Physical Nature of Irreversible Deformation of Plant Cells

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Summary. Etiolated mung bean hypocotyl segments were incubated in 0.25 M mannitol solutions with indoleacetic acid. They were then deformed mechanically with a longitudinal tensile force at a constant strain rate. The magnitudes of the mechanical forces were comparable to those of the hydrostatic forces existing in normally growing tissues. Each segment was repeatedly deformed and returned to zero force. The total deformation was increased at each cycle.

The irreversible and elastic changes in length and diameter were measured for each deformation and the changes in surface area and volume calculated. In addition the applied stress and the work of irreversible and of elastic deformation were determined as functions of deformation.

It was found that irreversible elongation, irreversible change in surface area and total change in surface area all were linear functions of total imposed elongation. However, very little change in volume occurred during the deformations.

The work of irreversible deformation was found to be independent of temperature between 8° and 25°. It was also virtually independent of rate of deformation measured over a 5-fold range of deformation rates.

From these results it is concluded that the irreversible deformation of mung bean hypocotyl tissue occurs by plastic deformation rather than by viscous flow. Thus, the irreversible deformation occurred as a result of breaking cross-links of a cross-linked polymer system.

It is now well established that elongation of plant stem, hypocotyl and coleoptile tissues occurs as a result of the hydrostatic pressure within the cell together with the physical yield of the cell wall (4, 6, 8, 9, 10, 12). Normally, the rate of wall deformation is a function of the rates of the metabolic processes that act on the cell wall and of the turgor pressure existing within the cell. The turgor pressure is, in turn, a function of the osmotic pressure of the tissue and the water conductivity of the system (7).

The effects of whatever metabolic processes are necessary to maintain cell wall deformability may be studied by measuring the changes that occur in the physical properties of the walls as a result of this metabolic action. In order to separate the metabolic effects and the deformation process the turgor pressure can be reduced. This will allow the metabolic processes that act on the wall to continue without concurrent wall yield. After incubation for an appropriate time the physical properties can be measured under known applied forces (hydrostatic or mechanical forces) (4, 8, 9). For the present studies the hydrostatic pressure was reduced by incubation in approximately isotonic mannitol solutions.

Whatever the details of the structure, the physical properties of plant cell walls are due to some type of polymer structure. In principle, a polymer system is either cross-linked or linear. To understand the mechanism of irreversible deformation it is important to determine whether the polymers that control the physical properties of the cell wall are linear or cross-linked. On the basis of indirect evidence various authors have already concluded that the cell wall is a cross-linked polymer system (2, 10). Most recently Cleland (3) has presented evidence strongly supporting plastic deformation as the mechanism of deformation of methanol-killed Avena coleoptile segments.

However, since the present author proposes to use the mechanical properties of the walls to study the mechanism of wall deformation it was considered important to confirm the cross-linked nature of the polymer system. Irreversible deformation of linear polymers occurs by processes described generally as viscous flow, while irreversible deformation of cross-linked polymers occurs only when cross-links are broken, i.e., by plastic deformation. In principle, viscous flow differs from plastic deformation in that the work (i.e., the energy) required to cause plastic deformation is independent of deformation rate, while the work required for viscous flow is directly proportional to the rate of deformation. An additional distinction is that the work required for viscous flow

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varies with the temperature of the material (during deformation) while the work required for plastic deformation is essentially independent of temperature (1, 5).

In a polymer system the cross-links are forces acting between the individual polymer elements. True cross-links are forces between polymer elements with sufficient potential energy to remain continuously intact (within a particular temperature range). Thus a sufficient number of cross-links will make a polymer system a true solid. If the forces between polymer elements are such that the bonds are continually forming and opening in a thermal equilibrium relationship, the polymer system is a linear polymer and has the properties of a viscous liquid.

One of the most useful techniques to study the deformation properties of solid polymer systems is to impose a constant rate of deformation on the material and monitor the force during deformation. The constant strain rate technique was first used on plant tissue by Olson, et al. (9) and Cleland (3) has used this technique extensively.

**Methods and Materials**

Dark-grown seedlings of mung bean (*Phaseolus aureus*) 4 or 5 days old were used as the experimental material. The seedlings were germinated and grown in a dark room at approximately 2°C. A 10 mm segment of hypocotyl (the portion from 7 to 17 mm below the apical hook) was used for the experimental studies reported here.

