ELASTIC-MATHEMATICAL THEORY OF CELLS AND MITOCHONDRIA IN SWELLING PROCESS

I. THE MEMBRANOUS STRESSES AND MODULUS OF ELASTICITY OF THE EGG CELL OF SEA URCHIN, *Strongylocentrotus purpuratus*

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ABSTRACT To the revolution-ellipsoidal and spherical membranous shell (cell mitochondrion) are introduced the equations for the calculation of both the modulus of elasticity (Young's modulus) and the stresses, which exist at the membrane. The existing pressure difference between the inner and outer surface of the membrane is calculated in the dilution of seawater media in the osmotic steady state. The experimental results are obtained by using egg cells of the sea urchin, *Strongylocentrotus purpuratus*. Up to the specific volume of the egg cell ($V_B \approx 35 \cdot 10^{-8}$ cm^3) Boyle-van't Hoff's law is valid (defined as the subelastic range) beyond that the elastic stresses exist (elastic range). For the maximum value of the stresses existing at the cell wall one obtains $\sigma \approx 5.5 \cdot 10^6$ dyne/cm^2 and for the modulus of elasticity $E = 1.0 \cdot 10^7$ dyne/cm^2, which is constant when the value of relative strain $\epsilon_v > 15\%$. The breaking limit by an approximate calculation is $\sigma_U \approx 11 \cdot 10^6$ dyne/cm^2. The membrane is assumed to be convoluted and its hypothetical degree of folding was calculated $\mathcal{H}_a = 34\%$. The results are compared with the values existing in the literature and other types of cells are found to have values of elasticity in the same range as values of the membrane of *S. purpuratus*. Both compression and cell elastometer methods are criticized and in certain cases results of these methods are considered to belong to the subelastic domain.

INTRODUCTION

Elasticity studies of the cell as well as of the mitochondrial membranes have been objects of relatively little interest in the otherwise rather wide field of cell and mitochondrial research. However, knowledge of the elastic phenomenon of the membrane makes many structural properties of the membrane clear. The elastic properties of membranes or the whole cells have been studied, for instance, starting from surface energy principles using the Laplace equation (1) or depending on the method of a combination of the Laplace equation and Poiseuille's law (2). Many other investigations have been done in this area (3–5).

In studies of materials the principles which are used in the elasticity theory are more natural than those which are used in the surface tension theory. By using theory of elasticity it is possible to determine certain constants and functions, which are typical of the material. To this group belong, among others, the modulus of elasticity (Young's modulus), Poisson's ratio, and different types of stresses. By means of the elasticity theory, the modulus of elasticity of the material in myxomycetes has been determined using a tension test (6). Other experiments based on the elasticity theory (7, 8) should be mentioned. Rikmenspoel (8) has been able to determine the modulus of elasticity using the simplified equation of the deflection curve for the bending tail of the spermatozoa. Theories for viscoelastic bodies have also been derived (9, 10). Osmotic properties of living cells have been discussed (11, 12). When using the osmotic properties of cell membranes for examining the dependence of the swelling (the deformation) of the cell on the osmotic difference pressure (difference of the pressure inside and outside the membrane), the elasticity of the membrane can be studied.

The purpose of Part I is to develop a basis for the uniform elastic-mathematical principles, which regardless of the type of cell are practical in studying the behavior of the membrane of cells (mitochondria, etc.) under stresses. According to this treatment, the theory is formulated for an elliptic shell of revolution (in particular, spherical), which gives more extensive potentiality to study, e.g., mitochondria with similar shape. However, the limitation of the theory is, besides postulates mentioned later, that the (outer) membrane is freely deforming. This means that mitochondrial christae do not prevent the deformation.

The State of Stress and Strain of the Membranous Shell

The Shape of the Shell. The cell or the mitochondrion is called in this paper simply the "shell." The theory is made more general by considering the shell as an ellipsoid of revolution. In the case where the shell exists as a sphere, the equations can be obtained easily by setting eccentricity equal to zero.

The axis of rotation of an ellipsoid is taken as the x-axis; thus, in the plane section ($z = 0$) the contour is a simple ellipse:

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} = 1 \tag{1}$$

where a and b ($a > b$) are semiaxes of the ellipse. If one assumes that the swollen shell keeps its original shape, then *eccentricity*, e, given by

$$e = \left[1 - \left(\frac{b}{a}\right)^2\right]^{1/2}, \tag{2}$$

remains constant.

The Stress Components in the Membranous Shell. To study the stress field in the membrane one makes the following simplifying assumptions concerning structure:

1. The membranous shell is freely deforming.
2. In the membrane existing stresses are caused by hydrostatic, i.e. osmotic, pressure.
3. One assumes that the membrane has uniform thickness and does not have any pores large enough to affect stress-strain calculations; the membrane is isotropic. The word "membrane" is implied here *generally*. It means, if not specifically mentioned, cell wall, e.g., membrane plus cortex (sea urchin egg cell) or exclusively (outer) membrane (mitochondrion, erythrocyte).
4. When the membrane is thin, one can analyze the stresses as membrane stresses. The membrane does not have flexural rigidity and the symmetrically distributed pressure p, which is perpendicular to the membrane, produces the stress $\sigma_n = -p$ on the inner surface of the membrane while the stress on the outer surface is zero. Thus, the so-called *normal stress* (σ_n) perpendicular to the shell surface is very small.
5. General elastic principles, such as superposition principle and Hooke's (generalized) law, are assumed to hold in the membranous shell.

