Induction of Coleoptile Elongation by Carbon Dioxide

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ABSTRACT

The ability of CO2 to induce elongation of Avena sativa coleoptile segments was examined with the use of a high resolution growth-recording device. CO2-saturated water causes an 8- to 16-fold promotion in the rate of elongation within 1 minute. This elongation is insensitive to a variety of metabolic inhibitors that suppress auxin-induced elongation, and the CO2 effect cannot be prevented by pretreatment with these inhibitors. Buffers of pH 3 to 4 also stimulate elongation quickly, and it seems that at least a major part of the action of CO2 depends upon its ability to reduce pH. The rate of elongation of auxin-promoted segments can be further enhanced by treatment with CO2 but not vice versa.

The response to CO2 can be inhibited by mannitol at osmotic concentrations that inhibit normal growth, by calcium, and by brief pretreatment with heavy water (D2O). The elongation rate that results from CO2 treatment is sensitive to temperature, but the induction by CO2 itself appears to be almost temperature-independent.

Elongation following treatment with CO2 may be a physical flow phenomenon, essentially independent of immediate biochemical participation, which occurs when cell polymer interactions that normally restrict strain in the cell wall are weakened or broken by CO2 in a manner that in effect substitutes for the role of metabolism in normal auxin-inducible cell enlargement.

The literature contains a number of reports on the promotion of elongation, cell enlargement, or water uptake by CO2 (3, 7–9, 19, 29, 35, 36) and by acid pH (1, 2, 16, 18, 22, 27, 34, 35). Reinhold and Glinka (29) provided evidence that induction of water uptake by CO2 in sunflower hypocotyl tissue involves a lowering of turgor stress. The brevity of the CO2 treatment required (10 sec) and the fact that the effect can be obtained at 0°C led them to suggest that the CO2 effect was a physical one. They speculated that the lowering of turgor stress by CO2 might be due to cell wall loosening resulting from alterations in cell wall structure induced by low pH.

The purpose of the present work was to characterize the elongation response of coleoptiles to CO2 and to examine the mechanism involved, using a high-resolution growth-recording device.

MATERIALS AND METHODS

Plant Material. The experiments were done with 8-mm segments taken beginning 3 mm below the tip of 3-day-old etiolated oat coleoptiles (Avena sativa L., var. Victory). Oats were grown as described elsewhere (6).

Growth Measurements. The growth-recording device used is these experiments is described in detail elsewhere (6). Briefly, 13 hollow coleoptile segments are strung on a thread and rest on a supporting platform at the bottom. A piece of red Pyrex glass capillary tubing weighing about 90 mg is also strung on the thread so that it rests upon the uppermost coleoptile segment and serves as a weight. The thread assembly is then inserted into a tubular glass chamber 14 ml in volume, and the chamber is clamped into a vertical position and filled with a particular growth medium. A small arc lamp is used to cast a sharp shadow of the glass weight onto a narrow vertical slit in a baffle placed about 1 m from the chamber. Immediately behind the slit a long piece of photographic paper is drawn horizontally by a kymograph drum turning at a rate of 3.08 mm/min. This arrangement allows continuous shadowgraphic recording of the elongation of the entire column of coleoptile segments by recording the vertical displacement of the shadow of the glass weight as the latter is pushed upward along the thread by the growing segments below. All curves shown in this work are direct tracings of original shadowgraphic records with time scales indicated. Since the magnification differs slightly for each record, a marker representing a 1-mm increase in length of the column of segments is shown at the end of each curve.

Unless otherwise noted, the growth medium surrounding the coleoptile segments was continuously gassed with oxygen. Solutions could be drained and replenished within 15 sec through the appropriate outlet and connecting funnel. All experiments were done under dim red light and at 26°C, except as noted.