To apply a tensile force to a tissue segment each end of 2 strips of cotton cloth roughly 1 X 5 cm were smeared with Elmer’s Contact Cement, a Borden Corporation organic solvent adhesive. Portions of the stem above and below the experimental segment were also smeared with adhesive. The adhesive was allowed to dry for 5 minutes. The hypocotyl was then cut off about 4 cm below the apex and the ends of the cloth strips were firmly pressed to the ends of the hypocotyl to form 2 loops of cloth. The adhesive was allowed to set for 5 minutes in air, then the tissues were placed in a mannitol solution for incubulation. Gluing and incubation was done in the darkroom with minimum exposures to green light.

Since the purpose of these studies was to measure the deformation of living tissues under known forces it was necessary to neutralize the normal turgor pressure of the tissue. This was done by incubating the tissues in 0.25 M mannitol (Atac Powder Company, Wilmington, Delaware) solutions for approximately 1.5 to 2.0 hours. Each segment remained in the mannitol solution until it was placed in the analyzer. Indoleacetic acid (IAA) was normally added to the mannitol as the potassium salt to make a concentration of 1.0 mg/liter.

For deformation, those portions of the segment to which the cloth had been glued were clamped by vises faced with several layers of leather leaving the 10 mm experimental segment between the vises. The glued cloth tape prevented the tissue from slipping within the vises. A constant rate of elongation was imposed on the tissue by lowering at a constant rate an elevator on which was mounted the lower vise. The deformation rate was 0.75 mm/min, i.e., 7.5% per min, unless otherwise specified. In the early experiments the lower vise and the tissue segment were immersed in a 0.25 M solution of mannitol during deformation. In the later experiments the tissues were deformed in air after it was found that the responses were the same in air and in mannitol solutions.

The force imposed on the tissue section was continuously measured by a Statham force transducer connected to the upper clamp by a lever system. The output of the transducer system drove the y-axis of an x-y recorder. Usually deformation was continued until a selected force or deformation was reached, then the direction of elevator movement was reversed and the vise was raised at the same rate to measure the deformation-force curve of recovery. A 20-turn potentiometer was geared to the elevator drive shaft, and the voltage drop across the potentiometer measured the position of the clamp and elevator and drove the x-axis of the recorder.

In the experiments reported here an additional measurement was made. The diameter of the tissue was measured at the beginning of the experiment and before and after each deformation with a horizontal microscope and a Vickers “Image-Splitting Eyepiece”. Thus, both irreversible and elastic changes in the surface area and volume of the tissue segment could be calculated and related to the total change in length and work required for deformation. These parameters provide a much more complete picture of tissue deformation than the measure of length alone (8). The relative changes in area and volume of tissue segments were calculated from the relationships: \( \frac{dA}{A} = \frac{dl}{l} + \frac{dr}{r} \) and \( \frac{dV}{V} = \frac{dl}{l} + 2\frac{dr}{r} \), where cylinder volume is denoted by \( V \), lateral surface area by \( A \), length by \( l \) and radius by \( r \).

The area under the force-deformation curve was measured with a planimeter. It will be shown below that this area represents the work of deformation of the tissue segment.

Deformations have been expressed here as percentage change, e.g., \( (\Delta l/l) \times 100 \), unless otherwise specified. The experimental variability is expressed either as the standard error or as the coefficient of variability, i.e., the standard deviation divided by the mean.

**Experimental Results**

**Irreversible and Elastic Tissue Deformation.** Preliminary constant strain rate experiments gave some surprising results. The irreversible elongation of the tissue was not linear with applied stress, as might have been anticipated. Instead, irreversible deformation, both \( \Delta l/l \) and \( \Delta A/A \), was found to be directly proportional to the total elongation imposed on the
system. Therefore, in subsequent experiments, equal increments of stem elongation rather than equal increments of tensile stress were imposed on the tissue.

Total changes in wall area and tissue volume as a function of the imposed changes in length are presented in figure 1. The effects of the imposed deformations on irreversible elongation and irreversible changes in wall area and tissue volume are presented in figure 2.

It is evident that under the experimental conditions used here, the decrease in tissue diameter was linearly related to the imposed increase in length. Furthermore, the total decrease in diameter was equal to approximately one-half the total increase in length. Evidently, then, there was little change in tissue volume during the imposed deformations and recoveries (figs 1 and 2), since $\Delta V/V = \Delta l/l + 2\Delta d/d$. The significance of the lack of substantial volume changes will be discussed below.

