Ethylene Action and the Ripening of Fruits

Ethylene influences the growth and development of plants and is the hormone which initiates fruit ripening.

Stanley P. Burg and Ellen A. Burg

Ethylene is only one of many volatile substances evolved by ripening fruits, and the small amount produced would have attracted little attention if it were not for the fact that the gas stimulates fruits to ripen (*1*, *2*). When early workers (*3*, *4*) found that young fruits produce small quantities of ethylene and ripening fruits much larger amounts, they proposed that ethylene might be the endogenous ripening hormone. However, the action of ethylene cannot be understood solely in these terms, because the gas causes equally striking morphogenetic and physiological changes in all types of plant tissue (*5–13*). Frequently these changes are associated with alterations in the pattern of growth (Fig. 1), and they would appear to be unrelated to the ripening of fruit; yet there are many reasons to believe that the markedly different responses which ethylene evokes in fruits, flowers, and vegetative tissue are a reflection of specific capacities for secondary changes in metabolism rather than a fundamental variation in the basic mechanism of the action of ethylene (*14–19*, *2*). Therefore we refer to experiments with vegetative tissue whenever they clarify some aspect of the role of ethylene in fruit ripening. For an excellent discussion of other aspects of the biochemistry and physiology of fruit ripening, the reader is referred to the publications of J. B. Biale (*19–22*).

Is Ethylene a Ripening Hormone?

The role of ethylene in the metabolism of fruits has been obscured by the suggestion that the gas is a byproduct rather than the cause of ripening (*20*, *23*). The proponents of the by-product theory stated (*23*) that ethylene cannot be the ripening hormone because prior to the climacteric (*24*) it is not always present in a large enough quantity to stimulate the fruit. However, this conclusion was based entirely on experiments which measured the rate of emanation but not the intercellular concentration of ethylene. These workers stated that they did not know what intercellular concentration accrued for any particular rate of ethylene emanation, and they admitted that until this relationship was established "one can advance the argument that small quantities sufficient to induce ripening are produced prior to the rise of respiration but measurable amounts are detected only after the onset of the climacteric" (*23*). With the manometric technique in use at that time it was not possible to detect a rate of ethylene production less than approximately 5 microliters per kilogram per hour, but with the advent of gas chromatography the limit of analysis was advanced almost a million-fold, and it was found (*15*) that more than ten parts of ethylene per million were always present within the intercellular spaces of a fruit before any emanation could be detected by the manometric method. Since in most fruits the threshold (*25*, *15*, *23*) for ethylene action is between 0.1 and 1 ppm (Table 1), as the proponents of the by-product theory estimated, it is clear that the assay which they were using did not approach the sensitivity needed to measure the minimum rate of synthesis which produces enough ethylene to stimulate fruit ripening. To illustrate this point, the data in Fig. 2 are presented (left) with a scale on the ordinate corresponding to amounts of ethylene which can be measured by manometry and (right) with an expanded scale illustrating the sensitivity obtained with gas chromatography. The pattern of emanation of ethylene in mangoes is clearly indicated in the gas chromatographic study (Fig. 2, lower right), but is below the limit of manometric detection (lower left); this explains why the proponents of the byproduct theory were led to assert incorrectly that mangoes and certain other fruits (citrus and pineapples) do not produce ethylene (*23*). The manometric procedure reveals the overall relationship between ethylene evolution and the CO_2 rise in avocados and bananas, but only gas chromatography shows that well in advance of the respiratory climacteric the intercellular content of ethylene increases to a level which stimulates these fruits to ripen. The same pattern has been demonstrated in cantaloupes (*26*) and honeydew melons (*27*) (see Table 1). In fact, no one has ever shown a climacteric fruit to ripen before it has accumulated enough ethylene to initiate its respiratory rise. Thus the proponents of the by-product theory have no experimental basis for questioning the concept that ethylene is a ripening hormone.

Evidence that ethylene is a ripening hormone has been reviewed elsewhere (*28*), and here we only mention a few recent studies which reinforce this view. As ethylene which accumulates in a fruit causes it to ripen, ripening might be delayed by ventilating the fruit if it were not for the fact that no amount of ventilation can remove the ethylene from the intercellular spaces where it accumulates as a result of endogenous production and a slow rate of diffusion through the tissue. However, if the fruit is placed in a partial vacuum, the escape of ethylene is accelerated because in the tissue the gas is moving through an air phase (*29*) in which its diffusivity is inversely related to the atmospheric pressure. As a result bananas can be stored in a flowing air stream at a pressure of 0.2 atmosphere (100 percent relative humidity, 16°C) for 3 months, and some fruits even longer. When the atmospheric pressure is lowered, not only ethylene but also CO_2 and O_2 become depleted within the tissue (for example, at 0.2 atmosphere the partial pressure of O_2 is approximately 0.04). The decrease in CO_2 might enhance ripening,

---

The authors are affiliated with the University of Miami School of Medicine, Miami, Florida, where Stanley P. Burg is assistant professor in the departments of physiology and medicine.

but lowering the O_2 concentration should have the opposite effect (*17*, *21*, *30*); thus it could be argued that the increase in storage life results from a depletion of O_2 rather than of ethylene. However, if pure O_2 is flowed at 0.2 atmosphere its partial pressure is the same as in air at 1 atmosphere, and ripening is still delayed. The simplest explanation of this behavior is that the ripening hormone ethylene has been removed. As would be expected, if a trace of ethylene is introduced into the ventilating stream, even in a partial vacuum the fruit responds by ripening immediately. Another piece of evidence which supports the theory that ethylene is the ripening hormone is the observation, mentioned above, that CO_2 retards ripening whereas O_2 enhances it. We present data below which indicate that CO_2 is a competitive inhibitor of ethylene action, and O_2 a substrate in the reaction activated by ethylene; therefore the observed effects of CO_2 and O_2 on the ripening process are consonant with ethylene's being the ripening hormone. A similar argument is applicable in the case of ethylene oxide, which delays fruit ripening and hinders the action of ethylene in flowers and vegetative tissues (*31*). If the oxide acts as an antagonist to ethylene, as has been suggested (*31*), the fact that it delays ripening is an indication that ethylene is a ripening hormone. However, this argument must be viewed as tentative, for we will show below that the idea of an antagonism between ethylene and its oxide may be an unwarranted conclusion. Finally, experiments based on gas chromatography (summarized in Table 1) show that ample ethylene accumulates before the onset of the climacteric in all fruits which have been investigated, and, in the one case where we have studied the phenomenon (*32*), the time which elapses between the appearance of an amount of gas sufficient to stimulate ripening and the onset of the respiratory rise is the same regardless of whether the gas is artificially applied or naturally produced just before ripening begins.

