Eur Biophys J (1990) 19:93–99

European Biophysics Journal

Local mechanical oscillations of the cell surface within the range 0.2–30 Hz

A. Yu. Krol, M. G. Grinfeldt, S. V. Levin*, and A. D. Smilgavichus

Institute of Cytology of the Academy of Sciences of the USSR, Leningrad, USSR

Received May 30, 1989/Accepted in revised form July 16, 1990

Abstract. This paper describes transverse oscillations, within the range 0.2–30 Hz, of the surface of different animal cells: human and frog erythrocytes, human lymphocytes and monocytes, cultured 3T6 fibroblasts, and rat cardiomyocytes. The minimal area of the cell surface which undergoes unidirectional transverse movement is equal to or less than 0.5×0.5 μm. The amplitude of the oscillations recorded on larger surface areas is lower than on the smaller ones because of the averaging of solitary oscillations. The oscillation amplitude is different in different cells. The highest amplitude is recorded in human erythrocytes (350–400 nm), the lowest one, in fibroblasts, lymphocytes and monocytes (20–30 nm). The oscillations of the human erythrocyte are suppressed on hypotonic swelling, after hardening of the cell membrane owing to adsorption at the surface of the impermeable dye Heliogen Blue, by treatment of the cell with 0.01% glutaraldehyde, by treatment with 0.5 m*M* 4-hydroxymercurybenzoate, and after crenation caused by 1–2 m*M* 2,4-dinitrophenol. The amplitude of the surface oscillations is decreased in spectrin deficient erythrocytes obtained from patients with hereditary spherocytosis, which indicates an essential role for spectrin in the rapid oscillations of the erythrocyte surface.

Key words: Cell motility – Cell surface light scattering – Erythrocytes – Cultured cells – Spectrin abnormality – Microspherocytosis – Echinocytosis – Membrane cross-linking

Introduction

In recent years an interesting type of cell surface motility has been discovered: fast movements of the cell surface of about 1–5 ms in duration (Levin and Golfand 1980; Iwasa et al. 1980; Kachar et al. 1986; Levin et al. 1986; Levin and Malev 1987). They are distinguished from other known non-muscle cell movements by higher velocities and by direct dependence on electrochemical processes in the cell membranes. Surface movements with intermediate velocities (50 ms–1 s in duration) were discovered on Paramecium caudatum (Levin et al. 1984).

In the present work we have attempted to detect this kind of motility in non-muscle animal cells: frog erythrocytes, human erythrocytes, lymphocytes and monocytes and in cultured 3T6 fibroblasts and rat cardiomyocytes. We have also made a more detailed analysis of the erythrocyte surface motility to test the effect of hypotonicity and of reagents that cross-link the membrane. Finally, we have examined spectrin-deficient erythrocytes obtained from patients with hereditary microspherocytosis.

Materials and methods

Fresh frog and human erythrocytes were washed by two-fold centrifugation in phosphate buffered saline (PBS): 130 m*M* NaCl, 10 m*M* glucose, 9 m*M* phosphate buffer, pH-7.4, supplemented with human serum albumin (1 mg/ml) and settled on the bottom glass of an experimental chamber. Lymphocytes and monocytes were obtained from human blood: the mononuclear cell fraction was isolated in a Ficoll-verographin gradient at 400 g and the cells were resuspended in RPMI medium (Difco) supplemented with 10% heat inactivated serum (Bøyum, 1968). The lymphocytes and monocytes were allowed to settle on a coverslip at 37 °C for 4–8 h. The fibroblasts and cardiomyocytes from rat heart were grown on coverslips (Borisov and Khairutdinova 1986) and placed in the chamber together with the coverslips. All the drugs used (Heliogen Blue, 1 m*M* 2,4-dinitrophenol (DNP) (Reachim USSR), 0.01% glutaraldehyde (Reanal), 0.5 m*M* 4-hydroxymercurybenzoate (4-HOMB)) were added to the PBS. The experiments were performed at 18–20 °C.

Mechanical oscillations of the cell surface were recorded using an optical method (Levin et al. 1984) on cells attached to the coverslip. The method detects the

* *Present address:* Tel-Aviv University, Tel-Aviv, Israel
Offprint requests to: M. G. Grinfeldt

