Biochimica et Biophysica Acta, 554 (1979) 76—89

BBA 78394

INSTABILITY DEVELOPMENT IN HEATED HUMAN ERYTHROCYTES

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(Received October 2nd, 1978)

Key words: Stress; Hyperthermia; Surface wave; Liquid jet; Surface tension; (Erythrocyte membrane)

Summary

Heated human erythrocytes gradually lose their form-maintaining structure as the temperature is increased to 50°C and can behave in some respects as a viscous fluid. We have developed a technique for heating and stressing these cells that is novel, simple and quantitatively precise. We have applied this technique to heated human erythrocytes and have measured instability development in the cells. We have employed instability growth theory to calculate a value for an effective surface tension which, in contrast to other methods of membrane surface tension measurement sought to minimize the effects of membrane supporting structural elements. The value obtained for the surface tension of the heated erythrocyte membrane was $0.9 \cdot 10^{-6}$ N/m with a range of variation from $0.4 \cdot 10^{-6}$ N/m to $1.4 \cdot 10^{-6}$ N/m. The methods described may be useful for determining fundamental physical parameters such as internal viscosity and interfacial tension in other systems.

Introduction

Since the work of Schultze [1] in 1865, it has been known that morphological changes occur in human erythrocytes that have been heated to temperatures near 50°C. These morphological changes are probably due to the inactivation of the sub-membrane reticulum [2,3] so that the originally elastic erythrocyte behaves in a manner similar in some respects to a viscous drop [4]. We have

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previously examined fragmentation patterns, haemolysis and microvesiculation of human erythrocytes heated to temperatures in the range 48°—75°C [5]. We also showed [6] that heated erythrocytes that were attached to glass could be stressed by a flow of liquid over the cells to form long membrane-bound tethers that were beaded along their length. It was suggested that these varicosities were due to the development of instabilities in the liquid-like tether as it was drawn out, much like the break-up into droplets of jets of one liquid immersed in another. Measurements were made from scanning electron micrographs of the ratio of length to diameter of the periodically spaced vesicles. The measured ratio was less than that predicted by theory for the break-up of a cylindrical liquid jet.

In the present paper we describe a new experimental technique for stressing cells in isolated conditions at various temperatures. Consequently we can now completely describe the hydrodynamic parameters of interest when the cells are stressed. The technique also allows us to obtain cinephotomicrographs of the cells while under stress so that a greater understanding has been obtained of the processes leading to the production of these vesicles, previously examined in scanning electron micrographs of fixed samples [6]. The theory for the breakup of a cylindrical liquid form is presented in some depth. From measurements of the growth rate of instabilities in the stressed erythrocyte we have been able to calculate values for the surface tension of the heated erythrocyte.

Materials and Methods

Fresh human blood was collected from donors into phosphate buffered saline (150 mM NaCl in 5 mM sodium phosphate buffer, pH 7.4). Samples of the cell suspension were diluted to $5 \cdot 10^7$ cells/ml, stored at room temperature, and used within 1 h.

In order to obtain cinephotomicrographs of the response of the erythrocytes to stress a specially designed heating stage was constructed. This stage consisted of two T-piece electrodes attached to a microscope slide; between the electrodes was positioned a precision microcapillary that contained a short length of the cell suspension (Microslides, Camlab, Ltd.). Near the viewing area was located a small bead thermistor (U23UD, Farnell, Ltd.) that enabled us to monitor the temperature of the cell suspension. Degassed buffer served as an electrolyte, the viewing area was covered with a cover slip, and the liquid between the electrodes was heated by 20 kHz current from a 6 W oscillator (Dawe Ltd., type 440B) with a 600 Ω output impedance. Fig. 1 shows a block diagram of the experimental arrangement. As the erythrocytes were heated to temperatures above 49°C morphological changes could be observed to commence. At this stage, a drop of buffer or a solution of 6% glutaraldehyde in buffer was brought to touch the liquid column in the partially filled microcapillary. The surface tension forces would then pull the liquid through the microcapillary, stressing the cells in a controlled manner.

An important aspect of this experiment was the controlled means of stressing the heated erythrocytes. An appropriate analysis of the various hydrodynamic parameters associated with flow through the microcapillary is presented in an appendix.

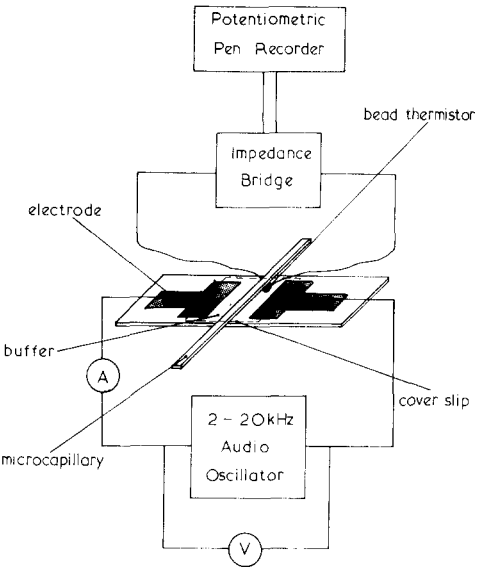

Fig. 1. Block diagram of experimental arrangement for the controlled heating and stressing of cells.

