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STUDIES ON THE CONTROL OF CELL-WALL EXTENSION

Three Year Progress Report

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PROGRESS REPORT

Contract: DE-AM06-76RLO2225, TA DE-AT06-76ER73019

Principal Investigator: Robert E. Cleland, Professor of Botany
Botany Department, University of Washington

Period: September 1, 1978 - June 30, 1981

I. INTRODUCTION AND BACKGROUND

The research in this laboratory is centered around the question as to how plant cell enlargement is controlled and regulated at the cellular level. Cell enlargement is influenced by many factors, but most of them act indirectly. For example, growth is altered by such environmental factors as light and temperature, and by internal factors such as the genetic complement of the cells, yet none of these directly control cell enlargement. The metabolism of cells, including the production of ATP and the synthesis of RNA, can have a major impact on cell enlargement, but again the effects are indirect. Theoretical considerations, and the research of Lockhart, Green and myself have shown that the growth rate (dV/dt) of cells can be defined in terms of four basic cellular parameters:

$$dV/dt = \frac{L'p \cdot WEx (\Delta\Psi_s - Y)}{L'p + WEx} \quad (1)$$

where $L'p$ is the hydraulic conductivity, WEx is the wall extensibility, $\Delta\Psi_s$ is the osmotic potential gradient between the cell and the water source, and Y is the wall yield stress. When a cell is induced to expand, the expansion must occur by a change in one or more of these four parameters. Likewise, when growth ceases it must be because of changes in these parameters. The basic goal of my research is to assess each of these parameters under conditions where growth is being enhanced or inhibited, with the purpose of explaining at the cellular level just how growth is being controlled.

In the past most of our research has been concentrated on one single situation; the induction of rapid cell elongation in the oat coleoptile by the hormone auxin. Addition of auxin causes the growth rate of coleoptile sections to increase by at least 5-fold after a lag of only about 10 minutes, making it one of the fastest hormonal responses known in plants. For a number of years interest centered on the question as to whether auxin causes

an increase in WEx. Our Instron measurements have been generally accepted as indicating that auxin does alter WEx, although some doubt has existed in our minds as to the actual relation between Instron extensibility and WEx (for a resolution of this problem, see IIC-1).

It became apparent a number of years ago that if the ultimate effect of auxin, which is believed to act within the cell, is to cause a loosening of the extracellular cell wall (i.e., an increase in WEx), it must do so by exporting some wall loosening factor (WLF) from the cell to the wall, but the identity of the WLF was unknown. Then in 1970-71, Hager and coworkers in Germany and Rayle and myself proposed that the WLF was simply hydrogen ions. The "acid-growth theory" which Hager and ourselves formulated states that auxin causes the cells to excrete protons, and that the resulting lowered pH of the walls activates some polysaccharide degrading enzymes in the walls, with the result that the wall becomes loosened and cell expansion takes place. It should be noted that this mechanism is similar to the one involved in protein digestion in the stomach, where the hormone gastrin induces the excretion of protons (HCl) into the stomach, and the lowered pH activates the enzyme pepsin.

In the years following the formulation of the acid-growth theory we were principally involved in obtaining evidence as to whether the theory was correct or not. We have now accumulated an impressive amount of data which seems to indicate that protons are the WLF in auxin-induced growth of stem and coleoptile tissues. These data are summarized in the accompanying review article from the last International Plant Growth Substance meeting (manuscript #9). The acid-growth theory is by no means universally accepted, however. Significant objections to it have been raised on several counts. One part of our research has been to examine some of the more important problems, to see if they can be resolved. For example, a question arose concerning the permeability of the cuticle to protons. We examined this, and showed for the first time that the cuticle is a significant barrier to proton movement. This research is described in IIA-1.

In 1973 Marré and coworkers showed that acid-excretion can be induced, not only by auxin, but also by the fungal phytotoxin fusicoccin (FC). This FC effect was of particular interest because the magnitude of the FC-induced growth and proton excretion was considerably greater than that induced by optimal auxin, and because many auxin-insensitive tissues would grow and excrete protons in response to FC. This led to the hope that studies on FC would provide a clue as to how auxin works. We found, however, that there were some significant differences in their action. For example, while

auxin-induced proton excretion was blocked completely by inhibitors of protein synthesis, FC-induced proton excretion was only slightly affected. There was a possibility that this was not a real difference because of the much greater H^+ -excretion induced by optimal FC. To check this, Dr. B. Rubinstein and I have compared optimal auxin with a concentration of FC which causes comparable rates of proton excretion, and have shown that the important differences still persist (IIB-2).

