Plant Physiol. (1975) 55, 663-669

Ethylene-enhanced Ion and Sucrose Efflux in Morning Glory Flower Tissue[1]

Received for publication October 8, 1974 and in revised form November 25, 1974

ANDREW D. HANSON AND HANS KENDE
Michigan State University-Atomic Energy Commission Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824

ABSTRACT

Rib tissue segments excised from open flowers or buds of *Ipomoea tricolor* Cav. and floated on aqueous media responded to ethylene treatment by rolling up after 2 to 3 hours; a simple method for quantitating the rolling up is presented. The rolling up response was temperature- and oxygen-dependent and was critically affected by the pH of the medium. The ethylene concentration giving a half-maximal response was 0.1 μl/l; continuous ethylene treatment was not required for the response as a 1-hour ethylene exposure enhanced rolling up.

Rib segments rolling up during ethylene treatment unrolled when transferred to 0.5 M sucrose, indicating that rolling up was due to asymmetric turgor changes in the segments. Compartmental analysis of $^{36}Cl^-$ efflux from rib segments showed a fast and a slow phase; the slow phase, with a half-time of about 6 hours, is tentatively identified as efflux from the vacuolar compartment. During ethylene treatment, the rate of $^{36}Cl^-$ efflux in the slow phase rose markedly as the rolling up response developed. A similar result was obtained with the efflux of $^{86}Rb^+$. The release of ^{14}C-metabolites, labeled either by a period of $^{14}CO_2$ fixation in darkness or by exposure to ^{14}C-(U)-glucose, also increased during ethylene-induced rolling up.

These results suggest that ethylene causes an increase in membrane permeability in certain cells of the rib tissue.

Flowers of the morning glory *Ipomoea tricolor* Cav. are ephemeral, opening early in the morning and fading in the afternoon of the same day. The first signs of senescence appear about 8 hr after flower opening with rolling up of the corolla, which is borne inwards by curling of the ribs. The rolling up is accompanied by a change of corolla color from blue to purple and a rapid rise in the activities of several hydrolases (12). As hydrolase activities increase, autophagic digestion of organelles in the vacuoles is apparent in electron micrographs; in the final stages of flower withering the tonoplast disintegrates and the cell autolyzes completely (12).

Kende and Baumgartner (8) have shown that aging in *Ipomoea tricolor* is regulated by ethylene. In untreated flowers, rolling up of the corolla coincides with a sharp increase in the rate of endogenous ethylene production. A 20- to 40-min pretreatment of flowers with ethylene induces premature rolling up of the corolla and provokes a rise in ribonuclease activity. Ethylene pretreatment also induces a high rate of endogenous ethylene synthesis that parallels the premature rolling up of the corolla. A model based on compartmentation changes has been proposed to account for this ethylene-induced ethylene synthesis; ethylene treatment could increase the permeability of the tonoplast, favoring flow of vacuolar substrate for ethylene production to an ethylene-generating system located in the cytoplasm.

This paper reports the effects of ethylene on rolling up of rib segments excised from morning glory flowers and buds and on efflux rates of $^{36}Cl^-$, $^{86}Rb^+$, and ^{14}C-metabolites from such tissue. The compartmental analysis procedure of MacRobbie and Dainty (11) and Pitman (14) was used to interpret the ion efflux data.

MATERIALS AND METHODS

Plant Material. Seeds of *Ipomoea tricolor* Cav. were obtained from three sources; one seed lot (cv. *rubro-coerulea praecox*) was purchased from Samen Mauser, Zurich, Switzerland. Two other lots (cv. Heavenly Blue) were from the Fredonia Seed Company (Fredonia, N. Y.) and Agway Inc. (Syracuse, N. Y.), respectively. No differences in flower morphology or physiology were observed among plants grown from these three lots. Seeds were sown singly in quart pots in a mixture of three volumes of Baccto Potting Soil (Michigan Peat, Houston, Texas) and one volume each of gravel, fine sand, sterile loam, and vermiculite. Pots were watered twice a week with half-strength Hoagland's solution, and with water alone on the other 5 days. After 6 weeks' growth, plants were given 1 week of 8-hr days and transferred to a growth chamber with a 16-hr photoperiod. The short day treatment was found to hasten flowering. Growth chamber day temperature was 23 C, night temperature 18 C, relative humidity 60 to 70%; days were from 5:00 AM to 9:00 PM with a light intensity of 4 to 6 $\times 10^4$ erg cm^{-2} sec^{-1} at the level from which flowers were harvested. Vigorous flowering continued for at least 10 weeks in these conditions.

Flowers were routinely harvested at 8:30 to 9:00 AM and used for preparation of rib segments; one segment (10 $\times$ 4 mm) was excised from each of the five ribs, starting 3 to 5 mm from the outer edge of the corolla. A narrow (<1 mm) band of blue flower tissue was present on both long edges of the segments. Flower buds were taken at 7:00 to 8:00 AM on the day before flower opening, the corolla unrolled gently, and the rib segments then excised as described for open flowers. Fresh weights for single rib segments were about 5 mg for flowers, 7 mg for buds.

[1] This research was supported by the United States Atomic Energy Commission under Contract AT(11-1)-1338.