Treatment with CO2. CO2 treatment was given by emptying the chamber and refilling it with a solution of CO2-saturated water. The CO2-saturated water remained in the chamber for 3 min. During this period, gassing of the solution was with CO2 instead of oxygen. After 3 min, oxygenation of the CO2-saturated water within the chamber was begun. CO2 treatment was given in this manner instead of continuously, since a continuous supply of CO2 is not necessary for the achievement of the full stimulatory effect, and complications due to anaerobiosis and to irreversible damage to membranes may thus be avoided (29). CO2 concentrations below saturation were made by mixing CO2-saturated water with the appropriate amount of distilled water and immediately adding the mixture to the growth-measuring chamber. During the 3 min that such solutions were in the chamber there was no gassing.

1 Supported by National Science Foundation grants to P. M. Ray, M. L. Evans, and K. V. Thimann. Much of the material presented here is drawn from the Ph.D. thesis of M. L. Evans (5).
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Received for publication May 28, 1970

Plant Physiol. (1971) 47, 335–341
In some experiments the segments were pretreated with 10⁻⁴ M KCN at pH 6.5. This solution was prepared by adding a small amount of HCl to 10⁻⁴ M KCN in 10⁻⁴ M phosphate buffer (initial pH, 6.5) so that the final pH remained at 6.5. The HCl was added to the KCN solution just prior to using the solution in the growth-measuring chamber. CO₂-saturated 10⁻⁴ M KCN was prepared by diluting 10⁻⁴ M KCN (pH adjusted to 6.5) with CO₂-saturated water.

In experiments in which it was desired to follow the time course of expansion of segments whose turgor had been reduced by evaporating water from them, the evaporation was accomplished by draining the chamber and forcing a stream of air around the segments. When the segments had shrunk the desired amount, the chamber was filled with water, and their expansion was recorded.

In order to measure the temperature dependence of the CO₂ response, a constant temperature bath with a cooling unit was used. By suitable methods (5) cold water could be circulated through the growth-measuring chamber when desired. A copper-constantan thermocouple was used to measure temperature. Temperature changes in the chamber were completed within 1 min.

RESULTS

Time Course of Growth Response to CO₂. Figure 1 shows the response of oat coleoptile segments to CO₂-saturated water (pH 3.8). After a lag of about 1 min the elongation rate increases abruptly to about 15 times the rate of elongation in water alone. The average CO₂-promoted rate of elongation for 20 experiments was 1.1 mm per hr per segment as compared to 0.07 mm per hr per segment in water alone. With a single CO₂ treatment, the stimulated rate of elongation continues for about 20 min before dropping abruptly to a value only moderately greater than the rate of elongation before CO₂ treatment. Figure 1 also shows that it is possible to obtain a second CO₂ response by treating with CO₂ again after the initial CO₂ response has declined. In all cases, however, the second response to CO₂ is weaker than the first. The duration of promotion by CO₂ can be extended by giving CO₂ continuously, but under these circumstances the rate of elongation begins to decline sooner than when a brief CO₂ treatment is given, possibly because a decline in turgor sets in because of deterioration of cellular membranes (29).

Figure 2 shows the dependence of the magnitude and duration of growth promotion on concentration of CO₂. Solutions below about 35% of saturation with CO₂ (pH 4.25) produce stimulations which are reduced in magnitude but continue during the usual 20-min period. The total elongation induced by CO₂ treatment is therefore less at CO₂ concentrations below 35% of saturation.

Interaction with Auxin-promoted Elongation. The elongation rate response of coleoptile segments to CO₂ is greater than that induced by indole-3-acetic acid. With IAA the normal lag period in the response is 10 to 12 min (6) and the growth promotion is about 8-fold. Figure 3A shows that IAA does not further accelerate elongation when the rate has already been promoted by CO₂. On the other hand, CO₂ treatment can raise the elongation rate of segments already responding to IAA, but only to the level of a typical CO₂ rate (Fig. 3B).

Elongation Induced by Buffers of Low pH. Curve B in Figure 4 shows that citrate buffer at the same pH as CO₂-
saturated water (pH 3.8) promotes elongation just as quickly as does CO2. However, the resultant elongation rate is somewhat less than that obtained with CO2. Other buffers such as malate and acetate at pH 3.8 were found to promote growth just as quickly, but not as strongly as citrate buffer. Curve C in Figure 4 shows that growth promotion by citrate buffer at pH 3.8 is not caused by the citrate molecule itself since citrate buffer at pH 6.5 has no growth-promoting effect. The duration of elongation promoted by buffers at low pH is many times greater than when elongation is promoted by CO2. Segments stimulated by buffers at low pH continue to elongate at a steady rapid rate for as long as we have continued recording (up to 90 min).

