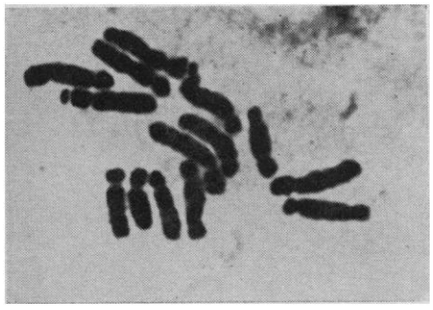

Fig. 1. Chromosomes of *Avena ventricosa*.

have rather symmetrical chromosomes and two pairs of satellited chromosomes (*3*).

In having these distinct morphological and cytological characters, *A. ventricosa* approaches closely the hexaploid oats (the group comprising *A. sativa* and *A. sterillis* and having $2n=42$). Hexaploids have awnless denticulate lemmas. They also show seven pairs of chromosomes with subterminal centromeres (*4*). Since these characters are absent in other diploid *Avena* (as well as in the tetraploid oats), the hypothesis is advanced that *A. ventricosa* participated in the formation of the hexaploid oats.

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Red Cell Slip at a Wall in vitro

Abstract. *Interferometric microscope observations of flowing blood indicate that the absolute number of red cells maintaining sliding contact with a wall is of the order 10^4 per square centimeter at low flow rates. Contacting cell oscillation and orientation results are given. The near wall local hematocrit is found to be relatively low.*

The purpose of this investigation was to explore the reality of blood slip, an issue significant to such matters as the local shear stress experienced by the containing vessel, the exchange of substances through the red cell membrane, and the total work performed by the heart.

It is generally accepted that real Newtonian fluids do not slip freely at a boundary, regardless of the flow velocity at some distance from the wall. Tested fluids include those that wet the wall, such as air (*1*) and water (*2*); and those that do not, such as mercury (*3*).

Unlike Newtonian fluids, the measured apparent viscosity of blood is sensitive to the roughness and chemical nature of a capillary wall (*4*) and to the vessel diameter (*5*), which suggests the possibility of slip. Velocity profiles taken several microns from the wall have been deemed (*6*) strongly suggestive of real slip, when the shear rate is low. Conversely, a number of hypotheses, with supporting evidence, have been advanced to explain the anomalous flow properties of blood without recourse to true slip. These include the sigma effect (*7*), the wall effect (*8*), the mechanical effect (*9*), and variable concentration (*10*).

To resolve the issue of slip it is sufficient to measure the velocity of blood, both plasma and cells, on planes of depth vanishingly close to a wall. Classic difficulties arising in such measurements are refraction and reflection of light, cell optical density variation as a function of velocity (*11*), and resolution limitations. The latter is particularly serious; the order of magnitude of lateral resolution with white light when employing practical lenses (in contrast to idealized values) has been given by Michelson (*12*) as 0.5 μ. Practical resolution in depth is further reduced, for additional error sources are introduced, such as the extent of accommodation within the eye and fine-focus backlash. Thus the results of Bloch (*13*), who reported red cells "in very close proximity to the wall, apparently touching the wall or 1–2μ from it," may be regarded as representing the limit of conventional microscopic depth perception. In order to establish the state of slip, a resolution improvement of at least one order of magnitude is necessary; an improvement of two orders of magnitude is useful.

The technique chosen employs a form of Newton's interference rings known as Fizeau bands. Widely known in metrology (*14*), minute differences in path length, within a thin film under test, are utilized to establish interference fringes of known separation in

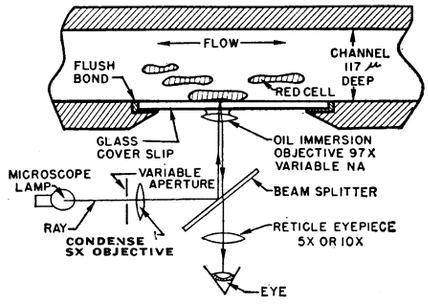

Fig. 1. Experimental arrangement schematic. Fizeau fringes are established on those cells within 1.4 μ of the channel floor.

depth. Through a fringe counting procedure, absolute depth is established, based on the wavelength of light.

