A MODEL OF BLEBBING IN MITOTIC TISSUE CULTURE CELLS

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ABSTRACT A preliminary study of blebbing in tissue cultures has been made. The tubal epithelium of fetal mouse oviduct was cultured at 37°C in Rose chambers. A cinematographic record was obtained of phase microscope observations of mitotic cells. Measurements of the size of both cells and blebs were made on the film using a "traveling" microscope. The duration and the rise and decay times of blebs were determined simply by counting frames on the film. Detailed observations are reported on blebbing in four cells undergoing mitosis. The results indicate that the frequency of blebbing as well as the duration of individual blebs exhibits a maximum during telophase. A model is proposed to account for blebbing in mitotic cells. The model attributes to *local* regions of the cell membrane the property of constant tension independent of stretch over some restricted range of stretch. This property implies that the cell membrane is locally unstable. Further assumptions stated explicitly in the model are that (i) cell division occurs at constant volume, (ii) the cell membrane stretches during cleavage, (iii) there is a positive pressure drop across the cell membrane. Evidence is cited in support of these assumptions as well as independent evidence that the cell membrane may be locally unstable. A physical model is described which would be expected to exhibit blebbing given the above assumptions.

INTRODUCTION

A number of investigators have described a curious movement of the cell surface, variously termed "bubbling," "ballooning," or "blebbing," which frequently, but not invariably, is exhibited by mitotic cells during late anaphase and telophase. Strangeways (1922) describes his observations of blebbing in tissue culture cells as follows:

"Small balloons of cytoplasm project from the surface of the cell, these remain for a few seconds and then collapse. The granules in the cytoplasm can be seen flowing in when the balloons are formed and streaming out when they collapse. This movement continues for about six minutes, new balloons being formed as others collapse. This balloon formation is unlike amoeboid movement, and appears due to local changes of surface tension."

Similar observations were made by Speidel (1935) of nerve fibers in the tadpole undergoing retraction after tail section. He observes that "often during retraction of a nerve fibre there are visible small knob-like excrescences, both at the top and also along the fibre. These are associated with violent agitation of the retracting fibre." Hughes (1952) reports some details of blebbing in mitotic tissue culture fibroblasts. He states that "a clear bleb of cytoplasm is extruded from the cell surface and assumes a rounded outline. For several seconds it grows rapidly in size to a diameter of 3–5 μ and then the process is reversed. The bleb decreases in size, usually at a lower rate. There is a strong tendency for repeated "bubbling" at the same site. The blebs grow larger as cleavage of the cell proceeds; the largest become virtually pseudopodial expansions of the cell wall. Treatment of the culture with any number of chemical agents exaggerates the degree of bubbling, some to a fantastic extent." Hughes suggests, in fact, that a bleb may result from pressure acting in a "local weakness" in the cell membrane. Boss (1955) reports that "during mitosis, especially in late anaphase, the surface of the newt fibroblasts have small rounded processes, 1–5 μ in diameter, which were protruded and retracted asynchronously." He further states that blebbing is "not peculiar to tissue culture cells." Boss suggests that blebbing is due to an agent released by the chromosomes. Palmer, Hodes, and Warren (1961) report blebbing in suspensions of Ehrlich ascites cells treated with surfactants, temperature shock, etc. This blebbing appears to be at least qualitatively similar to that described by the authors quoted above. They suggest and cite evidence to the effect that blebbing is "an index of cell membrane damage."

The mainly qualitative observations cited above are perhaps generally familiar now as a result of the wide distribution of films dealing with cell mitosis. But, perhaps surprisingly, there does not appear to have been a systematic study of blebbing per se nor a close consideration of possible mechanisms. The present preliminary study was undertaken in order to obtain detailed measurements of the frequency of blebbing during the mitotic cycle, as well as measurements of bleb size, rise time, duration, and decay time for a few cells. A tentative model of blebbing is proposed on the basis of these observations.

METHODS

The biological source material consisted of oviducts taken from 17–18-day mouse fetuses. The tubal epithelium of the oviduct had been cultured at 37°C from 3 to 5 days prior to the observations reported here. The tissues were cultured in Rose chambers which are designed especially to facilitate microscopic observation of growing cells. At the time the observations were made, an appreciable fraction of the cells in culture were undergoing mitosis.

