VISCOELASTIC PROPERTIES OF PLANT CELL WALLS-I.
MATHEMATICAL FORMULATION FOR STRESS RELAXATION
WITH CONSIDERATION FOR PRE-EXTENSION RATE

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ABSTRACT - Stress relaxation analysis was used to measure
mechanical properties of the cell wall. Although a pre-
extension period is needed to give the initial stress or
strain to a cell wall specimen in the experiments, studies
reported in literature have assumed its length to be zero.
Thus, the parameters obtained may not represent character-
istic properties of the cell wall, which vary depending upon
the pre-extension conditions. A mathematical formulation
for stress relaxation with consideration for pre-extension
was established by introducing a correction factor $K$ in order
to obtain parameters which represent characteristic mechanical
properties of the cell wall. The validity of this treatment
was examined by comparing the calculated values with experi-
mental data.

INTRODUCTION

Auxin-induced cell wall loosening has been measured by several techniques (1,2,8,
11,13). Only a few suitable methods to measure rheologically defined mechanical
properties of the cell wall have developed. Recently, Yamamoto *et al.* (14) and
Cleland and Haughton (3) attempted to measure stress relaxation properties of the
cell wall of higher plants. Yamamoto *et al.* (15) introduced the following semi-
empirical equation which represents the stress relaxation process of the cell wall:

$$S = b.\ln \{(t+T_m)/(t+T_o)\} + c,  \qquad (1)$$

where $S$ is stress, $t$ is time, $T_o$ and $T_m$ are minimum and maximum relaxation times,
and $b$ and $c$ are constants with the $c$ value not equal to zero in a cross-linked
polymer (4). They found that auxin pretreatment causes a decrease in the $T_o$ value.
Yamamoto *et al.* (16) and Masuda *et al.* (12) found that the decrease in the $T_o$ value
of the cell wall correlates well with the rate of cell elongation to occur.

For the method we developed, a wall specimen is stretched at a constant rate (pre-
extension). After the specimen has received a certain amount of initial stress or
strain, stretching is stopped and the stress decay for the fixed strain produced by
the specimen is recorded. Stress relaxation analysis of natural or synthetic fibers

generally has been done by assuming that the specimen is stretched instantaneously, i.e. pre-extension time is zero. However, a pre-extension period of a certain length is necessary to give an initial stress to a cell wall specimen in the stress relaxation method. The average time needed for pre-extension in the case of Avena coleoptile cell wall is in the order of 1 sec (14,15). On the other hand, the $T_o$ value of the cell wall is in the order of $10^{-2}$ sec (14,15). Thus stress relaxation, especially of the elements of the model having relaxation times shorter than the $T_o$ value, occurs during pre-extension. Iwayanagi (10) pointed out from a theoretical point of view that pre-extension should be considered in analyzing stress relaxation of synthetic or natural polymers. However, a formulation for stress relaxation which takes into consideration the condition of pre-extension had not been available for analysis of observed data of stress relaxation of the cell wall or other polymers.

The parameters $T_O, T_m, b,$ and $c$ in equation (1) representing stress relaxation properties of the cell wall vary with the pre-extension conditions (5) and hence may not represent characteristic properties of the cell wall. Thus, the condition of pre-extension needs to be mathematically expressed. This paper deals with some details of such a formulation and compares calculated values obtained using it with experimental values.

## PHYSICAL MEANING OF THE PARAMETERS $T_O, T_m, b,$ AND $c$

Yamamoto et al. (15) pointed out that $T_O$ and $T_m$ represent the times when the stress starts to decrease and reaches an asymptotic stress $c$, respectively [Fig. 1(a)]. We did the following calculation in order to confirm this idea. First, we classified the relationship among $T_O$, $T_m$, and $t$:

(1) when $t \ll T_O$, then equation (1) is approximated as

$$S \simeq b.\ln(T_m/T_O) + c \tag{2}$$

(2) when $T_O \ll t \, T_m$, then equation (1) is approximated as

$$S \simeq b.\ln(T_m/t) + c \tag{3}$$

(3) when $T_m \ll t$, then equation (1) is approximated as

$$S \simeq c. \tag{4}$$

Equations (2) and (4) represent the horizontal straight line in the $S - \log t$ diagram and equation (3) represents the oblique straight line with a minus gradient [Fig. 1(a)]. The intersection point of the first two straight lines represented by equations (2) and (3) gives $t=T_O$. The intersection point of the straight lines represented by equations (3) and (4) gives $t = T_m$. Thus, we agree exactly with the suggestion of Yamamoto et al. (15) that the values of $T_O$ and $T_m$ approximately give the times when the stress in a fixed strain starts to relax and reaches a constant $c$, respectively. The value of $b$ represents the rate of relaxation, i.e. the gradient of the middle straight line shown in Fig. 1(a). On the other hand, $T_O$ and $T_m$ practically determine the lower and upper limits of the box-type distribution of relaxation times as pointed out previously (14) [Fig. 1(b)].

## MATHEMATICAL FORMULATION FOR STRESS RELAXATION
## WITH A CONSTANT PRE-EXTENSION RATE

To measure stress relaxation of a cell wall, the initial stress is produced by stretching a specimen at a constant rate (14,15). When the stress relaxation process
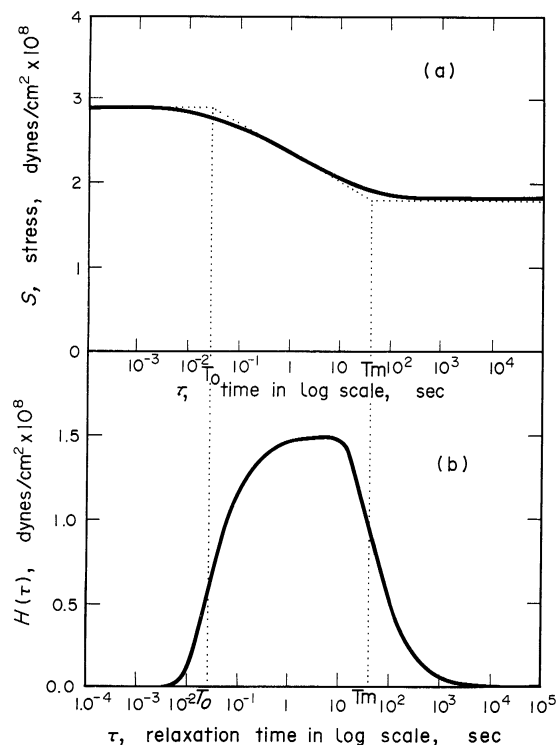
FIG. 1

(a)    Schematic stress relaxation curve of the pea
        epidermal cell wall represented in equation (1).
(b)    Almost bos-type $H(\tau)$ distribution of the pea
        epidermal cell wall (see Fig. 6a).

is represented by equation (1), the estimated values of the parameters $T_o$, $T_m$, $b$
and $c$ are affected by the pre-extension rate $\dot{\gamma}_p$ or the pre-extension time $t_p$ (5).
If the influence of $\dot{\gamma}_p$ and $t_p$ is eliminated, the parameters probably represent
characteristic properties of the specimen, although the influence of temperature
on the parameters should also be considered.

Stress relaxation experiments are conducted with a constant strain; hence, a
generalized Maxwell model is used in this paper. A generalized Voigt model is
related to a generalized Maxwell model, and they are interchangeable with each other
(6).

When the single Maxwell element shown in Fig. 2(a) is extended at a certain rate,
the change in stress is expressed as a function of time $t$:

$$S = G\dot{\gamma}_p\tau \ \{1 - \exp(-t/\tau)\}, \tag{5}$$

where $G$ is the elastic modulus, $\dot{\gamma}_p$ is the strain rate of pre-extension, $\tau$ is relaxa-
tion time. We use the time scale where pre-extension starts at $t = -t_p$ (<0) ($t_p$:
pre-extension time). The stretching stops at $t = 0$ and stress relaxation at a

constant strain starts. The stress at $t = 0$ is:

$$S = G\mathring{\gamma}_p\tau \{1 - \exp(-t_p/\tau)\}. \tag{6}$$

Therefore, the stress relaxation of the element at $t$ ($\geq 0$) is represented by:

$$S = G\mathring{\gamma}_p\tau \{1 - \exp(-t_p/\tau)\}.\exp(-t/\tau). \tag{7}$$

Thus, the stress relaxation of the continuous viscoelastic model shown in Fig. 2(b) is represented by:

$$S = \int_0^\infty G(\tau).\mathring{\gamma}_p\tau\{1 - \exp(-t_p/\tau)\}.\exp(-t/\tau)d\tau + G_e.\gamma_p, \tag{8}$$

where $G(\tau)$ is the distribution density function of relaxation times (14), $G_e$ is the elastic modulus of the spring connected in parallel with Maxwell elements, and $\gamma_p$ is pre-extension strain. This equation is the general formulation of stress relaxa--tion which takes into consideration pre-extension at a constant rate.
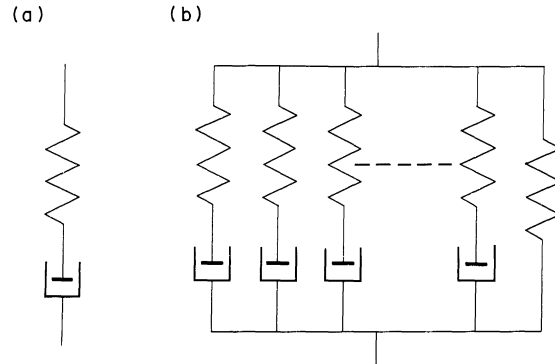
(a)          (b)



FIG. 2

(a)    Maxwell element.
(b)    Generalized Maxwell model with only a spring in
             parallel with the Maxwell elements. This is a
             continuous viscoelastic model consisting of an
             infinite number of Maxwell elements, especially
             with only one spring (a degenerated Maxwell element)
             in parallel with the Maxwell elements.

When $0 < t_p \ll \tau$, substitution of $\mathring{\gamma}_p t_p = \gamma_p$ into equation (8) gives:

$$S = \int_0^\infty G(\tau).\gamma_p.\exp(-t/\tau)d\tau + G_e.\gamma_p. \tag{9}$$

This equation is well known as a general expression of the stress relaxation process without consideration for pre-extension (14).

The distribution density function of relaxation times (7,9,14) in equation (9) corresponding to equation (1) is:

$$G(\tau) = \frac{b}{\gamma_p\tau}\frac{1}{} \left\{ \exp\left(-\frac{T_m}{\tau}\right) - \exp\left(-\frac{T_o}{\tau}\right) \right\}. \tag{10}$$

Maxwell elements with short relaxation times in the continuous viscoelastic model shown in Fig. 2(b) may contribute little to the stress relaxation process, because they relax during pre-extension. In order to represent the modification suitable for the distribution density function of relaxation times prior to pre-extension, the correction factor effective only at small values should be introduced into $G(\tau)$ in equation (10). For this purpose, a new parameter $K$ is introduced and $G(\tau)$ in equation (10) is multiplied by the correction factor $(1 + K/\tau)$. Then the new distribution density function of relaxation times is:

$$G_k(\tau) = \frac{b_k}{\gamma_p} \frac{1}{\tau}\left(1+\frac{K}{\tau}\right)\left\{\exp\left(-\frac{T_{mk}}{\tau}\right) - \exp\left(-\frac{T_{ok}}{\tau}\right)\right\},\tag{11}$$

where $T_{ok}$, $T_{mk}$, and $b_k$ are constant parameters. The parameters $T_{ok}$, $T_{mk}$, and $b_k$ correspond to $T_o$, $T_m$, and $b$, respectively in equation (1).