In 1977 Dohrmann et al found that FC-binding sites could be demonstrated to exist on plant plasma membranes (PM). Since it had been proposed that the action of FC might be to activate a PM-associated ATPase, Richard Stout and I attempted to solubilize the FC-binding site and the ATPases from oat root plasma membrane-enriched preparations. This we successfully accomplished, and showed that the two exist as different proteins (IIB-1). Although FC may still modify PM-ATPase activity, it must do so through association with some other protein rather than by any direct effect on the ATPase. This line of research is being continued by Dr. Stout under a USDA grant.

The export of a WLF can lead to wall loosening and growth only if the wall has the capacity to respond to the WLF and undergo wall loosening. We recognized that this capacity (CWL) might vary, and therefore might be a factor in the control of cell elongation. We devised a test for the CWL which consists of placing frozen-thawed sections under constant applied force, giving them a pH 3 solution, and measuring the rate of extension one hour later. We showed that the CWL was increased by auxin over a 6-8 hour period, and that this might be a direct response to auxin. The increase in CWL might be due to increased activity of the wall loosening enzymes, either by increased concentration of enzyme or by greater activity of existing enzymes, or it could be due to increased availability of sites for action by the enzyme. Unfortunately, our original method for measuring CWL did not allow us to distinguish between these two possibilities. The answer may come, instead, from our studies on the shape of the acid-extension curves.

Dr. Mark Tepfer attempted to fit the acid-induced extension curves for coleoptile walls to various equations, and determined that the curves best fit the first-order kinetic equation:

$$l = b - ae^{-kt} \quad (2)$$

where l is the length, t the time after addition of acid, and a, b and k are constants. By analogy with enzyme action, we would predict that the constant

a would be a measure of the number of sites available for attack by the wall loosening enzymes, while k would reflect the activity of the wall loosening enzymes. Since current theories state that protons act by activating wall loosening enzymes, we should predict that a decrease in pH would lead to an increase in k with no change in a. The rather surprising preliminary results that we have obtained from these studies are described in IIC-2.

In the course of these studies on auxin- and FC-induced proton excretion it became apparent that while enhanced proton excretion might explain the initiation of rapid cell elongation, it was not sufficient to explain how rapid elongation was controlled and maintained over long time periods. We therefore directed our attention to the other three cellular parameters in order to learn more about their control, and the ways in which each might contribute to the regulation of the rate of auxin-induced cell elongation.

Three years ago we initiated a study of the role of $\Delta\Psi_s$ in the control of growth. The driving force for wall extension is the turgor pressure (TP), which is proportional to the osmotic concentration (OC) of the cell as long as $L'p$ is not limiting (which we believe to be the case for Avena coleoptile sections in solution). As a cell takes up water during growth, it dilutes out the OC with the result that both the TP and the growth rate fall unless osmoregulation takes place; i.e., unless there is the uptake or generation of new osmotic solutes so as to maintain the OC. It had already been demonstrated by Ordin et al in 1956 that osmoregulation occurs during auxin-induced Avena coleoptile growth, but it was not known what effect auxin has on this process, or how it is controlled.

Tom Stevenson and I have examined the osmoregulation of both intact coleoptile sections, in which solutes entry is restricted to the cut ends, and peeled sections (i.e., sections whose epidermis has been removed), where solute entry is far less restricted. We have shown that in intact sections, osmoregulation appears to be controlled by the rate at which solutes enter the tissue; auxin enhances osmoregulation because auxin-induced water uptake results in greater entry of solutes through the cut ends. With peeled sections, however, the cells simply take up any available solute until some flux equilibrium for that solute is reached. Auxin depresses the internal concentration at which this equilibrium is established. Details of this work are given in IID-1-3.

The second turgor-related parameter which has been examined is the wall yield stress, Y , which is defined as the TP which must be exceeded in order for rapid cell elongation to take place. In theory Y can be determined by reducing turgor with an external osmoticum such as mannitol, measuring the resulting growth rate, plotting growth rate vs OPe and determining the point where the curve levels off. The problem is that upon addition of osmoticum, the growth rate does not fall to a constant new value, but undergoes change over a considerable time period before a steady-state rate is reached. This is because the walls undergo a long period to time-dependent viscoelastic shrinkage. We have developed ways to correct for this shrinkage, and have used our procedure to determine that Y is unaffected by auxin. Details are given in IIE. The third turgor-related parameter, the hydraulic conductivity $L'p$, has yet to be examined in this system.

If *Avena* coleoptiles are treated with FC, rapid elongation occurs for 2-6 hours, after which the growth rate falls to below the control level. We have examined the reasons why this decline in growth rate occurs. It is not due to any decrease in export of protons, or to a lack of OC within the cells, but seems to be due to a dramatic loss in the capacity of the walls to undergo wall loosening. Details are given in IIB-3.

