# STUDIES ON THE GROWTH PROMOTING SUBSTANCE OF THE EXCISED WHEAT ROOTS III. EFFECTS OF TRYPTOPHAN AND SOME RELATED SUBSTANCES

# STUDIES ON THE GROWTH PROMOTING SUBSTANCE OF THE EXCISED WHEAT ROOTS

## III. EFFECTS OF TRYPTOPHAN AND SOME RELATED SUBSTANCES

By

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(*Received December 26, 1961*)

In previous papers (28, 29), the beneficial effect of peptone on the growth of excised wheat roots was demonstrated and some characteristics of the active substance were described. An attempt was made in this paper to clarify the beneficial constituent of peptone and a further purpose of the work was to illustrate the mechanism of its action on the growth of excised wheat roots.

The experimental materials were the root tips of wheat (*Norin No.* 55). General culture techniques of the excised roots are not described here, as they are the same as in the previous paper (28). The other methods necessary for the experiments are given elsewhere.

### Methods and Results

1. *Influences of amino acids, especially dl-tryptophan on the growth of excised wheat roots.*

Amino acid composition of peptone is presented in Table 1, of which the right column gives the amounts of individual amino acids supplied by 300 mg peptone. The effect of amino acid mixture on the growth of excised roots, at first, was investigated in culture conditions of the dark and the light (Table 2). Thirteen amino acid tested were as follows; *l*-arginine (Arg.), *l*-histidine (His.), *l*-lysine (Lys.), *l*-phenylalanine (Phe.), *dl*-threonine (Thr.), *dl*-tryptophan (Try.), *dl*-methionine (Met.), *l*-leucine (Leu.), *l*-isoleucine (Isoleu.), *dl*-valine (Val.), *l*-glutamic acid (Glu.), *l*-aspartic acid (Asp.) and glycine (gly.). Amino acids were employed in amounts corresponding to 150 and 300 mg of peptone per liter which is the optimal amount for the growth. Cultures in the light were run in the intensity of 500~1,000 Lux from day light fluorescent tubes. As

shown in Table 2, amino acid mixture is notably beneficial in the light for the increment of root weight, which mainly is due to the development of lateral roots and to corpulence of the main axis. In darkness, however, the mixture seems not to exert satisfactorily its beneficial effect. If the excised roots are continuously exposed to the light immediately after inoculation with the root tips, however, harmful effect on the elongation of main axis occurs evidently in the concentration of mixture corresponding to the optimal amount of peptone. This harmful effect by amino acid mixture occurs no longer, if the older excised roots grown previously in darkness for the first six days or more are exposed to the light.

**Table 1.** Amino acid composition of peptone hydrolysate*

| Amino acids | Dry weight (%) | Dry weight in 300mg pepton (mg) |
|---|---|---|
| Total $\alpha$-amino acid nitrogen | 8.9 | 26.6 |
| Arginine | 4.8 | 14.4 |
| Histidine | 0.7 | 2.1 |
| Lysine | 7.7 | 23.1 |
| Phenylalanine | 4.7 | 14.1 |
| Threonine | 2.6 | 7.8 |
| Tryptophan | 0.84 | 2.5 |
| Methionine | 0.84 | 2.5 |
| Leucine, Isoleucine | 4.5 | 13.5 |
| Valine | 5.2 | 15.6 |
| Other amino acid nitrogen | 3.5 | 10.5 |

* The data were supplied by the *Kyokuto Medical Co., INC*, to which the writers' thanks are due.

**Table 2.** Influence of 13 amino-acid mixtures on the growth in the dark and the light. Culture time ; three weeks.

| Light condition | In the dark | | | In the light | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Illuminated after 6 days of inoculation | | | Illuminated immediately after inoculation | | |
| Concentration of the mixture | 0 | 1/2×SD | SD* | 0 | 1/2×SD | SD* | 0 | 1/2×SD | SD* |
| Main axis length (cm) | 16.9 | 18.2 | 19.0 | 17.5 | 19.2 | 22.0 | 18.7 | 23.9 | 4.9 |
| Root diameter (mm) | 0.21 | 0.28 | 0.26 | 0.28 | 0.36 | 0.40 | 0.23 | 0.43 | — |
| Fresh weight per 10 roots(mg) | 188 | 246 | 321 | 190 | 402 | 1043 | 265 | 615 | 851 |
| Dry weight per 10 roots(mg) | 16.8 | 29.0 | 36.2 | 17.4 | 46.3 | 130.7 | 28.2 | 81.8 | 120.5 |

* Concentration of amino acid mixture presented in the right column of Table 1. In place of "other amino acid nitrogen" in Table 1, aspartic acid, glutamic acid and glycine were respectively employed in concentration of 10 mg per liter. Leucine and isoleucine were added in the equal amount.

The effects of the sole addition of amino acids to the basal medium and of sole omission from the control of 13 amino acid mixture were shown in the experiments recorded in Fig. 1 and 2 respectively. Individual amino acid were employed in amount corresponding to 150 mg peptone per liter. The light from fluorescent tubes was continuously illuminated in intensity of 500~1000 Lux from the fifth day of inoculation with root tips.

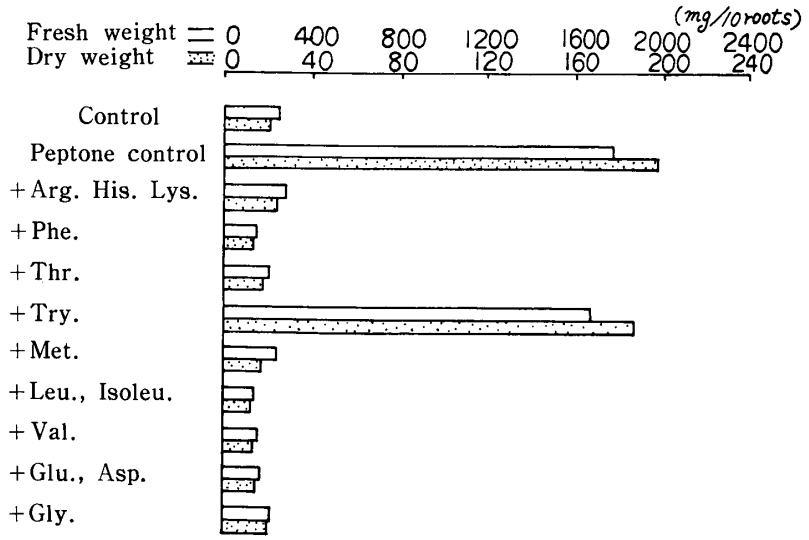



Fig. 1. Effects of individual amino acids on the growth of excised wheat roots: One or few amino acids were added to the basal medium. Culture time; one month. Cultures in peptone control were run in the dark.

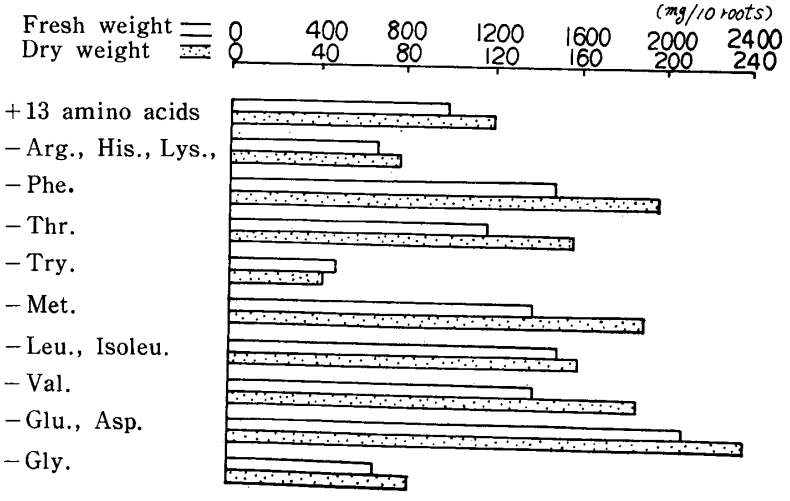


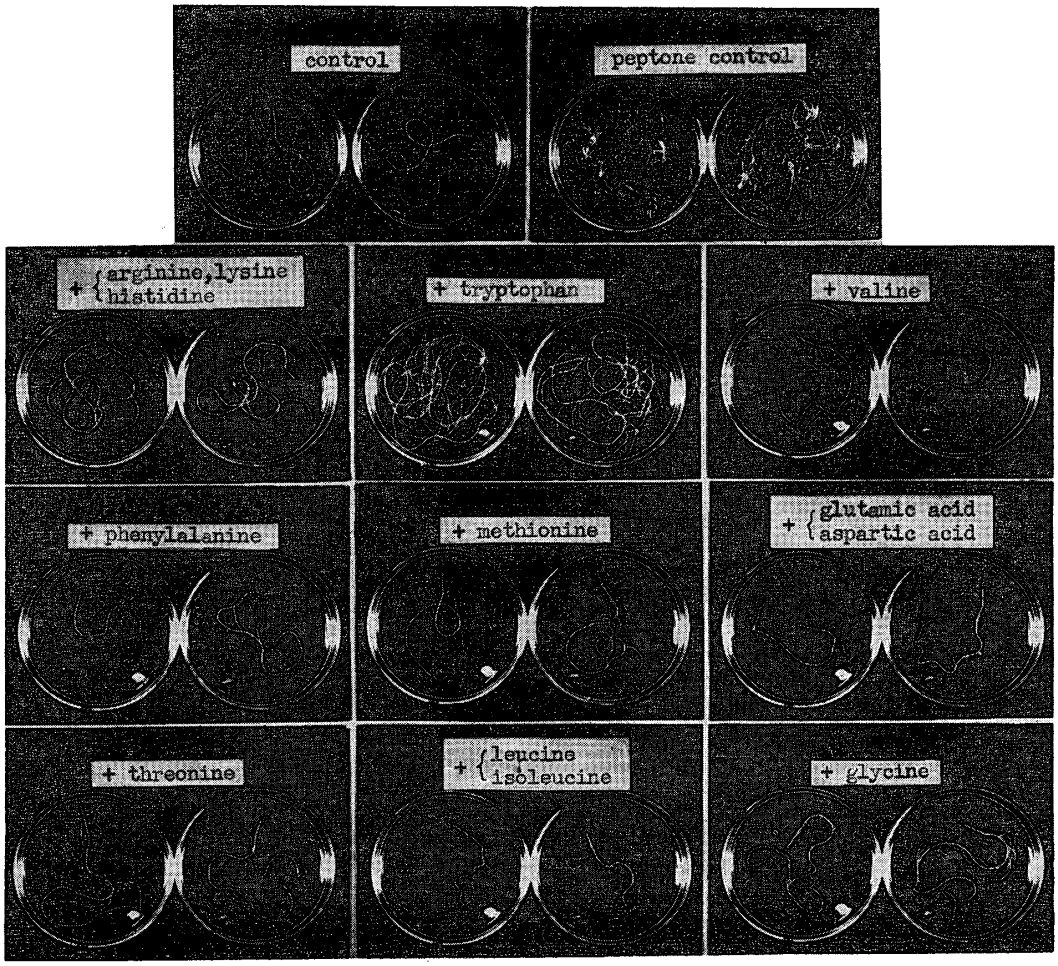

Fig. 2. Effects of individual amino acids on the growth of excised wheat roots; One or few amino acids were omitted from the control of 13 amino acid mixture. Culture time; one month.