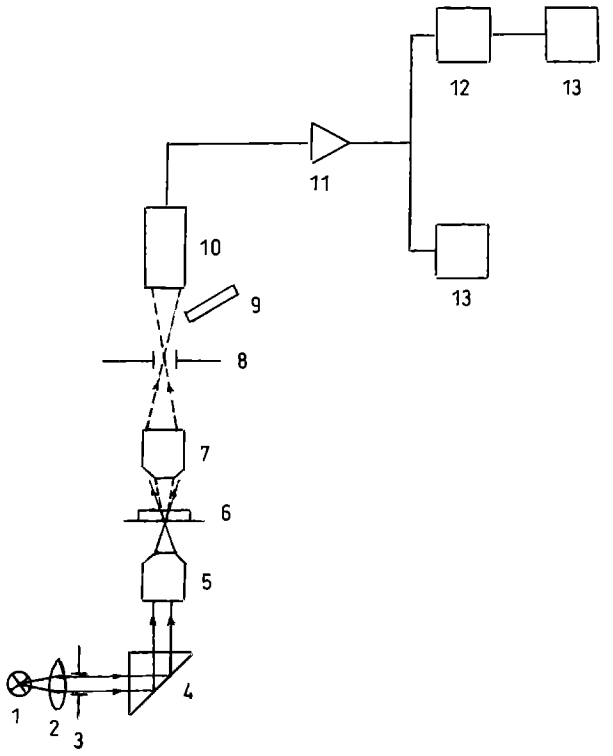

Fig. 1. Schematic diagram of a photometric unit. *1.* Light source, *2.* Collector lens, *3.* Field slit, *4.* Rectangular prism, *5.* Condenser-objective, *6.* Stage and preparation, *7.* Objective, *8.* Photometric diaphragm, *9.* Eye piece, *10.* Photomultiplier, *11.* Amplifier with low and high frequency filters, *12.* Spectrum analyser, *13.* Recorder

surface movement by the fluctuation of light scattered by a small area of the cell surface which changes its position relative to the annular cone of incident light.

Figure 1 shows a schematic diagram of the apparatus used. A special device provided small-spot dark-field illumination of the lateral cell surface (Korolev and Levin 1963). A catodioptric (lens-mirror) objective 115 × 0.7 was used as a dark-field condenser to image the field diaphragm on the lateral surface of the cell so that only scattered light could reach the detector (the objective was 40 × 0.75).

Displacement of the cell surface relative to the light scattering zone (LSZ) caused a change in intensity of the recorded light; entry of the cell margin into the LSZ resulted in an increase in light intensity. Fluctuations in scattered light intensity might also be caused by other processes; changes in refractive index, size of light-scattering particles, as well as by a whole cell displacement as a result of vibrations of the chamber, the apparatus, the building etc. In order to analyse the causes of fluctuations in scattered light intensity the following method was applied: the ratio of alternating/direct components of the photocurrent ($\Delta F/F$) was estimated for two different lengths of the LSZ. If the fluctuations were caused by changes in refractive index, membrane thickness or displacement of the whole cell, the $\Delta F/F$ ratio would be independent of the length of the LSZ. Such a dependence bears evidence of the local character of these oscillations which are averaged when light scattered by a large area is recorded. Owing to mechanical damping of the apparatus and the use of stable light sources and power supply, the fluctuations of intensity of light scattered by immovable defects, e.g., those in the coverslip, did not exceed 0.2–0.4%, the latter being the sensitivity threshold of the device. The power spectrum of the cell surface oscillations was recorded on a spectrum analyser *X* 6–8 (Fig. 1).

Calibration of the experimental device for the measurement of cell surface movement

To determine $\Delta F/F$ as a function of Δl, where Δl is the displacement of the cell surface causing fluctuations of scattered light, we used a vibrator kindly supplied by A. S. Mirkin (Institute of Machine Engineering, Academy of Sciences of the USSR). First, to the rod of the vibrator we attached a mask which partly covered the slit for a known length l. The vibrator was powered by rectangular current pulses and the $\Delta F/F$ ratio was recorded, this was assumed to be proportional to the $\Delta l/l$ ratio. If l is known, Δl in nm can be determined from $\Delta F/F$ at a given current pulse. These measurements were performed on a special device (Levin and Golfand 1980, 1984). From these data it was possible to find Δl in nm at a known current pulse. Then a small chamber formed by two horizontally arranged coverslips, 1.5 × 3 mm, was attached to the same vibrator and the latter was mounted on the stage of the installation shown in Fig. 1. The cells, prefixed with 2% glutaraldehyde, were placed on the lower coverslip of the chamber. The chamber together with the cells was put in motion by the vibrator. Light was directed to the cell margin and the intensity of light scattered by the cell surface was recorded. Figure 2 shows the dependence of $\Delta F/F$ on the displacement of the margin of the human erythrocyte. As can be seen, a linear dependence between the displacement of the cell margin and the scattered light intensity is observed within the range 10–300 nm. The minimum fluctuation of scattered light, $\Delta F/F$, was 0.4%, which corresponds to a surface movement of 6–8 nm. This value was close to the basic noise of the device.

