TISSUE TENSIONS IN *BRYOPHYLLUM CALYCINUM* SALISBURY

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[*Manuscript received March* 11, 1953]

Summary

Turgor changes following the isolation of various tissue systems from internodes of *Bryophyllum calycinum* Salisbury plants have been examined and related to the new environmental conditions. The structural changes in cambium-phloem parenchyma strips have been analysed.

In tissue preparations the effect of auxins on subturgid cells seems to consist of the lowering of the equilibrium volume (maximal turgidity) in water. This effect is concomitant with an increase in permeability which is particularly noticeable in plasmolysis.

As most of the effects observed are almost instantaneous and reversible, "growth" (as defined as an irreversible increase in cell size) is probably not involved.

I. Introduction

The presence of tensions in plants can be inferred from certain nastic movements of organs and from sudden, often irreversible, reactions to moisture and touch; they may be illustrated by the changes in shape following the separation of plant tissues. These tensions can be considered in two classes: those arising from turgor differences (e.g. pulvinar tensions) and those dependent upon imbibitional or hygroscopic effects, as in various dehiscence mechanisms.‡

F. W. Went (1934) showed that certain tensions dependent on turgor changes and on growth were sensitive to auxin; and this may be the key to reaction in the pea test. Recent studies have stressed the role of auxin in the development and resolution of tissue tensions—in the torsions developed in the growing apex (Snow 1950) and in the mechanism of the pea test itself (Schneider 1942). Even though it is a convenient object for auxin determination, the pea epicotyl is made up of a complex of tissues and consequently presents a system very difficult to analyse. In this paper some tissue preparations from mature internodes of *Bryophyllum calycinum* Salisbury are discussed.

If we consider a plant organ in physiological equilibrium with its environment, the sum of tensions at any surface is zero; however, if a force is applied, or if the organ is cut up, there will be a readjustment of tensions and a resultant change in shape. This change will continue until equilibrium is resumed. The shape at equilibrium will depend upon the structure of the isolated tissues, their former physiological condition in the plant, and the nature of their new environ-

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‡ In Bünning's monograph (1948) the subject of the "paratonic" movements is adequately treated. For hygroscopic effects the classical paper of Steinbrinck (1906) is stimulating, see also Ziegenspeck (1943).

ment. For an analogy we may compare the effect of an increase in temperature upon a bimetallic strip with the effect of water on a thin strip of subturgid tissue consisting of two types of cells (or on a detached epidermis with its cuticle). In such a simplified tissue-tension system we may consider the two cell types as antagonists.

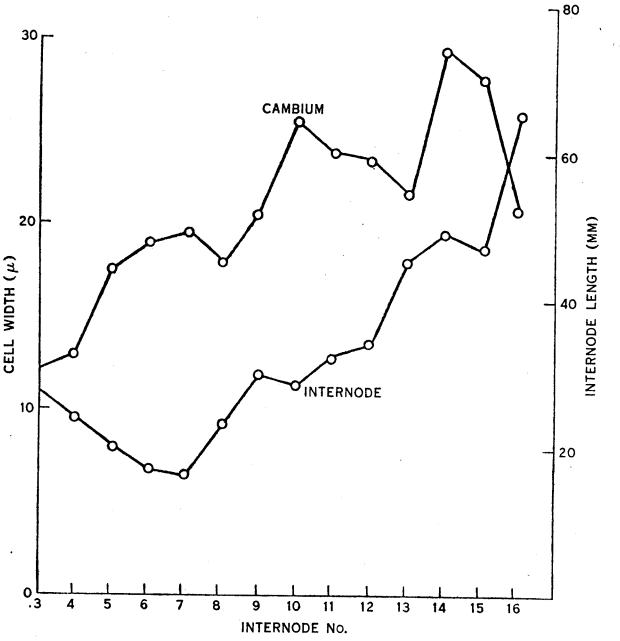

Fig. 1.—Width of the cambium cells and length of internodes. The numbers of the internodes on the abscissa refer to distance from apex.

Excised tissues of many plants show a distension of surface cells when put into culture on a moist medium. This hyperhydric increase in size has been linked with auxin content as well as the release of tensions (White 1951), nevertheless it is clear that a local release of tension may be followed by considerable modifications in the size and shape of cells, which, when irreversible, resemble growth. How much the balanced tensions contribute to the hydrostatic pressure in cells of an intact plant is not known—apart from the fact that they no doubt contribute to the effective wall pressure, and according to Broyer (1950) indirectly contribute to the "extrinsic turgor pressure."

An analysis of tissue-tension systems and their behaviour might yield some evidence as to the earlier phases in the action of auxins and also of the processes of morphogenesis and intracellular translocation of solutes in the intact plant. But there appears to be no necessity to go to such complex systems as those already in use for assaying growth substances; accordingly we have examined some simpler tissue tension systems in *Bryophyllum* and noted effects of indole-3-acetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D). In particular we have been interested in the cambium-phloem system; and an attempt at analysis of its mechanism is presented.

II. Material and Methods

Bryophyllum calycinum Salisbury,* a perennial herb, is a member of the Crassulaceae; it is endemic in Africa but of almost cosmopolitan tropical and subtropical distribution. The stem has short internodes with two opposite leaves at each node. A central leaf-trace is flanked on each side by two and sometimes three subsidiary traces. Except in the upper internodes a closed woody cylinder surrounds a central medulla.

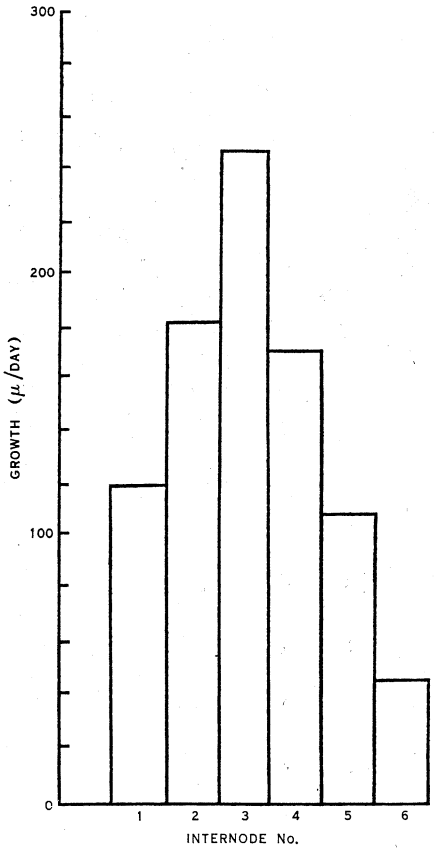

Fig. 2.—Distribution of growth (μ/day) over the stem of *Bryophyllum*. Average for four plants.

