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ELASTIC-MATHEMATICAL THEORY OF CELLS AND MITOCHONDRIA IN SWELLING PROCESS. PART V

TRANSFORMATION OF CORTICAL GRANULE MEMBRANE OF EGG CELL OF *STRONGYLOCENTROTUS PURPURATUS* IN SUBELASTIC AND ELASTIC SWELLING

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SUMMARY

Some elastic properties of the cell membrane have been determined by parallel studies of (1) the swelling function, $V = V(1/\pi_m)$, of the cell and (2) electronmicroscopic study of the cortical granule membrane of the egg cell of the sea urchin (*Strongylocentrotus purpuratus*) at 13.5°. The "free" subelastic swelling of the egg cell (obeying Boyle–Van 't Hoff's law) is attributed to swelling of the cortical granules. The beginning of the elastic range in the $V = V(1/\pi_m)$ plot has been shown to coincide with the point at which subelastic swelling of the granules ends. The degrees of folding of the cell membrane and the granule are $\mathscr{H}_a = 38.4\,\%$ and $\mathscr{H}_{ga} = 28.9\,\%$, respectively. On the basis of reduction of membrane thickness, $h = h(1/\pi_m)$, Poisson's ratio, $\nu \approx 0.5$, has been estimated. Comments have been made on error factors associated with ultrastructural dimensions in electronmicrographs and with granulolysis caused by membrane stresses. The magnitude of membrane stress and modulus of elasticity of the cortical granule membrane have been calculated.

INTRODUCTION

In Parts I (ref. 1) and II (ref. 2) of this series of studies on the elasticity of the cell membrane, the theoretical background, methods of study and terminology were explained.

In Part I two working hypotheses were formulated, one concerning the degree of folding of the cell membrane, $\mathscr{H}$, and the other Poisson's ratio of the membrane, $\nu = 0.5$.

It was found useful to employ the definition of the degree of folding, because there exists a certain limit (volume V_E of the cell) in the $(1/\pi_m; V)$ graph above which Boyle–Van 't Hoff's relationship is no longer valid. Thus V_E distinguishes between the subelastic (Boyle–Van 't Hoff's range) and elastic ranges.

The degree of folding was defined in ref. 1 as universally as possible, using corresponding areas of shell surface in different states of subelastic swelling:

$$\mathscr{H} = \frac{A_E}{A} - 1 \qquad (1)$$

when $A_E > A$. For spherical cells, $\mathscr{H}$ can be calculated from formula (28) (ref. 1). For cells in their natural medium, *e.g.* sea water, the degree of folding of the cell membrane has been denoted by $\mathscr{H}_a$; in this state $V = V_a$.

As the cell swells in the subelastic range the hydrostatic pressure difference, p, generated inside the cell is, according to Boyle–Van 't Hoff's law, equal to zero. As the volume of the cell approaches the value $V = V_E$, $p \approx 0$, and $\mathscr{H} = 0$. At this particular volume the "free" swelling of the cell ceases; the "folds" of the cell membrane have become straightened.

As far as the cortical granule membrane is concerned, it is possible, by referring to the equation that defines $\mathscr{H}$, to use the expression "degree of folding . . .", even though the actual phenomenon is a superposition of the granules' ability to swell subelastically, change form and orientate in a certain manner, together with the straightening of macroscopic folds of the cell membrane.

For Poisson's ratio, the value $\nu = 0.5$ was chosen without further proof. This value is the theoretical maximum value for ν. It implies that the material maintains a constant volume during elastic deformation. The theoretical minimum value, $\nu = 0$, on the other hand, would imply that the volume of the material increases (and the density correspondingly decreases) in direct proportion to the relative strain, as is practically the case with cork.

In Parts I and II, it was further assumed that the thickness of the membrane, h, remains approximately constant ($h_a \approx h_s \approx h_E$) during the subelastic swelling process of the cell, and that during the elastic swelling the thickness decreases in a certain manner defined by Eqn. 30 (ref. 1). The formula is a direct consequence of the assumption that $\nu = 0.5$.

