INFLUENCE OF PHYSICOCHEMICAL FACTORS ON RHEOLOGY OF HUMAN NEUTROPHILS

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ABSTRACT The effects of variations in temperature, pH, and osmolality on the rheological properties of human neutrophils were determined by studying the cell deformation in response to aspirational pressure applied via a micropipette. The time history of the deformation was analyzed by the use of a standard solid viscoelastic model consisting of an elastic element K_1 in parallel with a Maxwell element (an elastic element K_2 in series with a viscous element μ). With changes in temperature over a range of 9–40°C, only μ varied inversely with temperature, while K_1 and K_2 did not show significant alterations. Variations in pH over the range of 5.4–7.8 did not significantly affect the viscoelastic coefficients, but K_1 and μ rose at pH 8.4. An increase in osmolality caused all three coefficients to rise, but a decrease in osmolality had relatively little effect on the coefficients. These changes in response to physicochemical variations serve to provide insights into the viscoelastic properties of neutrophils and their possible roles in health and disease.

INTRODUCTION

The rheological behavior of blood cells in the microcirculation plays a significant role in governing blood flow dynamics and material transport. Although erythrocytes constitute the major volume fraction of formed elements in blood, the leukocytes, by virtue of their larger volume and lower deformability (Lichtman, 1973; Miller and Myers, 1975), may exert significant rheological influences on blood flow in the microcirculation (Chien, 1975; Skalak and Brånemark, 1969; Bagge et al., 1977; 1980). Knowledge of the microrheological properties of individual leukocytes is needed for the understanding of the dynamic behavior of these cells during flow and deformation, as well as their activities in physiological and pathophysiological states, e.g., interaction with the vascular endothelium, pseudopodia formation, chemotaxis, and phagocytosis (Gallin and Quie, 1978).

Neutrophils are most numerous among white blood cells. The neutrophil at rest is spherical in shape and has a segmented nucleus, which constitutes ~22% of the cell volume (Schmid-Schönbein et al., 1980). The other major intracellular particles are the granules (occupying ~15% of cell volume) and mitochondria (~ 0.6% of cell volume). The granules can be seen to undergo Brownian movement in the cytoplasmic matrix. The cell membrane has numerous crestlike foldings, and the cell contains many other types of membranes in its cytoplasm which may have significant implications on the viscoelastic properties of neutrophils (Schmid-Schönbein et al., 1980). In the present study, the rheological properties of neutrophils in response to changes in temperature, pH, and osmolality were investigated by the experimental and theoretical methods recently developed in our laboratory (Sung et al., 1979; Schmid-Schönbein et al., 1981).

MATERIALS AND METHODS

Preparation of Leukocytes

Fresh blood samples (10 ml) were obtained from five healthy human volunteers (age 22–49 yr) with EDTA as an anticoagulant. EDTA was chosen as the anticoagulant because the neutrophils retain their nearly spherical shape with minimum pseudopod formation in this medium. When heparin was used as the anticoagulant, neutrophils underwent spontaneous, active deformation which rendered it impossible to measure the passive-deformation behavior of the cell.

The erythrocytes were allowed to sediment at room temperature for 25–40 min. Leukocyte-rich plasma was aspirated from just above the sedimented erythrocytes and diluted with a buffered saline-albumin solution. The buffered saline-albumin solution contained 0.9 g/dl NaCl, 0.25 g/dl bovine serum albumin, and 12 mM Tris, with pH adjusted to 7.4 by the dropwise addition of 1 N HCl. The buffer solution was filtered through a 0.2-μm sterile Nalgene Filter Unit (Nalge Sybron Corp., Rochester, NY) before usage. The final volume concentration of leukocytes was ~0.005%. The prepared leukocytes were studied as soon as possible, and the experiment was usually completed within 3 h after sampling. The studies were performed at a temperature of 21–23°C, except in experiments in which the temperature was altered. The temperature of the cell suspension in the experiment chamber was monitored with a thermistor probe. The pH of the suspending medium was adjusted by changing the amount of Tris and determined by using a digital pH meter (model 3,500, Beckman Instruments Inc., Fullerton, CA). The osmolality of the medium was varied by changing the concen-