**Role of pH in CO2 Effect.** Buffers saturated with CO2 at pH 7.8 caused no promotion of elongation. CO2-saturated 10 mM bicarbonate buffer (pH 6.3) or CO2-saturated 10 mM histidine buffer (pH 6.1) caused no, or only a very slight, elongation response (Fig. 5C). On the other hand, 10 mM succinate buffer, saturated with CO2 and adjusted to pH 5.6 with NaOH, promotes elongation rate by about 40% of the effect of CO2-saturated water (Fig. 5B). A similar response is obtained with CO2-saturated malonate buffer (10 mM), pH 6.1. However, the pH of the CO2-saturated succinate buffer rose from 5.6 to 6.8, and that of the malonate buffer from 6.1 to 7.4, during the experiments (about 30 min). This indicates that the conjugate acid of these buffer systems enters the tissue more readily than the conjugate base so that the pH within the tissue during CO2 treatment must have been lower than that of the buffer itself. In contrast, the pH of the bicarbonate and histidine buffers, mentioned above, rose only slightly during treatment of the tissue (from 6.3 to 6.6 with bicarbonate buffer and from 6.1 to 6.5 with histidine buffer).

These results indicate that CO2 promotes elongation only in the acid pH region (below pH 5.6) in which acidic buffers themselves promote elongation. We feel that the reported (27) induction of elongation by CO2 at neutral pH may have been due to inadequate buffering at the site of action of CO2 within the tissue.

**Reversibility.** The stimulation of elongation by brief treatment with CO2-saturated water was not affected for 10 min after transfer to millimolar neutral buffers, but the duration of the response was shortened somewhat (Fig. 6, B and C). Transfer instead to 10 mM phosphate buffer, pH 7.5, stopped elongation more rapidly (Fig. 6D). However, the osmolarity (0.025) of this buffer is in the range that causes an appreciable osmotic effect on the CO2 response (Fig. 12B), so the inhibition in Figure 6D may not be entirely a pH effect. Whether the CO2 effect is in fact immediately reversible by raising the internal pH, as Rayle and Cleland (27) have concluded regarding the acid pH response, seems to us to remain in doubt.

**Effect of Metabolic Inhibitors.** As illustrated in Figure 7, the elongation that is induced by CO2-saturated water is completely unaffected by 10-4 M KCN (curve B) whereas KCN rapidly and completely inhibits auxin-promoted elongation (curve C).

Pretreatment with millimolar KCN prevented the elongation response to CO2. This seems to be due, however, to the alkalinity (pH 9.8) of unbuffered KCN solution, since an
HgCl₂, the metabolic inhibitors tested were incapable of preventing the CO₂ response even when the segments were pretreated with the inhibitors. All of these inhibitors, on the other hand, effectively prevented auxin-induced elongation. Partial inhibition of CO₂-induced elongation by HgCl₂ at 5 × 10⁻⁴ M is suspect because of general toxicity and damage to the cell membranes at this concentration; the treated segments were observed to lose turgor soon and to shrink.

Osmotic Relations. Mannitol inhibited CO₂-induced elongation by about 35% at 0.03 M and by about 75% at 0.1 M. The osmotic sensitivity of this elongation is, therefore, comparable to that of auxin-stimulated growth (Table I).

In order to distinguish between alternative hypotheses regarding the osmotic and mechanical relations of the CO₂ effect to be considered in the "Discussion," segments were transferred to 0.35 M mannitol at two different points during the CO₂ response (Fig. 8). The elastic shrinkage of the tissue was similar whether treated with mannitol early in (curve C) or toward the end of (curve B) the CO₂ response, and the final length of the segments in the latter case was, therefore, greater.