In Fig. 1 and 2 are presented the data of this experiment, showing that of amino acids tested *dl*-tryptophan is peculiarly stimulous, and that the growth in the presence of tryptophan is approximately comparable to that in the control

solution containing peptone. Phenylalanine, leucine-isoleucine and valine, however, are toxic at the concentration tested (Fig. 1). In spite of the presence of *dl*-tryptophan, on the other hand, the growth in omission of glycine or arginine-histidine-lysine is inferior to that in amino acid mixture (Fig. 2), although noneffective by the sole addition of them.

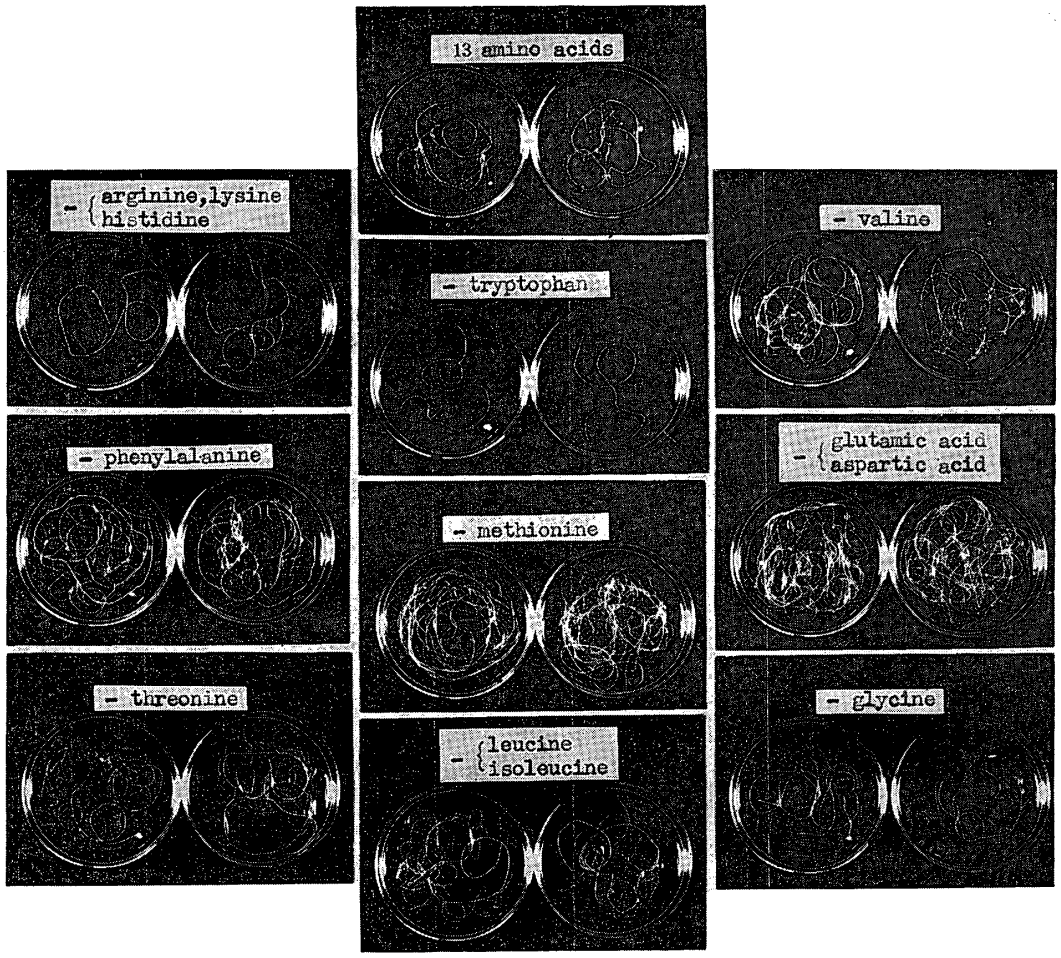
Skinner and Street (43) have reported that the optimum growth of the excised groundsel roots is obtained in media containing glycine together with alanine or lysine. According to Fries (9), in decotylized pea seedling maintained aseptically in darkness, root growth very soon ceased, but is maintained normally by adding arginine, glycine and adenine to the nutrient medium. On the effects of some amino acids exclusive of tryptophan on the growth of the excised wheat root, further experiments accordingly may be necessary.

From a comparison of the data between Fig. 1 and 2, the amino acids other than tryptophan also appear to be slightly stimulous in their mixture, which are individually noneffective or toxic. Sander and Barkholder (41) have reported that the growth values of Datura embryos for lesser mixtures and for single




Photograph 1. Growth of excised wheat roots in the medium containing amino acids. Culture time ; one month.

amino acid are well below those for the 20 amino acids. Our experimental results on amino acid effects described above also may be regarded as a complementary action or an antgonistic action among amino acids.




Photograph 2. Growth of excised wheat roots in omission of amino acids from the control of 13 amino-acid mixture. Culture time ; one month.

2. *Relation between concentrations of added tryptophan and intensity of illumination during culture.*

It was found that continuous low intensity illumination from fluorescent tubes enhances the benefical effect of tryptophan on the excised root growth. It was often observed, however, that a few main axis tips in the light are abnormally thickened or swollen by the addition of tryptophan, and that the growth ceases by the elapse of culture time. Then the relation through the root growth between concentrations of added tryptophan in the medium and intensity of illumination was investigated. The results of the experiment are given in Fig. 3. The retardation of the main axis growth by the excessive amount of tryptophan is more serious in the higher intensity of illumination. The amount of tryptophan 0.5 mg per liter, for example, does not inhibit the

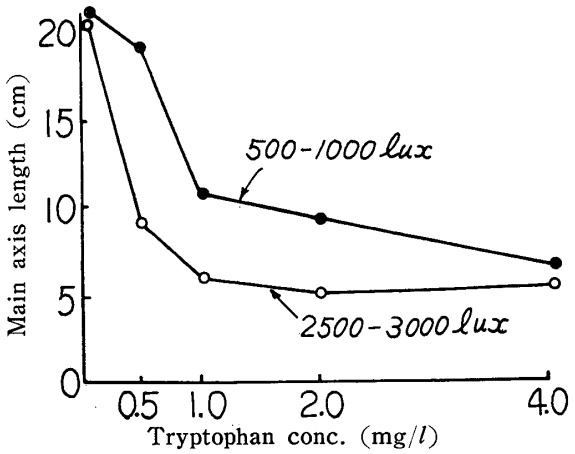



Fig. 3. Influence of various concentrations of tryptophan in different intensity of the light. Illumination was continuously carried out after 2 days of inoculation with 1 cm root tips. Culture time ; three weeks.

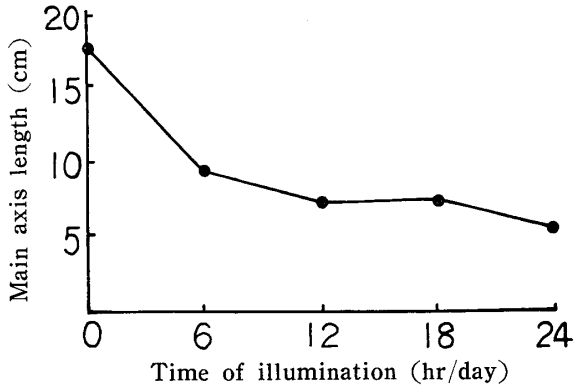



Fig. 4. Influence of lengh of illumination time per day on the tryptophan effect. Starting illumination of light (500~1000 Lux) was same as that in Fig. 3. Tryptophan concentration; 2.0mg per liter. Culture time; Three weeks.

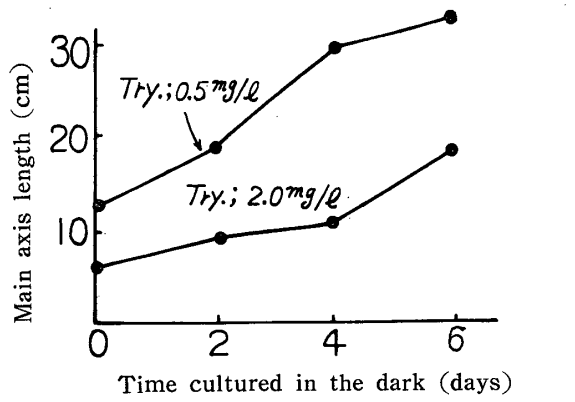



Fig. 5. Relation between the time of culture to initiate the illumination and the tryptophan effect. Culture time ; three weeks

growth of the main axis in the light intensity of 500~1000 Lux, but retards evidently in a higher intensity of 2500~3000 Lux.

Fig. 4 shows the influence of lengh of illumination time per day on tryptophan effect. Retardation of the main axis growth in the presence of tryptophan occurs evidently by exposure to the light of six hours per day, increasing in proportion to length of illumination time per day.

It is apparently inferred from two experimental results demonstrated above that inhibition in the main axis growth by excessive addition of tryptophan is apt to bring about with an increasing degree of illumination. Hence, in order to obtain active growth of main axis and lateral without the abnormal corpulence of main axis tips, relatively weak light for higher concentration of tryptophan or strong light for its lower concentration should be illuminated during the culture.

As shown in Fig. 5, the tryptophan effect also is changed by the difference of illumination period. If the illumination is continuously carried out imediately after the inoculation witn the root tips, the main axis tips swell abnormally and the growth ceases in a relatively early stage. Excised from 2 or 3 day-old seedling and grown previously in darkness during about five days or more, retardation of the main axis

growth in a given amount of tryptophan occurs no longer by illumination in the subsequent stage of culture time. This seems to indicate that the physiological nature of the excised roots changes with age of root tissue.

Sensitivity of the main axis tips to peptone as well as tryptophan is also increased by the illumination. In amount of 300 mg per liter, peptone does not inhibit the growth of the main axis tip in darkness, but exerts very effectively. The same amount of peptone, on the contrary, inhibits highly the growth of the main axis in the light. A great difference in effectiveness between peptone and tryptophan therefore may be in the point that the former exerts stimulously even in darkness and the latter does not exert in such a culture condition.