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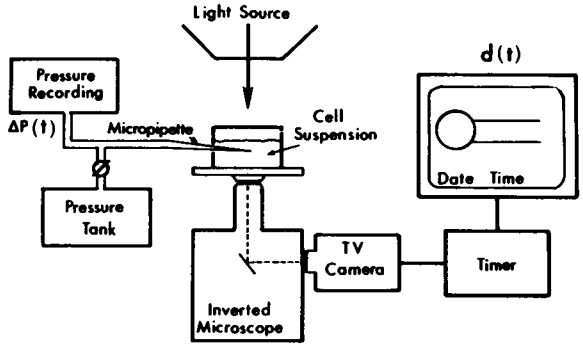

FIGURE 1 A schematic drawing of the system used for the micropipette experiment.

tration of NaCl, and the final osmolality was determined by freezing point depression (Fiske Osmometer, Fiske Associates, Inc., Bethel, CT).

Experimental Setup

The experimental setup and procedure were basically the same as those used for the studies on erythrocytes (Sung and Chien, 1978; Chien et al., 1978). About 0.5–1 ml of the cell suspension was loaded in a small round chamber located on the stage of an inverted microscope (Fig. 1). The chamber was surrounded by a copper ring filled with a circulating fluid of methanol-water (1:3) for temperature control. The fluid circulated between the copper ring and a reservoir containing the temperature control system. A thermistor probe was present in the sample chamber to monitor the temperature of the cell suspension. Individual leukocytes were viewed through the bottom of the chamber with an 100× objective (NA 1.25, oil immersion) and a 20× eyepiece. Neutrophils were identified by the presence of granules, which are smaller and show less contrast than those present in basophils and eosinophils. Through a video camera connected to the eyepiece, the viewing field was displayed on a TV monitor with a final magnification of 5,000×. Calibration was made with the use of a stage micrometer (50 × 2 μm, Graticules LTD, Towbridge Kent, England). The video image was recorded on a video recorder, together with the time output from a video timer.

Micropipettes were prepared with the use of a micropipette puller (Narishige Scientific Instrument Lab., Tokyo, Japan). The internal radius of the micropipette tip ranged from 1.1 to 1.7 μm. On several occasions, the size of the pipette tip was checked by scanning electron microscopy, and the results were in close agreement with light microscope measurements made on the TV monitor. The micropipette was filled with the buffered saline-albumin solution and mounted on a hydraulic micromanipulator.

The wide end of the micropipette (~0.8 mm i.d.) was connected to a pressure regulation system, which consisted of a damping chamber (~10 ml total volume) and two partially filled water reservoir bottles. By adjusting the relative heights of the water levels in the two reservoir bottles with the aid of a micrometer indicator (Montgomery Co., Chatham, NJ), desired pressure levels could be set in the system and then imposed on the micropipette by turning a three-way stopcock. The pressure level was monitored with the use of a transducer (Model 23 BC transducer, Statham Instrument, Inc., Oxnard, CA) connected to a Gould recorder (Gould Inc., Cleveland, OH). The time required for the pressure to reach full amplitude was 15–20 ms.

Deformation of Cells in the Micropipette

To analyze the time course of deformation of the leukocytes, sequential photographs were taken from the video image during single-frame replay on the TV monitor; the time between successive video frames is ~16 ms. The distance between the outer edge of the dark cell boundary and the tip of the micropipette was measured. The displacement of the cell surface into the pipette was determined by subtracting the distance that the cell reaches into the pipette without deformation. The displacement data were entered into a PDP 11/10 minicomputer (Digital Equipment Corp., Maynard, MA) and analyzed by using our theoretical model (Schmid-Schönbein et al., 1981), in which the neutrophil is treated as a standard