When equation (11) is substituted into equation (8), it is integrated as follows (see Appendix):

$$S = \frac{\dot{\gamma}_p t_p}{\gamma_p}b_k \cdot \ln \frac{(t+T_{mk})\left(1+\dfrac{t_p}{t+T_{mk}}\right)^{1+\frac{t+T_{mk}^{-K}}{t_p}}}{(t+T_{ok})\left(1+\dfrac{t_p}{t+T_{ok}}\right)^{1+\frac{t+T_{ok}^{-K}}{t_p}}} + G_e\cdot\dot{\gamma}_p \tag{12}$$

Since equation (12) is also too complicated, the following function is introduced for simplicity:

$$\phi(K,A;B) \equiv B\left(1 + \frac{A}{B}\right)^{1+ \frac{B-K}{A}}. \tag{13}$$

Using this notation, $G_e\gamma_p = c_k$ ( $= c$ in equation (1)) and $\dot{\gamma}_p\cdot t_p = \gamma_p$, then equation (12) becomes:

$$S = b_k \cdot \ln \frac{\phi(K, t_p; t+T_{mk})}{\phi(K, t_p; t+T_{ok})} + c_k. \tag{14}$$

If we assume that pre-extension is applied instantaneously $(t_p \to 0)$, then equation (14) in $K = 0$ is represented by:

$$S = \lim_{t_p\to 0}\left\{b_k \cdot \ln \frac{\phi(0, t_p; t+T_{mk})}{\phi(0, t_p; t+T_{ok})} + c_k\right\}$$

$$= b_k \cdot \ln\frac{t+T_{mk}}{t+T_{ok}} + c_k \quad, \tag{15}$$

because $\lim_{t_p\to 0}\phi(0, t_p; t) = e\cdot t$ ($e = 2.71828...$). Obviously, equation (15) is equation (1) itself so that equation (14) includes equation (1).

## LEAST SQUARES METHOD

The parameters $T_o$, $T_m$, $b$, and $c$ in equation (1) have been estimated by the method of least squares (14,15). In the same way, since equation (14) is nonlinear with respect to the parameters, they can not be estimated by the ordinary least squares method. If the initial values of parameters $T_{ok}^*$, $T_{mk}^*$, $K^*$, $b_k^*$, and $c_k^*$ are given, then we have

$$T_{ok} = T_{ok}^* + \Delta T_{ok}$$
$$T_{mk} = T_{mk}^* + \Delta T_{mk}$$
$$K = K^* + \Delta K \qquad\qquad\qquad (16)$$
$$b_k = b_k^* + \Delta b_k$$
$$c_k = c_k^* + \Delta c_k ,$$

where $\Delta T_{ok}$, $\Delta T_{mk}$, $\Delta K$, $\Delta b_k$, and $\Delta c_k$ are correction terms. When equation (14) is expanded by Taylor's expansion in the neighborhood of the initial value, we have

$$S \simeq \left\{ b_k^* \cdot \ln\frac{\phi(K^*, t_p; t+T_{mk}^*)}{\phi(K^*, t_p; t+T_{ok}^*)} + c^* \right\}$$

$$- b_k^* \left\{ \frac{1}{t_p} \cdot \ln\left(1+ \frac{t_p}{t+T_{ok}^*}\right) + \frac{1}{t+T_{ok}^*} \cdot \frac{K^*}{t+T_{ok}^*+t_p} \right\} \cdot \Delta T_{ok}$$

$$+ b_k^* \left\{ \frac{1}{t_p} \cdot \ln\left(1+ \frac{t_p}{t+T_{mk}^*}\right) + \frac{1}{t+T_{mk}^*} \cdot \frac{K^*}{t+T_{mk}^*+t_p} \right\} \cdot \Delta T_{mk}$$

$$+ \frac{b_k^*}{t_p} \left\{ \ln\left(1+ \frac{t_p}{t+T_{ok}^*}\right) - \ln\left(1+ \frac{t_p}{t+T_{mk}^*}\right) \right\} \cdot \Delta K$$

$$+ \left\{ \ln \frac{\phi(K^*, t_p; t+T_{mk}^*)}{\phi(K^*, t_p; t+T_{ok}^*)} \right\} \cdot \Delta b_k + \Delta c_k . \qquad\qquad (17)$$

This equation is linear with respect to the correction terms. Thus, we can determine the correction terms using equation (17) by the least squares method. Substituting the correction terms into equation (16), we obtain the parameters $T_{ok}$, $T_{mk}$, $K$, $b_k$, and $c_k$, which are used as the new initial values $T_{ok}^*$, $T_{mk}^*$, $K^*$, $b_k^*$, and $c_k^*$. This procedure is iterated until the value of the following equation reaches minimum:

$$\Omega = \Sigma(S_{obs} - S_{cal})^2 ; \qquad\qquad (18)$$

where $S_{obs}$ is the observed value of stress, $S_{cal}$ is the calculated value of the first term on the right side of equation (17), $\Sigma$ means summation taken over all stress data. When the $\Omega$ value reaches minimum, the values of the parameters $T_{ok}$, $T_{mk}$, $K$, $b_k$, and $c_k$ take the estimated values.

When equation (10) is substituted into equation (8), the following equation equal to equations (12) and (14) at $K = 0$ and $\dot{\gamma}_p \cdot t_p = \gamma_p$ is obtained:

$$S = b \cdot \ln \frac{(t+T_m)(1+\frac{t_p}{t+T_m})^{1+\frac{t+T_m}{t_p}}}{(t+T_o)(1+\frac{t_p}{t+T_o})^{1+\frac{t+T_o}{t_p}}} + G_e \cdot \gamma_p$$

$$= b \cdot \ln \frac{\phi(0,t_p;t+T_m)}{\phi(0,t_p;t+T_o)} + c. \tag{19}$$

The equation used for equation (19) by the least squares method of the nonlinear equation is:

$$S \simeq \left\{ b^* \cdot \ln \frac{\phi(0,t_p;t+T_m^*)}{\phi(0,t_p;t+T_o^*)} + c^* \right\} - \left\{ \frac{b^*}{t_p} \cdot \ln (1 + \frac{t_p}{t+T_o^*}) \right\} \cdot \Delta T_o$$

$$+ \left\{ \frac{b^*}{t_p} \cdot \ln \left(1+ \frac{t_p}{t+T_m^*}\right) \right\} \cdot \Delta T_m + \left\{ \ln \frac{\phi(0,t_p;t+T_m^*)}{\phi(0,t_p;t+T_o^*)} \right\} \cdot \Delta b$$

$$+ \Delta c. \tag{20}$$

In the same way, the equation used for equation (1) by the least squares method of the nonlinear equation is:

$$S \simeq \left( b^* \cdot \ln \frac{t+T_m^*}{t+T_o^*} + c^* \right) + \left( \frac{-b^*}{t+T_o^*} \right) \cdot \Delta T_o$$

$$+ \left( \frac{b^*}{t+T_m^*} \right) \cdot \Delta T_m + \left( \ln \frac{t+T_m^*}{t+T_o^*} \right) \cdot \Delta b + \Delta c. \tag{21}$$

## RESULTS AND DISCUSSION

Tables 1 and 2 show the values of the parameters in equations (1) and (14) determined by equations (21) and (19) using the stress relaxation data of pea epidermal cell wall reported by Yamamoto $et$ $al$. (Fig. 2 in Ref. 15). In this case, the time $t_p$ needed for pre-extension was 1.5 sec.

Although we attempted using the least squares method to determine the parameters $T_o$, $T_m$, $b$, and $c$ with equation (20), their values, especially that of $T_o$, did not converge in the iterating procedure. To find why the parameters did not converge, we calculated the values of $T_m$, $b$, and $c$, using the pre-extension time $t_p = 1.5$ sec from the experimental data and a fixed value of $T_o$. We obtained the minimum value, $\Omega\min(T_o)$, of $\Omega$ at the fixed $T_o$. When the fixed value of $T_o$ was changed, then the $\Omega\min(T_o)$ was calculated. Curve $A$ of Fig. 3 is the $\Omega\min(T_o)$ − $T_o$ diagram at $t_p = 1.5$ sec; the smaller the $T_o$ value, the smaller the $\Omega\min(T_o)$. In other words, $\Omega\min(T_o)$ at $t_p = 1.5$ sec does not reach the extremum in the range of $T_o \geq 0$.
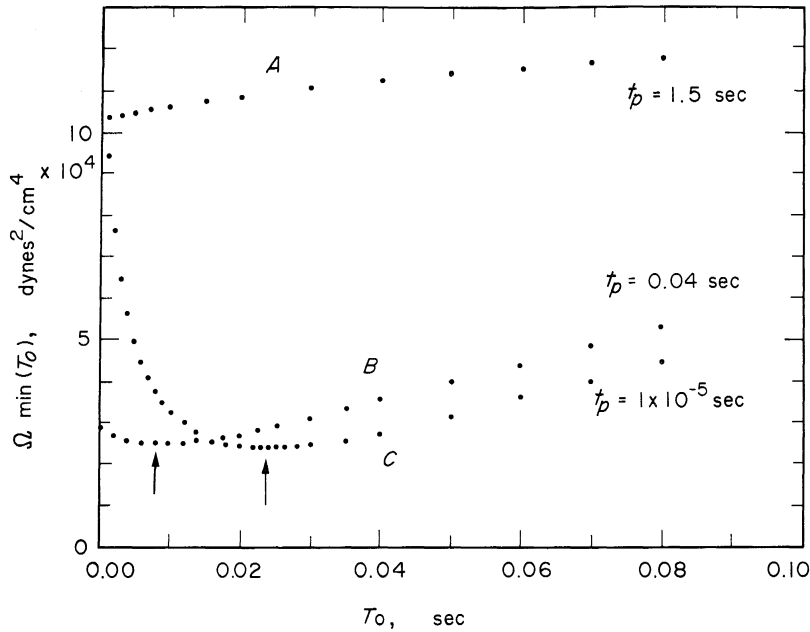
FIG. 3

Relationship between the minimum value, $\Omega_{\min}(T_O)$,
of $\Omega$ at fixed $T_O$ and minimum relaxation time $T_O$
for a pea epidermal cell wall. Arrows indicate the
extreme values of $\Omega_{\min}(T_O)$. At the pre-extension
time $t_p$ = 1.5 sec (curve $A$), the extreme values of
$\Omega_{\min}(T_O)$ is not in the range of $T_O \geq 0$; it is in
this range at $t_p$ = 0.04 sec (curve $\bar{B}$) and at $t_p$ =
1x10$^{-5}$ sec (curve $C$).

