ON THE MECHANISM OF THE TRANSVERSE DISTRIBUTION OF AUXIN IN GEOTROPICALLY EXPOSED PEA ROOTS

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SUMMARY

Carboxyl-labeled and methylene-labeled IAA-^{14}C were applied to the tips of two-day-old horizontal pea roots var. Alaska, and the tissue was subsequently halved and counted. In every experiment the lower halves of the subapical 4 mm became more radioactive than the upper halves. With an experimental period not over two hours the upper and lower halves contained an average of 33 and 67 per cent of the total radioactivity in the tissue respectively. Decapitation of the roots at 0.5 mm or more (removal of the entire root cap) prevented the transverse distribution of the applied auxin almost entirely.

If the roots were allowed to absorb caffeic acid or 2,4-dichlorophenol prior to the application of the IAA-^{14}C, the difference between the amounts of IAA-^{14}C present on the two sides was greatly decreased.

The IAA-oxidase activity of homogenates made from the lower halves of the apical 3 mm of horizontal roots was less than that of homogenates from the upper halves. Decapitation did not affect this phenomenon.

Apparently the unequal distribution of applied auxin and the difference between the activities of the IAA-oxidase on the two sides are not connected.

1. INTRODUCTION

It has been demonstrated several times, first by measurements based on bioassay (DOLK 1930; DIJKMAN 1934; GILLESPIE & BRIGGS 1961 and others) and later with the aid of IAA-^{14}C (GILLESPIE & THIMANN 1963; GOLDSMITH & WILKINS 1964), that when IAA is applied to the apical end of decapitated, horizontal coleoptiles and shoots it becomes distributed asymmetrically so that the lower side receives more auxin than the upper side. This asymmetry was proved with IAA-^{14}C to be the result of the lateral migration of the auxin towards the lower side of these organs. A parallel situation in roots has generally been accepted, but in fact a lateral transport of auxin in horizontal roots has not been convincingly demonstrated. HAWKER (1932) showed that a greater positive curvature could be induced in an unstimulated root stump of *Vicia faba* by the diffusate from the lower halves of root tips placed horizontal than by that from the upper halves of the same root tips. This suggested that growth hormones accumulated in the lower halves on geotropic stimulation. BOYSEN-JENSEN (1933), THIMANN (1936), and VAN RAALTE (1937) showed that the substance which diffused out of the root tips of *Vicia faba* and *Avena* respectively into sugar-containing agar was indeed an auxin, as measured by the *Avena* curvature test. Boysen-Jensen showed that more of this diffused out of the lower halves of

the horizontal root tips than out of the upper halves, and suggested that the transverse distribution was the result of lateral migration of the hormone. So, although an accumulation of some auxin in the lower half of a horizontal root tip was shown, it was not clear how this was achieved. In more recent experiments, CHING & FANG (1958) submerged the roots in solutions of IAA-^{14}C before they were placed horizontally, and found no support for the occurrence of lateral transport of auxin in roots; the roots failed to distribute the isotope unequally. However, the rather high concentration of auxin with which the roots were treated probably overloaded the mechanism which controls the unequal distribution. Similar failures to find asymmetric auxin distribution in horizontally placed coleoptiles have been attributed by GILLESPIE & THIMANN (1963) at least in part to overloading.

The experiments described below were therefore aimed at determining whether externally applied IAA-^{14}C, when applied to the tips of horizontal roots, is in fact transversely distributed in these tips, and if so, to study the mechanism of this distributing. It will be shown that pea roots are indeed capable of distribution applied auxin unequally, but only if the root cap is present, and further that certain changes occur in the horizontal root which are not affected by the presence or absence of the root cap. Apparently gravity has multiple effects on the physiology of the apical part of the root.

2. MATERIAL AND METHODS

In all experiments the roots of two-day-old seedlings of *Pisum sativum* var. Alaska have been used. The seeds were soaked in aerated tap water for 16 to 20 hours, then placed in moist sand over previously prepared vertical holes to ensure straight growth, and grown for two days in a room with a relative humidity of about 95 per cent and a temperature of 25 °C in darkness. Selected seedlings with roots 3 to 4 cm long were held by their seeds on pins, fixed in a wooden block. The agar blocks (0.014 ml each) were placed with one of their narrow sides on a plexiglass block and moved till they touched the root tips (fig. 1). During their elongation the roots were kept in contact with the agar blocks.

A similar set up was used for vertical roots, but with the agar blocks adhering to the plexiglass block by one of their flat sides (fig. 1). Although the agar blocks were blotted with filter paper before the experiments, they occasionally slipped along the plexiglass and lost contact with the roots. These roots were discarded. Special care was taken to avoid the formation of a meniscus of moisture between

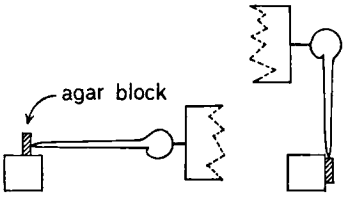

Fig. 1. Set up used for the application of IAA-^{14}C to horizontal and vertical roots.

the root apex and the agar block. The data in the tables are mostly based on means of 24 to 48 roots with any given duration of contact time. All manipulations were carried out under weak red light.

