Regional rheological differences in locomoting neutrophils

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Regional rheological differences in locomoting neutrophils. Am J Physiol Cell Physiol 287: C603–C611, 2004. First published May 26, 2004; 10.1152/ajpcell.00347.2003.—Intracellular rheology is a useful probe of the mechanisms underlying spontaneous or chemotactic locomotion and transcellular migration of leukocytes. We characterized regional rheological differences between the leading, body, and trailing regions of isolated, adherent, and spontaneously locomoting human neutrophils. We optically trapped intracellular granules and measured their displacement for 500 ms after a 100-nm step change in the trap position. Results were analyzed in terms of simple viscoelasticity and with the use of structural damping (stress relaxation follows a power law in time). Structural damping fit the data better than did viscoelasticity. Regional viscoelastic stiffness and viscosity or structural damping storage and loss moduli were all significantly lower in leading regions than in pooled body and/or trailing regions (the latter were not significantly different). Structural damping showed similar levels of elastic and dissipative stresses in body and/or trailing regions; leading regions were significantly more fluidlike (increased power law exponent). Cytoskeletal disruption with cytochalasin D or nocodazole made body and/or trailing regions ~50% less elastic and less viscous. Cytochalasin D completely suppressed pseudopodial formation and locomotion; nocodazole had no effect on leading regions. Neither drug changed the dissipation-storage energy ratio. These results differ from those of studies of neutrophils and other cell types probed at the cell membrane via β2-integrin receptors, which suggests a distinct role for the cell cortex or focal adhesion complexes. We conclude that 1) structural damping well describes intracellular rheology, and 2) while not conclusive, the significantly more fluidlike behavior of the leading edge supports the idea that intracellular pressure may be the origin of motive force in neutrophil locomotion.

MECHANICAL DEFORMATION OF LEUKOCYTES, and neutrophils in particular, is an essential feature associated with their function. Examples from the inflammatory response include chemotactic locomotion and transendothelial and transepithelial migration (21). The mechanical forces responsible for pseudopodal protrusion during locomotion and the cell’s resistance to deformation remain controversial. In particular, whether the origin of the protrusive force in pseudopods is causally associated with pushing from polymerizing microfilaments and/or cytoskeletal swelling forces (2, 10, 18, 26), whether it is associated with Brownian ratchets (16), and whether it is the result of hydrostatic pressure with associated fluid flow of cytoplasm into the pseudopod (13, 24) remain open questions. Probes of the rheological properties of cells are an appropriate tool with which to address these issues.

We previously measured intracellular elastic and dissipative properties in neutrophils from 0.3 to 3 Hz by oscillating intracellular granules trapped with optical tweezers, and we interpreted the results in terms of a viscoelastic body (23). Because of this narrow frequency range, it was not possible to determine whether the elastic properties of the cell were independent of the dissipative properties or whether the two were intrinsically coupled. Recent reports (7–9) have strongly suggested that the elastic and dissipative components arise from the same underlying molecular mechanism and are therefore necessarily coupled. This requires measurement of the rheological properties over a significant frequency range. In this article, we report displacement measurements of optically trapped granules after a step change in trap position, which in principle contains all frequencies in the response function. In this manner, we were able to extend our previous frequency range to about three decades (~0.3–500 Hz). We characterized our measurements in terms of simple viscoelasticity and the structural damping model, as recently applied to a variety of cell types, in which stress relaxation is associated with a power law in time (7). These measurements were taken separately in the leading edge, body, and trailing region of locomoting neutrophils to assess regional differences in intracellular rheology. To determine the contribution of the cytoskeletal polymers actin and microtubules, we also measured regional rheology after treatment of the neutrophils with the cytoskeletal disruptors cytochalasin D (actin filaments) and nocodazole (microtubules).

METHODS

Reagents. Krebs-Ringer phosphate with dextrose (KRPD) was constituted as (in mM) 115 NaCl, 14 dextrose, 6 KCl, 4.6 MgSO4·7H2O, 3.5 NaH2PO4·2H2O, and 16 Na2HPO4 in water. We used normosmotic RPMI 1640 medium with l-glutamine (GIBCO-BRL, Grand Island, NY). For separation of blood cells into mononuclear and polymorphonuclear (PMN) leukocytes, we used Mono-Poly resolving medium (Dainippon Pharmaceutical, Osaka, Japan). Fetal bovine serum was obtained from Cambrex International (Etonic, ON, Canada). The cytoskeletal disruptors used were cytochalasin D and nocodazole (Sigma Chemical, St. Louis, MO).