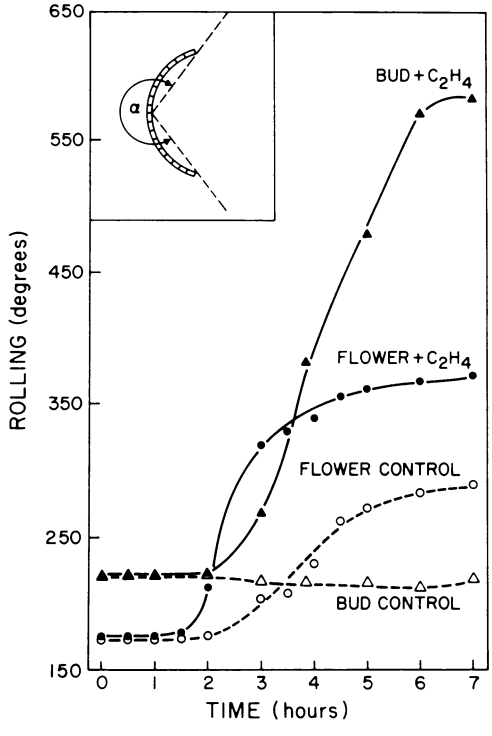

FIG. 1. Effect of ethylene on rolling up of rib segments isolated from open flowers and buds. Segments were incubated under a gas phase containing air only (control) or air to which ethylene was added to give a concentration of 10 μl/l at zero time. Inset shows the angle α used to quantify rolling up; the cross-hatched area represents an edge-on view of the side (10 mm) of a segment.

Incubation of Rib Segments. In most experiments, batches of 10 rib segments were floated on 5 ml of unbuffered (5 mM) KCl solution in 50-ml Erlenmeyer flasks closed with serum vial caps; when required, ethylene was injected through the caps with a gas-tight syringe. Modifications to this standard procedure are detailed in the text. Flasks were shaken in darkness at 27 C on a reciprocating shaker (80 cycles/min) and observed briefly at 30- to 60-min intervals to assess the extent of rolling up.

Measurement of Rib Segment Rolling Up. Angular measurements (α, Fig. 1 inset) were taken throughout the rolling up process by aligning the sides of the segments in a flask with a protractor. Immediately after excision, segments from flowers were straight ($\alpha = 180°$) or slightly curved towards the abaxial (white) surface of the rib ($\alpha = 170°$). Rolling up occurred towards the adaxial (blue) vein surface, often reaching a maximum of $\alpha > 360°$ with the segment ends overlapping. Segments from buds normally showed a slight curvature toward the adaxial surface after cutting ($\alpha = 220$–$230°$) and rolled up tightly in a spiral fashion reaching a maximum of close to two complete turns ($\alpha = 720°$).

Ion Efflux Measurements. Loading of rib segments from buds and from open flowers required the use of two different techniques. Batches of 20 rib segments prepared from buds were loaded in 50-ml flasks by floating on 5 ml of KCl solution (7–10 mM, pH 6) containing tracer amounts of $^{36}Cl^-$ or $^{86}Rb^+$. The flasks were shaken in darkness for 2.5 hr as described above. The specific radioactivities of the loading solutions were 0.15 μCi/μeq for $^{36}Cl^-$, 0.2 μCi/μeq K^+ for $^{86}Rb^+$.

Rib segments cut from open flowers could not be loaded by this method as they started to roll up about 3 hr after cutting (Fig. 1). To load such segments, it was necessary to feed excised buds for 20 to 24 hr through the cut pedicel ends. Buds were excised early in the morning on the day before opening by cutting the pedicel obliquely under water. The buds were incubated in the growth chamber with the cut pedicels in 0.25 ml $K^{36}Cl$ solution (70–100 mM, pH 6) of 0.15 μCi/μeq specific radioactivity. By 9:00 AM the following day most of the labeling solution had been absorbed and the buds had at least partially opened. Rib segments were then prepared from the radioactive corollas.

Batches of 10 washed or unwashed rib segments were transferred to 5 ml of 5 mM KCl solution in 50-ml flasks which were then stoppered with serum vial caps. Ethylene was injected into certain flasks to give a final concentration of 10 μl/l, and the flasks incubated as described above. Samples (0.1 ml) of the incubation solution were withdrawn at intervals through the caps with a microsyringe. Radioactivity remaining in the tissue at the end of the experiment was extracted by freezing, thawing, and boiling the rib segments in the remaining incubation solution. Radioactivity in the incubation solution and final extract samples was determined with a Packard liquid scintillation spectrometer Model 3375 operating at about 90% efficiency for ^{36}Cl and 55% for ^{86}Rb. All ion efflux experiments were repeated at least three times with similar results; figures in the text are results from single experiments.

Treatment of Efflux Data. A simple form of the procedure first applied to higher plant tissues by Pitman (14) was used in analysis of efflux data. In this method, the logarithm of the amount of radioactive tracer ion remaining in the tissue is plotted against time; a final, linear portion of the graph is taken to represent loss of tracer ion from the slowest exchanging compartment, equated with the vacuole. When such a straight line is extrapolated to zero time, the intercept represents the amount of isotope initially present in that compartment. From the slope of the line the first-order rate constant k can be calculated ($-k$ = slope $\times$ 2.303); k is proportional to the permeability coefficient for the ion in the particular membrane bounding the compartment and to the surface area A of this membrane; it is inversely proportional to the volume V of the compartment, and to the total chemical concentration of the ion within the compartment.

The ratio A/V for the vacuolar compartment remained fairly constant during the early stage of flower fading, as shown in the electron micrographs of Matile and Winkenbach (12). Determinations of total tissue concentrations of K^+ and Cl^- were made using Orion Research electrodes; in the highly vacuolate flower tissue, these concentrations approximate vacuolar concentrations. In both flower and bud rib segments, K^+ remained at 40 to 50 μeq/g fresh tissue throughout loading and efflux. Initial and final concentrations of Cl^- for flower and bud rib segments were 5 to 6 μeq/g, although apparent increases were observed during the loading period. These increases were ascribed to the relatively higher (up to 20-fold) Cl^- concentrations of the loading solutions remaining in intercellular spaces.

^{14}C-Efflux Measurements. Batches of 20 bud rib segments were ^{14}C-labeled either through $^{14}CO_2$ fixation in darkness or with ^{14}C-glucose. For $^{14}CO_2$ fixation, 20 rib segments were floated on 5 mM KCl and exposed to 0.5% $^{14}CO_2$ (57 μCi/μmole) in air for 2 hr in darkness. For ^{14}C-glucose uptake, 20 segments were floated for 2.5 hr in darkness on 2 ml ^{14}C-(U)-glucose solution (5 mM, 1.5 μCi/μmole) in 5 mM KCl.