Longitudinal growth follows the general pattern of plants with well-defined internodes (van Burkom 1913). Internodal length reflects temperature conditions, and the stem with its varying internodes presents a rough record of the climate. A strong negative correlation was found between internodal length and the width of the cambium cells (Fig. 1). In short winter days the plant flowers and the new internodes grow slender and much longer than usual before terminating in an inflorescence.

* Syn. *Kalanchoe pinnata* Persoon.

A batch of five 2-year-old plants was studied for evidence of maturation and new growth. A histogram (Fig. 2) shows the mean growth in μ/day (in winter). The full number of primary xylem bodies is usually formed by internode 5; cortical anthocyanin disappears at internode 7 where subepidermal cork formation is initiated. Secondary growth starts between the eighth and tenth internodes, and by the twelfth interfascicular xylem has completed the cylinder. There is considerable variation between individual plants, however.

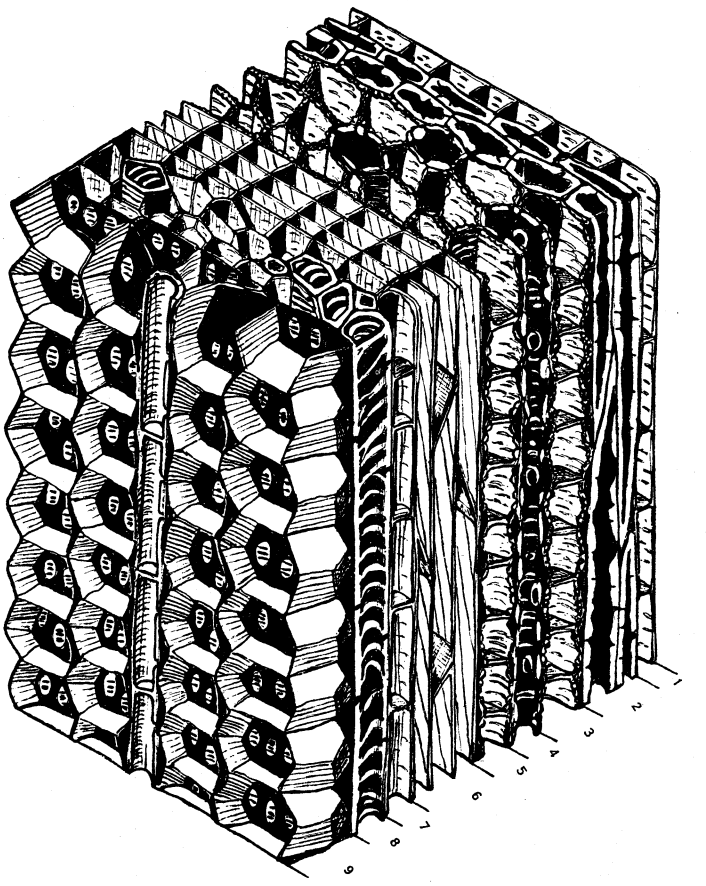

Fig. 3.—Diagrammatic representation of the tissues inside the endodermis. 1, Endodermis; 2, pericycle; 3, phloem parenchyma; 4, sieve-tubes; 5, phloem parenchyma; 6, cambium; 7, xylem parenchyma; 8, vessels; 9, central medulla. In the medulla, visible on the tangential plane, so-called tannin cells. Notice the "pit-field" on the tangential walls of the medullary parenchyma.

Figure 3 gives a diagrammatic representation of the tissues inside the cortex. The stem of *Bryophyllum* has three distinct pairs of antagonistic layers which may be isolated without much difficulty. Ability to remove the cambium and all outer tissues, which, below the first couple of internodes, yield easily from the xylem cylinder, was exploited by Loeb (1924) in his study of regenera-

tion.* First an internode of suitable length is isolated and two parallel incisions are made along it; a piece separates easily with epidermis outside and cambium inside. Then with a new razor blade a sharp tangential cut is made between the cambium and the pericycle (i.e. about 2 mm from the inner surface), the small flap is gently pulled away with flat forceps or the fingers, and a clear strip is obtained consisting of cambium and phloem parenchyma. The ends are trimmed and this preparation is ready for use (Fig. 4). Microscopic examination, vital staining, and plasmolysis have rarely shown apparent damage to the cambium, while on the phloem side there are only a few torn cells. For physiological studies these strips are very useful, they are readily obtained, rapidly penetrated by solutions, and fairly simple in structure. From one internode 8-12 strips may be prepared.

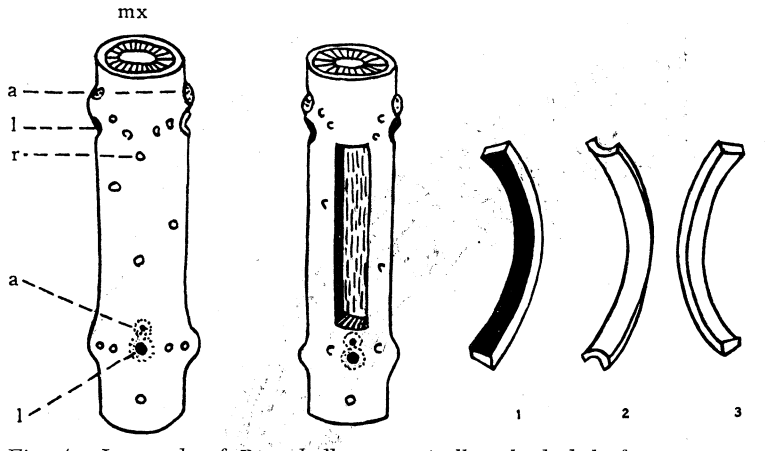

Fig. 4.—Internode of *Bryophyllum*. *a*, Axillary bud; *l*, leaf traces; *mx*, medulla-xylem; *r*, root primordia. 1, cortical strip removed from wood cylinder; 2, inner part of the strip (cambium plus phloem parenchyma); 3, outer part of strip (pericycle plus cortical parenchyma).