Preparation of cells. Human neutrophils were isolated from whole blood by using a density gradient technique described in detail in Yanai et al. (23). Briefly, 24 ml of peripheral blood were drawn from healthy volunteers with a heparinized syringe. Platelets were removed and replaced by KRPD solution, Mono-Poly resolving medium was

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added, and samples were centrifuged. This procedure resulted in four layers comprising, in order from top to bottom, KRPD solution, a monocyte/lymphocyte layer, a PMN cell layer, and a red cell layer. The PMN cell layer was collected and rinsed with KRPD solution. It was then centrifuged, and the PMN cells were resuspended with 10 ml of medium (RPMI 1640–5% fetal bovine serum).

Chamber preparation. A chamber was prepared with a clean, uncoated slide glass as the top surface and a clean, uncoated coverslip as the bottom surface, separated by ~20 μm using thin plastic sheet spacers. Neutrophils suspended in the medium were introduced into the chamber, the sheet spacers were removed, and the chamber edge was sealed with nail polish. The sample was placed on a microscope stage and maintained at room temperature. Many of the cells remained only loosely adherent to the glass surface and appeared round and inactivated. A modest fraction adhered to the glass strongly and began to spread and locomote. Neutrophils in the process of lamellipodial protrusion and locomotion were used for this study.

Inhibition of F-actin formation and microtubule assembly. To disrupt F-actin or microtubules, we introduced, respectively, cytochalasin D (2 μM) or nocodazole (10 μM) into the chamber in medium containing 0.1% DMSO. The sample was then incubated at 37°C for 5 or 10 min, respectively.

Optical trap and displacement detection. An inverted microscope (IX-70 Olympus) and other optical instruments were set on a vibration-free table (TDI-189LA; HERZ, Yokohama, Japan) as described previously with some modifications (12, 14). As shown in Fig. 1, an intracellular granule in the neutrophil was trapped by an infrared yttrium-aluminum-garnet laser trap (1,064 nm, 2 W; Spectra-Physics), the position of which was controlled by mirror 2. The images of the neutrophil and enlarged intracellular granule illuminated by a halogen lamp were projected on charge-coupled device cameras 1 and 2, respectively. The center of the granule was projected on the center of a quadrant photodiode detector (1-mm diameter, S1557–01; Hamamatsu Photonics). Displacements of the granule in orthogonal directions were determined from the differential outputs of the detector (differential amplifier OP711A; Sencore, Japan). The stiffness, or equivalent spring constant, of the optical trap, was determined from the granular Brownian motion using the equipartition law (19),

\[ k_{\text{trap}} <x^2> = k_B T \]

where \( <x^2> \) is mean square displacements of trapped vesicles, \( k_B \) is the Boltzmann constant (1.38 \times 10^{-23} \text{J/K})

and \( T \) is absolute temperature 300K. In the present work, the trap stiffness was 0.068 ± 0.018 pN/nm (mean ± SD, n = 37). Note that this calibration was done with intracellular granules because the optical properties of the intracellular milieu are different from those of the extracellular medium, and therefore the trap stiffness is different as well.

The position of center of trapping was moved quickly by changing the angle of mirror 2 by means of piezo actuator 1 (see Fig. 1). The 100-nm calibration of the granule displacements was accomplished by moving eyepiece 2 by means of piezo actuator 2. Data of displacement of the granules and the input voltage of piezo 1 and 2 were collected with a computer equipped with a laboratory interface (PowerLab; ADInstruments).

Protocol. A locomoting neutrophil was selected, and a granule in the leading edge, the body, or the trailing region of the cell was optically trapped. An example of granule selection in each region is shown in Fig. 2. This was followed by the step displacement protocol, in which each complete period lasted 2 s. At the beginning of each period (t = 0), the trap’s position was given a 100-nm step in position, driven by the mirror (piezo 1, mirror 2 in Fig. 1) (95% rise time, <1 ms), and held there for 1 s (Fig. 3, top left). During the period in which the step is on, the quad detector is moved the equivalent of 100 nm and then back again (motor X in Fig. 1), from \( t = 750 \) ms to \( t = 900 \) ms, measured from the beginning of the step. This allows essentially real-time self-calibration of the displacement signal against the known quad detector step. The trap’s position is then returned to baseline for 1 s. This cycle is automatically repeated.