If the value of $t_p$ decreases, then $\Omega_{\min}(T_O)$ can reach minimum, as shown in Fig. 3
($B$ for $t_p$ = 0.04 sec, and $C$ for $t_p$ = 1 x 10$^{-5}$ sec, when equation (19) can be regarded
as equation (1)). As the $t_p$ value increases step by step from 1 x 10$^{-5}$ sec, the
minimum value of $\Omega_{\min}(T_O)$ becomes sharply larger. As the $t_p$ value increases ca.
0.09 sec, the $T_O$ value at the minimum value of $\Omega_{\min}(T_O)$ becomes negative. Then the
parameters do not converge in the range of $T_O \geq 0$. Thus, the parameters of equation
(19) do not converge in the least squares method. This suggests that equation (19)
does not fit the experimental data and is not suitable for analysis of the stress
relaxation process with consideration for pre-extension of the plant cell wall.
Hence, direct introduction of the condition of pre-extension is not appropriate for
the formulation of pre-extension. Equation (19) was modified into equation (20).

We compared the stress curve from equation (1) with one from equation (14). Curves
$A$ and $B$ in Fig. 4 show the stress relaxation process represented by equations (1)
and (14) substituted with the values of the parameters shown in Tables 1 and 2.
Although curve $B$ differs little from curve $A$, curve $B$ fits the experimental data
better. This better fit of curve $B$ can also be understood by the fact that the
minimum value calculated with equation (14) is 0.4 times that calculated with equa-
tion (1).

Figure 5 shows the distribution of $G(\tau)$ and $G_k(\tau)$. The values of the parameters
in Table 1 were used for $G_k(\tau)$, and the values in Table 2 for $G(\tau)$. If we define
$H_k(\tau) \equiv \tau G_k(\tau)$ , then $H_k(\tau)$ is not a box-type distribution, though $H(\tau)$ [ $\equiv \tau G(\tau)$]
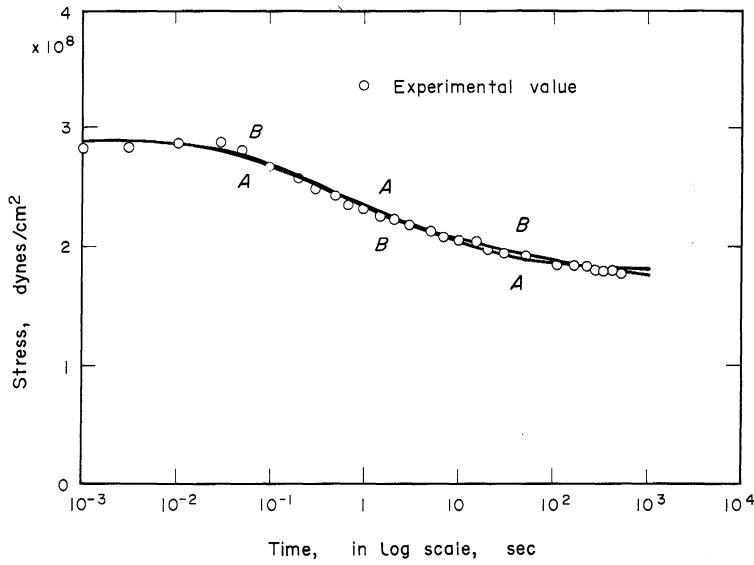approximately is (14).

Time,   in Log scale,   sec

FIG. 4

Stress relaxation curves from equations (1) and
(14). Curve $A$ was calculated from equation (1);
the parameters $T_O$, $T_m$, $b$, and $c$ took the values
given in Table 2. Curve $B$ was calculated from
equation (14); the parameters $T_{Ok}$, $T_{mk}$, $K$, $b_k$,
and $c_k$ took the values given in Table 1. Curve $B$
was closer to the experimental values than curve $A$.

TABLE 1

Values of parameters of the pea epidermal cell
wall in equation (1) calculated by least squares
method

| Parameter | Calculated value |  |
|---|---|---|
| $T_O$ | $2.75 \times 10^{-2}$ | sec |
| $T_m$ | $4.24 \times 10^{1}$ | sec |
| $b$ | $1.50 \times 10^{7}$ | dynes/cm$^2$ |
| $c$ | $1.80 \times 10^{8}$ | dynes/cm$^2$ |

Figure 6 compares $H_k(\tau)$ with $H(\tau)$. The parameter $T_{Ok}$ has been mathematically
proved to approximately represent the value of $\tau$ at the peak of $H_k(\tau)$ distribution,
whereas $T_O$ practically represents the value of $\tau$ at the lowest end of the $H(\tau)$ dis-
tribution. On the other hand, the values $T_{mk}$ and $T_m$ practically represent the values
of $\tau$ at the upper limits of the $H_k(\tau)$ and $H(\tau)$ distributions, respectively.

We conclude that the modification used in equation (11) is suitable as the for-
mulation of the stress relaxation process with consideration for pre-extension of
cell wall, although the number of parameters increases with the addition of $K$.

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TABLE 2

Values of parameters of the pea epidermal cell
wall in equation (14) calculated by least squares
method

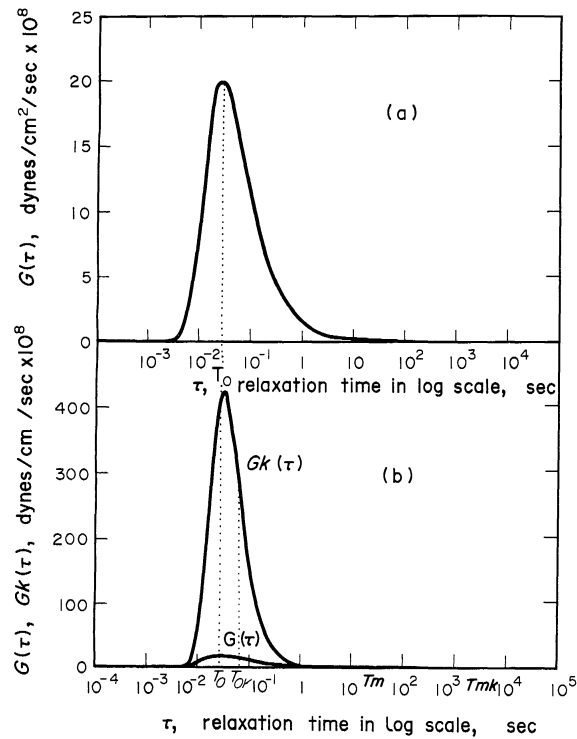| Parameter | Calculated value | |
| --- | --- | --- |
| $T_{ok}$ | $6.44 \times 10^{-2}$ | sec |
| $T_{mk}$ | $1.49 \times 10^{-3}$ | sec |
| $K$ | $4.87$ | sec |
| $b_k$ | $6.59 \times 10^6$ | dynes/cm$^2$ |
| $c_k$ | $1.70 \times 10^8$ | dynes/cm$^2$ |



FIG. 5

Curves of $G(\tau)$ and $G_k(\tau)$. The curve of $G_k(\tau)$ is
strengthened especially in the range of short re-
laxation time ($\tau$) compared to the curve of $G(\tau)$.

The physical and biological meanings of parameter $K$ are being studied and will be
reported elsewhere. Stress relaxation has been measured after pre-extension at
20 mm/min (14,15). Stress relaxation parameters have not been compared yet with
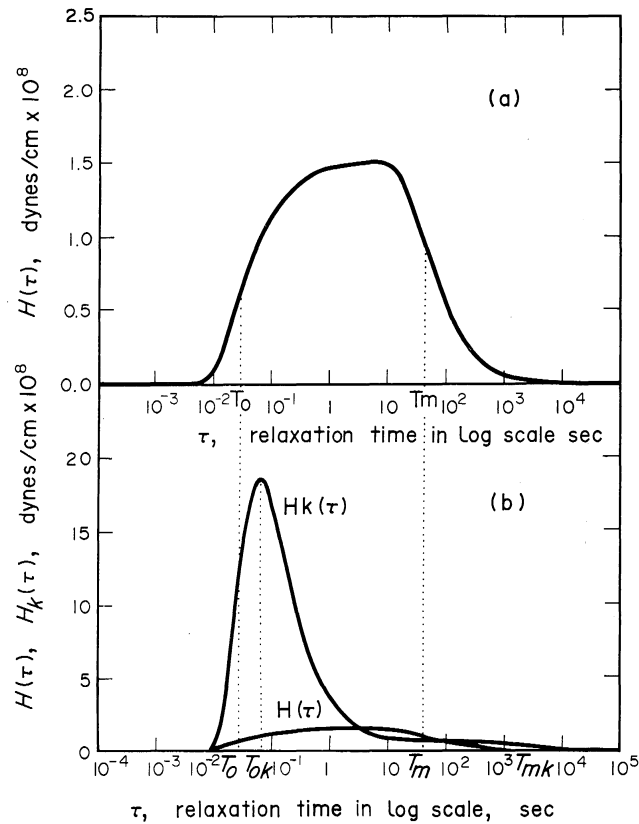
FIG. 6

Curves of $H(\tau)$ and $H_k(\tau)$. The curve of $H_k(\tau)$ is
strengthened especially in the range of short
relaxation time ($\tau$) compared to the curve of $H(\tau)$.

those measured at different pre-extension rates.