The purpose of this paper is to prove the validity of the assumptions concerning $\mathscr{H}$ and ν and to analyze further the biophysical phenomena associated with them. In order that the results obtained in Parts I and II might be utilized, egg cells of the sea urchin (*Strongylocentrotus purpuratus*) were used as experimental material.

The electronmicrographs from which the data used in the present paper were obtained will be presented elsewhere[3].

EXPERIMENTAL MATERIAL AND METHODS

Besides the swelling experiments (shedding, swelling and recording explained in ref. 1) with egg cells of *Strongylocentrotus purpuratus**, an electronmicroscopic study of the cell was carried out. Out of egg cells of several specimens, the egg cells of a single individual were chosen for the experiments; the criterion used in selection is explained in ref. 2.

In swelling experiments the media employed were 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.45, 0.4 and 0.35 of sea water. A portion of the egg cells in each of the swelling

* The specimens were supplied by Pacific Bio-Marine Supply Company, Venice, Calif., U.S.A.

media mentioned above was transferred to fixing media. The natural salinity of the sea water was $S = 32.65\,^{\circ}/_{\circ\circ}$ and the temperature during the experiments $13.5 \pm 0.5°$. This temperature was selected so that the modulus of elasticity of the cell membrane should remain constant during elastic swelling[2]. The eggs were fixed in glutaraldehyde–sea water and dilutions so arranged that every medium contained 3 % glutaraldehyde. The osmotic pressures of these solutions were approximately the same as in the swelling media mentioned before.

Fixation was done at the temperature of the swelling experiments, the fixing time being 2 h 15 min in each case. No buffer was added to the fixing media, because its possible effect upon the elastic properties of the cell membrane structure could not be confidently estimated, and it was decided to be content with the buffering action of sea water. (This action is rather pronounced: In 1.0-medium (abbreviated 1.0-m.) the pH value was 8.0 and decreased linearly to 7.7 in 0.35-m.) The pH value of the fixing media also decreased linearly from 5.2 in 1.0-m. to 4.1 in 0.35-m. Before staining, or post-fixing with OsO_4, the cells were rinsed with corresponding sea water media. For staining, the concentration of osmium tetroxide in different media was 0.5 % (fixation with OsO_4 in 1.0-m. was first presented in ref. 4), and the time of staining was 4 h.

It has been found that calcium chloride added to fixatives containing osmium tetroxide has the effect of preserving the structure of the cell membrane[5–7]. However, no additional $CaCl_2$ was used, for the reason explained above concerning usage of buffer in fixing media. Dehydration was achieved using a series of water–ethanol mixtures, followed by propylene oxide treatment, and embedding in epoxy resin, Epon.

Glutaraldehyde solutions were prepared about 10 min and osmium solutions about 6 h before use.

For electronmicrography, a Siemens Elmiskop I was used and the magnification was calibrated with latex particles.

RESULTS

$(1/\pi_m; V)$ *graph*

The swelling function of the cell in the $(1/\pi_m; V)$ plane[1] was normalized using the V_E and π_E values as reference point (Fig. 1). Normalization was performed in

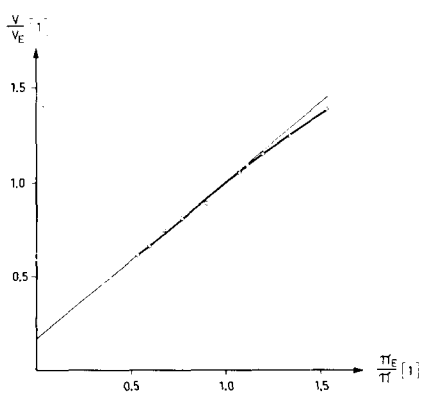

Fig. 1. Normalized swelling function (the egg cell of *Strongylocentrotus purpuratus*) at $13.5 \pm 0.5°$; $V_E : V_a : V_b = 1 : 0.614 : 0.162$; $\pi_a : \pi_E = 1.865$; $\pi_a = 22.08 \cdot 10^6$ dyne/cm²; $\mathscr{H}_a = 38.4\,\%$. (Each experimental point represents the average diameter of 20–40 eggs measured in two directions. At each experimental point the distribution is $< 1\,\%$.)

order to facilitate comparisons between cells of different size and type, regardless of their natural medium (sea water, extracellular fluid or blood plasma, *etc.*). For normalization the value V_E of the swelling function is considered the most natural reference point, and the corresponding argument is then $1/\pi_E$. At this point the degree of folding, $\mathscr{H}$, reaches the value zero.