Results

1. Local oscillations of the cell surface

Figure 3 A shows the results obtained on 3T6 fibroblasts. Records are given of photocurrent changes resulting from the fluctuations of light scattered by the cell surface at LSZ 0.5 × 0.5 μm (curve 1). It is seen that at long LSZ (curve 2) the amplitude of the photocurrent is nearly 3 times less than at short LSZ. This testifies to the fact that a significant part of the fluctuation of the scattered light is caused by local displacement of small areas of the cell surface comparable in size to the LSZ. A decrease in the signal recorded at long LSZ is the result of averaging of the signals generated by neighbouring areas moving in opposite directions. The change of the photocurrent amplitude at short LSZ corresponds to a displacement of a fixed cell in a calibrating device of 10–20 nm. Since the minimum wavelength of local oscillations remains un-

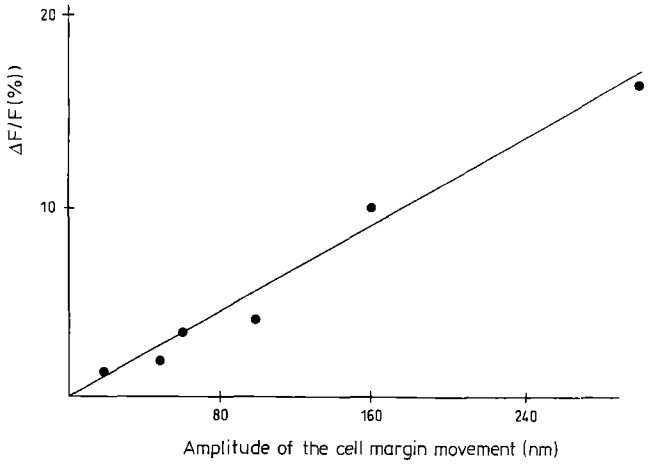

Fig. 2. The intensity of light scattered by the margin of the glutaraldehyde-fixed human erythrocyte as a function of the displacement of the whole cell by a vibrator

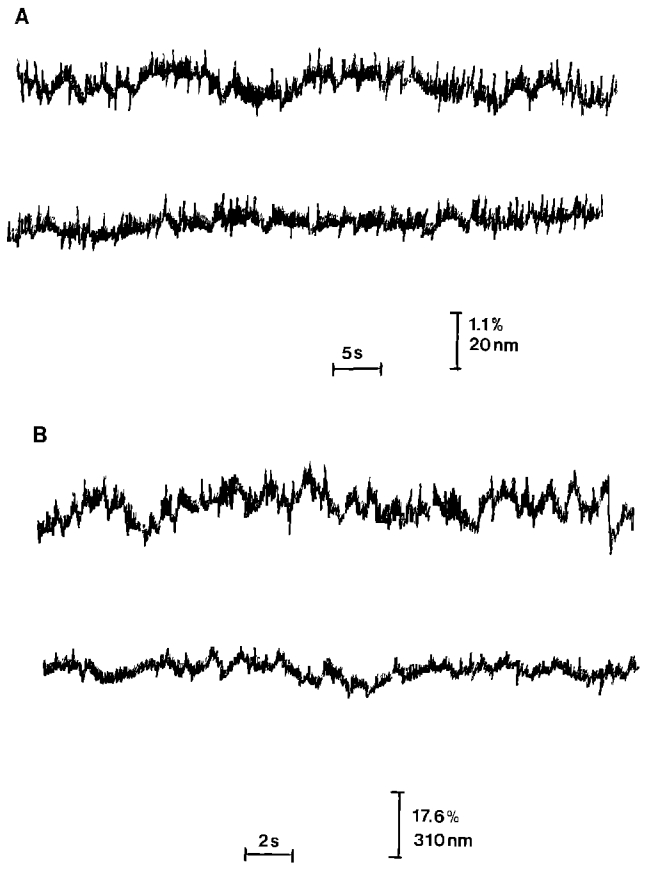

Fig. 3. Fluctuations of intensity of light scattered by a small area of the surface of fibroblast (**A**), human erythrocyte (**B**). Data were recorded for a short (0.5 × 0.5 μm), curve 1, or long, curve 2, light scattering zone. The size of "long" light scattering zone was: 0.5 × 5 μm

known, their true amplitude can be higher than that obtained from the "average" signal.

Similar data have been obtained on rat cardiomyocytes, human monocytes and lymphocytes. A dependence of scattered light fluctuation upon the length of the LSZ is observed here too. The fluctuations measured at short LSZ (0.5 × 0.5 μm) lie in the range 1–2%, which corresponds to a surface displacement of 20–30 nm. In the case of long LSZ (0.5 × 4–5 μm) the fluctuation of the photocurrent is close to the intrinsic noise of the device.

