Plant Physiol. (1969) 44, 1371-1377

PLANT PHYSIOLOGY

Vol. 44 No. 10

October 1969

Comparative Studies of Effect of Auxin and Ethylene on Permeability and Synthesis of RNA and Protein[1]

Joseph A. Sacher and Seppo O. Salminen[2]

Department of Botany, California State College, Los Angeles, California 90032

Received January 7, 1969.

Abstract. The effects of ethylene on permeability and RNA and protein synthesis were assayed over a 6 to 26 hr period in tissue sections from avocado (*Persea gratissima* Gaertn. F., var. Fuerte), both pulp and peel of banana (*Musa sapientum* L., var. Gros Michel), bean endocarp (*Phaseolus vulgaris* L., var. Kentucky Wonder Pole beans) and leaves of *Rhoeo discolor*. Ethylene had no effect on permeability in 4 of the 5 tissues, but sometimes enhanced solute uptake in banana peel; it had either no effect or an inhibitory effect on synthesis of RNA and protein in sections from fruits of avocado and banana. Auxin (α-naphthalene acetic acid) stimulated synthesis of RNA and protein in bean endocarp and *Rhoeo* leaf sections, whereas ethylene inhibited both basal and auxin-induced synthesis. It is concluded that in these tissues the auxin effect is not an ethylene effect.

The results of many investigations have shown that auxin may stimulate synthesis of ethylene (12, 16). It was concluded that auxin-induced synthesis of ethylene is the basis for the effect of high concentrations (10^{-5} M or greater) of auxin on inhibition of growth of etiolated sections of pea and sunflower stems (10). The stimulation of RNA synthesis in senescent bean abscission zone explants by ethylene has been reported (1, 2), and led to the suggestion that some effects of auxin on RNA synthesis may be owed to auxin enhancement of ethylene synthesis.

In line with the facts that enhanced ethylene synthesis is associated with the respiratory climacteric of some fleshy fruits, and that application of ethylene initiates the climacteric, ethylene has been regarded as a product of ripening by some (5) and as a ripening hormone by others (7, 18). Ethylene production above the threshold level precedes onset of the respiratory climacteric by 4 hr in banana (9). For banana tissue, however, marked changes in membrane permeability are initiated about 2 days before the onset of the climacteric, manifested first by a gradual and then a rapid increase in the percentage of the tissue volume that is free space (22, 25). This evidence was considered supportive of the Blackman and Parija (6) hypothesis that permeability changes are causative of the climacteric. The onset of the increase in free space in avocado, however, coincides with the onset of the respiratory increase (27, and Wong and Sacher, unpublished data). Other workers have also reported a large increase in leakage or free space during the climacteric in avocado (4, 20, 29) and banana (3, 21).

Some investigators have examined the causal relations between ethylene and membrane permeability. Isolated mitochondria are induced to swell by a high concentration of ethylene (15). Also, ethylene affects the rate of volume change induced in mitochondria by ADP and ATP and increases ATPase activity of isolated mitochondria from a

[1] Supported by National Science Foundation Grant GB-6985.

[2] Present address: Department of Agronomy, University of California, Riverside.

variety of tissues. For this effect the integrity of the mitochondria is essential, as ethylene does not affect activity of the purified enzyme (19). Ethylene had no effect on leakage of solutes from pea stem sections (10), while treatment of canteloupe tissue discs with ethylene caused an increased permeability to water (26).

The present investigations were conducted to ascertain the effects of ethylene on RNA and protein synthesis and membrane permeability in tissue sections from 5 kinds of fruit and leaf tissues. Ethylene had no adverse effect on membrane properties of these tissues to uridine or L-leucine, and either had no effect on RNA and protein synthesis or caused inhibition of synthesis in some experiments. The results also indicate that auxin-induced synthesis of RNA and protein is not owed to an effect of auxin on ethylene synthesis.

Materials and Methods

Tissue sections were prepared from fruits of avocado (*Persea gratissima* Gaertn. F., var. Fuerte), banana (*Musa sapientum* L., var. Gros Michel) and pole beans (*Phaseolus vulgaris* L., var. Kentucky Wonder) and leaves of *Rhoeo discolor*.

