ELASTIC-MATHEMATICAL THEORY OF CELLS AND MITOCHONDRIA IN SWELLING PROCESS

II. Effect of Temperature upon Modulus of Elasticity of Membranous Material of Egg Cells of Sea Urchin, *Strongylocentrotus purpuratus*, and of Oyster, *Crassostrea virginica*

M. J. MELA

From the Laboratory of Experimental Embryology, Department of Zoology, University of Helsinki, Helsinki, Finland

ABSTRACT The elastic behavior of the cell wall as a function of the temperature has been studied with particular attention being given to the swelling of egg cells of *Strongylocentrotus purpuratus* and *Crassostrea virginica* in different sea water concentrations at different temperatures. It was found that the modulus of elasticity is a nonlinear function of temperature. At about 12–13°C the modulus of elasticity (E) is constant, independent of the stress (σ) and strain (ϵ_v) which exist at the cell wall; the membranous material follows Hooke's law, and $E \approx 3 \times 10^7$ dyn/cm^2 for *S. purpuratus* and *C. virginica*. When the temperature is higher or lower than 12–13°C, the modulus of elasticity increases, and the membranous material does not follow Hooke's law, but is almost directly proportional to the stresses existing at the cell wall. On increasing the stress, the function $E_\sigma = E(\sigma)$ approaches saturation. The corresponding stress-strain diagrams, $\sigma = \sigma(\epsilon_v)$, and the graphs, $E_\sigma = E(\sigma)$ and $E_\sigma = E(t)$ are given. The cyto-elastic phenomena at the membrane are discussed.

INTRODUCTION

General

In comparing properties of the *membrane* (cell wall or membrane)[1] of different cells, mitochondria, etc., one of the characteristic quantities is the modulus of elasticity, E (Young's modulus) of the material. In comparing different kinds of cells, one can thus use the "absolute" measure, in which case shape, dimensions,

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[1] The word "membrane" is employed here as a general term, c.f. postulate 3, p. 97 of reference 1.

and type are not important. This description of the cell membrane in terms of E enables a stress-strain diagram to be constructed.[2] In this paper the important connection between the states of stress and deformation within the elastic range are explained and an empirical study on the effect of temperature on the modulus of elasticity is presented.

In cytokinetics, in cases where elastic deformation of the membrane exists, the interdependence of elasticity and temperature is important. For instance, in a crystalline material, modulus of elasticity increases with a decrease of temperature. The nature of the phenomenon in the case of membranous material is not known.

The thermal effect on the modulus of elasticity of the cell wall will be studied at various temperatures by using swollen egg cells of the oyster, *Crassostrea virginica*, and of the sea urchin, *Strongylocentrotus purpuratus*. The method of calculation has been presented in part I of this series of investigations (1).

The egg cell of *S. purpuratus* has been found to be a useful experimental material (1). To obtain an extensive comparison of results, egg cells of different species have been used. The egg cells of *C. virginica* have been chosen because their diameter (45–50 μm) is smaller than that of *S. purpuratus* egg cells (71–73 μm), but they are large enough for the photomicrography used here. The required time for steady state of swelling in different sea water concentrations is also much shorter (only 8–10 min) than in the case of *S. purpuratus* (1–3 hr; the time depends on the temperature and concentration of the medium. In addition, ripe oysters are both easy to obtain in summer months and inexpensive. The osmotic properties of egg cells of *C. virginica* obey Boyle-van't Hoff's law (in subelastic range) as Lucké and Ricca first verified (2).[3] However, the spherical shape and diameters of these egg cells cannot be maintained in tolerances as narrow as in the case of *S. purpuratus*.

Stress-Strain Diagram

The abscissa of the stress-strain diagram, i.e. the relative "volume-change strain" (ϵ_v), is calculated from Equation 29 in reference 1:

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

where

V = the volume of the shell ($V > V_E$)

V_E = the volume of the shell in the state where the elastic range begins

α = $2/3$, when eccentricity is $e = 0$ (for sphere) and Poisson's ratio $\nu = 0.5$, Equation 26 in reference 1.

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[2] Terminology used here explained in reference 1.

[3] Denomination "*Ostrea virginica*" used in reference 2 is at present obsolete.

The ordinate of the diagram, i.e. the state of largest stress in two dimensions existing at the membrane and which is equivalent to the reduced stress (in one dimension) $\sigma_{\mathrm{red}M}$, is evaluated from Equation 20 in reference 1. The state of stress in two dimensions is homogeneous in this case, ($e = 0$), and when $\nu = 0.5$ the stress is

$$\sigma_{\mathrm{red}M} = 0.31 \frac{p}{h} \sqrt[3]{V}, \tag{2}$$

where

p = the pressure difference across the membrane
h = the thickness of the membrane.

