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Translocation and Metabolism of Endosperm-Applied [2-^{14}C] Indoleacetic Acid in Etiolated *Avena sativa* L. Seedlings

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ABSTRACT

The role of free indole-3-acetic acid (IAA) in the endosperm of *Avena sativa* L. seedlings was investigated to determine its contribution to free IAA in the shoot. [2-^{14}C]IAA was injected into the endosperm of dark-grown seedlings and the transport and metabolism of the [^{14}C]-labeled compounds determined. It was concluded that translocation of free IAA directly from the endosperm is probably not a significant source of free IAA in the shoot, mainly because even small amounts of [^{14}C]IAA introduced into the endosperm were rapidly metabolized. This suggested that, in *Avena*, free IAA does not normally exist in the liquid endosperm.

Recently, the role of the coleoptile tip as the site of auxin production in cereal seedlings has been re-examined (3, 9, 10, 18, 23, 26). Sheldrake (23) found IAA and alkali-labile IAA compounds were present in the ascending xylem sap of *Avena*, *Zea*, *Triticum*, and *Hordeum* seedlings. He showed that [^{14}C] IAA injected into the endosperm of *Avena* seedlings was later detectable at the coleoptile tip, and in the xylem sap. This, and other evidence, led Sheldrake to conclude that IAA and 'inactive' auxins move acropetally in the xylem from the grain to the coleoptile tip, where they accumulate and where the 'inactive' auxins are converted to IAA. Similarly, Whitehouse and Zalik (29) have detected radioactive IAA in *Zea* shoots following injection of [^{14}C]IAA into the endosperm.

Bandurski's group, however, have demonstrated that, in *Zea* at least, the transport of free IAA directly from the endosperm is probably not a significant source of IAA in the shoot (10, 18). Through a series of isotope dilution experiments, they have calculated both the amounts and the rates of turnover of the indolyl compounds in the developing *Zea* seedling. Radioactively labeled IAA-*myo*-inositol was found to be transported from the grain into the shoot at 400 times the rate of labeled IAA, and at 40 times the rate of IAA derived from labeled tryptophan (18). The latter two sources of IAA were shown to be insufficient to provide the needs of the developing shoot (10, 18). Bandurski and co-workers (9, 18) have presented a body of evidence to support their view that IAA-esters are the immediate precursors of IAA in the endosperm and shoot, and that the *myo*-inositol ester is the major transport form of IAA from endosperm to shoot, not free IAA.

In *Avena*, however, the composition of the indolyl compounds in the endosperm is distinctly different from *Zea* (19). The bulk of the *Avena* esters are high mol wt compounds, similar to the nontransportable IAA-glucans which comprise approximately 50% of the ester pool in the *Zea* endosperm (9, 19). Low mol wt esters similar to the readily transported IAA-*myo*-inositols are virtually absent in *Avena* grains (19). Moreover, Sheldrake (23) found that the free IAA comprised a much higher proportion of the auxin in the xylem sap of *Avena* seedlings than *Zea*.

Therefore, it is possible that, in *Avena*, transport of free IAA directly from the endosperm to the shoot might be more important than in *Zea*. Experiments similar to those of Sheldrake were performed, whereby [^{14}C]IAA was injected into the endosperm and the fate of the label determined. Although Sheldrake (23) claimed that [^{14}C]IAA injected into the endosperm accumulated at the coleoptile tip, his experiments in fact do not show this. A gradient of radioactivity down the coleoptile was illustrated following application of radioactive IAA to the roots, or to the cut base of the coleoptile. However, it was not shown that such a gradient also occurred following injection of [^{14}C]IAA into the endosperm (23). Furthermore, the proportion of radioactivity transported into the shoot which remained as IAA was not determined in Sheldrake's experiments. This knowledge is necessary to ascertain whether the transport of IAA from endosperm to shoot occurs in physiologically significant amounts in *Avena*.

MATERIALS AND METHODS

Avena sativa L. cv 'Terra' grains (Dr. McKenzie, Agricultural Research Station, Winnipeg, Canada) were surface sterilized in 1.5% aqueous NaOCl (v/v) for 5 min, rinsed in distilled H_2O and soaked in 0.01 N HCl for a further 10 min to remove traces of adsorbed NaOCl (1). The grains were then washed thoroughly in distilled H_2O and inserted, embryo-downwards, between heat-sterilized, folded blotting paper strips within 7- × 60-mm lengths of glass tubing (16). Three tubes were placed vertically in small glass vials containing approximately 6 ml of distilled H_2O and incubated in darkness at 24 ± 1°C (80–90% RH). For the transport studies, seedlings with shoots between 30 and 35 mm were selected 63 h after planting. Manipulations were carried out under a dim green safelight consisting of a 40-w incandescent bulb filtered through one layer 'Yellow', one layer 'Deep Blue', and two layers 'Primary Green' 'Cinemoid' (Strand Electric and Engineering Co., U.K.). Light intensity at the work surface was 12.9 $\mu w \cdot cm^{-2}$.

Injection of [^{14}C]IAA. Four μl [2-^{14}C]IAA (1.998 GBq·mmol^{-1}, radiochemical purity 99.8%, Amersham) in 10% aqueous ethanol (v/v) was injected into the center of the endosperm, from the ventral side of the grain, using a 25-μl syringe (Unimetrics). (A delay of approximately 5 s was allowed for dispersion of the injected fluid before the needle was withdrawn.) The 4-μl aliquots contained 2 kBq (177 ng), 200 Bq (17.7 ng), or 20 Bq (1.77 ng) [^{14}C]IAA.