A whole fruit of avocado or banana was taken on successive days prior to the onset of the climacteric. Tissue discs were sectioned (1.5 × 5 mm), washed for 20 min in running tap water, blotted and duplicate or triplicate 1 g samples weighed out for each treatment. Sections 1.5 mm thick were similarly prepared from bean endocarp and the basal midrib region of mature Rhoeo leaves. For radioassay of RNA and protein synthesis, based on the rate of incorporation of labeled precursors, the tissue sections were arranged on a dry filter paper in 125 ml flasks and then 2.5 ml of the dual labeled solutions were added. For trapping CO_2 during the incubations 1.0 ml 10 % KOH was added to a center well and a fluted paper added. In all experiments the thickness of the sections allowed the upper cut surface to be freely exposed to the gaseous environment, and the lower surface in contact with the labeled solution; therefore it was not necessary to shake the flasks. The incubation solutions contained 25 μg/ml streptomycin sulfate buffered to pH 6.1 with phosphate buffer and dual labeled with 5 μc uridine-5-T (28.8 C/mM) and 1 μc of L-leucine-U-^{14}C (305 mc/mM). For some experiments auxin (α-naphthalene acetic acid, NAA) was added to a final concentration of 10 μg/ml.

Incubation in Ethylene. Ethylene was provided using either a closed flask or a continuous flow system. For the former the flasks were sealed with serum caps through which a volume of an air-ethylene mixture could be injected to give a final concentration of 7 or 45 ppm ethylene. An equal volume of air was injected into the control flasks. In the continuous flow system the flasks were ventilated with humidified, ethylene-free air or an air-ethylene mixture which provided a turnover of the gaseous environment about every 1.5 min. Periodic monitoring with a Beckman GC-4 gas chromatograph showed no detectable ethylene (<0.01 ppm) in the continuous flow-control flasks. The ethylene mixture provided was 30 ppm.

After incubation at 25° the tissue was washed for 1 hr in running tap water, blotted and placed into 3 volumes of absolute ethanol. Extraction and assay of RNA and protein and radioassay of dual labeled samples were conducted as described previously (23).

Results

Effect of Ethylene on Membrane Permeability. We have found that ethylene (7–45 ppm) has no effect on permeability of sections of *Rhoeo* leaves, bean endocarp or banana or avocado pulp tissue to labeled uridine and leucine. An exception has been observed in the case of banana peel, which will be taken up later. Data for uptake of labeled leucine are shown in table I. Uptake is measured as radioactivity retained in both the ethanol soluble and insoluble fractions of tissue homogenates after an exhaustive washing in running tap water to remove radioactivity from the free space. Sections from these 4 tissues undergo a 4- to 50-fold increase in solute uptake during a 15-hr period of aging (23).

Table I. *Effect of Ethylene on Permeability of Sections From Leaf and Fruit Tissues to Leucine-U-^{14}C*

Samples (1 g fresh wt) of tissue sections incubated in 125 ml Erlenmeyer flasks on filter paper wetted with 2.5 ml of dual labeled solutions containing 25 μg/ml streptomycin sulfate and buffered to pH 6.1. Incubations were 15 hr for bean, avocado, and banana sections, and 6 hr for *Rhoeo* leaf sections, which were pre-treated as described in table III. For bean and *Rhoeo* tissue ethylene was added with a syringe through serum caps to a final concentration of 7 or 45 ppm and 1 ml of 10 % KOH with fluted paper added to the flask center well. Banana and avocado sections were provided with a 90 cc/min flow of humidified air or a 30 ppm ethylene-air mixture. NAA was 10 μg/ml.

Expt.	Tissue	Total uptake of leucine-U-^{14}C[1] Control	Ethylene	NAA
		dpm×10^{-3}		
1	Bean	1730 ± 80	1722 ± 12	1820 ± 170
2	Rhoeo	1195 ± 17	1141 ± 11	1137 ± 10
3	Avocado	1248 ± 41	1296 ± 17	...
4	Avocado	1440 ± 20	1470 ± 30	...
5	Banana	1280 ± 12	1350 ± 30	...
6	Banana	1628 ± 3	1627 ± 20	...
7	Banana peel	990 ± 70	1027 ± 29	...