The carboxyl-labeled indoleacetic acid used had a specific activity of 16.9 curies per mole (97% pure). The specific activity of the methylene-labeled IAA-^{14}C was 13.3 curies per mole (not checked for purity). Both samples were stored in acetonitrile. The method of purification was as follows: one ml of the radioactive sample was put in 5 ml distilled water in a beaker and the acetonitrile was allowed to evaporate. The pH of the solution was adjusted to 3.0 with 1% H_3PO_4, the solution was extracted 4 times with 2.5 ml freshly distilled peroxide-free ether each time, the ether fractions were combined and extracted twice with 5 ml each time of 0.1 M $NaHCO_3$, brought to pH 8.0. The ether fractions were discarded, the pH brought to 3.0 with 1 N H_2SO_4, the solution extracted with ether 4 times, the ether fractions collected and dried overnight over Na_2SO_4. This ether was then added drop by drop to 5 ml distilled water in a test tube in a beaker of hot water. The solution thus obtained was autoclaved, stored in darkness in the cold and diluted as required. The agar was soaked in distilled water and washed daily for one week with decantation to remove solutes. The blocks of 2% agar (0.162 ml each) were soaked in a solution of IAA-^{14}C for 16 hours at 3 °C, then cut into 12 equal parts and used. The radioactivity of the agar blocks was determined as described by GOLDSMITH & THIMANN (1962). At the end of the experiments the root tips, usually 4 mm long, were split, cut off and dried on tared planchets for 30 minutes at 80 °C in a vacuum oven. The tissue was then ground on the planchet in a few drops of freshly redistilled chloroform. After evaporation of the chloroform the pulverized tissue was spread over the planchet in a few drops of distilled water and dried. The planchets with tissue were then weighed again, and the samples counted either in a Baird Atomic (manual) counter with an efficiency of 23 per cent and a background of 19 cpm or in a Nuclear Chicago automatic counter with an efficiency of 33 per cent and a background of 4 cpm. Self-absorption factors of the pulverized tissue and agar were determined in most experiments. Samples were counted for 10 minutes or at least 1000 counts.

3. RESULTS

3.1. The transverse distribution of carboxyl-labeled IAA-^{14}C in the tips of horizontal pea roots

Carboxyl-labeled IAA-^{14}C was supplied to the roots as described. After various periods of time the agar blocks were removed and the ^{14}C content of both blocks and halved root tips determined. In every experiment the lower halves of the horizontal root tips contained more ^{14}C than the upper halves (*table 1*). The data of this table were partly obtained from experiments with intact roots and partly from experiments with roots which had been decapitated at a distance of 0.2 mm. This cut was made to determine whether the resulting greater surface of contact allowed more auxin to enter. Comparison of the data from the two

procedures reveals that the total radioactivity was considerably higher in the intact tips, but that the transverse distribution was more pronounced in those that had been decapitated. The ratio of the amounts of ^{14}C present in the upper and the lower halves was 40/60 in intact roots and about 33/67 in the decapitated roots. The removal of the most apical 0.2 mm did not influence the geotropic curvature. (The curvatures of the roots in five experiments, including 50 roots, recorded every hour up to 7 hours, averaged 11, 23, 21, 24, 25, 31 and 35 degrees respectively for intact roots, and 10, 23, 18, 22, 24, 30 and 33 degrees for roots decapitated at 0.2 mm.)

The total amount of radioactivity in the root tips increased only roughly proportional to time of treatment (*table 1*, column 6), and a relation to the amounts of auxin applied was only found if they differed widely (e.g. 0.03 and 0.46 μg applied for two hours, *table 1*).

When the roots were kept horizontal longer than two hours the asymmetry of distribution became less, probably because the curvature became large enough

Table 1. The transverse distribution of carboxyl-labeled IAA-^{14}C in the apical 4 mm of intact or 0.2 mm decapitated horizontal pea roots.

time of exposure hours	position	μg IAA-^{14}C applied per 24 roots	cpm/mg dry weight in upper halves	in lower halves	total cpm in upper + lower halves	total cpm average	% of total cpm in upper halves	% recovery
0.5	hor.	0.22	17.4	34.6	52.0	52.0	33.5	33
1.0	„	0.22	28.3	59.0	87.3	87.3	32.4	26
1.5	„	0.21	29.4	59.8	89.2		32.9	40
1.5	„	0.25	30.6	69.0	99.6	105.0	30.7	24
1.5	„	0.21	41.7	84.5	126.2		33.0	23
1.5	vert.	0.24	36.1	36.0	72.1		50.0	36
1.5	„	0.30	40.2	41.4	81.6	79.3	49.2	28
1.5	„	0.20	42.6	41.7	84.3		50.5	26
1.5[1]	hor.	0.20	71.1	107.4	178.5		39.8	–
1.5[1]	„	0.14	56.2	83.0	139.2	181.6	40.3	42
1.5[1]	„	0.25	80.8	116.1	196.9		41.0	30
1.5[1]	„	0.19	84,3	127.5	211.8		39.8	55
1.5[1]	vert.	0.20	71.1	74.6	145.7		48.7	45
1.5[1]	„	0.14	55.0	56.1	111.1	139.0	49.5	48
1.5[1]	„	0.18	81.0	79.4	160.4		50.4	50
2.0	hor.	0.03	5.6	10.6	16.2		34.5	–
2.0	„	0.46	67.5	147.6	215.1	119.0	31.3	27
2.0	„	0.17	37.2	88.2	125.4		29.6	26
3.0	„	0.17	72.3	84.6	156.9	156.9	46.0	33
4.0	„	0.39	156.0	182.8	338.8		46.0	25
4.0	„	0.45	177.7	218.4	396.1	367.4	44.8	19

All data are for a group of 24 roots.

