RHEOLOGICAL BEHAVIOUR OF *CHARA AUSTRALIS*

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Summary

The viscoelastic behaviour of the cell wall of *Chara australis* was studied by following the relaxation of stress after deformation by (*a*) imposing a "step function" change in length and (*b*) varying the osmotic pressure of the external bathing solution, and hence changing the turgor pressure within the cell. It was found that (i) a sudden decrease in the original elongation of the cell produced an unexpected transient increase in stress and (ii) an interaction between water movement in the cell due to osmosis and stress relaxation produced a maximum stress with respect to time. The results are discussed in terms of a cross-linked amorphous polymer model.

I. INTRODUCTION

The rheological properties of giant algal cell walls have received attention from several workers recently. Probine and Preston (1962) found that because of the anisotropy of the cell wall and the nature of its constituents, the mechanical properties are different in the longitudinal direction to those in the radial or tangential directions. They demonstrated that when a cell wall was subjected to a constant load, the elongation of the cell was a function of time, i.e. creep resulted. Their results showed that the strain rate increased markedly when the load exceed a critical value.

It was also suggested that irreversible deformations produced upon extension were critically dependent on turgor pressure; the suggestion being further developed by Probine and Barber (1966) who postulated a model of a mat of cellulose microfibrils embedded in a plastic material in order to explain the observed irreversible deformations in the various directions.

Kamiya, Tazawa, and Takata (1963) investigated the relationship between turgor pressure and cell volume. They found a time dependency on the volume of a cell wall tube when subjected to various internal pressure changes. These results revealed a hysteresis effect; the volume and length change in response to increasing pressure did not follow the same path as that of decreasing pressure. They attributed this to the cell wall having elastic components which differ depending on the direction of turgor pressure change and which depend on the magnitude of the strains involved. Living cells also followed a hysteresis pattern in response to external osmotic pressure changes.

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In the present paper, similar experiments on *Chara australis* cells were performed. However, stress relaxation methods were chosen rather than creep for the following reasons:

(1) The material in which the microfibrils of *Nitella* and presumably *Chara* are embedded consists of an amorphous polymeric substance consisting of pectins, hemicellulose, protein, etc. (Probine and Preston 1961). Any amorphous polymer when subjected to an applied strain responds with the typical viscoelastic behaviour of stress relaxation, i.e. the induced stresses will decay with time because of various molecular configurational rearrangements.

(2) Owing to the anisotropy of the cells, any change in the turgor pressure will induce longitudinal tension effects. These changes in tension can be readily followed using an electro-mechanical transducer.

The experiments on the *Chara* internodal cells were specifically designed to investigate the possibility that irreversible deformations do not occur below a critical turgor pressure and to examine in closer detail the hysteresis effects found by Kamiya *et al.*

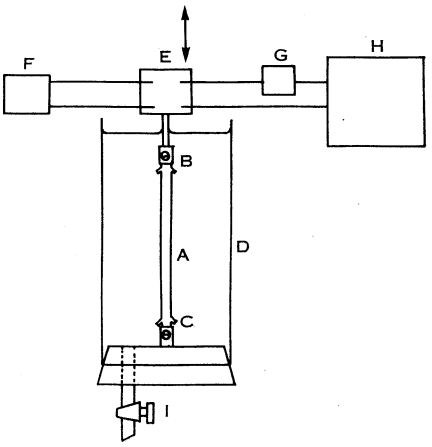

Fig. 1.—Schematic diagram of experimental apparatus. Plant cell *A*, in container *D*, is clamped between *B* and *C*. End *B* is attached to transducer *E* which is vertically adjustable. Excitation voltage is provided by *F*, a 9 V battery. Potentiometer *G* provides a biasing voltage for the transducer output which is displayed on recorder *H*. The bathing solution may be drained at *I*.

II. Experimental

The plant cell was firmly clamped immediately above the nodes. One end was attached to a Statham force-transducer and the other rigidly fixed to the base of the container (see Fig. 1). The force-transducer was racked vertically with a lead screw, the change in position being measured with a calibrated micrometer. The excitation voltage for the transducer was provided by a 9 V battery whilst a potentiometer was included in the output from the transducer to provide biasing voltages. The transducer output was displayed on a Speedomax, 0–10 mV, strip chart recorder.

