A Comparative Study of the Ability of Methionine or Linolenic Acid to Act as Precursors of Ethylene in Plant Tissues

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A comparative study has been made of the ability of three plant tissues to incorporate ^{14}C into ethylene from ^{14}C -labelled methionine and derivatives and from ^{14}C -labelled linolenic acid. Incorporation of label occurs readily from methionine and its derivative 4-methylmercapto-2-oxobutyric acid with apple, tomato or cauliflower floret tissue. No incorporation of label occurred, however, from uniformly ^{14}C -labelled linolenate.

Two substrates have been implicated as precursors of ethylene in plant tissue, methionine and linolenate. Tracer evidence indicates strongly that ethylene may be formed from C-3 and C-4 of methionine (Lieberman, Kunishi, Mapson & Wardale, 1966; Burg & Clagett, 1967), and this concept has been supported in enzymic studies (Mapson & Wardale, 1967; Ku, Yang & Pratt, 1968). Although there seems no doubt that methionine can serve as a precursor of ethylene in a number of plant tissues, other studies by Galliard, Rhodes, Wooltorton & Hulme (1968a) have emphasized the possibility of linolenate being a precursor. The latter workers demonstrated that with apple tissue linolenate stimulated the production of ethylene, and that a cell-free system could be extracted from the tissue that in the presence of ascorbate produced ethylene from linolenate. In this enzymic system ethane was also produced, especially under conditions of low oxygen partial pressure.

The possibility that ethylene may originate *in vivo* from two very different substrates therefore existed. The present work was planned to compare under similar conditions the abilities of both substrates to act as precursors of ethylene in three very different plant tissues, the apple, the tomato and cauliflower florets.

EXPERIMENTAL

Briefly, the general plan was to supply the ^{14}C -labelled substrates to tissue 'slices' and determine the extent to which the label was incorporated into ethylene. Linolenate was introduced as the potassium salt at pH 8.5 and the amino acids and 4-methylmercapto-2-oxobutyric acid were introduced as the free acids. The latter acid was prepared as described by Mapson, March & Wardale (1969). Experimental conditions varied somewhat with each tissue, so the procedures used are described separately.

Apple

Tissue. Discs of peel from apples of various varieties were prepared by the method described by Rhodes, Galliard, Wooltorton & Hulme (1968).

Incubation. Thirty discs were incubated in 3.0 ml of 0.05 M-potassium phosphate buffer, pH 4.5, containing 1-2 μCi of the precursor under study at 25°C for 3 h under an atmosphere of pure O_2 . The incubations were carried out in Warburg flasks (volume about 35 ml) each containing 0.2 ml of 30% KOH and strips of filter paper in the centre well to aid absorption of any radioactive CO_2 produced by the discs. The Warburg flasks were sealed with rubber stoppers through which a narrow glass tube, sealed off with polythene tubing and a screw clip, was passed. The reaction flasks were agitated in a constant-temperature water bath throughout the incubation period.

Tomato

Tissue. Discs of tissue 10 mm in diameter were cut from the flesh of the tomato walls, care being taken to ensure a clean cut, thus allowing the skin to remain attached to one face of the disc.

Incubation. Twelve discs were immersed in 10 ml of buffered liquor containing 2 mM-EDTA and the appropriate labelled substrate. The pH of the selected liquor medium in which the tissue was immersed had a marked effect on the absorption of either methionine or 4-methylmercapto-2-oxobutyric acid (oxo acid) and on the production of ethylene by the tissue. Separate experiments established that at pH 7.4 absorption of methionine was about 18% of the amino acid added, but at the optimum pH of 5.5 this increased to 58%. Corresponding values for the oxo acid are 4 and 34% respectively. Similarly the ethylene produced by the tissue in the medium at pH 7.4 was only about one-third of that produced at pH 5.5. Accordingly with tomato tissue the medium used was 0.2 M-mannitol with 0.2 M-potassium-sodium phosphate buffer, pH 5.5.

The incubation liquor containing the labelled substrate was prepared and thoroughly mixed before the introduction of the tissue. The reaction was carried out in a 50 ml

conical flask. Each flask contained a CO₂ trap consisting of a suitable vial containing 0.5 ml of 30% KOH and a strip of filter paper to aid absorption of the gas.

Incubation was carried out in an atmosphere of O₂ at 25°C. The reaction flasks were agitated throughout the incubation period.