In calculations one must be satisfied for the present to assume that Poisson's ratio, $\nu = 0.5$. A logical conclusion from this is that the thickness of the membrane (h) is a function of swelling level, Equation 30 in reference 1:

$$h = h_E \left(\frac{V_E}{V}\right)^{2/3}, \tag{3}$$

where

h_E = the thickness of the membrane, when $V = V_E$, or when the elastic deformation is just beginning.

On the boundary of the elastic range one has $V = V_E$ or $h = h_E$, but obviously h_E is approximately constant over the whole subelastic range ($h_E \approx h_s$), in which case one can identify h_E with the thickness of the membrane, h_a, under isotonic (100% sea water in this case) condition. For a more accurate analysis of h, see Mela.[4]

Equation 2 and the working hypothesis, Equation 3, can be combined:

$$\sigma_{\mathrm{red}M} = \frac{0.31}{h_E V_E^{2/3}} pV. \tag{4}$$

The stress-strain diagram is then easily constructed by using Equations 1 and 4. In the isotonic state, i.e. in sea water, the degree of folding of the membrane (for a spherical shell) is evaluated by means of Equation 28 in reference 1:

$$\mathcal{K}_a = \left(\frac{V_E}{V_a}\right)^{2/3} - 1, \tag{5}$$

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[4] Mela, M. J. Part V. In preparation.

where

V_a = the volume of the shell in the isotonic state. The working hypothesis, Equation 5, will be discussed in more detail elsewhere.[4]

The term p, of Equation 4 or 2 is calculated from Equation 27 of reference 1:

$$p = \frac{\phi nRT}{V - V_b} - \pi_m\,, \tag{6}$$

where

R = gas constant
T = absolute temperature
V_b = osmotic inactive volume in the shell ("osmotic dead space")
ϕ = osmotic coefficient
n = number of moles of solute
π_m = osmotic pressure of medium, see Appendix in reference 1.

The product, ϕn, is assumed constant under the present experimental conditions during the reversible swelling process. The product, ϕnRT, is estimated graphically, p. 103, (1).

In the stress-strain diagram, the slope of the curve $\sigma_{\text{red}M} = \sigma_{\text{red}M}(\epsilon_v)$ gives directly the modulus of elasticity, E, if the membranous material follows Hooke's law.[5] (The function $\sigma = \sigma(\epsilon_v)$ is linear.) When the material does not follow Hooke's law, a variable modulus of elasticity, E_σ, evaluated at the point ϵ_v is given by the slope of the tangent to the stress-strain curve (see Appendix). The latter variation can exist in the elastic range, where the previous equations are valid,[6] when the stress, σ, at the membrane is $\sigma_P < \sigma < \sigma_Y$, or if the material, also under the proportional limit (σ_P), does not follow Hooke's law. The stress-strain diagram is thus quite useful, because the method of calculation for the modulus of elasticity is not restricted to the normal Hooke's law. The diagram can be employed in the general case and gives in addition, the behavior of the material under variable stresses and deformations.

From the stress-strain diagram one obtains also $E_\sigma = E(\sigma)$ and $E_\sigma = E(\epsilon_v)$ graphs. When stresses exist in the elastic area, either of the mentioned graphs may be used. In the present paper, the former diagram is used.

We have had to discuss and examine extensively the elastic principles and possible errors in the conclusions.

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[5] Strictly speaking Hooke's law is a mathematical abstraction.

[6] Within the yield range ($\sigma > \sigma_Y$) and particularly, when $\sigma \approx \sigma_U$, the results of using the previous equations give only rough estimation, p. 103 of reference 1.

EXPERIMENTAL MATERIAL AND METHODS

Crassostrea virginica[7]

We extend the data (2) on the osmotic swelling beyond the subelastic range by allowing the egg cells of *C. virginica* to swell in sea water concentrations 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.45, 0.4, 0.35, 0.3, and 0.25. The salinity was $S = 32.65‰$. The osmotic pressure (π_m) of the different dilutions at different temperatures was calculated by the method described in the Appendix of reference 1.

Spawning was induced by using the thermal shock method of Galtsoff (3) or by opening the gonad. The egg cells were collected in sea water, washed three times and each time the lightest sedimentation in suspension was removed. In each experiment, only egg cells from one individual were used; the selection of the individual from many animals was made microscopically using as criteria, the roundness of the egg cells, the homogeneous appearance of the population, and the smoothness of the contour of the egg cell.