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## APPENDIX

$$S = \int_0^\infty G_k(\tau) \cdot \dot{\gamma}_p \tau \{1 - \exp(-t_p/\tau)\} \cdot \exp(-t/\tau) d\tau + G_e \cdot \gamma_p$$

$$= \int^\infty \frac{b_k}{d\gamma_p} \frac{1}{\tau} \left(1 + \frac{K}{\tau}\right) \left\{\exp\left(-\frac{T_{ok}}{\tau}\right) - \exp\left(-\frac{T_{mk}}{\tau}\right)\right\} \left\{1 - \exp\left(-\frac{t_p}{\tau}\right)\right\} \cdot \exp\left(-\frac{t}{\tau}\right) d\tau + G_e \cdot \gamma_p$$

$$= \frac{\dot{\gamma}_p \cdot b_k}{\gamma_p} \int_0^\infty \left\{\exp\left(-\frac{T_{ok}}{\tau}\right) - \exp\left(-\frac{T_{mk}}{\tau}\right)\right\} \left\{1 - \exp\left(-\frac{t_p}{\tau}\right)\right\} \cdot \exp\left(-\frac{t}{\tau}\right) d\tau$$

$$+ \frac{\dot{\gamma}_p \cdot t_p}{\gamma_p} \cdot K \int_0^\infty \left\{\frac{1}{\tau} \exp\left(-\frac{T_{ok}}{\tau}\right) - \exp\left(-\frac{T_{mk}}{\tau}\right)\right\} \left\{1 - \exp\left(-\frac{t_p}{\tau}\right)\right\} \cdot \exp\left(-\frac{t}{\tau}\right) d\tau$$

$$+ G_e \cdot \gamma_p.$$

The first term is:

$$(\dot{\gamma}_p \cdot b_p/\gamma_p) \int_0^\infty \{\exp(-\mu T_{ok}) - \exp(-\mu T_{mk})\} \{1 - \exp(-\mu t_p)\} \cdot \exp(-\mu t)(d\mu/\mu^2)$$

$$= (\dot{\gamma}_p \cdot b_k/\gamma_p) \int_0^\infty \int_{t_p}^0 \frac{\exp\{-\mu(t+T_{mk}+x)\} - \exp\{-\mu(t+T_{ok}+x)\}}{\mu} dx d\mu$$

$$= (\dot{\gamma}_p \cdot b_k/\gamma_p) \int_{t_p}^0 \int_0^\infty \frac{\exp\{-\mu(t+T_{mk}+x)\} - \exp\{-\mu(t+T_{ok}+x)\}}{\mu} d\mu dx$$

$$= (\dot{\gamma}_p \cdot b_k/\gamma_p) \int_{t_p}^0 \ln \frac{t+T_{ok}+x}{t+T_{mk}+x}$$

$$= (\dot{\gamma}_p \cdot t_p/\gamma_p) b_k \cdot \ln \frac{(t+T_{mk})\left(1+\dfrac{t_p}{t+T_{mk}}\right)^{1+\frac{t+T_{mk}}{t_p}}}{(t+T_{ok})\left(1+\dfrac{t_p}{t+T_{ok}}\right)^{1+\frac{t+T_{ok}}{t_p}}}.$$

In the same way, the second term is:

$$- \frac{\dot{\gamma}_p \cdot b_k}{\gamma_p} \cdot K \cdot \ln \frac{1 + \dfrac{t_p}{t + T_{mk}}}{1 + \dfrac{t_p}{t + T_{ok}}}$$

$$\therefore S = \frac{\dot{\gamma}_p \cdot t_p}{\gamma_p} b_k \cdot \ln \frac{(t + T_{mk}) \left(1 + \dfrac{t_p}{t + T_{mk}}\right)^{1 + \frac{t + T_{mk} - K}{t_p}}}{(t + T_{ok}) \left(1 + \dfrac{t_p}{t + T_{ok}}\right)^{1 + \frac{t + T_{ok} - K}{t_p}}} + G_e \cdot \gamma_p.$$

VISCOELASTIC PROPERTIES OF PLANT CELL WALLS — II. EFFECT
OF PRE-EXTENSION RATE ON STRESS RELAXATION

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ABSTRACT — The stress relaxation parameters $T_o$, $T_m$, $b$, and
$c$ vary depending upon the pre-extension rate when a cell
wall specimen is stretched using a tensile tester. The ef-
fect of the pre-extension rate on the stress relaxation par-
ameters of pea epidermal cell wall was investigated using a
newly established equation. The change in the parameters
due to the pre-extension rate can be corrected by the equa-
tion qualitatively and approximately quantitatively. The
newly established equation appears to validly express the
stress relaxation process with consideration for the pre-
extension rate and the parameters $T_{ok}$, $T_{mk}$, $b_k$, $c_k$, and $K$
in the equation seem to represent the characteristic mech-
anical properties of the cell wall, although the parameters
$T_o$, $T_m$, $b$, and $c$ are valid for practical use to analyze the
auxin effect on the mechanical properties of the cell wall.

INTRODUCTION

Stress relaxation at a fixed strain has been employed to study the mechnical prop-
erty of plant cell walls (3,4,11). In theoretical analysis of stress relaxation of
natural or synthetic fibers, the pre-extension period of a specimen has been assumed
to be instantaneous. However, a pre-extension period actually is needed before the
initial strain or stress can be produced. If the mechanical property of the cell
wall is simulated by a generalized Maxwell viscoelastic model, the elements having
relaxation times shorter than the pre-extension time must have relaxed during the
pre-extension process and may not contribute to the stress relaxation process after
the pre-extension (4). Based on general theory, an attempt has been made to correct
stress relaxation for the pre-extension condition by eliminating from the model the
elements with relaxation time shorter than the pre-extension time (5). However, the
usefulness of the formula was not fully confirmed. Thus, a formulation for stress
relaxation is needed, which includes correction for the pre-extension condition and
agrees well with experimental data.

  The stress relaxation formulation taking into consideration the pre-extension con-
dition was obtained by modifying the distribution density function of relaxation
times (4). The stress relaxation of the plant cell wall was reported to be repre-
sented by the following semiempirical equation:

$$S = b \cdot \ln \frac{t + T_m}{t + T_O} + c \; , \tag{1}$$

where $S$ is stress, $t$ is time, and $T_O$, $T_m$, $b$, and $c$ are constant parameters (11). Then a new parameter $K$, representing the influence of the pre-extension condition on stress relaxation, was introduced to the distribution density function of relaxation times of stress relaxation shown by equation (1) and the stress relaxation formula with consideration for pre-extension obtained was (4):

$$S = \frac{\dot{\gamma}_p \cdot t_p}{\gamma_{op}} \cdot b_k \cdot \ln \frac{(t+T_{mk})\left(1 + \frac{t_p}{t+T_{mk}}\right)^{1 + \frac{t+T_{mk}-K}{t_p}}}{(t+T_{ok})\left(1 + \frac{t_p}{t+T_{ok}}\right)^{1 + \frac{t+T_{ok}-K}{t_p}}} + \frac{\dot{\gamma}_p \cdot t_p}{\gamma_{op}} c_k$$

$$= \frac{\dot{\gamma}_p \cdot t_p}{\gamma_{op}} \cdot b_k \cdot \ln \frac{\phi(K, t_p; t+T_{mk})}{\phi(K, t_p, t+T_{ok})} + \frac{\dot{\gamma}_p \cdot t_p}{\gamma_{op}} \cdot c_k \tag{2}$$

$$\phi(K,A;B) = B \left(1 + \frac{A}{B}\right)^{1 + \frac{B-K}{A}} , \tag{3}$$

where $t_p$ is pre-extension time and $T_{ok}$, $T_{mk}$, $b_k$, $c_k$, and $K$ are constant parameters. Parameter $\gamma_{op}$ is the pre-extension strain of the standard experiment which gives the particular values of the $k$-series parameters $T_{ok}$, $T_{mk}$, $b_k$, $c_k$, and $K$. On the other hand, $\gamma_p$ is the pre-extension strain of specimens to be analyzed. We found that equation (2) fits the experimental data of stress relaxation of the pea epidermal cell wall as well as equation 1 (4). Thus, we concluded that equation (2) is one of the formulations for stress relaxation corrected for the pre-extension condition.

The parameters $T_O$, $T_m$, $b$, and $c$ in equation (1) vary with the pre-extension conditions. If the formulation is valid, the dependence of the change in the parameters in equation (1) on the pre-extension condition may be explained by comparing equation (1) with equation (2). The parameters $T_{ok}$, $T_{mk}$, $b_k$, $c_k$, and $K$ in equation (2) rather than the parameters $T_O$, $T_m$, $b$, and $c$ in equation (1) represent the characteristic mechanical property of the cell wall. We examined the validity of equation (2) using the experimental data of stress relaxation when pea epidermal cell wall was stretched at various pre-extension strain rates, such as 4, 2, 1, 0.8, 0.4, and 0.2 min$^{-1}$.

## MATERIAL AND METHODS

*Plant material: Pisum sativa* L., cv. Alaska, seeds were soaked in running tap water overnight at 26°C then transferred to plastic trays. After being allowed to germinate for one day in the dark, seedlings with uniform root length were selected and transplanted in plastic trays for water culture. Seedlings were grown under continuous light, 3000 lux, at 26°C for 8 days. One-cm-long segments were excised from the upper region of the fifth internodes, 30-35 mm long, and pooled in cold water. Next, the segments were incubated in test solution with or without $10^{-5}$ M indoleacetic acid (IAA) for 3 hr. The test solution solvent was 0.01 M K-phosphate, pH 6. After incubation, the epidermis was peeled off, boiled for 5 min in methanol then stored in methanol at 4°C until use. We used the epidermis instead of the

whole stem segments since pea epidermal tissue is much more uniform than whole stem segments and the epidermal cell wall has been reported to be part controlling stem segment elongation due to auxin (15).

*Stress relaxation analysis:*  Stored segments were washed three times with water and fixed between the two clamps of a Tensilon tensile tester (Toyo Measuring Instrument Co., Model UTM-II).  This distance between the clamps was 5 mm.  The segments were stretched by lowering the bottom clamp at a strain rate of 4, 2, 1, 0.8, 0.4, and 0.2 $min^{-1}$.  After the segment had received a certain amount of initial load (*ca.* 20 grw), the clamp was stopped and the decay of the load produced by the segment was recorded on a chart paper of the tensile tester.  The stress relaxation process was read at time intervals of at least 1 sec.

The stress relaxation process at a fixed strain of the cell wall of higher plants has been reported to be represented by equation (1) and the stress relaxation property of the cell wall can thus be represented by the parameters $T_o$, $T_m$, $b$, and $c$. However, the values of these parameters vary depending upon the pre-extension rate. Equation (1) provides no information about the change in the parameters due to the pre-extension rate, since it does not explicitly include the pre-extension condition.

Equation (1) is similar to equation (2) which includes the pre-extension time in its terms:  both equations are composed of a logarithmic term with a factor $b$ or $b_k$, a denominator and a numerator, and a constant term, $c$ or $c_k$.  As indicated previously (4), the parameters $T_o$, $T_m$, $b$, and $c$ in equation (1) are equivalent to the parameters $T_{ok}$, $T_{mk}$, $b_k$, and $c_k$ in equation (2), respectively, if the pre-extension is applied instantaneously.  Thus, the parameters $T_o$, $T_m$, $b$, and $c$ can be expressed by the parameters $T_{ok}$, $T_{mk}$, $b_k$, $c_k$, and $K$ as follows:

$$\alpha \cdot \phi(K, t_p; t+T_{ok}) \simeq t + T_o \qquad (4)$$

$$\alpha \cdot \phi(K, t_p; t+T_{mk}) \simeq t + T_m \qquad (5)$$

$$\frac{\dot{\gamma}_p \cdot t_p}{\gamma_{op}} \cdot b_k \simeq b \qquad (6)$$

$$\frac{\dot{\gamma}_p \cdot t_p}{\gamma_{op}} \cdot c_k \simeq c \quad , \qquad (7)$$

where $\alpha$ is a proportional constant.  The value of the proportional constant $\alpha$ is determined as follows.  If the strain rate $\gamma_p$ tends to reach infinity with $\dot{\gamma}_p \cdot t_p$ = constant (instantaneous extension), then equation (2) tends to reach equation (1). Therefore, $T_{ok}$ tends to reach $T_o$ when $t_p$ tends to reach zero.  Thus, from equation (4) we have

$$\lim_{t_p \to 0} \{\alpha \cdot \phi(0, t_p; t+T_o)\} = t + T_o$$

$$\therefore \ \alpha \cdot (t + T_o) \cdot e = t + T_o \ .$$

Therefore,  $\alpha = 1/e$  .