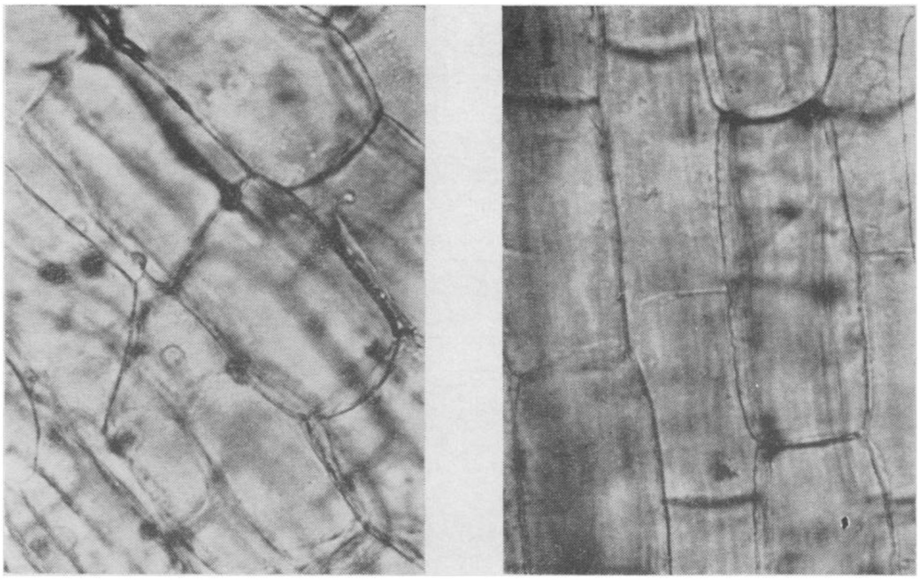

Fig. 1. Freehand sections (× 165) taken 2 mm below the apex of pea-stem sections which were incubated for 20 hours in a medium containing growth hormone, salts, and sugar. Ethylene treatment (left) has decreased the rate of elongation and induced radial swelling, but the average cellular volume is approximately the same as in the control tissue (right). A similar effect on intact plants (*6–10*) underlies many of the growth abnormalities induced by ethylene.

Response to Harvest

Although the ethylene content of mangoes (Fig. 2) is not markedly augmented until just prior to the climacteric peak, enough of the gas is present before ripening commences to initiate the process by autostimulation (*15*). In fact the data in Table 1, which show that from 0.1 to 1 part of ethylene per million are always present in this and other fruits before ripening commences, also show that a nearly stimulatory concentration (0.04 to 0.2 ppm) persists in fruits throughout most of their lifetime. Why do fruits remain preclimacteric for so long in the presence of an ethylene concentration which seemingly approaches the threshold for autostimulation? (See *33*, *34*.) This question naturally poses the problem of whether sensitivity to ethylene is constant throughout the life of a fruit, and experiments with bananas and mangoes would lead us to believe that it is not (*32*). These studies suggest that the threshold for ethylene action decreases after fruits are harvested, so that the amounts of ethylene which must be present for ripening to occur may be lower in detached fruits (Table 1) than the amounts required to stimulate ripening in comparable fruits left on the tree.

Many fruits enter the climacteric phase soon after harvest, whereas if left on the tree they might not ripen for several months, if at all. This behavior is compatible with the concept of a changing sensitivity to ethylene. The mechanism of response to harvest has been investigated by means of experimental interference with the conduction of food material to the fruit (*35*), and it has been shown that some substance transported from the shoot system is required to keep fruits in a preclimacteric state. When the fruit is harvested

Table 1. Internal content of ethylene and the threshold for ethylene action in various fruits (*53, 2, 15, 20, 26, 27, 32, 54*).

Fruit	Concentration (ppm)		
	Prior to respiratory rise	At onset of respiratory rise	Threshold (*25*, *15*, *23*)
Avocado (var. *Choquette*)	0.04	0.5–1	
(var. *Fuerte*)			0.1
Banana (var. *Gros Michel*)	0.1	1.5	0.1 –1
(var. *Lacatan*)	0.2		0.5
(var. *Silk fig*)	0.2	0.9	0.2 –0.25
Cantaloupe (var. *P.M.R. No. 45*)	0.04	0.3	0.1 –1
Honeydew melon	0.04	3	0.3 –1
Lemon (var. *Fort Meyers*)	0.1	†	0.1
Mango (var. *Kent*)	(0.14)*	0.08	0.04–0.4
Orange (var. *Valencia*)	0.1	†	0.1
Tomato (var. *VC-243-20*	0.08	0.8	

* An average value of 1.6 ppm has been reported during one growing season (*32*). † After harvest citrus fruits do not normally pass through a climacteric phase unless ethylene is applied (*20*).

it is removed from the source of supply of this material, and ripening ensues possibly because the threshold for ethylene action is lowered in the absence of the translocated substance. A similar mechanism may be involved in the natural ripening of fruits on the tree, for the transport of food material to attached fruits ceases when their growth is completed, and it is only then that the climacteric is initiated.

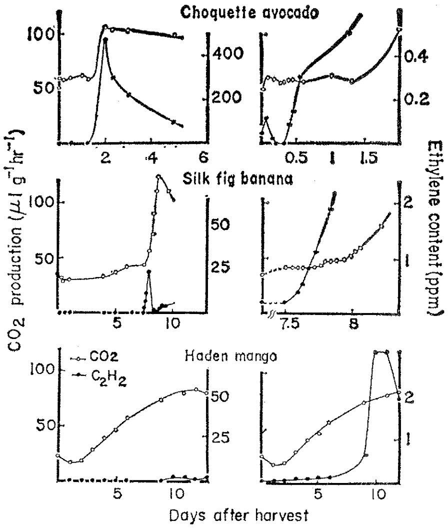

Fig. 2. Relation between respiration and the endogenous content of ethylene. (Left) The gross pattern is illustrated; (right) the ethylene scale is magnified to reveal small changes which occur at the onset of ripening. [Adapted from *15, 32,* and *54*].