The equations of state for the stress on a general shell of revolution, modified from Timoshenko (13, p. 365), are as follows:

$$\sigma_1 = \frac{\rho_2}{2h} p \tag{3}$$

where σ_1 = the meridional stress, ρ_2 = the radius of the curvature of the section perpendicular to the meridian, h = the thickness of the membrane, and p = pressure difference between inner and outer side of the membrane.

$$\sigma_2 = \frac{\rho_2}{2h} p \left(2 - \frac{\rho_2}{\rho_1}\right) \tag{4}$$

where σ_2 = the hoop stress and ρ_1 = the meridional radius of curvature.

The radii ρ_1 and ρ_2 of the curvatures for ellipsoid in equations (3) and (4) are given, when the coordinates of the reference point are $x = x_0$ and $y = y_0$, by

$$\rho_1 = \frac{1}{(ab)^4} (a^4 y_0^2 + b^4 x_0^2)^{3/2}, \tag{5}$$

$$\rho_2 = \frac{1}{a^2} (a^4 y_0^2 + b^4 x_0^2)^{1/2} \tag{6}$$

or

$$\rho_2 = b\sqrt{n}, \tag{6'}$$

where

$$n = 1 - \left(\frac{e}{a}\right)^2 x_0^2. \tag{7}$$

When

$$\frac{\rho_2}{\rho_1} = \left(\frac{b}{a}\right)^2 \frac{1}{n}, \tag{8}$$

equations (3) and (4) may be written:

$$\sigma_1 = \frac{b}{2h} p\sqrt{n} \tag{9}$$

$$\sigma_2 = \frac{b}{2h} p\sqrt{n}\left(2 - \left(\frac{b}{a}\right)^2 \frac{1}{n}\right). \tag{10}$$

In the case $e = 0$ (spherical shell) equations (9) and (10) contract to the form

$$\sigma_1 = \sigma_2 = \frac{r}{2h} p \tag{11}$$

where $r = a = b =$ the radius of the sphere.

Reduced Stress. The two-dimensional state of stress (σ_1 and σ_2) has, in the following treatment, been combined to a one-dimensional or *reduced stress,* which is equivalent in its effect to the former ones. The reduced value (σ_{red}) of the stress components has been calculated using the *constant strain energy hypothesis* (14, 15), when normal stress perpendicular to the surface of the shell is approximately zero. This stress when compared with σ_1 and σ_2 (the membrane stresses parallel to the surface of the shell) certainly can be neglected and thus:

$$\sigma_{\text{red}} = [\sigma_1^2 + \sigma_2^2 - 2\nu\sigma_1\sigma_2]^{1/2} \tag{12}$$

where $\nu =$ *Poisson's ratio.* Substitution of equations (9) and (10) into equation (12) produces:

$$\sigma_{\text{red}} = \frac{b}{2h} p \left[n(5 - 4\nu) - 2(1 - e^2)(2 - \nu) + \frac{1}{n}(1 - e^2)^2 \right]^{1/2}. \tag{13}$$

In the case of a sphere, one obtains from equation (13):

$$\sigma_{\text{red}} = \frac{r}{2h} p[2 - 2\nu]^{1/2}. \tag{14}$$

The use of hypothesis (12) is valid, if the volume expansion (dilatation), k, [see, for example, Timoshenko (16, p. 10)] is greater than zero; so

$$k = \frac{1 - 2\nu}{E}(\sigma_1 + \sigma_2) > 0, \tag{15}$$

where E = *the modulus of elasticity.*

To find the sign of k it is sufficient to examine equation (7) and the bracket part of equation (16), which has been obtained from equation (15):

$$k = \frac{1 - 2\nu}{E}\frac{b}{2h}p\sqrt{n}\left[3 - \left(\frac{b}{a}\right)^2 \frac{1}{n}\right]. \tag{16}$$

One finds, then, that always $k > 0$, regardless of e and x_0. If one can consider h, e, p, and ν to be approximately constant while the stress is varied, one can see from equation (13), that σ_{red} then depends only on the volume and shape of the shell as given by semiaxis and eccentricity.

In studying the distribution of stress (13) on the membrane, it is sufficient to calculate only the ratio of stresses at the position $x_0 = 0$ (maximum stress) and $x_0 = a$ (minimum stress). Then one obtains:

$$\frac{\sigma_{\text{red}}(x_0 = a)}{\sigma_{\text{red}}(x_0 = 0)} = \left[\frac{2(1 - e^2)(1 - \nu)}{2(1 + e^2)(1 - \nu) + e^4}\right]^{1/2} \approx \left[\frac{1 - e^2}{1 + e^2}\right]^{1/2}, \qquad \text{(17) and (17')}$$

when e is small. The ratio of $\sigma_{\text{red}}(x_0 = a)/\sigma_{\text{red}}(x_0 = 0)$ (17′) is plotted in Fig. 1 as a function of e. From this one can find that, with small values of e, stress concentrations on the membrane of ellipsoidal shell are minor. In the case $e = 0$ the "equator and polar" stresses are equal.

