Bound Indoleacetic Acid in Avena Coleoptiles[1]

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Summary. When C^{14} carboxyl indoleacetic acid (IAA) is transported through *Avena* coleoptile sections a fraction of the activity becomes bound. The nature of this bound IAA has been investigated. Upon extraction with solvents and chromatography a substance having the R_F of IAA in 4 solvents was detected. No evidence could be found for the formation of indoleacetyl conjugates. In pea stem sections subjected to a similar experimental regime good evidence was obtained for the occurrence of conjugates. When IAA was supplied exogenously to coleoptile sections floating in solutions the occurrence of conjugates was shown to be dependent on the presence of the primary leaf. In its absence no conjugates could be detected.

On grinding coleoptile sections and subsequent centrifugation at 240 × *g* the radioactivity was found to be in the tissue fraction as opposed to the supernatant. The radioactivity cannot be removed from the tissue by extraction with water, buffer solution or treatment with ribonuclease. It is readily removed by 10 % urea, crystalline trypsin and chymotrypsin. It is therefore concluded that IAA becomes bound to a protein. Bound IAA does not appear to be able to cause growth in *Avena* coleoptile sections.

When indoleacetic acid (IAA) labeled with carbon-14 in the carboxyl group is applied at the apex of decapitated *Avena* coleoptile sections and is transported through the tissue, a fraction of the activity becomes bound or immobilized and is not exported even after a prolonged export period. This phenomenon of the immobilization or binding of IAA has been described by Goldsmith and Thimann (7), who were able to show that the percentage of immobilized IAA increased with increasing donor concentrations, and also that after removal of the donor block containing radioactive IAA and its replacement with a block containing unlabeled IAA the immobilized IAA could not be exchanged with unlabeled IAA.

This behavior indicates that IAA becomes very tightly bound or else is irreversibly converted to another substance.

That there are various forms of bound auxin has of course been known for many years, though their nature has not been established. They may be important as potential sources of free auxin or as sinks into which IAA becomes lost. They greatly complicate the interpretation of data on the auxin content of tissues. In studies of the tropisms of *Avena* coleoptiles, the immobilization of IAA in polar transport tends to obscure the lateral gradients of mobile radioactivity (5, 6), and hence is an important factor in evaluating the results of such experiments.

The purpose of the present investigation was to characterize in more precise terms the nature of the bound IAA of the auxin transport system in the *Avena* coleoptile.

Materials and Methods

Preparation of Plants. Seedlings of *Avena sativa* L. var. Victory were grown as has been previously described (7). The experiments were carried out at 25°. The plants were handled under red light and coleoptiles were cut just above the apical end of the mesocotyl; the primary leaf was gently removed from the basal end of the section. The apical 3 mm of the tip were discarded and subapical sections of 7.5 mm were cut. The experimental arrangements were very similar to those previously described (7).

Groups of 20 sections were supported upright in a small lucite frame with their basal ends down with respect to gravity. At each end the cut surfaces were in contact with the appropriate block of 1.5 % agar (7) with or without IAA. The block supplying IAA to the sections (the donor) was placed on the apical cut surface, and the block collecting the auxin transported through the sections (the receiver) was placed at the basal cut surface. After the donor block had been removed, receiver blocks continued to be set in place for varying lengths of time; this time is referred to as the export period.

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Frames supporting 20 sections rested on glass microscope slides and were placed within lucite boxes containing a dampened paper towel.

Etiolated pea seedlings *Pisum sativum* var. Alaska were grown as described by Wickson and Thimann (13). Seven-mm sections were cut from the third internode directly below the terminal bud of 7-day-old plants.

Extraction of Tissue. Immediately after the export period the tissue was ground with a little sand and extracted with a solvent. Various solvents have been used for extraction. Unless stated otherwise these solvents have been freshly distilled from reagent grade samples just prior to use.

Chromatography. Ascending paper chromatography on Whatman No. 1 chromatography paper has been used throughout. The chromatograms were developed in the dark, and the solvent allowed to run 20 to 24 cm. The following solvents have been used: isopropanol-28 % ammonia-water (8/1/1, v/v) butanol-glacial acetic acid-water (5/1/2.2, v/v), 40 % ethanol and 20 % aqueous KCl (w/v).

Determination of Radioactivity. The radioactivity in donor and receiver blocks and in the tissue was determined by the methods previously described (7). Chromatograms were assayed for radioactivity using liquid scintillation techniques. In some of the earlier work a liquid scintillation counter with an efficiency for C^{14} of approximately 30 % and a background of 7 cpm was used; later on this was replaced by a counter with an efficiency of 82 % and a background of approximately 50 cpm.

