Hormonal Relations in the Phototropic Response
IV. Light-Induced Changes of Endogenous Auxins in the Coleoptile

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Summary. Beside indoleacetic acid (IAA), 3 auxins were found by chromatographic resolution of acidic fractions of *Avena* and *Zea* coleoptile tips. One of these auxins, designated P, occurred at levels of activity approaching those of IAA. The other 2 auxins, termed F and M, occurred at lower levels of activity. When the auxins of the excised coleoptile tips were isolated immediately after equilateral or unilateral irradiation with blue light at first positive energies, the ratio of IAA to the other auxins increases. This rise is the result of a decrease in P and F, and probably an increase in IAA. Light did not affect materially the total auxin content. It is suggested that P and F might be associated with the basipetal transport inequalities of IAA in phototropism.

P has been partially characterized. Its $R_f$ on chromatograms developed in ammoniacal isopropanol is about 0.65. It is converted to IAA in vitro by heat. The ultraviolet absorption spectrum of chromatographically resolved P also suggests an indolyl complex. P is not readily transported basipetally, and the slope of its relative concentration-response curve (*Avena* section test) is lower than that of IAA. P does not appear to be any of the chemically characterized native auxins.

This paper reports further on a reexamination of the hormonal changes in the coleoptile exposed to phototropic stimulation. The first work of the series (7), using $^{14}$C-labeled indoleacetic acid ($^{14}$C-IAA), reaffirmed the existence of basipetal transport rates consistent with the kinetics of the tropic response, and the strict basipolarity of the transport. The second study (8) found no indication of a radiation-induced lateral movement of $^{14}$C-IAA. The third (17) examined the effect of unilateral irradiation on the distribution of both introduced $^{14}$C-IAA and endogenous IAA in transport assemblies (coleoptile tips placed on bisected receiver blocks of agar). Again a lateral equality of IAA content in the plane of the light gradient was found for the half tip-plus-block assembly. However, it was also found that the shaded side of a tip transported more IAA basipetally than did the side illuminated. It was therefore suggested (8, 17) that a lateral difference in the capacity to transport IAA basipetally could account for lateral inequalities in the amounts of auxin transported from the tip. On the basis of barrier experiments, such as those of Oppenooorh and of Briggs (cf. 17), it was also suggested that the above photoinduced inequalities of IAA transport might be mediated by another hormone.

In the present paper we will show that auxins other than IAA occur in the coleoptile, and that irradiation alters the concentration relationships between these auxins.

Materials and Methods

Preparation and Irradiation of Plants. Coleoptiles were obtained from seed of *Avena sativa* (Svalöf Victory I) and *Zea mays* (Burpee Snowcross). Planting procedures and the growth environment for the seedlings were described previously (17). Five-mm tips were cut from etiolated coleoptiles of *Avena* and *Zea*, 72 and 96 hours, respectively, after planting. For irradiation, the tips were aligned on a moistened microscope slide, care being taken to maintain normal geotropic orientation. The slide was placed at 1 end of a light-tight, rectangular box, 1.3 m from the light source. For equilateral irradiation, the light beam was reflected downward from a mirror mounted directly above the tips. Only the tips of *Zea* were used for unilateral irradiation (see Methods in 17).

In 1 experimental series, described by table I, a red work light was used to facilitate manipulation of the plants (15 w tungsten filament plus 6.5 mm Corning Glass filter No. 3480, Red Shade Yellow). For the remaining experiments, manipulations were carried out under light from green fluorescent tubes.

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1 Work supported by the United States Atomic Energy Commission.
Table I. Effect of Equilateral Irradiation of Avena Tips on the Auxins Obtained by Aqueous Diffusion

<table>
<thead>
<tr>
<th></th>
<th>IAA, P,F</th>
<th>IAA</th>
<th>P</th>
<th>F</th>
<th>IAA/P,F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1300 tips</td>
<td>3.26 (100)</td>
<td>2.45 (75)</td>
<td>0.71 (22)</td>
<td>0.10 (3)</td>
<td>3.02 I</td>
</tr>
<tr>
<td>Nonirradiated</td>
<td>2.20 (100)</td>
<td>0.87 (40)</td>
<td>0.97 (44)</td>
<td>0.36 (16)</td>
<td>0.65 N1</td>
</tr>
<tr>
<td>1500 tips</td>
<td>2.77 (100)</td>
<td>2.21 (80)</td>
<td>0.56 (20)</td>
<td>0   (0)</td>
<td>3.95 I</td>
</tr>
<tr>
<td>Nonirradiated</td>
<td>3.18 (100)</td>
<td>1.90 (60)</td>
<td>1.04 (33)</td>
<td>0.24 (7)</td>
<td>1.48 N1</td>
</tr>
</tbody>
</table>

