STUDIES ON THE MECHANISM OF PLANT CELL EXPANSION

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ABSTRACT

The mechanism of elongation of segments of hypocotyl of light-grown seedlings of lupin (Lupinus angustifolius cv. N.Z. Bitter Blue) has been investigated. The approach was three-fold: biophysical analysis of growth responses; an investigation of the role of individual tissues in elongation; and tests of predictions of the acid-growth hypothesis.

In biophysical studies, a method was developed to measure the half-times of transients in elongation rate in response to application of a compressive load. For loads of 4-18g (equivalent to applied pressures of about 0.1-0.5 bars) half-times for the return of elongation rate to a steady value after loading were 3-15 minutes for segments incubated without IAA, and 6-13 minutes for IAA-treated segments. Half-times after removing the load were 2-7 minutes for non-IAA-treated segments. Results were analysed according to the diagnostic scheme of Cosgrove (1981, Plant Physiol. 68:1439-1446), and suggested that IAA promoted elongation through an effect on either the tissue free energy diffusivity of water (D), or on extensibility. It was not possible to distinguish between these alternatives on the evidence available.

In studies on the role of different tissues in elongation, the effect of removing specific tissues from non-IAA-treated segments was first determined. The epidermis apparently limited elongation of intact segments, since a burst of extension occurred when it was removed by peeling. In peeled segments, the stele (vascular tissue and pith) apparently limited the rate of extension since its removal resulted in very rapid extension of the remaining cylinder of cortex. On IAA treatment, the response of segments with the stele removed was initially similar to that obtained with intact segments, suggesting that the epidermis and cortex only were involved in the initial response. In segments where the epidermis had previously been removed this initial response to IAA was absent, but there was a longer term response. These results suggest that the response of intact segments to IAA consisted of two superimposed phases. The first was the result

of epidermal "relaxation", and the second was an independent elongation controlled by the cortex.

The acid-growth hypothesis predicts that treatment with acid solutions will promote elongation to IAA-induced levels. Tests of this prediction with hollow cylinders and peeled segments of lupin hypocotyl showed that the most IAA-responsive preparation (hollow cylinders with the epidermis intact) was the least acid-responsive, with little elongation response at pH 5. Treatment at pH 4 was needed to promote elongation to IAA-induced rate. The cortex alone responded strongly to acid treatment (pH 5), suggesting that the epidermis was limiting response when it was present. Peeled segments elongated in response to IAA treatment, but did not elongate in response to acid treatment (pH 5) (if pretreated in water), perhaps because response was limited by restricted diffusion of hydrogen ions through the starch sheath and into the stele. However, peeled segments elongated rapidly initially after treatment with acid if first pretreated in buffer (1mM K_2HPO_4-citric acid, pH 6.6). These results show that acid-induced elongation of segments may be influenced by differential response of tissues, by barriers to diffusion of hydrogen ions, and by treatment with buffered solutions. The results suggest that unless IAA action in intact segments causes pH in the walls of the outermost cell layers to fall to to about pH 4, then it is unlikely that IAA-induced elongation is mediated (initially) by hydrogen ions.

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1: INTRODUCTION

Plant growth is traditionally viewed as being governed by two processes - cell division and cell expansion. Of the latter, Cleland (1971) stated: "The importance of cell enlargement has long been recognised, and the papers dealing with the process number in the tens of thousands. From these papers there has been distilled a body of basic information ... The problem is that it is unclear as to how to fit these pieces together into any coherent picture of cell enlargement." This statement alludes to the difficulty in understanding the mechanism of expansion of single intact cells, when available techniques have dictated that measurement of expansion be made at a macroscopic level (using segments of tissue) or that the cells are disrupted.

A plant cell consists of a protoplast bounded by a cell wall. In a mature (non-growing) cell the protoplast can be viewed as an osmometer which will swell with water uptake and exert a pressure on the cell wall, causing it to stretch in response. The pressure on the wall is termed turgor pressure; it is the difference between the external pressure and the actual hydrostatic pressure of the cell vacuole (Dainty 1976). When turgor pressure is zero, the cell is at minimum (irreversible) volume. With water uptake into the cell the cell wall is stretched elastically (reversibly). The observed cell volume at any pressure will then be a reflection of the initial (irreversible) volume, and the elastic properties of the cell wall.

In a growing (expanding) cell, irreversible volume is increasing with time. At any point in time, the observed volume is a function of the irreversible volume at that time, and the elastic properties of the cell wall (Lockhart 1965). Increase in observed cell volume is due to water uptake into the protoplast, which is driven by the difference in water potential ($\Delta\psi$) between the cell vacuole and the external medium. Increase in irreversible cell volume is driven by turgor pressure. When cell volume is increasing at a steady rate, the rate of water uptake is equal to the rate of cell wall expansion (Lockhart 1965, Ray et al. 1972).

These two facets (water uptake and wall extension) of the cell expansion process have been recognised in early models. The role of turgor pressure was appreciated by Sachs (1874, cited by Heyn 1940) in the first published model of cell elongation. On the role of water uptake, Heyn (1940) stated "... actual enlargement is ... dependent, in the first place, on uptake of water by the vacuole, elongation being impossible if this is prevented. The uptake of water generally is not a limiting factor, however...". Given this assumption, cell expansion is limited by cell wall extension. In a summary of early models of cell wall extension, Heyn (1940) distinguished three types of hypothesis. The first was that cell wall enlargement was initiated by an active process of wall deposition, independent of any other force. This has not been supported by the results of many studies which suggest that turgor pressure is the driving force for cell expansion (Cleland 1959, Green et al. 1971, Green and Cummins 1974). The other two models have been discussed by Cleland (1971). In each, enlargement is initiated by a biochemical "wall loosening" step, and subsequent turgor-driven extension is irreversible, in one model; or reversible and then rendered irreversible by a second biochemical step (for example, wall synthesis), in the other.

The first formal analysis of cell expansion (Lockhart 1965) incorporated statements for both water uptake and cell wall extension. This analysis forms the basis of current thinking in studies on cell expansion. The model assumes that the plant cell wall responds to stress in the manner of a Bingham solid material. That is, the relative elongation rate is linearly proportional to stress above a yield threshold. Cosgrove (1981) expresses this relationship for the relative rate of irreversible cell volume expansion as

$$(1/V_o)(dV_o/dt) = \phi(P-Y) \qquad (1)$$

where V_o = cell volume at zero turgor

ϕ = wall extensibility

P = cell turgor pressure

Y = yield threshold (minimum turgor required for wall expansion)

Cell expansion also involves water uptake, and to account for this the additional assumption is made that the growing cell behaves as a simple osmometer. Hence the equation for the relative rate of volume change due to water influx is (Cosgrove 1981)

$$(1/V)(dV/dt) = L(\sigma.\Delta\pi - P) \quad (2)$$

where V = cell volume at a particular moment of growth

L = hydraulic conductivity of the cell, equal to membrane hydraulic conductivity Lp, times membrane surface area A, divided by cell volume V ($L = Lp.A/V$)

σ = solute reflection coefficient

$\Delta\pi$ = difference in osmotic potentials between the inside of the cell and the external medium

In steady-state growth, the relative rates of water influx and irreversible wall expansion are equal. When equations (1) and (2) are set equal to each other, the equation for steady state growth rate becomes (Cosgrove 1981)

$$\dot{v}_s = L\phi(\sigma.\Delta\pi - Y)/(L+\phi) \quad (3)$$

When water uptake is not limiting (cf. Heyn 1940) then $L >> \phi$, and equation (3) becomes

$$\dot{v}_s = \phi(\sigma.\Delta\pi - Y) \quad (4)$$

and since $\Delta\psi$ will be close to zero, $\sigma.\Delta\pi = P$. Hence

$$\dot{v}_s = \phi(P - Y) \quad (5)$$

which shows that in this situation, cell expansion is dependent on the rate of irreversible wall extension [equation (1)]. This is the case of extensibility-limited expansion discussed in the descriptive models (Cleland 1971).

It is not obvious in the biophysical model how expansion is initiated or maintained. In terms of the model proposed by Lockhart (1965), the

cell wall would "flow", in the manner of a Newtonian fluid, at stresses above the yield threshold. The rate of flow is proportional to stress, and is governed by the viscosity, which is directly related to extensibility. However, cell walls which have been isolated and subjected to a creep test (where extension is measured over a period of time in response to an applied tensile load) do not flow, instead showing a declining rate of extension with time (Cleland 1971). Apparently, and not surprisingly, simply replacing turgor stress (with an applied stress) is not sufficient to cause isolated cell walls to yield as in growing intact cells. Association with the protoplast is necessary for the wall to be maintained in a state where continuing extension will have the appearance of flow. It is this association which provides the means of initiating and maintaining cell expansion - the process of "stress relaxation" (Ray et al. 1972), "wall loosening" (Cleland 1971), or the "primary cause" (Heyn 1940). This is the "biochemical step" in the descriptive models (Cleland 1971), and shows how those models and the biophysical model (Lockhart 1965; Ray et al. 1972; Cosgrove 1981) may be related. The processes involved in the biochemical step determine the parameters of cell wall expansion in the biophysical model.

The biophysical model of Lockhart (1965) has been used as a basis of interpretation in many studies of plant cell expansion. Single cells (of Nitella) have been used in one study (Green et al. 1971). In others, segments of coleoptile (Green and Cummins 1974, Cleland 1977) or hypocotyl (Boyer and Wu 1978), or seedlings (Boyer and Wu 1978, Cosgrove and Green 1981) have been used. The model can be applied in unmodified form to the growth of segments in solution, if it is assumed that the main pathway for water uptake into the segment is through the epidermis, and that the epidermis provides the limiting resistance to water flow (Lockhart 1965, Cosgrove 1981). The segment is then regarded as analogous with a single cell, with the model parameter L (equal to Lp.A/V) given by the hydraulic conductivity of the epidermis, the segment surface area, and the segment volume. $\emptyset$ and Y for the segment will be average values of $\emptyset$ and Y for the cells of the tissue which limits elongation of the segment. The model must be modified to apply to intact plants, or in other cases where the main resistance to

water flow does not occur at one barrier, but is instead distributed through the tissue. A modified analysis which accounts for radial water transport in a growing cylindrical stem has been provided by Cosgrove (1981).

Although in widespread use as a working hypothesis, the model of Lockhart (1965) has been tested in only two studies (Lockhart 1965, Green et al. 1971). A formal test of the model requires that estimates be made of all the parameters in equation (3) and these values substituted in the general solution of equation (3) to yield a complete solution. This will be an expression for length as a function of time, and is a prediction of the model which can be compared with experimentally observed responses. Five parameters need to be measured: L, ϕ, σ, $\Delta\pi$, and Y.

In short-term studies, it is usually assumed that the osmotic pressure (π) of the cell is constant, and that σ is equal to one (i.e., that the cell membrane is impermeable to solutes). The term $\sigma.\Delta\pi$ [equation (3)] is therefore equal to $\Delta\pi$, and is constant if the external osmotic pressure is constant. There are several methods of measuring π (cell) (Dainty 1969). Only a few measurements of σ have been made (see Dainty 1976), in giant algal cells, and Penny and Penny (1978) observe that there do not appear to have been any attempts to measure σ in auxin-treated higher plant tissues.

The yield threshold Y has been measured in one study with single cells of Nitella (Green et al. 1971) and in several studies with segments of higher plant tissue (Cleland 1959, Green and Cummins 1974). Green et al.(1971) measured Y as the turgor pressure below which expansion apparently ceased, and suggested that the value of this parameter might depend on the time scale of measurement. In short-term measurements, Y was very close to cell turgor P, since only a small reduction in turgor pressure would apparently stop elongation (in Nitella). However, elongation subsequently recovered, and if stabilised values of elongation rate were considered (long-term) the yield threshold was quite low. This observation illustrates a general point about the model, namely that values of the parameters may change with time.

Unless this is recognised in a modification of the model (for example, Green et al. 1971), any set of parameters will apply in only one situation (Ray et al. 1972).

Hydraulic conductivity of the cell (L) can be estimated from measurements of Lp (hydraulic conductivity of the cell membrane), cell surface area and cell volume. Methods of measuring Lp in single cells are discussed in Dainty (1976); measurement of Lp in single cells of higher plant tissue has only been accomplished using the pressure probe technique (Cosgrove and Steudle 1981).

Measurement of extensibility, ϕ, has also proved difficult. Only one measurement of ϕ [as defined in the biophysical model (Lockhart 1965)], has been made (Green et al. 1971) in single cells of Nitella. In that case, turgor pressure was measured and it was shown that water uptake was not limiting growth. Equation (5) was the appropriate model, and ϕ was then estimated from the relationship between elongation rate and (P-Y), as $\phi = (1/L_o)(dL_o/dt)/(P-Y)$. No clear measurement of ϕ has been made for segments of higher plant tissue. In principle, the method of Green et al. (1971) could be used, but this requires that the condition of extensibility-limited extension applies. If this has not been shown, then equation (3) applies. Other measurements of "extensibility" have been made with various physical tests and are discussed in Cleland (1971) and Penny and Penny (1978). There are several problems in relating results of these tests to the model of Lockhart (1965). The major problem is conceptual. It is clear that extensibility (ϕ) is not simply a physical property of the cell wall (Green and Cummins 1974, Cleland 1977, Green et al. 1977). It is defined as a function of cell expansion rate and turgor pressure, and I have already suggested that an influence (possibly biochemical) of the protoplast is at least partly involved in controlling the rate of cell expansion. Even assuming that extensibility was a physical property of the wall, no physical test yields measurements of "extensibility" which can be clearly related to the biophysical model (Penny and Penny 1978).

These points illustrate some of the difficulties in testing the model of Lockhart (1965). However, in a particular situation, in the absence

of such a test, the model can still be used as a working hypothesis. Cosgrove (1981) shows how certain measurable features of growing cells or segments [namely $\dot{v}_s$ (the steady state expansion rate at a particular pressure); P_s (the value of turgor pressure at the steady state); and the half-time for transients in growth rate between steady states] can be used to diagnose (a) whether or not expansion is limited by water uptake; and (b) the mechanism of action of any factor affecting growth rate, in terms of the parameters (L, ϕ, $\sigma.\Delta\pi$, and Y) of the model.

However, there remains a possibly important problem in the use of the model as a basis of analysis of results of experiments on the growth of plant segments. Whether the segment is regarded as analogous with a single cell, or whether account is taken of distributed resistance to water flow, in neither case has the morphological and functional differentiation of cells within the segment been acounted for. The assumption is made, usually tacitly, but explicitly by Cosgrove (1981) in his analysis for the case of distributed resistance to water flow, that all cells in a segment are alike. An example of evidence which suggests that differential response of cells might need to be accounted for is the differential responsiveness of tissues in auxin-treated split stems (Thimann and Schneider 1938), where the outermost tissues apparently elongated more rapidly than the innermost tissues. The effects of any differential responsiveness in the rapid straight-growth response of segments has not been investigated.

Given that all cells in a segment may not respond equally to any factor affecting growth, one crucial question remains about the process of cell expansion. What is the "primary cause", the biochemical mechanism by which expansion is initiated? One of the means of investigating this has been to use auxin to promote expansion, and seek correlated changes in properties of the cell wall (reviewed by Penny and Penny 1978); or in hydraulic conductivity (Boyer and Wu 1978) or osmotic pressure (Penny et al. 1972). Results have suggested that auxin affects extensibility (Green and Cummins 1974, Boyer and Wu 1978); and does (Boyer and Wu 1978) or does not (Dowler et al. 1974) affect hydraulic conductivity. The use of techniques for high resolution

measurement of growth (Penny and Penny 1978) has proved valuable in critical assessment of hypotheses of auxin action. These have shown that rapid elongation of segments begins about 10-15 minutes after auxin treatment. For a change in a cellular parameter to be the cause of enhanced elongation that change must occur before, or coincident with, the increase in elongation rate. Penny and Penny (1978) document many such rapid responses to auxin treatment (not all in growing tissues). These include effects on cell wall xyloglucan (Labavitch and Ray 1974), membrane potential (Cleland et al. 1977) and permeability (Loros and Taiz 1982), water uptake in protoplasts (Gregory and Cocking 1966), and reduction of extracellular pH (Cleland 1976, Jacobs and Ray 1976). A currently favoured explanation for the mechanism of auxin action is the acid-growth hypothesis, which states that auxin acts on the cell protoplast to cause a reduction of pH in the cell wall which activates a wall-loosening enzyme (or enzymes), which initiates turgor-driven cell expansion (Rayle and Cleland 1980). Many experimental observations support this hypothesis (Cleland and Rayle 1978) but some do not (Penny and Penny 1978). A critical test of the hypothesis requires demonstration of an auxin-induced reduction of cell wall pH, to an elongation-promoting value, before the rate of elongation is increased. A prerequisite is to establish that treatment with acidic solutions will promote elongation rate to the auxin-induced value. In some studies (for example, Cleland 1976, Jacobs and Ray 1976) results of such tests yield results which support the acid-growth hypothesis. However, results of experiments with lupin hypocotyl segments (and Avena coleoptiles) (Penny et al. 1975) do not support the hypothesis. Reasons for these apparently contradictory results remain to be resolved.

The approach in this study to the problems outlined has been three-fold. Firstly, I have used the analysis of Cosgrove (1981) as a basis to diagnose the mechanism of action of auxin on elongation of lupin hypocotyl segments, in terms of the parameters of the Lockhart model. Secondly, I have shown how the rapid elongation response of hypocotyl segments, after auxin treatment, might depend on the interaction of differentially auxin-responsive tissues which are initially in different states of stress. From a qualitative point of

view, these results illustrate the need for caution in interpreting the responses of segments in terms of cellular behaviour. The third line of approach involves an investigation of an aspect of the biochemical mechanism of auxin action on segment elongation. Tests of the acid-growth hypothesis have shown that both the epidermis (peeled from segments and subjected to an applied load) and peeled segments will elongate in response to treatment with acid (for example, Durand and Rayle 1973). However, the assumption that all tissues of a segment are equally responsive to hydrogen ions at in vivo levels of stress has not been tested. This assumption is tested here with segments of lupin hypocotyl. Results suggest that differential responsiveness to acid is a feature which should be accounted for in tests of the acid-growth hypothesis; and also provide a possible explanation of the apparently contradictory results of Penny et al. (1975) and Cleland (1976).

2: BIOPHYSICAL ANALYSIS OF GROWTH RESPONSES

2.1 INTRODUCTION

Expansion of a plant cell involves water uptake into the protoplast, and yielding of the cell wall (Lockhart 1965, Ray *et al*. 1972, Cosgrove 1981). The driving force for wall extension is turgor pressure, as shown in experiments where elongation of single cells of *Nitella* has been measured as a function of turgor pressure (Green *et al*. 1971), and where elongation of segments of plant tissue has been measured as a function of external osmotic potential (Cleland 1959, Green and Cummins 1974). Our present understanding of the relationship between turgor pressure and cell expansion is expressed in the hypothesis first described in mathematical form by Lockhart (1965), restated by Ray *et al*. (1972), and further developed by Cosgrove (1981). This is outlined in the equations (1)-(3) on pages 2-3.

When the rate of expansion is steady, the rate of irreversible volume expansion [equation (1)] is equal to the rate of volume increase due to water uptake [equation (2)]. When set equal to each other, these equations yield equation (3), which is a model for steady-state expansion. This equation is given below and also outlined graphically in Fig. 1.

$$\dot{v}_s = L\phi(\sigma.\Delta\Pi - Y)/(L+\phi)$$

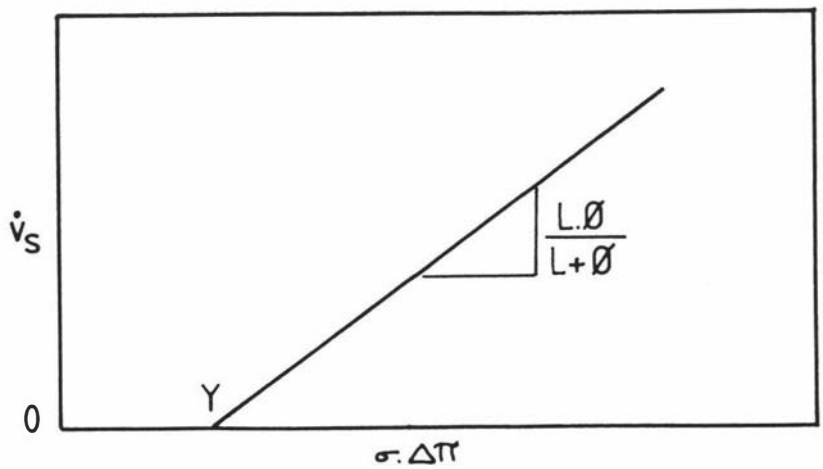

Fig. 1: The steady-state model of Cosgrove (1981).

Cosgrove (1981) and others (Green and Cummins 1974) have shown how measurement of $\dot{v}_s$ [equation (3)] as a function of external water potential [which is directly related to $\sigma.\Delta\pi$ of equation (3)] can be used in the diagnosis of the mechanism of action of any factor affecting growth, in terms of L, ϕ, and Y. For example, if auxin promotes expansion through reducing Y, then the slope [$L\phi/(L+\phi)$] of the plot of $\dot{v}_s$ against the external water potential (Fig. 1) would be the same for auxin-treated segments as for non-treated segments, but the X-intercept (which is the yield threshold Y) would be changed. If L or ϕ was increased by auxin then the slope of the plot would be increased, but the X-intercept would remain the same.

Cosgrove (1981) also shows how measurement of P_s (the steady-state turgor pressure) can help in determining the mechanism of action of auxin. P_s will increase if the rate of water uptake [equation (2)] is increased as a result of an increase in L, π(cell), or σ. This is most simply explained as follows. The rate of increase in the actual cell volume (V), becomes greater than the rate of increase of irreversible volume (V_o) [which is given by equation (1)]. Since turgor pressure P is related to cell volume according to the formula (Dainty 1976, Cosgrove 1981)

$$P = \epsilon(V-V_o)/V_o \qquad (6)$$

where ϵ = the volumetric elastic modulus

then P will increase with the increase in $(V-V_o)$. Using similar reasoning, P will decrease if the rate of irreversible wall expansion [equation (1)] increases as a result of an increase in ϕ or a decrease in Y.

As implied above, when growth rate* is changing (not in steady-state) equations (1) and (2) are not equal. This is shown by Cosgrove (1981)

* [The term "growth rate" used here (and by Cosgrove 1981) refers to the rate of actual volume increase $(1/V)(dV/dt)$, and not to the rate of increase of irreversible volume $(1/V_o)(dV_o/dt)$ which is the definition of growth given on page 1].

in an extension of the earlier analysis of Lockhart (1965). From equations (1), (2) and (6), Cosgrove (1981) derives an expression for turgor pressure as a function of time and the parameters L, $\sigma.\Delta\pi$, ϕ, Y and ϵ. After a change in growth rate, or in any of the parameters governing growth rate, turgor pressure changes exponentially towards a new steady state value. Since the relative rates of water influx [equation (2)] and of irreversible wall expansion [equation (1)] are linked to turgor pressure, each will also change towards a new steady state value, but with one increasing while the other decreases. The observed growth rate of the segment during the transient is given by $(1/V)(dV/dt)$, the relative rate of actual volume change. The time constant (and hence the half-time) of this change in rate is dependent on the parameters for both water influx and wall extension (Cosgrove 1981) and can thus be used as a third diagnostic feature of the biophysical mechanism of auxin action. Cosgrove (1981) shows that if growth rate is doubled, then the half-time is unchanged (or changed little) if a change in either $\sigma.\Delta\pi$, Y, L, or ϕ is the cause of the doubling in rate; but is reduced by half if both L and ϕ have changed.

To determine the half-time of growth rate transients, rate must be sharply displaced from the steady state, and the course of subsequent adjustment in rate measured (Cosgrove 1981). The method commonly used to alter the growth rate of cells or segments in solution has been to induce water flux and turgor change by altering the external water potential (Green et al. 1971, Green and Cummins 1974). However, unless segments are only a few cells thick (as in coleoptiles), and unless special measures are taken to increase the rate of entry of the osmoticum into the segment (Green and Cummins 1974), then it is unlikely that this method will meet the requirement of causing an abrupt change in growth rate. The time constant for diffusion of osmoticum into the segment is likely to be greater that the time constant of the change in turgor pressure which follows the imposed change in growth rate (Cosgrove 1981). Therefore it would not be possible to measure the required time constant (or half-time) of the transient in growth rate. This will be a particular problem in solid cylinders of plant tissue, such as stems and hypocotyls. Consequently, an alternative method was required for perturbing growth rates in the

segments of lupin hypocotyl which were used in this study.

The objectives of this study were as follows:

(1) To develop a method which would permit the elongation rate of lupin hypocotyl segments to be

(a) changed abruptly from a steady-state value; and

(b) measured through this change and the subsequent adjustment in rate.

A method was developed which allowed measurement of the response of elongating segments to an applied pressure. The technique of altering the elongation rate of plant segments through application of a compressive load has not previously been used in studies involving high resolution measurements of growth. The larger part of this study has been concerned with establishing the effectiveness and limitations of this technique.

(2) To compare the time course of rate adjustment with that predicted from the analysis of Cosgrove (1981), and to determine the half-time of this response.

(3) To determine the effect of IAA on the half-time.

(4) To determine the steady state elongation response as a function of applied pressure, in IAA-treated and non-treated segments.

(5) From (3) and (4), to assess the biophysical mechanism of action of IAA on elongation, in terms of the parameters of the model of Cosgrove (1981).

2.2 MATERIALS AND METHODS

2.2.1 Plant materials

Four-day old lupin seedlings (Lupinus angustifolius cv. N.Z. Bitter Blue) were grown in continuous low light at 22C (Penny 1969). The light was a mixture of fluorescent and incandescent, with PAR 60-70 μeinsteins $m^{-2}s^{-1}$. Seedlings for experiment were selected with hypocotyl 50-60mm long, and 10mm segments of hypocotyl (cut 2-3mm below the cotyledonary node) were used in experiments.