The tissue after extraction has also been counted using liquid scintillation techniques. In this case the tissue was dried at 80° for 30 minutes, ground as finely as possible and suspended in Cab-O-sil. A standard counting time of 10 minutes per sample was adopted for liquid scintillation counting.

Chemicals. C^{14}-*Carboxyl*-labeled IAA of a specific activity of 16.9 c/mole was synthesized by Dr. Bruce B. Stowe (11). This IAA was purified as described previously (7) and the resultant product was chromatographed; 98 % of the radioactivity was found in the IAA spot on the chromatogram, the remaining activity being uniformly distributed along the paper.

Unlabeled IAA was obtained from Mann and Company.

All enzymes utilized in these experiments were crystalline. Trypsin and chymotrypsin were obtained from the Worthington Biochemical Corporation. Ribonuclease was supplied by Sigma Chemical Company. The enzymes were employed at a concentration of 0.5 mg/ml. Trypsin and chymotrypsin were incubated at 37° for 2 hours in a 0.067 M phosphate buffer of pH 8.3, and ribonuclease was incubated at 30° for 2 hours in a veronal buffer of pH 6.1.

Growth Experiments. The coleoptile sections were placed in 5-cm petri dishes containing 4 ml of solution made up as follows: 2 % sucrose, 10^{-4} M Penicillin G and 0.006 M K_2HPO_4. The sections were incubated in the dark at 25°, for either 3 or 20 hours. In the former case they were measured with the aid of a dissecting microscope and in the latter by using a bench lens.

Results

Repetition of Experiments of Goldsmith and Thimann. In this experiment (7) the donor block, containing radioactive IAA at a low concentration (0.08 mg/liter), is in place for the first hour of the experiment and is then replaced by an agar block containing no radioactive IAA for the subsequent 2-hour export period. In our repetition the receivers were first changed at the end of the first hour, when the IAA donor was removed, and then again after 15 minutes, after a further 45 minutes, and again for the final hour of the export period. The results (table I) show that the greater part of the radioactivity is exported from the coleoptiles in the first hour of the export period. In the second hour (R_1) only 1 % more is exported. The remaining counts in the tissue after the export period represent the bound auxin. The experiment also shows that the addition of unlabeled IAA to the donor block during the export period does not alter the amount of bound IAA present in the sections, which is about 15 % of the IAA taken up by the section.

Extraction of Coleoptiles Containing Bound IAA. In these experiments the coleoptiles were subjected to the same experimental regime as in section 1. At the end of the export period the coleoptiles were ground and extracted in 10 ml of ether for 2 hours at 3°. The ether was then removed by filtration, evaporated in vacuo, and the residue chromatographed in a number of solvents. For each extraction 160 coleoptile sections were used. The results are shown in figure 1 A–D. The positions of synthetic indoleacetic

Table I. *Immobilization and Export of Radioactivity*

Dpm per group of 20 coleoptile sections. 1-hour uptake followed by 2 hours' export. Donor concentration 0.08 mg/liter IAA (2474 dpm in first group, 2534 in second).

No. of replicates	Transport period: Dpm uptake by difference	Dpm in receiver 1	Export period: 0–15′ R_2	15–60′ R_3	60–120′ R_4	Dpm remaining in tissue	Recovery %
4	708	259	205	134	25	112	101.5
6*	819	288	188	163	19	122	98.5

* Unlabeled IAA present in donor block during export period at a concentration of 1 mg/liter.