It was not felt necessary to repeat the shrinkage studies on reversible swelling carried out in ref. 2. It was considered sufficient to return the cells, swollen in different media, to 1.0-m. and to record the resultant cell diameters (Table I).

TABLE I

CONTROL OF REVERSIBILITY OF SWELLING PROCESS

V_a' = mean volume of the egg cells returned to 1.0-m, V_a = mean volume of the control egg cell. The number of cells returned from each medium: * = 4, † = 15–30 and § = > 30 egg cells.

Medium	*0.7*	*0.6*	*0.5*	*0.45*	*0.4*	*0.35*
V_a'/V_a	1.01§	1.03†	1.03†	1.04†	1.05†	0.98*

The ratio V_a'/V_a was always slightly greater than unity except when the cells were returned from 0.35-m. The reason for the discrepancy may be the small number of eggs measured. However, in view of the changes in cortical granule structure to be discussed later, it seems probable that leakage had taken place.

$(1/\pi_m; v_g)$ graph

The swelling function of the granule, $v_g(1/\pi_m)$, whose argument is equal to the argument in the swelling function of the cell, is presented in Fig. 2.

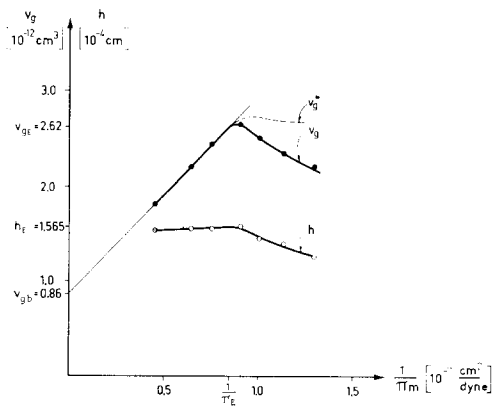

Fig. 2. The swelling function of the cortical granule, $v_g(1/\pi_m)$, and the thickness of the granule layer, $h(1/\pi_m)$. The corrected v_g function, v_g^* (Eqn. 9), is plotted as a dotted line. The abscissa of the functions is equal to that in Fig. 1. $v_{gE}:v_{ga}:v_{gb} = 1:0.691:0.328$; $\mathscr{H}_{ga} = 28.9\%$.

The average volume (v_g) of the granule was calculated from elliptical cross sections (axes a_i and b_i, $a_i > b_i$, b_i rotation axis), assuming the granule to be an oblate spheroid:

$$v_{gi} = \frac{\pi}{6} a_i^2 b_i \qquad (2)$$

In each medium, the average volume v_g was calculated using the method of normal distribution.

In 1.0-m., the granule is nearly spherical (eccentricity $e = 0.2$), but concurrently with the swelling of the cell and the granule, the form of the granule changes more and more towards what can be considered approximately an oblate spheroid (*e.g.* in 0.5-m., $e = 0.5$). The position of the granules, which were earlier oriented in different directions, also changes during the course of swelling, so that the longer axis, a_i, of the elliptical cross section turns in the direction of the tangent of the cell contour. This orientation is actually a result of the directive swelling of the granule. Only in 0.45–0.35-m. does this parallelism approach completion.

In electronmicrographs, where the section plane passes through the calotte of the cell, deformation of the granule in the elastic range can be observed to take place as well, so that the oblate spheroid changes slightly to assume the form of a cushion. The corners of the "cushion" are the junctions of the granule with other granules (4-point junction)[3]. Maximum elongation occurs in the region of the junction.

The thickness h of the cortical granule layer

The thickness of the cortical granule layer, h, as a function of $1/\pi_m$, is also shown in Fig. 2. This thickness is calculated by measuring the thickness of the layer perpendicular to the cell contour, and then making use of the method of normal distribution. The swelling, deformation and orientation of the granule are thus included in the result.

