Passive Mechanical Behavior of Human Neutrophils: Effect of Cytochalasin B

Mientao A. Tsai,* Robert S. Frank,‡ and Richard E. Waugh*
*Department of Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642, and
‡Applied Research Department, Coulter Corporation, Hialeah, Florida 33010 USA

ABSTRACT Actin is a ubiquitous protein in eukaryotic cells. It plays a major role in cell motility and in the maintenance and control of cell shape. In this article, we intend to address the contribution of actin to the passive mechanical properties of human neutrophils. As a framework for assessing this contribution, the neutrophil is modeled as a simple viscous fluid drop with a constant cortical ("surface") tension. The reagent cytochalasin B (CTB) was used to disrupt the F-actin structure, and the neutrophil cortical tension and cytoplasmic viscosity were evaluated by single-cell micropipette aspiration. The cortical tension was calculated by simple force balance, and the viscosity was calculated according to a numerical analysis of the cell entry into the micropipette. CTB reduced the cell cortical tension in a dose-dependent fashion: by 19% at a concentration of 3 μM and by 49% at 30 μM. CTB also reduced the cytoplasmic viscosity by ~25% at a concentration of 3 μM and by ~65% at a concentration of 30 μM when compared at the same aspiration pressures. All three groups of neutrophils, normal cells, and cells treated with either 3 or 30 μM CTB, exhibited non-Newtonian behavior, in that the apparent viscosity decreased with increasing shear rate. The dependence of the cytoplasmic viscosity on deformation rate can be described empirically by $\mu = \mu_c(\dot{\gamma}_m/\dot{\gamma}_c)^{-b}$, where μ is cytoplasmic viscosity, $\dot{\gamma}_m$ is mean shear rate, μ_c is the characteristic viscosity at the characteristic shear rate $\dot{\gamma}_c$, and b is a material coefficient. The shear rate dependence of the cytoplasmic viscosity was reduced by CTB treatment. This is reflected by the changes in the material coefficients. When $\dot{\gamma}_c$ was set to 1 s^{-1}, $\mu_c = 130 \pm 23$ Pa·s and $b = 0.52 \pm 0.09$ for normal neutrophils and $\mu_c = 54 \pm 15$ Pa·S and $b = 0.26 \pm 0.05$ for cells treated with 30 μM CTB. These results provide the first quantitative assessment of the role that Pa·s-actin structure plays in the passive mechanical properties of human neutrophils.

INTRODUCTION

Neutrophils are an integral part of the cardiovascular system, and neutrophil deformability can have important effects on blood flow, especially in the microvasculature. There is growing evidence that neutrophil deformability plays an important role in cell retention in capillaries and concomitant tissue damage in the lung and other tissues (Downey and Worthen, 1988; Worthen et al., 1989; Downey et al., 1990; Erzurum et al., 1992). Furthermore, it is becoming increasingly evident that neutrophils not only affect the microcirculation because they are less deformable than erythrocytes, but also participate in the signaling processes that regulate blood flow in the microvasculature (Mantovani and Dejana, 1989; Mantovani et al., 1992; Johnson et al., 1992). The degree of neutrophil participation in these processes is determined by the interaction between neutrophils and endothelial cells, an interaction which depends in part on neutrophil deformability. However, a great deal is still unknown about how the mechanical properties of human neutrophils are regulated.

Actin is a ubiquitous protein in all eukaryotic cells. It is the most abundant structural protein (~8 pg/cell or ~4.6% of the total proteins) in human neutrophils (Wallace et al., 1987) and is thought to play the major role in cell motility (Stossel, 1988; Sheterline and Rickard, 1989; Cooper, 1991). Actin has a remarkable ability to polymerize reversibly from globular monomers (G-actin) into filaments (F-actin). F-actin in association with a wide variety of actin-binding proteins can be bundled and cross-linked into networks (Hartwig et al., 1985). Ultrastructural evidence obtained from detergent-extracted neutrophils indicates that most of the polymerized actin within the cell is localized in a thin cortical submembranous layer. The detergent-insoluble residue (cytoskeleton) retains the overall shape, size, and structural features of the intact cell. In these studies, actin appears to be largely confined to a thin cortical filamentous meshwork, and no actin-like structures are evident in the region between this cortex and the nucleus (Sheterline and Hopkins, 1981). This view of cell structure is further supported by the localization of NBD-phallacidin, a fluorescent marker of polymerized actin, to the cell periphery (Sheterline et al., 1984, 1986; Stossel, 1988; Sheterline and Rickard, 1989). This actin cortex is thought to be the essential determinant of neutrophil chemotaxis and phagocytosis (Stossel, 1984; Hartwig et al., 1985; Stossel, 1988, 1990).

Immunofluorescence microscopy suggests that microtubules and intermediate filaments are few in number compared to actin filaments and are restricted to the cell body, although they do penetrate the actin-rich peripheral cytoplasm to some extent (Stossel, 1988). Chemicals that affect tubulin assembly, i.e., colchicine, podophyllotoxin, and

Received for publication 6 August 1993 and in final form 18 February 1994.
Address reprint requests to Dr. Mientao A. Tsai, Department of Biophysics, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14624. Fax: 716-275-6007.