2.2.2 Measurement of growth

Segments were held in a chamber similar to those described by Penny et al. (1974), and shown in Fig. 2. The chamber was made from a block of perspex through which holes were bored. Solution was pumped in at A, flowed past the segment (C), and drained at B. Arrows show the direction of flow. The segment was placed on a pin (D) embedded in a perspex plug (E) which was screwed into the incubation chamber. The foot (F) of the apparatus which was used to apply a force to the segment (Fig. 3), rested on the top of the segment, and was connected to a linear displacement transducer, which was used to measure elongation of the hypocotyl segment (Penny et al. 1974). Resolution was 0.63μm.

Segments were incubated in the chamber in flowing aerated buffer (1mM K-phosphate buffer pH 6.6), with or without 30μM IAA. Solutions were recirculated from flasks held in a water bath at 25C. The flow rate was 10ml/min.

Experiments were conducted in normal laboratory lighting, with the chamber illuminated in addition with a 40W tungsten lamp placed about 15 cm away.

2.2.3 Alteration of growth rate

The method of altering growth rate while simultaneously measuring elongation of a lupin hypocotyl segment involved use of apparatus designed by Drs P.Penny and R.O'Driscoll (Fig. 3). This was originally designed to apply a continuously varying force to a segment to maintain it at a fixed length, but had not been tested. In experiments in this study, it was used to apply a predetermined force which remained unchanged, until removed.

Apparatus

Figure 3 shows the equipment used. A coil (A), formed on a rectangular brass frame (B), was held horizontally in a brass frame (C). The coil could pivot freely on bearings at D and E. Ends of the coil wire were

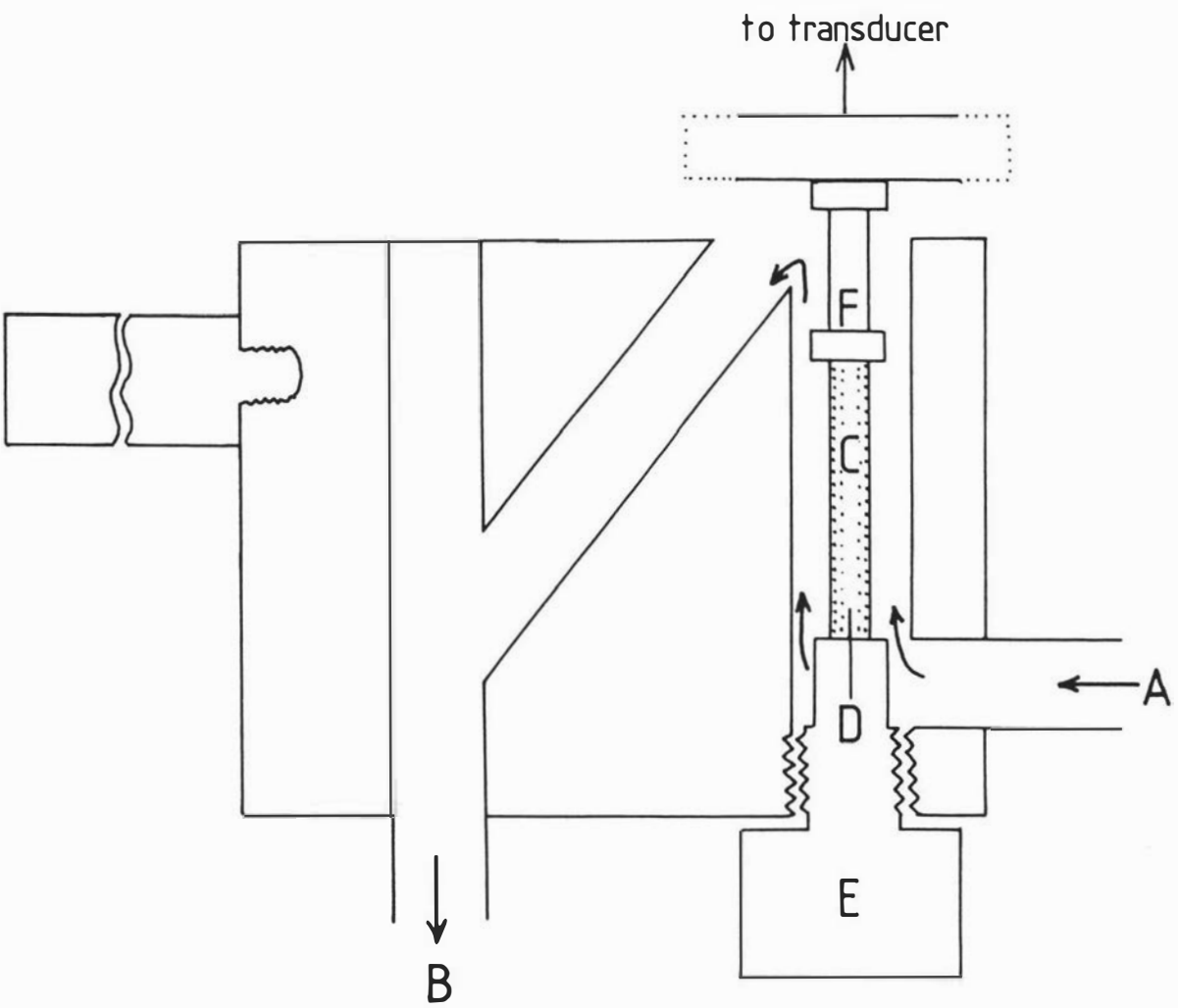

Fig. 2: Diagram of the incubation chamber (mid-section).

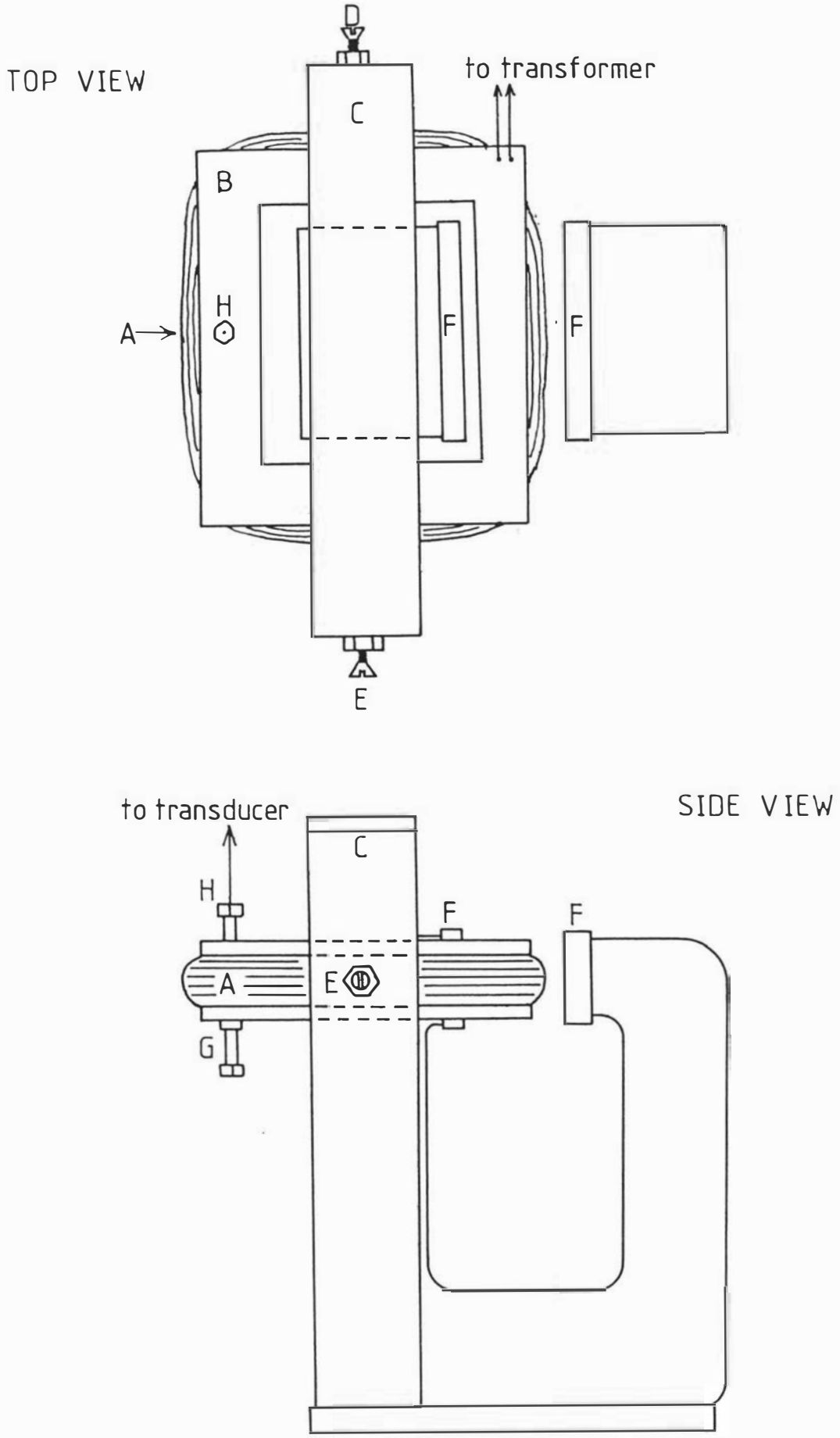

Fig. 3: Diagram of the apparatus for applying a force to the hypocotyl segment.

attached to a variable transformer. One side of the coil passed between the poles of a magnet (F). A short brass rod with a perspex foot (G) was attached to the underside of the opposite side of the coil, and rested on the top of the hypocotyl segment in the incubation chamber (Fig. 2). A polyester thread was attached to the upper side of the coil at H, and passed over a pulley to a linear displacement transducer.

Operation

A current was passed through the coil by applying a d.c. voltage across the ends of the coil. A current carrying wire in a magnetic field experiences a force (Linsley 1974)

$$F = I \beta l$$

where F = force

I = current

β = magnetic flux density

l = length of wire

The direction of this force is perpendicular to the current direction. If not opposed, the coil was deflected from its original horizontal position. In experiments, movement of the coil was opposed by the hypocotyl segment, and a compressive force was exerted on the top of the segment.

Calibration

The calibration curve for the coil-magnet apparatus is shown in Fig. 4. The force exerted by the foot of the coil (G in Fig. 3) on an electronic top-weighing balance was measured as a function of voltage across the coil, for the range 0 - 5 volts. (In this balance, readings represent the force required to keep the weighing pan stationary when a weight is added). The coil was initially balanced to give a load of about 2g, at zero volts. Voltage was increased in one volt increments to five volts and the weight recorded at each step. This procedure was repeated five times. Force increased linearly with voltage over the range tested, with a standard error at any point of less than 0.2g. The regression equation of force on voltage is shown in Fig. 4; a

change of one volt is equivalent to an increment in force of 17 g.

Controls

Care was taken to minimise movement in the apparatus when applying and removing load. Both the coil-magnet apparatus and the segment incubation chamber were rigidly mounted. Some movement did occur, mainly of the coil on its bearings. Measurement of this movement was made with the plant segment replaced with a brass substitute. Voltage was increased from zero to 1, 2, 3, 4, or 5 volts, and transducer readings recorded at 10 second intervals over the next minute. Voltage was reduced to zero, and readings again recorded. The procedure was repeated five times at each voltage. Most movement occurred in the first 10 seconds after changing the voltage, and is shown in Table I. Comparison with the deformation recorded in plant segments at 10 seconds after changing the load [for example, Fig. 6(B, C) - where an applied pressure of $20 g/mm^2$ results from a load of about 85g] suggests that movement in the apparatus may be the source of a major error in these results. Little additional movement was observed (at times up to one minute) (Table I).

Experimental procedure

Pretreatment: Segments were incubated in the chamber for about 270 minutes. By this time the mean elongation rate of segments treated with IAA was 5.5µm/minute. After 100 minutes the mean rate had declined by only 0.3µm/minute, in 11 control segments (which were not loaded). The mean elongation rate of segments incubated without IAA was 2.2µm/minute, and fell to 2.0µm/minute over the next 100 minutes, in 7 control segments.

Load application: Before applying load the frequency of elongation readings was increased from one minute to ten second intervals. Load was applied by increasing the voltage to a predetermined value, beginning as one elongation reading was initiated, and being complete before the next reading (i.e. within ten seconds). Load was maintained at this initial value until elongation rate had stabilised (up to 100 minutes). Applied loads covered the range zero to 85 g (the equivalent of a five volt change).

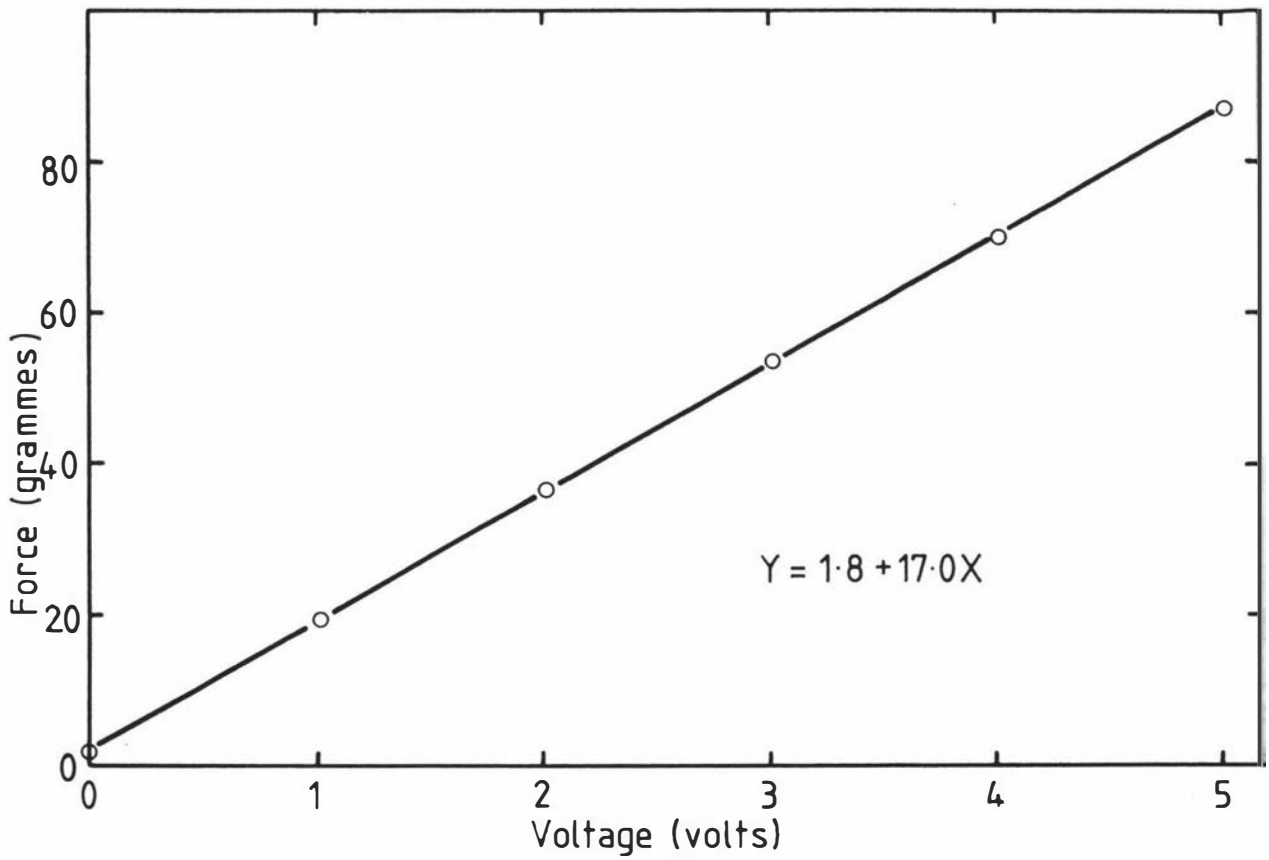

Fig. 4: Calibration curve for the coil-magnet apparatus.

Table I: Movement in the apparatus

Load	Displacement			
	After loading		After unloading	
	10sec	60sec	10sec	60sec
17	7.9	7.9	6.3	7.3
34	15.0	16.4	12.6	14.2
51	18.6	20.2	17.3	19.2
68	27.7	30.9	23.9	28.3
85	34.0	37.8	31.0	35.3

Units: Load = grammes
Displacement = μm

For each segment, an estimate was made of applied pressure (load/area). Mean cross-sectional area was estimated from the segment weight and length (measured before the segment was placed in the chamber), assuming a segment density of one g/cm^3, as area = mass/(length x one).

Load removal: The procedure was similar to that for load application. Voltage was rapidly reduced to zero (over 1-2 seconds).

Nitrogen treatment: The elongation of growing segments which were loaded and unloaded consisted of mechanical deformation superimposed on growth rate. Since I wished to know the time course of growth rate alone some means of estimating the amount of mechanical deformation was needed. In some experiments, after loading and unloading of the segment incubated in aerated buffer, the solution was gassed with oxygen-free nitrogen, and supplementary illumination of the segment stopped. (Preliminary experiments had indicated that reduction of growth rate with nitrogen treatment was more rapid without additional light). After 60-80 minutes elongation rate had fallen to a mean value of 0.9μm/minute, in IAA-treated segments; and to 0.6μm/minute in segments incubated without IAA. Load was applied and later removed, as described above. The response observed in the nitrogen-treated segment was assumed to be almost entirely mechanical (since the growth rate was very low), and was used as an estimate of the mechanical deformation which occurred when the growing segment was loaded.

This approach is the same, in principle, as that used to estimate the growth of Nitella cells (Green et al. 1971) or segments of rye coleoptile (Green and Cummins 1974) in response to turgor change. In each of those studies the response of a non-growing (or slowly growing) segment to a change in turgor was measured, and compared with the response of a growing segment to the same change. The difference between the two responses was interpreted as the response of growth to the imposed turgor change.

2.3 RESULTS

Results of experiments with segments incubated with and without IAA are discussed separately, and then compared. Within each section, responses of segments treated in aerated buffer or with nitrogen are discussed separately. In order to determine the "growth" response after loading or unloading in growing segments, it was necessary to estimate the extent of mechanical deformation which was superimposed on growth. This was done by comparing the response of growing segments with that of non-growing (nitrogen-treated) segments.

2.3.1 Segments incubated without IAA

Responses of nitrogen-treated segments

Segments which had been treated with nitrogen were subjected to a range of applied loads. Fig. 5 shows the results of one experiment, where change in length was recorded in response to application and removal of a 34 g load. [This load is equivalent to a pressure of 7.8g/mm^2 (or approximately 0.76 bar), calculated on the estimated mean cross-sectional area of the segment]. This result will be used as a model for discussion of results of the other experiments.

Response after loading: Since the elongation rate of the segment before loading was low (0.5μm/minute), the observed change in length was largely independent of growth, and the response depended on the mechanical properties of the segment. Fig. 5 shows that after loading, there was a large "instantaneous" compression of the segment followed by a more gradual approach towards an equilibrium length. The instantaneous compression (that occurring in the first ten seconds after loading) is shown in Fig. 6(C), for experiments covering a range of applied pressures. Fig. 6(D) shows "equilibrium" compression (the maximum compression recorded over the time that load was applied). Compression had reached a maximum (or was not less than 93% of the maximum recorded) within 20 minutes after application of load.

Response after unloading: Fig. 5 indicates that on removal of the compressive load, a complete reversal of the original compression

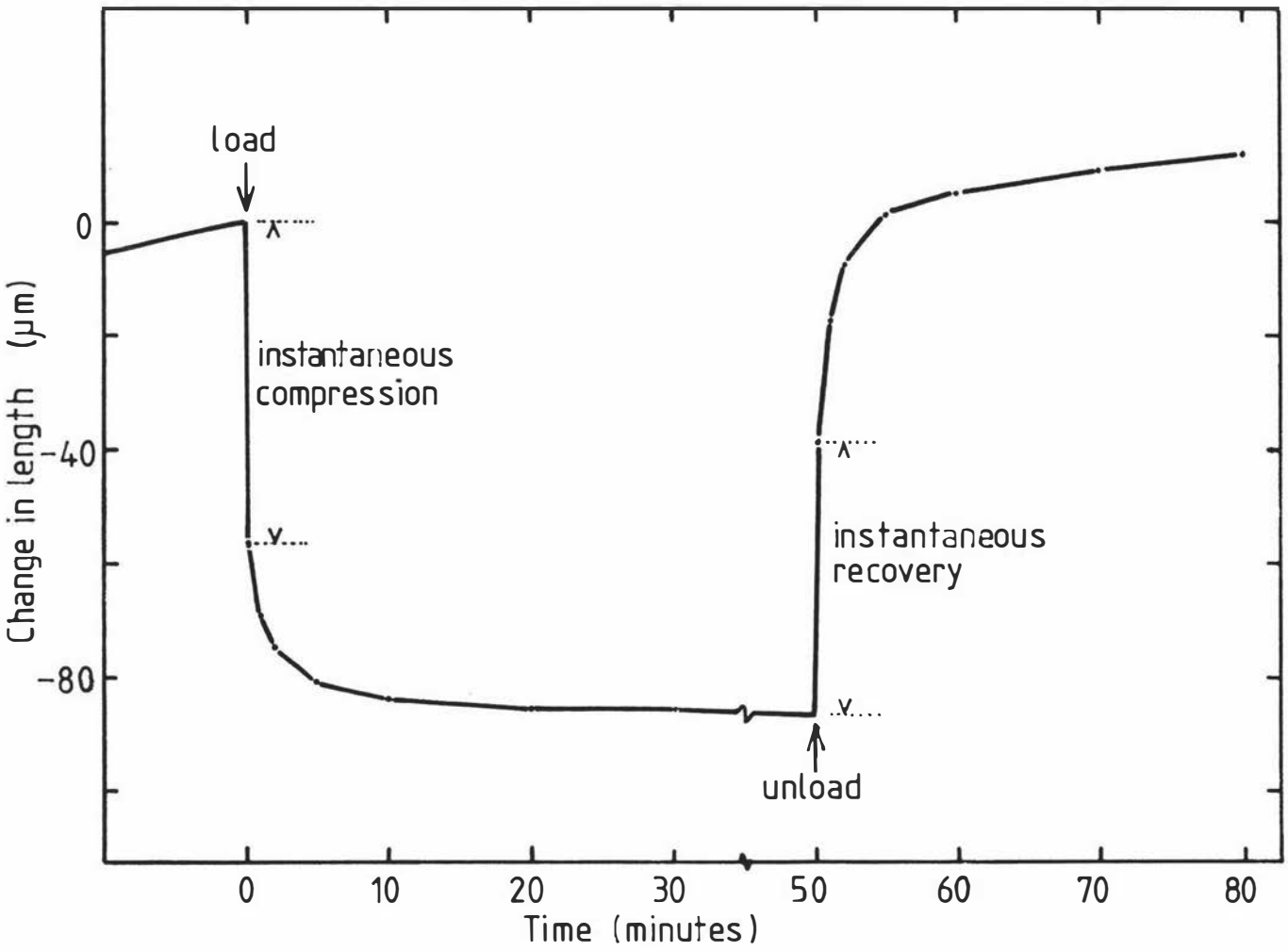

Fig. 5: Change in length of a nitrogen-treated segment after loading and unloading.

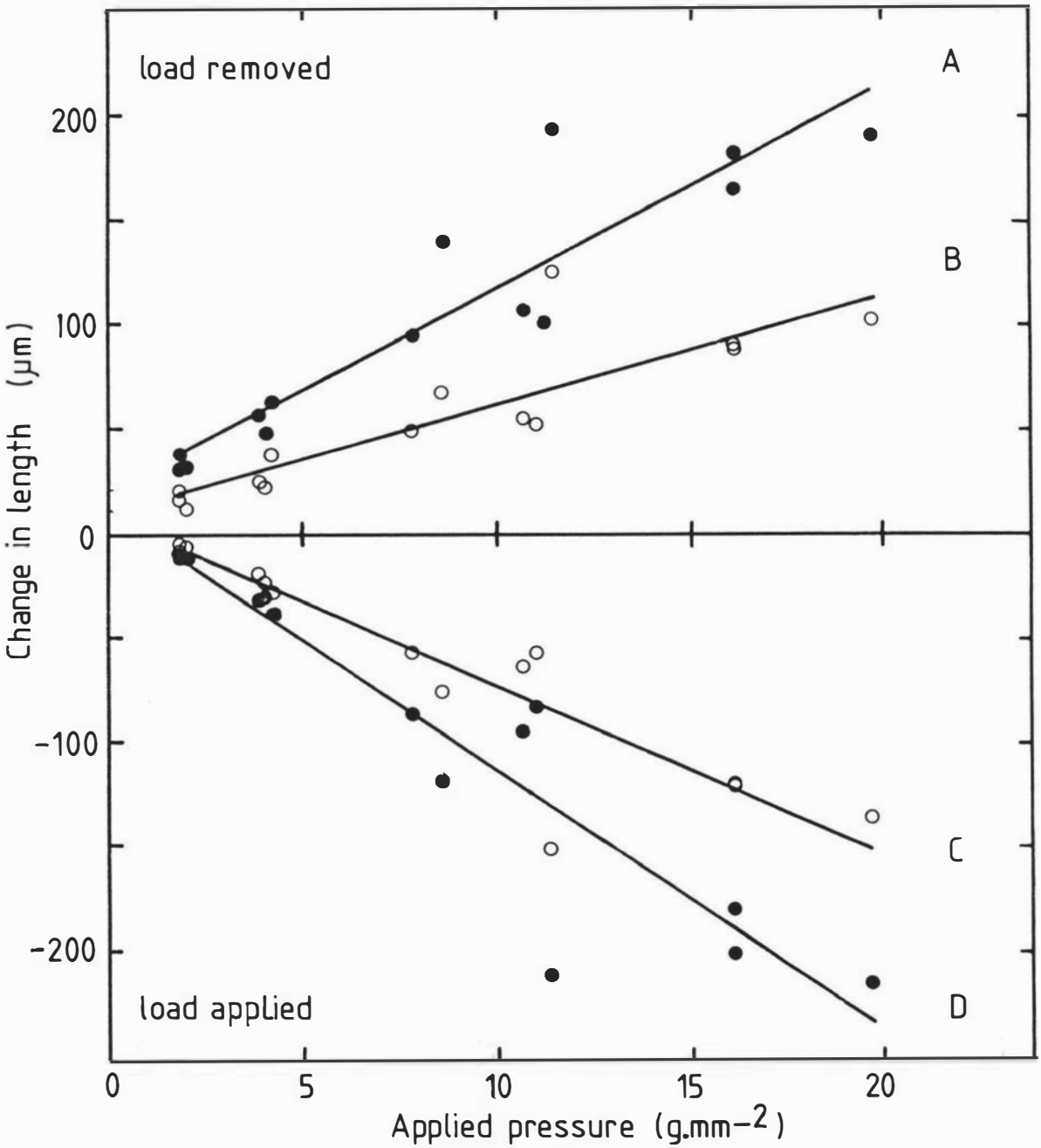

Fig. 6: Change in length of nitrogen-treated segments after loading and unloading, as a function of applied pressure. Each point is the response of one segment, measured 10 seconds after loading (C) or unloading (B), and at 20 minutes after unloading (A). (D) is the maximum compression recorded after loading.

occurred. The "instantaneous" recovery (that occurring in the first ten seconds of the response) is shown in Fig. 6(B), for all experiments. The change in length (recovery) at 20 minutes after removing the load is shown in Fig. 6(A).

