It is difficult to relate extractable auxin directly to the growth of plant tissues. The diffusible auxin, on the other hand, can be correlated with the growth in younger stem tissues (2,6). The chemical nature of this important substance is poorly characterized. Using paper chromatography Raadts and Söding (5) identified part of the diffusible auxin from the Avena coleoptile tip as IAA and reported the presence of another diffusible auxin (Wanderung auxin). By paper chromatography Shibaoa and Yamaki (9) could not find the Wanderung auxin. They found only IAA. The diffusible auxin from sunflower leaf (8) and Coleus stem tip (7) has been reported to be IAA also by paper chromatography. The present study was undertaken to examine the nature of diffusible auxin in etiolated and green plants, and to determine the effect of light on the diffusible auxin.

Materials and Methods

Both etiolated and green plants were used as the source of diffusible auxin. Diffusion from etiolated tissue took place at 24° for 3 hours in a humidity chamber under total darkness. Diffusion from green plants took place at 25° for 3 hours in a humidity chamber under 100% relative humidity (cool white). Under these conditions the maximum amount of diffusible auxin is obtained (10).

Etiolated Plants. Seeds of Avena sativa L. var. Victory were germinated under diffuse light for 1 day to prevent the growth of the first internode. The seedlings were transplanted into sand and grown in the darkness at 24° and a relative humidity of 85% for 52 hours. When the coleoptiles were 2 cm long, 2 mm of the tips were removed and placed on 1.5% agar blocks 2 × 2 × 2 mm.

Green Plants. Seeds of sunflower (Helianthus annuus L. var. Russian Mammoth) were sown in sand and kept in the greenhouse with the minimum temperature of 21°. The experiments were done during the period from October to February. When the epicotyl began to grow, the first nodes were excised and placed on 2 × 2 × 2 mm blocks of 1.5% agar. Seeds of Pisum sativum L. var. Alaska were soaked in distilled water with aeration for 12 hours, sown in sand and grown in the greenhouse with the minimum day temperature at 21° and the minimum night temperature at 16°. Knop's nutrient solution was applied to the sand every 4 days, otherwise growth is retarded and little diffusible auxin is obtained. The diffusible auxin from peas was obtained by placing the sixth node with leaf and apex on a 2 × 2 × 2 mm block of 1.5% agar. Seedlings of long-day plants, Centaurea cyanus L. var. Blue Boy, were transplanted into wooden flats 4 days after sowing and grown under 8 hours of daylight for 21 days. After this period 1 set of plants was placed under long-day conditions of 12 hours of light combining daylight with incandescent light. Apices from plants having 12 to 15 leaves and 2 to 3 g of fresh weight were excised. The stem apices with 3 expanding leaves and a fresh weight of ca. 120 mg were placed in 1.5% agar blocks 2 × 4 × 4 mm.

Avena Curvature Test. For the auxin bioassay the Avena curvature test was used exclusively. The test was performed in the standard manner (12) except that the time of the second decapitation was 2 hours after the first decapitation. The test was carried out under red light at 24° with a relative humidity of 85%. The standard curvature responses was determined each time with agar blocks which had equilibrated for 5 hours in 2 × 10^{-7} M, 6 × 10^{-7} M, and 2 × 10^{-6} M solutions of IAA. Degrees of curvature are given with the standard error.

Paper Chromatography. The number of agar blocks used to obtain the diffusible auxin varied for different plant tissues, 200 for Avena coleoptile tips, 100 for sunflower stem apices, 100 for stem apices of pea, 50 for short-day Centaurea and 25 for long-day Centaurea. The agar blocks were extracted twice with 30 ml of absolute ethanol at 4° for 12 hours. The ethanol extracts were combined and concentrated in vacuo to roughly 0.1 ml. The ascending technique with untreated Whatman No. 1 paper (2 cm in width) was used. After the concentrate was streaked on the paper it was allowed to equilibrate for 12 hours with the developing solvent system. It was then developed with either a basic solvent system of isopropanol : ammonia (1:1 v/v) (11) or a neutral solvent system of isopropanol : water (10:1 v/v). The RF of IAA in a neutral solvent system will vary depending upon the paper used, the distilled water, and the atmospheric conditions. Under ideal conditions the RF of IAA in the neutral solvent system is 0.8 to 0.9. If both solvent systems in separate chromatographic jars are used in the same room, then the RF of IAA is 0.4 to 0.5.

