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DO COLEOPTILE TIPS PRODUCE AUXIN?

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Summary

A re-examination of the evidence for auxin production by coleoptile tips reveals that it is not conclusive and that several important problems remain unresolved. The possibility that auxin and auxin precursors move acropetally in the xylem was tested by analysing guttation fluid from intact coleoptiles, decapitated coleoptiles and primary leaves of *Avena sativa*. In all cases two zones of auxin activity were detected on chromatograms of the acidic ether-soluble fraction, one of which corresponded to the R_F of indol-3-yl acetic acid (IAA). Similar auxin activity was found in guttation fluid from seedlings of *Zea mays*, *Triticum aestivum* and *Hordeum vulgare*. Evidence that guttation fluid also contains alkali-labile auxin complexes was obtained. Experiments on the movement of dyes and radioactive IAA introduced into the xylem of transpiring or guttating coleoptiles showed that these substances accumulate at the tip of the coleoptile, or at the apical region of decapitated coleoptiles. The hypothesis that IAA and 'inactive' auxins move acropetally in the xylem from the seed to the coleoptile tip where they accumulate and where the 'inactive auxins' can be converted to IAA is shown to be consistent with the classical work on coleoptiles; it can also explain the autonomous curvature of coleoptiles and the influence of the roots on the auxin content of coleoptile tips. An analogous accumulation of auxin probably occurs at the tips of primary leaves. The anomalous auxin economy of coleoptile tips is discussed.

Introduction

The coleoptile is a specialized seedling structure of limited growth whose tip is non-meristematic. In text books of plant physiology the coleoptile tip is variously described as a site of auxin production or auxin activation; but the auxin economy of coleoptile tips is anomalous in that it is known to depend on the presence of the seed (Went and Thimann, 1937). The work which led to the conclusion that coleoptile tips actually produce auxin was carried out over 25 years ago and has not been added to significantly since then. However, an examination of the original literature in the light of more recent ideas and techniques reveals that the evidence is by no means conclusive.

The classical findings fall into three main categories.

(1) Coleoptile tips contain more extractable auxin than more basal regions of the coleoptile. There is in fact a gradient of auxin from the tip downwards (Thimann, 1934; van Overbeek, 1938; Wildman and Bonner, 1948). More diffusible auxin can also be obtained from the tip than from other parts of the coleoptile, but the often-cited evidence of Went (1928) that only the extreme tip, less than 0.7 mm in length, produces auxin was not confirmed by the more detailed studies of van Overbeek (1941) who obtained more diffusible auxin from 3-mm than from 2-mm or 1-mm tips.

(2) Skoog (1937) showed that removal of the seed led to a decline in the amount of auxin

obtainable from the tip; and that deseeded plants were no longer capable of 'regeneration of the physiological tip' (the phenomenon by which the apical region of the stump of a decapitated coleoptile becomes a source of auxin several hours after decapitation). Pohl (1935, 1936) produced evidence that the seed, which is rich in auxin, acted as a source of auxin which moved acropetally and accumulated at the coleoptile tip, but this possibility was rejected by Skoog (1937) who found that no auxin could be detected in agar blocks placed on the stumps of decapitated coleoptiles. He concluded that the seed was acting as the source of an auxin precursor which moved acropetally but that auxin itself did not move in this way. Similar conclusions were reached by Voss (1939).

(3) More auxin can be collected from isolated coleoptile tips by diffusion into agar blocks than can be obtained by extraction of the tips immediately after isolation (Thimann, 1934; van Overbeek, 1941; Wildman and Bonner, 1948), indicating that auxin is produced during the period of diffusion.

The contention of Pohl (1935, 1936) that auxin moves acropetally from the seed and accumulates at the coleoptile tip is opposed only by Skoog's (1937) experiment which depends on the assumption that any auxin which might be moving acropetally will diffuse into an agar block on the stump of a decapitated coleoptile. Skoog's experiments, like most of the other classical work on coleoptiles, were carried out in standard *Avena* growth chambers with a relative humidity adjusted to minimize guttation (Went and Thimann, 1937). The guttation fluid from coleoptiles grown in a more humid atmosphere contains auxin (Sheldrake and Northcote, 1968a) and it is therefore possible that auxin could be moving acropetally in the xylem but that the method used by Skoog would fail to detect it. The evidence for the production of auxin by isolated coleoptile tips which depends on a comparison of diffusible and extractable auxin is based on the assumption that diffusion and extraction take place with equal efficiency. But while the diffusion technique may involve minimal losses, extraction of auxin can result in low recoveries (e.g. Mann and Jaworski, 1970). The figures for extractable auxin could therefore be seriously underestimated. The amounts of diffusible auxin could have been overestimated as a result of bacterial contamination, which can account for a large proportion of the auxin recovered from non-sterile plant tissues (Libbert *et al.*, 1966; Kaiser, 1967). The exhaustive diffusion of auxin from coleoptile tips was carried out for periods of up to 18 hours under non-sterile conditions. Therefore the conclusions drawn from these experiments cannot be regarded as unequivocal.

