# EFFECT OF ETHYLENE TREATMENT ON AUXIN METABOLISM OF POTATO TUBERS

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## Introduction

Following the first investigation by ROSA (16), possible role of ethylene on potato sprouting has been attracted considerable attention for many years. The majority of available evidence, which indicated that ethylene exerted effects not only on either breaking or prolonging of the tuber dormancy (14) but also on induction of tuber formation of potato (3, 5), has been accumulated. Especially, one of the most interesting question as to a physiological significance of ethylene concerns the regulation of their dormancy. Burton (14) has suggested that an effect of the ethylene on the dormancy seems to depend on the duration of its treatment; brief treatments at intervals stimulated sprouting, whereas the treatment of longer duration suppressed it. In this context, RYLSKI *et al* (17) have proposed recently an advanced opinion that ethylene exerts a dual effect on potato tuber of which dormant period is markedly shortened but elongation of the sprout is inhibited. In spite of such extensive studies on multitudinous effects of ethylene on the growth of potato plants, virtually no adequate explanation has been given on the direct effects of ethylene on their developmental processes. It has been pointed out in the preceding paper (11) that the effect of ethylene treatment appeared to result in a considerable lowering of extractable auxin level in the potato tubers. In view of a well established fact that indole-3-acetic acid (IAA) as a naturally-occurring auxin is underwent decarboxylation as well as conjugation with aspartic acid in many plant tissues (1, 2, 12, 20, 23), the inevitable question arises concerning how the ethylene-induced decrease in auxin level occurs in the potato tuber tissues. Therefore, it will be necessary to take into account changes in auxin metabolism of potato tissues with ethylene treatment. The present study was undertaken to ascertain whether or not the ethylene plays a causal role in activation of auxin metabolism and additionally to discuss in connection with changing in the sprouting

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activity of potato tubers. The experiments were performed to know whether or not ethylene really has a promoting ability to destroy or conjugate the IAA in the potato tuber tissues. A precise studies for this purpose can be pursued only with the help of radio isotope, *i. e.* IAA-1-$^{14}$C.

## Materials and Methods

**Plant Materials**: Potato tubers (*Solanum tuberosum* L. cv. Irish Cobbler) harvested at the Experimental Farm of Hokkaido University had been stored in cellar at 3°C. After sorting the tubers averaging 300 g in weight, they were surface-sterilized with 0.5% sodium hypochlorite solution for 10 min, followed by thoroughly washing with running tap water and dried. These tubers were divided into some groups for being subjected to several treatments (see Results).

**Analysis of IAA and Its Metabolism**: The tubers were dissected into individual tissues, and disc-shaped slices, 5 mm in diameter and 2 mm in thickness, were prepared from them (as designated in Fig. 1). These tissue discs were washed with sterilized distilled water. Eight pieces of discs were aseptically incubated in 2 ml of reaction mixture with gentle shaking for 2 hr at 25°C in darkness. The reaction mixture consisted of 50 mM phosphate buffer (pH 4.5) and 0.1 $\mu$Ci carboxyl-labeled $^{14}$C-IAA (specific activity 52 mCi per mM IAA; equivalent to 0.34 $\mu$g IAA) was added chloramphenicol as a safeguard against bacterial contamination in a final concentration of 50 $\mu$g/ml. The carbon dioxide released from the discs during the incubation time was absorbed with 0.2 ml of 20% KOH solution in a well suspended over the mixture from the rubber seal. Aliquots of the mixture before and after incubation were assayed for total radioactivity with a liquid scintillation counter. Immediately after the termination of the incubation period, the discs

