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THE ENZYMATIC INACTIVATION OF INDOLE ACETIC ACID. II. THE PHYSIOLOGY OF THE ENZYME [1]

Y. W. Tang and James Bonner

IT HAS BEEN shown (Tang and Bonner, 1947) that etiolated pea epicotyls contain an enzyme system which inactivates indole acetic acid (IAA). The inactivation can be followed either by the standard *Avena* test of Went and Thimann (1937) or by a chemical method using ferric chloride. Since IAA is a substance which occurs naturally in higher plants (Locke *et al.*, 1939; Stewart, 1939; van Overbeek, 1940; Haagen-Smit *et al.*, 1941; Dandliker, 1945; Wildman *et al.*, 1947) the IAA-inactivating enzyme would, therefore, appear to possess physiological significance for the plant. Although several investigators (van Overbeek, 1935, 1938; de Haan and Gorter, 1936) have suggested this possibility, still correlation of enzyme activity with physiological function has not yet been accomplished. This paper concerns certain aspects of the physiology of the IAA-inactivating enzyme.

MATERIALS AND METHODS.—*Pea seedlings.*—The seedlings used for these experiments were grown from seed of the variety *Alaska* planted in washed river sand contained in flats. The seeds were soaked for 2 hr. before planting. The flats were placed in a dark room maintained at 23°C. and the plants harvested for use after approximately 7 days when the epicotyls had reached a height of *ca.* 10–15 cm. For the production of etiolated plants it is essential that no light be permitted in the room. Even the weak orange light used in *Avena* dark rooms causes appreciable inhibition of growth of peas. All subsequent operations such as grinding of the tissue, filtration, centrifugation *etc.* were carried out in a cold room at 0°C.

Determination of IAA.—The colorimetric method used for the determination of IAA has been described in detail elsewhere (Tang and Bonner, 1947). It is based on the reaction of IAA with ferric chloride in concentrated H_2SO_4 to form a typical pink color which may be determined by quantitative colorimetry. The ferric chloride reagent contains: 15 cc. of 0.5 M $FeCl_3$, 500 cc. distilled water, 300 cc. of H_2SO_4, sp. gr. 1.84. To 2 cc. of the sample containing 5–100 μg. of IAA are added 8 cc. of reagent. The color is allowed to develop for 30 min. and is then determined in a photoelectric colorimeter.

THE INHIBITOR OF THE IAA-INACTIVATING ENZYME.—*The existence of the inhibitor.*—In preliminary experiments concerned with purification it was found that the IAA-inactivating enzyme is in some way activated by acetone precipitation since the acetone precipitated material contains approximately 20 per cent more total activity than the initial pea juice. This result could be interpreted as indicating the presence of some material in the whole protoplasm which partially masks the activity of the IAA-inactivating enzyme.

In order to demonstrate the presence of an inhibitor of the IAA-inactivating enzyme, an experiment was set up as follows: Fifty cc. of whole protoplasm was boiled for 5 min., filtered, and the filtrate concentrated to 15 cc., the concentrated filtrate representing 80 g. of fresh etiolated pea epicotyls. Five cc. of this preparation were added to 15 cc. of buffer solution containing purified enzyme representing 30 g. of fresh etiolated pea epicotyls. The reaction mixture further contained 300 μg. of IAA. As a control, 5 cc. of distilled water was used in place of the concentrated heat stable filtrate. Samples were removed after incubation at 25°C.

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TABLE 1. *The presence of a substance in whole protoplasm inhibiting the activity of IAA-inactivating enzyme. Twenty cc. reaction mixture, pH 6.6, containing acetone purified enzyme preparation representing 30 g. of fresh pea epicotyls.*

Condition	Incubation time in hours	IAA added μg.	IAA recovered μg.	% inhibition	IAA protected per g. tissue[a] μg.
Enzyme + buffer (control)	0	300	299	...	..
	1	...	101	...	..
	3	...	57	...	.
Enzyme + buffer + filtrate of boiled protoplasm	0	300	299	...	..
	1	...	279	89.8	6.7
	3	...	191	55.4	5.1

[a] This column refers to the amount of IAA protected from destruction per g. fresh weight tissue represented by the inhibitor preparation.

for 0, 1 and 3 hr. and the amounts of IAA remaining in the reaction mixture determined colorimetrically. The results are presented in table 1. In the presence of the heat stable filtrate the amount of IAA destroyed was reduced by 87.5 per cent after 1 hr. incubation, and by 55.4 per cent after 3 hr. as compared to the control in which no filtrate was added. It is clear that whole protoplasm contains an inhibitor which greatly reduces the enzymatic inactivation of IAA. This inhibitor may be removed by acetone precipitation of the enzyme. In no case did a second precipitation result in any further separation of enzyme from inhibitor.