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2 This investigation was supported by funds from National Science Foundation Grant 16336.
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on both chromatograms. If a separate room in which ammonia is excluded is used for chromatography with the neutral solvent system, the Rf for IAA is 0.8 to 0.9, illustrating the important effect of ammonia vapor on chromatography with the neutral solvent system. Further precaution against its effect was taken by allowing evaporation of glacial acetic acid to take place in the room prior to developing with the neutral solvent system. An acidic solvent system was not used since the activity of diffusible auxin is lost with a solvent system of isopropanol: acetic acid (0.02 M): water (10:1:1 v/v). In each experiment 1 sample of diffusible auxin was chromatographed directly. To another sample 0.3 ml of 2 × 10⁻⁶ M IAA was added and chromatographed similarly. By this method the Rf of the IAA which was added to the diffusate could be determined precisely each time. If the Rf value of the added IAA was less than 0.7, the chromatogram was discarded, because of the possibility of contamination by alkaline materials. This procedure was used instead of color development of the added IAA, because it requires only 0.1 γ of IAA which is unlikely to affect the Rf of diffusible auxin. After drying the chromatogram the portion between the origin and solvent front was cut into 10 equal parts, each of which was placed in a glass vial and eluted with 0.3 ml of water. To this eluate 15 blocks of 1.5% agar 2 × 2 × 2 mm were added and allowed to equilibrate for 5 hours. Afterwards the blocks were removed and excess moisture was absorbed by the filter paper before using them in the test. When the dried chromatogram was not used immediately, it was stored at -20° in vacuo.

Results

Chromatography of Diffusible Auxin in Basic Solvent System. Diffusible auxin from Avena, sunflower, short-day and long-day treated Centaurea plants was chromatographed using a basic solvent system and the auxin activities of the ten portions of the chromatogram are shown in figure 1. In all chromatograms developed with a basic solvent system a portion of the diffusible auxin was found at an Rf of 0.4 to 0.5. In Centaurea this is also the Rf of the synthetic IAA added to the diffusate. In Avena the Rf of diffusible auxin is a little less than the Rf of synthetic IAA. This slight difference may be due to materials in the diffusate since the Rf of synthetic IAA may be changed slightly when it is chromatographed with an extract (4). The diffusible auxin from sunflower had an Rf of 0.4 to 0.6, but in this case the Rf of the synthetic IAA was also higher. In other trials the Rf of diffusible auxin was 0.4 to 0.5 and the Rf of synthetic IAA was 0.55. In sunflower and Centaurea plants another portion of the diffusible auxin was found to have an Rf between 0.1 and 0.3. This diffusible auxin is clearly different from IAA as such. Since it is more hydrophilic than IAA, a neutral solvent system is best for separating the substance from IAA.

Fig. 1. Chromatograms of diffusible auxin developed with isopropanol: ammonia (1.1 M): water (10:1:1 v/v). The arrows show the Rf of synthetic IAA alone on separate chromatograms which were developed in the same jars as the chromatograms of the diffusates. For Avena, 200 coleoptile tips were used for diffusion. A concentration of 2 × 10⁻⁷ M, 6 × 10⁻⁷ M and 2 × 10⁻⁹ M IAA gave 3.0 ± 0.5, 9.3 ± 0.4 and 20.5 ± 0.9 degrees curvature, respectively. For sunflower, 100 stem apices were used. A concentration of 2 × 10⁻⁷ M, 6 × 10⁻⁷ M and 2 × 10⁻⁹ M IAA gave 3.0 ± 0.6, 11.2 ± 0.7, and 26.2 ± 1.1 degrees curvature, respectively. For long-day treated Centaurea, 25 stem apices were used for diffusion. A concentration of 2 × 10⁻⁷ M, 6 × 10⁻⁷ M and 2 × 10⁻⁹ M IAA gave 4.5 ± 0.5, 10.9 ± 0.5, and 20.6 ± 0.6 degrees curvature, respectively. For short-day treated Centaurea, 50 stem apices were used for diffusion. A concentration of 2 × 10⁻⁷ M, 6 × 10⁻⁷ M, and 2 × 10⁻⁹ M IAA gave 3.0 ± 0.6, 8.6 ± 0.6, and 20.0 ± 1.1 degrees curvature, respectively.