Reversibility: Compression of segments after loading was completely recovered on unloading. This is illustrated in Fig. 6, where the change in length 20 minutes after unloading (A) can be compared with the maximum compression after loading (D). However, results suggest that the time course of recovery is not simply a reversal of the course of compression after loading. While the instantaneous recovery after unloading [Fig. 6(B)] was not significantly different ($t = 1.624$, $p > 0.05$) from instantaneous compression after loading [Fig. 6(C)], the subsequent increase in length did not reach an equilibrium, but rather continued increasing. This was probably due to continuing (albeit slow) growth of the segments.

Response of growing segments

Fig. 7 shows the change in length recorded in a growing segment in response to application and removal of a 34g load. The same segment was later treated with nitrogen and again loaded (Fig. 5).

Response after loading: Fig. 7 shows that although the segment was initially compressed, there was subsequently a recovery with growth. The initial compression appears similar to that observed after loading in the nitrogen-treated segment (Fig. 5). The instantaneous compression is shown in [Fig. 8(B)], with results for other experiments for the range of applied pressures. The compression observed up to the time when length started increasing again is shown in Fig. 8(C), with results for all other experiments.

Response after unloading: Fig. 7 indicates that on removal of the load compression was recovered, and that this recovery was superimposed on an additional response. The separate courses of the two responses were not immediately apparent; separation of growth from the total response is discussed in the next section. Instantaneous recovery is shown in Fig.8(A), with results of the other experiments with different applied

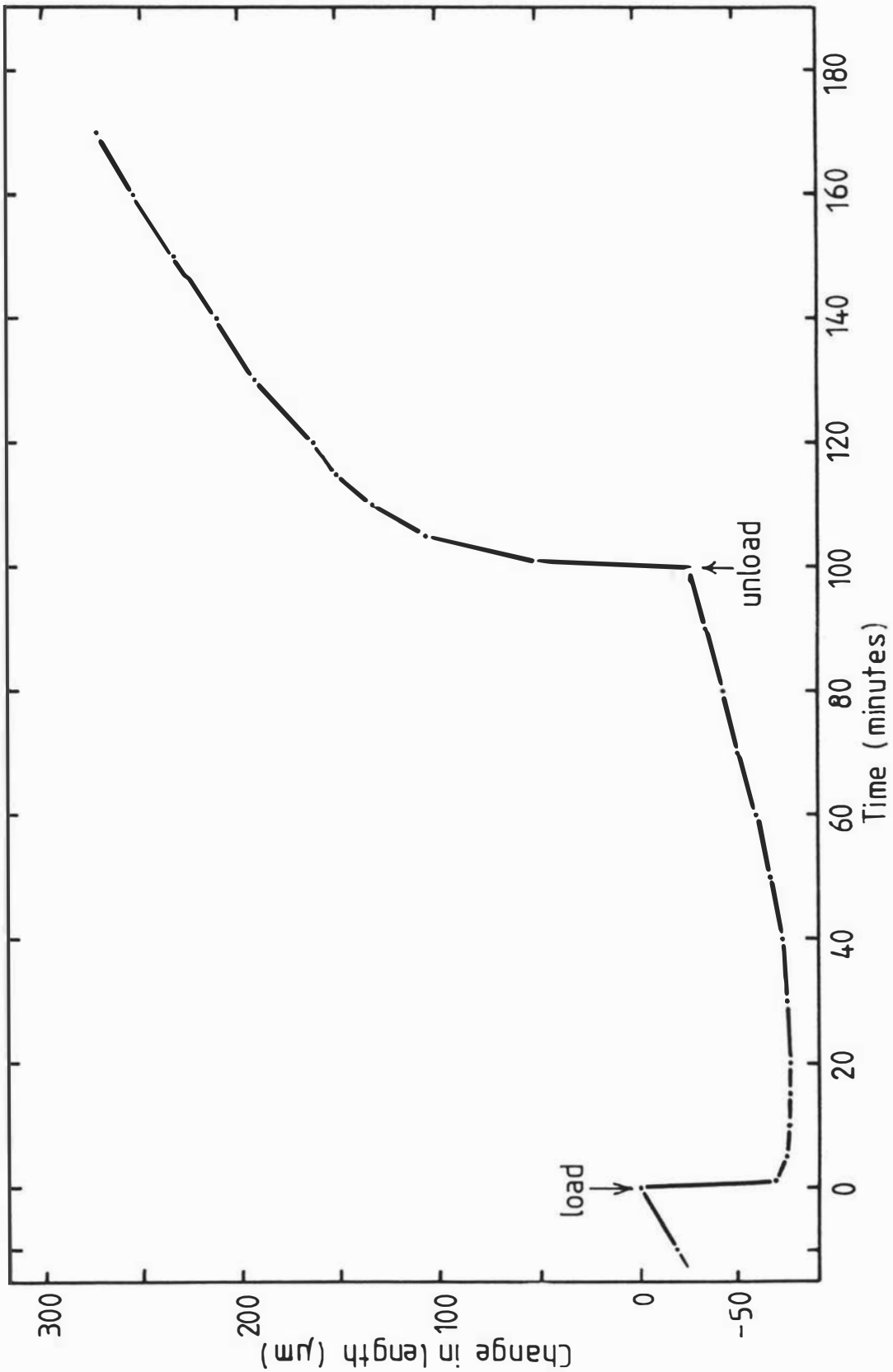

Fig. 7: Change in length of a growing segment after loading and unloading.

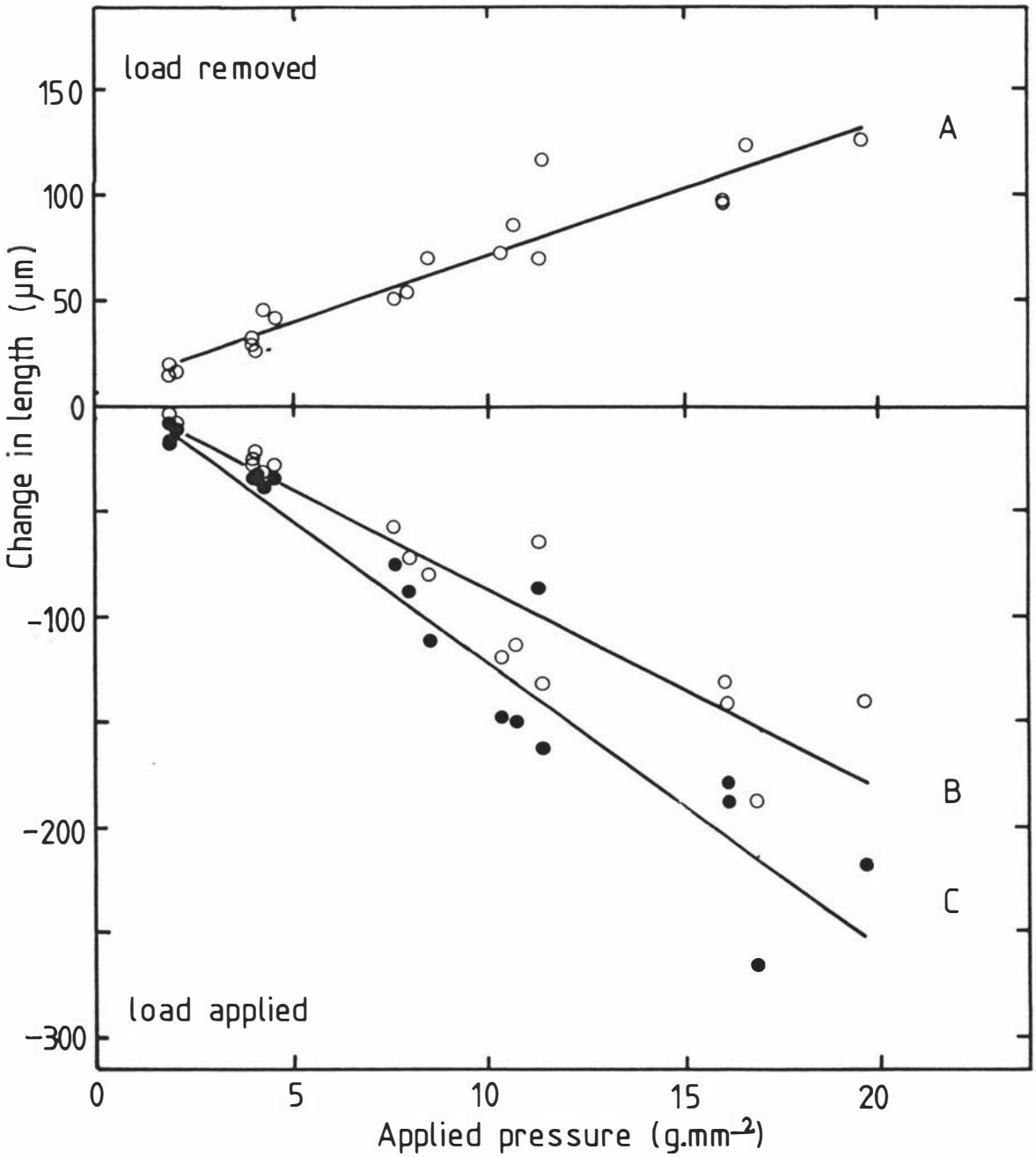

Fig. 8: Change in length of growing segments after loading and unloading, as a function of applied pressure. Each point is the response of one segment, measured 10 seconds after loading (B) or unloading (A), or at the time when compression had reached a maximum.

pressures. Although I might expect the instantaneous recovery after unloading to differ from the instantaneous compression after loading [Fig. 8(B)] (because of the effect of growth), comparison of the responses (paired) suggested that they were not significantly different ($t = 1.929$, $p>0.05$).

Procedure for determining the growth response

The change in length after loading and unloading in a growing segment (Fig. 7) represents physical deformation superimposed on elongation due to growth. The growth response alone was required, and this was obtained as follows.

Response after loading: Results suggested that compression after loading in the growing segments was the same as in nitrogen-treated segments. This was shown by these observations.

(1) The instantaneous compression in growing segments [Fig. 8(B)] was not significantly different ($t = 1.841$, $p > 0.05$) from that of nitrogen-treated segments [Fig. 6(C)] in comparison of results (paired) over the range of loads tested.

(2) The compression observed up to the time when length started increasing again in growing segments [Fig. 8(C)] was not significantly different ($t = 0.889$, $p > 0.20$) from the maximum compression of nitrogen-treated segments [Fig. 6(D)], in comparison of results (paired) over the range of loads tested. Growth responses were therefore estimated by subtracting from the observed response of the growing segment, the compression recorded when the loading procedure was repeated after the segment had been nitrogen-treated. The result of subtracting the data of Fig. 5 for loading, from that of Fig. 7 for loading, is shown in Fig. 9(D). This result suggests that growth rate was rapidly reduced to a low value after loading, and then slowly recovered. This observation was confirmed in many of the results of the other experiments, examples of which are included in Fig. 9. However, at higher loads (51g and greater) "growth" rate was not immediately reduced, but instead declined gradually over 10-20 minutes. This might be a real (growth) response, or might indicate that the nitrogen-treated segments were compressed more rapidly than the growing segments.

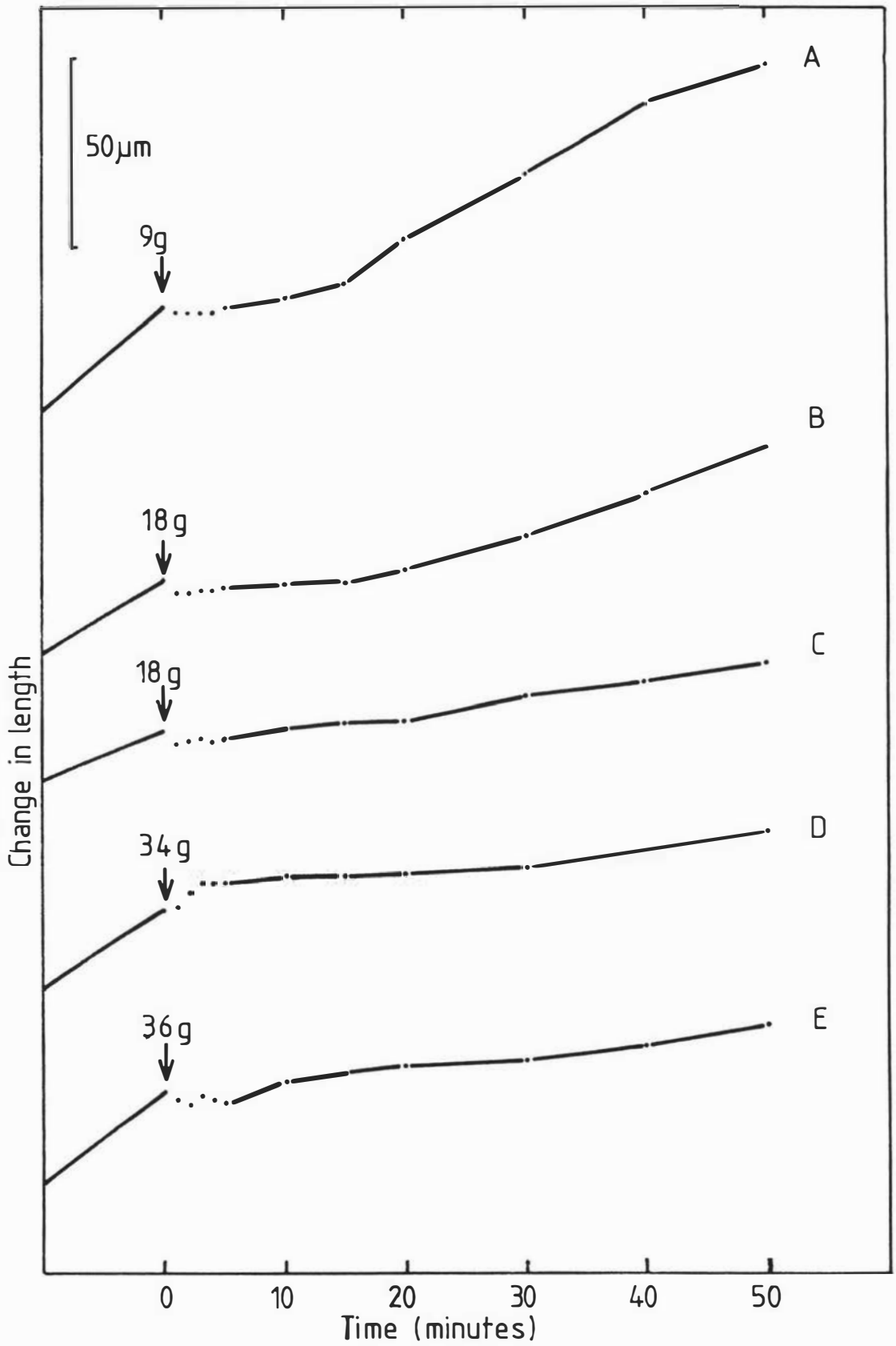

Fig. 9: Growth responses after loading, estimated from the difference of responses of growing and nitrogen-treated segments.

In many experiments the growing segment was loaded and unloaded, but was not subsequently nitrogen-treated for the loading procedure to be repeated. An alternative procedure was used for estimating the growth response in these experiments. If compression in the growing segment was the same as that in the nitrogen-treated segment, then it would approach equilibrium in the same way. In nitrogen-treated segments compression had reached maximum (or was within 93% of the maximum recorded value) at 10 minutes after loading (for loads of 18g or less); at 15 minutes (for loads of 34g); or at 20 minutes (for loads of 51-85g). It follows that at the same times in growing segments, compression would be complete or close to equilibrium. Therefore, at longer times the response in the growing segments would be (almost) entirely "growth". It is this part of the response which was used in further analysis, in all cases.

The second approach to estimating the growth response was the preferred method. It had a major advantage in obviating the need for nitrogen treatment, thus representing a saving in resources and time.

Response after unloading: Results suggested that compression after loading was recovered after unloading (experiments with nitrogentreated segments, page 21). I assume that the time course of recovery reversed the course of compression. There is no good evidence for this assumption, however, since the course of recovery of compression in nitrogen-treated segments (Fig. 5) was obscured by the small amount of growth occurring. On the basis of this assumption, at times after unloading greater than those given in the previous section, the response of growing segments would exclude most of the recovery from compression and thus be almost entirely "growth". The magnitude of the growth response was estimated by subtracting the maximum value of compression after loading, from the observed response after unloading. Examples of the growth responses obtained by this method are included in Fig. 12. Responses are those of the five segments whose responses after loading are shown in Fig. 9. The value of change in length (for the growth response) at 10 (or 15) minutes after unloading is also given.

Analysis of the growth response

The analysis of Cosgrove (1981) predicts that the turgor pressure, and therefore the growth rate, will show an exponential approach to a new steady-state value after growth rate has been changed from its original steady-state.

For the case where growth rate has been reduced from steady-state to a lower value (as happens when load is applied), this prediction can be expressed as

$$dL/dt = r_o + (r_s - r_o)[1 - \exp(-t/t_c)] \quad (7)$$

where L = length

r_s = new steady-state elongation rate

r_o = elongation rate at t=0

t_c = time constant

This equation is shown graphically in Fig. 10.

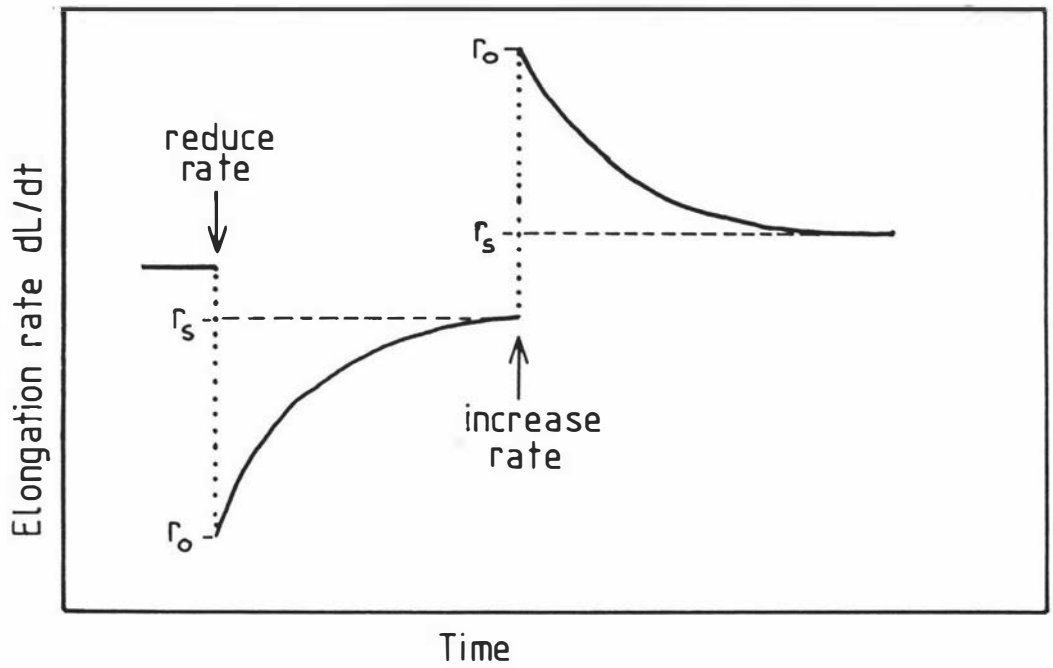

Fig. 10: Predicted response of elongation rate.

The equation is based on two simplifying assumptions. One is that diameter is not increasing, and so "growth rate" (1/V)(dV/dt) is equal to (1/L)(dL/dt). The second is that 1/L is constant, and if L=1 then

(1/L)(dL/dt) is equal to dL/dt. These are both reasonable assumptions for the segments of lupin hypocotyl used here, since segment radius increases only slowly in comparison with increase in length (Perley et al. 1975), and measurements were made over short times so that L increased at most by about 5%.

Integration of equation (7) gives

$$L(t) = r_s t + (r_s - r_o) t_c [\exp(-t/t_c) - 1] \qquad (8)$$

which is the prediction of the course of change in length as a function of time.

To test that growth responses after loading were of the predicted form, the fit of each response to equation (8) was estimated using a non-linear least squares procedure (Numerical Algorithms Group 1982). In all cases, a good fit was found; Fig. 11(D) shows the response of Fig. 7 replotted, with the fitted curve also shown. Examples of responses at different loads (those shown in Fig. 9), are also included in Fig. 11.

The fitted curves give values for the new steady-state rate (r_s), the initial rate (r_o), and the time constant (t_c) which are shown in Table II, as a function of applied pressure. Values of t_c have been used to calculate half-times, according to

$$\text{half-time } (t_{1/2}) = t_c . \ln 2$$
$$= 0.693 t_c \qquad (9)$$

and these are also given in Table II. Since the half-time increased with load, values of half-time at loads of 17-18g were used for comparison with results of IAA-treated segments. The mean half-time at this load was 11.2 minutes, with standard error 1.1 minutes.

A possible explanation of the increase in $t_{1/2}$ with increasing load is that the part of the observed response used in analysis still included a significant compression component. This would have the effect of

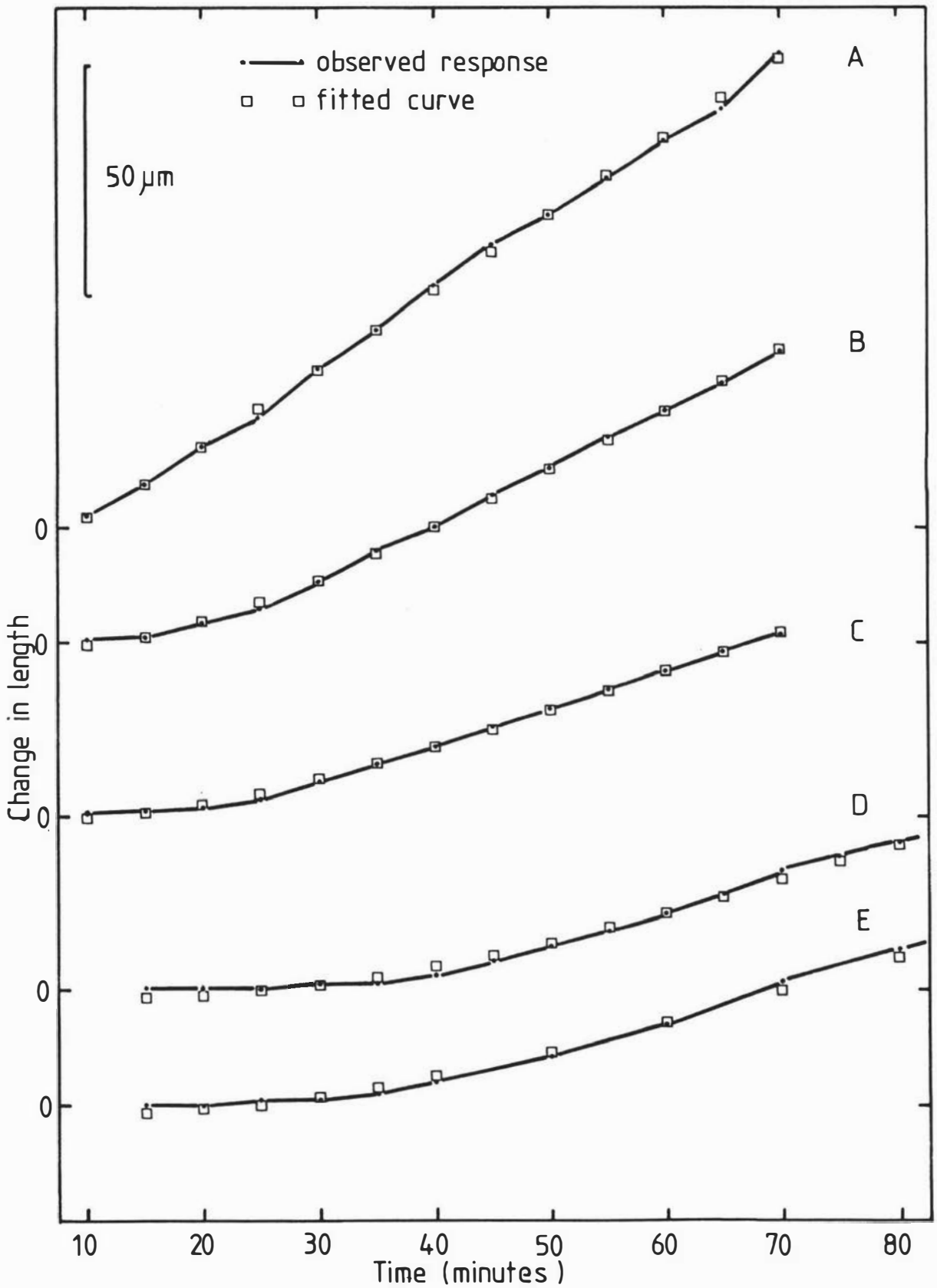

Fig. 11: Growth responses after loading, with fitted curves also shown.

Table II: Parameter values for the growth responses.

