MEASURING THE BLOOD-FLOW VELOCITY IN MICROVESSELS WITH A PULSE COUNTER

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A photometric trigger method of measuring the velocity of red blood cells, by means of which the linear velocity and the cell flow in microvessels can be measured for a long period of time, is described. The use of a pulse counter to time the intervals at which the red cells pass enables the signals of the measuring trigger to be transformed directly into a digital code. The results of trials of the apparatus on microvessels of the frog mesentery are described.

KEY WORDS: microcirculation; velocity of red blood cells; pulse-counter chronometer.

The velocity of the blood flow in microvessels is measured under the microscope by using the red blood cell as marker [6]. Photometry of adjacent segments of a blood vessel and determination of the time interval between indentical areas of the photographs can also be used [3]. Wayland and Johnson [7] developed an automatic method of processing the photometric information by correlation analysis on a computer. The same workers showed that the time taken for a red blood cell to cover a fixed distance could be measured directly [8] and they designed an analog device which would record the velocity and cell flow continuously on the tape of an automatic writer (trigger method). Other experiments [4, 5, 9] have shown that the trigger method is suitable for measuring the blood-flow velocity not only in capillaries, but also in vessels up to 137 μ in diameter.

This paper describes a photometric trigger device in which a pulse counter is used to measure the transit time of the red blood cell, so that an averaged velocity can be obtained in the course of long-term measurment.

The optical part of the apparatus (Fig. 1) is based on the ML-2 microscope, to which a rotating barrel is fixed. In the lower part of the barrel there is a sighting device with camera (2), by means of which the object can be selected and photographed and the detector, located in the upper part of the barrel, can be oriented. The detector consists of two parallel slits (3a, 3b), separated by a distance equivalent to 5 μ on the object, and placed behind the slits of a type FÉU-35 photomultiplier (4). A dc incandescent lamp (1) is the source of light.

The image of the vessel, magnified by the microscope, is projected on the screen of the detector, the slits of which are oriented perpendicularly to the axis of the vessel. The image of a moving red cell, as it passes the first and then the second slit, induces signals separated by time t on the outputs of the photomultipliers. If the distance between the slits is $l = 5\mu$, the red cell will darken the second slit before the next one darkens the first slit. Signals of the photomultipliers are amplified by the amplifiers (5a, 5b) and led to the inputs of Schmitt's triggers (8a, 8b), tuned to the same level of discrimination. The amplitude and stability of the signals are monitored through an oscilloscope (6) and loudspeaker (7). Pointed pulses of the Schmitt's trigger are led to the inputs of a symmetrical trigger (9), which emits a square pulse with a duration of t. The time t is measured by a pulse-counter method, based on determining the number of pulses with an exact frequency falling within the interval to be measured [1]. Pulses from a quartz generator (11) with a period $\tau = 10^{-5}$ sec are led to one input of an electronic key (10) and the pulse from the trigger

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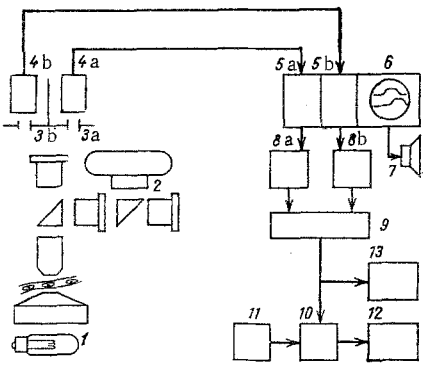

Fig. 1

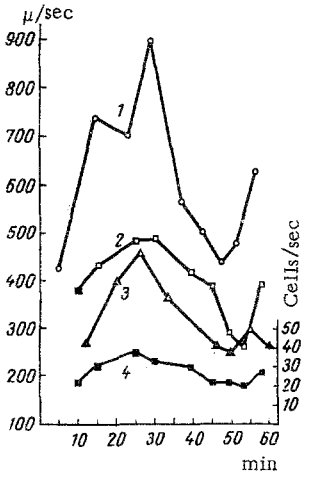

Fig. 2

Fig. 1. Scheme of apparatus for measuring velocity of blood flow in microvessels (explanation in text).

Fig. 2. Linear velocity of blood flow measured in turn in each of three vessels forming a bifurcation: 1) velocity in a trunk 32 μ in diameter; 2) velocity in a branch 27 μ in diameter; 3) velocity in a branch 19 μ in diameter; 4) cell flow in branch 27 μ in diameter. Abscissa, time (in min); ordinates: left – velocity of blood flow (in μ/sec), right – cell flow (in cells/sec).

(9) to the other input. The latter closes the key which allows n pulses with a period of τ to pass in the course of time t, and their number is recorded by a PST-100 counter (12). The velocity of the red cell is:

$$V = \frac{l}{t} = \frac{l}{n\tau}.$$

To obtain the mean harmonic velocity of the blood flow over a short time interval, the intervals t_i ($i = 1, 2, \ldots, k$) are measured continuously and the counter (12) accumulates a total of k intervals during the measuring time:

$$\sum_{i=1}^{k} t_i = \tau \sum_{i=1}^{k} n_i.$$

The number of measurements of k (in the case of movement of a single row of cells in the capillaries this will be equal to the number of red cells passing through) is recorded by another counter (PST-100) (13). The measuring time is set by an electronic timer, so that the mean cell flow can be calculated. The mean harmonic velocity is calculated by the equation:

$$\bar{V}_h = \frac{k}{\sum_{i=1}^{k} \frac{1}{V_i}} = \frac{k}{\frac{1}{l}\sum_{i=1}^{k} t_i} = \frac{l \cdot k}{\tau \sum_{i=1}^{k} n_i},$$

where k is the reading of the counter (13) and $\sum_{i=1}^{k} n_i$ the reading of counter (12).

It will be clear from this equation that if the control device stops the measurement on reaching $\sum_{i=1}^{k} n_i = l \cdot 10^5$, $\bar{V}_h = k$ and the value of the mean velocity can be read on the indicators of the counter (13).

The relative error of measurement of the velocity is given by:

$$\delta_{\bar{V}_h} = \delta_l + \delta_t + \delta_k,$$

where $\delta_l = 0.5\%$ is the relative error of making the slit system under the control of an MBS-1 microscope with ocular scale; $\delta_t = \tau/t$ is the instrumental error of the counting method [2]; $\delta_k = 1/k$ is the error due to the fact that the measurement may end at the beginning of t_k and the counter (12) does not record n_k completely, whereas the counter (13) records k. For a velocity of 500 μ/sec, a cell flow of 30 cells/sec, and a

measuring time of 10 sec, $\delta \bar{V}_h = 0.5\% + 0.01\% + 0.33\% = 0.84\%$. If necessary, δ_l can be reduced and δ_k abolished altogether by using a more complex control circuit. The pulse counters themselves introduce no additional error.

The apparatus was tested on microvessels of the mesentery of hibernating grass frogs weighing about 40 g. The mean velocity of the blood flow in the vessel over a period of 10 sec was measured during 5-10 min at intervals of about 1 min. For vessels 10-28 μ in diameter the measured values of velocity lay within the range 100-1100 μ/sec and the cell flow was between 9 and 58 cells/sec. The coefficient of correlation between the velocity of blood flow and the cell flow was 0.67 and between the velocity and diameter of the vessel 0.54. The results of measurement of the velocity in each of three microvessels forming a bifurcation are given in Fig. 2.

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