[1] Intact roots; the others were decapitated at 0.2 mm.

to bring the upper halves of the tips into a more favourable position to take up the auxin than the lower halves. No unequal distribution of the applied auxin was found in the tips of vertical roots, and the close agreement between the amounts of isotope in the vertical halves provides a good check on the method.

3.2. The distribution of carboxyl-labeled IAA-^{14}C in horizontal pea roots as function of distance behind the apex

In the aforementioned experiments tips of 4 mm length were cut off at the termination of the experimental period. In these parts about 96 per cent of the total absorbed radioactivity was localized, as can de deduced from the data in *table 2* and the *figs. 2* and *3*. In these experiments the transverse distribution and the longitudinal translocation were determined up to 8 mm behind the apex, in sections 0.5 to 2 mm long. The data of *table 2* show that an asymmetric distribution of the radioactivity extended to at least 6 mm behind the apex. The auxin distribution showed a steep gradient in the basipetal direction in the tissue and no radioactivity was detected in the 6–8 mm sections after a transport period of two hours. Indeed *fig. 3* shows that in the apical 0.5 mm, i.e. the root cap, 62 per cent of the entire radioactivity present in the 5.5 mm tip was found. It is evident that the asymmetry of the applied auxin must be already present in the root cap. *Fig. 2* shows the transport in vertical roots (i.e. upward) to be somewhat more rapid than in horizontal roots, since relatively more ^{14}C was found in the 2–4 and 4–6 mm sections and less in the apical 2 mm, when vertical.

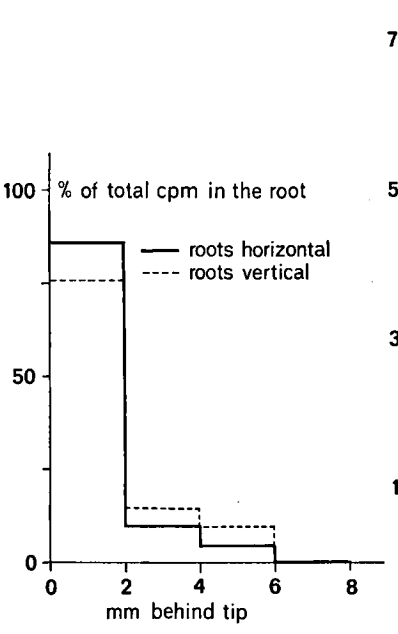

Fig. 2. The translocation of IAA-^{14}C in the apical zones of horizontal and vertical pea roots, when applied to the tips.

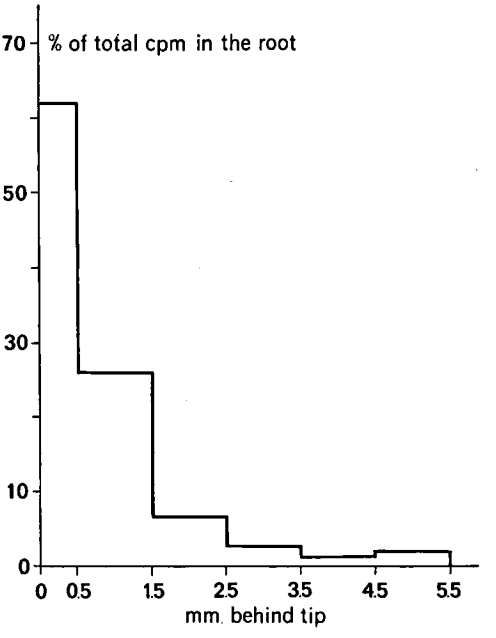

Fig. 3. The distribution of IAA-^{14}C in the apical zones of horizontal pea roots, when applied to the tips.

Table 2. The transverse distribution of carboxyl-labeled IAA-^{14}C in horizontal pea roots at various distances behind the apex.

time of exposure, hours	sections (length in mm) cut at:	cpm/mg dry weight		total cpm in upper + lower halves	% of total cpm in upper halves
		in upper halves	in lower halves		
1.0[1]	0–1	95.2	148.0	243.2	39.1
	1–2	26.6	33.2	59.8	44.4
1.5[1]	0–1	112.3	190.0	302.3	37.1
	1–3	30.0	42.0	72.0	41.6
	3–5	12.7	15.4	28.1	45.1
2.0	0–2	94.1	118.8	212.9	44.1
	2–4	10.4	13.9	24.3	42.7
	4–6	3.2	7.0	10.2	31.3
	6–8	0.0	0.0	0.0	–
3.0	1–4	63.0	104.9	167.9	37.5
3.0	2–4	9.7	25.1	34.8	27.8

All data are for a group of 48 roots.
[1] Intact roots; the others were decapitated at 0.2 mm.
The quantity of auxin applied ranged from 0.06 to 0.46 μg per 48 roots.