The absolute value of the stress-*maximum,* $\sigma_{\text{red}M}$, is obtained from equations (7) and (13) inserting $x_0 = 0$:

$$\sigma_{\text{red}M} = \frac{b}{2h}p[2(1 + e^2)(1 - \nu) + e^4]^{1/2}. \tag{18}$$

By substituting the semiaxis b with the volume of the ellipsoid and solving numerical calculations one can express the stress as a function of the shell volume:

$$\sigma_{\text{red}M} = \frac{1}{2}\sqrt[3]{\frac{3}{4\pi}}\frac{p}{h}\sqrt[3]{V}\sqrt[6]{1 - e^2}\sqrt{2(1 + e^2)(1 - \nu) + e^4} \tag{19}$$

which is in the more practical form, when e is small,

$$\sigma_{\text{red}M} \approx 0.31\frac{p}{h}\sqrt[3]{V}\left[\frac{1}{2} + (1 - \nu)(1 + e^2)\right]. \tag{20}$$

The Change of Volume of the Shell. The tangential elongation is in accordance with the generalized Hooke's law in plane stress $\epsilon_\phi = (1/E)(\sigma_2 - \nu\sigma_1)$. By substituting equations (9) and (10) in this equation and writing for the elongation, $u = b\epsilon_\phi$ (at the position $x_0 = 0$), one obtains:

$$u = \frac{b^2}{2Eh} p[e^2 + 1 - \nu]. \tag{21}$$

When one assumes that the eccentricity will be approximately constant as the volume expands, then one obtains from equation (2) that the major semiaxis of the expanded ellipsoid is $a_n = (b + u)(1 - e^2)^{-1/2}$, when the minor semiaxis is $b_n = b + u$. Taking the fractional volume change of the ellipsoid as

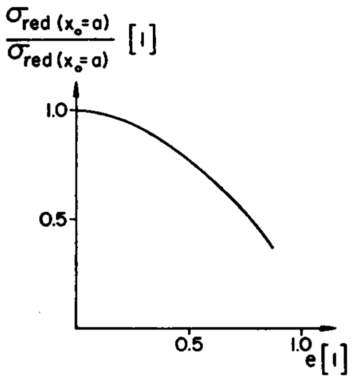

FIGURE 1 The ratio of reduced stresses as a function of eccentricity. When $e = 0.78$, stress existing on the equator is two times the stress existing on the poles. When the stress exceeds the breaking limit, the rupture of the shell occurs along the equator. When $e = 0$ (sphere), stresses are equal and state of stress is homogeneous.

$$\frac{\Delta V}{V} \approx \frac{a_n}{a} + 2\frac{b_n}{b} - 3$$

or

$$\frac{\Delta V}{V} \approx 3\frac{u}{b}$$

(when ratio u/b is small), one obtains from equation (21):

$$\frac{\Delta V}{V} = \frac{3}{2}\frac{b}{Eh} p[e^2 + 1 - \nu]. \tag{22}$$

By replacing the semiaxis b with the volume of ellipsoid, one gets from equation (22):

$$E = \frac{3}{2}\sqrt[3]{\frac{3}{4\pi}}\frac{1}{h}\sqrt[6]{1 - e^2}[1 + e^2 - \nu]p\sqrt[3]{V^*}\frac{V^*}{\Delta V}.\text{[1]} \tag{23}$$

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[1] The asterisk (V^*) distinguishes here between volumes in equations (19) and (20) and (23) and (24); the volume quantities will be discussed later for equations (23) and (24).

A more useful approximate form of this is, when e is small,

$$E \approx 0.90\frac{1}{h}[1 + e^2 - \nu]p\sqrt[3]{V^*}\frac{V^*}{\Delta V}. \tag{24}$$

Stress-Strain Diagram of Membrane. From equations (9) and (10) or (11) one can calculate the stresses σ_1 and σ_2 existing at any arbitrary point of the membrane. With equations (19) or (20) one can calculate the maximum value of the reduced stress, which exists at the membrane in the position $x_0 = 0$ (at the equator of the shell). From equations (23) or (24) the modulus of elasticity can be calculated. However, the more perspicuous comprehension of deformation of the membrane is obtained by using the *stress-strain diagram.*

When one introduces to Hooke's law $\epsilon_v = \sigma_{\mathrm{red}M}/E$, the stress and modulus of elasticity equations (19) and (23), respectively, one obtains:

$$\epsilon_v = \alpha\left(\frac{V}{V^*}\right)^{1/3}\frac{\Delta V}{V^*}. \tag{25}$$

where ϵ_v = *relative* "volume-change strain" in the direction of stress $\sigma_{\mathrm{red}M}$. The expression

$$\alpha = \tfrac{1}{3}[2(1 + e^2)(1 - \nu) + e^4]^{1/2}/(1 + e^2 - \nu) \tag{26}$$

can be evaluated, e.g. $\alpha = \tfrac{2}{3}$, when $e = 0$ (for sphere) and $\nu = 0.5$.[2]

The stress-strain diagram can now be drawn when the abscissa, ϵ_v, equation (25) and the ordinate, $\sigma_{\mathrm{red}M}$, equation (20), are known. By drawing a tangent for the curve $\sigma_{\mathrm{red}M} = \sigma_{\mathrm{red}M}(\epsilon_v)$, its slope, as is well-known, gives directly the value of the modulus of elasticity.