Theory. Visual inspection of the displacement data revealed a common feature in all measurements. After the step change in position of the optical trap, there was a very fast rise in the displacement response, although definitely not a step jump in position. This was followed by a much slower approach to a limiting behavior in displacement, which approximated a gently rising plateau. This suggested two potential models to characterize the data. First, the initial large and subsequent decreasing velocity of the granule, together with the presence of an approximate plateau asymptote, suggested consideration of the simplest possible linear viscoelastic description, consisting of an elastic element with stiffness \( G \) in parallel with a viscous element with viscosity \( \mu \). The advantage of such a description is its simplicity. Second, we modeled the cytoplasmic milieu with the structural damping idea, which is known to characterize a variety of biological tissues and cells (8, 9, 11). (Non-Newtonian fluid characterizations of cell rheology, which share features with structural damping, have also been investigated (5, 20).) In pure structural damping, stress relaxation after a displacement step behaves as a power law in time and is parameterized by an overall scale of stress and the exponent in the power law. Note that both Voigt viscoelasticity and structural damping are two-parameter models.

Both of these models are simple to implement when measurements of force are made after step displacements or when measurements of displacements are made after step changes in force. However, when the position of the trap is a step function, the trapping force applied to the granule is more complicated because the trap’s force on the
granule and the granule’s position are coupled (i.e., as the granule moves in response to the trap’s force, the force also falls because of the granule’s position moving closer to the trap’s center). In the case of the simple viscoelastic model, this presents no difficulty, because the force on the granule remains proportional to its displacement. The equations of motion and their solution are described in the APPENDIX. By contrast, the equations of motion are quite complex for a granule in a structural damping medium after a step change in the optical trap’s position. The equations of motion and their solution for this case are also provided in the APPENDIX.

All measures of stiffness and viscosity scale with the trap stiffness, which, as determined above, has dimensions of a spring constant, i.e., force/length. This yields essentially one-dimensional stiffness and viscosity measures in characterizing the step response data. As described at the end of the Appendix, it is easy to convert these measures to the more usual elastic moduli and viscosity by using the Stokes formulation for the displacement or velocity of a spherical particle in an elastic or viscous medium. This factor depends only on the particle radius, for which we take a typical granular radius of 300 nm.

All fits to the data were done by least-squares fitting of the measured $x(t)$ against the formulas in Eqs. A2 (viscoelastic model) and A10 (structural damping model). This was done for the first 500 ms of data collection (the trap step-on position). Data for times after 1 s, when the trap returned to the origin, were not used because of the confounding effects due to the presence of cellular motion. That is, the model assumptions include stasis of the intracellular milieu, which is variably valid for time scales longer than, in our case, ~1 s. We did choose data runs in which the return transient showed at least an approximate return to the origin, but it was clear that, because of

Fig. 2. Differential interference contrast image of a locomoting neutrophil, with arrows denoting granules located at each of the three regions of interest. L, leading edge; B, body; T, trailing region. Scale bar, 4 μm.

Fig. 3. A: schematic of the trap position as a function of time (note the self-calibration jump step and the return to baseline over the 2-s protocol) and three raw data tracings from granular displacement responses in the leading edge, body, and trailing region of a normal locomoting neutrophil. B: segments of data analyzed, with fits to the viscoelastic model (shaded traces) and to the structural damping model (solid lines).
locomotion, the reliability of data recorded past 1 s was questionable. The models were compared by examination of the sum of squared residuals. We compared the stiffness and viscosity (viscoelastic model) and stiffness and power law exponent (structural damping model) between the body, trailing, and leading edge of the locomoting neutrophils, and between control conditions and after cytoskeletal disruption, with cytochalasin D (actin filaments) or with nocodazole (microtubules). All data are expressed as means ± SE. Comparisons were done by t-test, with significance set at either \( P < 0.05 \) or \( P < 0.01 \), as shown in the figures.

