REFLECTION CHARACTERISTICS OF HEMODYNAMIC SYSTEMS UNDER COHERENT OPTICAL RADIATION

by

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I. INTRODUCTION

A. Physiology of the Cardiovascular System

The primary function of the cardiovascular system is to transport nutrients, metabolic intermediates, and waste products to and from the body tissues. The prime mover of the system is the heart which pumps the fluid component of the system, i.e., the blood, around the closed vascular system and allows the transportation function to be carried out. One of the principal nutrients carried by the blood is oxygen, which is picked up by the blood through a membrane diffusion process in the lungs.

B. Composition and Properties of Blood

Blood is a colloidal suspension of cells in an aqueous solution called plasma. Of the cellular components, by far the most numerous are the erythrocytes, the mature red blood cells. There are approximately 5,400,000 red blood cells per cubic millimeter of blood in a normal human male, with the number being slightly less in the normal human female. In human blood the red blood cells represent about 99 percent of the cellular components, which in turn represents about 45 percent of the total blood volume. The cellular percent of total blood volume is commonly called hematocrit. Other cellular components and their respective numbers per cubic millimeter of blood are: platelets[1] (350,000), reticulocytes[2]

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[1] Platelets are round oval discs 1/3 to 1/2 the size of the red blood cells.

[2] Reticulocytes are immature red blood cells containing a network of filament or granular material.

(37,500), and leucocytes[1] (7,500). The plasma portion of blood consists of the proteins fibrinogen, globulin, and albumin in aqueous solution. Also present in the plasma are small amounts of sugar, cholesterol and urea along with several inorganic ions. Detailed lists of the chemical composition of blood can be found in Guyton (13), Ruch and Fulton (26), and Cantarow and Schepartz (4).

Normal mature human red blood cells are non-nucleated cells in the shape of biconcave discs having a mean diameter of about eight microns. The thickest portion of the cell is about two microns with the center being about one micron thick. Figure 1 depicts a typical cross section of a red blood cell. The membrane of the cell consists of a protein-lipid material approximately 20 millimicrons thick. The cell interior consists of a spongelike stroma, which is also a protein-lipid material. Although the membrane of the red blood cell is inelastic, the membrane is sufficiently flexible that the cell is easily deformed as required for its passage through the capillary system. The principal function of the red blood cells is the transportation of the gases oxygen and carbon dioxide.

Hemoglobin is the active component of the red blood cell. There are approximately 34 grams of hemoglobin present in every 100 milliliters (ml.) of red blood cells.

The importance of hemoglobin in transporting oxygen results from its ability to combine readily and reversibly with oxygen. Each gram of hemo-

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[1]Leucocytes are white cells which are slightly larger than the red blood cells. Of the cellular components of blood, leucocytes are the only ones containing a nucleus. There are several types of leucocytes, some of which have a granulated cytoplasm.

globin can combine with 1.34 ml. of oxygen. Since there are 14 to 16 grams of hemoglobin per 100 ml. of blood, this results in a hemoglobin oxygen capacity of about 21 ml. of oxygen per 100 ml. of blood. At 95 mm. Hg. oxygen partial pressure there are about 19.5 ml. of oxygen carried in combination with hemoglobin and about 0.29 ml. of oxygen in solution per 100 ml. of blood. On reaching the tissues, the hemoglobin in 100 ml. of blood releases about 5 ml. of oxygen, while about 0.17 ml. of oxygen is released from the dissolved state. This means that hemoglobin is responsible for about 97 percent of the blood oxygen transport to the tissues.

The chemical composition and properties of hemoglobin are discussed in detail by Cantarow and Schepartz (4). It is a chromoprotein with molecular weight of about 68,000 consisting of four heme groups attached to the protein globin. The chemical structure of a heme group is shown in Figure 2. Globin is a complex protein composed of 574 amino acids which form four chains. There are about 10,000 atoms in the hemoglobin molecule. X-ray diffraction studies indicate the hemoglobin molecule is spheroidal in shape with approximate dimensions in angstroms of 50 x 55 x 64. The oxygenation of one heme group does not seem to be independent of the others, since the attachment of oxygen to one group appears to enhance the oxygen affinity of the others. This causes hemoglobin to have a tendency to be either completely oxygenated or completely reduced. The ratio of oxyhemoglobin to total hemoglobin times 100 is called percent oxygen saturation of the blood, a term commonly used in medical literature.

C. Importance of Determining the Percent Oxygenation

Percent oxygen saturation is a clinically important parameter used to indicate cardiac defects such as shunting, adequacy of lung function,

cardiac output, and oxygen utilization of various tissues and organs.

D. The Laser as an Optical Light Source

Schawlow (28) gives an excellent lay discussion of the operation and advances of lasers. To date the medical uses of lasers have been limited to those which utilize the focusability of the optical energy to obtain high energy densities and have used pulsed ruby or solid state lasers. Repair of retinal detachment and the destruction of certain cancerous tissues are two examples. Gas lasers utilizing a mixture of helium and neon in the optical cavity can have an output wavelength of 632.8 mμ with continuous power output levels in the order of magnitude of from one to ten milliwatts.

The possibility of using a continuous operating gas laser as a light source for reflection oximetry is indicated by the extinction coefficients for oxygenated and reduced hemoglobin obtained by Gordy and Drabkin (12). The extinction coefficients they obtained, when interpolated to 632.8 mμ, yield values of 0.194 and 1.11 for oxygenated and reduced hemoglobin respectively. There would appear to be little danger in using the unfocused output of a continuous operating gas laser to illuminate living tissue, since solar output in the visible range has an intensity of 10 mw/cm^2 at sea level. There are, however, no data to indicate the levels of visible optical radiation which might be harmful.

E. Purpose of This Study

It was the purpose of this study to investigate the reflection characteristics of hemodynamic systems using a coherent light source with a wavelength of 632.8 mμ. The objective of the investigation was to determine the feasibility of using a single wavelength to determine the percent

of oxygen saturation *in vivo*[1], and to study the artifacts produced by the hemodynamic variables flow and pressure.

[1] A Latin phrase commonly used for "in the living body."

II. REVIEW OF THE LITERATURE

A. Historical Development of Methods of Determination of Percent of Oxygen Saturation

According to Hayden (15), the discovery of hemoglobin and its physiologically important ability to readily take up and release oxygen was made by Hoppe-Seyler sometime between 1871 and 1876. Following this important discovery, much interest developed in the measurement of the amount of hemoglobin in blood and the ability of hemoglobin to combine with oxygen. Many methods evolved for estimation of the hemoglobin content in blood. Among them was a chemical method developed in 1898 by Haldane (14), in which the hemoglobin bound oxygen was released by adding potassium ferricyanide to the blood and measuring the volume of oxygen released.

It was natural that the above method would be adapted to a gasometric determination of percent oxygen saturation when this parameter became medically important. VanSlyke and Neill (33) in 1924 developed the basis of the method now used by many investigators as a reference standard for other methods of determining the percentage of oxygenated hemoglobin in blood. They used a saponin solution to hemolyze[1] the red blood cells and then added potassium ferricyanide to release the hemoglobin bound oxygen. A vacuum was used to withdraw the gas into a manometric apparatus in which the volume of gas was measured. This method has been widely accepted although it requires an experienced operator, and there is a considerable time delay between sample taking and the final result.

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[1]To hemolyze is to rupture the cell membrane and release its contents into the solution.

All photometric determinations of blood oxygen saturation are due to the differences in absorption of light by oxygenated and reduced hemoglobin and are based on Beer's law. Beer's law relates the intensity of the incident and transmitted light by the expression

$$I = I_0 e^{-\epsilon c d} \tag{2.1}$$

where I_0 = incident light intensity

I = transmitted light intensity

ϵ = molar extinction coefficient of the absorbing medium at a specific wavelength

c = concentration of the absorbing medium

and d = the optical path length.

For a solution with two absorbing components one can write $\epsilon c = \epsilon_o c_o + \epsilon_r c_r$ where ϵ_o and ϵ_r represent the extinction coefficient for the two pigments and c_o and c_r are their respective concentrations. Substituting this into Equation 2.1 and taking the logarithm of both sides gives

$$D = -\log \frac{I}{I_0} = (\epsilon_o c_o + \epsilon_r c_r) \tag{2.2}$$

where D is called the optical density.

If two wavelengths λ and λ' are used, and their respective densities determined, the ratio of the two densities gives

$$\frac{D}{D'} = \frac{\epsilon_o c_o + \epsilon_r c_r}{\epsilon'_o c_o + \epsilon'_r c_r} \tag{2.3}$$

where ϵ and ϵ' refer to the extinction coefficients at wavelengths λ and

λ' respectively. Equation 2.3 can be solved for the fractional ratio c_o/c_T by utilizing the fact that $c_T = c_o + c_r$. The solution yields

$$\frac{c_o}{c_T} = \frac{\epsilon_r - \frac{D}{D'}\epsilon'_r}{\frac{D}{D'}(\epsilon'_o - \epsilon_o) - (\epsilon_o - \epsilon_r)} . \qquad (2.4)$$

Equation 2.4 was the basis for early spectroscopic work by Singer and Drabkin (32), who made an extensive study of the transmission characteristics of blood in the region between 500 and 600 mμ. By 1942, Millikan (21) had developed an oximeter[1] using two wavelengths to determine oxygen saturation of arterial blood in the ear. From 1944 to 1957 Drabkin and Schmidt (7), Hickam and Frayser (16), Nahas (22), Roos and Rich (25), Huckabee (18) and Gordy and Drabkin (12) all had reported improvements in the techniques of taking and preparing samples for easier spectrophotometric analysis. Gordy and Drabkin (12) found the extinction coefficients at 660 mμ for oxygenated and reduced hemoglobin to be 0.1 and 0.820 respectively. They had also obtained a value of 0.196 for the extinction coefficient of both oxygenated and reduced hemoglobin at 805 mμ. These numerical values, when substituted in Equation 2.4, give, as an expression for the percent of oxygenated hemoglobin to total hemoglobin in blood, the formula

$$\text{percent oxygenation} = 100\,\frac{c_o}{c_t} = \frac{4.18 - \frac{D_{660}}{D_{805}}}{3.67} \times 100. \qquad (2.5)$$

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[1] An oximeter is any device used to continuously measure oxygen saturation.

Here, D_{660} and D_{805} are the optical densities of the unknown sample of blood, which are to be determined on a spectrophotometer such as the Beckman DU[1]. Hickam and Frayser (16) developed a method of withdrawing samples into syringes in which the dead space had been filled with heparin[2]. The syringes were then capped immediately with a soldered hub, and blood hemolyzed by injection of a 30 percent saponin solution through a temporary rubber cap. The unknown samples were then transferred to the cuvette and a layer of mineral oil placed over the cuvette to prevent oxygen exchange between the sample and the atmosphere. Spectrophotometric techniques in blood oxygen saturation analysis have changed little since 1957, with the only changes being slight modifications in methods of obtaining and handling samples.

The introduction of reflection oximetry by Brinkman and Zijlstra (3) in 1949 represented a new approach to the determination of oxygen saturation. The method developed from experimental observations which indicated that the intensity of reflected light at certain wavelengths is dependent upon the degree of oxygen saturation. The wavelengths involved are again the interesting ones of spectrophotometry, i.e., the wavelengths where the extinction coefficients of oxyhemoglobin and reduced hemoglobin are considerably different.

Dreosti (8) gave as the relationship between the reflected and incident light intensities for granulated media the expression

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[1]Beckman Instrument Company, Inc., Fullerton, California.

[2]Heparin is an anticoagulant which prevents blood clotting by inhibiting the conversion of prothrombin to thrombin.

$$R = \frac{I_R}{I_0} = p - \sqrt{p^2 - 1}. \qquad (2.6)$$

where I_R = reflected light intensity

I_0 = incident light intensity

and $p = k\epsilon + 1$

and where k = a constant depending on the particle size, shape and composition

ϵ = absorption coefficient of the granular material.

Rodrigo (24) showed that for large p, R could be approximated by

$$R = \frac{I_R}{I_0} = \frac{1}{2p}. \qquad (2.7)$$

He then attempted to show that 1/R was linearly related to the percent oxygen saturation of blood. To do this he took a sample of human venous blood, oxygenated a portion of it, and then mixed varying percentages of this with the remaining venous blood. Although a linear relationship of 1/R to calculated oxygen saturation was obtained, the method used to determine percent oxygen saturation is questionable.

The first reflection oximeters, as well as most in use today, require that the blood sample be drawn into a cuvette outside the body. It was also discovered that non-specific effects due to variations in hematocrit, pulsatile flow and the like could be effectively eliminated through use of a second wavelength of light, much as had been done in spectrophotometry to compensate for variations in concentration and optical depth. The wavelength used in most cases in an isobestic point, a point where the extinction coefficients for oxyhemoglobin and reduced hemoglobin are equal.

Enson, Briscoe, Polanyi, and Cournand (9) applied Equation 2.7 to a two component system in order to show a linear relationship between oxygen saturation and the ratio of reflected light at the two wavelengths. Using Equation 2.7 they obtained

$$I_{R\lambda} = I_{O\lambda} \frac{d_\lambda}{2c_T\epsilon_\lambda} \tag{2.8}$$

in which the subscript λ refers to the wavelength used and d_λ and ϵ_λ refer to the specific diffusion and extinction coefficients respectively. c_T is the total concentration of the two components involved. The relationships of Equations 2.9 and 2.10 exist for a two component system consisting of oxyhemoglobin and reduced hemoglobin.

$$c_T\epsilon_\lambda = c_r\epsilon_{r\lambda} + c_o\epsilon_{o\lambda} \tag{2.9}$$

$$c_T = c_o + c_r \tag{2.10}$$

c_r and c_o are the concentrations of reduced and oxyhemoglobin respectively, while c_T is the total concentration of hemoglobin. $\epsilon_{r\lambda}$ and $\epsilon_{o\lambda}$ refer to the specific extinction coefficients of reduced and oxygenated hemoglobin at wavelength λ. They applied Equation 2.9 to Equation 2.10 at the two wavelengths 660 mμ and 805 mμ. They found that

$$I_{R_{805}} = I_{O_{805}} \frac{d_{805}}{2\left(c_r\epsilon_{r_{805}} + c_o\epsilon_{o_{805}}\right)} \tag{2.11}$$

and

$$I_{R_{660}} = I_{O_{660}} \frac{d_{660}}{2\,(c_r \epsilon_{r_{660}} + c_o \epsilon_{o_{660}})} \cdot \qquad (2.12)$$

Upon division of Equation 2.11 by Equation 2.12 and substitution of $c_r = c_t - c_o$ from Equation 2.10, they found that

$$\frac{c_o}{c_T} = \frac{I_{R_{805}}}{I_{R_{660}}} \cdot \frac{d_{660}\,\epsilon_{805}}{d_{805}\,(\epsilon_{o_{660}} - \epsilon_{r_{660}})} - \frac{\epsilon_{r_{660}}}{(\epsilon_{o_{660}} - \epsilon_{r_{660}})} \qquad (2.13)$$

or

$$\frac{c_o}{c_T} = A \frac{I_{R_{805}}}{I_{R_{660}}} - B, \text{ where A and B are constants.} \qquad (2.14)$$

Another innovation made by Enson et al. (9) was the use of fiber optic catheter to allow in vivo measurements of reflected light. Some of the characteristics of glass fibers, similar to those used, are discussed in the following section. Their catheter consisted of two bundles of fibers, the ends of which were interspersed with one another at the catheter tip. Light was focused on the end of one bundle. A rotary filter alternately filtered the light to 660 and 805 mμ 20 times per second. This bundle of fibers transmitted the light to the catheter tip where the light emerges and is scattered and absorbed by the blood. Some of the back scattered light is picked up by the second bundle of fibers and transmitted to a photomultiplier tube, the output of which is alternately a measure of the back scattered light at 660 and 805 mμ. He then determined the ratio of $I_{R_{660}}/I_{R_{805}}$ and plotted this versus oxygen saturation

and obtained a linear relationship, as predicted by Equation 14. They determined oxygen saturation by the Van Slyke manometric method and obtained a standard deviation of 1.9 percent between 20 and 100 percent oxygen saturation. This method of reflection oximetry, for the first time, allowed the measurement of percent oxygen saturation at selected sites *in vivo* as well as *in vitro*[1].

B. Relative Accuracies

An excellent comparison between the gasometric method of analysis and the spectrophotometric procedure of Gordy and Drabkin (12) is given in a note added to that same paper by Julian B. Marsh (20). He reported that results from four different groups showed mean differences between the gasometric and spectrophotometric methods ranging from 0.41 percent and 1.97 percent. Enson, Briscoe, Polanyi, and Cournand (9) report that their two wavelength method of reflection oximetry and the gasometric method agree within a standard deviation of 1.9 percent between 20 percent and 100 percent oxygen saturation. Zijlstra and Mook (35) report the mean difference between their reflection method and the gasometric method of 1.7 percent in the range of 25 to 88 percent. It is concluded that any of these methods, when used properly, can result in roughly equivalent accuracies.

C. Anomalous Properties of Blood

The process of blood coagulation is essential to the condition of hemostasis[2] within the human body. Without this coagulation, or clotting

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[1] A Latin phrase meaning "outside the living body."

[2] Hemostasis means prevention of blood loss.

process, one would bleed to death from blood loss due to internal bleeding. Ultimately, the coagulation process involves the conversion of the plasma protein, fibrinogen, into long chains forming gossamer networks which trap the cellular components, and prevent excessive loss of blood. The enzyme which causes the fibrinogen to fibrin reaction to proceed is thrombin, which itself is activated from prothrombin by procoagulants released when trauma or foreign materials are present in tissues or blood stream. The inter-relationships of these factors are shown in Figure 3. Clotting is a positive feedback mechanism, since thrombin also stimulates the production of additional procoagulent. Two factors prevent the clotting of blood in the normal vascular system. First, the endothelium[1] of the vascular walls is too smooth to allow adherence of clots and clotting factors. (They are carried away by flowing blood.) Secondly, there are normally anticoagulants present which prevent the conversion of prothrombin to thrombin. Among the natural anticoagulants found is heparin. The addition of abnormal amounts of heparin to blood can effectively prevent clot formation, even when conditions are present which would normally create clotting. This method has found popularity in prevention of clotting in both *in vivo* and *in vitro* situations. Another method of prevention of clotting *in vitro* is to defibrinate the blood (remove the fibrinogen from the plasma). Defibrination can be accomplished by gently stirring the blood with an object to which fibrin will adhere. The object's presence in the blood catalyzes fibrin formation which in turn attaches

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[1]The endothelium is the inner lining of the vessel walls.

to the foreign object and is removed when it is withdrawn. The latter method, however, has the disadvantage that it affects other properties of blood in addition to preventing clotting.

When blood is not undergoing shear, the red blood cells have the tendency to assume positions with their concave faces adjacent, much like a stack of coins. The formation of these stacks of cells is called rouleaux formation. Zijlstra (35) attempted to correlate the rate of rouleaux formation with the amount of light reflected from blood. It seems reasonable to suspect that the decrease in exposed surface area would result in a decrease in the light reflecting ability of blood. Cokelet (6) studied the effects of rouleaux on blood flow properties. Some of his results will be discussed in the following section on blood flow.

Another property of blood which alters its physical properties is the alteration of shape of the red blood cells due to ion concentration changes and pH. Salmon, Stish, and Visscher (27) studied the effect of P_{CO_2}[1] on the optical density of dog blood at 660 and 805 mμ. They found that, for 100 percent oxygenated blood with constant hematocrit, there was a decrease in optical density with an increase in carbon dioxide tension. They performed duplicate experiments with hemolyzed blood and found no change in optical density with P_{CO_2}. The conclusion reached is that the change in optical density is due to alteration of the shape of the red blood cells. They argue that, due to the Donnan effect and the permeability differences of the red cell membrane, the higher P_{CO_2} causes anion

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[1] P_{CO_2} indicates partial pressure of carbon dioxide.

shifts into the cell and cause it to swell. Sinclair, Sutterer, Fox, and Wood (31) studied the alteration of the optical density of blood due to the injection of transparent hypertonic and hypotonic solutions. They found that the injection of hypotonic solutions had little effect on the optical density while hypertonic solutions tended to cause an increase in the optical density. They concluded that the hypertonic solutions caused the red cells to shrink and that the more highly curved surfaces reflect more light and so increase the optical density. While this seems reasonable at first glance, there is some doubt about the validity of this conclusion due to the relative sizes of the particle edges with respect to the wavelengths involved.

Another effect of the tonicity of dilutents is, in extreme cases, hemolysis of the red blood cells. The mechanism involved in hemolysis is not completely understood, but most theories involve changes in the permeability of the red blood cells. A change in the permeability is suspected to result in the shift of anions into the cell, accompanied by swelling and eventual rupturing. Another theory is that hemolytic agents enter into combination with the protein-lipid material of the cell membrane and cause a loss in its ability to concentrate the hemoglobin. In any event, hemolysis can be induced by the addition of saponin, distilled water, or many other known agents and also by mechanical action of freezing and thawing.

D. Blood Flow

The pulsatile flow of blood through the distensible arterial system of the body has been the subject of much study. As yet, a detailed mathematical analysis of *in vivo* blood flow is not possible. It was stated

earlier that blood can be thought of as being a colloidal suspension of the cellular components, (mainly red blood cells), in plasma. The addition of the colloids makes blood non-Newtonian throughout at least part of its physiological range. The ability of the red blood cells to form rouleaux creates additional problems since the size and shape of the colloids become shear dependent.