Theory

The analysis of the breakup of a liquid jet due to growth of instabilities has been extensively developed since the original paper by Lord Rayleigh in 1879 [7]. Recently, Meister and Scheele [8] have examined the generalized problem and have presented criteria for selecting which solution for limiting combinations of high and low viscosities and densities of the continuous and disperse phases is most appropriate for particular cases. They show that one can expect the theory of Tomotika [9], who studied the breakup of a high viscosity jet in a high viscosity liquid, to apply within an error of 5% provided the following equations are obeyed:

$$(\gamma\rho D)/(\eta)^2 < 0.133 \text{ and } (\gamma\rho' D)/(\eta')^2 < 0.4 \quad (1)$$

where γ = interfacial tension, ρ', ρ are the densities of the jet and surrounding liquid respectively, D is essentially the diameter of the jet and η', η are the dynamic viscosities of the jet and the surrounding liquid respectively. Although the correct values of γ and η' to be used for an erythrocyte at 50°C are not precisely known, we shall use $\gamma = 10^{-6}$ N/m and $\eta' = 2.75 \cdot 10^{-2}$ P and comment on their appropriateness later. If these values are used, together with $D = 0.5$ μm, one finds that the requirements of Eqn. 1 are easily met and the special approach of Tomotika [9] is applicable.

In order to solve the problem of the breakup of a cylinder of liquid, Tomotika assumed that random density and pressure fluctuations would lead to small periodic disturbances in the radius r of the cylinder given by

$$r = a + \epsilon_0 e^{(\alpha+i\omega)t+ikz} \quad (2)$$

where a is the undisturbed radius, ϵ_0 is a small amplitude fluctuation, α is the rate of growth of the disturbance, ω is the angular frequency and $k = 2\pi/\lambda$

where λ is the wavelength of the disturbance. Tomotika found that the growth rate α depended upon the interfacial tension γ, the radius of the jet a, and the viscosities of the two liquids η, η' through the equation

$$\alpha = \gamma(2\eta a)^{-1}(1 - k^2a^2)\phi(ka, \eta'/\eta) \tag{3}$$

where $\phi(ka, \eta'/\eta)$ is a complicated function of ka and η'/η involving the modified Bessel functions I_0, I_1, K_0 and K_1.

We have evaluated the function $\phi(ka, \eta'/\eta)$ with a computer for various values of the viscosity ratio η'/η. Dintenfass [10] has estimated the internal viscosity of the human erythrocyte at 37°C to lie within the range $1.0 \cdot 10^{-2}$ P to $6.0 \cdot 10^{-2}$ P. Assuming the same temperature dependence for the viscosity of the erythrocyte as for water, we find that for a temperature of 50°C, η'/η should lie between 1.4 and 8.6 with an average value of 5.0. Fig. 2 shows the predicted variation of the dimensionless parameter $\alpha/(\gamma/2\eta a)$ with ka and λ/D for three values of the viscosity ratio η'/η. Note that the Tomotika theory predicts a maximum growth rate for intermediate values of ka. In particular one should expect that for a viscosity ratio of 5.0, disturbances with $ka = 0.46$, or a wavelength to diameter ratio of 6.8 should be those that grow most rapidly. Thus, if cylindrical erythrocytes behave like liquid cylinders they should break up into beads by the growth of waves with a wavelength to diameter ratio of 6.8. Allowing for the range of variation of η'/η one should expect the λ/D ratio to lie somewhere between 5.7 and 7.4. It is to be noted that even for extreme values of η'/η the lowest value of λ/D possible is 5.5.

In order to estimate values of the experimental growth rate we need only specific values for the surface tension γ and radius of the cylinder a. Adams [11] has estimated the surface tension of human erythrocytes to be approximately 10^{-6} N/m. A typical radius of an erythrocyte tether would be 0.25 μm. For a viscosity ratio of $\eta'/\eta' = 5.0$, one would then expect a growth rate α on the order of 100 s^{-1}; a growth time of three time constants would thus be about 30 ms.

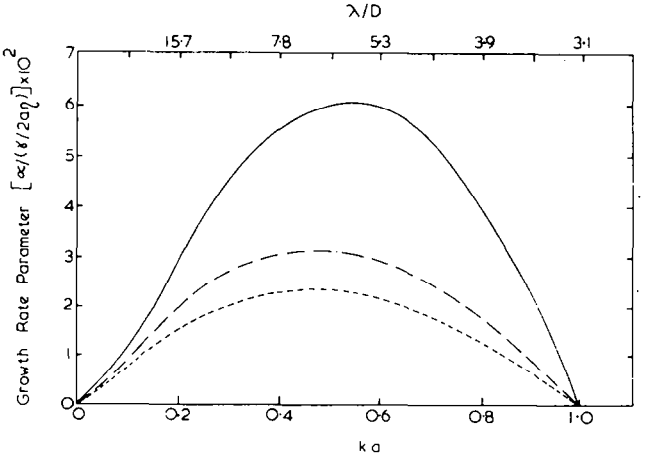

Fig. 2. Variation of the dimensionless growth-rate parameter $\alpha/[\gamma/2a\eta]$ with ka and λ/D for three values of the viscosity ratio; ——— $\eta'/\eta = 1.4$; · · · · · $\eta'/\eta = 5.0$ and - - - - - - $\eta'/\eta = 8.6$.

Experimental results

The incubation times and temperatures as well as the values of the stressing parameters strongly influence the response of the cells to stress. Regularly beaded tethers were produced when cells were incubated for approximately 120 s at 52°C, and then were stressed with an initial velocity of $1.7 \cdot 10^{-2}$ m/s and a shear stress of 0.46 N/m^2. When the stressing buffer contained glutaraldehyde: the times for which the cells were stressed before the glutaraldehyde reached the point at which the cells were examined was approximately 2 s. Further details of the specific requirements for producing tethers in heated erythrocytes will be presented elsewhere [12].