The recovery, i.e. the ratio between the radioactivity present in the tips and the total amount lost from the donor blocks, was usually not over 50 per cent. It was clearly higher in intact roots (average 45.0%) than in roots which had been decapitated at 0.2 mm (average 28.3%) as can be seen in *table 1*, last column. In any case a great deal of the applied IAA-^{14}C vanished. It is well known that breis or homogenates made from pea roots can degrade IAA, and also that such destruction can take place at cut surfaces, but it is not known whether this degradation also occurs in undamaged pea root tissue. It seemed wise therefore to check the transverse distribution of methylene-labeled IAA-^{14}C, because the ^{14}C of the methylene group is more stable than that of the carboxyl group (ANDREAE c.s., 1961).

3.3. The transverse distribution of methylene-labeled IAA-^{14}C in the tips of horizontal pea roots

Data on the asymmetric distribution of methylene-labeled IAA-^{14}C are summarized in *table 3*. They show the same features as those obtained with the carboxyl label, i.e. (a) a clear transverse distribution of the applied auxin, which is more pronounced when 0.2 mm of the tip has been removed than in intact tips, (b) a decreased asymmetry when the roots have been kept horizontal longer than two hours, and (c) no asymmetry of the absorbed IAA-^{14}C in the tips of vertical roots.

The recovery of the ^{14}C in both intact and decapitated roots was higher on the average than the recovery of carboxyl-^{14}C in decapitated roots, but equal to the recovery of this label in intact roots. The smaller recovery of carboxyl-labeled IAA-^{14}C in the roots decapitated at 0.2 mm is thus the result of some degrada-

Table 3. The transverse distribution of methylene-labeled IAA-^{14}C in the most apical 4 mm of intact or 0.2 mm decapitated pea roots.

time of exposure, hours	position	cpm/mg dry weight		total cpm in upper+lower halves	% of total cpm in upper halves	% recovery
		in upper halves	in lower halves			
1.5	hor.	20.3	35.6	55.9	36.3	–
1.5	„	19.0	34.0	53.0	35.8	–
1.5	„	81.5	121.1	202.6	40.2	–
1.5[1]	„	90.0	126.6	216.6	41.5	35
1.5[1]	„	80.0	120.0	200.0	40.0	44
1.5[1]	vert.	81.3	85.1	166.4	48.8	40
1.5[1]	„	84.4	93.2	177.6	47.5	42
2.0	hor.	110.0	165.0	275.0	40.0	40
2.0	„	22.1	41.5	63.6	34.7	45
3.0	„	17.6	17.9	35.5	49.5	76
3.0	„	18.0	21.5	39.5	45.5	–

All data are for a group of 24 roots.
[1] Intact roots; the others were decapitated at 0.2 mm.
The auxin application ranged from 0.04 to 0.21 μg per 24 roots.

tion at the cut surface. The fact, however, that methylene-labeled IAA-^{14}C was transversely distributed to about the same extent as the carboxyl-labeled compound makes any participation of IAA-degrading enzymes in the transverse distribution very unlikely.

3.4. The degradation of IAA-^{14}C in pea root homogenates

It was tacitly assumed in the previous section that IAA oxidase-peroxidase cannot liberate the methylene group. Since, however, recovery of methylene-^{14}C was almost the same as that of carboxyl-^{14}C, in the intact roots, it was necessary to determine whether in fact the methylene-^{14}C did not disappear through the action of the indoleacetic acid oxidizing system. Carboxyl-labeled and methylene-labeled IAA-^{14}C were therefore mixed with homogenates of tips of pea roots. The results (*table 4*) clearly demonstrate that within two hours the label of the methylene group did not disappear, whereas much of the ^{14}C in the carboxyl group vanished in a few minutes. (The figures in column 4 of the last experiment of *table 4*, which show no increase with time, are probably due to a systematic error of about 5% in the determination).

Hence, if the action of the IAA-oxidizing enzyme in vitro reflects its action in vivo, then the transverse distribution cannot be the result of decreased degradation on the lower side of the horizontal root tip.

3.5. The effect of decapitation on the transverse distribution of IAA-^{14}C

The facts that (a) a large fraction of the ^{14}C present in a 4 mm tip was found in the root cap (*fig. 3*), and (b) unequal amounts of radioactivity were present in

Table 4. The degradation of IAA-^{14}C in pea root homogenates.

expt	incubation time, min	cpm in sample		total cpm disappeared	
		^{14}C in carboxyl	^{14}C in methylene	^{14}C in carboxyl	^{14}C in methylene
1	0	380	422	0	0
	10	311	422	69	0
	20	257	422	123	0
	30	230	422	150	0
2	0	573	480	0	0
	3	493	472	80	8
	6	393	474	180	6
	12	355	474	218	6
	30	327	474	246	6
3	0	730	750	0	0
	15	410	750	320	0
	30	390	748	340	2
	60	322	747	408	3
4	0	1419	987	0	0
	5	443	946	976	41
	10	438	950	981	37
	20	320	935	1099	52
	30	317	940	1102	47
	120	278	942	1141	45

