Biochimica et Biophysica Acta, 512 (1978) 318—330

BBA 78139

VESICLE PRODUCTION ON HEATED AND STRESSED ERYTHROCYTES

WILLIAM TERENCE COAKLEY, ANTHONY JACK BATER and JOHN OWEN THOMAS DEELEY

Microbiology Department, University College, Newport Road, Cardiff CF1 1TA (U.K.)

(Received February 27th, 1978)

Summary

Human erythrocytes develop vesicles by budding when heated to temperatures close to the thermal transition for spectrin. Regularly spaced strings of vesicles also develop if cells heated to 51—54°C are pulled into unstable shapes by flow of liquid between cover slips. These strings of vesicles develop when cells which had attached to the glass are restrained in the flow by a long membrane-bound tether which maintains a connection with the attachment site on the glass. Breakup into regularly spaced vesicles suggests the breakup of a liquid-like cylinder by growth of Rayleigh instabilities. The ratio of length : diameter of the fragments of cylinder on which each disturbance grew ranged from 2.2 to 5.4 to 1 with a peak of 3.2, as measured from scanning electron micrographs. The upper limit of the range is slightly less than the ratio for the disturbance most likely to grow if surface tension and viscosity alone controlled the vesicle formation. Similar vesicle formation when the form-maintaining structures were weakened has been reported in other systems.

Introduction

The morphological changes which can occur in heated human erythrocytes have been described by Ham et al. [1] and by Ponder [2]. These changes include (i) the appearance of small bud-like protrusions on some cells, (ii) multiple buds which are usually connnected by a broad base or filament, (iii) the conversion of the majority of the cells to spherical fragments on continued heating, (iv) occasional ghosts and many polymorphic forms and, (v) the accomplishment of cell subdivision without haemolysis. Ponder [2] suggested that the fragmentation took place during a transition of the red cell from a body with elasticity through a plastic stage to a cell which behaved like a highly viscous fluid. In more recent work protein transitions in heated human erythrocytes [3], the effects of thermal transitions on the elasticity of human erythrocyte membranes [4,5], and the effects of shear stress on heated cells

[6] have been examined. However, the mechanisms involved in the process of fragmentation of heated erythrocytes have received little attention. We are presently engaged in a study of the fragmentation of heated erythrocytes using cinephotomicrography and the technique of scanning electron microscopy which was not available to the earlier investigators of fragmentation [1,2].

We pay particular attention in this report to those cases where chains of vesicles develop on parts of cells which have been drawn into cylinder-like unstable shapes. For the first time measurements have been made of the spacing of such vesicles and the results have been compared with theoretical predictions for the breakdown, by the growth of surface instabilities, of a cylinder of one liquid immersed in a second liquid. We also bring together a number of reports of similar beading on the membranes of other cells and suggest that the beading in these systems may also arise from Rayleigh instability growth when the form-maintaining structures are weakened.

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). The cell suspension was diluted to 10^7 cells/ml, stored at 37° C and treated within one hour. Samples of 3 ml were incubated in 10-ml test tubes at various temperatures in an aluminium heating block. After an interval the cells were fixed while in the heating block, by addition of an equal volume of phosphate-buffered saline containing 6% glutaraldehyde. The fixed cells were washed and aliquots were air dried and prepared for scanning electron microscopy examination (Cambridge Stereoscan 600). For cinephotomicrography two drops of the diluted blood were heated under a glass cover slip on a microscope slide mounted onto a heating block on a microscope stage. The temperature of some samples was measured by placing a fine copper/constantan thermocouple between the cover slip and microscope slide. Temperature equilibrium was attained within 45 s. However, since temperature gradients existed across the samples a temperature range is quoted which gives the upper and lower limits of the temperatures measured with the thermocouple.

To apply gently flow stress to heated cells two drops of cell suspension were placed between two cover slips on the heated microscope stage and, after temperature equilibration, a drop of heated phosphate-buffered saline containing 6% glutaraldehyde was brought into contact with the side of the cover slips. The resulting flow of glutaraldehyde solution both stressed and fixed the red cells. The stressed samples were air dried. The cover slips were sectioned with a glass cutter and the sections were prepared for scanning electron microscopy.

Results

Cells heated on the microscope stage to temperatures in excess of 50° C rapidly lost their biconcave shape. Vesicles were soon observed budding from the cells but remaining attached to the cell body. A single vesicle on cell i is

shown in Fig. 1a. A Stokes drag force from thermal currents between the cover slips helped the development of a second vesicle during the 12 s progression to Fig. 1b. The influence of local thermal flow (not externally applied flow) on the development of the second vesicle was clear since the chain developed in the direction of the flow, as determined on the cinephotomicrographs by the direction of movement of free erythrocytes. In Fig. 1c the second vesicle on cell i has been drawn out into a cylindrical shape while a large vesicle has been produced from the fragmenting cell ii. In Fig. 1d the cylindrical form of the vesicle on cell i has broken down into two vesicles while the elongation of cell iii has progressed to the development of a neck. This neck elongated in Fig. 1e and a vesicle has developed following the collapse of the ends of the neck in Fig. 1f. The transition from a neck to a clear vesicle occurred in about 2 s. As many as four spherical vesicles were observed by light microscopy along the necks of cells which were fixed following heating in a test tube at 50°C for 10 min.