Other preparations are obtained by sectioning the xylem-medulla core, by taking transverse sections of the sheath removed from this core—"cortical rings"—or by pulling free the pericyclic and attached cortical tissue to form longitudinal strips as before.

Features of the cambium include the appearance of near-vertical striations (angle of 15-20°) in still-growing internodes, the presence of slightly wider cells opposite groups of primary xylem where interfascicular xylem has not yet formed, and the presence of air in the subcambial intercellularies; the air may find its way there during the preparation of the strip. It is important to use only cambium with conspicuously pointed cells; plants appear to vary in this regard.

Figure 5 shows a transverse section from the fourth internode of a plant with 24 groups of primary xylem indicating the regions where the preparations

* For other studies, also on hormonal action, in this plant, see Went (1930), Mrkos (1933), Uhrova (1934).

described are obtained. Prosenchyma present in this section includes spiral and ring tracheids and vessels in the xylem, the cambium, pericyclic sclerenchyma, tannin cells in the central medulla, and phloem sieve-tubes; while parenchyma is found in the central medulla, the collenchyma, and thin-walled cells in the xylem, phloem, and cortex. It is to be noted that in expansion and contraction the sieve-tubes behave like parenchyma. When tissues are isolated and placed in water it is possible to infer from the change in shape whether they were under compression or extension in the intact plant. Prosenchyma,

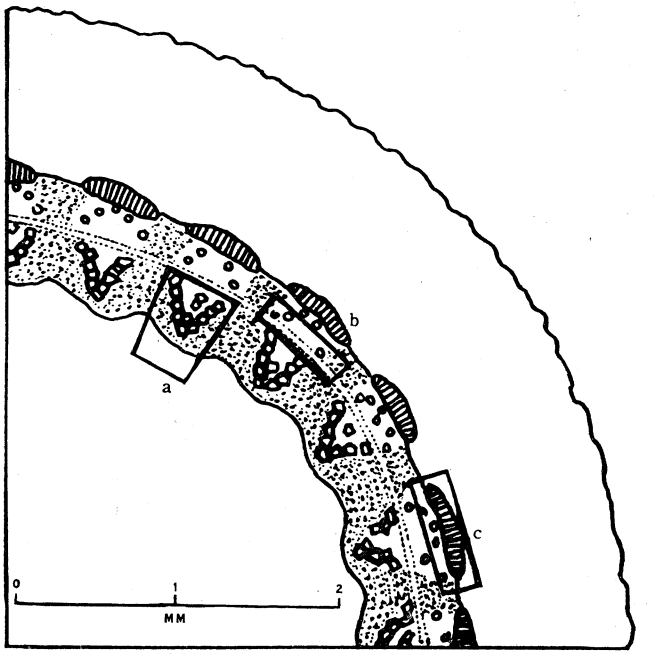

Fig. 5.—Part of transverse section through a young stem. *a*, Medulla-xylem strip; *b*, cambium-phloem strip; *c*, parenchyma-sclerenchyma strip. Fourth internode from the top.

with its longitudinal striations, is predisposed to changes in width, and is a logical antagonist for parenchyma, with its transverse striations, fitted for changes in length; however, lignified tissues like xylem, with no turgor reaction, cannot be considered this way. The stresses and strains in the tissues in *Bryophyllum* are depicted diagrammatically in Figure 6.

Apart from differences in striation and turgor, the initial shape of a cell fits it for (and governs) any subsequent change in shape. We shall discuss briefly this difference with respect to two types of cell, both of prismatic shape, but with different outline in the tangential plane. A cambium cell and phloem parenchyma cell are shown in Figure 7. The tangential surface may be assumed to be proportional to the volumes; this is approximately so for the cambium, but for slight discrepancies in phloem parenchyma (Meeuwse 1941).

(*a*) *The Cambium Cell*

In transverse section the outline is rectangular: in the tangential plane the cells look like parallelopipeds, although pointed hexagons are often seen.* Let the sides of the parallelopiped be a and b, then the total length, L of the cell is

$$L = b + a \cos \theta,$$

where θ is the apical angle, and the width W is

$$W = a \sin \theta,$$

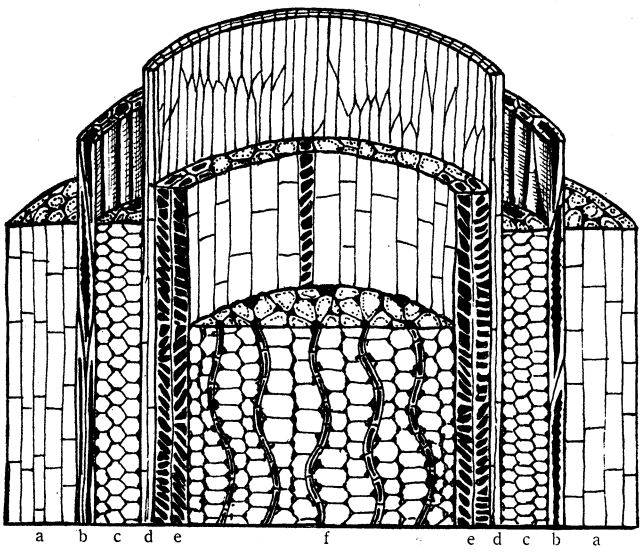

Fig. 6.—Distribution of tissue tensions in the stem (diagrammatic). *a*, Cortical parenchyma (slightly compressed); *b*, pericycle (under stress); *c*, phloem parenchyma (slightly compressed; *d*, cambium (stretched); *e*, xylem (slightly stretched); *f*, central medulla (with "tannin cells," much compressed).

from which it follows that

$$\left(\frac{L-b}{a}\right)^2 + \left(\frac{W}{a}\right)^2 = 1,$$

and it may be derived that the surface S in the tangential plane is

$$S = bW = ab \sin \theta,$$

which is maximal at

$$S = ab \text{ (rectangle)}.$$

* A similar system, the rhombic meshwork of cortical sclerenchyma in *Carica papaya* L., which is distended by secondary growth, was investigated by Arnold and Baas Becking (1949).