The data are relevant to 0.1 ml samples of the reaction mixture.
The reaction mixture contained the homogenate of 50 root tips of 3 mm length, IAA-^{14}C, and buffer pH 6.0 to a final volume of 1.5 ml.

the upper and the lower halves of the extreme mm of the apex (*table 2*), strongly suggested that the asymmetry of the applied auxin occurred in the root cap, i.e., that the mechanism which is responsible for the transverse distribution is localized in the cap. To test this, both carboxyl-labeled and methylene-labeled IAA-^{14}C were applied to roots from which the cap, or part of it, had been removed. The results are given in *table 5*. They show that when the auxin was applied to roots decapitated at 0.3 mm the asymmetry of ^{14}C distribution was less than in the tips of intact roots. Decapitation at 0.5 mm or more (removal of the entire cap) prevented the transverse distribution almost entirely; there was even a slight shift to the upper side in the last experiment of this table. The results were the same for both types of labelling. Apparently, therefore, the root cap is required to bring about the transverse distribution of the auxin.

Table 5 also shows, as did *table 1*, that the total amounts of ^{14}C taken up by decapitated roots were somewhat less than those absorbed by intact tips in the same period. The radioactivity of either the upper or the lower halves of the decapitated root tips was no more, or only very slightly more, than that found in the upper halves of horizontal intact root tips (*table 5*). The act of decapitation evidently decreases the auxin uptake. It could, therefore, be concluded that horizontal intact root tips take in more auxin and then distribute it asymmet-

Table 5. The transverse distribution of IAA-^{14}C in the apical 4 mm of intact and decapitated horizontal pea roots.

expt	decapitation mm	cpm/mg dry weight		total cpm in upper + lower halves	% of total cpm in upper halves
		in upper halves	in lower halves		
1	0.0	60.0	89.4	149.4	40.1
	0.3	57.0	64.8	121.0	46.7
	0.5	58.4	62.5	120.9	48.3
2	0.0	62.5	100.0	162.5	38.4
	0.3	52.2	60.0	112.2	46.5
	0.5	57.3	53.6	110.9	51.6
3	2.0	40.0	42.1	82.1	48.7
4[1]	0.0	80.0	120.0	200.0	40.0
	0.5	84.4	93.2	177.6	47.5
	1.0	92.8	79.4	172.2	53.8

All data are for a group of 24 roots.
[1] Methylene-labeled IAA-^{14}C was used in this experiment; carboxyl-labeled in the three others.
The quantity of auxin applied averaged 0.23 μg per 24 roots.
The exposure time was 1.5 hours in all cases.

rically so that the lower side thus obtains considerably more than the upper side.

3.6. The effects of caffeic acid and 2,4-dichlorophenol (DCP) on the transverse distribution of IAA-^{14}C in horizontal pea roots

The physiological difference between the upper and the lower sides of the root cap, which is responsible for the transverse distribution of the auxin, is not known. However, from earlier experiments on roots immersed in solutions of caffeic acid or 2,4-dichlorophenol, it was concluded that caffeic acid (10^{-6} g/ml) either inhibited or promoted a growth difference between the upper and the lower sides of the root tip, dependent on whether the roots had been horizontally exposed or not. 2,4-Dichlorophenol (10^{-5} g/ml), on the other hand, prevented the appearance of such a difference or wiped it out (KONINGS 1964). Both compounds were therefore restudied in connection with the transverse distribution of applied auxin (carboxyl-labeled IAA-^{14}C in all cases). Results with caffeic acid, presented in *table 6*, show that caffeic acid strongly prevented the asymmetric distribution of the applied IAA-^{14}C, whether given simultaneously with IAA in the agar or when applied in solution before the IAA. The total counts in the cases where direct comparison can be made, column 5 of *table 6*, show that in roots decapitated at 0.2 mm the total amount of ^{14}C was decreased by treatment with caffeic acid. However, when intact, the roots treated with caffeic acid contained more isotope than the controls. Caffeic acid therefore apparently decreased uptake through the cut surface, to an extent which outweighed its moderate inhibition of the IAA decarboxylation.

Roots which were immersed in 2,4-dichlorophenol (10^{-5} g/ml) for 15–60

Table 6. The effect of caffeic acid (CA) on the transverse distribution of IAA-^{14}C in the apical 4 mm of horizontal pea roots.

time of exposure, hours	(pre-)treatment	cpm/mg dry weight		total cpm in upper+lower halves	% of total cpm in upper halves
		in upper halves	in lower halves		
1.5	CA with IAA in agar	39.2	50.7	89.9[2]	43.6
	no CA	40.2	66.4	106.6[2]	37.7
2.0	15 min vert. in CA	37.8	40.9	78.7[2]	48.0
	15 min vert. in water	38.6	59.7	98.3[2]	39.2
2.5	15 min vert. in CA	32.8	35.1	67.9	48.3
3.0	CA with IAA in agar	32.3	38.6	70.9	45.5
1.5[1]	20 min hor. in CA	66.8	74.0	140.8[2]	47.4
	20 min hor. in water	45.6	88.7	134.3[2]	33.9
1.5[1]	30 min vert. in CA	140.0	156.4	296.4[2]	47.2
	30 min vert. in water	73.2	128.1	201.3[2]	36.3
	none	84.3	127.5	211.8[2]	39.8

All data are for a group of 24 roots.
The amount of auxin applied was either 0.15 or 0.20 μg per 24 roots.
The concentration of caffeic acid used was 10^{-6} g/ml.
[1] Intact roots; the others were decapitated at 0.2 mm.
[2] Pairs for direct comparison; influence of CA on total uptake.

minutes and then allowed to take up IAA-^{14}C could not bring about as great an unequal distribution as did the controls treated with distilled water (*table 7*).