Substitution of this equation into equations (4) and (5) gives

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$$\frac{1}{e} \cdot \phi(K, t_p; t+T_{ok}) \simeq t + T_o$$

$$\frac{1}{e} \cdot \phi(K, t_p; t+T_{mk}) \simeq t + T_m .$$

When $t = 0$,

$$T_o \simeq \frac{1}{e} \cdot \phi(K, t_p; T_{ok})$$

$$= \frac{T_{ok}}{e} \left( 1 + \frac{t_p}{T_{ok}} \right)^{1 + \frac{T_{ok}^{-K}}{t_p}} \tag{8}$$

$$T_m \simeq \frac{T_{mk}}{e} \left( 1 + \frac{t_p}{T_{mk}} \right)^{1 + \frac{T_{mk}^{-K}}{t_p}} . \tag{9}$$

$T_o$, $T_m$, $b$, and $c$ are defined by the right sides of equations (10)–(13) where $\gamma_p$ is substituted for $\dot{\gamma}_p \cdot t_p$,

$$T_o \equiv \frac{T_{ok}}{e} \left( 1 + \frac{\gamma_p}{\dot{\gamma}_p \cdot T_{ok}} \right)^{1 + \frac{\dot{\gamma}_p (T_{ok}^{-K})}{\gamma_p}} \tag{10}$$

$$T_m \equiv \frac{T_{mk}}{e} \left( 1 + \frac{\gamma_p}{\dot{\gamma}_p \cdot T_{mk}} \right)^{1 + \frac{\dot{\gamma}_p (T_{mk}^{-K})}{\gamma_p}} \tag{11}$$

$$b \equiv \frac{\gamma_p}{\gamma_{op}} \cdot b_k \tag{12}$$

$$c \equiv \frac{\gamma_p}{\gamma_{op}} \cdot c_k . \tag{13}$$

Thus, the parameters $T_o$, $T_m$, $b$, and $c$ in equation (1) can be estimated by the para-meters $T_o$, $T_m$, b, and c, respectively. Using these relationships, the values of $T_o$, $T_m$, $b$, and $c$ were compared with those of $T_o$, $T_m$, b, and c in the present study.

## RESULTS AND DISCUSSIONS

Experiments using a pre-extension strain rate of 4 1/min were chosen as standard experiments. Parameter values were obtained by the least squares method. The mean values of $T_o$, $T_m$, $b$, and $c$ of 6 to 12 duplicate experiments with S.D. are shown in Table 1; parameters $b$ and $c$ were tabulated in their ratios to the initial stress $S_o$ at $t = 0$ in order to avoid the effect of variation in the pre-extension strain $\gamma_p$ on their values because $b/S_o$ and $c/S_o$ were calculated from equations (2,12) and (13) as follows:

$$\frac{b}{S_o} \simeq \frac{b_k}{b_k \cdot \ln \frac{\phi(K, t_p; T_{mk})}{\phi(K, t_p; T_{ok})} + c_k}$$

$$\frac{c}{S_O} \simeq \frac{c_k}{b_k \cdot \ln \frac{\phi(K, t_p; T_{mk})}{\phi(K, t_p; T_{ok})} + c_k} \quad .$$

The values of the $k$-series parameters $T_{ok}$, $T_{mk}$, $b_k$, $c_k$, and $K$ were obtained from seven duplicate experiments and their mean values with the standard deviation are shown also in Table 1. We assumed that the cross section area of the cell wall of all the specimens with no extension is $3.75 \times 10^{-5}$ $cm^2$ and the volume of the cell wall is not changed by extension under the load 20–30 grw (7). The value of the area was determined from electron micrographs.

TABLE 1

Values of parameters of the pea epidermal cell wall

| IAA pre-treatment | Parameter | Calculated value of parameter in equation (1) | Parameter | Calculated value of parameter in equation (2) |
|---|---|---|---|---|
| Without IAA | $T_o$ | $2.95 \times 10^{-2} \pm 1.37 \times 10^{-2}$ sec | $T_{ok}$ | $4.20 \times 10^{-2} \pm 1.35 \times 10^{-2}$ sec |
| | $T_m$ | $6.11 \times 10^1 \pm 1.01 \times 10^1$ sec | $T_{mk}$ | $1.20 \times 10^2 \pm 2.2 \times 10^1$ sec |
| | $b/S_O$ | $5.38 \pm 6.2 \times 10^{-1}$ % | $b_k/S_O$ | $3.90 \pm 1.7 \times 10^{-1}$ % |
| | $c/S_O$ | $6.58 \times 10^1 \pm 1.1$ % | $c_k/S_O$ | $6.58 \times 10^1 \pm 1.2$ % |
| | | ($n = 11$) | | $1.36 \pm 2.1 \times 10^{-1}$ sec |
| | | | | ($n = 7$) |
| With IAA | $T_o$ | $9.02 \times 10^{-3} \pm 1.58 \times 10^{-3}$ sec | $T_{ok}$ | $9.15 \times 10^{-3} \pm 3.35 \times 10^{-3}$ sec |
| | $T_m$ | $7.08 \times 10^1 \pm 1.58 \times 10^1$ sec | $T_{mk}$ | $8.18 \times 10^1 \pm 1.42 \times 10^1$ sec |
| | $b/S_O$ | $4.81 \pm 9 \times 10^{-2}$ % | $b_k/S_O$ | $4.40 \pm 2.2 \times 10^{-1}$ % |
| | $c/S_O$ | $6.74 \times 10^1 \pm 1.2$ % | $c_k/S_O$ | $6.73 \times 10^1 \pm 1.4$ % |
| | | ($n = 12$) | $K$ | $1.29 \pm 3.3 \times 10^{-1}$ sec |
| | | | | ($n = 7$) |

The effect of the pre-extension rate on stress relaxation was studied by respectively comparing the parameters $T_o$, $T_m$, b, and c calculated using equations (10–13) with the parameters $T_o$, $T_m$, $b$, and $c$ at various pre-extension rates.

The change in $T_o$ with the pre-extension rate is illustrated in Fig. 1(a). The pre-extension rate is given on a reciprocal scale in Fig. 1, since the inverse of the strain rate has the dimension of time. The relationship between $T_o$ and the inverse of the pre-extension rate is empirically nearly linear as can be seen in Fig. 1(a), indicating that the pre-extension rate is in inverse proportion to the pre-extension time, since the pre-extension strain is nearly constant in each experiment.

$T_o$ may represent the minimum relaxation time of the elements which do not relax during the pre-extension. The elements of short relaxation time in the viscoelastic model may have relaxed during the pre-extension. Thus, $T_o$ should increase with the pre-extension time. This assumption agrees with the experimental result shown in Fig. 1(a). As the $T_o$ value varies greatly depending upon the pre-extension rate, changes in the pre-extension rate may mask the effect of auxin on the decrease in the $T_o$ value.
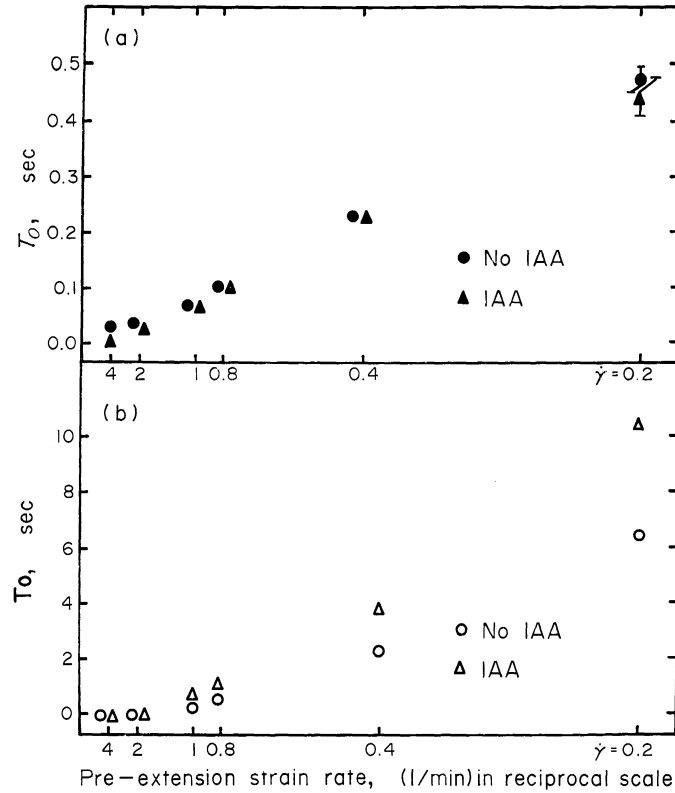
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(a)

$T_O$, sec

0.5
0.4
0.3
0.2
0.1
0.0

● No IAA

▲ IAA

4  2   1 0.8        0.4              $\dot{\gamma}$ = 0.2

(b)

To, sec

10
8
6
4
2
0

○ No IAA

▲ IAA

4  2   1 0.8        0.4              $\dot{\gamma}$ = 0.2

Pre—extension strain rate, (I/min)in reciprocal scale

FIG. 1

Effect of pre-extension strain rate on the $T_O$ and
$T_O$ values of the pea epidermal cell wall.
(a) The $T_O$ values are averages of those calculated from
experimental data ($n$ = 11, without IAA; $n$ = 12,
with IAA).
(b) The $T_O$ values are averages of those calculated from
experimental data ($n$ = 7, without IAA; $n$ = 7, with
IAA).

The values of $T_O$ at various pre-extension rates calculated using equation (10)
are shown in Fig. 1(b). As the pre-extension rate increases, the value of $T_O$ as
well as changes in $T_O$ decrease monotonously (Fig. 1(a)). Although the values of
$T_O$ are one order larger than those of $T_O$ , the values of $T_O$ at a changed pre-exten-
sion rate may be estimated by the values of $T_O$. The factors causing the quantita-
tive difference between the values of $T_O$ and $T_O$ are not clear yet. Further studies
are needed.

The $T_O$ value has been reported to decrease with auxin pre-treatment (8,10,11,14).
The effect of IAA on the decrease was observed at the pre-extension strain rate of
4 $min^{-1}$. However, when a lower pre-extension rate is used, the degree of the IAA
effect on $T_O$ value may be masked by the pre-extension time. On the other hand, IAA
caused a substantial decrease in the $T_{Ok}$ value as shown in Table 1. Thus, the value
of $T_O$ measured at a high pre-extension rate reflects the value of $T_{Ok}$ which may rep-
resent a characteristic property of the cell wall. Therefore, we can use the value

of $T_O$ measured at a high strain rate of pre-extension such as 4 min$^{-1}$ (20 mm/min) in lieu of $T_{Ok}$, as far as the auxin effect is concerned.

