References and Notes

1. C. W. Thayer, *Science* **182**, 1242 (1973); T. J. M. Schopf, *J. Geol.* **82**, 129 (1974).
2. J. S. Levinton, *Geology* **1**, 157 (1973); P. W. Bretsky, *ibid.*, p. 157; J. W. Valentine and F. J. Ayala, *ibid.* **2**, 69 (1974); P. W. Bretsky, S. S. Bretsky, J. S. Levinton, D. M. Lorenz, *Science* **179**, 1147 (1973).
3. J. S. Levinton, *Science* **180**, 75 (1973).
4. P. W. Bretsky and D. M. Lorenz, *Geol. Soc. Am. Bull.* **81**, 2449 (1970); in *Proceedings of the North American Paleontological Convention* (Chicago, 1970), part E, p. 522.
5. P. W. Bretsky, *Science* **159**, 1231 (1968); *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **6**, 45 (1969).
6. C. W. Thayer, *Lethaia* **7**, 121 (1974).
7. H. L. Sanders, P. C. Manglesdorf, Jr., G. E. Hampson, *Limnol. Oceanogr.* **10**, R216 (1965); A. L. McAlester and D. C. Rhoads, *Mar. Geol.* **5**, 383 (1967); J. B. C. Jackson, *Mar. Biol.* **14**, 304 (1972).
8. E. Nevo, *Nature (Lond.)* **244**, 573 (1973); ———, Y. J. Kim, C. R. Shaw, C. S. Thaeler, Jr., *Evolution* **28**, 1 (1974).
9. Rodents are known since the Eocene [A. S. Romer, *Vertebrate Paleontology* (Univ. of Chicago Press, Chicago, 1966)].
10. Bivalve mollusks date back to the Cambrian, according to J. Pojeta, Jr., B. Runnegar, and J. Kriz [*Science* **180**, 866 (1973)].
11. See S. M. Stanley [*Geol. Soc. Am. Mem. No. 125* (1970)] for criteria.
12. To be included, a superfamily or order had to contain numerous genera, all of which could be assigned with reasonable confidence to a single habit (epifaunal or infaunal).
13. I used the two-tailed *t*-test, assuming no uncertainty in the chronostratigraphic ranges of genera, which were taken from the *Treatise* (*14*). If the reported time span included a given chronostratigraphic unit, I assumed that the genus lived for the entire duration of the unit. Absolute dates are linear interpolations from F. W. B. Van Eysinga, *Geological Time Table* (Elsevier, New York, 1972). Ranges and genera preceded by a question mark, "unrecognizable" genera, and all subgenera were omitted. *Labrostrea* and *Oretia* were included. In the Hippuritacea "subfamily uncertain" was included, "family uncertain" omitted.
14. R. C. Moore and C. Teichert, Eds., *Treatise on Invertebrate Paleontology* (Geological Society of America, Boulder, Colo., and Univ. of Kansas Press, Lawrence, 1969, 1971), part N, Mollusca 6.
15. D. M. Raup [*Science* **177**, 1065 (1972)] has discussed the sources of error in such data.
16. D. M. Rohr and A. J. Boucot [*Geol. Soc. Am. Bull.* **85**, 665 (1974)] also deal with the limitations of *Treatise* data.
17. G. G. Simpson, *Major Features of Evolution* (Simon & Schuster, New York, 1953).
18. J. L. Cutbill and B. M. Funnel, in *The Fossil Record*, W. B. Harland *et al.*, Eds. (Geological Society of London, London, 1967), p. 791.
19. P. W. Bretsky, *Geol. Soc. Am. Bull.* **84**, 2079 (1973).
20. In the Veneracea, zero variance for the two cosmopolitans precluded the calculation of *t*.
21. E. G. Kauffman, in *Atlas of Palaeobiogeography*, A. Hallam, Ed. (Elsevier, New York, 1973), pp. 353–383.
22. For example, on Ordovician crinoids, see C. R. Scotese, *Geol. Soc. Am. North-Central Sect. Abstr. Programs Annu. Meet.* (1974), p. 543; on Paleozoic brachiopods, see (*19*).
23. A. J. Boucot, *Geology* **2**, 204 (1974).
24. ———, *Evolution and Extinction Rate Controls* (Elsevier, Amsterdam, in press).
25. P. W. Bretsky [*Geol. Soc. Am. Bull.* **85**, 667 (1974)] cautions that number of individuals is not necessarily proportional to geographic range.
26. J. B. C. Jackson, *Am. Nat.* **108**, 541 (1974).
27. G. J. Vermeij, *ibid.* **106**, 89 (1972).
28. S. M. Stanley, *Syst. Zool.* **22**, 486 (1973).
29. As inhabitants of physically "stable" shallow tropical waters and reefs [C. M. Yonge, *Phil. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **252**, 49 (1967)], the rudists were analogs of living *Tridacna*, in which substantial genetic variability has been reported [F. J. Ayala, D. Hedgecock, G. S. Zumwalt, J. W. Valentine, *Evolution* **27**, 177 (1973); J. W. Valentine, D. Hedgecock, G. S. Zumwalt, F. J. Ayala, *Geol. Soc. Am. Bull.* **84**, 3411 (1973)]. If the rudists had similar genetic variability, their rapid evolution is counter to the depauperate gene pool hypothesis.
30. J. S. Levinton, *Geol. Soc. Am. Abstr. Programs Annu. Meet.* (1973), p. 712.
31. T. H. Suchanek and J. S. Levinton, *J. Paleontol.* **48**, 1 (1974).
32. Some environmental "noise" remains even when taxa from generally similar environments are considered; infauna in very shallow water may be subject to greater physicochemical fluctuations than the epifauna of deeper waters. The available data do not permit finer resolution of environmental differences. However, many infaunal bivalves living in very shallow water have relatively extensive geographic ranges (*26*). Elimination of cosmopolitan genera thus reduces the effect of this "noise."