In addition, the total amount of ^{14}C in the DCP-treated roots was only slightly less, or even a little more, than in the water-treated roots. When, however, DCP in this concentration was added to pea root homogenates it enhanced the degradation of IAA-^{14}C by about 50 per cent. The effect of DCP in the intact roots therefore, is much weaker than in the tissue homogenates of these roots.

3.7. The IAA oxidase activities of homogenates from the upper and lower halves of horizontal intact roots

It was shown above that both caffeic acid and 2,4-dichlorophenol partially prevented the transverse distribution of IAA-^{14}C. Earlier (KONINGS 1964) it was found that the IAA degrading activity of homogenates made from the upper halves of horizontal root tips was greater than that of similar preparations made from the lower halves. These experiments were now repeated and extended with the aid of IAA-^{14}C in an attempt to obtain a complete picture of the causes of the geotropic curvature of the root tip. The procedure was as follows: The roots were placed horizontally for about an hour, then the 3 mm tips were split into upper and lower halves which were collected in solid CO_2 and ground in a mortar in 0.8 ml buffer, pH 6.0. To 0.45 ml of the homogenate was added 0.3 ml solution of IAA-^{14}C. The IAA-degrading activity of the crude homogenates was determined. The results (*fig. 4*) show that the activities of the homogenates made from the upper halves were at first consistently higher than those of the prepara-

Table 7. The effect of 2,4-dichlorophenol (DCP) on the transverse distribution of IAA-^{14}C in the apical 4 mm of horizontal pea roots.

time of exposure, hours	pretreatment	cpm/mg dry weight		total cpm in upper+lower halves	% of total cpm in upper halves
		in upper halves	in lower halves		
1.5	15 min. vert. in DCP	90.0	118.3	208.3	43.2
	none	80.0	150.0	230.0	34.7
1.5	60 min. vert. in DCP	45.2	60.0	105.2	42.9
	60 min. vert. in water	40.0	80.9	120.9	33.0
2.0	30 min. vert. in DCP	55.4	73.3	128.7	43.0
	30 min. vert. in water	35.5	84.6	120.1	29.5
1.5[1]	20 min. vert. in DCP	52.6	61.8	114.4	45.9
	20 min. vert. in water	55.7	86.8	142.5	39.0

All data are for a group of 24 roots.
The auxin application ranged from 0.16 to 0.41 μg per 24 roots.
The concentration of 2,4-dichlorophenol used was 10^{-5} g/ml.
[1] Intact roots; the others were decapitated at 0.2 mm.

tions from the lower halves. However, the difference between the activities of the two preparations largely disappeared after an incubation time of 3 minutes or longer, so that the amounts of ^{14}C which disappeared during 15 minutes incubation were about equal. When the crude homogenates were partly purified by centrifugation in the cold, the difference usually was not found. This suggests that the factor which is responsible for the initial difference is labile or volatile. Whether this phenomenon and the transverse distribution of the applied auxin are related or not, will be seen in the next section. No difference was found between the activities of preparations made from the halves of the vertical root tips.

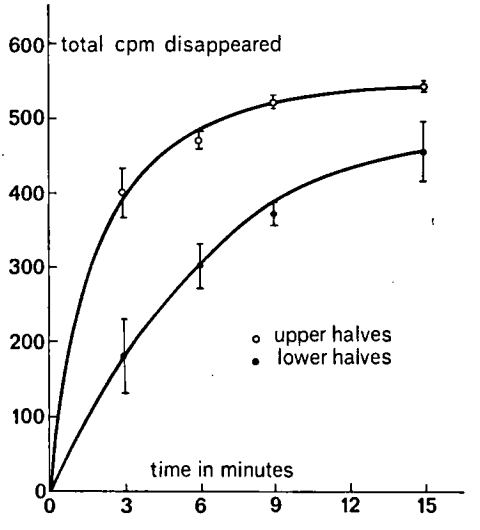

Fig. 4. The degradation of IAA-^{14}C in crude homogenates from the upper and lower halves of the apical 3 mm of horizontal pea roots.

3.8. The IAA oxidase activities of homogenates from the upper and lower halves of decapitated roots

Since no transverse distribution of the applied IAA-^{14}C occurred in the tips of roots which were decapitated at 0.5 mm or more (*table 5*), it follows that the root cap controls the lateral distribution. We must therefore ask: is the development of different IAA oxidase activities in the upper and lower halves of horizontal roots also controlled by the root cap? The experiments were carried out as described in section 7, but now with roots decapitated at 0.5 or 1.0 mm. *Table 8* shows that the results were similar to those obtained with intact root tips. The activity of the homogenates from the upper halves was much the greater at first, but the difference almost disappeared after 30 minutes' incubation. Hence the difference between the IAA oxidase activities of the homogenates from the upper and the lower sides is not controlled by the root cap.