Stability of the enzyme-inhibitor complex.—It is of interest to know whether the factor which inhibits IAA destruction is capable of irreversibly combining with the enzyme. The following experiment was performed to test this point. To 60 cc. of whole epicotyl juice were added 15 cc. of a concentrated inhibitor solution which had been prepared from dried green pea seedlings. It will be shown later that such material is a rich source of inhibitor. The mixture was allowed to stand at 25°C. for 15 min. As a control, 15 cc. of distilled water was used instead of inhibitor. In both cases, the protoplasm was precipitated by 32 cc. of acetone, centrifuged, and then the precipitate suspended in 36 cc. of phosphate-citrate buffer, pH 6.6. After centrifuging the suspension, the supernatant was decanted and the precipitate tested for IAA inactivation activity. At the same time, the ability of the extract of green seedlings to produce inhibition of the inactivating enzyme was tested by adding concentrated inhibitor to an enzyme preparation previously purified with acetone. All incubations were carried out in 20 cc. of phosphate-citrate buffer, pH 6.6, at 25°C. Samples were removed after 0 and 3 hr. and the amounts of IAA determined colorimetrically.

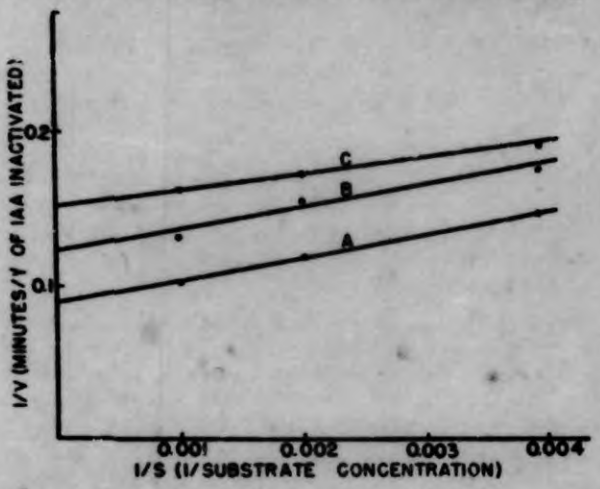
Table 2 summarizes the results obtained from this experiment. The data show that the presence of inhibitor in the reaction mixture results in almost complete inhibition of the IAA-inactivating enzyme but it is also shown that the added inhibitor can be almost completely separated from the enzyme by one acetone precipitation. These facts indicate that the inhibitor is neither capable of destroying the enzyme nor of combining with the enzyme to form a stable complex.

TABLE 2. *Absence of formation of a complex between the IAA inactivating enzyme and the inhibitor of this enzyme. Twenty cc. reaction mixture, pH 6.6, 25°C. Enzyme preparation representing 30 g. of fresh pea epicotyls. Incubation time 3 hr.*

Condition	IAA added μg.	IAA recovered μg.	% inhibition
Acetone purified enzyme	300	34	...
Acetone purified enzyme + inhibitor	300	290	96.2
Acetone purified enzyme-inhibitor complex	300	38	1.5

Nature of the enzyme-inhibitor-substrate reaction.—It is of interest to determine whether the naturally occurring inhibitor of IAA inactivation acts as a substrate competitor or whether its action is non-competitive. It is possible to distinguish between these two types of inhibition on the basis of a treatment proposed by Lineweaver and Burk (1934). Data for this treatment consist of the initial reaction velocity in the presence of a series of substrate concentrations, at each of two or more inhibitor concentrations. Due to the nature of the enzyme-substrate reaction (Lineweaver and Burk, 1934) the curves relating the reciprocal of the initial velocity and the reciprocal of the substrate concentration are straight lines with intercepts on the velocity axis. Each inhibitor concentration yields a separate straight line. In case of competitive inhibition, the intercept remains constant while the slope of the curve is increased with increasing inhibitor concentration whereas in the case of non-competitive inhibition the slopes remain uniform while the intercepts are increased.

For this experiment, three conditions were set up. An acetone purified enzyme, in each instance representing 34 g. of fresh pea epicotyls, was added to 20 cc. phosphate-citrate buffer, *p*H 6.6, containing 250, 500, and 1000 μg. of IAA, and incubated at 25°C. Samples were removed after 0 and 15 min. and IAA disappearance measured colorimetrically. In another series 0.5 cc. inhibitor solution representing 5 mg. dry weight of green pea seedlings was added. In another series the amount of inhibitor was increased to 1.0 cc. In all cases, additions were made in such a way that the volume and molarity of the buffer remained constant. The results of this experiment are plotted according to the directions given by Umbreit *et al.* (1945).



