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Effect of Ethylene on the Uptake, Distribution, and Metabolism of Indoleacetic Acid-1-^{14}C and -2-^{14}C and Naphthaleneacetic Acid-1-^{14}C[1]

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ABSTRACT

The effect of ethylene on the uptake, distribution, and metabolism of indoleacetic acid (IAA)-1-^{14}C, IAA-2-^{14}C, and naphthaleneacetic acid (NAA)-1-^{14}C in cotton stem sections (*Gossypium hirsutum* L., var. Stoneville 213) was studied. Stem sections excised from plants pretreated with ethylene for 15 hours transported significantly less ^{14}C-IAA and ^{14}C-NAA than control sections. Concomitant features of the reduction of ^{14}C-IAA transport were an increase in decarboxylation and a trend toward a reduction in total uptake. With ^{14}C-NAA, however, total uptake was significantly increased, and decarboxylation was unaffected.

^{14}C-IAA was rapidly converted to indoleacetylaspartic acid and many other metabolites in both control and ethylene-pretreated stem sections. Following transport, similar amounts of ^{14}C-IAA were recovered in the apical absorbing portion of the control and ethylene-pretreated sections. Significantly more ^{14}C-IAA metabolites, however, were recovered in this region of the ethylene-pretreated sections.

Conversely, ^{14}C-NAA was metabolized more slowly than ^{14}C-IAA under identical experimental conditions, with the only major metabolite being naphthaleneacetylaspartic acid. Following transport the apical absorbing portion of ethylene-pretreated stem sections contained significantly more ^{14}C-NAA than the controls. These results suggested that the disruption of auxin transport by ethylene cannot be explained in terms of a more rapid metabolism of auxin in the treated sections. The increased ^{14}C-IAA metabolites in the absorbing portion of ethylene-pretreated sections appear to be the result, rather than the cause, of the ethylene-mediated disruption of IAA transport.

The inhibition of auxin transport by ethylene is well documented (5–7, 10, 14, 15), yet the manner in which this inhibition occurs is unknown. At the present time it is not clear whether ethylene acts directly on the auxin transport system or on some other system which indirectly affects auxin transport. The effects of ethylene on the uptake (5, 7, 14, 15), decarboxylation (5–7, 14, 15), and rate of auxin movement (6, 7), as well as the gross morphology of the transport tissue (5, 7), have been eliminated as significant components in the inhibition of auxin transport by ethylene.

In spite of the number of studies on how ethylene inhibits auxin transport, two possible explanations have not been thoroughly investigated. First, ethylene could be increasing the rate of auxin destruction by mechanisms which do not involve decarboxylation. Second, the gas could be increasing auxin immobilization by some type of binding. The need for in depth studies of these two possibilities was suggested by the following: (*a*) pulse labeling studies (6) indicate that ethylene reduces auxin transport by stimulating removal of auxin from the transport stream, (*b*) both destruction and immobilization of auxin are known to prevent its transport (see reviews in Refs. 8, 12).

The inadequate nature of available information on the effect of ethylene on auxin metabolism and immobilization is quite apparent. Binding and conjugation of IAA and NAA[3] are well known (2–4, 16, 18–21), but the effect of ethylene on these processes has not been studied. Study of the role of auxin metabolism, other than decarboxylation, as related to the effect of ethylene on auxin transport has been limited. The only consideration of auxin metabolites did not include insoluble ^{14}C fractions and employed strip counting procedures which would have not detected minor components (15). The identity of all of the ^{14}C recovered in ethylene-treated tissue has not been established.

This study was undertaken to investigate whether or not auxin destruction and auxin immobilization play a causal role in the inhibition of auxin transport by ethylene.

MATERIALS AND METHODS

The methods used to study auxin uptake, decarboxylation, and transport simultaneously in cotton stem sections (*Gossypium hirsutum* L., var. Stoneville 213) have been previously described (5). These methods and their modifications will therefore be reviewed only briefly.