Blood-flow conditions chosen are those that minimize optical problems and maximize the possibility of slip detection. Specifically, a two-dimensional channel, offering the same Newtonian fluid parabolic flow velocity distribution as that of a cylindrical vessel, but few refraction difficulties, was employed in preference to the latter. To assure nearly vertical illumination, I used only central rays from the microscope (*15*) lamp, by means of a small, variable aperture placed a considerable distance from the bulb, and a 5-power objective, serving as a condenser. Alignment proved critical; see Fig. 1. Rays entering the microscope are routed conventionally.

With the instrument focused on the underside of a red cell, a series of interference bands of alternate light and

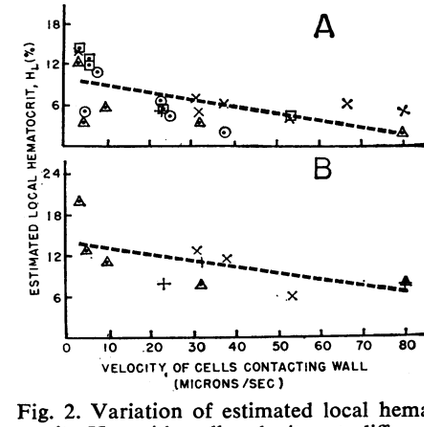

Fig. 2. Variation of estimated local hematocrit, H_L, with cell velocity at different depths from a wall. The dashed lines are least-squares regressions. Each symbol indicates a given blood donor. (A) Red cells in sliding contact with a wall. (B) Cells occupying some portion of the initial 1.4 μ depth with respect to a wall.

dark rings is discerned. Each ring represents the locus of all points on the cell contour at a fixed distance from the wall, given (*16*) by $D = d + (k\lambda/2N)$, where d is a constant (tare) determined experimentally, k is the order of interference (ring count), λ is the wavelength of light taken as 5000 Å for white light, and N is the index of refraction of plasma taken as 1.34 (*17*). Thus, the difference in depth between like rings (light to light or dark to dark) is found to be approximately 0.19 μ per count. As a considerable degree of interpolation between rings is possible, ultimate resolution is limited by the oscillation of the red cell membrane. Under optimum observation conditions (zero flow rate) a resolution of $\pm$ 0.04 μ in depth is practicable.

Observed cell depths were sorted into two categories: zero depth (optical contact with the wall) and a depth less than 1.4 μ. The latter value reflects those cells displaying fringes in any number; it is obtained from considerations of longitudinal coherence and color discrimination limitations of white light interference bands (*18*).

The blood used in this work is acid citrated human whole blood, obtained from a blood bank and used before the noted expiration date. All tests were conducted at room temperature, after filtering to remove possible microclots. Total filtering and test time length was less than 2 hours per blood sample.

Observations of moving cells in contact with the wall indicate that the majority display symmetrical fringes superimposed on a disc projection of the cell. The axes of such cells are aligned parallel and perpendicular to the wall. As the cell moves in the direction of flow, the fringe pattern oscillates radially so that the central bright spot varies in diameter from approximately 2 to 4 μ. At the latter value a dark fringe, of zero order, appears centrally. In displacement terms, this signifies an oscillation of 0.1 μ of the cell center in the direction of depth. The period appeared to be a function of velocity; at 10 μ/sec the mean period was approximately 2 seconds; at 40 μ/sec, less than 1 second. Oblique cells, moving in contact with the wall, are relatively rarer, as are rouleaux. Rouleaux stacking alignment is sufficiently imprecise that rarely are all the cells within a clump in simultaneous contact with the wall; typically one-half to two-thirds of the clumped cells make contact.

Table 1. Attitudes and configurations of those moving red cells in contact with a wall. Cell velocity 10 to 20 μ/sec.