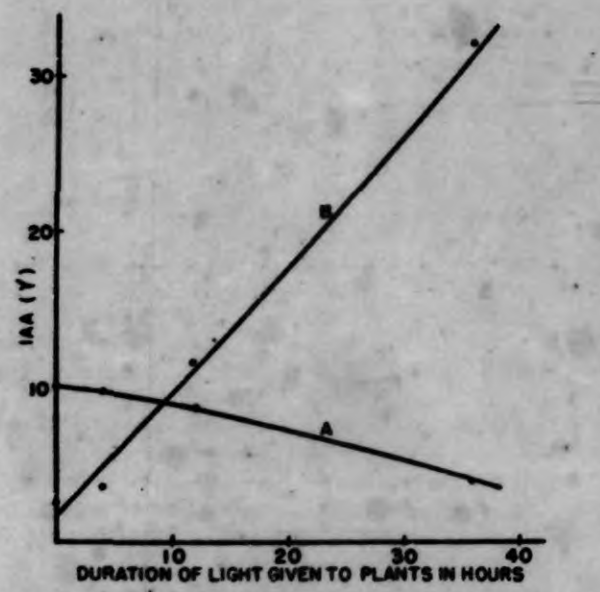


Fig. 1–2.—Fig. 1 (above). Noncompetitive nature of the inhibition of indole acetic acid inactivating enzyme by inhibitor from green pea seedlings. A. No inhibitor added. B. 0.5 cc. inhibitor preparation added. C. 1.0 cc. inhibitor preparation added.

Fig. 2 (below). Effect of illumination on indoleacetic acid destroying enzyme and enzyme inhibitor content of pea seedlings. A. Effect of light on IAA inactivating enzyme. IAA inactivated per gram fresh weight of epicotyls. B. Effect of light on inhibitor. IAA protected from inhibition per gram fresh weight of epicotyls.

When the reciprocal of the velocity constant is plotted against the reciprocal of the substrate concentration at three different concentrations of substrate and two concentrations of inhibitor, it is evident that the intercepts of the three curves are different (fig. 1), while the slopes of the curves are essentially equal. It can be concluded that the action of the inhibitor on the pea enzyme is not competitive.

DISTRIBUTION OF ENZYME IN ETIOLATED AND GREEN PEAS.—*Etiolated pea seedlings.*—In all the preceding experiments, whole epicotyls were used as the source of enzyme. In order to study the enzyme distribution in the plant, 1-week-old pea seedlings were separated into epicotyl, terminal bud, root and cotyledons. The fresh weight of each part was determined before extraction of the enzyme. Each part was ground with distilled water in a blendor. Forty cc. of whole protoplasm representing each part was then precipitated by 16 cc. of acetone and after centrifugation the precipitates were resuspended in 20 cc. of phosphate-citrate buffer, *p*H 6.6, for 30 min. After recentrifugation, the clear supernatant was decanted and used as the enzyme preparation. Ten cc. of this supernatant were added to 10 cc. of water containing 300 μg. IAA. Samples were removed after 0 and 1 hr. of incubation at 25°C., the enzymatic reaction was checked by boiling the sample for 5 min., and the amount of IAA remaining determined colorimetrically. Table 3 shows that root tissue of etiolated pea seedlings is the richest source of inactivating enzyme, that cotyledons also contain the enzyme but in a lesser quantity, and no activity is found in the buds.

Enzyme and inhibitor content of pea seeds.—The experiment of table 3 shows that the cotyledons of germinated seeds contain the IAA-inactivating enzyme. It would be of interest to test whether ungerminated seeds contain this enzyme. One hundred g. of pea seeds were soaked in distilled water over night, and then ground in the blendor. The milky juice obtained was subjected to centrifuging and the supernatant then precipitated with acetone. Phosphate-citrate buffer, *p*H 6.6, was used to suspend the precipitate. After centrifuging, the clear solution was used as the enzyme for the activity test. To 10 cc. of the enzyme, equal to 15 g. of fresh pea seeds, were added 10 cc. of IAA solution containing 300 μg. Ten cc. of distilled water were used instead of IAA in the control. Samples were collected after 0, 1, 3, and 5 hr. incubation and the IAA in each sample was determined colorimetrically. Summarized results are given in table 4, showing that the concentration of IAA remains constant even after 5 hr. incubation. This indicates that resting seeds are devoid of IAA-inactivating enzyme.

To test for the presence of the inhibitor, the

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THE RELATION OF METAPHLOEM TO THE TYPES OF VASCULAR BUNDLES IN THE MONOCOTYLEDONEAE [1]

Vernon I. Cheadle and Natalie Whitford Uhl

THIS IS THE second of two papers on vascular bundles in the Monocotyledoneae. Six types of vascular bundles were defined and figured in the first paper (Cheadle and Uhl, in press) and were discussed in relation to the elements in the late metaxylem.