It has been shown elsewhere that heated erythrocytes become significantly more susceptible to the effects of flow stress at temperatures in excess of 48°C [4—6] and it has been suggested that a thermal alteration in the structure of spectrin would account for the observed effect [3—5]. When a cell loses its biconcave shape the normal erythrocyte area and volume of 138 μm^2 and 94 μm^3, respectively, [7] are such that two or more main spherical bodies and a number of spherical vesicles of a smaller order than the main products could be formed. There is an abundance of elongated doublet and triplet cell forms which have developed as on cell iii in Fig. 1, in micrographs of cells heated in a test tube at 51°C (Fig. 2, a and b). Fig. 2c shows a micrograph of a divided erythrocyte from a sample heated in a test tube at 51°C for 4 min and fixed with glutaraldehyde. The two spherical ends are joined by a tubular neck on which perturbations are visible.

The development of vesicles along a neck, particularly when a single vesicle grows on the centre of the neck as in Fig. 1f, suggested that we might be observing the growth of an instability on a cylinder of one fluid immersed in another. To further investigate the possibility that such instabilities could grow on membrane-bound cylinders, cells between cover slips on a heated stage were stressed by flow of phosphate-buffered saline containing glutaraldehyde and prepared for scanning electron microscopy as previously described. Since the extent of the flow stress between the cover slips varied from point to point on the cover slip, response of the cells to the flow varied with position. Cells in any one area of the cover slip showed similar behaviour so that the variation in response was due more to physical variation in the flow patterns than to biological variation in the cell structure. At certain points on the cover slips cells which had attached to the glass surface were pulled along in the flow and some developed long tethers which restrained them. Examples of the break-down of such tethers into vesicles are shown in Fig. 3. Fine connections can be seen between some of the vesicles. The vesicles were commonly ovoid or dumb-bell in shape. The beaded tethers of Fig. 3 were not artefacts of the air drying procedure employed in the preparation for scanning electron microscopy since beaded tethers were observed both in cinephotomicrographs of cells while being stressed by flow of phosphate-buffered saline alone, and when wet