(*b*) *The Phloem Parenchyma Cell*

The parenchyma cell has transverse pits in the tangential and radial walls. The tangential aspect of a water-saturated cell is an equilateral hexagon, placed on its side. Again, from Figure 7, the length of the cell

$$L = 2p \sin \frac{\theta}{2}$$

and its width

$$W = p + 2p \cos \frac{\theta}{2},$$

from which it follows that

$$\left(\frac{L}{2p}\right)^2 + \left(\frac{W-p}{2p}\right)^2 = 1,$$

and the surface in the tangential plane is

$$S = \tfrac{1}{2}L(W+p) = 2p^2 \sin \frac{\theta}{2} + p \sin \theta,$$

maximal at

$$S = \frac{3}{2}p^2 \sqrt{3} \text{ (regular hexagon).}$$

The above only applies when the cell is approximately prismatic in shape.

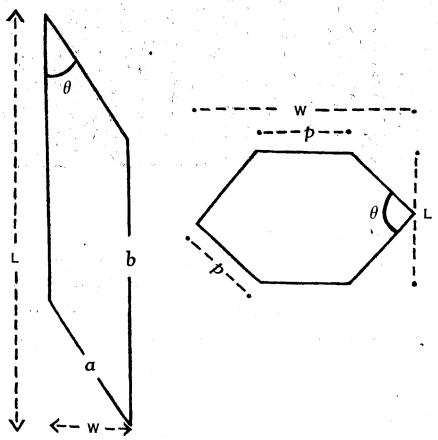

Fig. 7.—Schematical representation of cambium cell (left) and parenchyma cell (right).

Values of W and L for cambium and parenchyma cells are given in Table 1 and graphically represented in Figure 8.

It appears that in the cambium, an increase in width of the cell from 15 to 30 μ (100 per cent.) will cause a shortening from 197.6 to 193.3 μ—only 2.2 per cent. This means that, as a meshwork, the cambium, like the cortical sclerenchyma of *Carica,* may be distended or compressed a great deal without appreciable change in length. On the other hand, suppose a subturgid parenchyma cell to have a width of 30 μ and length 13.8 μ; these measurements will change

on saturation (to a regular hexagonal outline) to 23 and 19.6 μ, changes of 24 and 70 per cent. respectively.

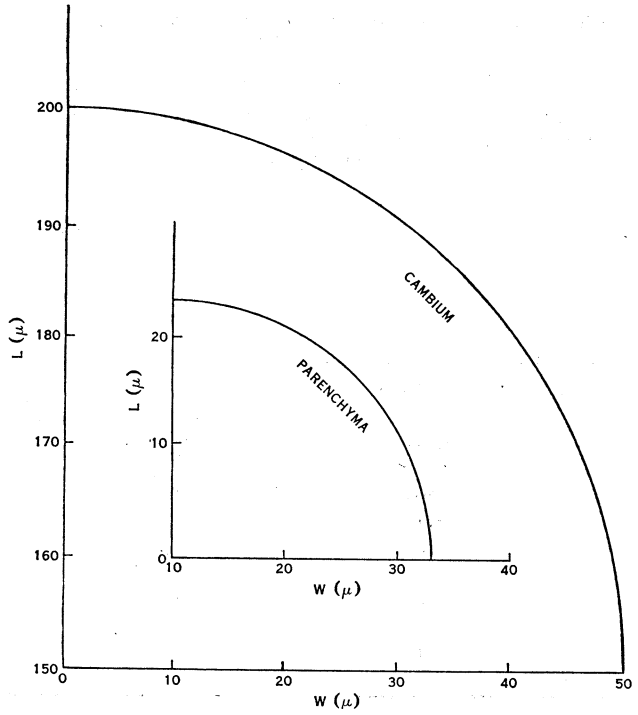

Fig. 8.—Width as a function of length in cambium cells and in parenchyma (see Table 1).

(*c*) *Cambium: Phloem Parenchyma Antagonism*

For a strip containing a layer of cambium against a layer of parenchyma changes in the overall shape of the strip will accompany changes in the turgor of the cells. The cambium will manifest most of any volume change by lateral expansion or contraction; but the behaviour of the parenchyma is not so readily predicted, the change in shape being a complex function of turgor. For if we calculate the change in tangential surface as a function of volume and increasing cell length, we obtain as the first differential of

$$S = \tfrac{1}{2}L(p+W)$$

$$\frac{S}{L} = \frac{(c+2p)(c-p)}{c}, \text{ where } c^2 = 4p^2 - L^2,$$

or

$$\frac{S}{L} = \frac{(W+p)(W+2p)}{(W-p)}.$$

The value of this differential varies from $2p$ to $-\infty$ as shown in Table 2.

This means that an originally water-saturated parenchyma cell (maximum surface $S = 3p^2$) may either shorten or lengthen, depending on the tissue tension.

Conversely a non-saturated cell may either lengthen or shorten when taking up water. It will lengthen if $L > p\sqrt{3}$ and shorten if $L < p\sqrt{3}$. As observation of this change of shape is difficult in the plant we do not know whether there is any normal preference; but as the parenchyma is apparently granted "two

TABLE 1

W AND *L* VALUES CALCULATED FOR CAMBIUM AND PARENCHYMA

(*a*) Cambium

W	*L*	Assuming $a = 50\mu$ and $b = 150\mu$	
		W (μ)	*L* (μ)
0·0 *a*	1·000 *a*+*b*	0	200
0·1	0·995	5	199·7
0·2	0·978	10	198·9
0·3	0·952	15	197·6
0·4	0·916	20	195·8
0·5	0·866	25	193·3
0·6	0·800	30	190·0
0·7	0·715	35	185·8
0·8	0·600	40	180·0
0·9	0·138	45	169·0
1·0	0·000	50	150·0

(*b*) Parenchyma

W	*L*	Assuming $p = 11{\cdot}5\mu$	
		W (μ)	*L* (μ)
3·0 *p*	0·00 *p*	34·5	00·0
2·8	0·88	32·2	10·1
2·6	1·20	30·0	13·8
2·4	1·43	27·6	16·5
2·2	1·60	25·4	18·4
2·0	1·73	23·0	19·6
1·8	1·83	20·8	21·0
1·6	1·92	18·4	22·1
1·4	1·96	16·2	22·6
1·2	1·99	13·8	22·9
1·0	2·00	11·5	23·0

degrees of freedom" and the cambium only one, it seems reasonable to assume that in the strips, at least, it is the cambium that determines changes in orientation, and the phloem parenchyma (within limits) accommodates itself to these changes.