Gas Storage

The ideal atmosphere for preserving fruits after harvest contains between 5 and 10 percent CO_2, 1 to 3 percent O_2, and as little ethylene as possible. This condition, known as gas storage, is achieved commercially by confining fruits in an airtight room so that through their own respiratory processes they may deplete the atmospheric O_2 and enrich their surroundings with CO_2; if the CO_2 content becomes too high the excess is "scrubbed" out of the atmosphere by being dissolved in water; if the O_2 concentration becomes too low, air is admitted; and if excessive ethylene is produced it is eliminated by means of brominated charcoal filters. Kidd and West, who developed the process of gas storage (*17*), thought that its ability to preserve fruit could be accounted for solely in terms of the ripening hormone ethylene. They proposed (*34*) that the sensitivity of fruits to ethylene is diminished at a low O_2 tension or high partial pressure of CO_2 and that the production of ethylene is restricted when O_2 is deficient. This explanation is in accord with findings that fruits do not ripen as quickly (*17, 21, 30*), produce as much ethylene (*4, 17, 34, 36, 37*), or respond to applied ethylene as readily (*2, 17–19*) if the atmosphere is enriched with CO_2 or made deficient in O_2. However, excess CO_2 inhibits respiration and O_2 is a substrate for that process, so it may be argued that the respiratory climacteric is delayed not because of any effect on the ethylene threshold, but rather because gas storage conditions directly depress the respiration rate. In order to resolve this problem, a kinetic study of the effects of CO_2 and O_2 on respiration and on sensitivity to ethylene was undertaken with stem sections from pea plants grown in the dark. When these sections are incubated in the dark in a medium containing growth hormone (auxin), sugar, and various salts, they almost double in length in 24 hours; but if ethylene is present in a concentration greater than 1 ppm, the rate of elongation is reduced by about 50 percent and the tissue swells. Carbon dioxide alone has no effect on the growth of pea plants unless its concentration exceeds 10 percent, but even much smaller quantities of the gas markedly reduce the efficacy of ethylene. This relationship is illustrated in Fig. 3 by the method of Lineweaver and Burke (*38*). The kinetic model which best describes this behavior is one in which CO_2 competes with the activator (ethylene) for the receptor site which ethylene must occupy to exert a biological effect (*38*). At the molecular level this is in accord with the fact that CO_2 is a close structural analogue of allene, a compound which mimics the effect of ethylene (see Table 2) by binding to the same receptor site.

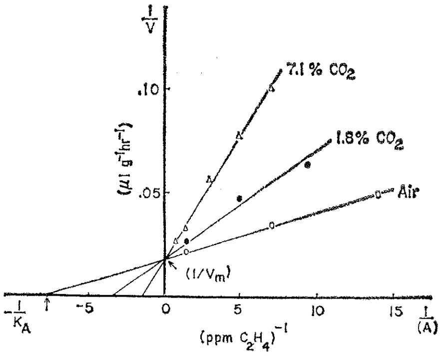

Fig. 3. Effect of CO_2 on ethylene action. The response to ethylene, expressed as percentage inhibition of elongation in the pea-stem-section assay, is plotted as a function of the ethylene and CO_2 concentrations by the method of Lineweaver and Burke (*38*). The Michaelis-Menten constant, K_A, is the concentration of ethylene (A), the activator, which causes a half-maximum response, and V_m is the maximum response at an infinite concentration of ethylene and 21 percent O_2.

$CH_2 = C = CH_2$	$O = C = O$
allene	carbon dioxide

Competition is governed by the law of mass action, and since the affinity of ethylene for the receptor site is one million-fold greater than that of CO_2, if enough ethylene is present no amount of CO_2 will prevent it from acting. Kidd and West (*34*) concluded that "moderate amounts of CO_2 (about 10 percent)—do not render the fruit insusceptible to stimulation by ethylene—but may act by raising the threshold value for ethylene stimulation."

Ostensibly the effect of lowering the concentration of O_2 in the atmosphere surrounding pea-stem sections is closely similar to that which results when the CO_2 tension is enhanced, but when the kinetics of the two cases are compared (compare Figs. 3 and 4) certain interesting differences are revealed. A steady-state kinetic model developed by Friedenwald and Maengwyn-Davies (*38*) for a dissociable activator exactly fits the data for O_2, and, in particular, the case which they term "coupling activation of the second type" is applicable. The criterion for this model is that the activator (ethylene) cannot bind to the enzyme (receptor site) unless the substrate (O_2) has already attached. The lowered response to applied ethylene which occurs when O_2 is deficient is accompanied in pea-stem sections by a slight inhibition of growth and respiration; however, this does not alter the interpretation of these experiments since, if the same inhibition is imposed on the tissue by applying respiratory poisons such as azide or iodoacetate or nonspecific inhibitors such as ethylene oxide, the sensitivity to ethylene is not changed. The results in vegetative tissue are in close agreement with those obtained in experiments with fruits (*2, 17–19*) and indicate that, when the CO_2 concentration is raised and the O_2 and ethylene tensions lowered, every factor is adjusted to yield the maximum threshold for ethylene action and the minimum concentration of ripening hormone, just as Kidd and West postulated (*34*).

Physiology of Ethylene Formation

The quantities of O_2 required for ethylene synthesis and for respiration are similar (*37*), and while this might mean that O_2 is directly used in the manufacture of ethylene, it is equally possible that the result simply reflects the fact that respiration is required to provide energy or substrate for ethylene synthesis. The latter interpretation is supported by the observation that respiratory poisons invariably inhibit both the synthesis of ethylene and consumption of O_2 to a closely similar extent (*39*). The other major metabolic gas, CO_2, has very little influence on the rate of ethylene formation aside from that which could be readily accounted for by its ability to inhibit respiration when present at a very high concentration (*4, 39*). At 28°C the rate of ethylene production reaches a maximum in fruits; at lower temperatures both ethylene synthesis and O_2 consumption increase about 2.5-fold for every 10-degree rise, whereas at temperatures only 5 to 10 degrees higher than the optimum, no ethylene is formed (*39*). When the temperature is raised and then lowered, it takes several hours for the tissue to recover its initial capacity for manufacturing ethylene; this may be of some physiological significance since the production fails at a temperature which the pulp of tropical fruits must approach on hot days.