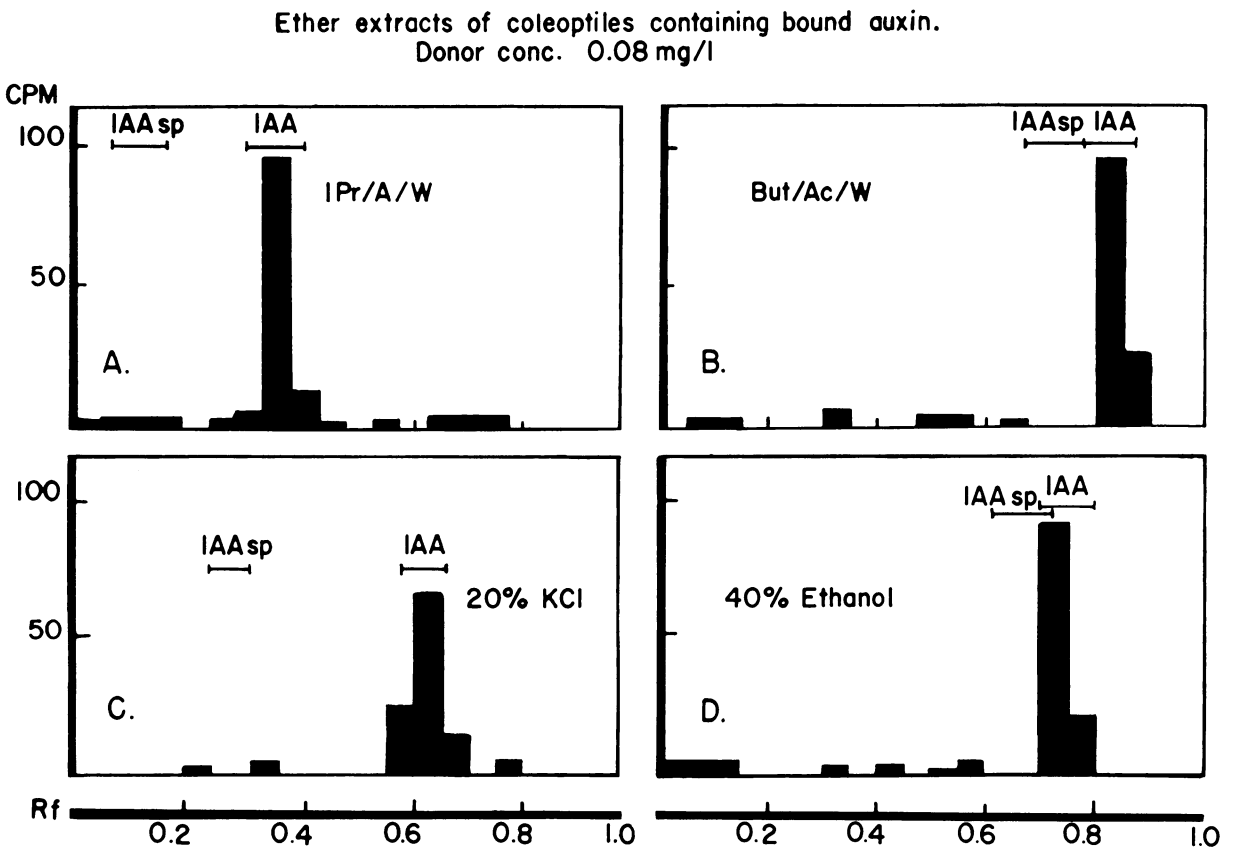

FIG. 1. Ether extracts of coleoptiles containing bound auxin; donor concentration 0.08 mg/liter. Solvents: A, isopropanol-ammonia-water; B, butanol-acetic acid-water; C, 20 % KCl; D, 40 % ethanol. IAA = indoleacetic acid. IAAsp = indoleacetylaspartic acid; chromatographed in parallel.

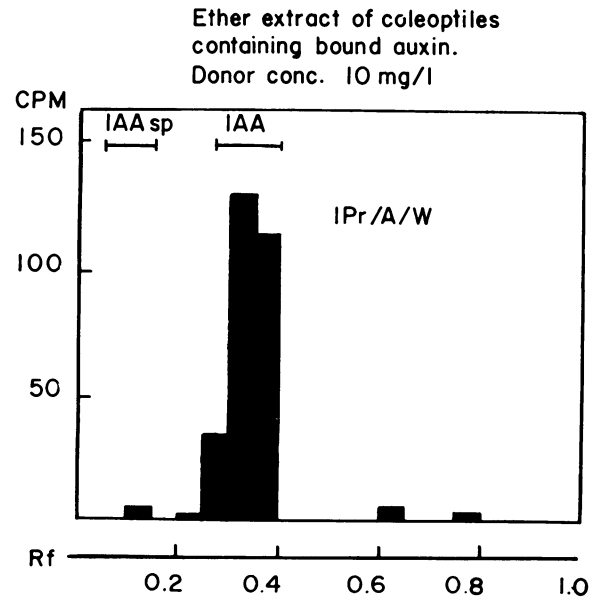

FIG. 2. Ether extract of coleoptiles containing bound auxin; donor concentration 10 mg/liter. Extract chromatographed in isopropanol-ammonia-water.

acid (IAA) and of synthetic indoleacetylaspartic acid (IAAsp) run in parallel are indicated on the figure. In all 4 solvents the radioactivity is in the IAA position; there is no evidence for the formation of any conjugates. Subsequent extraction of the ground tissue after ether extraction with ethyl acetate, 70 % alcohol (reagent grade) and water revealed no detectable radioactivity in these extracts. Determination of the radioactivity in the tissue after ether extraction and after extraction by the additional solvents showed no counts significantly above background level.