Cauliflower

Floret tissue. The stalk of each individual floret was cut so that each piece was approx. 5–6 mm across the mushroom-shaped head; the floret itself was not cut.

Incubation. Cauliflower florets (3.0 g) were partially immersed in 3.0 ml of liquor buffered with 0.1 M-sodium-potassium phosphate to pH 6.5 or 8.5. The liquor medium contained 2.0 mM-EDTA and the appropriate labelled substrate. The incubation and the method of fixing the CO₂ were as described for the tomato. As with the tomato, the floret tissue was added last to the complete incubation medium.

Post-incubation treatment

With all three tissues samples were assayed for ethylene production by using a technique described by Mapson *et al.* (1969). The tissue was separated from the liquor by filtration and washed with water. The liquor and washings were made up to a known volume. The CO₂-trap liquor volume was also made up to a known volume with water. These two solutions were checked for radioactivity by using a scintillation-counting technique. The absorption of labelled substrate was calculated from the radioactivity recovered in the washings.

RESULTS

The general objective of these experiments was to determine, on three very different tissues, the extent to which ¹⁴C was incorporated into ethylene from various labelled compounds supplied to tissue slices. It has been established (Lieberman *et al.*

1966; Burg & Clagett, 1967) that the carbon atoms of ethylene can originate from C-3 and C-4 of methionine, and this has been fully supported in the present experiments in which no labelling of ethylene occurred when either [1-¹⁴C]- or [5-¹⁴C]-methionine was supplied to these different tissues. In the experiments with 1,2,3,4-¹⁴C-labelled methionine or 4-methylmercapto-2-oxobutyric acid the degree of incorporation of label into ethylene has been calculated on the basis of (1) the conversion of C-3 and C-4 only into ethylene and (2) the actual amount of labelled compound absorbed by the tissue. Our results, however, take no account of the amount of labelled substrate absorbed but not metabolized, or metabolized into compounds other than CO₂ or ethylene. No volatile compounds other than CO₂ or ethylene were detected in these experiments.

Apple. Ethylene originating from discs of apple peel is readily labelled when the tissue is supplied with [1,2,3,4-¹⁴C]methionine. By the same criterion the 4-methylmercapto-2-oxobutyric acid (oxo acid) is even more readily converted into ethylene than is methionine (Table 1). Of the other amino acids tested, valine, aspartate and homoserine, only homoserine was converted, and then to a much smaller extent than was methionine. Amino acids, such as valine, that are utilized in a pathway distant from that of methionine were not converted. The results (Table 2) also show that label from [U-¹⁴C]linolenate was not introduced into ethylene when this fatty acid was administered. However, although linolenate is not itself converted into ethylene, it was capable of stimulating in this tissue the conversion of methionine into ethylene. The

Table 1. Comparison of the conversion into ethylene of labelled methionine, 4-methylmercapto-2-oxobutyric acid, homoserine, valine and linolenate by apple peel discs

Thirty discs (1.5 g fresh wt.) of the peel of apple fruit were incubated in 3.0 ml of 0.05 M-phosphate buffer containing the labelled substrate for 3 h at 25°C under an atmosphere of pure O₂. Results are calculated on the basis that only C-3 and C-4 of the active substrates are converted into ethylene.

Expt. 1	Labelled substrate	Absorption of labelled material by tissue (% of supplied radioactivity)	Incorporation of label into C ₂ H ₄	
			Total (% of that absorbed)	Rate (% converted by 100 g of tissue/h)
Var. Catshead	L-[1,2,3,4- ¹⁴ C]Methionine (0.402 mM, 0.96 μCi)	66	0.45	10
	1,2,3,4- ¹⁴ C-labelled oxo acid (0.382 mM, 0.91 μCi)	35	0.62	13.8
	L-[U- ¹⁴ C]Aspartic acid (0.038 mM, 1.0 μCi)	88	0.02	0.45
	L-[U- ¹⁴ C]Homoserine (0.009 mM, 1.05 μCi)	61.5	0.12	2.2
	[U- ¹⁴ C]Linolenate (0.003 mM, 9.0 μCi)	60	0	0
Expt. 2 Var. Cox's Orange Pippin	L-[1,2,3,4- ¹⁴ C]Methionine (0.402 mM, 0.96 μCi)	60	0.80	18.2
	L-[U- ¹⁴ C]Homoserine (0.009 mM, 0.91 μCi)	65	0.26	5.8
	L-[U- ¹⁴ C]Valine (0.022 mM, 0.98 μCi)	62	0	0

Table 2. *Effect of unlabelled linolenate on the conversion of various ^{14}C -labelled precursors into ethylene by apple peel discs*

Conditions of incubation were as described in Table 1, and any additions to this standard incubation mixture are indicated in the body of the table.