Examination of films of the cells under stress showed that cells which showed signs of undergoing morphological change before application of the stress were most likely to pull out in the flow. A reorganization of cell shape in the stress field often occurred over a period of the order of 0.5 s before a tether was produced and the main cell body moved along in the flow.

Fig. 3 shows a scanning electron micrograph of an area of stressed erythrocytes from a part of an opened microcapillary. There was some variation in the response of the cells to stress and some cells have produced long beaded tethers. Fig. 4, with a higher magnification, shows regularly spaced vesicles along an erythrocyte tether. We have produced over a hundred microcapillaries that have regularly spaced vesicles and have examined them with light microscopy immediately after production as well as with a scanning electron microscope after preparation. The results of this study indicate that the λ/D ratio obtained

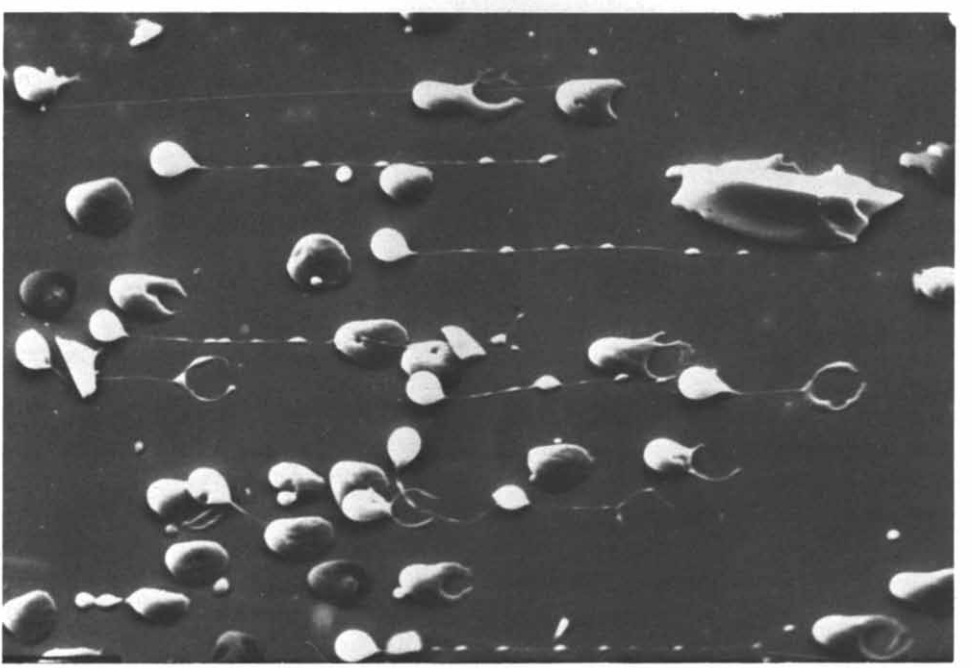

Fig. 3. Scanning electron micrograph of an area of erythrocytes stressed in a microcapillary. Some cells have pulled out long beaded tethers while others are in different stages of development.

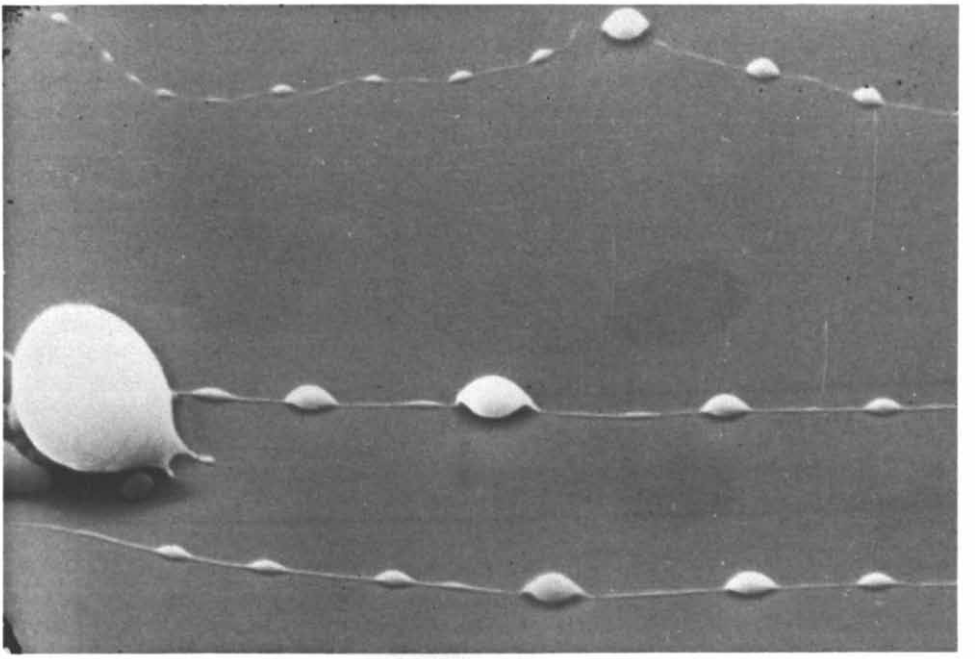

Fig. 4. Scanning electron micrograph of a group of tethers with regularly spaced beads.

from various measurements is in the range of 12—15; this contrasts with measurements reported previously [6] of a λ/D ratio in the range 2.5—4.5 when a different stressing technique was employed.