[1] Measured as radioactivity in the ethanol soluble and insoluble fractions of homogenates from tissue sections which had been incubated in labeled solutions and then washed in running water for 1 hr. Means and standard errors for duplicate or triplicate samples.

Table II. *Comparison of Effects of Ethylene and Auxin on Synthesis of RNA and Protein by Sections of Bean Endocarp*

Tissue sections were incubated for 15 hr as described in table I. NAA was 10 μg/ml and ethylene was 45 ppm.

Treatment	dpm incorporated: dpm total uptake Uridine		dpm incorporated: dpm total uptake Leucine		RNA[1]		Protein[1]	
						% of zero time		% of zero time
	ratio		*ratio*		*μg/g*		*μg/g*	
Zero time	...		...		593 ± 15	100	3816 ± 31	100
Water	0.224 ± 0.005		0.705 ± 0.005		486 ± 3	82	3523 ± 63	92
Ethylene	0.217 ± 0.012	0	0.678 ± 0.007	— 5 %	458 ± 0	77	3315 ± 0	87
NAA	0.570 ± 0.030	+ 139 %	0.899 ± 0.006	+ 25 %	700 ± 32	118	4336 ± 216	114

[1] Means and standard errors for duplicate samples of tissue.

and this rapid development of uptake is not affected by ethylene. From these data it may be concluded that ethylene does not have an adverse effect on membrane-integrity (*i.e.*, cause leakiness) of tissue slices during incubations of tissue sections for 6 to 26 hr. For 2 of these tissues (bean endocarp and *Rhoeo* leaf sections), in which auxin prevents senescence and maintains integrity of membranes, there is also no difference in active uptake between auxin- and ethylene-treated tissues. For these experiments and those following in this paper the non-response to ethylene was the same both with and without trapping of CO_2, which has been shown to competitively inhibit the response to ethylene (8).

Bean Endocarp; RNA and Protein Synthesis. An assessment of whether ethylene stimulates RNA and protein synthesis, and whether effects of auxin on RNA and protein synthesis are owed to auxin-induction of ethylene synthesis, can be made by investigations with tissues in which auxin stimulates synthesis of RNA and protein. A comparison of the effect of auxin on synthesis of RNA and protein by bean endocarp is shown in table II. Auxin greatly stimulated synthesis of these macromolecules, whether assayed on the basis of the rate of incorporation of labeled precursors or measurements of the amount of RNA and protein. In contrast, ethylene had a slight inhibitory effect on synthesis of RNA and protein. There was no detectable ethylene accumulated (thus <0.01 ppm) from 1 g fresh weight of bean endocarp maintained on water-wetted filter paper in a 125 ml sealed flask over a period of 24 hr. In another experiment in which auxin had effects very similar to those in table II (enhanced incorporation of uridine 126 % and leucine 21 %), 7 ppm ethylene inhibited uridine incorporation 7 %, did not affect leucine incorporation and the decline in RNA was 25 % as in the water controls.

Rhoeo Leaf Sections: RNA and Protein Synthesis. Auxin prevents senescence of *Rhoeo* leaf sections and enhances synthesis of RNA and protein (24, 25). When *Rhoeo* leaf sections were incubated for 15 hr with and without auxin (NAA, 10 μg/ml) and with and without ethylene (7 ppm), auxin enhanced incorporation of uridine and leucine, but ethylene had no effect as compared with the water- or auxin-controls (data not shown). Monitoring of ethylene production by 1 g fresh weight of *Rhoeo* leaf sections in a sealed 125 ml flask showed none detectable at 12 hr (<0.01 ppm), and an accumulation of 0.06 and 0.08 ppm in 21 and 24 hr respectively.

For bean abscission zone explants an effect of ethylene on RNA and protein synthesis was observed only in senescent explants which had been aged for 24 hr on agar (1, 2). To ascertain if senescent *Rhoeo* sections become sensitive to ethylene they were aged for 18 hr on water-wetted filter paper and then washed in running tap water for 20 min and incubated for 6 hr in dual labeled solutions. As is shown in table III auxin enhanced incorporation of both uridine and leucine, but ethylene had no effect. Although during aging of *Rhoeo* sections for 24 hr there occurs a decline in protein of about 12 %, a subsequent application of auxin causes a large enhancement of RNA and protein synthesis (24). When tissue sections were exposed to ethylene (7 ppm) for 26 hr, however, ethylene inhibited

Table III. *Effect of Ethylene and/or Auxin for 6 Hr on Incorporation of Uridine-5-T and L-leucine-U-^{14}C in* Rhoeo *Leaf Sections*

Samples of tissue sections aged 18 hr, washed for 20 min, blotted and incubated in dual labeled solutions for 6 hr as described in table I, with and without ethylene (7 ppm) and NAA (10 μg/ml).