Condition	Occurrence (%)
Aligned with wall	79
Rouleaux	5
Oblique	16
Leading edge down	2
Trailing edge down	10
Lateral edge down	4

Cell attitude information (see Table 1) was based on observations of 200 erythrocytes contained within five blood samples. No apparent difference in attitude between samples was noted. As an index to attitude eccentricity, an arbitrary offset of 2 μ between the cell geometric origin and the interference band origin was chosen to represent the maximum excursion of an aligned cell. Those cells exceeding this limit are deemed oblique cells; in turn, these are separated into leading-edge-down and lateral-edge-down categories as a function of contact location with respect to the direction of motion. This process of judging origin eccentricities of moving cells is one of low accuracy, owing to cell oscillation. Nonetheless, occurrence values, rounded off to the nearest whole percentage point, are believed accurate to $\pm$ 5 percent.

To determine red cell concentration at a given depth, the number of cells passing an arbitrary fixed point (eyepiece reticle witness mark) per unit of time, m, at some known velocity was compared with the maximum number of undistorted aligned cells capable of passing a fixed point per unit of time, M, at the identical velocity. The value M was determined with a hexagonal sheet packing criterion. It has been shown (*19*) that for the ratio $m/M = 1$, the approximate hematocrit is 58 percent. Therefore a crude (the assumption of aligned undistorted cells is highly idealized) index of local hematocrit, H_L, may be obtained from $H_L = 0.58\ m/M$.

Results, in the form of H_L versus cell velocity, were determined for zero depth, that is, wall optical contact (Fig. 2A) and the entire region extending from the wall through a distance of 1.4 μ normal to the wall (Fig. 2B). The scatter is considerable, reflecting (i) the blood specimen sampled, (ii) the location and cleanliness of the wall segment examined, and (iii) the time variant nature of local hematocrit; the latter is due to a period of concentration oscillation sufficiently great with respect to run length (typically 1 minute) to preclude smoothing.

All estimated local hematocrit values were below hematocrits representative of the whole blood (37 to 54 percent), which confirms the existence of a plasma-rich layer of fluid in immediate contact with the wall. Of those cells occupying some portion of the initial 1.4 μ depth with respect to the wall, roughly 60 percent are in moving contact with the wall. In the range of cell velocities tested, there is a trend to decreased wall contact and a thicker "plasma sheath" with increased velocity.

While the population of red cells inhabiting the wall region is thus seen to be relatively sparse, the number in absolute terms is large. If we imagine a vessel wall area consisting of 1 cm^2 subject to a local cell velocity of about 40 μ/sec, and apply local hematocrit results in combination with the standard value for red cell count, 74,000 cells are estimated to occupy some portion of the initial 1.4 μ in depth; 42,000 are estimated to maintain sliding contact with the wall. Cell population magnitudes of this order bring into question analytical attempts to treat the general flow of blood by means of the classical no-slip boundary condition, or an assumed cell-free peripheral plasma sheath condition, or both. A more realistic model might be obtained by adding an energy loss term, based on red cell sliding contact, to the usual Poiseuille flow pressure losses.

To examine qualitatively the effect of surface roughness on wall flow characteristics, an obstacle in the form of a mound some 50 μ high was placed on the smooth glass wall. The effect produced was one of intense turbulence and apparent local phase separation with attendant dead water regions. It is conceivable that reported (*20*) erratic in vivo plasma sheath behavior reflects local roughness.

Blood slip status is a function of the slip characteristics of both red cell and plasma components. It has been shown that red cells slip freely while manitaining contact with a wall. The slip state of the surrounding plasma is not yet fully understood.

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Fast Light-Evoked Potential from Leaves

Abstract. *When a leaf is illuminated with an intense flash of light, an electrical response with a time course in milliseconds can be recorded. This response was obtained between two wick electrodes placed at different positions on top of the leaf, with the entire leaf uniformly illuminated by the flash. During the first millisecond or so, the electrode nearer the apex of the leaf always became negative with respect to an electrode at the base, which indicates that the voltage-generating source is fixed longitudinally in the leaf.*

It was recently suggested (*1*) that fast electrical responses, such as those elicited from the vertebrate eye (*2, 3*) by a bright flash of light, may be a phenomenon widespread in all oriented biological pigment systems. This has now been confirmed by the discovery of similar fast light-evoked responses from the pigment epithelium-choroid complex (*4, 5*), from skin (*6*), and from the invertebrate eye of the *Limulus* (*7*). My report shows that such a fast light-evoked potential can also be recorded from leaves (*8*).