The Statham force-transducer is of the Wheatstone bridge type. A strain on one of the arms of the bridge changes the resistance and produces an out-of-balance voltage proportional to the strain. The "spring" in the transducer has a maximum displacement of about 0·038 cm for a maximum output of 45 mV (when the excitation

voltage is 9 V), which corresponds to a load of 50 g weight. The stiffness of the "spring" ensured that its extension was negligible when compared to the extensions produced in the cells.

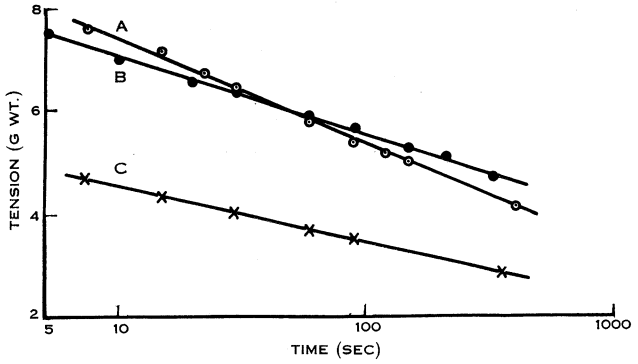

Fig. 2.—Typical stress relaxation of internodal cells and cell wall strips. *A*, internodal cell of length approximately 70 mm, extended 0·18 mm. *B*, same cell as in *A*, but extended a further 0·23 mm. *C*, longitudinal cell wall strip of length 50 mm extended 0·13 mm.

The experiments were divided into two sections: (1) A "step-function" change in length of the sample was applied and the change in tension followed as a function of time. (2) With both ends of the sample fixed in position, changes in

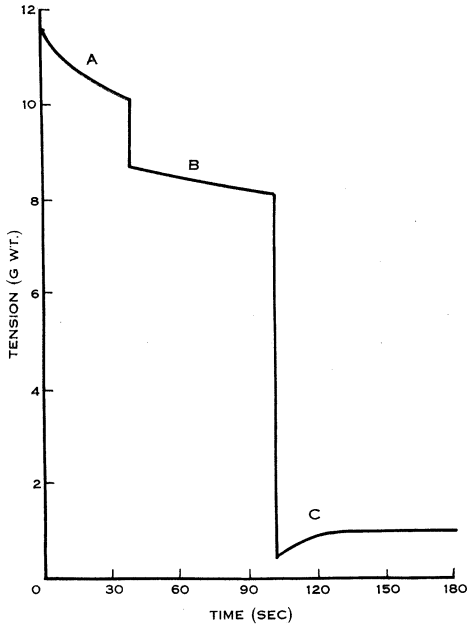

Fig. 3.—Cell wall response to extension and partial and total return to original length. *A*, stress relaxation after sudden extension. *B*, the continuation of relaxation of residual tension after partial release of extension. *C*, increase in tension after almost total return to original length.

longitudinal tension due to various osmotic pressure changes of the bathing solution were followed as a function of time. The osmotic pressure differences were obtained using sucrose concentrations varying between 0 and 0·16M.

The experiments were performed on single internodes and longitudinal strips of cell wall. Elongation of both cell wall strips and whole cells was initially carried out in distilled water.

III. Results

(1) An increase in length of the sample produced a longitudinal tension which decayed exponentially with time (Fig. 2). This occurred for both whole cells and cell wall strips.

(2) A sudden decrease in the original extension produced two effects. If the reduction in extension was small, the continuing relaxation of the residual stresses in the sample was observed. However, large reductions in the original elongations resulted, after the initial decrease, in a gradual increase in tension (Fig. 3). The experiment was repeated with a thoroughly dried strip of cell wall in a moisture-free atmosphere and the same type of result was obtained.

(3) An increase in sucrose concentration (corresponding to a lowering of turgor pressure) resulted in an increase in the longitudinal tension in whole cells but had no discernible influence on cell wall strips. Figure 4 shows a typical result for whole cells only.