The phase microscope employed was enclosed in a warm box so as to keep the culture at essentially constant temperature (30.3–30.4°C). Numerous mitotic cells were observed visually before photographs were taken. Photographs of mitotic cells were taken with a moving picture camera mounted over the microscope. A shutter mechanism, which interrupted the microscope illumination at precise intervals, served to put time markers on the film. The magnification was determined by photographing a substage micrometer. Thus, both length

and time calibrations were recorded directly on the film. Photographic recordings were made either continuously (approximately 11 frames per sec) for short periods or by time lapse for long periods.

Measurements of the size of both cells and blebs were made (where possible) using a "traveling" microscope. In most instances both major and minor diameters were determined. The duration and the rise and decay times of blebs were determined simply by counting frames.

The observations reported here were made on four cells undergoing mitosis. In the case of cells 1 and 2, filming was done continuously but for short intervals when blebbing was most prominent. Mitosis in cells 3 and 4, on the other hand, was recorded by time lapse photography and for much longer periods.

At the magnification employed, not all the blebs on a given cell at any one instant could be in focus. Furthermore, visual observations suggested that some few blebs were of a different character from the rest. The following arbitrary criteria were adopted in determining which blebs to measure (i.e., which blebs were "normal"). To be included in the measurements, the blebs must exhibit an approximately spherical or elliptical outline, occur in a more or less unobstructed area, be in good focus, exhibit a fast rise time (i.e., a few seconds), and later disappear completely. Similarly arbitrary criteria were adopted in measuring the dimensions of blebs. Two dimensions were determined, one of these being a measure of the maximum displacement of the membrane and the other a dimension perpendicular to this. Some of the blebs were approximately spheres on the ends of short stalks (i.e., resembling a Florence flask), in which case the major and minor diameters were taken as the diameter of the sphere, and the stalk was neglected.

RESULTS

Some general features of the results, such as filming technique, cell diameters, etc., are summarized in Table I.

The data obtained by continuous filming (i.e., from cells 1 and 2) were used mainly to make accurate determinations of rise time. Parameters which varied or might have been expected to vary during the mitotic cycle were more readily determined from the time lapse data (i.e., from cells 3 and 4). These parameters included frequency of blebbing (see Fig. 1), duration of individual blebs (Fig. 2), and size of blebs (see Fig. 3).

TABLE I
SUMMARY OF MEASUREMENTS

	Cell			
	1	2	3	4
Filming technique	Continuous	Continuous	Time lapse	Time lapse
Magnification	308×	125×	125×	125×
No. frames examined	5367	3677	180	187
Length of anaphase cell (microns)	—	20	26	21
No. of blebs measured	15	15	51	18
Mean diameter of blebs (microns)	3.1 ± 1.0	3.3 ± 0.5	3.1 ± 0.7	4.2 ± 0.8
Mean rise time (seconds)	6.3 ± 1.9	5.5 ± 1.3	—	—
Mean duration time (seconds)	30.7 ± 7.3	30.5 ± 7.1	14.0 ± 2.8	18.1 ± 4.5

Qualitative Observations

Blebbing was often especially prominent on the strand connecting two daughter cells as well as on various small projections from the cells. In these instances the blebs were too numerous to allow measurements of the type described above. Another observation concerns an apparent coupling between blebbing activity on the two sides of the cell. In several striking sequences blebs rise and fall on one side of the cell just out of phase with the rise and fall on the other side. Finally, we observe intense blebbing in cells in which there is obvious chromosomal disarrangement, this blebbing presumably being correlated with imminent death of the cell. We did not observe "a strong tendency for repeated bubbling at the same site," but otherwise our observations agree by and large with those made by other investigators (see Introduction).