An unusual aspect of ethylene synthesis is the striking and varied effect which tissue damage (*39, 40*), disease (*41*), and application of growth hormone (auxin) (*42*) have on the rate of the process. These three conditions sometimes induce tissues, whose production of ethylene is too small for detection by vapor-phase chromatography, to evolve quantities of the gas, and it is interesting to speculate on the possibility that a similar mechanism triggers the climacteric rise in ethylene synthesis. It is not clear in some of these studies whether the response to auxin is basically different from the wound reaction, because often a nearly toxic amount of growth hormone must be applied to vegetative tissue before the rate of ethylene evolution is altered. If a fruit initially has a high rate of production (as is the case with ripening apples), then cutting the tissue into sections or applying auxin may adversely affect emanation of ethylene, and usually when any tissue is homogenized or cut into very small pieces ethylene synthesis is abolished. The exceptions to this rule are two recently reported cases in which cell-free extracts that produce ethylene were prepared (*43–46*). However, these still are couched in uncertainty (*43–45*), and it has not been demonstrated that the same reaction is responsible for ethylene synthesis in vitro and in intact tissue.

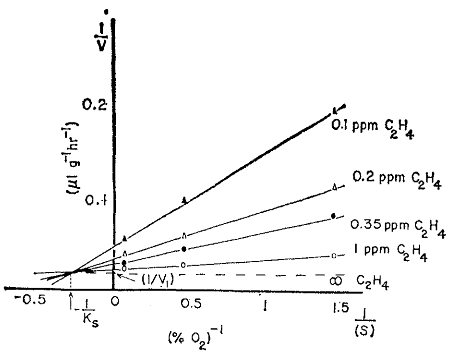

Fig. 4. Effect of O_2 on ethylene action. The response to ethylene, expressed as percentage inhibition of elongation in the assay with pea-stem sections, is plotted as a function of the concentrations of ethylene and O_2. The Michaelis-Menten constant (K_S) is the concentration of O_2 (S), the substrate, needed for a half-maximum response, and V_m is the maximum response at an infinite concentration of ethylene and O_2. The curves have been corrected for a 2.8-percent O_2 gradient between the tissue and ambient atmosphere.

Biochemistry of Ethylene Formation

Studies with specifically labeled C^{14}-sugars show that apple slices (*47*) and the fungus *Penicillium digitatum* (*48*) preferentially utilize the carbons at positions 5 and 6 of glucose to produce ethylene; some ethylene is formed from the carbons at 1 and 2, whereas little or none is produced from those at 3 and 4. During the glycolytic cleavage of sugar, pyruvic acid is formed with the labeling pattern illustrated in Fig. 5, but in addition a quantity of pyruvate is also derived exclusively from the carbons at positions 4, 5, and 6 of glucose if the direct oxidative pathway of sugar metabolism is functioning. By comparing the yield of C^{14}-labeled CO_2 after the addition of 1-C^{14} glucose with that obtained after the addition of 6-C^{14} glucose, it is possible to estimate the relative contributions of the direct oxidative and glycolytic pathways, and, although this method must be used with certain reservations, it still suffices to indicate that the direct oxidative pathway contributes significantly to the respiration of both apple tissue (*47*) and *P. digitatum* (*48, 49*). Accordingly it would be expected that the carbons at positions 4, 5, and 6 of glucose would find their way into pyruvate more readily than the carbons 1, 2, and 3, and if pyruvate were converted to ethylene this would explain why the lower half of the glucose molecule is more effective than the upper in forming ethylene. When pyruvic acid is decarboxylated to yield acetate (Fig. 5), the carbon which is released as CO_2 originates from the carbons at positions 3 and 4 of glucose, so the fact that the carbons at these positions do not contribute significantly to ethylene formation is an indication that acetate is an intermediate in the conversion of glucose to ethylene. Both carbons of C^{14}-acetate contribute equally to ethylene formation in short-term experiments with apple slices, but when studies with apples or *P. digitatum* are prolonged, the carboxyl carbon becomes far less effective than the methyl carbon in forming ethylene. It is likely that this effect is explained by depletion of the carboxyl carbon in the tissue, for it is lost as CO_2 much more rapidly than the methyl carbon. A similar result is obtained with the 4-carbon acid fumarate (see Fig. 5) which is derived indirectly from acetate via the Krebs cycle; in short-term experiments with apples the C^{14} yield in ethylene is similar after feeding either 1,4-C^{14} or 2,3-C^{14} fumarate, but in long-term experiments with

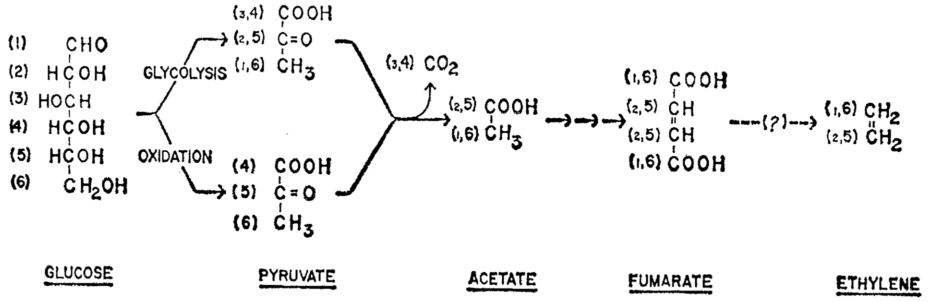

Fig. 5. Proposed pathway for the conversion of C^{14}-glucose to ethylene.

apples and *P. digitatum* the transfer of label from the carbons No. 2 and No. 3 to ethylene is much more rapid than from the carboxyl positions which are more labile. These results indicate that in the conversion of glucose carbon atoms to ethylene, the sugar is transformed to pyruvate, which is decarboxylated to acetate; this may enter the Krebs cycle before it is transformed into ethylene.