The utilization of higher donor concentrations of IAA also shows the same results. One such experiment with a donor concentration of 10 mg/liter is shown in figure 2. In order to conserve the radioactive IAA, unlabeled IAA was added to it for this experiment. There is no evidence for radioactivity on the chromatogram other than that at the IAA position. Determination of the radioactivity in the ground tissue following ether extraction showed no counts above background level.

Metabolism of IAA Applied to Apical Ends of Pea Stem Sections. For comparison with the results with *Avena*, C^{14}-IAA in agar blocks was applied to the apical ends of pea stem sections under identical experimental regimes as described above. With this

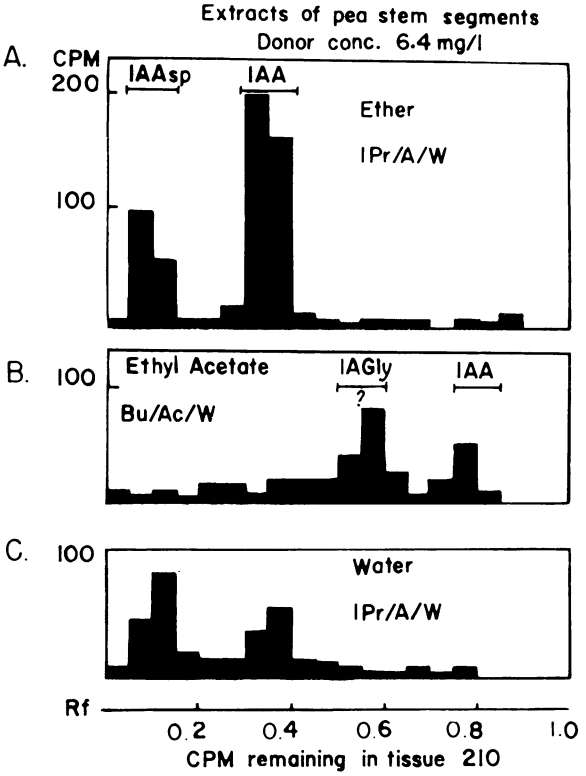

FIG. 3. Chromatograms of extracts of pea stem segments. Donor concentration 6.4 mg/liter. A, ether extract chromatographed in isopropanol-ammonia-water. B, ethyl acetate extract chromatographed in butanol-acetic acid-water. C, water extract chromatographed in isopropanol-ammonia-water. IAGly = expected position of indoleacetylglycoside. Note that in figure C the locations of IAA and IAAsp would be the same as in figure A.

material, using high donor concentrations of IAA, good evidence for the presence of conjugates was obtained. Figure 3 shows the distribution of radioactivity on the chromatograms after such an experiment. There is radioactivity associated with the position of free IAA in figure 3A, in which an ether extract was chromatographed in isopropanol-ammonia-water, and there is also radioactivity at a lower R_F than IAA which corresponds well with synthetic IAAsp. The ethyl acetate extraction (fig 3B) following the ether extraction shows a distribution of radioactivity along the chromatogram with a peak in the position where indole acetylglycosides are to be expected (15). There is also some radioactivity in the IAA position. Upon extraction with water for 18 hours 2 peaks of activity corresponding in position with synthetic IAAsp and IAA can be detected (fig 3C). After these 3 extractions the pea tissue was dried and counted and found still to contain 210 counts above background.

Metabolism of IAA by Coleoptile Sections under the Conditions of the Avena Straight Growth Test. Since Good et al. (8) had reported the occurrence of the conjugates of IAA in *Avena* coleoptile sections, it seemed desirable to repeat some of their experiments using stimulatory as well as inhibitory concentrations of IAA, and also to ascertain the effect of the presence of the primary leaf, since in the work of Good et al. (8) the primary leaf was retained inside the coleoptile section. The coleoptile sections have been incubated as for the straight-growth regime outlined in the Materials and Methods section. The methods of extraction followed those of Good et al. (8) for the acid-ether fraction; 200 10-mm coleoptile sections were utilized in each experiment. Unlabeled IAA was added to the radioactive IAA to achieve the desired concentration.