Quantitative Results

The measurements are summarized in Table I. It may be noted that the majority of blebs in these cells were of the type designated "normal." Therefore, but for those blebs which were out of focus, the majority of blebs observed are included in Table I. The term mean diameter refers to the geometric mean of the two measurements of

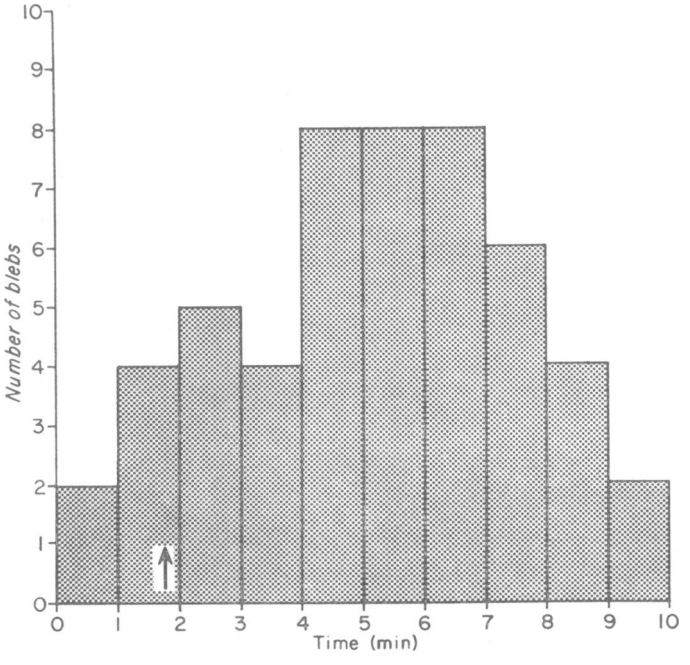

FIGURE 1 Frequency of blebbing. The arrow indicates the time at which constriction in the furrow region first became evident. The data, derived from cell 3, indicate the number of blebs observed per minute.

bleb size described above. The majority of blebs we observed fall in the range 2–5 μ in diameter. All the deviations in Table I represent standard deviations. The blebs typically rise to their maximum size in 4–7 sec. Judging from our measurements on these four cells, the total duration time of blebs is quite variable (10–40 sec). The rise time appears to represent about 20% of the total duration time.

Frequency. The data (see Fig. 1) illustrating the variation in number of blebs per unit time (i.e., per minute) are derived from cell 3, which happened to exhibit a large number of blebs. The maximum blebbing activity (i.e., blebs per minute) occurred when cleavage was about half completed. This is consistent with our qualitative observations of other cells.

Duration. The length of time each bleb lasted (i.e., initial appearance to final disappearance) on cell 3 is plotted in Fig. 2. There is apparently a maximum in the duration time about halfway through cleavage.

Size. The size of the blebs on cell 3 is plotted in Fig. 3. There does not appear to be a statistically significant variation in the size of blebs during cleavage.

Curvature. The radii of curvature of different parts of the cell surface are quite variable, being a few microns in some places but very large in others, where the membrane is substantially flat.

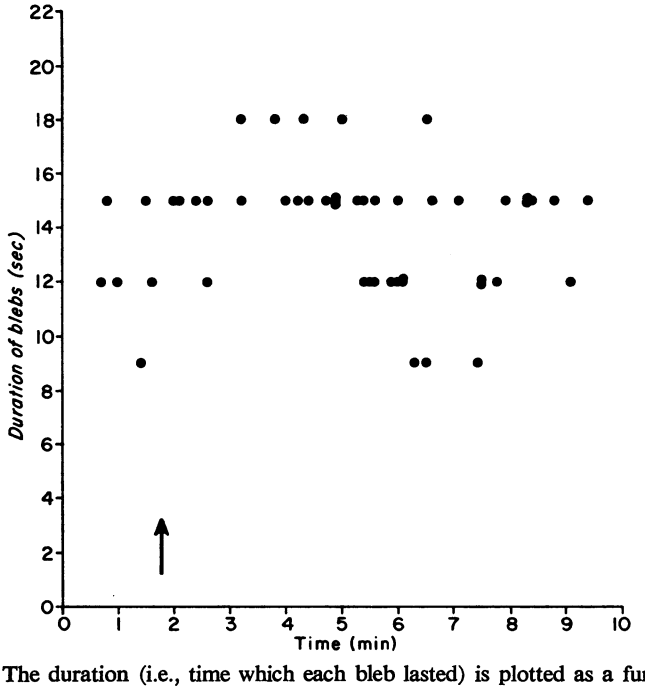

FIGURE 2 The duration (i.e., time which each bleb lasted) is plotted as a function of the time after which constriction first became evident, as indicated by the arrow. Data from cell 3.