Fig. 1. Phototropic exposure-response curves for unilaterally irradiated coleoptiles. A) *Avena* (7), B) *Avena*, and C) *Zea*, present work. Irradiation time: 15 seconds for *Avena*, 22 seconds for *Zea*. *Zea* coleoptiles were exposed to red light, incident energy about 0.6 mw·cm⁻², for 1 hour before phototropic stimulation.

filtered through green and amber Plexiglas (fig 1A, 17). The *Zea* seedlings were exposed to red light for 1 hour prior to experimental treatment. The source of red light was a G. E. Ruby Red tungsten filament lamp, 7.5 w, yielding an incident irradiance of 0.6 mw·cm⁻². Blue light (fig 1B, 17) was used for photo-stimulation of the coleoptile tips, with an irradiance incident to the tip of 32 μw·cm⁻². Figure 1 shows the coleoptile curvatures induced by various unilateral exposures. Based on these exposure-response curves, radiant densities eliciting maximum first positive curvature, 5 and 7 kiloergs·cm⁻² for *Avena* and *Zea*, respectively, were used; the exposure times were 15 seconds for *Avena* and 22 seconds for *Zea*.

**Auxin Isolation and Assay.** After irradiation, groups of 500 coleoptile tips were floated in 15 ml distilled water in a shallow glass dish and allowed to remain in the dark at 25° for 3 hours. The aqueous diffusates were frozen in liquid nitrogen and kept at −20° until enough auxin had been collected for fractionation (usually within 2 days). One ml of 8% NaHCO₃ solution was added to each 15 ml of diffusate solution. The neutral and basic components were extracted with diethyl ether and discarded, since they showed no auxin activity. The aqueous phase was acidified to pH 3.0 with HCl and extracted with ether to separate the ether-soluble acidic fraction for chromatography in isopropanol-ammonia (28%)-water, 10:1:1, v/v/v (17). In a number of experiments with *Zea* coleoptiles, the tips were extracted in the dark at 2 to 4° with diethyl ether for 16 hours; the acidic components chromatographed as above. The chromatograms were sectioned and each section was eluted with absolute methanol. Blank paper strips were also chromatographed, sectioned, and eluted with methanol; these eluates were used as bioassay controls. The ether was purified before use by shaking with and distilling from an aqueous mixture of CaO-FeSO₄ (16). The methanol was purified by distillation over Zn-KOH.

The various chromatogram eluates were evaporated in 5 ml culture dishes or glass vials at 7° by a stream of air. Auxin contents of the eluates were determined by either *Avena* section test (3) or by *Avena* curvature test (16). For the section
test, 3-mm coleoptile sections were cut 2 mm below the apex. These sections were soaked in $10^{-5}$ M MnCl$_2$ for 1 hour in the dark at 25° and transferred to the culture dish to which had been added 2 ml phosphate-citrate buffer, $10^{-3}$ M, pH 5.0, containing 2 % sucrose. The dishes were placed on a shaker platform (1 cycle/sec) and kept in the dark at 25°. After 20 hours, the sections were measured at 20x magnification. All section-test data are based on net mm elongation, i.e., the measured increment in length minus that of the sections incubated with eluates of the blank chromatogram. For the curvature test, 0.35 ml of 1.5 % agar was added to each vial containing an eluate residue, stirred, and equilibrated for at least 1 hour at room temperature before the agar block was poured.

To evaluate the effect of light on the relationship between the auxins, it was necessary to describe quantitatively the auxin activities at different loci on the chromatograms. Summation of the net increase in length of the test sections upon assay of the chromatogram segments about 1 locus is not valid, since response to known auxins in the section test is usually a logarithmic function of the concentration. Where the auxin is available in relatively pure form, viz. IAA, concentration-response curves can be constructed. Biological responses of individual chromatogram segments at the RF of IAA may then be summed as IAA equivalents. But for uncharacterized auxins, whose concentration-response curves (slopes) and thresholds (intercepts) were not known, no such determination could be made. A method for the summation of the activities of chromatogram segments was therefore derived (see Appendix) and applied to all the section tests. This derivation enables one to evaluate more precisely the relative changes in auxin activities that are induced by experimental treatment, without knowing the absolute amounts of auxin involved. It should be stated that qualitatively similar inferences may be drawn from comparison of the summed net growth increments of the test sections assaying the segments about one chromatogram locus. For the curvature test, responses are an approximately linear function of auxin concentration within the proportionality range. Hence, for quantitative comparison, we have summed the individual average degrees curvature obtained by assay of the different segments about one locus, since each datum was within the linear range.

