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Stimulation of Ethylene Production in Apple Tissue Slices by Methionine

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Summary. Methionine can induce more than a 100 % increase in ethylene production by apple tissue slices. The increased amount of ethylene derives from carbons 3 and 4 of methionine. Only post-climacteric fruit tissues are stimulated by methionine, and stimulation is optimum after 8 months' storage. Copper chelators such as sodium diethyl dithiocarbamate and cuprizone very markedly inhibit ethylene production by tissue slices. Carbon monoxide does not effect ethylene production by the slices. These data suggest that the mechanism for the conversion of methionine to ethylene, in apple tissues, is similar to the previously described model system for producing ethylene from methionine and reduced copper. Therefore, it is suggested that one of the ethylene-forming systems in tissues derives from methionine and proceeds to ethylene via a copper enzyme system which may be a peroxidase.

Two model systems which generate ethylene have recently been described. The first utilizes linolenate as substrate (1) and the second requires methionine, ethionine, or the α-hydroxy analogue of methionine as substrate (2). Both systems are catalyzed by cuprous ions and involve an intermediate peroxidation step. Since the biosynthetic pathway which gives rise to ethylene is unknown, it is of some interest to determine whether or not there is a relationship between the ethylene-forming model systems and ethylene biosynthesis in tissues.

It was not possible to study effectively the action of exogenous linolenate on tissue slices because of difficulties inherent in the incorporation of the fatty acid into tissues. However, incorporation of methionine into tissues, with a concomitant increase in ethylene production, was readily achieved. This report will describe these experiments.

Materials and Methods

Tissue Slices. Tissue slices were prepared from samples of post-climacteric Rome Beauty or Bramley's Seedling apples by slicing whole apples to a 1 cm thickness in a meat-slicing machine. Cylinders 1.0 cm or 1.5 cm in diameter were cut from these slices with a cork-borer and placed in solutions as follows: 3 g to 5 ml in 25 ml flasks, 6 g to 15 ml in 50 ml flasks, 15 g to 25 ml in 125 ml flasks, and 20 g in 20 ml or 30 g to 50 ml in 250 ml flasks. The solutions used as incubation mixtures contained 0.4 M sucrose, 0.4 M sucrose and 0.1 M sodium bicarbonate, or 0.4 M sucrose with various buffers as indicated. The flasks were sealed with 1 hole stoppers with clamped capillary tubes and atmospheres in the flasks were sampled periodically or at a fixed time after incubation at 25° or 30° in a shaker bath.

In some experiments the tissues were vacuum infiltrated. Vacuum infiltration was achieved by placing flasks in a desiccator and alternately evacuating and returning to normal pressure. In later experiments infiltration was reduced to 1 evacuation lasting only 60 to 90 seconds, and the vacuum released gradually, since prolonged infiltration reduced ethylene production. This procedure had the advantage, over noninfiltrated treatments, of removing the preformed ethylene from the slices. In some later experiments air was replaced by O_2, since it was found that ethylene production by apple slices immersed in liquid media was stimulated by O_2.

Gas Analysis. Gases evolved by the tissue slices were sampled by syringe and determined by gas chromatography with a flame-ionization detector in a system using either alumina or silicone (30% silicone oil on celite) columns (3).

Tracer Experiments. Tracer studies were carried out with ^{14}C, CH_3 labeled L-methionine, DL-methionine carboxy ^{14}C, DL methionine 2, ^{14}C, or DL methionine 3, 4, ^{14}C, added to the sucrose-bicarbonate incubation mixture. A 2 ml aliquot of the gases evolved by the apple tissue slices was first assayed for ethylene on the gas chromatograph, and then 50 % of the gaseous atmosphere in the incubation flask was removed with a 50-ml gas-tight syringe for ^{14}C analysis. Details of the technique by which the gaseous samples were

absorbed and finally counted have already been described (2). Radioactive ^{14}C ethylene was determined in a Packard Tri-Carb liquid scintillation counter, at an efficiency of approximately 50 % with backgrounds of about 10 cpm.

Further experimental details are described in tables, figures, and in the text where applicable.