Even though irreversible deformation was presumed to be a function of the imposed total change in stem length, it is probably equally valid to consider

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**FIG. 1.** The effect of an imposed elongation at a constant strain rate on average irreversible changes in tissue length, surface area and volume.

**FIG. 2.** The effect of an imposed elongation on the average total change in tissue diameter, surface area, and volume.

**FIG. 3.** Average elastic deformations of the tissue segments as a function of the imposed deformations.
the total change in surface area as the independent variable, i.e., as the imposed experimental treatment. However, since total changes in length and in surface area were linearly related, it made little difference which parameter was used as the independent variable in the present analysis. (Conversely, since they were linearly related, it was impossible to determine whether the change in length or change in surface area was the most direct cause of irreversible deformation.)

Various authors have reported that elastic deformation of hypocotyl and coleoptile segments was not linear with applied force (8, 11). However, elastic deformation of both length and surface area was found to be essentially linear with total deformation (fig 3). Evidently, a constant proportion of the total deformation is irreversible so that the remaining, reversible deformation is a constant proportion of total deformation.

Work of Tissue Deformation. The constant strain rate technique results in an output that continuously plots change of tissue length against applied tensile force (see fig 4). Evidently the area under such a curve represents the work of deformation, i.e.,

\[ W = \int F dx \]

Furthermore, when the imposed deformation is decreased (by reversing the elevator drive) until the applied force decreases to zero, the net change in length represents the irreversible elongation resulting from the treatment. Subsequently, a positive deformation can be reapplied. A second, equal deformation is completely reversible (see below). Thus, the area under this second curve measures the work required for a given elastic deformation.

Evidently, the difference between the total work required for the first deformation, and the elastic (reversible) work required for an equal elastic deformation gives the extra irreversible work required when a given deformation is imposed on the tissue for the first time. Since only the first deformation results in significant irreversible deformation, the extra work required for the first deformation is the work (i.e., the energy) necessary to cause the given amount of irreversible deformation.

The work required to reversibly deform tissue segments was measured as described above. The work of elastic elongation was found to be linear with elongation at elastic deformations equal to or greater than approximately 1% (fig 5). It would be expected that the first increment of elastic elongation would involve taking up slack in the system which would require relatively little work. Evidently the slack occurs because the tissue segment cannot be aligned precisely linearly in the direction of deformation. Test experiments indicated that the analyzer has virtually no slack or backlash.

Work of Irreversible Deformation. While elastic work was found to be a linear function of elastic deformation, work of irreversible deformation was far from linear with irreversible deformation (fig 5). Each successive increment of irreversible deformation required much more work than the previous increment.

FIG. 4. A tracing of the recording of the force-deformation curve of a stem segment elongated at a constant strain rate. When the force reached 200 gms the strain was reversed until the force was equal to zero. The strain was reversed again to measure elastic work. The significance of the various output parameters is indicated.

FIG. 5. The work of irreversible deformation as a function of irreversible change in length and as a function of irreversible change in surface area. Also shown is the work of elastic deformation as a function of elastic change in length and in surface area.
It is evident that, following a period of wall relaxation, a small irreversible deformation can occur with little work input, while greater deformations require more energy per unit deformation.

The plot of irreversible work against irreversible deformation plots total irreversible deformation against the total irreversible work required to effect that deformation, i.e., the sum of the values for the work against the sum of the irreversible deformations measured at each of the successive imposed deformations. The net work required for successive increments of irreversible deformation is shown in Table I.

If the wall is, in fact, a cross-linked polymer system, only a relatively few cross-links need be broken during the first increment of irreversible deformation. The work required for a given increment of irreversible deformation should be a direct measure of the energy required to break the cross-links that will be broken during an imposed deformation. This will be discussed further below.

**Effect of Rate of Deformation on Work of Irreversible Deformation.** As pointed out above, the definitive distinction between irreversible deformation by viscous flow and by plastic deformation is given by the relationship between work required (for irreversible deformation) and the rate of irreversible deformation.

During the present investigations the work of deformation of hypocotyl segments as a function of deformation rate was studied. Normally the hypocotyl segments were deformed at a rate of approximately 0.75 mm/min, i.e., 7.5 %/min. The elevator that imposed the deformations was driven by a synchronous motor geared to allow a choice of gear ratios permitting various deformation rates. Deformation rates of 0.33 mm/min and 1.65 mm/min were used to study the influence of deformation rate on the work of deformation. The selection of these rates was arbitrary but the experimental results suggested this was an appropriate range.