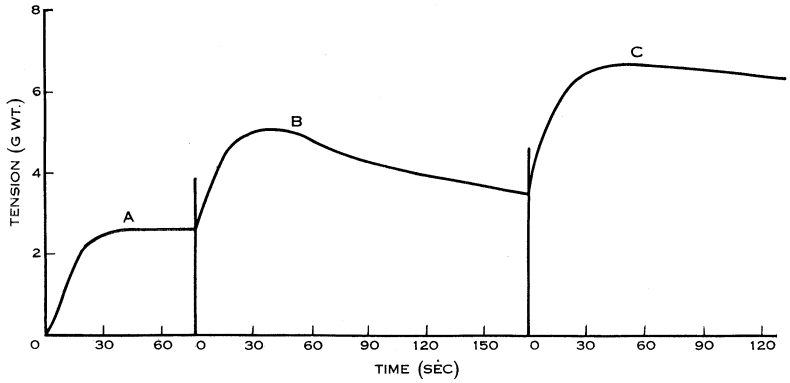

Fig. 4.—Longitudinal tension changes in response to incremental changes in osmotic pressure of bathing solution. *A*, a transient rise in tension upon an osmotic pressure change of 1·24 atm. *B*, a change in osmotic pressure from 1·24 to 2·48 atm produced a maximum value of tension as a result of "opposing" stress relaxation effect. *C*, continuation of stress relaxation after osmotic pressure change of 2·48–3·72 atm. The procedure involved in changing the sucrose solution did not affect the cells.

IV. Discussion

Result (1) reveals the marked viscoelastic properties of the cell wall in the longitudinal direction, the stress relaxation being quite linear over three decades of logarithmic time.

Before the experiment on the dried cell wall in a moisture-free environment was performed, it appeared that the increase in tension after release from the original elongation might be due to water movement in and out of the cell wall. That is, the effect of increasing the tension was to "squeeze" water out of the wall, whilst

a reduction in elongation led to water moving back into the wall, thus producing swelling and a resultant increase in tension. However, the result of the experiment on dried cell wall disproved any explanation based on water movement. Because of its relevance, the postulation of a more plausible explanation will be deferred until discussion of result (3).

In result (3), a change in turgor pressure produces a gradual change in longitudinal tension. It was expected that there should be two effects occurring simultaneously. As water moves out of the internodal cell (corresponding to an increase in external sucrose concentration), the turgor pressures and the cell volume decrease. Because the cell is fixed in length, however, there should be a resultant increase in longitudinal tension. Hence any water movement out of the cell should produce longitudinal stresses and possibly stress relaxation.

It must be pointed out that the above effect is the "inverse" operation to that of Kamiya, Tazawa, and Takata (1963) who followed the change in length of the cell with osmotic pressure differences. There are several important differences, however.

In the present result, a change in external osmotic pressure from 0 to 1·24 atm produced a new equilibrium value of tension (which persisted for a much longer period than indicated). A further change from 1·24 to 2·48 atm and higher osmotic pressures produced a tension which had a maximum value in time. It is now postulated that the two processes discussed above are operating at this level of stress in the wall. Decrease in volume and internal pressure tend to increase the tension (because the ends of the cell are fixed), whilst the stress relaxation "opposes" the increase.

If the osmotic pressure difference is lowered the tension in the walls is reduced and no stress relaxation occurs. The existence of stress relaxation partially explains the results obtained by Kamiya, Tazawa, and Takata (1963) who found that there was a hysteresis loop in a cycle of length–osmotic pressure change.

If the cycle is repeated, stress relaxation does not occur, even when the incremental change in osmotic pressure difference is 3·72 atm. This suggests that in keeping with many viscoelastic solids and liquids, the cell wall "remembers" its previous strain history.

In order to examine Probine's suggestion that creep does not appear to occur below a critical strain, the incremental change in the osmotic pressure of the sucrose solution was reduced to 0·31 atm. No stress relaxation was observed over a 0–3·1 atm range. However, when the osmotic pressure of the bathing sucrose solution was returned to zero, the tension was less than the original value. Subsequent cycles did not change this level and there appeared to be a permanent set in the material. Several other cells, however, of approximately the same length and age when subjected to an increase in osmotic pressure of 3·72 atm showed stress relaxation. The conclusion appears to be that a permanent set exists independently of stress relaxation and the stress relaxation process itself does not occur below a particular magnitude of strain.