Results

Stimulation of Ethylene Production by Methionine. Apple tissue slices from post-climacteric fruit stored about 9 months at 0°, incubated with L-methionine (10^{-3} M) at 30° in 0.4 M sucrose or 0.4 M sucrose–0.1 M bicarbonate buffer, produced significantly more ethylene than controls (table I). With methionine, a 33 % increase in ethylene production

Table I. *Effect of Methionine, Bicarbonate Buffer, and Infiltration on Ethylene Production by Apple Tissue Slices*

Three g of slices and 5 ml of solution were used in this experiment. Flasks were incubated 1 hour at 30° before sampling. Tissue was obtained from Rome apples stored 9 months at 0°.

Sample	Ethylene production: Noninfiltrated mμl/hr	% Change	Infiltrated mμl/hr	% Change
0.4 M Sucrose	120	...	60	...
0.4 M Sucrose + 10^{-3} M methionine	160	+33	80	+33
0.4 M Sucrose + 0.1 M $NaHCO_3$	130	...	90	...
0.4 M Sucrose + 0.1 M $NaHCO_3$ + 10^{-3} M methionine	280	+115	220	+144

was observed in sucrose solution and more than 100 % in sucrose-bicarbonate solutions. Compared to no external buffers, alkaline buffers greatly enhanced stimulation of ethylene production by apple slices, from 9-month-old apples, incubated with methionine. Vacuum infiltration, on the other hand, reduced ethylene production in apple slices. However, the percentage increase in ethylene caused by methionine was the same in both infiltrated and noninfiltrated tissues. Stimulation of ethylene production by methionine was not accompanied by any significant increase in respiration (table II).

The most critical factor associated with stimulation of ethylene production by methionine was the age of the fruit from which slices were made. Tissue slices from immature fruit, young fruit, and freshly harvested mature fruit of Rome Beauty and Bramley's Seedling apples were not stimulated by methionine. Stimulation of ethylene production appeared only after about 1 month's storage of the fruit at 0°, and then rose to a plateau during December through March (3–6 months) when about 30 to 35 % stimulation was obtained (fig 1). Greatest stimulation of ethylene production by methionine was obtained with fruit stored at 0° until April and May (8 months) when about 70 % stimulation was observed.

Table II. *Respiration and Ethylene Production of Apple Tissue Slices Incubated in Presence and Absence of Methionine*

Approximately 15 g of apple slices and 30 ml of solution were incubated in 115 ml Warburg-type flasks at 25°. The flasks were equipped with sidearms which contained 1 ml of 20 % KOH and fluted filter paper to facilitate absorption of CO_2. O_2 uptake was determined by Warburg manometric techniques.

Incubating solution	μl C_2H_4 in 5 hrs	μl O_2/hr per g tissue*
0.4 M Sucrose + 0.1 M $NaHCO_3$	0.67	11.1
0.4 M Sucrose + 0.1 M $NaHCO_3$ + 10^{-3} M methionine	0.94	11.7

* Tissue from Rome apples stored 6 months at 0°.

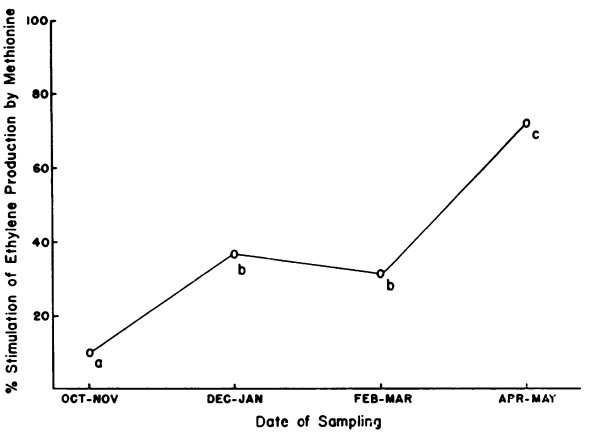

FIG 1. Percent stimulation of ethylene production by methionine in apple tissue slices as related to age of apples (length of storage at 0° and 90 % relative humidity). Tissue slices (15 g or 7 g) were incubated in 0.4 M sucrose–0.1 M bicarbonate buffer and 0.4 M sucrose–0.1 M bicarbonate–10^{-3} M methionine. Letters under points on graph indicate Duncan Multiple Test Range groupings at 5 % level (12). Each point on graph represents an average of 24 paired readings. One member of each pair is a control and one a methionine treatment. Points on graph underscribed by the same letters are not significantly different at the 5 % level. Points on graph underscribed by no letters in common are significantly different at 5 % level.