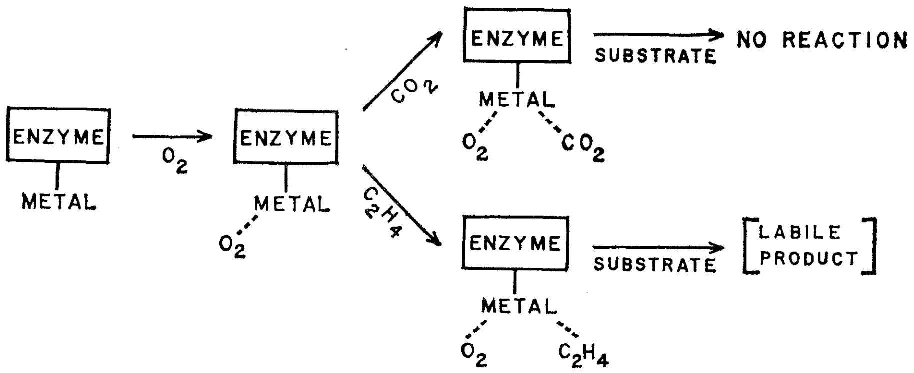

Fig. 6. Scheme for activation of the receptor site by ethylene.

The Mechanism of Ethylene Action

The chemical structure required for ethylene-like action in vegetative tissues, flowers, and fruits has been investigated (*11, 12*), and the results obtained are in fair agreement, indicating that the order of activity is ethylene > propylene > acetylene or carbon monoxide > butylene (unspecified isomer). Alkanes, halogenated ethylenes, and over 100 other compounds were found to be ineffective, and it was concluded that activity requires unsaturation and is inversely related to molecular size. We have recently investigated this problem using the pea-stem-section assay and chromatographically pure gases, and on the basis of the results (Table 2) a series of empirical rules governing ethylene action can be formulated:

1) All active compounds are unsaturated.

2) In any homologous series, smaller molecules tend to be more active than larger ones. (The effect of alkyl substitution is much less in the case of alkynes than with alkenes because in acetylenic compounds the carbons are colinear with the triply bonded carbons, and groups substituted on them are held out of the region of the multiple bond.)

Table 2. Biological activity of ethylene and other unsaturated compounds as determined by assay with pea-stem sections.

Compound*	K_A relative to ethylene†
Ethylene	1
Propylene	130
Vinyl chloride	2,370
Carbon monoxide	2,900
Vinyl fluoride	7,100
Acetylene	12,500
Allene	14,000
Methyl acetylene	45,500
1-Butene	140,000
Vinyl bromide	220,000
Ethyl acetylene	765,000
Vinyl methyl ether	1,175,000
Butadiene	1,200,000
1,1 Difluoro ethylene	2,060,000
Vinyl ethyl ether	5,440,000

* For a list of inactive and toxic compounds see *55*. † The Michaelis-Menten constant (K_A) is the molarity in solution required for half-maximum biological activity. Its value for ethylene at 24°C in the presence of an infinite concentration of O_2 is $6 \times 10^{-10}M$.

3) The more the bond order resembles that in ethylene, the greater the activity. (Electron delocalization due to hyperconjugation or other forms of resonance lowers activity.)

4) The unsaturated linkage must be adjacent to a terminal carbon atom. (Nitrogen will not substitute—compare acetonitrile and methyl acetylene.)

5) The terminal carbon next to the unsaturated position must not bear a positive charge (compare formaldehyde with carbon monoxide).

These requirements for ethylene action bear a marked resemblance to the rules for metal-binding to olefins as derived from experiments in which the stability constants of silver-olefin complexes were determined by gas chromatography (*50*). When this method was applied to the compounds listed in Table 2, there was a close correlation, within each homologous series, between biological activity and the stability of the silver complex which a substance forms. This and several other observations suggest that ethylene must bind to a metallic receptor site in tissues. Formation of complexes with a metal is a characteristic of olefins, acetylenes, carbon monoxide, cyanide, and a few aromatic molecules; all have ethylene-like activity except the aromatic molecules, which presumably are too large, and cyanide, which so inhibits growth and respiration that it renders the bioassay useless. The affinity of carbon monoxide for the receptor site is closely similar to its affinity for cytochrome oxidase, and it is known that carbon monoxide inhibits cytochrome oxidase and various other metal-containing enzymes by forming complexes with the metal. There is no reduction in the efficacy of ethylene at high concentrations, and since in enzyme systems inhibitions in the presence of a large amount of substrate are an indication that two substrate molecules can bind at the two sites of attachment which normally are occupied by a single substrate molecule, it follows that the lack of such an effect suggests a single point of attachment. In this context, it is important to note that carbon monoxide and cyanide are known to bind to metal through a single point of attachment at the carbon end; and in accord with the concept of metal-binding, the data in Table 2 show that the terminal carbon next to the unsaturated linkage must not bear a positive charge (if it did, a positively charged metal ion would be repelled). Finally, the kinetic studies which demonstrate that O_2 and ethylene attach to the same receptor are a further indication that this site contains metal, for enzymes able to bind O_2 typically contain iron or copper.

Any substance which is produced as a result of the reaction activated by ethylene must be labile, and any that is destroyed must be rapidly replaced, for in vegetative tissue ethylene produces no lasting effect unless it is continually present. [In fruits the response to ethylene is irreversible, but Kidd and West (*34*) have pointed out that this is because when ethylene is applied the tissue increases its own production of the gas]. From these facts and the kinetic studies with CO_2 and O_2, a model for ethylene activation of the receptor site emerges; this is illustrated schematically in Fig. 6.