In addition to the role of the seed in the auxin economy of the coleoptile tip the roots play a part which has never been explained. van Overbeek (1937) found that removal of the roots reduced by nearly one-half the amount of auxin which could be obtained from coleoptile tips 20 hours later. The role of the roots could perhaps also account for the fact that sand-grown *Avena* seedlings contain considerably more auxin in their coleoptile tips than seedlings grown in unaerated distilled water (van Overbeek, 1941). These results suggest that root pressure may be involved in some way in the movement of auxin and/or auxin precursor from the seed to the coleoptile tip. The presence of auxin in the guttation fluid of *Avena* (Sheldrake and Northcote, 1968a) indicates that auxin itself may move acropetally in the xylem. I have investigated this possibility in the light of the classical evidence in favour of auxin production by coleoptile tips.

MATERIALS AND METHODS

Seeds of *Avena sativa* L. cv. Condor, *Triticum aestivum* L. cv. Cappelle-Desprez,

Hordeum vulgare L. cv. Proctor and *Zea mays* L. cv. Inra 200 were obtained from the National Institute of Agricultural Botany, Cambridge. After soaking in water for 3 hours, they were sown on sand in plastic boxes covered with aluminium foil and grown in darkness at 22° C. Guttation fluid was collected from both the coleoptiles and young primary leaves (unless otherwise stated) of the seedlings at regular intervals with a Pasteur pipette and stored in the deep freeze. For the extraction of auxin, sodium hydrogen carbonate was added to the guttation fluid to a concentration of 0.1 M and the fluid was partitioned three times with peroxide-free ether, giving the neutral + basic fraction. With methyl orange as internal indicator the guttation fluid was then acidified to pH 3 by the addition of hydrochloric acid and partitioned three times with ether to give the acidic fraction. Ether extracts were concentrated to a small volume at atmospheric pressure and applied to the origins of cellulose thin-layer plates which were developed with isopropanol/ammonia/water (10/1/1, v/v/v). Zones of these chromatograms were scraped off and assayed by the *Avena* mesocotyl extension bioassay using plants of *A. sativa* cv. WW 16253 (Weibullsholm, Sweden) as described by Sheldrake (1971a). Marker spots of indol-3-yl acetic acid (IAA) were revealed by a $FeCl_3$/perchloric acid spray (Larsen, 1955).

[1-^{14}C]IAA (52 mCi/mM, Amersham) was used in tracer experiments with seedlings and also for the estimation of percentage recoveries. Four millilitres liquid scintillator (Bray, 1960) was added to samples which were counted on a Nuclear Chicago Unilux scintillation counter for at least 10 minutes each. Background counts (25–30 ct/minute) were subtracted from all results.

Auxin was extracted from plant tissues with peroxide-free ether for two periods of 2 hours at 2° C in the dark.

Results and discussion

Auxin in guttation fluid

Guttation fluid of *Avena* and of *Zea* was made alkaline by the addition of sodium bicarbonate and partitioned with ether. Bioassays of chromatograms of this fraction, containing basic and neutral ether-soluble substances, showed little auxin activity, although minor zones of activity with a high R_F were often observed (Fig. 1a, b). Ether extracts of guttation fluid acidified to pH 3 showed two pronounced zones of auxin activity, one of which corresponded to the R_F of IAA; the other was in the region of R_F 0.7–1.0 (Fig. 1c, d). Zones corresponding to the R_F of IAA were eluted from chromatograms of *Avena* guttation fluid and rechromatographed in three further solvent systems: pyridine/ammonia (3/1, v/v); ethanol/water (7/3, v/v) and chloroform/acetic acid (95/5, v/v). In each case bioassays revealed auxin activity only at the R_F of IAA, indicating that this auxin is in fact IAA.

Chromatograms of the acidic ether-soluble fraction of guttation fluid of *Triticum* and *Hordeum* showed patterns of auxin activity similar to those of *Avena* and *Zea* (Fig. 2a, b).