Effect of pH on Ethylene Production. As already suggested by the effect of buffer (table I), the pH of the external incubating medium plays an important role in the level of ethylene formed by apple tissue slices. The maximum amount of ethylene was produced at a pH of 9.5 and fell off rapidly above this pH (fig 2a, 2b). Below pH 9.5 there was a linear rise in ethylene production from pH 4.5 to 9.5 with Rome Beauty apples. Bramley's Seedling apples,

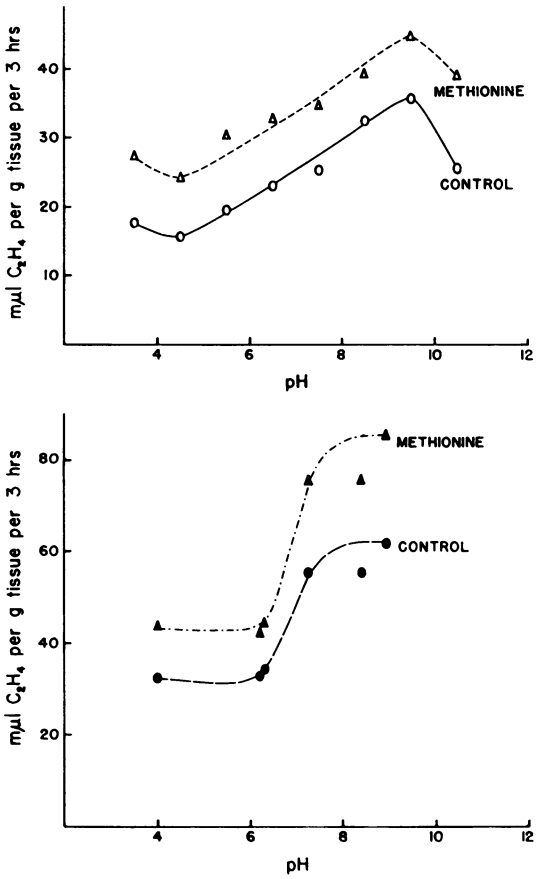

FIG. 2a (*top*). Ethylene production by Rome apple tissue slices, incubated in various buffers of different pH, in the presence and absence of methionine. Approximately 15 g of slices were incubated in 30 ml solution (0.4 M sucrose–0.1 M buffer). Buffers were as follows: Citrate-phosphate buffers pH 3.5 to 6.5, Tris-HCL pH 7.5 to 8.5, bicarbonate-carbonate buffers pH 9.5 to 10.5. Samples were taken from apples stored 6 months at 0°.

FIG. 2b (*bottom*). Ethylene production by Bramley's Seedling apple tissue slices, incubated with 0.4 M sucrose–0.1 M phosphate-citrate buffers, and 0.4 M sucrose-0.1 M phosphate-citrate buffer with 2×10^{-3} M methionine. Samples were taken from apples stored 5 months at 5°.

which had been vacuum infiltrated and the vacuum released in O_2, exhibited a sharp rise in ethylene formation between about pH 6.0 to pH 7.0, and then continued to rise less sharply to pH 9.0 (fig 2b). Essentially the same effects of pH were observed in the methionine stimulated systems with Rome Beauty (fig 2a) and Bramley's Seedling (fig 2b) tissues. In contrast to previous data (table I), approximately the same amount of stimulation by methionine was observed at all pH's. The discrepancy in the level of stimulation by methionine in unbuffered and bicarbonate-buffered solutions may be due to the different dates on which the tissues were sampled. The tissue slices used for the pH curve came from 6-month-old apples (stored at 0°), whereas the tissue slices used in obtaining data for table I were from 9-month-old apples (stored at 0°).