The unusual rheological behaviour of the *Chara* cell wall in regard to irreversible deformations, stress relaxation, etc. is also exhibited by natural rubbers and synthetic

elastomers (Bueche 1962; Meares 1963). These substances, after release from a state of tension do not recover completely to their original shape and the residual deformation is referred to as permanent set. It is independent of crystallization and slow relaxation effects, and, because of the cross-linked networks involved, is not associated with plastic flow.

The intrinsic mechanism involved is as follows: the polymer chains, when under too great a tension, break and the broken ends reform cross-links under unstressed conditions. The original strained network is thus replaced progressively with one which is entirely relaxed in the deformed state of the sample. Upon releasing the sample from its strained condition, the original network, in attempting to regain its "old" dimensions, extends the new network and thus produces a tension in the opposite direction. Clearly a point is reached when the opposing tensions in the two networks are equal and the material is then in equilibrium.

The above mechanism could explain all the observations in the present paper. Permanent set, as distinct from stress relaxation, was certainly found in the turgor pressure experiments. Also the experiment performed on the cell wall strip in a moisture-free atmosphere appears to follow the process exactly.

There have been several speculations on the type of rheological model needed to characterize the cell wall's response to applied strains. Probine and Barber (1966) proposed various plastic models to establish a relationship between turgor pressure and the plastic flow in the longitudinal direction. Their final choice was the quasi-plastic relation,

$$V = (1/a)(P-P_0)^r,$$

where V is the rate of shear strains, P is the stress, and a, P_0, and r are constants. However, this equation does not include any elastic terms which must exist if the matrix surrounding the microfibrils is composed of amorphous polymeric material and exhibits viscoelasticity. Kamiya, Tazawa, and Takata (1963) tend to regard the change in volume in response to internal pressure changes as due to the cell wall elasticity having two different components which are responsible for the two different components of the volume change. The transient rise in elongation due to changes in turgor pressure, they suggest, is because of a difference in relative proportion of instantaneous and retarded components between the longitudinal and transverse elasticities.

The present evidence suggests that the most appropriate rheological model is probably intermediate between the two mentioned above. It appears that: (1) the stress is, in general, a non-linear function of strain; (2) the cell wall exhibits both permanent set and viscoelastic effects; and (3) stress relaxation depends on the magnitude of the strains involved and (probably) the rate of strain.

It is difficult to see how an exact mathematical expression can describe this type of behaviour. A possible approach, however, has been made by Lodge (1964) in his mathematical description of similar effects observed in elastic solutions. In dealing with the theoretical implications of elastic recoil in rubber-like liquids, he found that after steady shear flow the liquid "recovers" to a state in which it has never previously existed. As mentioned above, permanent set also occurs in

rubber-like solids containing composite networks, owing to changes in cross-linking during deformation. Thus the rheological equations of state describing the behaviour of rubber-like solids and liquids may have application to the rheology of cell walls.

V. Acknowledgments

I wish to thank Professor E. P. George for his encouragement and interest, Mr. H. Coster for his very helpful advice on the physics and physiology of the *Chara* cell, and to Dr. G. E. Hibberd for valuable discussions and criticism on the rheological aspects of the work.

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Appendix

The purpose of the model to be described below is to relate the rheological response of the *Chara australis* plant cell to changes in turgor pressure. Specifically, it is designed to describe the interaction between an increase in turgor and the opposing stress relaxation in the cell wall. The model is founded on the following assumptions:

(1) The *C. australis* cell is a long cylindrical hollow cylinder with anisotropic rheological properties.

(2) Planes of symmetry exist which are perpendicular to the long axis of the cell.

(3) The moduli in the longitudinal directions are time dependent whereas those in the tangential and radial direction are purely elastic.