General Experimental Design. Intact 23- or 25-day-old greenhouse-grown plants were exposed to 100 μl of ethylene per liter of air or to room air (control) for 15 hr at 30 $\pm$ 1 C in the dark. Stem sections 15 mm in length were then excised. Randomly selected sections from both groups were oriented vertically in Plexiglas holders with the morphological base resting on receiver agar cylinders. Agar cylinders containing 20 μM IAA-1-^{14}C (Nuclear-Chicago, 33 mc/mmole), IAA-2-^{14}C (Nuclear-Chicago,

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[3] Abbreviations: NAA: naphthaleneacetic acid; IAAsp: indoleacetylaspartic acid; NAAsp: naphthaleneacetylaspartic acid.

47.2 mc/mmole), or NAA-1-^{14}C (Nuclear-Chicago, 8.27 mc/mmole) served as the auxin donors and rested on the apical cut surface of each section.

The decarboxylation of auxin was determined simultaneously with auxin transport by placing stem sections inside glass chambers. The chambers were sealed and purged with compressed air at a constant flow rate, and the effluent air was scrubbed in 20% KOH to trap ^{14}C released as $^{14}CO_2$.

Two separate experiments were conducted with this experimental arrangement. The first experiment included four chambers each containing 25 stem sections. Two of the chambers contained control sections and two contained ethylene-pretreated sections. IAA-1-^{14}C donor agar cylinders were applied to the control and ethylene-pretreated sections in two of the chambers while NAA-1-^{14}C was simultaneously applied to the sections in the remaining two chambers. In a second experiment donor agar cylinders containing IAA-2-^{14}C were applied to 35 control and 35 ethylene-pretreated sections in separate chambers. Donor agar cylinders were in contact with the sections for 6 hr in the first experiment and 2 hr in the second experiment. Stem sections were incubated in the dark at 28 C during transport.

Immediately following transport all stem sections were cut into three successive 5-mm segments designated as the apical, middle, and basal segments. Five stem sections from each chamber were used to determine the distribution pattern of ^{14}C activity in the sections after transport. The apical, middle and basal 5-mm segments from these sections were grouped into separate lots, weighed, frozen, and subsequently homogenized in 5 ml of 40% ethanol. Duplicate 1-ml aliquots of the tissue homogenates were infrared-dried and planchet-counted. The receiver agar cylinders from these sections were counted by the method of McCready (13).

The remaining 5-mm stem segments from each chamber were grouped into separate apical, middle, and basal lots; weighed; frozen; and subsequently extracted. The donor and receiver agar cylinders were treated in a similar manner. The ^{14}C recovered in the KOH traps was scintillation-counted following diffusion into hyamine hydroxide (17).

Extraction Procedure. The method of extraction was similar to that described by Good *et al.* (9). Each lot of 20 or 30 apical, middle, or basal segments from each chamber was ground three successive times in 5-ml portions of cold 0.3 N $NaHCO_3$ for 2 min on a Virtis grinder. After the initial grinding with the first 5-ml portion of $NaHCO_3$, the supernatant was poured off, and fibrous residue was reground with a second 5-ml portion of $NaHCO_3$. This operation was repeated, and the supernatants were combined with a final 5 ml of $NaHCO_3$ used to rinse the flask to yield 20 ml of homogenate. The homogenate was saturated with $(NH_4)_2SO_4$ (15 g), and Kieselguhr G (1 g) was added. After shaking for 2 min, the samples were filtered through Whatman No. 1 filter paper in a Buchner funnel. The donor and receiver agar cylinders were ground once in 20 ml of cold 0.3 N $NaHCO_3$ and filtered without adding $(NH_4)_2SO_4$ and Kieselguhr G. The filter papers containing residues of tissue or agar cylinders were placed in Petri dishes, frozen, and subsequently assayed for ^{14}C activity.

Extraction of filter papers with distilled water followed by planchet counting of duplicate 1-ml aliquots of the extracts indicated that the percentages of ^{14}C removed from the control and ethylene tissue samples and donor and receiver agar cylinders were similar. Approximately 30% of the activity present in the tissue homogenates and less than 8% of the activity present in the donor and receiver agar cylinder homogenates was recovered on the filter papers.