After $^{14}CO_2$ or ^{14}C-glucose treatment, the rib segments were washed twice for 10 min in 5 mM KCl or 5 mM KCl with 5 mM glucose, respectively; batches of 10 segments were transferred to 20-ml Warburg vessels and floated on 4 ml of KCl (5 mM) or 5 mM KCl with 5 mM glucose. The center wells contained 0.3 ml 10% (w/v) KOH solution. After closing with serum vial caps, ethylene was injected into certain flasks to give a concen-

tration of 10 μl/l, and the vessels were incubated as described above. At intervals, 0.1-ml medium samples and 0.02-ml KOH samples were withdrawn. The medium samples were acidified, evaporated to dryness, and redissolved in water before scintillation counting. Samples of KOH were transferred to 2-cm disks of Whatman No. 1 filter paper wetted with 0.02 ml of saturated $Ba(OH)_2$ solution; the disks were air-dried, and trapped $^{14}CO_2$ was determined by scintillation counting. At the end of the experiments, the rib segments were extracted by boiling in 80% (v/v) ethanol; ^{14}C in the extract was determined by scintillation counting. Radioactivity remaining in the ethanol-insoluble residue was measured following combustion in a Packard Tricarb sample oxidizer. Ethanolic extracts and labeled material in the medium were analyzed further by the Dowex column technique of Canvin and Beevers (4) and by paper chromatography. The identity of sucrose was checked by inversion. The ^{14}C-metabolite efflux data in the text are results from single experiments which were repeated twice (^{14}C-glucose) and three times ($^{14}CO_2$), respectively, with similar results.

RESULTS

Effect of Ethylene on Rolling Up of Rib Segments. Rib segments isolated from flowers began to roll up spontaneously but slowly after about 3 hr. When ethylene was applied to such segments rolling up occurred rapidly after a lag of 1.5 to 2 hr, finally reaching a higher value than rolling up in untreated segments (Fig. 1). Both the spontaneous rolling up and the fast, premature rolling up induced by ethylene closely paralleled the behavior of the rib tissue during the fading of the intact flower (8).

Rib segments taken from buds showed no tendency to roll up spontaneously, although ethylene application resulted in extensive rolling up after a lag of 2 to 2.5 hr (Fig. 1). Thus, the sensitivity to ethylene already existed at least 1 day before corolla opening, again reflecting the situation in the intact bud (8).

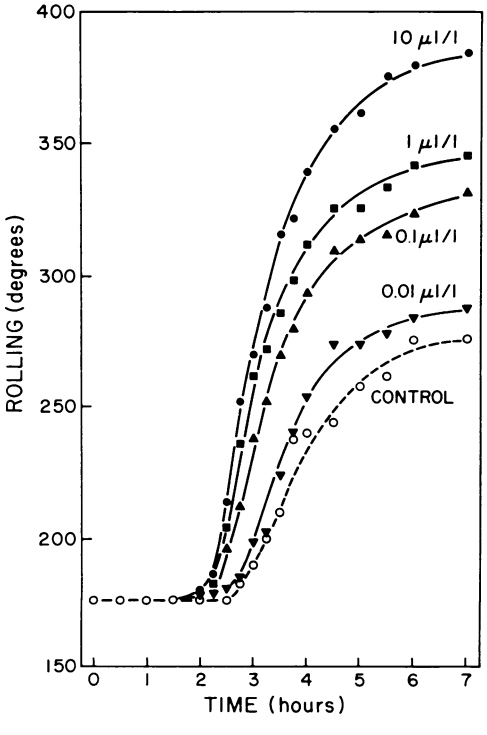

FIG. 2. Effect of various ethylene concentrations on rolling up of flower rib segments. The gas phase was air only (control) or air to which ethylene was added at zero time to give concentrations of 0.01, 0.1, 1 and 10 μl/l.

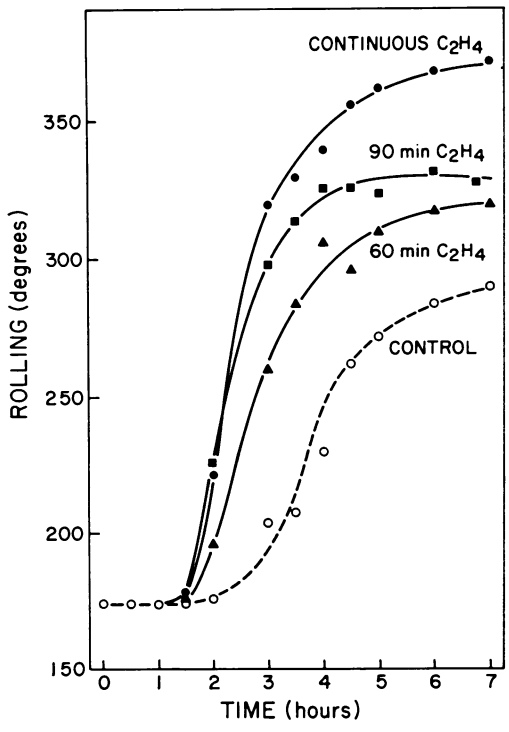

FIG. 3. Effect of duration of ethylene exposure on rolling up of flower rib segments. One batch of segments (control) was incubated under air only; ethylene was added to give a concentration of 10 μl/l at zero time to three further batches. After 60 and 90 min, one batch of segments was transferred from ethylene to fresh medium and incubation continued in air only.

Maximal rolling up with flower rib segments was obtained at 10 μl/l of ethylene; concentrations of 30 and 100 μl/l neither reduced the length of the lag period nor altered the maximal extent of rolling up. Results with a range of lower ethylene concentrations (Fig. 2) indicated that half-maximal response occurred at 0.1 μl/l.