**Table 3.** Influence of various concentrations of tryptophan on the growth of excised wheat roots. Culture time; three weeks.

| | Try. conc. (mg/*l*) | Main axis length (cm) | Fresh wt. (mg/10roots) | Dry wt. (mg/10roots) | Nitrogen content (mg/10roots) | Nitrogen content (%) |
|---|---|---|---|---|---|---|
| In the dark | 0 | 22.0±3.5 | 184 | 15.7 | 0.54 | 3.74 |
| | 0.5 | 25.5±2.8 | 518 | 43.0 | 0.71 | 3.99 |
| | 1.0 | 24.6±3.6 | 452 | 35.5 | 1.4 | 3.97 |
| | 2.0 | 23.0±4.5 | 737 | 64.3 | 2.4 | 3.86 |
| | 4.0 | 15.3±1.1 | 807 | 91.7 | 2.36 | 2.58 |
| In the light | 0 | 24.2±2.7 | 348 | 23.6 | 0.95 | 4.00 |
| | 0.5 | 35.5±6.2 | 1103 | 118.0 | 4.37 | 3.70 |
| | 1.0 | 25.8±3.2 | 975 | 102.4 | 4.06 | 3.96 |
| | 2.0 | 16.5±2.0 | 1083 | 116.0 | 3.43 | 2.95 |
| | 4.0 | 10.8±1.2 | 580 | 77.5 | 1.96 | 2.52 |

As the relationship between tryptophan effect and illumination was clarified, the optimal concentration of tryptophan was subsequently investigated under a given light condition to proceed the next expriment. The results are given in Table 3. The light from fluorescent tubes was illuminated in the intensity of 500~1000 Lux after grown previously for the first six days in the dark. The beneficial effect of tryptophan in the dark is not very notable as compared with that in the light, although the root weight increases somewhat with the amount of tryptophan. Inferring from the main axis length and nitrogen contents of the excised roots, the optimal concentration of *dl*-tryptophan seems to be in the relatively narrow range of 0.5 to 1.0 mg per liter in the light, while in approximatelly 2.0 mg per liter in the dark. Injurious effect in the excessive amount of tryptophan on the main axis growth is observed somewhat at 2.0 mg per liter and heavily at 4.0 mg per liter in the light, whereas observed at 4.0 mg per liter in darkness. Root weight, which represents predominantly the lateral root growth, increases in proportion to the amount of tryptophan, in which the growth in length of the main axis is inhibited. This fact suggests

that the optimal concentration of tryptophan for the growth of main axis tips is defferent from that for the development of laterals.

According to the results obtained above, subsequently the excised roots were grown in the medium containing tryptophan in a concentration of 1.0 mg per liter, and the respiratory rate of cultures, which is marked as a synthetical indication of metabolic activity, was estimated at the second week and fourth week of culture with a Warburg manometer. As shown in Fig. 6, supplement of tryptophan allow the respiration activity of the excised roots to maintain more constantly over the culture time of the second to the fourth week. Not only the superficial growth, but also energy metabolism in the excised root tissue would be highly enhanced through the longer time of culture by supplement of tryptophan.

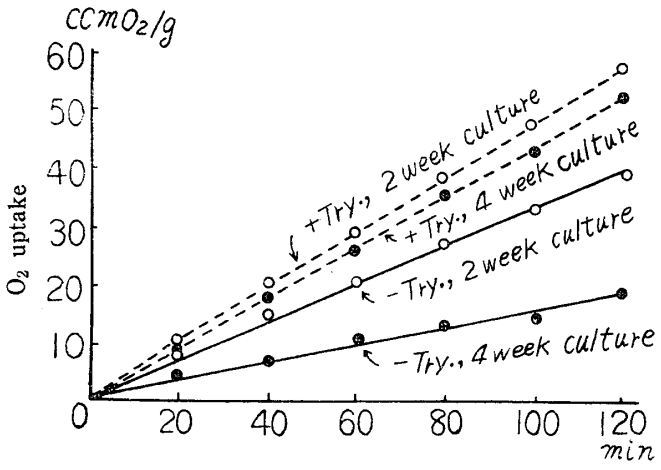



Fig. 6. Influence of tryptophan on respiration of the excised roots. Illumination (500~1000 Lux) was continuously carried out after grown previously for the first five days at 25°C in the dark. Concentration of tryptophan ; 1.0mg per liter.

Studies on the influence of light on the root growth have been reported in many papers, but the results obtained are not always the same. Active production of laterals in the light was observed by White (58) and Almestrand (2). According to Torrey (54), however, lateral roots were highly produced in darkness and inhibited by illumination of white light. Street (47) reported that the main axis of excised tomato root tips grew actively under continuous weak light, but lateral formation was inhibited. In addition to these, there are some reports that the light is beneficial (7, 24, 36), noneffective (37) or harmful (35, 42) for root growth. This discrepancy may be caused by unequalities in way to express the effect and probably also by the difference in the various plant materials or the culture conditions. According to our experimental results, the different effects of the light obtained by these workers may be partially able to explain as dependence on amount of endogenous tryptophan or its derivatives in root tissue employed as experimental materials.

3. *Stability of tryptophan on heating and illuminating in aqueous solution.*

Tryptophan is generally known as one of labile amino acids on heating or illuminating in aqueous solution. Influence of heating on tryptophan has been studied very well in connection with the investigations on the acid hydrolysis

of protein (1, 19, 30, 39, 60). These results may be summarized as follows. Tryptophan is stable in the usual condition of acid hydrolysis, but is unstable in the presence of glucose or some amino acids, changing to new substance such as other amino acids or sugar-tryptophan complex.

Tryptophan in aqueous solution can be destroyed by illuminating as well as heating. Obata and Sakamura (27, 40) have isolated several products resulted by the photolysis of tryptophan. Berthelot *et al.* (3) reported on the conversion of tryptophan to β-indoleacetic acid (IAA) by photochemical action.

In our experiment, sterilization of the medium containing glucose and tryptophan with inorganic substances and B-vitamins has been carried out at 115°C for 15 min by autoclaving. Furthermore, the excised roots were grown in the light from day light fluorescent tubes. Hence it should be suspected whether the tryptophan effect described in the previous section is due to the secondary action through chemical change of tryptophan in the medium by two artificial factors, heating and illuminating.

(a) *Decomposition of tryptophan on autoclaving in the medium*

Modifying the basal medium, glucose or sucrose was added in concentration of two per cent to aqueous solution containing *dl*-tryptophan. Hydrogen ion concentration of the solution was adjusted to pH 6.4 by phosphate buffer. The amount of tryptophan which was remained in the solution after autoclaving, was determined by colorimetory using *p*-dimethylamino benzaldehyde (45).

**Table 4.** Decomposition of *dl*-tryptophan in sugar solution on autoclaving. Numerals in table show the remaining amount (mg/cc) of tryptophan after autoclaving. Numerals in parenthesis show decomposed ratio (%) of tryptophan.

| Autoclaving | In water | | In 2% glucose | | In 2% sucrose | |
|---|---|---|---|---|---|---|
| | Tayptophan concentration (mg/cc) | | | | | |
| | 0.50 | 1.00 | 0.50 | 1.00 | 0.50 | 1.00 |
| 110°C | — | — | 0.45 | 0.87 | 0.50 | 0.96 |
| 10 min | — | — | (9.1) | (13.2) | (0.0) | (3.4) |
| 120°C | 0.50 | 1.0 | 0.24 | 0.49 | 0.48 | 0.96 |
| 20 min | (0.0) | (0.0) | (52.8) | (50.8) | (3.2) | (3.4) |

The results are presented in Table 4. In the presence of sucrose as well as in water, tryptophan is not almost decomposed by autoclaving at 120°C, for 10 min. In the glucose solution, on the other hand, about ten per cent and about 50 per cent of added tryptophan are decomposed by autoclaving at 110°C for 10 min and at 120°C for 20 min respectively. In addition to tryptophan glucose is correspondingly decomposed by autoclaving (Table 5).

Fig. 7 shows the growth of the excised roots in media which are previously sterilized by filtration through Berkefeld Candle and by autoclaving. The

**Table 5.** Influence of the presence of *dl*-tryptophan on heat decomposition of sugar. Numerals in table show the remaining amount (mg/cc) of sugar after autoclaving. Numerals in parenthesis show decomposed ratio (%) of sugar.

| Autoclaving | Glucose (20.0 mg/cc) | | | Sucrose (20.0 mg/cc) | | |
|---|---|---|---|---|---|---|
| | Tryptophan concentration (mg/cc) | | | | | |
| | 0.0 | 0.80 | 1.00 | 0.0 | 0.50 | 1.00 |
| 110°C 10 min | 2.0 (−1.0) | 19.5 (2.5) | 19.2 (4.0) | 19.9 (1.5) | 20.2 (−1.0) | 20.4 (−2.0) |
| 120°C 20 min | 18.1 (9.5) | 18.2 (9.0) | 17.5 (12.5) | 20.0 (0.0) | 19.8 (1.0) | 19.9 (0.5) |

growth in the autoclaved medium is evidently inferior to that in the filtrated medium. Decrement in the growth is more notable in the medium seriously autoclaved at 120°C, for 20 min, and appears to relate on the amount of tryptophan destroyed previously in the medium. This finding suggests that the tryptophan effect is not due to the production of new secondary substance like other amino acid or tryptophan-glucose complex which may occur from tryptophan by autoclaving.

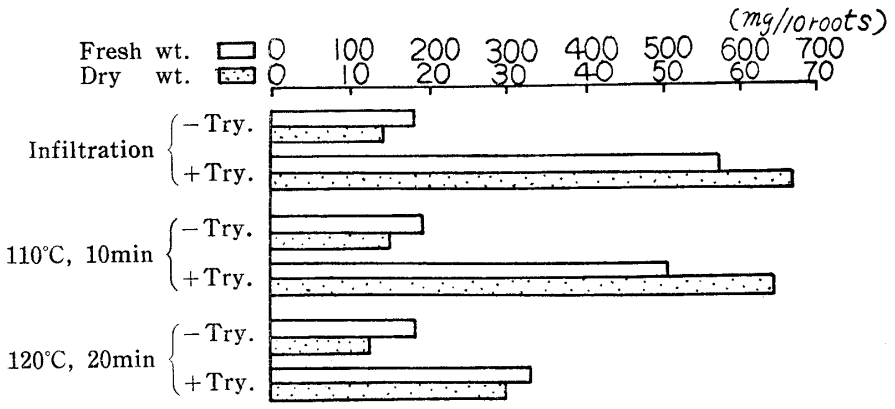



Fig. 7. Change in the tryptophan effect by autoclaving.

According to Roberts and Street (38), tryptophan is very stimulous for the growth of excised rye root, being activated during autoclaving or when refluxied in distilled water. Our result on the excised wheat roots seems to be different from theirs. Naylar and Rappaport (25) have reported that *l*-cystine and *l*-tryptophan in combination support growth of excised pea roots at a level above the control. According to Sahyum (39) dehydration of tryptophan by heating is cospicuous in the presence of cystine. Hence the result of Naylar *et al.* as well as that of Roberts *et al.* may be due to result of activation of tryptophan by heating.

