Simultaneous measurement of steady-state and pulsatile optical density changes*

Daniel D. Upthegrove Gunter N. Franz Kenneth C. Weber

Department of Physiology and Biophysics, West Virginia University Medical Center, Morganstown, West Virginia 26505, USA and Appalachian Laboratory for Occupational Respiratory Diseases, National Institute for Occupational Safety and Health, Public Health Service, Department of Health, Education and Welfare, Morgantown, West Virginia 26505, USA

Abstract—*A highly stable, easily built and operated circuit was designed to linearise the output of a particular photocell operating at the isobestic point of blood. In addition, the system was tested* in vitro *by simultaneously recording the total optical-density (o.d.) changes, and pulsatile o.d. changes observed when blood was perfused through a rigid tube and tubing capable of volume changes. The results showed different pulsatile o.d. changes, both in waveform and temporal sequence, for the rigid and distensible tubes.*

Keywords—*Optical density of blood*

PHOTORESISTIVE CELLS have repeatedly been used in photoelectric plethysmography (D'AGROSA and HERTZMAN, 1967; LIEBMAN *et al.*, 1962; UPTHEGROVE *et al.*, 1966; UPTHEGROVE *et al.*, 1968; WEINMAN and MANOACH, 1962). In this paper we studied the response of a particular photocell at the isobestic point of blood. The isobestic point of blood is the wavelength of light (800 nm) at which oxygenated and reduced haemoglobin have identical absorption coefficients. After this information was collected, a circuit was designed for linearising the response characteristic. We also present *in vitro* demonstrations of pulsatile variations of the optical density of blood due to volume changes and flow-related phenomena in distensible and rigid tubes.

Apparatus

Photoelectric sensor—The clamp-like sensing unit (Fig. 1) can be fitted around an exposed blood vessel, tubing or other substrate for *in vitro* experiments. A photocell† and a tungsten light source‡ are mounted on opposite sides of the adjustable clamp (Fig. 1). For peak sensitivity at the isobestic point of blood, an 800 nm interference filter§ covered the photocell. A regulated d.c. supply (less than 1 mV ripple) provides power for the light source, thus ensuring a constant light output.

Electronic circuitry—The signal from the photoconductive sensor is processed in four operational-amplifier stages (Fig. 2): measurement of the

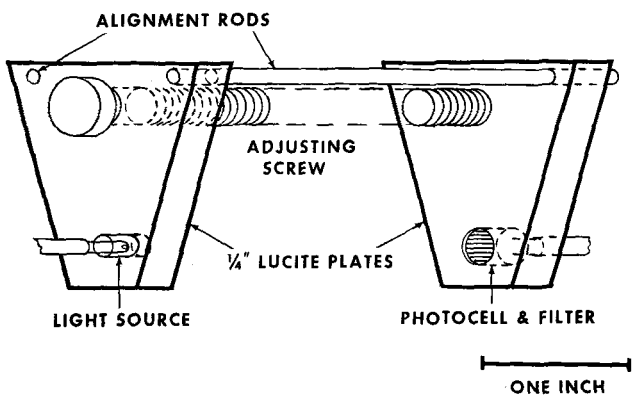

Fig. 1 Drawing of photosensitive detector unit showing photocell and light-source placement

† *Clairex type 604L, Clarex Corp., New York, NY, USA*

‡ *Chicago Miniature Lamp type 666 CM8; Chicago Miniature Lampworks, Chicago, Ill., USA*

§ *Wratten No. 88A; Eastman Kodak, Rochester, NY, USA*

* *First received 9th June and in final form 20th December 1972*

photocell resistance by a bridge circuit (operational amplifier A_1); linearisation of the bridge output with a logarithmic convertor, A_2; and d.c. and a.c. amplification, A_3 and A_4.

The photocell P is in one arm of the bridge circuit around amplifier A_1 (Fig. 2). Bridge excitation e_i is set by a Zener diode at $-3{\cdot}7$ V. The potential at the positive input terminal is one-half of the bridge excitation voltage. The high gain of the operational amplifiers ensures that the potential at the negative input terminal assumes the same value. The output voltage e_{o1} is then equal to the potential at the negative input terminal minus the voltage drop across the bridge arm containing the photocell resistance r:

$$e_{o1} = (e_i/2) - (e_i/2R_0)(R_0 + r) = -e_i r/2R_0 \ . \quad (1)$$

With $e_i = -3{\cdot}7$ V and $R_0 = 5\,k\Omega$, e_{o1} is directly proportional to the photocell resistance r:

$$e_{o1} = 0{\cdot}37r \text{ (in volts; } r \text{ in k}\Omega)$$

The photocell can be shunted by closing switch S. In this condition, the bridge is balanced, and the offset controls of A_1 should be set for $e_{o1} = 0$.