33. I thank P. W. Bretsky, J. B. C. Jackson, J. S. Levinton, A. L. McAlester, D. C. Rhoads, and S. M. Stanley for the comments on the manuscript.

18 June 1974 ■

Blood Velocity Measurements in Human Retinal Vessels

Abstract. *Laser Doppler velocimetry was used to measure the velocity of blood in human retinal vessels. The mean flow velocities obtained were 1.9 centimeters per second in a retinal vein and 2.2 centimeters per second in a retinal artery. Scattered light from a weak helium-neon laser beam focused on the vessel was detected by a photomultiplier, and the temporal correlation of the intensity fluctuations was measured with a photon counting autocorrelator. Autocorrelation functions for blood flowing through glass capillaries were used for calibration.*

The feasibility of using laser Doppler velocimetry to measure blood flow velocity in retinal vessels of rabbits was previously demonstrated (*1*). It was speculated then that such measurements could be performed in humans if the recording time could be short enough to minimize the effects of slow eye drifts and if the retinal irradiance could be decreased to permissible levels. These requirements have now been satisfied, and we report here in vivo measurements of the flow velocity of blood in human retinal vessels.

Laser Doppler velocimetry is a conventional technique for measuring the velocity of particles suspended in a fluid (*2*). It is based on the Doppler effect. The laser light scattered from a moving particle is shifted in frequency by an amount f according to the Doppler relation

$$f = (1/2\pi)\ (\mathbf{K}_s - \mathbf{K}_i)\cdot\mathbf{V}$$

where $\mathbf{K}_i$ and $\mathbf{K}_s$ are the wave vectors of the incident laser beam and the scattered beam, respectively, and $\mathbf{V}$ is the velocity of the particle. The magnitude of the frequency shift can be measured by optical heterodyning. With this method, the light scattered from the moving particles is combined at the surface of a photodetector with a local oscillator or reference beam which is, in general, a portion of the laser beam initially incident on the scattering re-