The clear filtrate from the tissue and agar cylinder homogenates (pH 7.3) was extracted three times with 19-ml portions of freshly opened diethyl ether (peroxide as H_2O_2 0.00005%). The filtrate was acidified to pH 2.5 with 1 M H_3PO_4 and re-extracted with ether as before and then three times with 19-ml portions of 1-butanol. This procedure removed over 90% of the activity in the filtrates and yielded an alkaline ether, acid-ether, and an acid-butanol extract from each lot of stem segments or donor and receiver agar cylinders. The percentage of the ^{14}C remaining in the control and ethylene-treated filtrates was similar. After the acidic extracts had been made slightly basic with NH_4OH, all extracts were evaporated to dryness; the ether extracts were dried in a stream of N_2 and the butanol extracts in a rotary evaporator. The residues were redissolved in 1 ml of 0.3 N $NaHCO_3$ and stored in the dark at 4 C.

Radioautography of Extracts. Within 48 hr after extraction, 20-μl aliquots of each extract were spotted on 33- x 38-cm sheets of Whatman No. 1 filter paper and chromatographed for approximately 16 hr at 22 C by the ascending technique. To avoid decomposition and to promote evaporation of the extracts, N_2 was forced up through the area of the filter paper to which the extracts were applied.

Each extract was chromatographed once in each of the following solvent systems (9): (*a*) 2-propanol (containing 1.25% acetic acid)–20% aqueous solution of ammonium acetate (8:2, v/v), (*b*) 2-propanol–8 N NH_4OH solution (8:2, v/v). Equivalent extractions from the control and ethylene-treated segments were chromatographed in adjacent lanes to insure their development under identical chromatographic conditions.

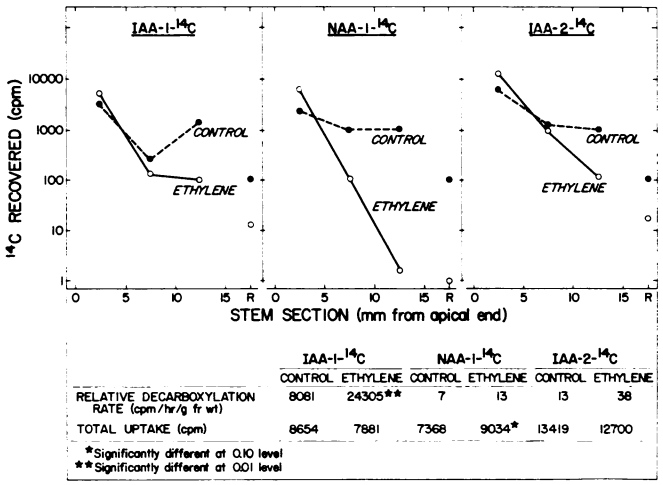
For purposes of comparison and identification, the extracts were cochromatographed with ^{14}C-IAA or NAA-1-^{14}C from the stock solutions used in preparing donor agar cylinders and indoleacetylaspartic acid (Calbiochem) or naphthaleneacetylaspartic acid (sample supplied by Dr. M. A. Venis, London, Ontario).

After development, chromatograms were dried, and the lanes containing nonlabeled IAAsp or NAAsp were cut from the chromatograms. IAAsp was located with either the Gordon and Weber (11) or Erhlich's (9) reagents while NAAsp was located by viewing its characteristic fluorescence under short wave ultraviolet light. The chromatograms containing the labeled extracts were radioautographed for 7 weeks on 27.9- x 35.6-cm Kodak No-screen x-ray film. After the films were developed, the entire paper chromatograms were sectioned and assayed for ^{14}C activity in a liquid scintillation system as previously described (5). Chromatograms were sectioned so as to isolate areas of highest activity as revealed by radioautography. The dimensions of the chromatogram pieces were 1.27 cm x 1.27 cm with no more than four pieces per scintillation vial.