The data represented in Fig. 3B were obtained on human erythrocytes. It is seen that in these cells the fluctuations of the light scattered by the lateral cell surface amounts to 18% of the direct photocurrent component. This value is recorded in the case of short LSZ (0.5 × 0.5 μm). With a ten-fold increase in LSZ (which corresponds to an increase in the cell surface examined) the amplitude of the photocurrent fluctuation decreases by nearly a factor of 4. The results obtained testify that the erythrocyte surface undergoes transverse oscillations with an amplitude of 310 nm, which exceed, by 10–20 times, the amplitude of those in other cells studied. An increase in the length of the cell surface area tested does not lead to a complete disappearance of the fluctuations of scattered light in contrast to that observed on monocytes and fibroblasts. What might be the reason for such high fluctuations of the human erythrocyte surface? Can it be due to the displacement of the whole cell because of vibration of the device, or to incomplete averaging of single high-amplitude waves of opposite direction? Some of our preliminary data show that in hypotonically swollen erythrocytes, oscillations of the surface are reversibly suppressed ($\Delta F/F = 0.2–0.4\%$). Thus, vibrations of the whole cell are small in amplitude (not more than 3.5–7 nm), and therefore, it can be concluded that the motion recorded at long LSZ represents incompletely averaged oscillations of the erythrocyte surface.

The power spectrum of transverse oscillations at LSZ 0.5 × 0.5 μm (Fig. 4) shows that oscillations occur within the range of (0.2–1)–30 Hz. The spectral curve has its maximum at 0.2–1 Hz, and tends to zero at higher frequencies. The half-maximum power frequency (Δf 1/2) falls at frequencies of 2–3 Hz. The data available at present are not sufficient for a quantitative description of the oscillation spectrum since higher accuracy of Fourier analysis and a larger number of measurements are required.

In nucleated frog erythrocytes the amplitudes of transverse oscillations were ten times lower than in human erythrocytes. As in other cells, transverse oscillations become averaged when recorded at long LSZ. The power spectrum of oscillations of the frog erythrocyte surface (Fig. 4B) is similar to that described for human erythrocytes. These data point to a relationship between the oscillation amplitude and the shear elastic modulus of the erythrocyte surface. In nucleated erythrocytes, to which the frog erythrocytes also belong, this modulus is 50–100 times higher than in the un-nucleated ones (Evans and Skalak 1980).

2. The effect of cross-linking reagents, hypotonicity and spectrin deficiency (hereditary spherocytosis) on human erythrocyte surface oscillations

Effect of hypotonicity. One and half fold dilution of PBS with water leads to a significant reduction in the oscilla-

tion amplitude. A two-fold hypotonicity change causes a complete suppression of transverse waves on the erythrocyte surface. After the hypotonic solution is replaced by the isotonic one, the surface movements recover (Fig. 5).

The anionic phthalocyanine dye, Heliogen Blue (HB) does not penetrate the cell membrane and is absorbed by the erythrocyte surface (Kirpichnikova et al. 1981). After washing out the poorly bound dye, the residual tightly bound dye occupies no more than 1.5–4.5% of the erythrocyte surface (Brudnaya et al. 1984). It was of interest to know if in this case the motility of the erythrocyte surface is changed. Physiological properties of such erythrocytes are changed: their hypotonic ghosts lose the ability to reseal (Brudnaya et al. 1984). The amplitude of local surface oscillations of such erythrocytes appeared to be a 2.5–3 times lower than those in the control (Fig. 6).

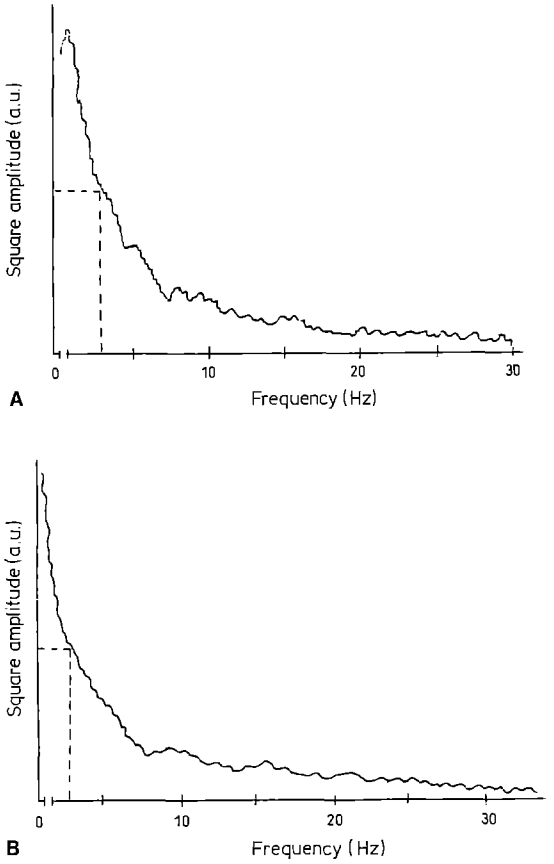

Fig. 4. The power spectrum of oscillations of a small surface area of the human erythrocyte (**A**, 0.5×0.5 μm) and frog erythrocyte (**B**, 2×2 μm). The dashed line designates the values of half-maximum power frequency of surface oscillations ($\Delta f = 1/2$) amounting in frog and human erythrocytes to 2.5–3 Hz