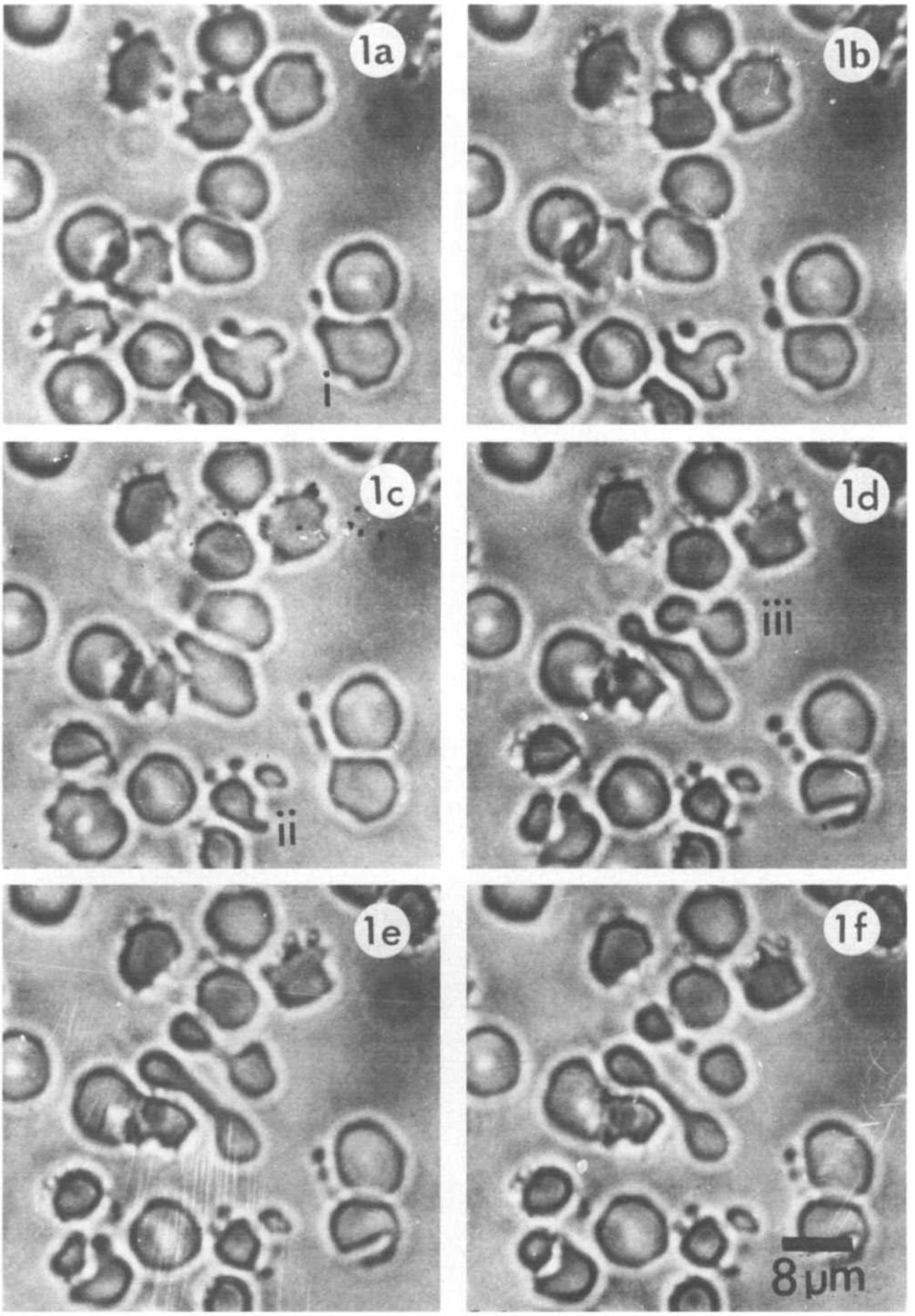

Fig. 1. Cinephotomicrographs of erythrocytes heated to 51—54°C, frame speed 2 frames/s; magnification ×1300 (a) time t taken as zero. A vesicle has developed on cell i. (b) t = 12 s. A second vesicle has been pulled from cell i. (c) t = 27 s. The second vesicle on cell i has been pulled out into a cylindrical form, a vesicle has been formed from a protrusion on cell ii. (d) t = 55 s. The cylindrical form of the second vesicle on cell i has broken into two vesicles, a neck is developing on cell iii. (e) t = 70 s. The neck on cell iii increases in length. (f) t = 90 s. A vesicle has developed on the neck of cell iii.

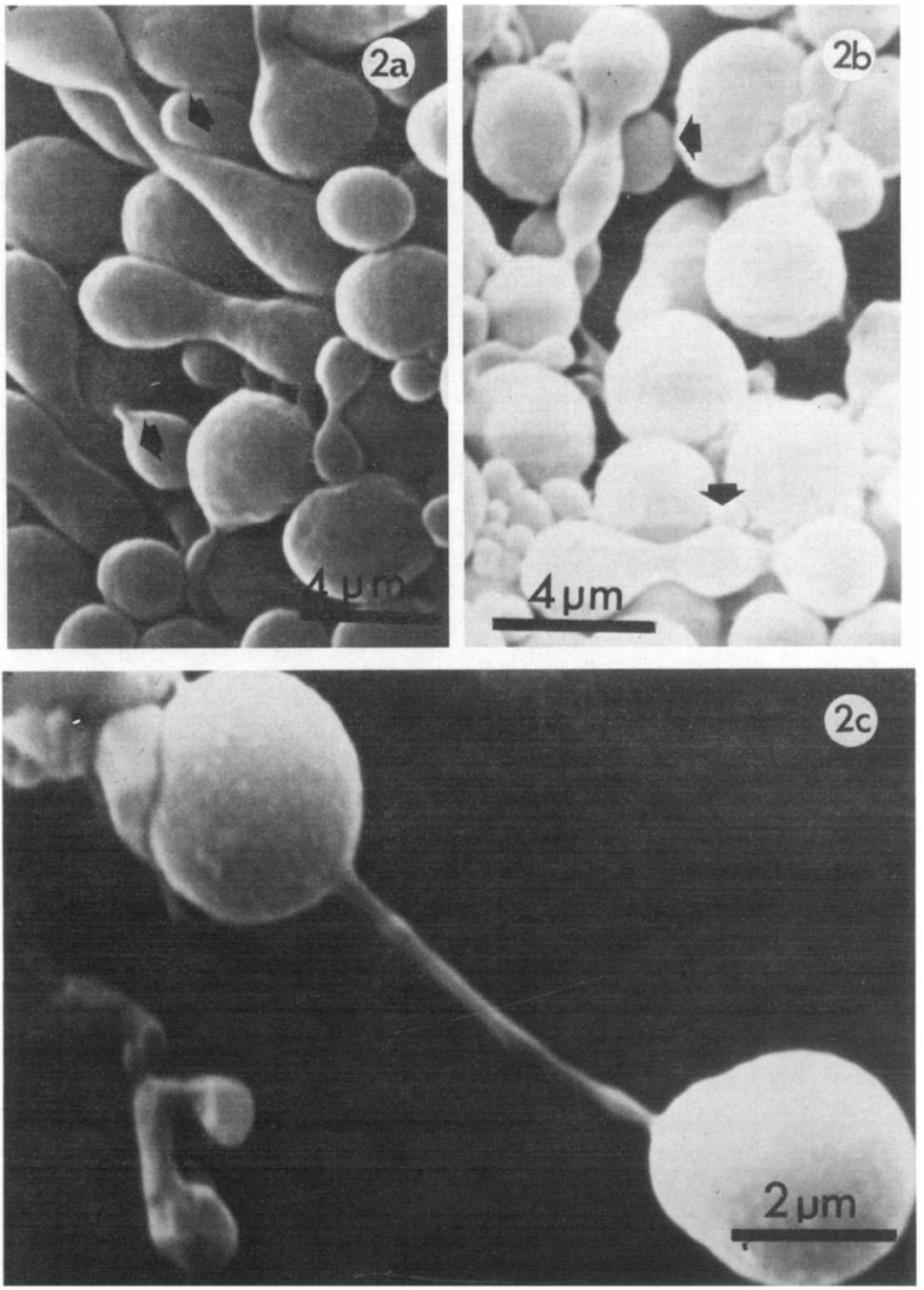

Fig. 2. Scanning electron micrographs of cells heated in a test tube at 51°C, (a) 4 min incubation. Some cells have been drawn out into dumb-bell shapes with narrow connecting necks. Magnification ×5500, bar = 4 µm. (b) 10 min incubation. Two examples of cells which have broken down to form three distinct portions. Magnification ×6000, bar = 4 µm. (c) 4 min incubation time. A cell showing two main portions connected by a tubular neck on which swellings can be observed. Magnification ×12 000, bar = 2 µm.