Load	Applied pressure	Load applied				Load removed			
		r_s	r_o	t_c	$t_{1/2}$	r_s	r_o	t_c	$t_{1/2}$
8.2	1.8	1.7	-2.3	4.8	3.3	1.3	5.3	10.3	7.2
9.0	1.8	1.7	-2.0	4.5	3.1	1.9	7.3	5.1	3.5
9.0	2.0	1.2	-2.0	3.7	2.6	1.5	10.0	2.5	1.7
17.7	3.8	1.3	-0.6	14.7	10.2	2.3	9.9	6.4	4.4
17.7	4.0	1.4	-0.6	16.9	11.7	2.2	15.7	4.6	3.2
17.7	4.1	1.0	-0.7	15.4	10.7	1.7	6.6	7.8	5.4
17.9	4.3	1.0	-0.3	21.5	14.9	2.8	12.9	4.4	3.0
17.5	4.6	1.4	-0.6	12.2	8.5	2.5	16.8	3.6	2.5
33.8	7.6	1.2	-0.3	54.1	37.4	2.0	12.3	8.2	5.7
33.8	8.0	*2.6	-0.1	245	170	2.3	12.1	7.6	5.3
36.2	8.5	0.8	-0.4	28.6	19.8	2.6	9.5	10.5	7.3
51.2	10.3	*1.1	-0.1	136	94.4	1.5	9.4	12.3	8.5
51.9	10.7	*3.7	-0.4	165	114	***			
51.2	11.3	1.3	-0.4	30.5	21.1	2.8	30.8	5.3	3.7
51.5	11.3	*2.0	-0.1	267	185	2.6	11.0	7.1	5.0
68.3	16.0	**				1.2	9.7	10.6	7.3
68.7	16.1	**				1.3	2.6	27.0	18.7
85.0	16.6	**							
85.0	19.7	**				1.2	8.6	20.4	14.1

Units are: Load = grammes

Applied pressure = g/mm^2

r_s, r_o = μm/minutes

t_c, $t_{1/2}$ = minutes

* see text page 34

** loading responses not fitted

*** satisfactory fit to equation (11) not obtained

slowing the approach of the observed response towards steady rate. The actual growth response might have reached steady rate much sooner, and would therefore have a shorter half-time than suggested by these results. Given this explanation, values of half-time at low loads might be the most reliable indication of the half-time of the growth response.

The fit of responses at loads of 68g or 85g was not tested, since it seemed likely that the apparent course of approach to the very low steady rate (less than 0.3μm/minute, at 90-120 minutes after loading) would be significantly affected by continuing compression (which was 0.1μm/minute on average over the period 20-50 minutes after loading in nitrogen-treated segments at the same loads).

Values of steady rate given by the fitted curves (r_s in Table II) are plotted as a function of applied pressure in Fig. 16, with the exception of those values marked (*). These values differed from values of rate calculated from the final 30 minutes of the observed response by more than 100%, suggesting that steady rate had not been reached in the time of measurement. This might be the case, but it is also possible that the course of approach to steady rate was affected by continuing compression. The values of steady rate estimated from the final 30 minutes of the observed response in these experiments and those at higher loads (68g and 85g) have been shown in Fig. 16, but were not included in the regression of steady rate on applied pressure.

For the case where growth rate is increased from steady-state to a higher value (as happens when load is removed), the prediction for the course of subsequent rate adjustment (Cosgrove 1981) can be expressed as

$$dL/dt = r_o + (r_o - r_s)[\exp(-t/t_c) - 1] \qquad (10)$$

which is shown graphically in Fig. 10, and which is integrated to give

$$L(t) = r_s t + (r_o - r_s) t_c [1 - \exp(-t/t_c)] \qquad (11)$$

To test that growth responses after unloading were of this predicted form, the fit of each response to equation (11) was estimated as described for the responses after loading. With one exception, good fit to equation (11) was found. Fig. 12(D) shows the response of Fig. 7 replotted with the fitted curve also shown. Other examples are also shown - these are the responses of the same segments whose responses after loading are shown in Fig. 11.

The fitted curves give values of r_s, r_o and t_c which are shown in Table II, with values for half-times calculated from t_c [equation (9)]. Half-times after unloading were lower than those after loading - the mean half-time for loads of 17-18g was 3.7 minutes. If the reduction in half-time represents a real change in the half-time of the growth response, then it could occur through a change in any of the parameters $L, Y, \sigma, \Delta\pi, \emptyset$, or ϵ during the time that load was applied.

Higher values of half-time at higher loads (Table II) might be explained in the same way as for the responses after loading.

2.3.2 IAA-treated segments

Results of experiments with segments incubated without IAA suggest that the method of applying load will cause sudden changes in growth rate and that the subsequent responses are consistent with predictions. The primary objective of the study has been satisfied. The secondary aim was to compare responses of segments incubated with and without IAA. In this section, results of experiments with IAA-treated segments are presented.

The responses of IAA-treated segments have been analysed in the same way as those of non-treated segments. In general they support conclusions already made about the method. A comparison of the results of IAA-treated and non-treated segments is made in the next section.

Responses of nitrogen-treated segments

Segments which had been treated with nitrogen were subjected to a range of applied loads. Responses were similar to those of segments

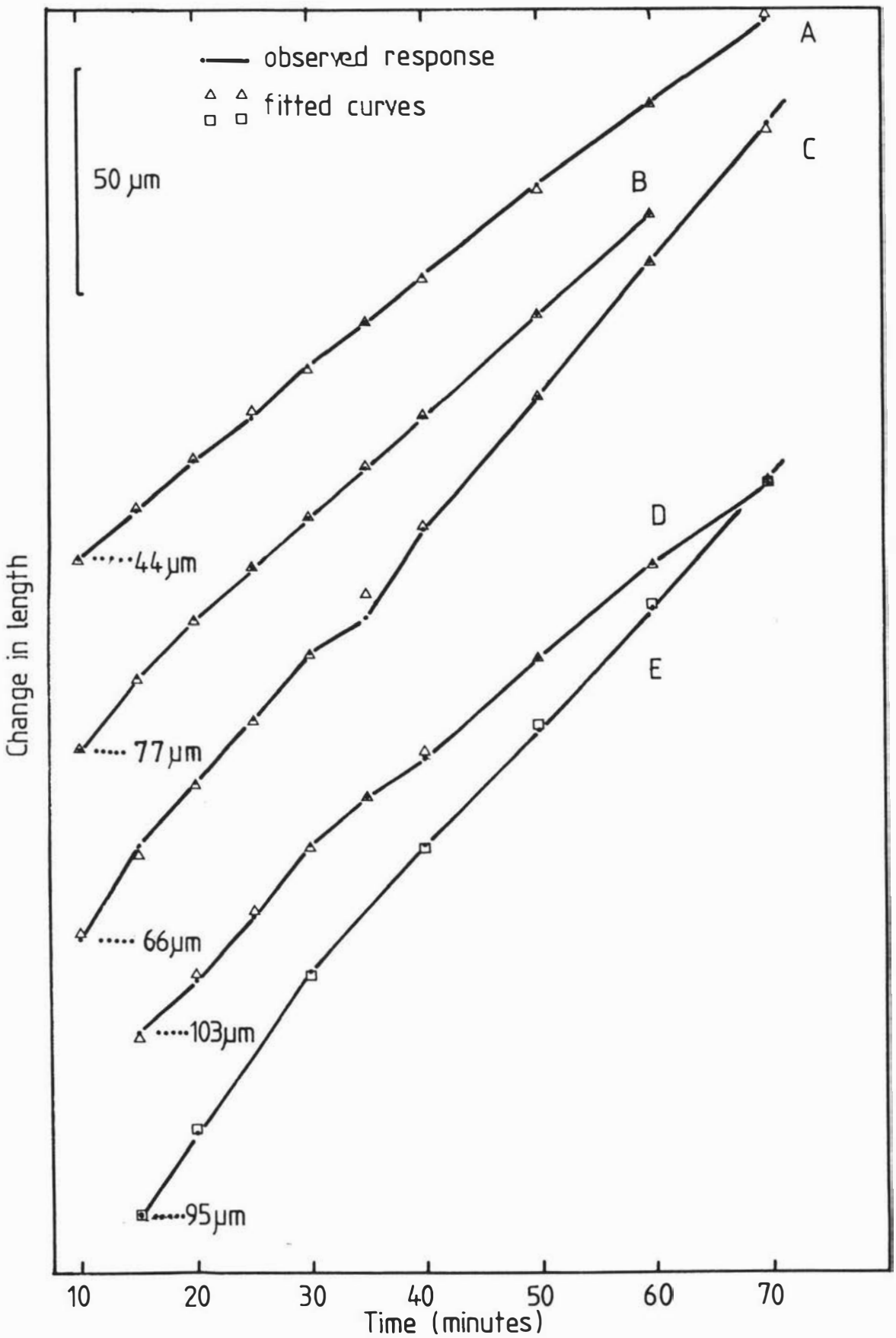

Fig. 12: Growth responses after unloading, with fitted curves shown.

incubated without IAA (Fig. 5), in that a large instantaneous compression was followed by a more gradual approach towards an equilibrium length.

The instantaneous compression (in the first ten seconds after loading) is shown in Fig. 13(C), as a function of applied pressure, for all experiments. The instantaneous recovery after unloading is shown in Fig. 13(B).

Values of maximum compression after loading are shown in Fig. 13(D). As with segments incubated without IAA, after unloading the segment length did not reach equilibrium but rather continued increasing, presumably because the segment was still growing slowly. The change in length at 20 minutes after unloading is shown in Fig. 13(A), to indicate that by this time the compression which occurred after loading [Fig. 13(D)] had been recovered. Instantaneous compression after loading was recovered "instantaneously" after unloading, since there was no significant difference ($t = 0.548$, $p > 0.5$) between the responses shown in Fig. 13(B and C) (paired responses were compared).

Responses of growing segments

The responses of growing segments after loading and unloading were similar in form to those of segments incubated without IAA (Fig. 7). After loading segments were initially compressed, and later recovered. The instantaneous compression (in the first ten seconds) is shown in Fig. 14(B), for experiments covering a range of applied pressures. The compression recorded up to the time when length started increasing again is shown in Fig. 14(C).

The instantaneous recovery after unloading [Fig. 14(A)] was significantly greater ($t = 2.816$, $p < 0.02$) than the instantaneous compression after loading [Fig. 14(C)] (paired responses compared). This presumably reflects the effect of growth.

Determination of the growth response

To separate the mechanical response from the response of growth after loading and unloading, the same methods were used as for segments

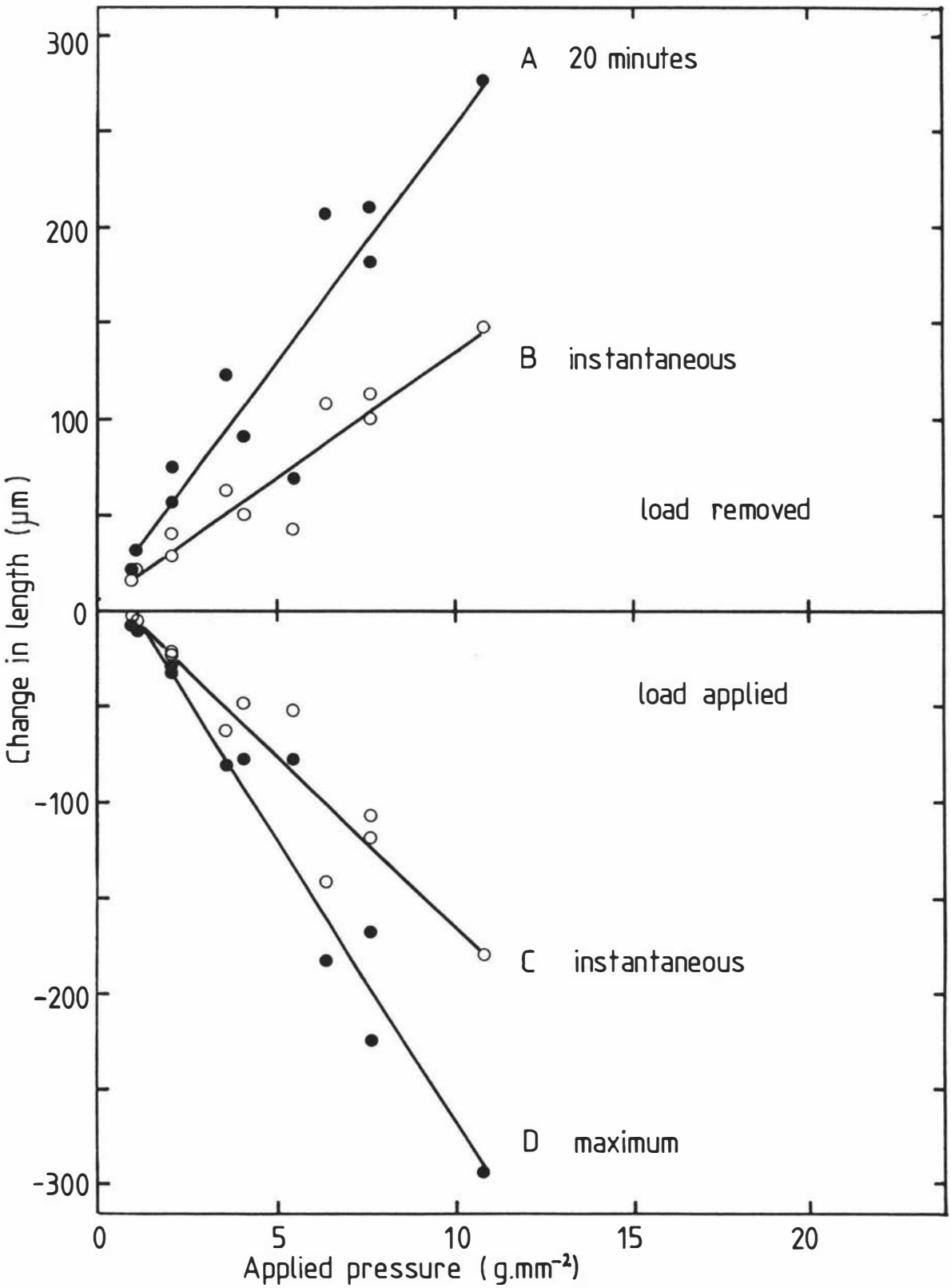

Fig. 13: Change in length of nitrogen-treated segments (IAA-treated) after loading and unloading, as a function of applied pressure.

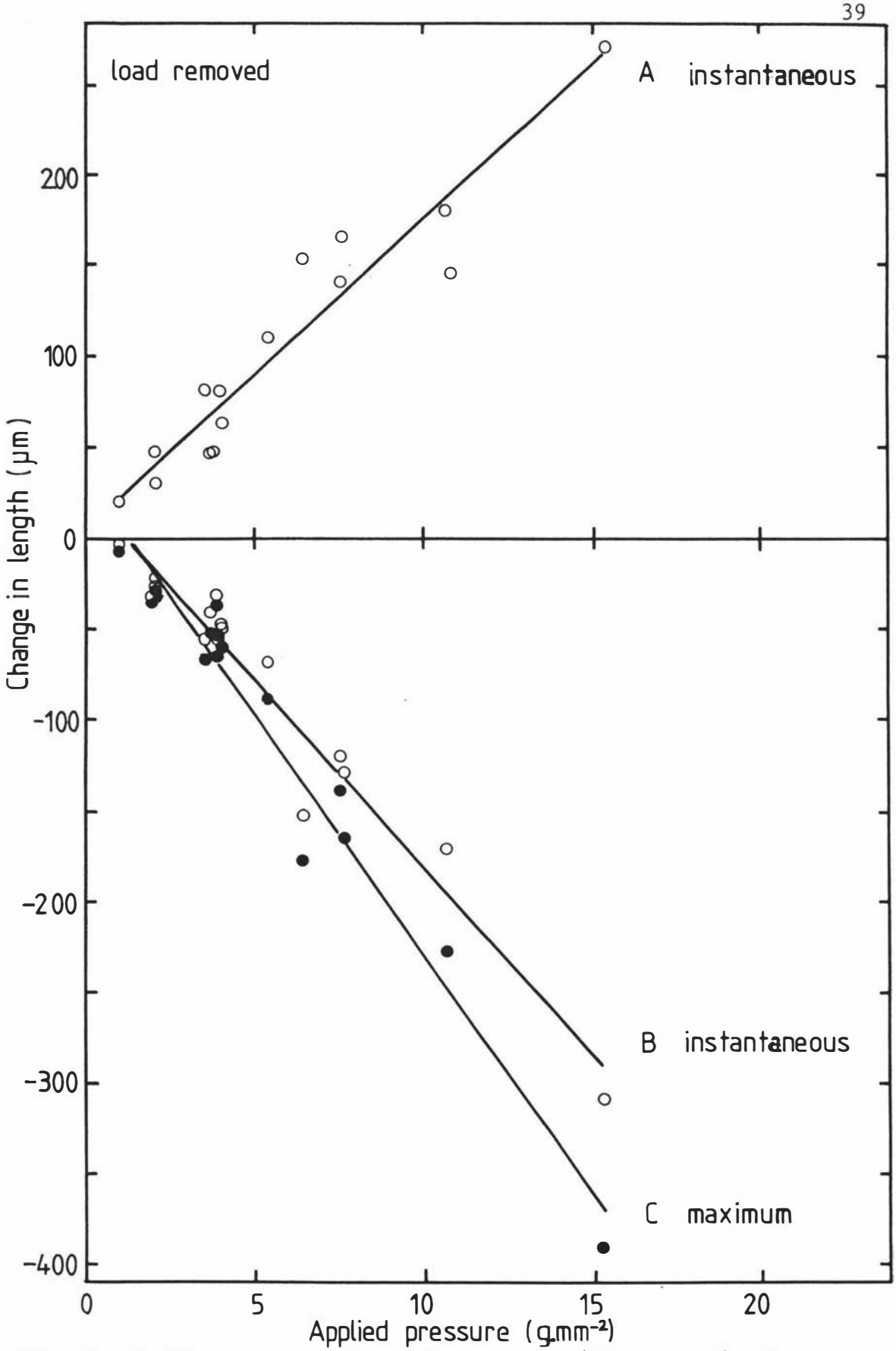

Fig. 14: Change in length of growing segments (IAA-treated) after loading and unloading, as a function of applied pressure.

incubated without IAA.

Response after loading: In those experiments where segments were loaded both when growing rapidly and also after treatment with nitrogen, the growth response was estimated by subtracting the latter response from the former response, assuming that the compression in the nitrogen-treated segment was the same as that in the growing segment. Since values of maximum compression in the nitrogen-treated segment [Fig.13(D)] were not significantly different ($t = 1.649$, $p > 0.10$) from values of compression up to the time when length started increasing again in growing segments [Fig. 14(C)], this assumption appeared justified. However, the instantaneous compression in nitrogen-treated [Fig.13(B)] and growing [Fig.14(B))] segments was not the same - paired comparisons indicated significantly greater ($t = 2.484$, $p < 0.05$) compression in the latter.

The results of subtracting the responses of nitrogen-treated segments from the responses of growing segments were similar to the results for non-IAA-treated segments, in that growth rate was rapidly reduced to a low value, from which it subsequently recovered. The one exception was the single experiment at a load of 51g, where rate apparently declined over a period of 15 minutes after loading.

If compression in the growing segment followed the same course as in the nitrogen-treated segment, then it would be complete (or largely complete) at the same time. In nitrogen-treated segments, compression had reached a maximum (or was not less than 96% of the maximum recorded compression) at 10 minutes, for loads of 18g or less; at 15 minutes, for loads of 34g; and at 20 minutes, for loads of 51-68g. Therefore, that part of the response in growing segments after these times was considered to be "growth" response, and was used in further analysis.

Responses after unloading were not analysed, since in most cases elongation rate did not stabilise but instead continued decreasing. A similar decline in rate was also seen in control segments (not loaded) over the same period.

Analysis of the growth response

Responses after loading have been fitted to equation (8). The fit was good in all cases; Fig. 15 shows examples of responses with fitted curves also included.

Fitted values of the new steady-state rate (r_s), the initial rate (r_o), and the time constant (t_c) are given in Table III, with half-times calculated from t_c [equation (9)]. The results at loads of 17-18g were used for comparison with the results of segments incubated without IAA. The mean half-time of those results was 9.1 minutes, with standard error 1.2 minutes. Values of new steady rate (r_s) are shown in Fig. 16 as a function of applied pressure.

2.3.3 Comparison of responses of IAA-treated and non-treated segments

Mechanical properties

The mechanical properties of IAA-treated and non-treated segments differed. This was seen in comparison of compression after loading. In nitrogen-treated segments, the slopes of regression lines of instantaneous compression on applied pressure [Figs. 6(C) and 13(C)], and of maximum compression on applied pressure [Figs. 6(D) and 13(D)], were significantly greater in IAA-treated segments.[For instantaneous compression, $t = 4.329$, $p < 0.001$; for maximum compression, $t = 5.519$, $p < 0.001$. The regression coefficients were compared using the method of Bailey (1981)]. As expected, in growing segments the slopes of the regression of instantaneous compression on applied pressure [Figs. 8(B) and 14(B)], and of maximum compression on applied pressure [Figs. 8(C) and 14(C)], were significantly greater in IAA-treated segments. (For instantaneous compression, $t = 7.116$, $p < 0.001$; for maximum compression, $t = 8.445$, $p < 0.001$).

Growth responses:

(1) Half-times: The half-times of the growth rate transients after loading (for loads of 17-18g) were lower in IAA-treated segments, but not significantly so ($t = 1.327$, $p > 0.20$).

(2) The slope of the plot of steady rate against applied pressure is greater for IAA-treated segments than for segments incubated without

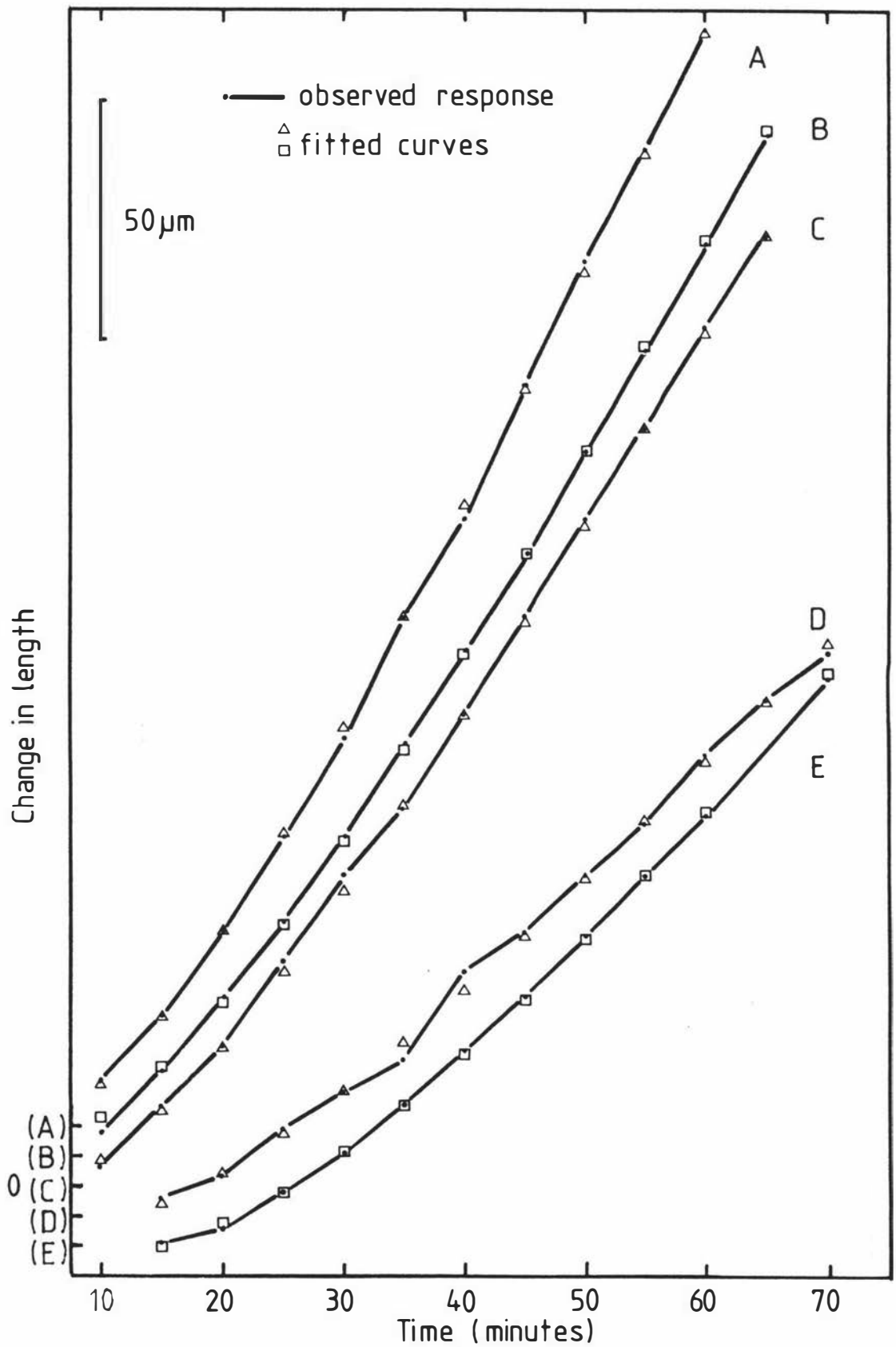

Fig. 15: Growth responses after loading (IAA-treated segments), with fitted curves also shown.