The possibility that the auxin detected in guttation fluid was formed by bacteria growing in the fluid was investigated by dividing samples of *Avena* guttation fluid into two aliquots, one of which was stored in the deep freeze while the other was incubated for 8 hours at 22° C. The amount of auxin detected in the incubated guttation fluid was found to be slightly less than that in the control; if bacteria were responsible for producing the auxin in guttation fluid the incubated samples would be expected to contain considerably more.

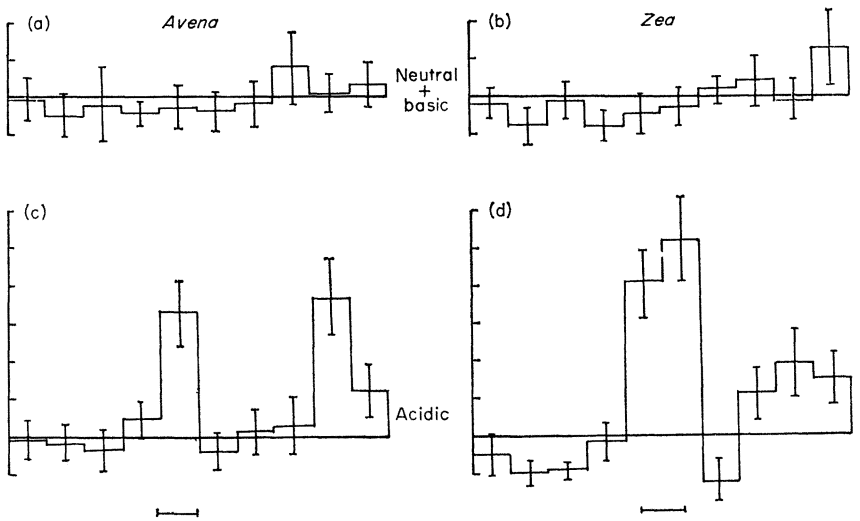

Fig. 1. Auxin activity on chromatograms of the neutral + basic and acidic fractions of guttation fluid of *Avena* (16.0 ml) and *Zea* (18.5 ml). The origins of the chromatograms are shown at the left, the solvent front at the right. The positions of marker spots of IAA are indicated. Each division on the vertical axis represents a mesocotyl extension of 0.2 mm.

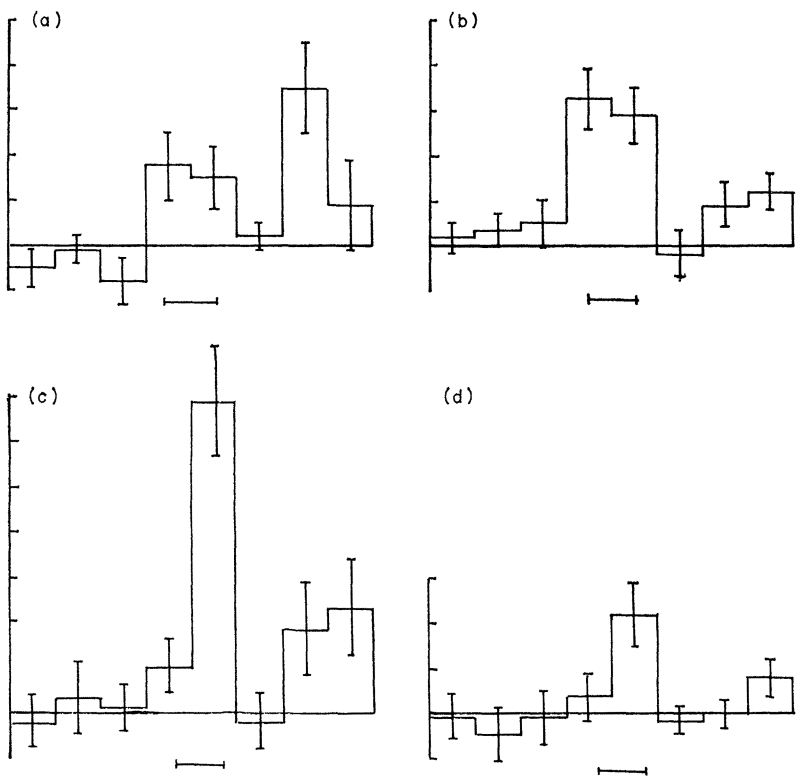

Fig. 2. Auxin activity on chromatograms of the acidic fraction or guttation fluid of *Triticum* (a) (11.0 ml), *Hordeum* (b) (10.5 ml), decapitated *Avena* coleoptiles (c) (10 ml) and *Avena* primary leaves (d) (11.5 ml). Conventions as in Fig. 1.