Much of the past work on the rheological properties of blood had been summarized by Bayliss (1). A fluid undergoing laminar flow in a circular tube will undergo shear along concentric circular sleeves. This occurs because the velocity is a function of the distance from the tube axis and for a tube of radius R is

$$U_r = \frac{2Q}{\pi R^2}\left(1 - \frac{r^2}{R^2}\right) \tag{2.15}$$

where U_r = axial flow velocity at radius r

and Q = volume rate of flow through the tube.

The rate of shear is equal to the magnitude of the velocity gradient, and can be found by differentiating Equation 2.15. This yields

$$\dot{\gamma} = \left| -\frac{4Qr}{\pi R^4} \right| = \frac{4Qr}{\pi R^4} \tag{2.16}$$

where $\dot{\gamma}$ = rate of shear.

The shear stress is equal to η times the rate of shear, where η is the viscosity of the fluid, and thus is

$$\tau_r = \frac{4\eta Qr}{\pi R^4} . \tag{2.17}$$

Cokelet (6) also concluded that the yield shear stress exhibited by blood was a result of the ability of the red cells to form rouleaux. The relationship between the length to diameter axial ratio, J, and the shear rate, $\dot{\gamma}$, for dilute suspensions was developed by Casson (5) for an axial ratio very much greater than unity and is given by Equation 2.18.

$$J = \left(\frac{9F_A}{48\eta_0 a \dot{\gamma}} \right)^{\frac{1}{2}} \tag{2.18}$$

where η_0 = suspending medium viscosity

F_A = cohesive force between particles

a = a constant depending on the orientation of the aggregates with respect to the fluid streamlines

and $\dot{\gamma}$ = shear rate.

For shorter aggregates he assumed the aggregate axial ratio to be given by

$$J = \alpha + \beta \left(\frac{1}{\eta_0 \dot{\gamma}} \right)^{\frac{1}{2}} \tag{2.19}$$

where α and β are constants.

The technique does not take into account particle and aggregate interactions occurring in high packing densities in blood. The interesting point to be noted here, however, is that the predicted axial ratio varies as the inverse square root of shear rate in both cases. Cokelet also showed that human blood fulfilled the shear stress-shear rate relationship

$$\tau^{\frac{1}{2}} = b + s\dot{\gamma}^{\frac{1}{2}} \tag{2.20}$$

where s and b are constants depending on hematocrit.

It should be noted that Equations 2.15 to 2.17 are valid only for Newtonian fluids undergoing laminar flow. The Reynolds number, R, of a flowing fluid is an indication of whether or not the flow will be laminar and is defined as

$$R = \frac{\bar{U}a\rho}{\eta} \tag{2.21}$$

where $\bar{U}$ = the average flow velocity

a = the radius of the tube

ρ = the density of the fluid

and η = the viscosity of the fluid.

The greater the value of the Reynolds number obtained, the greater the possibility of turbulence occurring. Strictly speaking, the Reynolds number is applicable only to long straight tubes. However, it can be of use as an indication of whether turbulence will occur in other instances as well. For instance, for a Reynolds number of 200 or greater, turbulence is likely to occur when there is branching in the tube, but the turbulence will die out as the liquid flows further down the tube. The critical value of the Reynolds number in a smooth straight tube is about 1000, a value which is attained in vivo only in the aorta. Values of R of several hundred can be attained in the larger arteries, so that turbulence at branches is likely.

Benis (2) observed some extraordinary transient phenomena when blood flow was started from rest. He found that on start-up the red cells formed a tight core around the tube axis, with the core breaking up as the flow rate increased. This phenomenon was observed only on blood samples

which formed agglomerates under static conditions. Another observation was "plasma skimming," in which flow into a tributary branch was seen to have a lower hematocrit than that of the larger vessel. This effect was attributed to the momentum of the red cells carrying them past the tributary inlet.

Goldsmith and Mason (11), in observing the rotation of discs in suspensions undergoing laminar flow through rigid tubes, found that the angular velocity of rotation was not constant. The angular velocity they considered was in the plane formed by the axis of flow and the center of the discs. They found that the angular velocity was greatest when the discs were orientated with their axis parallel to the axis of flow and a minimum $\pi/2$ radians from that position. These results were for dilute suspensions and laminar flow, and as such there is some doubt as to their applicability to in vivo blood flow. However, it is an indication that there may be a statistical orientation of red cells in flowing in vivo.

E. Fiber Optics

The conduction of light through a cylindrical rod by total internal reflection is not new, but the application of this principle to what is now known as "fiber optics" did not become widely used until late in 1958. Siegmund (30) gives excellent background material on the history and application of fiber optic principles. Basically, a glass fiber is a smooth filament of glass of index of refraction, n_1, which has been clad with a second glass of a lower index of refraction, n_2. The effect of the cladding glass is to produce a uniform surface at which total reflection can take place.

For the case of a meridional ray (one which passes through the axis

of the fiber) entering the fiber at an angle, α, Snell's law gives the smallest angle at which total internal reflection can take place. This in turn can be used to determine the maximum angle, α, at which a meridional ray can be accepted for transmission along the fiber. This yields:

$$\alpha = \arcsin \sqrt{n_1^2 - n_2^2} \qquad (2.22)$$

Any light entering the fiber at an angle less than α will thus undergo total internal reflection.

Most glass fibers used can transmit light of wavelengths 400 to 2000 mμ. A typical fiber will absorb about 50 percent of the light it receives for every seven feet of length. This loss is due to the slight loss incurred at each internal reflection. There are additional losses at the ends of the fibers due to imperfections in polishing. The flexibility of a bundle of glass fibers is dependent upon the size of the individual fibers and the closeness of confinement of the bundle. (A loosely packed bundle will allow considerable flexing without breaking individual fibers.)

Enson, Briscoe, Polanyi, and Cournand (9) demonstrated the usefulness of the flexible fiber optic light guides in the field of reflection oximetry. The advantage of this catheter is that it allows the measurement of percent oxygenation at any point in the vascular system to which the catheter tip can be maneuvered.

III. APPARATUS

A. Optical System

A fiber optics catheter was constructed which was similar to the one described by Enson, Briscoe, Polanyi and Cournand (9). The glass fibers used to construct the catheter were taken from a four foot bundle of incoherent fibers manufactured by American Optical Company[1]. Individual fibers were about 0.003 in. outside diameter and consisted of an inner glass core of index of refraction equal to 1.62 and an outer cladding of glass of index of refraction of 1.52. The fibers will transmit light of wavelengths from 400 to 2000 mμ. They absorb about 50 percent of the light intensity for every seven feet of length.

Construction of the catheter was begun by taking a group of approximately 100 fibers of equal length and sliding them into a three foot length of Intramedic[2] polyethelene tubing. The outside diameter of this tubing was about 0.080 in. The inside diameter was approximately 0.050 in. This allowed the catheter to flex to a radius of about one and one-half inches without serious danger of fiber breakage.

Epoxy resin was then spread over one end of the fibers for a length of about 1/4 in. after which a 1/4 to 3/8 in. length of Alphex[3] 3/64 in. diameter irradiated polyvinyl shrinkable tubing was placed over this section. The shrinkable tubing was heated until it had shrunken down to tightly enclose the bundle of fibers. This process forced the fibers at

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[1]American Optical Company, Southbridge, Massachusetts.

[2]Clay-Adams, Inc., New York, New York.

[3]Alpha Wire Corp., New York, New York.

the tip into a closely packed bundle approximately circular in cross section and left the spaces between the fibers filled with epoxy compound. A thin coating of the adhesive was then placed on the surface of the shrinkable tubing and the catheter tubing forced over this to create a water tight seal at the catheter tip.

The tip thus formed was then polished using a succession of abrasives. The final polishing was with jewelers rouge. This process reduced the end losses created by internal reflections and also created a smooth surface to minimize the possibilities of clot formation at the tip.

After the end of the catheter tip had been formed and polished, the loose end remaining was divided into two bundles in such a manner that the fiber ends of each bundle were uniformly dispersed at the polished end. Each of these two bundles was inserted into a ten inch length of the polyethelene tubing and the tips of each formed as described above. These two tips will hereafter be referred to as the transmitting hub and receiving hub and designated as hub T and hub R respectively. The "Y" junction of the catheter was then reinforced by shrinking a three inch length of larger shrinkable tubing over it so that the shrinkable tubing and catheter prevented any stress from being applied to the fibers. Each of these hubs was then polished to complete the catheter. In later experiments a light shield was placed around the catheter tip to prevent light reflected from sidewalls of the vessel from entering the catheter. This shield was formed from a 3/4 in. length of 0.1 in. O.D. shrinkable tubing. The catheter tip was placed about half way into the shrinkable tubing the end of which was then shrunk onto the catheter for a length of about 1/4 in. Two rectangular sections were cut away from opposite sides of the

uncontracted polyvinyl tubing. The openings thus formed extended from the level of the catheter tip back to the shrunken portion, allowing blood to flow freely through the shield and past the catheter tip. Side reflection variations were prevented by the portion of the shield adjacent to and beyond the catheter tip. A photograph of a completed catheter with shield is shown in Figure 4.

The light source used in these experiments was a Spectro-Physics[1] Model 130 gas laser. This is a direct current (d.c.) excited helium-neon laser and was operated in a confocal[2] configuration. Output of the laser is about 0.5 mw at a wavelength of 632.8 m . The laser was operated such that it produced a multimode pattern, and as such, phase coherence between modes was not present. The diameter of the light spot was about 0.1 in. at the exit aperture.

An adapter was made to hold the catheter transmitting bundle hub T in place on the laser such that the laser beam illuminated the fiber bundle uniformly. This adapter consisted of a piece of 1/4 in. thick plastic with a hole the size of the catheter tip drilled in it. This piece was attached to the laser such that catheter hub T was held directly in the laser beam. Two clamps then held the catheter in place in the adapter.

The photomultiplier tube used was a Du Mont[3] 6467 ten stage photomultiplier tube with an S-11 photocathode response. The circuit for the

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[1]Spectra-Physics, Inc., Mountainview, California.

[2]Reflectors for the cavity have radii of curvatures equal to the separation distance.

[3]Allen B. Du Mont Laboratories, Inc., Clifton, New Jersey.

division of dynode voltages is shown in Figure 5. Typical anode to cathode voltages used were from 1200 to 1400 volts (v.), depending on the size catheter employed. The power supply used was a John Fluke[1] Model 412A, capable of supplying 0 to 10 milliamperes (ma.) at up to 2000 v.

B. Steady Flow

The pumping system used in the steady flow experiments is pictured in Figure 6. It consists of two pumps with piston-cylinder arrangements which could be filled with blood and driven at constant flow rates from one cylinder to another. Each cylinder consisted of a 3 1/2 in. inside diameter plexiglass cylinder fitted with a Bellofram[2] seal over a 3 1/4 in. diameter piston. This system, by avoiding the use of a tight fitting piston-cylinder arrangement, reduced friction and potential trauma to blood normally associated with the sliding parts. A rod was fitted to each cylinder so that each could be driven either manually or by one of the drive systems described below. The cylinders were connected together by 1/4 in. inside diameter glass tubing. Since both the cross sectional area of the glass tubing and the effective area of the piston are known, the flow velocities of the blood in the tubing can be easily calculated when the linear velocity of the piston is known.

Two drive systems were used to drive the blood from one cylinder to the other, one being used for velocities from 0.75 to 18.95 cm./sec. and the other for velocities from 10 to 70 cm./sec. The drive system used to

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[1] John Fluke Co., Inc., Seattle Washington.

[2] Bellofram Corporation, Burlington, Massachusetts.

attain the lower velocities consisted only of a Harvard Infusion Pump[1]. This pump has a linear drive mechanism which operates from a synchronous motor through a gear drive. The gear ratio can be changed in order to obtain different speeds, with the upper limit on speed being 1/24 in./sec. Only the five upper speeds on the drive were used. These provided fluid flow velocities in the tubing of 18.95, 7.55, 3.77, 1.895 and 0.75 cm./sec.

For higher velocities, the drive mechanism was removed from the infusion pump and mounted so it could be driven by a variable speed motor. The fluid velocities attainable with this arrangement ranged from about nine to 65 cm./sec. A microswitch was mounted near the shaft of the drive mechanism so that it operated once per revolution. By connecting this switch to a battery, an impulse per revolution was obtained. From a recording of these impulses the linear velocity of the piston and the fluid flow velocities could be calculated.

C. Pulsatile Flow System

The pump used to create pulsatile flow in this study was one designed in the Biomedical Engineering Program at Iowa State University as part of an artificial heart system. Basically it consists of an air driven diaphragm which drives blood from a reservoir past a one-way valve and into an "arterial" system. A second one-way valve prevents backflow into the venous system during pumping and yet allows the cavity to refill with blood from the "venous" side of the system. The pulse duration and pulse rate of the pump can be controlled electronically.

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[1]Harvard Apparatus Co., Dover, Massachusetts.

Connections to various parts of this system were made with distensible tubing so that the system would be a reasonable analog to an actual arterial system. Provisions were made in tubing connections to facilitate insertion of the catheters to measure pressure and reflected light, as well as an electromagnetic flow probe. Distal to these connections, the blood in the system was run through a gas bubbling apparatus before returning to the pump. The gases used in the oxygenator were either nitrogen or oxygen, depending on whether it was desired to reduce or oxygenate the hemoglobin.

D. Flowmeter

The flowmeter used was a square-wave electromagnetic type similar in design to the one described by Scher, Zepeda, and Brown (29). The flowmeter contains the components shown in the block diagram of Figure 7. The flow probe consists of two coils in a Helmholtz configuration with electrodes placed perpendicular to the magnetic field created by the coils. The flow probe used throughout the experiment was a Statham[1] OH-6100A with a lumen diameter of 10 mm. When a conductive fluid flows through the magnetic field created by the coils, a voltage is developed at right angles to the field and is picked up by the electrodes. The coils of this probe were driven by a 400 cps. square wave so that the output voltage at the electrodes was square wave modulated by the alternating magnetic field. The 400 cps. square wave used to drive the probe coils was synchronized with the 400 cps. voltage which drives the chopper coil in the preamplifier, an Offner[2] Model 481B.

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[1] Statham Physiological Division, Los Angeles 25, California.

[2] Beckman Instruments, Inc., Fullerton, California.

Normally, this preamplifier utilizes a set of chopper contacts to chop the incoming d.c. signal and create a square wave modulated a.c. signal for amplification. This signal is then amplified by a high gain a.c. preamplifier, the output of which is demodulated by a second set of chopper contacts operating in synchronism with the input contacts. In this application, the incoming signal from the flow probe was already square wave modulated in synchronism with the chopper so the input chopper was bypassed. Thus the signal was amplified and then demodulated by the output chopper as usual.

Synchronism of the square wave coil current with the chopper of the preamplifier was accomplished using the circuit shown in Figure 8. This circuit provides a variable phase shift for a 400 cps. input sine wave, taken from the same source that drives the chopper. A saturable amplifier amplifies the phase-shifted sine wave and creates a square wave. This square wave voltage is used to control the transistors driving the probe coils, alternately driving the probe current first in one direction and then the other. Thus the square wave current in the probe coils is phase locked to the 400 cps. voltage driving the output chopper. The mechanical output chopper demodulates the amplified signal from the flow probe electrodes. This provides an output which is proportional to the instantaneous mean flow velocity between the electrodes.

E. Recorder

The recorder used throughout this study was a Grass[1] Model 5 ink writing polygraph, equipped with chopper modulated preamplifiers for recording low level signals. The input to the preamplifier provides excitation for strain gauge transducers, such as the Statham[2] P23AA which was used to record blood pressures. The pen driven amplifiers have switchable high frequency filters of 60, 15, 3, 0.5 and 0.1 cps. with the amplitude of the voltage down 50 percent at the specified frequencies. Paper drive speeds are also selectable in nine steps between 0.25 to 100 mm. per sec.

While preamplification was not necessary for the signal from the photomultiplier tube, the signals from the pressure transducer and the flowmeter did require preamplification.

F. Spectrophotometer Materials

The spectrophotometer used to determine the percent oxygen saturation was the Beckman Model DU. Some modifications were made to the standard equipment to facilitate anaerobic handling of samples and to allow determinations to be made on whole hemolyzed blood.

A matched pair of one mm. optical path length cuvettes[3] were used and fitted to the standard one cm. cuvette holder by an adapter[4]. The volume of sample held by these cuvettes was approximately 0.3 ml. Special rubber caps were molded for the cuvettes. This was accomplished by constructing

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[1] Grass Instrument Company, Quincy, Massachusetts.

[2] Statham Instruments Company, Inc., Hato Rey, Puerto Rico.

[3] Beckman Instruments number 75196.

[4] Beckman Instruments number 72085.

a rectangular cavity about 3/8 in. deep and slightly larger than the cuvette cross section. The cavity was filled with Silastic[1] RTV No. 382 liquid potting rubber. A rectangular plexiglass plug with a cross section identical to that of the cuvette was inserted part way into the cavity during the curing process. Removal of the plug and rubber from the mold after the curving process resulted in a tight fitting rubber cap for the cuvettes. Anaerobic filling and flushing of new samples into the cuvettes could then be accomplished by the needle puncture technique described in Chapter IV.

Silastic rubber caps were also molded for the five ml. syringes used to take and hold blood samples. The mold for the caps was made by cutting a piece of flexible plastic tubing the same size as the syringe hub base and long enough to extend from the base to beyond the hub tip. This section of tubing was then placed around the hub base on the syringe and the cavity filled with Silastic RTV 382. Removal of the plastic tubing left a close fitting syringe cap which could be punctured to allow injection of the hemolyzing solution under anaerobic conditions.

A 30 percent[2] aqueous saponin solution was prepared weekly. The solution was kept under refrigeration until use.

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[1] Dow Corning Corp., Midland, Michigan.

[2] 30 grams of saponin to 100 ml. distilled water.

IV. METHODS

A. Spectrophotometer Sample Handling

The technique used to obtain and process blood samples for spectrophotometric determination of percent oxygen saturation was designed to insure as nearly as possible that no change in oxygen content in the blood took place in the time interval between sample withdrawal and analysis. The method used to obtain and hemolyze samples was modified from that of Hickam and Frayser (16), while the spectrophotometric procedure was modified from that of Gordy and Drabkin (12).

While the procedures used are described in detail below, it would be well to mention here the modifications made to accepted procedures and techniques and to give justification for the changes made. Distilled water was used to fill the dead space of the syringes in place of the heparin solution others have used. This was not a significant departure since the same volume of dead space fluid, and hence the same amount of oxygen in solution, was involved in either instance. Also, there was no need to heparinize the samples obtained since the blood was already heparinized in all the experiments performed.

No mercury was added to the sample syringe to facilitate mixing of the injected saponin solution and blood sample since it was found that complete hemolysis could be obtained by vigorously shaking the syringe immediately after injection of the saponin solution. The saponin solution used was not made anaerobic since at 25° C the 0.8 ml. of saponin solution mixed with the sample would contain only about 4.64×10^{-3} ml. of oxygen in solution. This amount, when compared with a hemoglobin oxygen capacity of 0.84 ml. per 4 ml. of blood, would cause errors in percent oxygen

saturation of at most 0.5 percent, the greatest error occurring at low oxygen saturations. This compromise is even less drastic than it appears since an anaerobic saponin solution would have a tendency to extract oxygen from the hemoglobin. This would lead to inaccuracies at higher levels of saturation.

Syringes were prepared in advance by lightly lubricating their plungers and filling them with distilled water. All air bubbles were then expelled from the syringes as well as all but about one ml. of the distilled water. Following this each syringe was capped with the molded rubber cap and placed in a rack until needed. Samples were taken in two ways, viz., by needle puncture and through a cannula attached to a three way valve. The needle puncture method was used for in vitro experiments while the cannula method was always used for in vivo experiments.

When needle puncture was used to withdraw samples, the needle was attached to the syringe and the syringe plunger pressed in as far as possible. This left the dead space of the needle and syringe filled with distilled water and eliminated all air bubbles. A direct puncture was then made and a 4 ml. sample withdrawn. The needle was then withdrawn from the blood system, removed from the syringe, and a careful examination was made for air bubbles in the sample. Any air bubbles found were immediately expelled and the syringe was then recapped with the rubber cap. Hemolysis was accomplished by injecting 0.8 ml. of the 30 percent aqueous saponin solution through needle puncture of the syringe cap. Vigorous shaking of the sample syringe hemolyzed the blood sample in a matter of seconds after which the sample was stored in ice water until spectrophotometer analysis was made.

When samples were withdrawn through a three way stopcock, the syringe hub was first loosely fitted into the stopcock connection. The stopcock valve was then partially opened to allow some of the blood to leak past the syringe hub and the syringe plunger depressed. This procedure washed away any air bubbles from the syringe-stopcock connection and at the same time prevented distilled water from entering the blood system. The connection was then tightened and the sample withdrawn and treated in the manner described above.