A brief description of the six types of bundles follows. Type I is characterized by late metaxylem elements that are all rather similar in size and which meet the phloem along a straight or slightly curved line. Type II has a V-shaped xylem area with several larger elements in the arms of the V. Types IIIA and IIIB are featured by the presence of a single large element on each side (or wing) of the bundle, but in IIIB the xylem generally meets the phloem along a more curving line which may almost be V-shaped in some bundles. Type IV has a large element in the center, and type V is the amphivasal form. The evidence presented in the first paper indicates that type I is primitive and that it has given rise phylogenetically to type II and that in turn to type IIIB, either directly or through type IIIA. Type IIIA may have arisen directly from type I. Type IV was derived phylogenetically from type I, and type V may have arisen separately from any one of the other five types.

The objective of the present paper is to ascertain whether the phylogenetic specialization of the metaphloem can be correlated with the phylogenetic specialization of bundle form as determined by evidence derived from the metaxylem elements.

The literature is scanty and generally not pertinent to the present paper. Necessary references to the literature will be made throughout the paper.

[1] Received for publication April 5, 1948.

MATERIALS AND METHODS.—Available parts of the shoot system of 216 species in 158 genera of 33 families as defined by Hutchinson (1934) were investigated. The families and the numbers of genera and species investigated given in the previous paper (Cheadle and Uhl, in press) are approximately the same as those utilised in the present study and therefore need not be tabulated again. The two additional families are the Dioscoreaceae and Zosteraceae. The data were obtained from the same preparations used for the study of the metaxylem discussed in the first of the two papers on bundles.

OBSERVATIONS AND DISCUSSION.—It was pointed out in the earlier paper that the six bundle forms were defined on the basis of the appearance of the metaxylem in cross section and that the appearance of the phloem in each of these bundle forms would be considered later. The bundle types as originally defined, therefore, will be used in the present discussion of phloem.

The fact that the phloem may have a characteristic appearance in cross section was first noted in members of the Gramineae. The well-known regular arrangement of sieve tubes and companion cells in corn is more or less typical for the stems of the grasses. Other members of the Monocotyledoneae were then examined and the phloem areas were eventually separated into three categories, based upon the arrangement and variations in size of the elements of the metaphloem as seen in cross section. The arrangement first noted in the grasses was defined as "regular" and is characterized by similarity in size of the sieve tubes in the metaphloem and by the orderly occurrence of companion cells (fig. 1). The arrangement characterized by dissimilar sizes of sieve tubes and by the lack of any easily recog-

TABLE 3. *Distribution of IAA-inactivating enzyme in etiolated pea seedlings. Twenty cc. reaction mixture, pH 6.6, containing 300 μg. IAA, 25°C.*

Plant organ	Fresh wt. (g.) of tissue used for enzyme preparation	IAA added μg.	IAA recovered μg. 0	IAA recovered μg. 1 hr.	IAA inactivated μg.	IAA inactivated g. fresh wt. of tissue μg.
Roots	19.2	300	300	8	292	15.2
Epicotyls	23.0	300	300	170	130	5.6
Cotyledons	32.0	300	300	191	109	3.4
Buds	2.4	300	300	300	0	0

original milky juice obtained in the previous experiment was boiled for 5 min. and filtered, the filtrate being used as the inhibitor preparation. To 10 cc. of an acetone purified enzyme preparation from pea epicotyls were added 5 cc. of a solution containing 300 μg. of IAA and 2.5 cc. or 5 cc. of inhibitor. As a control, 2.5 cc. or 5 cc. of distilled water were substituted for the boiled pea seed extract. The total volume of the reaction mixture was adjusted to 20 cc. Samples were removed after 0 and 3 hr. of incubation, and the IAA determined colorimetrically. The results of this experiment are given in table 5. A boiled extract equal to 7.5 g. of pea seeds completely inhibited the IAA-inactivating enzyme while one-half of this quantity caused 50 per cent inhibition. It can be concluded that while the pea seeds apparently do not contain the IAA-inactivating enzyme, they nevertheless contain a considerable amount of the inhibitory factor.

