Plant Physiol. (1967) 42, 144–152

Molecular Requirements for the Biological Activity of Ethylene[1]

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Received October 17, 1966.

Summary. The molecular requirements for ethylene action were investigated using the pea straight growth test. Biological activity requires an unsaturated bond adjacent to a terminal carbon atom, is inversely related to molecular size, and is decreased by substitutions which lower the electron density in the unsaturated position. Evidence is presented that ethylene binds to a metal containing receptor site. CO_2 is a competitive inhibitor of ethylene action, and prevents high concentrations of auxin (which stimulate ethylene formation) from retarding the elongation of etiolated pea stem sections. It is suggested that CO_2 delays fruit ripening by displacing the ripening hormone, ethylene, from its receptor site. Binding of ethylene to the receptor site is also impeded when the O_2 concentration is lowered, and this may explain why fruit ripening is delayed at low O_2 tensions.

Several gases in addition to ethylene elicit the triple response in etiolated seedlings, and the concentration of each required to produce a just discernible effect has been determined (9, 25). Included are propylene, butylene (isomer unknown), acetylene, and CO. Since these vapors also substitute for ethylene in causing epinasty (9, 10), fruit ripening (11, 27), and other effects (8, 9, 29), it would appear that the molecular requirements for biological action are similar in each case. We have attempted to delineate these requirements using as an assay for ethylene action the effect which the gas has on the growth and tropistic behavior of etiolated pea stem sections.

Materials and Methods

Peas (*Pisum sativum,* var. Alaska) were soaked in water for 5 hours and germinated in moist vermiculite. The seedlings developed in darkness at 23° receiving an occasional exposure to dim red illumination, and were used on the seventh day. Sections 10 mm long were cut from the third internode just below the leaf hook and incubated in a medium containing 1 μM indole-3-acetic acid (IAA), 2 % sucrose (w/v), 0.05 M potassium phosphate buffer (pH 6.8), and 5 μM $CoCl_2$ in glass distilled water. Ten ml of media and 10 sections were placed in a 125 ml Erlenmeyer flask, this was sealed with a vaccine cap, the gas phase adjusted as described below, and the tissue slowly shaken at 23° in the dark. After 3 hours the sections were visually scored in red light for curvature and 15 hours later the gas phase was analyzed by gas chromatography before the tissue was removed, blotted dry, weighed and measured. Details of the procedure have been published elsewhere (4).

Gas Chromatography and Preparation of Gas Mixtures. All gases were chromatographed on a 3 foot alumina column to determine whether ethylene or other low molecular weight contaminants were present. The instrument, a Perkin-Elmer flame ionization detector-Cary Model 31 electrometer combination, could detect a few ppb ethylene in a 5 ml air sample. If a gas was contaminated several 5 ml samples of it were purified by passage through the alumina column, or a shorter length of column packed with alumina or silica gel in the case of compounds with retention times much greater than that of ethylene. A stream splitter inserted between the column and detector diverted about 95 % of the desired chromatographic band which was collected by displacement of water. This sample was recovered admixed with N_2 carrier gas in a ratio of about 1 : 4 and an aliquot of the mixture was analyzed to assure its purity and determine the exact proportions of N_2 and sample. Then a measured amount of the mixture was added to the air phase above the tissue by injecting it through the vaccine cap. When high concentrations were required about half of the air in the flasks was removed before a measured amount of the mixture was added, followed by a known volume of O_2, and enough N_2 to restore atmospheric pressure. In this way mixtures containing 20 % O_2, up to 15 % sample gas and the balance N_2 were prepared.

[1]This investigation was supported by research grants EF-00214 and EF-00782 from the United States Public Health Service, Division of Environmental Engineering and Food Protection, and was carried out while S. P. Burg was the recipient of Career Research Development Award 1-K3-GM-6871 from the USPHS.

CO_2 was chromatographed on a 1 foot silica gel column, and analyzed with a thermal conductivity detector. To adjust the CO_2 level in the gas phase above the tissue 20 ml of air was removed from the sealed container, an appropriate amount of CO_2 added, and air readmitted until atmospheric pressure was restored. The CO_2 concentration was checked at the start and conclusion of the incubation, and the pH of the media at the end by injecting pH indicator dye into the flask. The initial O_2 content was adjusted by flushing the sealed flasks with N_2 for several minutes to instate anaerobic conditions, after which a measured amount of N_2 was removed and the same volume of O_2 added. The O_2 concentration was determined at the start and finish of the incubation by means of gas chromatography, using a 6 foot 5A molecular sieve column and thermal conductivity detector.