We can conclude from our studies that auxin initiates rapid cell elongation of *Avena* coleoptiles by causing the cells to excrete protons, and this leads to an increase in WEx and thus the growth rate. Auxin, over long periods of time, increases the capacity of the walls to respond to protons, and helps to maintain the osmotic potential gradient by enhancing solute movement into the tissues, but does not affect the yield stress. The effect of auxin on $L'p$ must still be determined in order to complete the picture as to the relation between auxin-induced growth initiation and the cellular growth parameters. In FC-treated coleoptile sections, on the other hand, rapid cell elongation is first induced by an acid-mediated increase in WEx, but then falls again when the CWL becomes limiting for growth.

In order to complement and extend our studies on the *Avena* coleoptile, we have begun a series of studies on the control of cell enlargement in other systems. At this point we are focusing our attention on three different systems. The first is the initiation of rapid cell enlargement in bean leaves by bright light. Elizabeth Van Volkenburgh and I developed a system in which

we could study the light-induced leaf expansion of bean leaves without the complication of cell divisions, by growing seedlings in dim red light for 10 days and then transferring them to bright white light. The initial studies on this system were supported by these contracts, although subsequent work was supported by an NSF grant.

The second system is the endogenous and FC-induced growth of corn roots. Apical root segments grow rapidly without addition of any hormones; added auxin primarily inhibits this endogenous growth. In 1950 Burström found that the antiauxin p-chlorophenoxyisobutyric acid (PCIB) stimulated wheat root growth over a several-day period, and suggested that endogenous auxin acted primarily to slow down root growth. Moloney, Elliott and I have asked two questions concerning corn root growth. First, will PCIB promote root growth over short periods of time? Secondly, is proton excretion required as part of the endogenous growth, the PCIB-induced growth or the FC-induced growth of these segments? As indicated in IIF-2, the answer to both questions is yes. We conclude that the endogenous growth does require proton excretion in order for growth to occur, that endogenous auxin interferes with some part of this mechanism, and that FC promotes root growth by enhancing proton excretion.

The third system we have studied is the growth of soybean hypocotyl sections. Boyer and coworkers have shown that in intact soybean hypocotyls auxin increases both WEx and L'p, and that the tissues maintain their turgor by osmoregulation. We are conducting parallel studies with sections, where the path of solute and water entry is different from that of intact tissues. Here, auxin causes a large increase in WEx, apparently by inducing acid excretion. Studies are currently underway on the role of osmoregulation and Y in the control of auxin- and FC-induced growth of these sections. Details of these studies are presented in IIF-3.

II. DETAILS OF SPECIFIC RESEARCH PROJECTS

During the past three years a number of aspects of the general problem have been investigated. In some cases the research has resulted in papers which are either already published, in press, or in preparation. In those projects I have simply appended the manuscript or reprint. In those projects in which no manuscript has yet been produced, some details and data will be given in this section.

A. Hydrogen Ions and the Growth of Coleoptiles

1. The role of the cuticle as a barrier to proton movement.

Ms. Ann Dreyer, Dr. Virginia Seymour and I have conducted a study of the proton conductance of the cuticular layer of stems and leaves. Details are given in accompanying manuscript #1.

2. Is the epidermis the site of auxin-action in the Avena coleoptile?

In dicotyledonous stems only the outer several layers of cells respond in a significant fashion to auxin, but in coleoptiles it has been assumed that all cells are auxin-receptive. Pope has recently questioned this idea, and stated that if the epidermis is completely removed from a coleoptile section, it cannot undergo auxin-induced growth. Since the interpretation of much of our data concerning auxin-induced proton excretion assumes that all coleoptile cells are able to pump protons in response to auxin, we decided that we should determine the relationship between the amount of epidermis which is removed in peeling and the response of the section to auxin. One reason this information has not been available is the difficulty in determining how much of the epidermis has actually been removed during peeling. We have solved this problem in the following way. If Avena coleoptile sections are incubated in neutral red for 30 seconds and then quickly rinsed, peeled areas show up as bright pink, while unpeeled areas are yellow. It is thus easy to determine the extent of removal of the epidermis.

Three types of evidence indicate that the epidermis is not needed for auxin-induced growth of Avena coleoptiles. First, when sections are carefully peeled on just one side and incubated in auxin plus sucrose for 24 hours, they grow almost 100%, but do not curve either towards or away from the peeled side. When we correlate the amount of growth in individual sections with the percent epidermis removed, there is no indication that removal of the epidermis reduces the auxin-induced growth (Fig. 1). Finally, peeled sections grow as much as unpeeled sections if given optimal conditions (0.5% sucrose rather than 2%, for example). However, in order for peeled sections to show a maximum growth response to auxin, the auxin must be added immediately after removal of the epidermis, as shown in Table I. It is safe to conclude from our data that epidermal cells are not the only auxin-sensitive cells in the Avena coleoptile.