Table 8. The degradation of IAA-^{14}C in crude homogenates from the upper and lower halves of the apical 3 mm of decapitated horizontal pea roots.

expt	incubation time, min	total cpm disappeared		difference, upper halves minus lower halves
		upper halves	lower halves	
1	3	1035	550	485
	6	1120	810	310
	9	1130	870	260
	15	1175	916	259
	30	1175	1174	1
2	3	726	349	377
	6	763	565	198
	9	842	669	173
	15	882	749	133
	30	882	792	90
3	3	490	226	264
	6	539	320	219
	9	–	515	–
	15	614	–	–
	30	618	545	73

The roots in experiment 1 were decapitated at 0.5 mm; 1.0 mm in the two other experiments. The exposure time was one hour in all cases.
The tips were ground in 1.0 ml buffer pH 6.0; to 0.6 ml of the homogenate were added 0.1 ml IAA-^{14}C solution (various concentrations) and 0.3 ml buffer; 150 tips were used in each experiment.

The cause of the difference is not known, but it is evidently due to some compound which in vitro inhibits the IAA oxidase activity, and accumulates on the lower side of a horizontal root tip.

IAA, when applied in vivo, has been reported to affect the IAA degradation in vitro (PILET 1964), and to increase the peroxidase activity in cells of the elongating zone of roots of *Vicia faba* (JENSEN 1955). Finally, therefore, the

effect of IAA on the IAA oxidase activity was investigated. Exposure of the roots to IAA in vivo appeared to lower the velocity of its subsequent breakdown in vitro. When roots decapitated at 1.0 mm (to remove their own source of auxin) were placed for an hour in a solution of IAA (10^{-6} g/ml), next ground and the homogenate tested for its IAA degrading activity, then the homogenate from the IAA-treated root tips had a consistently lower activity than that from water-treated roots (*table 9*).

Table 9. The effect of preincubation in IAA on the degradation of IAA-^{14}C in pea root homogenates.

expt	incubation time, min	total cpm disappeared		difference, water treated minus IAA treated
		water treated	IAA treated	
1	3	148	136	12
	6	150	164	-14
	9	192	165	27
	15	284	170	114
2	3	729	440	289
	6	775	619	156
	9	804	675	129
	15	804	675	129
3	3	550	404	146
	6	591	594	-3
	9	608	596	12
	15	656	606	50

The roots were decapitated at 1.0 mm, then placed vertically in distilled water or in a solution of IAA, 10^{-6} g/ml, for one hour. They were then placed horizontally in moist air for one hour in the experiments 1 and 2, for 1.5 hours in the last experiment.
The reaction mixture contained 0.6 ml of the homogenate, 0.1 ml solution of IAA-^{14}C (various concentrations), and 0.3 ml buffer pH 6.0; 80 apical 3 mm root sections were used in each experiment.

4. DISCUSSION

The results show unmistakably that auxin (IAA-^{14}C) applied in physiological concentrations to the apex of intact or 0.2 mm decapitated horizontal pea roots is transversely distributed. The ratio between the amounts of ^{14}C found in the upper and lower halves was 33/67 in the roots decapitated at 0.2 mm and 40/60 in the intact tips. These data agree well with those obtained by GILLESPIE & THIMANN (1963) with corn coleoptiles, in which about 40 per cent of the ^{14}C found in the tissue was localized in the upper halves. When more than 0.2 mm of the root cap was removed, however, the transverse distribution became less, and when 0.5 mm or more was cut off no lateral distribution at all was found. Evidently therefore, the root cap is indispensable for the transverse distribution of applied auxin to occur.

The parallelism between the asymmetric distribution of the applied auxin and

geotropic curvature is clear and obviously significant; the root cap controls both. For in other experiments (to be published) it has been shown, in agreement with earlier workers, that removal of the root cap only (i.e. 0.5 mm of these roots) does not decrease elongation but completely prevents geotropic curvature.

Caffeic acid and 2,4-dichlorophenol, which both greatly modify the transverse distribution, probably directly affect the mechanism responsible for the unequal distribution in the root cap. It is attractive to suggest that the physiological difference between the upper and lower halves of the root cap, which caused the transverse distribution of the applied auxin, is or includes the unequal distribution of some phenolic compound. Caffeic acid and 2,4-dichlorophenol would then have obscured this unequal distribution.

While the experiments do not show how the asymmetric auxin distribution occurs, they at least eliminate one possible mechanism. For carboxyl-labeled and methylene-labeled IAA-^{14}C were transversely distributed to the same extent. No disappearance of ^{14}C occurred in the homogenates when the label was in the methylene group, whereas that in the carboxyl group disappeared rapidly. Hence, although a decreased degradation of the auxin in the lower half of the root cap would, with carboxyl-labeled IAA-^{14}C result in its apparent accumulation in the lower half, this process does not play a role in the transverse distribution of the auxin.

Since the recovery from the tips decapitated at 0.2 mm was less when carboxyl-labeled IAA-^{14}C was applied than when methylene-labeled IAA-^{14}C was given, some decarboxylation of the auxin doubtless occurred at the cut surface.