It has been claimed (*31*) that ethylene oxide is an antagonist of ethylene action in fruits, flowers, and vegetative tissue, including pea seedlings, but we find that the oxide does not compete with ethylene in the straight-growth test on peas. The concentration of ethylene oxide which "antagonized" ethylene action in pea seedlings was not given (*31*), but the growth of the plants was stunted, and it was reported that between 0.1 and 0.5 percent ethylene oxide was required in other tissues. Our kinetic studies were carried out in the presence of 0.3 percent ethylene oxide, a concentration which inhibits the rate of elongation of pea-stem sections by 25 percent. At a concentration of 1 percent, ethylene oxide almost completely stops pea-stem elongation, and since the action of ethylene in vegetative tissue is recognized by its effect on growth, without growth no response to ethylene can be perceived. Therefore it seems likely that the reported lack of a "visible" ethylene effect in pea seedlings treated with ethylene oxide must be a result of growth inhibition rather than of any direct antagonism of ethylene action. Even if ethylene oxide prevents ethylene from affecting fruits and flowers, this is not in itself a demonstration of an antagonism between the two molecules, for to demonstrate such an antagonism it must also be shown that the criteria of competitive inhibition are met. A high concentration of ethylene must be fully effective in the presence of an amount of ethylene oxide which reverses the action of a lower concentration of ethylene. If this does not occur it must be concluded that the "antagonism" between ethylene and its oxide is noncompetitive, possibly no different in kind from that which might be obtained with various metabolic and enzymatic poisons. So far all studies on the molecular structure required for ethylene action indicate that ethylene oxide does not compete with ethylene for the receptor surface since only unsaturated molecules are able to attach to the surface.

The mainstream of thought concerning the mode of ethylene action in vegetative tissue has centered on an interplay between ethylene and auxin because so many processes known to be controlled by auxins are also influenced by ethylene. Some think that this interplay consists of a similarity between the action of auxin and that of ethylene (*12*); others have presented evidence that ethylene causes a reduction in the rate of synthesis of auxin and a breakdown in the system by which the auxin is transported laterally in the stem under the influence of gravity (*6*); still others have concluded that ethylene enhances the destruction (*7, 51*) or inactivation (*13*) of auxin, or that it influences the spread of auxin from the conduction system (*8*) and upsets the auxin-induced polarity of plant cells (*10*). Recent studies in our laboratory have eliminated several of these possibilities (*52, 6, 7, 13*), but it remains to be seen whether the concept of an interplay between growth hormone and ethylene which has evolved from experiments with vegetative tissues will prove useful in interpreting the action of ethylene in mature fruits where growth has ceased.

Summary

Recent studies employing gas chromatography show that an amount of ethylene large enough to stimulate ripening is always present within a fruit before the respiratory climacteric begins. This fact and data from experiments in which fruits were exposed to a partial vacuum or varying concentrations of O_2, CO_2, and ethylene oxide reinforces the view that ethylene is a ripening hormone. The respiratory climacteric begins soon after the fruit is harvested because the tissue no longer receives from the shoot system a substance which inhibits ripening; this substance may act by lowering the sensitivity of the fruit to ethylene. The threshold for ethylene action is also influenced by the composition of the atmosphere, for O_2 is a substrate in the reaction activated by ethylene and CO_2 inhibits the action of ethylene by competing with the olefin for the receptor site. Experiments indicate that ethylene is derived from acetate or acids of the Krebs cycle and acts by binding to a metal receptor site in the tissue.

References and Notes

1. A. E. Sievers and R. H. True, *Bull. U.S. Dept. Agr. Bur. Plant Ind.* **232** (1917); R. B. Harvey, *Bull. Minn. Agr. Exp. Sta.* **104** (1930); R. P. Hibbard, *Tech. Bull. Minn. Agr. Exp. Sta.* **104** (1930); H. S. Wolfe, *Botan. Gaz.* **92**, 337 (1931); E. Hansen and H. Hartman, *Plant Physiol.* **12**, 441 (1937).
2. F. E. Denny, *J. Agr. Res.* **27**, 757 (1924).
3. F. Kidd and C. West, *Gt. Brit. Dept. Sci. and Ind. Res. Rept. Food Invest. Bd.* **1932**, 52 (1933); R. Gane, *J. Pomol.* **13**, 351 (1935); ———, *New Phytologist* **36**, 170 (1937); E. Hansen, *Proc. Amer. Soc. Hort. Sci.* **43**, 69 (1943).
4. E. Hansen, *Botan. Gaz.* **103**, 543 (1942).
5. Ethylene causes leaves to abscise, chlorophyll to blanch, and flowers to fade; in seedlings it induces leaf petioles to overgrow (a condition known as epinasty), reduces the rate of elongation of the stem, and causes the stem to swell; in its presence plants lose their ability to orient normally with respect to gravity, the stem assumes a horizontal position (horizontal nutation), as do the secondary roots (plagiogeotropism), and normal growth movements (circumnutations) cease; it inhibits the growth of roots and the formation of lateral roots, induces roots to form on cuttings, breaks dormancy in bulbs and cuttings, promotes development of buds and intumescences, causes pineapples to flower and plants to exude water (guttation), enhances protoplasmic permeability, and has many other effects (*6–13*).
6. P. A. van der Laan, *Rec. Trav. Bot. Neerl.* **31**, 691 (1934).

→ D. H. Michener, *Amer. J. Bot.* **25**, 711 (1938).