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Chromatography of Diffusible Auxin in Neutral Solvent System. When the diffusible auxin from 200 coleoptile tips of Avena grown in the dark room was chromatographed in the neutral solvent system, the RF was found to be 0.6 to 0.7 as shown in figure 2. Little or none of the diffusible auxin from coleoptile tips has an RF of 0.8 to 0.9 and most of the diffusible auxin must be something other than IAA as such.

The diffusible auxin from stem apices of Alaska pea grown in light has an RF of 0.5 to 0.6 in the neutral solvent system and IAA in the presence of diffusate has an RF of 0.7 to 0.8 (fig 3). Very little diffusible auxin is obtained from stem apices of Centaurea grown under short-day conditions and in the neutral solvent system it has an RF of 0.5 to 0.8. The RF of the synthetic IAA in the presence of this diffusate is 0.7 to 0.8 (fig 4). Large amounts of diffusible auxin are obtained from the stem apices of Centaurea plants grown under long-day and the RF of this diffusible auxin is 0.3 to 0.4 (fig 4). When the diffusible auxin was chromatographed with IAA, the RF of synthetic IAA was 0.6 to 0.9 with a peak at 0.7 to 0.8. The diffusible auxin of Centaurea plants under long-day conditions is not simply IAA.

Chromatography of diffusible auxin from the

Fig. 2. Chromatograms of diffusible auxin from 200 Avena coleoptile tips developed with isopropanol: water (10:1 v/v). A concentration of $2 \times 10^{-7} \text{M}$, $6 \times 10^{-7} \text{M}$ and $2 \times 10^{-6} \text{M}$ IAA gave 1.9±0.6, 8.9±0.7, and 21.0±1.0 degrees curvature, respectively.

Fig. 3. Chromatograms of diffusible auxin from 100 stem apices of Alaska pea developed with isopropanol: water (10:1 v/v). A concentration of $2 \times 10^{-7} \text{M}$, $6 \times 10^{-7} \text{M}$ and $2 \times 10^{-6} \text{M}$ IAA gave 2.8±0.5, 8.1±0.1, and 20.6±0.6 degrees curvature, respectively.

Fig. 4. Chromatograms of diffusible auxin from 50 apices of short-day treated Centaurea and from 25 apices of long-day treated Centaurea developed with isopropanol: water (10:1 v/v). For chromatogram at upper left, a concentration of $2 \times 10^{-7} \text{M}$, $6 \times 10^{-7} \text{M}$, and $2 \times 10^{-6} \text{M}$ IAA gave 4.5±0.5, 10.9±0.5, and 20.6±0.6 degrees curvature, respectively. For chromatogram at lower left, a concentration of $2 \times 10^{-7} \text{M}$, $6 \times 10^{-7} \text{M}$, and $2 \times 10^{-6} \text{M}$ IAA gave 2.7±0.5, 9.3±0.6, and 20.4±0.7 degrees curvature, respectively. For chromatogram at upper right, a concentration of $2 \times 10^{-7} \text{M}$, $6 \times 10^{-7} \text{M}$, and $2 \times 10^{-6} \text{M}$ IAA gave 3.0±0.6, 8.6±0.6, and 20.0±1.1 degrees curvature, respectively. For chromatogram at lower right, a concentration of $2 \times 10^{-7} \text{M}$, $6 \times 10^{-7} \text{M}$, and $2 \times 10^{-6} \text{M}$ IAA gave 2.3±0.6, 9.1±0.5, and 20.6±0.6 degrees curvature, respectively.
stem apex of sunflower in the neutral solvent system revealed 2 unusual aspects. The RF of synthetic IAA added to the dif fusate was found to be 0.0 to 0.3 and the RF of diffusible auxin varied from one determination to another. If the agar blocks were leached with petroleum ether (bp 66-75°) for 24 hours at 4° in the dark, the peculiar effect on the RF of synthetic IAA was eliminated as shown in figure 5. The RF of synthetic IAA in the presence of dif fusate in then 0.6 to 0.8, while the RF of diffusible auxin is not affected by leaching. The nature of the substances which interfere in the chromatography are unknown. They are soluble in petroleum ether, they have no auxin activity per se and on the chromatogram they have a region of ultraviolet absorption corresponding to an RF of 0.0 to 0.5. Although the RF of diffusible auxin is the same as the RF of synthetic IAA, some difference must exist since the RF of synthetic IAA is so radically changed by the contaminating materials while the diffusible auxin is not affected.