Table I. Effect of Inhibitors on Promotion of Elongation by CO₂ and IAA

<table>
<thead>
<tr>
<th>Inhibitor (pH 6.5)</th>
<th>Concn</th>
<th>Duration of Pretreatment</th>
<th>Inhibition by Pre-treatment</th>
<th>Inhibition by Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCN</td>
<td>10⁻³ M</td>
<td>60</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>NaF</td>
<td>3 × 10⁻³ M</td>
<td>90</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>10⁻⁴ M</td>
<td>30</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>2 × 10⁻⁴ M</td>
<td>30</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>5 × 10⁻⁴ M</td>
<td>50</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>10 µg/ml</td>
<td>40</td>
<td>95</td>
<td>10</td>
</tr>
<tr>
<td>Ethylene</td>
<td>5 µl/liter</td>
<td>20</td>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td>Mannitol</td>
<td>0.03 M</td>
<td>30</td>
<td>78</td>
<td>100</td>
</tr>
<tr>
<td>Mannitol</td>
<td>0.1 M</td>
<td>30</td>
<td>78</td>
<td>70</td>
</tr>
<tr>
<td>Low temperature</td>
<td>(4 C)</td>
<td>60</td>
<td>70</td>
<td>70</td>
</tr>
</tbody>
</table>

1 10 mg/liter.

Fig. 6. Effect of pH 7.5 buffers in reversing the elongation response to CO₂. At the first arrow on each curve medium was changed from water to CO₂-saturated water; at the second arrow, to: B: 1 mM phosphate, pH 7.5; C: 1 mM citrate, pH 7.5; D: 10 mM phosphate, pH 7.5 (0.025 osmolar).

Fig. 7. Cyanide insensitivity of elongation induced by CO₂. A: Normal response to CO₂; B: growth medium changed from water to CO₂-saturated water at the first arrow and to 10⁻⁴ M KCN at the second arrow; C: growth medium changed from water to 10 mg/liter IAA at the first arrow and to IAA + 10⁻⁴ M KCN at the second arrow; D: growth medium changed from water to 10⁻⁴ M KCN (pH 6.5, 1 mM phosphate) at the first arrow and to CO₂-saturated 10⁻⁴ M KCN (pH 3.8) at the second arrow.

elongation response to CO₂ could be obtained after pretreatment with 10⁻⁴ M KCN that was buffered to pH 6.5 with phosphate (Fig. 7D). This CO₂ response was slower to develop and the maximal elongation rate was somewhat reduced, compared with the control, but these features were probably due to the gradual exodiffusion and residual pH effects of buffer that is inevitably carried over in the tissue's free space. Brief (10-min) pretreatment with the same concentration of KCN completely prevents the elongation response to IAA, whereas it does not prevent the induction of elongation by acidic buffers (27, and unpublished data of the authors).

Table I summarizes the results of similar tests with a variety of inhibitors. With the exception of high concentrations of
Table II. Temperature Dependence of CO₂-promoted Elongation

<table>
<thead>
<tr>
<th>Temperature</th>
<th>CO₂-Induced Elongation Rate (mm per hr per segment)</th>
<th>Q₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>26.3</td>
<td>0.79</td>
<td>2.24</td>
</tr>
<tr>
<td>14.8</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>31.6</td>
<td>1.43</td>
<td>2.02</td>
</tr>
<tr>
<td>14.9</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>26.1</td>
<td>1.11</td>
<td>1.86</td>
</tr>
<tr>
<td>8.0</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>26.0</td>
<td>1.90</td>
<td>1.69</td>
</tr>
<tr>
<td>4.0</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>25.5</td>
<td>1.20</td>
<td>1.50</td>
</tr>
<tr>
<td>5.3</td>
<td>0.53</td>
<td></td>
</tr>
</tbody>
</table>

1 The first temperature listed is the temperature at which the CO₂ stimulus was given. The second temperature listed is the temperature to which segments were transferred after the initial rate had been recorded.

Induction of elongation by CO₂ (Table II). This indicates that low temperature does not inhibit the action of CO₂ itself, although the elongation process that results from CO₂ action is temperature-sensitive. The total elongation that results from treatment with CO₂ is similar at 4°C and 26°C but simply occurs more slowly at the lower temperature.