Figs. 5 and 6 are cinephotomicrographs showing the history of the response of some cells to stress. In Fig. 5 it is seen that the cell is at first distorted into a tear-drop shape and then rapidly pulls out a long cylindrical tether that breaks up as the tether is extended. This behaviour explains the high λ/D ratios in this experiment; there is extension after breakup. The extended tether is then fixed by the glutaraldehyde. The extended cell would retract considerably leaving a line of proximate vesicles, when the flow stress fell to zero. The cinemicrographs suggest an explanation for the differences in λ/D ratio obtained from cells such as those in Fig. 4 and the results obtained previously [6] in that the cells appear to have been fixed at different stages of the stress history of the tethers.

The typical sequence of tether production in Fig. 5 shows that drops are already forming as the cell moves rapidly down field. The time required to pull out a tether of 10 μm (20 D) is of the order of 100 ms for the flow rates used in this study. Our fastest available filming rate with high speed film was approximately one frame per 30 ms which again restricted our ability to resolve growth times. The ring of material left behind on the glass as the cell was swept away (Fig. 5) was usually of larger diameter than the tether, the growth times were slower, and since the ring was not being translated in the flow the growth times and spacings were measurable. A ring of diameter much greater than its thickness can be treated approximately as a cylinder. We have obtained 8 measurements of λ/D and T_g from the breakdown of rings such as in Fig. 5. Further,

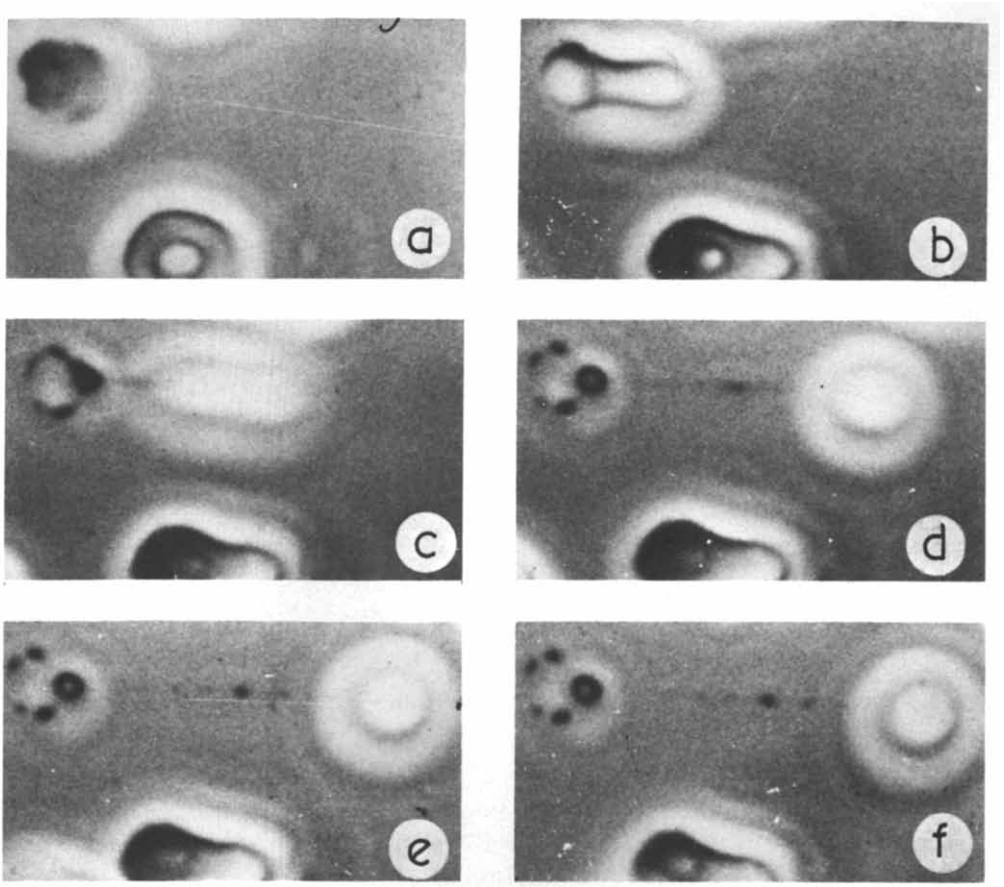

Fig. 5. Cinephotomicrographs of erythrocytes being stressed in a microcapillary, speed 16 frames/s. The frames are sequential with a time interval of approximately 62.5 ms between frames. (a) Stress has just commenced, (b) cell is distorted by flow, (c) cell pulls loose from glass and rapidly pulls out a tether; note the partially broken ring, (d) tether is difficult to resolve; ring has mostly broken up, (e) beads are now discernible in tether (f) cell will soon be fixed in this position by arrival of glutaraldehyde.

Fig. 6 shows a large tether observed in the process of breakup, enabling a measurement to be made of the λ/D ratio and the growth time. From measurements of the 8 rings and the single tether we obtain an average value of λ/D = 3.6 with a S.D. of 0.4. With these values λ/D and T_g it was possible to obtain an experimental estimate for the surface tension of the heated erythrocyte with its form-maintaining structures weakened. In Fig. 7 are plotted values of the growth time, T_g, based on an estimate of the time required for an instability to grow to completion, versus the diameter of the cylinder in which the instability grew. The error bars represent an estimate of the precision of the measurements and are related to the framing rate of the camera. We have obtained a best fit straight line that goes through the origin by averaging the slopes of the individual points. Using this value for the slope, the average of the measured values of λ/D and Eqn. 3, we can obtain a value for the surface tension. For a viscosity ratio of 5.0 our estimate of γ was $0.93 \cdot 10^{-6}$ N/m; for $\eta'/\eta = 8.6$ we obtain $\gamma = 1.4 \cdot 10^{-6}$ N/m; for $\eta'/\eta = 1.4$ we obtain $\gamma = 0.40 \cdot 10^{-6}$ N/m.