The values of $T_m$ and $T_m$ at various pre-extension rates are shown in Fig. 2. As the pre-extension rate increases, the value of $T_m$ decreases nearly monotonously, though the value of $T_m$ remains constant. IAA seems to cause no change in the values of $T_m$ and $T_m$.
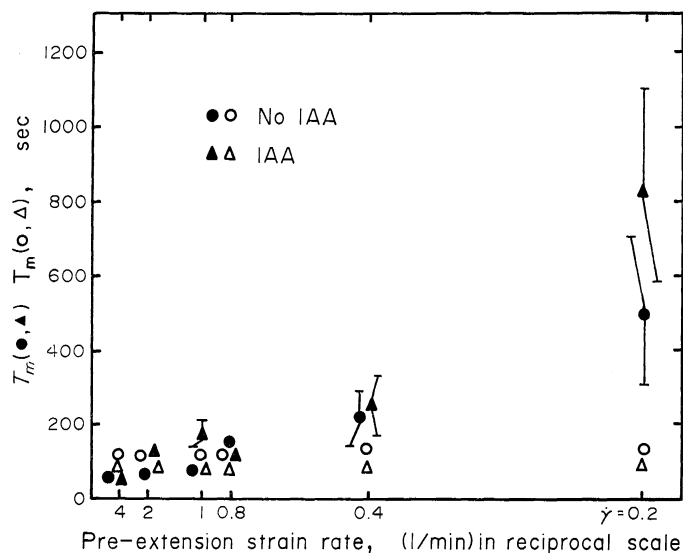


FIG. 2

Effect of pre-extension strain rate on the $T_m$ and $T_m$ values of the pea epidermal cell wall. The $T_m$ values are averages of those calculated from experimental data ($n$ = 11, without IAA; $n$ = 12, with IAA). The $T_m$ values are averages of those calculated from experimental data ($n$ = 7), without IAA; $n$ = 7, with IAA).

Changes in the values of $b$ and b with the pre-extension rate are illustrated in Fig. 3. The values of $b$ and b are represented as percentage of $S_O$ (initial stress), that is, $(b/S_O)$ x 100 and (b/S$_0$) x 100 (cf. 11). The values of $b/S_O$ seem to be nearly constant, although those of b/S$_0$ decrease with the pre-extension rate. The values of b/S$_0$ are approximately equal to those of $b/S_O$.

The values of $c$ and c at various pre-extension rates are shown in Fig. 4. They also are given in the form of $(c/S_O)$ x 100 and (c/S$_0$) x 100. The values of $c/S_O$ at different pre-extension rates in the case without IAA pretreatment can be represented by those of c/S$_0$ qualitatively and approximately quantitatively. However, IAA seems to cause an increase in c/S$_0$, but not in $c/S_O$.

In the present study, the parameters $T_O$, $T_m$, b, and c were derived from equation (2) to estimate the values of $T_O$, $T_m$, $b$, and $c$. The changes in the values of $T_O$, $T_m$, $b$, and $c$ due to the pre-extension rate could be represented qualitatively and approximately quantitatively by using the values of $\gamma_{op}$, $\gamma_p$, $T_{ok}$, $T_{mk}$, $b_k$, $c_m$, and $K$. Thus, the introduction of parameter $K$ into equation (2), which was described in the first paper of this series (4), can be concluded to be appropriate. In addition,
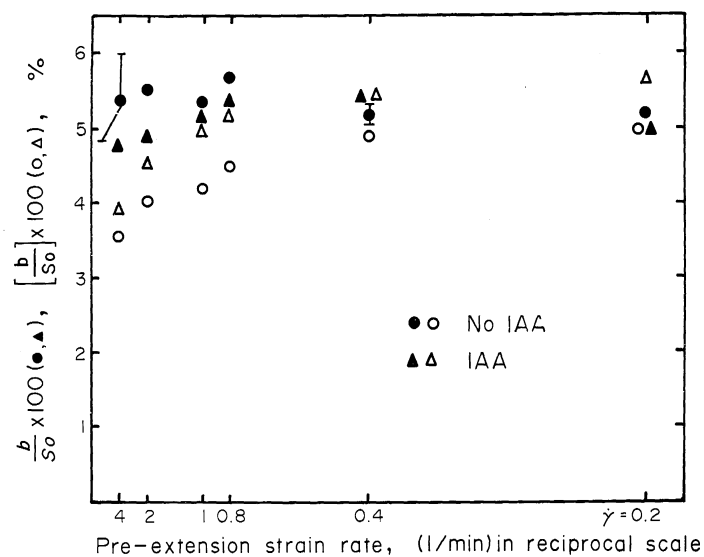
FIG. 3

Effect of pre-extension strain rate on the $b/S_O$ and (b/So) values of the pea epidermal cell wall. The $b/S_O$ values are averages of those calculated from experimental data ($n$ = 11, without IAA; $n$ = 12, with IAA). The (b/S$_o$) values are averages of those calculated from experimental data ($n$ = 7, without IAA; $n$ = 7, with IAA).
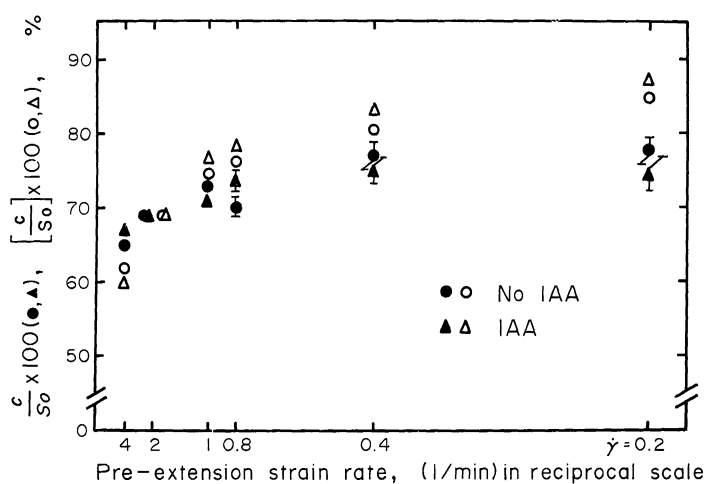


FIG. 4

Effect of pre-extension strain rate on the $c/S_O$ and (c/S$_o$) values of the pea epidermal cell wall. The $c/S_O$ values are averages of those calculated from experimental data ($n$ = 11, without IAA, $n$ = 12, with IAA). The (c/S$_o$) values are averages of those calculated from experimental data ($n$ = 7, without IAA, $n$ = 7, with IAA).

we suggest that even a complex phenomenon such as the change in stress relaxation with pre-extension can be simulated by the linear model.

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VISCOELASTIC PROPERTIES OF PLANT CELL WALLS — III.
HYSTERESIS LOOP IN THE STRESS-STRAIN CURVE AT CONSTANT STRAIN RATE

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ABSTRACT — When the cell wall of pea epidermis is extended
and returned at a fixed rate, the stress-strain curves form
a hysteresis loop. The mechanical properties of the pea
epidermal cell wall in a constant rate experiment can be
simulated by a model consisting of an infinite number of
Maxwell elements and a single spring in parallel with them,
as in stress relaxation experiments. The mathematical for-
mulation for a constant rate experiment is closely related
to that for a stress relaxation experiment throught its con-
stant parameters.

   In a constant rate experiment, auxin pretreatment increases
strain at a small stress. The effect of auxin on the stress-
strain curve in the constant rate experiment is qualitatively
and quantitatively reproduced well by the constant parameters
representing the effect of auxin on the stress-time curve
in the stress relaxation experiment.

INTRODUCTION

In studies on auxin action on extension of the planet cell, mechanical properties
of cell walls have been investigated by several techniques. One useful method is
the stress relaxation experiment (3,7,17,18). The specimen is stretched at a con-
stant rate, then the stress produced by it at a fixed strain decays with time (17).
The stress-time curve in stress relaxation of plant cell walls is very effectively
expressed by the equation of Yamamoto *et al.* (17, 18).

  Pre-extension prior to stress relaxation at a fixed strain is needed before init-
ial stress can be produced in a specimen, although the equation of Yamamoto *et al.*
did not take the pre-extension into consideration. The first paper (5) of this
series proposed a mathematical formulation for stress relaxation with consideration
for pre-extension, using a new constant parameter $K$. The second paper (6) showed
that the values of the constant parameters $T_0$, $T_m$, $b$, and $c$ in the equation of Yam-
amoto *et al.* (17,18), which represent the mechanical properties of the specimen,
were effected by the pre-extension rate. The influence of the pre-extension rate
on these parameters was explained qualitatively using the mathematical formulation
in the first paper (5).

In stress relation experiments, stress-time curves are used (4,5,7,11,17,18). On the other hand, in constant strain rate experiments such as compliance determinations, stress-strain curves are used (1,2,4,7-10,12,13).

Hence, we try to analyze the stress-strain curves at a constant rate by developing the method reported in the first paper of this series (5). If a constant strain rate is applied to a cell wall specimen repeatedly, then a hysteresis loop in the stress-strain curve is obtained (1,2,4,7-9). Thus, we attempted to analyze the hysteresis loop using the formulation of an extension process at a constant rate which was reported also in the first paper (5).

There is a possibility that the hysteresis loop of the stress-strain curve can be obtained by changing the sign of the extension rate parameter in the formulation depending upon whether the process is an extension or a return one. This paper discusses this possibility, namely, simulation of the hysteresis loop by a generalized Maxwell model and also discusses the effect of auxin pretreatment on hysteresis loops of the cell wall obtained experimentally and theoretically.


## MATERIALS AND METHODS

*Plant Material:* As reported previously in this series (6), *Pisum sativa* L., cv. Alaska was used. Segments were incubated in test solution with or without $10^{-5}$ M indoleacetic acid (IAA) for 3 hr. We used the epidermis instead of the whole stem segments since the pea epidermal tissue is much more uniform than whole stem segments and the epidermal cell wall has been reported to be the part controlling stem segment elongation due to auxin (16).

*Cell wall extension study:* The stress relaxation process and the stress-strain curve were measured by methods reported previously (1,2,17,18).


## Constant-rate extension

When a single Maxwell element is extended at a constant rate, the relationship between the stress (= force/cross section area) and the strain (= extension/initial length) is:

$$S = G\dot{\gamma}\tau \{1 - \exp(-\gamma/\dot{\gamma}\tau)\} \, ,$$

where $S$ is the stress, $G$ is the elastic modulus of the spring, $\dot{\gamma}$ is the strain rate (= extension rate/initial length), $\gamma$ is the strain, $\tau$ is the relaxation time.

The mechanical properties of the cell wall of pea epidermis are simulated by a continuous viscoelastic model consisting of an infinite number of Maxwell elements and a single spring in parallel with them, as illustrated in Fig. 1 (5,6,17,18). Then the formula above is modified as follows:

$$S = \dot{\gamma}\int_0^\infty G_k(\tau) \cdot \tau \{1 - \exp(-\gamma/\dot{\gamma}\tau)\}d\tau + G_e \cdot \gamma \quad , \tag{1}$$

where $G_k(\tau)$ is the distribution density function of relaxation times in the generalized Maxwell model in Fig. 1, $G_e$ is the elastic modulus of the spring which is the element without a dashpot (see Fig. 1) (4,5,6).