Incubation	dpm incorporated[1]: dpm total uptake Uridine-5-T		dpm incorporated[1]: dpm total uptake L-leucine-U-^{14}C	
Air controls	0.472±0.027		0.852±0.006	
Ethylene	0.500±0.028	0	0.853±0.008	0
NAA	0.588±0.002	+25 %	0.909±0.001	±7 %

[1] Means and standard errors.

Table IV. *Effect of Ethylene and/or Auxin for 26 Hr, on Incorporation of Uridine-5-T and* L-*leucine-U-*^{14}C *in* Rhoeo *Leaf Sections*

Tissue sections aged for 20 hr on filter papers wetted with water or a solution of NAA in presence or absence of ethylene The sections were then washed for 15 min and transferred to 125 ml flasks onto filter papers wetted with dual labeled solutions for 6 hr. NAA was 10 μg/ml and ethylene 7 ppm.

Treatment of tissue			dpm incorporated[1]	
20 Hr aging	+	6 hr incubation in dual labeled solutions	dpm total uptake Uridine-5-T	dpm total uptake L-leucine-U-^{14}C
			ratio	
In air		In air	0.299 ± 0.026	0.780 ± 0.005
In C_2H_4		In C_2H_4	0.265 ± 0.003 — 12 %	0.782 ± 0.002 0
NAA in air		NAA in air	0.357 ± 0.008 + 19 %	0.835 ± 0.003 + 7 %
NAA in C_2H_4		NAA in C_2H_4	0.325 ± 0.009 + 9 %	0.822 ± 0.013 + 5 %

[1] Means and standard errors.

uridine incorporation 12 % and inhibited the auxin-stimulation of uridine incorporation by 47 % (table IV).

Thus, in both bean and *Rhoeo* ethylene caused a significant inhibition of RNA and/or protein synthesis. In *Rhoeo* leaf sections the auxin-induced synthesis of RNA was partially inhibited by ethylene. A similar inhibitory effect of ethylene on both auxin- and kinetin-induced RNA synthesis occurs in senescent bean petiolar explants (see table I in reference 2).

Avocado and Banana Tissue: RNA and Protein Synthesis. Preclimacteric banana and avocado appeared suitable tissues to investigate the effect of ethylene on RNA and protein synthesis because of their high sensitivity to ethylene; its application may induce the onset of the climacteric in as little as 3 hr (9). Also, recent studies (13) indicate that 30 ppm ethylene increased incorporation of ^{32}P in sections of green banana peel during a 6-hr period. An increase in the rate of leucine incorporation has been observed in avocado tissue sections during the climacteric rise (20), when ethylene production is accelerated. Other studies (27), however, show that in avocado the amount of RNA and protein (80 % ethanol insoluble) declines from 12 to 25 % during the climacteric, paralleling a decline in the rate of incorporation of leucine and glycine. In banana the level of protein remains unchanged during ripening, although the rate of incorporation of 0.05 M leucine-^{14}C decreases 50 % from the preclimacteric through the respiratory peak (22, 25). It has been suggested (25) that this decrease could be a consequence of the fact that the tissue is essentially 100 % free space at the climacteric, which would result in a 3-fold dilution of all other amino acids and cofactors essential to protein synthesis, due to a diffusive mixing of solutes between the tissue and the ambient solution.

The rate of ethylene production by 1 g samples of tissue sections from banana and avocado pulp and banana peel was assayed in 125 ml flasks sealed with syringe caps. Toward this end the sections were washed for 20 min after cutting and then incubated in the flasks on filter paper wetted with 2.5 ml of a buffered streptomycin solution, just as we are using in studies on incorporation of radioactive precursors. Banana pulp sections produced 0.3 ppm by 9 hr, after which the rate appeared to decline so that assays at 21 and 24 hr showed 0.4 ppm. Sampling of flasks containing banana peel showed 0.04 ppm ethylene at 7 hr. Avocado sections produced ethylene at a linear rate after a 3-hr lag period, increasing from 0.3 ppm at 3 hr to 8.8 ppm at 14 hr. Because even the lowest concentration of ethylene produced could have an effect, for all experiments done with banana and avocado a continuous flow system was used.