Blank Determinations. A blank determination was conducted to determine whether or not the auxin degradation products appearing on the chromatograms were formed during the transport period or during the grinding, extraction, or chromatographic procedures. A sample of 20 ethylene-treated stem sections, 5 mm in length, which had not been previously treated with labeled auxin, was placed in 5 ml of 0.3 N $NaHCO_3$. A 2-μl volume of the stock solution of IAA-2-^{14}C (0.1 mM) was added to the sections, and the sections were immediately ground, extracted, chromatographed, and radioautographed as described above. This determination was designated as XC. A parallel determination designated as XB was also conducted. This determination differed from XC in that the 20 stem sections were boiled for 5 min before IAA-2-^{14}C was added.

RESULTS

Effect of Ethylene on the Uptake, Distribution and Decarboxylation of ^{14}C-IAA and -NAA. In an earlier paper (5) we reported that ethylene: (*a*) reduced the basipetal transport of ^{14}C from labeled IAA and NAA, (*b*) increased the rate of release of ^{14}C from IAA-1-^{14}C but not NAA-1-^{14}C or IAA-2-^{14}C (*c*), occasion-



	IAA-1-^{14}C		NAA-1-^{14}C		IAA-2-^{14}C	
	CONTROL	ETHYLENE	CONTROL	ETHYLENE	CONTROL	ETHYLENE
RELATIVE DECARBOXYLATION RATE (cpm/hr/g fr wt)	8081	24305**	7	13	13	38
TOTAL UPTAKE (cpm)	8654	7881	7368	9034*	13419	12700

*Significantly different at 0.10 level
**Significantly different at 0.01 level

FIG. 1. The effect of a 15-hr ethylene pretreatment period on the distribution, total uptake, and relative decarboxylation rate of IAA-1-^{14}C and NAA-1-^{14}C following 6 hr of basipetal transport and of IAA-2-^{14}C following 2 hr of basipetal transport. Total uptake data represent only that ^{14}C activity recovered from the stem sections and receiver agar cylinders (R). Decarboxylation cpm are not directly comparable to stem section or total uptake cpm. Decarboxylation rates are expressed as the average number of cpm of ^{14}C recovered as $^{14}CO_2$ per hr of transport per g of fresh weight.

ally decreased the total uptake of IAA-1-^{14}C but consistently increased the total uptake of NAA-1-^{14}C, and (*d*) generally caused an accumulation of ^{14}C in the apical absorbing segment. The ^{14}C total uptake, distribution, and decarboxylation data presented in Figure 1 confirm these results.

Effect of Ethylene on the Metabolism of IAA-1-^{14}C. The distribution of the radioactivity on the paper chromatograms of the extracts from the control and ethylene-pretreated sections following 6 hr of basipetal IAA-1-^{14}C transport are shown in Figure 2A. In Figure 2 histograms of chromatograms of the extracts from the receiver agar cylinders are not shown since the activity present in these extracts was too low to allow meaningful comparisons. Three chromatograms were obtained from each lot of IAA-1-^{14}C, NAA-1-^{14}C, and IAA-2-^{14}C donor agar cylinders and stem segments. These corresponded to the chromatograms obtained from the alkaline ether, acid ether, an acid butanol extractions of the donor cylinder and stem segments. For purposes of presentation, the radioactivity recovered at the same R_F value on these three chromatograms was pooled in Figure 2 to yield a single histogram for each lot of donor cylinders and stem segments.

The distribution of radioactivity on the chromatograms of the IAA-1-^{14}C donor agar cylinder extracts (Fig. 2A) indicate that only about 65% of the ^{14}C recovered from the donor cylinders of the control and ethylene-pretreated sections was associated with the R_F zone of authentic IAA. This was considerably lower than than the 90% radiochemical purity of the cochromatographed stock solution from which these agar cylinders were prepared (chromatogram not shown). Chromatography of extracts from similar cylinders which had not been in contact with stem sections revealed that approximately 88% of the ^{14}C present in these donor cylinders chromatographed as IAA-1-^{14}C. Therefore, the reduction noted in the percentage of ^{14}C chromatographing as IAA-1-^{14}C in the donor cylinders following transport (Fig. 2A) was probably due to enzymatic breakdown or conjugation at the cut surface.