Spectrophotometer measurement of optical densities at 805 and 660 mμ were made by the standard procedures. A matched pair of one mm. optical path length cuvettes were fitted to the cuvette holder by an adapter. The reference cuvette was filled with distilled water and both cuvettes capped with the molded rubber caps. A five inch No. 22 gauge needle was placed on the first sample syringe and the syringe plunger depressed sufficiently to expel the air in the needle. The cap of the sample cuvette was then punctured and the needle inserted so that the tip of the needle rested on the bottom of the cuvette. A short No. 22 gauge needle was placed on an empty five ml. syringe and the sample cuvette cap punctured again. The needle tip was extended to just below the rubber cap. Injection of the sample into the cuvette and withdrawal of the other syringe plunger were then begun until the cuvette had filled and been flushed with at least three ml. of the blood sample. Following optical density determinations on the spectrophotometer, the next sample was flushed through the cuvette in a manner similar to that described above and this procedure was continued until optical densities had been determined for all samples.

The flushing of new samples into the cuvettes in the above manner is

not a new technique, but has not been justified with respect to inaccuracies involved. The errors involved in flushing the new sample into the cuvette can be approximated by assuming a constant volume chamber and perfect mixing within the chamber. The resulting relationship between actual concentration in the chamber, c, and the present and preceding sample concentrations, c' and c'', respectively, is

$$c = c' + (c'' - c') \exp(-V_f/V_c) \qquad (4.1)$$

where V_f = volume of new sample flushed through the cuvette

V_c = volume of the cuvette.

For a 0.3 ml. cuvette and a 3 ml. flushing volume one obtains

$$c = c' + (c'' - c') \exp(-10). \qquad (4.2)$$

The result indicates that the concentration of the cuvette is very nearly c' and that there is no error of practical consequence. Measured optical densities were substituted into Equation 2.5 and the percent oxygen saturations were then calculated. Data obtained in this manner were used as standards for comparison with reflected light intensities at 632.8 mμ.

B. Rigid System for Constant Flow

The purpose of the experiments described in this section was to determine the effects of constant flow velocities on the intensity of light reflected from blood of a constant degree of oxygen saturation. The block diagram of the experimental set up is shown in Figure 9. Blood was driven through the tube connecting the two pumps at average cross sectional velocities ranging from 0.75 to 70 cm./sec. The fiber optic catheter was

inserted into the glass tubing through a glass "T" connector located near pump B and situated so that the incident light would be directed into the flow from pump A.

Approximately a liter of blood was obtained from canine donors and was heparinized with 24 mg. of heparin per liter of blood. The blood was divided into two equal portions in beakers; one portion was reduced and the other fully oxygenated. Oxygenation and reduction of the blood was accomplished by bubbling oxygen and nitrogen respectively into the blood through tubing with a fritted termination. Foaming of the blood was prevented by capping the beakers with stainless steel sponges which had been treated with an antifoam agent[1]. By mixing quantities of oxygenated and reduced blood, blood of any desired percent oxygenation could be obtained for use in the pumping system. This allowed the determination of constant flow effects for various levels of oxygen saturation.

In a typical experimental run one portion of treated blood was placed into the pump system and residual air expelled as rapidly as possible. The flow velocities were then varied in steps over the range specified. As stated in Chapter III, it was necessary to change driving mechanisms in order to span the spectrum of velocities desired. Before and after each series of velocity runs at one level of oxygen saturation, a sample was drawn anaerobically. The oxygen saturation of these samples were determined spectrophotometrically and the average of the two oxygen saturations taken as the oxygen saturation for that series of runs. Oxygen saturation of the blood in the system was then altered by addition of a quantity of

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[1]Dow Corning Silicone XC-2-0033, Dow Corning Co., Midland, Michigan.

blood from one remaining portion and the above procedure repeated. Results obtained from this procedure are shown in Figures 10 and 11. Both of these Figures show the output of the photomultiplier tube as a function of this velocity of the blood at several levels of oxygen saturation. Output of the photomultiplier tube as a function of percent oxygen saturation for constant flow velocities are shown in Figures 12 and 13.

Blood flow velocities were determined from the relationship

$$\bar{U} = U_p (A_p/A_t) \tag{4.3}$$

where $\bar{U}$ = average blood velocity in the tube

U_p = linear velocity of the piston

A_p = cross sectional area of the piston = 8.78 sq. in.

and A_t = cross sectional area of the tube = 0.049 sq. in.

The velocity of the piston was calculated from the recorded rotary speed and pitch of the lead screw of the drive mechanism. These are related by

$$U_p = p(rps) \tag{4.4}$$

where p = pitch of lead screw in inches

rps = the number of the revolutions per second of the lead screw.

When the infusion apparatus was being used as a drive mechanism, the rotary speed of the lead screw were recorded by noting the speed setting on the pump. In the case of the modified drive, the microswitch connected to a battery was activated once each revolution of the lead screw. This was recorded by the Grass recorder.

C. Rigid System for Pulsatile Effects

The purpose of these experiments was to determine the effects on reflected light intensity of intermittent flow, reversal of flow and pressure. The system used was similar to that of Figure 9 except that an electromagnetic flowprobe was placed between pump B and the glass "T". A pressure catheter was inserted along with the fiber optic catheter and the tip of the former displaced about one inch downstream from the fiber optic catheter tip. The pressure catheter was connected to a strain gauge, the output of which was recorded along with the flowmeter output and photomultiplier tube output. Blood was obtained and oxygenated as before and the degree of oxygenation kept constant throughout the procedure.

To determine the effects of pressure on reflected light intensity, a clamp was placed on the rubber tubing between the flow probe and pump B in order to eliminate flow. The piston of pump A was then manually driven to vary the pressure of the blood. Since no flow was possible, any variations in the intensity of reflected light would have to be pressure induced.

The clamp was then removed from the tubing and the pistons manually driven so as to attain intermittent forward flow, intermittent reverse flow, and reversal of flow directions. Pressure, flow, and reflected light intensity were again recorded. Results are shown in Figures 14 through 17.

D. Distensible System for Pulsatile Effects

The objective of this experiment was to observe the artifacts present during pulsatile flow through distensible and rigid tubes and compare them with the results obtained in previous experiments. A block diagram of the system used is shown in Figure 18. The pump used was one under development

at Iowa State University and consisted of the pumping cavity and control system for varying pumping rate and duration as described in Chapter III. Provisions were made for changing the section of tubing in which the catheter tips rested so that pulsatile flow effects could be observed for tubes of different diameters.

Catheters were inserted as illustrated and the flow probe set in place. Pumping was then begun and reflected light intensity, blood flow and bloodpressure recorded. The section of tubing was then tightly wrapped with an inelastic sleeve which in turn was wrapped with inelastic tape. This process effectively made the tubing inelastic and allowed pulsatile effects in distensible and rigid tubing of the same diameters to be compared. Tubing with inside diameters of 0.375, 0.25 and 0.187 in. were used and the above procedure followed for each. Typical results for the 0.375 in. tubing, distensible and rigid, are shown in Figures 19 and 20 respectively. Figures 21 and 22 and Figures 23 and 24 display results obtained for the 0.25 and 0.187 in. tubing respectively.

E. In Vivo Experiments Recording Pressure

Two in vivo experiments were run to observe the reflected light intensity variation with oxygen saturation and pulsatile flow. Dogs were anesthetized with 30 mg. sodium pentobarbitol per kilogram of body weight. After exposing the left common carotid artery and the left and right femoral arteries, the animal was heparinized by injecting 3 mg. heparin per kilogram of body weight into the cephalic vein.

The exposed carotid artery was cannulated with a Horsley needle attached to a three way stopcock. A 50 ml. syringe was connected to one of the stopcock openings and the other opening was left for blood sample

withdrawal. The fiber optic catheter was inserted into the left femoral artery so that the catheter tip was located in the thoracic portion of the descending aorta. The pressure catheter was inserted into the right femoral artery far enough so that the pressure catheter tip was just behind the fiber optic catheter tip. Provisions were made so that the dog could be made to rebreathe, or breathe atmospheric air or oxygen. Rebreathing was accomplished by cannulation of the trachea and attachment of the tracheal tube to a rubber bag. Oxygen breathing was accomplished by attachment of the trachea tube to an oxygen supply, while for atmospheric air breathing the tracheal tube end was left exposed to the atmosphere.

Blood samples were withdrawn through the three way stopcock after about 30 ml. of blood had been withdrawn into the 50 ml. syringe. This procedure was designed to remove stagnant blood from the dead space just below the cannula and make fresh arterial blood available for sampling. The blood in the 50 ml. syringe was returned to the dog's system after the sample had been withdrawn. Sample handling after withdrawal was identical to that described in Section A.

Pressure and intensity of reflected light were recorded continuously throughout the experiments with the photomultiplier tube output filtered to about 0.1 cps. During the rebreathing maneuver a slow decrease in blood oxygen saturation resulted. This caused a decrease in the amplitude of the photomultiplier tube output. Blood samples were taken at intervals during the rebreathing process. After each episode the dog was allowed to breathe pure oxygen. A rapid increase in the photomultiplier tube output was noted when oxygen breathing was begun following which a sample taken as rapidly as possible. The purpose of the above procedure was to obtain

a correlation between reflected light intensity and oxygen saturation throughout a wide range of oxygen saturations in vivo.

In the second in vivo experiment, the procedure was nearly the same except that at each sampling time, a five to ten second recording of the unfiltered photomultiplier tube output was obtained. The unfiltered output was also recorded during the entire rebreathing cycle in order to observe pulsatile amplitude variations with the degree of oxygen saturation. Blood samples were again taken for spectrophotometric analysis.

A typical unfiltered output is shown in Figure 26 and filtered photomultplier outputs for the two experiments are plotted against percent oxygen saturation in Figure 27. Peak to peak variation of the pulsatile artifact was plotted versus percent oxygen saturation and is shown in Figure 28. Percent artifact in the output was plotted versus percent oxygen saturation and the results shown in Figure 29.

F. In Vivo System with Flowmeter

The purpose of the in vivo studies using a flowmeter was to determine the relationships of in vivo flow to the artifact imposed on the intensity of reflected light. Two flow probes were used. One was the flow through probe which was used earlier in the in vitro studies while the other was a clamp-on probe.

The procedure followed was to anesthetize the dogs as before and to expose the left and right femoral arteries. In one case, the flow through probe was inserted in the thoracic aorta and in another, the clamp-on probe was used in the abdominal aorta. Access to the thoracic aorta was gained via an incision in the left fifth intercostal space. The animal was heparinized following this, and the two catheters inserted so that

their tips were located distal to the point at which the flow probe was inserted. The intercostal arteries were ligated in the area of insertion of the flow probe, the aorta clamped proximal and distal to this point and an oval section of the aorta removed so that the flow probe could be inserted. The aorta was slipped over both ends of the probe and held in place by ligatures. Clamps were then removed, permitting flow through the probe, and the catheters were advanced so that their tips were just posterior to the flow probe, with the pressure catheter tip slightly anterior to the fiber optic catheter tip.

When the abdominal aorta was cannulated, access to the artery was gained through a midline incision in the abdomen. The catheters were inserted after heparinizing the animal as before, and the flow probe was clamped on. It was necessary to use extreme care with the clamp-on probe due to electrode contact problems, induced by motion of the blood vessel wall with respect to the transducer. Blood flow, pressure and reflected light intensity were recorded in each experiment. Figure 30 shows the decrease in artifact amplitude with decrease in percent saturation. The relationship between flow, pressure, and reflected light intensity are shown by Figure 31.

V. RESULTS AND DISCUSSION

A. Rigid System

Figures 10 and 11 illustrate that below a certain flow velocity the intensity of reflected light decreases with flow velocity. Zijlstra and Mook (35) have observed that reflected light intensity is inversely related in an unspecified manner to the length of a rouleaux. Equation 2.19, repeated below shows that for a dilute suspension the axial ratio of a rouleaux is inversely related to the square root of the shear rate $\dot{\gamma}$ by

$$J = \alpha + \beta \left(\frac{1}{\eta_o \dot{\gamma}}\right)^{\frac{1}{2}} \tag{2.19}$$

The relationship between shear rate and total flow Q for laminar flow was given by Equation 2.15. The average cross sectional velocity is related to total flow by

$$Q = \pi R^2 \bar{U} \tag{5.1}$$

where R is the radius of the tube.

Substituting this relationship for Q in Equation 2.15 yields

$$\dot{\gamma} = 4r\, \bar{U}/R^2 \tag{5.2}$$

where r is the radial distance from the axis of the tube.

The average shear $\bar{\dot{\gamma}}$ across the tube is then

$$\bar{\dot{\gamma}} = 2\bar{U}/R \tag{5.3}$$

Substituting this value for shear rate in Equation 2.19 gives

$$J = \alpha + \beta \left(\frac{R}{2\eta_o \bar{U}}\right)^{\frac{1}{2}} \qquad (5.4)$$

It is obvious that Equation 5.4 is not completely valid since the axial ratio J would be infinite for zero flow. However, under static conditions, Isaacs (19) reports rouleaux with as many as 16 red blood cells. Equation 5.4 does predict that the length of a rouleaux would vary inversely as the square root of the velocity. This then explains the increase in reflected light intensity with increasing velocity up to a velocity of about 20 cm./sec. Above this velocity, no change in output is seen. At these velocities cells apparently exist only as individuals and not in rouleaux.

The relationship between photomultiplier tube output and percent oxygen saturation for constant flow velocities is shown in Figures 12 and 13. Above a velocity of approximately 20 cm./sec., oxygen saturation and reflected light intensity are linearly related. The deviation of the point for 66.5 percent oxygen saturation from the otherwise straight line plot shown in Figure 12 is probably due to experimental error in the determination of oxygen saturation. Below 20 cm./sec., the oxygen saturation reflected light intensity relationship cannot be approximated as well by a straight line. This can probably be attributed to a difference in the rouleaux forming ability of the red blood cells at different levels of oxygen saturation. An interesting point arising from these experiments was the fact that in the region where few or no rouleaux exist, oxygen saturation and reflected light intensity are nearly linearly related. One might anticipate from this that a nearly linear relationship may exist

in vivo since it is probable that there is little rouleaux formation due to the pulsatile nature of the flow through the distensible arterial system.

Figure 14 is a recording of the relationship between pulsatile pressure variations and reflected light intensity in a rigid system under no flow conditions. There are no corresponding pulsatile variations in reflected light intensity. A decrease in reflected light intensity with time can be seen in the recording. This is in agreement with the observations of Zijlstra and Mook (35). The decrease can be attributed to the formation of rouleaux during the experimental procedure. The fact that there are no pulsatile variations in the reflected light intensity indicates that there is no first order effect due to pressure. These results are a definite indication that there is no change in the shape of the red blood cells due to pressure variations inasmuch as a change in shape would result in a change in rouleaux formation rate thereby giving pulsatile variations in reflected light intensities.

The effects of pulsatile changes in flow on reflected light intensity in a rigid system are shown in Figures 15, 16 and 17. These effects can be attributed to the streaming of the red blood cells along streamlines in the flow past the catheter tip. The output of the photomultiplier tube is slightly greater during forward flow than during reverse flow. A typical example of this is Figure 17. This result is probably due to the reverse flow streamlines carrying the red blood cells past the catheter tip and creating a volume immediately in front of the tip in which there are fewer red blood cells than normal, i.e., a volume with low hematocrit. As flow undergoes a change from forward to reverse, this effect is more noticeable

and is quite likely due to the difference in flow patterns during the acceleration process. The change from reverse to forward flow results in a temporary increase in reflected light intensity due to an accumulation of red blood cells in the volume in front of the catheter prior to the establishment of the normal streamlines. Intermittent reverse flows and intermittent forward flows result in variations of reflected light intensities similar to those discussed above.

B. Distensible System

In vitro experiments using pulsatile flow through distensible and rigid tubing were performed to determine the effects of pulsatile flow on the intensity of reflected light. Some of the problems associated with measuring flow are apparent from the amount of noise associated with the signal from the flowmeter. This creates difficulty in interpreting the results of these experiments as well as the in vivo experiments with the flowmeter.

Figures 19 through 24 display results obtained in these experiments. Figures 19 and 20 are from 0.375 in. I.D. tubing, distensible and rigid respectively. The reversal of flow artifact, that is a decrease in amplitude during reversal of flow is seen in both the rigid and distensible tubing. While the figures for distensible and rigid tubing in each case show some difference in the artifact observed, there is also a corresponding difference in the flow patterns.

C. In Vivo Experiments

Figure 25 shows a typical recording of pressure and photomultiplier output, filtered to 0.1 cps., obtained from in vivo experiments showing the variation of the reflected light intensity during a rebreathing

procedure. This indicates that most of the imposed artifact can be removed by filtering without seriously interfering with the ability to record relatively rapid changes in oxygen saturation. Wood, Sutterer and Cronin (34) and Enson, Jameson and Cournand (10), using other methods of detecting oxygen saturation, show that clinically useful results can be obtained with a frequency response of this order. A typical *in vivo* record showing unfiltered photomultiplier tube output and pressure is shown in Figure 26. A cyclic artifact with a period the same as that of cardiac activity can be seen. The cyclic sharp reduction in reflected light intensity is similar to that seen during flow reversal in the *in vitro* studies.

Figure 27 shows the filtered photomultiplier tube output for two *in vivo* experiments plotted against oxygen saturation as determined by the spectrophotometric procedure. In the range of oxygen saturations from 40 to 100 percent, a single straight line, as indicated by the solid line in Figure 27, can be used to approximate the results of both experiments. Below 40 percent oxygen saturation, reflected light intensity decreases less rapidly with a decrease in oxygen saturation. The two dashed lines in Figure 27 form bands of five percent oxygen saturation above and below the values indicated by the solid line. All experimental data in the range of 40 to 100 percent oxygen saturation lie within these bands.

Light reflectance from blood is a function of hematocrit, reaching a maximum at about 45 percent. In the normal range of hematocrits, i.e., from 40 to 50 percent, there is little change in reflectance. Above and below this range, however, reflectance drops off quite rapidly. This requires that a single wavelength system be recalibrated for use on blood

with a hematocrit outside the 40 to 50 percent range.

The peak to peak variation in output artifact was measured at several levels of oxygen saturation. These results are shown in Figure 28. This illustrates that the artifact amplitude is dependent upon oxygen saturation. The ratio of the peak to peak artifact amplitude to the average photomultiplier output for each oxygen saturation level was determined and the results plotted against oxygen saturation as shown in Figure 29. This shows that the percent of artifact in the output remains relatively constant. Figure 30 illustrates the decrease in both the artifact amplitude and average photomultiplier tube output with a decrease in oxygen saturation for an *in vivo* recording.

The relationships of pressure and flow to artifact are shown in Figure 31. A decrease in reflected light intensity is seen during the *in vivo* reversal of flow. One inherent difficulty in observing the artifact-flow relationship *in vivo* is the disturbance to normal flow patterns caused by the flow probe itself. One possible way to circumvent this problem might be to implant a loose fitting clamp-on probe in an animal for three to four weeks before performing the experiments. This would allow scar tissue to fix the probe in place and permit measurements to be made with a probe which would minimize flow disturbances.

VI. SUMMARY

A. Results

The data from these experiments show that the intensity of light reflected from blood is altered by pulsatile flow, but is independent of pressure variations. The intensity of light reflected from blood undergoing constant flow is dependent upon flow velocity up to a critical value. Above this value reflected light intensity is independent of velocity.

The intensity of light undergoing steady flow is dependent upon the length of rouleaux formations present. The shear rate of the blood determines the length of rouleaux present, and it in turn is determined by the velocity of blood flow.

Variations in reflected light intensity due to pulsatile flow are probably due to flow streamlines being set up around the catheter tip. During the time required to set up streamlines, periods of abnormal hematocrit are thought to exist in the vicinity of the catheter tip.

The amplitude of the artifact remains a constant fraction of the d.c. level of the photomultiplier tube output. A low-pass filter with a cut off frequency of 0.1 cps. removes nearly all of the pulsatile variations. Even with this filtering the system response is such that it can adequately follow the oxygen saturation changes normally encountered. The intensity of reflected light at 632.8 mμ is linearly related to the oxygen saturation of whole blood in the range of 40 to 100 percent. Oxygen saturation determined for blood of normal hematocrits compare within five percent saturation with those determined spectrophotometrically.

B. Recommendations

The pulsatile variation in reflected light intensity requires further study. The streaming properties of the red blood cells around the catheter tip should be studied to delineate the origin of the artifact. The effect of the shape of the red blood cells on the intensity of reflected light should be investigated since this factor apparently causes a change in their reflectance.