Osmotic Pressure Difference. When assuming the cellular and mitochondrial swelling to be due only to the osmotic pressure difference, in the osmotic steady state one can simply write:

$$p = \frac{\phi nRT}{V - V_b} - \pi_m \tag{27}$$

where p = osmotic pressure difference [which is equivalent to p in equation (3)], R = gas constant, T = absolute temperature, V_b = osmotic inactive volume in the shell ("osmotic dead space"), n = number of moles of solute, ϕ = osmotic coefficient, and π_m = osmotic pressure of medium (for seawater, see Appendix). The product ϕn is assumed to be constant.

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[2] In the present paper one uses for Poisson's ratio the value $\nu = 0.5$, which is rather generally used for cell membranes. The question of the value for Poisson's ratio $\nu = 0.5$, which also is the theoretical maximum value, and whether it is constant regardless of deformation will be treated in later parts of this series of studies.

Definitions of the State of the Membranous Shell as a Function of Stress

Subelastic Membrane. When one studies a cellular or mitochondrial swelling process starting from its isoosmotic state and going in a hypotonic direction, one verifies a well-known phenomenon, that the swelling process follows Boyle-van't Hoff's law. The swelling process is reversible, the deformations in the membrane are elastic. The pressure difference, p, in the previously obtained data has been approximately zero.[3] The existing volume of the shell is indicated with V_s.

Elastic Membrane. When transferring to more hypotonic media, it can, however, be observed (by calculating) that at one state of deformation of the membrane the pressure difference is $p > 0$. This limit point, where p just exceeds practically the value zero, is defined here as the "beginning of the elastic behavior of the membrane." At this point the existing volume of the shell is denoted V_B. The V vs. $1/\pi_m$ graph is no longer linear and the swelling process does not follow Boyle-van't Hoff's law. This phenomenon was illustrated by Shapiro and Parpart (17, p. 150) in studies of the swelling of leukocytes: "Up to a dilution of 0.4 R.-L. (Ringer-Locke) the cells appear to follow Boyle's law moderately well, but after this volume (about 1000 μm^3) has ben attained, further dilution seems to have no effect in increasing the volume. What happens at this point is quite conjectural." The volume change from normal (isoosmotic) value V_a to V_B can hypothetically be considered as smoothing of the "folded membrane." *The degree of folding of the membrane* ($\mathcal{K}$) can then be defined $\mathcal{K} = A_B/A - 1$ and for spherical shells:

$$\mathcal{K} = \left(\frac{d_B}{d}\right)^2 - 1 = \left(\frac{V_B}{V}\right)^{2/3} - 1 \qquad (28)$$

where A and A_B are the areas ($A < A_B$), d and d_B are the diameters ($d < d_B$), and V and $V_B (V < V_B)$ the volumes, respectively. Particularly $\mathcal{K}_a$, when $A = A_a$ (or $d = d_a$, $V = V_a$), expresses in the *isoosmotic* state a degree of folding of the membrane.

When the swelling process is continued by making the media more hypotonic, the stresses at the membrane increase and attain first the *proportional limit* (when $V = V_P$) and later the *yield limit* ($V = V_Y$).[4]

The deformation of elastic membrane is reversible.

Plastic membrane. In continuing the swelling process and deformation of membrane beyond the yield limit the nature of the deformation becomes plastic. The swelling process has now irreversible features. In the region of the yield limit exist numerous blebs at the cells, which indicate that the field of stress at the membrane is not homogenous. Stress concentrations generate the local plastic deforma-

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[3] With the latter compression method pressures have been measured obviously in subelastic domain.

[4] Since it is not known how the membranous material behaves in the field of stress, this question will be treated in future parts of this study.

tions and after that attenuate. The increase of deformation terminates with the rupture of the membrane. The *breaking limit*[5] (σ_U) has been reached.

By using the above mentioned subindex for other parameters of the process, one obtains:

1. Sub-elastic range: $V_s < V_E$, $p = 0$. Boyle-van't Hoff's law is valid.
2. Elastic range: $V_E \leq V < V_P$ or V_Y (see footnote 4), $0 \approx p_E \leq p < p_P$ or p_Y. The equation (25) can now be defined correctly with the form:

$$\epsilon_v = \alpha \left(\frac{V}{V_E}\right)^{1/3} \left(\frac{V - V_E}{V_E}\right) = \alpha \left(\frac{V}{V_E}\right)^{1/3} \left(\frac{V}{V_E} - 1\right). \tag{29}$$

   The previously derived equations are valid in the elastic range and are most accurate when $\epsilon_v \lesssim 15\%$. It should be noticed that the *elastic* deformation of the membrane has been defined to take place when V_E is exceeded.
3. Yield range: When $p = p_Y$ and $V = V_Y$, the yield limit is reached and when $p = p_U$ and $V = V_U$, the breaking limit is reached. The stresses σ_Y and σ_U are the yield and ultimate stresses of material, respectively. The equations give very roughly an estimation of stresses and modulus of elasticity in this range.