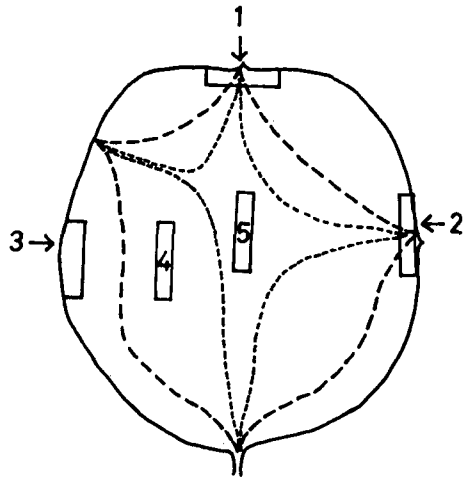


**Fig. 1.** Representative portions of potato tuber tissues selected for analysis of IAA metablism. Disc-shaped tissues, 5 mm in diameter and 2 mm in thickness, were excised from different parts of tuber which were designated as apical bud (1), lateral bud (2), cortex (3), perimedullary pith (4) and central pith (5), respectively.

were removed and rinsed thoroughly with 50 mM phosphate buffer containing cold IAA at 10 mg/l. The washed discs were homogenized and extracted first with 95% ethanol, followed with 80% ethanol three times successively. The entire alcohol extract was combined and evaporated to dryness *in vacuo* and redissolved in 1 ml of 80% ethanol. A 50 $\mu$l aliquot of the extract was counted and another 50 $\mu$l was streaked on Toyo No. 50 chromatographic paper (40 × 2 cm), then developed in ascending fashion using isopropanol : 28% ammonia : water at 10 : 1 : 1, v/v/v. After drying the developed chromatogram was cut into 20 equal sections corresponding to Rf, and each of them was placed in 10 ml of scintillation medium and counted radioactivity.

Decarboxylation of $^{14}$C-IAA was measured by counting $^{14}CO_2$ evolved. The $^{14}CO_2$ absorbed by 20% KOH during the incubation was precipitated by saturated Ba-acetate and centrifuged. The resultant precipitate was placed on glass filter (Whatman GF/G) to remove the excess of Ba with water by suction filtration. The dried filter with precipitate was placed in 10 ml of scintillation medium and counted.

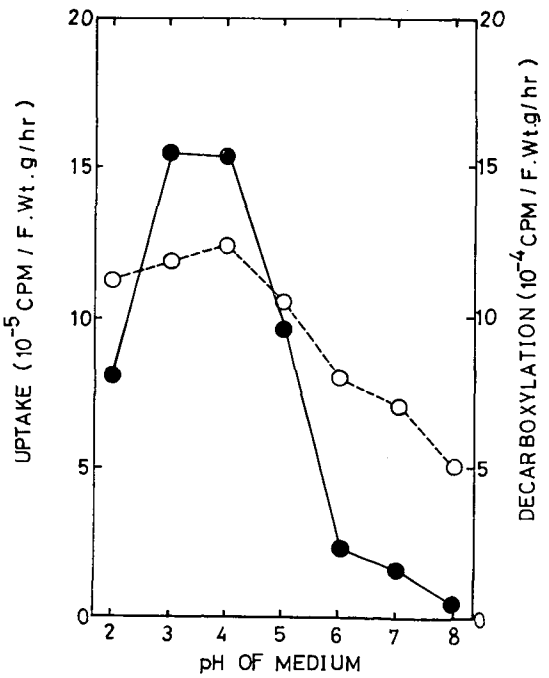


**Fig. 2.** Comparison of uptake (—●—) and decarboxylation (···○···) of IAA-1-$^{14}$C by the potato tuber tissues incubated at various values of pH for 2 hr.

Although the figures and the tables contain data from one typical determination, all experiments were done at least twice, and the results agreed to within 15% of the average.

## Experimental Results

**Optimum pH Value for Incubation**: The preliminary experiment to obtain the optimal pH value of the incubation medium for auxin metabolism was done using McIlvaine buffer over the whole pH range 2.0–8.0. From Fig. 2, it can be seen that uptake of $^{14}$C-IAA by the discs showed an optimum between pH 3.0 and 4.0, but the maximum activity of decarboxylation of $^{14}$C-IAA appeared to be at pH 4.0. The highest levels of $^{14}$C-IAA and its $^{14}$C-conjugate in the incubated tissues also showed at pH 4.0 (Fig. 3). Therefore, optimum pH for incubation medium can be decided at around pH 4.0. For convenience, 50 mM $KH_2PO_4$ (pH 4.5) was used as buffer for all subsequent experiments.