VII. FIGURES

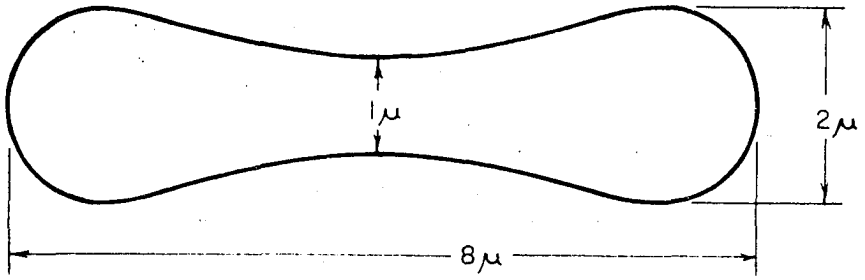


FIGURE 1 TYPICAL HUMAN RED CELL CROSS SECTION

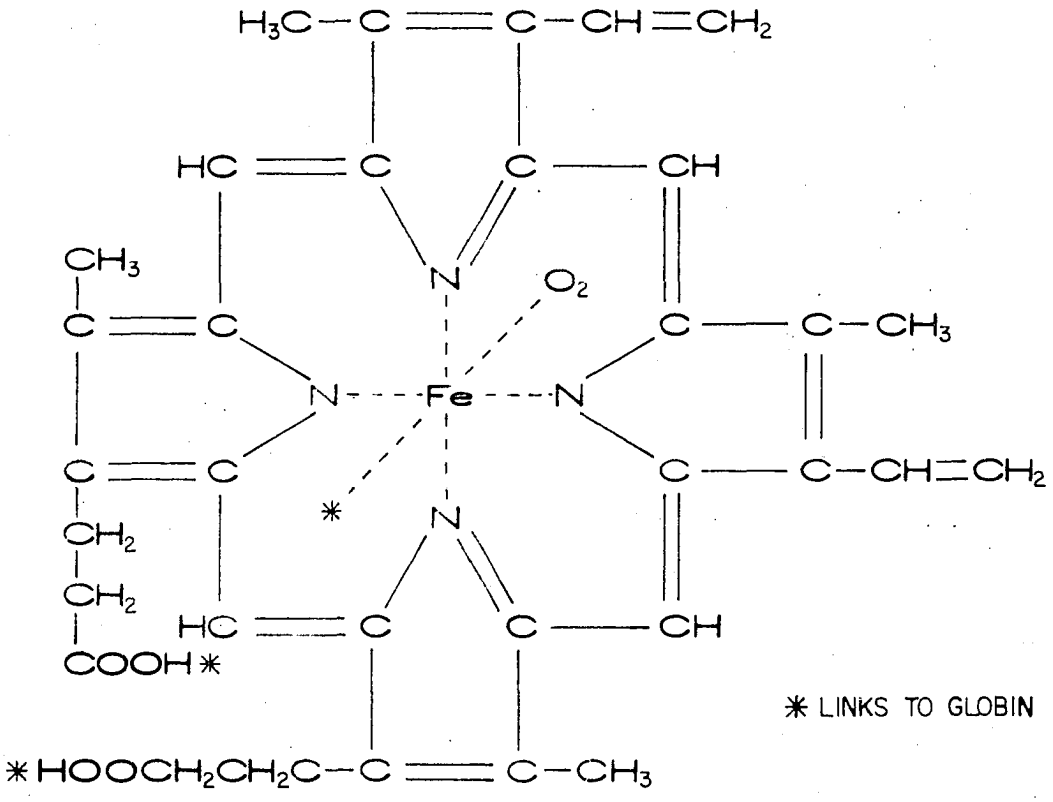


FIGURE 2 HEME GROUP OF HEMOGLOBIN

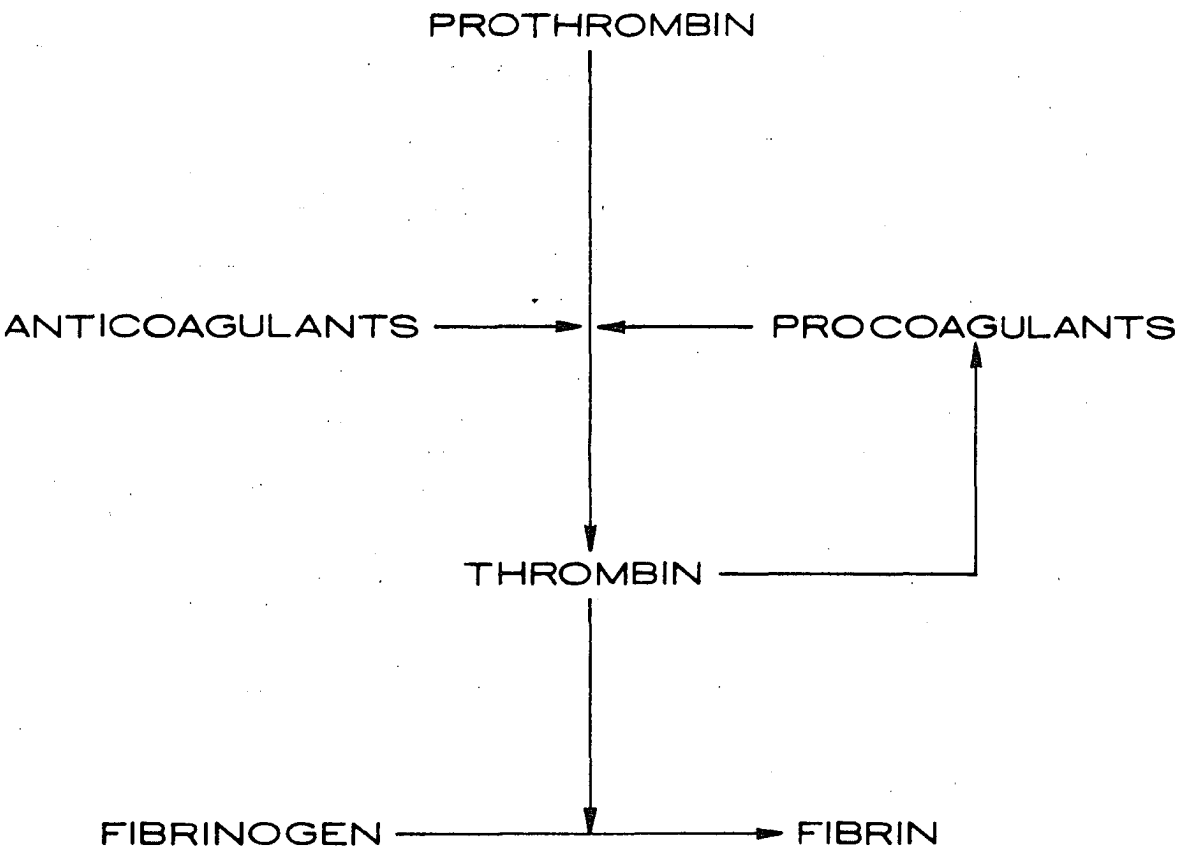
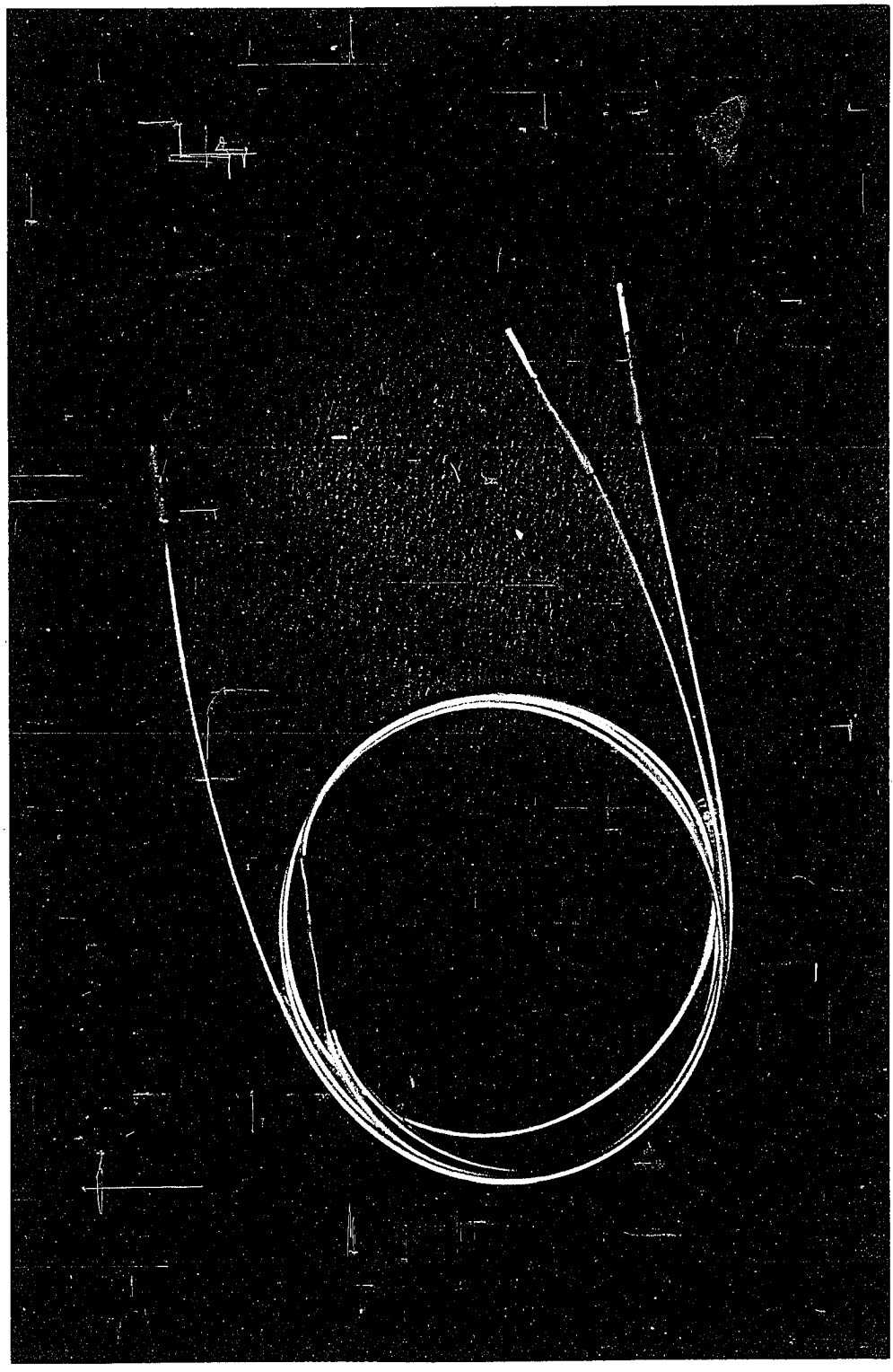


FIGURE 3 THE CLOTTING MECHANISM

Figure 4. Fiber optic catheter with tip cage



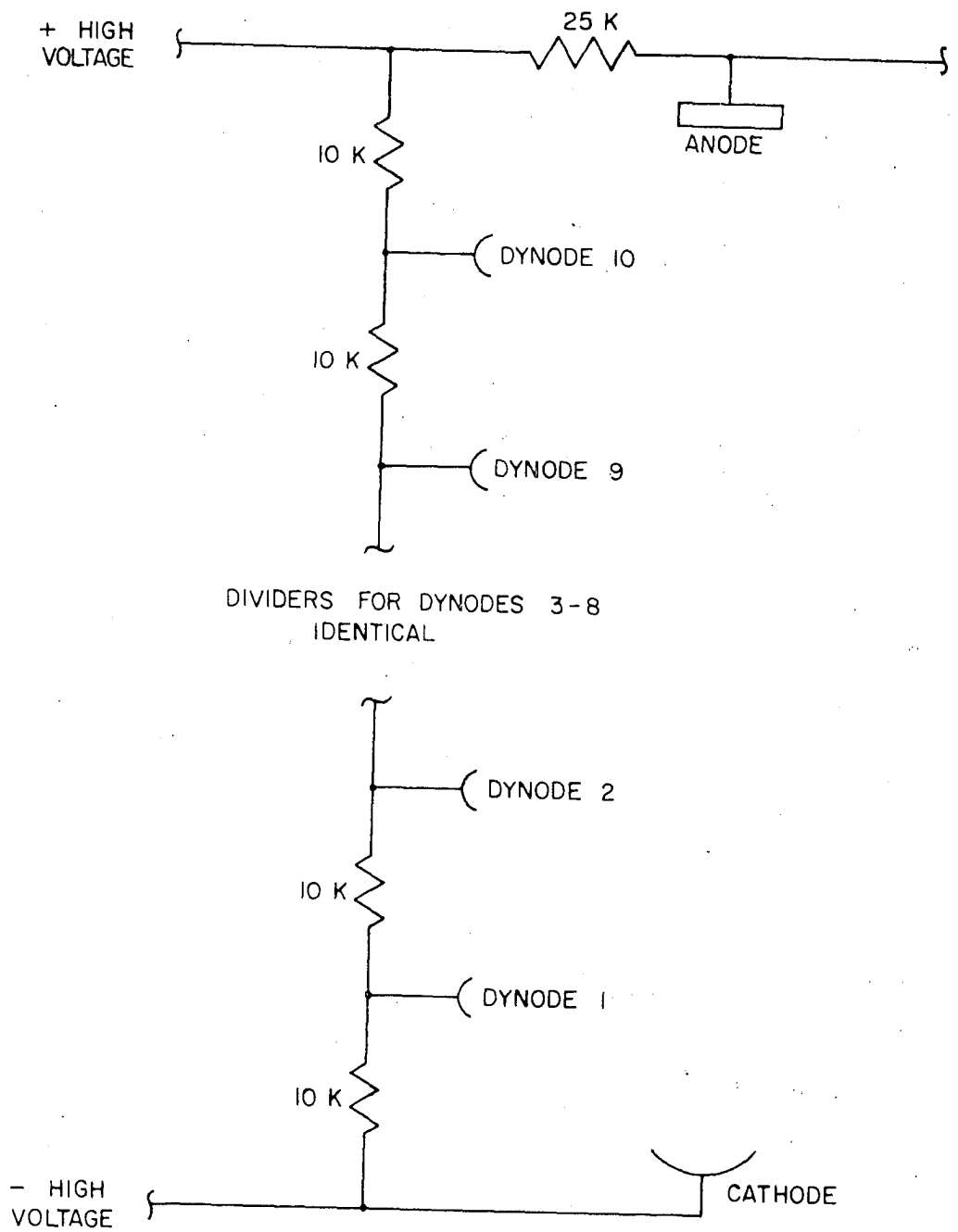
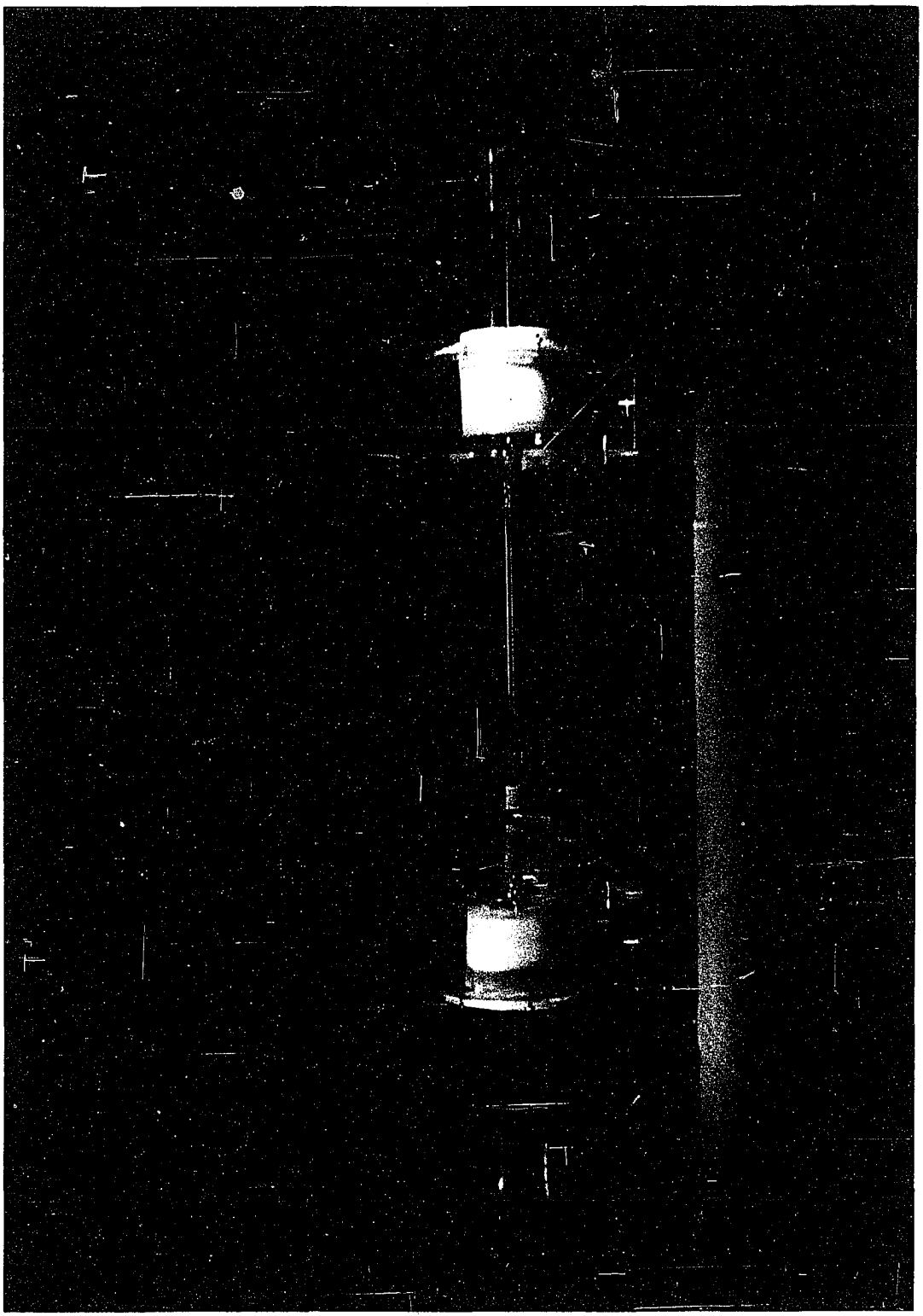