TABLE 4. *The absence of IAA-inactivating enzyme in ungerminated pea seeds. Twenty cc. reaction mixture, pH 6.6, 25°C.*

Condition	Incubation time in hours	IAA added μg.	IAA recovered μg.
Enzyme + buffer	0	0	0
	1	...	0
	3	...	0
	5	...	0
Enzyme	0	300	310
	1	...	300
	3	...	310
	5	...	310

IAA-inactivating enzyme in green pea seedlings.—Thus far the activity of the IAA-inactivating enzyme has been studied in etiolated pea seedlings. The relations of the inactivating enzyme in light grown pea seedlings will next be considered. Pea seeds were germinated in washed river sand and grown in an air-conditioned greenhouse at a continuous temperature of 80°F. for 10 days. Six hundred g. of fresh green pea shoots thus obtained were blended with distilled water, the juice filtered, and precipitated with acetone. Phosphate-citrate buffer, pH 6.6 was used to suspend the precipitate obtained. The clear suspension obtained after centrifugation was used as the enzyme preparation. A sample of whole protoplasm or of acetone purified enzyme equal to 25 g. of fresh green pea shoots was incubated with 300 μg. of IAA contained in 20 cc. of phosphate-citrate buffer, pH 6.6. Samples were removed after 0, 1, and 3 hr. incubation at 25°C. The colorimetric method was used for IAA determination. The results are presented in table 6. No IAA-inactivating activity was found in whole protoplasm, and only a very small amount in protoplasm purified by acetone precipitation. Since as shown previously in table 2 the inhibitor of the IAA-inactivating enzyme is almost quantitatively removed by a single acetone precipitation, the results of the present experiment suggest that the inactivating enzyme is essentially absent from green pea epicotyls.

Inhibitor content of buds and stems of green pea

TABLE 5. *Inhibitor content of ungerminated pea seeds. Twenty cc. reaction mixture, pH 6.6, 25°C. Acetone purified enzyme equal to 30 g. of pea epicotyls. One cc. of inhibitor (boiled seed) extract equals 1.5 g. of pea seeds.*

Condition	Incubation time in hours	IAA added μg.	IAA recovered μg.	IAA inactivated μg.	% inhibition
Enzyme + buffer	0	300	300	...	...
	3	...	34	264	...
Enzyme + buffer + 2.5 cc. boiled pea extract	0	300	303	...	...
	3	...	180	123	53.4
Enzyme + buffer + 5 cc. boiled pea extract	0	300	300	...	...
	3	...	300	0	100

seedlings.—Although as shown in table 6 green pea seedlings contain negligible amounts of IAA-inactivating enzyme, they are a rich source of inhibitor as will be shown in the following experiments.

TABLE 6. *IAA-inactivating enzyme content of green pea seedlings. Twenty cc. reaction mixture, pH 6.6 25°C.*

Condition	Incubation hours	IAA added μg.	IAA recovered μg.	IAA inactivated per g. tissue μg.
Whole protoplasm	0	300	285	..
	1	...	285	0
	3	...	285	0
Acetone purified enzyme	0	300	290	..
	1	...	270	0.8
	3	...	260	1.2

Seeds were germinated in darkness for 4 days and then transferred into the greenhouse for another 3 days. The harvested seedlings were separated into buds and stems, and each portion blended with distilled water. The juice thus obtained was boiled, filtered, and the filtrate used as the inhibitor preparation. To 10 cc. of an acetone purified enzyme solution, representing 18 g. of fresh etiolated pea epicotyls, were added 7.5 cc. of a solution containing 300 μg. of IAA, and 2.5 cc. of the boiled juice from green peas. Two and one-half cc. of distilled water were used instead of inhibitor preparation for a control. Samples were removed after incubation at 25°C. for 30 and 180 min. In table 7 are presented the results of this experiment which show that buds of green pea seedlings are a richer source of the inhibitor than the stems.

It is interesting to note that in both green and etiolated pea seedlings, the buds contain much larger amounts of inhibitor than the stems. When the buds and epicotyls of green and etiolated pea seedlings are compared, it is found that green peas contain nearly three times more inhibitor than etiolated peas on a fresh weight basis.

A more directly quantitative experiment for comparison of the inhibitor content of etiolated and green pea seedlings was made as follows. Epicotyls from green or etiolated pea seedlings were dried at 70°C. in a forced draft oven for 48 hr. and were then ground in a Wiley mill. Two g. of the powder were extracted by refluxing with 100 cc. of distilled water for 1 hr. These extracts were filtered and the filtrates concentrated to 50 cc. To 10 cc. of acetone purified enzyme were added 5 cc. of a solution containing 300 μg. of IAA, and 3.2 cc. or 5 cc. of the inhibitor, the final volume of the reaction mixture being adjusted to a total of 20 cc. by addition of distilled water. Samples were removed after 0 and 3 hr. incubation and IAA determined colorimetrically. Table 8 shows that green pea seedlings contain several times more inhibitor than etiolated pea seedlings.