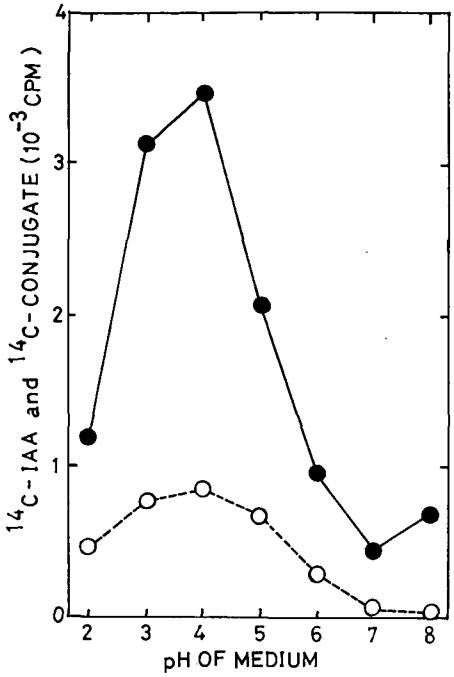


**Fig. 3.** Effect of different pH values of the incubation medium on the levels of free $^{14}$C-IAA (—●—) and $^{14}$C-conjugate (···○···) in the potato tissues incubated for 2 hr.

**IAA Metabolism of Different Part of Tuber**: The discs of tuber tissue were divided into five groups depending on the origin of tissues derived from the tubers, as illustrated in Fig. 1, and each of which was incubated with $^{14}$C-IAA, in order to obtain information as to the degree of activity of these discs to metabolize IAA. From a typical data listed in Table 1, while the ethanolic extract obtained from discs of the central pith showed the maximum in its radioactivity, the extracts from those of the lateral bud and the cortex resulted in exceedingly low activities which were about one half of that of the central pith. When the discs were arranged in order of degree of IAA decarboxylating activity, the lateral bud was highest; cortex came second; next in order were the apical bud, the central pith and the perimedullary pith. The paper radiochromatography of all discs revealed distinctively two radioactive regions. The one had Rf value of 0.3 which

corresponded to IAA, but the other had Rf of 0.01 which being expected to be IAA-conjugate as indole-3-acetylaspartic acid (IAAsp), citing evidence of others (4, 12, 21). There was no additional region with a significant radioactivity other than the major two peaks stated above. The subsequent experiments were carried out to ensure this assumption. When the strips

TABLE 1 Comparison of auxin metabolism in different part of potato tuber tissues labeled with 0.1 $\mu$Ci IAA-1-$^{14}$C. Activity applied as determined by liquid scintillation counting = 213,000 cpm.

| Tissue | Total activity recovered* (cpm/g, fresh wt.) | Recovery (%) | Decarboxylation ($^{14}CO_2$) (cpm/g/hr) | Conjugation** IAA (cpm/g) | IAAsp (cpm/g) | IAAsp/IAA |
|---|---|---|---|---|---|---|
| Apical bud | 92,148 | 16.1 | 2568 | 2758 | 1573 | 0.57 |
| Lateral bud | 132,499 | 24.7 | 3292 | 4244 | 1647 | 0.39 |
| Cortex | 145,631 | 27.3 | 2758 | 4500 | 811 | 0.18 |
| Perimedullary pith | 90,213 | 18.0 | 1287 | 3842 | 445 | 0.12 |
| Central pith | 271,517 | 48.3 | 1617 | 5134 | 1592 | 0.31 |

* Fifty microliters of ethanol extract was counted in Bray's scintillation medium.
** Each section of chromatograms was placed into toluene scintillation medium and counted.