EXPERIMENTAL MATERIALS AND METHODS

In order to extend the range of the available experimental data [McCutcheon et al. (19) and Lucke et al. (20)] and to emphasize deviation from the van't Hoff relationship, the experiments on the eggs of sea urchin, *Strongylocentrotus purpuratus*, were carried out over a very wide range of π_m values. The shedding was induced with KCl solution (0.5 M).[6] The eggs were collected in sea water, washed four times, and the slower sedimenting part of the suspension was removed each time. The work was done in temperature 12.0 ± 0.5°C. The salinity of sea water was $S = 32.65‰$,[7] the diluted media were 0.9, 0.8, 0.7, 0.6, 0.5, 0.45 0.4, 0.35, 0.3, 0.25, and 0.2 of sea water. When changing eggs from one medium to another, the mixing of media was kept as small as possible by centrifuging egg suspension three times and removing eggs by micropipette each time. The time, when eggs were swelling in different media, was over 90 min (21), at which time the osmotic equilibrium was reached practically well.

The diameters of the eggs were registered under the microscope by photography (objective $M = 43\times$, $NA = 0.45$, dry, but water immersion was employed) so that the magnification on the film was *circa* 100. The measurements of diameters were made by enlarging the pictures on the screen, so that the total magnification was *circa* 900.

RESULTS

The Constants V_b, V_E, and ϕnRT

For the measurements of the volume V_b one uses the well-known method in Lucke and Ricca (22 p. 217) to extrapolate volume (V_b) to the point $1/\pi_m = 0$ (Fig. 2).

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[5] V_U and p_U correspond to the critical volume V_h and tonicity T_h in condition where hemolysis occurs (18). Here, however, the subindex "U" has been employed, corresponding to the word "ultimate."

[6] Dr. David Wilson. Personal communication.

[7] Mr. John Valois, Assistant Manager. Marine Biological Laboratory, Woods Hole. Personal communication.

For determining the constant $\phi n\mathrm{R}T$ in equation (27) one also uses the well-known method in Tedeschi (23 p. 162) of calculation from the slope of the curve in the region where $p = 0$. Volume V_E can be obtained graphically.

Stress-Strain Diagram

By using values of the "smooth" curve, in Fig. 2, stress-strain diagram of membrane has been plotted in Fig. 3 by calculating the maximum reduced stress ($\sigma_{\mathrm{red}M}$) and relative strain (ϵ_v) from equations (20) and (29), respectively, when $e = 0$, $\nu = 0.5$, $\alpha = 0.75$ (equation 26), and $h_E = 3.0 \cdot 10^{-4}$ cm[8] (24). In consequence of the presently used value of Poisson's ratio, $\nu = 0.5$, one can write in the elastic range the expression for the thickness of the membrane as a function of the volume (V):

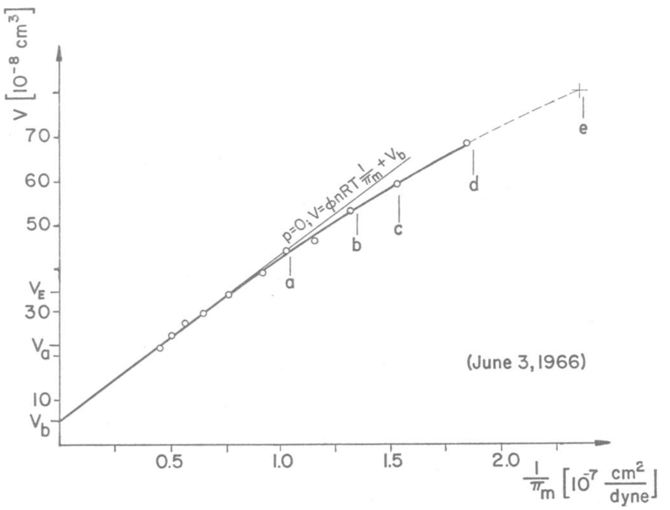

FIGURE 2 The volume of the eggs of *Strongylocentrotus purpuratus* as a function of the inverse value of the osmotic pressure of media. ($t = 12 \pm 0.5$°C, $V_a = 22.5 \cdot 10^{-8}$ cm^3, $V_b = 5.3 \cdot 10^{-8}$ cm^3, $V_E = 35.0 \cdot 10^{-8}$ cm^3, $V_b/V_a = 23.6\%$, $\mathcal{H}_a = 34.1\%$, and $\phi n\mathrm{R}T =$ 3.75 dyne cm). (*a*) Blebs are noticeable in an insignificant fraction of cells indicating the onset of cytolysis. (*b*) →(*d*) Frequency of cytolysis increases. (*e*) All cells are cytolyzed in this medium; the volume is extrapolating graphically estimated. (Each experimental point represents the average value of the diameter of 15–50 eggs measured in two directions).

$$h \approx h_E \left(\frac{V_E}{V}\right)^{2/3}. \qquad (30)$$

From the curve, in Fig. 2, one obtains $V_E = 35.0 \cdot 10^{-8}$ cm^3[9] and $\phi n\mathrm{R}T = 3.75$

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[8] The literature also mentions other values for the thickness of the cortex of egg cell: 1.6 μm (25) and 6 μm (26).