THE ANALYSIS OF RESULTS

Measurement of the ultramicroscopic structure

A frequently used method for checking the effect of fixation and dehydration is to measure the size of the cell before and after the process. This procedure has not been adopted here, because to judge from the volume changes of the cell, under the present experimental conditions, it would apparently be impossible to make any assumptions about ultrastructural stability even if, for example, the hardening of the resin and its effect on the volume of the cell[8] were known. The reason for this conclusion is as follows: Cells which have swollen in very hypotonic media contain much more fluid in the neighbourhood of the cell wall than in the inner part of the cell. Dehydration is particularly effective in decreasing the volume of such cells, and this shrinkage is revealed as macroscopic wrinkles of the cell wall. On the other hand, no such fluid spaces could be observed inside the granules in the electronmicrographs and hence dehydration probably has little or no effect on the volume of the granule.

Because the manner in which fixation changes ultrastructure cannot be determined reliably (as indirect methods of study have been employed) one has to be content with the assumption that fixation has the same relative effect in all the different media.

In electronmicroscopy the collision of electrons with the ultrathin section[9] has the effect of decreasing the dimensions of the structure. This effect is not considered to be of any significance as regards the relative results. Deformations due to the specimen holder and the grid[10] produce errors that can be assumed to be smoothed statistically.

When the dimensions of the structure of the cell membrane are measured from the electronmicrographs, some systematic errors are incurred. Of these, the dependence of the results of the measurements on the location of the section plane in respect to the ultramicroscopic structure is considered to be the most important. For instance, it is owing to this source of error that the average of the measurements of the dimensions of the granules is smaller than their actual average*. On the other hand, because of the granular structure of the cell wall, the location of the section plane in respect to the whole cell does not give rise to significant errors provided the plane goes through the central region of the cell.

It is believed, however, that the values obtained in the ultramicroscopic studies reported in this paper, yield correctly shaped experimental curves demonstrating the effects of the different media. This conclusion is based on the fact that the systematic errors made in the various sections may be considered to be proportionally the same; and that the effect of the errors caused by the fixing process and other systematic error factors may be taken as approximately equal in the different media.

The error caused by the breaking of the granules in the elastic range will be discussed later.

The degree of folding of the membrane

It seems apparent that the swelling of the cortical granule in a medium which lies between 0.6- and 0.5-m. is still subelastic. On the linear part of the function $v_g(1/\pi_m)$, the hydrostatic pressure difference, p, generated inside the granule by osmosis is equal to zero. The fact that the linear part of the function $v_g(1/\pi_m)$ is sufficiently correct for conclusions can be verified simply by examining the correctness of the slope of the linear part of the $v_g(1/\pi_m)$ function (Fig. 2). The slope (k_m) of the linear part of the volume function of the cortical granule membrane $V_m(1/\pi_m)$ in Fig. 5, discussed in greater detail later, is selected for reference. The reason for this choice is that for calculation of the $V_m(1/\pi_m)$ function values of the swelling function of the cell (Fig. 1) are used which are not affected by errors arising from the fixation or the electronmicrographs, *etc.* Though V_m includes h (Eqn. 8), it should be noted that v_g also implicitly contains h, so that its effect is cancelled out in the comparison of V_m and v_g made in the following calculations.

As $V_{mE} = 5.76 \cdot 10^{-8}$ cm^3 and $1/\pi_E = 0.845 \cdot 10^{-7}$ cm^2/dyne a value of $k_m = 0.43$ dyne·cm is obtained (Fig. 5). Since V_m is actually kV_m, where $k = 1.23$ is the correction factor for h**, the actual slope is $k_m = 0.43\ k$ dyne·cm. The integrated volume of the granules is

$$V_g = nAk^3 v_g(1/\pi_m) \tag{3}$$

where $nA = N$, or, granule density (1/cm^2) times corresponding cell area (cm^2) =

* An approximate estimate of absolute values (the only error eliminated being that caused by the location of the section plane in respect to the ultrastructure of the cell, *i.e.* in respect to different granules) can be made by the following elementary method: The correction factor k is written so that $kd_1 = d_2$, where d_1 = average value of measured dimensions, and d_2 = average value of actual dimensions. Apparently d_2 equals the average value of the dimensions measured in the granules which have been sectioned through their center (8 shells visible in electronmicrographs). By comparing the values obtained from these center-sectioned granules with d_1, it was found that $k = 1.23$. In the present experiments k could be determined only in 1.0-medium.