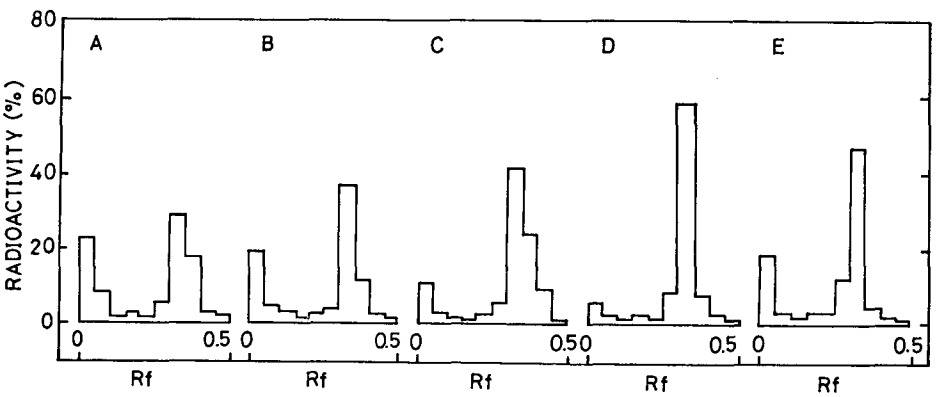


**Fig. 4.** Paper chromatograms of the ethanol extracts of tissues incubated with IAA-1-$^{14}$C. Tissues of potato tuber were obtained from apical bud (A), lateral bud (B), cortex (C), perimedullary pith (D) and central pith (E), respectively. Extracts prepared from those tissues were chromatographed on paper developed with iso-propanol: 28% ammonia: water (10 : 1 : 1; v/v/v). The radioacive regions with Rf of 0.01 and 0.3 are IAA-conjugate and IAA, respectively. There were no other radioactive regions on the the chromatogams.

of chromatogram at Rf 0 to 0.1 were eluted in 30% ethanol and rechromatographed in n-butanol : acetic acid : water (4 : 1 : 4, v/v/v), a single radioactive peak was present at Rf 0.7. This radioactive component was eluted into 30% ethanol again and the combined eluate was divided into two portions. One aliquot was hydrolyzed with 1 N HCl for 6 hr at 120°C, then neutralized and finally extracted with five successive equal volumes of ethyl acetate. The other aliquot was subjected to the similar treatment without 1 N HCl. Both resultant extracts were chromatographed using ammoniac isopropanol as solvent system (see Methods). It yielded a radioactive compound which had the same Rf value as authentic IAA being cochromatographed (Fig. 5). These results indicated that the major component of IAA-conjugate was IAAsp.

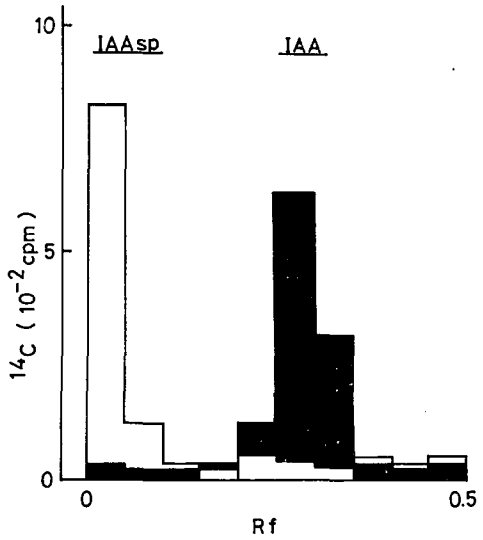


**Fig. 5.** Distribution of radioactivities on the chromatograms, which shows comparison between IAA conjugate fractions of being subjected by acid hydrolysis (black bar) and control (white bar). Details of procedure is represented in the text.

**Effect of Ethylene Treatment on Auxin Metabolism of Intact Tubers:** The present experiments were performed to investigate whether and how ethylene treatment really produces some effect on the metabolism of IAA in the potato tubers. The tubers were divided into five groups for several treatments which were described briefly as follows: (A) Tubers were placed in dark room without air ventilation as control. (B) Tubers were placed in

dark room subjecting continuous air ventilation for 4 days. (C) Tubers placed in darkness were previously ventilated with air alone 2 days and succeeded by continuous ventilation with ethylene (2 ppm) for additional 2 days. (D) After pretreatment of ethylene fumigation (2 ppm) for 2 days, tubers were subjected to air ventilation for additional 2 days to remove ethylene. (E)