[9] Estimation has been done calculating from the values of Fig. 2.

dyne cm. The pressure p for equation (20) is calculated from equation (27), when the osmotic pressures (π_m) of the media are known (see Appendix).

According to the results, the existing parameters of cell membrane can be verified in different domains:

1. Subelastic range: $22.5 \cdot 10^{-8}$ cm$^3 \leq V_s < 35.0 \cdot 10^{-8}$ cm^3, $V_a = 22.5 \cdot 10^{-8}$ cm^3, $V_b = 5.3 \cdot 10^{-8}$ cm^3, $V_b/V_a = 23.6\%$, $\mathcal{H}_a = 34.1\%$.
2. Elastic and yield range: $V_B = 35.0 \cdot 10^{-8}$ cm^3 (which corresponds between 0.6 and 0.5 media), $E_{\max} = 1.0 \cdot 10^7$ dyne/cm^2 (constant when strain is $\epsilon_v > 15\%$).

In Fig. 3 one cannot specify the transition points, σ_P or σ_Y. But account of cytolysis, one can assume that $\sigma_Y \approx 5.5\text{–}8.5 \cdot 10^6$ dyne/cm^2. Below this is the elastic range $\sigma_{\text{red}M} \approx 0\text{–}5.5 \cdot 10^6$ dyne/cm^2 and the corresponding strain $\epsilon_v \approx 0\text{–}63\%$. The rupture of the membrane occurs *roughly* in the range of $\sigma_U \approx 11 \cdot 10^6$ dyne/cm^2.

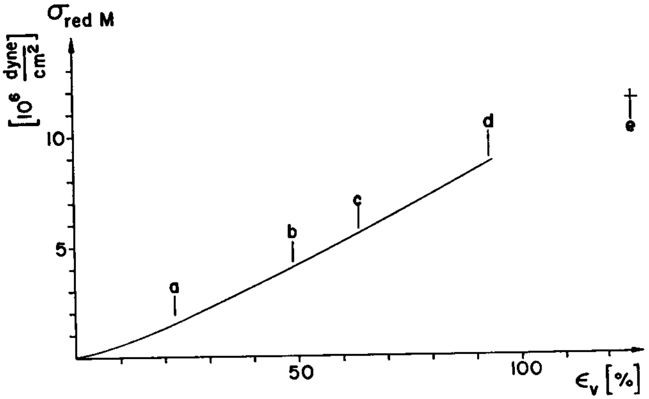

FIGURE 3 Stress-strain diagram. Point (*a*)–(*e*), see Fig. 2.

Corresponding Results

Rikmenspoel (8) has, by making good use of the differential equation of the deflection curve, obtained for the fibrous sheet of the tail of bull spermatozoa $E = 3 \cdot 10^7$ dyne/cm^2. Some other values of elastic modulus for biological tissues, which Rikmenspoel mentions, are for collagen $E_3 = 3 \cdot 10^7$ dyne/cm^2 [10] and elastic $E = 1 \cdot 10^7$ dyne/cm^2 (27) and extracted muscle fibers $E = 6 \cdot 10^7$ dyne/cm^2 (28). These values of the modulus of elasticity correspond with the author's value.

Also Rikmenspoel has calculated that the maximum tensile stress which develops at the surface of the earlier mentioned fiber is $\sigma = 4 \cdot 10^6$ dyne/cm^2 (8). There is a reason to assume a priori that in the fibrous membrane of the sperm tail as a consequence of its natural dynamics, it does not develop stress which exceeds the yield limit, but is rather at a certain distance of the safety margin from it. Thus, it is obvious that the stress $4 \cdot 10^6$ dyne/cm^2 is at least below the yield limit. Accord-

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[10] The subindex, E_3, means the value of the modulus of elasticity, when strain is 3%.

ing to the results of the author the reduced stress, in the elastic area, is $0 \cdots 5.5 \cdot 10^6$ dyne/cm^2, which, compared to the above mentioned value, seems reasonable. The maximum reduced stress corresponds to the strain $\epsilon_v \approx 60\%$. Within 15–60% strain (elastic range) the modulus of elasticity is constant. The stress mechanism developed in the cell wall looks like the stress mechanism in the strands of myxomyceta, which has the constant modulus of elasticity up to 40% elongation (6) ($E \approx 9 \cdot 10^4$ dyne/cm^2 at *circa* 23°C).