** This limit has also been observed with the egg cells of *Arbacia punctulata* in fertilization tests: ". . . except those in 50% (= 0.5-m) solution; these underwent an atypical cleavage and therefore were slightly injured"[11].

number of granules, and k^3 is the correction factor of the volume. N was estimated from electronmicrographs of cells in which the calotte had been sectioned and was found to have a value of $N \approx 1.4 \cdot 10^4$. The slope using Eqn. 3 is thus $k_g = 0.295\ k^3$ dyne·cm (Fig. 2). So it can be seen that $k_m \approx k^2 k_g$, when $k = 1.23$, which means that the linear part of the function $v_g(1/\pi_m)$ is sufficiently correct to be used for further conclusions.

In the foregoing calculations the significance of the matter filling the spaces between the granules, the "interplasm", has been assumed to be negligible. Since changes in interplasm volume are apparently minute as compared with the volume of the subelastically swelling granule and the relative interplasm volume (as compared with the entire volume of the granule):

$$i \approx \frac{Ahk - v_g N k^3}{Ahk} = 1 - \frac{v_g N k^2}{36\pi \Gamma^{2/3}} \tag{4}$$

is $i \approx 5$–$10\ \%$, there are good reasons to consider that the effect of the interplasm on k_m is minor and to neglect it in slope calculations.

The point where subelastic swelling of the granule ceases has values $v_{gE} = 2.62 \cdot 10^{-12}$ cm^3 and $1/\pi_E = 0.845 \cdot 10^{-7}$ cm^2/dyne. The latter value is the same as the corresponding value of the normalized swelling function of the cell (Fig. 1) at the point $\pi_E/\pi = 1$ or $1/\pi_E = 1.865/22.08 \cdot 10^6$ cm^2/dyne $= 0.845 \cdot 10^{-7}$ cm^2/dyne. Thus it can be seen that the subelastic swelling of the granule ends at the same $1/\pi_E$ value as the subelastic swelling of the cell. (See 2nd footnote, p. 546.)

The subelastic swelling of the cell can be physically explained on the hypothesis that the subelastic swelling of the granules produces the necessary increase in cell membrane area concurrently with the expansion of cell volume. In such a case there would appear to be no force to prevent cell volume enlargement and thus the cell could swell subelastically. If this hypothesis is accepted, an equation can be written between cell volumes, the corresponding volumes of the granule and the thickness of the cortical granule layer:

$$\frac{\Gamma}{\Gamma_E} = \alpha \left(\frac{v_g}{h}\right)^{3/2} \tag{5}$$

when the radius of the cell is $R \gg h$, and

$$\alpha = \left(\frac{h_E}{v_{gE}}\right)^{3/2} = \text{constant}$$

The term on the right in Eqn. 5 is plotted in Fig. 3 (values $v_g = (1.81$–$2.62) \cdot 10^{-12}$ cm^3

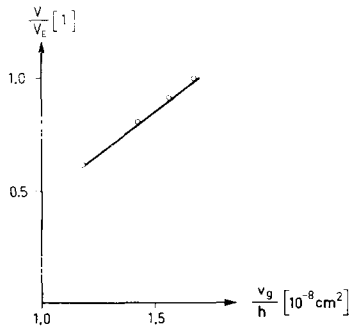

Fig. 3. Correspondence between area necessary for swelling of the cell and area produced by the swelling granule in the subelastic domain (see Eqn. 5).

and h (1.53–1.56)·10^{-4} cm are obtained from Fig. 2). For the ratio V/V_E on the left in Eqn. 5, the values for the dots (4) are obtained from Fig. 1. It will be observed that the dots and the graph of the term on the right in Eqn. 5 nearly coincide. (The fact that the V/V_E dots are slightly above the $\alpha(v_g/h)^{3/2}$ curve probably results from inaccurate location of V_E in Fig. 1.) Thus it can be seen that the swelling of the granules is sufficient to produce the additional area of the cortical granule membrane necessary for the cell to swell subelastically. (Later, in connection with the $\mathscr{H}$-term, it will be shown that there are also other factors that contribute to the subelastic swelling of the cell.)