Since the yield of egg cells in a given individual of *C. virginica* was noticeably less than that in *S. purpuratus*, and because the egg cells did not withstand gentle centrifuging without damage, the transfer of eggs from one medium to another was made with a micropipet (volume $= 2\lambda$). To minimize the mixing of different media, e.g. between 1.0 and swelling media, the eggs were placed in a large volume of swelling medium after concentration of egg-precipitate in a micropipet by absorbing excess medium with paper tissue. This transfer operation was repeated twice before the eggs were placed in the final swelling medium. The eggs were kept in 1.0 medium and used in experiments approximately 2 hr after they had been removed from the animal. The swelling process reaches the state of osmotic stability within 10 min at the temperature scale employed even for the lowest concentrations of the medium. To ascertain the steady state, however, the swelling time 15–25 min was used depending on temperature. The scale of temperature was 4.0 ± 0.5, 13.5 ± 0.5, 21.0 ± 0.5 ("room temperature"), and 26.5 ± 0.5°C. The diameters of egg cells were recorded photomicrographically (1).

Strongylocentrotus purpuratus[8]

The temperature scale was 5.0 ± 0.5, and 24.6 ± 0.5°C. Swelling time, depending on temperature was 1.5–2.5 hr. The procedure for handling the eggs has already been presented (1).

ANALYSIS OF RESULTS

$\left(V; \frac{1}{\pi_m}\right)$ - Graph

The swelling process (Figs. 1 and 2) follows Boyle-van't Hoff's law for up to about 0.6 medium. However, one can verify that at the higher temperatures, Boyle's law is valid even to 0.4 medium (Fig. 1 at 26.5°C). The boundary (V_E) between subelastic and elastic range has been normalized on the basis of degree of folding ($\mathcal{H}_a$), and as can be seen in Figs. 1 and 2, it is a function of temperature.[4, 9]

In Fig. 1 the curved part of the function at 26.5°C is approximate; thus the *p*

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[7] Oysters are from Marine Biological Laboratory, Woods Hole, Mass.

[8] The sea urchins are from Pacific Bio-Marine Supply Company, Venice, Calif.

[9] Mela, M. J. Part III. In preparation.

value calculated from this is a very rough approximation. (It has not been possible to repeat this experiment following the latter observation because oysters are not ripe at the time of this writing.)

In Figs. 1 and 2, points representing so-called "collapse volumes", V_C, have also been plotted. For these points the cell, i.e. the average value of the volumes of the cells has collapsed or become flattened and spread, possibly owing to their own weight and large plastic deformations. This phenomenon has also been observed in permeability curves.[9]

During experiments shrinking studies were performed to verify whether or not the swelling process was reversible. The egg cells were returned to two control media. When the cell wall was deformed strongly in a very hypotonic medium corresponding to 1.0 medium, depending on its attaining value of the stress, it was deformed either elastically or plastically. In both cases, leakage of the intracellular fluid is possible but particularly so in the latter case. When the cell membrane was deformed elastically (the present study considers only elastic phenomena) and the leakage occurred, then obviously the volumes in both control media were smaller than the control volumes in these media. Table I presents a case at 21.0°C.

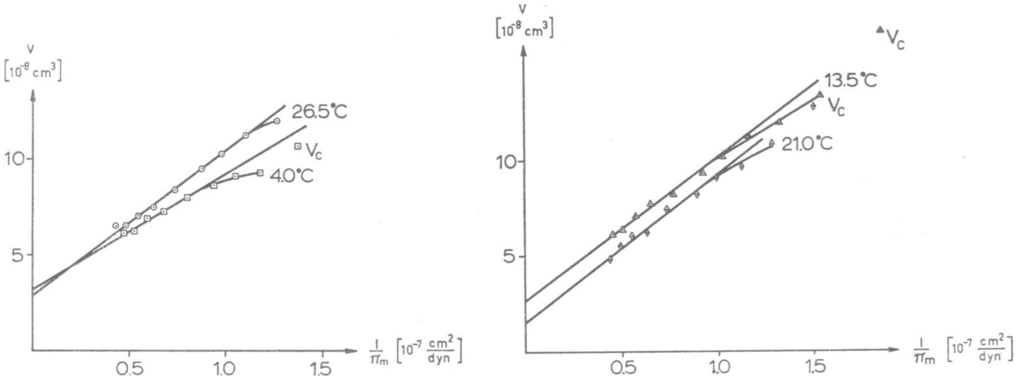

FIGURE 1 The swelling function, $V = V\left(\frac{1}{\pi_m}\right)$, of the egg cell of *Crassostrea virginica* at different temperatures

$$t = 4,\ 13.5,\ 21,\ \text{and}\ 26.5^{\circ}\text{C}$$
$$V_b/V_a = 53,\ 43,\ 31,\ \text{and}\ 47\%$$
$$V_B = 8.0,\ 9.0,\ 8.1,\ \text{and}\ 11.4 \cdot 10^{-8}\ \text{cm}^3$$

(Each experimental point represents the average value of the diameter of 10–40 egg cells measured in two directions.)