Previous experiments showed that about 90 % of ^{14}C-leucine taken up is incorporated when tissue sections are incubated in a solution of high specific activity. Therefore the concentration of exogenous leucine was adjusted to 5 mM, which results in a reduction of the ratio of dpm incorporated/dpm total uptake to a lower value more favorable to measurement of stimulation of synthesis. In separate experiments (Sacher, unpublished data), tissue sections with normal membrane properties were assayed for the rate of incorporation of leucine over a range of concentrations of exogenous leucine from 0.1 to 5 mM. When 5 mM leucine is used there is no difference in the number of μg of ^{14}C-leucine incorporated in tissue that was preincubated for 3 hr in water or in 20 mM unlabeled leucine. In the tissue (0.5 g fresh wt) preincubated in unlabeled leucine the size of the endogenous pool of leucine was augmented 159 to 237 μg. Thus, using a high concentration of exogenous leucine essentially eliminates any possible dilution of the isotope precursor at the site of synthesis by endogenous unlabeled leucine, whether it arises from proteolysis or endogenous synthesis. The latter is inhibited by a high concentration of exogenous leucine (17).

In the experiments following, tissue sections were cut from pulp tissue of preclimacteric avocados and bananas and banana peel, and incubated in dual labeled solutions in presence and absence of 30 ppm ethylene with a continuous flow system. In the air controls no ethylene could be detected (thus <0.01 ppm). As pointed out previously, there was no adverse effect of any of the incubation procedures (± ethylene) on permeability characteristics of the tissue. On the contrary, these tissues undergo the same rapid development of uptake that occurs during aging of storage tissues of carrot and potato for about 15 to 24 hr (23).

In table V are shown the results of 2 experiments each with discs of banana and avocado pulp tissue (Expt. 1–4), in which measurements of the effect of ethylene on synthesis of RNA and protein are based on assays of the rate of incorporation of labeled precursors. Ethylene caused a small but significant inhibition of either RNA or protein synthesis in discs of pulp tissue from both banana and avocado. In the 2 experiments we did with peel from green banana (table V), 30 ppm ethylene inhibited uridine incorporation 12 % in a 15-hr incubation, and 8 % in an experiment of 7 hr duration. Ethylene had no effect on the rate of incorporation of leucine.

Discussion

Ethylene has no effect on, or inhibits, RNA and/or protein synthesis in sections of bean endocarp and *Rhoeo* leaves during incubations of 6 to 26 hr, while auxin enhances such synthesis in both tissues. Ethylene also inhibits auxin-induced synthesis of RNA in *Rhoeo*. Thus for bean endocarp and *Rhoeo* the effect of auxin on RNA and protein synthesis is not mediated by auxin causing synthesis of ethylene. If auxin-induced synthesis of RNA and protein were owed to auxin-induced synthesis of ethylene it could be expected that auxin would not cause enhancement of synthesis of RNA and protein over that of the ethylene controls, in which ethylene was provided at 7 or 45 ppm. That the auxin-effect in bean endocarp is not an ethylene effect may be deduced also from previous studies (23) in which it was demonstrated that treatment of bean endocarp for 20 hr with concentrations of NAA, ranging from 10^{-7} to 10^{-4} M caused an increase of up to 13 % for RNA and 42 % for protein. Semi-log plots of the amount of RNA and protein against the concentration of auxin showed a linear relationship. It seems unlikely that this relationship is owed to 2 different modes of action of auxin, with the effect of auxin at the 2 higher concentrations (10^{-5} and 10^{-4} M) being owed to auxin-induced synthesis of ethylene.