FIGURE 5 PHOTOMULTIPLIER TUBE CIRCUIT

Figure 6. Pumping system used for studies involving rigid tubing



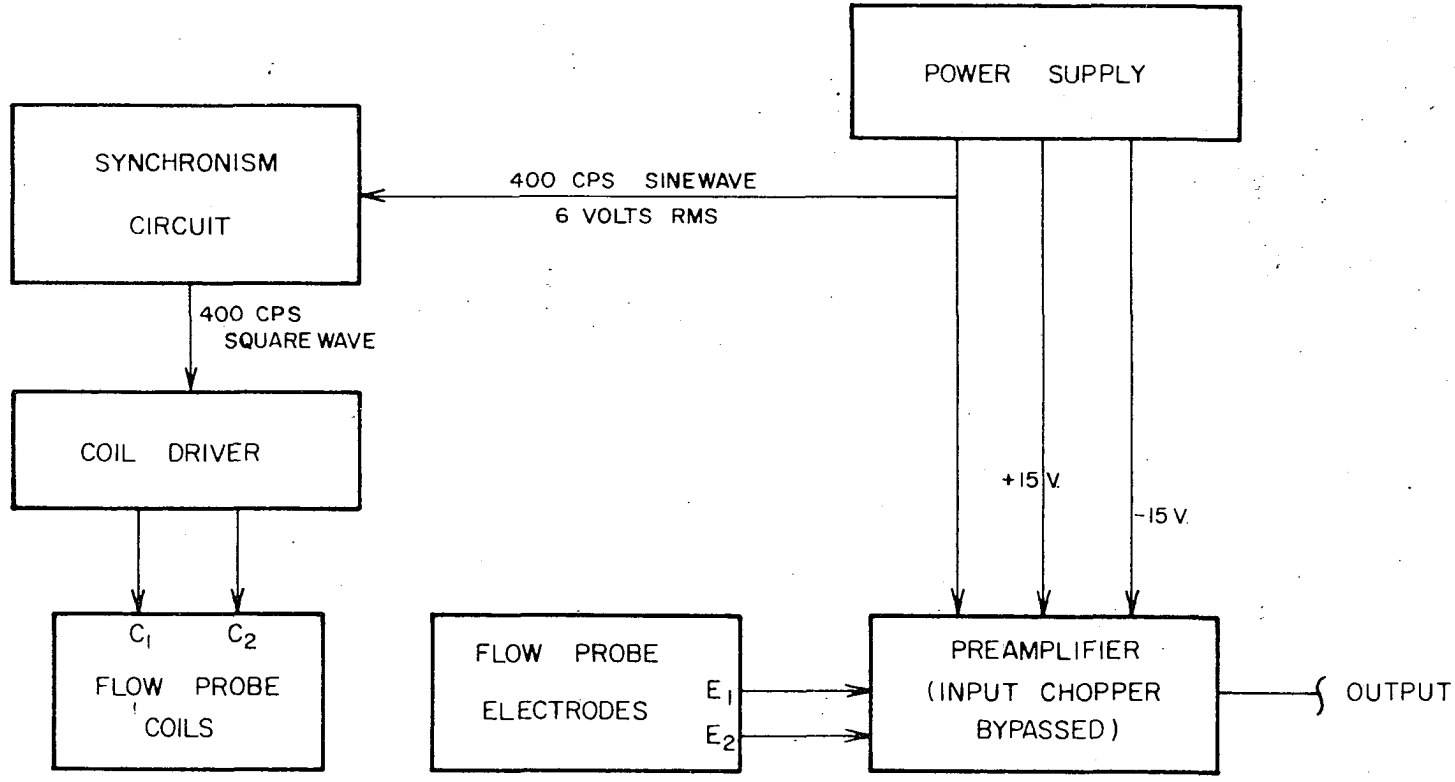


FIGURE 7 BLOCK DIAGRAM OF FLOWMETER

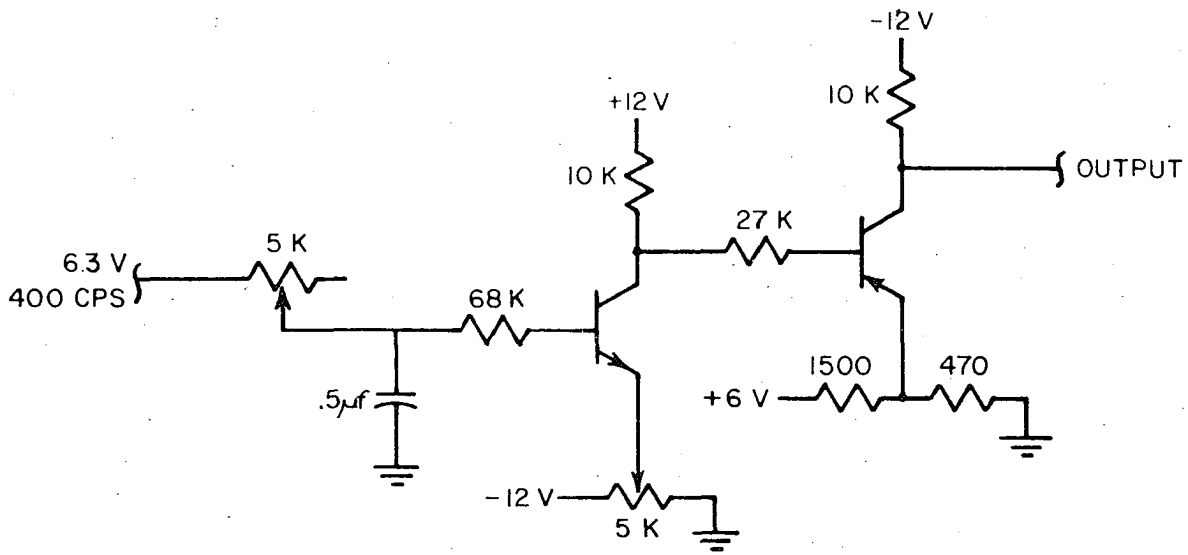


FIGURE 8 SYNCHRONISM CIRCUIT

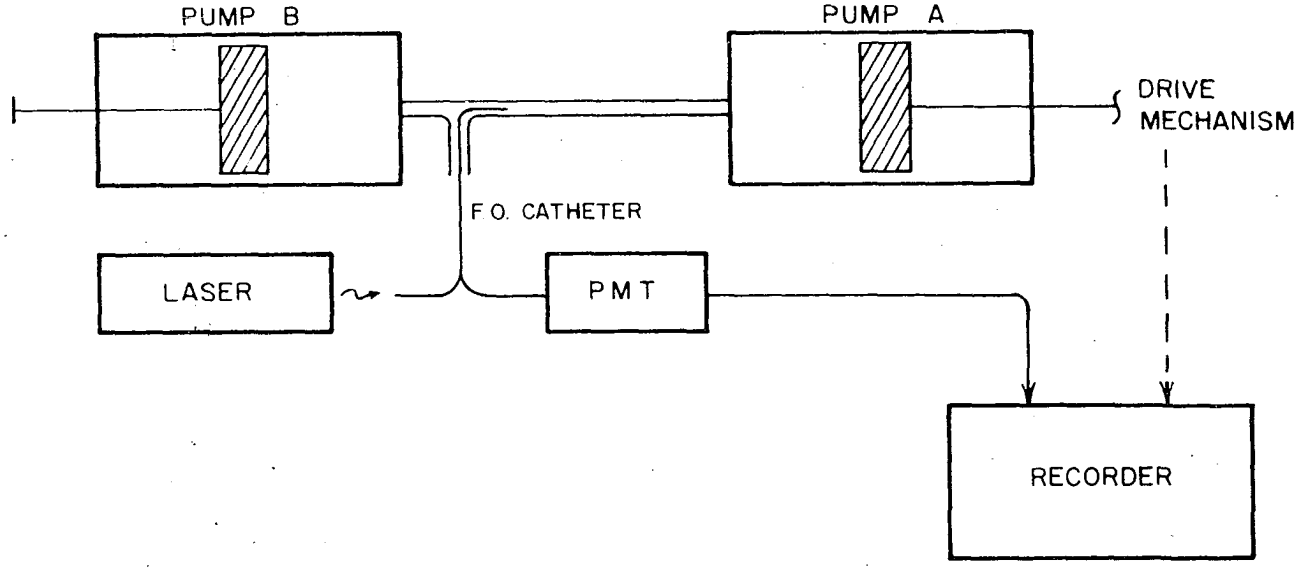


FIGURE 9 BLOCK DIAGRAM OF RIGID PUMP SYSTEM

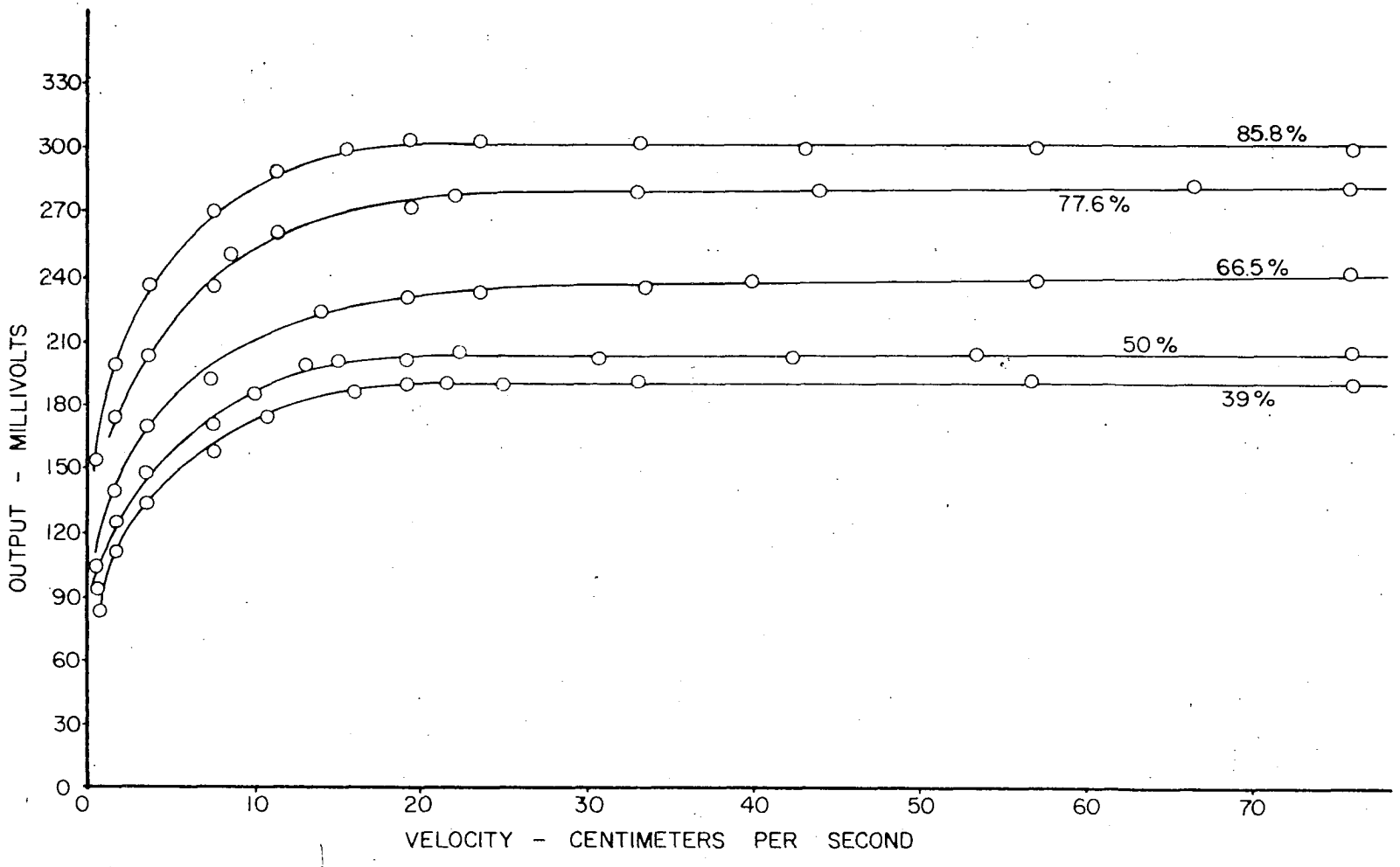


FIGURE 10 VARIATION OF OUTPUT VS. VELOCITY

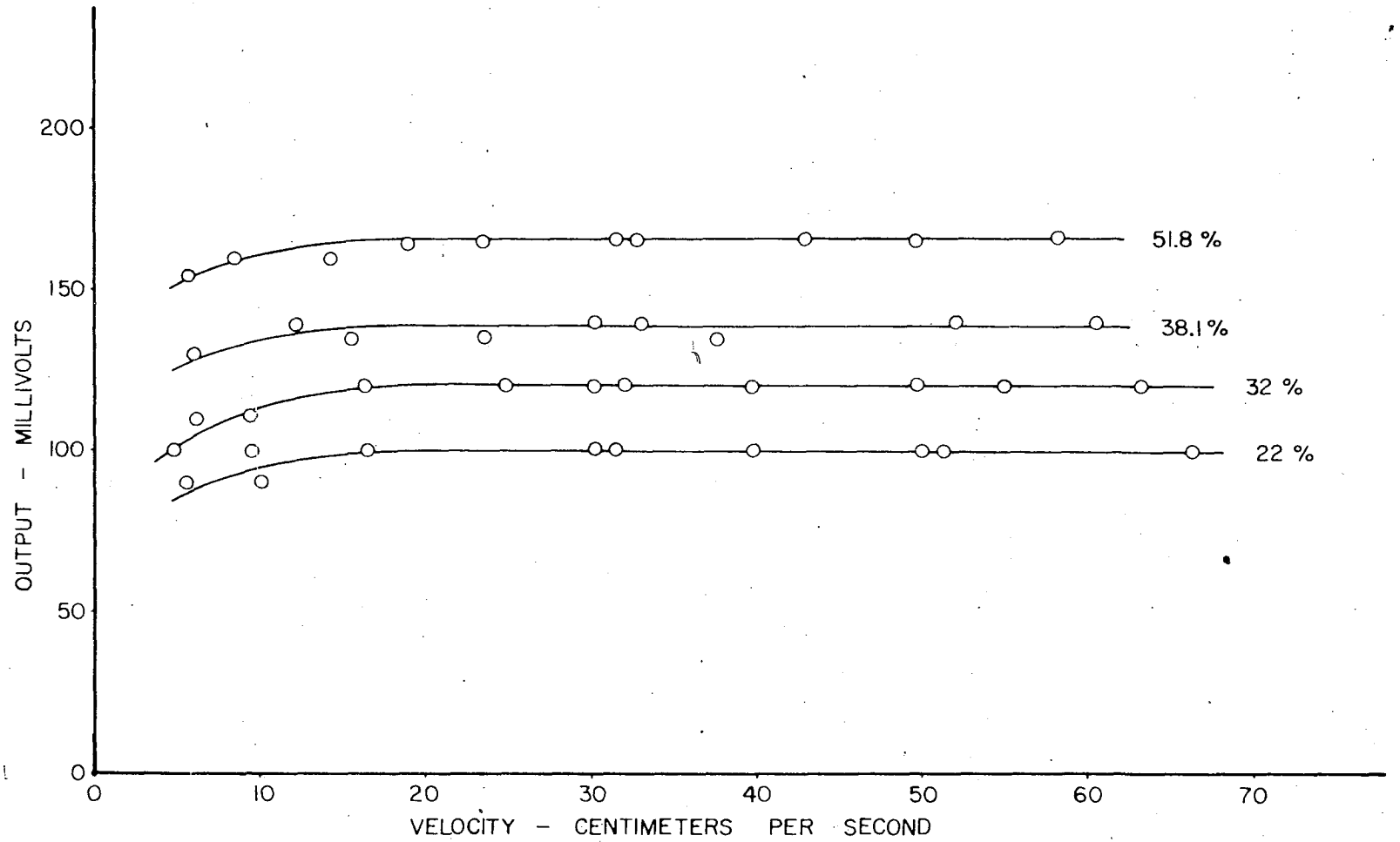


FIGURE 11 VARIATION OF OUTPUT VS. VELOCITY FOR CONSTANT SATURATIONS

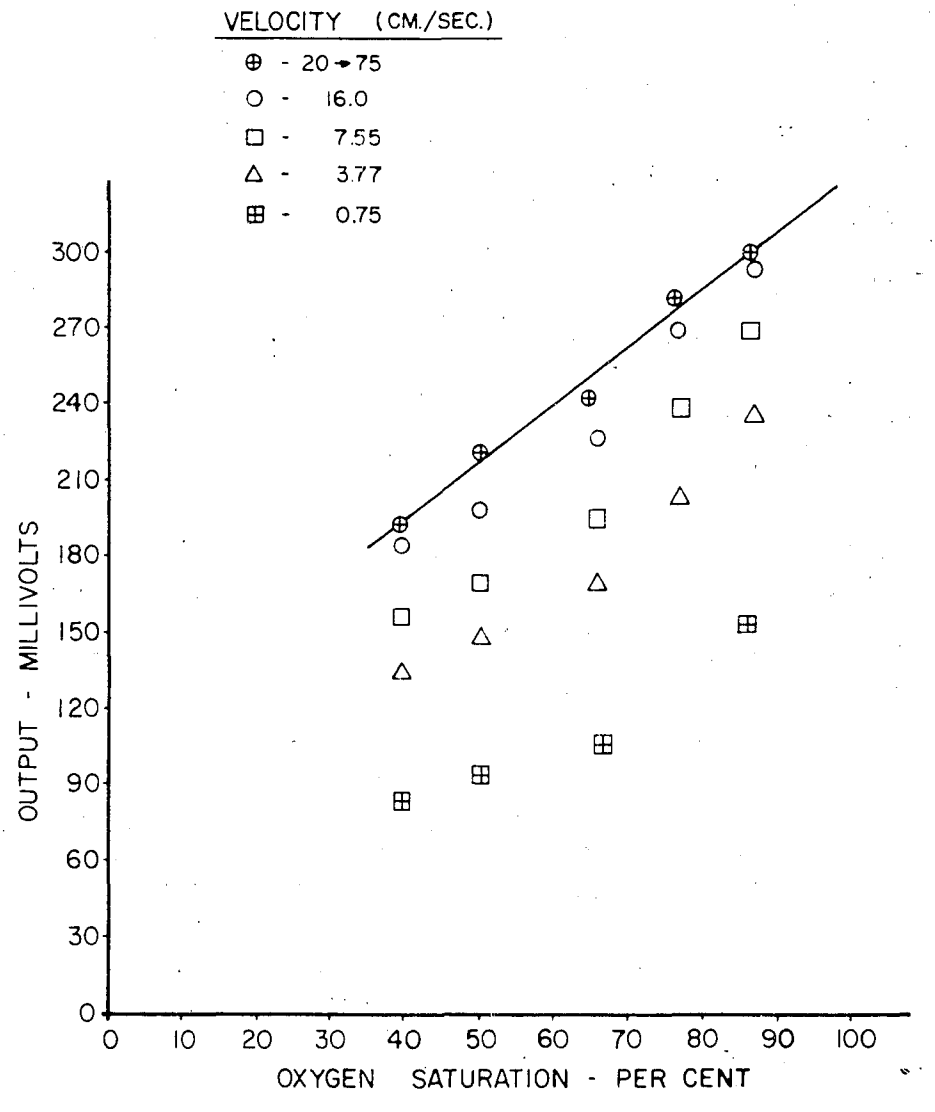


FIGURE 12 REFLECTED LIGHT VS. OXYGEN SATURATION FOR CONSTANT VELOCITIES

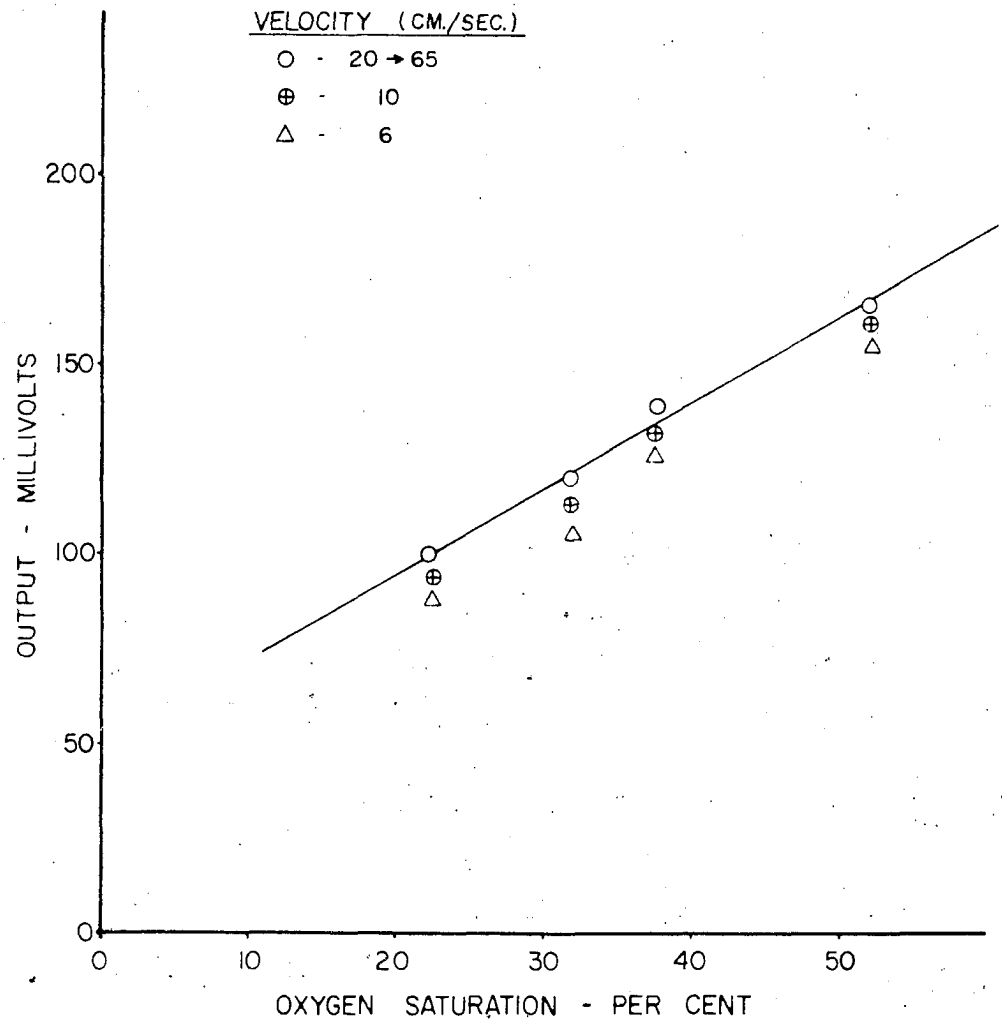