In the range of interest, the resistance of this photocell is approximately an exponential function of the optical density of the substrate covering the photocell. The output e_{o1} follows the same exponential law. For linearisation, the bridge output e_{o1} is applied to a logarithmic convertor A_2. The convertor exploits the logarithmic relationship between voltage drop and current through a transistor connected as a diode (Philbrick Researchers Inc., 1966). The output e_{o2} is a negative voltage proportional to the logarithm of e_{o1}. Its absolute value is therefore directly proportional to optical density.

Usually, the changes in photocell resistance are measured as a deviation from a given initial level. The d.c. amplification stage A_3 provides an adjustable bucking voltage (1 kΩ potentiometer) for cancellation of that part of e_{o2} corresponding to the given initial level. The remaining portion of e_{o2} is further amplified with sign inversion. The amplifier output e_{o3} is therefore directly proportional to the change in optical density from the initial level.

Small rhythmic variations of light intensity due to pulsatile changes of optical density of the experimental substrate are detected by a.c. amplification (A_4). The low-frequency cut-off of this stage begins at approximately 0·16 Hz.

Input-output characteristics

The output of the photoelectric sensor at 800 nm is illustrated by the open-circle curve in Fig. 3. The amount of light striking the photocell was changed by covering the constant light source with identical

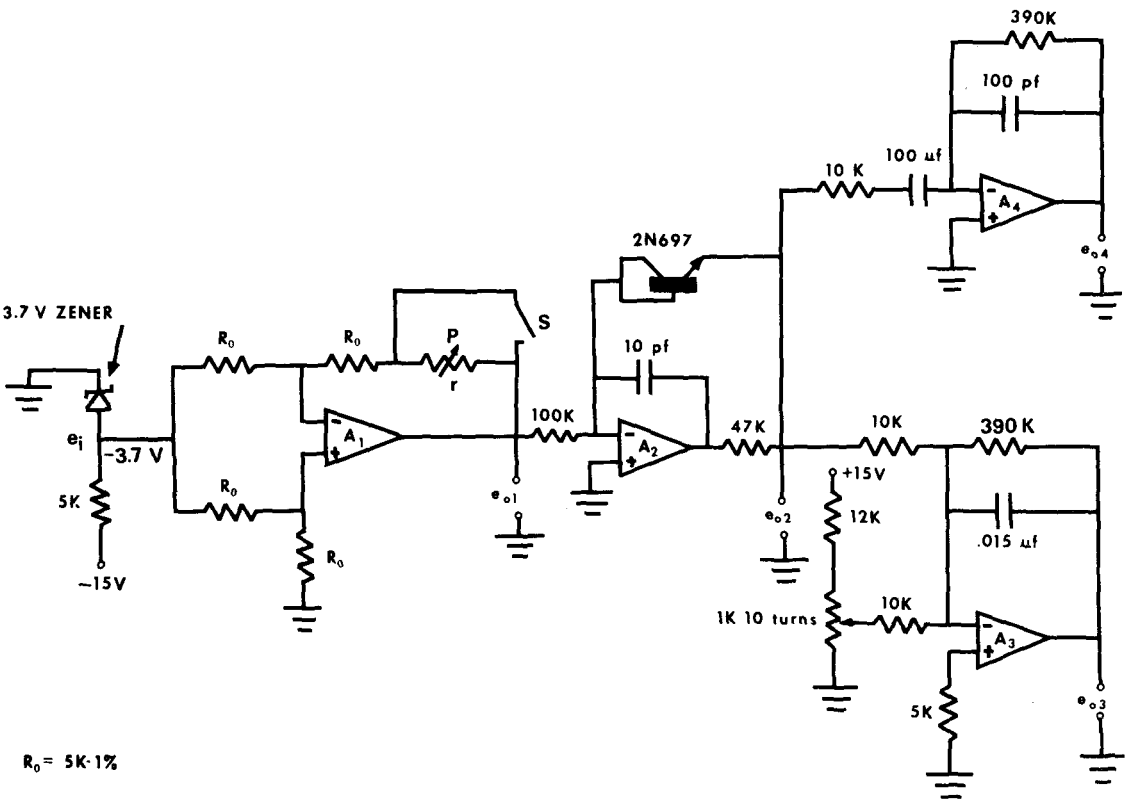

Fig. 2 Schematic of circuitry used for detection and linearisation of photocell output
K = k Ω; f = farad