The work of irreversible deformation at the 2 deformation rates is presented in Figure 6. It is evident that when the deformation rate is 5-fold more rapid, the work of irreversible deformation is increased by less than fifty percent. This small increase in the work of irreversible deformation at the faster deformation rate is hardly greater than the increase in the work of elastic deformation under the same condition also shown in Figure 6. The work of simple elastic deformation is, in principle, independent of rate of deformation, but Cleland has presented evidence for a significant retarded elastic deformation in killed *Avena* tissue (3), and we have observed relatively small but significant retarded elastic deformations in the present study. This response could account for most or all of the differences observed at the 2 strain rates. In any case the work of irreversible deformation of hypocotyl segments was virtually independent of deformation rate.

It was shown above that in this system irreversible deformation is linear with total deformation. Figure 7 shows that this linear relationship holds for all strain rates used here. In addition, the results presented in Figure 7 illustrate that the magnitude of irreversible deformation is essentially independent of rate of strain. Thus, both the magnitude of irreversible deformation and the energy per unit irreversible deformation are independent of strain rate.

**Repeated Deformations.** After a hypocotyl segment has been stretched to a given total length and the imposed tensile force removed, irreversible deformation will be found to have occurred. If the stem segment is then stretched to a greater total length, additional irreversible deformation will occur, as shown above. However, if the stem is stretched a second time to the same total length, virtually no...
additonal irreversible deformation will occur. Figure 8 is a tracing of a chart showing the behavior of a single, typical hypocotyl segment. It may be seen that during subsequent imposed deformations the force-deformation curve traces virtually the same elastic deformation curve each time, and no further irreversible deformation occurs. Incidentally, these results validate the determination of work of irreversible deformation at 1 point: i.e., when no irreversible deformation occurs, irreversible work equals zero.

While the second and subsequent equal deformations result in very little additional irreversible deformation, it is evident that small and decreasing irreversible deformations do occur, consuming proportionally small quantities of irreversible work. Generally, the assumption has been made here that the first deformation results in all the irreversible deformation that will occur for a given deformation.

Effect of Temperature on the Work of Irreversible Deformation. In order to measure the work of deformation at low temperatures the stress-strain analyzer (except the recorder) was placed in a refrigerator. After a normal incubation, hypocotyl segments were brought into the laboratory and transferred to a mannitol solution that had been prechilled to about 4 to 5°. The tissue segments were kept in the cold mannitol solution in the refrigerator for at least 20 minutes before deformation was begun. During deformation the refrigerator door remained open to allow diameter measurements. A piece of Plexiglas fitted into the refrigerator doorway retarded mixing the cold air in the refrigerator with room air. The temperature in the refrigerator remained at 8 to 10° throughout a series of experimental runs. To insure comparable controls, 2 to 3 segments were run in the cold, then the machine was moved out of the refrigerator and 2 to 3 tissue segments that had been kept at room temperature were run. The analyzer was equilibrated in the refrigerator before additional cold-treated tissues were run, even though heat con-

Fig. 6. The work of irreversible deformation as a function of imposed elongation at 2 deformation rates.

Fig. 7. The effect of total elongation on irreversible deformation at 2 deformation rates.
This assumption has been studied previously (8). It was shown that a good qualitative correlation exists between these 2 responses. In addition, some of the quantitative relationships were discussed. Nothing significant can be added to that discussion at this time, but no results have been found here that would make the same mechanism unlikely.

Certainly if the normal growth process is being measured the magnitude of the mechanical forces used must be comparable to the normal forces existing in growing stems. The available values for the forces are compared in table II. A rough estimate of the cross-sectional area of the cell walls has been made and this estimate was used to estimate the unit stress on the cell walls. The comparison of hydrostatic force with mechanical force does not depend on this estimate, of course.

Evidently, the mechanical forces imposed on the tissue in this study were completely comparable to the forces exerted on the tissue under normal growing conditions.

It should be noted that the magnitudes of irreversible deformations resulting from the 2 forces will not necessarily be comparable even if the longitudinal tensile forces are equal. This is due to the fact that, in principle, a deformation is a function of all the stresses acting on the body.

**Discussion**

Several investigators (2, 3, 10) have proposed that the cell wall is a cross-linked polymer system (i.e., that deformation is by plastic flow) based on limited evidence. The results presented here fully confirm that assumption. Cell wall deformation evidently occurs by breaking polymer cross-links rather than by viscous flow.