(b) *Destruction of dl-tryptophan by illumination.*

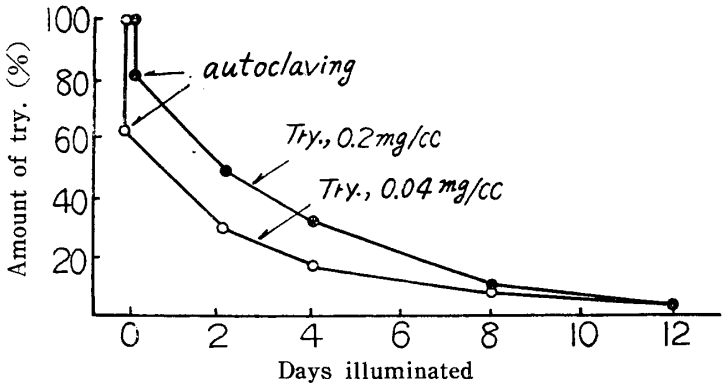



Fig. 8. Destruction of tryptophan on the light from daylight fluorescent tubes.

Destruction of tryptophan in aqueous solution by illumination is shown in Fig. 8. Flasks containing the sterilized tryptophan solution were exposed to the artificial light of about 5000 Lux. The amount of tryptophan diminishes gradually with time and is not detected at all in the solution after 12 days of continuous illumination.

Subsequently whether tryptophan is activated by exposure the medium to the light was investigated (Fig. 9). The culture medium containing tryptophan in the concentration of 1 mg per liter was exposed to the light of 500~1000 Lux during various periods of time, being inoculated with the excised root tip and incubated at 25°C in darkness for three weeks. Tryptophan in the solution, however, is almost stable under such a light condition as shown in Fig. 10.

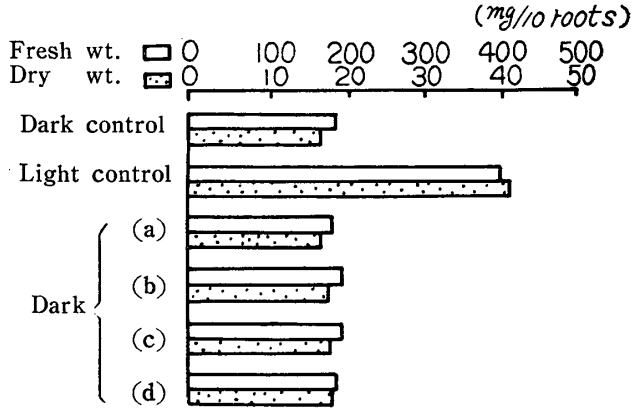



Fig. 9. Excised root growth in the medium to which the light was previously exposed. (a), (b), (c) and (d) in Fig., show medium illuminated previously during 1, 2, 4, and 8 days respectively. "Dark" and "Light" indicate the light condition during culture.

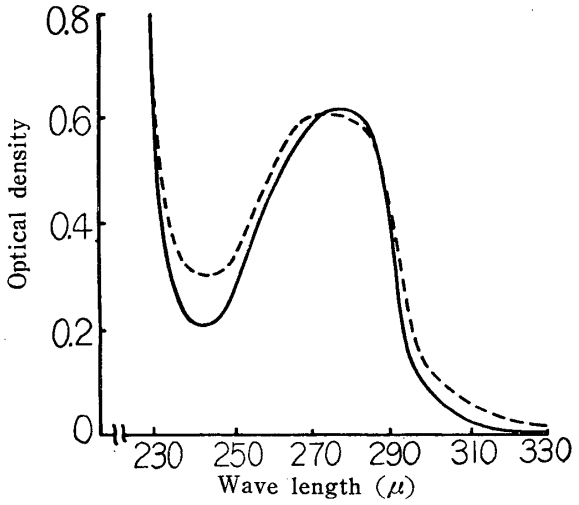



Fig. 10. Ultraviolet absorption spectrum of tryptophan. A dotted line: curve of tryptophan exposed to light of 500~1000 Lux for eight days.

As shown in Fig. 9, tryptophan is noneffective in darkness in spite of the previous illumination to the medium, but only in the light during culture.

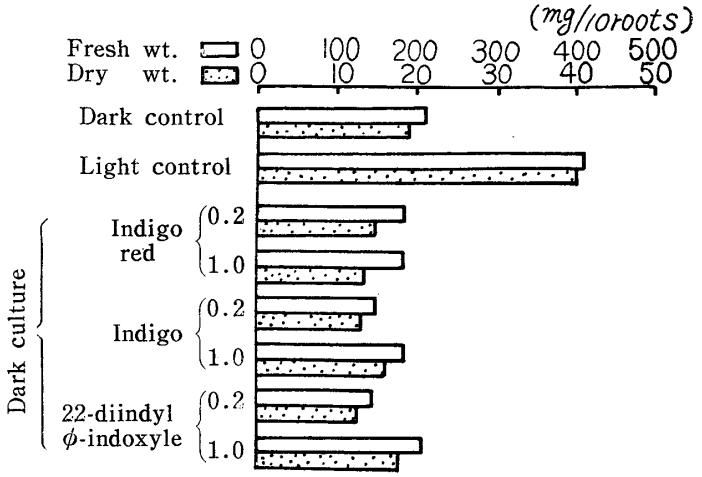



Fig. 11. Influences on the growth of some photochemical products of tryptophan.

Fig. 11 shows influences on the growth of some substances isolated as photochemical products of tryptophan. In darkness all of them appear to be noneffective on the growth. In addition, β-indoleacetic acid, which is regarded as a photochemical product of tryptophan (3), is not beneficial within the range of concentrations of $10^{-5}$ to $10^{-1}$ μg per liter in darkness.

It may be concluded that the beneficial effect of tryptophan on the growth of the excised roots is not due to the indirect action through chemical change of tryptophan in the medium by illuminating during culture.

4. *Effects of some substances related to tryptophan on the excised root growth.*

If the promoting effect of tryptophan on the excised root growth was not due to the secondary substance possibly produced by heating or illuminating, tryptophan taken up by roots would exert its promoting effect through some metabolic pathway. To clarify the biochemical movement in organism, effects of several substances related metabolically to tryptophan were previously investigated.

The excised root tips were grown in darkness during the first five days and then were continuously exposed to the light of 500~1000 Lux from day light fluorescent tubes during the final 17 days of three weeks culture time. The basal media containing tryptophan in the amount of 1 gm per liter were always employed as the control series.

(a) *L-kynulenine, anthranilic acid and nicotinic acid.*

In microorganism and animal tissue, enzymatic conversions of tryptophan to anthranilic acid or nicotinic acid, in which kynulenine is present as a intermediate, have been well known (44). In higher plants, on the other hand,

Nason (22, 23) has demonstrated that germinating corn embryos can synthesize nicotinic acid when supplied with tryptophan, and Gustafson (16) also has obtained the similar result in the embryo culture of maize.

In the basal medium, in which nicotinic acid is contained at a concentration of 0.5 mg per liter, the growth is evidently inferior to that in the same medium containing tryptophan. Then it is previously assumed that the tryptophan effect would not be due to the result of conversion of tryptophan to nicotinic acid.

Omission of nicotinic acid from the basal medium does not result in the decrement of the growth in the culture time of three weeks. Nicotinic acid is noneffective at a concentration equivalent in molarity to the optimal amount of tryptophan (about $5 \cdot 10^{-6}$ Mol) and is harmful in increasing the amount of nicotinic acid by eight times. Anthranilic acid and *l*-kynulenine are also non-effective within the range of 0.1 to 4.0 mg per liter.

Thus it seems to be difficult to illustrate the beneficial effect of tryptophan through the metabolic pathway of tryptophan → kynulenine → anthranilic acid or tryptophan → kynulenine → nicotinic acid.

(b) *Indole and serine*

Tatum and Bonner (52, 53), using a strain of Neurospora mutant, demonstrated the course of the synthesis of tryptophan by condensation of indole and serine. Wildmann *et al.* (56) suggested that the tryptophan synthesis in the higher plant also may follow the pathway determined for Neurospora.

**Table 6.** Influence on the growth of indole and *dl*-serine. Culture time ; 16 days.

| Concentration $\times 5 \cdot 10^{-6}$M | Main axis length (cm) | Fresh wt. (mg/10 roots) | Dry wt. (mg/10 roots) |
|---|---|---|---|
| Control | 19.2 | 185 | 15.8 |
| Try. 1.0 | 20.4 | 389 | 40.5 |
| Serine | | | |
| 0.1 | 18.5 | 182 | 16.0 |
| 0.5 | 15.5 | 143 | 13.8 |
| 1.0 | 16.4 | 159 | 14.8 |
| 2.0 | 14.5 | 161 | 14.3 |
| 4.0 | 15.7 | 157 | 16.0 |
| Indole | | | |
| 0.1 | 19.5 | 179 | 15.4 |
| 0.5 | 12.4 | 127 | 11.1 |
| 1.0 | 12.9 | 135 | 12.2 |
| 2.0 | 11.4 | 120 | 13.5 |
| 4.0 | 10.4 | 108 | 11.7 |
| Indole + Serine | | | |
| 0.1 | 17.9 | 185 | 15.6 |
| 0.5 | 16.5 | 150 | 13.4 |
| 1.0 | 9.4 | 88 | 8.3 |
| 2.0 | 6.3 | 96 | 10.4 |
| 4.0 | 8.2 | 102 | 12.1 |

Table 6 shows the influences of indole and *dl*-serine on the growth. The cultures were exposed to the light after five days. Even if added in mixture they were noneffective or injurious in any of the concentration tested during 16 days of culture time. Wheat root tissue may be absent in ability to synthesize tryptophan from indole and serine. Even if synthesized, its amounts may not be so much as exerting the promoting effects.