B. Action of Fusicoccin

1. Isolation and solubilization of FC-binding proteins.

There is considerable indirect evidence to suggest that FC promotes proton excretion by activating a PM-associated ATPase. One might therefore expect that the ATPases might be the FC receptors. Richard Stout and I have attempted to determine this by solubilizing and characterizing the PM-associated K^+ -stimulated ATPase and the FC-binding proteins from oat roots. We show in manuscript #2 that FC binds to a protein which is different from the ATPase.

2. Comparison of FC- and auxin-induced proton excretion at equal proton efflux rates.

It has been suggested by Marré that FC and auxin activate the same PM-associated ATPases, leading to proton efflux in both cases. However, there seemed to be two significant differences between FC- and auxin-induced proton efflux; FC has little or no lag while auxin promotes proton efflux only after a 10 minute lag, and FC action does not require protein synthesis while auxin-action is rapidly inhibited by protein synthesis inhibitors such as cycloheximide. These differences might be more apparent than real, caused by the much greater rate of proton export in the presence of optimal FC. To settle this, we have now compared auxin- and FC-induced proton efflux under conditions in which the rate of proton efflux is the same, and show in manuscript #3 that the important differences still persist.

3. Changes in cellular growth parameters during initiation and cessation of FC-induced growth.

Treatment of Avena coleoptile sections with FC leads to a very rapid cell elongation for 2-6 hours, after which the growth falls to below the control level. We have asked the question as to what change in the cellular growth parameters leads to the initiation of cell elongation, and what change leads to its subsequent decline.

The Instron technique has been used to demonstrate that an increase in WEx occurred simultaneously with the induction of cell elongation (Fig. 2). When the growth rate declines, WEx as measured by the Instron also falls (data not shown). FC does not enhance the growth rate by increasing the OC of the cells; the OC of control tissues is always as high or higher than the OC of FC-treated tissues (data not shown). If the external

solution contains sucrose, uptake of osmotic solutes takes place. While auxin increases this uptake by about 50%, FC has no effect on this process (Table II).

The decline in growth rate after 2-6 hours of FC treatment is not due to any lack of OC or to a decrease in rate of proton excretion (data not shown). What does decrease dramatically is the capacity of the walls to undergo acid-induced wall loosening (Fig. 3). The CWL remains nearly constant for the first few hours, but then falls sharply at just the time that the growth declines. We conclude that the CWL is the parameter which controls the growth rate in this case, and is the cause of the decrease in WEx which accompanies the decline in the growth rate.

C. Studies on Wall Loosening

1. The relationship between Instron extensibility and WEx

In the Instron technique, sections are incubated in the desired solution, killed in boiling methanol, and extended twice to 30 g load in an Instron extensometer. From the slope of the two load-extension curves one can calculate elastic (DE) and plastic (DP) compliance values. We originally suggested that DP was a measure of the WEx parameter, but in recent years we have come to wonder about this interpretation. We have now investigated this matter.

If DP is a measure of WEx, it should increase and decrease in parallel or before the changes in growth rate. To test this we incubated Avena coleoptile sections in auxin for varying lengths of time and determined the time course for changes in DP (Fig. 4). Although the growth rate reaches a maximum about 20 minutes after addition of auxin, DP reaches its maximum only after 60-90 minutes. Upon addition of KCN to auxin-treated sections, the growth rate falls to near zero within 10 minutes, but the DP values reach a minimum only after about 60 minutes (Fig. 5). From these and a series of other experiments we have concluded that DP values indicate the average WEx that has existed in the cells over the previous 60-90 minutes, rather than any potential of the walls for future extension.

What is the relationship between DP and wall loosening which leads to enhanced WEx? We have found that we can measure DP on the same tissue several successive times if we simply alter the force vectors slightly each time, but if the force vectors remain unchanged, only a single plastic

extension can take place. We have concluded that when the wall is loosened by bond cleavage, plastic extension can occur until entanglements within the polymers prevent further extension. If the force vectors are changed, the entanglements are altered so that additional extension can occur. In living tissues this capacity for extension will persist until new wall synthesis covers up this section of wall.

A manuscript detailing this research is currently being prepared and will be submitted to Planta.

2. Analysis of acid-induced extension curves.

When frozen-thawed Avena coleoptiles are placed under constant applied tension at pH 7, they undergo viscoelastic extension at a rate which is proportional to log time. If the pH is now lowered, they undergo additional extension the amount of which depends upon the applied force, the pH and the capacity of the walls to undergo acid-induced wall loosening (CWL). We have arbitrarily used the rate of extension at 20 g force and 1 hour after addition of a pH 3 solution as a measure of the CWL, and have shown that changes in CWL can contribute to the control of the extension rate in coleoptiles (see IIB-3). Details of these CWL studies have been given in previous progress reports, and are in a manuscript which will shortly be submitted to Planta.