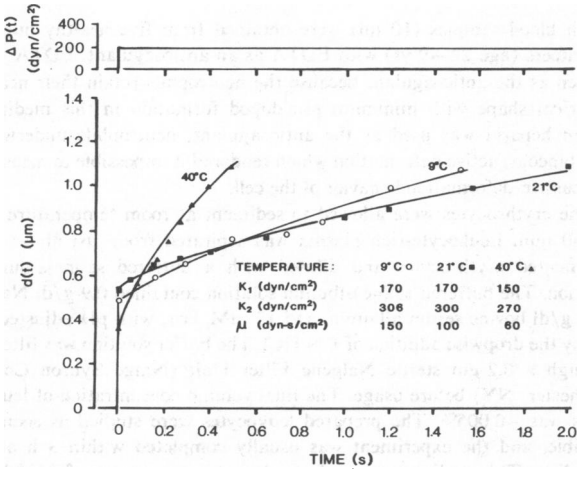

FIGURE 2 Time courses of cell deformation, $d(t)$, and aspiration pressure, $\Delta P(t)$, for three human neutrophils at different temperatures. The pressure amplitude (200 dyn/cm^2) and internal pipette radius (1.45 μm) were kept constant. The data points at each temperature were fitted with the theoretical curves (lines) by the use of the K_1, K_2, and μ values tabulated in the figure.

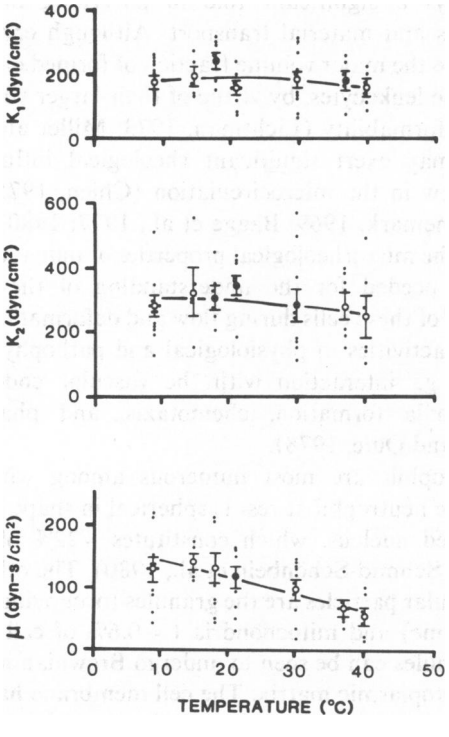

FIGURE 3 The viscoelastic coefficients K_1, K_2, and μ for human neutrophils as a function of temperature. The cells were suspended in isotonic medium (310 mosmol) and at pH 7.4. Open circles represent the mean values at different temperatures, and the vertical bars represent the standard errors of the mean. The lines were least-square fits with a second order polynomial.

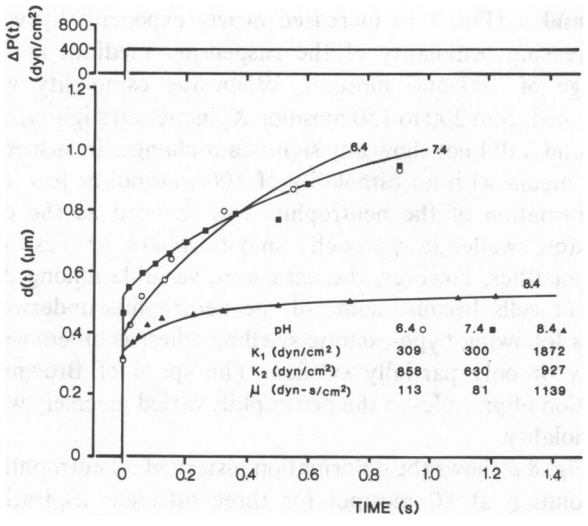

FIGURE 4 Time courses of cell deformation, $d(t)$, and aspiration pressure, $\Delta P(t)$, for three human neutrophils at different values of pH. The pressure amplitude (400 dyn/cm^2) and pipette internal radius (1.6 μm) were kept constant. The data points at each pH were fitted with the theoretical curves (lines) by the use of the K_1, K_2, and μ values tabulated in the figure.