(c) *β-indoleacetic acid*

It has been well known that in higher plants tryptophan converts enzymatically to β-indoleacetic acid (IAA). Table 7 shows the effect of IAA on the excised root growth in the dark and in the light. IAA as well as

**Table 7.** Influence on the growth of β-indoleacetic acid. The excised roots were exposed to the light (500~1000 Lux) after five days of inoculation with root tips. Cuture time; 40 days.

| IAA conc. ($\mu g/l$) | Cultured in the light | | | Cultured in the dark | | |
|---|---|---|---|---|---|---|
| | Main axis (cm) | Fresh wt. (mg/10roots) | Dry wt. (mg/10roots) | Main axis (cm) | Fresh wt. (mg/10roots) | Dry wt. (mg/10 roots) |
| 0.5 | 23.2±2.1 | 319 | 25.7 | 24.5±3.4 | 175 | 13.7 |
| 1.0 | 29.3±4.5 | 341 | 36.5 | 20.5±4.1 | 162 | 15.1 |
| 2.0 | 47.5±7.4 | 901 | 98.2 | 15.1±2.2 | 140 | 14.7 |
| 4.0 | 38.5±8.3 | 1215 | 141.4 | 10.0±2.5 | 204 | 22.0 |
| 6.0 | 19.8±2.3 | 643 | 68.0 | 12.2±1.2 | 194 | 21.6 |
| 8.0 | 8.8±1.3 | 403 | 44.5 | 10.4±2.5 | 230 | 26.0 |
| 10.0 | 7.8±1.5 | 298 | 31.5 | 8.4±0.7 | 204 | 26.3 |
| Try.(mg/$l$) | | | | | | |
| 0 | 24.3±1.9 | 314 | 24.6 | | | |
| 0.5 | 41.4±6.5 | 1114 | 124.5 | | | |

tryptophan is very beneficial for the growth of excised wheat roots in the light from fluorescent tubes, but almost not beneficial in darkness. The optimal concentration of IAA in the light seems to be in a narrow range of 2.0 to 4.0 $\mu$g per liter and to be lower as compared with that of tryptophan 0.5—1.0 mg per liter. In time course of growth, the lag period is observed to occur at a appropriate concentration of IAA as well as peptone or tryptophan. IAA is not almost effective on the growth in amount below 1.0 $\mu$g per liter, while at and above a concentration of 10 $\mu$g per liter the tips of excised roots swell abnormally and produce unusually many root hairs to cease the growth.

Effect of auxin on excised root growth has been studied by many workers (59). A detailed description on this problem is not given here. There are some evidences that various roots tissues have efficiency to produce auxin by the roots themselves (21, 31), and so that the excised roots are able to grow

during a longer time without IAA in the basal mdium. Geiger-Huber and Burlet (12) and Fiedler *et al.* (8), however, have demonstrated that the growth of excised maize roots are slightly enhanced by the addition of IAA within the concentration range of $10^{-9}$ to $10^{-12}$ Mol. The beneficial effect on the excised maize root growth is more slight than that found on the excised wheat roots by us. Although a similar stimulous effect of IAA on the excised rye root have been found by Roberts and Street (38), they have not emphasized the necessity of illuminating during culture.

(d) *d-* and *l-tryptophan*

To determine the effect of tryptophan on the growth of excised roots, racemic mixture of tryptophan was employed up to the present. *l-* and *d-*tryptophan which are components of racemec mixture exert the same beneficial effect as *dl*-tryptophan in concentration of 0.5 to 1.0 mg per liter. Two optical isomers are unable to be distinguished by the differences in the physiological action.

5. *Enzymatic conversion of tryptophan to auxin by excised wheat roots.*

It was demonstrated that on the beneficial effect on the growth of excised wheat roots tryptophan is replaced with IAA which is closely related with tryptophan in metabolic pathway. Numerous authors have reported to produce auxin from tryptophan in a wide variety of plant tissues such as leaves, stem, coleoptile, ovaries etc. (13, 17, 20, 26, 57). It is said that the tissue in which a higher concentration of auxin is contained is particularly rich in the enzyme system on conversion of tryptophan to IAA (4). In the root tissue in which auxin is generally contained in lower concentration, so that the enzyme system is inferred to be relatively scarce (45). The results employing the excised root cultures as the experimental material, however, have not been reported so far as we know. In this section, therefore several experiments were performed to determine if tryptophan is converted enzymatically to IAA in the wheat root tissue.

The experimental materials and methods were as follows. Ten g fresh roots cut from 5-day-old wheat seedling were placed in 100 cc of 0.1 *M* phosphate buffer (pH 8.8), containing 10 mg of *l*-tryptophan. They were thoroughly infiltrated with repeated evacuation, after which were allowed to incubate with the solution for five hr at 25°C. According to the excised root culture condition, the roots in the 300 cc Erlenmeyer flask were exposed to the light from fluorescent tubes during incubation at 25°C. At the end of the periods of time, the roots were thoroughly washed with deionized water and lyophilized. The dry matter samples of 200 mg were cut finally with scissors, and then ether extractable substances in acid- and neutral-fraction were chromatographed separately in isopropanol-ammonia-water (90 : 10 : 10) (V/V) solvent.

Fig. 12 and 13 show the location on the paper chromatograms of auxin which is assayed with the avena straight method. In the acid fraction of Fig. 12, around *Rf* 0.3—0.5 corresponding with the Rf of the IAA spot, avena test positive substance is located more in the tryptophan-treated roots than in the untreated roots, in which endogenous auxin only is found. In the neutral fraction of Fig. 13, on the other hand, the region promoting avena elongation in the test is hardly marked in any part of the paper chromatogram.

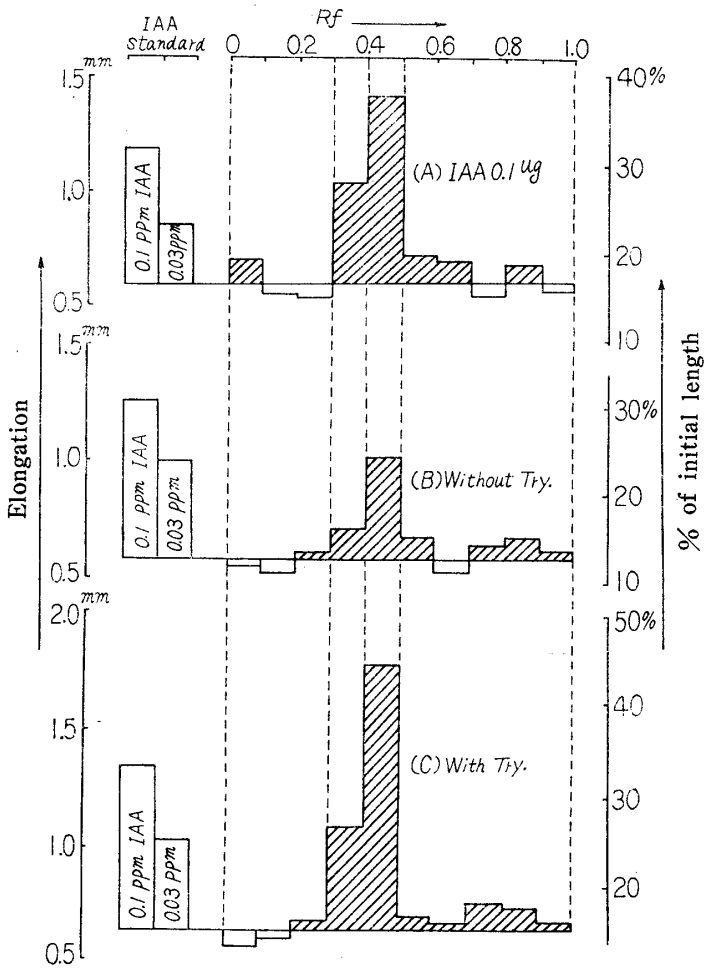



Fig. 12. Location on the chromatograms and activity in the coleoptile sections on the growth substance extracted from wheat root tissue with ether. —Acid fraction—

Using cultures of excised wheat roots as experimental material, subsequently, the formation of auxin from added tryptophan was confirmed as follows. After three weeks of the culture in the light, different amounts of tryptophan were sterilizingly added to the media to make various concentrations, and the excised roots were grown furthermore during four days. In such high concentrations as $10^{-1}$ mg and 1.0 mg per cc, a number of excised roots were brown in color and dead, and those which were alive did not grow apparently during

this time. As shown in Fig. 14, the formation of auxin from added tryptophan is observed only in the excised roots supplied with much amount of tryptophan. Not only in root tissue, but also in the medium, auxin is detected is an amount corresponding with that in the root tissue. This phenomenon would be due to

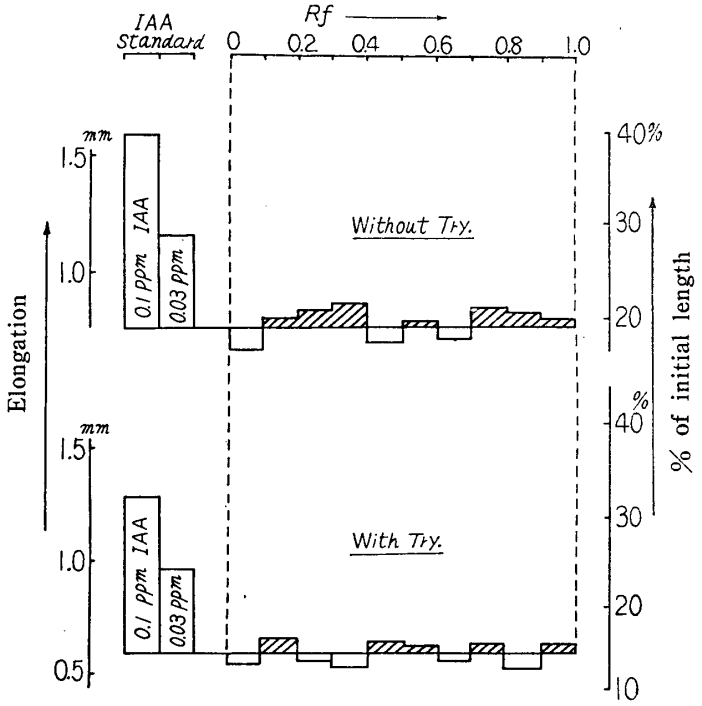



Fig. 13. Location on the chromatograms and activity in the coleoptile sections of the growth substance extracted from wheat root tissue with ether. —Neutral fraction—

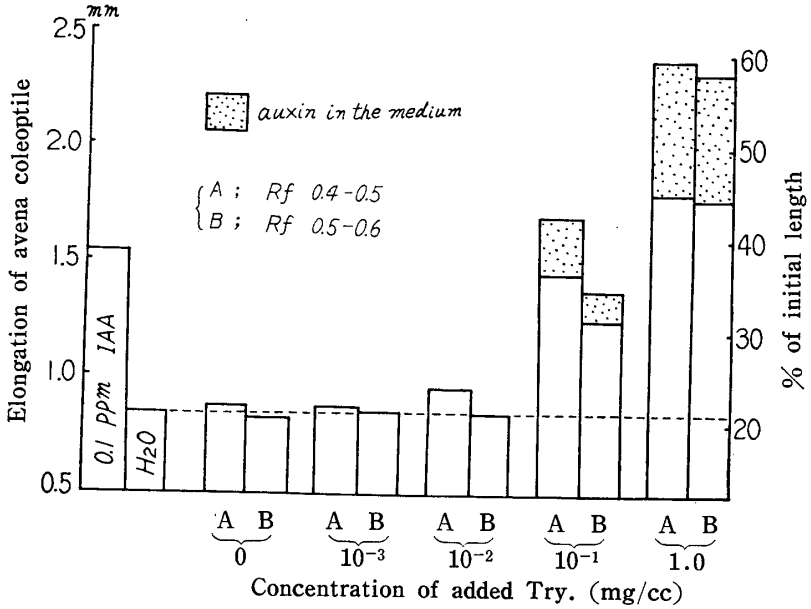



Fig. 14. Formation of auxin from added tryptophan by the excised growing roots.

release from the growing roots to the medium of auxin, which was probably formed from added tryptophan in the root cells.