Our results show that ethylene causes some significant inhibition of the rate of incorporation of RNA or protein precursors in sections of pulp tissue of banana and avocado and banana peel, under the conditions of incubation used, and with a continuous flow system. The effect of added ethylene (7 or 45 ppm) could not be demonstrated in several experiments each with banana and avocado discs in sealed 125 ml flasks, whether of 7, 15 or 24 hr duration. This is consistent with the interpretation that the amount of ethylene produced by the tissue discs is sufficient to elicit its maximal response. It is not certain, however, whether the effects observed with added ethylene as compared with the continuous flow air-controls are truly maximal, because of the possible effect of the amount of ethylene that is in the tissue (see 8). What is known only is that the continuous flow of air maintained an outward gradient and held the accumulation of ethylene to less than 0.01 ppm within the flasks.

We cannot explain how, in banana and avocado, ethylene may inhibit incorporation of uridine at one time and leucine at another (table V); the inhibition of uridine incorporation, however, is more consistent. One possible interpretation is that these results manifest an ethylene-inhibition of synthesis, or en-

Table V. *Effects of Ethylene on Incorporation of RNA and Protein Precursors in Sections of Pulp Tissue From Avocado and Banana Fruits and Banana Peel*

Avocado and banana tissue incubated 15 hr (except Expt. 6, which was 7 hr), in dual labeled solutions as described in table I. Experiments were with different batches of avocados and bananas. Ethylene was 30 ppm. The concentration of exogenous leucine was adjusted to 5 mM.

		dpm incorporated/dpm total uptake[1]					
		Uridine-5-T			Leucine-U-^{14}C		
Expt.	Tissue	Control	Ethylene	Effect of ethylene	Control	Ethylene	Effect of ethylene
		ratio		%	*ratio*		%
1	Banana	0.800 ± 0.006	0.778 ± 0.011	0	0.100 ± 0.007	0.082 ± 0.006	—18
2	Banana	0.708 ± 0.003	0.675 ± 0.002	— 5	0.0921 ± 0.0006	0.0943 ± 0.0012	0
3	Avocado	0.469 ± 0.007	0.419 ± 0.016	—11	0.147 ± 0.006	0.143 ± 0.004	0
4	Avocado	0.449 ± 0.008	0.427 ± 0.020	0	0.158 ± 0.002	0.140 ± 0.002	—10
5	Banana peel	0.508 ± 0.004	0.448 ± 0.013	—12	0.0560 ± 0.0017	0.0548 ± 0.0018	0
6	Banana peel	0.436 ± 0.002	0.402 ± 0.010	— 8	0.0249 ± 0.0004	0.0254 ± 0.0004	0

[1] Means and standard errors for triplicate samples of tissue. All differences significant at the 1 % level.

hancement of degradation, of RNA or protein. Precautions were taken to provide a high concentration of labeled leucine to prevent or greatly diminish any isotopic dilution effect. Also, in bean endocarp ethylene increased degradation of both RNA and protein, as compared with the water-controls. That the decrease in the rate of incorporation of labeled precursors and in the amount of RNA and protein caused by ethylene is relatively small could be owed to ethylene inhibiting synthesis of some rather than all RNA and protein. Alternatively, the results observed with avocado and banana could be explained if ethylene in some way affected the relative distribution of labeled and endogenous, unlabeled precursors at the site of synthesis, so as to alter the specific activity of the metabolic pool at the site of synthesis. The fact that the tissue sections were provided with 5 mM labeled leucine would make the rate of leucine incorporation less susceptible than the rate of uridine incorporation to any such effect of ethylene. There is previous evidence (15, 19, 26) that ethylene may affect permeability and possibly other characteristics of cellular membranes, whether or not this is its primary effect. We will discuss further ahead the ethylene-enhancement of uptake of uridine and leucine by sections of banana peel.

The physiological significance of the effects of ethylene observed on tissue discs of pulp tissue will not be clear until we have learned more of how the conditions of aging of tissue discs affects the response of the tissue to ethylene. Such information is important to an understanding of the relations between the biochemical behavior of tissue discs and intact fruits. Similarities in this respect have been demonstrated (14) between discs of apple peel from preclimacteric apples during a period of aging up to 24 hr in buffered solutions, and peel discs removed on successive days from climacteric apples. During aging of avocado discs in moist air, changes in softening, respiration and taste resemble changes in the intact fruit more closely than discs aged in water (4). A recent article (28) cites personal communications from J. K. Palmer that banana slices aged in moist air show a response like intact fruit in respect of a respiratory climacteric, in presence and absence of ethylene. In our experiments we attempted to achieve the advantages of moist air and still provide conditions favorable for accurate radioassay procedures, by incubating discs on filter paper wetted carefully with dual labeled solutions, so as to leave the upper half of the discs exposed to the air.