0006-3495/94/06/2166/07 $2.00

vinca alkaloids, have been used to assess the importance of microtubules in neutrophil function. These agents inhibit tubulin polymerization and cause net depolymerization of microtubules in leukocytes. Chien and Sung (1984) examined the effects of colchicine on the passive mechanical response of neutrophils. Unfortunately, these studies were confined to extremely small deformations of the cell surface, and it is not clear how their findings relate to neutrophil function, which typically includes large deformations of the cytosol. Other published reports indicate that microtubule reagents do not have profound effects on neutrophil motility (chemotaxis and phagocytosis) (Keller et al., 1984; Stossel, 1988), leading to the expectation that actin filaments play the predominant role in controlling the mechanical behavior of human neutrophils.

In this article, we examine how the passive mechanical properties of human neutrophils are affected by changing cellular structure, and in particular, what role F-actin (microfilaments) plays in determining cellular mechanical properties. Changes in mechanical properties have been quantitatively assessed after altering F-actin structure with cytochalasin B. These results shed new light on the relationship between cellular structure and the mechanical properties of human neutrophils.

MATERIAL AND METHODS

Neutrophil preparation

The cell separation technique has been described in detail elsewhere (Tsai et al., 1993). Briefly, about 10 ml of whole blood was drawn from a healthy adult donor in a sodium heparin-containing tube (Vacutainer, Becton Dickson, Rutherford, NJ). Neutrophils were separated with Ficoll-Hypaque media (Mono-Poly Resolving Medium, Flow Laboratories, Inc., McLean, VA). The separated pellet of polymorphonuclear leukocytes (>95% neutrophils) was collected and suspended in HEPES buffer (NaCl, 135.8 mM; KCl, 4.8 mM; HEPES, 11.3 mM; HEPES sodium salt, 9.3 mM; glucose, 1 g/l; pH 7.40 ± 0.05; 290 ± 5 mOsm/kg). Cells were washed twice and suspended in fresh buffer at a concentration of $\sim 10^5$ cells/ml. Experiments were performed within 7 to 8 h after the blood was drawn.

CTB treatment

Cytochalasin B was purchased from Sigma Chemical Co. (St. Louis, MO). 5 mg of CTB was dissolved in 1.066 ml of DMSO into 3.00 M stock solution and was kept frozen at −20°C. When used for an experiment, the stock solution was thawed and diluted with fresh buffer to twice the desired concentrations. An equal v/v dilution with the cell suspension yielded the desired concentrations. Except during experiments investigating effects of long-term exposure to CTB, measurements were begun 5 min after cells were exposed to the reagent and were finished within 1 h.

Cortical tension measurement

The neutrophil cortical tension was determined by simple force balance. The liquid drop model includes the assumption that neutrophils possess a constant "surface" tension. Thus, there exists a threshold pressure, Δp_{cr}, for cells to continuously enter a micropipette. According to the law of Laplace, cortical tension is determined by (cf. Fig. 1)

$$T = \Delta p_{cr} \cdot R_p R_c / 2(R_c - R_p), \quad (1)$$

where T is the cell cortical tension, R_p is the pipette radius, and R_c is the cell radius.

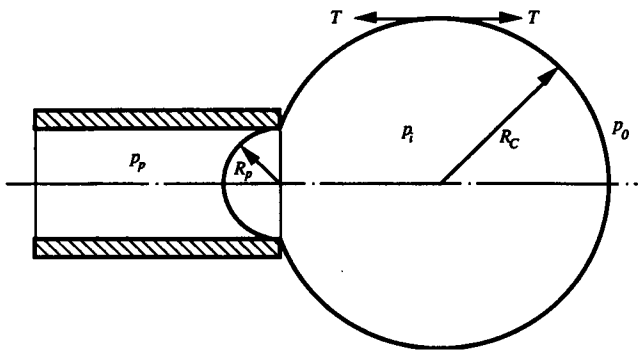

FIGURE 1 Illustration of the threshold pressure resulted from the cell cortical tension, where p_o is the ambient pressure, p_i is the pressure inside the cell, p_p is the pressure inside the pipette, T is the cell cortical tension, R_p is the pipette radius and R_c is the cell radius.

The threshold pressure, Δp_{cr}, is the minimum aspiration pressure for a cell to form a hemisphere inside a micropipette. To determine Δp_{cr}, an aspiration pressure was set to a small value and maintained for 2 min for the cell to respond fully. The pressure was then increased by 2.45 Pa (0.025 cmH_2O) and maintained again for ~2 min. This procedure was repeated until the cell was aspirated into a hemispherical bulge and the projection remained stationary. The pressure at this point was measured as the threshold pressure Δp_{cr}. The pressure was increased by additional one or two steps of 2.45 Pa to make sure that the cell did not adhere to the pipette wall and that it would keep flowing into the pipette if the threshold pressure was exceeded.