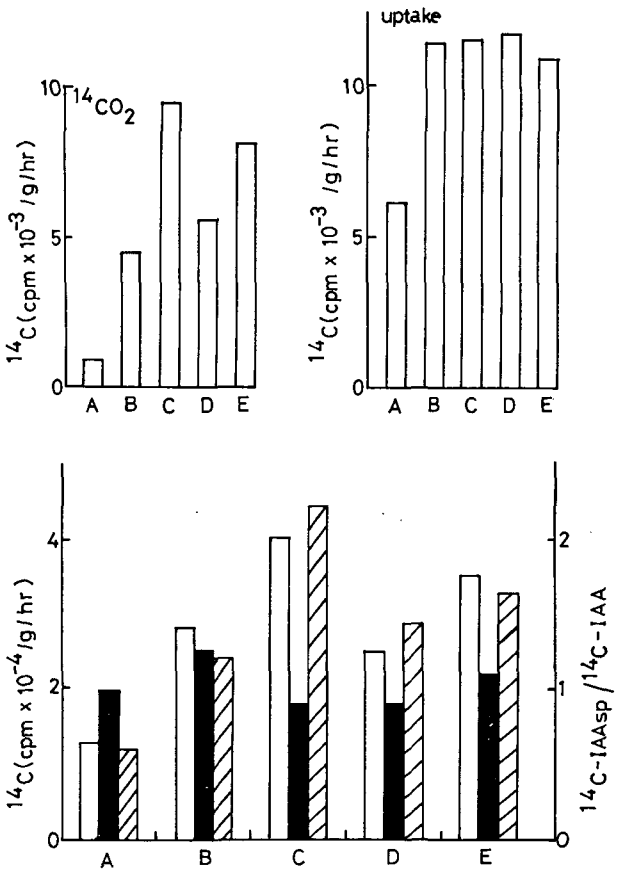


**Fig. 6.** Effect of air ventilation with and without ethylene on the IAA metabolism of tissue discs of potato tuber. A=control, B=air ventilation without etnylene, C=air ventilation with ethylene for first 2 days, D=air ventilation with ethylene for later 2 days, E=air ventilation with ethylene throughout the experimental period.

Upper: Comparison of decarboxylation (left) and uptake (right) of $^{14}C$-IAA.

Lower: Comparison of $^{14}C$-IAA (black bar) and $^{14}C$-IAAsp (white bar) of the ethanol extracts, and the ratio of $^{14}C$-IAAsp to $^{14}C$-IAA (hatched har).

Tubers were continuously subjected with air ventilation supplemented with ethylene (2 ppm) throughout the experiment (4 days).

At the end of the experiment, all tubers were sampled for analysis as described in Methods, and the results were shown in Fig. 6. When the tubers were subjected by air ventilation even in the absence of ethylene, stricking double to five folds increase in not only uptake but decarboxylation of $^{14}$C-IAA was resulted in the discs excised from the ventilated tubers. Especially, a stimulating effect of ethylene fumigation on the decarboxylation of the discs was greatly prominent. Although there was no visual increase in uptake of $^{14}$C-IAA caused by the air ventilation with ethylene, level of $^{14}$C-IAAsp in the discs displayed a tendency to rapidly increase by the treatment. It was also appeared that such stimulating effect of ethylene on IAA metabolism was mitigated by resulting from removal of ethylene. It is also evident from Fig. 6 that the ethylene fumigation is more effective for the conjugation of IAA, thereby the highest ratio of $^{14}$C-IAAsp to $^{14}$C-IAA was observed.

**Time Course Experiment on IAA Metabolism of Potato Tuber Tissues**: As stated above, ethylene produced a significant effect on IAA metabolism of potato tubers, and subsequently time course experiments were performed to obtain further detailed information as to effect of ethylene on tuber tissues for a longer period, especially a special attention was paid for diverting treatment of the ethylene application at the 7th day after incubation. From the result showing in Fig. 7, a slight increase in uptake of $^{14}$C-IAA was consistently noticed in control discs and the increasing rate showed no significant change until the end of incubation period, even if the ethylene treatment started at the later stage of the incubation period (on the 7th day). However, the ethylene treatment throughout the period occurred in a somewhat increase of the uptake within a day after starting the incubation, thereafter remained more or less constant. With regard to the change in degree of decarboxylation of $^{14}$C-IAA, an ascending phase was noticeable in the curve of ethylene-treated discs during the course of the experiment, while there was no substantial increase in $^{14}CO_2$ liberated from control discs. On the 7th day when exogenous ethylene application diverted to be subjected control tubers, the tendency of above curves altered. Namely, considerable improvement of $^{14}CO_2$ liberation in the control discs and noticeable lowering off of it from the discs which had been previously ethylene treatment for 7 days were recognized.