FIGURE 13 REFLECTED LIGHT VS. OXYGEN SATURATION FOR CONSTANT VELOCITIES

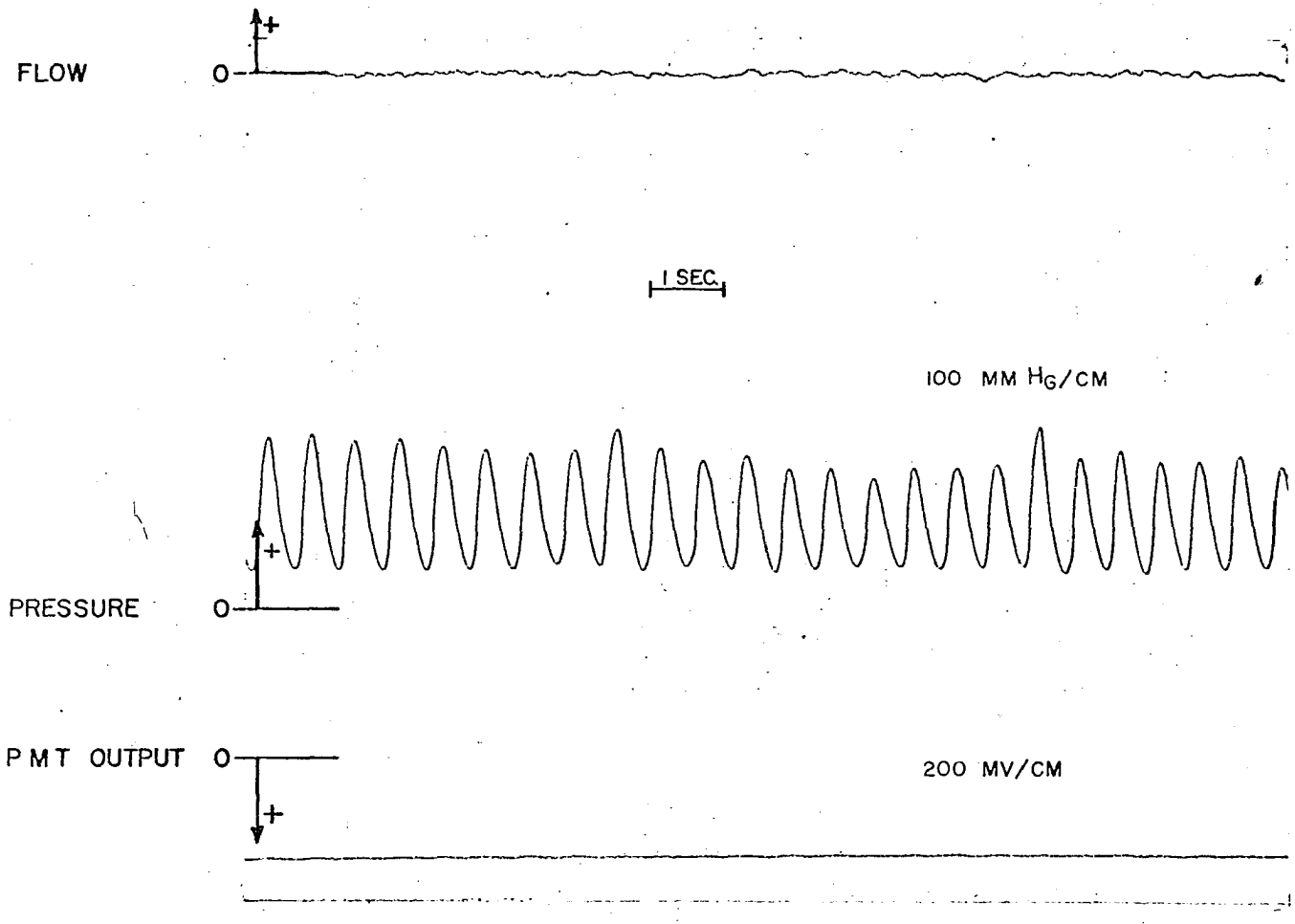


FIGURE 14 PULSATILE PRESSURE - REFLECTED LIGHT RELATIONSHIP FOR ZERO FLOW

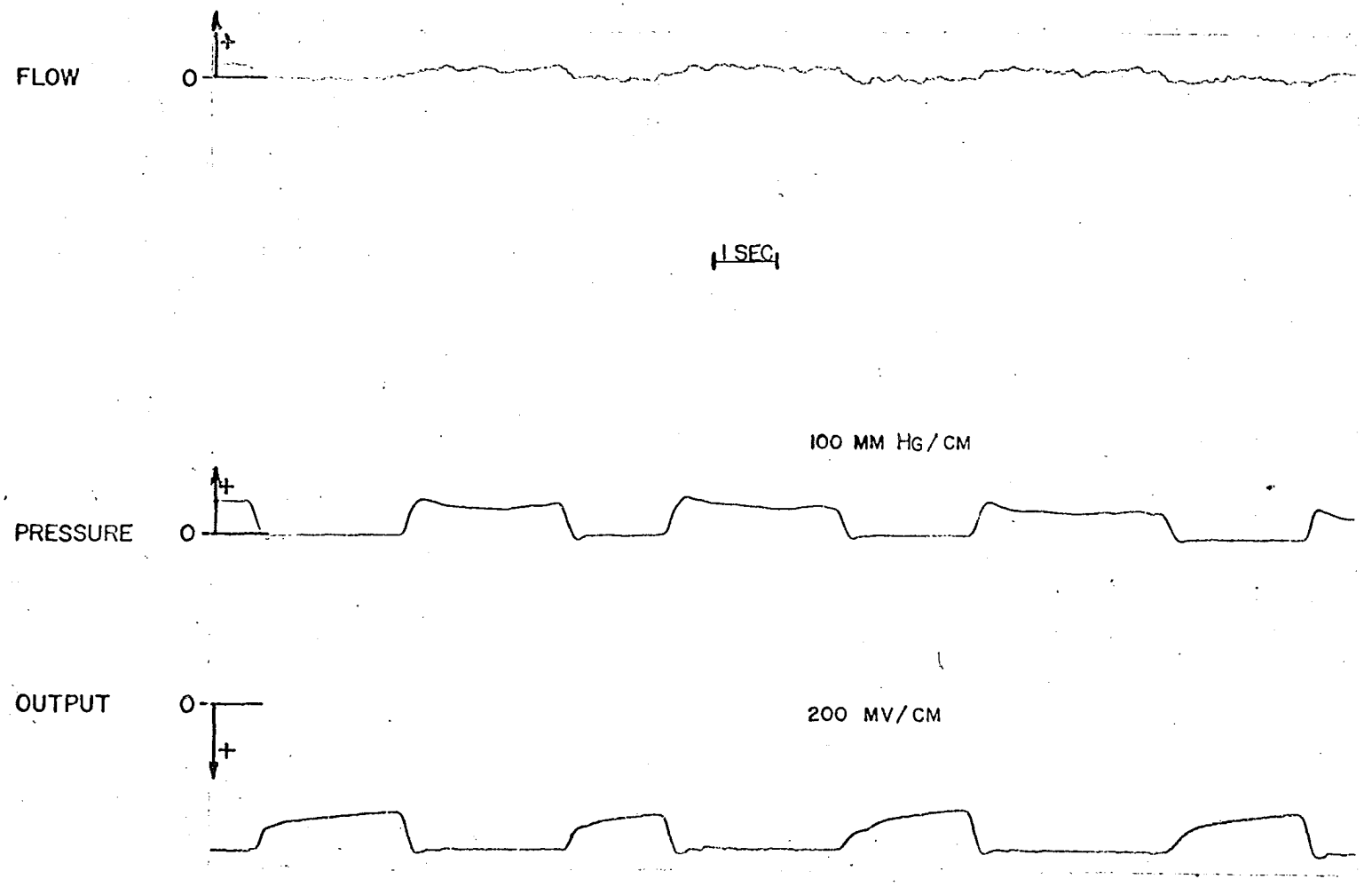


FIGURE 15 OUTPUT VARIATION WITH FLOW : INTERMITTENT FORWARD FLOW

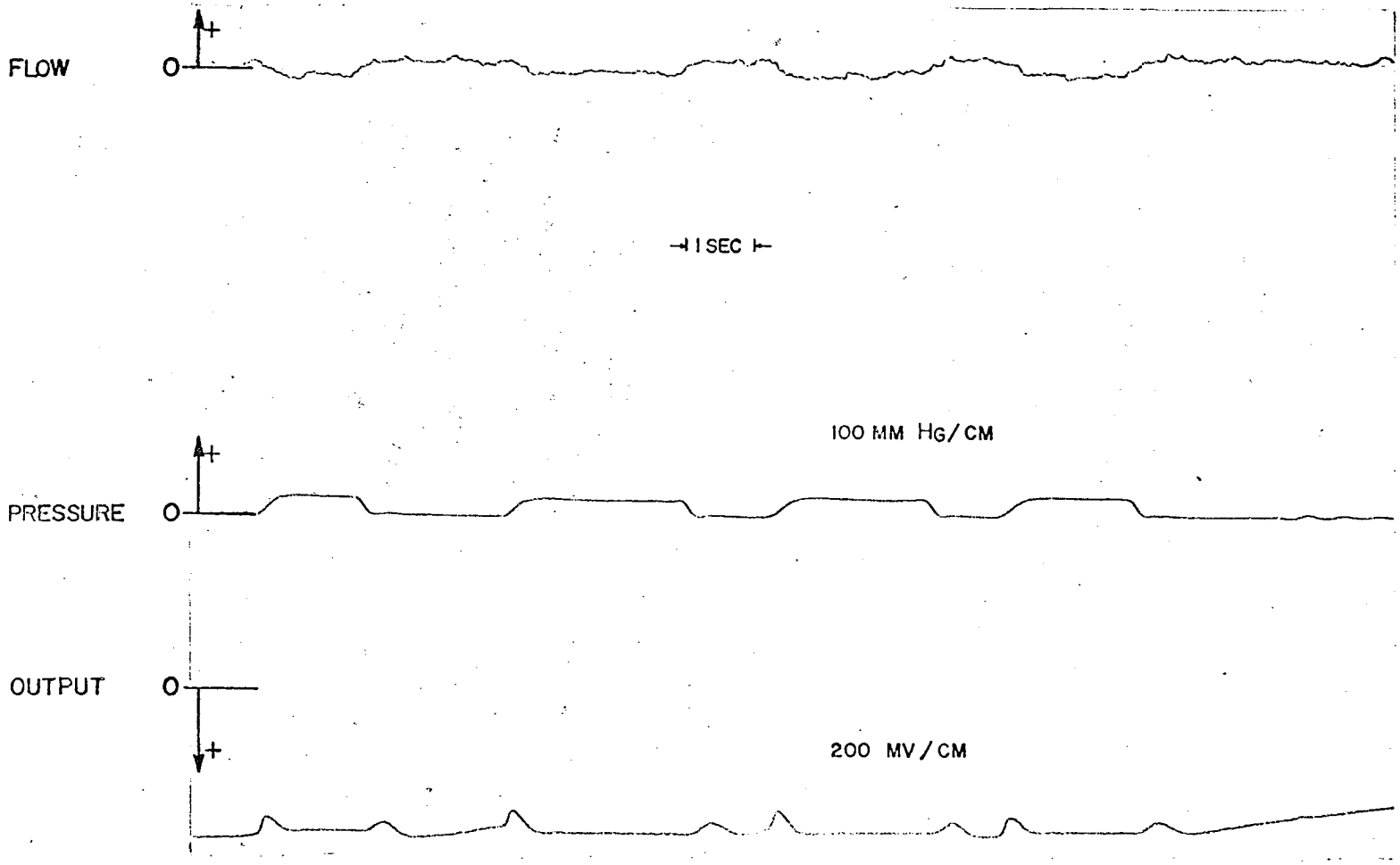


FIGURE 16 OUTPUT VARIATION WITH FLOW : INTERMITTENT REVERSE FLOW

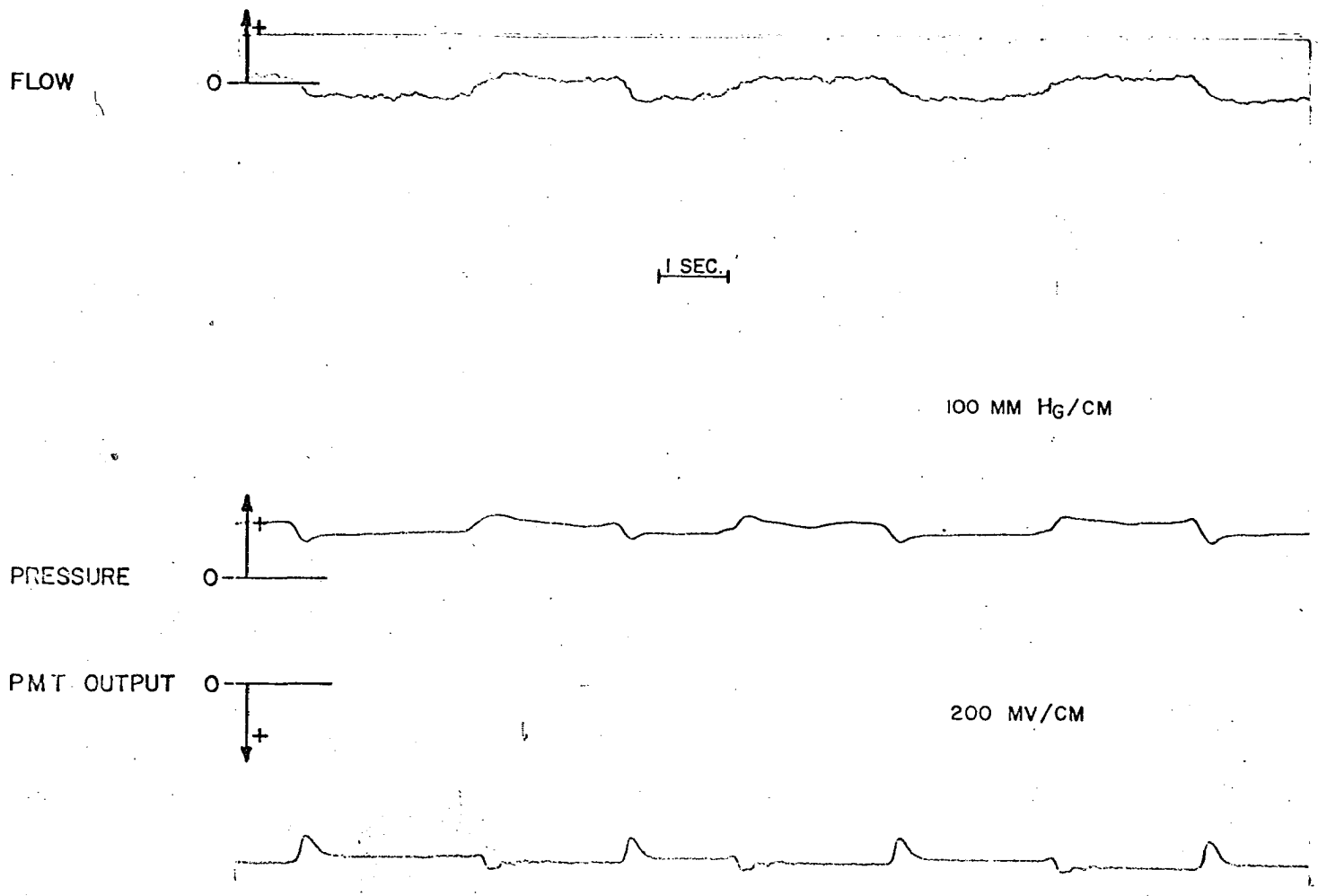


FIGURE 17 OUTPUT VARIATION WITH FLOW : REVERSAL OF FLOW

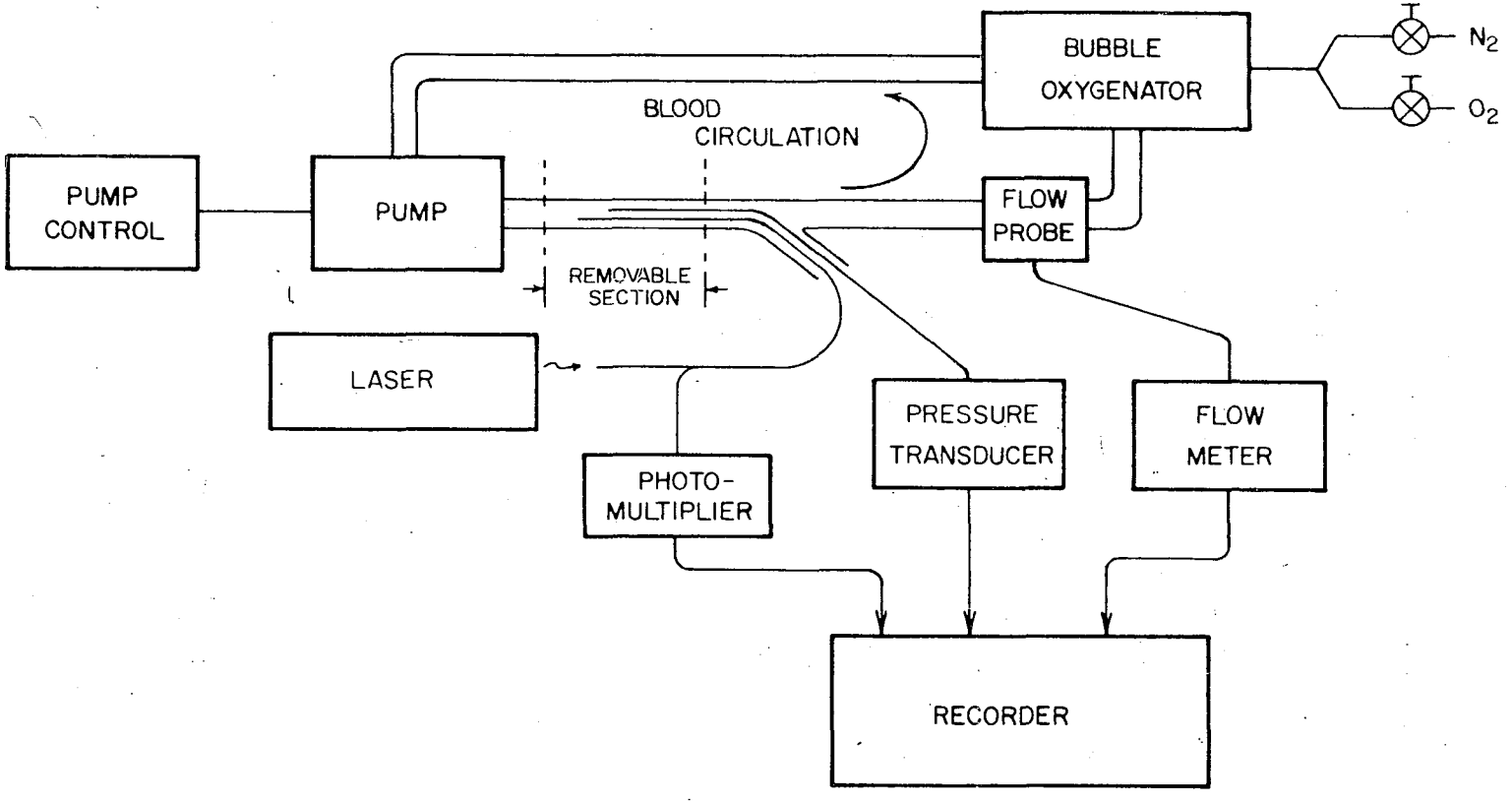


FIGURE 18 PULSATILE FLOW SYSTEM

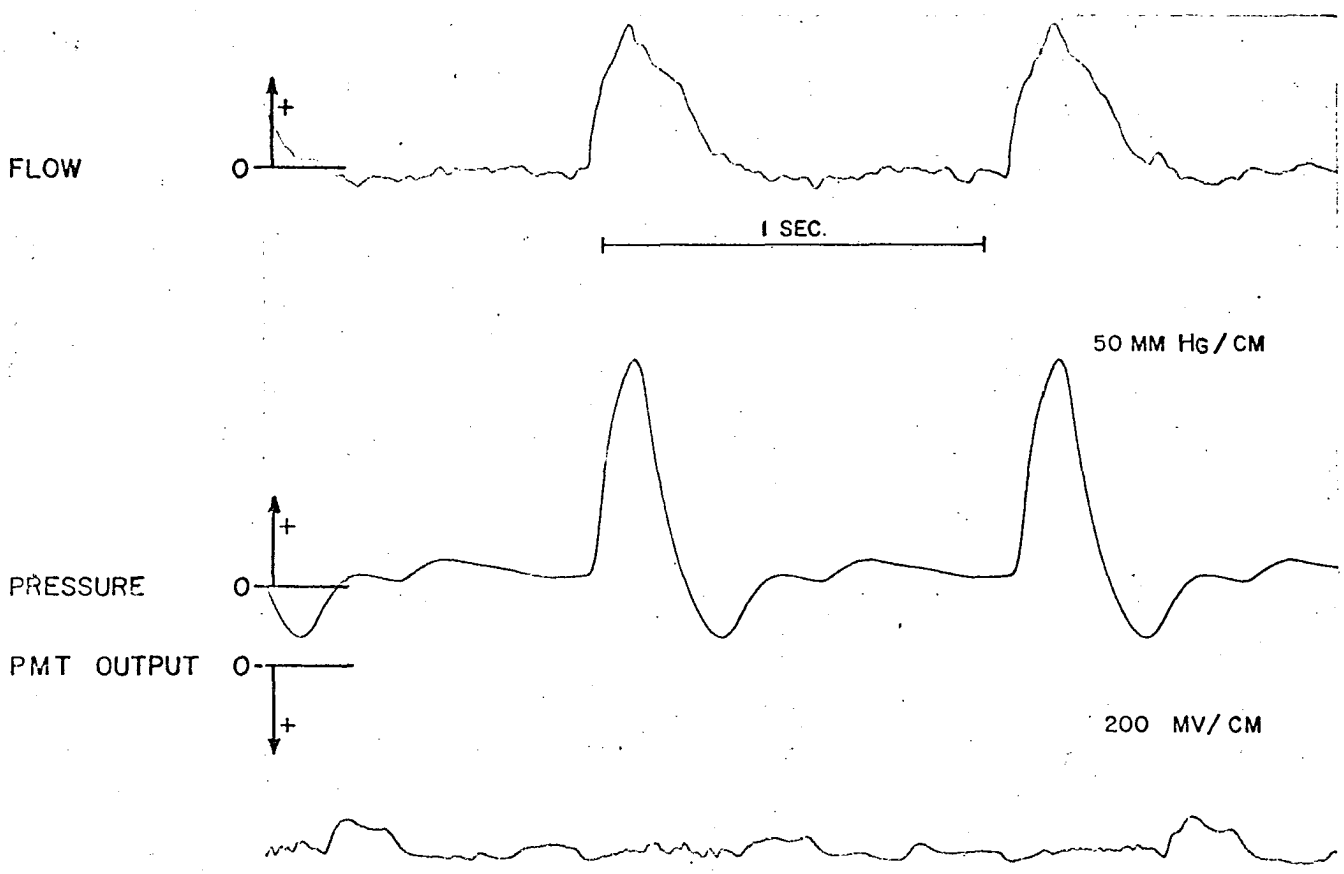


FIGURE 19 IN VITRO PULSATILE FLOW : 0.375 I. D. DISTENSIBLE TUBING