As a measure of respiration (except when CO_2 was added) the CO_2 content of the flasks was determined by gas chromatography at the end of the incubation period. If an effect on respiration was indicated or if it was particularly important to determine whether any had occurred, O_2 consumption and CO_2 production were measured using a Warburg respirometer.

Determination of Henry's Law Constants. The Henry's law constants for many of the gases studied in these experiments are not available, so a simple procedure was devised to determine them. A 125 ml flask (A) and a 1000 ml flask (B) were sealed with vaccine caps and their volumes calibrated. Into (A) was injected 10 ml of distilled water and a 0.3 ml gas sample, the flask was shaken for an hour, and the concentration of gas in the air phase determined by gas chromatography. Flask (A) was then inverted and 5 ml of water removed with a hypodermic syringe. This was injected into (B), the flask shaken for an hour, and the concentration of gas in the air phase determined. Because the vapors tested are relatively insoluble in water, and since the volume of the air phase in (B) is very large by comparison to that of the liquid phase, at equilibrium essentially all of the gas is found in the air phase. Therefore the amount of gas dissolved in 5 ml of the liquid phase of (A) is equal to the total gas content of the air phase in (B), and by comparing this value to that for the concentration of gas in the air phase of (A) the Henry's law constant was calculated. Reliability of the procedure is evidenced by the fact that experimentally determined values for ethylene (0.80×10^7) and acetylene (0.0985×10^7) at 23° are within a few percent of published figures (20).

Stability Constants of Metal Complexes. The methods of Muhs and Weiss (28) and Gil-Av and Herling (18) were used. In the first a stationary phase consisting of a measured weight of 0, 0.85 and 1.59 M $AgNO_3$ dissolved in ethylene glycol was coated on 60/80 mesh Gas Chrom R at a concentration of 40 % (w:w) and packed into a 6 foot column. The temperature, flow rate, pressure at the column inlet and outlet, density and amount of liquid phase were measured, and from these values and the retention time of a compound on each column a value for the stability constant of the silver-olefin complex was calculated (28). In the second method retention time on an ethylene glycol column containing 1.59 M $AgNO_3$ was compared to that on a column containing ethylene glycol and 1.59 M $NaNO_3$ (18).

Testing Gases for Their Ability to Ripen Fruits. Gros Michel bananas were kindly supplied by a local shipper (Banana Supply Co. of Miami). A single hand of green fruit was divided into 5 lots of 4 fingers and each lot placed in a 10 liter desiccator at 15°. One desiccator served as a control, into another was injected 1 ml of ethylene, and into the remaining chambers quantities of the gas to be tested in the ratio 1:10:100 respectively. After 18 hours the fruits were removed, and thereafter they were kept at 15° until ripening occurred. No attempt was made to purify the gases by chromatography because of the large volumes required. However, the ethylene content of each was determined and results are only included for gases whose activity cannot be due to contamination with ethylene.

Use of Michaelis-Menten Kinetics and Lineweaver-Burk Plots. These familiar techniques and their associated symbols, interpretation, and nomenclature have been applied to other physiological problems (1, 15, 26), and therefore we have adopted them without modification. It is assumed, although not proven, that the specific terminology employed (16) has the same meaning in the present physiological context as it does in the study of enzyme kinetics.

Results
Discussion and Conclusions

The morphogenetic changes which occur when ethylene is present during the pea straight growth test have been described in detail (4), and are only briefly reviewed here. Within the first few hours of the assay, before the gas begins to retard elongation, control sections curve by about 30° whereas segments exposed to ethylene remain straight. Subsequently ethylene treated sections begin to swell, and ultimately they elongate 50 % less than controls. Both elongation and curvature display the same dependence upon ethylene concentration, half-maximum inhibition resulting when ethylene is present at a concentration of about 0.1 ppm. A double reciprocal plot of the effect (V = % change in rate of elongation) vs ethylene concentration (A = ppm ethylene) yields a straight line (fig 1, lower curve) with an intersect on the ordinate at $1/V_m$ where V_m is the maximum percent inhibition caused by ethylene and $1/V_m$ equals 0.02. Any substance which substitutes for ethylene in

the pea straight growth test should produce the same maximum effect as ethylene, so that a double reciprocal plot describing its activity at various concentrations must intersect the ordinate at 0.02. The inhibition of elongation must not be accompanied by a significant change in fresh weight however, for ethylene causes pea tissue to swell but does not inhibit its increase in volume (4). Upon applying an optimal or supra-optimal concentration of the vapor, the ratio between percentage increase in weight vs percentage increase in length should change from the control value, 1.3, to a maximum of 2.6, just as it does when the ethylene concentration exceeds 1 ppm (4). In addition an active compound must prevent the nastic curvature from developing during the first few hours of the assay. Growth inhibitors do not prevent curvature development unless they are present at sufficient concentration to retard elongation by more than 70 %, and substances such as benzimidazole and colchicine which retard elongation without decreasing the fresh weight of pea tissue do not prevent curvature development. That concentration of an active compound which half-inhibits curvature should also produce a half-maximum retardation of elongation (4). Finally an active compound should not decrease either O_2 consumption or CO_2 evolution when it causes a maximum effect on elongation and curvature, for ethylene has no effect on the respiration of pea tissue (table I).