8. G. Borgstrom, *Kgl. Fysiograf. Sällskap. Lund Förh.* **9**, 135 (1939).
9. E. M. Harvey, *Botan. Gaz.* **60**, 193 (1951).
10. E. Bunning and H. Ilg, *Planta* **43**, 472 (1934).
11. L. I. Knight, R. C. Rose, W. Crocker, *Science* **31**, 635 (1910); L. I. Knight and W. Crocker, *Botan. Gaz.* **55**, 337 (1913); F. E. Denny, *Contrib. Boyce Thompson Inst.* **7**, 191 (1935); United Fruit Co., unpublished data.
12. W. Crocker, P. W. Zimmerman, A. E. Hitchcock, *Contrib. Boyce Thompson Inst.* **7**, 231 (1935).
13. H. von Guttenberg and E. Steinmetz, *Pharmazie* **2**, 17 (1947).
14. For example, in all tissues the threshold for ethylene action is closely similar [see Table 1 and the data compiled in table III of reference *15*]; the same gases mimic the effect of ethylene [see Figs. 3 and 4 and H. Fitting, *Jahrb. Wiss. Botan.* **49**, 187 (1911); W. B. Mack, *Plant Physiol.* **2**, 103 (1927); and *16* for experiments with vegetative tissue; for studies with fruits see *2, 17, 18*].
15. S. P. Burg and E. A. Burg, *Plant Physiol.* **37**, 179 (1962).
16. F. E. Denny, *Contrib. Boyce Thompson Inst.* **7**, 341 (1935).
17. F. Kidd and C. West, *Gt. Brit. Dept. Sci. and Ind. Res. Rept. Food Invest. Bd.* **1933**, 51 (1934).
18. ———, *ibid.* **1937**, 101, 108 (1938).
19. J. B. Biale and R. E. Young, *Endeavour* **21**, 164 (1962).
20. J. B. Biale, *Advan. Food Res.* **10**, 293 (1960).
21. ———, in *Handbuch der Pflanzenphysiologie* (Springer, Berlin, 1960), p. 536.
22. ———, *Ann. Rev. Plant Physiol.* **1**, 183 (1950); *Science* **146**, 880 (1964).
23. J. B. Biale, R. E. Young, A. Omstead, *Plant Physiol.* **29**, 168 (1954).
24. The respiration rate reaches a maximum in fruits shortly after they are formed and subsequently decreases steadily as the cells pass through successive phases of division and enlargement. This decline continues even if the fruit is harvested, but in many varieties, when ripening commences, the trend is abruptly reversed by a respiratory upsurge which F. Kidd and C. West [*Gt. Brit. Dept. Sci. and Ind. Res. Rept. Food Invest. Bd.* **1925**, 27 (1926)] termed the climacteric.
25. The threshold is the minimum concentration of applied ethylene which induces a biological response. Because the activity of ethylene is related to the log of the gas concentration (*15, 23*), and the preclimacteric content of ethylene is augmented by exactly the amount applied (in accord with Fick's law), there can be no response unless the applied concentration equals or exceeds that within the fruit. The data in Table 1 show this to be the case, and in addition they suggest that under appropriate conditions the threshold value may be closely similar in all fruits, ranging from 0.1 to 1 ppm ethylene. Kinetic studies with pea-stem sections (see Figs. 3 and 4, and the discussion of gas storage) indicate that there the threshold is about 0.02 ppm at 21 percent O_2 and a very low concentration of CO_2, but in the presence of several percent CO_2 the value is closer to 0.1 ppm, just as is the case with fruits. Since fruits contain at least several percent CO_2 when they begin to ripen, the results suggest that the threshold for ethylene action is closely similar in fruits and seedlings.
26. J. M. Lyons, W. B. McGlasson, H. K. Pratt, *Plant Physiol.* **37**, 31 (1962).
27. H. K. Pratt and J. D. Goeschl, *Proc. Plant Physiol.* **39**, ix (1964).
28. S. W. Porritt, *Sci. Agr.* **31**, 99 (1951); S. P. Burg, *Ann. Rev. Plant Physiol.* **13**, 265 (1962).
29. We have proven this for apples by demonstrating that the partial pressure of ethylene in the intercellular spaces is inversely related to the diffusivity of the gas mixture surrounding the fruit. Thus if air is replaced by a helium-oxygen mixture, ethylene escapes from an apple even though the rate of ethylene production is not changed; the final concentration of ethylene within the tissue depends

on the diffusivity of ethylene in the particular helium-oxygen mixture which is applied. Similarly, when the atmospheric pressure is reduced the diffusivity of ethylene per unit volume of air is increased; this does not change the rate at which the fruit produces ethylene, but the partial pressure of ethylene in the intercellular spaces declines to the extent that the atmospheric pressure is lowered. Since the rate of escape of ethylene from the fruit is dependent upon the diffusivity of ethylene in the ambient gas phase, it follows that ethylene moves through the tissue and escapes from it in a gas phase.

30. R. E. Young, J. Romani, J. B. Biale, *Plant Physiol.* **37**, 416 (1926).
31. M. Lieberman, S. Asen, L. W. Mapson, *Nature* **204**, 756 (1964).
32. S. P. Burg and E. A. Burg, *Botan. Gaz.*, in press.
33. Kidd and West (*34*) concluded that "throughout its growth the apple produces ethylene in very small quantities, but a minimum or threshold concentration in the tissue is necessary before respiratory activity is affected." They speculated that ripening might be induced by "a change with age in the threshold for ethylene stimulation"; also see E. Hansen, *Proc. Amer. Soc. Hort. Sci.* **43**, 69 (1943).
34. F. Kidd and C. West, *Plant Physiol.* **20**, 467 (1945).
35. S. P. Burg, in *Régulateurs Naturels de la Croissance Végétale*, Intern. Colloq., Gif, France, 1963 (Centre National de la Recherche Scientifique, Paris, 1964), p. 719.
36. R. Gane, *Nature* **134**, 1008 (1934).

→ S. P. Burg and K. V. Thimann, *Proc. Natl. Acad. Sci. U.S.* **45**, 335 (1959).