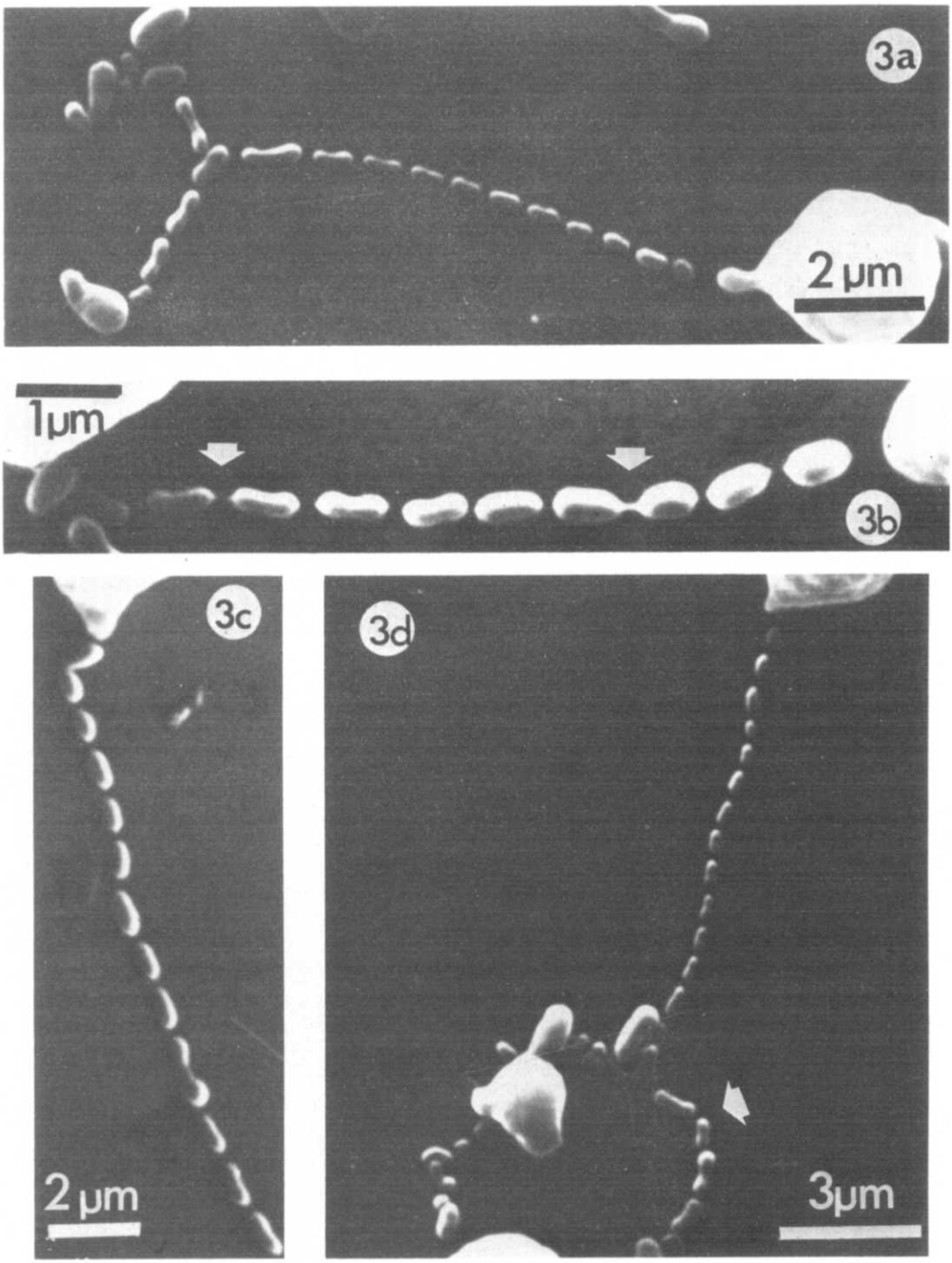

Fig. 3. Scanning electron micrographs of vesicle chains developed when cells heated to 51—54°C were stressed by flow of phosphate-buffered saline and 6% glutaraldehyde. (a), Magnification X7200, bar = 2 μm. (b) shows fine connections between vesicles, magnification X15 000, bar = 1 μm. (c) Magnification X6500, bar = 2 μm. (d) Line of vesicles together with outline of attachment site (arrowed) which has also broken into vesicles. Magnification X7200, bar = 3 μm.