RESULTS

A total of 52 cells were studied under control conditions, with 20, 17, and 15 measurements in body, tail, and leading edges of the cell, respectively. Because we found no significant differences between the body and tail regions, these data were subsequently pooled. Body regions were studied in 29 cells after treatment with cytochalasin D; because there was no locomotory activity, there are no data for any leading edge in this treatment group. Pooled body and trailing regions and leading edge regions were studied in 16 and 9 cells, respectively, after treatment with nocodazole.

The three data panels in Fig. 3A show typical granular displacement curves vs. time after a step change in the position of the optical trap. These data are from a normal control neutrophil, with the granule located in the leading edge, body, or trailing region, respectively, in the three panels. Note the 150-ms self-calibration step that occurs at 0.75 s. The adjacent panels in Fig. 3B show the data segments analyzed, together with the curves fitted to the viscoelastic model and to the structural damping model. Note the shape differences; the body and trailing regions appear stiffer (less granular displacement for the same trap displacement) and more solidlike (generally faster response). Curves from cells treated with nocodazole showed patterns qualitatively similar to these. As noted above, treatment with cytochalasin D completely inhibited any leading edge, and motility was stopped. We thus have no data for the leading edge in cytochalasin D-treated cells; data from the body and trailing regions of these treated cells were similar to data from these regions, shown in Fig. 3.

Figure 4 shows the results of the analysis of control neutrophil rheology as a function of cell region. Figure 4A corresponds to the simple viscoelastic model, and Fig. 4B corresponds to structural damping. In both models, there was no significant difference in any parameter between the body and trailing region of the cell. These regions were therefore pooled for later analysis and for comparison with the rheology after pharmacological intervention. By contrast, both measures of stiffness \( G \) (in the viscoelastic model and the stress scale \( F_{100\text{ms}} \) in structural damping) in the leading edge were significantly lower. Similarly, both measures of fluidity (the viscosity \( \mu \) in the viscoelastic model and the power law exponent in structural damping) were significantly different in the leading edge compared with the body or trailing region. Both measures (lower \( \mu \), higher \( k \)) support the idea of a more fluidlike characteristic of the cytoplasm in the leading edge.

With cytochalasin D treatment, we found that the cells were virtually stationary; because there was no locomotion, there was no leading edge. Comparisons were therefore made between the cytochalasin D-treated cells and the pooled body and trailing regions of control cells. Disruption of microtubules with nocodazole did not prevent locomotion; in this case, comparisons were possible against both the pooled body and trailing regions of control cells, as well as against the leading region. These results are shown in Fig. 5. Fig. 5, A and B, shows the viscoelastic stiffness and viscosity in control conditions and after treatment. Figure 5, C and D, shows the structural damping stiffness and power law exponent in control conditions and after treatment. Figure 6 shows the results displayed as % change from control conditions, with Fig. 6A showing the viscoelastic parameters and Fig. 6B showing the structural damping parameters. We found that in the body and trailing regions, with either cytochalasin D or nocodazole treatment, the stiffness of the cell as assessed in both models was significantly reduced. Similarly, both treatments reduced the apparent viscosity in the pooled body and trailing region. By contrast, disruption of microtubules with nocodazole did not significantly change any parameter in the leading region of the cell. Perhaps most surprisingly, the power law exponent in structural damping was not changed by any treatment in any cellular region.

The sum of squared residuals for the simple viscoelastic model was averaged over all measurements, separately by cell region and by drug treatment. In all cases, we found that the structural damping characterization fit the data better than did
the simple viscoelastic model ($P < 0.01$). However, we choose to report the analyses of both methods because, first, classic viscoelastic parameters are easily understood and can be compared with other such common measures in the literature, and second, there is complete agreement between both methods regarding the similarities or significant differences among cell regions and after cytoskeletal disruption.