During the CWL studies, it became apparent that the shape of the acid-induced extension curve was different from the extension curve at pH 7. Dr. Mark Tepfer and I fitted the acid-extension data to a series of equations and determined that there was a close fit to the first-order kinetic equation:

$$l = b - ae^{-kt}$$

where l is length, a , b and k are constants. By analogy with enzymatic reactions, we would predict that a should be a measure of the number of sites in the wall available for attack by the wall loosening enzymes while k should be a measure of the activity of the wall loosening enzymes. If so, changes in temperature should alter k without affecting a ; such results have been obtained (data not shown). As predicted, increases in tension cause increases in a with no change in k except at the very low tensions (Fig. 6). But to our surprise, as the pH is lowered, it is a which increases, not k (Table III). And when the CWL is increased by preincubation of live sections with auxin, it is again a which is increased rather than k (data not shown). There are two interpretations for these results.

Either a is not a measure of the available sites but is a measure of enzyme activity, or the effect of acid and of auxin is to increase the number of sites available for attack, rather than altering the activity of the wall loosening enzymes. To test this, we have been treating walls for short periods (e.g. 30 minutes) with trypsin, then measuring the change produced by this treatment on a and k. Our results are so preliminary that we cannot place any real confidence in them as yet, but they suggest that proteolytic attack decreases a rather than k (data not shown). It is clear that additional experiments are needed to settle this matter.

D. Studies on Osmoregulation

1. Osmoregulation in intact Avena coleoptiles.

Tom Stevenson and I have examined the ability of Avena coleoptile sections in which the cuticle is intact, to take up osmotic solutes and osmoregulate, and have determined the effect of auxin on this process. The details are given in manuscript #4. We have concluded that the limiting factor in osmoregulation is the ability of solutes to reach the cells, and that auxin enhances osmoregulation indirectly, by increasing the flow of water into the tissues.

2. Osmoregulation in peeled Avena coleoptiles.

When the epidermis with its cuticle is removed, the osmoregulation of Avena coleoptiles is markedly altered. Now uptake of solutes is increased, and cells increase their OC until some internal concentration is reached, at which point the OC plateaus. Auxin decreases the plateau OC level, and this appears to be, in part, a direct effect of auxin. If more than one absorbable solute is present, each is taken up until its own flux equilibrium is reached. Thus in coleoptiles there is no overall sensing of the total OC of the cell, but each solute acts independently to contribute to osmoregulation. Details of this study are presented in manuscript #5 which has been submitted to Plant Physiology.

3. Further studies on osmoregulation of Avena coleoptile sections.

Information has been obtained on a number of other points concerning osmoregulation. These include:

a) The sugars sucrose, glucose and fructose are equally effective as sources of solutes for osmoregulation. Arabinose is only about 20% as

effective, and galactose kills the cells. NaCl and KCl are equally effective, while CaCl_2 is about 1/3 as effective. For potassium, the chloride and nitrate are equally effective, but sulfate, bicarbonate and citrate prevent any significant osmoregulation from occurring (data not shown).

b) The external pH does not affect the equilibrium OC reached with NaCl within the range of 4.0 to 8.0. The pH does influence the osmoregulation from sucrose, however (Table IV). As the pH decreases from 7 there is a decrease in the equilibrium OC, and the reduction in this steady-state OC by auxin is diminished.

c) Studies on the rate of uptake of ^{14}C -sucrose indicate that the uptake rate is unaffected by auxin when either freshly harvested sections are used, or when sections are used which have reached their steady-state OC. The rate of uptake appears to be unaffected by the age of the section. Thus changes in the uptake rate of sucrose do not appear to be responsible for the leveling off of the OC, or for the reduction in steady-state OC which occurs in the presence of auxin.

d) Studies on cyanide-sensitive and CN-insensitive respiration suggest that the alternative respiratory pathway develops in Avena coleoptile sections after they are cut. When initially harvested, most of the respiration is inhibited by KCN, but by 24 hours, only 25% is sensitive to cyanide. The remainder of the respiration is inhibited by SHAM or propyl gallate (Table V). The exact timing of the development of the SHAM-sensitive respiration must still be determined.

E. Yield Stress and its Relation to Auxin-induced Growth

The yield stress, Y , is defined as the turgor that must be exceeded in order for turgor-driven wall extension to occur. In theory it can be determined by reducing turgor with varying amounts of mannitol (O_{Pe}), determining the growth rate at each O_{Pe} and then finding the O_{Pe} which causes the growth rate vs O_{Pe} curve to flatten out. The problem is that after addition of mannitol, the growth rates undergo prolonged periods of change (Fig. 7). At what time does one measure the growth rate? It seems that Avena coleoptiles undergo prolonged periods of viscoelastic contraction whenever the turgor is reduced (Fig. 8), with the result that

the observed growth rate is the sum of cell elongation plus viscoelastic contraction. We have devised a method for correcting for this viscoelastic contraction; groups of sections are incubated at both 25C (where both viscoelastic contraction and cell elongation will be occurring) and at 0C (where only viscoelastic contraction will occur), and the change in length between these two is plotted as a function of time (Fig. 8). Now a nearly constant rate of extension can be obtained starting at time zero.