Substances which cause membrane cross-linking increase the rigidity of both the membrane and the whole erythrocyte. This effect is observed with the action of low concentrations of glutaraldehyde which crosslink the aminogroups of proteins and lipids and with thiol poisons which form S-S bridges in the membrane proteins.

All these substances were found to suppress surface oscillations. Thus, 0.01% glutaraldehyde irreversibly blocks them in 1–1.5 minutes (Fig. 7). It was reported that erythrocytes treated in such a way fail to pass through the capillaries (Chien 1987).

Other agents which harden the erythrocyte membrane owing to the formation of S-S bridges in the membrane proteins are the thiol poisons. We used 4-HOMB, an analogue of parachloromercurybenzoate. At a concentration of 0.5 m*M* this drug hinders the penetration of erythrocytes through the capillaries (Chien 1987). We found that 0.5 m*M* 4-HOMB suppresses surface oscillations (Fig. 8). This process develops in 10–15 min and results in a 5-fold decrease in the amplitude of oscillations.

Transformation of normal erythrocytes into echinocytes was brought about by adding 2,4-DNP to the medium (Alhanaty and Sheetz 1981). All the discocytes rapidly turned into echinocytes: the cells became rounded and spicules appeared. Measurements of surface oscillation were made between the spicules. Figure 9 shows the results of the experiment in which the amplitude of surface oscillations decreased from 340 to 160 nm.

Spectrin plays a principal role in determining the mechanical properties of the erythrocytes and in maintain-

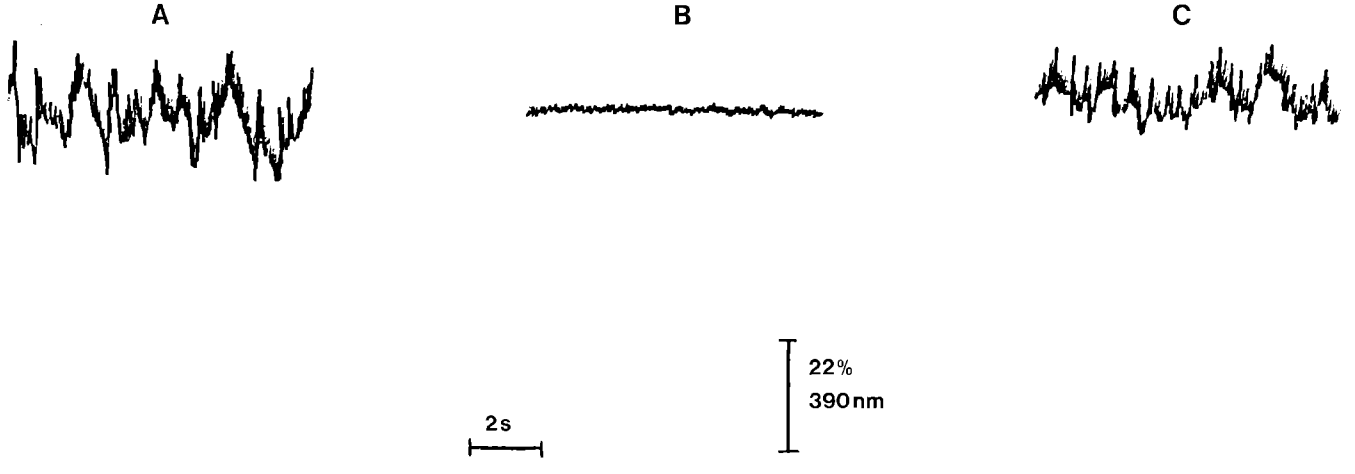

Fig. 5A–C. Reversible suppression of human erythrocyte surface oscillations in hypotonic medium. **A** Normal erythrocyte in isotonic PBS. **B** The same cell after a two-fold dilution of the PBS. **C** Recovery in isotonic PBS