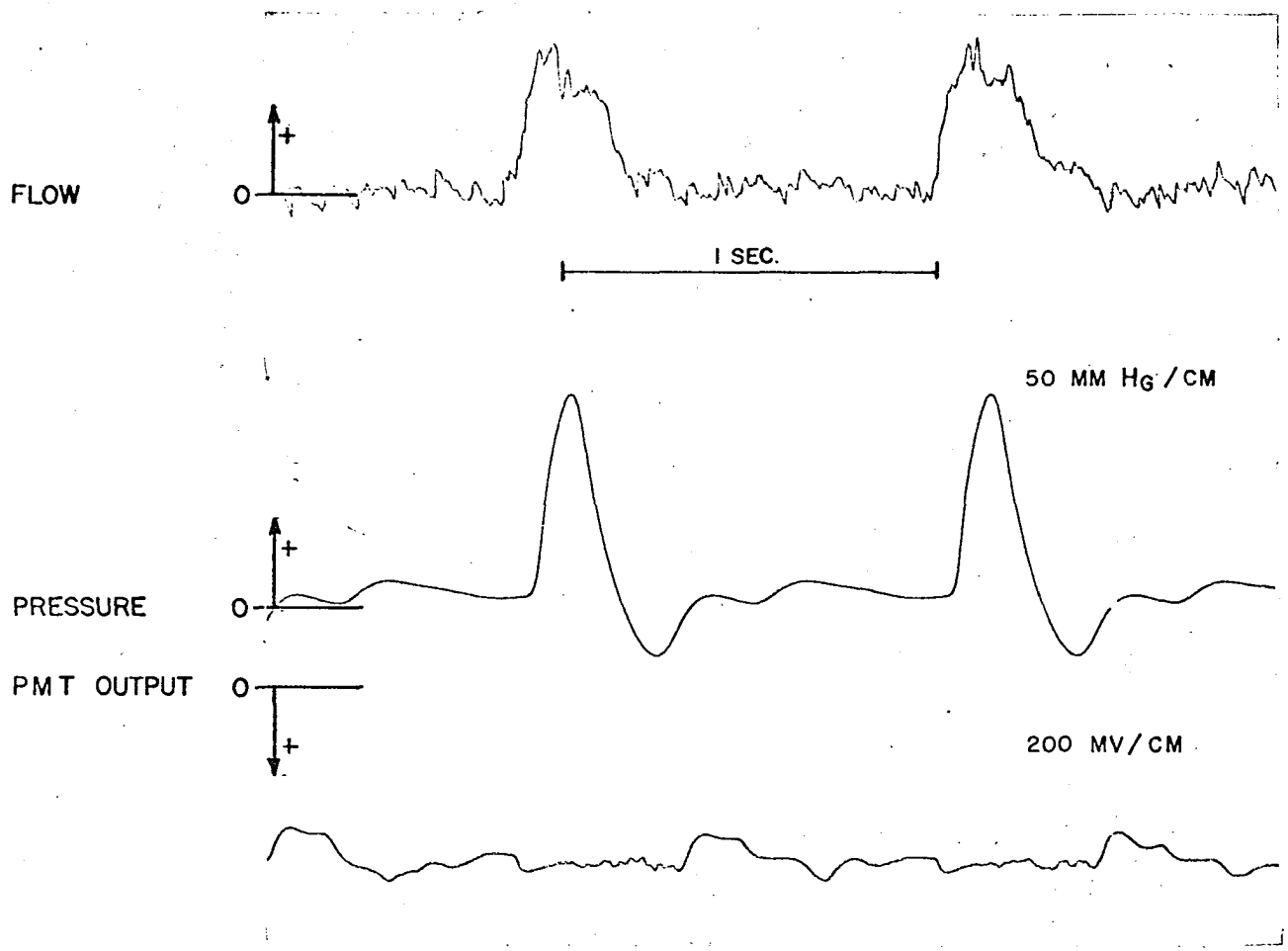


FIGURE 20 IN VITRO PULSATILE FLOW : 0.375 I. D. RIGID TUBING

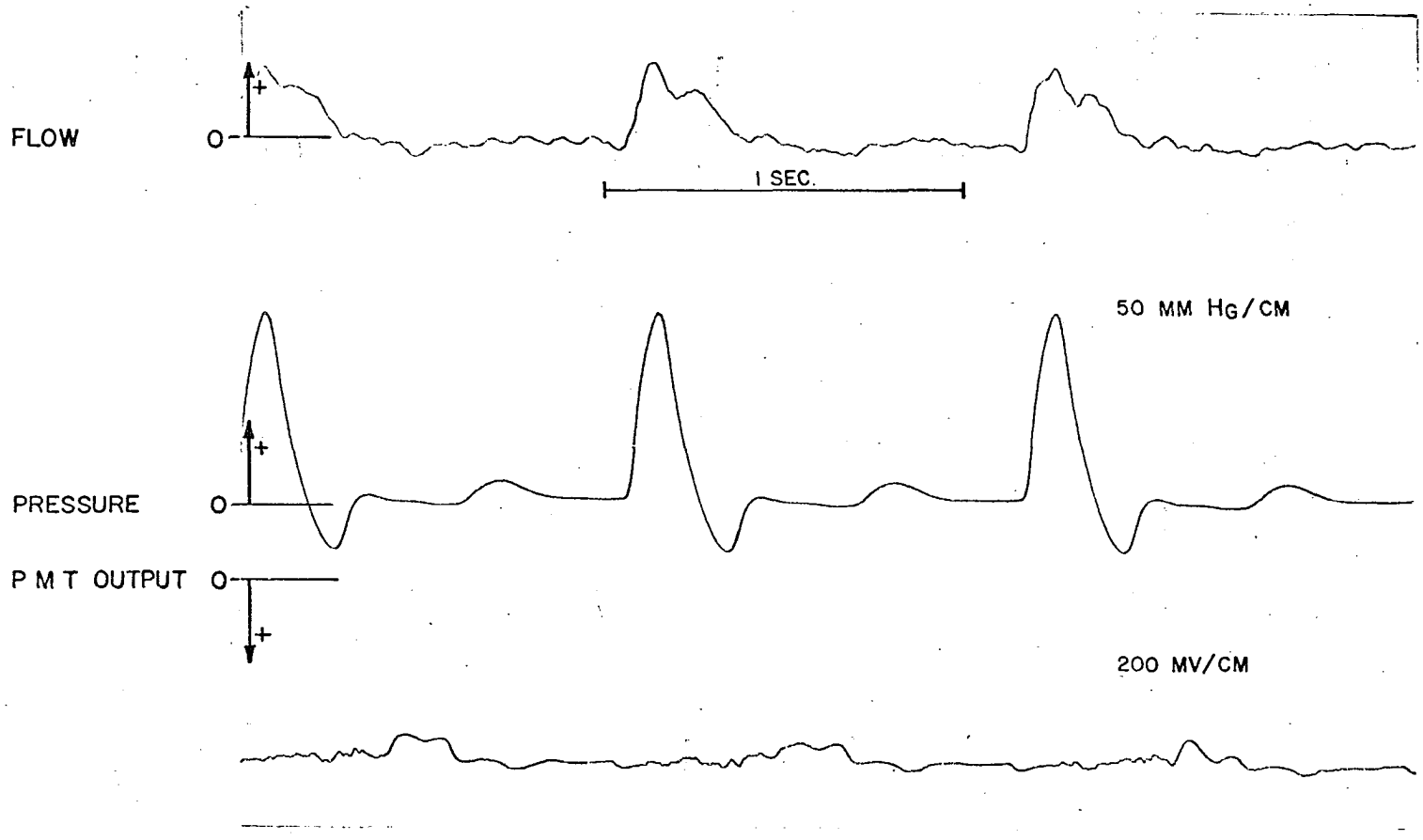


FIGURE 21 IN VITRO PULSATILE FLOW : 0.25 I. D. DISTENSIBLE TUBING

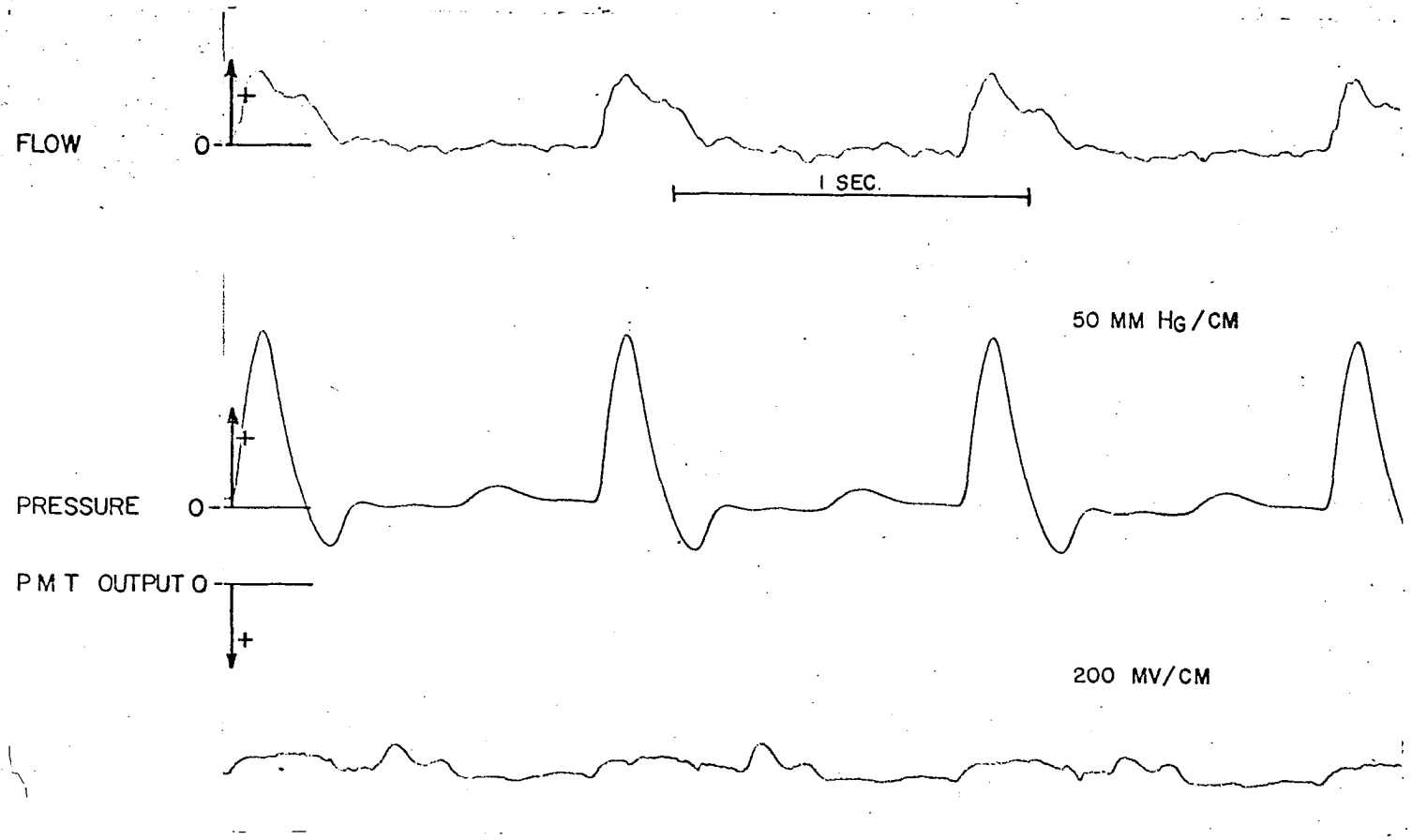


FIGURE 22 IN VITRO PULSATILE FLOW : 0.25 I.D. RIGID TUBING

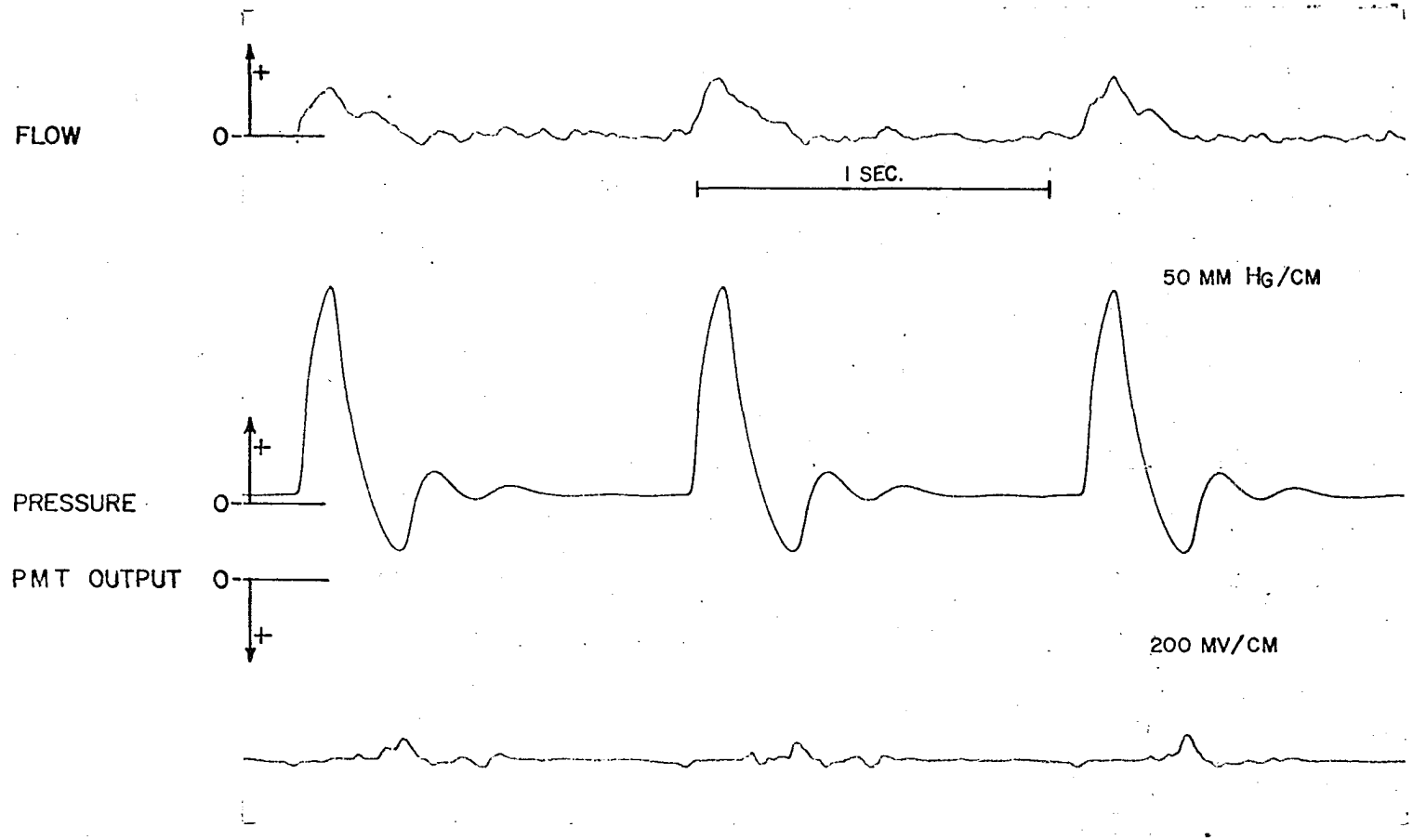


FIGURE 23 IN VITRO PULSATILE FLOW : 0.187 I. D. DISTENSIBLE TUBING

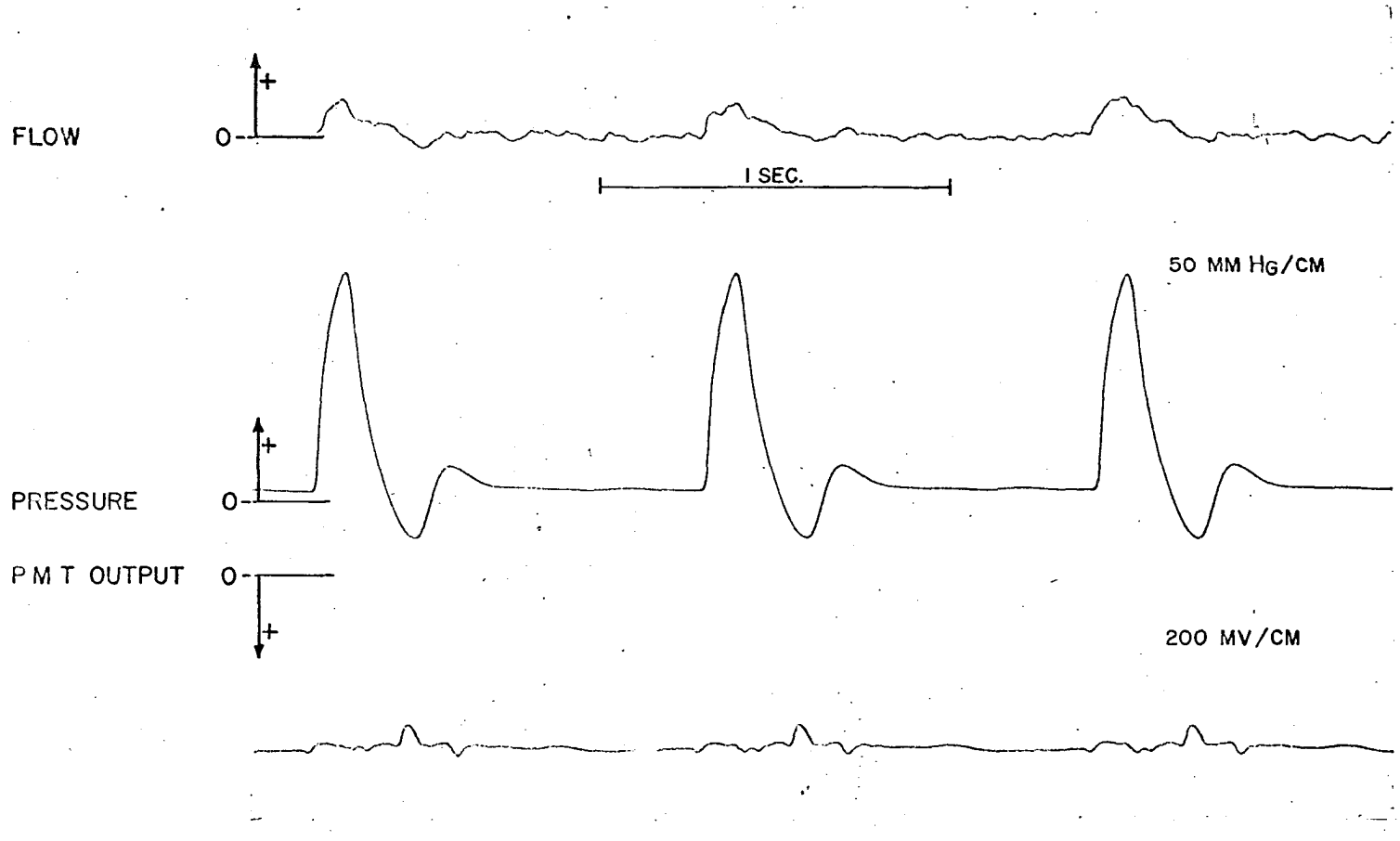


FIGURE 24 IN VITRO PULSATILE FLOW : 0.187 I. D. RIGID TUBING

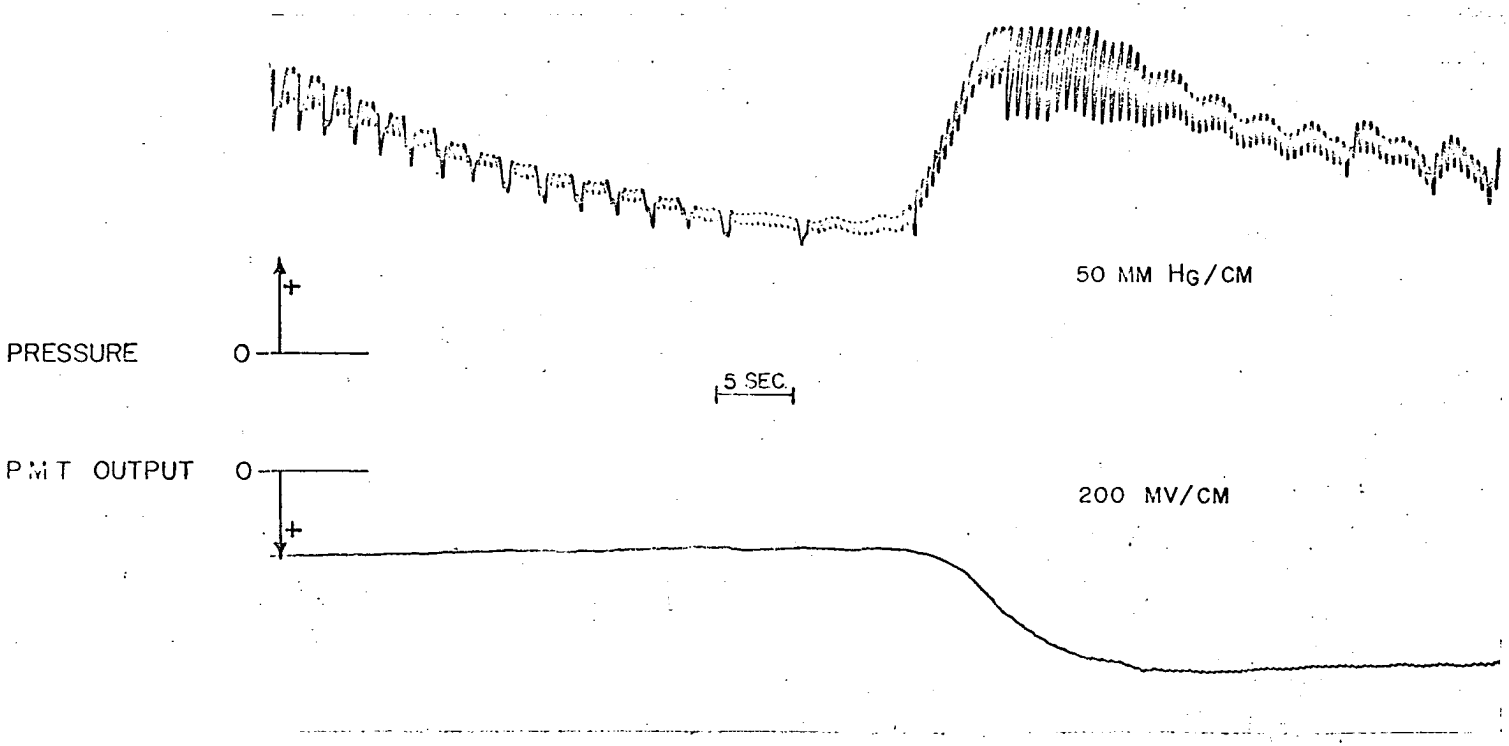


FIGURE 25 PRESSURE AND FILTERED PMT OUTPUT DURING REBREATHING

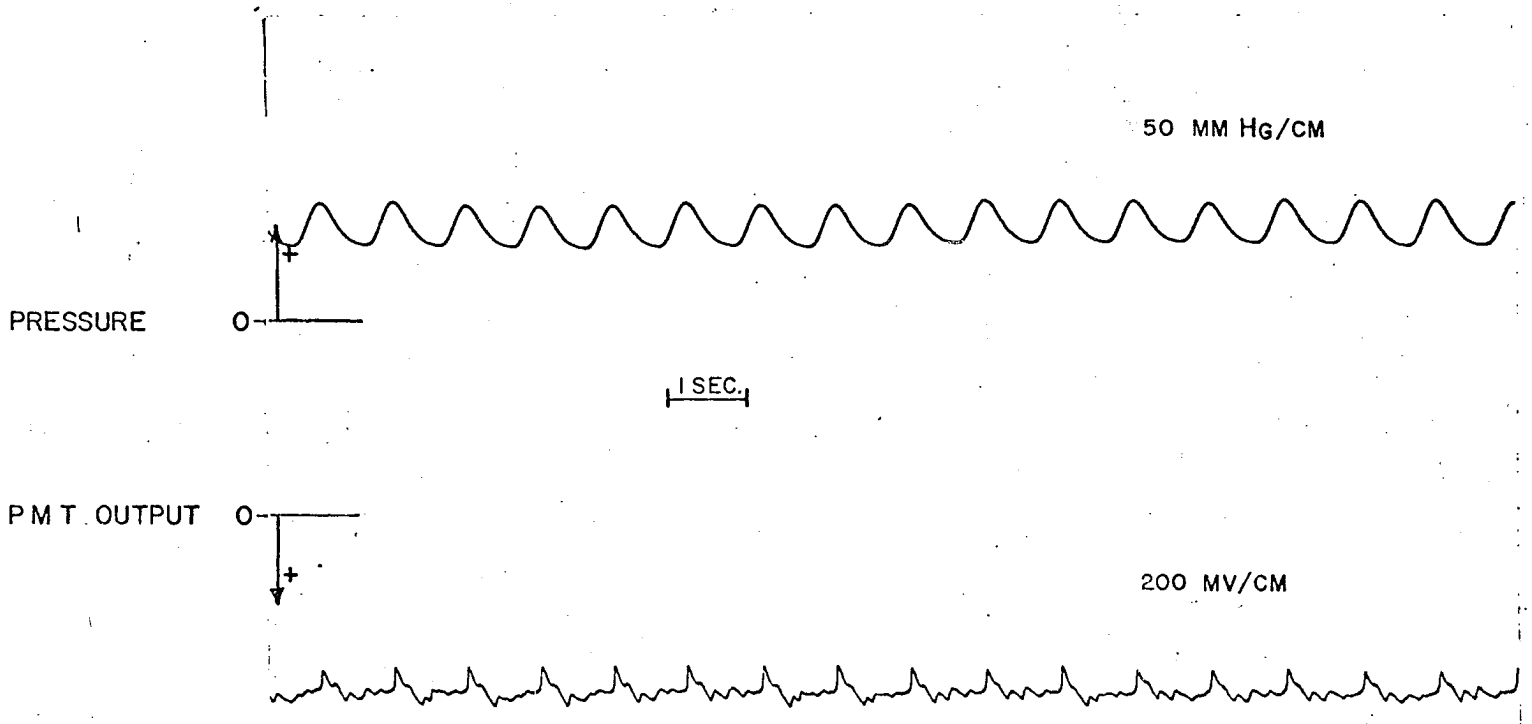