If the coleoptile tip is a site of auxin production rather than a site of auxin accumulation, it could be argued that the auxin detected in coleoptile guttation fluid had been eluted from the coleoptile tip. This possibility was checked in two ways. Guttation fluid was collected from decapitated coleoptiles within 2 hours of decapitation; the coleoptiles were then decapitated again and further guttation fluid was collected, and so on. Guttation fluid was also collected from the young primary leaves of *Avena* seedlings. In both cases a distribution of auxin activity was found on chromatograms of the acidic ether-soluble fraction which was similar to that found for guttation fluid from intact coleoptiles (Fig. 2c, d). This evidence indicates that auxin is present in the xylem sap and is not merely eluted from coleoptile tips as guttation takes place.

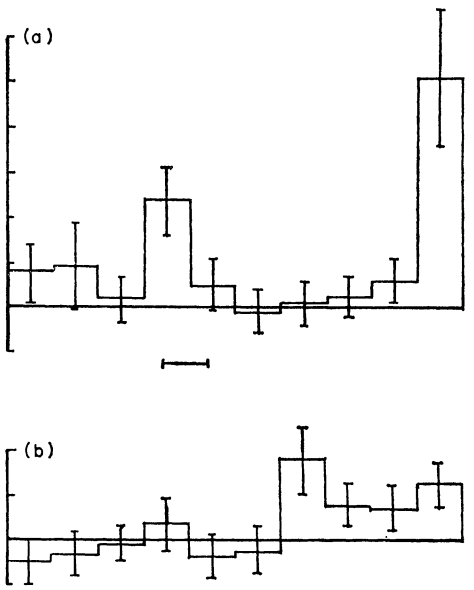

Fig. 3. (a) Auxin activity of the rechromatographed high R_F (0.7–1.0) zone of the acidic fraction of *Avena* guttation fluid (40.0 ml). The ether eluate was dried, heated for 2 minutes at 100° C, taken up in a small volume of ether and applied to the origin of the chromatogram shown. (b) Auxin activity on chromatogram of the neutral fraction of *Avena* guttation fluid (40.0 ml) after acidification to pH 3 for 10 minutes. The initial neutral + basic fraction was removed by partitioning with ether before acidification.

Söding and Raadts (1953) found that aqueous diffusates from *Avena* coleoptile tips contained an auxin which was not identical to IAA, and later showed that this auxin could be separated by chromatography from IAA, which was also present. This second auxin was inactive in the coleoptile curvature bioassay but could be activated by mild acid treatment and was sometimes converted to active auxin spontaneously (Raadts and Söding, 1957). The quantities of this second auxin decreased on incubation of coleoptile tissue. Raadts and Söding concluded that this auxin was not produced from IAA in the tip, but that the reverse might be true. Ramshorn (1955) also detected IAA and another compound with auxin activity in diffusates of *Avena* tips, using a straight growth bioassay. Shen-Miller and Gordon (1966) examined the auxin present in aqueous diffusates of coleoptile tips with similar results. Three main zones of auxin activity were detected on chromatograms developed in ammoniacal isopropanol of the acidic ether-extractable fraction of the diffusates. One corresponded to IAA; another ('F') was present only in

relatively small amounts and ran near the solvent front; the third ('P') was present in larger amounts and had an R_F between that of IAA and F. Only the IAA zone was active in the coleoptile curvature bioassay, but all were active in the coleoptile straight-growth bioassay. The compound P could be converted to IAA by mild heat treatment. From its R_F and its presence only in the acidic extractable fraction Shen-Miller and Gordon concluded that it was either weakly acidic or a neutral substance which was produced on mild acidification. They were unable to identify it further. It seems likely that at least some of the auxin activity found on chromatograms of the acidic ether-extractable fraction of guttation fluid with a high R_F (Fig. 1c, d; Fig. 2) is due to the substance investigated by Shen-Miller and Gordon since mild heating leads to the appearance of auxin activity close to the R_F of IAA (Fig. 3a). Very little auxin activity was found in the neutral + basic ether-extractable fraction of guttation fluid (Fig. 1a, b) but if the guttation fluid was acidified to pH 3 then made alkaline again with sodium bicarbonate and re-extracted with ether, slight but significant auxin activity with a high R_F could be detected on chromatograms of this extract (Fig. 3b), suggesting that it is due to one or more neutral substances formed on acidification.