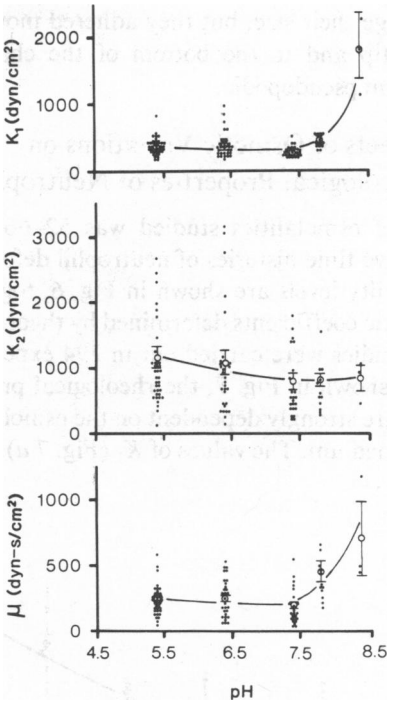

FIGURE 5 The viscoelastic coefficients K_1, K_2, and μ for human neutrophils as a function of pH. The cells were suspended in isotonic medium (310 mosmol) at room temperature (21–23°C). Open circles represent the mean values at different pH, and the vertical bars represent the standard errors of the mean. The lines were fitted by eye.

solid consisting of an elastic element K_1 in parallel with a Maxwell element (an elastic element K_2 in series with a viscous element μ).

The neutrophil sealed the pipette as it was drawn toward the pipette tip by the aspiration pressure. In some of the experiments on osmotically shrunken neutrophils, which were much stiffer with many membrane foldings (Schmid-Schönbein et al., 1980), the micropipette was not sealed, as evidenced by the motion of platelets toward the pipette tip. These cases were not included in the study because, in the presence of fluid flow into the micropipette, the exact stress distribution over the surface of the cell is unknown and would be different from that assumed in the model.

RESULTS

Effects of Temperature on Rheological Properties of Neutrophils

The range of temperature studied was 9–40°C. Representative time histories of neutrophil deformation at three different temperatures are shown in Fig. 2. The experimental data can be fitted with the theoretical curves by the use of different K_1, K_2, and μ values. Such analysis was carried out in 112 experiments on 26 neutrophils; the results are summarized in Fig. 3. The elastic behavior of the neutrophil did not vary significantly with temperature, as K_1 and K_2 values remained essentially constant in this temperature range. The μ value of the neutrophil, however, varied inversely with temperature (Fig. 3). The speed of Brownian motion of granules in the neutrophils varied directly with temperature.

Effects of pH Variations on Rheological Properties of Neutrophils

The range of pH studied was 5.4–8.4. Representative time histories of neutrophil deformation at three different pH levels are shown in Fig. 4, together with the viscoelastic coefficients determined by theoretical analysis. The results of 115 experiments on 21 neutrophils are summarized in Fig. 5. The K_2 values remained essentially constant in different pH media, but K_1 and μ increased with increasing pH (Fig. 5). In the higher pH media (pH 7.8 and 8.4), the cell diameter increased, and the granules also became swollen. In the lower pH media (pH 5.4 and 6.0), the cells

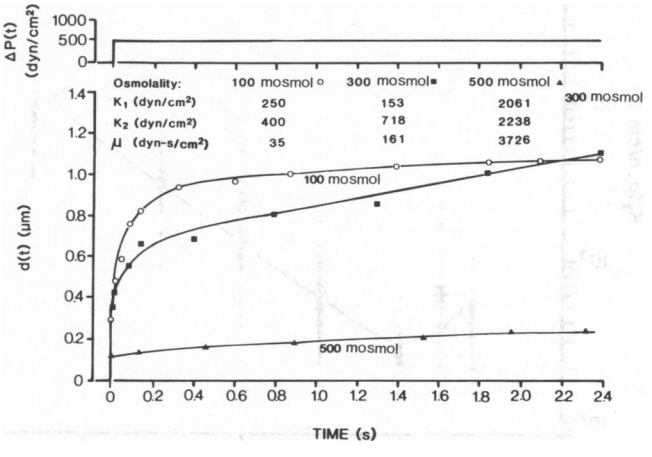

FIGURE 6 Time course of cell deformation, $d(t)$, and aspiration pressure, $\Delta P(t)$, for three human neutrophils at different values of osmolality. The pressure amplitude (500 dyn/cm^2) and pipette internal radius (1.2 μm) were kept constant. The data points at different osmolalities were fitted with the theoretical curves (lines) by the use of the K_1, K_2, and μ values tabulated in the figure.

did not change their size, but they adhered more easily to the pipette tip and to the bottom of the chamber and tended to form pseudopodia.