The major observation to be made about these experiments (fig 4) is that the presence of the primary leaf has a profound effect on the formation of conjugates of IAA. When it is absent no significant formation of acid-ether soluble conjugates occurs (fig 4A, 4C), and from the number of counts remaining in the tissue there is no evidence for any other metabolites that do not involve decarboxylation. In the presence of the primary leaf there is a clear indication of the formation of conjugates of IAA at both growth stimulatory (fig 4B) and growth inhibitory (fig 4D) concentrations. The substance at low R_F is evidently IAAsp, that at R_F 0.8 to 0.9 may be

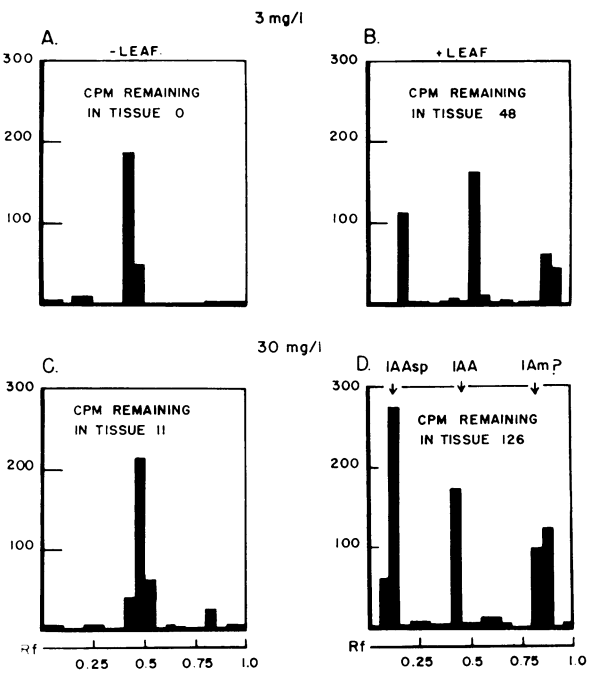

FIG. 4. A comparison of the acid-ether soluble products of coleoptiles with and without the primary leaf, supplied with exogenous IAA for 20 hours. Chromatography solvent isopropanol-ammonia-water. A, IAA concentration 3 mg/liter. Primary leaf removed. B, IAA concentration 3 mg/liter. Primary leaf retained. C, IAA concentration 30 mg/liter. Primary leaf removed. D, IAA concentration 30 mg/liter. Primary leaf retained. IAm = expected position of indoleacetamide.

indoleacetamide. Furthermore, the tissue retains considerable radioactivity after extraction with acid-ether indicating the formation of IAA metabolites that are not soluble in acid-ether. The presence of these compounds can also be ascribed to the primary leaf, since there is no evidence for their presence in more than faint traces when the primary leaf is absent (fig 4A, 4C).

Distribution of Radioactivity between the Soluble and Insoluble Fractions of Coleoptile Sections. Coleoptile sections after the export period were ground up with a little sand and centrifuged at 240 × *g* for 10 minutes. The soluble supernatant or cell liquid fraction was pipetted off and counted and the remaining tissue fraction was dried and counted. In this experiment the duration of the export period was varied but the donor concentration was held constant at 0.1 mg/liter IAA, and the transport period was 1 hour. The results, table II, show that the radioactivity in the insoluble fraction remained fairly constant whatever the length of the export period, while the radioactivity in the soluble fraction declined with the increase in length of the export period.

Table II. *Comparison of Dpm in Cell Liquid Fraction with Dpm in Tissue Fraction after Different Export Periods*

Donor concentration 0.1 mg/liter. One hour transport period was followed by the export period. 80 coleoptile sections were used for each treatment.

Export time hrs	0	1/2	1	2
Cell liquid	710	290	49	41
Tissue	375	396	342	363

This shows that the bound auxin is in the insoluble fraction and presumably is associated in some way with a relatively large structural component of the cell.

Effect of Urea and of Enzymes on the Binding of IAA in the Tissue Fraction. The discovery that IAA was associated with the tissue fraction led to experiments designed to determine the nature of this binding. The experiments of table I and figures 1 and 2 had shown that the bound IAA was readily extractable with ether and other solvents, and all available evidence showed the extracted form to be identical with free IAA. The indications were therefore that the binding did not involve formation of an acyl link.

In these experiments the coleoptiles were subjected to the following experimental regime: the concentration of IAA in the donor blocks was 0.1 mg/liter, the transport period was 1 hour and was followed by a 2-hour export period. At the end of this time the sections were ground with a little sand and centrifuged as described in the Metabolism section above. The cell liquid fraction, referred to hereafter as supernatant, was pipetted off, and the tissue fraction then subjected to its particular treatment for 2 hours. This material was then centrifuged, and the liquid fraction, supernatant, pipetted off. The tissue was then dried and all fractions counted.

The various treatments and the distribution of radioactivity between the fractions are shown in table III. Treatment with 10 % urea at room temperature for 2 hours removes virtually all the radioactivity from the tissue, as does incubation with trypsin and chymotrypsin under the conditions described in Materials and Methods. Water, ribonuclease and phosphate buffer (as used with trypsin and chymotrypsin) do not remove the radioactivity. A number of other hydrolytic enzymes have been considered but have not been used since they have all been shown to possess tryptic activity when assayed by the standard methods for measuring this activity. The results indicate clearly that IAA is associated in some way with a protein.