**Results**

**Equilateral Irradiation of Avena and Zea.** Two auxins were repeatedly encountered upon chromatography of the aqueous diffusate from both non-irradiated and irradiated excised coleoptile tips. Results of one experiment with Avena are shown in figure 2. One auxin has the migration rate of IAA (RF 0.3). The second has an RF of about 0.65 and will be designated F. Small amounts of a third auxin F were frequently found at the solvent front (fig 2A). The activity in this region of the chromatogram was generally less when the tips were exposed to light (fig 2B). A more quantitative evaluation of the effect of light is given in figure 2A, which shows that irradiation decreased the activities of P and F. The ratio of IAA to P + F was raised 3- to 4-fold by irradiation.

Coleoptile tips of Zea, which are larger and more easily manipulated than those of Avena, were used for most of the subsequent experiments. Aqueous diffusates from the coleoptile tips of Zea were fractionated, chromatographed, and bioassayed. The distribution of auxin activity on the chromatogram (table I) was similar to that obtained from Avena tips. When the seedlings were not pre-exposed to red light, activity was found at the RF's corresponding to IAA and P, but not F. Though F was not always detected, it was found only when red light had been used. As with the Avena, equilateral irradiation of Zea tips with blue light resulted in an increase in the ratio of IAA to P and F. This occurred both when the coleoptiles had and had not been previously exposed to red light. Omission of the pre-exposure to red light appeared not to affect materially the activities associated with IAA or P.

To investigate more effectively the effect of irradiation on F, P and IAA, we increased the yields of these auxins by extracting the tips, at low temperature, with ether. The distribution of auxin activities on chromatograms of the ether-soluble acid fraction of Zea tips not exposed to blue light is shown in figure 3A. Beside IAA, P and F, auxin activity appeared at a new locus, RF 0.4 to 0.45. This activity will be termed M. In addition, a strong growth inhibitor is extracted by ether; it remains localized at the origin of the chromatogram.
Table 11. Effect of Equilateral Irradiation of Zea Tips on the Auxins Obtained in Aqueous Diffusion

<table>
<thead>
<tr>
<th></th>
<th>IAA, P, F</th>
<th></th>
<th>IAA</th>
<th></th>
<th>P</th>
<th></th>
<th>F</th>
<th></th>
<th>IAA/P, F</th>
<th>I/N1</th>
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<td>700 tips*</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irradiated</td>
<td>2.13 (100)</td>
<td>1.95 (92)</td>
<td>0.18 (8)</td>
<td>0 (0)</td>
<td>10.8 I</td>
<td>1.8</td>
<td></td>
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<tr>
<td>Nonirradiated</td>
<td>3.16 (100)</td>
<td>2.70 (85)</td>
<td>0.46 (15)</td>
<td>0 (0)</td>
<td>5.9 N1</td>
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<td></td>
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<tr>
<td>750 tips*</td>
<td></td>
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<td></td>
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<tr>
<td>Irradiated</td>
<td>3.50 (100)</td>
<td>3.24 (93)</td>
<td>0.26 (7)</td>
<td>0 (0)</td>
<td>12.5 I</td>
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<tr>
<td>Nonirradiated</td>
<td>2.98 (100)</td>
<td>2.68 (90)</td>
<td>0.30 (10)</td>
<td>0 (0)</td>
<td>8.9 N1</td>
<td></td>
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<td>700 tips</td>
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<tr>
<td>Irradiated</td>
<td>3.32 (100)</td>
<td>3.10 (93)</td>
<td>0.22 (7)</td>
<td>0 (0)</td>
<td>14.1 I</td>
<td>1.2</td>
<td></td>
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<tr>
<td>Nonirradiated</td>
<td>3.55 (100)</td>
<td>3.28 (92)</td>
<td>0.27 (8)</td>
<td>0 (0)</td>
<td>12.1 N1</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>750 tips</td>
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<td></td>
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</tr>
<tr>
<td>Irradiated</td>
<td>3.70 (100)</td>
<td>3.10 (84)</td>
<td>0.43 (12)</td>
<td>0.17 (4)</td>
<td>5.17 I</td>
<td>1.3</td>
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<tr>
<td>Nonirradiated</td>
<td>4.51 (100)</td>
<td>3.60 (80)</td>
<td>0.61 (13)</td>
<td>0.30 (7)</td>
<td>3.96 N1</td>
<td></td>
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</tr>
</tbody>
</table>

* Seedlings not pre-exposed to red light.