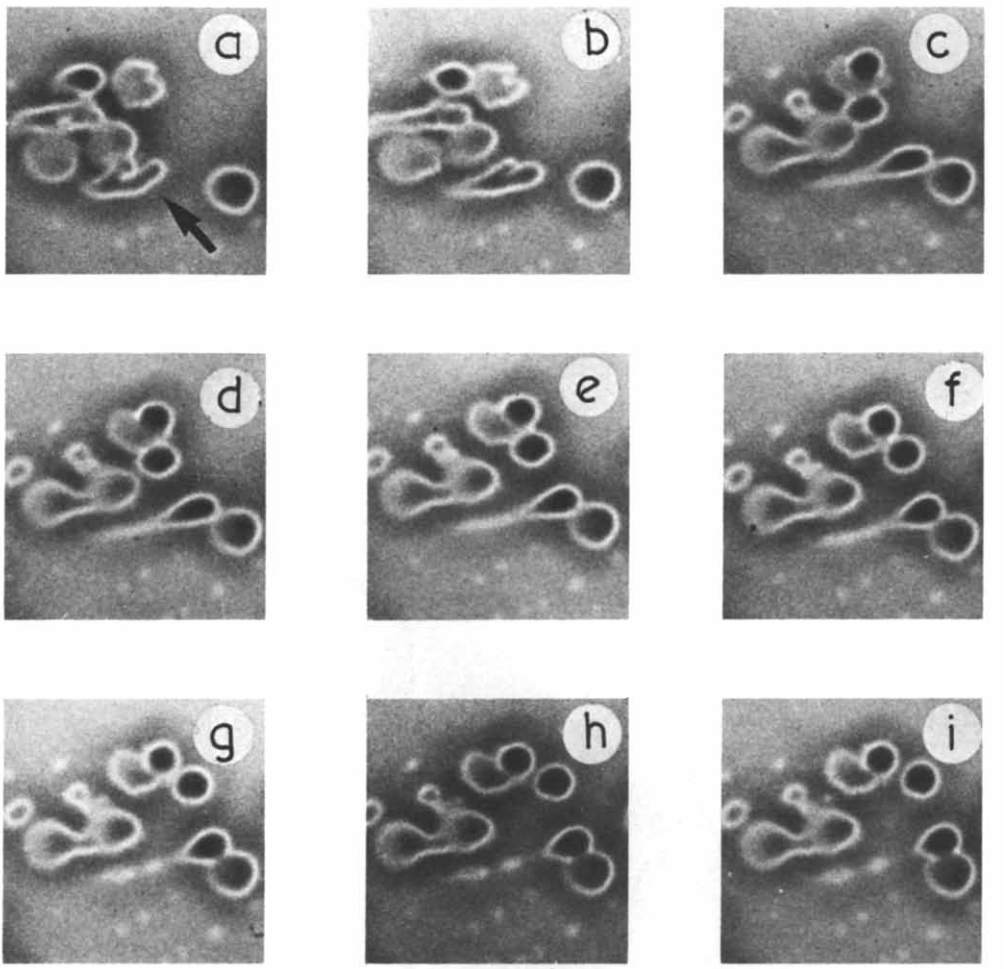

Fig. 6. Cinephotomicrographs of erythrocytes being stressed in a microcapillary, speed 32 frames/s. The frames are sequential with a time interval between frames of approximately 31 ms. In frames a, b, and c the erythrocyte has been drawn out into a short thick tether. In frames d, e, and f indications of beading are beginning to appear. By frames h and i the instability has grown through the tether and the beads are beginning to round. The cells were stressed by flow of PBS.

An observation reported earlier [4,5] that was consistently made in examining the fragmentation of heated erythrocytes was that they often fragmented into two or three pieces. Fig. 8 shows such a fragmentation sequence. It is noted that this particular mode of fragmentation is much like the breakup of a cylinder. Many similar observations were made of erythrocyte fragmentation sequences that seemed to be governed by liquid cylinder breakup. It was noted in a number of cases that the development of instabilities did not occur on the extended portions of the cell until the ratio of wavelength to diameter was of the order of $\pi : 1$. The strong dependence of α on λ/D when $\lambda/D \approx \pi$ (Fig. 2) made quantitatively analysis of this form of fragmentation impractical.

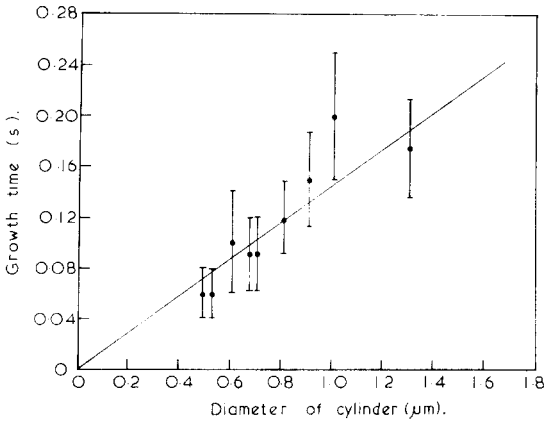

Fig. 7. The time required for erythrocyte cylinders of different diameters to break up into regular beads. The error bars are an estimate of the uncertainty in the time measurement due to the relatively low framing rate. The straight line is a best fit to the measurements with the requirement that it passes through the origin. Eqn. 3 predicts a linear relationship between these variables.