Using this technique, we have determined the OPe which is at Y for sections incubated in optimal, suboptimal and no auxin (Fig. 9). In each case Y appears to occur at the same OPe, suggesting that auxin has no influence on Y. It must be pointed out, however, that until more direct measurements of TP are made, this conclusion must remain tentative. This research is being written up for publication.

F. Control of Cell Enlargement in Other Systems by Cellular Growth Parameters

1. Light-induced bean leaf expansion.

Dicotyledonous leaves expand to only a limited extent in the dark, but when illuminated by bright light they undergo massive enlargement. This expansion involves both cell division and cell enlargement. We wished to study the cell enlargement without the complication of division, and so Elizabeth Van Volkenburgh and I devised a system in which cell enlargement can be studied without any cell division. This consists of growing bean seedlings in dim red light for 10 days, by which time all division activity is completed, and then placing them in bright white light, whereupon the epidermal cells enlarge 6 to 10-fold over the next four days. This work is published (manuscript #6). Subsequent work on this system was supported by an NSF grant.

2. Growth of corn roots.

The role of auxin and proton excretion in the endogenous growth of roots has been obscure. Added auxin usually inhibits root growth, although under some circumstances it can promote growth to some extent, making it difficult to determine the role of endogenous auxin. Moloney, Elliott and I have shown that the growth of corn root segments can be promoted over short time periods with the antiauxin PCIB, suggesting that endogenous auxin is normally inhibiting endogenous growth. In addition, we have demonstrated that neutral buffers inhibit the endogenous growth, the PCIB-induced growth, and the FC-induced growth of corn roots, indicating that in each case, proton excretion must

play an integral role in the growth process. This research is detailed in manuscript #7, which has been accepted for publication in *Planta*.

3. Auxin-induced soybean hypocotyl growth.

Vanderhoef and coworkers suggested a few years ago that proton excretion is not required for continued auxin-induced growth of soybean hypocotyl sections, and reported that they could not detect any auxin-induced proton excretion in this tissue. Dr. David Rayle and I have reexamined this question, and have shown that auxin-induced proton excretion can be detected, and have presented evidence that proton excretion is required throughout auxin-induced growth in this tissue. This research is contained in manuscript #8.

In the absence of absorbable solutes auxin and FC both induce rapid growth of soybean hypocotyl sections for only about 6 hours, after which the growth rate falls to the control level. In both cases the OC reaches a steady-state level of about 185 mOs/kg, suggesting that Ψ for this tissue is 4.5 bars. In the presence of 2% sucrose, FC-induced growth proceeds at a nearly constant rate for over 12 hours. Again the OC drops to a plateau after 3 hours, but in this case it is about 215 mOs/kg. If the water potential were zero, the TP would be 4.3 bars, only 0.8 bars above Ψ . In such a rapidly-growing tissue the Ψ is almost certainly below zero, with the result that the TP will be even lower than 4.3 bars. These results suggest that in soybean hypocotyls a combination of sucrose plus FC may cause a decrease in Ψ . This possibility is currently being studied.

III. PERSONNEL CONNECTED WITH THIS RESEARCH

Principal Investigator: Dr. Robert E. Cleland

Visiting Scientists and Postdoctoral Fellows:

Dr. B. Rubinstein, Botany Dept., Univ. of Massachusetts

Dr. David Rayle, Botany Dept., San Diego State Univ.

Dr. Mark Tepfer, Botany Dept., Univ. of British Columbia

Dr. Daniel Cosgrove, Botany Dept., Univ. of Washington

Students:

Elizabeth Van Volkenburgh. Ph.D., UW, 1980

Richard Stout. Ph.D., UW, 1980

Maurice Moloney, Ph.D., Leicester Polytechnic, 1978 (with M. Elliott)

Thomas Stevenson, M.S., UW, 1980.

SaraLynn Mandel. M.S., UW, 1981.

Virginia Seymour. Ph.D., UW, 1980 (with R.B. Walker)

S. Ann Dreyer. B.S., UW, 1979

Technician:

S. Ann Dreyer, since 1979.

IV. PUBLICATIONS

Publications Resulting from this Contract, 1978-81.

Goldsmith, M.H.M. and R.E. Cleland. 1978. The contribution of tonoplast and plasma membrane to the electrical properties of a higher-plant cell. *Planta* 143: 261-265.

Tepfer, M. and R.E. Cleland. 1979. Comparison of acid-induced cell wall loosening in *Valonia ventricosa* and in oat coleoptiles. *Plant Physiol.* 63: 898-902.