Immediately after injection, seedlings, complete in their blotting paper/glass tube assemblies, were placed in 2-ml vials containing 0.5 ml distilled H_2O. The vials were suspended by string in individual, stoppered, 25- × 150-mm test tubes. Two ml of 20% aqueous KOH (w/v) was included in the bottom of each test tube to trap evolved $^{14}CO_2$. The seedlings were incubated in the darkroom at 24°C for 1, 3, 6, or 24 h.

Harvesting and Extraction—2 kBq and 200 Bq [^{14}C]IAA Applications. Twenty-five plants were injected for each incubation period. The labeled plants were harvested in the same sequence in which they were injected. Each plant was sectioned in dim light into 10 parts (Fig. 1), and the radioactivity from each part was extracted and analyzed separately. The grain was gently pulled loose at the scutellum, and the roots excised at the junction with the shoot. An incision was made approximately 2 mm from the apex of the shoot, and the coleoptile tip gently broken loose. Incisions were made 1.5 to 2.0 mm above, and below, the coleoptilar node and the 'nodal segment' (N), 3 to 4 mm long, removed. The primary leaves were withdrawn from within the remainder of the coleoptile. These, along with the small amount of leaf material removed from the coleoptile portion of the nodal segment, constituted the leaf fraction (designated L in Fig. 1). The remainder of the coleoptile comprised fraction C. The mesocotyl was cut into quarters; designated MA for the apical segment, to MD for the basal, scutellum-containing segment.

The lengths of the coleoptile, leaf, and mesocotyl segments varied, depending on the time of harvesting after injection. However, the lengths of the coleoptile tip and nodal segments excised from the plant were kept approximately constant at each harvest. In a separate experiment, the fresh weights of the eight different shoot portions were determined for each harvest time. These data were used to express the radioactivity in each segment on a fresh weight basis.

On harvesting, each seedling part was immediately placed in separate 20-ml vials containing 1.5 ml of chilled (7°C) absolute ethanol. Five replicate sets of vials were used, with five segments (or roots or grains) per vial (allocated on a randomized basis). Roots were cut into 1-cm lengths whereas grains and shoots were extracted intact, except at the 24-h harvest time when the coleoptile segment and the primary leaves were cut into three pieces to facilitate handling.

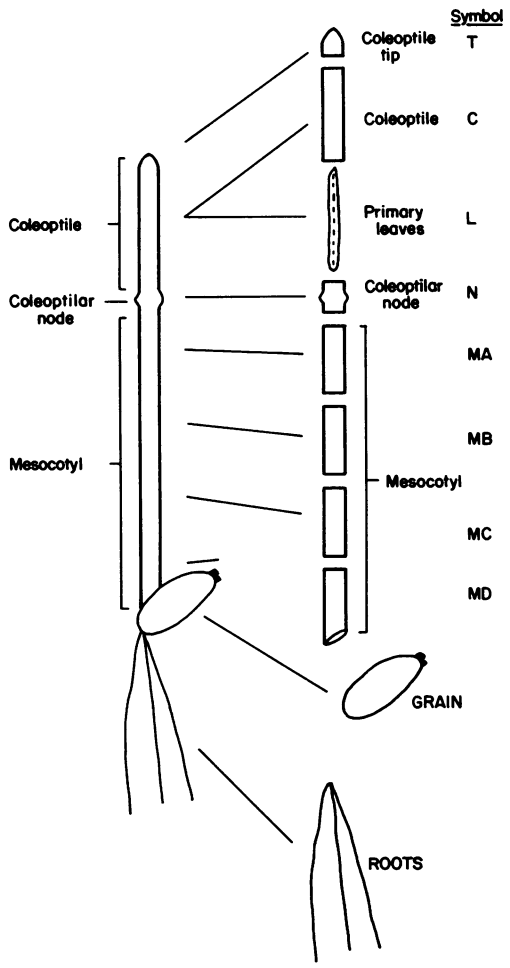

FIG. 1. Sectioning of seedlings for extraction of radioactivity.

Extraction was in darkness using the multiple solvent method of Davies (8) modified as shown in Figure 2. This yielded four radioactive fractions soluble in 95% ethanol, water, NaOH, and Soluene 100 (a tissue solubilizer, Packard Instrument Co.). The extraction schedule employed 1.5-ml changes of 95% ethanol and 3-ml changes of water. For grains and roots, the subsequent extractions in NaOH and Soluene involved 2-ml and 0.5 -ml volumes, respectively. For shoot segments, 1 ml NaOH and 0.2 ml Soluene were used. Following NaOH-extraction, the plant tissue was rinsed twice with 1.5 ml distilled H_2O and the rinsings combined with the NaOH extract. Each fraction was decanted directly into a 20-ml liquid scintillation vial.

Liquid Scintillation Analysis. Radioactivity was counted using a Philips PW 4540 liquid scintillation spectrometer operating on the external standard-channels ratio mode. For all water-containing samples, 10 ml toluene:Triton X-100 (2:1 v/v) containing PPO (4 g·l^{-1}) and POPOP (0.1 g·l^{-1}) was used as the scintillation fluid. Ten ml toluene with PPO (4 g·l^{-1}) was employed with the Soluene fraction.