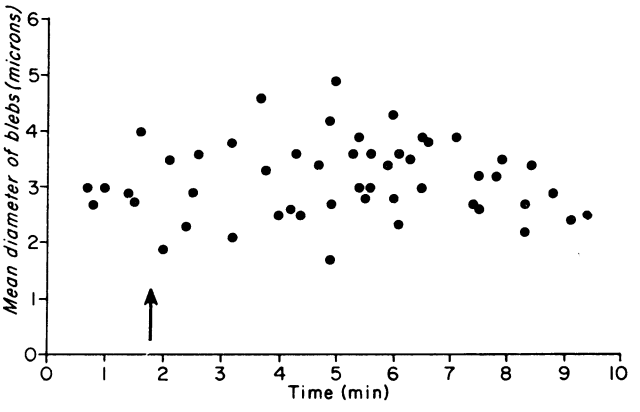

FIGURE 3 Mean diameter of bleb is plotted against stage of telophase, the beginning of which is indicated by the arrow. Data from cell 3.

DISCUSSION

We note that the quantitative results obtained for four cells, as described above, are consistent with our qualitative observations with respect to frequency, duration, and size of blebbing in several dozen cells of the same type under the same conditions.

Blebbing has generally been attributed to changes in membrane tension, with the inference that a chemically mediated decrease in tension is involved (e.g., release of a surfactant). Though this is an attractive hypothesis, it proves less simple upon closer examination. The following points appear to be relevant: (*a*) If, as electron micrographs suggest, the membrane is mechanically homogeneous in its own plane, then the effects of a uniformly distributed surfactant would be expected to be a function of the surface-to-volume ratio. Thus blebbing should be least where the ratio is greatest. On the contrary, the most intense blebbing is observed on the strands projecting from the cell body. Therefore, if blebbing is due to release of a surfactant, the latter must be nonuniformly distributed throughout the cell. (*b*) The release of a surfactant does not appear, on present knowledge, to relate in a natural way to the other events of cell cleavage. (*c*) If blebbing is due to a surfactant, there must be some relatively rapid and, as yet, unspecified repair process. (*d*) It would have to be supposed that on occasion, at least, the surfactant is released rhythmically, in order to explain the rhythmic blebbing occasionally observed. (*e*) The tension in cell membranes is in any case very low (see, e.g., Rand, 1964).

An economical model of blebbing in mitotic cells would presumably relate naturally to the events of cell cleavage, particularly to changes in cell shape. It is

known that before cleavage cells generally "round up" and then divide approximately symmetrically into two spheroidal daughter cells.

It has been demonstrated by Hiramoto (1958) in the case of sea urchin eggs and by Ishizaka (1966) in the case of grasshopper spermatocytes that cell cleavage occurs at constant volume. Assuming that this is true of mammalian cells, then cleavage must involve an increase in surface area of about 26%. This increase could arise by (*a*) unfolding of previously plicated membrane; (*b*) synthesis of new membrane; (*c*) stretching of original membrane; (*d*) any combination of these factors.

Electron micrographs of dividing cells (Roosen-Runge, 1967, personal communication; Buck and Tisdale, 1962) do not suggest that the increase in area occurs by unfolding. In order to account for the observed movement of markers on the surface of grasshopper spermatocytes (Ishizaka, 1966), one would need to postulate a uniformity of folding of a quite unlikely character. Nor do we know of any positive evidence which favors the hypothesis that the increase in area occurs by unfolding. Accordingly, we favor mechanisms (*b*) and (*c*). Mayhew (1966) has observed that the electrophoretic mobility of cells (human osteogenic sarcoma) increases during mitosis. On the assumption that the cell volume remains constant during cleavage, he states that "the hypothesis can be advanced that cell surface *N*-acetylneuramic acid is synthesized during later interphase and/or mitosis and the resulting higher density of ionized carboxyl groups causes a higher electrophoretic mobility during mitosis; after division a drop in charge density occurs, possibly caused by stretching of the cell surface. The results presented in this paper would support this hypothesis although they do not prove it." Thus Mayhen invokes both the mechanisms of insertion of new material and stretching at different stages in the life cycle. Also bearing on the membrane changes associated with cleavage is the observation of Mitchison and Swann (1955) that, at least in sea urchin eggs, there is an order-of-magnitude increase in the stiffness of the membrane during late anaphase. Finally, there are the recent experiments by Hiramoto (1965) which demonstrate that cleavage can proceed substantially unaltered even though the mitotic apparatus is destroyed in late anaphase. A reasonable inference to draw from this fact is that cleavage is brought about by forces generated and acting at or near the cell membrane.