**Light-type and Dark-type Diffusible Auxin.** In the first determinations the RF of the diffusible auxin from sunflower was found to vary from 0.1 to 0.8. The diffusible auxin from plants harvested on bright sunny days had lower RF values and the diffusible auxin from plants harvested on cloudy days had higher RF values. The effect of light on the RF of diffusible auxin was examined by placing plants in the dark room after 7 hours exposure to sunlight. The RF of the diffusible auxin was then determined at 0, 5, 12, 24, and 48 hours. As shown in figure 6, the diffusible auxin had an RF of 0.1 to 0.2 for 0 and 5 hours of darkness. After 12 hours in the dark the RF of the diffusible auxin was changed to 0.5 to 0.8 with a peak at 0.6 to 0.7. After 24 hours in darkness the only change is a diminution in the amount of diffusible auxin. After 48 hours an insignificant amount of diffusible auxin was present. In each instance the RF of synthetic IAA added to the dif fusate was found to be 0.7 to 0.9. When a group of plants was placed under sunlight for 5 hours after 24 hours dark treatment (fig 6) an increase in diffusible auxin was found and the RF was 0.5 to 0.8 with the peak at 0.6 to 0.7. The low RF of diffusible auxin from sunflower plants in sunlight was checked by rechromatographing the diffusible auxin from 100 sunflower apices. The diffusible auxin on the first chromatogram had an RF of 0.2 to 0.3 and when rechromatographed the RF was 0.3 to 0.4 with the RF of synthetic IAA at 0.9 to 1.0.

**Ehrlich Color Reaction of Diffusible Auxin.** In general the chromatography of diffusible auxin in a neutral solvent system indicates this auxin to be different from IAA and raises the question of the degree of difference. As a first approach to this question the color development of diffusible auxin with Ehrlich reagent was used to establish the indole char-

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**Fig. 5.** Chromatograms of diffusible auxin from sunflower apices with and without leaching by petroleum ether developed with isopropanol: water (10:1 v/v). For chromatograms of upper and lower left a concentration of 2 x 10^{-7} M, 6 x 10^{-7} M, and 2 x 10^{-6} M IAA gave 2.3±0.6, 8.3±0.5, and 20.5±0.4 degrees curvature, respectively. For chromatograms of upper and lower right a concentration of 2 x 10^{-7} M, 6 x 10^{-7} M, and 2 x 10^{-6} M IAA gave 3.1±0.4, 7.7±0.5, and 19.3±0.5 degrees curvature, respectively.