The stem segment chromatograms (Fig. 2A) indicate that only a small amount of the ^{14}C present in either the control or ethylene-pretreated sections following 6 hr of basipetal transport represented IAA-1-^{14}C. Of the total ^{14}C recovered from the extracts of the apical control and ethylene-pretreated segments, approximately the same amount of ^{14}C chromatographed as as IAA-1-^{14}C. Significantly more ^{14}C-IAA metabolites, however, were recovered in this segment of the ethylene-pretreated sections. The majority of these metabolites, as well as those present in the apical segments of the controls, were in the R_F region 0 to 0.23.

Similar amounts of ^{14}C remained at the origin in the extracts of the apical segments of the control and ethylene-pretreated sections. Immediately above the origin, in the R_F zone 0.02 to 0.09, significantly more ^{14}C was recovered in the apical segment of the ethylene-pretreated sections. The majority of the ^{14}C recovered in this R_F region was in the acid-butanol extract. The R_F value of the labeled metabolite(s) of IAA from the ethylene-pretreated sections in this R_F zone was slightly higher than the R_F value of the labeled metabolites(s) in the adjacent lane of the control acid-butanol extracts. These results suggested that ethylene induced changes in the auxin degradative enzyme complex.

Ethylene pretreatment increased the total amount of IAAsp-^{14}C in the apical segments. On a percentage basis, however, approximately 28% of the total ^{14}C recovered from the extracts of the control and ethylene apical segments appeared as IAAsp-^{14}C. The majority of the IAAsp-^{14}C was recovered in the acid-ether fraction.

The similarity between the distribution of ^{14}C on the control apical, middle, and basal segment chromatograms indicated that many of the ^{14}C-IAA metabolites present in the apical segment extracts were also present in the middle and basal segment extracts. On a percentage basis more IAA-1-^{14}C was present in the control, middle, and basal segments than in the apical segments.

Effect of Ethylene on Metabolism of NAA-1-^{14}C. The histograms summarizing the chromatograms of the NAA-1-^{14}C donor cylinders and stem segment extracts are presented in Figure 2B. Inspection of the distribution of radioactivity in these chromatograms indicates that NAA-1-^{14}C is much less susceptible to degradation in cotton stem sections than IAA-1-^{14}C. The only major labeled compound other than NAA-1-^{14}C which appears on any of the chromatograms has an R_F value identical to that of the aspartic acid conjugate of NAA.

In the donors very little breakdown or conjugation of NAA occurred. Of the total activity present in the control and ethylene donor cylinder extracts, 95% chromatographed as NAA-1-^{14}C. This was only slightly lower than the 97% radiochemical purity of the stock solution (chromatogram not shown).

The apical segment of the ethylene-pretreated segments contained more NAA-1-^{14}C than the controls following 6 hr of transport (Fig. 2B). On a percentage basis, 58% of the ^{14}C extracted from the apical segments of the ethylene-pretreated sections and 51% of the ^{14}C extracted from the control apical segments chromatographed as NAA-1-^{14}C. In contrast, considerably less NAAsp-^{14}C was present in the apical segments of the ethylene-pretreated sections on a percentage basis (control 39%, ethylene 26%).

Ethylene pretreatment severely inhibited the transport of ^{14}C from NAA-1-^{14}C. This reduction in transport resulted in very little activity being present in the extracts from the middle and basal segments of the ethylene-pretreated sections. The extracts from the corresponding control segments, however, contained an appreciable amount of activity. About 88% of the ^{14}C in these extracts chromatographed as NAA-1-^{14}C and about 7% as NAAsp-^{14}C.