filters made from uniformly exposed X ray film. One layer of film is arbitrarily set to equal one filter unit. The bridge output e_{o1} and the photocell resistance r increase exponentially with the number of filter units (Fig. 3). With the chosen values for e_i and R_0 (Fig. 2), the highest measurable photocell resistance is 30 kΩ, corresponding to the maximum output voltage ($e_{o1\,max}$ = 10 V) of the operational amplifier. According to eqn. 1, if $e_{o1\,max}$ is constant at 10 V, the measuring range of the bridge can be extended to higher values of r by either decreasing e_i or increasing R_0.

The linearisation of the exponential characteristic by means of the logarithmic convertor is demonstrated by the closed-circle plot of Fig. 3. Here the output e_{o3} is plotted as a function of the number of filter units. At zero filter units, i.e. the highest light level, the output e_{o3} is set to zero voltage by adjusting the bucking potentiometer connected to amplifier A_3. From this reference level, e_{o3} rises linearly as the optical density increases with the addition of uniform filters (Fig. 3).

Application to pulsatile blood flow

The applicability of the instrumentation to pulsatile blood flow was tested on an *in vitro* perfusion arrangement. Blood or saline was pumped through transparent plastics tubing of 6 mm inside diameter. The tubing was made rigid by cementing it into a block of clear Perspex drilled to the tube's outside diameter. The first half of the length of embedded tubing was used to measure optical density (o.d.) during flow through a rigid tube. For measurements during flow through a tubing capable of volume changes, the other half of the embedded tubing was used. A distensible window was produced in this latter portion of the rigid tubing by drilling a 4 mm window through one side of the Perspex block and the encased tubing. A thin rubber membrane was cemented on the inside of the tubing, extending from the rigid portion of the tube to a point past the window. Precautions were taken during this step not to create a significant source of turbulence by making sure the rubber membrane was flush with the inner wall of the tubing. The sensing clamp could then simply be shifted along the Perspex block for comparison of o.d. changes during flow in a rigid tube and in a tubing capable of volume changes.

Using both blood and saline, the tubing was perfused with a pump giving pressure pulses similar to those in large arteries (Fig. 4). The mean flow was 100 cm³/min. The 800 nm filter covering the sensor permits the measurement of o.d. change of blood independent of the degree of oxygenation of the blood.

O.D. changes during perfusion of the rigid tube with saline and blood are shown in Fig. 4*a*. No changes in o.d. are visible when saline is the perfusate, and total o.d. stays at the reference level set by the bucking voltage. However, during perfusion with blood, the total o.d. rises and small pulsatile o.d. variations appear. The pulsatile o.d. changes must be due to flow-related phenomena, since volume remains constant in the rigid tube. The flow-related phenomena could be a combination of axial accumulation and orientation of red cells during increased flow velocity (BAYLISS, 1959; TAYLOR, 1955). Turbulence was kept to a minimum by using a long length of tubing so that the o.d. was measured some 4 ft (505·5 mm) away from the turbulence produced by the pump. In addition, axial streaming was observed when changing from saline to blood.

In the portion of the tube with the distensible window, perfusion with blood causes larger changes in total and pulsatile o.d. than for the rigid section (Fig. 4*b*). The pulsatile o.d. variations are now a combination of both flow and volume effects. Total o.d. reaches a slightly higher level than the o.d. level in the rigid tube because of the larger volume caused by the bulging out of the window. The small o.d. pulsations visible during perfusion with saline (Fig. 4*b*) are the result of reflections from the surface of the pulsating rubber membrane, and would