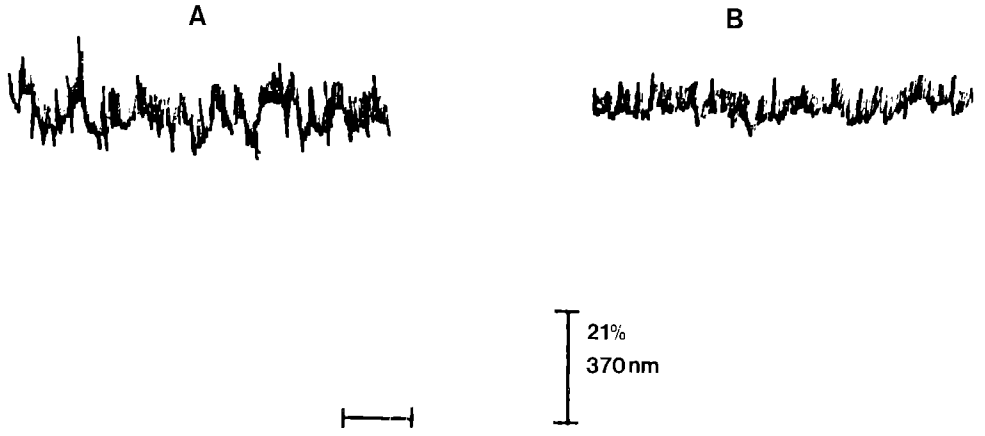

Fig. 6 A, B. Decrease in the erythrocyte surface movement induced by the phthalocyanin dye Heliogen Blue. **A** Normal erythrocyte. **B** The same cell after treatment with 0.002% Heliogen Blue for 60 min and subsequent washing in PBS for 50 min

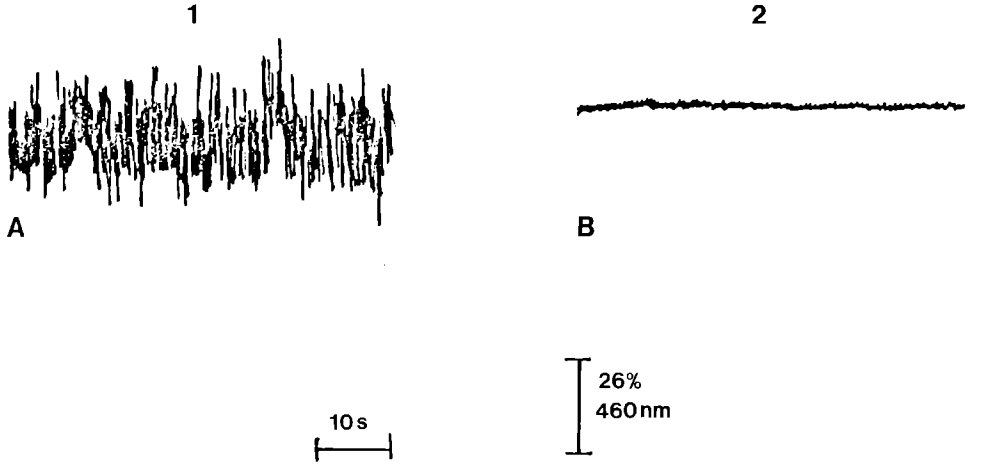

Fig. 7. Suppression of erythrocyte surface movement by 0.01% glutaraldehyde. **A** normal erythrocyte. **B** The same cell after treatment with glutaraldehyde for 5 min and subsequent washing with PBS for 20 min