O_2 Concentration as a Factor in Stimulation by Methionine. The ability of O_2 to stimulate and nitrogen to completely inhibit ethylene production by whole fruit has long been known (4, 5, 6). These gases similarly affect apple tissue slices (fig 3). Stimulation of slices by methionine occurs at all concentrations of O_2 but the amount of ethylene produced is dependent on O_2 concentration. Thus, the ethylene formed by the tissues as a result of stimulation by methionine is affected in the same way by O_2 and nitrogen as the ethylene formed by the endogenous system. The temperature sensitivity of the methionine stimulated ethylene system is also the same as the endogenous ethylene system, with severe inhibition occurring at 40°.

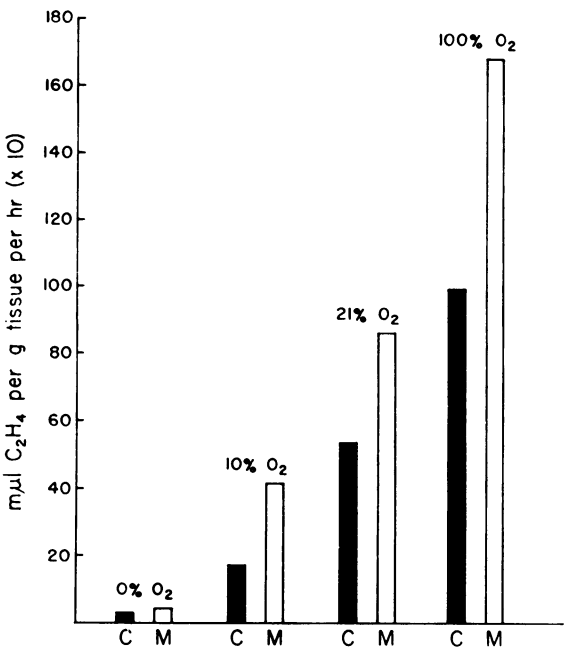

FIG. 3. Ethylene production as influenced by concentration of O_2, in the presence and absence of methionine. Approximately 7 g of Rome tissue slices incubated at 25° in 15 ml 0.4 M sucrose–0.1 M bicarbonate or 0.4 M sucrose–0.1 M bicarbonate and 10^{-3} M methionine during a period of 6 hours. C = control; M = methionine.

Stimulation as Related to Methionine Concentration. Stimulation of ethylene production in tissue slices was obtained over a range of methionine concentrations from 10^{-2} M to 10^{-4} M (table III). The amount of stimulation was approximately the same from 10^{-2} M to 10^{-4} M methionine, but dropped markedly at a concentration of 10^{-5} M. This is an indication that the ethylene forming enzyme can be saturated at 10^{-4} M and further concentration of the substrate does not inhibit the system. Such a concentration effect is reminiscent of the action of ex-

Table III. *Effect of Concentration of Methionine on Stimulation of Ethylene Production by Apple Tissue Slices*

Rome tissue slices (7 g) were incubated at 30° for 3 hours, in 15 ml of 0.4 M sucrose–0.1 M bicarbonate and 10^{-3} M methionine and compared to control, 0.4 M sucrose–0.1 M bicarbonate. Absolute control values for ethylene produced in mμl per hour per g fresh weight was 6, 8 and 14 for the 7, 8 and 9-month apples respectively.

Conc of methionine	% Stimulation of ethylene production Expt 1 7-month apples	Expt 2 8-month apples	Expt 3 9-month apples
10^{-2} M	+13	+37	+100
5×10^{-3} M	...	+28	+126
10^{-3} M	+32	+35	+116
10^{-4} M	+25	+28	+106
10^{-5} M	0	...	+ 16