In homogeneous two-tissue strips, if they are long compared with the individual cells, curvature accompanying turgor changes will be constant throughout. As a result, strips placed in water curve towards a circular form — curvatures other than circular occur only if the strip is of uneven thickness or an antagonist has been damaged. In addition to longitudinal curvature due to a contraction in length of the cambial cells (Table 1), there will be a lateral curvature due to their expansion in width. Measurements directly made on the cambium cells can be used to assess the lateral curvature while a method for measuring the longitudinal curvature is given below. The appearance of strip, say 30 by 2 mm, turgid in water is like the circumference of a circle; the inner surface (cambium) is convex in cross section.

TABLE 2

L AND *S*/*L* VALUES CALCULATED FOR PHLOEM PARENCHYMA AS A FUNCTION OF *p*

L	*S*/*L*
0·0 *p*	2·00 *p*
0·2	1·99
0·4	1·94
0·6	1·86
0·8	1·75
1·0	1·57
1·2	1·35
1·4	1·03
1·6	0·53
1·73 ($\sqrt{3}$)	0·00
1·8	−0·043
1·9	−0·161
1·95	−0·300
2·00	$-\infty$

Only if the strip is homogeneous throughout will the radius of curvature be constant along the strip; then to measure this radius is a means of assessing the longitudinal curvature. Suppose the strip originally to have had a length L; now if it becomes a chord D of a circular circumference, it is evident that

$$D = 2r.\sin(\pi - L/2r),$$

where r is the radius of curvature.
For a circle, the curvature

$$C = 1/r,$$

so that

$$D = \frac{2\sin(\pi - \frac{1}{2}LC)}{C}.$$

(*d*) *Other Preparations*

Other preparations were considered in much the same way as cambium-phloem; however, they all proved rather rigid except the xylem-medulla system which produces a tight, helicoidal structure not unlike a tendril in certain climbing plants. The linear expansion of medullary cylinders was also measured. Details are presented with the results.

III. Experimental Results

(*a*) *Cambium-phloem Strips*

The strips are sufficiently thin to arrive at an equilibrium in water within 15 or 20 min, although after 24 hr there may be some readjustment. There was usually no significant trend in tensions between internodes, but variability in the youngest internodes is higher, presumably because of immaturity in the cambium. From a plant with 11 internodes 10-12 strips could be prepared from internodes numbered 2-9 inclusive. The length of each strip was 30 mm. Results in this case are given as the length of the chord D (Table 3).

Table 3

DISTRIBUTION OF TENSION ALONG THE STEM

D (mm)	Internode Number, from Top								Totals
	2	3	4	5	6	7	8	9	
9-10	1								1
11-12	1								1
13-14		1		1		4	1		7
15-16	1		1			3		3	8
17-18	2	1		1		1	1	4	10
19-20	2	3	1	4	3	1	1	1	16
21-22	3	3	4	2	5	1	3	2	23
23-24		1	1	4	4	1	4	2	17
25-26	1		4						5
27-28	1	2	1						4
N	12	11	12	12	12	11	10	12	92
D	19·0	20·8	22·8	20·5	21·7	16·6	20·9	18·7	20·15
$3\sigma_D$	4·38	3·41	2·82	2·0	1·44	3·36	2·94	2·64	2·62

That the nature of the release is dependent on the physiological condition of the material is illustrated in Table 4, where the reaction of an "acclimatized" plant is compared with a control. The effect of the osmotic pressure of the solution is also illustrated. Here curvature is expressed as the reciprocal of the radius.

As the turgor of the cambium controls curvature, the pretreatment *B* must increase osmotic pressure or alter the plasticity of the cambium cell walls, so increasing suction pressure and producing greater volume at turgidity than in control tissue.

TABLE 4

VALUES OF $1/r$

Treatment of Strips	Paraffin Oil	Water	Glucose 0·1M	Glucose 0·3M	Glucose 0·5M
Plant *A* outside at about 16°C	0·08	0·39	0·32	0·12	0·00
Plant *B* 48 hr incubated at 27°C	0·13	0·47	0·38	0·12	0·07

It is clear from Table 4 that the osmotic gradient affects the value of $1/r$. The osmotic systems of cells only affect the shape of tissues when turgor pressure is actually present. As we are concerned with volumes rather than pressures it is desirable to use the following terminology to cover changes in volume between the point of incipient plasmolysis and full tissue turgor. Any isolated tissue can be considered as saturated to a degree corresponding to its turgor; the volume of cells as isolated minus the volume when plasmolysed is the *saturation factor* (S_f). The volume at full saturation (turgid) minus the volume as isolated gives the *saturation deficit* (S_d). And the difference in volume between the fully saturated and plasmolysed states may be considered as the *total saturation capacity* (S_c) of the tissue, and represents the full change in volume that can be brought about by the turgor pressure of the protoplasts. Experimentally, the tissues may be measured in paraffin oil, to give V_o, in water (V_w), and in a plasmolyte such as glycerol to give V_p. We can then determine the saturation states as follows:

$$S_f = V_o - V_p \quad , \quad S_d = V_w - V_o \quad , \quad S_c = V_w - V_p.$$

As the release of tensions amounts in fact to an adjustment to new volume relationships, these being partly determined by the osmotic gradient, we may consider a strip about to become turgid in water to be capable of a certain tension release corresponding to the factor

$$S_f/S_c\,.$$

This may be expressed as a percentage release as in Table 5. In the experiments volumes were not obtained, but as already pointed out they have been assumed to be proportional to linear dimensions. Our two methods of measurement, by the calculation of $1/r$ for longitudinal curvature, and by calculating mean cell width by direct counting of cambium cells on a calibrated field for transverse curvature, correlate well in calculating tension releases.

Table 5 gives the results of measurements by both methods in different solutions, including some done in 10 p.p.m. 2,4-D. Apart from the lack of systematic distribution of the tensions, previously seen in Table 3, the effect of 2,4-D in releasing tensions is of interest. Before considering further results

obtained with 2,4-D on cambium-phloem strips, some observations on the behaviour of other tissue preparations are presented.