Continuous presence of ethylene was not required for the enhancement of rolling up (Fig. 3). When the segments were removed from ethylene after 1 or 1.5 hr, before any response was apparent, the onset of rolling up was advanced and the maximum extent increased, although the maximum response observed with short treatments was less than with continuous treatment.

Factors Affecting the Rolling Up Response. An optimal rolling up response was obtained with rib segments floated on distilled H_2O or unbuffered 5 mM KCl solution. Addition of $CaCl_2$ in concentrations as low as 0.1 mM strongly inhibited both spontaneous and ethylene-induced rolling up; at 5 to 10 mM, $CaCl_2$ inhibited rolling up almost completely.

The pH of the incubation medium also modified the rolling up response. Representative results for spontaneous rolling up are shown in Figure 4; results for ethylene-induced rolling up were quite similar (not shown). When the medium was buffered at pH 7 both spontaneous and ethylene-induced rolling up were reduced while at pH values of 6 or below both were enhanced. As unbuffered 5 mM KCl generally gave the best separation of spontaneous and ethylene-induced rolling up, it was adopted as a standard incubation medium; the pH of this medium rose 0.1 to 0.4 pH units from an initial value of 6.1 to 6.3 during typical experiments.

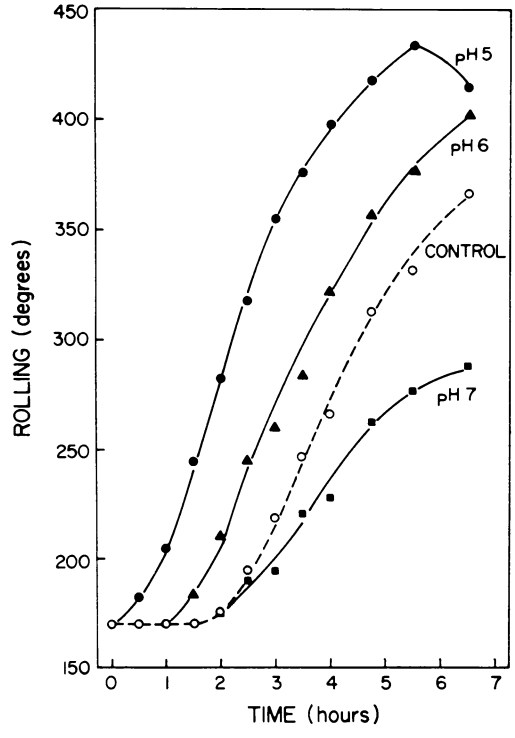

FIG. 4. Effect of pH of incubation medium on rolling up of flower rib segments. Segments were incubated under a gas phase of air only in unbuffered 5 mM KCl (control) or in 5 mM KCl containing citrate (1 mM)-phosphate (2 mM) buffers of initial pH 5, 6, and 7.

Both spontaneous and ethylene-induced rolling up required O_2; incubation of flower rib segments under N_2 or N_2 containing 10 μl/l ethylene gave no rolling up after 5 hr. Lowering the temperature from 27 C to 17 C retarded the onset of spontaneous and ethylene-induced rolling up by 3 to 4 hr; at 7 C very little rolling up had occurred after 24 hr.

Effect of Plasmolysis on Rolling Up. Plant movements are often mediated by changes in tissue turgor; bending movements may be due to either uniform turgor loss within a tissue, allowing expression of differences in cell wall elasticity, or to asymmetric turgor changes. Imposition of a general turgor loss on the whole tissue allows a choice between these possibilities. Such a treatment should cause a rapid rolling up with a mechanism involving an over-all turgor loss. On the other hand, such a turgor loss should prevent a response dependent on asymmetric turgor changes when imposed before response development, and should reverse it when applied during or after response development. Figure 5 shows the effect of transferring rib segments at various stages of rolling up to a plasmolyzing concentration of sucrose. When the transfer was made at the time of ethylene addition the segments became flaccid, curled slightly towards the abaxial surface, and began to roll up in the usual direction very slowly as deplasmolysis occurred. Transfer to sucrose after partial or almost complete rolling up caused immediate unrolling of the segments.

Effect of Ethylene on $^{36}Cl^-$ and $^{86}Rb^+$ Efflux during Rolling Up. A complete efflux curve for $^{36}Cl^-$, obtained with bud rib segments, is presented in Figure 6. After an initial rapid loss of $^{36}Cl^-$, efflux reached a constant rate at about 20 min which was then maintained for 7 hr. The slow component had a half-life of about 6 hr, and extrapolation of the slope of the slow phase to zero time showed that it accounted for some 80% of the $^{36}Cl^-$ absorbed. As the following experiments concerned only

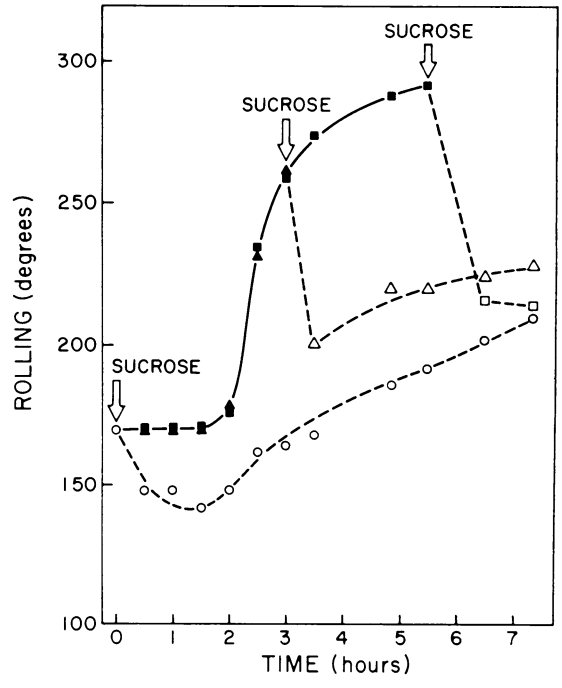

FIG. 5. Effect of plasmolysis on rolling up of flower rib segments. Ethylene was added to give a concentration of 10 μl/l to the gas phase of all treatments at zero time. ○---○: Segments incubated in 5 mM KCl containing 0.5 M sucrose from zero time; ▲——▲, ■——■: segments incubated initially in 5 mM KCl only; after 3 hr (△---△) or 5.5 hr (□---□) the KCl medium was withdrawn and replaced by 0.5 M sucrose in 5 mM KCl, the gas phase remaining unchanged.