38. J. S. Friedenwald and G. D. Maengwyn-Davies, in *A Symposium on the Mechanism of Enzyme Action*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Univ. Press, Baltimore, 1954), p. 154.
39. S. P. Burg and K. V. Thimann, *Plant Physiol.* **35**, 24 (1960).
40. D. F. Meigh, K. H. Norris, C. C. Craft, M. Lieberman, *Nature* **186**, 902 (1960).
41. C. W. Fischer, *N.Y. State Flower Growers' Bull.* **49**, 9 (1949); **61**, 1 (1950); C. E. Williamson, *Phytopathology* **40**, 205 (1950); W. H. Smith, D. F. Meigh, J. C. Parker, *Nature* **204**, 92 (1964).
42. P. W. Zimmerman and F. Wilcoxon, *Contrib. Boyce Thompson Inst.* **7**, 209 (1935); E. Hansen, *Plant Physiol.* **21**, 588 (1946); P. W. Morgan and W. C. Hall, *Physiol. Plantarum* **15**, 420 (1962); F. B. Abeles and B. Rubenstein, *Plant Physiol.* **39**, 963 (1964).
43. M. Lieberman and C. C. Craft [*Nature* **189**, 243 (1961)] reported that subcellular particles from apples and tomatoes produce ethylene if they are prepared at an acid *p*H and fortified with certain thiol-containing compounds. Subsequently the gas was identified as ethane (*44, 45*), and its production was shown to be dependent on the presence of linolenic acid [M. Lieberman and L. W. Mapson, *Nature* **195**, 1016 (1962)] and demonstrable in a nonenzymatic system containing iron (*41*). Recently M. Lieberman and L. W. Mapson [*Nature* **204**, 343 (1964)] have described a nonenzymatic system which produced predominantly ethylene in the presence of copper, ascorbic acid, and "aged" linolenic acid, and they report that cytoplasmic particles from apples evolve ethylene in the presence of copper and ascorbic acid.
44. S. P. Burg and E. A. Burg, *Nature* **191**, 967 (1961).
45. D. F. Meigh, *ibid.* **196**, 345 (1962).
46. M. S. Spencer [*Nature* **184**, 243 (1959)] reported that subcellular particles derived from tomatoes produce ethylene, but others (*44, 45*) were unable to duplicate these results. G. R. Chandra, M. Spencer, and M. Meheriuk then pointed out [*Nature* **194**, 361 (1962)] that the production only occurs after the particles are "aged" or treated with high-frequency sound, but D. F. Meigh (*45*) noted that ethylene is formed when a nonenzymatic mixture containing sucrose and adenosine triphosphate is sonically treated. Spencer's subcellular system may be similar to that of Lieberman and Mapson (*43*), for the former must be aged before ethylene is produced, whereas in the latter case linolenic acid must be aged to be "activated." Subsequently, R. Chandra and M. Spencer have claimed [*Nature* **197**, 366 (1963)] that by their methods it is possible to prepare ethylene-producing subcellular particles from rat liver, rat intestinal mucosa, and the mold *Penicillium digitatum*.
47. S. P. Burg and K. V. Thimann, *Arch. Biochem. Biophys.* **95**, 450 (1961); S. P. Burg and E. A. Burg, *Nature* **203**, 869 (1964).
48. C. H. Wang, A. Persyn, J. Krackov, *Nature* **195**, 1306 (1962).
49. D. J. Reed and C. H. Wang, *Can. J. Microbiol.* **5**, 59 (1959).
50. M. A. Muhs and F. T. Weiss, *J. Amer. Chem. Soc.* **84**, 4697 (1962).
51. W. C. Hall and P. W. Morgan, in *Régulateurs Naturels de la Croissance Végétale*, Intern. Colloq., Gif, France, 1963 (Centre National de la Recherche Scientifique, Paris, 1964), p. 727.
52. For example, during ethylene action in pea-stem sections the uptake and destruction of C^{14}-indole-3-acetic acid (the native auxin) is not altered, and its transport from the apex to the base of the section proceeds at a normal rate (also see *6* and *7*). Although it seems likely (see, for example, *6, 7, 13*) that production of auxin is reduced when ethylene is present, this reduction cannot be a requisite for ethylene action because the gas is equally effective in the pea-stem assay (where the site of auxin production has been removed) in the presence of widely differing concentrations of applied indole-3-acetic acid. Ethylene does not duplicate the effect of auxin in enhancing the growth of the tissue and does not prevent auxin from stimulating growth (measured as water uptake).
53. The data in this table have been compiled from the reports cited in *2, 15, 20, 26, 27, 32, 54*, and from R. Gane, *Gt. Brit. Dept. Sci. and Ind. Res. Rept. Food Invest. Bd.* **1935**, 123 (1936); J. M. Lyons and H. K. Pratt, *Proc. Amer. Soc. Hort. Sci.*, in press; J. B. Neiderl, M. W. Brenner, J. N. Kelley, *Amer. J. Botany* **25**, 357 (1938); United Fruit Co., unpublished data; S. P. Burg and E. A. Burg, unpublished data.
54. S, P. Burg and E. A. Burg, *Nature* **194**, 398 (1962).
55. 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 $10^{-1}M$. The following were inactive at all concentrations below the toxic level which is indicated in parentheses: dichloromethane, *cis*-dichloroethylene, *trans*-dichloroethylene, trichloroethylene, tetrachloroethylene, hydrogen sulfide, ethylene oxide, allyl chloride (all approximately 10,000 ppm); carbon dioxide (100,000 ppm); hydrocyanic acid ($3 \times 10^{-5}M$); acrylonitrile ($1.7 \times 10^{-4}M$); hydrogen peroxide ($10^{-2}M$); potassium azide ($4 \times 10^{-3}M$); allyl alcohol ($10^{-3}M$); and formaldehyde ($10^{-4}M$).
56. The studies cited in references *15, 32, 51, 35, 44*, and *47* were supported by Public Health Research Grant EF-00214 from the Division of Environmental Engineering and Food Protection, and this review was written while S.P.B. was the recipient of Research Career Development Award 1K3GM6871 from the PHS.

Women in Science: Why So Few?

Social and psychological influences restrict women's choice and pursuit of careers in science.

Alice S. Rossi

Where women are concerned, the late 1940's and the 1950's were marked by a national mood of domesticity demonstrated by the rapid rise in the birth rate and the flight of families to the suburbs. It was a period of high praise for woman's domestic role. That mood has shifted in the 1960's. Educators, employers, government officials, and manpower specialists are urging women to enter more fully into the occupational life of the nation. A President's Commission on the Status of Women has recently issued a set of wide-ranging recommendations to this end (*1*). Particular stress has been put on the need for women in fields in which there is a critical shortage of manpower—teaching, science, and engineering—and conferences on women in science have been held under federal auspices, at Marymount College in 1963 and at the Massachusetts Institute of Technology in 1964.

What can we expect as a result of this campaign? Working women in the industrial, service, and clerical occupations will probably experience an improvement in status. The implementation of the Equal Pay Act and the retraining possible under the Manpower Development and Training Act will be of help to such women, as will all attempts to improve community child-

---

The author is a research associate on the Committee on Human Development, University of Chicago. This article is adapted from an address at the Conference on American Women in Science and Engineering, at the Massachusetts Institute of Technology, 23–24 October 1964.