Effect of Deuterium Oxide. Brief pretreatment with 100% D₂O strongly reduced the rate of elongation in response to CO₂ (Fig. 10C), but the response that occurs develops as quickly as in the control. The elongation response to auxin is completely prevented by 100% D₂O (cf. Ref 4). Upon transfer from either D₂O or D₂O + CO₂ to water, a strong burst of elongation occurs (curves B and C), which makes it difficult to determine whether any residual potential for elongation is induced by treatment with CO₂ in the presence of D₂O.

Effect of Ca²⁺. In Figure 11, curve B shows that, although

Fig. 9. Promotion of elongation at low temperature by CO₂. A: Growth medium changed from water (26°C) to CO₂-saturated water (26°C) at the arrow; B: growth medium changed from water (4°C) to CO₂-saturated water at 4°C (first arrow) and then to water at 26°C (second arrow). In this experiment the segments were floated on water at 4°C for 30 min before being inserted into the growth-measuring chamber and submerged in water at 4°C.

Further pertinent evidence was obtained by measuring the time course of a purely elastic osmotic swelling as can be seen when tissue is given water after a period of evaporation that has reduced the turgor pressure of its cells (26). Elastic osmotic expansion of coleoptile segments in water (Fig. 8A) followed an exponential course with a half-time of about 3 min. Osmotic equilibration thus occurs much more quickly than the duration of CO₂-induced elongation, and the time course of the latter differs further in being linear rather than exponential.

Effect of Temperature. Table II shows the effect of temperature on an already established CO₂ response. Below 26°C the CO₂-induced elongation rate exhibits a Q₀ of approximately 1.5 to 2.2, considerably lower than the value of 3.5 observed for auxin-induced growth (25).

Figure 9 shows the influence of low temperature on the inception of the CO₂ response. CO₂ stimulates elongation just as quickly when given at 4°C (curve B) as at 26°C, and the resulting elongation rate at 4°C is about the same as when segments are transferred from 26°C to 4°C after induction of elongation by CO₂. This indicates that low temperature does not inhibit the action of CO₂ itself, although the elongation process that results from CO₂ action is temperature-sensitive. The total elongation that results from treatment with CO₂ is similar at 4°C and 26°C but simply occurs more slowly at the lower temperature.

Fig. 10. Effect of heavy water on the response to CO₂. A: Growth medium changed from water to CO₂-saturated water at the arrow; B: growth medium changed from water to 100% D₂O (first arrow) and then back to water (second arrow); C: growth medium changed from water to 100% D₂O (first arrow) to CO₂-saturated D₂O (second arrow) and then back to water (third arrow).

Fig. 11. Effect of CaCl₂ pretreatment on the response to CO₂. A: Normal response to CO₂; B: growth medium changed from water to 2 × 10⁻⁴ M CaCl₂ at the first arrow and to CO₂-saturated water at the second arrow; C: growth medium changed from water to 2 × 10⁻⁴ M CaCl₂ at the first arrow and to CO₂-saturated CaCl₂ (2 × 10⁻⁴ M) at the second arrow.
Fig. 12. Inhibition of the CO₂ response by calcium. Growth medium changed from water to CO₂-saturated water at the first arrow in each curve. In curve B the growth medium was changed to 10⁻⁴ M CaCl₂ at the second arrow. In curve A the growth medium was changed to 3 × 10⁻⁴ M mannitol at the second arrow.

0.02 M CaCl₂ strongly inhibits normal elongation, transfer from this medium to CO₂-saturated water leads to a CO₂ response of normal slope and duration. On the other hand, when CO₂ treatment is given in the continuous presence of CaCl₂ (curve C), the elongation response is slow to develop and does not attain a normal magnitude with respect to either rate or duration.

In Figure 12, curve B shows that 0.01 M CaCl₂ inhibits an already established CO₂ response maximally within 10 min after its addition. That this inhibition was not due simply to osmotic action of the CaCl₂ solution is shown by comparison with the effect of a mannitol solution of slightly higher osmolality (Fig. 12A).