(*b*) *Medulla Cylinder*

The living pith in the plant is under compression (Fig. 6) as seen by the wavy outline of the tannin cells. In very old internodes the pith cells are sometimes dead, and the tannin ducts show brown contents. Radial sections exposed to the air gradually turn brown where these ducts are present. If a

TABLE 5

Internode (from top)		Curvature of Strip		Average Width of Cambium Cells (μ)				Percentage Release of Tension	
No.	*L* (mm)	Water 1/*r*	2,4-D 1/*r*	Water	2,4-D 10 p.p.m.	Glycerol 50%	Paraffin Oil	Water (compared widths)	2,4-D 10 p.p.m. (compared widths)
3	27			11·8	10·0	9·0	9·2	7	33
4	26	0·52	0·46	13·2	9·6	7·6	9·2	28	36
5	20	0·58	0·54	18·0	13·8	13·0	12·7	6	51
6	16	0·48	0·52	19·6	14·5	11·6	13·0	18	36
7	17	0·40	0·40	19·5	19·4	14·5	17·2	51	98
8	24	0·41	0·40	18·0	15·5	11·4	12·7	20	64
9	31	0·57	0·55	20·6	18·0	15·0	16·5	28	38
10	29	0·55	0·45	25·4	23·6	19·5	21·2	29	70
11	32	0·44	0·44	24·0	21·2	16·8			61
12	35	0·24	0·24	24·0	21·0	13·8	20·6	68	70
13	45	0·68	0·59	21·5	18·0	16·0	19·0	55	36
14	50			28·2	24·0	14·2	20·6	46	70
15	47	0·62	0·57	25·4	14·0	13·4	14·4	8	8
16	66	0·58	0·62	21·0		12·7	13·8	13	
Average		0·52	0·48					29	49

TABLE 6

PERCENTAGE INCREASE IN LENGTH OF MEDULLARY CYLINDERS

(Temperature 24·5°C)

Time (min)	0	20	55	85	125	215
Water	0	13	24	30	34	40
10 p.p.m. 2,4-D	0	10	15	15	15	13

pith cylinder is removed with a narrow cork-borer (we used 2.5 mm diameter) it will elongate as a rule over 10 per cent. of its original length in situ. Care must be taken not to take any adjacent xylem with the cylinder or the preparation becomes very contorted. Swelling in water continues for several hours.

Considering its striations the medullary parenchyma is highly disposed to increase in length rather than breadth. Results obtained are given in Table 6 and are also shown in Figure 9.

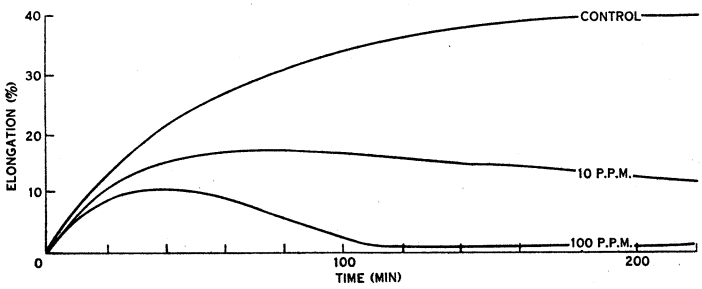

Fig. 9.—Influence of various concentrations of 2,4-D on the elongation of medullary cylinders as a function of time at 24.5°C.

Measurements were made both by direct observation, measuring, and by recording on a kymograph; however, no extensive work was done on these preparations.

(*c*) *Medulla-xylem Strips*

These show a much greater tendency to curl up on saturation than do the cambium-phloem strips; this is because of the initially high compression of the pith and the inextensible nature of the xylem tissue. Satisfactory preparation is possible only before secondary thickening. The strips suffer disadvantages of heterogeneity and slow adjustment. Results are given in Table 7.

TABLE 7

LENGTH (MM) OF CHORD *D* OF MEDULLA-XYLEM STRIP AFTER VARIOUS TIMES

Five strips/batch

Time (min.)	0	20	50	130	300
Water	25	3·4	−4·0	−8·2	−10·8
100 p.p.m. 2,4-D	25	4·0	−1·3	−1·5	−1·8

(*d*) *Cortical Preparations*

By taking transverse sections of the tissues sheathing the xylem cylinder, small slit circlets are obtained, and their reactions can be examined. These rings open out a little in water and less in 2,4-D and glucose solutions. In one experiment the mean opening between the ends of five rings in water was twice that of a replicate set in 10 p.p.m. 2,4-D, after 20 min. Although equilibrium had not occurred, there was a similar difference after 1 hr. Complexity and heterogeneity are again disadvantages.

Longitudinal strips of pericycle and adhering cortex show only slight curvatures in water and, as errors in the estimation of $1/r$ in the range 0-0.1 are

large, these preparations were not studied further. The great rigidity of the pericyclic fibrous tissue is responsible.

TABLE 8

LENGTH (MM) OF CHORD *D* FOR THREE TISSUE-TENSION SYSTEMS IN WATER AT 17°C

Time (min.)	0	5	15	30	60	90
Medulla-xylem	25	20·6	11·8	6·1	1·8	0·1
Cambium-phloem	25	10·3	8·5	8·1	8·3	8·2
Pericycle-cortex	25	23·0	22·3	22·8	22·5	22·8

Table 8 compares the curvatures of three tissue-tension systems in water, five strips per figure, temperature 17°C. These values are the basis of the curves in Figure 10.

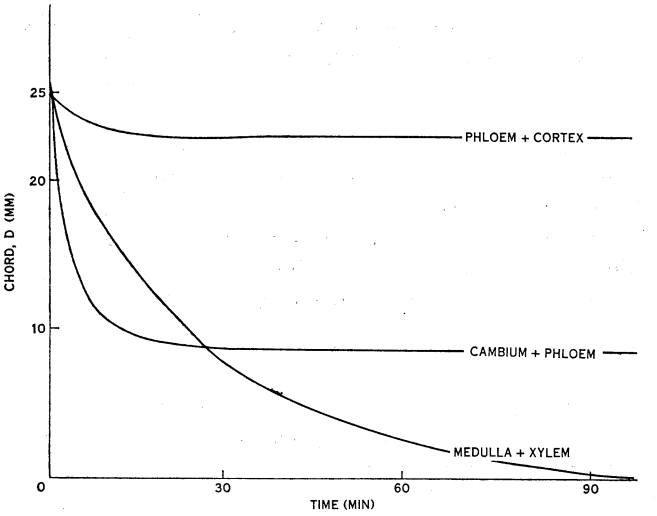

Fig. 10.—Comparison of curvature (measured as chord) as a function of time in various preparations from the same internode at 17°C.