Measurements of the cytoplasmic viscosity

Neutrophil cytoplasmic viscosity was determined by single-cell micromechanical measurements. Individual cells were sucked into a micropipette at fixed aspiration pressures. The aspiration process was observed microscopically and recorded on video tape. The cell projection inside the micropipette was measured as a function of time at different constant aspiration pressures, Δp_h, by using a computer-based video imaging analysis system. The total transit time of the cell entry into the micropipette, t_e, was noted. According to a numerical analysis based on the Newtonian liquid drop model (Tsai et al., 1993), the cytoplasmic viscosity, μ, was determined by

$$\mu = \frac{\Delta p_h \cdot t_e}{4P_1(\bar{R}_0)\sqrt{\bar{R}_0^2 - 1}}, \quad (2)$$

and the mean shear rate during the cell entry, $\dot{\gamma}_m$, was approximated by

$$\dot{\gamma}_m = \frac{\Delta p_h}{4\mu(\bar{R}_0^2 - 1)^{7/20}} P_2(\bar{R}_0), \quad (3)$$

where $\bar{R}_0$ is the ratio of the initial cell radius, R_0, to the pipette radius, R_p,

$$\bar{R}_0 = R_0 / R_p, \quad (4)$$

and $P_1(\bar{R}_0)$ and $P_2(\bar{R}_0)$ are third order polynomials in $\bar{R}_0$. The coefficients of the polynomials are functions of the ratio of the threshold pressure to the hydrostatic aspiration pressure and have been described in detail in a previous publication (Tsai et al., 1993).

RESULTS AND DISCUSSION

Neutrophil responses to small aspiration pressure: cortical tension

The response of a normal neutrophil to small stepwise increases in aspiration pressure is shown in Fig. 2, *top*. The

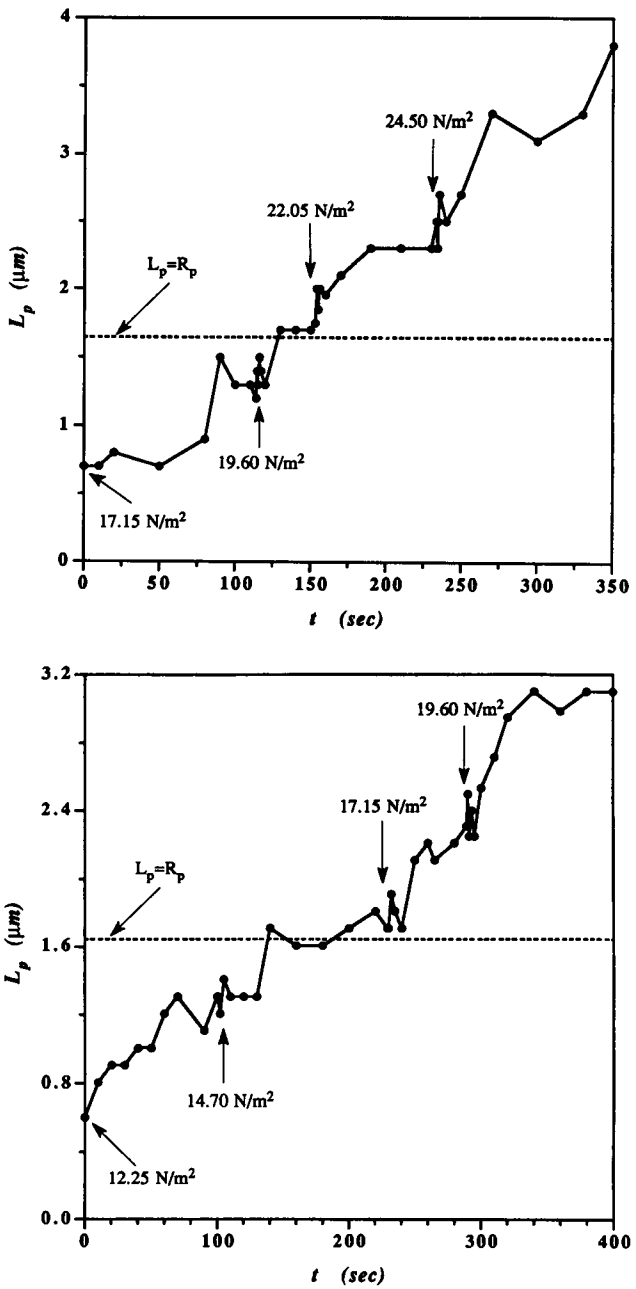

FIGURE 2 Response of neutrophils to aspiration pressures with step augmentation and pipette size of 3.31 μm in diameter. (*Top*) Response of a normal neutrophil. The aspiration pressure ranged from 17.15 to 24.50 N/m² with the increment of 2.45 N/m². The threshold pressure for this cell was measured to be 19.60 N/m² for the cell to form a hemisphere. (*Bottom*) Response of a neutrophil treated with 30 μM CTB. The aspiration pressure ranged from 12.25 to 19.60 N/m² with the increment of 2.45 N/m². The threshold pressure was measured to be 14.70 N/m². It is evident that once the threshold pressure was exceeded, the cell continuously deformed into the micropipette.

aspiration pressure was changed in steps of 2.45 Pa from 14.70 to 24.50 Pa. The threshold pressure was taken to be 19.60 Pa. The similar response of a neutrophil treated with 30 μM CTB is shown in Fig. 2, *bottom*, but with the aspiration pressure varying from 12.25 to 19.60 Pa. For this CTB-treated cell, the threshold pressure was taken to be 14.70 Pa. It is evident that the cell projection reaches equilibrium if the aspiration pressure is less than threshold and the cell is given enough time to respond. It is also apparent that cells continuously flow into the micropipette once the threshold pressure is exceeded. This observation is consistent with the results reported by Evans and co-workers and recent work by Needham and Hochmuth (Evans and Kukan, 1984; Evans and Yeung, 1989; Yeung and Evans, 1989; Needham and Hochmuth, 1992) and supports the view that the neutrophil behaves as a viscous liquid drop, i.e., the neutrophil cortex maintains a persistent "surface" tension, and the cytoplasm continuously flow into the micropipette once the aspiration pressure exceeds the threshold pressure created by the cortical tension.

