

P (mmHg)
150
100
50
0
v (mm/s)
5
2
1
0
A12 v = 2.6 mm/s
Φ = 14.3 μ
A13 v = 1.7 mm/s
ϕ = 11 μ
A14 v = 1.4 mm/s
ϕ = 8.7 μ
A15 v = 0.4 mm/s
ϕ = 10.14 μ
Rat 195g ♀

Figure 4.



T_t = Transit time = time delay for which cross correlation $C_{ud} = \int u(t-T_d)d(t)dt$ is maximum

velocity = D/T_t ; D = effective distance bet. photodiodes

Feedback operation: VCO is continuously adjusted so that $P_3 = P_1$ in which case

$T_t = 8T$

Figure 5. Block diagram and Principle of Operation of cross correlator-velocimeter.

❶ $v = \frac{x}{\tau}$

❷ $\phi(t) = \int_{t_0}^{t_0+\tau} f_u(t)\, f_d(t+\tau)\, dt$

$f_u(t)$ = signal from upstream diode

$f_d(t+\tau)$ = signal from downstream diode

$\phi(t)$ = "similarity" of two patterns

❸ $\dot{Q} = \bar{v}_{blood} \times \pi r^2$ = volume flowrate