Although ethylene-enhancement of RNA synthesis in sections of banana peel has been reported (13), based on assays of incorporation of ^{32}P, in experiments performed similarly we find that ethylene inhibits the rate of incorporation of uridine and has no effect on leucine. In 1 experiment with banana peel sections (Expt. 6, table V) uptake (dpm $\times 10^{-3}$) in absence and presence of ethylene respectively was 763 ± 6 and 861 ± 21 for uridine, or an ethylene-induced increase of 13 %, and 329 ± 3 and 371 ± 6 for leucine, or an increase of 10 %. Assays based on dpm incorporated only would show ethylene-enhancement of incorporation of both precursors, although, in fact, ethylene inhibited uridine incorporation and had no effect on leucine (Expt. 6, table V), based on the ratio of incorporation/total uptake.

Ethylene apparently does not act on membranes to cause the loss of membrane-integrity and thus the increase in free space, which is well established for climacteric fruits (3, 4, 20, 21, 22, 25, 29). Such irreversible changes may represent impaired metabolism of the kind occurring in bean endocarp and *Rhoeo* leaf sections (25), where loss of membrane-integrity is preceded by a rundown in RNA and protein synthesis. Rather, the evidence does indicate that ethylene at high (15) or low concentrations (19, 26) can influence the rate of permeation of water and/or solutes into cells or isolated mitochondria. That this effect can vary with different tissues is shown by the absence of any effect of ethylene on permeability of sections of avocado and banana pulp tissue, bean endocarp or *Rhoeo* leaves to uridine and leucine, while it may enhance uptake of these solutes by banana peel. Hormones may have effects on uptake which are distinct from and do not affect their more primary action. Auxin (NAA) severely inhibits uptake of glucose, L-phenylalanine and orotic acid, stimulates leucine uptake, independent of its enhancement of synthesis of RNA and protein in bean endocarp (23).

These preliminary studies with tissue sections from 5 different tissues, all from determinate organs, indicate that the concept of ethylene-enhancement of RNA and protein synthesis (1, 2) does not have general application, even for senescent tissues. From comparative studies of the effects of auxin and ethylene on synthesis of RNA and protein in bean endocarp and *Rhoeo* leaf sections it is concluded that auxin-induced synthesis of RNA and protein is not mediated by an effect of auxin on inducing ethylene synthesis.

Acknowledgments

We thank Mrs. Elizabeth Anderson for skillful technical assistance.

Literature Cited

1. ABELES, F. B. AND R. E. HOLM. 1966. Enhancement of RNA synthesis, protein synthesis, and abscission by ethylene. Plant Physiol. 41: 1337–42.
2. ABELES, F. B., R. E. HOLM, AND H. E. GAHAGAN. 1967. Abscission: the role of aging. Plant Physiol. 42: 1351–56.
3. BAUR, J. R. AND M. WORKMAN. 1964. Relationship between cell permeability and respiration in