Time course of the whole cell aspiration process

In Fig. 3, *top* and *bottom*, the cell projection length inside the micropipette, L_p, is shown as a function of time t during the aspiration process for a normal neutrophil and a neutrophil treated with 30 μM CTB, respectively. Generally, normal cells and cells treated with different concentrations of CTB exhibited similar patterns of behavior as they flowed into the micropipette under aspiration pressures ranging from 98 to 882 Pa. The time course of the whole cell aspiration was sigmoidal and could be divided into three phases. At the initial phase, cytoplasm flowed rapidly into the micropipette. At the middle phase, cell entry rate decreased and approached a quasi-steady regime as the cytoplasm crept into the pipette with a nearly constant velocity. At the third phase, the cytoplasmic flow dramatically accelerated as the last part of the cell entered the pipette. After the neutrophil was fully aspirated, it flowed rapidly through the micropipette. This indicated that the fluid resistance of the suspension medium in the thin layer between the cell and pipette was negligible in comparison with the neutrophil cytoplasmic viscosity.

Effects of cytochalasin B on cortical tension

The reagent CTB reduced cortical tension of neutrophils in a dose-dependent fashion. A single pipette, 3.3 μm in diameter, was used to measure the cell cortical tension in three separate experiments. Each experiment included three groups of cells, i.e., normal neutrophils, neutrophils treated with 3 μM CTB, and neutrophils treated with 30 μM CTB. Each group consisted of five or six cells. For each neutrophil, its threshold pressure was measured at the point that the cell formed a hemispherical bulge, and cortical tension was calculated via Eq. 1. The results of the cortical tension measurements for three different groups of neutrophils from the three experiments are shown in Fig. 4. The vertical bars represent SE. The results of these three experiments are also summarized in Table 1, where the ± values represent SE. The mean value of the cortical tension of normal neutrophils from the three experiments is 2.68×10^{-5} N/m ($n = 21$) and is in good agreement with the findings of other laboratories

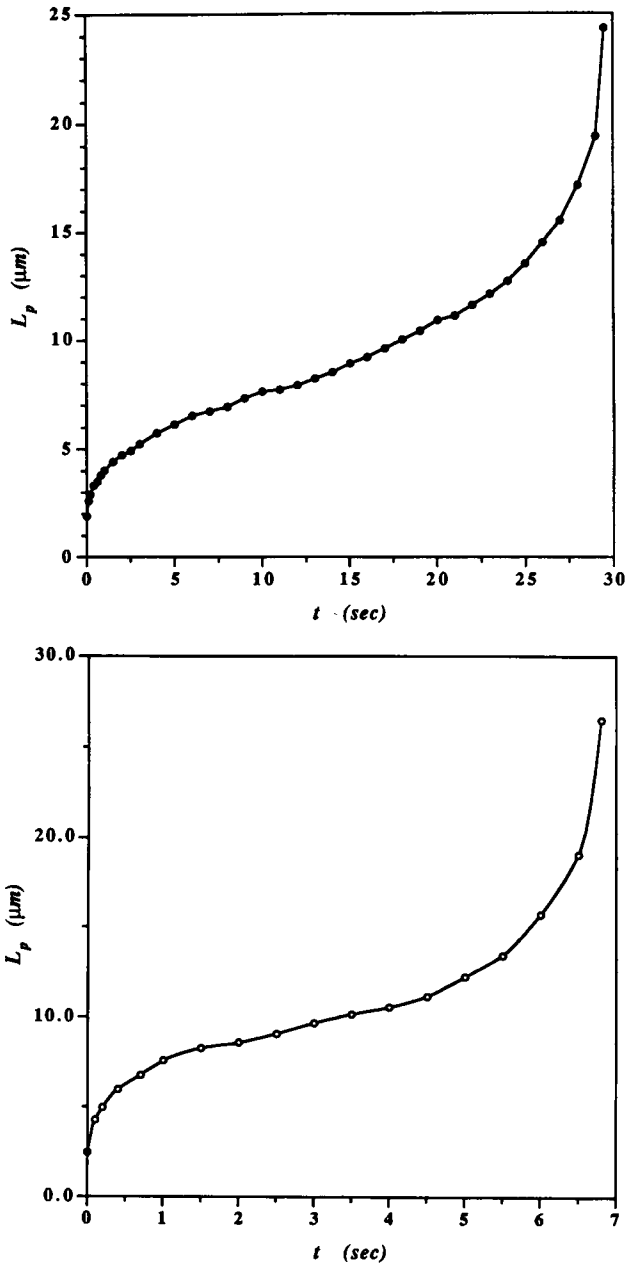

FIGURE 3 Aspiration process of human neutrophils into a micropipette represented by the cell projection length inside the pipette, L_p, as a function of time. The diameter of the pipette was 4.03 μm, and the aspiration pressure was 294 Pa. (*Top*) Time course of a normal neutrophil. (*Bottom*) Time course of a neutrophil treated with 30 μM CTB.