To obtain *in vitro* conversion of tryptophan to auxin, the following experiment using cell free wheat root extracts was performed (Table 8).

**Table** 8. Formation of auxin from added tryptophan by cell-free extracts of wheat seeding roots.

| Boiling | Addition of Try. (1mg/cc) | Length of avena coleoptile(×0.1mm) | $\times 10^{-4}$mg IAA |
|---|---|---|---|
| (A) Cell free extract; 2 cc. Reaction time; 5 hr | | | |
| − | − | 46.5±1.5 | 0 |
| − | + | 56.8±3.0 | 0.57 |
| + | − | 47.8±1.0 | 0 |
| + | + | 47.6±1.1 | 0 |
| (B) Cell free extract; 5 cc. Reaction time; 2 hr | | | |
| − | − | 48.2±1.8 | 0 |
| − | + | 60.8±2.5 | 0.77 |
| + | − | 47.6±1.6 | 0 |
| + | + | 50.8±0.9 | 0.20 |

Ten g fresh roots from 6-day-old wheat seedling were ground with a little amount 1/10 *M* phosphate buffer (pH 8.0) in a blendor, centrifuged for 10 min at 4,000 RPM and made up to 100 cc with the same buffer solution. This solution was applied as a crude enzyme preparation. All of the preparations such as grinding of the tissue, centrifugation etc. were carried out in a cold room at 0°C.

The data presented in Table 8 show that added tryptophan is rapidly converted to an auxin by cell free wheat root extracts. This conversion of added tryptophan would be enzymatic process, because auxin was not formed by preparations which had been heated for 20 min at 95°C to destroy enzyme activity.

Experimental results shown above suggest that enzyme to form auxin from added tryptophan is present in the excised wheat root tissue as well as in the other tissue of higher plants.

6. *The effect of light on the sensitivity of the excised roots to added auxin.*

It was shown that tryptophan is effective for the excised root growth and is able to act as a precursor of IAA in the root tissue. Both tryptophan and IAA, however, were beneficial in the light during culture, but not in the dark. To clarify the biological significance of illumination during culture, physiological relation between illumination and the added auxin, at first, was investigated.

Fig. 15 shows the influence of added IAA on the excised root growth in the

dark and the light. In a given amount of IAA, the number of inhibited roots, whose main axis tips are abnormally swollen and the growth cease by the way of culture time of two weeks is more in the light than in the dark. In IAA concentration of $10^{-7}M$, for example, ratio of the inhibited roots to the normal roots is only 20 per cent in darkness, but 100 per cent in the light. In the case

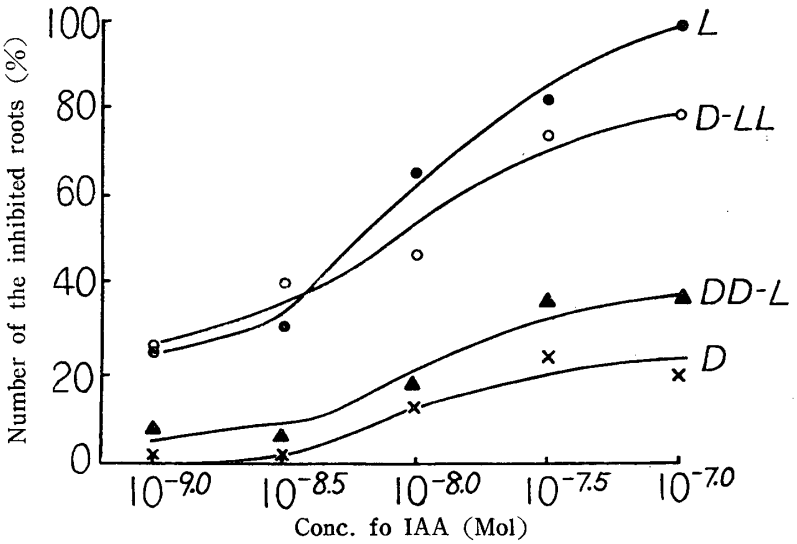



Fig. 15. Relation between illumination during culture and added auxin. Number of the excised roots grown in each plot is 12 roots. Culture time; two weeks.
D; Cultured in the dark. L; In the light (500~1000 Lux).
DD-L; In the dark for the first 7 days and thereafter in the light.
D-LL; In the dark for the first 3 days and thereafter in the light.

of illuminating in periods of culture time, the treatment in the earlier stage of culture periods (L or D-LL) enhances more the auxin inhibition than that in the subsequent stage (D or DD-L). Sensitivity of the excised roots to the presence of IAA superficially seems to be higher in the light than in the dark and this tendency is evident in the earlier stage of the culture time or younger stage of the excised roots.

**Table 9.** Sensitivity of the excised roots in the dark and the light to added IAA. D; Culture in the dark. L; Culture in the light. Culture time; 8 days. The light was continuously illuminated in intensity of 500~1,000 Lux after 3 days.

| IAA conc. | Main axis longth (cm) | | | Number of laterals | | | Length of lateral (cm) | | |
|---|---|---|---|---|---|---|---|---|---|
| (Mol) | L | D | L/D | L | D | L/D | L | D | L/D |
| 0 | 10.5 | 9.2 | 1.14 | 7.5 | 3.0 | 2.50 | 9.4 | 4.0 | 2.36 |
| $10^{-9.0}$ | 10.8 | 8.9 | 1.23 | 8.3 | 3.3 | 2.51 | 10.8 | 3.1 | 3.48 |
| $10^{-8.5}$ | 9.6 | 8.3 | 1.16 | 7.6 | 3.8 | 2.00 | 9.0 | 2.9 | 3.12 |
| $10^{-8.0}$ | 10.2 | 10.3 | 0.99 | 6.7 | 5.3 | 1.27 | 13.3 | 6.3 | 2.11 |
| $10^{-7.5}$ | 7.8 | 8.8 | 0.89 | 6.2 | 6.6 | 0.94 | 11.5 | 6.9 | 1.65 |
| $10^{-7.0}$ | 4.6 | 6.6 | 0.77 | 8.2 | 6.7 | 1.22 | 11.2 | 6.0 | 1.87 |

Table 9 shows the sensitivity of excised roots to concentrations of added IAA. Growth in length of main axis is inhibited in proportion to increase of IAA concentration. This inhibition is more notable in the light than in the dark as indicated by values of L/D in the table.

Development of laterals in length or number, on the other hand, is enhanced by the addition of IAA as seen in figures in darkness, whereas illumination also exerts the similar effect to IAA as shown by difference of figures in the absence of IAA between in the dark and in the light. It is shown furthermore that in the amount to be excessive for the growth of main axis, IAA acts stimulously for laterals. This difference in responsibility to added IAA suggests that endogenous auxin in the laterals is more suboptimal than that in the main axis.

Segelitz (42) reported a possibility of increasing the endogenous auxin production in excised corn roots by exposure to white light, and Naundorf (24) also showed that the formation of endogenous auxin in the roots of *Helianthus* is increased by illumination. Our results obtained on excised wheat roots, that is, increase of sensitivity to added IAA by illumination, may be interpreted as due to the fluctuation of endogenous auxin level by illumination, as suggested by Segelitz and Naundorf.

### 7. *Influence of illumination on IAA-destroying enzyme and its inhibitor in wheat roots.*

It was suggested that illumination during culture may result in increasing the endogenous auxin level, since the excised roots in the light show the high sensitivity to added auxin. This suggestion was subsequently confirmed from a different angle of auxin economy in the root tissue.

It has been generally considered that IAA-destroying enzyme or IAA oxidase determines the endogenous auxin level, high oxidase meaning low auxin *Vice versa* (33). In this section, then, influence of illumination during culture on IAA destroying activity of the excised roots, at first, was investigated.

The IAA-destroying enzyme has been isolated in 1947 from pea tissue by Tang and Bonner (50) and thereafter has been found in several plants (15, 18, 51). It has been reported that this enzyme abounds more in the root as compared with the other organs of higher plants (10, 11, 51, 55). The problems on IAA oxidase have been reviewed in detail by Ray (34). In addition to the IAA-destroying enzyme, according to recent evidence, an inhibitor of the enzyme is occurred in higher ylants (15, 18, 51).

*Materials and methods—Wheat roots*—Experimental materials were 5-day-old seedling roots and excised roots cultured during about four weeks. The seedling roots were obtained as follows. Seeds were soaked in tap water in the dark for 4 hr at 25°C, and were then sown on vinyl net floated on tap

water in enameled iron-trays. The seedlings were grown in complete darkness in a room maintained at 25±0.5°C.

—*Preparation of IAA-destroying enzyme*—Determinations of IAA-destroying enzyme were mainly performed by converting the tissue to a brei. The havested root tissue was ground thoroughly with deionized water in a blendor, and brei was decanted into 30×90 mm centrifuge tubes. The tubes were centrifuged at 3,000 g for 10 min, and made up to volume with the buffer solution. Thus the extract obtained was applied as crude enzyme of IAA destruction. The enzyme obtained was purified with acetone according to the method of Tang and Bonner (51) as follows. Forty cc of extract was precipitated by 20 cc of cold aceton and centrifuged in 3,000 g for 10 min, and the precipitate was suspended in 40 cc of phosphate-buffer, pH 4.4. After centrifuging, the clear solution was used as the enzyme for the activity test. One cc of the crude or the purified enzyme was prepared to be equal to 200 mg of fresh roots.

—*Assay for IAA oxidase—In vitro* IAA destroying enzyme assays were performed in 50 cc Erlenmeyer flasks, An aliquot of extract was incubated with IAA at 28°C. In addition to 1 cc of enzyme solution and 350 or 500 $\mu$g of IAA, 10 cc of the reaction mixture contained 1 cc of $10^{-3}$M 2,4-dichlorophenol, 1 cc of $10^{-3}$M $MnCl_2$ and 2 cc of 0.1 M phosphate buffer, pH 4.2. These conditions depended upon results previously tested on wheat roots. The final hydrogen ion concentration of reaction mixture was pH 4.4, which was optimal for IAA-destroying enzyme action of wheat roots. At the end of incubation time of 30 min or 1 hr, initial and residual IAA concentration of the reaction mixture were determined colorimetrically according to the method of Gordon and Weber (14).