We now wish to consider briefly the question of membrane stability in dividing cells. It is reasonable to suppose that there will be a positive pressure drop (ΔP) across the cell membrane during cleavage, inasmuch as contraction in the cleavage furrow must produce an outward flow of cytoplasm. For equilibrium there must be a tension (T) in the membrane which will be related to the pressure drop and the radius of curvature (r) of the cell by an equation of the form of LaPlace's law, i.e.:

$$T = \Delta P \cdot r \tag{1}$$

This equation is plotted in Fig. 4 for a range of radii and pressure drops which would appear to be reasonable for many mammalian cells. Note that during cleavage the average radius of curvature is decreasing (although the surface area is increasing). If the tension (T) of the membrane should remain constant over some range of stretch, then equilibrium would obtain only if the pressure drop (ΔP) increased in exact proportion as the average radius decreased. Thus under a constant surface

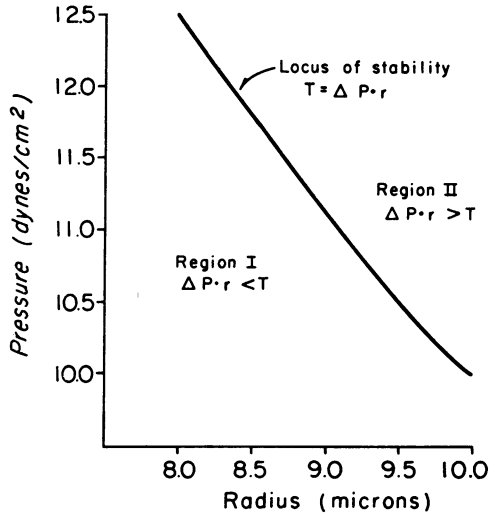

FIGURE 4 The solid line indicates the conditions for stability for a membrane having constant surface tension.

tension regime the solid curve of Fig. 4 is a locus of stability, whereas on either side of it (i.e., regions I and II) there are regions of instability. It is postulated in the following model that the membrane is locally unstable over a certain range of stretch.

Model

The model of blebbing in mitotic cells which we propose is based upon the following explicit assumptions:

(*a*) During cell division the total cellular volume remains constant (Hiramoto, 1958).

(*b*) The cell membrane is elastically stretched at some stages of cleavage (Mitchison and Swann, 1955).

(*c*) Over a certain range of stretch, however, the *local* membrane tesion is constant and independent of stretch.

(*d*) The radius of curvature of the cell membrane varies markedly in different directions. See Results.

(*e*) There is a positive (i.e., outward directed) hydrostatic pressure drop across the cell membrane during cleavage.

Predictions of Model

According to postulates (*b*) and (*c*), local regions of the membrane have constant tension over a limited range of extension, and increasing tension for further extension. We note parenthetically that the property of constant tension could, in principle, correspond to extension associated with the insertion of new material. In any

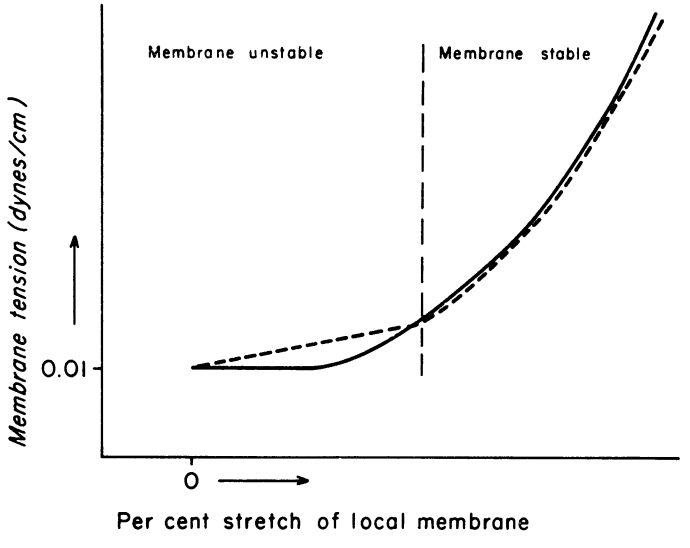

FIGURE 5 The solid line indicates the postulated tension-area diagram for local regions of the membrane. The dotted line indicates the postulated pressure-radius product. In the unstable region the pressure-radius product exceeds the postulated membrane tension and blebbing is predicted. In the stable region the membrane tension is equal to the pressure-radius product.