The specific functional form of $G_k(\tau)$ of the primary cell wall of higher plants was formulated in the first paper (5) as follows:

$$G_k(\tau) = \frac{b_k}{\gamma_{op}} \frac{1}{\tau} \left( 1 + \frac{K}{\tau} \right) \left\{ \exp\left( -\frac{T_{ok}}{\tau} \right) - \exp\left( -\frac{T_{mk}}{\tau} \right) \right\} \quad , \tag{2}$$
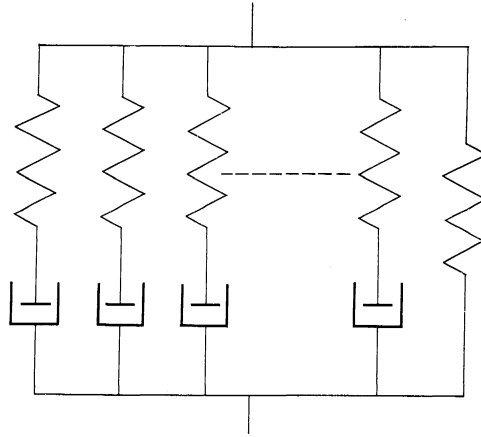
FIG. 1.

Generalized Maxwell model.  Continuous viscoelastic
model consisting of an infinite number of Maxwell
elements and a spring (a degenerate Maxwell element)
in parallel with them.

where $\gamma_{op}$ is the pre-extension strain in the stress relaxation experiment, and $b_k$,
$K$, $T_{ok}$, and $T_{mk}$ are constant parameters.

The relationship between the stress and the strain in constant-rate experiments
is obtained by substituting $G_k(\tau)$ of equation (2) into equation (1) as follows:

$$S = \frac{\dot{\gamma}t}{\gamma_{op}} \cdot b_k \cdot \ln \frac{\phi(K, t; T_{mk})}{\phi(K, t; T_{ok})} + \frac{\gamma}{\gamma_{op}} \cdot c_k \qquad (3)$$

$$\phi(K, A; B) = B \left(1 + \frac{A}{B}\right)^{1 + \frac{B-K}{A}} \qquad (4)$$

$$t = \frac{\gamma}{\dot{\gamma}} \quad , \qquad (5)$$

where $c_k = G_k \cdot \gamma_{op}$; $t$ is the time.

The extension of the specimen is stopped when $t$ arrives at $t_p$.  Then $t$ becomes
zero again.  Thereafter, the stress relaxes with the strain remaining constant.
Then the equation of a stress-strain curve was obtained in the first paper (5) as
follows:

$$S = \frac{\dot{\gamma}t}{\gamma_{op}} \cdot b_k \cdot \ln \frac{\phi(K, t_p; t+T_{mk})}{\phi(K, t_p; t+T_{ok})} + \frac{\gamma}{\gamma_{op}} \cdot c_k \quad . \qquad (6)$$

The independent variable $t$ in equation (3) was included in the position of $A$ in
$\phi(K, A; B)$, whereas $t$ in equation (6) was included in the position of $B$ in $\phi(K, A; B)$.


Return process at a constant strain rate

The extension of the specimen was stopped when $t$ reached $t_1$ and the strain reached

$\gamma_1$. Then $t$ became zero again. Thereafter, an immediate return of $t$ sec at the same constant rate as the extension rate occurred, as long as the stress was positive. Then the strain was:

$$\gamma = \gamma_1 - \dot{\gamma}t \quad .$$

The formula of the stress was obtained in the same way as equations (1), (4):

$$S = -\dot{\gamma}\int_0^\infty G_k(\tau).\tau\{1 - \exp(-t/\tau)\}d\tau$$

$$+ \gamma\int_0^\infty G_k(\tau)\cdot\tau\{1 - \exp(-t_1/\tau)\}\cdot\exp(-t/\tau)d\tau + G_e\cdot\gamma \quad , \tag{7}$$

When $G_k(\tau)$ of equation (2) was substituted into equation (7), we obtained a formula corresponding to equation (3):

$$S = - \frac{\dot{\gamma}t}{\gamma_{op}} \cdot b_k \cdot \ln \frac{\phi(K,t;T_{mk})}{\phi(K,t;T_{ok})}$$

$$+ \frac{\dot{\gamma}t}{\gamma_{op}} \cdot b_k \cdot \ln \frac{\phi(K,t_1;t+T_{mk})}{\phi(K,t_1;t+T_{ok})} + \frac{\gamma}{\gamma_{ok}} \cdot c_k \quad . \tag{8}$$

Note that the first term in equation (8) is the same functional form as that in equation (3), and the second and third terms in equation (8), are the same as the first and second terms in equation (6).


## Hysteresis loop in the stress-strain curve

When the hysteresis loops (1,2,9) in stress-strain curves at a fixed rate were recorded, we anticipated that the first loop would be represented by equation (3) and thus equation (8). Generally, when a plant cell wall is extended and returned repeatedly at a fixed rate, the relationship between the stress and the strain is calculated.

The times required for the extension process and the return process in hysteresis loops in stress-strain curves were counted. Then $t$ was set at zero again at reverse points. Thus, the formulation of the $n$ th movement was:

$$S = (-1)^{n+1}\cdot\dot{\gamma}\int_0^\infty G_k(\tau).\tau\{1 - \exp(-t/\tau)\}d\tau$$

$$+ \sum_{i=1}^{n-1} \left[ (-1)^{i+1}\cdot\dot{\gamma}\int_0^\infty G_k(\tau)\cdot\tau\{1 - \exp(-t_i/\tau)\}\cdot\exp\{(-t- \sum_{j=i+1}^{n-1} t_j)/\tau\}d\tau \right]$$

$$+G_e\cdot\gamma \tag{9}$$

$$\gamma = \dot{\gamma}\left( \sum_{i=1}^{n-1} \left\{ (-1)^{i+1}\cdot t_i \right\} + (-1)^{n+1}\cdot t \right) \tag{10}$$

where the positive integer $n$ represents the extension process in the $(n+1)/2$ th loop when $n$ is an odd number, and the return process in the $n/2$ th loop when $n$ is an even number.

When $G_k(\tau)$ of equation (2) is substituted into equation (9), we obtain the formulation expanding equation (3) and equation (8) on the first loop into the case of the $n$ th movement:

$$S = \frac{\dot{\gamma} b_k}{\gamma_{op}}\left[(-1)^{n+1}\cdot t\cdot \ln \frac{\phi(K,t;T_{mk})}{\phi(K,t;T_{ok})}\right.$$

$$\left.\div \sum_{i=1}^{n-1}\left\{(-1)^{i+1}\cdot t_i\cdot \ln \frac{\phi(K,t_i;t+T_{mk}+\sum\limits_{j=i+1}^{n-1}t_j)}{\phi(K,t_i;t+T_{ok}+\sum\limits_{j=i+1}^{n-1}t_j)}\right\}\right] + \frac{\gamma}{\gamma_{op}}\cdot c_k \qquad (11)$$

$$\gamma = \dot{\gamma}\left[\sum_{i=1}^{n-1}\{(-1)^{i+1}\cdot t_i\} + (-1)^{n+1}\cdot t\right].$$

The above formulation results from applying the method shown in the first paper of this series (5) to hysteresis loops in stress-strain curves.

We would like to discuss whether the hysteresis loops in stress-strain curves are simulated well by equation (11).

RESULTS AND DISCUSSION

Hysteresis loop in the stress-strain curve of the isolated cell wall of pea epidermis

A 5 mm long cell wall of pea epidermis isolated from segments pretreated without IAA was extended at 2 mm/min, and the experimental stress-strain curve in Fig. 2 was obtained. The stress relaxation experiment after pre-extension at 20 mm/min was carried out using seven specimens of this material. The data were treated using the least squares method (5,6); the estimated values of the constant parameters $T_{ok}$, $T_{mk}$, $b_k$, $c_k$, and $K$ in the No IAA column in Table 1 were obtained from equation (6).
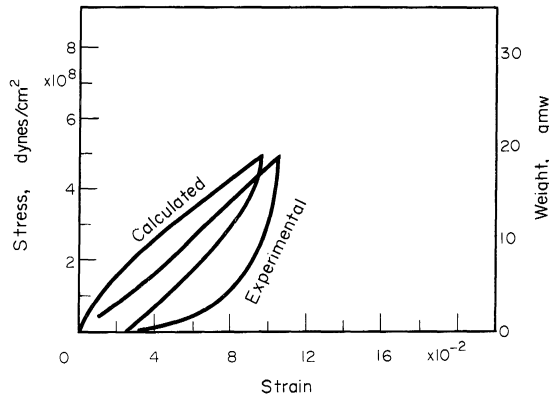


FIG. 2

Stress-strain curve of the pea epidermal cell wall in an extension process and a return process at 2 mm/min. Both the experimental and the calculated curves show a hysteresis loop.

These estimated values were substituted into equation (3) to obtain the calculated
curve of extension in Fig. 2. The cross-sectional area of the specimen in the ini-
tial length was estimated to be 3.75 x $10^{-5}$ $cm^2$.

When the specimen was extended to 0.013 strain and returned at the same rate, the
experimental curve of return in Fig. 2 was obtained. The calculated curve of return
was obtained by substituting the values in Table 1 into equation (8). The order of
the calculated values corresponded with that of the experimental values; both curves
had a similar shape except in the region of low strain. The difference of the two
curves at low strain seems to be due to the initial slack of experimental measure-
ments after the specimen was stretched (1,12). The calculated curve hysteresis
loop in Fig. 2 is similar in form to the experimental curves of mung bean hypocotyl
reported by Lockhart (9) and of *Avena* coleoptile reported by Preston and Hepton (13).

TABLE 1

Values of constant parameters of a pea epidermal
cell wall stretched at 20 mm/min

| Parameter | No IAA | Calculated value | Plus IAA |
|---|---|---|---|
| $T_{ok}$ | 4.20 x $10^{-2}$± 1.35 x $10^{-2}$ sec | | 9.15 x $10^{-3}$± 3.35 x $10^{-3}$ sec |
| $T_{mk}$ | 1.20 x $10^{-2}$± 2.2 x $10^{1}$ sec | | 8.18 x $10^{1}$ ± 1.42 x $10^{1}$ sec |
| $K$ | 1.36 ± 2.1 x $10^{-1}$ sec | | 1.29 ± 3.3 x $10^{-1}$ ·sec |
| $b_k$ | 2.21 x $10^{7}$ ± 1.0 x $10^{6}$ dynes/$cm^2$ | | 2.09 x $10^{7}$ ± 1.1 x $10^{6}$ dynes/$cm^2$ |
| $c_k$ | 3.74 x $10^{8}$ ± 7 x $10^{6}$ dynes/$cm^2$ | | 3.19 x $10^{8}$ ± 7 x $10^{6}$ dynes/$cm^2$ |
| Pre-exten-sion strain | 6.34 x $10^{-2}$± 1.17 x $10^{-2}$ | | 9.66 x $10^{-2}$± 1.20 x $10^{-2}$ |

### Effect of introducing the new constant parameter K on the hysteresis loop of the stress-strain curve at constant rate

When the pre-extension time of a stress relaxation experiment is assumed to be
zero, the stress relaxation process of the cell walls of *Avena* coleoptile or pea
epidermis is represented by (5,17,18):

$$S = b \cdot \ln \frac{t + T_m}{t + T_o} + c \, ,$$                              (12)

where $T_o$, $T_m$, $b$, and $c$ are constant parameters. The experimental curve of stress
relaxation fits equation (12) as well as equation (6). Using the least squares
method (cf. 5), the values of the constant parameters $T_o$, $T_m$, $b$, and $c$ in equation
(12) were estimated with the same data from which the values of the No IAA column
in Table 1 were obtained, and are shown in Table 2.