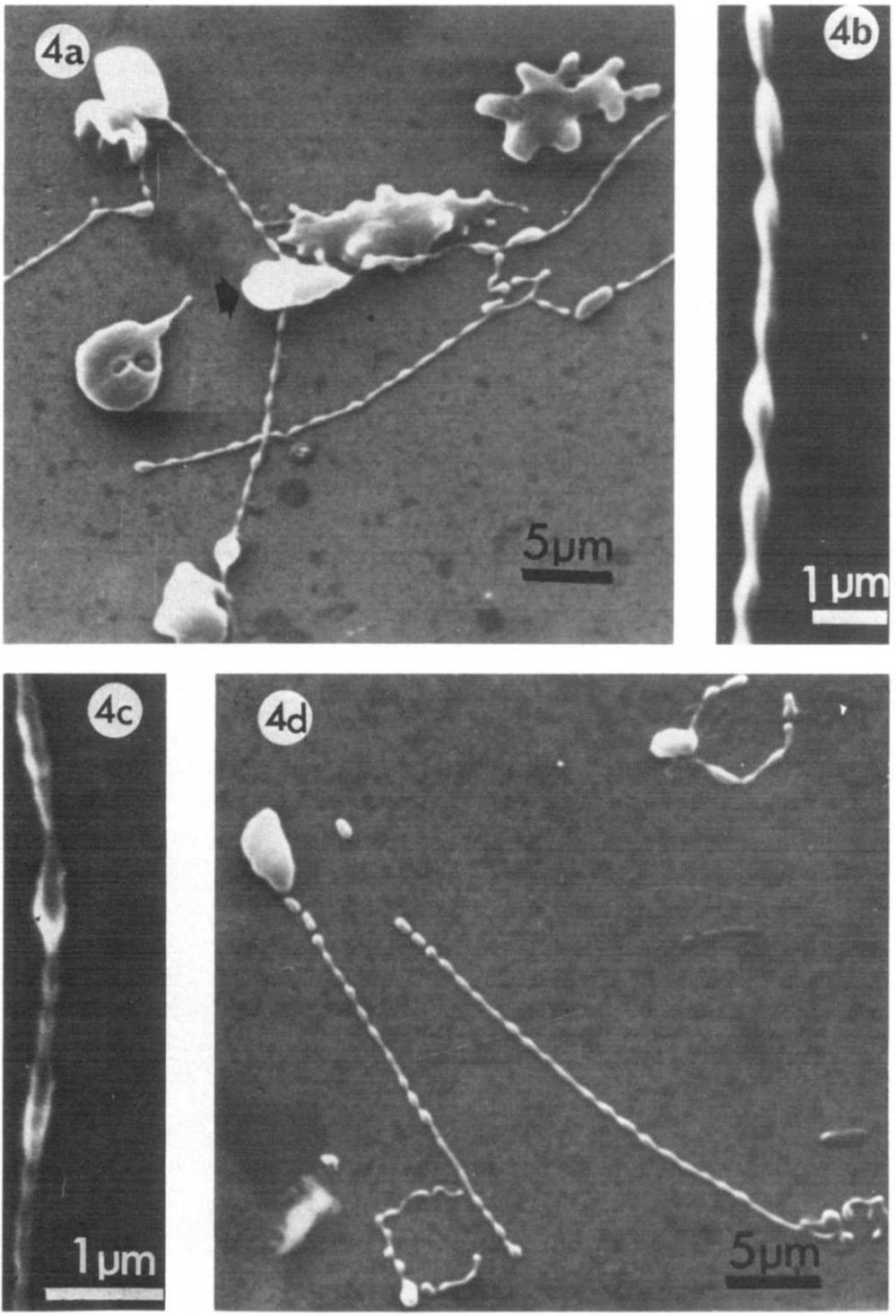

Fig. 4. Scanning electron micrographs of cells stressed by flow of phosphate-buffered saline and glutaraldehyde at 51—54°C. (a) A tether, developed in flow, has fallen across another cell (arrowed); Magnification X2700, bar = 5 μm. (b) A tether exhibiting a regular twist, magnification X11 000, bar = 1 μm. (c) An early stage of disturbance growth on a tether in the absence of twist; magnification X18 000, bar = 1 μm. (d) A beaded tether associated with a beaded attachment site; magnification X2700, bar = 5 μm.

preparations of cells stressed and fixed by flow of phosphate-buffered saline and glutaraldehyde were examined by phase contrast microscopy before air drying. Cinephotomicrographs showed that the production of a tether could occur in less than 0.5 s, a much shorter time scale than the 60 s required to produce the chain of the vesicles in Fig. 1. Fig. 4a shows that the cells were floating in suspension as they were restrained by the tether since the fixed tether fell across another cell. This observation rejects the possibility that the vesicles were produced by the cell rolling along the glass surface leaving fragments attached to the glass. Some of the tethers which were fixed before breaking into vesicle form showed clear signs of a twist along the tether (Fig. 4b). However, the twist was not responsible for the pinching of the tether into fragments because regular constrictions of low amplitude were observed on membrane-bound cylinders in the absence of twist (Figs. 2c and 4c). Furthermore, the ring of membrane retained on the glass where the cell had been attached showed constrictions similar to those along the tethers even though the ring had not been subjected to twist (Figs. 3d and 4d).

We have measured the dimensions of the vesicle forms produced as in Fig. 3

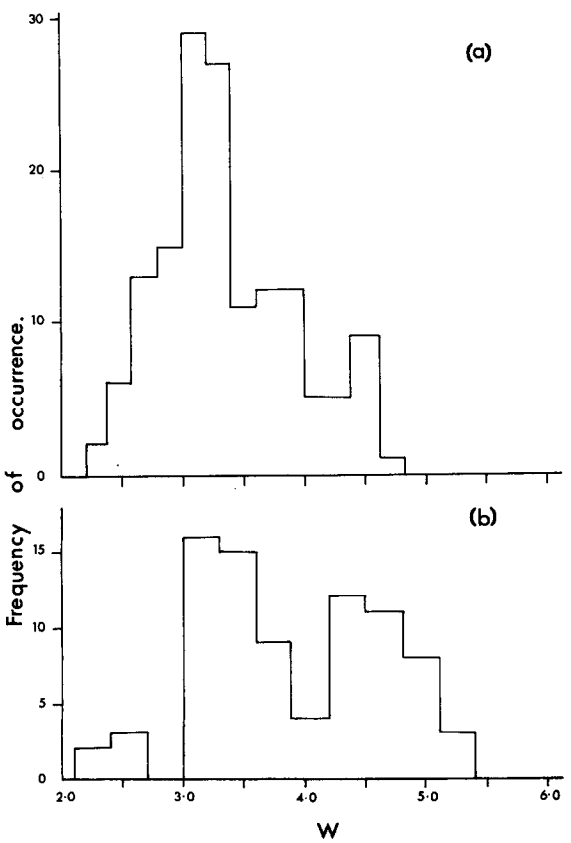

Fig. 5. Frequency distributions of W, the ratio of cylinder length to diameter involved in disturbance growth, based on (a) measurements on 153 vesicles and (b) measurements of 81 cases where the disturbance was at an early stage of growth.