Table III: Parameter values for growth responses
(IAA-treated segments)

Load	Applied pressure	r_s	r_o	t_c	$t_{½}$
4.3	1.0	3.5	0.3	9.3	6.4
9.4	1.9	5.0	-1.1	12.0	8.3
17.5	3.6	4.2	-0.8	14.7	10.2
17.7	3.7	2.7	-1.4	8.6	6.0
17.5	3.9	4.2	-2.0	8.7	6.1
17.3	4.0	4.7	-0.3	18.8	13.1
17.5	4.0	3.5	-1.7	11.2	7.8
34.5	7.6	2.6	-1.0	16.7	11.6
34.5	7.6	3.4	-0.9	30.3	21.0
51.2	10.7	2.9	-0.8	29.9	20.7
68.5	15.4	1.3	-0.5	37.9	26.3

Units: Load = grammes
Applied pressure = g/mm^2
r_s, r_o = um/minute
t_c, $t_{½}$ = minutes

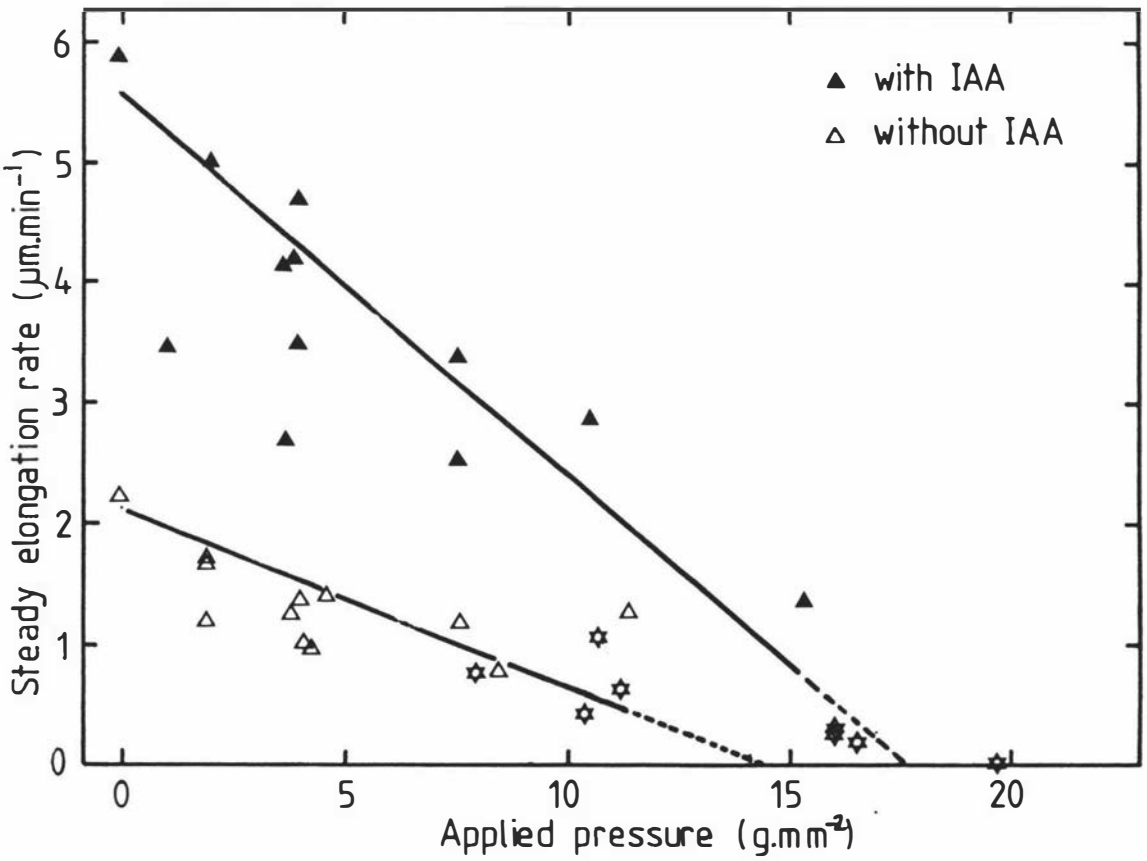

Fig. 16: Steady elongation rate of IAA-treated and non-treated segments as a function of applied pressure. Points shown as * have not been included in the regression analysis for non-treated segments.
The points at zero load are the means of the elongation rates before loading.

IAA (Fig. 16). Since there are no values for IAA-treated segments at higher pressures, and because there is some doubt about the values for non-IAA-treated segments at high pressures (page 34), it is not possible to make a comparison of the X-intercept for the two treatments. However, if the relationship between steady rate and applied pressure is linear over the whole range of applied pressures, extrapolation of regression lines suggests that the X-intercept might be the same for the two treatments.

These results suggest that L (hydraulic conductivity) or $\emptyset$ (extensibility) has been increased by IAA treatment, but that Y (yield threshold) has remained unchanged.

2.4 DISCUSSION

The first objective of this study was to establish a method which would enable the growth (elongation) of lupin hypocotyl segments to be suddenly changed from steady state, and the growth rate to be simultaneously measured. The applied force technique meets these requirements. In addition, the method yields results which are consistent with those obtained by other investigators.

Growth responses are of the form predicted by Cosgrove (1981) and shown to occur in the studies of Green and Cummins (1974), and Cosgrove and Green (1981). Responses of non-growing (nitrogen-treated) segments are of the form expected from the results of Ferrier and Dainty (1977) for compressive loading of non-growing onion epidermis. Such a response depends on tissue rigidity, which is a function of cell wall elasticity and turgor pressure (Falk *et al.* 1958); and also reflects shrinkage as a consequence of water efflux due to the increased hydrostatic pressure (Ferrier and Dainty 1977). The effect of IAA on responses of non-growing segments (in increasing the amount of compression which occurs after loading) might then be due to an increase in elastic compliance (strain/stress) of the cell walls, or to reduced turgor pressure. This is consistent with observations of increases in elastic compliance after auxin treatment, in other materials (Cleland 1971); and with the reduction in turgor pressure seen in IAA-treated hypocotyls of soybean seedlings (Boyer and Wu 1978).

The secondary aim was to use results to determine the effect of IAA on growth, in terms of the parameters of the biophysical model of Lockhart (1965) and using the criteria detailed by Cosgrove (1981). This requires that three quantities be measured: P_s (the steady-state turgor pressure); $\dot{v}_s$ (the steady-state growth rate); and the half-time for approach of growth rate to steady-state, after rate has been changed. I have measured two of these: $\dot{v}_s$, as a function of applied pressure; and the half-time. My results suggest that there is no significant difference between half-times of responses of segments treated with or without IAA. Also, the slope of the curve of $\dot{v}_s$ against applied pressure is steeper for IAA-treated segments than for segments incubated without IAA. In terms of the analysis presented by Cosgrove (1981), these two results suggest that in IAA-treated segments, either L (the hydraulic conductivity), or ϕ (extensibility), is higher than in segments not treated with IAA. These two alternatives could be distinguished by measuring P_s.

Some qualification of this summary of conclusions is necessary. Firstly, it is not obvious how applied pressure may be interpreted in terms of the analysis of Cosgrove (1981). A possible explanation of the relationship is as follows. Firstly, an applied external pressure will increase the hydrostatic pressure in (cells of) the segment (Ferrier and Dainty 1977). In terms of equations (1) and (2) this would result in an increase in $(1/V_o)(dV_o/dt)$ and a reduction in $(1/V)(dV/dt)$. My results which suggest an immediate reduction in observed growth rate $(1/V)(dV/dt)$ after loading are consistent with this prediction. Turgor pressure would subsequently decrease, and $(1/V_o)/(dV_o/dt)$ decrease while the observed growth rate $(1/V)(dV/dt)$ increased (Cosgrove 1981), as seen in my experiments. A second effect of applied pressure is to reduce the stress (in the longitudinal walls of cells of the segment) which is acting to drive longitudinal extension. This stress arises from the action of turgor pressure on the cell wall (Nobel 1974). This effect of applied pressure is then viewed as equivalent in effect to a reduction in turgor pressure, in terms of effects on stress in the longitudinal walls. [However, it is likely that applied pressure will have a greater effect than an equivalent reduction in turgor pressure (Ferrier and Dainty 1978)].

With conventional methods, a reduction in turgor pressure would be accomplished by reducing the external water potential (using an osmoticum). From a qualitative viewpoint, then, increasing applied pressure is the same as reducing the external water potential. The slope of the relationship between $\dot{v}_s$ and applied pressure is then qualitatively equivalent to the (-)slope of a plot of $\dot{v}_s$ against external water potential, and is thus a measure of $L.\phi/(L+\phi)$ (Cosgrove 1981). The quantitative relationship between applied pressure and external water potential need not be established, since all that is required is to compare $\dot{v}_s$ in IAA-treated and non-treated segments across a range of applied pressures.

The second point concerns the relationship of the biophysical model to lupin hypocotyl segments. If the epidermis (which has a cuticle, and stomata) is the major path for water uptake into the segment, and if it provides the limiting resistance to flow in that path, then the segment can be regarded as analogous with a single cell. The model given by equation (3) will then apply, with the hydraulic conductivity (L) that of the epidermis. If the epidermis is not the major path for water entry (i.e. water entry is mainly through the cut ends of the segment), then the modified form of the model, which acounts for distributed resistance to water flow (Cosgrove 1981), will apply. The rate of uptake of water for growth is governed by the rate of diffusion through the tissue, and L in the diagnostic scheme of Cosgrove (1981) is replaced by D (the tissue free energy diffusivity of water). Since the path of water uptake in lupin hypocotyl segments is not known, it is not clear which form of the model will apply.

While I cannot distinguish whether IAA affects L (or D) or ϕ, Penny (1977) has shown that the conductivity of the epidermis of lupin hypocotyls could be increased (by ether-dipping, which removes some of the cuticle) without an increase in elongation rate. If the epidermis is the major pathway for water flow into the segment, then this result suggests that an IAA-induced increase in growth rate could not be caused by an increase in conductivity of that barrier. If this is the case, then my results suggest that IAA has acted through increasing extensibility. However, if the major path for water entry is through

the cut ends of the segment then the possibility remains that IAA could cause an increase in rate through an effect on tissue free energy diffusivity (D). In other cases, reports of auxin-induced effects on conductivity in growing tissues are conflicting. For example, Dowler et al. (1974) have shown that IAA did not increase the conductivity of pea stem segments, while Boyer and Wu (1978) have suggested that auxin increased both hydraulic conductivity (of the path for water flow radially outwards from the xylem) and extensibility in soybean seedlings. However, Cosgrove (1981) has argued instead that the results of Boyer and Wu (1978) indicated that the effect was mainly on extensibility, since turgor pressure apparently decreased. Auxin-induced effect on extensibility has been suggested by Green and Cummins (1974), who measured the elongation of segments of rye coleoptile in response to step-changes in external water potential.

I have suggested that it is necessary to measure the steady-state turgor pressure (P_s) in order to distinguish between the two alternative possible mechanisms of IAA action (L or D vs. $\emptyset$). Methods for measuring turgor pressure are discussed by Cosgrove (1981). A more direct approach would be to measure L (or D). In principle the analysis of Ferrier and Dainty (1977, 1978) could be used with the results (for compression of nitrogen-treated segments) of this study. However, a preliminary inspection of results suggests that it might be difficult to separate the component of the response which is due to water efflux from the part of the response which depends on tissue rigidity. Nonetheless, it may be worthwhile persevering with this approach.

It is important to note that the conclusions of my study do not necessarily apply to the initial mechanism of IAA action, since the results were obtained only after a prolonged period of incubation of segments in IAA. It is also worth remembering that "extensibility" is not simply a physical property of the cell walls of the segment, but rather reflects the action of some process on cell walls (Green and Cummins 1974, Green et al. 1977). Measurements of physical "extensibility" (i.e. mechanical properties) of cell walls of lupin hypocotyl segments (Penny et al. 1972) show an increase in plastic

(irreversible) compliance (strain/stress) after auxin treatment. However, increase in elongation rate preceded this change, and so it is possible that increased compliance was an effect rather than a cause of the increase in elongation rate. In some other studies, though, there is evidence of changes in physical properties preceding auxin-induced changes in growth rate of segments (reviewed by Penny and Penny 1978).

In the absence of direct measurement of turgor pressure, it may be possible to use measurements of physical properties of cell walls in conjunction with measurements of tissue rigidity (using a compressive force) to determine whether the initial action of IAA is accompanied by a change in turgor pressure. If a decrease in tissue rigidity cannot be accounted for by an increase in elastic compliance, then a reduction in turgor (and hence IAA action on ϕ rather than on L or D) would be indicated.

The studies of Green and Cummins (1974) and Green et al. (1977) are examples of the use of osmotica to perturb growth rate. After a step-change in external water potential, elastic extension or shrinkage of the segment occurred (with discharge of the imposed water potential gradient) superimposed on the "growth" response of the segment. The rapid osmo-elastic transients observed suggested that the method was appropriate for the material used - rye coleoptiles, grown in penta-erythritol to suppress cuticle development, and with both the inner and outer surfaces of the coleoptile in contact with the osmoticum. The growth response was apparently an exponential approach of rate towards a steady value, with a half-time of 6-10 minutes (Green and Cummins 1974), or about 5 minutes (Green et al. 1977). Other measurements of the half-times of transients in growth rate have been made by Cosgrove and Green (1981), who measured the elongation of hypocotyls of sunflower and cucumber seedlings in response to an increase in hydrostatic pressure of the water around the roots of the seedlings. The half-times of growth rate transients were 70-150 seconds for sunflower, and 15-35 seconds for cucumber. In lupin hypocotyl segments, the half-times (at low loads) of growth rate transients after rate had been changed with an applied force are similar to those obtained by Green and Cummins (1974) and Green

et al. (1977); but higher than the half-times measured by Cosgrove and Green (1981).

Unless turgor pressure can be measured, there is no assurance that observed transients reflect a change in growth rate due to a corresponding change in turgor pressure. The observed behaviour could be accounted for by any process which limits elongation rate and whose rate shows first order behaviour [i.e., could be described by equations of the form of equations (7) and (10)]. Changes in turgor may be rapid, but changes in growth rate protracted due to change in the yielding properties of cell walls. Such a response has been shown in Nitella (Green et al. 1971) and suggested to occur in the rye coleoptiles in the studies of Green and Cummins (1974), and Green et al. (1977). The latter have speculated that a continuous activity which could vary in rate, such as flux of vesicles to the cell wall, could account for the change in growth rate. Since the basic model of Lockhart (1965) does not explicitly account for the effects of such metabolic activities on growth, additional assumptions must be made if the model is to be retained. For example Green et al. (1971) assume that the minimum yield threshold (Y) is under metabolic control, and incorporate in the model a statement showing Y as a time-dependent function of several parameters; and Lockhart (1965) provides additional solutions of the model which incorporate the assumptions that extensibility or osmotic pressure are functions of time.

If such modifications are to be made to the model for single cells on the basis of the behaviour of segments of tissue, it is firstly necessary to test the assumption that cellular behaviour can be inferred from the responses of segments. Results of such tests for segments of lupin hypocotyl are reported in the next chapter.

3: TISSUE INTERACTIONS IN IAA-INDUCED RAPID ELONGATION RESPONSES *

3.1 INTRODUCTION

The rapid elongation response of excised plant segments to auxin has been frequently observed to show two rate maxima (Vanderhoef et al. 1976, Penny and Penny 1978). One hypothesis which accounts for the observed response is that there is a single reaction mechanism, with oscillation in elongation rate due to the action of a negative feedback system (Penny and Penny 1978). A second is that there are two separate responses (Vanderhoef et al. 1976, Penny and Penny 1978). Another explanation is that rapid elongation is initiated by wall loosening, and then fluctuates with turgor changes (Cleland and Rayle 1978).

Implicit in these hypotheses is the assumption that cellular behaviour and molecular mechanisms can be inferred from the gross responses of segments of hypocotyl, stem, or coleoptile. Evidence that not all cells in a stem respond equally to auxin is seen in the inward bending of auxin-treated split stems (Thimann and Schneider 1938). There is also evidence of differential tissue response to auxin in results of straight-growth experiments with stem segments (Masuda and Yamamoto 1972, Brummell and Hall 1980). Using pea stem segments, peeled to remove the epidermis, or bored to remove the pith, Masuda and Yamamoto (1972) conclude "that the induction of stem elongation by auxin, at least in its initial stage, is brought about by the removal of restraint of the stem tissues by the epidermis." There is therefore the possibility that the two maxima observed in rapid responses could be the result of an interaction of tissue responses, with differences in timing or degree of response to auxin. This hypothesis is tested here with lupin hypocotyl segments. Results of similar experiments with mung bean hypocotyl segments have recently been reported (Prat and Roland 1980).

* This chapter has been accepted for publication substantially in this form.

3.2 MATERIALS AND METHODS

3.2.1 Plant materials

Four-day old lupin seedlings (*Lupinus angustifolius* cv. N.Z. Bitter Blue) were grown in continuous low light at 22C (Penny 1969). The light was a mixture of fluorescent and incandescent, with PAR 60-70 μeinsteins $m^{-2}s^{-1}$. Seedlings for experiment were selected with hypocotyl 50-60mm long, minor diameter 2.2-2.3mm, and major diameter 2.4-2.6mm. (The hypocotyl is elliptic in cross section).

3.2.2 Preparation of segments

Segments used in IAA treatments were prepared as follows.

(a) "Intact" segments, 10mm long, were excised from the hypocotyl 2-3mm below the cotyledonary node, using a double-bladed cutter.

(b) "Peeled" segments were prepared from 15mm segments which were peeled from both ends using a razor blade and fine forceps, and then trimmed to 10mm. The segment surface was kept moist with water during this procedure. Peeling removed the epidermis, the pigmented sub-epidermal layer, and in places one or two other cell layers. The cell layers removed will be referred to hereafter as "epidermis". Peeling was considered complete when there was no visual evidence for remaining pigmented cells.

(c) "Outer tissue cylinders" were obtained by cutting the hypocotyl 15mm below the cotyledons and pushing a thin-walled glass tube 1.50mm in external diameter through the centre of the hypocotyl from the cut end so that the "central tissues" (vascular tissue and pith) were separated from the outer tissues. The boring tube was withdrawn, a 10mm segment was cut and the central core removed and discarded. Microscopic examination confirmed that the hollow cylinder consisted of epidermis and cortex only.

(d) "Central tissue" cores were prepared as above.

(e) "Cortex cylinders", hollow cylinders 7-12 cells wide consisting solely of cortex parenchyma, were prepared by boring as in (c), and were then peeled as in (b), before being cut to 10mm.

3.2.3 Measurement of growth

Linear displacement transducers were used to measure elongation of individual segments. Readings were obtained at one minute intervals, and rates calculated from these. Resolution was 0.6μm. Segments were held in perspex chambers similar to those described by Penny et al. (1974), but which permitted perfusion of the central cavity of hollow cylinder segments in addition to bathing of the outer surface, as shown in Fig. 17. Segments were incubated at 25C in recirculating aerated buffer (1mM K-phosphate, pH 6.6). Sixty-eighty minutes after beginning incubation, the solution was changed to 30uM indoleacetic acid (IAA) in the same buffer. This IAA concentration gives maximal growth rates under these conditions (Penny and Penny 1978). Control segments remained in buffer. The pH of solutions (±IAA) changed by less than 0.1pH units during experiments.

3.3 RESULTS

3.3.1 Pretreatment responses

The elongation responses of the different segment preparations, following boring and/or peeling, are shown in Fig. 18.

The burst of elongation, which occurred in peeled segments in solution, is evidence of the restraining effect of the epidermis on the remaining tissues of the segment. Removal of the limiting epidermis apparently permits realisation of a "potential" for extension. Once this initial limitation has been removed, the central tissues (presumably the vascular tissues) apparently limit the rate at which extension occurs. Extremely rapid extension of cortex cylinders occurred once the central tissues were removed. This response was largely completed during peeling. The isolated central core showed a response similar to that of a peeled segment. Outer tissue cylinders showed a small initial burst of extension; thereafter, the epidermis presumably limits further extension. After pretreatment in buffer for 60 minutes, a burst of extension still occurred on peeling the epidermis from hollow cylinders or intact segments (Fig. 19).

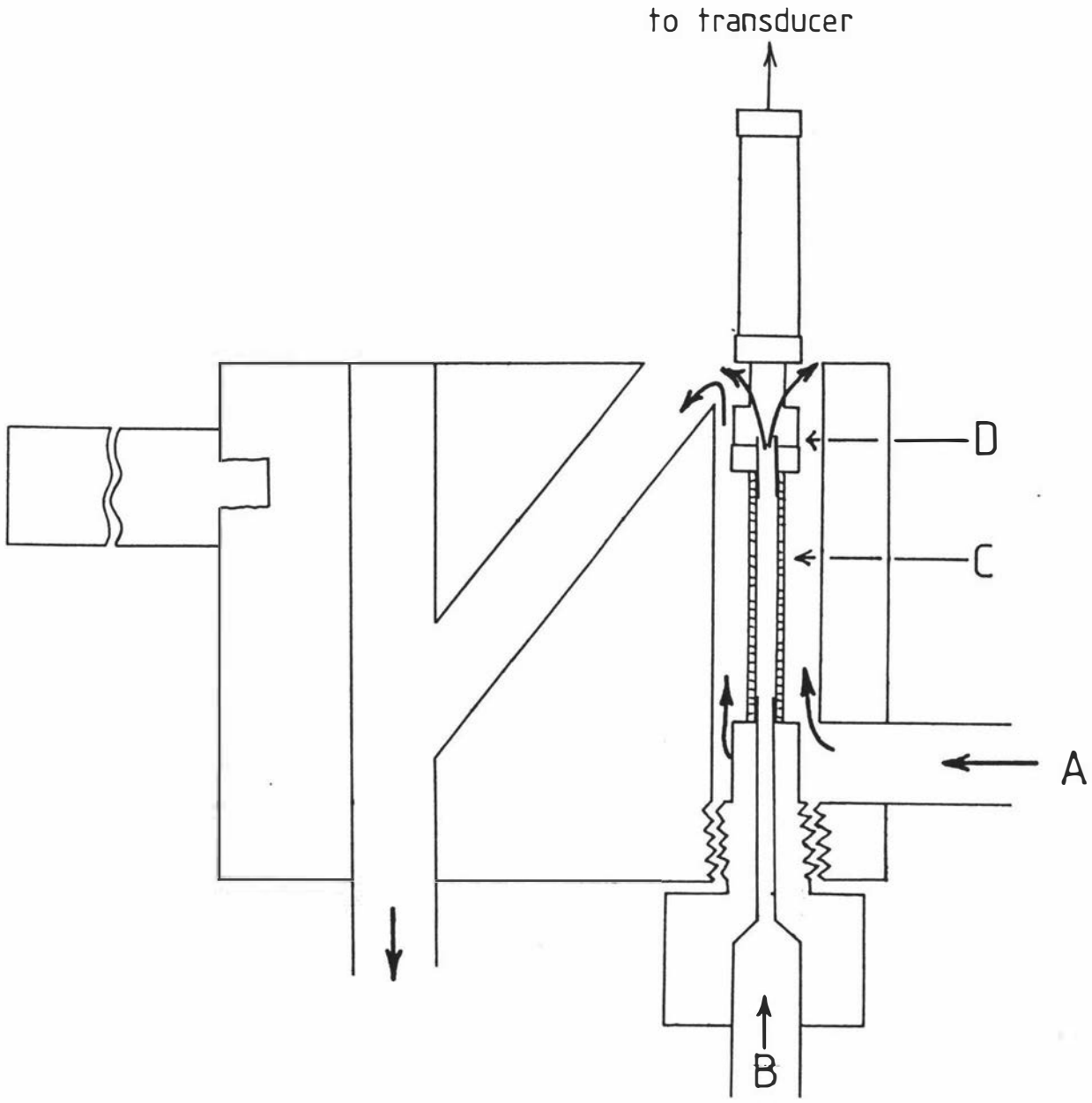

Fig.17: Diagram of the incubation chamber (mid-section), with a hollow cylinder segment in place. Solution was pumped in at A and B, and flowed past the outer surface (and through the centre) of the segment (C). A perspex "head" (D) which allowed solution to pass was placed on the top of the segment, and connected by a polyester thread over a pulley to a transducer. The head was counterbalanced with the transducer core so that the net weight on the segment was about 0.5g.
Where intact segments or central tissue cores were used, the head was replaced with one which was flat (intact segments), or which had a 1mm deep cavity which fitted over the top of the cores (and reduced bending).

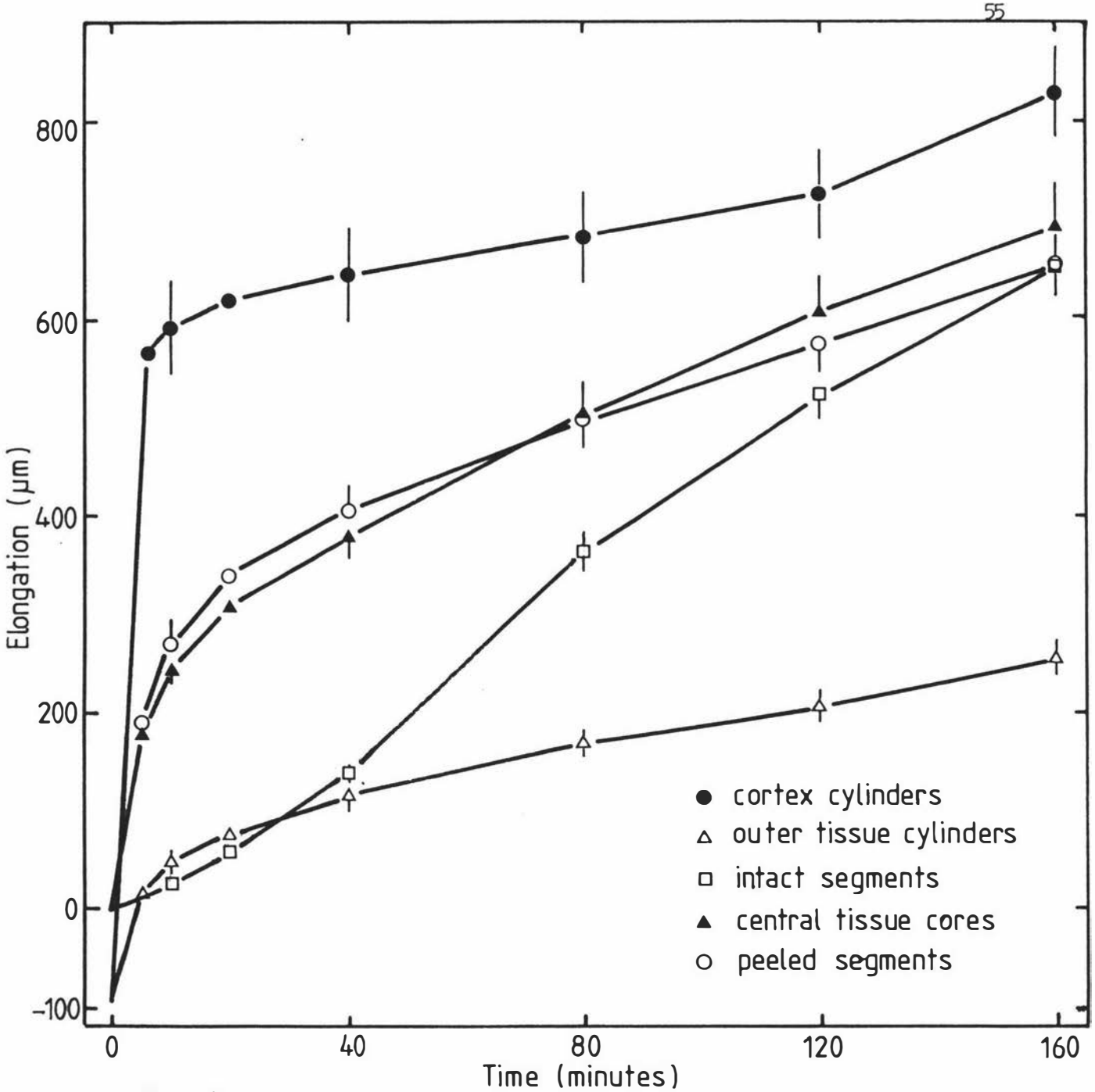

Fig. 18: Elongation of intact segments, and of segments after boring and/or peeling. Preparation of segments differed slightly from that described in Methods. Segments were cut, measured, bored and/or peeled, and elongation in buffer measured with transducers.