The volumes 8.12, 8.29, and $8.11 \cdot 10^{-8}$ cm³ (Table I) correlate with the control volume. However, the final volumes in 1.0 medium, 4.95 and $4.86 \cdot 10^{-8}$ cm³, are slightly smaller than the control volume $4.98 \cdot 10^{-8}$ cm³. If leakage has occurred, it has been very small. The volume $5.18 \cdot 10^{-8}$ cm³ is larger than the control volume but taking into account the tolerances (standard errors) of the volumes 4.95, 4.86, and $5.18 \cdot 10^{-8}$ cm^3, one can say that leakage and plastic deformations in the media 0.45, 0.4, and 0.35 are minor. Also, on the basis of cytolysis the volume corresponding with 0.35 medium has been chosen as the limiting volume for the beginning of the yield range. The volume $7.85 \cdot 10^{-8}$ cm^3 is smaller than the control volume $8.15 \cdot 10^{-8}$ cm^3. Possibly, leakage occurred; and it dominated over plasticity and was able to significantly decrease cell volume. Conversely, the latter leakage cannot be seen in 1.0 medium; the volume of $5.01 \cdot 10^{-8}$ cm^3 is very near to control. A possible reason for this is that in 0.3 medium, the average value of the volume exists as the collapse volume since the critical region of the cell wall has obviously deformed plastically, but without visible rupture or cytolysis. The leakage is, however, great. In returning the cell to the 0.5 medium, the pressure difference p (> 0) keeps the stretched cell wall bulging, but during shrinking in 1.0 medium the weakened wall might collapse.[10] Therefore the real volume is smaller than the volume calculated on the basis of measured diameters ($5.01 \cdot 10^{-8}$ cm^3).

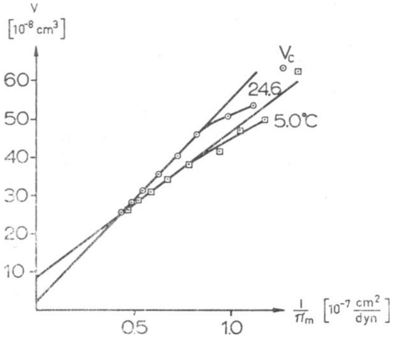

FIGURE 2 The swelling function. The egg cell of *Strongylocentrotus purpuratus*.

t = 5 and 24.6°C

V_b/V_a = 31 and 8.7%

V_E = 36.5 and $46.0 \cdot 10^{-8}$ cm^3

(Each point represents the average value of 12–27 cells.)

Since the point of view of leakage discussed above is important in calculations of the pressure difference, p, one must still examine in the light of Table I, the significance of a change of the product ϕn. Here the following simple criterion is used. In the isotonic (sea water) state the original volume of the cell is V_a, after returning the swollen egg to isotonic control medium the volume is V_a', ($V_a > V_a'$ because of leakage). Thus:

$$\frac{\phi n'}{\phi n} \approx \frac{V_a' - V_b}{V_a - V_b}, \qquad (a)$$

when $V_b \approx V_b'$, or the leakage has not affected "solid" intracellular material. (It has been assumed that plastic deformation is negligible.) By introducing $V_a = 4.98$ and $V_a' = 4.86 \cdot 10^{-8}$ cm^3 (Table I), and $V_b = 1.52 \cdot 10^{-8}$ cm^3 (Fig. 1) at 21.0°C,

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[10] The simple experimental arrangements did not allow measuring at the horizontal level.

one obtains: $\phi n'/\phi n = 96.5\%$. Since one can obviously estimate that in a hypotonic swelling medium of volume, V, the same ratio (or larger) existed, the leakage of the swollen egg is estimated to be about $\lesssim 3.5\%$. When $V \approx 9.5 \cdot 10^{-8}$ cm^3 (Fig. 1), the real (without leakage) volume, V'', must be larger. The corrected volume cannot be exactly calculated (E is now unknown), but a rough estimation is that $V'' < 1.035\, V$ or $V'' < 9.8 \cdot 10^{-8}$ cm^3. Swelling functions in Figs. 1 and 2 have not been corrected by the above mentioned method, because the swollen eggs can become flattened under their own weight, p. 109 of reference 1.

Stress-Strain Diagram

The value $h_E = 3.0$ μm (reference 1), has been used for the thickness of the egg cell of *S. purpuratus*. Preliminary experiments[4] have, however, supported the opinion of the author, that the most effective elastic component of the cell wall might be the cortical granular membrane. Some works, which will be mentioned in the Discussion (Elastic Membrane), support the latter conclusion. In particular, the studies of Afzelius (4) and of Wolpert and Mercer (5), provided the value of the thickness of the membrane, $h_E = 1.0$ μm, for the egg cell of *S. purpuratus*. The same value will also be used for the egg cell of *C. virginica*. It is to be observed that the variation of $h_E = 1.0^{+1.0}_{-0.5}$ μm, might still not be regarded as a too critical variation of the size class.