The compounds listed in table II demonstrate

Table I. *Effect of Ethylene on the Respiration of Etiolated Pea Stem Sections*

Measurements were made in a Warburg manometer using the dual flask method for CO_2. Ten 5 mm stem sections were incubated in 3 ml of media containing 2 % sucrose (w/v), 5 μM CoCl, 0.05 M potassium phosphate buffer (pH 6.8), and in some cases 1 μM IAA. Half the samples were gassed with air containing 0.1 % ethylene for 2 minutes before the flasks were sealed. Rates are calculated on the basis of the starting weights, and each value in the table is an average derived from 7 duplicate flasks. At the end of the incubation the flasks were opened and the sections weighed and measured. It was found that ethylene had caused its characteristic inhibition of elongation.

		No IAA		1 μM IAA	
	Hours	Control	Ethylene	Control	Ethylene
Q_{O_2} (μL/g/hr)	0–8	279	268	569	555
Q_{O_2} (μL/g/hr)	18–20	...	...	281	278
Respiratory quotient	0–8	...	...	0.95	0.95

Table II. *Biological Activity of Ethylene and Other Unsaturated Compounds as Determined by the Pea Straight Growth Test*

Compound*	K'_A relative to ethylene**	ppm in gas phase for half-maximum activity
Ethylene	1	0.1
Propylene	130	10
Vinyl chloride	2370	140
Carbon monoxide	2900	270
Vinyl fluoride	7100	430
Acetylene	12,500	280
Allene	14,000	2900
Methyl acetylene	45,000	800
1-Butene	140,000	27,000
Vinyl bromide	220 000	1600
Ethyl acetylene	765,000	11.000
Vinyl methyl ether	1.175,000	10 000
Butadiene	1,200,000 (?)	500,000 (?)***
1. 1 Difluoro ethylene	2,060,000	350 000
Vinyl ethyl ether	5,440,000	30 000

* Ethane, trans-2-butene, cis-2-butene, isobutene, and nitrous oxide were inactive at the highest concentration which could be tested (300,000 ppm) and acetonitrile at 0.1 M. The following were inactive at all concentrations below the toxic level which is indicated in parenthesis: dichloromethane, cis-dichloroethylene, trans-dichloroethylene, trichloroethylene, tetrachloroethylene, H_2S, ethylene oxide, allyl chloride (all approximately 10,000 ppm); CO_2 (100,000 ppm); HCN (0.03 mM); acrylonitrile (0.17 mM); H_2O_2 (0.01 M); KN_3 (4 mM); allyl alcohol (1 mM); and HCHO (0.1 mM)

** The Michaelis-Menten constant (K'_A) for ethylene at 24° has a value of 0.62 mμM in the presence of an infinite concentration of O_2. The term K'_A is used to indicate that ethylene is an activator (A) rather than a substrate (S).

*** This activity may be due to contamination of the butadiene with 0.2 ppm ethylene. So much butadiene was required to produce an effect that it was not possible to purify the requisite amount of gas by chromatography.

an ethylene-like action judged by all the criteria listed above. There is fairly good agreement between results obtained with pea stem sections (table II) and those reported for seedlings (9, 25). Several of these compounds were also tested in the banana ripening assay and it was found that 10 times the half-maximal concentration shown in table II was just as effective as any amount of ethylene, whereas the half-maximal concentration itself gave variable results and one-tenth that level never hastened ripening. Included in these tests were ethylene, propylene, CO, acetylene, methyl acetylene, ethyl acetylene, and allene. Therefore, it is clear that each compound has about the same efficacy in promoting fruit ripening, inhibiting the elongation of pea tissue, and causing epinasty (9). This suggests that triggering a single primary mechanism with ethylene gives rise to a wide variety of responses, just as light and the phytochrome system control a multiplicity of events.

Requirements for Ethylene Action. Based on the data in table II we propose the following requirements for ethylene action:

A) Only unsaturated aliphatic compounds are active (also see 9, 10, 25). The double bond (ethylene) confers far more activity than the triple bond (acetylene) whereas the single bond (ethane) is ineffective.