On the other hands, the incorporation of radioactivity into IAAsp markedly increased within 3 days after incubation, and it was observed a similar

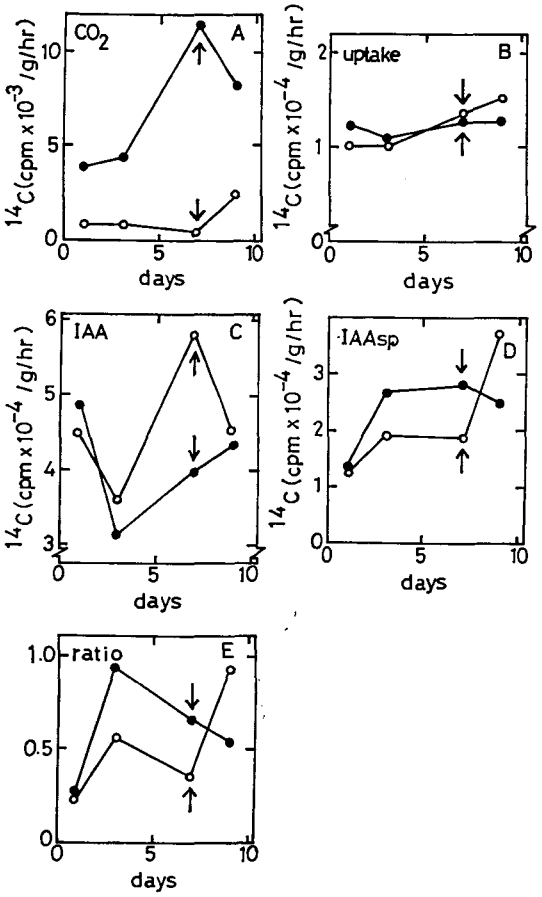


**Fig. 7.** Time course experiment of $^{14}$C-IAA metabolism of tissue discs of potato tuber, control (—○—) and ethylene treatment (—●—), A=liberated $^{14}$C-$CO_2$, B=uptake of $^{14}$C-IAA into the tissues, C=$^{14}$C-IAA in the ethanol extracts, D=$^{14}$C-IAAsp in the ethanol extracts and E=ratio of $^{14}$C-IAAsp to $^{14}$C-IAA.

tendency in the treated and control discs but a degree of the incorporation was apparently less in the control ones compared with the treated ones. Subsequently, these levels remained relatively constant until the diversion of ethylene application started at the 7th day, thereafter the situation was reversed, namely level of $^{14}$C-IAAsp in the control discs increased rapidly due to starting introduction of ethylene and that in the discs treated with ethylene previously occurred to contrarily decline due to removal of ethylene. On the other hands, changes in the levels of $^{14}$C-IAA in the discs were mirrored

in the variation of their conjugating activity, and it was found a reverse tendency of changing curves in level of $^{14}$C-IAA detected in the both groups of discs during the course of the experiment.

## Discussion

From the result of the present experiment concerning the pH value of incubation medium, it was found to be clear that a low pH value of medium (pH 4.5) is suitable for decarboxylation and conjugation of IAA when IAA-1-$^{14}$C was fed to the potato tuber tissues. ZENK and MÜLLER (24) and ANDREAE (1) have been already reported the similar facts and suggested that the effect of decreasing pH on decarboxylation of IAA appeared to result from increasing in uptake of IAA. Our data are in fully accordance with those of ANDREAE (1) and lend to support his contention. Additional support for this idea also come from the results presented by LAU and YANG (9).