The radioactivity in the aqueous, NaOH, and Soluene fractions was determined directly by counting the entire extract. Half (4.5 ml) of the 95% ethanol extracts of the coleoptile tip, upper quarter of the mesocotyl, and the grain were taken for TLC. The remainder of these extracts, and the 95% ethanol extracts of the other parts of the seedling, were radioassayed by LSA.[1]

Thin Layer Chromatography. The aliquots taken from replicates 1 and 2 were combined (solution A), as were the aliquots from replicates 3 and 4 (solution B). Aliquot 5 was divided equally between solutions A and B. These solutions (11.25 ml each) were dried by thin-film evaporation under reduced pressure at 35°C. The residue was taken up in a few drops of 80% aqueous ethanol (v/v) and spotted on 0.25-mm layers of silica gel G (Sigma), prepared on glass (5 × 20 cm) using 10 mM citrate-phosphate buffer (pH 5.2) containing 0.3% (w/v) carboxymethyl cellulose (Whatman CM 32) as the slurrying liquid. The latter functioned as a gel hardener (22). The plates were developed twice for 5 cm, and then twice for 10 cm, with methyl ethyl ketone:*n*-hexane (35:65 v/v). After autoradiography (Kodak X-ray film), the radioactivity in the main bands on the TLC plate was quantified by LSA (12). TLC was performed at all harvest times in the 2-kBq injection experiment, but only at the 6-h harvest in the 200-Bq experiment.

Application of 20 Bq [^{14}C]IAA. In these experiments, 50 plants were used for each harvest time. Seedlings were injected, incubated, and harvested as above, but in two batches of 25 for reasons of timing. The plants from both injection sequences were extracted together, with the 50 replicates of each shoot segment in a single series of vials (*i.e.* there were no replicate extracts of each shoot part). Roots and grains were extracted, 10 items per vial, in five replicate vials.

The multiple solvent extraction system as in Figure 2 was used, with 3-ml changes of 95% ethanol and 4 ml of water. All plant material was then extracted in 3 ml of NaOH, with two 2-ml washes of distilled H_2O. The final extraction used 0.5 ml Soluene.

From the 6-h harvest time, half the 95% ethanol extracts of the grain, coleoptile tip, and upper quarter of the mesocotyl were taken for TLC and autoradiography. The radioactivity in the H_2O, NaOH, and Soluene extracts, and in the 95% ethanol solutions sampled for TLC, was counted directly by LSA. The

[1] Abbreviation: LSA, liquid scintillation analysis.

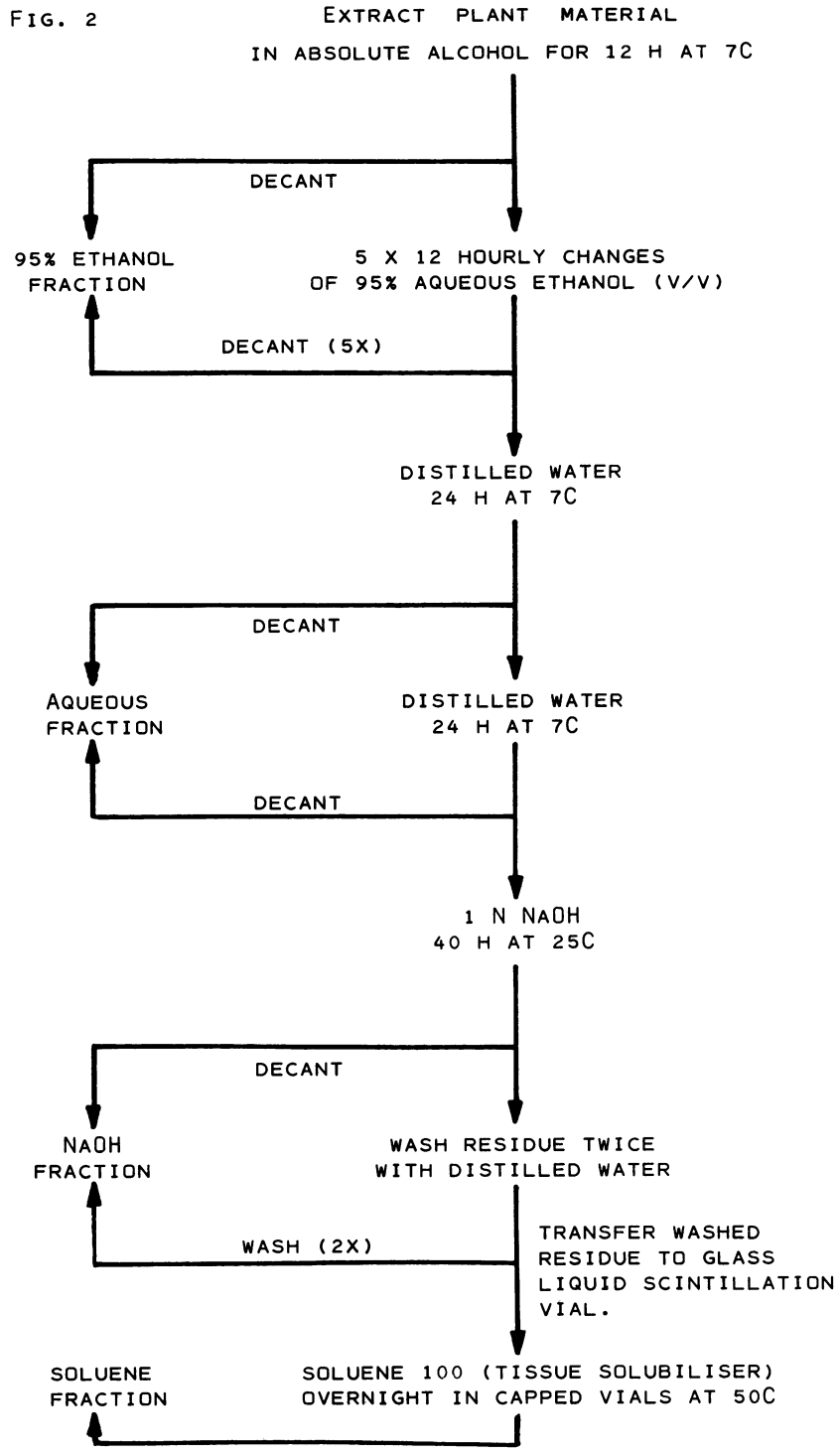

FIG. 2. Flow diagram for extraction of radioactivity from plant material (see "Materials and Methods").