**DISCUSSION**

**Characterization of rheological properties as simple viscoelasticity.** Over a limited frequency range, and when force/displacement Lissajous loops are approximately elliptical, or when a step response is roughly exponential (both being consistent with linearity), a classic description of the phenomena in terms of a simple viscoelastic medium is attractive. In our case, because the approximate displacement plateau that the granule reached after the trap step was systematically less than the trap step size, the standard solid, or Kelvin, model might be considered. On the other hand, we saw no evidence of a sharp jump or step in the displacement, suggesting that the spring constant in the Maxwell body component of the standard solid is very stiff compared with the parallel elastic element. We thus used the simpler parallel viscoelastic model, known as a Voigt body. Such a description is conceptually simple and parsimonious; its two parameters clearly distinguish between energy that can be stored elastically through displacement per se and energy that is dissipated viscously through the rate of change of displacement. On the other hand, as the frequency range over which measurements are made is increased, other behaviors can become apparent, as is the case here; the structural damping model systematically fits the data better than did simple viscoelasticity. Nevertheless, the viscoelastic model remains a useful descriptor. We also investigated other potential models, including a "cage" model of the cytoskeletal network wherein the granules reside in a fluid regime, but as they are displaced, they encounter the cytoskeleton at some particular displacement length. This model is manifestly nonlinear and is not consistent with the approximate

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**Fig. 5.** Rheology of neutrophils in control conditions and after cytoskeleton disruption effected by treatment with cytochalasin D (cyto-D) or nocodazole (noc). From the observation of no significant difference between the body and trailing region of the cell (see Fig. 4), the body and trailing region results were pooled. The neutrophils did not locomote after cytochalasin D, and therefore no data are shown for the leading edge. A and B: viscoelastic stiffness and viscosity (control and after treatment). C and D: structural damping stiffness and power law exponent (control and after treatment). * $P < 0.05$, ** $P < 0.01$.

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**Fig. 6.** Percent changes in rheological parameters from control conditions after treatment with either cytochalasin D or nocodazole. A: viscoelastic stiffness and viscosity. B: structural damping stiffness and power law exponent. ** $P < 0.01$. BT, pooled body and trailing regions.
mirror symmetry that we observed when the trap, while still on, returned to the coordinate origin.

Characterization of rheological properties with structural damping. The idea of structural damping arose from the observation, in a number of materials, of a nearly frequency-independent phase of the complex stiffness (or equivalently, a roughly frequency-independent fractionation of stress-bearing properties between those that are recoverable and those that are dissipative). This is an old phenomenological model that has been used successfully in the material science literature for more than a century; it has been applied to biological tissues in the past few decades (e.g., in lung tissue, see Refs. 8, 11; in neutrophils specifically modeled with non-Newtonian fluids that mimic some aspects of structural damping, see Refs. 5, 20). More recently, Fabry et al. (7) found strikingly consistent structural damping in several cell types, including neutrophils, with a variety of pharmacological interventions and over five decades in frequency. The unique feature of structural damping is that stress relaxation follows a power law in time (unlike simple viscoelastic materials, which show exponential behavior). That is, after a unit step displacement, the force falls proportional to \( t^{-k} \). Note that as \( k \to 0 \), the material is more elastic (this limit is trivial to verify), and that as \( k \to 1 \), the material is more viscous (this limit is more delicate and involves an unbounded apparent stiffness). In this formulation, as noted in the Appendix, there is no intrinsic time scale or time constant of the material. To obtain one-dimensional stiffness or elastic modulus with the usual units, one nondimensionalizes the force by the introduction of a suitable time scale \( \tau \) that is determined by the scale of the experiments rather than by the material. The stress relaxation then is written proportional to \( (t/\tau)^{-k} \). Our results have been scaled to 100 ms, a convenient choice representative of our experiments.

The numerical value that we found for the structural damping stiffness in the body and trailing region of the cell is \( \sim 7 \) Pa; this is scaled at 100 ms, for which the conversion to ordinary stiffness, given a structural damping exponent of 0.5, corresponds to roughly the same numerical value at 1 Hz. Note that this corresponds to an effective one-dimensional spring constant of \( \sim 0.06 \) pN/nm for a 300-nm radius granule and that therefore a 100-nm displacement implies a net force on the granule of \( \sim 6 \) pN. In our previous work (23), we found neutrophil stiffness on the order of 1 Pa at 1 Hz, but in that study, the granules were separated into fixed and free fractions, with the latter being the only ones in which measurements could be made. The exclusion of the very stiff granules from analysis may account for the almost 1 log increase in stiffness found in this study. It is also the case that the numerical value for stiffness deduced from structural damping measurements depends on the time scale used to nondimensionalize time (see Eq. A3). Extrapolations of our data to time scales of, for example, 10 s, reveal stiffness on the order of 0.7 Pa for the body and trailing regions and 0.04 Pa for the leading edge.