Variety of apple used	Labelled precursor	Addition of unlabelled material	Uptake of labelled compound by tissue (% of supplied radioactivity)	Incorporation of label into C_2H_4	
				Total (% of that absorbed)	Rate (% converted by 100 g of tissue/h)
Cox's Orange Pippin	[U- ^{14}C]Linolenate (9 μCi)	—	81	0	0
	[U- ^{14}C]Linolenate (9 μCi)	Linolenate (0.33 mM)	—	0	0
Hambling Seedling	L-[1,2,3,4- ^{14}C]Methionine (0.96 μCi)	—	59.6	0.85	18.9
	L-[1,2,3,4- ^{14}C]Methionine (0.96 μCi)	Linolenate (0.33 mM)	40.2	1.69	37.7
Cox's Orange Pippin	L-[1,2,3,4- ^{14}C]Methionine (0.96 μCi)	—	28	0.68	14.9
	L-[1,2,3,4- ^{14}C]Methionine (0.96 μCi)	Linolenate (0.33 mM)	30	1.09	24.3
Cox's Orange Pippin	L-[1,2,3,4- ^{14}C]Methionine (0.96 μCi)	—	38	0.8	17.7
	L-[1,2,3,4- ^{14}C]Methionine (0.96 μCi)	Linolenate (0.33 mM)	31	1.04	23.2
Catshead	1,2,3,4- ^{14}C -labelled oxo acid (0.91 μCi)	—	35	0.62	13.8
	1,2,3,4- ^{14}C -labelled oxo acid (0.91 μCi)	Linolenate (0.33 mM)	29	0.92	20.4

Fig. 1. Changes in the ethylene-producing capacity of discs of peel of pre-climacteric apples during their incubation ('aging') in dilute phosphate buffer in the presence (----) and absence (—) of cycloheximide (0.1 $\mu\text{g}/\text{ml}$) for periods up to 24 h. This figure shows two separate experiments, (a) and (b), in which discs were taken from the incubation media at intervals and both their ability to produce ethylene (upper set of curves) and their capacity to produce $^{14}\text{C}_2\text{H}_4$ from [1,2,3,4- ^{14}C]methionine (lower set of curves) was measured over 1 h (a) or 3 h (b) periods.

degree of stimulation observed was somewhat variable, depending mainly on the state of maturity of the tissue.

It has been shown previously (Galliard *et al.*,

1968a) that discs of peel of pre-climacteric apples, which are not themselves producing ethylene, can be induced to produce ethylene by periods of incubation in dilute aqueous buffer (termed 'aging').

Table 3. *Conversion of labelled L-methionine or 4-methylmercapto-2-oxobutyric acid into ethylene by tomato discs*

Twelve tissue discs (4–5 g) were incubated in 10 ml of liquor buffered to pH 5.5 with 0.2 M-mannitol-phosphate buffer containing 2.0 mM-EDTA and labelled substrate. Incubation was carried out in an atmosphere of O₂ for 22 h at 25°C.

Tissue condition	1,2,3,4- ¹⁴ C-labelled substrate	Total C ₂ H ₄ (μl)	Absorption of label by tissue (%)	Ratio ¹⁴ CO ₂ / ¹⁴ C ₂ H ₄	¹⁴ CO ₂ as % of absorbed label	Incorporation of label into C ₂ H ₄	
						Total (% of that absorbed)	Rate(% converted by 100 g of tissue/h)
Expt. 1							
Pre-climacteric	L-Methionine (0.053 mM, 0.45 μCi)	{ 0.80 5.0 3.96	74	13.4	10.2	1.5	1.6
Climacteric			61	3.1	18.8	12.1	13.8
Post-climacteric			46	3.6	20.3	11.2	11.3
Pre-climacteric	Oxo acid (0.153 mM, 1.15 μCi)	{ 0.68 5.15 4.50	32	17.6	10.4	1.2	1.3
Climacteric			34	3.5	17.8	10.3	13.5
Post-climacteric			29	4.0	17.8	8.9	8.8
Expt. 2							
Pre-climacteric	L-Methionine (0.053 mM, 0.45 μCi)	{ 1.3 14.9 4.0	78	6.6	9.5	1.3	1.2
Climacteric			76	1.3	13.9	10.3	9.4
Post-climacteric			28	1.8	12.7	7.2	6.1
Pre-climacteric	Oxo acid (0.12 mM, 0.92 μCi)	{ 1.3 6.2 3.6	34	9.6	11.2	1.2	1.1
Climacteric			35	2.0	16.9	8.4	7.8
Post-climacteric			19	3.1	14.9	4.8	4.2
Expt. 3							
Early climacteric	L-Methionine (0.053 mM, 0.45 μCi)	{ 6.4 8.9	82	1.9	9.5	10.2	9.5
Climacteric			69	1.9	16.6	17.2	14.8