case, extension over the range of constant tension will be stable only when the pressure drop varies exactly in inverse proportion to the radius of curvature (equation (1)). We consider this an unlikely contingency. More probably, perhaps, the pressure drop will rise more quickly than the average radius of curvature decreases, so that cleavage will take place in the unstable area denoted as region II of Fig. 4. This hypothesis is summarized in Fig. 5. The solid line represents the assumed tension area diagram for local regions of the membrane. The dotted line represents the assumed product of the pressure drop across the membrane and the local radius of curvature. Over the unstable region there will be a tendency for the local radius of curvature to decrease (i.e., for blebs to form) in order to satisfy equation (1). In the stable region the increasing tension in the membrane balances the $\Delta P \cdot r$ product, thus giving stability. Over the unstable region, the membrane would be metastable

if the surface were spherical. Given assumption (*d*) (i.e., surface nonspherical) and a positive pressure drop across the cell membrane (assumption (*e*)), the model then predicts that the membrane will be unstable. The positive internal pressure (assumption (*e*)) means that the instability (i.e., blebs) must be directed outward. The nature of the assumed tension-area diagram (Fig. 5) is such that the instability will be iimited in extent. Finally, the assumption that cellular volume is conserved (i.e., assumption (*a*)) means that blebbing in one part of the cell must be compensated for by alterations in the shape of the cell elsewhere. This latter point could, in principle, explain the "rhythmic" character which blebbing sometimes exhibits. The predic-lion that blebbing will be intense where great changes in the radii of curvature occur ts in part borne out by the observation that blebbing is intense in the strands connecting the daughter cells as well as on projections from the cell. Finally, the model relates blebbing naturally to cleavage, as this is the time when the membrane may reasonably be assumed to stretch, when the radius of curvature may be assumed to alter markedly, and finally when a positive internal pressure may plausibly be assumed to develop.

Comments on Model

The implied assumption that *local* regions of the cell membrane may have a tension to some extent independent of stretch while at the same time the *whole* cell is known to exhibit elastic tension may appear paradoxical. However, a model can be conceived of which would presumably so behave. Consider surrounding a spherical liquid drop by a slack and somewhat irregular fishnet constructed of elastic material which does not "wet" the drop. Now constrict the liquid drop slightly. Evidently the liquid surface will be unstable, since the drop is no longer spherical. The positive force producing the constriction, coupled with the variation in radius of curvature, would be expected to produce something like blebbing. With further constriction the whole system would exhibit elastic behavior as the slack in the net was taken up. It is not intended to suggest that the cell membrane has this structure, but merely that a structure having the assumed properties can be imagined.

The fact that "dying" cells frequently exhibit blebbing in tissue culture is not necessarily inconsistent with the model if it is assumed that dying cells take on water. In this case, the cells would presumably swell, develop a positive internal pressure, and stretch the surface membrane, at first through an unstable then through a stable region.

It might be thought that the model would predict blebbing whenever a cell was deformed from the spherical shape, say by pressing the cell between a cover slip and a glass slide. Although this may be the case, it can be argued that blebbing will not occur because no pressure drop is developed across the wall. In the case of the dividing cell, it is not unreasonable to assume that an appreciable internal pressure is developed owing to constriction of an elastic surface.

Implications of Model

If the model is confirmed by further work, it will have an interesting bearing upon theories of membrane structure. Classically, the membrane was considered to be a liquid-liquid interface, with presumably the property of constant surface tension independent of stretch. Subsequently, numerous experiments (e.g., Cole, 1932; Sichel and Burton, 1936) indicated that the cell membrane or large parts of it exhibit elastic tension. If the present model is confirmed, the two views of the membrane may both be partially correct. The possible nature of the elastic diagram of cell membranes seems to have been little considered in framing theories thereof, as indeed does the possibility that membranes apparently are able to stretch.

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