B) Activity is inversely related to molecular size. In the olefinic series the concentrations of ethylene, propylene, and 1-butene causing half-maximal responses are in the ratio 1 : 130 : 140,000. In contrast, the loss in activity associated with alkyl substitution is far less marked in the acetylenic series and the concentrations required are in the ratio 1 : 3.7 : 61 respectively for acetylene, methyl acetylene, and ethyl acetylene. These differences can be explained by assuming that biological activity is inversely related to molecular size because the unsaturated end of the molecule has to attach to a position of limited access. In the alkene series an alkyl substituent is positioned obliquely with respect to the carbon associated with the double bond and hence it would sterically hinder approach to such a site, whereas in the acetylenic series an alkyl substituent is held in line with the triple bond where it would not cause steric hindrance.

C) Substitutions which lower the bond order of the unsaturated position by causing electron delocalization reduce biological activity. On the basis of size the order of biological activity should be vinyl fluoride > vinyl chloride $\cong$ propylene > vinyl bromide. Instead it is observed that propylene is considerably more active than any of the vinyl halides, and vinyl chloride slightly more active than vinyl fluoride. The resonance form $(-):CH_2-CH = X(+)$ contributes to the structure of the vinyl halides, producing electron delocalization which might influence the biological activity of these compounds. The inductive effect is in the order F > Cl > Br so the fluoride derivative has the lowest bond order and highest dipole even though it has the smallest size. Electron delocalization may also alter the activity of alkyl substituted alkenes and alkynes since both undergo hyperconjugation; alkenes to form $(-):CH_2CH = R(+)$ and alkynes to $(-):CH = C = R(+)$. However, the hyperconjugated form does not contribute very strongly to the structure of the alkyl substituted olefines so their dipole is low, between 0.3 and 0.4 debye units, in contrast to values in excess of 1.4 debye units for the vinyl halides. Assuming that the biological activity of these unsaturated compounds is inversely related to their dipole (a measure of electron delocalization) the order of activity should be approximately opposite to that based on a consideration of molecular size alone. We suggest, therefore, that the observed activity is a compromise between these opposing tendencies and that both steric interference with the approach to the double bond and a lowering of the electron density in the double bond reduce biological activity. The low activity of butadiene relative to 1-butene could then be attributed to extensive electron delocalization throughout the conjugated system of butadiene, and the closely similar activities of methyl acetylene and allene might reflect not only nearly identical sizes, but also the fact that the hyperconjugated form of methyl acetylene has a structure which superficially resembles that of allene.

D) The unsaturated position must be adjacent to a terminal carbon atom. This is indicated by the activity of 1-butene as contrasted with the behavior of cis and trans 2-butene. Presumably in spite of the favorable position of its double bond isobutene is inactive because of extensive steric hindrance. Since acetonitrile is completely inactive, whereas methyl acetylene is highly active, it would appear that nitrogen will not substitute for the carbon adjacent to the double bond. Acetonitrile undergoes extensive hyperconjugation however, and this also might explain its inactivity.

E) The terminal carbon must not be positively charged. This requirement is suggested to account for the activity of CO and inactivity of formaldehyde. The resonance form $(-):C = O(+)$ contributes to the structure of CO, overcoming the inherent polarity of the C-O bond to yield a molecule in which there is a slight negative charge on the carbon. The polarity of the carbonyl group in formaldehyde causes its carbon to be strongly positive in charge.

Evidence that Biological Activity Requires Metal Binding. The ability to form complexes with metals is a property of unsaturated aliphatic compounds which is lacking in other aliphatic and all but a few aromatic molecules. The bonding in the complex is influenced by the availability of electrons in the filled π-orbitals of the unsaturated compound, and also by the ease of overlap of these orbitals with those of the metal as determined by

Table III. *Comparison of Biological Activity of Olefines with the Stability of Various Silver-Olefin Complexes at 23°*

Compound	Stability constant (K_1)* Method 1	Method 2	Relative concentration needed for half-maximal biological response
Ethylene	27.0	34.0	1
Propylene	14.4	17.0	130
1-Butene	13.4	...	140,000
Vinyl methyl ether	9 5	9 2	1,175 000
Butadiene	6.9	7.8	1 200 000 (?)**
Vinyl ethyl ether	7.2	...	5,400,000
Cis-2-butene	6.4	...	inactive
Trans-2-butene	1.9	3 2	inactive
Allene	1.4	1 5	14 000
Isobutene	0	0	inactive
Methane	0	0	inactive

* The stability constant $K_1 = (U.AgNO_3)_L/(U)_L(AgNO_3)_L$ where $(U)_L$ is the concentration of the compound in the liquid phase, $(AgNO_3)_L$ the concentration of $AgNO_3$ in the liquid phase, and $(U.AgNO_3)_L$ the concentration of the metal complex in the liquid phase. Method 1 is that of Muhs and Weiss (28), and values reported by them at a temperature of 40° are only slightly lower than those in the table. Method 2 is that of Gil-Av and Herling (18). The constants for acetylenic compounds and vinyl halides could not be determined as the alkynes did not pass through the column, and the halogenated olefines apparently were influenced by a strong salting out effect which was not corrected for by the methods employed.