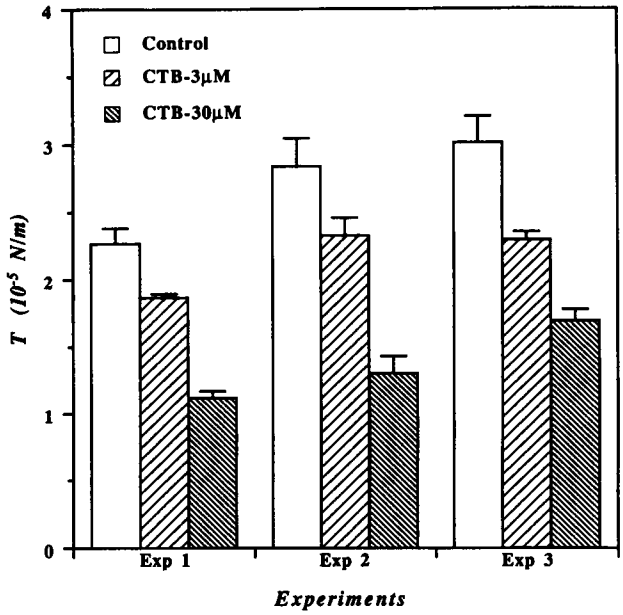

FIGURE 4 Comparison of cortical tensions of three neutrophil groups: normal neutrophils, neutrophils treated with 3 μM CTB, and neutrophils treated with 30 μM CTB. The vertical bars represent SE (n = 5).

TABLE 1 Statistical summary of cortical tension of neutrophils (all data)

Cell group	T ($\times 10^{-5}$ N/m)	ΔT (%)	n
Control	2.68 ± 0.12	—	21
3 μM CTB	2.17 ± 0.07	−19 ± 4	16
30 μM CTB	1.35 ± 0.07	−49 ± 4	16

(Evans and Yeung, 1989; Needham and Hochmuth, 1992). Neutrophils treated with 3 μM CTB had a mean cortical tension of 2.17×10^{-5} N/m (n = 16), and those treated with 30 μM CTB had a tension of 1.35×10^{-5} N/m (n = 16) (Table 1). A pooled t-test showed that the difference between any two populations within an experimental group was significant with $p < 0.01$. Thus, at a concentration of 3 μM, CTB caused a reduction of the cortical tension by ~20%, and at a concentration of 30 μM, the reduction was ~50%. Control experiments were performed to exclude the possibility of nonspecific effects of DMSO on cortical tension. (The 3 and 30 μM CTB treatments contained 0.07 and 0.7% carrier reagent, DMSO, respectively.) The results showed that DMSO, even at the highest possible concentration (0.7%), does not affect neutrophil mechanical properties. Cortical tension of neutrophils suspended in 0.7% DMSO was measured to be $2.66 \pm 0.09 \times 10^{-5}$ N/m (n = 5), whereas that of corresponding control cells was measured to be $2.56 \pm 0.07 \times 10^{-5}$ N/m (n = 5). This conclusion is further supported by the early work by Frank (1990) that DMSO, up to 0.4%, does not alter neutrophil deformability.

Effects of cytochalasin B on cytoplasmic viscosity

Cytochalasin B also produced a dose-dependent reduction in cytoplasmic viscosity. Shown in Fig. 5 are results of a complete series of experiments including measurements on a total of 120 cells at aspiration pressures ranging from 294 to 882 Pa. Results from three such series of experiments are summarized in Table 2. Comparing measurements made at the same aspiration pressure, CTB reduced the cytoplasmic viscosity by ~25% at a concentration of 3 μM and by ~65% at 30 μM. However, it is also evident from Fig. 5 and Table 2 that the value of the cytoplasmic viscosity is a function of

TABLE 2 Neutrophil cytoplasmic viscosity of three cell groups at different aspiration pressures

	Normal		CTB 3 μM		CTB 30 μM	
ΔP (Pa)	μ (Pa·s)	Δ (%)	μ (Pa·s)	Δ (%)	μ (Pa·s)	Δ (%)
294	200 ± 12	—	148 ± 11	−26	52 ± 2	−74
490	137 ± 13	—	94 ± 5	−31	44 ± 2	−67
686	99 ± 4	—	81 ± 4	−18	42 ± 2	−57
882	92 ± 6	—	72 ± 3	−22	41 ± 1	−55
Average	131 ± 12		98 ± 9	−25	46 ± 5	−65

The results were averaged from three experiments with a 4.03-μm micropipette. Each group included 120–130 cells with a total of 380 cells.