TABLE I

LEAKAGE AND PLASTICITY OBSERVATIONS OF THE MEMBRANOUS SHELL OF THE EGG CELL OF *CRASSOSTREA VIRGINICA* AT 21.0 ± 0.5°C.

Swelling medium...	0.45	0.4	0.35	0.3*	Control in
Returning in to 0.5 medium, V	▼8.12 ±0.24‡	▼8.29 ±0.23	▲8.11 ±0.13	◆7.85 ±0.48	◆0.5 medium, V = 8.15 ± 0.11
Returning in to 1.0 medium, V	◆4.95 ±0.12	◆4.86 ±0.13	▼5.18 ±0.34	▼5.01 ±0.13	▼1.0 medium, V = 4.98 ± 0.16

The nonfootnote symbols mean the number of the measured cells: ▼10–20; ◆20–30; ▲30–40.
* The collapse volume (V_C) exists in this medium.
‡ The complete marking is $V = (8.12 \pm 0.24) \cdot 10^{-8}$ cm^3, the indicated tolerance means standard error.

The stress-strain diagrams are shown in Fig. 3.[11] At 4.0, 13.5, and 21.0°C, the yield limit is $\sigma_Y \approx 8\text{–}12 \cdot 10^6$ dyn/cm^2, and at 26.5°C, cytolysis and blebs were not observed at $\sigma_{\mathrm{red}M} = 5.3 \cdot 10^6$ dyn/cm^2 (*C. virginica*). At 5.0, 12.0, and 24.6°C, one has $\sigma_Y \approx 14\text{–}17 \cdot 10^6$ dyn/cm^2 (*S. purpuratus*). Differences between these values are not significant because of the uncertainty factors mentioned earlier. In general,

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[11] The function $\sigma = \sigma\,(\epsilon_v)$ (*S. purpuratus*) at 12.0°C has been determined from Fig. 2 of reference 1 by multiplying the ordinate values with the factor $h_E'/h_E = 3/1 = 3$ for the correction of the thickness of the membrane.

one can estimate that the yield point is $\sigma_Y \approx 8 - 17 \cdot 10^6$ dyn/cm²; below this the elastic range exists. The transition points σ_P and σ_Y were not found on the curves (same as in reference 1).

Modulus of Elasticity, $(E_\sigma ; \sigma)$*-Graph*

The stress-strain diagrams for the cell wall do not follow Hooke's law; the exception exists only at 12.0° (over part of the range) and 13.5°C (Fig. 3). In other cases, the modulus of elasticity is a linear or approximately linear function of the stress (Fig. 4). Upon increasing the stress ($\sigma < \sigma_Y$) saturation is approached except for at 26.5°C. It may be possible that saturation is approached only at higher stress levels. The latter phenomenon could not be further studied because of the existence of V_C. At 5.0, 12.0, and 21.0°C one can find stabilization in the value of the modulus of elasticity. It should be noted in this context, that the value $\sigma_Y \approx 8 - 17 \cdot 10^6$ dyn/cm² for yield point has been estimated, for which reason, when $\sigma > \sigma_Y$ the results may include error factors of two kinds: the previously mentioned leakage effect and the effect described in footnote 6.

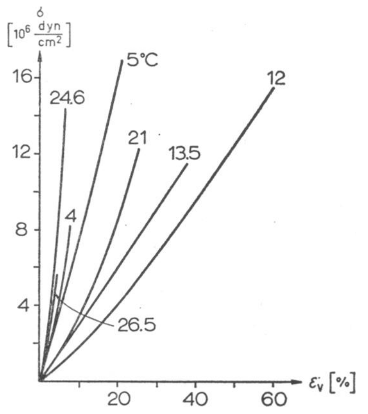

Figure 3 Stress-strain diagram, $\sigma = \sigma(\varepsilon_v)$, of the cell wall at different temperatures.
Only the part of the function ($\sigma < \sigma_Y$) has been drawn.

On the boundary of the elastic range ($\sigma \approx 8 \cdot 10^6$ dyn/cm²) one can verify as the modulus of elasticity $3–20 \cdot 10^7$ dyn/cm² (*C. virginica*) and $3–30 \cdot 10^7$ dyn/cm² (*S. purpuratus*), depending on the temperature. For small values of the stress ($\sigma \approx 0 - 1 \cdot 10^6$ dyn/cm²), the function $E_\sigma = E(\sigma)$ in Fig. 4 is unreliable owing to inaccuracy in graphical derivation in the neighborhood of the origin in Fig. 3.

In the temperature range 12.0–13.5°C, existing monotonic functions $[E = E(\sigma)]$ are obviously valuable in the experimental cases, where the elastic phenomenon affects the outcome of the experiment; however, one attempts to preserve the simplest possible elastic state by keeping the modulus of elasticity as constant as possible over the wide range of stress (or deformation).