95% ethanol extracts of those plant parts not sampled for TLC were concentrated to approximately 6 ml, by thin-film evaporation in 15- × 125-mm test tubes. The contents, including a 2-ml ethanol rinse, were transferred to 20-ml scintillation vials and radioassayed by LSA.

RESULTS

Distribution of Radioactivity in the Seedling. Figure 3 shows the time course of total radioactivity recoverable from various parts of the seedling following injection of 2 kBq, 200 Bq, or 20 Bq [^{14}C]IAA. Considering the range of amount of IAA injected, an essentially similar pattern of transport of radioactivity out of the grain into the shoot and roots was evident in all three cases. This pattern was typified by a rate of export from the grain which was rapid in the first 1 to 3 h after injection, but which slowed considerably thereafter, almost ceasing. In all three series of experiments, the roots gained radioactivity more rapidly than the shoot. However, accumulation of label in the roots ceased after 1 to 3 h, while the shoot continued to gain radioactivity slowly throughout the 24 h experimental period in all cases. Ultimately, the radioactive content of the shoot was equivalent

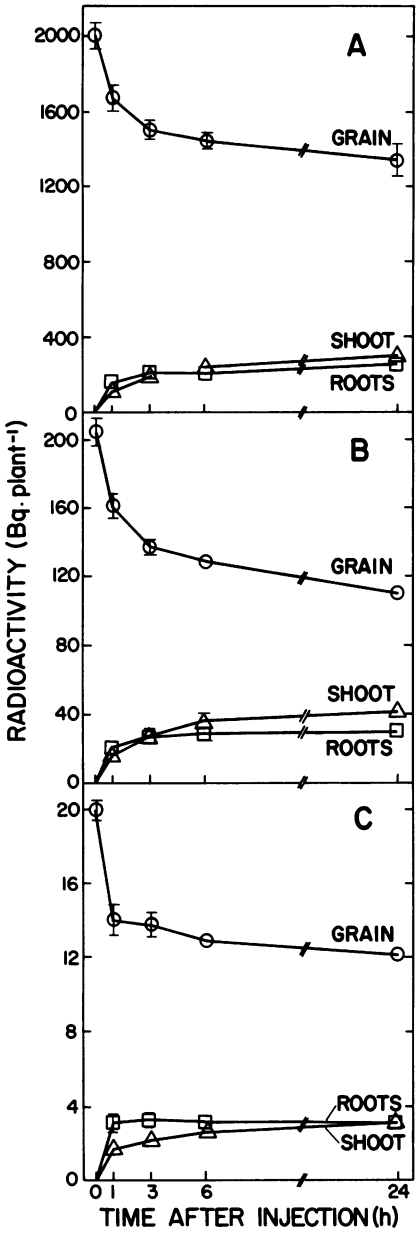

FIG. 3. Distribution of radioactivity within seedlings at various times after injection into the endosperm of 63-h-old dark-grown *Avena* seedlings of (A) 2 kBq; (B) 200 Bq; or (C) 20 Bq of [2-^{14}C]IAA. Total extractable radioactivity was determined as the sum of the activity extracted by each of the four solvents employed as in Figure 2. In A and B, values plotted represent the mean ± SE of five replicates each containing five items. In C, the five replicates of the grain and roots contained 10 items, whereas the shoot values are from single replicates containing 50 items each. Hence, for the latter, no SE can be shown. Elsewhere, the absence of a SE is an indication that the error term was less than or equal to the symbol used to designate the mean.

to or exceeded that of the roots. Some of the applied radioactivity was recoverable from outside the plants (in the blotting paper strips, in the water reservoir, and in the KOH trap). However, this never amounted to more than 10% of the total, even after 24 h incubation.

Distribution of Radioactivity within the Shoot. For each [^{14}C] IAA application rate, the amount of radioactivity in each segment of the shoot, at the various harvest times, was calculated as a function of the segment fresh weight, using the data in Table I. Again, when account was taken of the difference in the

Table I. *Fresh Weights of Shoot Segments*

Plants were selected for length at age 63 h and harvested at intervals thereafter. The values shown are the average weight per segment, computed from the total weight of 30 replicate segments. For the position of the segments, and the meaning of the abbreviations, see Figure 1.