Time zero is the time at which peeling started; or for unpeeled segments, the time at which continuous measurement started. Hollow cylinder segments contract when bored. Each curve is the mean of responses of at least five segments. Standard errors are shown.

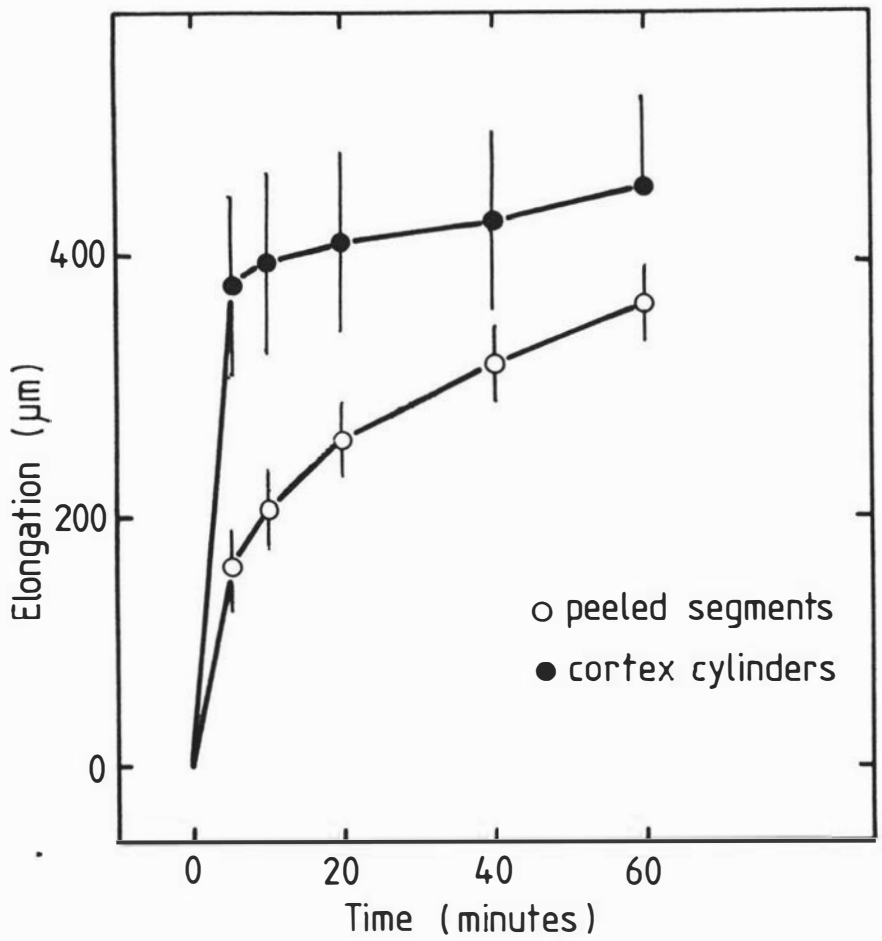

Fig. 19: Elongation of segments after peeling.
Segments were first incubated in buffer for 60–80 minutes, otherwise treatment was the same as outlined in Fig. 18. The curve for peeled segments is the mean of responses of six segments; that for cortex cylinders the mean of four responses.

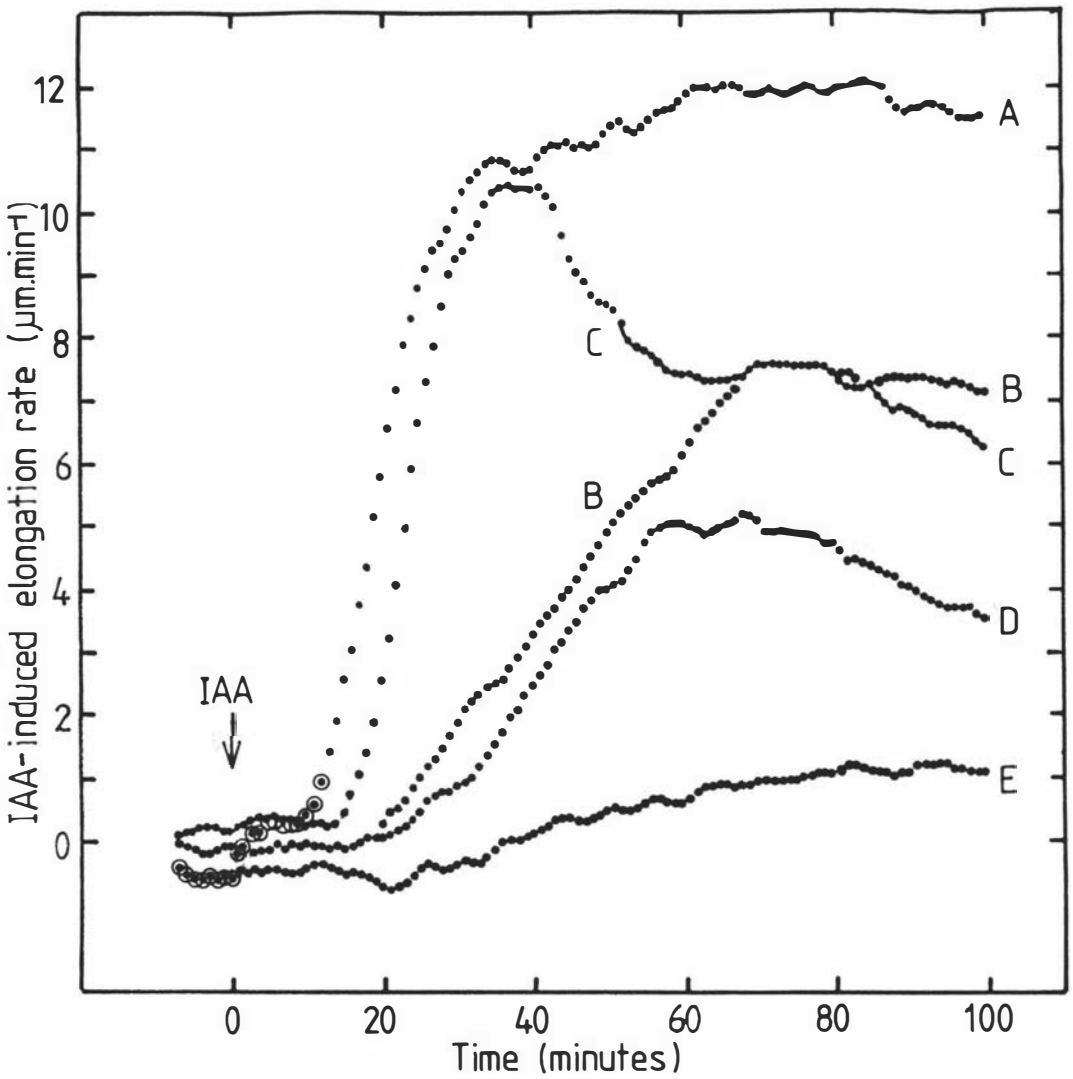

Fig. 20: IAA-induced elongation rate of 10mm hypocotyl segments. Intact segments (A), peeled segments (B), outer tissue cylinders (C), cortex cylinders (D), or central tissue cores (E) were pretreated in buffer for 60-80 minutes before IAA was added at time zero. Each curve is the mean of five segments, with mean control rates (for non-IAA-treated segments) subtracted. Data have been smoothed using a triangularly-weighted 5-point running average.

3.3.2 Responses to IAA treatment

The elongation rate responses to IAA treatment are shown in Fig. 20, as the average results from five segments of each type, minus mean control rate values. Times and rates cited in the text are calculated from responses of individual segments. Standard errors of means are given.

The response of intact segments was similar to that reported previously for 25mm segments of lupin hypocotyl (Penny and Penny 1978). The latent period was 13 ± 0.4 minutes. Rate increased to a first maximum of 11 ± 0.8μm/minute above control rate, at 36 ± 2 minutes. In some experiments, there was a minimum, and second maximum at about 78 minutes, but these were not as well defined as in previous experiments (Penny and Penny 1978). This oscillation is not apparent in Fig. 20; loss of detail with averaged results is discussed in Penny and Penny (1978). The response of outer tissue cylinders showed a latent period of 18 ± 1.3 minutes. The time and value of the first rate maximum were not significantly different from those of intact segments. In some experiments, a minimum and second maximum were apparent, but these were not sharply defined. These results show that removal of the central tissues from intact segments results in little change in initial response to IAA. I suggest that the central tissues are not significantly involved in this early response; this is supported by the poor response of the isolated central core. In contrast, the responses of peeled segments and cortex cylinders show that the effect of removing the epidermis is apparently to eliminate the first phase of the response seen in intact segments or in outer tissue cylinders. I suggest that the epidermis has a major role in the initial response to IAA. However, it has been reported that strips of epidermis peeled from lupin hypocotyls do not elongate in response to IAA treatment (Penny *et al*. 1972). These results then suggest that the epidermis responds indirectly to IAA. A direct response cannot be ruled out, though, and has been shown in one study with strips of *Helianthus* epidermis (Soll and Böttger 1982). Since peeled segments and the cortex in isolation showed a strong response to IAA, the cortex is apparently the major site of direct IAA action.

I suggest that in the intact segment, IAA response occurs as follows. Initial action is to cause relaxation of the epidermis (a "physiological peeling") and potential extension is expressed. This accounts for the first maximum observed at about 36 minutes. This response is superimposed on an independent response. It is due to continuing direct action on the cortex and either direct or indirect action on the epidermis and central tissues. This second response reaches its maximum rate at about 70-80 minutes.

3.4 DISCUSSION

Evidence of the epidermis apparently limiting elongation is found in excised segments of pea stem (Thimann and Schneider 1938, Masuda and Yamamoto 1972, Brummell and Hall 1980), *Avena* coleoptile (Thimann and Schneider 1938), *Helianthus* hypocotyl (Firn and Digby 1977), and mung bean hypocotyl (Prat and Roland 1980). These all show a phase of rapid extension following peeling. In this respect then, lupin hypocotyl segments do not differ from other materials investigated. I suggest that the hypothesis of epidermal relaxation advanced for the first phase of auxin action on lupins may apply generally. The hypothesis is supported by the observation that in intact segments, significant IAA-induced effects on wall properties can be detected only in the outer layers, after 90 minutes treatment (Penny *et al*. 1972). Also consistent with this hypothesis are observations of loss of auxin-sensitivity in peeled segments, which suggest that auxin action is on the epidermis (Brummell and Hall 1980, Prat and Roland 1980, Soll and Böttger 1982, Firn and Digby 1977). The contention that the central tissues are not significantly involved in the initial response to auxin is supported by results showing that removal of the pith from mung bean hypocotyl segments does not alter the early response to auxin (Prat and Roland 1980).

The "relaxation" of this hypothesis is analogous to the stress relaxation process described as the primary event in cell enlargement (Ray *et al*. 1972), which results in formation and discharge of a water potential gradient with consequent extension. However, the rapid

extension following peeling might also be due to discharge of an existing water potential gradient, and hence initial auxin action on water transport would be an alternative possibility (Cosgrove and Steudle 1981).

Peeled segments of lupin hypocotyl are strongly IAA-responsive. Other studies have shown a strong response to auxin in peeled segments of Avena coleoptile (Durand and Rayle 1973), and varying degrees of response in peeled pea stem segments (Thimann and Schneider 1938, Masuda and Yamamoto 1972, Durand and Rayle 1973). These results are consistent with the hypothesis that at least part of the response to auxin is controlled by the cortex. I am not aware of any other report which identifies the cortex as the major site of direct auxin action in peeled segments. Results which show loss of auxin response on peeling do not fit with this hypothesis, however (Brummell and Hall 1980, Prat and Roland 1980, Soll and Böttger 1982, Firn and Digby 1977). In these cases, either responsiveness is confined to the outer layers, or is lost from the inner layers as a consequence of peeling (Brummell and Hall 1980).

The explanation given here of the two maxima in the auxin-induced rate response, in terms of tissue interactions, is an alternative to hypotheses originally advanced. Within the hypothesis that tissue interactions are responsible for the oscillation in rate observed with intact segments, it is possible that one or two response mechanisms could be involved. One mechanism could cause epidermal relaxation (and lead to expression of potential extension) and also initiate the longer term response (Penny 1970). Alternatively, two different mechanisms could account for the two phases of the response. Since the "second" response occurs in the absence of the "first", they need not be linked. It is not possible to distinguish between these alternatives on the evidence presented here. The simplest explanation is that there is one reaction mechanism.

Hollow cylinders used in this work have advantages in studies of acid effects on growth since the cuticular barrier is overcome without recourse to treatment which removes or damages the epidermis. Results

of experiments which test predictions of the acid-growth hypothesis are given in the next chapter.

4: ACID-INDUCED RAPID ELONGATION RESPONSES

4.1 INTRODUCTION

Auxin treatment of stem, coleoptile and hypocotyl segments causes rapid elongation after a 10-15 minute latent period. One explanation of this stimulation of elongation is the acid-growth hypothesis. Rayle and Cleland (1980) provide a recent statement of this hypothesis: "...auxin causes cells to excrete protons into the wall solution. The lowered wall pH activates one or more enzymes which cleave load-bearing bonds in the cell walls, thus allowing for accelerated turgor-driven extension." Two predictions which arise from this hypothesis are:
(1) A reduction in cell wall pH will occur in tissue elongating in response to IAA treatment; and
(2) Treatment with hydrogen ions should "substitute for auxin in any auxin-sensitive tissue and cause rates of elongation equivalent to that produced by optimal auxin" (Cleland 1977).

Tests of these predictions have shown:
(1) Treatment with IAA caused free space pH in *Avena* coleoptile segments to fall to a minimum value of about 4.8 (Cleland 1976); and
(2) Treatment with acid solutions of pH 4.8-5.0 promoted elongation initially to the same rate as is induced by auxin, if peeled segments were used (Rayle 1973). A more acidic solution (pH 3) was needed to achieve the same result in intact segments (Rayle and Cleland 1970), probably because the cuticle was restricting access of hydrogen ions to the segment from the external solution (Dreyer *et al.* 1981). In peeled segments the cuticle was removed along with the epidermis.

Thus the measured IAA-induced reduction of pH was sufficient to account for the initial phase of IAA-induced elongation, at least in peeled *Avena* coleoptile segments. For the hypothesis to be satisified in intact segments, it is also necessary to show that the epidermis, as well as peeled segments, is responsive to hydrogen ions. Strips of epidermis peeled from *Avena* coleoptiles, elongated under applied load in response to acid treatment (Durand and Rayle 1973, Rayle and Cleland 1977).

In other materials, peeled segments show a similar dependence of elongation on pH, and strips of epidermis peeled from segments also elongate under applied load, in response to acid treatment (Durand and Rayle 1973, Yamagata *et al*. 1974, Yamamoto *et al*. 1974, Rayle and Cleland 1977).

However, the relationship of results of these experiments to responses which occur in intact segments is uncertain, since it is difficult to make qualitative comparisons between the effect of stress imposed by applying a load, and that generated by turgor pressure. In this study I have used hollow cylinder segments of lupin hypocotyl in an investigation of the acid-induced elongation response of the epidermis of lupin hypocotyls. These cylinders retain undamaged the auxin-responsive outer tissues (page 58) and permit access of hydrogen ions to these tissues from the central cavity. I am able to test the hypothesis that the outer cell layers and the inner tissues are equally responsive to acid, and can compare acid-induced and IAA-induced response of the limiting outer cells, at *in vivo* stress.

Results show that different tissues are not equally responsive to acid, and suggest that the most auxin-responsive preparation, which includes the epidermis, is the least acid-responsive. So that results could more easily be compared with those of other studies, I have also investigated the acid-induced elongation response of peeled segments. Results are similar to those obtained with other materials, but I show that response to acid treatment is largely dependent on the pretreatment regime.

4.2 MATERIALS AND METHODS

4.2.1 Plant materials

Four-day old lupin seedlings (*Lupinus angustifolius* cv. N.Z. Bitter Blue) were grown in continuous low light at 22C (Penny 1969). A detailed description of seedling selection and segment preparation has been given previously (page 52). Segments were 10mm long. Five different preparations were used in this study:

(a) Intact segments;
(b) Peeled segments, which had the outermost 2-4 cell layers removed;
(c) Outer tissue cylinders, which were hollow cylinders consisting of epidermis and cortex, the central tissues of the hypocotyl having been removed by boring;
(d) Cortex cylinders, which were outer tissue cylinders peeled to leave hollow cylinders consisting only of cortex parenchyma;
(e) Central tissue cores, the cores of vascular tissue and pith bored from hypocotyls in the preparation of hollow cylinders.

4.2.2 Measurement of growth

Elongation of individual segments was measured with linear displacement transducers (Penny et al. 1974). Digital readings of voltage were recorded at one minute intervals, and elongation rates were calculated from these. The data of rate responses presented here were smoothed with a triangularly weighted five-point running average. Unless otherwise noted, each rate curve is the mean of responses of at least five segments, with the mean response of control segments (at least five) subtracted. Segments were held in perspex chambers designed to allow perfusion of the central cavity of hollow cylinder segments in addition to bathing of the outer segment surface (Fig. 17, page 54). The volume of each chamber was 1.2ml. Aerated solutions were recirculated from flasks held in a water bath at 25±0.5C. Flow through each chamber was 10ml/minute. Where hollow cylinder segments were used, flow was 5ml/minute through both the central cavity and the outer chamber.

Unbuffered solutions were used in many experiments, since some studies have shown effects of buffer components on elongation, in addition to effects of low pH (Gabella and Pilet 1978, Edwards and Scott 1974, Moll and Jones 1981). Unbuffered solutions were prepared with distilled water with pH adjusted initially to 6.6 with NaOH. Acid solutions were prepared with HCl. IAA solutions were 30μM. Since acidic solutions were toxic at low pH, in some experiments calcium chloride was added to overcome toxicity. [Marschner et al. (1966) have reported that $CaCl_2$ would reduce acid-induced potassium loss and tissue

damage in Zea root tips].

In some experiments buffered solutions were used so that effects of the buffer on elongation of segments could be determined, and also so that results might be more easily compared with those of other studies where buffered solutions have been used. Buffered solutions were 1mM or 10mM K_2HPO_4 plus citric acid added to give pH 6.6 (pretreatment) or a lower pH (treatment).

Segments were pretreated for one hour in the chambers before solution was changed to acid, IAA, or fresh pretreatment solution (controls). Because of mixing of pretreatment and treatment solutions in inlet tubes on changeover, solution changed gradually in the segment chambers over four minutes from the times shown in figures.

4.3 RESULTS

4.3.1. Response of outer tissue cylinders

Unbuffered solutions

The responses of outer tissue cylinders to treatment with acid are shown in Fig. 21. The response to IAA is included for comparison. The response of segments treated with acid at pH 4.0 or 3.0 shows two distinct maxima. I interpret the first maximum (that occurring within 20 minutes) as extension of the innermost cells of the cortex, independent of extension of the outermost cell layers. This explanation is suggested for the following reasons. Firstly, the cortex is immediately adjacent to the central cavity of the cylinder and so will receive acid before the outermost cell layers, assuming that the cuticle restricts access of hydrogen ions to the cylinder from the outside. This assumption is justified by the observation that there was little elongation of intact segments in response to treatment with acid at pH 3 (Fig. 22). (The small response which was found might represent the action of hydrogen ions which had penetrated the epidermis, but might also reflect acid action at the cut ends of the segment). Secondly, the cortex in isolation responded strongly to acid treatment [Fig. 27(B)]. Thirdly, it is known that not all tissues in a

segment are constrained to elongate equally. For example, at the ends of IAA-treated intact segments of lupin hypocotyl the outer tissues were observed to have elongated more than the inner tissues.

Since elongation of the isolated cortex in response to acid treatment [Fig. 27(B)] was prolonged in comparison with the "first" response of acid-treated outer tissue cylinders (Fig. 21), and assuming that files of cells in the cortex could not slip ahead independently of the outermost cells, it is suggested that continuing elongation of outer tissue cylinders was limited by the outermost cell layers. I then interpret the second phase of the response as elongation of the whole cylinder (the cortex and outermost cells together), in response to acid action at the outer layers. The course of this response will presumably depend on the pH in the outermost cells changing gradually with time as hydrogen ions diffuse across the cortex, and also on the sensitivity of the outer cells to low pH. Since these outer cells are those on which IAA must act before the segment can elongate (page 58) then it is the second phase of the response which is compared with the IAA response, in order to determine the external pH which will substitute for IAA in inducing elongation.

At no stage during treatment did the acid-induced elongation rate reach the maximum IAA-induced rate (Fig. 21, Fig. 23). However, the decline in rate seen in the latter stages of experiments at pH 4.0 and 3.0 (Fig. 21) was probably caused by toxic effects of acidity, since sections cut from segments after the 150 minute treatment showed cells to have lost turgor and cell content to have coagulated. Toxicity apparently occurred first in cells adjacent to the central cavity of the cylinders, and progressed to the outermost cells as treatment continued. At pH 5.0 there was evidence of toxicity in the innermost 2-3 cell layers after the 150 minute treatment.

Since it was likely that promotive effects of hydrogen ions on elongation were confounded by toxic effects in the later stages of treatment, I used $CaCl_2$-supplemented solutions in an attempt to overcome acid-induced toxicity.

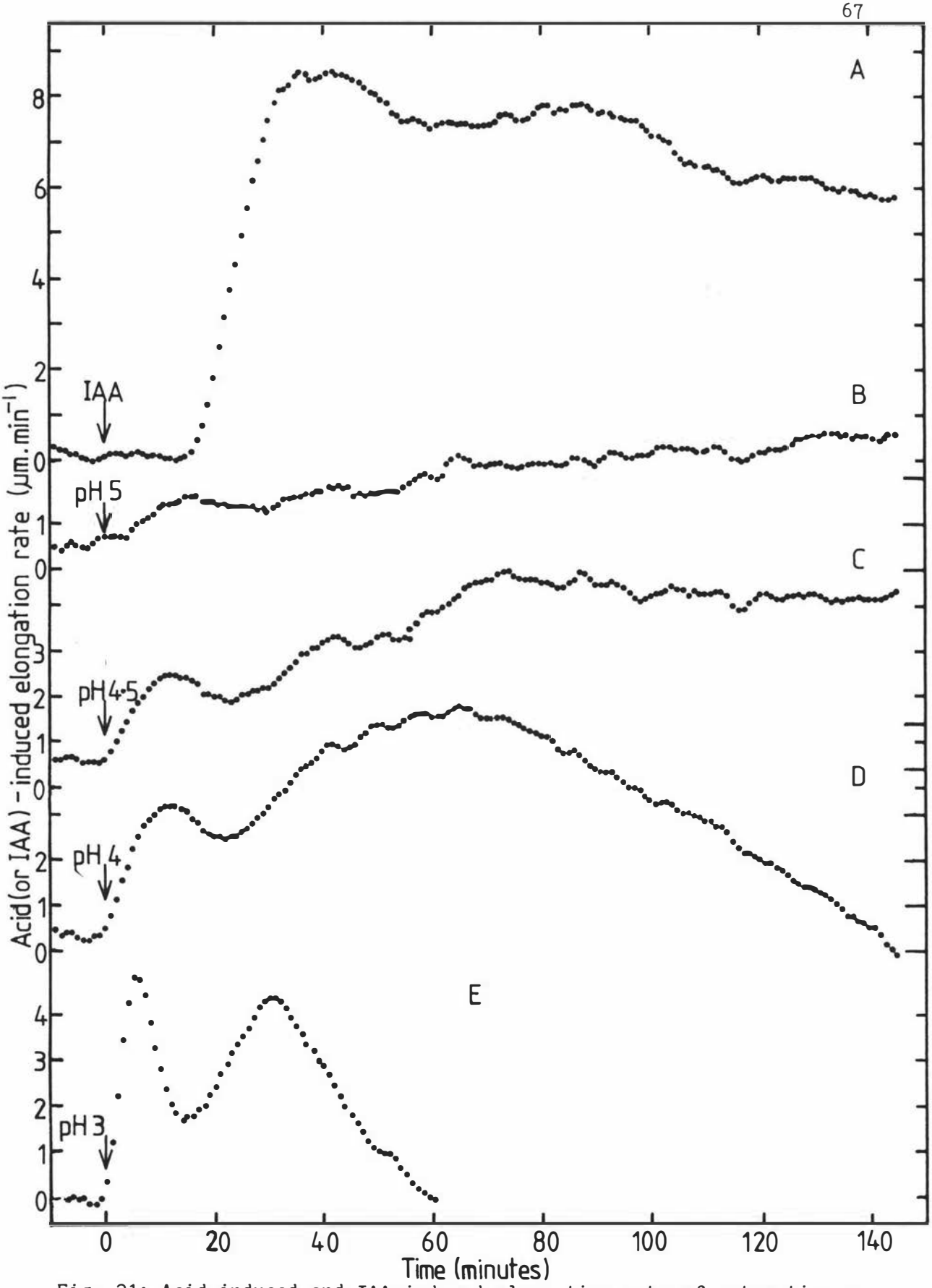

Fig. 21: Acid-induced and IAA-induced elongation rate of outer tissue cylinders, in unbuffered solution.