Effect of Ethylene on Metabolism of IAA-2-^{14}C. Histograms summarizing radioautographs of the extracts obtained from the control and ethylene-pretreated sections and the donor agar cylinders from these sections following 2 hr of basipetal transport of IAA-2-^{14}C are shown in Figure 2C. The chromatograms of the donor cylinder extracts were generally similar to those obtained

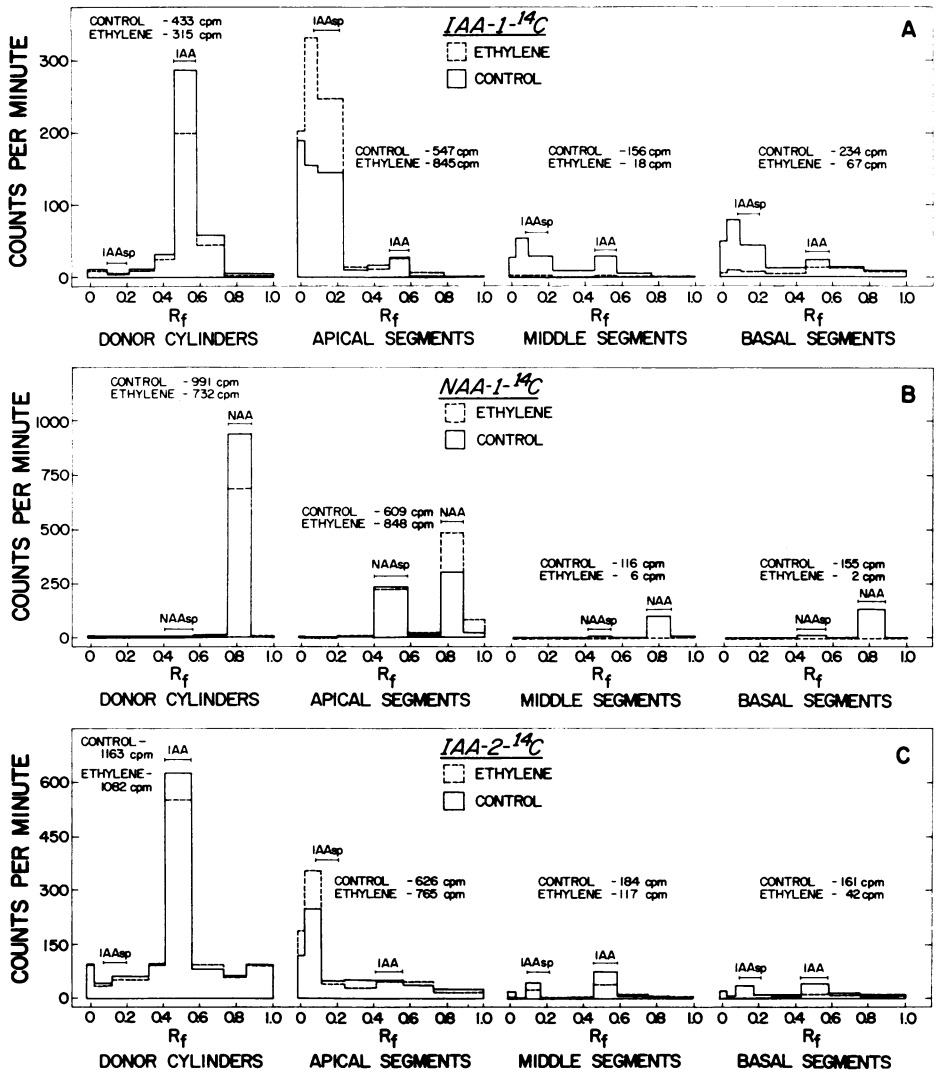


FIG. 2. The effect of a 15-hr ethylene pretreatment period on the metabolism of IAA-1-^{14}C (A) and NAA-1-^{14}C (B) during 6 hr of basipetal transport and of IAA-2-^{14}C (C) during 2 hr of basipetal transport. Donor cylinders, apical, middle, and basal stem segments were extracted with alkaline ether, acid-ether, and acid-butanol. Each extract was chromatographed separately and the results of the three chromatograms were combined to form the single composite histograms shown. The IAA-1-^{14}C and IAA-2-^{14}C chromatograms were developed in 2-propanol–8 N NH_4OH (8:2, v/v) while the NAA-1-^{14}C chromatograms were developed in 2-propanol (containing 1.25% acetic acid)–20% aqueous solution of ammonium acetate (8:2, v/v). The cpm data in the upper part of each composite histogram represent the total ^{14}C recovered from the three chromatograms.