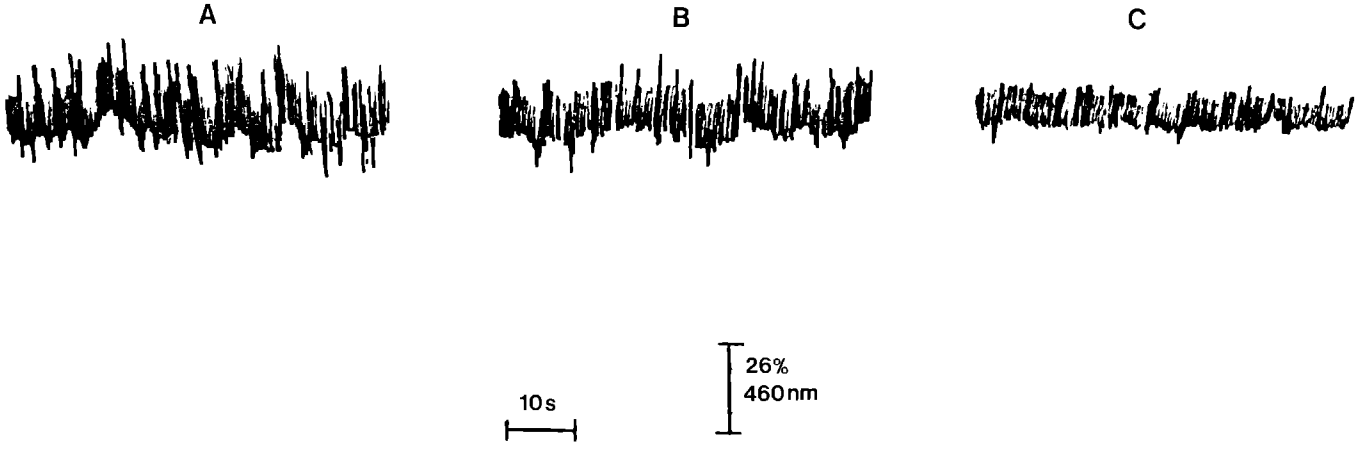

Fig. 8 A–C. Suppression of erythrocyte surface movement by 4-HOMB. **A** Normal erythrocyte. **B, C** The same cell after treatment with 4-HOMB for 7 min (**B**) or 15 min (**C**)

ing their shape. Therefore it was of interest to analyse its effect on the local oscillations of the cell surface. With this aim we tested the amplitude of surface oscillations of erythrocytes from patients with hereditary microspherocytosis caused by spectrin deficiency (Knowles et al. 1983; Agre et al. 1985). Spherocytes exhibit a decrease in membrane shear modulus as well as in membrane viscosity (Waugh 1987) and are more sensitive to osmotic shock. Table 1 gives averaged data on the amplitude of oscillations in defective and normal erythrocytes. In the former

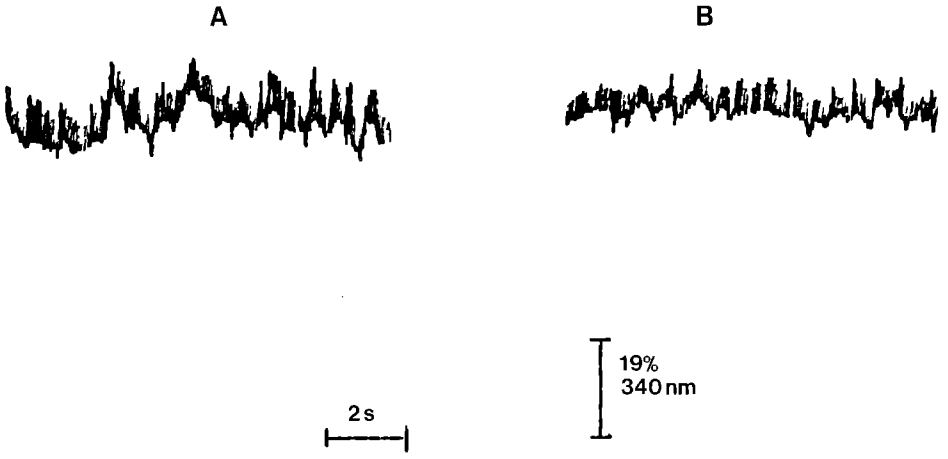

Fig. 9 A, B. Influence of discocyte-echinocyte transition on cell surface movement. **A** Normal erythrocyte (discocyte). **B** The same cell after echinocytosis caused by 1 m*M* 2,4-dinitrophenol

Table 1. The amplitude of the local oscillations of the human erythrocyte surface

Source of the blood	Amplitude, nm	
Healthy donors	310± 8	(77)
Patients with hereditary	195±16	(7)
microspherocytosis	140±52	(4)
(two sets of measurements	210±23	(12)
for two persons)	195±61	(4)
M.±s.d.	185±12	(n=27)

the amplitude of oscillations is on average 1.7 times lower than in the latter. This fact bears evidence of the participation of the spectrin in the oscillations recorded on the erythrocyte surface.

Discussion

Local oscillations of the surface of various cells described above bear evidence to the frequent occurence of this kind of motion of the cell surface. This type of motion occupies an intermediate position between the slow motion found in cell spreading, phagocytosis, morphogenic displacements, and rapid non-muscle movement caused by electrochemical disturbance of the cell membrane. The nature of the surface oscillations described in the present paper and their functional role still remains obscure. In human erythrocytes a phenomenon of "flicker" (shimmering) was described and interpreted as a change in erythrocyte thickness (Brochard and Lennon 1975; Fricke and Sackmann 1984; Fricke et al. 1986). In our experiments we directly recorded the local oscillations which occur on the cell surface. It is possible that in the case of erythrocytes the movements we observed might underlie the flicker phenomenon.

The membrane shear modulus of human erythrocytes ($\mu = 1 \cdot 10^{-2} - 5 \cdot 10^{-2}$ dyne/cm; Evans 1973; Evans and La Celle 1975) is 50–100 times lower than in nucleated amphibian erythrocytes. A decrease in the oscillation amplitude of the human erythrocyte surface induced by glutaraldehyde and 4-HOMB, which increase the rigidity of the membrane (La Celle et al. 1976), shows that the amplitude of the oscillations depends on membrane elasticity. It seems reasonable to associate lower amplitude of surface oscillations in frog erythrocytes, cultured fibroblasts and cardiomyocytes, as well as lymphocytes and monocytes, with higher values of the shear modulus of their membrane.