EFFECTS OF LIGHT ON THE IAA-INACTIVATING ENZYME SYSTEM OF ETIOLATED PEA SEEDLINGS.—The experiments of this section will consider the effect of light on the amounts of IAA-inactivating enzyme and inhibitor present in an etiolated seedling. Pea seedlings were grown in total darkness for 4 days. At this time the plants were divided into lots and one lot given 12 hr. of diffuse daylight per day for a further 3-day growth period. A further lot received 12 hr. of light on the final seventh day, one lot received 4 hr. of light on the final day, and one lot remained in total darkness. Thus all plants were of the same age, 7 days, when harvested. The amounts of IAA-inactivating enzyme and of inhibitor were determined as in previous experiments the enzyme with acetone precipitated material and the inhibitor in boiled whole protoplasm. The results of this experiment are given graphically in fig. 2. It can be seen that there is a nearly linear relationship between the duration of irradiation of the plant and the amount of inhibitor recovered

TABLE 7. *Inhibitor content of buds and stems of green pea seedlings. Twenty cc. reaction mixture containing 300 μg of IAA, pH 6.6, 25°C. Acetone purified enzyme representing 18 g. of fresh etiolated pea epicotyls.*

Condition	Incubation time in minutes	Inhibitor in terms of fresh wt. (g.)	IAA added μg.	IAA recovered μg.	IAA inactivated μg.	IAA prevented from enzymatic inactivation per g. fresh wt μg.
Enzyme	0	...	300	300	...	...
	30	...	...	190	110	...
	180	...	...	138	162	...
Enzyme + 2.5 cc. of inhibitor from buds	0	0.27	300	300	...	...
	30	...	...	300	0	...
	180	...	...	293	7	574
Enzyme + 2.5 cc. of inhibitor from stems	...	0.77	300	300	...	...
	...	...	...	265	35	...
	...	...	...	145	155	9

While the relationship between time of irradiation and the amount of enzyme activity is not simple, still longer periods of illumination cause a decrease in amount of IAA-inactivating activity found in the tissue.

TABLE 8. *Comparative inhibitor concentrations in green and etiolated pea seedlings. Twenty cc. reaction mixture, pH 6.6. Acetone precipitated enzyme (30 g. fresh pea epicotyls), incubation time 3 hr. One cc. of inhibitor represents 0.2 g. dry weight of pea seedlings.*

Addition to the reaction mixture	IAA added μg.	IAA recovered μg. 0	IAA recovered μg. 3 hr.	% inhibition
None	300	293	45	...
Inhibitor of green pea seedlings				
3.2 cc.	300	295	245	79.8
5.0 cc.	300	293	267	89.5
Inhibitor of etiolated pea seedlings				
3.2 cc.	300	300	80	11.3
5.0 cc.	300	295	120	27.4

IAA-INACTIVATING ENZYME SYSTEM IN PLANTS OTHER THAN PEAS.—*IAA-inactivating enzyme.*—The IAA-inactivating enzyme is found in plants other than etiolated peas. For this investigation, cabbage, lettuce, carrot, spinach and *Avena* were used as sources. One hundred and fifty g. fresh weight of carrot root and of the inside leaves of cabbage and lettuce were separately ground with 100 cc. of distilled water in the blendor and filtered. One hundred and ninety cc. of the filtered juice were obtained from lettuce, 170 cc. from cabbage and 100 cc. from carrot. In each case the fresh tissue was blended with water, filtered free of cell walls *etc.*, and 40 cc. of the filtered juice precipitated with 16 cc. of acetone. The precipitate removed by centrifugations was resuspended in phosphate-citrate buffer, pH 6.6, recentrifuged and the clear supernatant used as the enzyme preparation. Ten cc. of enzyme solution prepared from each source were added to 10 cc. of a solution containing 300 μg. of IAA. Samples were removed after incubation at 25°C. for 0 and 3 hr. (in the case of spinach and *Avena*, 4 hr.) and the residual IAA determined colorimetrically. In table 9 are summarized results which show that while neither carrot, lettuce nor spinach leaf contains the IAA-inactivating enzyme still activity was found in spinach roots and in both coleoptiles and roots of etiolated *Avena* seedlings. Cabbage leaves contained a very low activity. That spinach leaves are unable to inactivate even small amounts of IAA was also demonstrated by following the amount of residual IAA activity with the *Avena* test in which it was possible to follow the fate of as little as 5.0×10^{-7} μg. of IAA per cc. of reaction mixture.

Inhibitor content.—For the investigation of inhibitor content of plants other than peas two series of experiment were carried out. In series a, the lyophylized cell-free juice of spinach leaf was dissolved in water, boiled, filtered and the filtrate used as the inhibitor preparation. In the case of *Avena*, 10-day-old seedlings were cut into roots and coleoptiles (with mesocotyls) and blended separately with distilled water. The cell-free juice was boiled and the filtrate free from denatured protein was used as the inhibitor preparation. The inhibition experiments were carried out with precipitated enzyme preparation of etiolated pea epicotyls. To 10 cc. of enzyme dissolved in buffer and representing 40 g. of fresh tissue, 10 cc. IAA solution containing 300 μg. and inhibitor from the different sources were added as indicated in table 10. In order to demonstrate the presence of inhibitor in lettuce, cabbage and carrot, the experiment of series b was performed. In each case, 5 cc. of boiled whole protoplasm were filtered and the filtrate added to an acetone precipated preparation representing 30 g. of etiolated pea epicotyls. Distilled water replaced inhibitor dilution for the control in both series. The reaction mixtures were incubated at 25°C. and samples were removed after 0 and 30 min. In table 10 are summarized results showing that all species and tissues investigated contain the factor which inhibits the enzymatic inactivation of IAA.