As well as developing the ability to produce ethylene endogenously, their capacity to convert labelled methionine into ethylene increases in a very similar manner (Fig. 1). This increase in capacity to produce ethylene from labelled methionine is inhibited by low concentrations (0.1 μg/ml) of cycloheximide, an inhibitor of protein synthesis.

Tomato. The incorporation of label from methionine into ethylene was measured with this tissue in three different metabolic states. Discs of tissue were examined from fruit in a pre-climacteric condition, from fruit near the peak of the climacteric, a phase in which ethylene production was at its maximum, and from fruit in the post-climacteric phase, where the rate of ethylene production had declined to some extent.

The results of three experiments are recorded in Table 3. Incorporation of label from [1,2,3,4-¹⁴C]-methionine occurred most readily when ethylene production by whole fruit was near its maximum. This is evidenced by the conversion of 10–17% of the added labelled substrate absorbed by the tissue into ethylene at the peak of the climacteric, compared with values of only 1–1.5% in the pre-climacteric state and 7–11% in the post-climacteric phase. Further, decomposition of methionine leading to CO₂ by other pathways is at a minimum in the climacteric phase, as is evidenced by the lower ratio of ¹⁴C-labelled CO₂ to ¹⁴C-labelled ethylene (¹⁴CO₂/¹⁴C₂H₄). In the pre-climacteric stage values of 6.6–13.4 were obtained as compared with ratios of about 2 or less at the height of the climacteric. In one experiment, indeed, a value approximating to 1.0 was obtained (Table 6), which is the theoretical value for the complete

conversion of [1,2,3,4-¹⁴C]methionine into ethylene assuming that C-1 and C-2 are completely converted into CO₂. In the post-climacteric phase the fall in the efficiency with which methionine is converted into ethylene is paralleled by a rise in the ¹⁴CO₂/¹⁴C₂H₄ ratio.

Comparable values were obtained with the oxo acid, although in most experiments the degree of incorporation was marginally lowered, and this was accompanied by an increase in the ¹⁴CO₂/¹⁴C₂H₄ ratio, a result that must be attributed to the greater facility with which the oxo acid is metabolized in other pathways. It was noteworthy that absorption of the oxo acid by the tissue was also much less than that of methionine.

Both the total ethylene production and incorporation of label from either methionine or oxo acid were equally diminished in discs prepared from pre-climacteric, climacteric or post-climacteric tomato fruit by the presence of cycloheximide (1.2 μg/ml) (Table 4). The inhibitory effect of cycloheximide was greatest in the discs from the post-climacteric fruit.

Of the three other labelled amino acids tested, valine, aspartate and homoserine, only homoserine produced labelled ethylene, and here the percentage conversion was only one-third of that of methionine or oxo acid under comparable conditions (Table 5). This result agrees with that obtained with apple tissue, and confirms results originally found by Burg & Claggett (1967) that methionine is a more effective precursor than is homoserine. Evidence that all amino acids were metabolized is indicated by the amounts of ¹⁴C-labelled CO₂ liberated during the experiment.

Table 4. *Inhibiting effect of cycloheximide on the conversion of labelled L-methionine or 4-methylmercapto-2-oxobutyric acid into ethylene by tomato discs*
 Twelve tissue discs (4–5 g) were incubated in 10 ml of liquor buffered to pH 5.5 with 0.2 M-mannitol-phosphate buffer containing 2.0 mM-EDTA and labelled substrate. Incubation was carried out in an atmosphere of O₂ for 22 h at 25°C.