ripening banana fruit tissue. Plant Physiol. 39: 540–43.
4. BEN-YEHOSHUA, S. 1964. Respiration and ripening of fruit discs of the avocado fruit. Physiol. Plantarum 17: 71–80.
5. BIALE, J. B., R. E. YOUNG, AND A. J. OLMSTEAD. 1954. Fruit respiration and ethylene production. Plant Physiol. 29: 168–74.
6. BLACKMAN, F. F. AND P. PARIJA. 1928. Analytic studies in plant respiration. I. The respiration of a population of senescent ripening apples. Proc. Roy. Soc. Ser. B. 103: 412–45.
7. BURG, S. P. 1962. The physiology of ethylene formation. Ann. Rev. Plant Physiol. 13: 265–302.
8. BURG, S. P. AND E. A. BURG. 1965. Ethylene action and the ripening of fruits. Science 148: 1190–96.
9. BURG, S. P. AND E. A. BURG. 1965. Relationship between ethylene production and ripening in bananas. Botan. Gaz. 126: 200–04.
10. BURG, S. P. AND E. A. BURG. 1966. The interaction between auxin and ethylene and its role in plant growth control. Proc. Natl. Acad. Sci. U. S. 55: 262–69.
11. BURG, S. P. 1968. Ethylene, plant senescence and abscission. Plant Physiol. 43: 1503–11.
12. HANSON, E. 1946. Effect of 2,4-D on the ripening of Bartlett pears. Plant Physiol. 21: 588–92.
13. HOLM, R. E. AND F. B. ABELES. 1967. Abscission: the role of RNA synthesis. Plant Physiol. 42: 1094–1102.
14. HULME, A. C., M. J. C. RHODES, T. GALLIARD, AND L.S.C. WOOLTORTON. 1968. Metabolic changes in excised fruit tissue. IV. Changes occurring in discs of apple peel during the development of the respiration climacteric. Plant Physiol. 43: 1154–61.
15. LYONS, J. M. AND H. K. PRATT. 1964. An effect of ethylene on swelling of isolated mitochondria. Arch. Biochem. Biophys. 104: 318–24.
16. MORGAN, P. W. AND W. C. HALL. 1962. Effect of 2,4-dichlorophenoxyacetic acid on the production of ethylene by cotton and grain sorghum. Physiol. Plantarum 15: 420–27.
17. OAKS, A. 1965. The effect of leucine on the biosynthesis of leucine in maize root tips. Plant Physiol. 40: 149–55.
18. PRATT, H. K. AND J. D. GOESCHL. 1969. Physiological roles of ethylene in plants. Ann. Rev. Plant Physiol. 20: 541–84.
19. OLSON, A. E. AND M. S. SPENCER. 1968. Some investigations on the effect of ethylene on mitochondrial systems. In: Biochemistry and Physiology of Plant Growth Substances. F. C. Wightman and G. Setterfield, eds. 6th Intern. Conf. on Plant Growth Substances. Runge Press, Ottawa, Canada. p 1243–53.
20. RICHMOND, A. AND J. B. BIALE. 1966. Protein and nucleic acid metabolism in fruits. I. Studies of amino acid incorporation during the climacteric rise in respiration of the avocado. Plant Physiol. 41: 1247–53.
21. SACHER, J. A. 1962. Relations between changes in membrane permeability and the climacteric in banana and avocado. Nature 195: 577–78.
22. SACHER, J. A. 1966. Permeability characteristics and amino acid incorporation during senescence ripening of banana tissue. Plant Physiol. 41: 701–08.
23. SACHER, J. A. 1967. Dual effect of auxin: inhibition of uptake and stimulation of RNA and protein synthesis: assessment of synthesis. Z. Pflanzenphysiol. 56: 410–26.
24. SACHER, J. A. 1967. Control of synthesis of RNA and protein in subcellular fractions of *Rhoeo discolor* leaf sections by auxin and kinetin during senescence. Expt. Geront. 2: 261–78.
25. SACHER, J. A. 1967. Studies of permeability, RNA and protein turnover during ageing of fruit and leaf tissues. In: Aspects of the Biology of Ageing. H. W. Woolhouse, ed. 21st Symp. Brit. Soc. Exptl. Biol. Cambridge Univ. Press. p 269–303.
26. VON ABRAMS, G. J. AND H. K. PRATT. 1967. Effect of ethylene on the permeability of excised canteloupe fruit tissue. Plant Physiol. 42: 299–301.
27. WONG, B. L. 1967. The relationship between permeability and characteristics, active uptake, incorporation of amino acids and the amount of protein and RNA in senescing avocado fruit tissue. Master's Thesis, California State College, Los Angeles, California.
28. YOUNG, R. E. 1968. Regulation of respiration associated with slicing of plant tissue. In: Biochemical Regulation in Diseased Plants or Injury. Proc. Intern. Symp. Phytopath. Soc. of Japan, 1967. Tokuzo Hirai, ed. c/o Nat. Inst. Agr. Sci. Tokyo. p 213–21.
29. YOUNG, R. E. AND J. B. BIALE. 1967. Phosphorylation in avocado fruit slices in relation to the respiratory climacteric. Plant Physiol. 42: 1357–62.