*Experimental result* Fig. 16 shows the effect of varying concentration of IAA-destroying enzyme in wheat seedling roots. In the crude enzyme, the amount of destroyed IAA increases with increasing the added extract to 0.5 cc, decreasing reversely over the higher concentration and being negligible in the presence of extract, 3.0 cc or more. In the purified enzyme, on the other hand, the usual concentration activity curve without the diminution of IAA destruction in higher concentration of the enzyme is obtained.

This anomalous effect of varying concentration of crude enzyme appears to be due to a natural inhibitor which can be removed from the crude extract by acetone purification. This was confirmed in the experiments described below.

Cell free extract of wheat seedling roots, used as the crude enzyme above was boiled for 5 min and centrifuged, and then its supernatant was added to reaction system as an inhibitor of IAA-destroying enzyme.

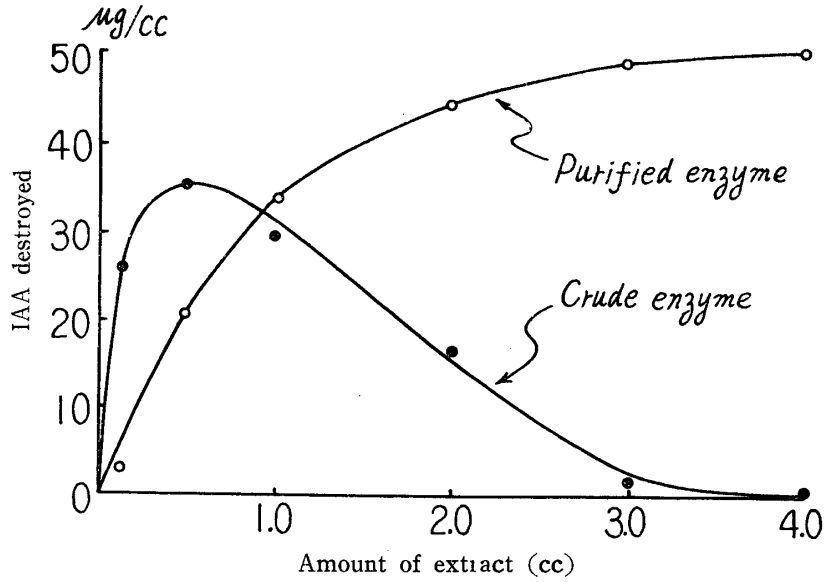



Fig. 16. Effect of concentration of the crude and the purified enzyme. In this experiment, 1.0cc of extract is equivalent to 250mg of the root tissue.

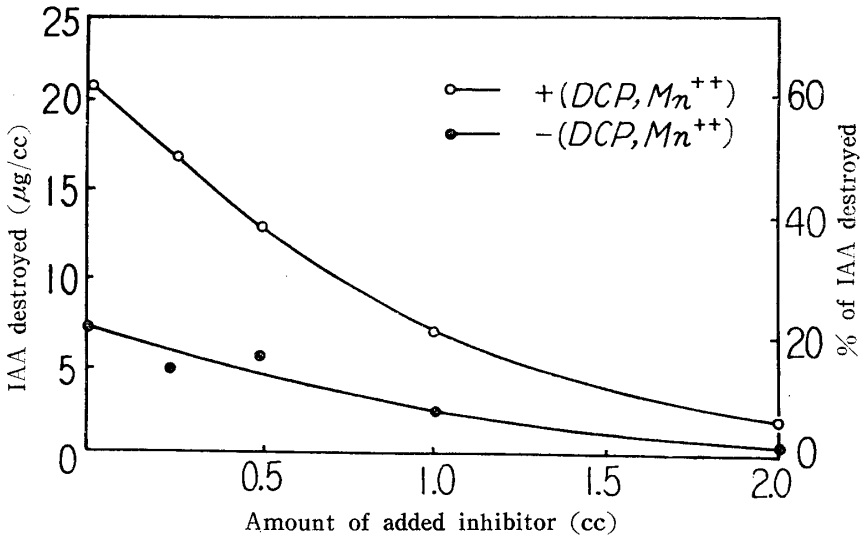



Fig. 17. The presence of inhibitor of the IAA-destoying enzyme in the wheat roots. One cc of inhibitor is equivalent to 200mg of the root tissue.

The result is presented in Fig. 17. The amount of destroyed IAA decreases according to increasing the inhibitor added to the reaction mixture. The effect of the inhibitor is not altered by addition of $Mn^{++}$ or 2,4-dichlorophenol, but is substrate-dependent.

Fig. 18 shows the presence of IAA-destroying enzyme in the excised roots cultured in the dark and the light. The activity of IAA destruction by crude enzyme is more powerful in the excised roots from the dark than those from the light over all ranges of enzyme concentration. This difference in the

activity between them is more evidently observed in higher concentrations of the crude extract. Expressed in terms of unit of nitrogen instead of unit of volume or fresh weight, this tendency develops more clear, since the nitrogen content of the extract is 0.47 mg per cc in the excised roots from the dark and 0.53 mg per cc in those from the light. It is suggested from the data of Fig. 18 that the difference in the activity of IAA destruction between two crude extracts may be due to containing the inbibitor more plentifully in the excised

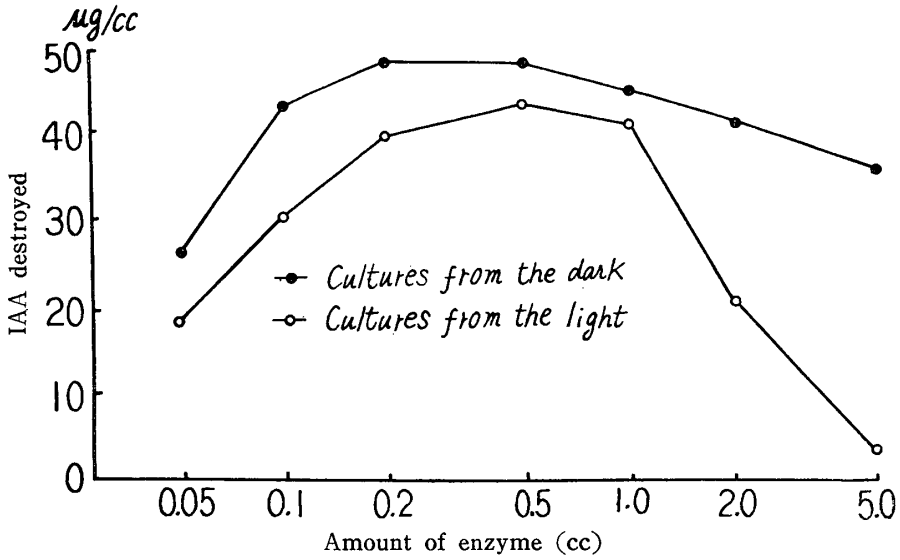



Fig. 18. The activity of crude IAA-destroying enzyme obtained from the excised roots. Excised wheat roots were cultured during four weeks in the dark and the light.

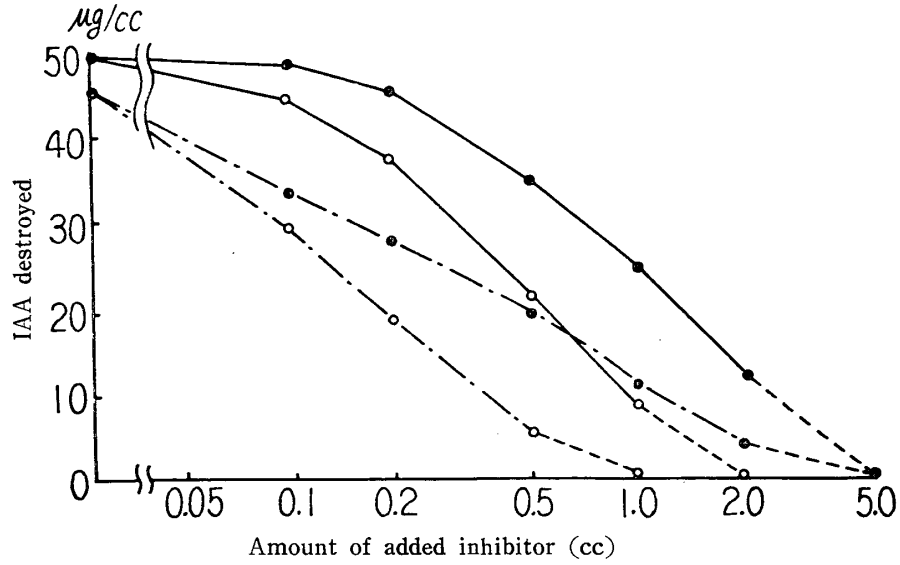



Fig. 19. Influence of various amount of inhibitor obtained from the excised roots on the activity of IAA-destroying enzyme.

Reaction time ; 20 min { —●— excised roots from the dark / —○— excised roots from the light

Reaction time ; 1 hr { -·-●-·- excised roots from the dark / -·-○-·- excised roots from the light

roots from the light than in those from the dark.

Fig. 19 shows the presence of an inhibitor of IAA-destroying enzyme in the excised root cultured during about four weeks. The boiled extract which was obtained from the excised root cultured in the dark, retards more effectively the activity of enzyme as compared with that from the comparable light cultures. The difference in inhibition of IAA-destroying enzyme between them develops progressively in the higher concentration of the boiled exracts, and two inhibition concentration curves in Fig. 19 are respectively similar in shape to those in the higher concentrations of the crude enzyme in Fig. 18. Hence the inhibition in the high concentration of crude enzyme apparently must be the result of excessive amounts of inhibitor.

In the experiments described above, the presence of IAA-destroying enzyme was demonstrated by employing *in vitro* preparations. It is possible, however, that IAA-destroying enzyme acts only when released by wounded cells (5, 8). The following experiment indicates that the present auxin destroying enzyme is able to act *in vivo*.

The excised roots were cultured in the light and the dark. When the excised roots were 28-days-old, they were immersed in a solution of IAA, 0.2 mg per cc (pH 4.4) and vacuum infiltrated. The infiltrated roots were thoroughly washed with deionized water and then placed in humid air at 30°C. Samples were collected after 0, 1 and 2 hr, residual auxin in each sample was extracted with ether and IAA was determined colorimetrically.

**Table 10.** Destruction of IAA which was infiltrated into the excised wheat root.

| Culture condition | | Reaction time (hr) 0 | 1 | 2 |
|---|---|---|---|---|
| Dark | IAA remained (μg*) | 58 | 29 | 19 |
| | IAA destroyed (%) | 0 | 50 | 67 |
| Light | IAA remained (μg*) | 49 | 33 | 27 |
| | IAA destroyed (%) | 0 | 36 | 45 |

* μg *per* 20 mg dry matter of excised wheat roots.