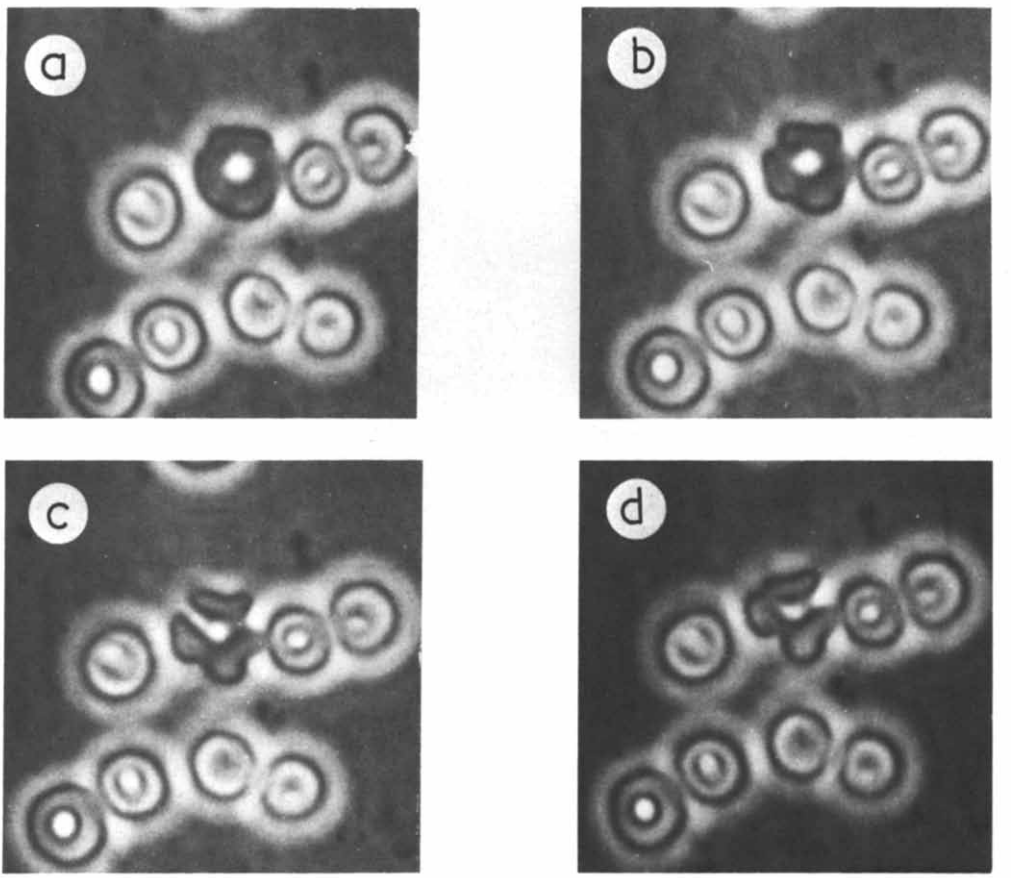

Fig. 8. Fragmentation sequence for heated erythrocytes in a microcapillary. These cells were not stressed except for small thermal currents that may have existed. (a) time t taken as zero; note that the dimple has collapsed and the cell shows indentation on its rim. (b) t = 1 s, fragmentation commences and the cell appears to be breaking into 3 pieces. (c) t = 3 s. The indentations have broken through the annular ring on the top. (d) t = 5 s. Fragmentation is nearly complete but the pieces are still connected by the membrane.

Discussion

If an erythrocyte is maintained at 52° C for 120 s the form-maintaining structures are weakened to such an extent that the erythrocyte may be rapidly deformed into an unstable configuration that lends itself to instability analysis. Instability analyses given in the literature concern cylindrical jets of liquid in a gas or another liquid. In the case of membrane tethers we deal with membrane tubes filled with solution rather than with cylinders made of membrane material. We take a 'black-box' view of the tether and see it as a system which behaves as though it had a particular effective interfacial tension at its interface with the buffer. When the tether diameter is large compared with the membrane thickness the viscosity of the haemoglobin solution can be taken as the viscosity of the cylinder. The same 'black-box' view of the tether enables us to include the contribution of the subreticulum structural elements to tether behaviour when relating viscoelastic behaviour of the tethers to the treatment of viscoelastic cylinders by Goldin et al. [13]. These authors have modified the approach of Weber [14] to the problem of the breakup of a viscous liquid cylinder in a gas to provide a general treatment of the breakup of viscoelastic jets in air. The neglect of the viscosity of the continuous phase makes their detailed results inapplicable to our case but some general features of their conclusions are of interest. In highly elastic liquids the jet breakup is dominated by non-linear effects and the disturbances appear as a series of droplets connected by random lengths of threads which thin with distance. Weakly elastic solutions initially show a growing wave with a clearly defined wavelength. The growth of the wave is arrested before breakup and a string of regularly spaced droplets connected by thin threads is formed indicating their formation from a wave of constant wavelength. The growth rates were found to lie between those predicted for an inviscid jet and those for a Newtonian fluid of the same zero shear viscosity. In our case the internal viscosity of the erythrocyte is considered to be independent of shear stress and we therefore expect the Tomotika analysis to be most appropriate for the analysis of those cases where structural elements have been weakened sufficiently to produce regularly spaced beads. Examination of the case where the elasticity has a larger effect is treated elsewhere [12]. The general instability theory of Tomotika for the breakup of one long viscous liquid cylinder immersed in another viscous liquid predicts that the heated erythrocyte pulled into a cylindrical tether of 0.5 μm diameter should break up with a λ/D ratio greater than 5.5. The major problem in obtaining reliable estimates of the λ/D ratio from fixed tethers was the observation, made with cinephotomicroscopy, that elongation and contraction of the tether occurred after breakup. We were successful in obtaining some measurements of the λ/D ratio from our cinephotos which gave us a value of 3.6 ± 0.4. This value is about half the value of 6.8 predicted by the Tomotika theory but is consistent with earlier results [6]. We submit three possible reasons for this low value of λ/D. First, calculations and measurements by Huebner and Chu [15] and by others indicate that the presence of surface charge may lower the ratio significantly. Our own calculations tend to reject this argument but it can not be fully discounted as a possible explanation. Second, harmonics of the fundamental wavelength have been measured by