** This activity may be due to a slight contamination with ethylene (see footnote to table II).

steric factors (28). Similarly, biological activity requires unsaturation, is affected by steric factors, and influenced by electron delocalization, and this suggests that ethylene and its analogues might bond to a metal containing receptor site. The empirical requirements for biological activity outlined in the preceding section bear a marked resemblance to the rules for silver binding to olefines (28) and accordingly there is a close correlation between biological activity and the stability constants of various olefin-silver complexes (table III). Even in the case of allene, ostensibly an exception (table III), the data on metal binding explains why the biopotency is only one-hundreth that of propylene, whereas just the opposite result might be predicted from rules 2 and 3 above. Another reason for postulating a metal containing receptor site is the fact that CO produces ethylene-like symptoms at concentrations as low as a few hundred ppm, whereas due to competition with O_2 it does not inhibit the respiration of pea and other plant tissues unless it is applied at a much higher concentration. Perhaps by coincidence the K_A for CO binding to the ethylene receptor (table II) and the K_I for CO binding to plant cytochrome oxidase (22) are closely similar. As CO and ethylene must attach to the same receptor to produce the same effect, and since CO characteristically inhibits only enzymes containing metal, it can be concluded that ethylene must bond to a metallic receptor when it produces a biological effect. It will be shown subsequently that the attachment of these gases to the receptor is enhanced by O_2, a further indication that the receptor contains metal, for proteins which bind O_2 characteristically have this property and CO inhibits particularly those enzymes which react directly with O_2. Finally it should be noted that none of the inactive substances (10, 25, and table II, footnote) possess the ability to complex metal except cyanide, and it was not really possible to test this compound in the pea straight growth test since it prevented almost all growth and respiration at a relatively low concentration.

The nature of the metal involved is not known, but there is circumstantial evidence that it may be zinc. We find that month old zinc deficient tomato plants hardly respond to ethylene even overnight, whereas plants deficient in copper, iron, phosphorous or nitrogen show strong epinastic symptoms within a few hours.

Competitive Inhibition of Ethylene Action by CO_2. To account for the observation that CO_2 delays and ethylene hastens fruit ripening, Kidd and West (24) proposed that CO_2 interferes with the action of ethylene. At the molecular level such an interaction is not difficult to envision because CO_2 is a close structural analogue of allene, a compound which substitutes for ethylene in both the pea growth and fruit ripening assays:

$O = C = O$ $H_2C = C = CH_2$ $H_2C = CH_2$

carbon dioxide allene ethylene

Since CO_2 possesses the essential structural features needed for ethylene action, except that it lacks the terminal carbon atom and is negatively charged on both ends, might it not act as a competitive inhibitor of ethylene action? To test this possibility the growth of pea stem sections was studied in the presence of differing concentrations of CO_2 and ethylene. With ethylene omitted, CO_2 did not affect growth except that concentrations greater than 10 % caused a progressive inhibition which was not accounted for by a pH change in