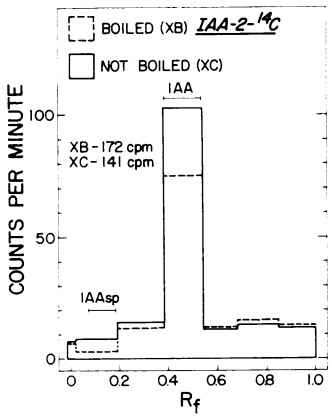


FIG. 3. Histogram of the chromatogram showing the effect of the grinding, extraction, and chromatographic procedures on the breakdown of IAA-2-^{14}C. IAA-2-^{14}C was added to 20 stem sections boiled (dotted line, XB) or not boiled (solid line, XC), the sections were immediately ground and extracted, and the extracts were chromatographed.

with IAA-1-^{14}C (Fig. 2A). Approximately 89% of the ^{14}C in the stock solution chromatographed as IAA-2-^{14}C (chromatogram not shown), while only 52% of the ^{14}C from the extracts of the donor cylinders of the control and ethylene-pretreated sections chromatographed as IAA-2-^{14}C.

Trends apparent after 6 hr of IAA-1-^{14}C (Fig. 2A) transport were also apparent after 2 hr of IAA-2-^{14}C transport (Fig. 2C). The major difference at the shorter transport period with IAA-2-^{14}C was the larger percentage of the total ^{14}C which chromatographed as IAA-2-^{14}C in extracts from both the control and ethylene-pretreated sections.

Blank Determination. The distribution of the radioactivity on the chromatogram of the blank determination with IAA-2-^{14}C is shown in Figure 3. Approximately 85% of the ^{14}C in the stock solution (chromatogram not shown) which was added to the segments that were boiled for 5 min (XB) and the segments not boiled (XC), chromatographed as IAA-2-^{14}C.

The percentage of ^{14}C recovered as IAA-2-^{14}C from the alkaline ether, acid-ether, and acid-butanol extracts of the boiled (XB) and not boiled (XC) sections was about 55%, indicating that

some breakdown or conjugation of IAA-2-^{14}C did occur. From the position of these labeled compounds on the chromatogram it is obvious that the low R_F value compounds shown in Figure 2, A and C, are not artifacts of the grinding, extraction, or chromatographic procedures.

Effect of Ethylene on Fresh Weight of the Transport Tissue. Ethylene was found to have no significant effect on the fresh weight of apical, middle, or basal stem segments used in the experiments of this study. Therefore, the differences noted in Figures 1 through 3 cannot be attributed to differences in fresh weight.

DISCUSSION

In an earlier paper (5) we concluded that an ethylene-mediated reduction of auxin uptake or increase in auxin decarboxylation does not contribute significantly to the effect of ethylene on auxin transport. The data presented here support this conclusion and further indicate that this reduction in transport cannot be explained in terms of an effect of ethylene on auxin breakdown other than decarboxylation or conjugation.

The histograms of the chromatograms of the NAA extracts shown in Figure 2B clearly demonstrate that the reduction in the amount of NAA-1-^{14}C transported was not due to an increased metabolism of NAA-1-^{14}C. More NAA-1-^{14}C was present in the apical segment of the ethylene-pretreated sections than in the apical segments of the control sections, yet very little was transported out of this segment in 6 hr. Thus, the reduction of NAA-1-^{14}C transported by ethylene was not due to a lack of auxin in the sections.

The data presented here and elsewhere (2, 5, 14) demonstrate that IAA is metabolized much more rapidly than NAA. Evidence *in vitro* (17) suggests that in cotton this difference is related to the inactivity of NAA in the IAA-oxidase system. Although no attempt was made to identify the major metabolites of ^{14}C-IAA, one metabolite had an R_F value identical with that of IAAsp, suggesting that it was this conjugate. IAAsp has been previously reported in cotton stem sections (16) as well as many other tissues (3, 9).