Shoot Segment	Fresh Weight			
	1 h	3 h	6 h	24 h
	mg/segment			
MD	4.6	4.9	5.2	8.2
MC	3.9	4.2	4.6	7.9
MB	4.3	4.6	5.2	8.2
MA	4.6	5.1	5.6	8.8
N	3.6	3.7	3.7	4.0
L	2.7	2.9	3.4	6.1
C	6.8	7.7	9.3	17.5
T	1.5	1.5	1.4	1.5

amounts of [^{14}C]IAA injected, the pattern of distribution of radioactivity was basically similar in all cases (Fig. 4). In the early stages of each experiment, radioactivity levels were generally highest in the mesocotyl and the coleoptilar node. Most of the radioactivity in the mesocotyl was concentrated in the basal, scutellum-containing segment. A later experiment, in which the lower quarter of the mesocotyl and scutellum were analyzed separately, showed that the bulk of the label was contained in the scutellum, and that the level of radioactivity in the mesocotyl segment alone was similar to the level observed in the neighboring mesocotyl tissue. These mesocotyl levels increased to a peak 6 h after [^{14}C]IAA injection, and then declined by 24 h to between the levels that occurred at the 1- and 3-h harvests. Within the coleoptile, radioactivity tended to be concentrated at the tip and, at most harvests, the levels were at least double those in the rest of the coleoptile. In all experiments by the final harvest, the radioactivity levels in the coleoptile tip exceeded those in the mesocotyl (with the exception of the scutellum-containing portion). Both the coleoptilar node segment and the primary leaves also contained particularly high amounts of radioactivity, relative to their fresh weight.

[^{14}C]IAA Metabolism. Davies (8) and others (14, 21) previously have used sequential extraction systems similar to that employed in this study to differentiate total extractable radioactivity into IAA and its immediate derivatives (soluble in 95% ethanol), two bound IAA fractions (insoluble in 95% ethanol but soluble in water or NaOH, respectively), and the residue (soluble only in Soluene 100). However, under the conditions employed here, this system did not give satisfactory separation of the various labeled compounds formed in the seedling. Some metabolites were found to be present in both the 95% ethanol and aqueous fractions (12). IAA, however, was confined only to the 95% ethanol fraction. But because this IAA had been quantified following TLC (see "Materials and Methods"), it was possible therefore to differentiate the total extractable radioactivity into free IAA, and metabolized IAA (*i.e.* the rest). Inasmuch as the transport and metabolism of IAA was of primary interest, and the nature of the metabolites of secondary importance, such a distinction was acceptable. Extensive control experiments were carried out to ensure that the IAA metabolites were not artifacts of the extraction or purification procedures (12). Only during silica gel TLC was any [^{14}C]IAA destruction observed. By developing plates immediately after spotting, this breakdown could routinely be kept to 6% of the applied [^{14}C]IAA (12). (The values of 'metabolites' in Figure 5 have not been adjusted for this small background level of IAA breakdown.)

Figure 5A shows the time course of [^{14}C]IAA metabolism in the grain following injection of 2 kBq [^{14}C]IAA. Over 41% of