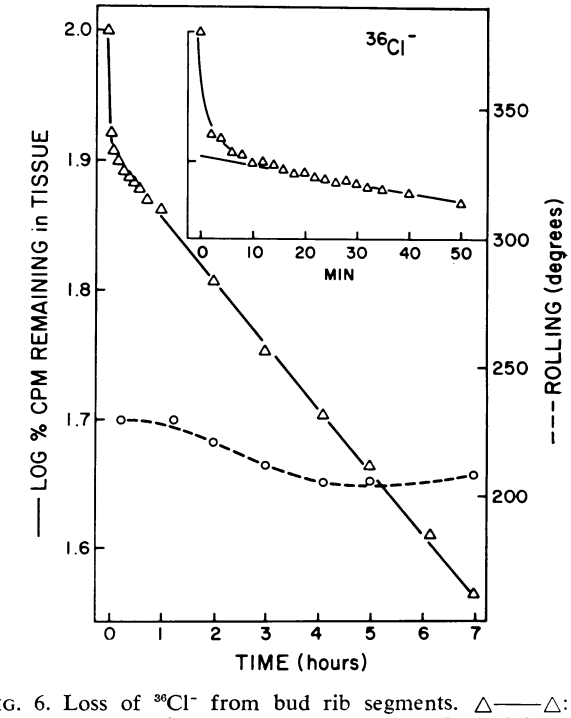

FIG. 6. Loss of $^{36}Cl^-$ from bud rib segments. △——△: $^{36}Cl^-$ efflux; ○---○: rolling up of segments. Inset shows initial efflux of $^{36}Cl^-$ plotted with an expanded time scale; extrapolation of the slope shown to zero time gives an intercept at 1.904. Total $^{36}Cl^-$ absorbed during loading period = 5.5×10^4 cpm per 10 segments.

the slow phase of $^{36}Cl^-$ efflux, segments were washed twice for 10 min in 10 ml of unlabeled 5 mM KCl in order to wash out the rapid phases.

Figure 7 shows the slow $^{36}Cl^-$ efflux component observed in both flower and bud rib segments as they rolled up. In flower rib segments (Fig. 7A), $^{36}Cl^-$ efflux increased sharply after 2 hr of ethylene treatment as premature rolling up was developing. In untreated segments, an increase in efflux rate occurred later, as spontaneous rolling up took place.

In contrast, with bud rib segments (Fig. 7B) in which there was no spontaneous rolling up, efflux from untreated segments was maintained at a fairly constant rate, as in Figure 6. After 2 hr of ethylene treatment, however, the efflux rate increased as the rolling up proceeded.

The efflux kinetics for $^{86}Rb^+$ from bud rib segments are shown in Figure 8; the graph is a smooth curve from which distinct fast and slow efflux components are absent. This deviation from first-order kinetic behavior may be explained by the structure of the rib segments in which most efflux probably occurs through the cut ends of the veins rather than directly through the waxy cuticle. After release from cells and before final loss to the medium, ions would have to travel up to several mm by diffusion through the surface film of water on cell walls since the air spaces do not become waterlogged during an experiment. As cell wall macromolecules carry many negative charges, a continual exchange of $^{86}Rb^+$ with unlabeled cations could occur along this diffusion path, retarding the appearance of radioactivity in the medium. Experimental support for this explanation was obtained from the efflux curves of rib segments only 2 mm in length. With these short segments, in which rolling up cannot be studied, initial $^{86}Rb^+$ loss occurred far more rapidly, and the efflux rate approached first-order kinetics after 4 hr.

The effect of ethylene on $^{86}Rb^+$ efflux and rolling up in normal 10-mm bud rib segments is seen in Figure 9; these segments

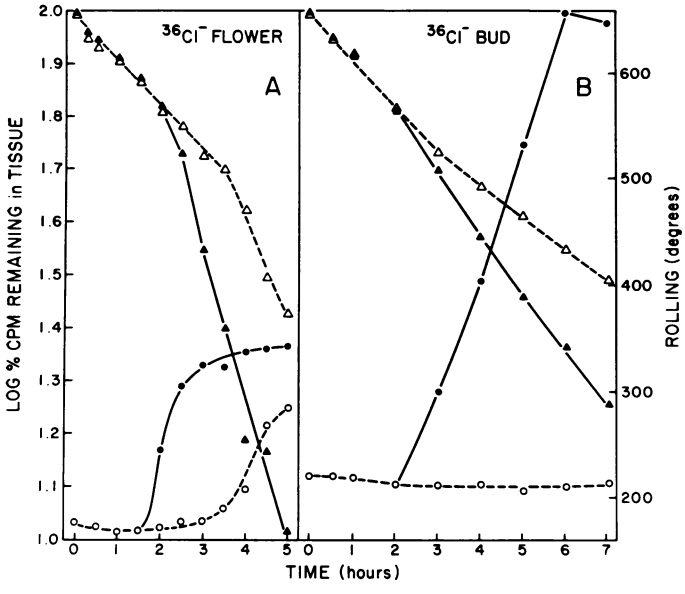

FIG. 7. Effect of ethylene on rolling up and loss of $^{36}Cl^-$ from rib segments of flowers (A) and buds (B). Segments were incubated under a gas phase of air only (control) or of 10 μl/l ethylene added at zero time. ○---○: Rolling up of control segments; ●——●: rolling up of ethylene-treated segments; △---△: $^{36}Cl^-$ efflux from control segments; ▲——▲: $^{36}Cl^-$ efflux from ethylene-treated segments. $^{36}Cl^-$ present in tissue at start of efflux period = 1.1×10^4 cpm (flowers), 3.5×10^4 cpm (buds) per 10 segments.