Modulus of Elasticity, (E_σ ; t)-Graph

Fig. 5 shows a set of values of the function $E_\sigma = E(t)$ taken from Fig. 4, using stress as a parameter. With this transformation, one obtains a more coherent picture of the variation of the modulus of elasticity as a function of temperature. Since only seven different temperature points are available, the trend of function $E_\sigma = E(t)$ has not been sketched.

DISCUSSION

$\left(V; \frac{1}{\pi_m}\right)$ - Graphs

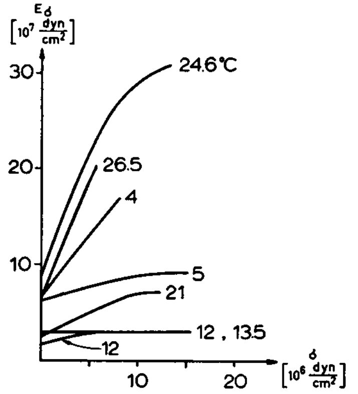

FIGURE 4 Modulus of elasticity as a function of stress, $E_\sigma = E(\sigma)$, when the temperature is as a parameter.

Among the V_b/V_a-values of Fig. 1 (*C. virginica*), there exists rather large differences; the variation is $V_b/V_a = 31\text{–}53\%$. The latter ratio V_b/V_a is large compared to the case of *S. purpuratus* (Fig. 2) which has $V_b/V_a = 9\text{–}24\%$ except for at 5°C when it reaches 31%. Lucké and Ricca (2) have at first measured $V_b/V_a \approx 44\%$ (at 22°C) for oyster eggs, but also reported calculated values in the range 31–56%. The results of the author correlate with the latter values. In the case of *S. purpuratus*, the V_b/V_a-value is in the normal range, p. 107 of reference 1.

Boyle's law is valid at 22°C for up to 0.4 medium (*C. virginica*) as noted in reference 2. At 21.0°C (Fig. 1), the boundary volume V_B, exists around 0.5 medium. This contradiction cannot be explained other than by referring to Fig. 2 of reference 2; however, it is difficult to estimate the V_B-value because of the scatter of experimental points.

The osmotic inactive volume, V_b, has been estimated using linear extrapolation (1). Is the error produced by this method larger than that produced by measuring the volume of cells swollen in hypertonic media? The answer is not known. It is assumed a priori, that in the neighborhood of origin ($\frac{1}{\pi_m}$ is proportionally small), a state of stress can be generated at the membrane, and also below the subelastic range, particularly if V_b is large. The phenomenon in the case of erythrocytes is well-known; the amount of shrinkage is always less than Boyle's relationship implies, p. 404 of reference 6.

$$\sigma = \sigma(\epsilon_v) \text{ and } E_\sigma = E(\sigma)$$

The swelling function, $V = V\left(\frac{1}{\pi_m}\right)$, has been estimated, within experimental error,

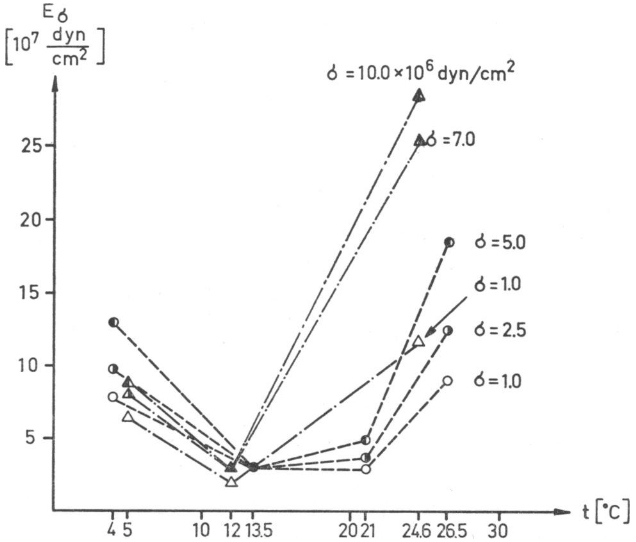

FIGURE 5 Modulus of elasticity as a function of temperature, $E_\sigma = E(t)$, when the stress is as a parameter.
(Corresponding points are connected by broken lines.) ● *Crassostrea virginica*; ▲ *Strongylocentrotus purpuratus*.

to be "smooth." It is difficult to find in the previous result (Figs. 1 and 2) critical points where the $V = V\left(\frac{1}{\pi_m}\right)$ changes its direction rapidly. For this reason, in the $\sigma(\epsilon_v)$- and $E(\sigma)$-graphs, transition points σ_P and σ_Y have not been found. It is possible that the membranous material does not have clear transition points, but it can also be supposed that the abscissa in Figs. 1 and 2 is inaccurate above $\frac{1}{\pi_E}$. It is possible, however, that transition points are slightly developed in the material and are subsequently hidden by the statistical smoothening effect. In this context, one can observe the same statistical phenomenon, i.e., the "anomaly" existing in permeability curves.[9] The elastic range has been estimated previously on the basis of cytolysis (since no transition points were found); $\sigma \approx 0\text{–}8 \cdot 10^6$ dyn/cm^2. This estimation corresponds to certain values in reference 1, of which only the study of Rikmenspoel (7) will be discussed again below.