According to Cristol and Benezich, in the human erythrocyte the existing osmotic pressure difference (p) exceeds the value zero when $V \approx 370$ cm^3 and rises to the value $0.12 \cdot 10^6$ dyne/cm^2 on the upper boundary of linear range, where the volume $V \approx 430$ cm^3; the corresponding osmotic pressure of the medium decreases from 6.0 to 4.5 atm, [(29) Fig. 1 and (30) Fig. 2).[11] Correspondingly, the volumes, reduced to one erythrocyte from the cited figures, change from $100 \cdot 10^{-12}$ cm^3 (V_E) to $118 \cdot 10^{-12}$ cm^3. The reducing was performed taking for the value of the volume of the erythrocyte in isotonic medium $87 \cdot 10^{-12}$ cm^3 (V_a) (32, p. 14, Table I). When one assumes that the biconcave shape of the erythrocyte in the area of the above mentioned media has changed approximately to a sphere, one can calculate the stress-strain diagram for erythrocyte. When the values of the volumes are $V = 109$, $V = 114$, and $V = 118 \cdot 10^{-12}$ cm^3 (the corresponding strains are $\epsilon_v \approx 7\%$, 10%, and 13%, respectively), and assuming that the thickness of the membrane is really $h_E \approx 10^{-5}$ cm (1000 A) (33), one obtains the following stresses, which one can assume to belong to the elastic range (see footnote 11):

$$\epsilon_v \approx 7\% \qquad \sigma \approx 0.5 \cdot 10^6 \text{ dyne/cm}^2$$

$$\epsilon_v \approx 10\% \qquad \sigma \approx 0.9 \cdot 10^6 \text{ dyne/cm}^2$$

$$\epsilon_v \approx 13\% \qquad \sigma \approx 2 \cdot 10^6 \text{ dyne/cm}^2.$$

The corresponding modulus of elasticity is:

$$E_7 \approx 1, \qquad E_{10} \approx 2, \quad \text{and} \quad E_{13} \approx 5 \cdot 10^7 \text{ dyne/cm}^2.$$

The corresponding values for erythrocyte membrane, like the author has calculated with present equations, also Katchalsky et al. (10) and Rand (34) have obtained with their own methods:

$$E = 2.4 \cdot 10^7 \text{ dyne/cm}^2 \quad \text{and} \quad E = 0.73\text{–}30 \cdot 10^7 \text{ dyne/cm}^2, \quad \text{respectively.}$$

The behavior of the membrane of the erythrocyte under stress will be discussed in detail elsewhere (35).

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[11] Studies (29, 30) do not satisfy the condition $V_U/V_a = 1.75 \cdots 1.54$ (31) but the ratio is $450/320 \approx 1.4$. The deviation might be methodical, being caused by hemolysis, and in the linear part of the curve 2 (30) the error is obviously smallest. Therefore, the author has not calculated values from the nonlinear part.

Kao and Chambers have measured (internal) hydrostatic pressure of puncture-actived Fundulus eggs (36) in 1.0 medium of seawater to obtain *circa* $p = 150$ mm Hg ($\approx 2 \cdot 10^5$ dyne/cm^2). When one calculates approximately the existing state of stress at the membrane by assuming that the hydrostatic pressure is distributed over the whole membrane, regardless of the effect of the size of the perivitelline space and using the value for the volume of the egg $V \approx 3 \cdot 10^{-3}$ cm^3, one obtains [when $h \approx 10^{-3}$ cm (chorion)], stress $\sigma \approx 9 \cdot 10^6$ dyne/cm^2. This is in the same class as earlier mentioned stresses.

According to the above mentioned comparisons it can be verified that at the cell wall existing stresses both in the egg cell of sea urchin (*Strongylocentrotus purpuratus*) and in the other mentioned membranes can reach values which are in the elastic range to $\sigma < 9 \cdot 10^6$ dyne/cm^2, and the ultimate stress is approximately $11 \cdot 10^6$ dyne/cm^2.[12]

Up to the volume $V_B = 35 \cdot 10^{-8}$ cm^3 the form of the function $V = V(1/\pi_m)$ in Fig. 2 is a straight line and Boyle-van't Hoff's law is valid. The calculated $\mathcal{H}_a = 34.1\,\%$. By using results of McCutcheon et al. (19, Table II), the author has calculated $\mathcal{H} = 50$–$55\,\%$[13] (for the egg cell of *Arbacia punctulata*) and for the same egg cell from results of Lucké et al. (20) $\mathcal{H} = 30\,\%$.

The ratio $V_b/V_a = 23.6\,\%$ in Fig. 2. For the egg cell of *Arbacia* $V_b/V_a = 6 - 20\,\%$ (20) and of *Strongylocentrotus intermedius* and *Strongylocentrotus nudus* 21 % and 28 %, respectively (37). Both values, $\mathcal{H}_a \approx 34\,\%$ and $V_b/V_a \approx 24\,\%$, have a good agreement with the above mentioned corresponding values.

Compression and Cell Elastometer Methods

For compression method (1) Hiramoto has derived equations and found the modulus of elasticity for sea urchin (*Hemicentrotus pulcherrimus*) egg $E \approx 4 \cdot 10^3$ dyne/cm^2 (38). The same kind of results many other workers have also obtained. This value is *circa* 10^4 times smaller than the value in the present paper for *Strongylocentrotus purpuratus*. The difference between the above mentioned values quite obviously comes from the dissimilarity of the measurement methods. One might have courage to suggest here a point of view which might cause the difference of the results. The volume of the compressed egg has to remain constant during the compression at all deformation levels by using Hiramoto's equations, but the area of the egg increases with compression. This constant condition is revealed also by the control experiments in Appendix, p. 73 of Hiramoto's paper. The author has calculated the volume and area of the egg at the different compression states used by Hiramoto starting from the values (at the moment of 5 sec) in Figs. 3, 4, and 5 and using equations 1–4 in reference 38. Results are in Table I.