(*e*) *Effects of 2,4-D and IAA on Cambium-phloem Strips*

The appreciable effect of 2,4-D in lowering the percentage release of tension in cambium-phloem strips (Table 5) was investigated with respect to concentration of 2,4-D and temperature. The effect on the value of $1/r$ is presented in Table 9. It is seen that while the effect of 2,4-D is reduced at lower temperatures, even here the curvature is determined by the amount of 2,4-D present (Fig. 11).

In Figures 12 and 13 the differences in the values of $1/r$ for strips in 2,4-D or IAA and water are plotted. Notable features are the rapidity with which differences become apparent and the tendency for the IAA set gradually to approach the water value; there is no such tendency to reversal in 2,4-D.

Strips plasmolysed in sucrose plus 2,4-D attain equilibrium much faster than those in sucrose alone. This effect is shown in Figure 14. The width of the cambium cell was established by counting the number of cells over a known distance on an irrigation slide. The concentration of 2,4-D was 0.5 p.p.m.*

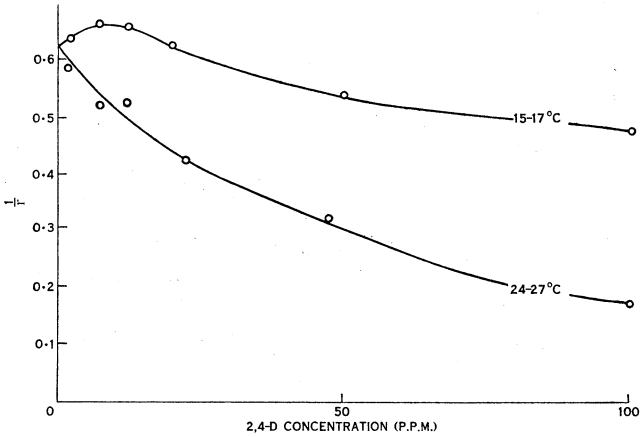

Fig. 11.—The effect of temperature on the release of tensions at various concentrations of 2,4-D.

In no case has tissue already turgid in water shown measurable alteration in volume on being transferred to solutions of 2,4-D; although work in progress indicates that this can occur. No such effect could be found for cambium in 10^{-3} to 10^{-9}M 2,4-D. Quite frequently, however, disappearance of air from intercellular spaces was observed.

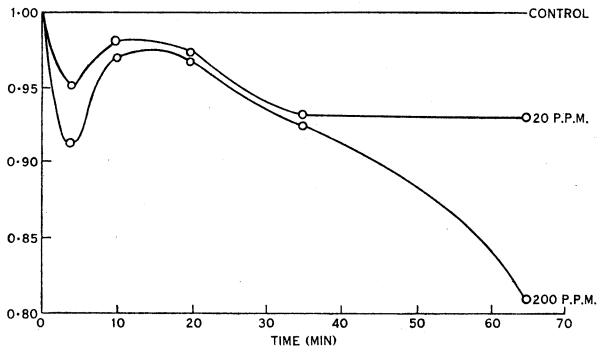

Fig. 12.—Curvature of strips as a function of time at 20.8°C for 20 and 200 p.p.m. 2,4-D expressed in fractions of the curvature of the control.

* As a bioassay, the cambium-parenchyma strip is much less sensitive than an assay by means of root growth (5×10^{-7} p.p.m. as against 10^{-12} p.p.m for the cress root) (Moewus 1948, 1951).

IV. Discussion

The fact that cell elongation in growth is dependent upon a temporary excess of turgor pressure over wall pressure was recently emphasized by Burstrøm (1948, 1951) and Haines (1951). While the systems described in this

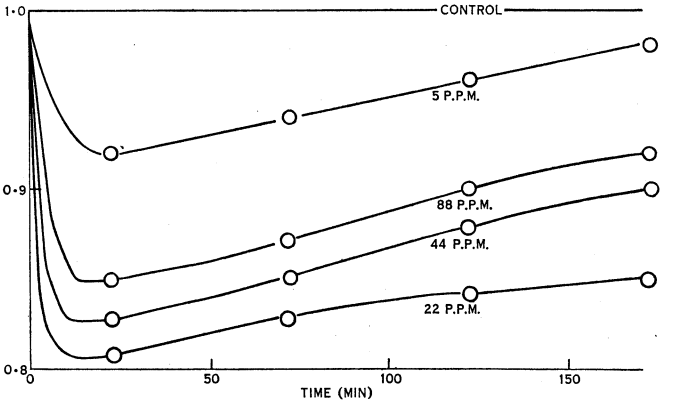

Fig. 13.—Curvature of strips as a function of time at 25°C for various concentrations of IAA expressed in fractions of the curvature of the control.

paper do not contain actually growing cells, they resemble growing tissues in their natural tendency to increase in volume and alter in shape, owing to the release or modification of the wall pressure through tissue tensions. The

Table 9

CURVATURES AS $1/r$, BROUGHT TO STANDARD FOR WATER (COMPOSITE OF MANY EXPERIMENTS)

2,4-D (p.p.m.)	Temperature (°C)						
	27	26	25·5	24	17	16	15
0·0	0·62	0·62	0·62	0·62	0·62	0·62	0·62
1·0	0·56		0·61	0·57			
1·5			0·61				
3·0		0·53			0·53		
4·0				0·56			
5·0		0·38				0·62	0·64
6·0		0·53		0·51			
10·0			0·56		0·60	0·66	0·66
12·5		0·54					
15·0				0·50			
20·0					0·52	0·60	0·67
25·0		0·49	0·34				
50·0			0·49				
100·0	0·12	0·15	0·28		0·49		

changes in volume are determined by the turgor pressure and the previous shape of the cells. Schneider (1942) recognized in the pea test the importance of cell shape to curvature; yet he ascribed the curvature to obscure "nastic" and "traumatic" responses which he considered affected growth. This seems to cloud the issue, however, for the nastic and traumatic responses seem to be inseparable theoretically from the change in tissue tensions accompanying the preparation of the epicotyl and its immersion in water, and the "growth" may be a local increase in turgidity accompanying water saturation.