Table III. *Distribution of Radioactivity Following the Treatment of the Tissue Fraction with Enzyme Preparations and with 10 % Urea*

Donor concentration IAA 0.1 mg/liter. Results expressed as dpm/160 coleoptile sections.

Treatment	Supernatant$_1$*	Supernatant$_2$	Tissue
10 % Urea	8	594	8
Water	0	5	686
Trypsin	26	772	5
Chymotrypsin	24	662	0
Phosphate buffer	61	6	712
Ribonuclease	21	30	735

* Corresponds to cell liquid fraction of table II.

Relation of Bound Auxin to the Growth of Avena Coleoptile Sections. The above findings raise the interesting question of whether the bound auxin is in a functional form, i.e., able to produce elongation. In order to answer this, the growth of coleoptiles which had been subjected to a one-hour transport period with donor blocks containing a graded series of IAA concentrations was measured, and the influence on the observed growth of allowing a subsequent export period was determined. The procedures outlined in the Growth section of Materials and Methods for a 3-hour growth period were followed. The results of 2 such experiments are shown in table IV. Coleoptiles subjected to a 1-hour transport period but no export period (NE) showed an increase in growth over that of control sections which had no IAA in the donor blocks. By contrast, the coleoptiles which received a 2-hour export period following the transport period (E in the table) showed no increase in growth over the controls.

A possible reservation, in relation to the apparent inability of bound auxin to cause growth, is that the total duration of transport followed by the export period is 3 hours, during which time the coleoptiles have been in air. It was therefore necessary to determine whether this treatment affects the response of the coleoptiles to added auxin. Accordingly, the

Table IV. *Comparison of the Growth of Coleoptiles With and Without an Export Period Following an IAA Transport Period.*

Transport period 1 hour. Export period 2 hours. Coleoptiles measured after a 3-hour growth period. Initial length of sections 7.4 mm. 40 coleoptile sections used per treatment.

	IAA mg/liter							
	0		0.1		1.0		10	
Expt I	NE*	E**	NE	E	NE	E	NE	E
Final Length mm	7.68	7.69	7.82	7.64	7.79	7.52	8.18	7.65
△ Control	...	...	0.14	0	0.11	0	0.50	0
Expt 2								
Final Length mm	7.57	7.48	7.83	7.39	8.04	7.51	8.23	7.47
△ Control	...	...	0.26	0	0.47	0	0.66	0

* NE, No export period.
** E, Export period.

growth responses of coleoptile sections maintained for the first 3 hours after cutting exactly as in the experiments described have been compared with those of coleoptiles placed directly in solutions of IAA. In this experiment the growth period in the solution was 20 hours. The results are presented in table V. This clearly shows that the coleoptile sections can respond to IAA after being subjected to the experimental regime adopted in this investigation, though their response is somewhat less than when the sections are placed directly in IAA solutions. Thus, the data of table IV are fully valid, and the bound auxin is not in growth-promoting form.

Table V. *Effect of Maintaining Coleoptile Sections Under the Conditions Employed in the Present Experiments on their Subsequent Response to Exogenously Supplied IAA*

Initial length of sections 7.5 mm. Growth period 20 hours.

	IAA conc. mg/liter			
	0	1	3	10
	Final lengths of sections, mm			
Sections placed directly in growth medium	8.9	12.3	13.3	14.7
Sections placed in growth medium after 3 hrs in moist air	8.3	10.6	11.2	12.5

Discussion

The finding (7) that a fraction of IAA transported in a polar manner by *Avena* coleoptile sections becomes bound in the tissue has been confirmed. In the experiments with *Avena* coleoptile sections described here the concentration of IAA in the donor block was close to 0.1 mg/liter. The reason for this is that this particular concentration in the donor block gives rise in the tissue and in the receiver block to that amount of IAA that is normally present in undecapitated coleoptiles (6, 7). This concentration of IAA is some 10 times below the saturation level of the transport system; when compared with the IAA concentrations that have often been applied to *Avena* coleoptile sections the physiological concentration of IAA in the coleoptile is seen to be exceedingly low. In order to perform experiments that had some basis with respect to the normal physiology of the plant a donor concentration of about 0.1 mg/liter ($= 6 \times 10^{-7}$ M) was used routinely; high concentrations were used only as a basis for comparison.