If equation (6) of the new formulation corresponding to equation (12) was used,
then equation (3) for the extension process and equation (8) for the return process
in the hysteresis loop in the stress-strain curve were introduced, respectively.
For the trial with the values in Table 2 substituted into the constant parameters
$T_{ok}$, $T_{mk}$, $b_k$, and $c_k$ and the parameter $K$ set at zero in equations (6) and (8), the
calculated curve illustrated in Fig. 3 was obtained. The calculated curve at $K \neq 0$
shown in Fig. 2 resembles the experimental curve more than the calculated curve at
$K = 0$ shown in Fig. 3.

TABLE 2

Values of constant parameters of a pea epidermal
cell wall without IAA pretreatment stretched at
20 mm/min

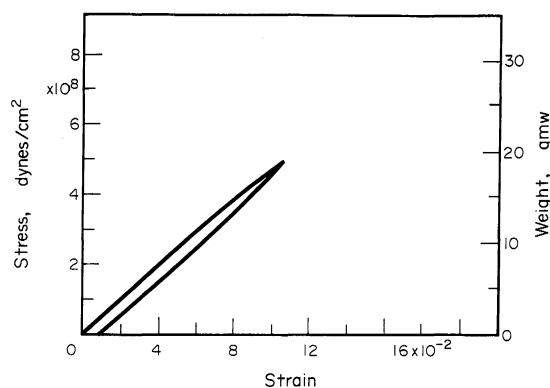| Parameter | Calculated value | | |
|---|---|---|---|
| $T_O$ | $1.53 \times 10^{-2} \pm$ | $2.4 \times 10^{-3}$ | sec |
| $T_m$ | $5.27 \times 10^{1} \pm$ | $8.0$ | sec |
| $b$ | $2.76 \times 10^{7} \pm$ | $1.0 \times 10^{6}$ | dynes/cm |
| $c$ | $3.81 \times 10^{8} \pm$ | $7 \times 10^{6}$ | dynes/cm |
| Pre-extension strain | $6.34 \times 10^{-2} \pm$ | $1.17 \times 10^{-2}$ | |



FIG. 3

Hysteresis loop calculated at $K = 0$ of stress–strain
curve at 2 mm/min. This calculated curve at $K = 0$
does not fit well the experimental curve shown in
Fig. 2.

## Hysteresis loops in the stress–strain curve at constant rate of pea epidermal cell wall

Figure 4 was obtained by repeating the extension and the return processes of the
pea epidermal cell wall at 2mm/min rate. The calculated values of the hysteresis
loops were obtained by using the constant parameters in Table 3 as in the case of
Fig. 2. The difference of the calculated curve with the low strain from the ex-
perimental curve seems likely to be due to the initial slack, as stated above. At
other points, both curves closely resemble each other qualitatively. The strain in-
dicating maximum stress in the loop becomes gradually larger each time, especially
between subsequent hysteresis loops. Moreover, the distance between the loops be-
comes gradually narrower. The form of the calculated curve closely resembles that
of the experimental curve in the neighborhood of the point at which the stress–strain
curve reverses from an extension process to a return process, in contrast with the
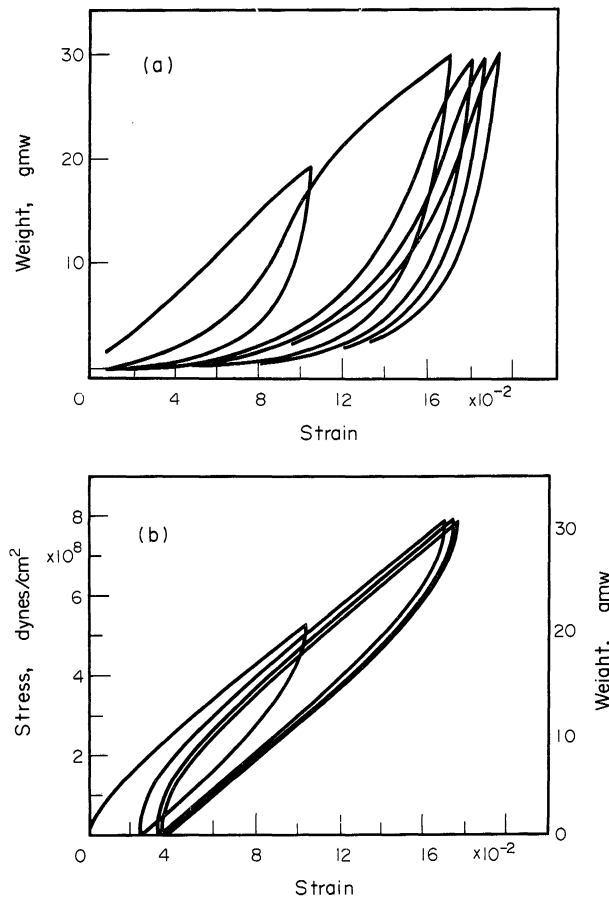difference of the form at the low strain.

FIG. 4

Hysteresis loops of stress-strain curves of the cell
wall specimen stretched and returned at 2 mm/min,
(a) experimental, (b) calculated.

In view of the fact that the form of the calculated curve was different from that
of the experimental curve at low strain, the following experiment was done. The
movement was reversed from the return process to the extension process at the point
above zero stress. The experimental and the calculated curves are shown in Fig. 5.
The calculated loops, except the first curve in Fig. 5, agree with the experimental
loops better than the fully returned hysteresis loops shown in Fig. 4.


Effect of IAA pretreatment on the stress-strain curve of plant cell wall

The effect of IAA on the plant cell wall has been investigated using the stress
relaxation experiment (3,6,11,14,17,18) and the constant-rate experiment (1,2,12).
As these experiments were carried out independently, the relationship between ef-
fects on stress-time properties at a fixed strain and those on stress-strain prop-
erties at a fixed rate in the cell wall have not been analyzed. We try to clarify
this relationship by developing the above methods.

TABLE 3

Values of constant parameters of a pea epidermal
cell wall stretched at 2 mm/min

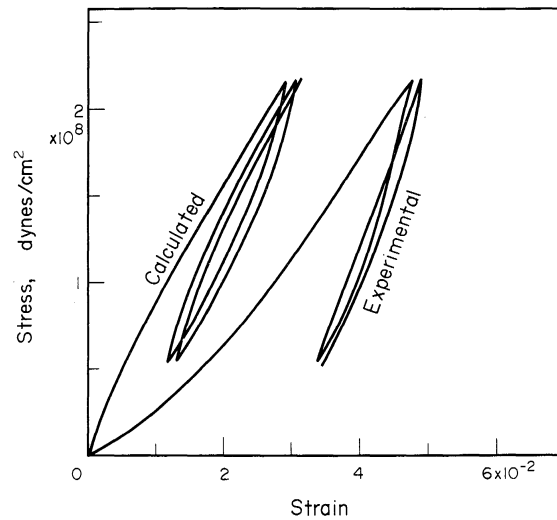| Parameter | Calculate Value | |
| --- | --- | --- |
| | No IAA | Plus IAA |
| $T_{ok}$ | $1.50 \times 10^{-1}$ sec | $1.84 \times 10^{-1}$ sec |
| $T_{mk}$ | $8.16 \times 10^{1}$ sec | $1.84 \times 10^{2}$ sec |
| $K$ | $1.38 \times 10^{1}$ sec | $2.48 \times 10^{1}$ sec |
| $b_k$ | $2.60 \times 10^{7}$ dynes/cm$^2$ | $1.02 \times 10^{7}$ dynes/cm$^2$ |
| $c_k$ | $3.82 \times 10^{8}$ dynes/cm$^2$ | $2.27 \times 10^{8}$ dynes/cm$^2$ |
| Pre-extension strain | $1.08 \times 10^{-1}$ | $1.24 \times 10^{-1}$ |



FIG. 5

Hysteresis loops of stress-strain curves at 5 mm/min.
The movements were reversed from the return process
to the extension process at ca. 0.5 dynes/cm$^2$.

The values of the constant parameters $T_{ok}$, $T_{mk}$, $b_k$, $c_k$, and $K$ were estimated using
the least squares method from the data of the stress relaxation experiment of pea
epidermal cell wall pretreated with or without IAA, and are shown in Table 1. The
stress-strain curves in Fig. 6 were calculated by substituting the values in Table
1 into equation (11). The gradient of the curve in the extension was steeper in
the pretreatment without IAA than in the one with IAA, regardless of how long the
extension time took in the range of 0.00-0.15 strain. The effect of IAA pretreat-
ment of Avena coleoptile by Cleland (2) was reproduced well by the results on pea
epidermis shown in Fig. 6, except at low strain. This indicated that the stress
relaxation property of the cell wall is closely related to the stress-strain prop-

erty (cf. 1,2) as far as the auxin effect is concerned. The biochemical background for the change in the mechanical property of the cell wall in terms of auxin action has been reported (11,14,15).
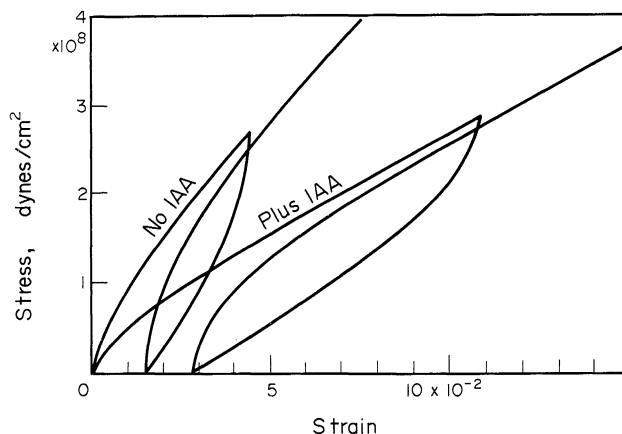


FIG. 6

Effect of IAA pretreatment on the hysteresis loop
of the stress-strain curve at 2 mm/min.

It is worth noting that both the stress-time curve in the stress relaxation experiment and the hysteresis loop of the stress-strain curve in a constant-rate experiment may be simulated systematically and theoretically by a generalized Maxwell model which, in a certain sense, is a definite simple model with linear elements consisting of springs and dashpots.

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