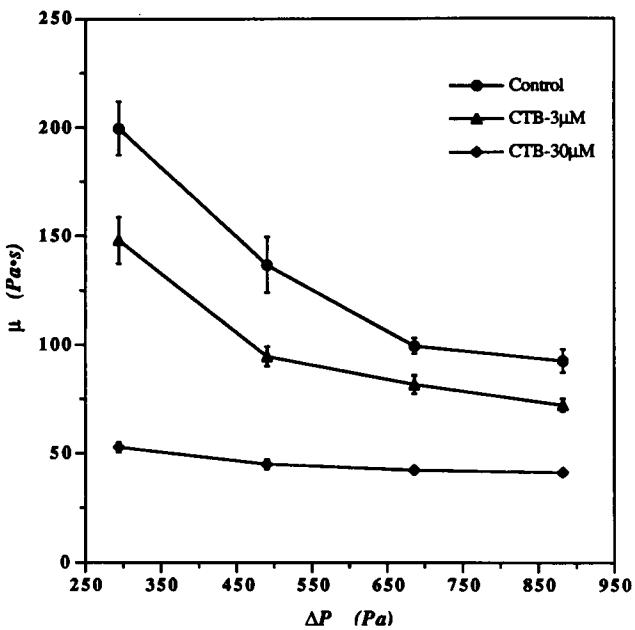

FIGURE 5 Apparent cytoplasmic viscosity under different pressures ranging from 294 to 882 Pa for different cell groups, calculated from whole cell aspiration experiment with pipette diameter of 4.03 μm. The vertical bars represent SE (n = 30).

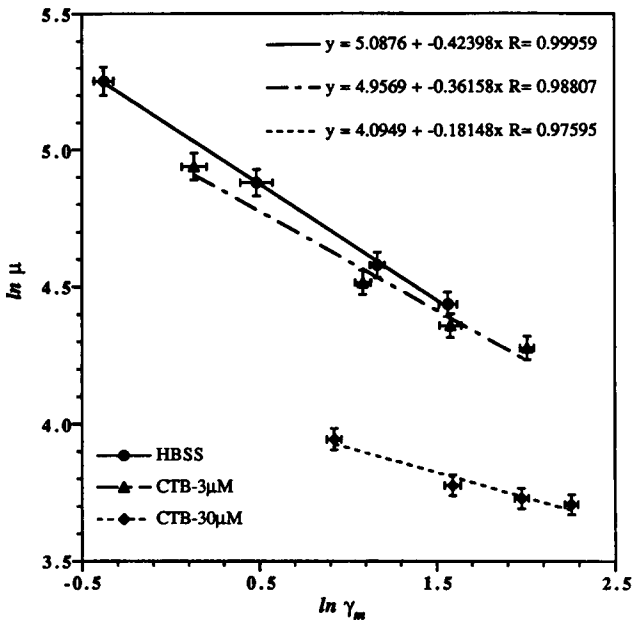

FIGURE 6 Apparent cytoplasmic viscosity as functions of mean shear rate for different cell groups, calculated from whole cell aspiration experiment with pipette diameter of 4.03 μm. The vertical bars represent SE (n = 30). HBSS indicates HEPES-buffered saline solution (control).

aspiration pressure within each group of cells. These results are consistent with our earlier report that the cytoplasmic viscosity depends on shear rate (Tsai et al., 1993). Therefore, simple comparison of different cell types at the same aspiration pressure may exaggerate differences in viscosity, because cells that deform more easily will exhibit even lower viscosity at a given pressure due to their more rapid deformation (high shear rate).

To evaluate the effects of CTB on the shear rate dependence of neutrophil viscosity, the results shown in Fig. 5 are replotted in Fig. 6 after natural logarithmic transformation. It is clear from Fig. 6 that the behavior of neutrophil cytoplasm both before and after CTB treatment can be characterized as that of a power-law fluid

$$\mu = \mu_c(\dot{\gamma}_m/\dot{\gamma}_c)^{-b}, \quad (5)$$

where μ is cytoplasmic viscosity, $\dot{\gamma}_m$ is mean shear rate, μ_c is the characteristic viscosity at the characteristic shear rate $\dot{\gamma}_c$, and b is a material coefficient. It is also clear that the shear rate dependence of the cytoplasmic viscosity was reduced by CTB treatment. This was most evident for cells treated with 30 μM CTB, and is reflected by the changes in the material coefficients as shown in Table 3. For $\dot{\gamma}_c$ set to 1 s^{-1}, μ_c = 130 ± 23 Pa·s and b = 0.52 ± 0.09 for normal neutrophils, and μ_c = 54 ± 15 Pa·s and b = 0.26 ± 0.05 for cells treated with 30 μM CTB.