other observers if the systems respond non-linearly [16] and a harmonic of the fundamental wavelength is consistent with our observations of $\lambda/D = 3.6$. Third, the experimental system studied here contrasts with the theoretical model in that the erythrocyte cylinder is covered by an essentially incompressible membrane [17] that must conserve surface area. Thus, suppose a cylinder does break up with the predicted wavelength to diameter ratio of 6.8. There is now a cylinder that is still unstable (any length greater than π times its diameter is unstable) and a cylinder of length 6.8 D has 50% more surface area than a sphere of the same volume. It thus seems reasonable that divisions would occur that would result in smaller values of λ/D. Previous studies have shown that larger vesicles in which λ/D tended to be as large as 4.5 were usually dumbell-shaped [6] as if they would have fragmented if sufficient surface area had been available. Vesicles with λ/D greater than 4.5 have sufficient membrane area to break into smaller fragments.

Difficulty was also encountered in determining the growth rate due to the short time required for the instability to develop. The time required to pull out a tether of 10 μm (20 D) is on the order of 100 ms for the flow rates used in this study. Since predicted growth times using our estimates of λ/D are on this order of magnitude, most tethers appeared to break up as they were pulled out. The limited number of data points obtained appear to be consistent with the theory in that the growth time increases with the diameter of the cylinders as shown in Fig. 7.

We have derived a value for an effective interfacial tension of the heated erythrocyte membrane. The membrane was in such a condition that the elements which normally bestow elasticity on the cell envelope were weakened to an extent which allowed regular instability growth. Any active sub-reticular structure which remained after heating could cause us to underestimate the surface tension. The effective surface tension is unlikely to be less than our estimate since a lower value of γ would lead to a higher value of T_g and such a higher value would have been detected in our experiments. Our experiments endeavoured to measure an effective surface tension which, in contrast to other methods of membrane surface tension measurement, sought to minimize the effects of membrane supporting structural elements. As a consequence it probably represents a better estimate of the surface tension of the membrane itself.

Evans and Hochmuth [17] have examined the responses of erythrocytes to stress and have derived a number of mechanical constants which they associate with the structural components of the intact membrane. They consider that the lipid bilayer exerts little influence on the response of the membrane to stress. Our studies examine a membrane property which is expressed when the structural elements are weakened. We have previously drawn attention to a similar expression of interfacial tension on unstable membrane shapes when the structural elements in a number of cells have been weakened by excess pressure, mechanical contact, exposure to high salt and exposure to rapidly applied stress at room temperature [6]. These phenomena suggest that we are not examining a membrane property which is expressed only at high temperatures. We argue that a complete description of the dynamic behaviour of membranes under all circumstances requires an understanding of the interfacial properties of the

membrane as well as the material properties of its structural elements.

In summary, we have presented an experimental technique for heating and stressing cells that is novel, simple and quantitatively precise. We have applied this technique to heated human erythrocyte and examined instability development in the cells. Modest confirmation of the theory was observed which suggest this method may be useful in determining fundamental physical parameters such as internal viscosity and interfacial tension in other systems.

Appendix

The microcapillaries described here provide an environment in which labile heated cells are protected from thermal currents which encourage cell fragmentation and haemolysis in more exposed systems [5]. This protective property has enabled us to study stress effects on cells at temperatures of 55°C [12] without difficulty while a previous study of stress effects on heated erythrocytes was limited to 50°C because of problems at higher temperatures with cell fragmentation in the flow channel used [18]. Because of the utility of the system we include here a general treatment of the hydrodynamic characteristics of flow in a rectangular capillary of length L containing an initial length of liquid z_0, and immersed to a depth h as shown in Fig. 9. When the liquid in the column is free to move under capillary action the equation of motion of the column of liquid is given by a balance of the forces exerted at a particular point, say the air-liquid interface. These forces are the surface tension force F_γ, the retarding liquid drag force F_d, the gravitation force F_g and the force of inertia F_i. The vector sum of the forces in then set equal to zero:

$$F_\gamma - F_d - F_g - F_i = 0 \tag{A1}$$

Without derivation, we express the composition of these forces as

$$2\gamma d \cos\theta - 12\eta z d a^{-1}\bar{z} - (z\cos\beta - h)(da\rho g) - da z\rho\ddot{z} = 0 \tag{A2}$$

where γ is the surface tension, θ is the contact angle of the liquid-glass interface, d is the width and a thickness of the gap in the rectangular capillary, η is the viscosity of the liquid, z is the position of the interface measured from the loaded end of the capillary, $\bar{z}$ is the average velocity of the column of liquid