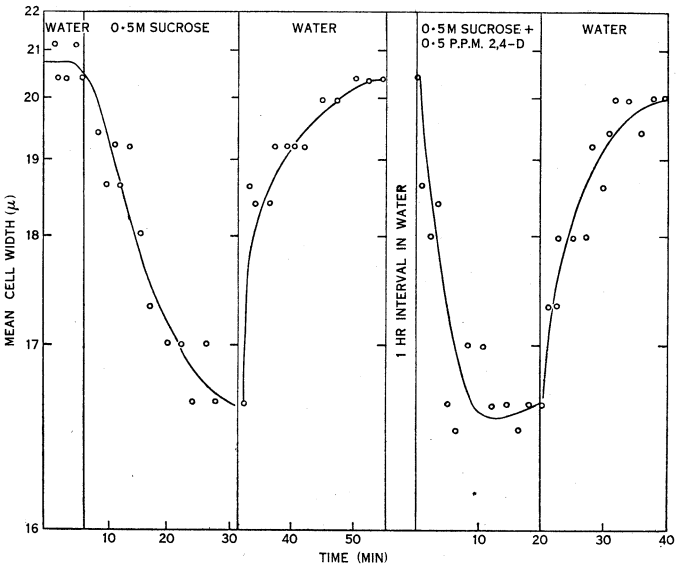

Fig. 14.—Plasmolysis and deplasmolysis as a function of time of cambium cells with and without 0.5 p.p.m. 2,4-D at 19-21°C. Logarithms of cell width are plotted to enhance the difference between plasmolysis and deplasmolysis.

In view of the effect of auxins on the release of tensions in *Bryophyllum* strips, we believe that their physiological action is directly on turgor adjustment, and consequently that their effects in plants will be felt wherever active turgor adjustment is going on. This also occurs throughout the plant to a limited extent, but is most obvious in growing regions and in photosynthetic cells and wounded tissue. Even in tissues apparently in equilibrium with their environment, such as thin potato discs washed in water for 24 hr, there is a gradual increase in turgor in auxin solution (Reinders 1938, 1942; Hackett 1951), and in unpublished experiments with beetroot discs there was sometimes a decrease in turgor. The "paralysing" effect of 2,4-D on pulvinar movements in *Oxalis* (Baas Becking and Everson 1952) and the familiar hypo- and epinastic movement in leaves can be considered in the light of turgor adjustments; while an "internal wilt" like that described by Gaümann (1949) and co-workers for parasitized plants, a flaccidity without loss in weight, has also been noticed by us in 2,4-D-treated leaves of *Bryophyllum.*

In Table 5 the greater percentage release of tensions in 10 p.p.m. 2,4-D is observed; in 2,4-D the tissues change their volume less in coming to equilibrium. This effect is comparable with that of placing strips in dilute sugar solutions (Table 4) where the smaller osmotic gradient produces less ultimate distension than in water for cells to attain equilibrium. And in experiments where tissue was plasmolysed in the presence of 2,4-D, the rapid plasmolysis suggests a higher effective osmotic pressure gradient than in the control. However, the effect could be due to any of the three following processes:

(*a*) Increased permeability of the protoplast,

(*b*) Increased wall pressure,

(*c*) Increased osmotic value of the vacuolar sap,

of which (*a*) seems the most feasible considering the rate of the adjustments concerned. von Guttenberg and Beythien (1951) and Pohl (1948) have demonstrated effects of auxins on the permeability of *Rhoeo* epidermis and the *Avena* coleoptile respectively; and Veldstra (1947) has suggested a possible mode of action in this respect by his study of the surface characteristics of auxin molecules.

The change in width of cambium cells in cambium-phloem strips is thought to be directly caused by turgor changes in the cambium rather than in the phloem, as discussed earlier; and while further experiment would be helpful, it is now suggested that an abnormal release either of water or of cell sap occurs when auxin acts on a protoplast already changing in volume. In the experiments with strips, subturgid cells were either becoming turgid or plasmolysed. If water is extruded from the protoplast, as is claimed to occur under pressures greater than 10 atmospheres (Bennett-Clark and Bexon 1940),* this could be brought about by a change in the surface area of the tonoplast with a concomitant increase in the osmotic pressure of its contents. If water were extruded this would require a great deal of energy. In this case the re-establishment of the equilibrium, however, would be exergonic. The opposite is the case when cell sap is extruded, where the re-establishment of the equilibrium, by intake of salt, would require energy. In the latter case, however, intracellular adjustment might play a role (e.g. starch-sugar equilibrium). The entry of vacuolar substance into the cytoplasm, moreover, might extend a wide influence on metabolic paths, due, in part, to an increase in available substrate with little change in osmotic pressure.

For these reasons we assume that there is more probability that cell sap is extruded rather than water. This cell sap may leave the cytoplasm and appear in the intercellularies. In this connection the experiments of Nicolai (1929), who obtained tumorous growths on roots mechanically stimulated and noticed a coincidental extrusion of air from the intercellular spaces, are recalled.†

The changes in shape of cells in the tissue tension systems studied may be contrasted with the effects of hygroscopic tensions observed by Diehl *et al.*

* This seems unlikely on thermodynamic grounds, however.

† On these and similar, as yet little-exploited phenomena, see Prillieux (1868), Hofmeister (1860), Janse (1926).

(1939). In systems dependent on tensions of osmotic origin the properties of the tonoplast are most important.

No effect of 2,4-D on the plasticity of the cell wall could be found with sagging, plasmolysed cambium-phloem strips or with cylinders cut from the medulla (after Heyn 1934). The short-term experiments described in this paper may be considered independent of possible subsequent alteration of the structure of the cell wall, which are inherent in growth processes.

V. Acknowledgment

This research was made possible by a grant from Timbrol Pty. Ltd., Sydney.

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