Ethylene caused an acculumation of ^{14}C in the absorbing segment (Figs. 1, 2A and 2B). The data presented in Figure 2, A and C, clearly indicate that very little of this ^{14}C represents ^{14}C-IAA. What is not clear is (*a*) whether ethylene increased the rate of IAA metabolism to such an extent that only a small amount of ^{14}C-IAA was available for transport or (*b*) whether ethylene inhibited auxin transport *per se*, resulting in an accumulation of ^{14}C-IAA in the absorbing segment, which ultimately resulted in the formation of additional ^{14}C IAA metabolites. While neither of these possibilities can be evaluated on the basis of the ^{14}C-IAA data presented in Figure 2, A and C, the NAA data (Fig. 2B) would strongly support the second possibility. If ethylene inhibits IAA and NAA transport in a similar manner, then IAA would be expected to accumulate in the apical segment since very little of the auxin taken up would be transported into basal tissue. Therefore, a larger quantity of IAA would be present in the apical segment of the ethylene-pretreated sections for a longer period of time than in the apical segment of the control sections. This condition could result in the greater quantity of IAA metabolites in the apical segment of the ethylene-pretreated sections since Veen (19) has shown an increase in NAA metabolites with increasing donor concentration and Andreae (2) has obtained data indicating an increase in auxin metabolites with time.

The results of a previous study (14) have indicated that ethylene does not reduce auxin transport by increasing binding of IAA-2-^{14}C or NAA-1-^{14}C to particles which can be precipitated by centrifugation at 20,000*g*. The extraction data obtained in the present experiments further indicate that auxin binding to a protein fraction is not involved in this effect of ethylene. In the extraction procedure described here, tissue homogenates were saturated with $(NH_4)_2SO_4$ and filtered through Whatman No. 1 filter paper. When the ^{14}C content of the residue samples was counted, no significant differences were noted between the percentage of ^{14}C removed by filtration from the ethylene *versus* the control homogenates. If ethylene treatment resulted in an increased amount of auxin binding to a protein sufficient to account for the inhibition of auxin transport, considerably more ^{14}C would be expected to be recovered in the precipitated protein fraction. Since this was not the case, protein binding does not appear to play a significant role in ethylene-mediated inhibition of auxin transport.

The present data indicate that even in short term experiments, assays of ^{14}C do not always give an accurate measurement of auxin distribution. Under conditions of relatively long transport periods (6 hr), a limited source of auxin (donor cylinders not replaced), a significant cut surface effect (5), and a rapid turnover of auxin in the transport tissue (Fig. 2, A and C), very little of the ^{14}C which remains in the tissue following transport is free ^{14}C-IAA. With synthetic auxins such as NAA (2, 19, present study) and 2,4-D (2) under similar conditions, ^{14}C activity is a more accurate indicator of the auxin in the tissue.

The data presented here and elsewhere (5–7, 10, 14, 15) clearly demonstrate the ability of ethylene to disrupt basipetal auxin transport *in vivo*. The disruption could involve any of the following: (*a*) a gradual increase in auxin destruction, (*b*) a gradual increase in the rate of auxin immobilization, (*c*) a gradual breakdown of the mechanism involved in actively transporting auxin from cell to cell, or (*d*) a gradual breakdown of the structural integrity of the pathway through which transport occurs. Our data would indicate that auxin destruction and immobilization processes involving conjugation or binding do not play a significant role in the disruption of transport by ethylene. At this time elimination of binding should be considered only tentative in that a critical evaluation of this factor was not included in this study.

In view of the possibility that ethylene may effect membrane permeability (1), ethylene may cause auxin immobilization by stimulating the "leakage" of auxin from the transport stream into areas where it is no longer available for transport. A modification of permeability may also disrupt the structural integrity of the membranes essential for the normal transport of auxin from cell to cell. The importance of such a permeability alteration, however, remains to be evaluated.

Previously we reported (6) that increased ethylene pretreatment periods from 1.5 to 3.0 hr progressively increased the rate of auxin loss from a pulse of auxin in transit. The auxin remaining in the pulse generally moved at a normal velocity and retained the same general appearance as the control pulse. These observations suggest that, whatever processes are involved in the disruption of auxin transport by ethylene, they must occur gradually in such a manner so as to leave a part of the system intact.

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