Incorporation of label into C ₂ H ₄									
Tissue condition	Cycloheximide (μg/ml)	1,2,3,4- ¹⁴ C-labelled substrate	Total C ₂ H ₄ (μl)	Absorption of label by tissue (%)	Ratio ¹⁴ CO ₂ / ¹⁴ C ₂ H ₄	¹⁴ CO ₂ as % of absorbed label	Rate(% converted by 100g of tissue/h)		
							Total (% of that absorbed)		
Expt. 1									
Post-climacteric	—	L-Methionine (0.053 mm, 0.45 μCi)	4.0	28	1.8	12.7	7.2	6.1	
Post-climacteric	1.2		0.67	12	7.5	12.7	1.7	1.5	
Pre-climacteric	—	Oxo acid (0.153 mm, 1.15 μCi)	1.3	34	9.6	11.2	1.2	1.1	
Pre-climacteric	1.2		0.51	27	12.4	7.9	0.64	0.62	
Climacteric	—		6.2	35	2.0	16.9	8.4	7.8	
Climacteric	1.2		4.5	28	3.1	12.0	3.9	3.6	
Post-climacteric	—	L-Methionine (0.053 mm, 0.45 μCi)	3.6	19	3.1	14.9	4.8	4.2	
Post-climacteric	1.2		0.58	18	5.2	6.2	1.2	1.0	
Expt. 2									
Early climacteric	—	L-Methionine (0.053 mm, 0.45 μCi)	6.4	82	1.9	9.5	10.2	9.5	
Early climacteric	1.2		2.9	51	2.2	6.0	5.6	5.0	
Climacteric	—		8.9	69	1.9	16.6	17.2	14.8	
Climacteric	1.2		3.7	35	2.0	10.1	11.4	10.2	

With tomato discs [U-¹⁴C]linolenate was supplied and direct comparison made with ¹⁴C-labelled methionine. The results (Table 6) show that, although linolenate was readily absorbed and about 8% of the acid absorbed by the tissue, was metabolized, as evidenced by the ¹⁴CO₂ production, no labelling of ethylene could be detected, whereas in the same experiment incorporation from methionine was equal to 25% of the amino acid absorbed by the tissue. We thus found no evidence that linolenate could serve as a precursor. In contrast with the results with apple tissue, no stimulation of ethylene production was observed. On the contrary, when non-labelled linolenate was supplied in addition to ¹⁴C-labelled methionine, in the higher concentrations used, it decreased the absorption of labelled methionine and the extent to which label was incorporated into ethylene.

Cauliflower. Some results on the conversion of ¹⁴C-labelled methionine and oxo acid into ethylene in cauliflower floret tissue have been published (Mapson *et al.* 1969). In the experiments there described no account was taken of the absorption of these labelled substrates, the conversion being expressed as a percentage of the amount of the labelled material added. These experiments were repeated, and the percentage conversion into ethylene of the amount of labelled substrate absorbed by the tissue was determined. The results confirm those already reported, in that the incorporation of label from the oxo acid into ethylene was four- to five-fold more efficient than from ¹⁴C-labelled methionine. The results (Table 7) also show that no label from [U-¹⁴C]linolenate was incorporated into ethylene, although evidence that it was readily absorbed was directly shown, and its subsequent metabolism confirmed from the large amount of radioactive CO₂ detected. The effect of linolenate on the incorporation of label from ¹⁴C-labelled methionine was also determined. The results show, as with the tomato, that whereas the fatty acid had no influence on the absorption of the labelled compound or on the production of labelled CO₂ the incorporation of label into ethylene was decreased by 45%. The overall metabolism of methionine therefore appeared to be unaffected by the presence of the fatty acid, though the specific reactions leading to ethylene were inhibited.

DISCUSSION

The present work has provided additional evidence supporting the concept that ethylene originates from methionine in plant tissues. With both apple and tomato tissue the ease with which ¹⁴C-labelled methionine was converted into ethylene was directly related to the endogenous ability of the tissue to synthesize ethylene. Thus in 'aging' apple

Table 5. *Comparison of the conversion into ethylene by tomato discs of labelled L-methionine and 4-methylmercapto-2-oxobutyric acid with that of labelled aspartate, homoserine and valine*

Twelve tissue discs (3g) were incubated in 10 ml of liquor buffered to pH 5.5 with 0.2M-mannitol-phosphate buffer containing 2.0 mM-EDTA and labelled substrate. Incubation was carried out in an atmosphere of O₂ for 21 h at 25°C.