Importantly, the stiffness that we found in the leading edge of the cell was roughly 10 times lower, and this is approximately true independent of the time scales used (this ratio increases to almost 20:1 for a 10-s time scale). Using the viscoelastic model, we note that both the stiffness and the viscosity in the leading edge are significantly less than in the body or trailing region but that the stiffness fell more than the viscosity. This is reflected in the equivalent viscoelastic time constant, given by the ratio of the viscosity to the stiffness. In the body and trailing regions, this viscoelastic time constant was \( \sim 0.15 \) s\(^{-1}\), doubling to 0.3 s\(^{-1}\) in the leading edge. This is consistent with our interpretation that the leading edge is significantly more fluidlike than the body or trailing region.

Other rheological techniques. Rheological measurements have also been made with micropipette aspiration techniques. Schmid-Schönbein et al. (17) and Chien and Sung (3) reported low-frequency stiffness in the 10- to 15-Pa range, similar to our results. This suggests that the intracellular properties (which we measured directly) are an important contributor to the stiffness as measured by the aspiration technique. These authors used the standard solid model to characterize their data, but it is very interesting to note that, for example, Schmid-Schönbein et al. (see Fig. 7, A and B, in Ref. 17) reported data that systematically departed from the standard model fit. In particular, their data showed a sharper rise and a slower tail than could be expressed in exponential terms. This is precisely the feature that structural damping exhibits and is fully consistent with our observations.

By contrast, Zahalak et al. (25), using a cell-poking technique, found stiffness in excess of 100 Pa. Again, this technique probes the cell from the plasma membrane and thus displays an average behavior that is necessarily strongly influenced by inhomogeneities of stiffness within the cortical region of the cell. Frequency sweeps using atomic force microscopy have also been done. Recently, Mahaffy et al. (15) measured the microrheology of polymer gels and fibroblasts. While the latter cell type is very stiff compared with neutrophils, we note two very interesting features of their data. First, there is a roughly equal division of stress between those that are elastic and those that are dissipative. This corresponds, in the structural damping theory, to a power exponent of 0.5, which is very similar to what we have found. Second, both these statements are supported by the fact that their stiffness measures show an approximate square root dependence on frequency (stiffness increases of roughly a factor of 2 for a 4-fold frequency increment). We find this to be further evidence of structural damping.

Finally, Yamada et al. (22) used laser-tracking microscopy of intracellular Brownian motion to characterize the mechanics of kidney epithelial COS-7 cells. This work most closely approximates our own insofar as it is a direct intracellular probe (albeit with a completely different cell type). Interestingly, they found elastic moduli to increase slowly with frequency, with a power law exponent slightly less than 0.5. This is further reflected in the approximately constant phase angle that they found, which is somewhat less than the phase angle of \( \pi/4 \), which would correspond to our power law exponent of 0.5.

The value that we measured for the structural damping exponent, roughly 0.5 for the body and trailing region of the locomoting neutrophil, is similar to the exponent found by Tsai et al. (20) and Drury and Dembo (5) in their investigations of shear thinning of the neutrophilic cytoplasm. However, these are models using the characteristics of pure non-Newtonian fluids without a necessary elastic component, and it is not clear whether this represents a coincidence of exponent in the two approaches or whether it is a deeper manifestation of underlying structure.
It is important to recognize that an exponent of $-0.5$ is significantly larger than any of those found by Fabry et al. (7), in differing cell types, and after treatment with contractile and relaxing agonists or with cytoskeletal disruptors. In the specific case of neutrophils, they found an exponent near 0.2 (meaning the neutrophil appeared much more rigid than we found). This large difference in the value of the exponent is interesting and potentially significant. Importantly, the measurements of Fabry et al. (7) were made by observing the lateral displacement of magnetic beads bound to the cell surface through $\beta_2$-integrins, for which there is ample evidence that the beads are thereby intimately connected to the cytoskeleton through focal adhesion complexes. But the characteristics of the cytoskeleton that are probed are thus necessarily associated with the cortical region of the cell and with the mechanical properties of the focal adhesion, because that is the immediate locale of all stress-bearing elements. There is no a priori reason to suppose that the rheological properties are uniformly distributed throughout the cell’s body, including the cortex and ligated integrin receptors, quite apart from the manifest differences between the leading edge behavior and the rest of the cell. We therefore interpret these different observations as evidence of inhomogeneous properties of the intracellular distribution of the cytoskeleton, with its behavior being much stiffer and more solidlike in the cortical region, especially near focal adhesions, and more dissipative in the interior. This is support for, and perhaps a reconciliation of, different models of cellular rheology, including those that envision an elastic cortical region surrounding a fluid or viscoelastic core (4, 6), which, when probed in the cortical vicinity, would appear more elastic than when probed in the interior.