and have calculated the ratio, W, of the length to diameter of the cylinder from which such vesicle forms could develop if the vesicle retained the volume and membrane surface area of the cylindrical fragment. A histogram of the results of 153 measurements on vesicles from 23 tethers is shown in Fig. 5a. The distribution of 87 measurements of W from 26 tethers on which the perturbation had not grown to the vesicle formation stage and where twist could be ignored is shown in Fig. 5b.

Discussion

It has been suggested that the dramatic increase in human erythrocyte deformability between 48 and 50°C may be attributable to conformational changes in the structural protein 'spectrin', a high molecular weight protein located on the inner surface of the membrane [3—5]. Beading processes similar to those reported here have been described previously in other systems. Kitching [8] found that beads developed on the axopodia of the protozoan *Actinophrys sol* under pressures of 35 kN/m^2 and that under favourable conditions the beads on each axopod could be seen to be arranged along a fine thread. Tilney et al. [9] later showed by electron microscopy that the microtubular structure, which plays an important role in the form stability of the axopodia of *Actinosphaerium nucleofilum*, disintegrated in vivo under high pressure and that beading extended along the axopodia leaving only delicate strands of connections between the beads. Marsland [10] describes how a pressure-induced shift in the sol-gel equilibrium in *Amoeba proteus* leads to the liquid-like breakdown of well extended pseudopodia into a series of separated beads. Hogue [11] incubated fibroblasts of embryo chick heart in hypertonic saline and found 'light and dark areas, like a striated muscle' along a cell process 'which quickly became constricted into a chain of protoplasmic beads.' In a study of the elastic properties of red cells, at 25°C, Hochmuth et al. [12] found that 'globules of haemoglobin' were observed in tethers when flow stress was suddenly applied. Tethers developed when cells were stressed slowly but no globules were observed. We have recently reported [13] that haemolysis of erythrocytes stressed in flow at room temperature occurs at a shear stress of 3.0 kN/m^2 if the stress is rapidly applied, i.e., in times as short as 0.45 ms, but exposure to 1.5 kN/m^2 for 100 ms produces no haemolysis. Our observations, taken with those of Hochmuth et al. [12], imply that the structural network on the erythrocyte membrane is particularly susceptible to rapidly applied stress. It appears then that if the structures which confer mechanical strength and form to cell membranes, either in the actinopodea, amoeba, fibroblasts or human erythrocytes, are disrupted then beading can occur on membrane-bound extensions of the cell. The examples above show that removal of the constraints exercised by form-maintaining structures, rather than the elevated temperature, is the condition required to enable the membranes to express that property which leads to beading.

The similarity in vesicle sizes in the tethers of Fig. 3 and the fact that the vesicle in Fig. 1f arose at the centre of the neck sugest that the vesicles develop from the growth of surface disturbances, as instabilities. Rayleigh [14] developed a treatment for the stability of a cylinder of a low-viscosity liquid in

air to a periodic disturbance at right angles to the cylinder axis. He showed that when the surface of a liquid cylinder of radius a was subjected to a displacement α at right angles to the axis of the cylinder, where α was a small quantity varying with time, then the potential energy, V, per unit length of the cylinder due to a surface tension, σ, was given by

$$V = -\sigma\pi\alpha^2(1 - k^2a^2)/2a \tag{1}$$

where $k = 2\pi/\lambda$ and λ is the wavelength of the surface disturbance. If α increases with time then V decreases if $(1 - k^2a^2) > 0$, i.e., if $\lambda > \pi d$, where d is the diameter of the cylinder at rest. If T is the kinetic energy per unit length due to the motion of the column at right angles to the axis then conservation of energy requires that if the potential energy, V, decreases with time the kinetic energy, T, must increase with time. Rayleigh showed that the disturbance will grow at a rate q where

$$q^2 = \sigma F(ka)/\rho a^3 \tag{2}$$

$F(ka)$ is a function of ka involving Bessell Functions and ρ is the density of the cylinder. The disturbance which will dominate and lead to breakdown of the cylinder is that for which $F(ka)$ has its maximum value. Rayleigh found that a cylinder of water in air would be most likely to disintegrate in response to a disturbance with $ka = 0.697$ or $\lambda = 4.5d$.