Thus it has been possible to confirm the existence of $\mathscr{H}$ by two methods: by microscopic measurements, in connection with the swelling function of the cell, and by electronmicroscopic methods, in connection with structural changes in the cell wall. A physical explanation can thus be offered for Boyle–Van 't Hoff's law. No conclusive answer to the question how the shells of the cortical granule can deform freely in the subelastic range can be given on the basis of electronmicrographs.

When the definition, Eqn. 1, is written for oblate spheroids, one obtains

$$\mathscr{H} = \frac{d_{eqvE}^2 - \frac{h_E^2}{2e_E}\ln\frac{1+e_E}{1-e_E}}{d_{eqv}^2 - \frac{h^2}{2e}\ln\frac{1+e}{1-e}} - 1 \qquad (6)$$

$$\mathscr{H} \approx \frac{d_{eqvE}^2 + h_E^2\left(1 - \frac{e_E^2}{3}\right)}{d_{eqv}^2 + h^2\left(1 + \frac{e^3}{3}\right)} - 1 \qquad (7)$$

where $d_{eqv} = (6v_g/\pi h)^{1/2}$ and the eccentricity $e = (1-(h/d_{eqv})^2)^{1/2}$. The d_{eqv} thus defined is the length of the mean granule (volume v_g, height h) in the direction of the cell contour tangent. When the dimensions of the ellipsoidal granule (Fig. 2) at points $1/\pi_a$ and $1/\pi_E$ are substituted into Eqn. 6, the degree of folding in 1.0-m., $\mathscr{H}_{ga} = 28.9\,\%$, is obtained. The $\mathscr{H}_a$ for the cell was 38.4 %*. The causes of the difference in the $\mathscr{H}$-values can be noticed in electronmicrographs: As the volume of the granule increases, the granule simultaneously is deformed more and more oblately, and even during subelastic swelling there is already a certain amount of orientation of the granules in the direction tangential to the cell contour. Whether or not the interplasma stretches cannot be distinguished from the electronmicrographs, but the macroscopically convoluted cell wall becomes smoother during the subelastic swelling of the cell, and at $1/\pi_E$ the membrane appears almost completely even[3].

* Here it can be noted that the degree of folding of the cell membrane appears to be a function of temperature, t. If $\mathscr{H}_a$-values are plotted from Fig. 2 (ref. 1), Figs. 1 and 2 (ref. 2) and from the present paper, Fig. 1, on semi-log-paper ($\mathscr{H}$ is on log-axis), the function $\mathscr{H}_a(t)$ can be estimated to be linear.

The thickness h of the cortical granule layer

In the subelastic range h remains nearly constant, or, $h_a \approx h_s \approx h_E$, Fig. 2. To be more accurate, during subelastic swelling, h increases slightly. This phenomenon can be explained by crowding of the granules. The crowding can be understood if the additional cell area produced by the swelling granules exceeds the area necessary for subelastic swelling of the cell. This assumption can be confirmed as follows: After differentiation of Eqn. 5 one obtains $\Delta h/h = \Delta v_g/v_g - (2/3)\Delta V/V$, and when values from Fig. 1 and Fig. 2 are introduced into the equation, the left and the right side of the equation are found to be approximately equal.

During the elastic swelling of the cell, $h(1/\pi_m)$ decreases rather markedly (Fig. 2) as a result of increasing membrane stresses.