**Implications of leading edge fluidity.** Our results strongly suggest that the leading edge of locomoting neutrophils contains a more fluidlike core than either the body or the trailing region. This is important for the identification of the motive force involved in pseudopodial protrusion; its fluidity in turn is consistent with the idea that cytoplasmic flow into the pseudopod is driven by a pressure gradient, although it is not conclusive evidence. While we appreciate that stresses can be borne by either elastic or viscous elements, we do not see how to reconcile polymerizing microfilaments in the advancing pseudopod with significantly increased fluidity. Of course, both F- and G-actin flow into the developing pseudopod, and important polymerization events follow. The presence of increased F-actin near or within the pseudopod, however, does not necessarily imply a role in the initiation or provocative event itself, as distinguished from subsequent traction and stress-bearing roles that have downstream importance. To the extent that pressure gradients are the origin of pseudopod formation and subsequent cytoplasmic flow (24), some kind of contractile machinery is implied that may be of cortical origin or perhaps even within the entire body of the cell.

**Cytoskeletal disruption.** Our results with regard to the cytoskeletal disruptors may be interpreted as follows. First, it is clear that disruption of the actin tension-bearing elements with cytochalasin D will lower the apparent stiffness, as will disruption of the microtubules with nocodazole. It is striking that there appears to be an approximately proportional fall in the viscosity (in the viscoelastic model) or, equivalently, that the structural damping exponent $k$ is approximately invariant. These observations are consistent with a coupling at the molecular level of those elements (e.g., actin binding proteins) that may be important contributors to both stiffness and energy dissipation (through bond breakage). In this sense, if the same molecular elements are the origin of both the energy storage and energy-dissipative mechanisms, then it follows that in the absence of other effects, a decrease in their concentration through cytoskeletal disruption will reduce both proportionately. To the extent that the structural damping power law exponent is a function only of the ratio of energy dissipation to energy storage, it is then a proper intensive variable that is independent of concentration. We note that these observations stand in sharp contrast to the work of Fabry et al. (7), who consistently found an increase in the structural damping exponent after treatment with cytochalasin D (i.e., the cell is more fluidlike). It remains unclear why the cell should look more fluidlike after microfilament disruption when viewed from the cell surface and yet retain its basically equal division of elastic and dissipative intracellular properties. Finally, we note that finding little change in any rheological property of the leading region with nocodazole treatment implies little role for microtubules in pseudopodial protrusion.

In summary, we have found that 1) structural damping is more faithful to observed data than is a viscoelastic parallel spring-dashpot model; 2) independent of the model, the body and trailing regions of the neutrophil are rheologically indistinguishable; and 3) also independent of the model, the leading or pseudopodial region is significantly less stiff, less viscous, and more fluidlike than is the body or trailing region. While not conclusive, this is consistent with intracellular pressure being the origin of the motive force that drives pseudopodial protrusion in locomoting neutrophils.

**APPENDIX**

Here we describe mathematically the displacement of a granule, given a step change in the optical trap position for the viscoelastic and structural damping models of the intracellular milieu.

**Viscoelastic model.** In this case, the cytoplasm is modeled as a simple viscoelastic medium of stiffness $G$ in parallel with a viscosity $\mu$. The force of the optical trap on the granule at position $x$ is $F = K(a - x)$, where $K$ is the trap stiffness and $a$ is the step displacement in the trap position. Force balance then yields the equation of motion

$$K(a - x) = Gx + \mu dx/dt. \quad (A1)$$

The solution to this problem is elementary:

$$x(t) = \frac{aK}{K + G} \left(1 - e^{-\beta t}\right), \quad (A2)$$

where $\beta = (K + G)/\mu$. ($1/\beta$ is the time constant.)