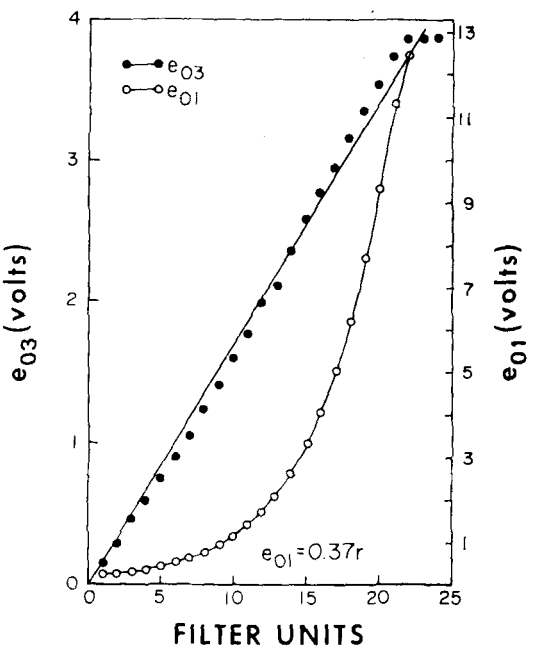

Fig. 3 Plot of photocell resistance (in ohms) versus the number of filter units in the light path (open-circle curve on right.) Note the nonlinear output characteristic. Plot of amplifier no. 3 output versus number of filter units in the light path (closed-circle plot on left). Note the relatively linear output as shown by the small deviations from the best visual line of identity

have to be subtracted to determine the true blood pulsatile changes.

The pulsatile o.d. traces for the rigid and distensible sections differ in amplitude, waveform and temporal sequence with respect to the pressure pulse. After investigating their differences further with the aid of simultaneous flow measurements, it may be possible to use pulsatile o.d. recordings for differentiating volume from flow-related events in various vascular beds.

Application—So far the unit designed has been used in a unique way in a preliminary study. The use of this *in vitro* perfusion arrangement eliminates the problem of vessel movement into and out of the optical fields as well as the problems of turbulence and high flow rates, which can be minimised to approximate more closely to biological conditions. The rigid structure of the photocell assembly and the use of 800 nm light might make this unit useful in studying the *in vivo* circulation of bone, since the unit could be firmly attached to the bone and since differences in arterial and venous blood o.d. are eliminated. However, additional studies need to be performed before this technique can be used for quantitating blood flow.

Acknowledgments—The authors wish to thank Miss Paula Phillips and Mr. James Bland for their excellent technical assistance. The work was supported in part by Grants Nos. 5501 FR 05347-8 and 5501 FR 05433-08 from West Virginia University Schools of Medicine & Dentistry.

References

BAYLISS, L. E. (1959). The axial drift of the red cells when blood flows in a narrow tube. *J. Physiol.* **149**, 593–613.