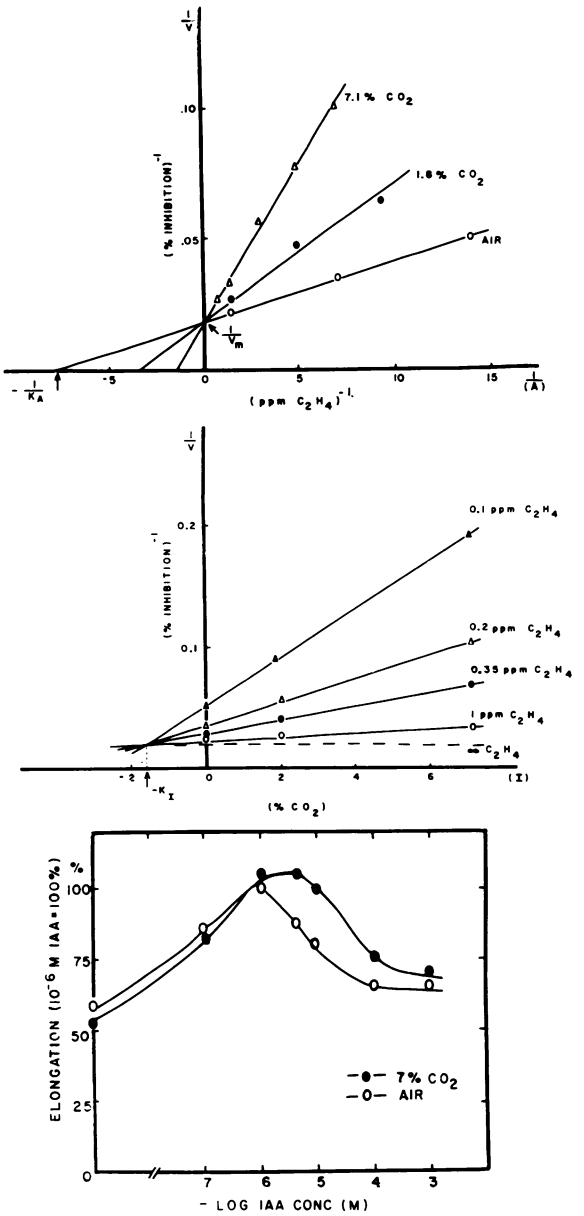


FIG. 1 (upper). A Lineweaver-Burk plot (1/V vs 1/A) relating the percent inhibition of growth (V) to the concentration of ethylene (A) at various levels of CO_2 (I). V_m is the maximum effect occurring when the ethylene concentration is infinite (1/A = 0).

FIG. B (middle). A plot of 1/V vs (I) at various concentrations of (A). The Michaelis-Menten constant for CO_2, K_I, has a value of 0.49 mM (1.55 % in the gas phase). The data in figure 1 shows $1/V_m$ to be independent of (I) so that the behavior of tissue at an infinite concentration of ethylene (1/A = 0 can be predicted by the lower dashed line.

FIG. 3 (lower). Reinstatement of auxin inhibited elongation in etiolated pea stem sections by CO_2. The fresh weight of the tissue was not increased by CO_2 at any auxin concentration in accord with the fact that neither auxin nor ethylene inhibit the water uptake of etiolated pea stem sections (4).

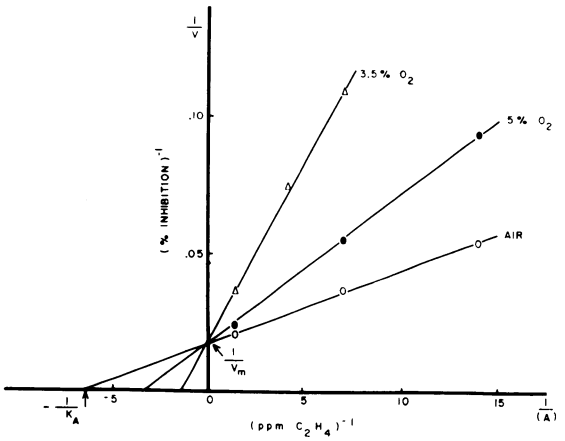
the buffered medium. However, concentrations less than 1.8 % competitively inhibit the action of ethylene (figs 1, 2). The K_I for CO_2 in this process is 0.49 mM (1.55 % in the gas phase) based on the data in figure 2. Similar results have been obtained with pea root sections (7).

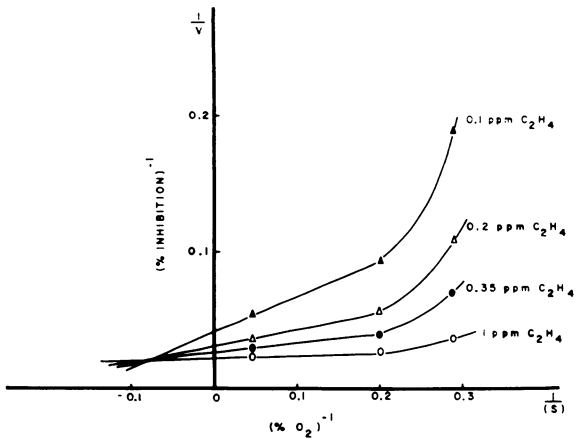
Many physiological effects can be explained in terms of competition between CO_2 and ethylene. For example, ethylene causes flower fading and CO_2 reverses this response and also delays normal fading (31, 32); ethylene accelerates and CO_2 delays abscission (21); ethylene induced epinasty is not expressed at high concentrations of CO_2 (13); and fruit ripening is hastened by ethylene and delayed by CO_2 (23, 34). It has recently been reported that the elongation of etiolated pea and sunflower stem sections is inhibited at supra-optimal IAA concentrations not because of any direct action of auxin but because of auxin-induced ethylene production (4). If this is true CO_2 should be able to reinstate growth at high concentrations of auxin, and the data in figure 3 shows this to be the case. CO_2 is highly effective in reversing the inhibition of elongation caused by 5 μM IAA, and becomes progressively less effective at higher auxin levels because it cannot counteract the action of more than a ppm ethylene (fig 1), the ethylene content of the tissue at 50 μM IAA (4). Similarly, CO_2 reinstates auxin inhibited growth in pea roots (7), and it has been demonstrated that auxin inhibits growth in this tissue by inducing ethylene formation (7). These numerous examples suggest that a response to CO_2 may be used as a fairly specific diagnostic test for the participation of ethylene in a physiological process.