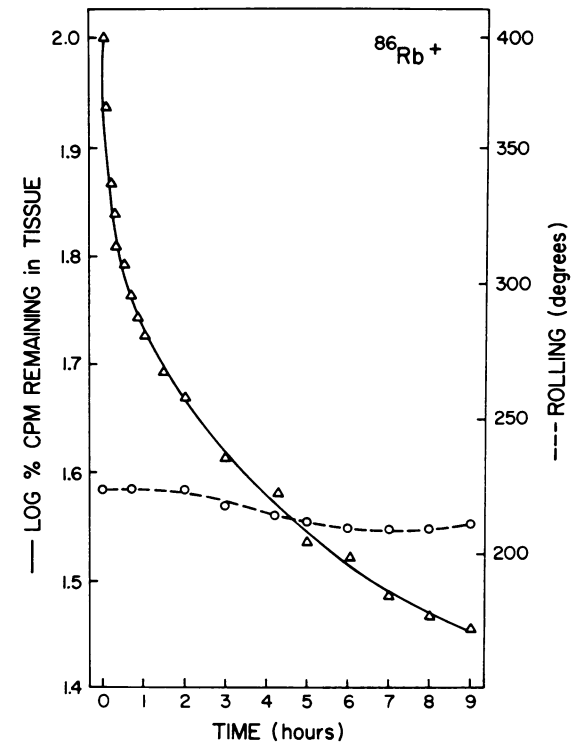

FIG. 8. Loss of $^{86}Rb^+$ from bud rib segments. △——△: $^{86}Rb^+$ efflux; ○---○: rolling up of segments. Total $^{86}Rb^+$ absorbed during loading period = 2.6×10^5 cpm per 10 segments.

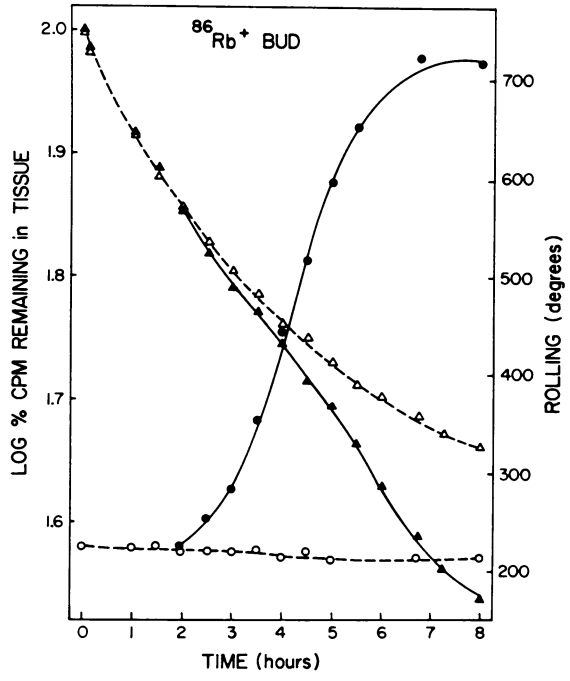

FIG. 9. Effect of ethylene on rolling up and loss of $^{86}Rb^+$ from rib segments of buds. Segments were incubated under a gas phase of air only (control) or of 10 μl/l ethylene added at zero time. ○---○: Rolling up of control segments; ●——●: rolling up of ethylene-treated segments; △---△: $^{86}Rb^+$ efflux from control segments; ▲——▲: $^{86}Rb^+$ efflux from ethylene-treated segments. $^{86}Rb^+$ present in tissue at start of efflux period = 2.2×10^5 cpm per 10 segments.

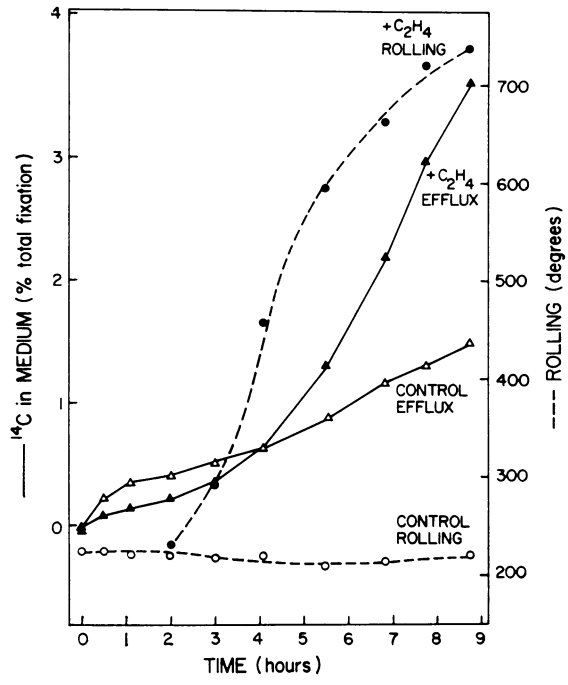

FIG. 10. Effect of ethylene on rolling up and the efflux of soluble products of dark $^{14}CO_2$-fixation. Bud rib segments which had been exposed to 0.5% $^{14}CO_2$ in air were incubated under a gas phase of air only (control) or 10 μl/l ethylene added at zero time. Efflux of soluble ^{14}C-metabolites is expressed as a percentage of the total ^{14}C originally absorbed. ○- - -○: Rolling up of control segments; ●- - -●: rolling up of ethylene-treated segments; △——△: efflux of soluble ^{14}C from control segments; ▲——▲: efflux of soluble ^{14}C from ethylene-treated segments. Total ^{14}C fixed during $^{14}CO_2$ exposure = 8.6×10^6 dpm per 10 segments.