(4) The changes in turgor may be described by a rate equation of the form

$$p(t) = (p_1+p_0)-p_1e^{-a_1t}.$$

There will be no loss in generality if the reference pressure p_0 is set equal to zero, that is,

$$p(t) = p_1(1-e^{-a_1t}). \qquad (1)$$

(5) The cell is effectively infinite in length so that volume changes are neglected.

Assumptions (1), (2), and (3) are justifiable if the cell wall is regarded as being composed of transversely oriented cellulose microfibrils of high tensile strength embedded in an amorphous polymeric material (Probine and Preston 1961). The inclination of the microfibrils in immature cells, which would introduce twisting moments into the analysis, is disregarded. The expression in assumption (4) is probably greatly oversimplified but it has the attribute of describing the pressure increase by a single rate constant, a_1. Assumption (5) avoids consideration of the interaction between pressure and volume, which would involve another and more difficult problem altogether.

The following analysis rests upon classical linear viscoelastic theory in which stress and strain within the material are characterized by the rheological equation of state

$$\epsilon_{rs}(t) = \int_0^\infty a_{ij}^{rs}(\tau)\frac{\mathrm{d}\sigma_{ij}}{\mathrm{d}\tau}(t-\tau)\mathrm{d}\tau, \tag{2}$$

which is an inversion of an equation given by Pipkin (1964). Equation (2) is the integral superposition law for infinitesimal deformations of anisotropic viscoelastic materials in which ϵ_{rs} is the strain tensor, σ_{ij} the stress tensor, a_{ij}^{rs} are the various viscoelastic moduli, τ is an arbitrary commencement time in the previous stress history of the material, and t is a time such that $\tau < t$.

The problem is facilitated by applying the well-known correspondence principle between viscoelastic behaviour and elasticity. By applying Laplace transforms to equation (2) and using the convolution theorem (Churchill 1958), the resulting equation (omitting the tensor notation) is

$$\bar{\epsilon}(s) = s\bar{a}\bar{\sigma}, \tag{3}$$

where

$$\bar{\epsilon}(s) = \int_0^\infty \epsilon(t)\mathrm{e}^{-st}\mathrm{d}t, \text{ etc.}$$

Hence any result obtained in an elastic analysis may be used in the corresponding viscoelastic case by multiplying the appropriate modulus by s and assuming that all stress and strain components have been transformed into the s-plane.

To illustrate the above principle, we note that the stress–strain relationship, in abbreviated tensor notation, is, by assumption (3),

$$\begin{aligned}
\epsilon_r &= a_{11}\sigma_r + a_{12}\sigma_\theta + \int_0^\infty a_{13}(\tau)\frac{\mathrm{d}\sigma_z}{\mathrm{d}\tau}(t-\tau)\mathrm{d}\tau,\\
\epsilon_\theta &= a_{21}\sigma_r + a_{22}\sigma_\theta + \int_0^\infty a_{23}(\tau)\frac{\mathrm{d}\sigma_z}{\mathrm{d}\tau}(t-\tau)\mathrm{d}\tau,\\
\epsilon_z &= \int_0^\infty a_{31}(\tau)\frac{\mathrm{d}\sigma_r}{\mathrm{d}\tau}(t-\tau)\mathrm{d}\tau + \int_0^\infty a_{32}(\tau)\frac{\mathrm{d}\sigma_\theta}{\mathrm{d}\tau}(t-\tau)\mathrm{d}\tau + \int_0^\infty a_{33}(\tau)\frac{\mathrm{d}\sigma_z}{\mathrm{d}\tau}(t-\tau)\mathrm{d}\tau.
\end{aligned} \tag{4}$$

Applying Laplace transforms and using the convolution theorem, equations (4) become

$$\begin{aligned}\bar{\epsilon}_r &= a_{11}\bar{\sigma}_r + a_{12}\bar{\sigma}_\theta + s\bar{a}_{13}\bar{\sigma}_z,\\ \bar{\epsilon}_\theta &= a_{21}\bar{\sigma}_r + a_{22}\bar{\sigma}_\theta + s\bar{a}_{23}\bar{\sigma}_z,\\ \bar{\epsilon}_z &= s(\bar{a}_{31}\bar{\sigma}_r + \bar{a}_{32}\bar{\sigma}_\theta + \bar{a}_{33}\bar{\sigma}_z).\end{aligned} \tag{5}$$