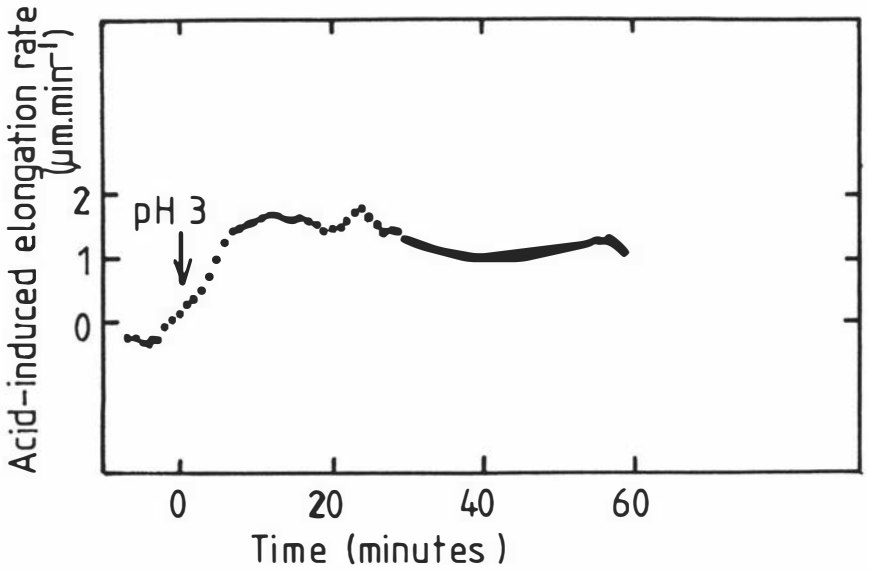

Fig. 22: Acid-induced elongation rate of intact segments, in unbuffered solution.

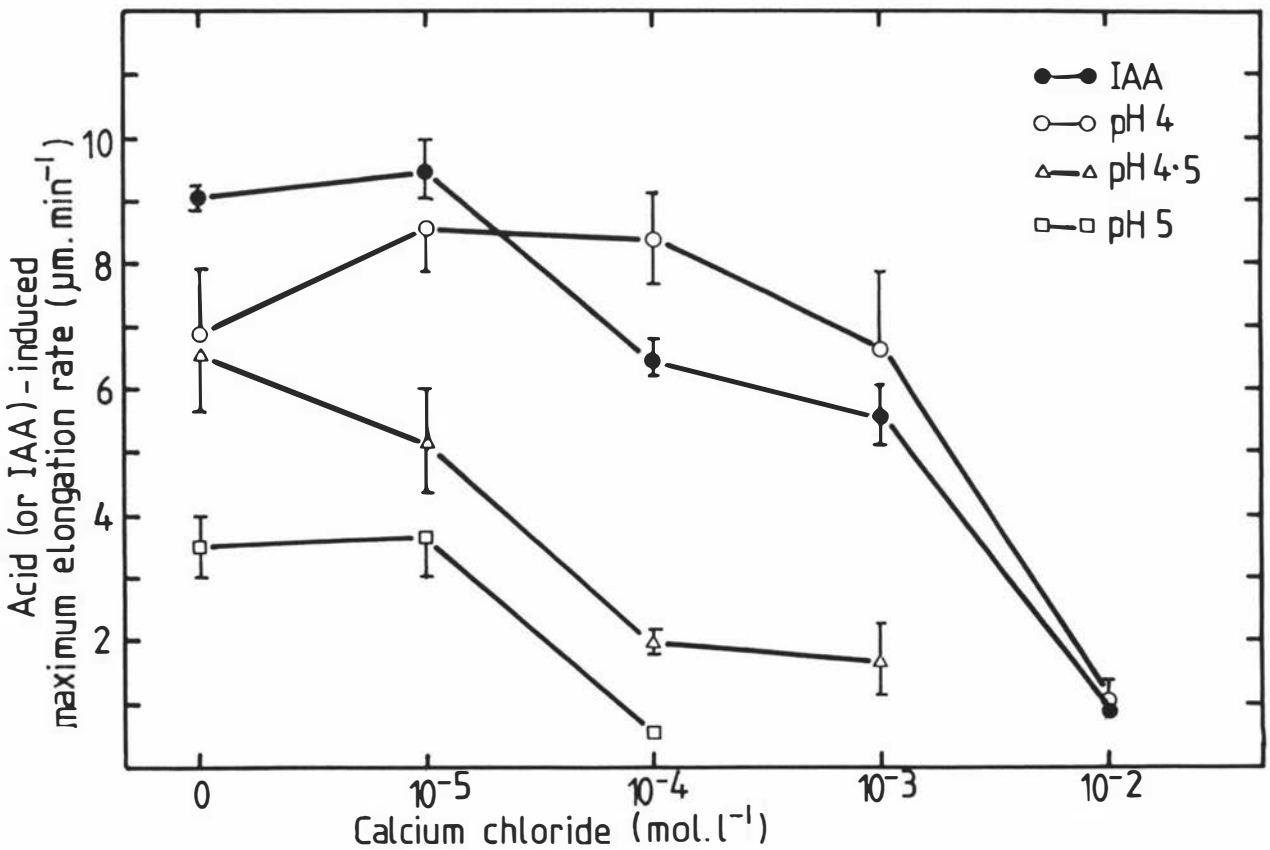

Fig. 23: Acid-induced and IAA-induced maximum elongation rates of outer tissue cylinders, in unbuffered $CaCl_2$ solutions.

Figure 23 shows acid-induced maximum elongation rates, at each $CaCl_2$ concentration used. IAA-induced maximum elongation rates are included for comparison. The value of maximum rate shown at each point is the mean of results of individual experiments, with standard errors shown. For each experiment the mean control rate at the time corresponding to that of the maximum, was subtracted from the maximum to give the acid-induced or IAA-induced maximum rate. The results shown in Fig. 23 suggest that on the basis of maximum elongation rates in $CaCl_2$-supplemented solutions, hydrogen ions at about pH 4.0 would replace IAA in promoting elongation in outer tissue cylinders. In sections cut from segments after 150 minutes of acid treatment I found little evidence of toxic effects at pH 4.0 in 0.001M $CaCl_2$, or at pH 4.5 in 0.0001M $CaCl_2$. Toxic effects although apparently reduced, were still obvious at lower $CaCl_2$ concentrations at either pH.

Buffered solutions

It is possible that unbuffered solutions might not maintain free space pH within the tissue at the external pH. To test the possibility that observed responses were a consequence of the use of unbuffered solutions, in some experiments I used 1mM or 10mM K_2HPO_4-citric acid buffers. Figure 24 (1mM buffer) and Fig. 25 (10mM buffer) show that segments elongated little in response to treatment with buffered solution at pH 5. The response was similar to that of outer tissue cylinders treated with unbuffered solutions at pH 5 [Fig. 21(B)]. If these buffers penetrate the tissue, then I conclude that these cylinders are only slightly responsive at this pH.

In some conditions, outer tissue cylinders responded more rapidly to acid treatment. Figure 26 shows the response of a cylinder treated at pH 5 after a five hour pretreatment in 1mM Na_2HPO_4-citric acid buffer. This response was not found consistently. I have not investigated further the apparent dependence of response on length of pretreatment.

4.3.2. Response of cortex cylinders

Outer tissue cylinders appeared less responsive to acidic solutions than I expected from the work of other investigators with peeled

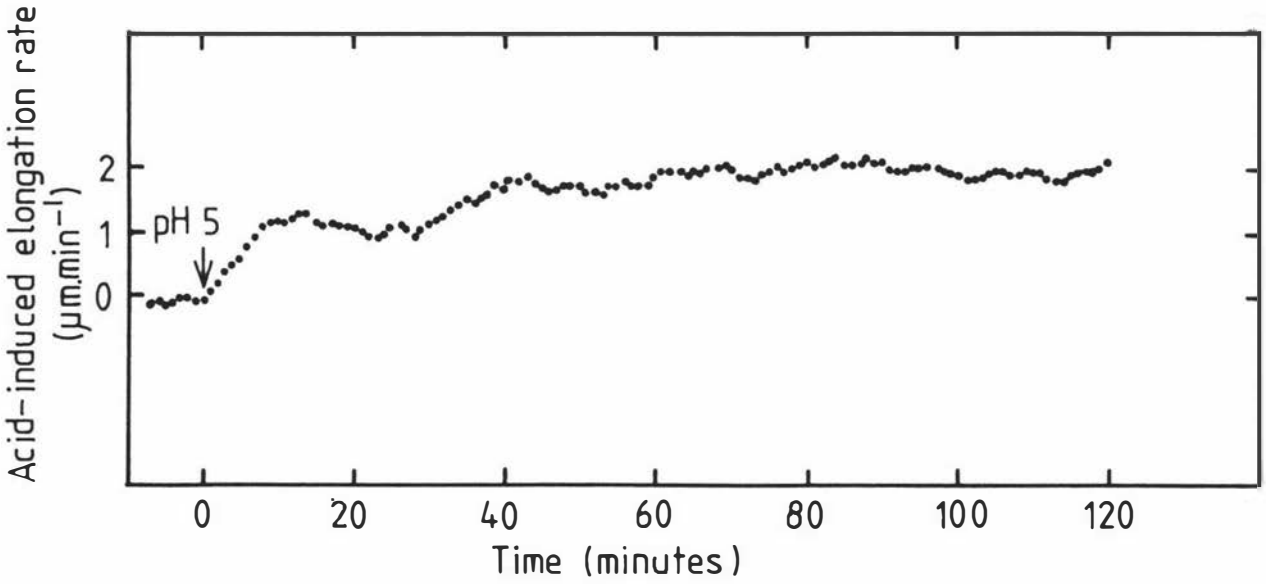

Fig. 24: Acid-induced elongation rate of outer tissue cylinders in response to acid (pH 5) treatment, in 1mM buffer.

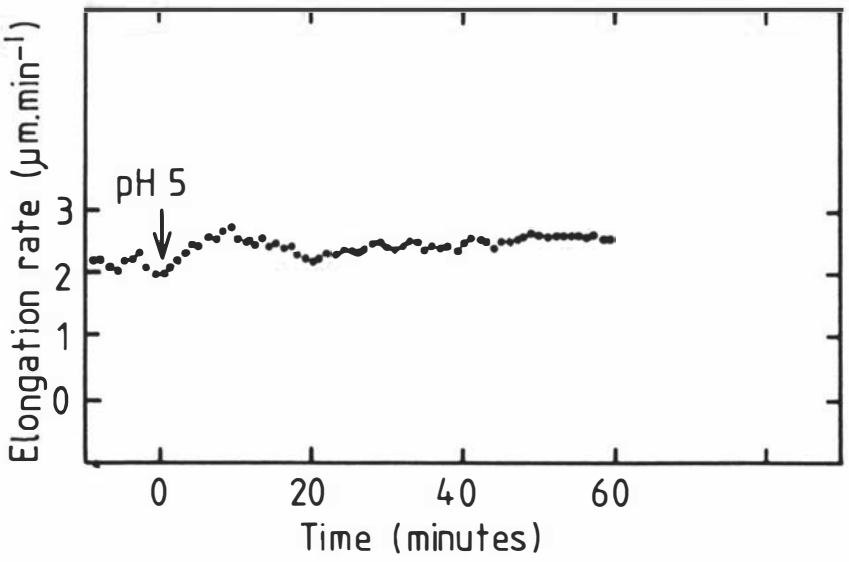

Fig. 25: Elongation rate of outer tissue cylinders in response to acid (pH 5) treatment, in 10mM buffer.

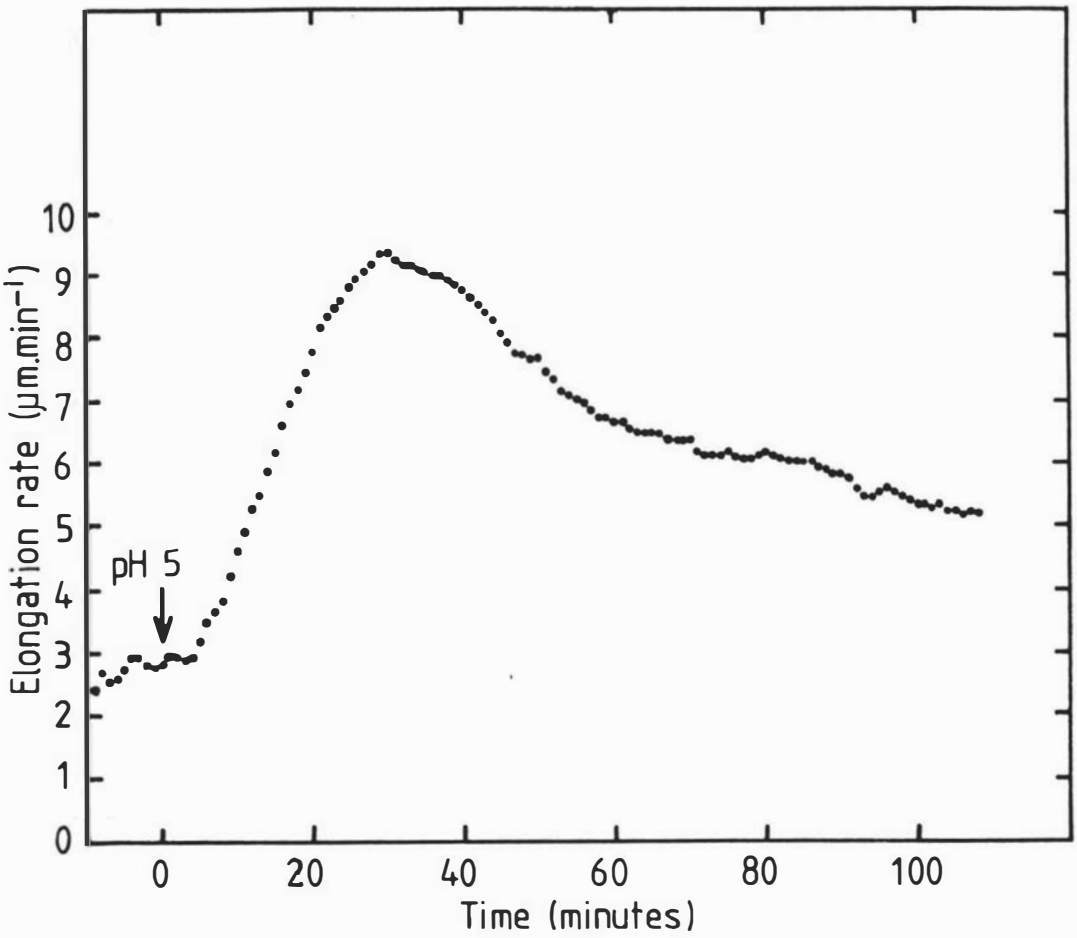

Fig. 26: Elongation rate of an outer tissue cylinder in response to acid (pH 5) treatment, after five hours pretreatment in 1mM Na_2HPO_4-citric acid buffer.

segments of other materials. This insensitivity was apparently not due to the use of unbuffered solutions. It seems more likely that it is the outermost cell layers in these cylinders which were comparatively unresponsive to hydrogen ions. The evidence for this is the rapid and strong elongation response of cortex cylinders in acid solution at pH 5.0 [Fig. 27(B)]. Compare this with the response of outer tissue cylinders at pH 5.0 [Fig. 21(B)]. Apparently, all that was required for outer tissue cylinders to respond rapidly at pH 5 was removal of the outermost cell layers. However, acid had access to the tissue from both inside and outside the cylinder, and so it was inevitable that overall penetration of the cortex would be faster than in outer tissue cylinders. The segments may have elongated rapidly because acid penetrated the tissue more quickly, rather than because elongation was no longer restricted by the less responsive outer cell layers. This hypothesis was tested by perfusing the central cavity with solution at pH 5, while the solution outside the segment remained unchanged. Segments responded similarly [Fig. 27(A)] to those whose outer surface was also exposed to acid [Fig. 27(B)]. This supports the conclusion that the outermost cell layers were less responsive to acid than the greater part of the cortex.

Comparison of the response of cortex cylinders to IAA [Fig. 27(C)], with the acid-induced response at pH 5 [Fig. 27(A) and (B)], suggests that a less acidic solution would substitute for IAA in meeting the prediction of the acid-growth hypothesis, if this tissue alone is considered.

4.3.3. Response of peeled segments

Unbuffered solution

Since cortex cylinders responded quickly to acid, I expected that peeled segments would also respond well. However, this was not the case. Little response to treatment with unbuffered acid at pH 5 was observed [Fig. 28(A)]. Since the central tissues probably limit elongation in peeled segments (page 53), two possible explanations for the poor response to acid of peeled segments are that either hydrogen ions did not penetrate to the central tissue, or that these tissues

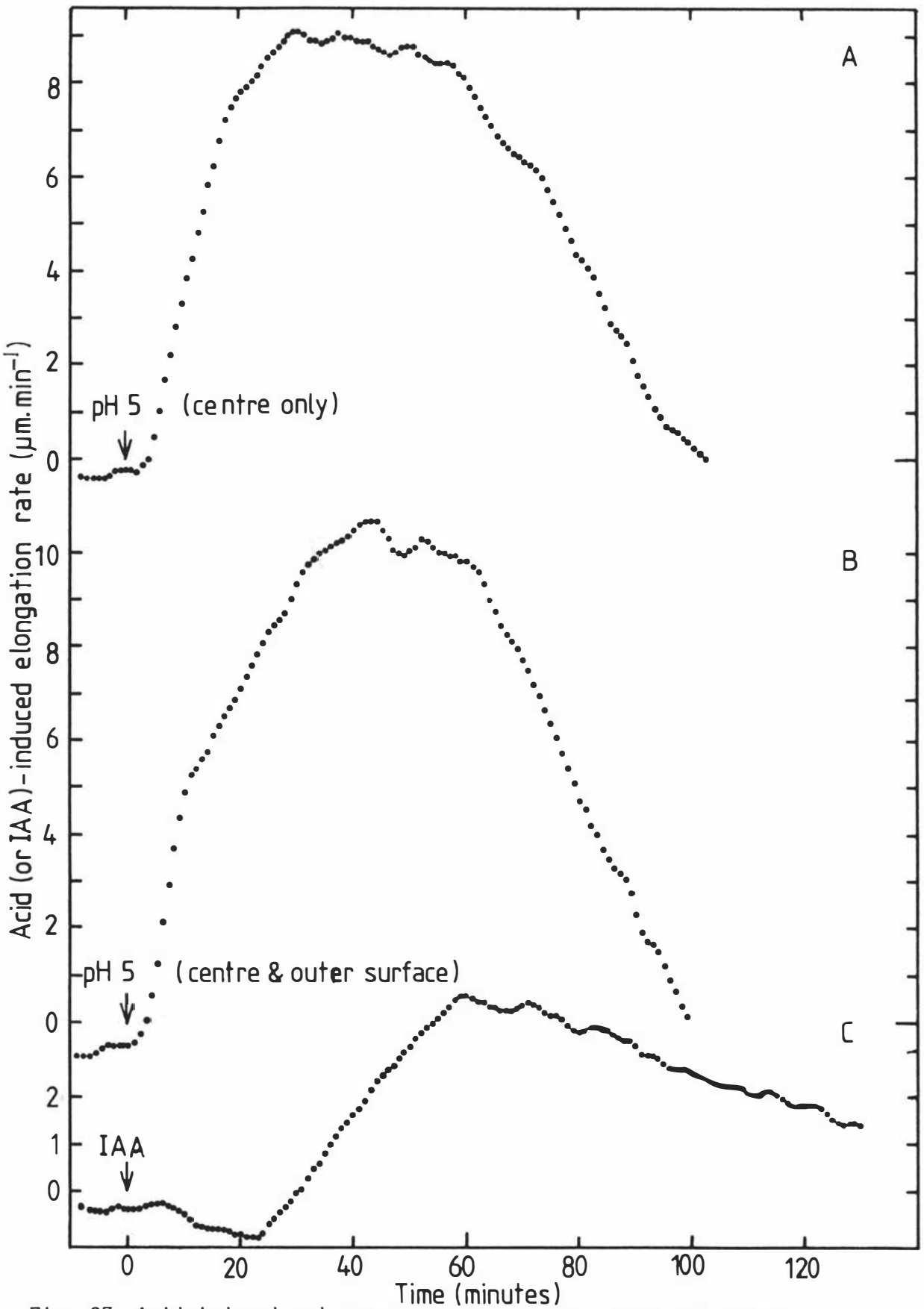

Fig. 27: Acid-induced and IAA-induced elongation rate of cortex cylinders, in unbuffered solution.

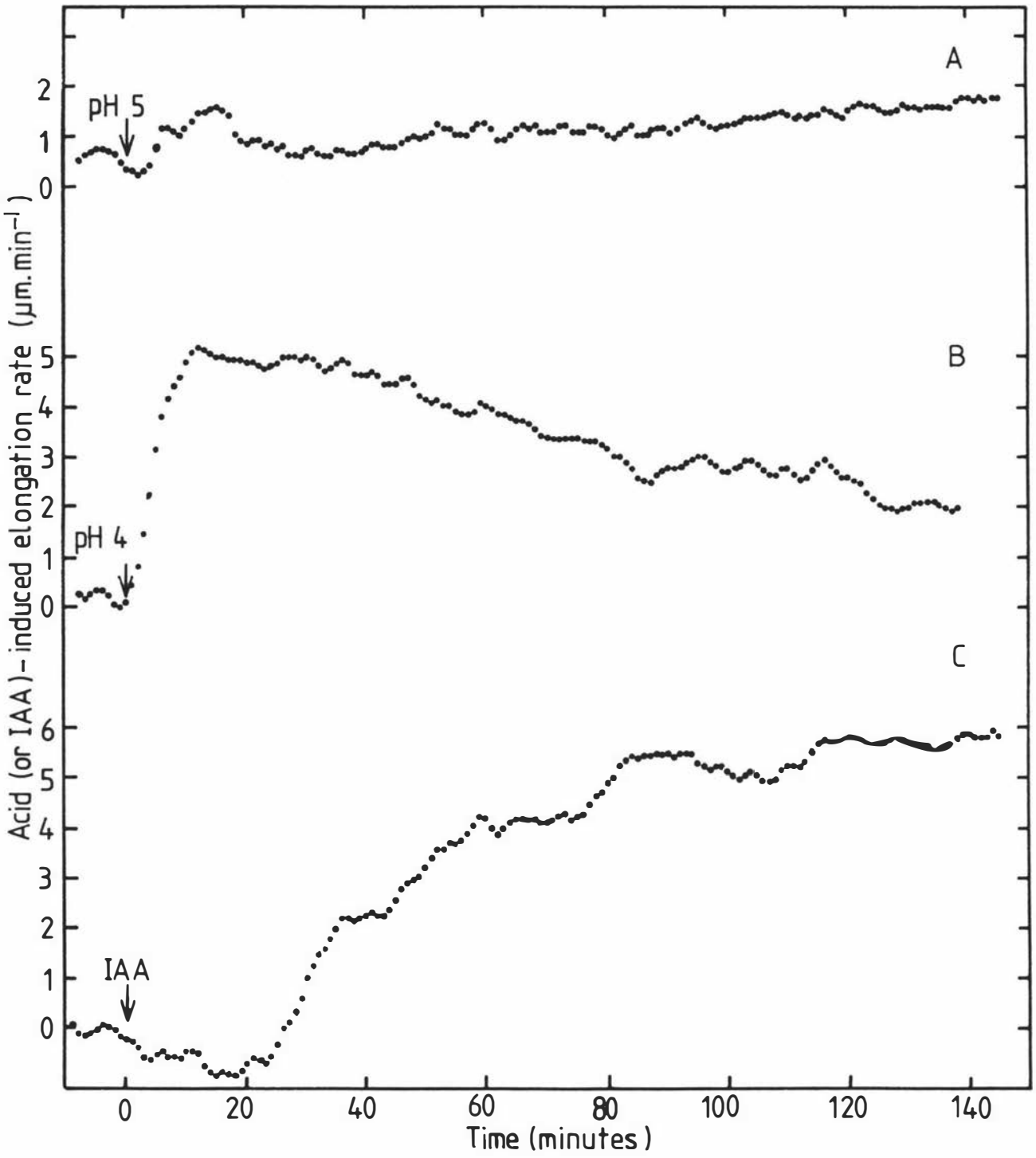

Fig. 28: Acid-induced and IAA-induced elongation rate of peeled segments, in unbuffered solution.

were less responsive to low pH, than was the cortex. While the central tissue cores were apparently less sensitive to acid than the cortex, nonetheless they responded more strongly to acid than did peeled segments [Fig. 29(B)]. This result implies that in peeled segments, hydrogen ions did not reach the central tissues from the external solution. How far did the acid penetrate? Observations of toxicity in peeled segments treated with acid for a prolonged period suggested that inward diffusion of hydrogen ions was restricted by the innermost layer of the cortex (the starch sheath). I noted that in the central tissue cores, the starch steath was invariably cut at some point. Any barrier it may have provided no longer existed.

To test the possibility that the starch sheath was limiting access of hydrogen ions to the stele, the response to acid treatment of "large" central tissue cores was determined. The response at pH 5 [Fig. 29(A)] was similar to that of peeled segments [Fig. 28(A)]. These cores consisted of the vascular tissue and pith plus about 2-5 layers of cortex cells. In sections cut from the cores and examined with a light microscope, the starch sheath appeared to be intact. Since the only apparent differences between these segments and the normal central tissue cores were the presence of an intact starch sheath and some additional layers of cortex cells in the former, I suggest that the small response of the "large" cores (and of peeled segments) at pH 5 was due to restriction of access of hydrogen ions to the stele by the starch sheath.