The mechanism of plastic flow of a cross-linked polymer is consistent with a number of experimental results reported in the past. For example, it has been shown that after incubation to allow metabolic wall relaxation, a tensile force caused substantial irreversible increase in length (9) and cell wall surface area (8). However, subsequent applications of equal tensile stresses caused very little additional irreversible deformation, a result fully confirmed here.

Table II. *A Comparison of the Applied Mechanical Force with Estimates of Maximum Normal Hydrostatic Forces That Would be Expected to Occur in the Tissues Used Here*

<table>
<thead>
<tr>
<th>Maximum applied mechanical force</th>
<th>Hydrostatic pressure when osmotic pressure is equal to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 m</td>
</tr>
<tr>
<td>Hydrostatic pressure (gm-mm^-2)</td>
<td>3.958</td>
</tr>
<tr>
<td>Cross-section area (mm^2)</td>
<td>212.0</td>
</tr>
<tr>
<td>Force (gm)*</td>
<td>1034.1</td>
</tr>
<tr>
<td>Stress, Longitudinal** (gm-mm^-2)</td>
<td>1,014,038</td>
</tr>
</tbody>
</table>

* Force = pressure × cross-sectional area of stem.
** Total longitudinal stress given by (force/cross-sectional area of walls) or (pressure × cross-sectional area of stem/cross-sectional area of walls). The cross-sectional area of the walls was estimated as 0.205 mm^2.
This phenomenon is well known in materials science as strain hardening. It is readily interpreted as the result of breaking those cross-links that were under the greatest internal stress in the absence of an imposed force. The frequency distribution of the cross-links as a function of the internal stress on the individual cross-link may be expected to show a large population of cross-links with low internal stresses and fewer cross-links under relatively high internal stresses. Thus, after the first group of cross-links are broken an applied force will be distributed among a larger number of cross-links so that ultimately there will not be sufficient total stress on any 1 cross-link to cause it to break.

Irreversible deformation of a cross-linked polymer is a plastic deformation. In an ideal cross-linked polymer, irreversible deformation must be the result of breaking bonds between elements of the system. In the case of cross-linked polymers, deformation can occur only when cross-links are broken. It should be noted that, in principle, the cross-links are simply those bonds that break under an imposed stress. They may, in fact, be distinct and more readily ruptured than the bonds within the polymer elements (e.g., vulcanized rubber). On the other hand, polymers without special links between polymer elements may act like (and be) cross-linked polymers, if the polymer chains are sufficiently long and intertwined. In this case, the cross-links that are broken are simply those bonds within the polymer chain on which the greatest stress falls. In the case of cross-linked polymers, then, irreversible deformation will occur only when energy is expended to break cross-links. Thus, the work required for deformation will be independent of the rate of deformation.

When a linear polymer (i.e., a polymer system without cross-links) is subjected to a constant stress the irreversible deformation is by viscous flow. The irreversible deformation will be a linear function of time and of the magnitude of the stress. Thus, a larger stress will cause more rapid deformation of such a polymer. Evidently, then, more work is required to give the same magnitude of deformation if deformation is rapid. Thus, the work necessary for a given deformation will be directly proportional to the rate of strain. If, instead of a constant force, a constant rate of strain is imposed on such a system the force necessary to maintain such a rate of strain will be a constant.

The (reversible) work of elastic deformation is the portion of the total work that is reversibly incorporated into the polymer elements as potential energy (e.g., crystals, or as a decrease in entropy, e.g., rubber) when the orientation of the polymer elements is deformed from configurations of minimum free energy to those having higher free energies. This portion of the total work will be recovered when the external force is removed and the elements return to their original minimum free energy configurations. It is, by definition, the elastic component of the work. This elastic deformation occurs both in materials that undergo viscous flow and also in those that undergo plastic deformation.

One of the advantages of the present experimental approach is that no assumptions are made about the identity of the effective components of the cell wall. It seems reasonable to suppose that the polymer elements studied here are actually the cellulose microfibrils known to be present. However, the conclusions arrived at here are true for the polymer elements that give the mechanical properties to the tissue even if it turns out later that some other long-chain chemical is responsible for the mechanical properties of the cell wall.

In the same way, no assumption has been made regarding the nature of the cross-links. The only conclusion that can be reached here is that there is, in fact, some form of cross-link between adjacent polymer elements. Furthermore, the energy to break these cross-links is sufficiently great so that they do not spontaneously break and reform at a significant rate at normal temperatures. If such spontaneous breaking and reformation occurred, deformation of the system would be a viscous flow process.

Acknowledgments

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Literature Cited