Ether extraction of coleoptiles containing bound auxin has failed to show any evidence for the occurence of indoleacetyl conjugates. Since carboxyl-C^{14}-IAA was used exclusively, the bound auxin must contain the carboxyl group and cannot be a decarboxylation product of IAA. The only radioactivity on the chromatograms coincides with the position of synthetic IAA run in parallel in 4 solvents. The fact that the tissue after ether extraction contained no significant counts above background indicates complete extraction of the auxin. It must therefore be concluded that bound IAA in *Avena* coleoptile sections, formed under the conditions of the present experiments, is extractable by ether and is in the chemical form of IAA. This is in agreement with the earlier observations of Thimann and Skoog (12) on the extractibility of auxin from *Avena* coleoptile tips and sections cut just below the tip. Using the standard *Avena* curvature test, these workers showed that 1 extraction with ether completely removed the auxin from the sections. By contrast, and in line with the findings in the present work, the primary leaf of *Avena* behaved differently from the coleoptile, in that repeated extractions were necessary to remove all the auxin.

Auxin transport in the pea stem is generally polar but this polarity is not as strict as in the *Avena* coleoptile; furthermore, the fraction of auxin transported is small (ca. 10 % of that taken up by the section), the bulk of IAA taken up by the sections does not appear in the receivers, and about 40 % of that taken up appears to be decarboxylated (13). In other (unpublished) experiments in this laboratory similar sections floating on dilute IAA solutions for 6 hours have been

shown to decarboxylate 45 % of the IAA taken up. The main reason for using the pea stem sections here was to compare them with *Avena* coleoptile sections. Since the characteristics of the auxin transport system in the pea stem have not been as exhaustively studied as in the *Avena* coleoptile, the choice of IAA concentration in the donor block is not based on such firm data, and to that extent is arbitrary. At a donor concentration of 6.4 mg/liter IAA there is clear evidence for the occurrence of indoleacetyl conjugates. Although the R_F values of these conjugates do coincide with those of synthetic markers, the coincidence in a 1-dimensional system can only be taken as a general indication of the identity of the compound. The experiment does demonstrate that conjugation represents an important fate for IAA during transport experiments in the pea stem. This fact is not surprising since Andreae and co-workers have shown the occurrence of indoleacetyl conjugates following the supply of exogenous IAA to pea epicotyls and seedlings (2, 3, 4). Thus the auxin bound during transport is different in its chemical identity in different plant species.

The inability of the *Avena* coleoptile sections to form conjugates of IAA when the primary leaf has been removed is emphasized in the experiments where IAA was supplied in solution to coleoptile sections. The earlier detection of conjugates of IAA in *Avena* coleoptile sections (8) can now be ascribed to the presence of the primary leaf, since only when the leaf is present are conjugates easily detectable. The experiments reported here agree with earlier observations (8) on the metabolism of exogenous IAA by *Avena* coleoptile sections containing the primary leaf.

The distribution of radioactivity between the cell liquid and the insoluble fraction shows that the bound IAA is located in the insoluble "tissue" fraction, whereas the IAA that is still being transported is located in the cell liquid fraction. This observation agrees with that of Yamaki (14) in his experiments on the intracellular localization of endogenous auxins, since he found that free auxin was not centrifuged down but remained in the supernatant even at very high speeds (100,000 × *g*). Yamaki was unable to detect any auxin activity in the sediment corresponding to the insoluble fraction of this investigation, when it was extracted with ether. This apparent discrepancy may be explained by the fact that the amount of auxin is too small to be detected by the *Avena* curvature test used by Yamaki for bioassay.

In view of the fact that the bound IAA is liberated from the tissue fraction by 10 % urea and by ether, as well as by 2 proteinases, it is tempting to suggest that IAA is bound to a protein via a hydrogen bonding mechanism. It is true that both ether and urea would alter the charge distribution on the protein and thus might cause the IAA to be released even if it were covalently bound. However, the very great stability of indoleacetyl peptides makes a peptide linkage very unlikely and on balance a linkage via hydrogen bonds seems indicated.

The binding of IAA to a protein has been described by several workers. The report by Siegel and Galston (10) of a protein-IAA complex formed in vivo has been shown on 2 occasions to be due to an artefact of the trichloroacetic precipitation procedure (4, 16). Nevertheless Zenk (16) has obtained good evidence for the occurrence of an IAA-protein complex, using more refined experimental methods. The physiological significance of this complex has yet to be determined. Ronnike (9) has shown that IAA present in human serum is to a large extent bound to its proteins. In later work (1) there is evidence that the IAA-protein complex can act like IAA as measured by a wheat root cell elongation test.