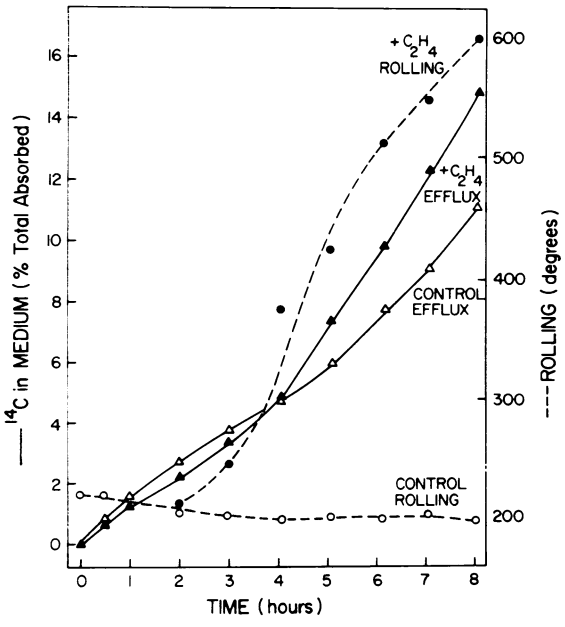

FIG. 11. Effect of ethylene on rolling up and the efflux of soluble products of ^{14}C-(U)-glucose metabolism. Bud rib segments were preincubated in 5 mM ^{14}C-(U)-glucose in air; the efflux medium contained 5 mM glucose. Otherwise as in Fig. 10. Total ^{14}C absorbed during preincubation in ^{14}C-(U)-glucose = 5.5×10^5 dpm per 10 segments.

had been washed briefly in KCl following the loading period as described for $^{36}Cl^-$ experiments. In untreated segments, no rolling up took place and the efflux rate declined steadily with time. In ethylene-treated segments, the initial rate of efflux was maintained for about 5 hr and then increased slightly; efflux curves for control and ethylene-treated segments began to diverge clearly as rolling up developed.

Effect of Ethylene on Efflux of ^{14}C-labeled Metabolites. When bud rib segments were exposed to $^{14}CO_2$ in darkness, ^{14}C was incorporated mainly into anionic products (presumably organic acids) with a lesser amount of label in cationic products (presumably amino acids). Figure 10 shows the effect of ethylene treatment on the appearance of these labeled metabolites in the incubation medium. Untreated segments released ^{14}C-metabolites to the medium at a rate which increased only slightly with time, whereas in ethylene-treated segments the release rate increased sharply after 3 hr as rolling up of these segments took place. Although only 3.6% of the total ^{14}C fixed by the tissue was released after 9 hr of ethylene treatment, this represented a large fraction of the total soluble material remaining in the tissue at this time, as 50 to 60% of the total ^{14}C had been lost as $^{14}CO_2$ and 11 to 12% incorporated into products insoluble in 80% ethanol.

After incubation of bud rib segments in ^{14}C-(U)-glucose, label in the tissue was mainly in glucose, fructose, sucrose, and ethanol-insoluble material. Release of soluble ^{14}C-metabolites from bud rib segments prelabeled with ^{14}C-(U)-glucose again increased as the segments rolled up in response to ethylene (Fig. 11). The only ^{14}C-labeled product released from both ethylene-treated and control tissues was sucrose; glucose and fructose were not released, although they accounted for about half the soluble radioactivity remaining in the segments at the end of the experiment.

DISCUSSION

In intact flowers, rolling up of the corolla is driven by curling of the ribs; since the rolling up of excised rib segments closely resembles that of the flower and bud ribs from which they have been isolated, rib segments can serve as a convenient model to study some aspects of flower aging.

Ethylene induces rolling up of the rib segments and, simultaneously, enhances efflux of solutes from the rib cells. Both of these processes may be the consequence of increased permeability of either the tonoplast or the plasmalemma or of both membranes. From our results, we cannot determine unequivocally which of these two membranes is affected. However, different lines of evidence discussed below suggest that permeability changes may occur first in the tonoplast.

The reduced rolling up of rib segments held under plasmolyzing conditions and the rapid reversal of rolling up by plasmolysis indicate that differential turgor changes in the tissue bring about curling up of the ribs. Underlying the adaxial rib surface are large, irregularly shaped cells with many intercellular air spaces, while the cells under the abaxial surface are small, regular and tightly packed (15). We suggest that in spontaneous and ethylene-induced rolling up a turgor differential arises between these cell types; as turgor is lost in the large cells, the adaxial rib surface contracts causing the rib to roll up. Since the tonoplast is probably the principal membrane determining the osmotic properties of plant cells (2), the results obtained with plasmolyzed rib segments suggest a loss of the semipermeable properties of the tonoplast during spontaneous and ethylene-induced rolling up.

The kinetics of $^{36}Cl^-$ loss from rib segments also points to an effect of ethylene on tonoplast permeability. In a number of

plant tissues, three components of ion efflux have been observed (10). The fastest component is extracellular in origin; two slower, linear components with half-times of 10 to 80 min and several hours are equated with the cytoplasm and the vacuole, respectively. In our experiments, the slowest component that could be seen in efflux curves for $^{36}Cl^-$ had a half-time of about 6 hr and accounted for about 80% of the label in the rib segments. We tentatively identify this component as efflux from the vacuole and assume that any changes in its rate under our experimental conditions reflect mainly changes of the permeability coefficient of Cl^- in the tonoplast. Ethylene treatment clearly resulted in an increase of the efflux rate of Cl^- as rolling up occurred, and the slow spontaneous rolling up of flower segments was associated with a similarly enhanced release of Cl^-.

Although a definite linear slow component was not found for $^{86}Rb^+$ efflux from 10-mm rib segments, $^{86}Rb^+$ loss was enhanced during ethylene-induced rolling up. This result can again be attributed to an increase in the permeability coefficient of the ion.