Effects of Osmotic Variations on Rheological Properties of Neutrophils

The range of osmolalities studied was 52–664 mosmol. Representative time histories of neutrophil deformation at three osmolality levels are shown in Fig. 6, together with the viscoelastic coefficients determined by theoretical analysis. Such studies were carried out in 294 experiments on 42 cells. As shown in Fig. 7, the rheological properties of neutrophils are strongly dependent on the osmolality of the suspending medium. The values of K_1 (Fig. 7 *a*), K_2 (Fig. 7 *b*) and μ (Fig. 7 *c*) increased nearly exponentially with increasing osmolality of the suspension medium in the range of 200–600 mosmol. When the osmolality was reduced from 200 to 150 mosmol, K_2 increased slightly, but K_1 and μ did not show any significant change. In suspending media with an osmolality of 100 mosmol or less, the deformation of the neutrophils was reduced as the cell became swollen to approach a smooth sphere. At these low osmolalities, however, the data were variable among different cells because some of the neutrophils underwent lysis following hypo-osmotic swelling whereas others were fully or only partially swollen. The speed of Brownian motion of granules in the neutrophils varied inversely with osmolality.

Fig. 8 *a* shows the deformation history of a neutrophil in a solution at 80 mosmol for three different aspiration pressures. The deformation in each case rapidly reached a steady state (d_{max}). As shown in Fig. 8*b*, d_{max} of this neutrophil swollen in an hyposmotic medium is independent of the applied stress above 4,000 dyn/cm^2, even up to 20,000 dyn/cm^2. Neutrophils that were tested in a 50 mosmol medium did not show any measurable deformation for all aspiration pressures that were applied without rupturing the membrane.

DISCUSSION

Our experiments on human neutrophils subjected to small deformations indicate that their protoplasm behaves like a viscoelastic material whose main features can be modeled