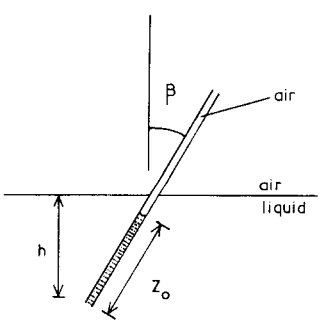

Fig. 9. The general case for the immersion of a microcapillary containing a column of liquid prior to application of flow stress.

and thus of the air-liquid interface and β is the inclination of the microcapillary from the vertical. The density of the liquid is denoted by ρ, g is the acceleration of gravity and $\ddot{z}$ is the acceleration of the liquid interface (we have assumed that $a/d << 1$). Since the effect of inertia in the microcapillary is quite small, we set $\ddot{z} = 0$, divide through by d in Eqn. A2 and rearrange to obtain the velocity of the air-liquid interface as a function of the position of the interface. This velocity is equal to the average velocity of the liquid in the column, and is given by

$$\bar{\dot{z}} = [(\gamma a \cos\theta)/6\eta + h\rho a^2 g/12\eta]z^{-1} - (\rho a^2 g \cos\beta)/12\eta \quad \text{(A3)}$$

Note that the liquid reaches an equilibrium height in the column given by setting $\bar{\dot{z}} = 0$ and solving for z, to be

$$z_e = h/\cos\beta + (2\gamma \cos\theta)/\rho a g \cos\beta \quad \text{(A4)}$$

This equation allows us to determine $\cos\theta$, where θ is the effective liquid-glass contact angle.

In order to obtain the shear stress, we need to know the velocity as a function of position across the thickness of the capillary. This velocity is related to the average velocity by

$$\dot{z} = 6\bar{\dot{z}}(ya^{-1} - y^2 a^{-2}) \quad \text{(A5)}$$

Eqn. A5 describes the velocity profile, which is expected to be parabolic, where y is measured from the wall of the capillary. The shear stress is obtained by the equation $\tau = \eta d\bar{\dot{z}}/dy$ and is thus given by

$$\tau = [(2\gamma \cos\theta + h\rho a g)z^{-1} - \rho a g \cos\beta](\tfrac{1}{2} - y/a) \quad \text{(A6)}$$

It should be noted that for distances near the wall such that $y << a$, the shear stress is essentially constant.

A final parameter of interest is the time required for the interface to advance from its initial position z_0 to a position z. In a situation where the incoming fluid contains a fixative this expression is related to the time required for the fixative to advance a distance $z - z_0$. This time can be obtained from a direct integration of Eqn. A3 to be given

$$t = \delta\xi(g\cos\beta)^{-2} \ln[(\xi - gz_0 \cos\beta)(\xi - gz\cos\beta)^{-1}] - \delta(g\cos\beta)^{-1}(z - z_0) \quad \text{(A7)}$$

where $\delta = 12\eta/\rho a^2$ and $\xi = (2\gamma\cos\theta)/\rho a + gh$

The value of t for the horizontal case can be obtained by taking limits as $g\cos\beta \to 0$. However, a simpler expression for t can be easily derived for the horizontal microcapillary when only the first two terms of Eqn. A2 are considered.

Experimental confirmation of the above equations has been obtained by cinephotography of the motion of the liquid interface.

Acknowledgements

J.O.T. Deeley is a research assistant supported by the Science Research Council. L.A.C. is grateful to the U.S. Naval Academy and the Office of Naval

Research for providing leave and financial support during his sabbatical enabling him to participate in this research. We are grateful to the Department of Zoology and the Centre for Educational Technology for advice and assistance with the microscopy.

References

1 Schultze, M. (1865) Arch. F. Mikorscop. Anat. 1, 1—42
2 Hainfeld, J.F. and Steck, T.L. (1977) J. Supramol. Struct. 6, 301—311
3 Brandts, J.F., Erickson, L., Lysko, K., Schwartz, A.T. and Taverna, R.D. (1977) Biochemistry 16, 3450—3454
4 Ponder, E. (1950) J. Exp. Biol. 27, 198—209
5 Coakley, W.T., Bater, A.J., Crum, L.A. and Deeley, J.O.T. (1979) J. Therm. Biol. 85—95
6 Coakley, W.T., Bater, A.J. and Deeley, J.O.T. (1978) Biochim. Biophys. Acta 512, 318—330
7 Rayleigh, Lord (1879) Proc. London Math. Soc. 10, 4—13
8 Meister, B.J. and Scheele, G.F. (1967) A.I. Ch.E. Journal 13, 682—688
9 Tomotika, S. (1935) Proc. R. Soc. (London) A150, 322—337
10 Dintenfass, L. (1968) Nature 219, 956—958
11 Adams, K.H. (1973) Biophys. J. 13, 209—217
12 Deeley, J.O.T., Crum, L.A. and Coakley, W.T. (1979) Biochim. Biophys. Acta 000, 000—000
13 Goldin, M., Yerushalmi, J., Pfeffer, R. and Shinnar, R. (1969) J. Fluid Mech. 38, 689—711
14 Weber, C. (1931) Z. Angew Math. Mech. 11, 136
15 Huebner, A.L. and Chu, H.N. (1971) J. Fluid Mech. 49, 361—372
16 Rutland, O.F. and Jameson, G.J. (1971) J. Fluid Mech. 46, 267—271
17 Evans, E.A. and Hochmuth, R.M. (1977) J. Membrane Biol. 30, 351—362
18 Williamson, J.R., Shanahan, M.O. and Hochmuth, R.M. (1975) Blood 46, 611—624