Implication about the role of F-actin in determining neutrophil mechanical properties

Cytochalasin B is a fungal metabolite and a membrane-permeable reagent. In vitro experiments indicate that CTB binds to the barbed ends of actin filaments and thus inhibits

TABLE 3 Material coefficients of three cell groups when the characteristic shear rate $\dot{\gamma}_c$ is set to 1 s^{-1}

Cell group	μ_c (Pa·s)	b
Control	130 ± 23	0.52 ± 0.09
3 μM CTB	128 ± 21	0.44 ± 0.09
30 μM CTB	54 ± 15	0.26 ± 0.05

both the association and dissociation of actin monomers at that end of the filaments (MacLean-Fletcher and Pollard, 1980; Cooper, 1987). CTB treatment acts to shorten the length of actin filaments and weaken F-actin networks (Flanagan and Lin, 1980; Cooper, 1987). Assays on living cells indicate that CTB causes cells to stop ruffling and translocating and to become round and less stiff (Yahara et al., 1982; Cooper et al., 1987). Based on electrophoretic measurements, Wallace and colleagues reported that CTB reduces the content of neutrophil cytoskeletal F-actin in a dose-dependent fashion but leaves the total content of neutrophil F-actin unchanged (Wallace et al., 1987). Although CTB was the most widely used reagent in the early studies of cell motility, other cytochalasins have been identified that have greater activity and better specificity for actin. Unfortunately, thorough characterization of the effects of these other cytochalasins on neutrophils has not been made. Only recently, Rao et al. (1993) reported increased levels of total F-actin in cells treated with cytochalasins D and E, but no assessment of the effects of these cytochalasins on cytoskeletally associated actin have been reported. Consistent with the findings of Wallace et al. (1987), Rao et al. (1993) found little effect of CTB on total cell actin. As reported by Wallace et al. (1987), the reduction in cytoskeletal F-actin by CTB was ~10% at a concentration of 3 μM and ~30% at 30 μM 5 min after exposure to the reagent.

These measurements coupled with our own suggested that the alteration of cortical tension by CTB is roughly proportional to the reduction in cytoskeletal F-actin. This is consistent with the notion that neutrophil cortical tension is generated by cytoskeletal F-actin network, or more precisely, by the cross-linking of actin filaments by actin-binding proteins. Changes in actin filament structure most probably account for the dramatic reduction in cytoplasmic viscosity and the weakening of its shear rate dependence. However, it is still unknown whether the dissociation of F-actin from the cytoskeleton, as indicated by the amount of cytoskeletal F-actin, or the shortening of filament length is the primary mechanism for the changes in neutrophil cytoplasmic viscosity.

One well known side effect of CTB is its inhibition of glucose transport (Kletzien and Perdue, 1973). This is of particular interest in light of the findings by Rao et al. (1993) that metabolic inhibitors have a synergistic effect on the action of cytochlalasin D on actin in neutrophils. It raises the possibility that the effects we have observed are due in part to metabolic depletion of the cells as a result of glucose deprivation. However, if this were the case, a progressive change in cellular properties with time would be expected as the cells became progressively more metabolically depleted. To test this possibility, cells were treated with CTB (30 μM), and their mechanical properties were measured over a period of 3–4 h. The cytoplasmic viscosity of neutrophils tested between 5 and 30 min of exposure was found to be 27.3 ± 3.6 Pa·s ($\dot{\gamma}_m = 8.3 \pm 1.2\ s^{-1}$), and the viscosity of those tested with the same pipette and at the same aspiration pressure between 180 and 220 min of exposure was 27.7 ± 3.8 Pa·s ($\dot{\gamma}_m = 7.4 \pm 0.8\ s^{-1}$). This lack of time dependence for the effect of CTB argues against the possibility that the changes in properties we have observed are the result of metabolic effects, but that they simply reflect the CTB-induced alteration of the actin structure within the cell.

CONCLUSION

Our experimental results support the view that the passive mechanical properties of human neutrophils are governed by their cellular structure, particularly by the actin-based cytoskeleton. Disruption of actin structure reduces cell cortical tension and cytoplasmic viscosity, and it weakens the dependence of cytoplasmic viscosity on shear rate. These results provide the first quantitative assessment of the contribution of F-actin structures to the passive mechanical properties of human neutrophils undergoing large deformations.

The authors would like to thank Dr. Charles H. Packman for the fruitful discussion in hematology.

This research project was supported by National Institutes of Health grant HL-18208. M. A. Tsai is an American Heart Association-New York Affiliate Research Fellow.

REFERENCES

Chien, S., and P.-L. P. Sung. 1984. Effect of colchicine on viscoelastic properties of neutrophils. *Biophys. J.* 46:383–386.

Cooper, J. A. 1987. Effects of cytochalasins and phalloidin on actin. *J. Cell Biol.* 105:1473–1478.

Cooper, J. A., J. Bryan, B. Schwab III, C. Frieden, D. J. Loftus, and E. L. Elson. 1987. Microinjection of gelsolin into living cells. *J. Cell Biol.* 104:491–501.

Cooper, J. A. 1991. The role of actin polymerization in cell motility. *Annu. Rev. Physiol.* 53:585–605.

Downey, G. P., and G. S. Worthen. 1988. Neutrophil retention in model capillaries: deformability, geometry, and hydrodynamic forces. *J. Appl. Physiol.* 65:1861–1871.

Downey, G. P., D. E. Doherty, B. Schwab, E. L. Elson, P. M. Henson, and G. S. Worthen. 1990. Retention of leukocytes in capillaries: role of cell size and deformability. *J. Appl. Physiol.* 69:1767–1778.

Erzurum, S. C., G. P. Downey, D. E. Doherty, B. Schwab, E. L. Elson, and G. S. Worthen. 1992. Mechanisms of lipopolysaccharide-induced neutrophil retention. Relative contributions of adhesive and cellular mechanical properties. *J. Immunol.* 149:154–162.