FIGURE 26 IN VIVO ARTIFACT - PRESSURE RELATIONSHIPS

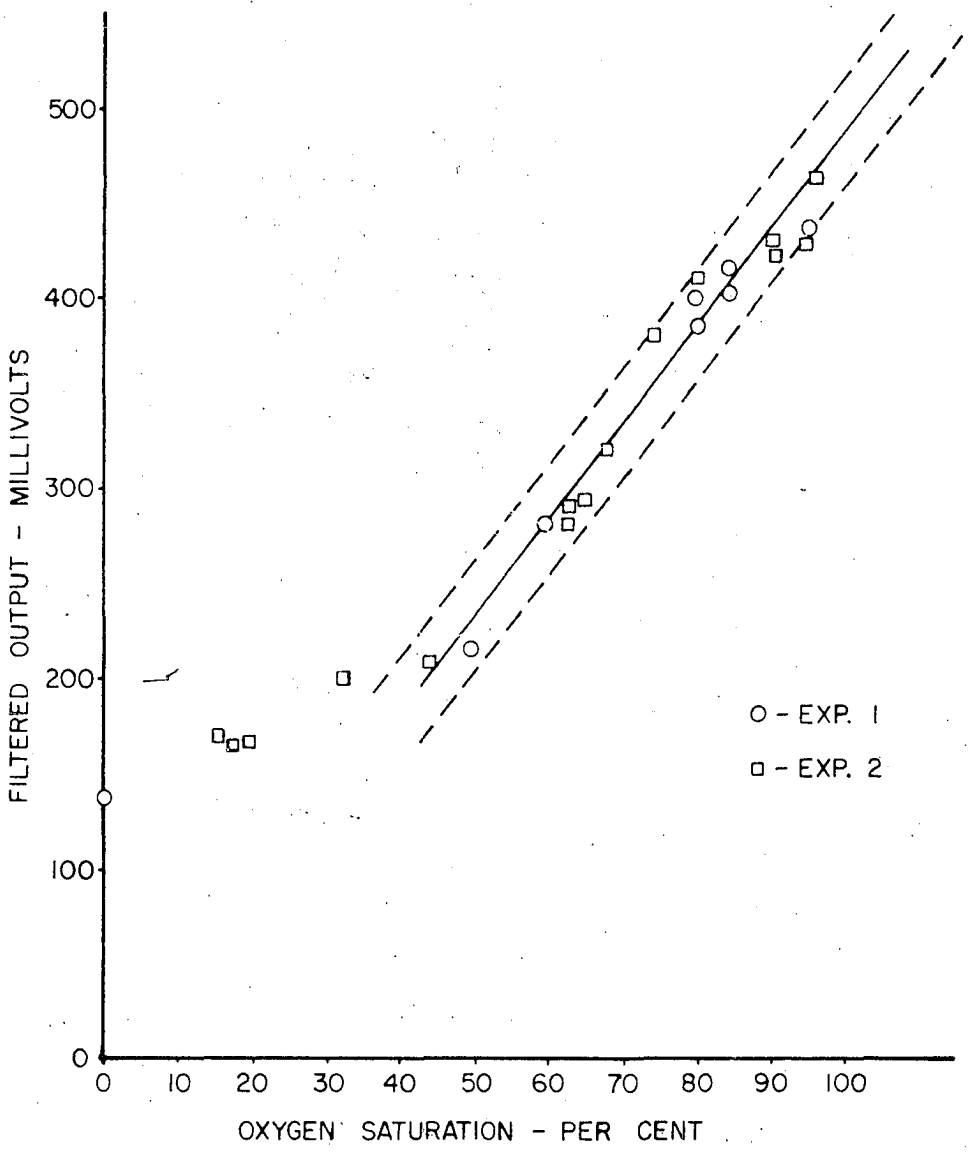


FIGURE 27 FILTERED OUTPUT VS. PER CENT OXYGEN SATURATION

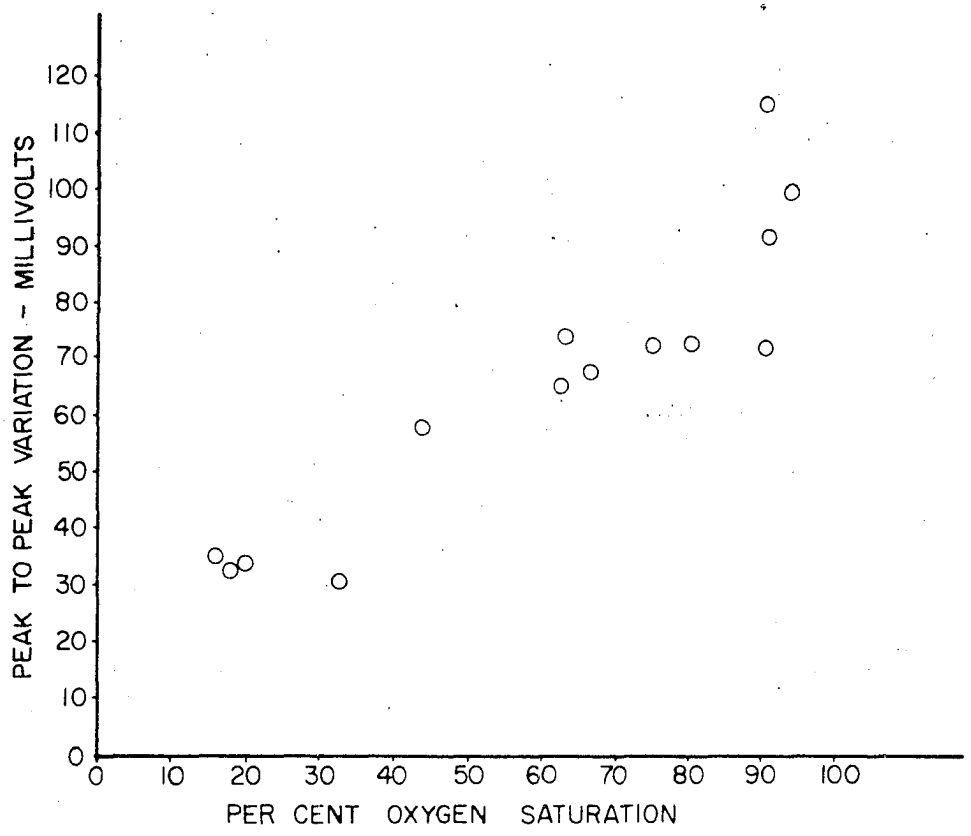


FIGURE 28 PEAK TO PEAK VARIATION VS. OXYGEN SATURATION

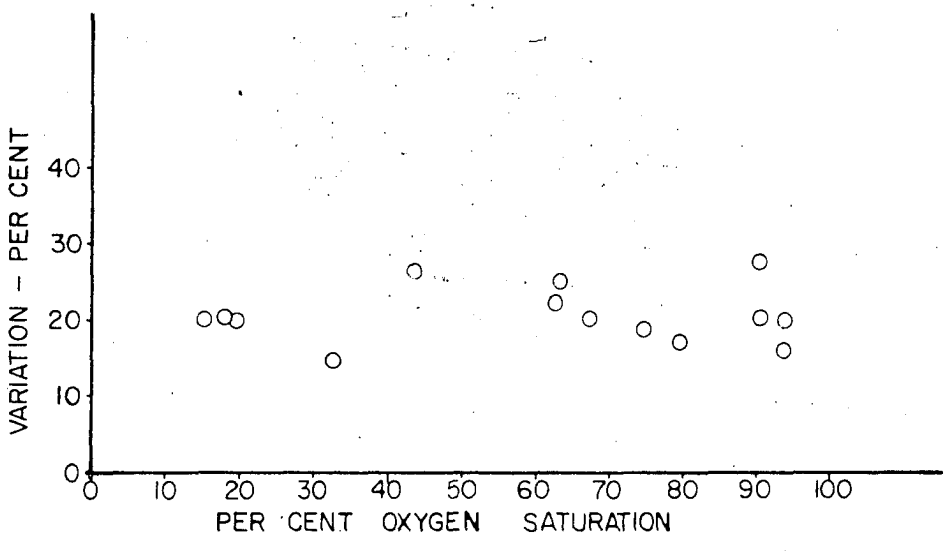


FIGURE 29 PER CENT VARIATION VS. OXYGEN SATURATION

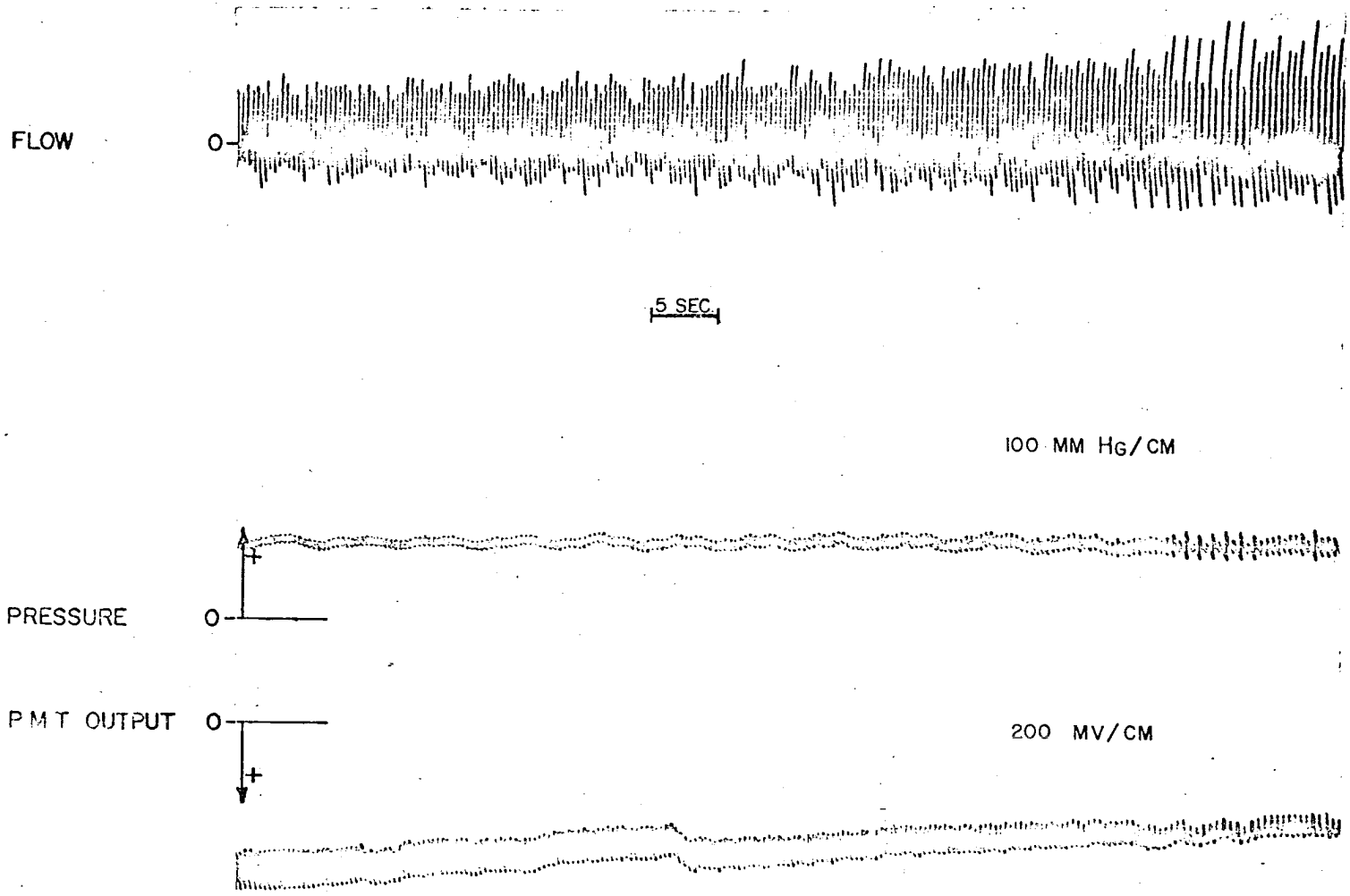


FIGURE 30 IN VIVO RECORDINGS : FLOW PROBE IN THE ABDOMINAL AORTA

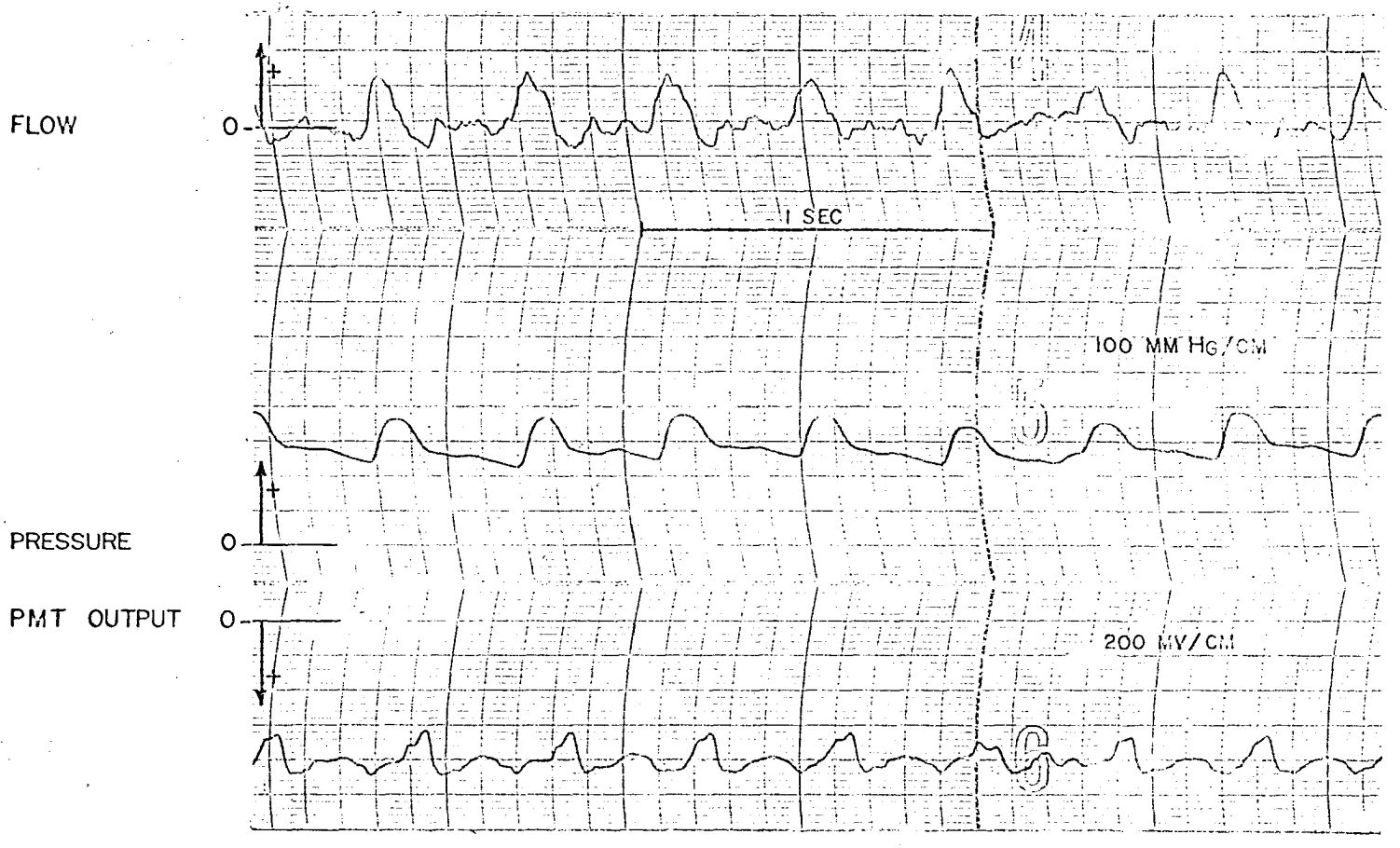


FIGURE 31 IN VIVO RECORDINGS : FLOW PROBE IN THE DESCENDING THORACIC AORTA

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IX. ACKNOWLEDGEMENTS

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