**Structural damping.** This model rests on a power law behavior of stress adaptation after a step response in displacement:

$$F_{step} = F_s(t/\tau)^{-k} \quad (A3)$$

for a unit step in $x$. The exponent in the power law is restricted to the range $0 < k < 1$. Here $F_s$ is stiffness (with the usual units of force/length) for a particular choice of time scale $\tau$, typically that of the experimental probe. Note the important point that $\tau$ does not represent a time constant of any kind that is characteristic of the medium; it is arbitrary and is conveniently chosen to fall somewhere in the middle of the time scales spanned by the experiments.
The force of the trap on the granule remains \( F = K(a - x) \), as in the viscoelastic model. For simplicity, choose \( K, a, \) and \( \tau \) as units of stiffness, length, and time, respectively. Then the trap force is \( F = 1 - x \). The restoring force of the medium on the granule is given by a convolution of the impulse response with the displacement. Thus

\[
F = F_{\text{impulse}} \otimes x = (dF_{\text{step}}/dt) \otimes x = 1 - x. \tag{A4}
\]

This may be solved in the Laplace domain, denoted by tildes. Thus

\[
sF_{\text{step}} \tilde{x} = s^{-1} - \tilde{x}, \text{ or}
\]

\[
\tilde{x} = s^{-1}(1 - sF_{\text{step}})^{-1}. \tag{A5}
\]

The Laplace transform of the step response is given by \( F_{\text{step}} = F_s\Gamma(1 - k)s^{k-1} \), where \( \Gamma(\cdot) \) is the Legendre \( \Gamma \) function (recall time is nondimensionalized by \( \tau \)). The solution for the displacement in the Laplace domain is therefore

\[
\tilde{x} = \frac{1}{s[1 + F_s\Gamma(1 - k)s^{k}]}.
\tag{A6}
\]

The time-dependent solution \( x(t) \) is given by the inverse Laplace transform of this equation, but unfortunately it does not exist in terms of the elementary transcendental functions. Padé approximations are appropriate in this case (1). In this method, one replaces the desired function with an approximation such that both the short and long time limits (equivalently, the large and small \( x \) behavior) are asymptotically matched.

The large \( x \) (short \( t \)) solution is given by a Taylor series expansion about \( x = \infty \):

\[
\tilde{x} = s^{-1} \sum_{n=1} \frac{(-1)^{n-1}(F_s\Gamma(1 - k)s^{k})^n}{n!}.
\tag{A7}
\]

which has the explicit inverse given by

\[
x(t) = \sum_{n=1} \frac{(-1)^{n-1}}{n!} (F_s\Gamma(1 - k)s^{k})^n. \tag{A8}
\]

This is a convergent series and is exact. Unfortunately, it converges very slowly for large times; it is suitable as written for the short time solution.

For small \( x \) (large \( t \)), note that the asymptotic limit is \( x = 1 \); thus we expand \( y = 1 - x, \tilde{y} = s^{-1} - \tilde{x} \) in a Taylor series about \( x = 0 \):

\[
\tilde{y} = s^{-1} - s^{-1}[(1 + F_s\Gamma(1 - k)s^{k}) - 1] = F_s\Gamma(1 - k)s^{k-1} \sum_{n=0} (-1)^n(F_s\Gamma(1 - k)s^{k})^n. \tag{A9}
\]

The inverse transform of this equation gives the long time behavior, but the series is asymptotic:

\[
x(t) = 1 - y(t) = 1 - \sum_{n=1} \frac{(-1)^{n-1}(F_s\Gamma(1 - k)s^{k})^n}{n!}. \tag{A10}
\]

The Padé approximation is constructed as follows. Denote \( \hat{t} = t \). Then the behavior near \( t = u = 0 \) is approximately quadratic: \( x(t) = bu + du^2 \), where \( b = 1/[F_s\Gamma(1 - k)\Gamma(1 + k)] \) and \( d = -1/[F_s\Gamma(1 - k)\Gamma(1 - 2k)] \). For long times, the solution approaches \( x(t) = 1 - F_s/u \). We approximate these two behaviors near \( u = 0 \) and \( u = \infty \) with the Padé rational expression:

\[
x(t) = \frac{Bu + Du^2}{1 + Cu + Eu^2}, \quad u = \hat{t}^2, \tag{A11}
\]

where \( B = b, C = (b + Du)/(1 - F_s), D = E = d + BC \). This choice of coefficients matches the two-limiting behavior near both \( t = u = 0 \) and \( t = u = \infty \).