Tissue condition	Labelled substrate	Total C ₂ H ₄ (μl)	Absorption of label by tissue (%)	Ratio ¹⁴ CO ₂ / ¹⁴ C ₂ H ₄	¹⁴ CO ₂ as % of absorbed label	Incorporation of label into C ₂ H ₄	
						Total (% of that absorbed)	Rate (% converted by 100 g of tissue/h)
Climacteric	L-[1,2,3,4- ¹⁴ C]Methionine (0.053 mM, 0.42 μCi)	6.1	51	2.3	23.2	20.0	27.2
Climacteric	1,2,3,4- ¹⁴ C-labelled oxo acid (0.063 mM, 0.47 μCi)	5.5	26	3.4	30.7	14.6	19.9
Climacteric	L-[U- ¹⁴ C]Aspartic acid (0.012 mM, 1.02 μCi)	5.6	26	—	27.5	0	0
Climacteric	L-[U- ¹⁴ C]Homoserine (0.003 mM, 1.10 μCi)	4.8	48	3.5	10.8	6.2	9.2
Climacteric	L-[U- ¹⁴ C]Valine (0.006 mM, 0.95 μCi)	5.0	44	—	9.7	0	0

Table 6. *Conversion of labelled linolenate into ethylene by tomato discs and the effect of linolenate on the conversion of L-methionine into ethylene by tomato discs*

Twelve tissue discs (4g), at or near the climacteric, were incubated in 10 ml of liquor buffered to pH 5.5 with 2.0M-mannitol-phosphate buffer containing 2.0 mM-EDTA and appropriate substrates. Incubation was carried out in an atmosphere of O₂ for 22 h at 25°C.

Substrate	Total C ₂ H ₄ (μl)	Absorption of label by tissue (%)	Ratio ¹⁴ CO ₂ / ¹⁴ C ₂ H ₄	¹⁴ CO ₂ as % of absorbed label	Incorporation of label into C ₂ H ₄	
					Total (% of that absorbed)	Rate (% converted by 100 g of tissue/h)
L-[1,2,3,4- ¹⁴ C]Methionine (0.053 mM, 0.42 μCi) added to each plus unlabelled linolenate as indicated:						
(1) —	15.1	59	1.1	14.0	25.6	29.1
(2) + 0.153 mM-Linolenate	10.6	47	1.9	21.0	22.9	26.7
(3) + 0.306 mM-Linolenate	8.7	41	1.4	15.0	20.9	23.7
(4) + 0.612 mM-Linolenate	7.1	34	1.6	16.0	19.8	23.1
[U- ¹⁴ C]Linolenate (0.01 mM, 10 μCi)	13.0	71	—	7.6	0	0

Table 7. *Conversion of labelled linolenate, L-methionine and 4-methylmercapto-2-oxobutyric acid into ethylene by cauliflower floret tissue*

Floret tissue (3.0 g) was incubated in 3.0 ml of liquor buffered to pH 6.5 or 8.5 with 0.1 M-phosphate buffer containing 0.2 mM-EDTA and the required substrate. Incubation was carried out in an atmosphere of O₂ for 15–20 h at 25°C.

Substrate	pH	Total C ₂ H ₄ (μl)	Absorption of label by tissue (%)	Ratio ¹⁴ CO ₂ / ¹⁴ C ₂ H ₄	¹⁴ CO ₂ as % of absorbed label	Incorporation of label into C ₂ H ₄	
						Total (% of that absorbed)	Rate (% converted by 100 g of tissue/h)
Expt. 1							
L-[1,2,3,4- ¹⁴ C]Methionine (0.23 mM, 0.85 μCi)	6.5	0.13	77	80	4.1	0.01	0.21
1,2,3,4- ¹⁴ C-labelled oxo acid (0.23 mM, 0.53 μCi) (16 h incubation)	6.5	0.20	72	41	11.7	0.54	1.12
Expt. 2							
L-[1,2,3,4- ¹⁴ C]Methionine (0.47 mM, 1.0 μCi)	8.5	0.18	57	—	*	0.16	0.33
[U- ¹⁴ C]Linolenate (0.01 mM, 10 μCi)	8.5	0.11	83	—	*	0	0
L-[1,2,3,4- ¹⁴ C]Methionine (0.47 mM, 1.0 μCi) (16 h incubation)	6.5	0.20	69	—	*	0.17	0.35
Expt. 3							
L-[1,2,3,4- ¹⁴ C]Methionine (0.45 mM, 1.1 μCi)	6.5	1.1	72	26.6	4.0	0.31	0.51
As above + unlabelled linolenate (0.45 mM) (20 h incubation)	6.5	0.53	82	46.8	3.9	0.17	0.28
Expt. 4							
1,2,3,4- ¹⁴ C-labelled oxo acid (0.23 mM, 0.53 μCi) (3 h incubation)	6.5	0.06	26	—	—	0.38	0.84
(15 h incubation)	6.5	0.56	81	16.3	15.9	1.95	4.33