Fig. 3. Bioassay of chromatograms of ether extract, acid fraction, of Zea coleoptiles. A) Nonirradiated, 250 tips. B) Equilaterally irradiated with blue light, 260 tips. (See data of Table III).

Comparison of figure 3A with figure 3B suggests that equilateral exposure to blue light here also caused an increase in the ratio of IAA to the other auxins (extraction of the tips began several minutes after irradiation). This ratio change is described quantitatively by the data in Table III. It may be recalled that equilateral irradiation of the *Avena* tip (Table I) and of the *Zea* tip (Table II) with blue light causes an increase in the ratio of IAA to P and F in the aqueous diffusates. Similarly, with ether extraction irradiation of *Zea* tips causes a reduction in the amounts of P and F and a rise in the ratios of IAA to the remainder of the auxin activities.

*Unilateral Irradiation of Zea.* The effect of unilateral irradiation with blue light on the various

Fig. 4. A) Bioassay of chromatogram segments, between Rf 0.15 and 0.45, of aqueous diffusate, acid fraction, of nonirradiated *Avena* coleoptiles (1200 tips). The broken line indicates the distribution of activity on chromatogram segments, Rf above 0.45, in a comparable fraction with the same number of tips. B) Solid lines: Bioassay of above chromatogram segments between Rf 0.45 and 0.95 that were eluted with ether, heated and rechromatographed. Broken lines: Bioassay of chromatogram segments between Rf 0.45 to 0.95 where gaseous N2 at about 7* was used instead of heat to reduce the volume of the elutes before rechromatography.
auxins in the irradiated and shaded tissues of the tip was then determined.

Within 1 to 2 minutes after irradiation each tip was halved by pushing it downward on the supporting blade. Each group of half-tips was extracted with diethyl ether. The acid fraction was chromatographed and bioassayed. As with the whole tips (fig 3), auxin activity was found at the F, P, M, and IAA loci. The effect of unilateral irradiation on these auxins is described quantitatively by the data in table IV from experiments with sets of paired half tips. Here again the ratios of IAA to the remaining auxins are increased in the tissues exposed to light.

In the preceding experiments, extraction of the tips began within several minutes after irradiation. In another series, the tips were left on the blade-block assembly in a moist atmosphere for a period of 2.5 hours after unilateral irradiation before splitting and extraction. With the extended interval between irradiation and extraction, we found IAA but no P or F in either the tips or blocks.

**Characteristics of P. I. Thermal Lability.** Heating of P results in the formation of an auxin with the chromatographic mobility of IAA. An acid fraction from the aqueous diffusate from 1200 unirradiated *Avena* tips was chromatographed. The segments between Rf 0.15 and 0.45, which encompassed the Rf of IAA, were eluted and assayed. The auxin distribution in these segments is shown in figure 4A, solid line. The remainder of the chromatogram between Rf 0.45 to 0.95 was eluted with ether. This eluate was reduced to dryness in a waterbath at 97°, and left in the bath for 3 minutes. It was then taken up in ether and spotted for chromatography. Auxin activities of the segments of this second chromatogram are shown in figure 4B. Significant activity was found only at the Rf of IAA; little remained at the higher Rf's. Without the heat treatment (broken lines, fig 4B) there was no conversion apparent of the faster moving components to activity at the Rf of IAA. Rechromatography in both experiments apparently resulted in a loss of some activity.

**II. Basipetal Transport.** *Avena* tips, which have a relatively higher content of P and less interfering pigments than the tips of Zea, were used as a source of the P component. The acid fraction of the aqueous diffusate from 8000 and from 9500 *Avena* tips was chromatographed, using 9 strips for each fraction. The P areas between Rf 0.50 to 0.85 were eluted with methanol. As a control, areas of
blank chromatograms corresponding to the P region were also eluted with methanol. All methanol eluates were evaporated at 7° by a stream of air. The residues were taken up in ether, and distributed equally among 6 agar blocks (1.5%, 1 × 8 × 11 mm); this was done by adding the ether dropwise onto the agar surface upon which a stream of nitrogen, that had been bubbled through water, was directed (16). The blocks were equilibrated by storage for 2 hours at 25° in the dark in a water-saturated atmosphere. They were then cut into 3 equal longitudinal strips and used as donor blocks.