What external pH will substitute for IAA in peeled segments? Results show that treatment at pH 4 caused a rapid elongation response [Fig. 28(B)] with a maximum similar to the IAA-induced maximum rate [Fig. 28(C)]. The initial response might be that of the cortex (i.e., as suggested in explanation of the initial response of outer tissue cylinders treated with acid) and continuing elongation a whole segment response due to slow penetration of the stele by hydrogen ions. The promotive effects of the acid were probably confounded by toxic effects as treatment continued, since toxic effects (similar to those observed in outer tissue cylinders treated with acid) were seen in sections cut from segments at the end of experiments.

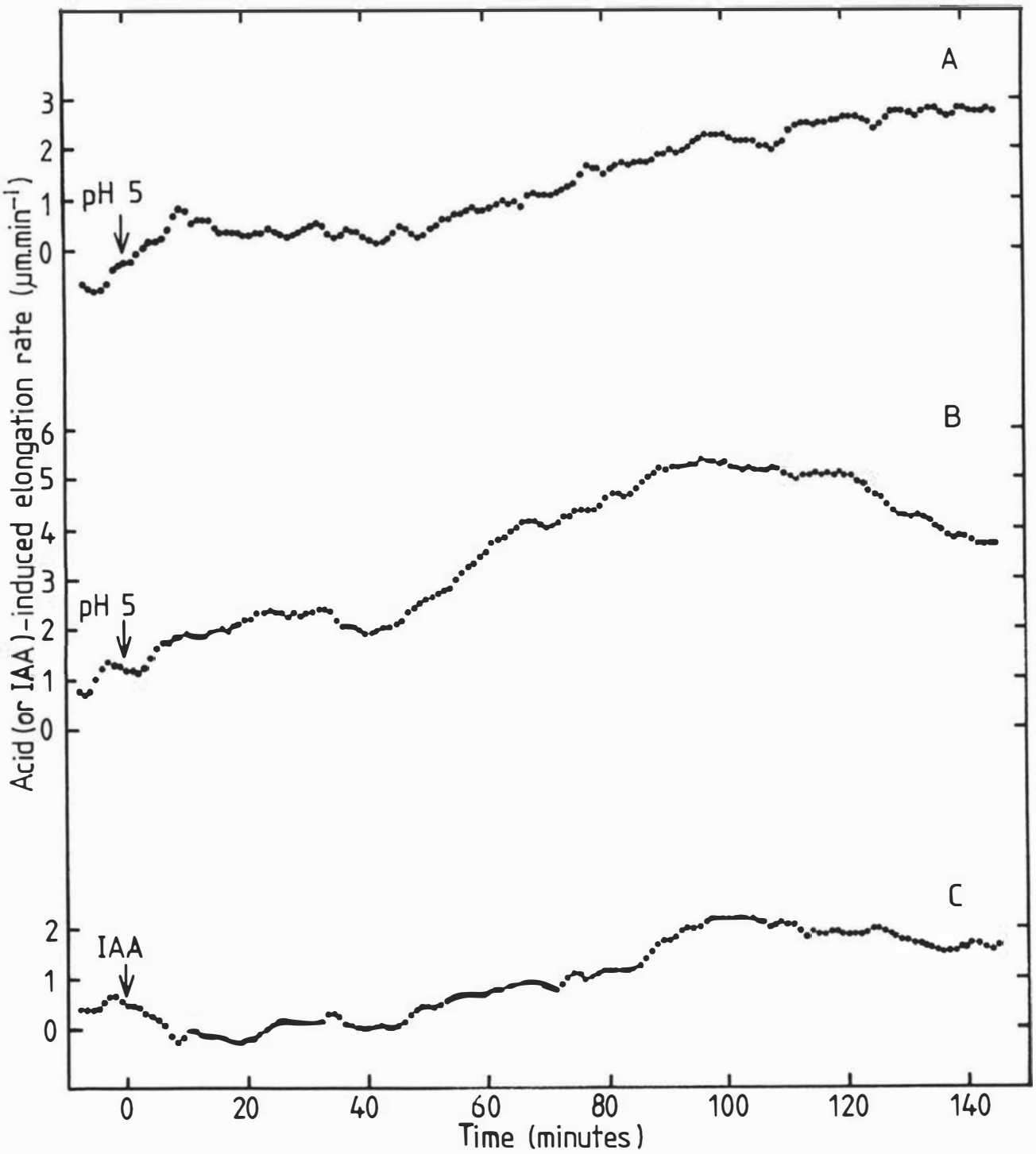

Fig. 29: Acid-induced and IAA-induced elongation rate of central tissue cores, in unbuffered solution.

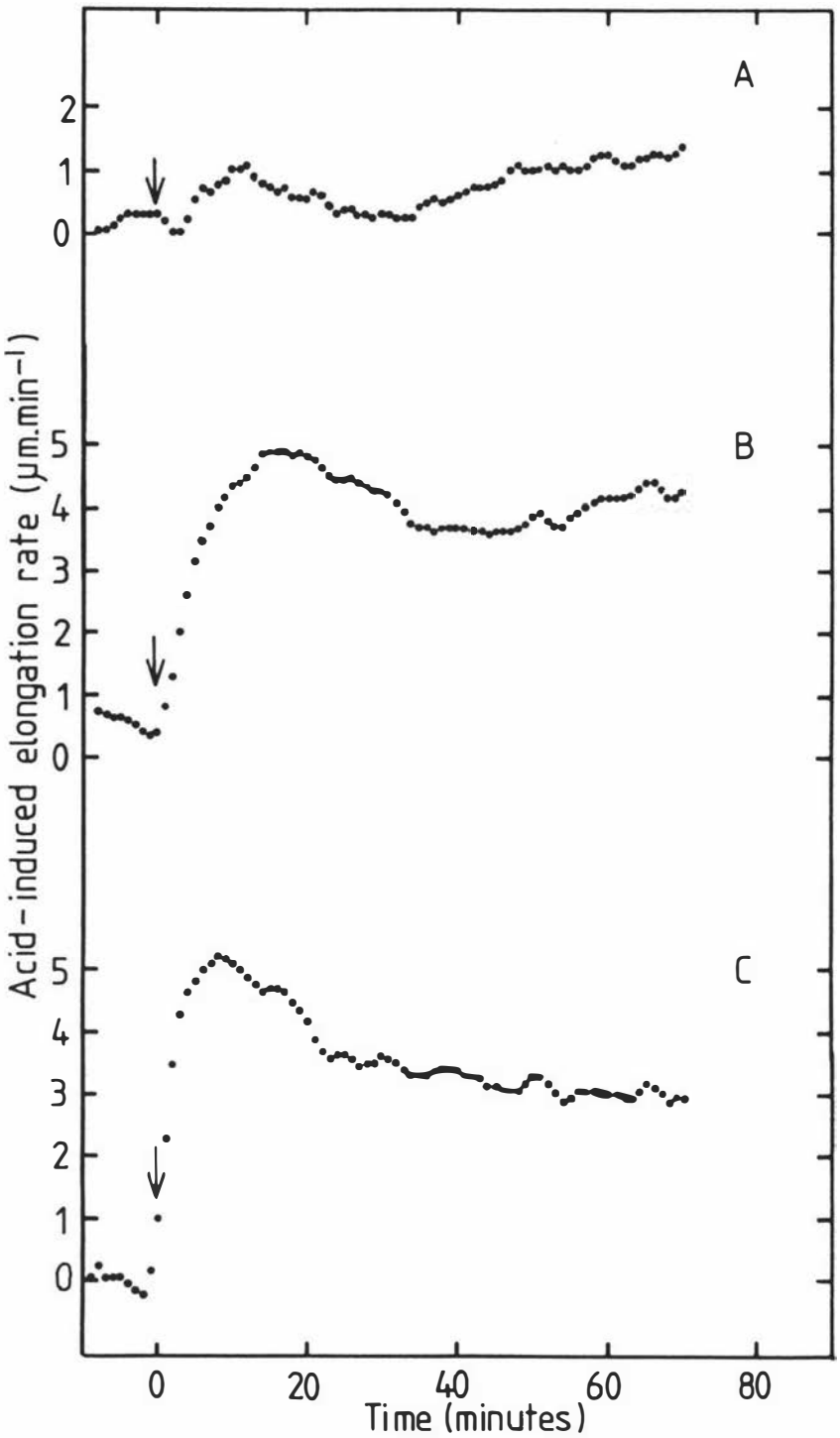

Fig. 30: Acid-induced elongation rate of peeled segments, pretreated in water (A), or buffer (B,C) and then treated at pH 5 with buffered (A, C) or unbuffered (B) solution.

Buffered solution

Buffered solutions were used in some experiments, so that results could be compared more directly with those of other studies. Peeled segments responded rapidly when treated with 1mM buffer pH 5.0 after pretreatment with buffer pH 6.6 [Fig. 30(C)]. The maximum rate was similar to the IAA-induced maximum rate [Fig. 28(C)]. Compare this result with the response in unbuffered solution [Fig. 28(A)]. How is the difference explained? I observed that the mean pretreatment elongation rate in buffer was only 70% of the rate in water. Possibly, then, acid-induced extension after pretreatment in buffer was dependent on that buffer pretreatment. This hypothesis was supported by the results shown in Fig. 30. After pretreatment in buffer, segments treated with either buffered [Fig. 30(C)] or unbuffered [Fig. 30(B)] solutions at pH 5 responded rapidly to reach similar maximum rate. After pretreatment in water, segments responded equally poorly when treated with either buffered [Fig. 30(A)] or unbuffered [Fig. 28(A)] pH 5 solutions. Apparently, it was the pretreatment, and not the method of treatment, which largely determined the response.

4.4 DISCUSSION

The first objective was to determine the *in vivo* response to acid of the outer cell layers of lupin hypocotyls. Apparently, the outermost layers were less responsive to acid than all other tissues. A possible explanation of at least part of this differential sensitivity is that the stress in longitudinal cell walls might be lower in cells of the outermost layers than in cells of the cortex. Stress in the longitudinal walls of an ideal cylindrical cell is given by Nobel (1974) as

$$\sigma_L = rP/2t$$

where σ_L = longitudinal stress
r = cell radius
P = turgor pressure
t = cell wall thickness

In lupins, cells of the outermost four layers of the hypocotyl have

smaller radii and thicker radial walls than cells of the inner cortex. The ratio r/2t for the outer cells is about one-third of that for the inner cells (K. Miller, unpublished). If the turgor pressure is the same in the different cell layers, it follows that σ_L will be lower in the outermost layers than in the inner cells. If the rate of extension is limited by stress borne equally throughout the thickness of the wall [rather than limited by stress in only part of the wall, as suggested by Taiz et al. (1981) for Nitella] then it might be expected that the rate of acid-induced extension of the outer cells be lower than that of the inner cells (because stress is lower in the walls of the former than in the walls of the latter).

I can now assess the possible role of hydrogen ions in IAA-induced elongation of lupins. I have previously suggested that in lupin hypocotyls, the outermost cell layers are limiting elongation of the whole segment, and that the initial elongation response to IAA is a consequence of action on these layers (page 58). If this response is mediated by hydrogen ions, then I predict that (1) IAA treatment will cause a reduction in pH in the walls of the outermost cell layers; and (2) treatment of outer cell layers with acid at the same pH will promote elongation to the same rate as induced by IAA.

My results with outer tissue cylinders suggest that an an acid solution of about pH 4 will substitute for IAA, if maximum acid-induced and IAA-induced rates in unbuffered solution are compared (Fig. 23). Given the assumption that IAA-induced reduction in pH causes the same elongation response as that caused by treatment with an acid solution, I suggest that an IAA-induced reduction of pH to about 4 must occur in the outer cell layers of lupin hypocotyls, if the IAA-induced extension is mediated by hydrogen ions.

Penny et al. (1975) have measured pH changes induced by IAA treatment of lupin hypocotyl segments, with a pH microelectrode inserted in a xylem vessel of an elongating segment. No reduction in pH below 6.0 could be detected before segments started elongating rapidly in response to IAA treatment. However, I have not investigated the possibility that IAA causes pH to be reduced in the outer cell layers

of lupin hypocotyls. In other plants which have been investigated, it has been observed that IAA treatment causes the pH of the free space of the outer cell layers to fall to values ranging between 4.8 and 5.4 (Cleland 1976, Jacobs and Ray 1976, Mentze et al. 1977, Rayle and Cleland 1980). My results (Fig. 23) suggest that treatment with acid solutions of pH in this range (4.8-5.4) would not be sufficient to promote elongation of outer tissue cylinders of lupin hypocotyl to rates observed in IAA-treated segments. If IAA treatment of lupin hypocotyls causes pH in the free space of the outer cell layers to fall to a value similar to that recorded in other studies (pH 4.8-5.4), then it seems unlikely that the major part of the early IAA-induced elongation response in lupins is mediated by pH lowering in the walls of the limiting outer layers.

Comparison of responses to acid treatment of outer tissue cylinders [Fig. 21(B)] and cortex cylinders [Fig. 27(A), (B)], suggests that the outermost cells of outer tissue cylinders respond less rapidly to acid than the inner cells of those cylinders. This conclusion is based on the assumption that hydrogen ions would effectively penetrate the cortex free space to reach the outer layers. However, I do not know how the free space pH in the outer layers changed in response to a pH change of the solution in the central cavity. The observed course of response of outer tissue cylinders was possibly governed by both tissue sensitivity to hydrogen ions, and by change of pH with time in the outer cell layers of these cylinders. Possibly, then, the poor response to acid at pH 5 seen in outer tissue cylinders did not reflect the response which would occur if IAA reduced pH in the outer cell layers.

It is difficult to further test this possibility. Access of protons to the outer cell layers from outside the segment was apparently restricted by the cuticle, since intact lupin hypocotyl segments responded only weakly to an acid treatment at pH 3 (Fig. 22), in line with results of experiments on light-grown peas (Barkley and Leopold 1973, Yamamoto et al. 1974). Cuticular resistance to diffusion of protons can be reduced by treatments such as ether-dipping. Lupin segments so-treated show an improved response to acid treatment at

pH 4, but which is still only about 70% of IAA-induced rate (Perley et al. 1975). However, it is likely that some restriction on proton diffusion remains. The same problem exists where abraded segments are used (Jacobs and Ray 1976, Taiz and Métraux 1979).

The second objective of this study was to compare the responses of lupins with those of other plants, so that the general applicability of the conclusions could be assessed.

I have shown that peeled segments of lupin responded rapidly to acid treatment, if they had first been pretreated in buffer. This short-lived response was similar to that found with peeled segments of etiolated Avena coleoptile (Rayle 1973), peeled segments of Helianthus hypocotyl (Firn and Digby 1977), and abraded segments of etiolated Zea coleoptile (Jacobs and Ray 1976). Similarly buffered solutions were used in those experiments. At least in these conditions, then, the behaviour of lupins was not different from that of other materials. Since it has been shown that acid solutions of pH 4.8-5.0 will cause an elongation response of maximum rate equivalent to that obtained with IAA treatment, it has been concluded that observed IAA-induced pH lowering is sufficient to account for the rapid IAA-induced elongation response (Rayle 1973, Jacobs and Ray 1976). However, I have shown that the acid-induced response in peeled segments of lupin hypocotyl was due to pretreatment in buffer, and segments responded poorly if pretreated in water. Similar effects of buffer treatment have been noted with segments of Zea root (Edwards and Scott 1974) and lettuce hypocotyl (Moll and Jones 1981). I have not further investigated the reason for inhibited pretreatment elongation rate in buffer. It might be an osmotic effect, or due to buffer pH or composition. The elongation response on pH change might be an expression of "stored growth" (Ray 1961).

I suggest that effects of buffer pretreatment on the response to subsequent acid treatment might account for the greater part of acid-induced elongation found in other investigations (Rayle 1973, Jacobs and Ray 1976, Firn and Digby 1977). If so, then only when segments have been pretreated with buffer will IAA-induced pH lowering

account for the initial rapid IAA-induced elongation response.

I have shown that buffer-treated outer tissue cylinders of lupin hypocotyls were less responsive to acid than peeled segments. This is consistent with my suggestion that the outermost cell layers are less sensitive to acid than the inner tissues. There is other evidence in other materials of such differential sensitivity. In a study similar to the present one, Yamamoto et al. (1974) present data which show that acid-induced elongation of hollow cylinders of light-grown pea stem, at pH 4.5, over one hour, was about 38% of that of similarly treated peeled segments. Cleland and Rayle (1975) give results which show that peeled segments of light-grown pea stem elongated initially about 50% more rapidly than segments with the epidermis slit but otherwise intact, at pH 3.3. These results suggest that the outermost cells of segments used in these studies might be less responsive to acid than the inner tissues.

I am not aware of any studies with etiolated plants where similar comparisons can be made. However, abraded segments of some etiolated materials will respond to pH 5 treatment (Jacobs and Ray 1976). Since the epidermis was retained, then these segments were apparently more sensitive to acid than are segments of light-grown lupin hypocotyl which also retained the epidermis, and to which acid had access. Possibly, segments of etiolated plants are more sensitive to acid than are segments of light-grown plants of the same species. I have not compared etiolated and light-grown lupins; however, Barkley and Leopold (1973) have reported that peeled segments of light-grown pea stem would not respond to acid treatment, but that intact segments of etiolated plants would respond.

I consider that my results do not support the hypothesis that hydrogen ions cause the initial phase of IAA-induced elongation in intact lupin hypocotyl segments. However, the association of free space pH lowering with IAA action is apparently a common phenomenon (Cleland 1976, Jacobs and Ray 1976, Mentze et al. 1977, Rayle and Cleland 1980), and might also occur in lupins. Penny et al. (1975) failed to detect any reduction in pH within the latent period for IAA-induced elongation, in

segments of both Avena and lupin. The pH microelectrode was positioned in a xylem vessel, however, and it now seems possible that any pH change in the free space outside the starch sheath would not be detected within it. I have shown that the stelar tissues of lupin hypocotyls in isolation are only weakly IAA-responsive [Fig. 20 and Fig. 29(C)], so little free space pH lowering because of direct IAA action on these tissues would be predicted. O'Brien and Carr (1970) have shown that there are suberised layers in walls of bundle sheath cells of Triticum, Zea and Avena leaves. The presence of any such layers in bundle sheaths of Avena coleoptiles may restrict proton diffusion into the vascular bundle from outside, and might explain why Cleland (1976) could show IAA-induced pH lowering in the outer cell layers of Avena coleoptiles, while Penny et al. (1975) could not detect any such rapid response in the pH of the solution inside a xylem vessel. Other explanations for this difference have been discussed elsewhere (Jacobs and Ray 1976).

5: DISCUSSION

In introducing this work I referred to the difficulty in studying the mechanism of cell expansion when measurement of expansion (in cells of higher plants) must be made on a macroscopic scale, using segments of tissue. I have illustrated some of the problems in interpreting the elongation responses of segments of lupin hypocotyl in terms of underlying cellular behaviour, in each of the three sets of experiments conducted. Since results have already been discussed in each chapter, this concluding discussion is limited to showing how the results might be interrelated.

There are two fundamental difficulties in interpreting the results. Firstly, the major pathway for water flow into growing segments in solution is not known; and secondly, it is not clear whether elongation of segments is limited by the rate of water uptake or by the rate of irreversible wall yielding. The path for water flow to expanding cells in the hypocotyl of intact seedlings is probably radially inwards and outwards from the xylem (Boyer and Wu 1978, Cosgrove 1981). In elongating segments in solution, it is possible that this remains the major path for water flow. If most water flow is from the xylem, it might be in the cell-cell path rather than through cell walls (Cosgrove and Steudle 1981). The alternative pathways for water flow are through the epidermis, and longitudinally through the tissue from the cut ends of the segment. Since it is not clear which is the main path for water flow, it is not obvious which form of biophysical model (Cosgrove 1981) will apply. Therefore, it is not clear which parameters are involved in control of water uptake.

In principle, elongation of segments in solution could be limited by the rate of water flow into the segment, if resistance of the pathway was sufficiently high. If limited by water uptake, it is predicted that turgor pressure (P) in cells of the hypocotyl would be lower when growing than when growth had ceased (Cosgrove 1981). Evidence that P is lower in hypocotyls of soybean seedings when elongating rapidly than when elongating slowly, has been provided by Boyer and Wu (1978). In segments of lupin hypocotyl in solution, evidence that the segment

might not be in water potential equilibrium with the external medium is seen in the rapid extension of segments after peeling (Fig. 18). This extension might represent discharge of a water potential gradient (page 60) and hence might indicate that P was low in the segments before peeling (and therefore that elongation was limited by water uptake). However, it is also possible that the segments were initially at a low water potential because the water potential in the xylem was low. This was probably the case, since preliminary experiments indicated that the root provided a limiting resistance to water flow into the hypocotyl (elongation rate of the hypocotyl was increased when the root was excised), and also suggested that the xylem sap was under tension (since dye immediately entered the xylem when the hypocotyl was cut under the dye solution). However, since segments which had been in solution for an hour also extended rapidly after peeling (Fig. 19), it is possible that some water potential disequilibrium persisted.

If elongation is limited by extensibility (the rate of irreversible wall yielding), then an alternative explanation of the rapid extension after peeling is required, and has been given on page 59. This other possibility is that removal of the epidermis effectively reduces the elastic modulus [equation (6), page 11] of cell walls in the peeled segment, thus resulting in a reduction in P and therefore a reduction in the water potential. The water potential gradient between the segment and the medium would then be discharged, and the segment would extend. This hypothesis arises from the possibility that the epidermis may limit extension of the intact segment, thus imposing a restriction on extension of the other tissues. Turgor pressure in the inner tissues would therefore be "high" because the effective elastic modulus of cell walls would be high (i.e., cell walls would be relatively inextensible). A possible explanation of why the epidermis might limit elongation of the intact segment is that the walls of the epidermal cells might not yield at the same rate as the walls of other cells in the segment. This might be due to differences in wall thickness (page 78) or in some other property of the cells which determines extensibility or the minimum yield threshold [equation (1), page 2]. If elongation is limited by the rate of wall yielding, then it is predicted that turgor pressure will be high (i.e., turgor pressure in

rapidly elongating segments will be similar to that in segments elongating only slowly) (Cosgrove 1981).

If elongation is limited by water uptake, then the initial action of auxin must be on this limiting step, if the rate of elongation is to be promoted. In principle the rate of water uptake could be increased by an effect on any of the parameters governing D, which is a function of protoplast and cell wall hydraulic and elastic properties (Molz and Boyer 1978); or by effects on L (hydraulic conductivity of the epidermis) or σ (the reflection coefficient); or π (the osmotic pressure).

The initial elongation response to IAA treatment seen in intact segments of lupin hypocotyl (Fig. 20) might then represent the effect of action on the rate of water uptake, with an increase in P in the outer cells and (partial) discharge of an existing water potential gradient. This hypothesis is consistent with the observations that the initial elongation response (after IAA treatment) was not seen in peeled segments, since it would be expected that these segments were already close to water potential equilibrium with the external medium. It would also be consistent with the evidence that the initial response was still found in outer tissue cylinders, if in these cylinders the pathway for water flow to the outer tissues was across the cortex, since the length of that path was changed only little in comparison with its length in the intact segment.

If the rate of water flow was increased so that it was no longer limiting, then elongation would become limited by the rate of wall yielding. A second effect of IAA might then be in enhancing extensibility (Green and Cummins 1974, Boyer and Wu 1978), and maintaining the gradient for water influx by reducing P (as seen in the results of Boyer and Wu). The elongation responses of peeled segments of lupin hypocotyl might then reflect IAA action on wall yielding.

However, if segment elongation is limited initially by the rate of irreversible wall yielding, then the first action of IAA must be on this process. In lupins, this effect need only be on the outer tissues

(initially, at least). In terms of the hypothesis already described, an effect on the rate of yielding of the outer tissues might result in a large initial elastic extension of cells of the other tissues of the segment. Since P would increase, the rate of water uptake would fall; and because of this, and also because of a continuing high rate of wall yielding, the turgor pressure would subsequently fall. Turgor might then be maintained by an increase in the osmotic pressure of the cells, as suggested by Cleland and Rayle (1978).

The elongation responses of segments treated with acid (Chapter 4) are now explained in the context of the points just discussed. According to the acid-growth hypothesis (Rayle and Cleland 1980) the action of IAA (and of hydrogen ions) is on wall properties. If elongation of the segment was limited by the rate of water uptake, then the acid-growth hypothesis could not account for the initial elongation response after IAA treatment. It would then be expected that treatment with acid would not promote elongation. If the elongation of outer tissue cylinders of lupin hypocotyl was limited by water uptake, then it is not surprising that they did not initially elongate rapidly when treated with acid. This is an alternative explanation of the failure of these cylinders to respond to acid treatment; on page 78 I suggested that differences in wall thickness might account for the difference in response between outer tissue cylinders and cortex cylinders. That explanation was based on the assumption that elongation was extensibility-limited. It is possible that the elongation of cortex cylinders might be limited by extensibility, if they were close to water potential equilibrium with the external solution.

These points illustrate the difficulties in drawing inferences about cellular behaviour from the responses of segments, and also suggest that caution should be exercised in drawing inferences about the responses of intact segments from the responses of different tissues in isolation. Clearly, some questions need to be answered before the elongation of segments is interpreted in terms of a particular biophysical model (Cosgrove 1981). What is the main path for water flow in the growing segment? Is it through the epidermis, or from the

xylem? Through cells, or through the apoplasm? What is the limiting resistance to flow in this path? For example, if water flows from the xylem radially outwards to growing cells, is resistance distributed equally along the path, or is there a limiting resistance at the starch sheath?

Whether the model chosen is that for single cells or that which accounts for distributed resistance to water flow (Cosgrove 1981), each predicts that turgor pressure will be low in growing segments if elongation is limited by extensibility (Cosgrove 1981). It would be useful to test this prediction in any study of elongation, before the assumption of extensibility-limited elongation is made. This would help in interpreting the elongation responses to IAA treatment, and would indicate whether or not the initial response to IAA treatment could in principle be accounted for by an effect on wall yielding properties. The advent of techniques which permit measurement of turgor pressure in single cells of higher plant tissues (Hüsken et al. 1978) would be useful in this context, and should greatly assist our understanding of the mechanism of cell expansion.

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