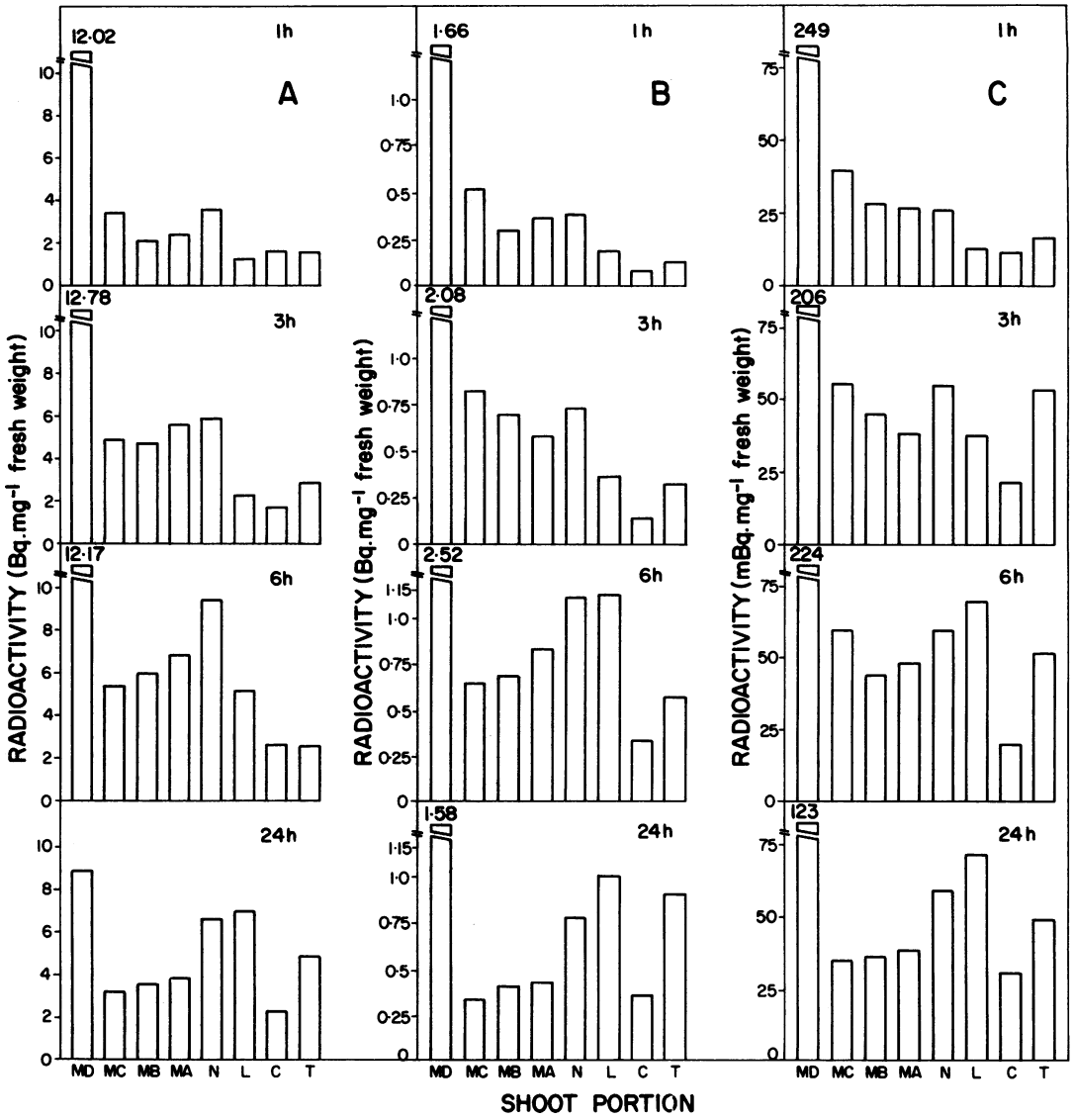

FIG. 4. Time course of amount of radioactivity in each shoot segment, expressed as a function of segment fresh weight, following injection into the endosperm of (A) 2 kBq; (B) 200 Bq; or (C) 20 Bq [^{14}C]IAA. The positions of the shoot segments are shown in Figure 1; fresh weights at each harvest time are in Table I. Other details are given in the legend of Figure 3.

the applied IAA was metabolized within 1 h of injection. The rate of metabolism slowed thereafter and in the subsequent 23-h period this proportion increased only to 54%. The export of radioactivity from the grain, and the slow but continued metabolism of [^{14}C]IAA, meant that the proportion of label in the grain comprised of free IAA fell steadily throughout the experiment to 17.6% at the end of the 24-h experimental period.

In the coleoptile tip and upper mesocotyl, both free and metabolized forms of [^{14}C]IAA were present initially (Fig. 5, B and C). However, free IAA disappeared rapidly, indicating that metabolism of [^{14}C]IAA was occurring within the shoot. Free IAA levels peaked in both tissues 3 h after injection and then declined to comprise approximately 5% of the total radioactivity in each by 6 h. By the end of the 24-h experimental period, no [^{14}C]IAA was detectable in either the coleoptile tip or the upper segment of the mesocotyl. In the coleoptile tip, the level of labeled metabolites increased steadily throughout the experiment, whereas in the mesocotyl segment these rose to a peak after 6 h, and then declined to near the 3 h level. In a later experiment, the nature of the radioactive compounds entering the shoot was investigated by analyzing the radioactivity in the scutellum 6 h after [^{14}C]IAA injection, using TLC. It was found that less than 2% of the total radioactivity in the scutellum consisted of free [^{14}C]IAA.

With the 200- and 20-Bq experiments, IAA metabolism was determined only at the 6-h harvest (Table II). In the 200-Bq experiment, the proportions of radioactivity present as free IAA in both the coleoptile tip and the mesocotyl segment were similar to those present at the same stage following injection of 2 kBq [^{14}C]IAA (Fig. 5, B and C). After application of 20 Bq, no free [^{14}C]IAA was detectable in either shoot tissue after 6 h. In both the 200- and 20-Bq experiments, approximately one fifth of the radioactivity remaining in the grain after 6 h was still as free [^{14}C]IAA. This compares with 29.1% in the 2-kBq experiment (Fig. 5A).

DISCUSSION

The present experiments confirm Sheldrake's (23) belief that [^{14}C]IAA applied to the endosperm of *Avena* seedlings will move acropetally from the endosperm and accumulate at the coleoptile tip. However, it is concluded that such movement is not physiologically significant, and that free IAA in the endosperm is not

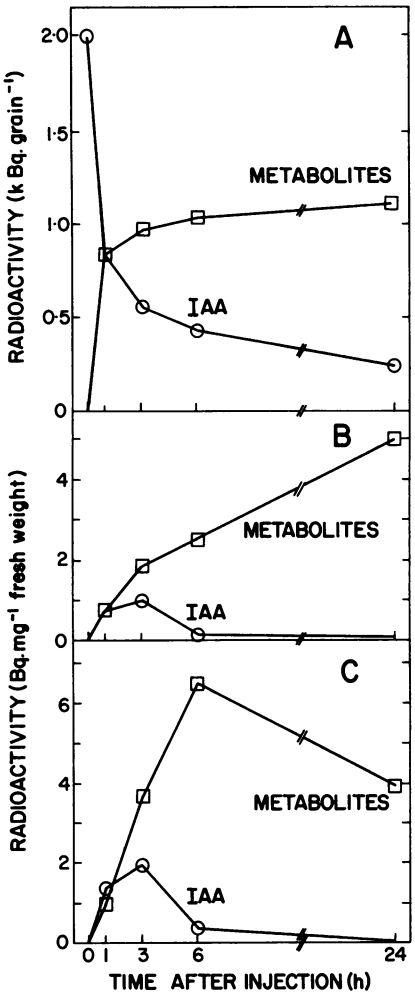

FIG. 5. Changes in levels of [^{14}C]IAA and labeled metabolites in (A) the grain, (B) the coleoptile tip, and (C) the upper quarter of the mesocotyl, following injection into the endosperm of 2 kBq [2-^{14}C]IAA. To determine free [^{14}C]IAA, the 95% ethanol-extractable radioactivity was subjected to TLC and the radioactivity in the IAA band was eluted and counted by liquid scintillation analysis. Labeled metabolites were designated as 'the rest'—*i.e.* that extractable in H_2O, NaOH, and Soluene 100, plus the non-IAA radioactivity soluble in 95% ethanol.

an important source of free IAA in the shoot in *Avena*.