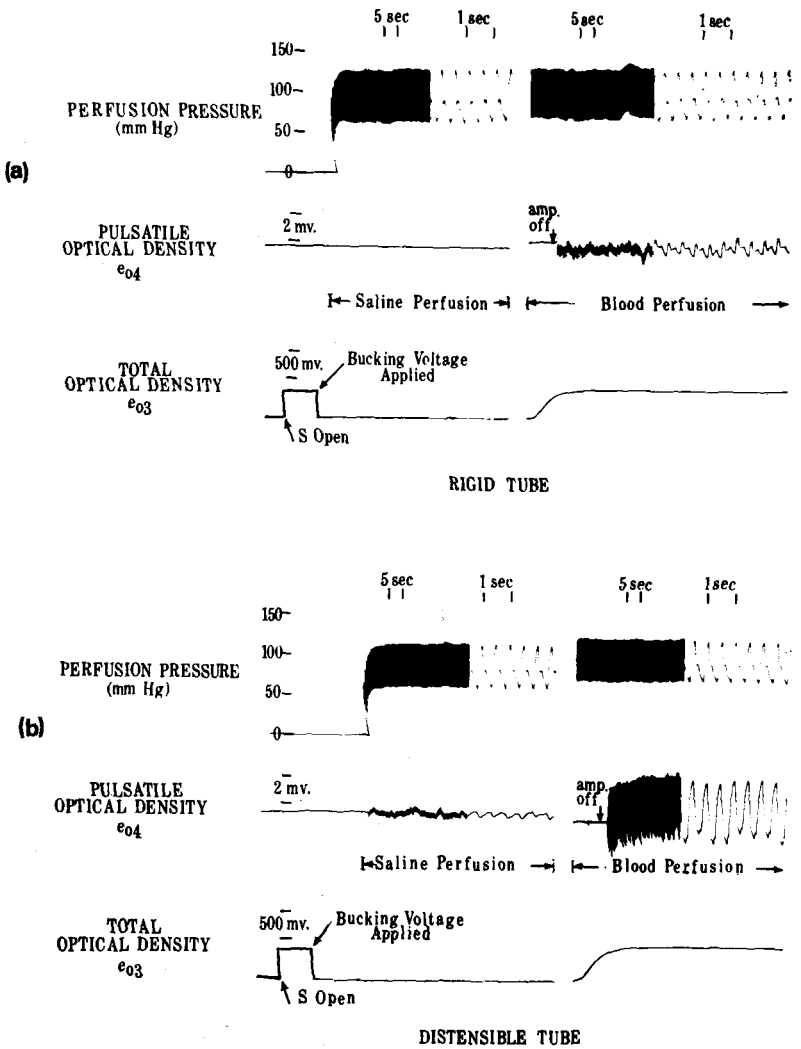

Fig. 4 Traces (a) *show the total optical density and pulsatile optical-density changes when saline and blood are perfused through a rigid tube. Traces* (b) *show the total optical density and pulsatile optical-density changes found when saline and blood are perfused through tubing with a distensible window in which volume changes occur. The pulse waveform and temporal sequence are different for the rigid and distensible tubes*

D'AGROSA, S. and HERTZMAN, A. B. (1967). Opacity pulse of individual minute arteries. *J. Appl. Physiol.* **23**, 613–620.

LIEBMAN, F. M., PEARL, J. and BAGNOT, S. (1962). The electrical conductance properties of blood in motion. *Phys. Med. Biol.* **7**, 177–194.

PHILBRICK RESEARCHERS, INC. (1966). *Applications manual for computing amplifiers*. 2nd ed., p. 50, Debham, Mass.

TAYLOR, M. (1955). The axial stream and its formation as determined by changes in optical density. *J. Exp. Biol.* **33**, 1–16.

UPTHEGROVE, D. D., BISHOP, J. G. and DORMAN, H. L. (1966). A method of detection of blood flow in the dental pulp. *J. Dent. Res.* **45**, 1115–1119.

UPTHEGROVE, D. D., BISHOP, J. G. and DORMAN, H. L. (1968). Indirect determination of the blood pressure in the dental pulp *Arch Oral Biol.* **13**, 929–935.

WEINMAN, J. and MANOACH, M. (1962). The photoelectric approach to the study of peripheral circulation. *Am. Heart J.* **63**, 219–231.

Mesure simultanée des variations d'intensité lumineuse de l'état fixe et de l'état pulsatif

Sommaire—Un circuit très stable facilement construit et utilisé, fut conçu afin de linéariser la sortie d'une cellule photoélectrique particulière fonctionnant au point isobestique du sang. Le système fut aussi testé in vitro en enregistrant simultanément les variations d'intensité lumineuse totale et les variations d'intensité lumineuse pulsatives observées lorsque du sang était filtré dans un tuyau rigide et dans un tuyau capable de changer de volume. Les réstultats présentèrent divers changements d'intensité lumineuse pulsative à la fois pour l'onde et pour la séquence temporelle du tuyau rigide et du tuyau extensible.

Gleichzeitige Messung der Veränderungen der optischen Ditchte im pulsierenden oder Dauerzustand

Zusammenfassung—Es wurde eine äusserst beständige Anlage entworfen, die liecht zu bauen und zu handhaben ist. Sie soll die Ausgabe einer besonderen Fotozelle linearisieren, die am isobestischen Blutpunkt wirkt. Ausserdem wurde das System in vitro getestet, indem es gleichzeitig alle Änderungen der optischen Dichte (o.D.) aufzeichnete und die pulsierenden o.D.-Änderungen feststellte, wenn Blut durch eine starre Röhre, sowie eine Röhre, die ihr Volumen ändern kann, durchsickerte. Die Ergebnisse zeigten unterschiedliche pulsierende o.D.-Änderungen in der Wellenform und in zeitlicher Folge und zwar sowohl für die starren als auch für die dehnungsfähigen Röhren.