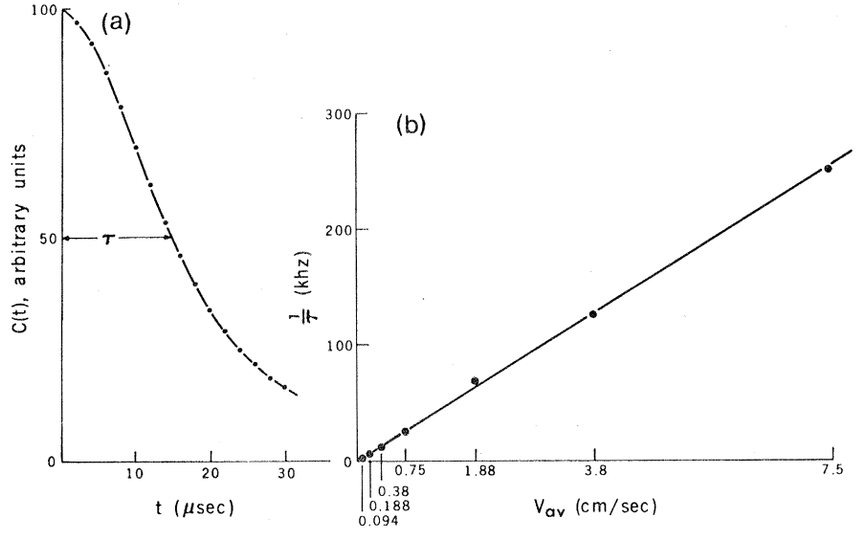

Fig. 1. (a) Correlation function for light scattered from the blood flowing in a glass capillary with an internal diameter of 160 μm; the flow velocity was 2.0 cm/sec. The half-width of the correlation function is denoted by τ. (b) Plot of $1/\tau$ as a function of average velocity of blood flowing in a glass capillary with an internal diameter of 160 μm.

gion. The photodetector mixes both signals to give in the output photocurrent a signal which fluctuates at the difference frequency.

Light from a He-Ne laser beam is focused on a retinal vessel through a contact lens applied to the cornea of the subject. In the fundus the beam is about 200 μm in diameter. The power of the beam is attenuated to 18 μw by a neutral density filter. As a result, the retinal irradiance is about 0.05 watt/cm^2. Such an irradiance corresponds to half of the maximum permissible level for continuous illumination (*3*).

The spot is observed through the microscopes of a conventional slit lamp. One of these microscopes is also used to collect the light scattered back by the retinal vessel. After deflection by a semitransparent mirror, this light is focused on a slit and detected by a photomultiplier. The output pulses of the photomultiplier are digitally amplified and discriminated by a pulse amplifier and then fed into an 18-channel digital autocorrelator.

The subject is positioned in front of the slit lamp in the same way as for a routine contact lens examination. The optical setup provides heterodyne detection of the Doppler shift. The light scattered from the vessel wall acts quite effectively as a local oscillator. To better define our scattering geometry for the experiments in humans, we measured velocity spectra only from vertical blood vessels around the optic disk.

When flowing particles move at various velocities, the frequency spectrum contains a range of frequencies corresponding to the range of velocities. For example, the frequency spectrum of polystyrene spheres suspended in water flowing through glass capillaries is characterized by a rectangular shape with a sharp cutoff at a frequency corresponding to the maximum velocity (*1*). With blood, on the other hand, the sharp cutoff disappears and we have instead a rather broad tail. This tail has been associated with the effect of multiple scattering (*1, 4*). Light can be scattered by two or more red blood cells before impinging on the photocathode, and this broadens the spectral profile, since two or more velocity combinations are possible in the multiply scattered light. Therefore, the frequency spectrum for blood is not a direct representation of the velocity spectrum. As a result, the mean flow velocity cannot be determined directly from the frequency spectrum and an experimental calibration procedure relating the frequency spectrum to the mean velocity of the red cells was adopted. Similar considerations are valid for the autocorrelation function, which is just a Fourier transformation of the frequency spectrum.

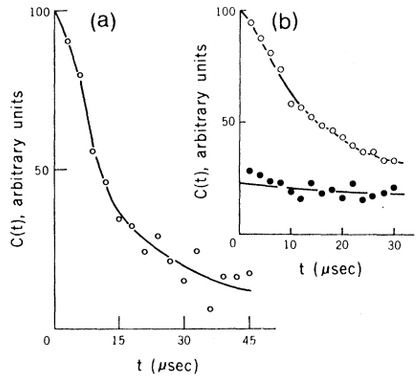

Fig. 2. Correlation functions for light scattered from blood flowing in (a) a human retinal artery 120 μm in diameter and (b) a human retinal vein 160 μm in diameter (open circles). The closed circles in (b) represent the correlation function for light scattered from the optic disk tissue.