**DISCUSSION**

The rapid but short-lasting elongation of coleoptile cells that CO₂ induces is remarkable in being, according to the foregoing evidence, independent of metabolic requirements and uncoupled from controls that govern auxin-induced growth. Somewhat comparable “explosive growth” phenomena are known in certain other plant cells (11, 20, 23, 30).

Our evidence indicates that the CO₂ response involves and requires acidification and may be a special case of the promotive effect of acid pH upon plant cell enlargement. The differences noted above between induction of elongation by CO₂ and by acidic buffers could be due either (a) to a more effective action of H⁺ ions resulting from CO₂ as compared with buffer treatment, for example, because of better penetration of carboxylic acid than of other acids to the sites where H⁺ ions act, or (b) to some action of CO₂ above and beyond its acidifying effect but which depends upon acidic pH. A conclusive decision between these alternatives cannot be made from present evidence, and in default of proof of a special action of CO₂, we prefer the hypothesis that CO₂ acts by acidification.

Since biosynthetic activity, including cell wall polymer synthesis, is suppressed by cyanide whereas CO₂-induced elongation is not, the latter clearly does not depend upon concurrent cell wall synthesis. This does not warrant the conclusion (28) that cell wall synthesis is not involved in the mechanism of wall expansion during normal growth, because the kinds of evidence presented above, especially the fact that the temperature coefficient of CO₂-induced elongation is much lower than that of auxin-induced growth, indicates that the mechanisms of these two elongation processes are different.

Noteworthy is the short time (1–2 min) that is required for CO₂ or low pH to bring about their maximal effect on elongation rate. Since some time must be allowed for CO₂ or other acids to diffuse at least into the free space of the tissue, and since diffusion of somewhat larger molecules than CO₂ into coleoptile free space has a half-time of several minutes (26), there is strong indication that the effect of CO₂ on elongation rate is exerted essentially immediately upon entry. This is true even at 4 C, and it contrasts with the effect of auxin, which under usual experimental conditions acts on elongation with a temperature-dependent latent period (about 10 min at 26 C), due primarily to processes that follow upon auxin uptake (6, 25). However, Nissl and Zenk (21) have reported an instantaneous promotion of coleoptile elongation by a very high concentration of IAA supplied at 40 C, which they believed to be an auxin effect. Since they supplied IAA in phosphate medium at pH 4.8, at which pH phosphate does not buffer effectively, the immediate promotion that they observed may actually have been an acid pH effect caused by rapid entry of undissociated IAA molecules into the tissue.

Regarding the nature of the effect of CO₂, acidity, or both upon elongation, the first question that must be asked is whether the elongation process induced by CO₂ constitutes an irreversible straining of the cell walls, as against an elastic extension of the walls that would result from osmosis if exposure to CO₂ (or H⁺) caused a sudden stress relaxation within the cell wall and thus a sudden drop in turgor pressure below the osmotic equilibrium or steady state value. The evidence given above (a) that the time course of an elastically controlled osmotic swelling is much more rapid and of quite different form than CO₂-induced extension, and (b) that when transferred to a nearly isotonic medium (0.35 m mannitol) the tissue shrinks no more at the conclusion of a CO₂-induced extension than at its start, and therefore retains a greater final length than before this extension, rules out the second alternative. The possibility can also be excluded that the CO₂ response results from a sudden increase in turgor due to an increase in either solute concentration or in osmotic permeability, because a osmotically provoked step-up in turgor does not cause a comparable elongation response (5) and there is no evident way by which such effects could cause the elongation process to become uncoupled from metabolism. It seems clear that the CO₂ response is the result of some kind of decrease in the normal resistance of cell walls to irreversible extension.