Tomotika [15] developed a theory for the case of one liquid immersed in another and included the influences of the densities and viscosities of both liquid phase. He was, however, unable to obtain a closed-form solution applicable to a wide variety of cases. Meister and Scheele [16] solved the equations of Tomotika by numerical methods and presented a series of curves from which the dominant wavelength could be derived for any combination of viscosities and densities of the continuous and disperse phases. The transition to unstable behaviour, because of surface tension, occurred for the general case when $\lambda \geqslant \pi d$. Meister and Scheele [16] derived a correlating parameter N_o^*, which was given by

$$N_o^* = (\sigma\rho D_N)^{1/2}(\mu' + 3\mu)^{-1} \tag{3}$$

where μ' and μ are the viscosities of the continuous and disperse phases, respectively, and D_N is the diameter of the nozzle from which the cylinder is formed. Dintenfass [17] gives the 'apparent' internal viscosity of the red cell at 37°C as lying in the range 10^{-3} to $6 \cdot 10^{-3}$ N · s · m^{-2}. If, at 37°C, we take $\mu = 3.5 \cdot 10^{-3}$ N · s · m^{-2} and the viscosity of water as 0.7 N · s · m^{-2} then $\mu/\mu' = 5.0$. The viscosity of water at 50°C is $5.5 \cdot 10^{-3}$ N · s · m^{-2} so that at 50°C we will assume that $\mu = 2.75 \cdot 10^{-2}$ N · s · m^{-2}. The cylinder diameter is approximately 0.5 μm and Adams [18] estimates σ for the erythrocyte as 10^{-6} N/m. Eqn. 3 then gives $N_o^* = 7 \cdot 10^{-3}$. When $\mu/\mu' = 5$ and $N_o^* \to 0$ Meister and Scheele [16] give $\lambda = 6.9d$. There is some variation in published estimates of the surface tension and internal viscosity of erythrocytes. It is instructive therefore to examine the effect of varying values of σ and μ on the wavelength for maximum growth. Since the value of 10^{-6} N/m quoted above for σ leads to an essentially negligible value of the correlating parameter N_o^* we can see from Eqn. 3 that taking a smaller estimate of σ would not affect N_o^* and,

therefore, would not affect λ. If we take a high estimate of σ at $1.2 \cdot 10^{-3}$ N/m [19] and take $\mu = \mu'$ at 50°C as the case most likely to decrease λ, then $N_0^* = 0.35$ and $\lambda = 5.5d$ [16]. A low value of N_0^* and a high value of μ/μ' would result in a high estimate of λ. Above, we took μ/μ' as 5.5, if we increase the estimate to 20 then $\lambda = 9.0d$ when $N_0^* = 0$ [16]. For the purposes of the present discussion the point of importance is that all of the above estimates of λ are greater than $5.5d$.

Inspection of the histogram of Fig. 5a shows a peak in the distribution of W at 3.2, which is less than the above values predicted by theory for breakup under surface tension forces. Our measurements from the beaded tether photograph presented by Hochmuth et al. [12] gave a value of 3.0 ± S.E. of 0.2 for W, in agreement with Fig. 5a. Vesicle production in the tether system differs from the case in an ordinary liquid since there is probably a limit to the extent to which the membrane can reduce its surface area as the droplets are formed. Essentially, σ in Eqn. 1 would then be a function of α and non-linearities leading to the growth of harmonics might account for the low values of W in Fig. 5a. Even for a pure liquid cylinder the growth of a second harmonic has been predicted because of neglect of terms involving α^2 in the classical theory [20] and has been observed experimentally [21]. The distribution of the values of W in Fig. 5b is wider than the distribution of W calculated from vesicle size in Fig. 5a. The minimum length of cylinder which can break into two spherical fragments conserving both volume and area is $4.5d$. Some of the lower values in the range of W from 2.3 to 4.7 in Fig. 5a may have arisen from fragmentation of cylindrical vesicles produced by long wavelength disturbances.

An alternative explanation for the observation that the ratio W is lower than that predicted by surface tension theory would arise if charge on the membrane cylinder contributed to the growth of the instabilities since charged liquid cylinders produce smaller droplets than the uncharged case. Basset [22] showed that charge destabilizes a cylinder against a radial disturbance of $\lambda < 5.2$. Huebner and Chu [23] examined the relative influence of surface tension and charge terms and showed how these terms can interact to reduce the droplet size.

The drawing out of the tethers shown in Figs. 3 and 4 takes place much more rapidly than the slow production of the chain of three vesicles shown in Fig. 1. The latter situation is analogous to the development of successive drops on a nozzle when liquid is slowly emerging, while the vesicles of Figs. 3 and 4 appear to develop on a cylinder similar to the jet which emerges from a nozzle when the liquid flow rate is increased.

The approaches developed above refer to the breakup of Newtonian fluid cylinders. Goldin et al. [24] have examined the breakup of jets of a number of different types of non-Newtonian solutions of polymers in water. In separating viscous and elastic effects they predicted that even for jets of an inviscid purely elastic liquid as well as viscoelastic liquids, instability will arise if the elastic modulus E is less than $\sigma(1 - k^2a^2)/6a$. The breakup of Newtonian and non-Newtonian jets was examined experimentally. As expected, the breakup wavelength of the Newtonian liquid was predictable and at low jet speeds was independent of jet velocity, i.e., the results apply equally well to jets and stationary threads. A solution of Carbopol, a non-Newtonian inelastic liquid

exhibiting a shear dependent viscosity but possessing very small normal forces, showed a regular breakup with a wavelength similar to that predicted for an inviscid fluid. Viscoelastic solutions of sodium carboxy-methyl cellulose showed a growing wave with a clearly defined wavelength. The growth of the wave was, however, arrested before breakup and a string of regularly spaced droplets connected by thin threads was formed. Secondly, instabilities of very small droplets also developed on the same threads. In considerably more elastic viscoelastic solutions of Separan and Polyox no wave formation was discernible and the first visible disturbance appeared as a large droplet, isolated in space from any systematic growth pattern. The distances between drops were randomly distributed and the drops were connected by threads which narrowed with distance eventually leading to the breakup of the liquid column.