TABLE 9. *IAA-inactivating enzyme content of plants other than peas. Twenty cc. of reaction mixture, pH 6.6, 25°C. Samples of enzyme equal to 10 g. of fresh lettuce, 17 g. of cabbage, 22.5 g. of carrot, 10 g. of fresh spinach root, 7 g. of spinach leaves, 8 g. of Avena roots and 6.8 g. of Avena coleoptiles.*

Source of enzyme	Incubation time hr.	IAA recovered μg.	IAA inactivated μg.	IAA inactivated per g. of fresh tissue per hr. μg.
Lettuce	0	299	..	..
	3	299	..	..
Cabbage	0	299	..	..
	3	273	26	0.5
Carrot	0	299	..	..
	3	299	..	..
Spinach roots	0	300	..	..
	4	240	60	1.5
leaves	4	299	..	..
Avena coleoptiles	0	299	..	..
	4	189	109	4.0
roots	0	295	..	..
	4	220	75	2.3

ENZYMATIC INACTIVATION OF IAA IN VIVO.—The IAA-inactivating enzyme thus far discussed has been demonstrated by *in vitro* preparations. It remains to be demonstrated that this same IAA-

TABLE 10. *Inhibitor content of plants other than peas. Twenty cc. reaction mixture, pH 6.6, containing 300 γ IAA, 25°C.*

Experiment series	Composition of reaction mixture	G. fresh wt. of tissue used for inhibitor preparation	IAA recovered 0	IAA recovered 30 min.	IAA inactivated	IAA prevented from inactivation per g. of fresh tissue
			γ	γ	γ	γ
A.	Acetone purified enzyme	..	300	153	143	...
	Acetone purified enzyme + 4 cc. of inhibitor of *Avena* roots	1.2	300	199	101	36.7
	Acetone purified enzyme + 4 cc. of inhibitor of *Avena* coleoptiles	1.2	300	276	24	100.8
	Acetone purified enzyme + 4 cc. of inhibitor of spinach leaves	2.1	300	287	13	62.8
B.	Acetone purified enzyme	..	294	178	116	...
	Acetone purified enzyme + 5 cc. of inhibitor of cabbage	4.4	302	299	...	...
	Acetone purified enzyme + 5 cc. of inhibitor of carrot	7.5	294	300	...	...
	Acetone purified enzyme + 5 cc. of inhibitor of lettuce	3.9	300	294	...	...

inactivating reaction actually takes place *in vivo* and is not for example produced by wounding the tissue. Thus Fiedler (1936), among others, has suggested that auxin inactivation may occur mainly at cut surfaces and has shown that in particular instances the disappearance of auxin can be largely prevented by coating the cut surface of roots with gelatin or lanolin. The experiments described below suggest, however, that the present auxin inactivating enzyme may be acting *in vivo*.

Pea seeds were germinated in Petri dishes in light or in dark. When the seedlings were 7 days old, they were immersed in a solution of IAA, 100 mg./l., and vacuum infiltrated. The infiltrated seedlings were thoroughly washed with distilled water and then placed with their roots in fresh distilled water at 25°C. Batches of twenty infiltrated seedlings were removed after a further 0, 1, or 4 hr. The epicotyls were then removed from cotyledons plus roots and used for the determination of the residual auxin after being dried in an oven at 70°C. The auxin was then extracted from the tissue with distilled ether and IAA determined in the extracts by the standard *Avena* test (Went and Thimann, 1937). Table 11 summarizes the results of this infiltration experiment and shows that added IAA rapidly decreases with time in etiolated seedlings, the reduction being 83.3 per cent within 1 hr., and 95.0 per cent in 4 hr., whereas the added IAA remains nearly intact in green pea seedlings.

Histochemical evidence also indicates that IAA-inactivation occurs in vivo. When etiolated pea seedlings are infiltrated they become transparent. If the superficial IAA is removed by washing the seedlings and $FeCl_3$–H_2SO_4 reagent is immediately infiltrated into the tissue, the characteristic color given by IAA in the presence of this reagent develops at once. If the IAA infiltrated seedlings are allowed to stand for 1–3 hr. before application of the reagent little or no color develops indicating that IAA has been inactivated by the living tissue.