Bates, G.W. and R.E. Cleland. 1980. Protein patterns in the oat coleoptile as influenced by auxin and by protein turnover. *Planta* 148: 429-436.

Stout, R.G. and R.E. Cleland. 1980. Partial characterization of fusicoccin binding to receptor sites on oat root membranes. *Plant Physiol.* 66: 353-359.

Rayle, D.L. and R. E. Cleland. 1980. Evidence that auxin-induced growth of soybean hypocotyls involves proton excretion. *Plant Physiol.* 66: 433-437.

Cleland, R.E. 1980. Auxin and H^+ -excretion: the state of our knowledge. In: *Plant Growth Substances, 1979*. Ed. F. Skoog. Springer: Berlin. pp. 71-78.

Stevenson, T.T. and R.E. Cleland. 1981. Osmoregulation in the *Avena* coleoptile in relation to auxin and growth. *Plant Physiol.* 67: 749-753.

Papers Accepted or Submitted for Publication

Dreyer, S.A., V. Seymour and R.E. Cleland. Low proton conductance of plant cuticles and its relevance to the acid-growth theory. *Plant Physiol.*, accepted for publication.

Rubinstein, B. and R.E. Cleland. Responses of *Avena* coleoptiles to suboptimal fusicoccin: kinetics and comparisons with IAA. *Plant Physiol.*, accepted for publication.

Moloney, M.M. , M.C. Elliott and R.E. Cleland. Acid growth effects in maize roots: evidence for a link between auxin-economy and proton extrusion in the control of root growth. *Planta*, accepted for publication.

Stevenson, T.T. and R.E. Cleland. Osmoregulation in the *Avena* coleoptile: control of solute uptake in peeled sections. Submitted to *Plant Physiol.*

Silk, W.K., L.L. Wang and R.E. Cleland. Mechanical properties of the rice panicle. Submitted to *Plant Physiol.*

V. TALKS AND SEMINARS GIVEN COVERING WORK CARRIED OUT UNDER THIS CONTRACT

1978-79: Invited symposium paper, X International Conference on Plant Growth Substances, Madison, WI, July 1979.

Invited discussant, International Workshop on Plant Membranes, Toronto, July 1979

Contributed papers (2). American Soc. Plant Physiol. Meetings, Columbus, Ohio.

Seminars: North Carolina State Univ., Botany Dept.

Western Washington State Univ., Botany Dept.

1979-80: Invited symposium talk, Conference on Plant Hormones, USDA and Washington Section, ASPP, Bethesda, MD.

Contributed paper. Amer. Soc. Plant Physiol. Meeting, Pullman, WA

Seminars: Univ. of Nebraska, Biology Dept. (2)

Washington Univ., St. Louis, Biology Dept and Physiol. Dept.

1980-81: Symposium organizer and speaker, International Botanical Congress, Sydney, Australia, August 1981

Invited speaker: Plant membrane workshop, Strasbourg, France, Sept. 1981.

Invited speaker: Gordon Conference, Agricultural Sciences, Ventura CA, Jan. 1981

Contributed papers (2): Amer. Soc. Plant Physiol. meeting, Quebec City.

Seminars: Stanford Univ., Plant Physiol. group

Univ. of California, Davis. Botany Dept. (2)

Univ. of Illinois, Botany Dept.

Washington State Univ., Botany Dept.

Table I. Decrease in the growth response of peeled Avena coleoptiles when auxin is added at intervals after peeling.

Hr auxin added	$\Delta L/24 \text{ hr} - \%$
0	85
2	60
4	54
No auxin	27

5 mm coleoptile sections were peeled and incubated in 1 mM K-Pi buffer, pH 6.0 + 2% sucrose, + 10 μM IAA. IAA was added at 0,2,4 hrs or not at all. Growth measured after 24 hours.

Table II. Fusicoccin does not enhance osmoregulation in intact Avena coleoptile sections.

Condition	$\Delta L/8 \text{ hrs}$ %	OC Osm/kg	ΔOS %
Initial	. . .	0.273	. . .
S	8	0.369	+44
AS	31	0.335	+61
FS	17	0.339	+45

Unpeeled 5 mm Avena coleoptile sections were incubated in 10 mM KPi, pH 6.0 + 2% sucrose, + 10 μM IAA or FC, for 8 hrs. Growth measured, and OC determined by vapor pressure osmometry of expressed sap. ΔOS is % increase in total solutes over initial. S: sucrose only; AS: auxin + sucrose; FS: fusicoccin + sucrose.

Table III. Effect of pH on parameters governing acid-induced extension.

pH	a (units)	k (min ⁻¹)
7.0	12	0.0656
5.0	18	0.354
4.0	31	0.350
3.6	29	0.0341
3.0	31	0.0388

Peeled Avena coleoptile sections frozen-thawed, then incubated under 10 g tension at pH 7 for 1 hr. The solution then changed to one at indicated pH, and the resulting extension curve was fitted to the exponential equation with a computer. Each value is the average of 8 runs.