Build-up of radioactivity at the tip tended to follow, rather than precede, accumulation elsewhere in the shoot. Therefore, the coleoptile tip probably does not act as the site of accumulation and redistribution of IAA and associated compounds as was suggested by Sheldrake (23). Furthermore, the observed distribution of radioactivity in the rest of the shoot does not correlate with the endogenous levels of IAA. The mesocotyl contained higher radioactivity levels than the coleoptile, whereas the distribution of endogenous IAA in the *Avena* shoot is almost the opposite of this; the coleoptile contains higher concentrations of IAA than the mesocotyl (15, 25, 27). It is possible to simply correlate the distribution of radioactivity in the shoot with the presence of vascular tissue. The highest levels of radioactivity occurred at the coleoptilar node and in the primary leaves, both of which contain numerous vascular bundles (2). Similarly, the higher concentration in the mesocotyl than the coleoptile can be accounted for by the fact that the *Avena* mesocotyl contains two major vascular bundles, while the coleoptile contains two minor bundles (2). Sheldrake (23) has shown that radioactivity can be detected in the xylem sap of *Avena* coleoptiles following injection of [^{14}C]IAA into the endosperm. Also, in preliminary experiments not described here, it was found that radioactivity was recoverable from the coleoptile tip within 5 min of injection into the endosperm. Since the radioactivity had to diffuse through 2 to 3 mm of endosperm and travel 30 mm up the shoot, this represents a translocation rate of at least 360 mm·h^{-1}. This velocity suggests translocation in the vascular system (5, 6, 17).

In each experimental series, approximately the same proportion of applied radioactivity was translocated, irrespective of the concentration of the [^{14}C]IAA solution applied. This suggests a certain proportion of the injected solution was transported into the shoot and roots in each case, rather than a certain quantity of IAA. Thus, it is unlikely that [^{14}C]IAA was being transported to fulfill specific growth requirements. It seems more likely that the radioactivity was carried along by the nutrient flow out of the endosperm, and that the amount transported merely reflects the proportion of radioactivity comprising the milky (liquid) endosperm at any particular time. This view is supported by the data of Hall and Bandurski (10) which, after correction for counting efficiency, show that in 8 h, similar proportions of the radioactivity were transported into the shoot when either 3670 Bq of [^{3}H]tryptophan or 35 Bq [^{14}C]IAA was applied to the endosperm of *Zea* seedlings.

The results of the 2 kBq experiment indicate that initially free IAA was transported into the shoot, but that once there it was metabolized rapidly. IAA was also metabolized rapidly in the grain. Much of this metabolism, and the initial transport of [^{14}C]IAA into the shoot, almost certainly is not representative of the normal situation in the *Avena* seedling. In all experiments, translocation of radioactivity out of the grain into the shoot and roots was rapid in the 1st h, but then declined to a much slower rate, even though the growth rate of the shoot (and presumably the roots) remained constant. From the 2 kBq experiment, it is apparent that a similar pattern of decline in the rate of IAA metabolism also occurred. The declines in the rates of transport and metabolism were not simply due to lack of IAA in the grain, because in all experiments, after 6 h, the amount of free IAA had only fallen to about one-sixth of the original, yet the rates of transport and metabolism had decreased by more than 20-fold.

Table II. *Proportion of [^{14}C]IAA and Labeled Metabolites in the Radioactivity Extractable from the Coleoptile Tip, the Apical Segment of the Mesocotyl, and the Grain*

The metabolites were analyzed 6 h after injection of 200 or 20 Bq [^{14}C]IAA.

Fraction	Amount of [^{14}C]IAA Injected					
	200 Bq			20 Bq		
	Coleoptile tip	Mesocotyl	Grain	Coleoptile tip	Mesocotyl	Grain
	%					
[^{14}C]IAA	5.4	6.1	20.9	0	0	18.7
Metabolites	94.6	93.9	79.1	100	100	81.3

Nor was the decrease due to saturation of the transport and metabolic capabilities of the seedling, as similar patterns were evident at all IAA application rates. It appears that somehow some of the applied IAA was becoming unavailable for transport and was also immune from metabolic attack. 'Auxin protectors' (24) cannot account for the reduction in metabolism, inasmuch as these compounds merely delay IAA oxidation for 1 to 2 h.

The most likely explanation is that, after injection, part of the applied [^{14}C]IAA finds its way into the intact cells of the endosperm. In cereal grains, dissolution of the cell walls in the endosperm occurs first near the scutellum, and gradually extends to the remainder of the endosperm (9, 20). Under malting conditions, complete degradation in barley may take 10 d (7). At the stage used in this investigation (63 h), the endosperm of the oat seedlings was still quite firm, suggesting that cell wall disintegration was only partial. The above explanation would account for the fact that, 6 h after injection, similar proportions of IAA remained unmetabolized at each application rate; and that the export of radioactivity from the grain decreased after a period, in all cases. The slow but continued metabolism of [^{14}C]IAA, and transport of labeled material into the shoot and roots in the last 20 h of the experiments, is interpreted as the gradual release of IAA (and its readily mobile metabolites) from the intact endosperm as cell wall degradation proceeded.