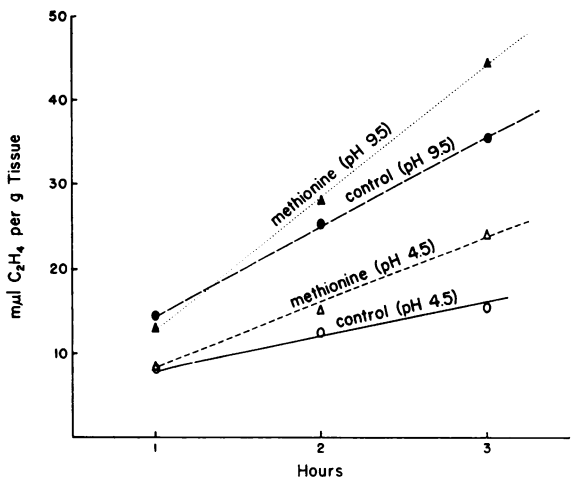

FIG. 4. Rate of ethylene production by tissue slices (not infiltrated) in presence and absence of methionine at pH 4.5 and 9.5. Rome apple tissue slices (15 g) were incubated at 25° in 30 ml 0.4 M sucrose–0.1 M buffer [either citrate-phosphate (pH 4.5) or bicarbonate (pH 9.5)] or 0.4 M sucrose–0.1 M buffer and 10^{-3} M methionine. Samples were taken from apples stored 6 months at 0°.

ogenous ethylene which can stimulate ripening in fruit over a thousand-fold or more range of concentration, from 0.1 ppm upward (7).

Rate of Ethylene Production by Tissue Slices With and Without Methionine. The rate of ethylene production with and without methionine is shown in figure 4. Significant stimulation by methionine is observed only after about 1 hour of incubation and thereafter rises steadily and diverges rapidly from the control. The stimulatory effect persists over a 23-hour period in tissue slices incubated with methionine (fig 5).

Effects of Other Substances Related to Methionine on Ethylene Production by Tissue Slices. The D-isomer of methionine was just as effective as the

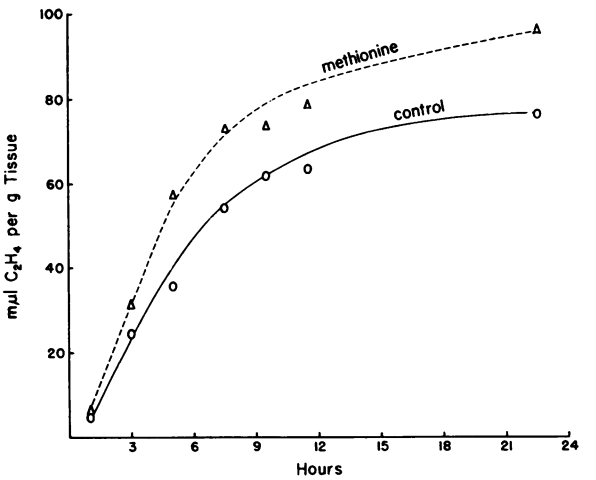

FIG. 5. Ethylene production by tissue slices in presence and absence of methionine, during a 24-hour period. Rome apple tissue slices (15 g) were incubated at 25° in 30 ml 0.4 M sucrose–0.1 M bicarbonate or 0.4 M sucrose–0.1 M bicarbonate-10^{-3} M methionine. Incubation flasks (115 ml volume) were Warburg-type flasks with side arms which contained 1 ml 10 % KOH and fluted filter paper to absorb CO_2 produced during incubation.

L-isomer in stimulating ethylene production (table IV) as was also the α-hydroxy analogue of methionine (α-hydroxy γ-methyl mercapto butyric acid). Ethionine which was active in the ethylene-forming model system (2), was ineffective with tissue slices, and actually inhibited ethylene production (fig 6a). Such evidence suggests that ethionine may be acting as a competitive inhibitor since the inhibitory effect of ethionine could be mitigated by the inclusion of methionine. Betaine and choline, 2 substances which function as methyl donors in metabolism like methionine, did not significantly stimulate ethylene production by apple tissue slices (table IV). *S*-methyl cysteine, homocysteine, and β-methyl mercapto propionic acid, all failed to stimulate ethylene production

Table IV. *Effectiveness of Various Compounds Related to Methionine as Stimulators of Ethylene Production by Apple Tissue Slices*

Thirty g of Rome apple tissue slices were incubated at 25° for 2 hours in 50 ml 0.4 M sucrose and 10^{-3} M of the substances indicated above. Comparisons were made with sucrose controls. Absolute control values were 6 mμl C_2H_4 per hour per g fresh weight.