Three subapical Avena coleoptile sections, 5 mm, cut 2 mm from the tip, were placed on an agar receiver block, 1 × 8 × 11 mm, maintaining normal vertical orientation. The coleoptile sections were held vertical through holes in a thin rigid plastic strip bridging the receiver block. A single donor was placed on the apical ends of each 3 coleoptile sections, and allowed to remain there, in the dark at 25°, in a moist atmosphere. Eighteen such assemblies were made with P component in the donor, matched by 18 control assemblies with the blank eluate in the donor. After 3 hours, the donor blocks, tissues, and receiver blocks were separated and immersed in ether for 15 hours, in the dark, at 2 to 4°. Each extract was chromatographed. The resultant chromatogram segments were assayed by the section test. Summed activities at the various chromatogram loci are given in table V. Some P, and in 1 experiment, I.AA, appeared in the tissue. Small amounts of F activity appeared in the receiver blocks. These data show no basipetal transport of P. If such transport did occur, either the velocity was less than 1.7 mm per hour, or the amount transported was too small to be measured by the section test.

The preceding transport inferences are further substantiated by the inactivity of P in the Avena curvature test. The acid fraction of the aqueous diffusate from 750 Avena tips was chromatographed. The developed chromatogram was divided into 10 segments. Each segment was eluted with methanol and the eluates assayed by the Avena curvature test. The distribution of curvature activity along the chromatogram is shown in figure 5. Virtually all of the activity was at the R_P corresponding to that of I.AA.

**III. Absorption Spectrum of P.** The acid fraction of aqueous extracts from 6600 Avena tips was chromatographed. Methanol eluates of the chromatogram segments between R_P 0.5 to 0.8 were pooled and dried at 7° by a stream of air. The residue was dissolved in 5 ml twice-distilled water for determination of absorption spectra in the visible and ultraviolet regions. No absorption bands in the visible spectrum were found. In the UV, there was a minimum absorption band at 257 m\(\mu\), a maximum at 272 m\(\mu\), with a secondary absorption band at 287 m\(\mu\) (fig 6). The absorption spectrum shows

<table>
<thead>
<tr>
<th>Table V. Basipetal Transport of P Through Coleoptile Sections of Avena</th>
</tr>
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<tbody>
<tr>
<td>Values represent activities found in transport assemblies 3 hours after application of donor block.</td>
</tr>
<tr>
<td>IAA</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>I. Donor block*</td>
</tr>
<tr>
<td>tissue</td>
</tr>
<tr>
<td>Receiver block</td>
</tr>
<tr>
<td>II. Donor block*</td>
</tr>
<tr>
<td>tissue</td>
</tr>
<tr>
<td>Receiver block</td>
</tr>
</tbody>
</table>

* Donor blocks contain P component isolated from aqueous diffusate of 8000 (I) and 9500 (II) Avena tips.
little fine detail, possibly because of underlying impurities. However, its general form is similar to those of uncomplexed 3-substituted indolyl compounds, but with a shift toward shorter wavelengths. It is of interest that some of the bound forms of IAA, which are suspected to be indolylacetyl glycosides, show such a hypsochromic shift (20), as does an indolyl-acetil-glucoside (23).

IV. Comparison of P with Other Native Auxins. Unidentified auxins of Rf similar to P have been reported to occur in plant extracts. One was found by Hemberg (10) on chromatography of the acidic fraction of corn-kernel extracts, but was not further characterized. However, in the neutral-basic fraction of the extract he found 3 auxins that appeared to be interconvertible. The 3 were ninhydrin positive, and one (f) appeared to resemble tryptamine in mobility and convertibility. In the present work with coleoptile diffusates and extracts, no auxin activity occurred in the neutral-basic fractions, only in the acidic.

We have considered P as an acidic component since it is found only on the chromatograms of the acidic fraction of the tissue extracts and diffusates. However, the Rf of P in the ammonia-isopropanol system indicates a mobility greater than those of acidic auxins (1, 12, 21), one more usually associated with neutral or basic substances. It is possible that the acidification of the aqueous phase (making the acids ether soluble) in fractionation caused P to form from an acid; or perhaps such a conversion was effected by the chromatography.