**Fig. 6.** Effect of darkness on the RF of diffusible auxin from sunflower apices. Plants were kept for 0, 5, 12, 24, and 48 hours in total darkness before diffusion. The chromatogram at lower right is of the diffusible auxin from sunflower plants that were kept in darkness for 24 hours and exposed to 5 hours sunlight before diffusion. Chromatograms were developed with isopropanol: water (10:1 v/v). The RF of synthetic IAA for comparison in all cases was 0.7 to 0.8. For upper left a concentration of 2 x 10^{-7} M, 6 x 10^{-7} M, and 2 x 10^{-6} M IAA gave 2.3±0.6, 7.7±0.8, and 19.0±0.4 degrees curvature, respectively. For middle left a concentration of 2 x 10^{-7} M, 6 x 10^{-7} M, and 2 x 10^{-6} M IAA gave 1.7±0.5, 8.3±0.7, and 19.0±0.5 degrees curvature, respectively. For lower left a concentration of 2 x 10^{-7} M, 6 x 10^{-7} M, and 2 x 10^{-6} M IAA gave 2.3±0.6, 8.1±0.6, and 18.6±0.7 degrees curvature, respectively. For upper right a concentration of 2 x 10^{-7} M, 6 x 10^{-7} M, and 2 x 10^{-6} M IAA gave 2.0±0.5, 7.8±0.5, and 18.9±0.4 degrees curvature, respectively. For middle right a concentration of 2 x 10^{-7} M, 6 x 10^{-7} M, and 2 x 10^{-6} M IAA gave 3.1±0.4, 7.7±0.5, and 19.3±0.5 degrees curvature, respectively. For lower right a concentration of 2 x 10^{-7} M, 6 x 10^{-7} M, and 2 x 10^{-6} M IAA gave 2.6±0.5, 8.2±0.5, and 19.5±0.5 degrees curvature, respectively.
acter. Sunflower plants were grown in sand under direct sunlight and the stem apices of 8534 plants were excised at the base of the first leaves when the internode was 1.0 cm or less and placed on the same number of agar blocks for diffusion. After leaching with petroleum ether, 8534 agar blocks were extracted with 1.5 liter of absolute ethanol at 4° for 24 hours. The ethanol extracts were concentrated in vacuo, streaked on a piece of Whatman No. 3 MM filter paper, 46.1 cm wide, and developed with the neutral solvent system for 7 hours. After the paper had dried, a strip 0.5 cm wide was cut off from each side of the chromatogram and assayed in the usual manner. The diffusible auxin was found at Rf of 0.1 to 0.2 corresponding to the Rf value of figure 6 at 0 and 5 hours. This region of the remaining chromatogram was then eluted with 100 ml of absolute ethanol at 4° for 24 hours and the eluate concentrated in vacuo. Approximately 1/50 of the concentrate was placed on one spot on Whatman No. 3MM paper and the remainder was placed as a second spot on the same paper. This chromatogram was then developed with the basic solvent system. From the first spot the Rf of diffusible auxin was shown to be 0.2 to 0.4 with a peak at 0.2 to 0.3 by the Avena curvature test. The chromatogram of the second spot did not show ultraviolet absorption in this region, but gave a very slight bluish-black color development at Rf 0.28 with the Ehrlich reagent. Since the synthetic IAA when chromatographed similarly gave a blue color at the Rf 0.28, the diffusible auxin is indicated to be an indole compound also.

Discussion

The chromatography of diffusible auxin with a basic solvent system has given fairly uniform Rf values of 0.4 to 0.5 in this investigation and in many others (3, 5, 7, 8, 9). Raadts and Söding (5) and Miller and Gordon (3) found 2 different auxins on the chromatograms, one with an Rf comparable to IAA, and another with a lower Rf than IAA in the first case and with a higher Rf in the second case. In their studies however, the auxin was obtained by placing the coleoptile tips in water and extracting the auxin from the water. When the auxin is diffused directly into agar blocks and extracted from them, the Rf value is the same as that of IAA as shown in this study and also in the work of Shibaoka and Yamaki (9).

Using a neutral solvent system it is possible to separate the diffusible auxin on the chromatogram from IAA that is added to the difusate for Avena, Alaska pea and Centaurea. The Rf values for the diffusible auxin of these 3 plants are slightly different and may reflect some chemical differences. Because of its possible significance, the light-type diffusible auxin of sunflower has been studied in greater detail. It is not acidic since, if it were, the salt formed in the basic solvent system should have a lower Rf than the Rf in the neutral solvent system. Thus acidic indole derivatives are excluded as possibilities for the diffusible auxin. The Avena curvature test shows that the light-type diffusible auxin is 10 times more soluble in the acidic water fraction than in the ether fraction when it is partitioned. Thus both the methyl and ethyl esters of IAA and indole-3-acetonitrile may be excluded as possibilities for the diffusible auxin on the basis of solubility as well as on the basis of their higher Rf values in a basic solvent system (11). The diffusible light-type auxin is also clearly different from N-(indole-3-acetyl)-aspartic acid for the Rf of this substance in the basic solvent system is extremely low (0.08). The light-type auxin cannot be 1-(indole-3-acetyl)-β-D-glucose (13) since this substance in the basic solvent system forms indole-3-acetamide (W. A. Andreae, personal communication) which has only a hundredth of the activity of IAA in the Avena curvature test. Thus most if not all of the biological activity of the glucose complex would be lost after chromatography in the basic solvent system.