Substance	% Change in ethylene production
L-Methionine	+66
D-Methionine	+63
DL-Ethionine	0
Betaine	+19
Choline	−15
α-Hydroxy analogue of methionine*	+74
Methional	0

* α-Hydroxy γ-methyl mercapto butyric acid.

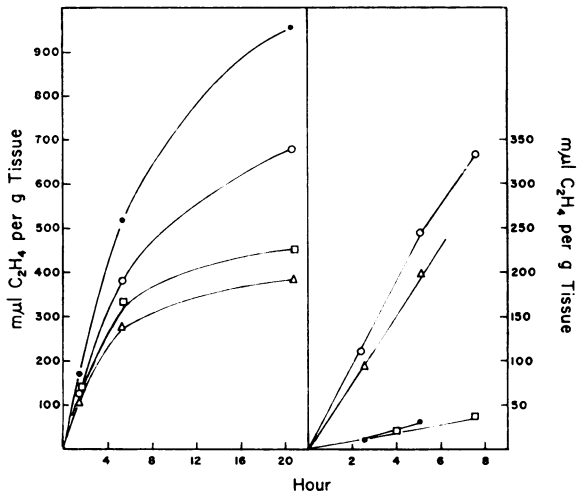

FIG. 6a (*left*). Effect of ethionine on ethylene production. Apple slices (30 g Bramley's Seedling) infiltrated with 50 ml 0.4 M sucrose–0.1 M bicarbonate or 0.4 M sucrose–0.1 M bicarbonate and ethionine or methionine. Tissue slices were incubated at 25° in 100 % O_2. ○, control; □, DL ethionine 5 mM; ●, DL-methionine 10 mM; △, DL-ethionine 10 mM.

FIG. 6b (*right*). Effect of copper chelating agents on ethylene production by Bramley's Seedling tissue slices. Conditions as in 6a. ○, control; △, EDTA 1 mM; ●, DIECA 1 mM; □, Cuprizone 1 mM.

by apple slices. The inactivity of these compounds with tissue slices is identical to their inactivity in the model system. On the other hand, methional, the most active precursor of ethylene in the model system (*2*) was totally inactive as a stimulator of ethylene in tissue slices.

Other amino acids were not effective as stimulators of ethylene production in apple tissue slices (table V) and in most instances actually inhibited ethylene production. Therefore, the stimulation of ethylene production in tissue slices by methionine is not associated with its function as either a methyl donor or a nonspecific amino acid.

Effect of Inhibitors on Ethylene Production by Apple Tissue Slices. Since the model systems which product ethylene are catalyzed by copper (*1, 2*) it was of interest to study the action of copper chelators on ethylene production by apple tissue slices. The reagents DIECA (sodium diethyl dithiocarbamate) at 10^{-3} M concentration and cuprizone (*bis* cyclohexanone oxalyl dihydrazone) at 10^{-3} M were very potent inhibitors of ethylene production by Bramley's Seedling tissue slices, inhibiting at approximately the 90 % level (fig 6b). However, EDTA at 10^{-3} M only inhibited in the order of 15 to 20 %. Much greater inhibition by EDTA (10^{-3} M) was obtained with tissue slices from Rome Beauty apples (stored 8 months at 0°), but inhibition by DIECA (10^{-3} M) was considerably greater (92 % compared to 59 % (table VI). Cuprizone at 10^{-4} M gave 68 % inhibition of the endogenous ethylene sys-

Table V. *Effect of Amino Acids Infiltrated into Apple Slices on Ethylene Production*

Amino acids at a concentration of 10^{-3} M were infiltrated into tissues in 50 ml of 0.4 M sucrose solution. Thirty g of Rome apple slices were incubated 2 hours at 25° before sampling. Slices were prepared from apples stored 8 months at 0°. Absolute control values were 6 mμl C_2H_4 per hour per g fresh weight.