Scanning electron micrographs of some areas of the cover slips on which we stressed erythrocytes showed tethers with beads which were less regularly spaced than those shown in Fig. 3, and resembled the forms reported for viscoelastic behaviour by Goldin et al. [24]. The regularly spaced beads reflect either Newtonian or viscoinelastic behaviour while the irregular beads reflect viscoelastic behaviour. The above results were obtained under conditions where there was variation of temperature and flow across the coverslip. We are presently developing a system which will enable us to stress our cells under controlled conditions of stress, temperature and heating duration. In concurrent work the effect of heating on the structure of spectrin is being examined by measuring changes in the circular dichroism with time. We also plan to extend previous investigations of thermally-decreased erythrocyte membrane phosphorylation [25] and then examine if the above molecular changes correlate with the transitions from stable to viscoelastic to viscoinelastic or Newtonian behaviour in the tethers.

We have shown that vesicles similar in character to those observed in other biological systems have been produced when erythrocyte membranes have been structurally weakened by heat treatment are pulled into unstable shapes. A common feature of vesicle production in all the biological systems considered above is that a property of the membrane which can produce vesicle fission can be expressed when the form maintaining structures are weakened. This property of the membrane is normally masked by the elastic properties of the supporting structure, as shown by the absence of beads on the tethers which Hochmuth et al. [12] produced slowly compared with the beads which result in their experiments when stress is rapidly applied. It would appear from the analysis of Goldin et al. [24] that the stable, no-beading case arises because the elastic modulus E is greater than $\sigma(1-k^2a^2)/6a$, while formation of irregularly spaced beads or a uniform pattern, as in Fig. 3, would depend on the extent to which E is reduced by a specific treatment. The necessary reduction in E does not always require the imposition of non-physiological conditions on the membrane since physical contact of two processes of forminifera under physiological conditions leads to beading [26].

We conclude from the above that beading of extended membrane forms arises from the growth of instabilities. These instabilities require a mechanical disturbance, however small, to initiate them. The spacing, regularity and growth rates of the instabilities provide information on the physical state,

during the beading process, of those structures which are normally form maintaining. In view of the wide scale of vesicle production in cells it is surprising that the process of vesicle formation in readily observable systems [8—12,26] has not received more attention to date.

Acknowledgements

The authors are grateful to Dr. L.A. Crum for his careful reading of the manuscript. We acknowledge the interest and encouragement of Professor D.E. Hughes. A.J.B. is grateful to the Science Research Council for support. The assistance of the Centre for Educational Technology for cinephotomicrography and the Zoology Department for scanning electron microscopy is gratefully acknowledged.

References

1 Ham, T.H., Shen, S.C., Fleming, E.M. and Castle, W.B. (1948) Blood 3, 373—403
2 Ponder, E. (1950) J. Exp. Biol. 27, 198—209
3 Brandts, J.F., Erickson, L., Lysko, K., Schwartz, A.T. and Taverna, R.D. (1977) Biochemistry 16, 3450—3454
4 Rakow, A.L. and Hochmuth, R.M. (1975) Biorheology 12, 1—3
5 Williamson, J.R., Shanahan, M.O. and Hochmuth, R.M. (1975) Blood 46, 611—624
6 Krizan, J.E. and Willams, A.R. (1973) Nat. New Biol. 246, 121—122
7 Jay, A.W.L. (1973) Biophys. J. 13, 1166—1182
8 Kitching, J.A. (1957) J. Exp. Biol. 34, 511—517
9 Tilney, L.G., Hiramoto, Y. and Marsland, D. (1966) J. Cell Biol. 29, 77—95
10 Marsland, D. (1964) in Primitive Motile Systems: Cell Biology (Allen, R.D. and Kamiya, N., eds.), pp. 173—185, Academic Press, New York
11 Hogue, M.J. (1919) J. Exp. Med. 30, 617—649
12 Hochmuth, R.M., Mohandas, N. and Blackshear, Jr., P.L. (1973) Biophys. J. 13, 747—762
13 Coakley, W.T., James, C.J. and Macintosh, I.J.C. (1977) Biorheology 14, 91—98
14 Rayleigh, Lord (1879) Proc. Lond. Math. Soc. 10, 4—13
15 Tomotika, S. (1935) Proc. Roy. Soc. (Lond.) A150, 322—337
16 Meister, B.J. and Scheele, G.F. (1967) A.I.Ch.E. Journal 13, 682—688
17 Dintenfass, L. (1968) Nature 219, 956—958
18 Adams, K.H. (1973) Biophys. J. 13, 209—217
19 Williams, A.R. (1970) Some Biophysical Effects of Ultrasound, Ph.D. Thesis, University of Wales
20 Yuen, M.C. (1968) J. Fluid Mech. 33, 151—163
21 Rutland, O.F. and Jameson, G.J. (1971) J. Fluid Mech. 46, 267—271
22 Basset, A.B. (1894) Am. J. Maths. 16, 93—110
23 Huebner, A.L. and Chu, H.N. (1971) J. Fluid Mech. 49, 361—372
24 Goldin, M., Yerushalmi, J., Pfeffer, R. and Shinnar, R. (1969) J. Fluid Mech. 38, 689—711
25 Matsumoto, N., Yawato, Y. and Jacob, H.S. (1977) Blood 49, 233—239
26 Allen, R.D. (1964) in Primitive Motile Systems: Cell Biology (Allen, R.D. and Kamiya, N., eds.), pp. 407—432, Academic Press, New York