The accelerated release of ^{14}C-sucrose and ionic assimilates during ethylene treatment is also consistent with an effect of ethylene on the tonoplast. Sucrose is thought to be stored in a vacuolar compartment in a number of plants (5, 6); ionic products of $^{14}CO_2$ dark fixation may also be held mainly in a cellular storage compartment (9). A similar increased efflux of ^{14}C-label originating from acetate or sucrose has been reported to occur during the turgor-driven coiling movements of pea tendrils (7).

Although increases in the efflux rates for individual ions could be explained by changes in electrical potentials rather than permeability coefficients, the observed increase in the efflux of cationic, anionic, and uncharged species is most simply accounted for by a general nonspecific increase in tonoplast permeability.

The ethylene-induced increase in efflux rate for all species studied ($^{36}Cl^-$, $^{86}Rb^+$, and ^{14}C-metabolites) was detected as the rib segments began to roll up. Although a rise in efflux rate prior to visible rolling up might be expected, it was never observed; two features of our experimental system could have obscured any such early rise. As only a fraction of the cells of the tissue appear to respond to ethylene, changes in the efflux rates of these cells were observed against a "background" of efflux from the remainder of the tissue; only after loss from ethylene-sensitive cells had risen substantially would such an increase have been detectable. As discussed for $^{86}Rb^+$ efflux, final loss of label from the rib segments probably involves diffusion from the site of cellular release to the cut ends of the segments; such a diffusion path could obscure small changes in efflux from the cell themselves.

The effect of ethylene on membrane permeability is mediated by metabolic changes, since at least 1.5 hr elapsed between ethylene application and the visible response, and since ethylene is unable to cause rolling up in the absence of O_2 or at low temperatures.

The view that ethylene increases the permeability of the tonoplast is consistent with observations by Matile and Winkenbach (12) on ultrastructural changes in aging flower tissue of the morning glory. During senescence of the flower, the vacuole first becomes highly irregular in shape: this change in form of the tonoplast is followed by its complete distintegration which occurs while the plasmalemma is still intact (12). Further evidence for increased tonoplast permeability during flower aging comes from work by Nichols (13) who reported the loss of vacuolar pigments from senescing carnation petals. Kende (unpublished data) observed a similar release of anthocyanins from petals of *Tradescantia* sp. senescing spontaneously or in response to ethylene treatment.

Our data provide some experimental support for the hypothesis put forward by Kende and Baumgartner (8) to explain ethylene-induced ethylene synthesis in morning glory flowers. An ethylene-induced increase in tonoplast permeability could well allow mixing of the components of an ethylene-generating system.

Ethylene has frequently been invoked as a permeability-increasing agent in fruit ripening although, as Abeles (1) and Burg (3) have pointed out, much of the evidence is equivocal and the arguments regarding cause (increased permeability) and effect (ripening) circular. In any event, experiments with ripening fruit extend over many days, and the tissues undergo only relatively slow metabolic changes. In contrast, morning glory flowers show rapid senescence that can clearly be triggered by ethylene at any time during the 24 hr preceding normal fading. The characteristics of the release of radioactive ions and metabolites from this tissue and the observed turgor changes suggest that an early result of ethylene action is an increase in the permeability of the tonoplast.

Acknowledgments—We wish to thank Dr. Yoash Vaadia for many valuable discussions, and Mrs. Renate deZacks for technical assistance.

LITERATURE CITED

1. Abeles, F. B. 1972. Biosynthesis and mechanism of action of ethylene. Annu. Rev. Plant Physiol. 23: 259–292.
2. Bennet-Clark, T. A. 1959. Water relations of cells. *In:* F. C. Steward, ed., Plant Physiology—A Treatise, Vol. II. Academic Press. New York. pp. 105–191.
3. Burg, S. P. 1968. Ethylene, plant senescence and abscission. Plant Physiol. 43: 1503–1511.
4. Canvin, D. T. and H. Beevers. 1961. Sucrose synthesis from acetate in the germinating castor bean: kinetics and pathway. J. Biol. Chem. 236: 988–995.
5. Hardy, P. J. and G. Norton. 1968. Sugar absorption and compartmentation in potato tuber slices. New Phytol. 67: 139–143.
6. Hawker, J. S. and M. D. Hatch. 1965. Mechanism of sugar storage by mature stem tissue of sugarcane. Physiol. Plant. 18: 444–453.
7. Jaffe, M. J. and A. W. Galston. 1968. Physiological studies on pea tendrils. V. Membrane changes and water movement associated with contact coiling. Plant Physiol. 43: 537–542.
8. Kende, H. and B. Baumgartner. 1974. Regulation of aging in flowers of *Ipomoea tricolor* by ethylene. Planta 116: 279–289.
9. Lips, S. H. and H. Beevers. 1966. Compartmentation of organic acids in corn root. I. Differential labeling of 2 malate pools. Plant Physiol. 41: 709–712.
10. MacRobbie, E. A. C. 1971. Fluxes and compartmentation in plant cells. Annu. Rev. Plant Physiol. 22: 75–96.
11. MacRobbie, E. A. C. and J. Dainty. 1958. Sodium and potassium distribution and transport in the seaweed *Rhodymenia palmata* (L.) Grev. Physiol. Plant. 11: 782–801.
12. Matile, P. and F. Winkenbach. 1971. Function of lysosomes and lysosomal enzymes in the senescing corolla of the morning glory (*Ipomoea purpurea*). J. Exp. Bot. 22: 759–771.
13. Nichols, R. 1968. The response of carnations (*Dianthus caryophyllus*) to ethylene. J. Hort. Sci. 43: 335–349.
14. Pitman, M. G. 1963. The determination of the salt relations of the cytoplasmic phase in cells of beetroot tissue. Aust. J. Biol. Sci. 16: 647–668.
15. Winkenbach, F. 1970. Zum Stoffwechsel der aufblühenden und welkenden Korolle der Prunkwinde *Ipomoea purpurea*. I. Beziehungen zwischen Gestaltwandel, Stofftransport, Atmung und Invertaseaktivität. Ber. Schweiz. Bot. Gesellsch. 80: 374–390.