The techniques used to investigate the leaf potentials are similar to those used in recording the fast response from the vertebrate eye (*3, 9*). The light source was a Honeywell Strobonar P600 photographic flash housed in a soft steel box to reduce electromagnetic artifacts. The strobe has an input energy of 65 joules. Duration of the flash was 800 μsec, with the peak occurring at 250 μsec. Large-diameter Fresnel and conventional lenses were used to collect light from the strobe and focus it to give a uniform spot of light about 3 cm in diameter. This is sufficient to illuminate a considerable portion of the surface of the typical leaf used in these investigations. The incident energy on the leaf surface was approximately 0.05 joule/cm^2 in the visible region. Wick electrodes, well shielded from the light, were used for recording. A CR-4 low-noise differential amplifier (Princeton Applied Research) was used to amplify the signals, which were then displayed on a Tektronix type 502 oscilloscope.

A response was found in a number of different types of leaves, but the type chosen for detailed study was that of the bean plant, *Phaseolus vulgaris* var. Black Valentine, since this is a species that has been well studied in connection with photosynthesis. All plants used in my experiment were 3 to 4 weeks old and seemed to give large and reproducible responses.

In recording from the *Phaseolus* leaf, both electrodes were placed on top of the leaf [such as positions 1 and 2 on leaf diagram (Fig. 1)]. If electrodes were placed about 1 cm apart and the leaf uniformly illuminated with an intense flash of light, the response in Fig. 1A was recorded. The sign of the response was such that, for the first millisecond or so, the electrode at the apex of the leaf became negative with respect to the one close to the base; then a slow positive response was seen which often took as long as 1 second to decay. The fast response did not have a detectable latency, and its peak time was about 0.6 millisecond. The amplitude of this slow response varied from preparation to preparation with respect to the amplitude of the fast response, which suggests that these responses have different sources or pigment pools, or are brought about by different mechanisms.

Electrodes at positions 3 and 4 on the underside of the leaf gave a response with the same waveform as that shown in Fig. 1A, except that it was smaller. With the electrodes at positions 1 and 4 or 2 and 3, exactly opposite each other, there was no response.

Figure 1C shows that a response similar to that recorded with both electrodes on top of the leaf can also be recorded with one electrode on top and one underneath. This response is presumably the same as that recorded with both electrodes on top of the leaf, since no response was found with one electrode exactly above the other. If the leaf was turned over while the electrodes remain fixed and the electrodes were then placed along the midrib, but on the underside of the leaf, the response obtained was that shown in Fig. 1B. This response is similar to the responses obtained with illumination from the top, but it is much smaller, which suggests that the response comes primarily from what is normally the top surface of the leaf.

If the positions of the electrodes along the midrib on top of the leaf were interchanged, the waveform of the response was reversed, which shows that the effect is not one of "photoconduction" connected with the accidental polarization of one of the electrodes. In fact, the electrode near the apex of the leaf always became negative with respect to an electrode at the base, which shows that the voltage source producing the response must be longitudinally oriented. The amplitude of the response became larger as the electrodes were moved apart along the midrib of the leaf within the area of uniform illumination; this also indicates longitudinal polarization of the voltage source. Indeed, it is possible to make a "map" of equipotentials on the leaf surface. It is a little surprising to find the signal-generating structure fixed longitudinally in the leaf, in view of the perpendicular orientation of the signal generators in the vertebrate retina (*10*).

That a leaf should give a light-evoked potential at all is somewhat unexpected. Chloroplasts containing the pigments are not usually considered to be oriented as the receptors in the eye are. On the other hand, there is no evidence for a high degree of orientation in many of the other systems in which these fast potentials have now been observed, such as skin and the pigment epithelium-choroid complex. In many of these cases the effect may be