* Large amount of ¹⁴CO₂ produced but not determined quantitatively.

tissue the rise in the endogenous production of ethylene was paralleled by a similar rise in the ability of the tissue to incorporate label from methionine into ethylene.

Similarly with tomato tissue the incorporation of label was lowest in the pre-climacteric state, rising sharply at the climacteric and falling somewhat in the post-climacteric phase. Therefore the ability of this tissue to convert methionine into ethylene also ran parallel to its ability to produce ethylene endogenously. The greater efficiency of the ethylene-synthesizing system at the climacteric is evident from the lower $^{14}\text{CO}_2/^{14}\text{C}_2\text{H}_4$ ratio obtained, which varied inversely with the conversion of labelled methionine to ethylene.

Our results also emphasize the role of 4-methylmercapto-2-oxobutyric acid as an intermediate in the synthesis. With both the apple and cauliflower tissue this labelled acid was converted to a greater extent than was methionine, although with the tomato the incorporation of label was slightly lowered. This appears to be accounted for by the greater ease with which the oxo acid is metabolized in other pathways, for the $^{14}\text{CO}_2/^{14}\text{C}_2\text{H}_4$ ratio was always greater than that observed with labelled methionine, a result that may perhaps be due to the design of the experiments. Where methionine is supplied the steady production of small amounts of the oxo acid may offer less chance of decomposition in other pathways and thus lead to more efficient conversion compared with conditions in which the oxo acid is supplied in higher concentrations at the start.

As expected, homoserine was much less readily converted into ethylene, and the other amino acids not at all. The fact that label from homoserine was incorporated, in conjunction with the fact that homoserine itself is not converted into ethylene either by model systems (Lieberman, Kunishi, Mapson & Wardale, 1965) or by isolated enzymic systems (L. W. Mapson & D. A. Wardale, unpublished work), suggests that homoserine must first be converted into methionine.

In contrast with the positive results achieved with methionine and its derivatives, no evidence was obtained that any of the carbon atoms of linolenate were converted into ethylene, although it was demonstrated that the fatty acid was absorbed and readily metabolized, as evidenced by the amounts of $^{14}\text{CO}_2$ detected. A considerable body of circumstantial evidence has been presented (Rhodes & Woollorton, 1967; Galliard *et al.* 1968a) linking the formation of ethylene with the metabolism of lipid constituents of fruit. In view of the disruption of chloroplast membranes, the large increase in lipoxidase activity (Woollorton, Jones & Hulme, 1965) and the breakdown of linolenate (Galliard, 1968) during the ripening of pome fruits,

it was suggested that the products of the hydroperoxidation of linolenate were the precursors of ethylene in fruits (Galliard *et al.* 1968a; Rhodes, Woollorton, Galliard & Hulme, 1970). Evidence for this suggestion was provided when it was found possible to prepare extracts of apple peel that would convert linolenate into ethylene (Galliard, Hulme, Rhodes & Woollorton, 1968b). It was shown by using radioactive linolenate that the ethylene produced in the system arose directly from the supplied linolenate. In view of the present findings it seems unlikely that this represents the operative system *in vivo*. However, the present finding that in the apple linolenate stimulates the conversion of methionine into ethylene suggests that linolenate may play some essential secondary role in ethylene biosynthesis in this tissue. Such a role may be the replacement of a glucose oxidase system shown to be operative in the cauliflower (Mapson & Wardale, 1967) by a lipoxidase system. The fatty acid hydroperoxide produced by this latter enzyme has already been shown to be capable of replacing hydrogen peroxide in the generation of ethylene from methionine or its derivatives (Mapson & Wardale, 1968), and preliminary experiments (L. W. Mapson & D. A. Wardale, unpublished work) indicate that in the tomato a lipoxidase enzyme rather than a glucose oxidase is involved in the synthesis of ethylene.

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