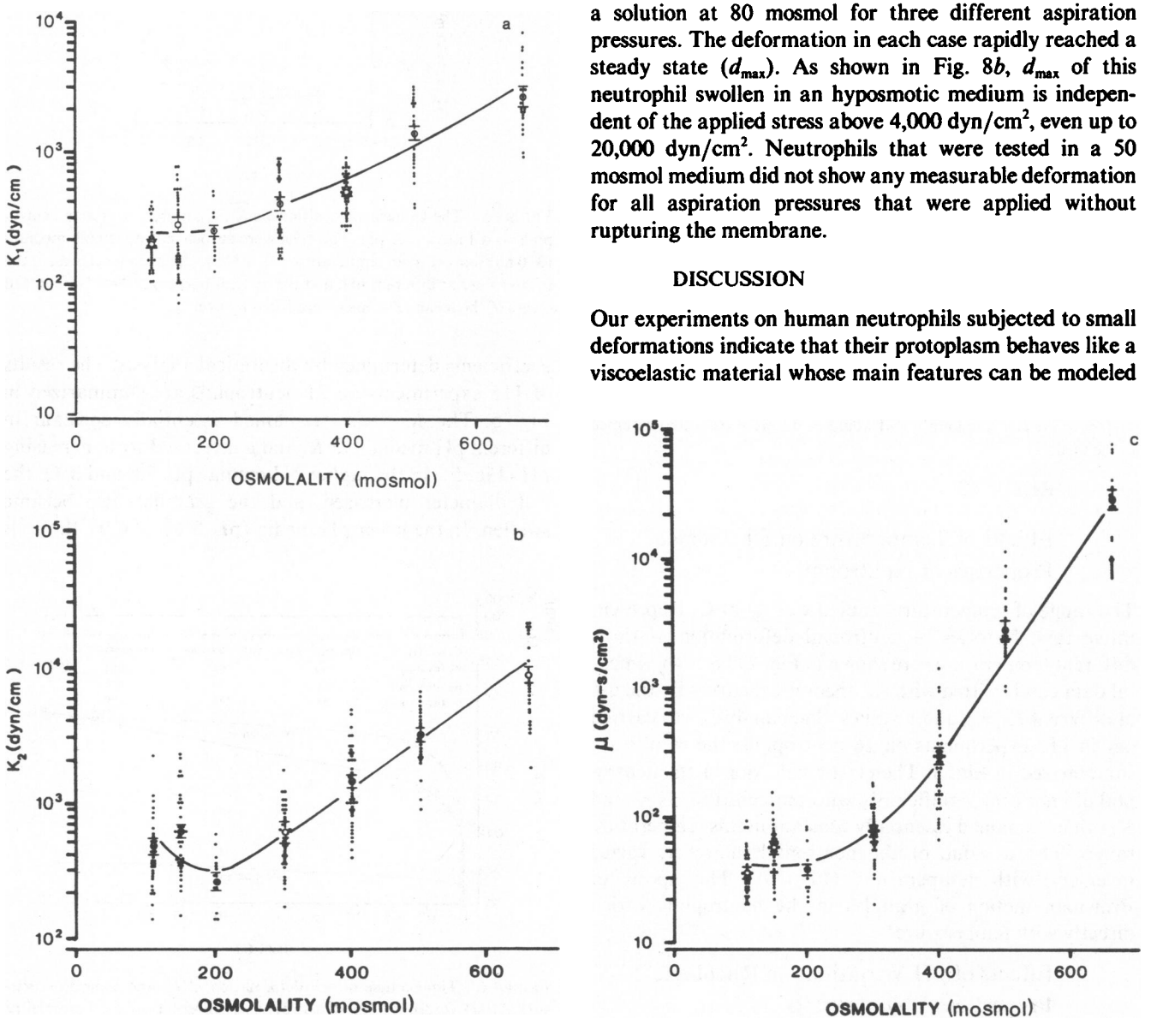

FIGURE 7 The viscoelastic coefficients K_1 (*a*), K_2 (*b*) and μ (*c*) for human neutrophils as a function of osmolality of the suspension media at room temperature and pH – 7.4. Open circles represent the mean values at different osmolalities, and the vertical bars represent the standard errors of the mean. The lines were fitted by eye.

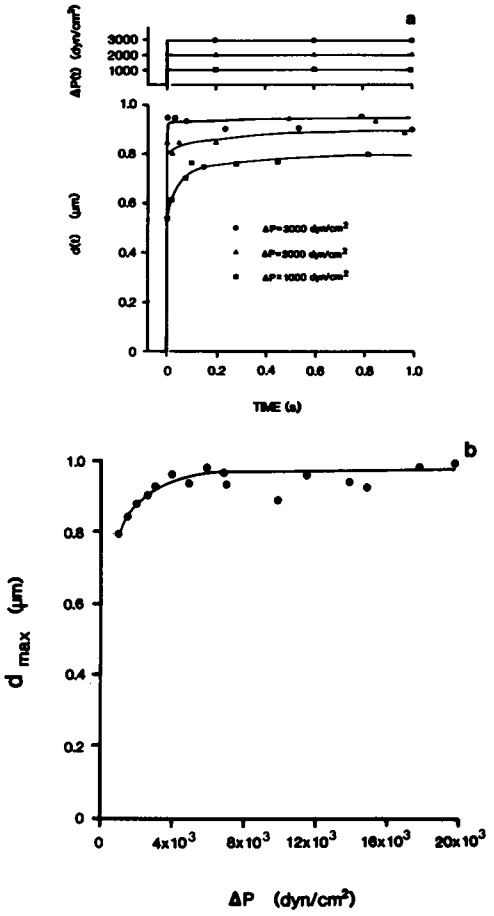

FIGURE 8(*a*) Time courses of deformation, $d(t)$, for a human neutrophil swollen in 80 mosmol suspension medium (22°C, pH = 7.4) in response to three different pressure amplitudes with constant pipette radius (1.5 μm). The cell diameter was 12.2 μm. The deformation $d(t)$ reached steady-state values d_{max} in <1 s. The lines have been drawn to fit the data by eye. (*b*) Variations of d_{max} as a function of pressure amplitude for the same cell as in (*a*). Note that for aspiration pressures greater than 4,000 dyn/cm² the cell showed an essentially constant d_{max}.

using a three-element standard solid material. Precise experimental determinations of these small deformations are limited by the resolution of optical microscopy. Analysis of the same photographs by different individuals yielded variations of the coefficients by <25%. With alterations in physicochemical conditions of measurements, the present study has shown that changes in rheological properties of neutrophils can be demonstrated by the micropipette method.