These two types of experiments indicate, therefore, that IAA is inactivated in living tissues even in the absence of cut surfaces or other injury. Since this disappearance of IAA is correlated with the presence of the enzyme, it may be concluded that the auxin inactivating enzyme is active in the intact plant.

DISCUSSION.—The foregoing experiments have brought out the fact that an enzyme capable of inactivating IAA is found in the tissues of a number of species of plants. In particular, the tissues of etiolated seedlings are highly active, while tissue of similar seedlings grown in the light contain either much less enzyme or none at all. The enzyme is absent from green leaves thus far investigated but is found in roots of etiolated and green plants alike. An inhibitor of the enzyme is found in plant tissues and the distribution of the inhibitor is roughly the converse of that of the enzyme, being found in high concentration in tissues poor in enzyme and vice

TABLE 11. *Inactivation of IAA which has been infiltrated into intact etiolated or green pea seedlings. Auxin determined by Avena test on ether extracted dry samples of epicotyls only.*

Condition of pea seedlings	Incubation time after infiltration	Degrees curvature
Etiolated	0 hr.	6.0 ± 0.07
	1 hr.	1.0 ± 0.17
	4 hr.	0.3 ± 0.08
Green	0 hr.	6.1 ± 0.56
	1 hr.	7.0 ± 0.84
	4 hr.	6.8 ± 0.43

versa. What then is the physiological significance of the IAA-inactivating enzyme? In the first place we may assume on the basis of the experiments involving IAA infiltration of intact seedlings that the enzyme does actually function in living uninjured tissue. Thus the IAA-inactivating enzyme may be expected to participate in auxin metabolism of the etiolated plant by inactivating auxin, while in the green parts of green plants no such inactivation would be anticipated. It is actually the etiolated plant however which shows what might be construed as symptoms of high auxin content, *e.g.*, hyperelongation, etc. The resolution of this paradox is not yet obvious but the following considerations are applicable. In general, etiolated plants contain higher levels of free auxin than comparable light grown green plants (Oppenoorth, 1941; Larsen, 1944; Gustafson, 1946; McIlvaine and Popp, 1940) although this is not invariably true. On the contrary light is essential to the continued formation of auxin as measured by diffusion (Thimann and Skoog, 1933, 1934; Navez, 1933; van Overbeek, 1936). It would therefore seem that light plays at least two roles in auxin metabolism; it increases auxin production but it must also increase destruction. Auxin destruction by light has been noted by many investigators beginning with Went (1928) and the subject has been reviewed by Oppenoorth (1941). Oppenoorth has additionally separated the influence of light on auxin production from that on auxin inactivation in the *Avena* coleoptile, the latter being the dominant reaction at low intensity and the former the dominant reaction at high intensities. A third factor possibly of great importance in etiolation is that etiolated tissues frequently show a greater response to auxin than comparable tissue from light grown plants, as has been shown by van Overbeek (1936) with *Raphanus*. In summary, in the etiolated plant light inactivation of auxin does not take place. The extent of light inactivation in green tissue is not known but must be considerable since light grown plants despite larger auxin production have in general lower auxin contents than etiolated plants. A possible role for the auxin inactivating enzyme of etiolated tissue may lie in maintaining the auxin level actually found, that is, without the enzyme the etiolated plant would presumably exhibit even more symptoms of auxin surplus.

One possible role of the auxin inactivating enzyme is suggested by the presence of the enzyme in roots. Root growth is inhibited by auxins (Nielsen, 1930; Kögl *et al.*, 1934) and applied auxin is rapidly destroyed by roots (Fedler, 1936; Faber, 1936). The auxin inactivating enzyme may then act as a protective mechanism by which the inhibiting effect of auxin on root growth is kept at a minimum.

SUMMARY

Etiolated pea seedlings contain an enzyme which inactivates indole acetic acid (IAA). In both etiolated and green peas there is also a water soluble, thermostable factor which inhibits the enzymatic inactivation of IAA. Light promotes the formation of the inhibitory factor. The inhibitor was found in all plant tissues investigated. The reaction between the substrate, inactivating enzyme and inhibitor is non-competitive. The IAA-inactivating enzyme occurs not only in etiolated tissues of peas but also in *Avena* and in roots. Green leaves of plants thus far used do not contain the active enzyme. Etiolated leaves such as those of cabbage contain the enzyme. The IAA-inactivating enzyme cannot be obtained from dormant pea seeds but the cotyledons of germinated pea seedlings contain the active enzyme. Exposure to light diminishes the content of the IAA-inactivating enzyme of etiolated pea seedlings. The longer the exposure to light the less the amount of the active enzyme found in the plant. The enzymatic inactivation of IAA takes place not only *in vitro* but also *in vivo* as judged by the inactivation of infiltrated IAA.

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