Table IV. Effect of external pH on osmoregulation when NaCl is the absorbable solute.

pH	OC (Os/Kg)	Decrease in OC + auxin (Os/kg)
4	0.274	0.025
5	0.352	0.067
6	0.443	0.065
7	0.501	0.048
8	0.463	0.020
Initial	0.219	

5 mm peeled Avena coleoptile sections were incubated for 21 hrs in 1 mM KPi-citrate buffers, pH 4.0 to 8.0, + 20 mM NaCl, + IAA. OC measured by vapor pressure osmometry on expressed sap.

Table V. Change from cyanide-sensitive to cyanide-insensitive respiration in Avena coleoptile sections.

Time (hrs)	Respiration (% of control)	
	+ KCN	+ KCN & SHAM
0	31	20
1 1/2	35	10
4	45	7
10	54	8
23	76	8

Rate of oxygen uptake measured with O_2 electrode on 5 5-mm Avena coleoptile section in 5 ml 1 mM K-Pi, pH 6.0, + 2% sucrose. After control rate established, 0.1 mM KCN added. After new rate established, 1 mM SHAM added. Values are for % original rate remaining after addition of inhibitor.

FIGURE LEGENDS

- Fig. 1. Effect of peeling on amount of auxin-induced growth. 5 mm Avena coleoptile sections peeled, then incubated in 0.1 mM KPi buffer, pH 6.0 + 0.5 % sucrose + 1 mM KCl + 0.1 mM CaCl_2 , with (●) or without (○) 10 μM IAA. After 24 hrs, growth of each sections measured, and % peeled determined by neutral red method. Note lack of correlation between % peeling and amount of growth.
- Fig. 2. Effect of FC on WEx as measured by Instron technique. Avena coleoptile sections were incubated in 1 mM KPi + 1 mM KCl, with 10 μM IAA (—●—), FC (—○—) or no hormones. At varying times, growth measured, sections killed, and plastic extensibility measured by the usual Instron technique.
- Fig. 3. Control of FC-induced growth rate by changes in capacity for wall loosening (CWL). Avena coleoptile sections were incubated for 0-11 hours in 1 mM KPi, pH 6.0, + 2% sucrose and with 10 μM IAA (A), FC (F) or with no hormones (S). Sections then frozen-thawed, subjected to 20 g force at pH 7 for 1 hr, then solution changed to pH 3 and rate of extension 1 hr later determined (CWL).
- Fig. 4. Changes in WEx as measured by the Instron technique, after treatment with auxin. Avena coleoptile sections incubated in 1 mM KPi + 2% sucrose for 2 hrs, then 10 μM IAA added, and sections harvested at varying times. DP determined as with usual Instron technique. In this experiment maximum growth rate was achieved by 30 minutes after addition of auxin.
- Fig. 5. Decrease in plastic extensibility when growth rate is inhibited by KCN. Avena coleoptile sections were incubated as in Fig. 4, and after 4 hrs, 1 mM KCN was added to some sections. Sections were then harvested at varying times, and DP was determined in the usual manner with an Instron. In this experiment, the growth rate reached a minimum within 10 minutes of addition of KCN.
- Fig. 6. Effect of tension on the parameters of acid-extension. Frozen-thawed coleoptile sections were incubated under 5-20 g tension at pH 7, then the pH was changed to 4.0 and the extension curve was recorded. The data were fitted to the exponential equation with a computer to give values of a (—○—) and k (—●—).
- Fig. 7. Effect of addition of mannitol to the growth of Avena coleoptile sections. Sections preincubated 2 hrs + 10 μM IAA, then 0 - 0.24 M mannitol added, and elongation measured over next 8 hrs. Note the length of time needed to achieve constant elongation rates.
- Fig. 8. Method of correcting for viscoelastic contraction following reduction in turgor. Avena coleoptile sections preincubated 2 hrs with auxin, then some kept at 25C while others give 10 minutes at 0C. 0.24 M mannitol added to AM sections, and elongation measured over the next 9 hours. AM (25 - 0) curve calculated by subtracting length at 0 from length at 25C.

Fig. 9. Effect of auxin on yield stress. Coleoptile sections were pretreated 90 minutes in 2.5 mM K-maleate buffer, pH 4.7 with 0-0.2 M mannitol. IAA at 5 uM ($\bullet$), 0.15 uM ($-\circ-$), 0.03 uM ($-\blacktriangle\cdot$) or no auxin added ($\cdots\Delta$) and elongation after 90 minutes determined.

Fig 8.

Fig 1

Fig 2.

Fig 3

Fig. 4

Fig 5

Fig 6

Fig 7

Fig 8.

Fig 9