Since the first studies conducted by ANDREAE and GOOD (2), it has been well known the presence of IAAsp as a conjugate product in many plants being supplied by IAA (4, 9, 10). The present data also confirmed the production of $^{14}$C-IAAsp in the incubated tuber tissues with $^{14}$C-IAA. Although IAA glucoside was also detected in some plants (23), IAAsp seems to be a sole IAA-conjugate in the potato tuber tissue, in considering the results of chromatographic analysis as showing in Fig. 4 and 5.

Regarding various aspects of IAA metabolism in the tissue discs excised from different part of the tubers, the discs from the peripheral parts of tubers either with or without eyes showed relatively higher degree in IAA metabolic activity, as compared with discs obtained from the inner part of tubers. A possible interpretation of such behavior is due to higher metabolic activity, citing the fact that higher respiratory activity distributed in the peripheral layer of the tuber (20).

The fact that a noticeable improvement of $^{14}$C-IAA uptake occurred in the discs consisted of the central pith tissues which concomitantly yielded a substantial amounts of $^{14}$C-IAAsp, should be received attention in relation to only a slight activity of IAA decarboxylation in these discs. The increase in $^{14}$C-IAA uptake is considered to be a consequence of relatively higher absorbing ability, depending on higher level of soluble sugar and less amount of stored starch in these discs as compared to that in another discs (19). Therefore, an appreciable increase in yield of $^{14}$C-IAAsp in these discs may be interpreted to be primary result of increase in accumulation of $^{14}$C-IAA in them. However, somewhat less activity of decarboxylation in these discs seems to be also depending on the low respiratory activity stated above.

Incubation of sweet potato root tissues in the atomosphere with ethylene occurred in stimulating peroxidase activity and oxygen uptake, and removal of ethylene resulted to lose this effect thereafter (8). Experiment using potato stolon also showed a similar evidence that peroxidase activity increased sharply during the initial days of incubation in the presence of CEPA which is ethylene generation agent (13). Peroxidase of several plants are known to show oxidative action of IAA (7, 18, 22). In spite of an evidence that decarboxylation of IAA was not affected by ethylene (4), it is reasonable to expect a possible involvement of peroxidase in oxidative decarboxylation of IAA in the potato tuber discs, being associated with the stimulating effect of ethylene on peroxidase activity (7). In any way, an apparent contradiction between the present data and ERNEST's ones might be attributable to use the intact tissue of potato tubers in the present experiment, unlike use of enzyme brei of Coleus plant by ERNEST (4).

On the other hands, REID and PRATT (15) found an ethylene stimulated respiration in potato tubers. Therefore, it can be suggested an assumption that such enhancement of respiration brought about an increase in ATP level, leading to stimulate formation of IAAsp, citing the fact that IAA conjugation required a continuous supply of ATP (10). By reference to the fact that the effect of auxin on potato tubers may exert to controlling apical dominance rather than of prolonging the rest period (6), it seems improbable that change in level of endogenous active auxin due to ethylene treatment links to directly regulation of their dormancy. In conclusion, a physiological role of ethylene on sprouting of potato tubers may be played to mediate through lowering of endogenous auxin in its active form.

## Summary

An investigation as to effect of ethylene treatment on several facets of auxin metabolism in potato tubers (*Solanum tuberosum* L.) revealed number of changes induced by this gas. Potato tubers receiving air ventilation markedly increased in rate of IAA decarboxylation and its stimulaing effect was more intensified by the ethylene fumigation. The air ventilated tubers either with or without ethylene resulted in a more amount of uptake of $^{14}$C-IAA into alcohol soluble fraction, as compared to that without ventilation, but there was no significant difference of the uptake rate between air ventilation with and without ethylene. An activity of IAA conjugation was also enchanced by the ethylene treatment. The fact that the ethylene fumigation resulted in lowevering of auxin level in the potato tubers would be due to the stimulating effect of ethylene on auxin catabolism. Therefore, this

idea enable us to draw an inference that there is a physiological importance of ethylene concerning in sprouting, not in breaking dormancy, of potato tubers.

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