From reference 7, the existing stress at the fibrous sheath of the tail of bull spermatozoa can reach the value $\sigma = 4 \cdot 10^6$ dyn/cm². The author has concluded that this stress of $4 \cdot 10^6$ dyn/cm² is obviously below the yield point (1). This supports the conclusion that the elastic range attains the value $\sigma \approx 8 \cdot 10^6$ dyn/cm². Rikmenspoel's later work reports a smaller stress value, about $1.6 \cdot 10^6$ dyn/cm², which has been determined from the sea urchin sperm flagellum (8). The modulus of elasticity as a function of stress gives the values: $E_\sigma \approx 2\text{–}9 \cdot 10^7$ dyn/cm² and $E_\sigma = 3\text{–}27 \cdot 10^7$ dyn/cm², when $\sigma = 0$ and $\sigma = 8 \cdot 10^6$ dyn/cm², respectively. Rikmenspoel calculates $E = 3 \cdot 10^7$ dyn/cm², $\sigma = 4 \cdot 10^6$ dyn/cm², (7), and $E \lesssim 10 \cdot 10^7$ dyn/cm², $\sigma = 1.6 \cdot 10^6$ dyn/cm², (8).

These values lie between the results given by the author. If one assumes that Rikmenspoel's work (7, 8) was done at room temperature, one obtains for the modulus of elasticity (from Fig. 4) at 21.0 and 24.6°C (employing the same values of stresses as Rikmenspoel mentioned): $E_\sigma = 4.4\text{–}19.5 \cdot 10^7$ dyn/cm², $\sigma = 4 \cdot 10^6$ dyn/cm², and $E_\sigma = 3.3\text{–}13 \cdot 10^7$ dyn/cm², $\sigma = 1.6 \cdot 10^6$ dyn/cm². These values now correspond more closely to the values mentioned in references 7 and 8.

$E_\sigma = E(t)$

From Fig. 4, one sees that the temperature is related directly to the modulus of elasticity of the membranous material and in addition through the stress (or strain) characteristics to the modulus of elasticity. When the value $\sigma = 0$, the material is only affected thermally; the "rest modulus" of elasticity ($E_{\sigma 0}$) is a function of temperature. Necessary conditions are that the thermal affect should be related only to the membranous material, not to the stress-mechanism, and that the family of curves $E\sigma = E(\sigma)$ has equal slope for every value of σ, when the parameter is t. However, such is not the case; the slopes (k) of the function $E_\sigma = E(\sigma)$ are not equal[12] (in the linear area).

For the linear part of the function $E_\sigma = E(\sigma)$ one can write the simple expression

$$E_\sigma = k\sigma + E_{\sigma 0} \tag{b}$$

where the superposition has been realized. The effect of temperature on k and $E_{\sigma 0}$ is not necessarily similar. The membranous material has, because of Equation (*b*), two different moduli of elasticity. The "rest modulus" $E_{\sigma 0}$, and the "work modulus of elasticity," $E_{\sigma w}$ ($= k\sigma$). In the flat portion, within the accuracy of the data in Fig. 5, in the range $t \approx 12\text{–}15$°C Equation (*b*), $E_\sigma = E_{\sigma w} + E_{\sigma 0}$, shrinks

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[12] The graphical derivation is taken to $\sigma = 1 \cdot 10^6$ dyn/cm²; the interval $1 - 0 \cdot 10^6$ dyn/cm² has been extrapolated.

to the form $E_\sigma = E_{\sigma 0} = E$, (Hooke's law is valid). The terms of Equation (*b*) were not evaluated here, because of the small number of results and as will be discussed later, some of the estimations may change the shape of the functions $E_\sigma = E(\sigma)$.

Norris (9) has measured the modulus of elasticity in a strand of Myxomyceta in the temperature interval 10–30°C and verified that Hooke's law is valid only at 23–24°C.[13] In Fig. 2 A of reference 9 (at 10°C), the experimental points form the curve, $\sigma = \sigma(\epsilon)$, similar to the one seen in Fig. 3. For the modulus of elasticity Norris has not used the generalization criterion (Appendix). This hinders the analysis of his results here in $(E_\sigma\,;\,t)$-graph. However, from his results one can draw the important conclusion, that the modulus of elasticity is not constant within the temperature range 10–30°C, but only at 23–24°C. The phenomenon has then the same kind of features as the results of this work.