The standard solid model that was proposed earlier (Sung et al., 1979; Schmid-Schönbein et al., 1981) gives overall properties of the cell including the contributions of all protoplasmic structures and the membrane. Since the deformations were small, the overall geometry of the neutrophil or the cell nucleus did not influence the viscoelastic behavior studied in the present experiments. The empirical coefficients K_1, K_2, and μ can be used to explain the recorded deformation history $d(t)$. At very short times after the initiation of deformation, the neutrophils behave like elastic bodies with a shear modulus equal to $(K_1 + K_2)/2$. The slope of $d(t)$ at $t = 0$ when it is approached from time $t > 0$ is proportional to $K_2^2/[(K_1 + K_2)^2\mu]$, and the maximum displacement for long times is proportional to $1/K_1$.

In experiments using different temperatures ranging from 9° to 40°C, the K_1 and K_2 values remain essentially constant, but the value of μ varies inversely with temperature. The high viscosity value at low temperature is associated with a slowing of the Brownian motion of the granules. Thus, the viscosity of neutrophils has a temperature dependence in the same direction as that of other liquids and erythrocytes (Barbee, 1973; Chien, 1975). The μ value of neutrophils increases by ~40% as temperature is reduced from 37 to 27°C. This is to be compared with the increases of viscosity by 23% for water (Handbook of Chemistry and Physics, 1962), 30% for plasma (Harkness, 1971) and 74% for erythrocyte membrane (Hochmuth et al., 1980) over the same range of temperature drop.

In experiments using suspending media with different pH, the cells show increases in K_1 and μ in alkaline solutions (pH 8.4). These rheological changes are associated with swelling of the cell and its granules and nucleus. The loss of cell-membrane folding with neutrophil swelling would reduce the excessive membrane surface area available for deformation (Schmid-Schönbein et al., 1980) and contribute to the observed changes in rheological properties. In acidic media with pH down to 5.4, the neutrophils do not exhibit significant changes in K_1, K_2, and μ. Studies on erythrocytes have shown that their elastic modulus increases in acidic media with pH <6.0 (Crandall et al., 1978). In the acidic media, the size of the neutrophil does not change, but the cells tend to adhere to the bottom of the glass chamber. This may contribute to an increase in neutrophil adhesion to the venular endothelium in low-flow states where metabolic acidosis reduces the pH in the microcirculation. The adhered leukocytes, which are less deformable than the erythrocytes, would then protrude into the flow field and cause an elevation of flow resistance (Lipowsky et al., 1980).

In experiments using suspending media with different osmolalities, all three coefficients of neutrophils increase markedly in response to hyperosmotic shrinkage. In media with normal osmolality (295–305 mosmol), the μ-value of neutrophils is about 10^2 poise (P); in a hyperosmotic medium of 660 mosmol, the μ-value of neutrophils increases by ~300-fold to 3×10^4 P. The elastic moduli K_1 and K_2 increase by lesser degrees. The high values of μ, K_1, and K_2 in the hyperosmotic medium probably reflect the increase in solid concentration due to cellular dehydration. When the osmolality is reduced from 300 to 200 mosmol, there is a slight decrease in μ of neutrophils. In comparison to erythrocytes, which began to exhibit hemolysis when the osmolality of the medium is reduced below 150 mosmol (Dodge et al., 1963; Katchalsky et al., 1960), the neutro-

phils have more tolerance to hypo-osmotic treatment, and most neutrophils are stable in 100 mosmol medium without rupturing. The cytoplasm of such neutrophils is diluted approximately fivefold with water, and the cell has an almost spherical shape with unfolded membrane (Schmid-Schönbein et al., 1980). The overall rheological properties of the neutrophil in response to variations in osmolality reflect a balance between the influences of the intracellular solid concentration and the geometric relation between cell surface area and cell volume. At low osmolalities near 200 mosmol, the relative constancy of the moduli values probably results from a balance of the decrease in solid concentration and the increase in sphericity, as the osmolality is lowered. At osmolalities of 100 mosmol or lower, when all the surface foldings in most cells disappear and the neutrophil becomes a smooth sphere, the cell becomes essentially nondeformable despite the increase in fluidity of the cell interior.

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