The correspondence principle is particularly useful in the present case, as the rather complicated problem of the distribution of stresses in an elastic anisotropic hollow cylinder under the influence of internal pressures has already been solved (Lekhnitskii 1963). The transformed longitudinal stress $\bar{\sigma}_z$ is given by the expression,

$$\bar{\sigma}_z = -[a^{\bar{K}+1}(\bar{a}_{13}+\bar{K}\bar{a}_{23})r^{\bar{K}-1} - b^{\bar{K}-1}(\bar{a}_{13}-\bar{K}\bar{a}_{23})r^{-\bar{K}-1}] \times [\bar{p}/\bar{a}_{33}(b^{2\bar{K}}-a^{2\bar{K}})], \tag{6}$$

where a and b are the inner and outer radii respectively, r is the radial distance from the central axis of the cylinder, $\bar{K}$ is given by

$$\bar{K} = \left(\frac{a_{11}}{a_{22}}\right)^{\frac{1}{2}} \left(\frac{\bar{a}_{33}-\bar{a}_{13}^2/a_{11}}{\bar{a}_{33}-\bar{a}_{23}^2/a_{22}}\right)^{\frac{1}{2}}, \tag{7}$$

and $\bar{p}$, the Laplace transform of $p(t)$, is given by

$$\bar{p} = p_0 a_1/s(s+a_1). \tag{8}$$

If it may be assumed that $\bar{a}_{13}^2/a_{11}$ and $\bar{a}_{23}^2/a_{22}$ compared with $\bar{a}_{33}$ are negligibly small, $\bar{K}$ may be approximated by $(a_{11}/a_{22})^{\frac{1}{2}}$ which, being independent of s, greatly reduces the problem of inverting the transforms.

The longitudinal tension $\bar{T}$ is related to $\bar{\sigma}_z$ by

$$\int_a^b \bar{\sigma}_z r \mathrm{d}r = \bar{T}/2\pi. \tag{9}$$

On carrying out the integration, we find

$$\begin{aligned}\bar{T} = -2\pi[&b^{K-1}(b^{K-1}-a^{K-1})a^2b^2(\bar{a}_{13}-K\bar{a}_{23})/\bar{a}_{33}(1-K)\\ &-a^{K+1}(b^{K+1}-a^{K+1})(\bar{a}_{13}+K\bar{a}_{23})/\bar{a}_{33}(1+K)] \times \bar{p}.\end{aligned} \tag{10}$$

To obtain specific results, a distinct relationship between the viscoelastic moduli and time must be assumed. For simplicity, the moduli are assumed to be Maxwellian in their time dependency. That is,

$$\begin{aligned}a_{13}(t) &= a'_{13}\mathrm{e}^{-a_2 t},\\ a_{23}(t) &= a'_{23}\mathrm{e}^{-a_2 t},\\ a_{33}(t) &= a'_{33}\mathrm{e}^{-a_3 t}.\end{aligned} \tag{11}$$

where a'_{13}, a'_{23}, and a'_{33} are constants and the radial and tangential shear components are assumed to have the same relaxation time $1/a_2$. Laplace transforms of equations (11) give

$$\begin{aligned}\bar{a}_{13} &= a'_{13}/(s+a_2),\\ \bar{a}_{23} &= a'_{23}/(s+a_2),\\ \bar{a}_{33} &= a'_{33}/(s+a_3).\end{aligned} \tag{12}$$

On substituting equations (8) and (12) into equation (10), we find that

$$\bar{T} = A\bar{f}, \tag{13}$$

where

$$A = -2\pi[b^{K-1}(b^{K-1}-a^{K-1})a^2b^2(a'_{13}-Ka'_{23})/a'_{33}(1-K) \\ -a^{K+1}(b^{K+1}-a^{K+1})(a'_{13}+Ka'_{23})/a'_{33}(1+K)], \tag{14}$$

and

$$\bar{f} = p_0a_1(s+a_3)/s(s+a_1)(s+a_2). \tag{15}$$

Using the method of partial fractions, it can be shown that the inverse transform of $\bar{f}$ is

$$f(t) = p_0(a_3/a_2)\{1+(a_2/a_3)[(a_3-a_1)/(a_1-a_2)]e^{-a_1t}-(a_1/a_3)[(a_3-a_2)/(a_1-a_2)]e^{-a_2t}\}. \tag{16}$$