In Fig. 4, the rising part of the curve is the normalized theoretical function $h/h_E = (V_E/V)^{2/3}$ for the thickness of the membrane, Eqn. 30 (ref. 1), shown in the $(V_E/V; h/h_E)$ plane, in which have been plotted the experimental points for the ratio h/h_E as calculated from Fig. 2, so that each normalized h/h_E corresponds to the V_E/V value of the same medium. From the figure it can be observed that the experimental points correspond rather well to the theoretical function. Hence, Poisson's ratio of the cortical granule membrane of the egg cell of *S. purpuratus*, is $\nu \approx 0.5$ and thus the use of formula (30) (ref. 1) leads to a relatively accurate approximation of the membrane thickness during the elastic swelling process of the cell.

Referring to the difference between the experimental points and the theoretical curve (30) (ref. 1), which is based on the assumption that $\nu = 0.5$, it may be difficult to observe readily from Fig. 4 whether the volume of the cortical granule layer remains constant or increases ($\nu < 0.5$). To examine this, a simple theoretical correspondence can be expressed between the volume and thickness of the cortical granule layer, on the one hand, and the volume of the cell, on the other:

$$\frac{V_m}{V_{mE}} = \left(\frac{V}{V_E}\right)^{2/3} \frac{h}{h_E} \tag{8}$$

When the values obtained from Figs. 1 and 2 are employed, and Eqn. 8 is plotted in the $(\pi_E/\pi; V_m/V_{mE})$ plane in Fig. 5, the ratio V_m/V_{mE} can be seen to remain constant in 0.5- and 0.45-m., but to decay slightly in 0.4- and 0.35-m. (see next paragraph).

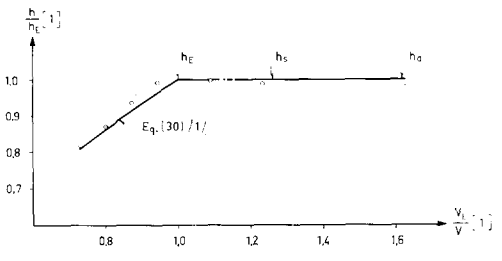

Fig. 4. Normalized function for the thickness of the cortical granule membrane in elastic deformation, the monotonic part belongs to the subelastic range. The experimental points represent the normalized thickness of the cortical granule membrane of the egg cell of *S. purpuratus*.

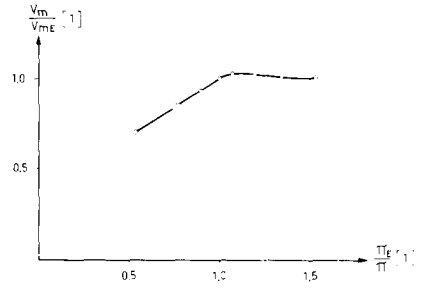

Fig. 5. Normalized volume of the cortical granule membrane as a function of normalized osmotic pressure of the medium.

However, it will be understood that the granule membrane maintains an approximately constant volume during elastic swelling.

v_g, V_m and h in the elastic range

It is still necessary to examine the error factor constituted by the breaking of the granules. During swelling of the cell in the elastic range some granules break, obviously because of membrane stresses (*cf.* ref. 3 also). On the basis of electronmicrographs it seems possible that this "granulolysis" occurs with larger granules, or, that stress-selective granulolysis is involved (*cf.* ref. 1, p. 109). Since the measurements were made only on granules which were not noticeably injured (the injured ones have a clearly spread shape), the values obtained are probably lower than they should actually be, especially as only a small number of granules were measured (average 30/medium). The granulolysis percentage (G) is shown in Table II. A direct result of this is that the values of the h and v_g functions in the elastic domain (Fig. 2) are too small. However, the error caused by granulolysis has most effect on v_g, because in its calculation the dimensions are raised to third power, and least on h, whose dimension is in first power. Thus the empirical function $v_g(1/\pi_m)$ is unreliable in the elastic domain, in Fig. 2. In 0.5–0.35-m., Eqn. 3 should be approximately equal to $kV_m(1/\pi_m)$. This is not the case, however. Granulolysis and failure to take into account the volumes of the stretched corners of the granules mentioned earlier (p. 545) are obviously the primary causes of the decay of $v_g(1/\pi_m)$. An exact correction for the v_g function cannot be obtained from the experimental results, but in the following an approximation for the actual v_g curve ($v_g^*(1/\pi_m)$) is presented.