Using a similar scattering geometry, we measured the correlation function for light scattered from blood flowing in glass capillaries, as a function of the mean flow velocity (Fig. 1a). The half-width τ of this correlation function provided the necessary calibration for the results for humans. The calibration curve we obtained consisted of a plot of $1/\tau$ against the mean flow velocity, V_{av}, in the tube. A typical calibration curve for a tube 160 μm in diameter is shown in Fig. 1b. Similar curves were constructed for glass tubes with diameters ranging from 60 to 200 μm. Since the time scale of the correlation function is, in general, determined by the reciprocal of the characteristic frequency of the spectrum, we expect that

$$(1/\tau) \propto f_{max} \propto V_{av}$$

This proportionality between $1/\tau$ and V_{av} is what we found and is represented in Fig. 1b.

Autocorrelation functions $C(t)$ for the light scattered by blood flowing in a retinal artery and a retinal vein of a human subject are shown in Fig. 2, a and b, respectively. The vein was about 160 μm in diameter and the artery about 120 μm, evaluated from fundus photographs (assuming a refractive power for the emmetropic subject of 57 diopters). These autocorrelation functions have practically the same shape as those obtained from blood flowing through glass capillaries.

The mean flow velocity in a vessel was determined from the half-width of the autocorrelation function and the calibration curve associated with the capillary tube of the same size as the vessel. In the human subject we found 1.9 cm/sec for a 160-μm vein, 1.6 cm/sec for a 130-μm vein, and 2.2 cm/sec for a 100-μm artery. The mean brachial arterial blood pressure was 90 torr and the intraocular pressure was 21 torr. As a blank test, we also measured the autocorrelation function of light scattered by a part of the optic disk that did not contain any large vessels. We found it to be practically constant, corresponding to the slow, multidirectional flow of blood in the capillaries of the optic nerve (Fig. 2b).

We were unable to find published data on the mean flow velocity in retinal vessels (*5*). Thus, we cannot confirm our measurements of V_{av}. Nevertheless, the close similarity between the correlation functions for blood flowing in the retina and in glass tubes gives us confidence in our results. These experiments show that laser Doppler velocimetry permits simple, safe, and quantitative determination of blood flow velocity in human retinal vessels. This technique, which can easily be adapted for clinical use, should prove to be a valuable tool for the diagnosis and management of circulatory disorders in the eye.

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References and Notes

1. C. Riva, B. Ross, G. B. Benedek, *Invest. Ophthalmol.* **11**, 936 (1972).
2. Y. Yeh and H. Cummins, *Appl. Phys. Lett.* **4**, 176 (1964).
3. D. H. Sliney and B. C. Freasier, *Appl. Opt.* **12**, 1 (1973).
4. T. Tanaka and G. B. Benedek, in preparation.
5. We have found various data related to the axial velocity of fluorescein in human retinal vessels. F. C. Delori, R. W. Airey, C. T. Dollery, E. M. Kohner, C. J. Bulpitt [*Adv. Electron. Electron Phys.* **33** (Part B), 1089 (1972)], using intracarotid dye injection, measured a speed of 3.5 cm/sec in one diabetic patient. P. Niesel, however [*Exp. Eye Res.* **17**, 388 (1973)], using the dye dilution technique, published values ranging between 1 and 2 mm/sec in young normal subjects.
6. We acknowledge the contribution of B. Ross in the development of the instrument and in the initial stages of these experiments. We also thank G. B. Benedek for helpful suggestions and discussions, R. Thompson for construction of the instrument, and W. Roberts for technical assistance. Supported by PHS grants EY-54465, EY-01242-01, EY-01303-01, and HL-14322-01, by Research to Prevent Blindness, Inc.–William Friedkin award, and by the General Electric Foundation. D. F. Dobies provided editorial assistance.

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