For a maximum value in time,

$$dT/dt = df/dt = 0, \tag{17}$$

which corresponds to a value of t given by

$$t = (a_1-a_2)^{-1}\ln[(a_3-a_1)/(a_3-a_2)]. \tag{18}$$

The analysis is thus completed.

Discussion

The method of inducing the turgor–stress relaxation interaction in the theoretical model is different to the experimental procedure. Experimentally, longitudinal stress relaxation occurred as a result of water movement out of the cell. Because the ends of the cell were fixed, the longitudinal tension increased as the turgor pressure decreased. In the present theoretical model, water movement into the cell increases the turgor pressure and because of the anisotropy of the cell wall increases the longitudinal tension. The ultimate result, however, is identical in both situations and the theoretical time dependency of the tension, if valid, should be applicable to the experimental findings.

Qualitatively, the expression within the curly brackets in equation (16) has the appropriate mathematical form necessary to describe the behaviour of the *Chara* cell as shown in *B*, Figure 4. The rapid process of turgor change represented by the first exponential term is opposed by the slower stress relaxation process described by the second exponential and a maximum value of tension [equation (18)] is thus obtained. In compliance with the initial conditions for pressure, the sum of the coefficients of the exponential terms equals -1.

To examine the model quantitatively, a CDC-3200 digital computer was programmed to calculate the theoretical time dependency of the tension for various values of the parameters involved within the curly brackets of equation (16). The comparison between the theoretical calculation and the result obtained for the particular cell in *B*, Figure 4, is graphically presented in Figure 5. In order to separate the magnitude of the change in tension from its time dependency, the

two sets of values were normalized by dividing each set by its respective maximum, thus eliminating the need to introduce scaling factors to bring the magnitudes into coincidence.

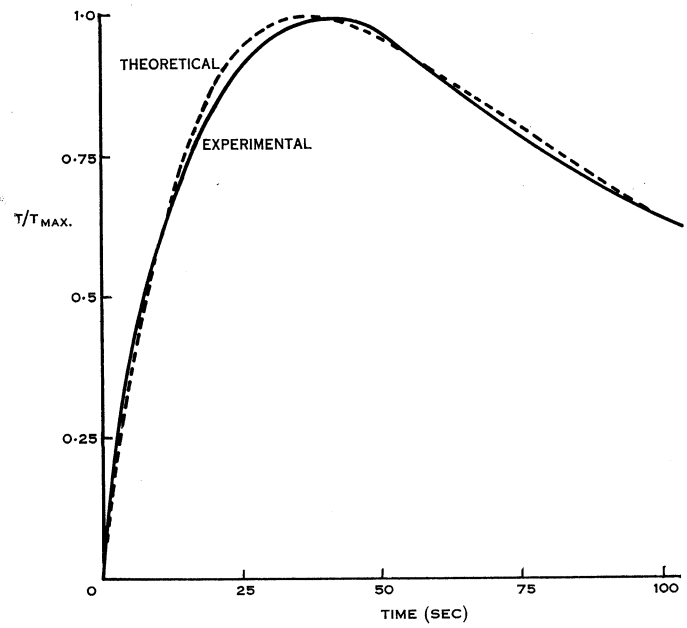

Fig. 5.—Time dependency of change in longitudinal tension in response to a turgor pressure change. The tension was normalized by dividing each result by its respective maximum value.

It may be seen from Figure 5 that there is quite good agreement between the theoretical and experimental time dependency of longitudinal tension. For the particular cell used, $1/a_1 = 20$ sec, $1/a_2 = 60$ sec, $1/a_3 = 340$ sec, and the theoretical maximum value of tension occurred at $t = 37$ sec.