The recovery of both types of molecules from the intact root tips was equal, but generally not over 50 per cent. This means that at least half of the ^{14}C lost from the donor blocks vanished. This cannot have been the result of degradation, because then the recovery would have been clearly higher with methylene-labeled IAA-^{14}C, since the metabolites of this molecule retain their label at least for a few hours (*table 4*, see also ANDREAE c.s. 1961; GERONIMO c.s. 1964).

Although some auxin from the agar blocks probably got lost to the plexiglass blocks, it is not known how far this could explain the low recovery data.

The steep gradient of the applied auxin in the root tips is noteworthy. The ^{14}C was translocated basipetally about 5 mm in 1.5 hours, a transport velocity which agrees well with those which can be deduced from the results of BONNETT & TORREY (1965) with *Convolvulus* roots and of PILET (1964) with roots of *Lens*, but which is higher than that found in roots of *Vicia faba* by YEOMANS & AUDUS (1964).

The meaning of the (in vitro rapidly decreasing) difference between the IAA oxidase activities of homogenates from the upper and the lower halves is obscure. In the first place it was evidently not controlled by the root cap, since decapped roots behaved in the same way as intact roots. In the second place, although the factor which accumulated in the lower halves of horizontal roots inhibited the degradation of IAA in vitro, the same may not occur in the intact root. In experiments (to be published) where the root cap was removed at

different stages during the downward bending of the roots, a rapid straightening followed because the lower side then elongated much faster than the upper side. The "IAA-oxidase difference" may be connected with this growth action. Apparently gravity has more than one effect on the physiology of the root tip. Investigations will now be aimed at finding the relation between the events occurring in the root cap and those in the elongation zone.

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REFERENCES

ANDREAE, W. A., J. R. ROBINSON & M. W. H. VAN IJSSELSTEIN (1961): Studies on 3-indoleacetic acid metabolism. VII. Metabolism of radioactive 3-indoleacetic acid by pea roots. *Plant Physiol.* **36**: 783–787.

BONNETT, H. T. & J. G. TORREY (1965): Auxin transport in Convolvulus roots cultured in vitro. *Plant Physiol.* **40**: 813–818.

BOYSEN-JENSEN, P. (1933): Die Bedeutung des Wuchsstoffes für das Wachstum und die geotropische Krümmung der Wurzeln von Vicia faba. *Planta* **20**: 688–698.

CHING, T. M. & S. C. FANG (1958): The redistribution of radioactivity in geotropically stimulated plants pretreated with radioactive indoleacetic acid. *Physiol. Plantarum* **11**: 722–727.

DOLK, H. E. (1930): Geotropie en Groeistof. Dissertation. Utrecht.

DIJKMAN, M. J. (1934): Wuchsstof und geotropische Krümmung bei Lupinus. *Rec. trav. bot. néerl.* **31**: 391–450.

GERONIMO, J., P. B. CATLIN & E. C. MAXIE (1964): Metabolism of indoleacetic acid-2-^{14}C in plum stem cuttings. *Physiol. Plantarum* **17**: 644–653.

GILLESPIE, B. & W. R. BRIGGS (1961): Mediation of geotropic response by lateral transport of auxin. *Plant Physiol.* **36**: 364–368.

GILLESPIE, B. & K. V. THIMANN (1963): Transport and distribution of auxin during tropistic response. I. The lateral migration of auxin in geotropism. *Plant Physiol.* **38**: 214–225.

GOLDSMITH, M. H. M. & K. V. THIMANN (1962): Some characteristics of movement of indoleacetic acid in coleoptiles of Avena. I. Uptake, destruction, immobilization, and distribution of IAA during basipital translocation. *Plant Physiol.* **37**: 492–505.

GOLDSMITH, M. H. M. & M. B. WILKINS (1964): Movement of auxin in coleoptiles of Zea mais L. during geotropic stimulation. *Plant Physiol.* **39**: 151–162.

HAWKER, L. E. (1932): Experiments on the perception of gravity by roots. *New Phytol.* **31**: 321–328.

JENSEN, W. A. (1955): The histochemical localization of peroxidase in roots and its induction by indoleacetic acid. *Plant Physiol.* **30**: 426–432.

KONINGS, H. (1964): On the indoleacetic acid converting enzyme of pea roots and its relation to geotropism, straight growth and cell wall properties. *Acta Bot. Neerl.* **13**: 566–622.

PILET, P. E. (1964a): Auxin transport in roots. *Nature* (London) **204**: 561–562.

PILET, P. E. (1964b): Processus d'induction ou d'adaptation auxines-oxydasiques. *C.R.Ac.Sc. (Paris)* **259**: 1183–1184.

RAALTE, M. H. VAN (1937): On factors determining the auxin content of the root tip. *Rec. trav. bot. néerl.* **34**: 278–332.

THIMANN, K. V. (1936): Studies on the growth hormone of plants. VI. The distribution of the growth substance in plant tissues. *J. Gen. Physiol.* **18**: 23–34.

YEOMANS, L. M. & L. J. AUDUS (1964): Auxin transport in roots. *Nature* (London) **204**: 559–561.