Indolepyruvic acid (IPyA), whose occurrence in the corn kernel has been verified recently (22), is labile upon alkaline chromatography or mild heating, breaking down to several substances with auxin activity, one of which is IAA (4, 5, 11). The Rf of IPyA itself in alkaline systems is uncertain (compare 4 and 18). Figure 7 shows the results of bioassay by the section test, and of a chromatographic spray of a chromatogram of crystalline IPyA developed in the ammoniacal solvent. Three areas out of the 6 that developed color were active in the section test, the most active area appearing at the Rf of IAA. Some activity remained at the origin, and low activity overlaid (Rf 0.6-0.9) the locus of P. The latter, area f, figure 7, developed a crimson color after treatment with dilute FeCl3-HClO4 reagent (9). The auxin indole-3-acetamide produces a similar color, has an Rf in ammoniacal isopropanol of about 0.8 (12, 23), is hydrolyzable to IAA, and is apparently one of the decomposition products of IPyA upon alkaline chromatography (11). Tryptophol, another decomposition product of IPyA (14), is a weak auxin also travelling to the same Rf region (0.84). Therefore area f (Rf 0.6-0.9) on chromatograms of IPyA was eluted with methanol, heated, rechromatographed, and the chromatogram assayed by the section test. All the activity was again found at Rf 0.6 to 0.9. Thus the breakdown product of IPyA, f, is apparently not P. If f is indoleaceta-

mide or tryptophol, we may infer that both the amide and the alcohol are also not converted to IAA by the heat treatment, and hence are not P.

To check this inference, as well as the implications of Hemberg's work (10) concerning tryptamine, crystalline preparations of tryptamine (Eastman), indole-3-acetamide (American Chem. Paint Company), and tryptophol (Regis) were purified by chromatography in the ammonia-isopropanol system, and eluted with methanol. The methanol eluates at Rf 0.86, 0.82 and 0.91, of the above compounds, respectively, were treated as follows: 1) (200 µg) evaporated and heated at 97° (see Methods) and rechromatographed; 2) (200 µg) evaporated by a stream of N2 gas at about 7° and rechromatographed; 3) (1 mg) fractionated into neutral-basic and acidic components and each fraction chromatographed. Chromatograms were examined under ultraviolet light (3660 Å source) and sprayed with the iron-perchloric acid reagent for spot localization.

Tryptamine and tryptophol remained wholly in the basic-neutral phase upon fractionation. The acidic phase from partitioning of the amine chromatographed to yield an area at the Rf of IAA (0.34) that formed a small crimson spot after being sprayed; nothing appeared at the Rf of tryptamine. Neither the amine nor the alcohol was transformed to IAA or any other substance by either the heat treatment or by evaporation under nitrogen. Indoleacacetamide appeared to distribute itself equally between the acidic and neutral-basic phases upon

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**Fig. 7.** Bioassay (A) and color development (B) of chromatograms of indolepyruvic acid (IPyA) developed in isopropanol: ammonium hydroxide: water (10:1:1). Solid-line histogram was developed from chromatogram spotted with 100 µg IPyA, broken-line from 10 µg IPyA. For B, the chromatogram was sprayed with FeCl3 in dilute HClO4; the broken lines indicate areas fluorescent under UV light, before treatment with the color reagent.
fractionation. But here again no IAA or other component was formed from the amide by treatments 1 and 2.

Similarly, indole-3-acetonitrile (Pal), appeared also in part in the acidic phase, unlike indole-3-acetaldehyde (Regis) and indole-3-ethylacetate (Cal. Biochem.). But these 3 auxins likewise were unchanged by the heat treatment that converts P to IAA.

It is unlikely that the IAA complexes, ascorbigen and the indolyl acetyl peptides are identical with P. Ascorbigen is inactive in the *Avena* section test and breaks down in the ammoniacal chromatographic system to several biologically active compounds (15); in these respects it differs from P. The peptides, indolylacetyl-aspartate and -glutamate are biologically active but have a solubility, stability and chromatographic mobility dissimilar to that of P (2). Moreover, the slope of the concentration-response curve of indolylacetyl glutamate parallels that of IAA (2, p 616), unlike the response curve of P (fig 8).

Srivastava (19) has shown that maize contains a number of IAA complexes, probably indolylacetyl glycosides, which appear to be partly soluble in ether, though this solubility was not demonstrated rigorously. Also, Zenk (23) reported that the naturally occurring 1-(indolyl-3-acetyl)-β-D-glucose (unlike P), is labile upon exposure to bicarbonate or ammoniacal chromatography, yielding indoleacetic acid and IAA. The previously described conversion experiment indicated that P is not indoleacetic acid. Additional evidence that P is not the glucoside, indoleacetic acid, or the “e” spot of Hemborg’s (10) are the observations that the concentrations of these compounds rise when tissues are incubated with IAA (6, 10, 23). Yet when we incubated *Avena* coleoptile sections with IAA, no change was observed in the levels of P. However, unresolved is the possibility that an ether and water-soluble glycoside was converted by the experimental manipulation into an auxin other than those considered, with the RF and thermal behavior of P.