The diffusible light-type auxin of sunflower cannot be identified with IAA or any of the proven IAA complexes in plant tissues. Its properties are those of a water-soluble auxin rather than an ether-soluble material such as IAA. Bentley (1) has already suggested that IAA is not the physiological auxin although it may occur in many tissues but in smaller amount than the non-ether soluble auxin which is the physiological substance.

Summary

The diffusible auxin and indoleacetic acid (IAA) were examined by paper chromatography with a neutral solvent system (iso-propanol: water (10:1 v/v)) in an acidic atmosphere to prevent effects of ammonia vapor. The identification of diffusible auxin as related to IAA was checked in each instance by adding synthetic IAA to the diffusate.

Under these conditions the Rf of IAA was either 0.7 to 0.8 or 0.8 to 0.9. The Rf of diffusible auxin from coleoptile tips of *Avena sativa* L. var. Victory grown in the dark room was 0.6 to 0.7. The Rf of diffusible auxin from stem apices of *Pisum sativum* L. var. Alaska grown in the greenhouse was 0.5 to 0.6. The Rf of diffusible auxin from stem apices of *Centaurea cyanus* L. var. Blue Boy under short-day conditions was 0.5 to 0.8, while the Rf of diffusible auxin of these plants under long-day conditions was 0.4 to 0.5. In each instance the diffusible auxin is different from IAA.

Auxin diffused from sunflower apices ( *Helianthus annuus* L. var. Russian Mammoth) grown under sunlight (light-type auxin) had an Rf of 0.1 to 0.2 and that from sunflower apices kept in the dark (dark-type auxin) had an Rf of 0.7 to 0.8. The light-type auxin diffused from 8534 sunflower apices and separated chromatographically was Ehrlich positive, indicating that it might be an indole compound.
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Literature Cited


Penetration of Ions through Isolated Cuticles

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Absorption of mineral nutrients by leaves of plants was demonstrated experimentally over 100 years ago (16). Since then numerous workers have studied the uptake of ions by aerial plant parts. In recent years it has been possible, by radioisotopic techniques, to assess accurately the extent of nonroot absorption, and subsequent transport of trace as well as the major nutrient elements (1,2,12).

Any study of the mechanism of foliar absorption must consider ion penetration of the cuticle. This is the first barrier to be penetrated before a chemical applied to a leaf can contact plant protoplasm. Although the permeability of the cuticular membrane to ions, herbicides, and pesticides has been discussed (3,7,9,11) their mode of entry through the cuticle is still not well understood.

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3 Journal Article 3152 of the Michigan Agricultural Experiment Station.
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Stomatal pores may or may not be present in cuticular membranes, depending on the characteristics of the plant from which the cuticle is isolated. Stomata may serve as a portal of entry into the leaf, however, if entry is gained through the stomata it is not equivalent to absorption. Further, stomatal absorption does not preclude the necessity of cuticular penetration, since substances in the intercellular spaces still must pass through a layer of cutin (10, 15).

Nutrient losses from above ground plant parts by action of rain and dew are also significant. Tukey et al. (13,14) found that some nutrients like Na and Mn were leached easily. Ca, Mg, S, K, Sr-Y, moderately, and Fe, Zn, P, Cl with difficulty. Studies thus far have been concerned either with foliar absorption or foliar leaching, but not both. In this report, the rate of cation and anion penetration from the outer to the inner surface and, from the inner to the outer surface of isolated cuticular membranes was studied. The permeability of a synthetic dialyzing membrane was compared with that of the biologically synthesized cuticular membranes.