The results we have obtained on the suppression of erythrocyte surface oscillations by hypotonicity are in accordance with those reported by Fricke and Sackmann (1984) in their studies of flicker phenomenon. Erythrocyte surface motility should evidently be associated with deformability of erythrocytes and similarly, enables them to pass through the capillaries.

Acknowledgements. The authors are greatly indebted to their colleagues: S. A. Ezhevsky, T. A. Vinogradova and A. B. Borisov for supplying them with lymphocytes, monocytes, fibroblasts and cardiomyocytes. We are also thankful to M. M. Vyasminskaya, V. M. Abdulkadyrov and E. A. Selivanov (Leningrad, Institute of Haematology and Blood Transfusion) for supplying us with blood samples of hereditary spherocytosis patients.

References

Agre P, Casella JF, Zinkham WH, McMillan C, Bennett V (1985) Partially deficiency of erythrocyte spectrin in hereditary spherocytosis. Nature 314:381–383

Alhanaty E, Sheetz MP (1981) Control of the erythrocyte membrane shape: recovery from the effect of crenating agents. J Cell Biol 91:884–888

Borisov AB, Khairutdinova YuA (1986) Regeneration and DNA synthesis in cultures of the rat cardiac ventricles after lesions of the cellular layer. Cytology 9:62–67

Bøyum A (1968) Isolation of leucocytes from human blood. Scand J Clin Lab Invest 21 [Suppl 97]:9–29

Brochard F, Lennon JF (1975) Frequency spectrum of the flicker phenomenon in erythrocytes. J Phys 36:1035–1047

Brudnaya MS, Kirpichnikova KM, Komissarchik YaYu, Levin SV (1984) Changes in the shape and ultrastructure of the white ghosts of erythrocytes upon their sealing and phthalocyanin dye sorption. Cytology 26:184–187

Chien S (1987) Red cell deformability and its relevance to blood flow. Ann Rev Physiol 49:177–192

Evans EA (1973) New membrane concept applied to the analysis of fluid shear and micropipette deformed red blood cells. Biophys J 13:941–954

Evans EA, La Celle PL (1975) Intrinsic material properties of the erythrocyte membrane indicated by mechanical analysis of deformation. Blood 45:29–43

Evans EA, Skalak R (1980) Mechanics and thermodynamics of biomembranes. CRC Press, Boca Raton, Fla

Fricke K, Sackmann E (1984) Variation of frequency spectrum of the erythrocyte flickering caused by aging, osmolarity, temperature and pathological changes. Biochim Biophys Acta 803:142–152

Fricke K, Wirthensohn R, Laxuber R, Sackmann (1986) Flicker spectroscopy of erythrocytes. Eur Biophys J 14:67–81

Iwasa K, Tasaki I, Gibbons RC (1980) Swelling of nerve fibers associated with action potentials. Science 210:338–339

Kachar B, Brownell WE, Altschuller R, Fex J (1986) Electrokinetic shape changes outer hair cells. Nature 322:365–368

Kirpichnikova KM, Levin SV, Shuvalova EV (1981) The spectrophotometric study of the surface of erythrocytes and of their ghosts using a phthalocyanine dye. Cytology 23:297–304

Knowles W, Marchesi SL, Marchesi VT (1983) Spectrin: structure, function and abnormalities. Semin Hematol 20:159–174

Korolev NV, Levin SV (1963) On microscopic examination of living biological test-objects by means of dark field lighting. Cytology 5:588–591

La Celle PL, Weed RI, Santillo PA (1976) Pathophysiologic significance of abnormalities of red cell shape. Membranes and disease. Raven Press, New York, pp 1–18

Levin SV, Golfand KA (1980) Reversible displacement of crab axon at the action potential. Cytology 22:717–721

Levin SV, Golfand KA (1984) A rapid transverse movement of the crab axon at action potential. Cytology 26:920–926

Levin SV, Malev VV (1987) The influence of elastic properties of the crab axon sheath on into movement during action potential. Cytology 29:569–576

Levin SV, Irlyna IS, Krol AYu (1984) The surface movement of the deciliated infusoria Paramecium caudatum. Cytology 26:1073 (in Russian)

Levin SV, Golfand KA, Malev VV (1986) The wave of local shortening and lengthening of crab axon during action potential. Cytology 28:1307–1315

Waugh RE (1987) Effect of inherited membrane abnormalities on the viscoelastic properties of erythrocyte membrane. Biophys J 51:363–369