**Possible Modes of Action.** At least three kinds of action may be considered as possible explanations of the effect of CO₂ on the cell wall. (a) CO₂ (or H⁺) may act on the wall structure in some physicochemical manner, for example, by altering the conformation of, or ionic interactions between, polymer molecules and/or between them and cations such as Ca⁺ and Mg⁺. (b) CO₂ or acid pH may cause acid hydrolysis of acid-labile linkages, as suggested by Rayle, Haughton, and Cleland (28), or may promote the action of wall-bound degradative enzymes such as polysaccharidases (13, 15, 17), as suggested by Menzel (18). (c) By altering plasma membrane permeability (7, 8, 14, 24) or promoting some kind of membrane transport such as export of vesicles, CO₂ may induce a release, from the cytoplasm, of enzymes (such as those supposed in b) or of substrates or metabolic products (such as new wall polymers) which interact with the wall to weaken its resistance to extension.

From the rheological point of view the strain process induced in the cell walls by CO₂ could be either a viscoplastic flow or a chemorheological extension, i.e., a strain process governed by continued occurrence of chemical reactions such as those visualized in modes b and c, above. A viscoplastic
strain process independent of chemical reactions is implicit in mode a. Such a strain process could, however, be induced by CO₂ by an action of types b or c if the visualized chemical event took place at the time of exposure to CO₂ and weakened normal restraints to wall expansion sufficiently that viscous properties of the wall material controlled the rate of subsequent extension. The CO₂ effect may, therefore, give experimental access to the viscous retardation mechanisms that in principle must operate in parallel with biochemical stress relaxation phenomena during normal growth.

In the light of the multiple possibilities for CO₂ action on elongation just reviewed, it is perhaps not surprising that much of the presently available experimental evidence can be construed as compatible with more than one kind of mechanism. For example, temperature dependence is obviously to be expected for mechanisms b and c but must be anticipated also for a because the viscosity of concentrated polymer solutions always exhibits strong temperature dependence. The inhibitory effect of Ca⁺⁺ seems to favor a but could be compatible with c, since Ca⁺⁺ influences membrane permeability, and effects of CO₂ on membranes have been inferred to involve exchange of Ca⁺⁺ (31). The inhibitory effect of D₂O seems to favor b or c since D₂O is known to affect the properties of certain proteins (10, 12, 32) and the permeability of certain cell membranes (33, and J. Varner, personal communication), but the possibility that D₂O stiffens the interactions between wall polymers due to increased hydrogen bond strength (cf. references cited in Ref. 10) cannot be excluded at this time. Mechanisms of types b and c demand that the particular enzymes whose action is visualized be resistant to the various enzyme poisons that were tested. Elongation can be induced by CO₂ after normal growth has been suppressed by inhibitors of energy metabolism and protein synthesis that presumably cut off the supply of enzymes, enzyme reaction products, or both that are needed for normal cell wall expansion. Therefore, it appears that if CO₂ acts by a biochemical mechanism of type b or c, the enzymes or products whose action or export is stimulated by CO₂ cannot be the agents that bring about normal auxin-inducible growth. This speaks against the involvement of the cytoplasmic pool of cell wall precursor molecules by a mechanism of type c since these are obviously secreted during normal growth and their production is shut off by inhibitors of energy metabolism.

Recent thinking that the action of CO₂ takes place largely or entirely at the time of exposure, implying that the actual extension occurs by a viscoelastic strain process, are (1) the rapid and especially the saturating nature of the CO₂ effect on elongation rate, (2) the fact that continued exposure to CO₂ is not necessary for continuance of the induced strain process, and (3) the indication that CO₂-induced extension may continue after treatment with neutral buffers that would prevent inception of the response. For reasons given in “Results,” point 3 is somewhat moot, but even if CO₂-induced extension ceased immediately upon neutralization, a physical mode of action of type a could be involved, and a chemomechanical strain mechanism would not necessarily be indicated.

In view of the observation that CO₂ induces elongation as quickly at 4 C as at 26 C, it is difficult to imagine how chemical mechanisms of types b or c could be involved if the action of CO₂ is indeed completed at the time of inception of the response. This speaks in favor of a physical mode of action as visualized in alternative a. However, a conclusive decision between the major kinds of possible action enumerated above cannot be made on the basis of present evidence and must be left for future research.

Acknowledgment. We thank Provost Kenneth V. Thimann for his interest and for support that made possible Dr. Reinhold’s participation.

LITERATURE CITED