Visco-Elasticity

A discussion of the relationship between elasticity and viscosity is not included in this paper since the cells are in osmotic steady state before measurements are taken. Katchalsky et al. (10) and Rand (11) have used visco-elastic bodies in swelling studies. In the elastic range and in osmotic steady state the equations in references 10 and 11 contract to Hooke's law. It is to be noted, that in the *visco*-elasticity, the modulus of elasticity is a function of strain rather than a function of stress (11).

Elastic Membrane

The stress-phenomenon is assumed to exist in the cell wall when the cell volume $V > V_E$. The degree of folding of the membrane at the isotonic level, or $\mathcal{K}_a$, is for the present an unknown structural transition in the membrane. The folding separates two areas from each other, namely the subelastic (Boyle's) and elastic ranges in the $\left(V;\,\frac{1}{\pi_m}\right)$-graph. Poisson's ratio has been assumed to be $\nu = 0.5$ and constant during the swelling process (in the elastic range). Therefore, it has been possible to calculate the thickness (*h*) of the membrane as a function of the swelling level using Equation 3. Future study might yield data supporting these assumptions. But it can be proved that the functions presented in this paper, e.g. in the $(\sigma;\epsilon_v)$-graph, retain their characteristic features when the deviation from the value $\nu = 0.5$ is not large.

The thickness (h_E) of the membrane affects the values of stress directly. The condition $h_E \approx h_s \approx h_a$ has been assumed to exist. In some cases in the literature the thickness of the cell wall has been rejected and substituted with the product $h_a \cdot E$ (12). In the present paper it has been satisfactory, however, to accept the

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[13] The notation in Figs. 1 and 2 of reference 9: the ordinata "F in dynes/cm^2" $\equiv \sigma$ (the tensile stress) and $Y \equiv E$, but "percent elongation" or $\epsilon = (l - l_0)/l$ has been defined differently in reference 1.

variation in the value of h_a, particularly since this variation does not affect the over-all distribution of the functions in Figs. 3–5.

Mercer and Wolpert (13) have verified the existence of a cortical granular layer in the egg cell of *Psammechinus miliaris*, which might be a fusion of the cortical granules and the cell membrane. Because it is necessary to know the strength of different layers of the cell wall in order to estimate the distribution of stresses in them, the observation of Mercer and Wolpert is very helpful. If some solid layer existed on the inside of the cortical granular layer of the cell, it would have little bearing on the evaluation of membrane strength. This can be verified by the following rough calculation.

The centrifugal operation described in reference 13 transfers the yolk granules to beneath the cortical granular layer. The force, which pushes the yolk granules toward the cortical layer during centrifugation, generates the surface pressure $p' \approx Dg'(\rho_1 - \rho_2)$. When $D \approx 0.6$ μm (the mean diameter of the yolk granules, calculated from Fig. 2 of reference 13) and $g' = 4400g$, and assuming that the density of a yolk granule is similar to that of protein, i.e. $\rho_1 \approx 1.3$ g/cm^3, and assuming that the "density" of cytoplasm is $\rho_2 \approx 1$ g/cm^3, then $p' \approx 50$ dyn/cm^2. This value is about 10^{-5} times the value of the stress existing at the membrane. If some assumed membrane has been punctured by yolk granules when the surface pressure is 50 dyn/cm^2, then the value's significance for calculating stress distributions is negligible.

The functions $\sigma = \sigma(\epsilon_v)$ and $E_\sigma = E(\sigma)$ further represent the structural properties of the membrane (cortical granular structure). How great their effect is in the results one cannot estimate in the present paper. However, the stress-strain diagrams of the "simple" membrane, similar for example to the one of the erythrocyte, give a value in the same class as presented here,[14] when the thickness of the membrane is in the region of 200–600 A.

APPENDIX

The derivation of equations of the state of strain in reference 1 has been for the sake of simplicity based primarily on postulate 5. The equations can be easily generalized: (*a*) when material does not follow Hooke's law or (*b*) E is not constant. When in the stress-strain diagram the function $\sigma = \sigma(\epsilon_v)$ will increase with an infinitely small value $d\epsilon_v$, the corresponding increase in stress is $d\sigma = \sigma'(\epsilon_v)d\epsilon_v$. This is analogous to Hooke's law, where the modulus of elasticity, E (constant), has been substituted with a variable modulus of elasticity $\sigma'(\epsilon_v)$ which will be marked here E_σ. In the stress-strain diagram the value of the variable modulus of elasticity can be obtained as the derivative (slope) of the function $\sigma = \sigma(\epsilon_v)$ evaluated at the point ϵ_v, and can thus be marked as E_3 which indicates the value of the variable modulus of elasticity, when the relative strain is 3%.

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