The initial rapid transport of radioactivity is an indication that much of the injected solution went into the liquid part of the endosperm adjacent to the scutellum. And the initial rapid rate of metabolism suggests that IAA is also metabolized there. Evidence of this comes from data published by Bandurski's group. Very little [^{14}C]IAA metabolism occurs in *Zea* grains which are commencing germination (13), when the endosperm is mostly solid, but [^{14}C]IAA is rapidly metabolized in grains of 4-d-old seedlings, where liquefaction of the endosperm is well advanced (9).

It is unlikely that the rapid metabolism of IAA in the grain was due to application of excess amounts of growth regulator. Percival and Bandurski (19) have estimated that dehusked grains of cv 'Lodi' oats contain 440 $\mu g \cdot kg^{-1}$ free IAA, but they give no indication of how much this represents per grain. One kg of (naked) grains of the cv 'Terra' oats used in this study contains approximately 30,000 grains. Using Percival and Bandurski's figures, this represents about 15 $ng \cdot grain^{-1}$ of free IAA. According to Zimmermann *et al.* (30), the free IAA content of cv 'Sorte Delphin' grains, with hulls intact, is about 1150 $\mu g \cdot kg^{-1}$. Since 1 kg of cv 'Mapua' grains (with husks) contains approximately 23,000 grains, an approximate estimate of the amount of free IAA per grain, on this basis, would be 50 ng.

Assuming a free IAA content of between 15 to 50 $ng \cdot grain^{-1}$, injection of 177, 17.7, and 1.77 ng [^{14}C]IAA into the grain would have increased the endogenous IAA levels by 350 to 1180%, 35 to 118%, and 3.5 to 11.8%, respectively. At the lowest application rate, even if only part of the endogenous IAA were in the liquid part of the endosperm, there would have been little disturbance from the [^{14}C]IAA injected, yet the proportion metabolized was similar to when higher amounts of IAA were applied. Furthermore, Epstein *et al.* (9) found a similar level of [^{14}C]IAA metabolism in *Zea* grains, even though the expected perturbation of the endogenous pool from their application was only 3%.

The fact that even small quantities of IAA are metabolized in the liquid portion of the endosperm suggests that IAA does not normally occur in the free form in this part of the grain. At the lowest injection rate, which represents amounts of IAA that are physiologically realistic, about 80% of the free IAA injected disappeared from the grain in 6 h. This represents a loss of about 0.25 ng $IAA \cdot h^{-1}$. If the labeled IAA were mixing with the 15 to 50 ng of endogenous IAA calculated to be present in the grain, then the total amount of IAA metabolized in 1 h would be in the order of 2.1 to 7.1 ng. For either Bandurski's or Zimmermann's figures, this would exhaust the supply of free IAA in the grain in approximately 7 h. Thus, the initial response to injection of [^{14}C]IAA clearly is not physiologically significant. The possibility that this rapid metabolism of [^{14}C]IAA represented merely a small pool of free IAA with rapid turnover owing to IAA-ester hydrolysis in the endosperm was discounted. Such a scheme has been proposed for *Zea* (9). Prior to germination, the amount of IAA-conjugate in the *Avena* grain is approximately 20 times that of free IAA (19, 30). If complete turnover of the free IAA pool was occurring in 7 h, hydrolysis of the ester pool could supply free IAA for 5.8 d before becoming exhausted. However, measurement of IAA-conjugate levels in germinating *Avena* grains over a 4-d period shows only a 12% diminution (30)—nothing approaching that necessary to supply the free IAA pool if rapid turnover was occurring at the rate calculated here. We therefore favor the explanation that the observed rapid metabolism of [^{14}C]IAA in the grain is an indication that the free IAA does not normally occur in the milky endosperm.

If endogenous free IAA exists in the endosperm, then it must do so in the intact cells. Therefore, the slower rate of IAA metabolism and transport of radioactivity in the 6- to 24-h period after injection, presumably as the cells are lysed, is probably more representative of endogenous IAA transport and metabolism. In all the labeling experiments, [^{14}C]IAA was absent or virtually absent from the shoot 6 h after injection, even though IAA still comprised about 20% of the radioactivity in the grain. Inasmuch as free IAA was disappearing from the grain, it was obviously being metabolized en route. Most of this metabolism probably occurred in the milky endosperm, because [^{14}C]IAA liberated from the intact endosperm cells had to migrate through the liquid portion prior to reaching the shoot. Furthermore, 6 h after injection of 2 kBq [^{14}C]IAA, only 2% of the radioactivity in the scutellum was found to be as free IAA, suggesting that by this stage most of the radioactivity being taken up from the endosperm had been metabolized already. On the basis of the above arguments, it is concluded that transport of endogenous free IAA, from the endosperm into the shoot and roots, does not occur to any significant extent. A similar conclusion was reached by Hall and Bandurski (10) following experiments in which radioactively labeled IAA was applied to the cut endosperm surface of 4-d-old *Zea* seedlings. They too found that only traces of [^{14}C]IAA could be detected in the radioactivity extractable from the shoot 8 h after IAA application.

A possibility that perhaps should be considered in future research is that endogenous IAA is not located in the starchy endosperm as has been assumed (9, 18, 19, 23), but rather is compartmentalized and protected elsewhere, possibly in the aleurone layer/pericarp. Hatcher (11) found 71% of the total auxin activity (free and bound) extracted from rye grains was localized in the aleurone layer. Similarly, rice polishings (*i.e.* aleurone and pericarp) have been shown to contain considerable auxin (28); and polished barley contains much lower amounts of IAA (both free and bound) than other cereal grains which have not been polished (4).

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