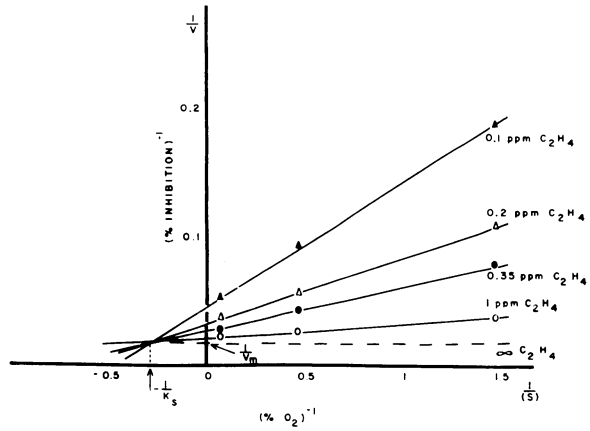
It is not likely that CO_2 frequently functions as a natural growth regulator because the concentration required to cause such an effect is probably higher than that present in most tissues. However, amounts of CO_2 in excess of a few percent typically accumulate in the intercellular spaces of preclimacteric fruits, and the concentration may approach 10 % during ripening and the postclimacteric phase. It is probably this endogenous CO_2 which raises the threshold for ethylene action in fruits to a slightly higher level than that in vegetative tissue (2). According to this view preclimacteric fruits, with their ethylene content of 0.04 to 0.2 ppm (3), have an internal atmosphere in which a just stimulatory quantity of ethylene is carefully held in check through competitive inhibition by endogenous CO_2.

Oxygen Requirement for Ethylene Action. To explain why fruit ripening is delayed when the O_2 concentration is reduced, Kidd and West (24) proposed that O_2 depletion might interfere with the production and action of ethylene. This suggestion is supported by the observation that fruits evolve less ethylene (6) and have a reduced sensitivity to applied ethylene (5, 11, 23) at low O_2 partial pressures, but it has also been argued that

O_2 depletion preserves fruits by reducing their rate of respiration (34). To distinguish between these possibilities pea sections were incubated in the presence of differing ethylene and O_2 concentrations and their growth, respiration and ability to respond to ethylene determined. O_2 consumption (14), CO_2 production, and elongation (19) are unaffected at 5 % O_2 and half-inhibited at 3 % O_2, whereas the efficacy of ethylene is markedly reduced even at 5 % O_2 (fig 4). Thus it is possible to lower this tissue's sensitivity to ethylene without affecting its respiration rate. The same mechanism probably accounts for the retardation of fruit ripening at low O_2 tensions, for 5 or 10 % O_2 has little effect on the rate of respiration and ethylene formation in apples (6, 23) and bananas (17, 34) but significantly delays the onset of the climacteric in both cases. Pea stem sections were also treated with various concentrations of fluoride, azide and ethylene oxide in order to determine whether a change in growth and respiration alters the tissue's ability to respond to ethylene. Even







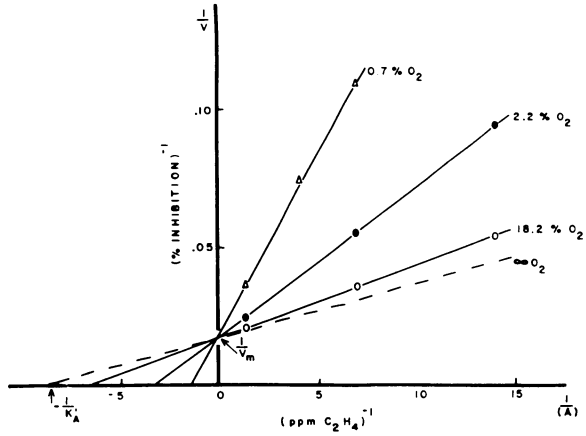


FIG. 4 (upper left). A Lineweaver-Burk plot (1/V vs 1/A) relating the percent inhibition of growth (V) to the concentration of ethylene (A) at various levels of O_2 (S). V_m is the maximum effect occurring when the concentration of ethylene is infinite (1/A = O). The data has not been corrected for an O_2 deficit in the tissue.