Amino acid	Ethylene production (% change)
L-Methionine	+73
DL-Ornithine	+10
L-Proline monohydrochloride	+ 8
DL-Citrulline	+ 5
L-Leucine	− 4
L-Histidine monohydrochloride	− 5
DL-Tryptophane	− 5
Glycine	− 9
DL-Arginine	− 9
DL-Alanine	−13
DL-Threonine	−17
DL-Lysine	−22
DL-Valine	−25
L-Glutamate	−32
DL-Serine	−37
DL-Cystine	−41
DL-Aspartate	−41
L-Cysteine hydrochloride	−53

Table VI. *Effect of Metal Inhibitors on Ethylene Production by Apple Tissue Slices*

Inhibitor	% Inhibition
EDTA (10^{-3} M)*	59
EDTA (10^{-4} M)	0
DIECA (10^{-3} M)*	92
DIECA (10^{-4} M)	7
Cuprizone (10^{-4} M)*	68
KCN (10^{-3} M)**	64
KCN (10^{-4} M)	4
Carbon monoxide***	0

* Rome apple slices (7 g) were incubated 4 hours at 30° in 15 ml 0.4 M sucrose–0.1 M bicarbonate infiltrated with inhibitors in concentrations indicated. DIECA = diethyl-dithiocarbamate; EDTA = ethylene-diamine-tetraacetic acid; cuprizone = *bis*-cyclo-hexanone-oxalyl dihydrazone. Inhibitions were calculated by comparison to a control. Absolute control values for ethylene production were 6 mμl per hour per g fresh weight. Samples were obtained from apples stored 8 months at 0°.

** Rome apple slices were incubated at 25° in 0.4 M sucrose with and without KCN, after vacuum infiltration. Other conditions were the same as in * above.

*** Atmospheres of 90 % CO and 10 % O_2 were compared to controls containing atmospheres of 90 % N_2 and 10 % O_2. Absolute control values for ethylene produced was 3 mμl per hour per g fresh weight. Seven g of Rome apple slices were incubated for 3 hours at 25° in 15 ml 0.4 M sucrose or 0.4 M sucrose–0.1 M bicarbonate, after vacuum infiltration with the CO-O_2 or N_2-O_2 atmospheres. Samples were obtained from apples stored 7 months at 0°.

tem, whereas insignificant inhibition was shown by 10^{-4} M EDTA or DIECA. Since the greatest inhibition of the ethylene system was obtained with inhibitors which are rather specific for copper [cuprizone and DIECA (8, 9)] these data suggest the involvement of copper in the endogenous ethylene-forming system in apples.

Cyanide at 10^{-4} M was virtually noninhibitory to ethylene production by apple tissue slices, but at 10^{-3} M, was a potent inhibitor. This may indicate that the metal associated with the ethylene-forming enzyme is not easily accessible to some chelators until a concentration of 10^{-3} M is reached. It is also possible that at 10^{-4} M the decomposition or fixing of cyanide by other constituents leave little or no cyanide to chelate with the enzyme. The ethylene-forming enzyme system in apple tissue slices is also resistant to CO (table VI). The lack of response to CO places the enzyme system responsible for ethylene production in the same category as ascorbic acid oxidase and some peroxidases (10, 11).

Tracer Studies with Labeled Methionine. It was not clear whether the stimulation of ethylene production in apple tissue slices was a direct effect of a precursor-substrate to product relationship, or an indirect effect of methionine on ethylene metabolism in the cell. To test the nature of the stimulation by methionine, apple tissue slices were incubated with ^{14}C methionine labeled in either the *S*-methyl group, the carboxy group, carbon number 2, or carbons 3 and 4, and the radioactivity of the ethylene formed in these systems was assayed. Only methionine labeled in carbons 3 and 4 produced significant amounts of radioactive ethylene (table VII). Therefore, methionine stimulates ethylene production by apple tissue slices directly and is itself converted to ethylene. Since only carbons 3 and 4 of methionine were converted to ethylene as in the model system (2), it is likely that the mechanism involved in conversion of methionine to ethylene in apple tissues is similar to that operating in the model system.