Evans, E., and B. Kukan. 1984. Passive material behavior of granulocytes based on large deformation and recovery after deformation tests. *Blood.* 64:1028–1035.

Evans, E., and A. Yeung. 1989. Apparent viscosity and cortical tension of blood granulocytes determined by micropipet aspiration. *Biophys. J.* 56: 151–160.

Flanagan, M. D., and S. Lin. 1980. Cytochalasins block actin filament elongation by binding to high affinity sites associated with F-actin. *J. Biol. Chem.* 255:835–838.

Frank, R. S. 1990. Time-dependent alterations in the deformability of human neutrophils in response to chemotactic activation. *Blood.* 76:2606–2612.

Hartwig, J. H., R. Niederman, and S. E. Lind. 1985. Cortical actin structure and their relationship to mammalian cell movements. *Subcell. Biochem.* 11:1–49.

Johnson, K. J., J. Varani, and J. E. Smolen. 1992. Neutrophil activation and function in health and disease. *In* Granulocyte Responses to Cytokines. R. G. Coffery, editor. Marcel Dekker, New York. 1–46.

Keller, H. U., A. Naef, and A. Zimmerman. 1984. Effects of colchicine, vinblastine and nocodazole on polarity, motility, chemotaxis and cAMP levels of human polymorphonuclear leukocytes. *Exp. Cell Res.* 153: 173–185.

Kletzien, R. F., and J. F. Perdue. 1973. The inhibition of sugar transport in chick embryo fibroblasts by cytochalasin B. Evidence for a membrane-specific effect. *J. Biol. Chem.* 248:711–719.

Mantovani, A., and E. Dejana. 1989. Cytokines as communication signals between leukocytes and endothelial cells. *Immunol. Today.* 10:370–375.

Mantovani, A., F. Bussolino, and E. Dejana. 1992. Cytokine regulation of endothelial cell function. *FASEB J.* 6:2591–2599.

MacLean-Fletcher, S., and T. D. Pollard. 1980. Mechanism of action of cytochalasin B on actin. *Cell.* 20:329–341.

Needham, D., and R. M. Hochmuth. 1992. A sensitive measure of surface stress in the resting neutrophil. *Biophys. J.* 61:1664–1670.

Rao, K. M. K., J. Padmanabhan, and H. J. Cohen. 1993. Cytochalasins induce actin polymerization in human leukocytes. *Cell Motil. Cytoskel.* 21:58–64.

Sheterline, P., and C. R. Hopkins. 1981. Transmembrane linkage of surface glycoproteins to components of the cytoplasm in neutrophil leucocytes. *J. Cell Biol.* 90:743–754.

Sheterline, P., J. E. Rickard, and R. C. Richards. 1984. Involvement of the cortical actin filament network of neutrophil leucocytes during phagocytosis. *Biochem. Soc. Trans.* 12:983–987.

Sheterline, P., J. E. Rickard, B. Boothroyd, and R. C. Richards. 1986. Phorbol ester induces rapid actin assembly in neutrophil leucocytes independently of changes in $[Ca^{2+}]_i$ and pH_i. *J. Muscle Res. Cell Motil.* 7: 405–412.

Sheterline, P., and J. E. Rickard. 1989. The cortical actin filament network of neutrophil leucocytes during phagocytosis and chemotaxis. *In* The Neutrophil: Cellular Biochemistry and Physiology. M. B. Hallett, editor. CRC Press, Boca Raton, FL. 141–165.

Stossel, T. P. 1984. Molecular architecture of the cytoplasmic matrix. *In* White Cell Mechanics: Basic Science and Clinical Aspects. H. J. Meiselman, M. A. Lichtman, and P. L. LaCelle, editors. Alan R. Liss, New York. 75–86.

Stossel, T. P. 1988. The mechanical responses of white blood cells. *In* Inflammation: Basic Principles and Clinical Correlates. J. I. Gallin, I. M. Goldstein, and R. Snyderman, editors. Raven Press, New York. 325–341.

Stossel, T. P. 1990. How cells crawl. *Am. Sci.* 78:408–423.

Tsai, M. A., R. S. Frank, and R. E. Waugh. 1993. Passive mechanical behavior of human neutrophils: power-law fluid. *Biophys. J.* 65:2078–2088.

Wallace, P. J., C. H. Packman, R. P. Wersto, and M. A. Lichtman. 1987. The effects of sulfhydryl inhibitors and cytochalasin on the cytoplasmic and cytoskeletal actin of human neutrophils. *J. Cell. Physiol.* 132:325–330.

Worthen, G. S., B. Schwab, E. L. Elson, and G. P. Downey. 1989. Mechanics of stimulated neutrophils: cell stiffening induces retention in capillaries. *Science.* 245:183–186.

Yahara, I., F. Harada, S. Sekita, K. Yoshihita, and S. Natori. 1982. Correlation between effects of 24 different cytochalasins on cellular structures and cellular events and those on actin in vitro. *J. Cell Biol.* 92:69–78.

Yeung, A., and E. Evans. 1989. Cortical shell-liquid core model for passive flow of liquid-like spherical cells into micropipets. *Biophys. J.* 56: 139–149.