TABLE II

GRANULOLYSIS (G %) IN DIFFERENT SWELLING MEDIA

The total number of granules: * = 28, † = 50–60 and § = 80–120.

Medium	1.0	0.7	0.6	0.5	0.45	0.4	0.35
G %	—§	—§	2†	13§	15§	14*	16†

The use of Eqn. 5 can be extended to cover the elastic range, since (when $i = 0$):

$$v_g^* \approx \frac{v_{gE}}{h_E} h \left(\frac{V}{V_E}\right)^{2/3} \qquad (9)$$

which is plotted in Fig. 2. If v_g^* is multiplied by the product Nk^2, it can be shown that $V_g^* = v_g^* Nk^2$ almost coincides with $V_m(1/\pi_m)$.

Stress–strain diagram

Eqns. 1 and 4 in ref. 2 permit a stress–strain plot of the cell membrane to be sketched in Fig. 6. The upper limit, σ_Y, of the elastic range is estimated in ref. 1 to be reached around 0.3-m. on the basis of the existence of blebs. From the electronmicrographs it can be seen that even in 0.35-m. there are local points of damage in the cortical granule membrane. Thus it is apparent that membrane stress may vary between the values $\sigma_{\mathrm{red}\,M} \approx 0 \ldots 8 \cdot 10^6$ dyne/cm^2 (depending upon the estimation of

membrane thickness). The modulus of elasticity is then $E = 3.5 \cdot 10^7$ dyne/cm^2 and linear, when $\varepsilon_v > 10\,\%$. Between $\varepsilon_v = 0 \ldots 10\,\%$ the variable modulus of elasticity can be calculated as in ref. 2 (Appendix).

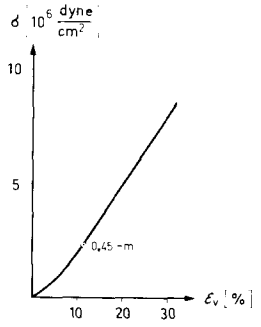

Fig. 6. Stress-strain diagram of the cell wall at 13.5°. (The h_E value evaluated = 1.8 μm.)

LIST OF SYMBOLS

A	cell area
N	number of granules
V	cell volume
V_b	osmotic inactive volume in the cell
V_m	volume of cortical granule membrane
a, b	axis of ellipse
h	thickness of membrane (or cortical granule layer)
k	correction factor
k_g, k_m	slope of function
n	granule density
p	hydrostatic pressure difference inside the cell
v_g	granule volume
$\mathscr{H}$	degree of folding
π_m	osmotic pressure of medium

e.g. 0.7-m. = medium contains 70% sea water and 30% water

Subindexes:

E	point between subelastic and elastic range
a	isotonic state (1.0-m)
g	granule
s	state inside subelastic range

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REFERENCES

1 M. J. MELA, *Biophys. J.*, 7 (1967) 95.
2 M. J. MELA, *Biophys. J.*, 8 (1968) 83.
3 M. J. MELA, *Biochim. Biophys. Acta*, 150 (1968) 553.
4 D. MCCULLOH, *Doctor Thesis in M.I.T., Dept. of Biology*, 1951.
5 G. D. PAPPAS, *Ann. N.Y. Acad. Sci.*, 78 (1959) 448.
6 L. E. ROTH AND E. W. DANIELS, *J. Cell Biol.*, 12 (1962) 57.
7 P. W. BRANDT AND G. D. PAPPAS, *J. Cell Biol.*, 15 (1962) 55.
8 P. J. NAPJUS, *Voordr. Ned. Veren. Electronenmicrosc.*, 1964, p. 11.
9 J. A. FREEMAN, *Scient. Instrum. News*, 8 (1963) 9.
10 P. F. ELBERS AND J. PIETERS, *J. Ultrastruct. Res.*, 11 (1964) 25.
11 M. MCCUTCHEON, B. LUCKÉ AND H. K. HARTLINE, *J. Gen. Physiol.*, 14 (1931) 393.