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[12] In the light of the above mentioned values of the membrane it is rubber-like. For the soft rubber $\sigma_p \approx 10 \cdot 10^6$ dyne/cm^2 and $E_{50} \approx 1.8 \cdot 10^7$ dyne/cm^2.

[13] The observation (19) that the cell wall of the cell is slightly injured in 0.5 medium signified that the state of deformation of the membrane is in the neighborhood of elastic range and, therefore, one can estimate $\mathcal{H}_a \approx 50$–55% in this case.

In light of the author's theory, when the eggs of *H. pulcherrimus* and *S. purpuratus* are assumed very similar in their mechanical and structural properties, it is likely that the values of modulus of elasticity measured by Hiramoto are from the subelastic domain. When the degree of folding ($\mathcal{H}_a$) in *S. purpuratus* is *circa* 34% or roughly speaking the area of the folded membrane of the egg has to "dilate" before the deformation exists in elastic area, it can be seen that using the compression method the maximum value of $\mathcal{H} \approx 10.6\%$ is below this.[14] It is to be noted, that the recalculated value of the volume ($V \approx 48.1 \cdot 10^{-8}$ cm^3), which corresponds to the area ($A \approx 313 \cdot 10^{-6}$ cm^2), is still larger compared to $V_a \approx 44.9 \cdot 10^{-8}$ cm^3 and, therefore, $\mathcal{H} \approx 10.6\%$ might be still smaller.

TABLE I

RECALCULATED VALUES FROM HIRAMOTO'S RESULTS. COMPRESSION AT THE MOMENT OF 5 SEC

(In recalculating by using the values of experimental points in figures, the original accuracy of measured values cannot, of course, be attained.)

F (10^{-3} dyne)	V (10^{-8} cm^3)	A (10^{-6} cm^2)	$\mathcal{H}$ (%)
0.0	44.9 (V_a)	283 (A_a)	—
0.5	42.1	277	?
1.0	46.7	297	4.9
2.5	44.6	297	4.9
4.0	48.1	313	10.6

The same artifact as before also exists in using the cell elastometer when the surface area of the membrane increases only *circa* 5% (39, p. 448).

About Source of Errors

The present elastic-mathematical theory consists of certain simplifications, which have been mentioned in the postulates of the theory. Also these equations are based on differential equations of the first order, although they are not explained here. This limits among other things the use of greater relative strain (ϵ_v). According to the postulate the membrane is isotropic and homogeneous. However, the membrane is porous and the calculated values of stress and modulus of elasticity are, when reducing them to the nonporous membrane, unlikely. Nevertheless, one can understand the stress-strain diagram as a macroscopic characteristic of the membrane and then one can speak also about modulus of elasticity.

The errors in the calculation of the osmotic pressure difference (p) are difficult to estimate because of the inadequate data of the swelling process. The possibility that the intracellular concentration would decrease because of leaking of material through the membrane when stresses on the membrane increase and, therefore, the

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[14] The same observation has been made for erythrocytes that the stresses at the membrane exist at significant levels only after sufficient swelling (10, 35).

volume of the cell would remain smaller than in the ideal case when leaking is zero, does not seem probable because of the performed experiments studying permeability [$V = V(t)$] and the demonstrated reversibility of the osmotic swelling from the media of 0.25–0.6 to 0.7, when all existing volumes (in each media the average value of 10 egg cells) were between the range of $V = 30.0^{+0.3}_{-0.9} \times 10^{-8}$ cm^3.

The deviation of the upper points from the line in Fig. 2 might be due to "stress-selective cytolysis," i.e., the stress on the membrane reaches the breaking limit (in the same medium in conditions of the osmotic steady state) earlier for large cells than for small cells. This cannot be the case, however, because the sample populations were selected for a uniform size and this possible error source was eliminated.

The flattening of the eggs under their own weight especially in hypotonic media is significant (19, 40). The correction of the volumes has not been made, because the apparent increase in volume decreases the pressure difference but increases the stress value. Thus, the factor of error has autocorrective features.

APPENDIX

Osmotic Pressure of Sea Water and Its Dilution

100% sea water or 1.0 medium, the salinity of which is $S = 32.65\%$, has been diluted to different concentrations. The osmotic pressure of media as a function of salinity can be obtained with curvilinear interpolation from "pioneering" paper[15] (41) or from a table in reference 42 made on the basis of this study. Knowing the chlorinity, Cl(‰), of media, which can also be calculated from the empirical equation (43), one can obtain the osmotic pressure (43 or 44, Table II, p. 451). The given values of the osmotic pressure in the cited papers can be transformed to the temperature of the experiment by using the approximate equation (44).

The values of the osmotic pressure of the medium calculated from references 42 and 44 are not exactly the same. In the present paper data has been used from reference 42.

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