**Discussion**

We have shown that the acidic fraction of aqueous diffusates and ether extracts of *Avena* and *Zea* coleoptile tips contains several auxins. Based on the section-test bioassay, the major activities resolved by paper chromatography in ammonia-isopropanol are at the RF of IAA, about 0.35, and a broader band, P, at RF about 0.65. Some activity occurs at the solvent front (F), and at RF about 0.43 (M) when ether is used as an extraction solvent.

P is active in the *Avena* section test and inactive in the curvature assay. Though P was found only in the acidic fractions, its RF suggests that it must be a relatively weak acid, if it is one. The concentration response curve for P is relatively flat (fig 8). Hence small changes in the activities assigned to P probably represent large differences in concentrations of the auxin. P shows little, if any, basipetal transport in vivo, though it is found in aqueous diffusates when the whole tip is immersed in water. It is readily converted to IAA in vitro by mild heating. The UV absorption spectrum of a partially purified preparation of P possesses spectral characteristics of indolyl compounds. On the basis of its solubility and partition characteristics, thermal conversion to IAA, chromatographic behavior, and relative biological activity, P is apparently not any of the chemically characterized native auxins.

P and F were found in both the ether extracts and the aqueous diffusates when isolation of the auxins began within several minutes after excision of the tip. When there was a lapse of 2.5 hours between excision of the tips and isolation of the auxins, IAA but no P or F was found. It may be suggested that the intact seedling is necessary for the maintenance of a P-F pool in the tip, and that immersion of the tip in water or ether enables escape of these auxins before they are transformed by the tissue.

Wide differences were encountered in auxin activities obtained per tip (perhaps caused by undefined variations in either chromatographic or extraction conditions, since the bioassays were within proportionality range). Because of these differences, we have stressed the effect of light on the relationship between auxins within each experiment, and grouped the experiments to show changes in ratios between the auxins.

When coleoptile tips were placed into water or ether within several minutes after being irradiated, light caused the ratio of IAA to the remaining auxins to rise about 3- to 4-fold in *Avena* (table I) and about 1.5-fold in *Zea* (tables II, III). In every one of 11 separate experiments, the ratio of IAA to the other auxins was higher in the equilaterally irradiated tip than in the nonirradiated, and was higher for the irradiated tissue halves than for the shaded after unilateral irradiation. As a binomial distribution, the
probability that this would occur by chance in every one of 11 experiments is less than 1 in 2000. The mean quotient of the auxin ratios, irradiated to non-irradiated (I/NI derived from all tables), was 2.0 ± 0.30, a ratio differing significantly from unity (p<0.01).

This irradiation-induced increase in the ratio of IAA to the remaining auxins may be ascribed largely to the effect of light on P, and to a lesser extent, on F. Table VI summarizes the changes in each of the 3 auxins expressed as ratios between the activity of auxin from irradiated to that from non-irradiated tissues. Considering the data as a whole, light significantly reduces the activities of both P and F. An apparent exception occurred with P when ether was the auxin extractant, where there was a single instance of a ratio materially larger than the mean ratio. The mean activities ascribed to IAA were increased by light with both isolation methods, though the increase, when aqueous diffusion was used, was not significant. Since light caused no material change in the total auxin, it is possible that the light-induced increase in IAA occurred at the expense of P and F. In view of the convertibility of P + F to IAA by mild heat treatment, the resemblance of the UV absorption spectrum of P to those of indolyl compounds, the growth promoting activity of P in the section but not curvature test, and the relatively rapid disappearance of P and F but not IAA in isolated tips, it is tempting to ascribe a precursor function to P and F.

In a previous study (17, fig 2), where 14C-IAA was applied to coleoptiles, a small amount of radioactivity was found at the locus of P when the tissue extracts were chromatographed. This suggests that P possibly arises from IAA; inhibition of such a transformation by light could account for the observed increase in both IAA and the ratio of IAA to P after irradiation. This possibility is unlikely, however, since soaking Avena tips in IAA solution results in no detectable increase of the P content in extracts of the tissues. Furthermore, the above-mentioned study (17, figs 2A, 2C) also showed that light did not change the amount of 14C activity occurring at the P region of the chromatograms of extracts of tips treated with 14C-IAA before irradiation.