The liberation of the radioactivity of the IAA by chymotrypsin is significant in another connection. It had been suggested more than once that when auxin activity was liberated by chymotrypsin in the early experiments (12), this was derived from the breakdown of tryptophan liberated by the enzyme, followed by oxidative deamination of the tryptophan, rather than the liberation of auxin as such. In the present work, auxin from such a source would not have been labeled. Chymotrypsin, therefore, does liberate truly bound auxin.

It was obviously of interest to determine the relationship of the bound auxin to the growth of the coleoptile sections. The experiments show that when auxin in transport through the coleoptile is present, growth is greater than when no auxin has been applied. If, however, there is a 2-hour export period so that the coleoptiles contain only bound auxins, then these coleoptiles supplied with auxin elongate no more than those that received none. This means that bound auxin by itself cannot cause a growth increase. The possibility that it may be involved in growth is not fully excluded, since one cannot remove the bound auxin from the coleoptile sections and then test to determine whether the auxin being transported is responsible for the observed growth increments. The proof that coleoptile sections are capable of responding to added auxin (though at a somewhat reduced level) after the lapse of time needed for the transport and export periods strengthens the conclusion that this type of protein-bound auxin is not active in growth.

Literature Cited

1. ANDERSON, A. S. AND F. RONNIKE. 1962. Plant hormonal effect of serum and urine before and after adding indole-3-acetic acid. Acta Pharmacol. Toxicol 19: 139–45.
2. ANDREAE, W. A. AND N. E. GOOD. 1955. The formation of indoleacetylaspartic acid in pea seedlings. Plant Physiol. 30: 380–82.
3. ANDREAE, W. A. AND M. W. M. VAN YSSELSTEIN. 1956. Studies on IAA metabolism. III. The uptake of IAA by pea epicotyls and its conversion to 3-indoleacetylaspartic acid. Plant Physiol. 31: 235–40.

4. Andreae, W. A. and M. H. W. Van Ysselstein. 1960. Indoleacetic acid uptake and metabolism by pea roots and epicotyls. Plants Physiol. 35: 225–32.
5. Gillespie, B. and K. V. Thimann. 1961. The lateral transport of IAA-C^{14} in geotropism. Experientia 17: 126–29.
6. Gillespie, B. and K. V. Thimann. 1963. Transport and distribution of auxin in geotropism. Plant Physiol. 38: 214–25.
7. Goldsmith, M. H G. and K. V. Thimann. 1962. Some characteristics of movement of indoleacetic acid in coleoptiles of *Avena* I. Uptake, destruction, immobilization and distribution of IAA during basipetal translocation. Plant Physiol. 37: 492–505.
8. Good, N. E., W. A. Andreae, and M. W. H. Van Ysselstein. 1956. Studies on 3-indoleacetic acid metabolism. II. Some products of the metabolism of exogenous indoleacetic acid in plant tissues. Plant Physiol. 31: 231–35.
9. Ronnike, F. 1959. Effects of serum proteins and certain salts jointly and separately on the amounts of free phytohormone in the blood. Acta Pharmacol Toxicol. 16: 97–112.
10. Siegel, S. M. and A. W. Galston. 1953. Experimental coupling of indoleacetic acid to pea root protein in vivo and in vitro. Proc. Natl. Acad. Sci. 39: 1111–18.
11. Stowe, B. B. 1963. Synthesis of high specific activity C^{14} carboxyl indoleacetic acid and of C^{14} nitrile indoleacetonitrile. Anal. Biochem. 5: 107–15.
12. Thimann, K V. and F. Skoog. 1940. The extraction of auxin from plant tissues. Am. J. Botany 27: 951–60.
13. Wickson, M. and K. V. Thimann. 1960. The antagonism of auxin and kinetin in apical dominance. II. The transport of IAA in pea stems in relation to apical dominance. Physiol. Plantarum 13: 534–54.
14. Yamaki, T. 1964. Intracellular localization of native auxins and gibberellins. In: Régulateurs Naturels de la Croissance Végétale. 5th Intern. Conf. Plant Growth Substances, Gif-sur-Yvette, 1963. C.N.R.S. Paris. p 319–31.
15. Zenk, M. H. 1961. 1-(Indole-3-acetyl)-β-D-Glucose, a new compound in the metabolism of indole-3-acetic acid in plants. Nature 191: 493–94.
16. Zenk, M. H. 1964. Isolation, biosynthesis and function of indoleacetic acid conjugates. In: Régulateurs Naturels de la Croissance Végétale. 5th Intern. Conf. Plant Growth Substances, Gif-sur-Yvette, 1963. C.N.R.S. Paris. p 241–49.