FIG. 5 (upper right). A plot of 1/V vs 1/s at various concentrations of (A). The data has not been corrected for an O_2 deficit in the tissue.

FIG. 6 (lower left). A plot of 1/V vs 1/S at various concentrations of (A). The O_2 values have been corrected for a 2.8 % deficit in the tissue. The data in figures 4 and 7 show $1/V_m$ to be independent of (S) so that the behavior of the tissue at an infinite concentration of ethylene (1/A = O) can be predicted by the lower dashed line. Ks, the Michaelis-Menten constant for O_2, has a value of 40 μM (2.8 % in the gas phase), which is comparable to values reported for many other oxidases (33) but considerably higher than that for cytochrome oxidase (33).

FIG. 7 (lower right). A plot of 1/V vs 1/S at various concentrations of (A). The O_2 values have been corrected for a 2.8 % O_2 deficit in the tissue. The inhibition of elongation (V) caused by each concentration of ethylene in the presence of an infinite amount of O_2 (1/S = O) is given by the ordinate intersect of each curve in figure 6, and has been used to construct the lower dashed line. K'_A, the Michaelis-Menten constant for ethylene at an infinite concentration of O_2, equals 0.62 mμM.

at inhibitor concentrations which retarded growth and respiration by more than 50 % the sensitivity to ethylene remained unchanged and the double reciprocal plot was indistinguishable from the control curve (fig 4, AIR).

The kinetic interpretation of the interaction between O_2 and ethylene is complicated by the fact that a plot of 1/V vs 1/S (where S = % O_2) at different ethylene concentrations does not yield intersecting straight lines, but rather intersecting curves which are skewed upward at low O_2 partial pressures (fig 5). Although many factors can lead to such an effect, in this case it is most likely due to an O_2 deficit in the center of the tissue. The factors which give rise to this deficit are well known (33) and it is only necessary here to note that on theoretical grounds the diffusion coefficient for CO_2 (Dco_2) should be 20 times Do_2 (30) so that while a correction may be needed in the case of the O_2 data, none is required for the CO_2 data in figures 1 and 2. The magnitude of the O_2 deficit can be estimated from the fact that the terminal oxidase in pea stem sections is cytochrome oxidase (14, 19), which is half-saturated at 0.2 % O_2 (33), whereas the O_2 consumption of the tissue is half-inhibited at 3 % O_2 (14). When a similar discrepancy has arisen with other tissues it has been possible ultimately to obtain half-inhibition of respiration at 0.2 % O_2 by eliminating the causes of the O_2 deficit (33). Thus it is clear that under the conditions of the pea straight growth test the stem sections have an internal O_2 content approximately 2.8 % below that of the ambient gas mixture. When the O_2 concentrations in figure 5 are corrected by subtracting 2.8 % from each, the resultant lines are now straight (fig 6).

A steady state kinetic model exactly accounting for the data in figures 6 and 7 is that termed coupling activation of the second type by Friedenwald and Maengwyn-Davies (16). The equations governing this model are derived by expansion of the Michaelis-Menten equation in a manner analogous to that used in the case of a dissociable inhibitor. Criteria for the model are that the substrate (S = oxygen) must bind to the receptor (metal containing site) before the dissociable activator (A = ethylene) can attach. When this is the case a plot of 1/V vs 1/A (fig 7) yields straight lines intersecting the ordinate at $1/V_m$, and a curve having a positive slope when $S = \infty$. A plot of 1/V vs 1/S (fig 6) yields straight lines intersecting beyond the ordinate at a point having a value of $-1/K_S$ on the abscissa, and a curve which is parallel to the abscissa when $A = \infty$. Various other steady state models describing interactions between a dissociable activator, a substrate, and receptor have been developed (16) but none are consistent with the data in figures 6 and 7.

It is not necessary to envision a simultaneous attachment of O_2 and ethylene to the metal of the receptor as previously proposed (3). The same kinetics should result if the metal of the receptor can be reversibly oxidized and reduced, provided that oxidation occurs rapidly after O_2 has attached and ethylene only binds to and activates the oxidized form. In fact it is even conceivable that O_2 does not attach directly to this receptor, for it might bring about oxidation of the receptor indirectly through a coupled O/R system. In any event we envision the ethylene receptor to be a metal containing compound which is oxidized directly or indirectly through the intervention of molecular O_2, and while in the oxidized form activated by ethylene to produce some fundamental change in the metabolism of plant tissue.

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