The lateral inequality in the amount of IAA translocated from the tip unilaterally irradiated with first positive energies appears not to derive from antecedent lateral transport of IAA (17). We concluded that the light-induced inequality arises primarily from laterally unequal reductions in capacity for basipetal transport of the auxin. Such transport differences might be associated with light-induced inequalities of P or F. However, as yet there is not enough information about these auxins to enable rigorous inference about their function in phototropism.

Acknowledgment

We are grateful to David Miller and Sylvanus A. Tyler of this Laboratory for their suggestions and comments on the summation of activities at chromatographic loci, and to H. M. Sell, Michigan State University, for a sample of crystalline indolepyruvic acid.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Method</th>
<th>Irradiation</th>
<th>Total</th>
<th>IAA</th>
<th>P</th>
<th>F</th>
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<tr>
<td>Avena</td>
<td>Water</td>
<td>Equilateral</td>
<td>1.48</td>
<td>2.82</td>
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<td>&quot;</td>
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<td>0.72</td>
<td>0.39</td>
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<td>&quot;</td>
<td>1.17</td>
<td>1.21</td>
<td>0.87</td>
<td>(0/0)</td>
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<tr>
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<td>&quot;</td>
<td>&quot;</td>
<td>0.94</td>
<td>0.95</td>
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<td>&quot;</td>
<td>0.82</td>
<td>0.86</td>
<td>0.70</td>
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<td>p</td>
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<td>&gt;0.9</td>
<td>0.4</td>
<td>&lt;0.01</td>
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<tr>
<td>Zea</td>
<td>Extraction</td>
<td>Equilateral</td>
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<td>1.27</td>
<td>0.89</td>
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<td>0.23</td>
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<td>&quot;</td>
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<td>&lt;0.1</td>
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<tr>
<td>Mean (diffusion and extraction)</td>
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<td>0.17</td>
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<td>&lt;0.01</td>
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</table>

Student's t test was used to obtain the probability estimates, p.
Appendix

We assume that the responses to the different auxins encountered are linear functions of the logarithm of their concentrations. Auxin response curves in the stoichiometric range (fig 8) may be expressed as

\[ r = b \log_{10} c + a \]  

(1)

where \( r \) = response (net mm elongation), \( b \) = slope, \( c \) = concentration of the auxin, and \( a \) = intercept.

Then

\[ \frac{r - a}{b} = \log_{10} c. \]  

(2)

Exponentiating (2),

\[ c = 10^{(r-a)/b}. \]  

(3)

Let

\[ k = 10^{-a/b}, \]

a constant for each auxin:

then

\[ c = k \cdot 10^{r/b}. \]  

(4)

The total concentration (\( C \)) of an auxin in one area of a chromatogram is the sum of the concentrations in the separate chromatogram segments (\( \sum c_i \)) within that area, i.e.,

\[ C = \sum_{i=1}^{s} c_i. \]

Since, by (4),

\[ c_i = k \cdot 10^{r_i/b}, \]  

(5)

\[ C = k \sum_{i=1}^{s} 10^{r_i/b}. \]

Let

\[ R = a + b \log C, \]

where \( R \) is the total response (mm) elicited by concentration \( C \). Then

\[ R = a + b \log \left( k \sum_{i=1}^{s} 10^{r_i/b} \right) \]

\[ = a + b \left[ \log k + \log \left( \sum_{i=1}^{s} 10^{r_i/b} \right) \right] \]

\[ = a + b \left( \frac{a}{b} \right) + b \log \left( \sum_{i=1}^{s} 10^{r_i/b} \right) \]

\[ = b \log \left( \sum_{i=1}^{s} 10^{r_i/b} \right). \]  

(6)

For example, if there are 3 chromatogram segments yielding responses of \( r_1, r_2, \) and \( r_3 \) at 1 locus of a chromatogram, then, by equation (6)

\[ R = b \log \left( \text{antilog } r_1/b + \text{antilog } r_2/b + \text{antilog } r_3/b \right). \]

We used the above procedure to sum the auxin activities of the grouped segments of a chromatogram at 1 locus. The slope, \( b \), of the concentration-response curve (within the proportionality range) for any auxin was obtained by determining the net mm elongation in the section test to serial dilutions of the auxin eluted from a single activity area. Figure 8, for example, shows the concentration-response curve for IAA and the relative concentration-response of the auxin I obtained from coleoptile tissues. Unless otherwise indicated, all tabular data were based on the adjusted total response, \( R \) (equation 6), of the *Avena* test sections.

**Literature Cited**