The data presented in Table 10, show that IAA infiltrated into excised root tissues is decreased more rapidly with time and this reduction of IAA is more rapid in cultures from darkness than those from the light. The former destroys 14 per cent more at 1 hr and 2 per cent more at 2 hr of incubation time the infiltrated IAA than the latter.

Fiedler (8) suggested that auxin inactivation may occur mainly at cut surfaces, and his suggestion allows to consider that IAA-destroying enzyme may be active only *in vitro*. Our results on wheat roots obtained above indicate,

however, that IAA inactivating reaction can take place *in vivo* as well as *in vitro*, as shown by Tang and Bonner (51) with pea seedling.

We demonstrated in experiments thus far described that seedling roots or excised roots of wheat contain IAA-destroying enzyme and a water soluble, thermostable inhibitor of the enzyme. Optimal pH for the IAA-destroying enzyme of wheat roots is at 4.4, being nearer to the optimum of pH 3.5 for pineapple leaf (15) than the pH 6 to 7 for pea (50) and bean plant (55).

It was furthermore demonstrated that the excised roots grown in the light contain more plentifully the inhibitor than the comparable dark grown roots. In the case of etiolated pea, the similar phenomenon that exposure of the plant to light promotes the formation of the inhibitory factor has been reported (51). Gortner and Kent (15), however, have stated that an inhibitor of IAA oxidase in pineapple is destroyed by the exposure of the extract to light, and we also have observed the same phenomenon with the wheat root extract. On formation of the inhibitor of IAA-destroying enzyme, our result obtained on wheat contrasts with that of Gortner *et al.* Why such contradictry results occur by difference of *in vivo* and *in vitro* status to be illuminated, is not clear yet.

## Discussions and conclusions

(a) *Relationship among peptone, tryptophan and IAA through the stimulous effect on the excised root growth.*

It was demonstrated in the first paper of this experimental series that the addition of peptone to the basal medium containing minerals, sugar and B-vitamins brings about the beneficial effect on the growth of excised wheat roots. The same effect also was exerted by tryptophan or IAA only in the light during culture. In place of peptone, B-vitamins, namely growth substances for the excised roots of many dicotyledonous plants, however, were unable to take place any stimulous effect for the excised wheat roots. In some chemical characteristics almost insoluble in alcohol or ether and labile to acid hydrolysis, the beneficial constituent in peptone is more similar to tryptophan rather than B-vitamins. Optimal concentration of peptone is 300 mg per liter and this amount contains about 2.5 mg of tryptophan as a constituent. Optimal concentration of tryptophan, on the other hand, is in 0.5 mg to 1.0 mg per liter. This quantitative difference between peptone and tryptophan seems not to be very large if the light condition during culture and effects of consituents other than tryptophan in peptone are entertained. It is inferred from these considerations that the superior growth obtained with peptone may be mainly attributed to tryptophan contained as a constituent of peptone. Since Stowes *et al.* (46) has reported that yeast extract contains IAA at a concentration of about 0.15 $\mu$g per g, it is possible that IAA instead of tryptophan may be a beneficial constituent of peptone which is such a natural substance as yeast. IAA certainly

is labile to acid hydrolysis, but the beneficial constituent has a nature insoluble in ether. Hence the beneficial constituent in peptone is unlikely to be IAA itself.

The fact that effect of casein hydrolysate is considerably inferior to that of peptone may be attributed to the reason why the former is treated by acid hydration and the latter by enzymatical hydration. Tryptophan contained in casein perhaps would be destroyed more easily with the presence of other amino acids during its hydrolysis (39). Not only added aseptically to previously autoclaved medium, but also autoclaved moderately with other components in the basal medium, tryptophan stimulated the growth in the light. In the previously illuminated medium containing tryptophan, the inferior growth was obtained in darkness during culture. Although tryptophan is known as one of unstable amino acids on heating or illuminating, possibly its beneficial effect would not be due to the indirect action through the chemical change of tryptophan by autoclaving to sterilize the medium or by illuminating during culture.

Of some compounds related metabolically to tryptophan, IAA exerted the approximately equivalent effect to tryptophan or peptone in concentration of 0.2~0.4 $\mu$g per liter. It was furthermore confirmed that enzymatical conversion of tryptophan to IAA can occur in the excised roots without receiving any artificial effects and would exert the beneficial effects through conversion to IAA in the root tissue.

Roberts and Street (38) have reported that tryptophan for excised rye root growth is activated during autoclaving and that this activation is not due to conversion of tryptophan to IAA heating. In their case it may be entertained that tryptophan converts by autoclaving to a beneficial substance which is a precursor of IAA or a intermediary member in the pathway from tryptophan to IAA and has negative property in the avena test.

(b) *On physiological significance of illumination during culture.*

It was demonstrated that illumination during culture retards activity of the IAA-destroying enzyme in the excised roots through probably the occurrence of its inhibitor. Applying the review that auxin level in plant tissue is determined by the extremely powerful activity of IAA-destroying enzyme (11, 33), the illumination during culture would play a possible role to keep constantly or highly auxin level in excised roots thruogh culture time. This consideration seems to illustrate reasonably the fact that the sensitivity of the excised root to added tryptophan or IAA is risen by illumination.

It naturally should be considered whether auxin level of the excised roots cultured in darkness is able to be maintained as highly as that of the excised roots in the light by external supplement of IAA. According to view of Galston and Dalberg (11) that IAA-oxidase is an adaptive enzyme, one would not be

able to maintain constantly or highly auxin level in the excised roots by external supplement of IAA under the condition of darkness with would bring about adaptable production of IAA-destroying enzyme. Physiological significance of illumination during culture, therefore, may be in the point that action of illumination to maintain constantly or highly auxin level in the excised roots is not be capable to replace only by the external supplement of IAA.

It is remained to be accounted for the beneficial effect of peptone in darkness in contrast with that of tryptophan or IAA in the light during culture. It was observed that sensitivity of excised roots to peptone is risen by illumination during culture, as well as the case of tryptophan or IAA. In the light, peptone was beneficial in lower concentration, and the optimal amount in darkness was excessive in the light. It is supposed from this finding that other components than tryptophan in peptone, for example, organic nitrogenous substances, may play the same role as illumination, which retards action of IAA-destroying enzyme. Thus auxin level of the excised roots in medium containing peptone assumed to de maintained highly even if cultured in darkness. Bosemark (6) has speculated that the amount of endogenous auxin in roots of wheat seedling increases correspondingly with the increasing supply of nitrogen, and Galston also has suggested that IAA-protein may act as sequestering agent to protect IAA from its oxidase (32). These considerations would be examples which maintain constantly or highly endogenous auxin level through nitrogen metabolism.

(c) *Relation between aging of the excised root and the auxin level.*

when the excised root of wheat were cultured in darkness, the main axis tips tapered off and the growth ceased during the short term of two to three weeks. What fluctuation of auxin level would occur in the excised roots during the growing process and furthermore what relation there is between auxin level and physiological age of the root, we are going to consider subsequently.

Street (48) noted the possibility that excised root growth may be limited by the endogenous rate of natural auxin production. There are other reports that fluctuation of auxin level can occur during growing periods of roots. We remenber the fact that tryptophan or IAA as well as peptone retarded temporarily the growth in the early stage of culture time and exerted the beneficial effect in the following stage. It has been found, on the other hand, that the inhibition in the excessive amount of tryptophan or IAA occurs more easily in the early stage than in the advanced stage of culture time. The fact that sensitivity of excised roots to added IAA or tryptophan is higher in the younger root tip excised from radicle, suggests that endogenous auxin level of the excised roots in the dark falls down with culture time. This consideration furthermore may be supported by the following comparison in the experimental results obtained. Experimental material in Fig. 16, which was obtained from 6-day-old

seedling, is regarded as relatively active and young roots corresponding with inoculating stage in cultures. On the other hand, experimental materials in Fig. 18 were the excised roots which such active young roots as those in Fig. 16 were run in culture during the longer time.

The IAA-destroying enzyme curve of the excised roots from the light in Fig. 18 shows similar shape to the curve of crude enzyme in Fig. 16, whereas the curve of the excised roots from the darkness shows different pattern from the two curves and indicates to be relatively higher in its activity. This suggests that IAA-destroying enzyme activity of the excised roots in darkness may increase more progressively with culture times. Pilet and Galston (33) have found that IAA oxidase is more active in the older tissue of base than in the young tissue of root tips and have drawn the same conclusion as ours. This suggests that cause of aging is in decreasing in auxin level which is assumed to be easily flactuated by IAA-destroying enzyme. Hence, physiological age of the excised wheat roots appears to proceed more raidly in the dark than in the light.

According to the general suggestion that IAA-destroying enzyme determines the endogenous auxin level, the auxin level of the excised wheat roots appears to be lower in the dark than in the light. In order to maintain the constant growth of the excised wheat roots, then, exposure of the root tissue to the low intensity light may be required to maintain constantly auxin level, which may naturally decrease in darkness with culture time, through active production of an inhibitor of IAA-destroying enzyme. The external supplement of IAA would be necessary to compensate for action of illumination to maintain optimal concentration of auxin in root tissue.

## Summary

(1) To clarify beneficial constituent of peptone for the growth of excised wheat roots and to illustrate physiological mechanism of its action, a series of experiments were carried out.

(2) Of 13 amino acids tested, *dl*-tryptophan exerted approximately the same effect as peptone in concentration of 0.5 to 1.0 mg per liter.

(3) $\beta$-indoleacetic acid as well as tryptophan was stimulous in concentration of 2.0 to 4.0 $\mu$g per liter. Anthranylic acid, *l*-kynulenine and indole plus serine, which are related metabolically to tryptophan, were noneffective.

(4) The beneficial effects of tryptophan and $\beta$-indoleacetic acid were conspicuous in the weak light from fluorescent tubes.

(5) The beneficial effect of tryptophan is not due to the artificial change of tryptophan in the solution by autoclaving to sterilize the medium or by illuminating during culture.

(6) It was evidenced that enzymatical conversion of added tryptophan to IAA

is able to occur in the excised roots.

(7) In the excised roots from the dark than in those from the light, activity of IAA-destroying enzyme was higher, and exogenous auxin was destroyed more actively.

(8) Sensitivity of the excised roots to added tryptophan or IAA was risen by illumination during culture. This phenomenon was illustrated as reference of increasing endogenous auxin level with retarding activity of auxin destroying enzyme.

(9) It was suggested that maintained appropriately the auxin level by illumination during culture and by external supplement of tryptophan or IAA, the excised wheat roots may be able to grow over the longer time of culture.

(10) The following points were synthetically discussed through experimental results obtained in the previous and present papers.

(a) Relationship among peptone, tryptophan and IAA on the excised wheat root growth.

(b) Physiological significance of illumination during culture.

(c) Relation between aging of the excised wheat roots and the auxin level.

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