The relative specific activity [specific activity of ethylene (0.53 μc/μmole)/specific activity of methionine (0.9 μc/μmole)] is approximately 0.6 when the total ethylene produced is used to calculate specific activity. However, since the tissue is producing ethylene from endogenous substrates as well as from exogenous ^{14}C methionine, there is dilution with the endogenous pools. Therefore, a precise value cannot be given for the relative specific activity, but 0.6 is a minimal value which indicates that methionine goes rather directly to ethylene.

Table VII. *Radioactivity in Ethylene Formed by Apple Tissue Slices Incubated in Solutions of ^{14}C Methionine Labeled in Various Positions*

Rome tissue slices (7 g) in 15 ml 0.4 M sucrose–0.1 M sodium bicarbonate and 10^{-3} M methionine (cold or ^{14}C labeled) (2.5 μc per flask in expt 1 and 13.5 μc per flask in expt 2) were incubated at 25° for 6 hours in both experiments. The 50 ml incubation flasks were fitted with side arms which contained 10% KOH with wicks to absorb the CO_2 evolved during the reaction. Samples of tissue slices were prepared from apples stored at 0°, 5 months in experiment 1 and 6 months in experiment 2. Details of the technique for collecting and determining radioactivity was as previously described (2).

Sample	mμmoles C_2H_4	Radioactivity in C_2H_4 cpm
Expt 1		
1) Tissue slices + cold L-methionine	20	...
2) Tissue slices + carboxy labeled DL-methionine	25	0
3) Tissue slices + C-2 labeled DL-methionine	24	73
4) Tissue slices + CH_3 labeled L-methionine	24	176
Expt 2		
1) Tissue slices + cold L-methionine	23	...
2) Tissue slices + C-3 and 4 labeled DL-methionine	30	34,566

Discussion

Methionine and its OH analogue are unique in their ability to stimulate ethylene production by apple tissue slices, but only post-climacteric tissue can be stimulated. An aging of the tissues must occur before a response to methionine is obtained. Experiments with ^{14}C methionine indicate that the utilization of methionine for ethylene formation is very direct and involves the same carbons that are converted to ethylene in the methionine model system. Therefore, it is probable that a similar mechanism operates in apple tissues. It is also possible that ethylene production in pre-climacteric tissues, or in tissues that do not respond to methionine, do not involve a pathway which converts methionine to ethylene.

Ethionine, which is very active as a precursor of ethylene in the model system, does not stimulate ethylene production by tissue slices, and in fact inhibits it. This suggests that ethylene in tissues is produced by an enzyme which is specific for methionine and its OH analogue. Since ethionine is a similar molecule it may act as a competitive inhibitor.

If the enzymatic ethylene-forming system in the tissues is similar to the model system, it is likely that the enzyme contains an active copper moiety, and by analogy to the model system an intermediate peroxide. Inhibition of ethylene formation by various copper chelators supports the probability of an active copper factor in ethylene biosynthesis. There is also some evidence which is consistent with the hypothesis that a peroxidase enzyme is involved in ethylene biosynthesis. The O_2 requirement for ethylene production and the lack of inhibition of the ethylene system by CO suggests some type of peroxidase enzyme per-

haps similar to catalase (11), peroxidase (11), or ascorbic acid oxidase (10). It thus seems possible that the enzymatic system forming ethylene uses a copper enzyme which may be a peroxidase.

There are 2 model systems for the production of ethylene and it is possible that more than 1 system for ethylene production exists in tissues. These may be associated with the different physiological functions of ethylene. Evidence is presented to implicate methionine as a precursor of ethylene in apple tissue slices, but methionine cannot stimulate ethylene formation in pre-climacteric apples, which are already producing some ethylene. This may mean that other precursors of ethylene are involved in ethylene production by pre-climacteric tissue. Methionine did not significantly stimulate ethylene production in post-climacteric tomato tissue slices or in avocado tissue slices, which indicates that either another precursor for ethylene production is operative in these tissues or that some factor or factors, other than precursors, prevent stimulation by methionine. Except for methionine and its α-hydroxy analogue there is no direct evidence for other precursors in the biosynthesis of ethylene.

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