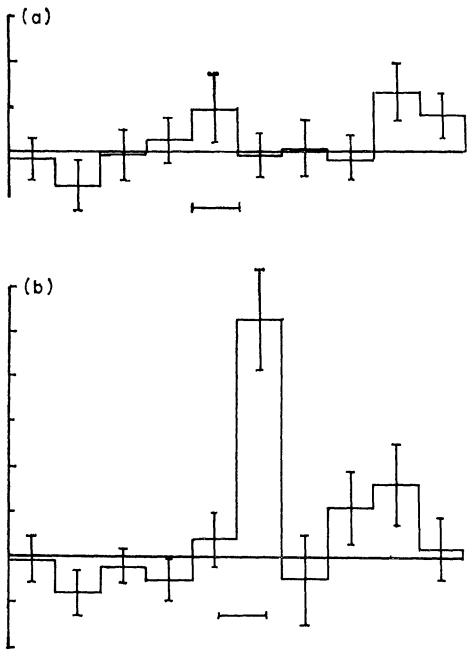

Fig. 4. Auxin activity on chromatograms of the acidic fraction of hydrolysed guttation fluid of *Avena* (a) (25.0 ml) and *Zea* (b) (24.0 ml). The guttation fluid was exhaustively partitioned with ether in the usual way before before hydrolysis with alkali.

Shen-Miller and Gordon found that P was probably a precursor of IAA, that it was not transported by the polar auxin transport system (which accounts for its inactivity in the coleoptile curvature bioassay) and that it could only be obtained from freshly harvested tips. They concluded that the intact seedling was necessary for the maintenance of a pool of P in the tip. This is consistent with the presence of P or P-like substance in guttation fluid. If P were present in the xylem sap in the coleoptile tip the maintenance of the P pool would depend on the intact seedling and P would appear in aqueous diffusates

even though it is incapable of moving through living cells in the polar auxin transport system; it will be shown in the next section of this paper that substances present in the xylem sap are concentrated at the coleoptile tip and can readily diffuse out of isolated tips into water.

The source of the auxins present in guttation fluid seems likely to be the seed. Seeds of *Avena* and *Zea* are known to be rich in auxin (Pohl, 1935, 1936; Hemberg, 1955; Hamilton, Bandurski and Grigsby, 1961). The acidic ether-extractable fraction of *Zea* seeds contains IAA and other zones of auxin activity on chromatograms developed with ammoniacal isopropanol which are similar to those found in aqueous diffusates of coleoptile tips by Shen-Miller and Gordon; the major one has an R_F corresponding to that of P (Hemberg, 1958). In addition to these ether-soluble auxins, the seeds contain considerable quantities of bound auxin. In *Zea* much of the bound auxin consists of IAA esters, particularly IAA-inositols, from which IAA is released by mild alkaline hydrolysis (Lambarca, Nicholls and Bandurski, 1965; Ueda and Bandurski, 1969). The possibility that guttation fluid might also contain alkali-labile IAA complexes was investigated by subjecting guttation fluid, which had previously been exhaustively extracted with ether, to alkali (1 N NaOH for 15 minutes at 20° C). After adjustment of the pH the fluid was partitioned in the usual way to give a neutral + basic and an acidic fraction. Chromatography and bioassay of these extracts showed that little or no auxin was present in the neutral + basic fraction, but that in the acidic fraction auxin activity was present at R_F 0.7–1.0 and also in the case of *Zea* at the R_F of IAA (Fig. 4). These results indicate that guttation fluid contains alkali-labile complexes of auxin which are probably esters.

Table 1. *Auxin in guttation fluid from coleoptiles (the results, estimated by bioassay, are expressed in terms of IAA equivalents, as* $\mu g\ l^{-1}$ *guttation fluid)*

	Avena	*Zea*
Acidic fraction		
IAA	0.52	0.35
R_F 0.7–1.0	0.34	0.10
Acidic fraction of hydrolysed guttate		
IAA	0.08	0.28
R_F 0.7–1.0	0.12	0.11
Total	1.06	0.84

A quantitative comparison in terms of IAA equivalents of the different forms of auxin in guttation fluid is shown in Table 1. This could be misleading since the non-IAA auxins may not have activity-concentration curves identical to that of IAA; for example Shen-Miller and Gordon (1966) found that P had a much shallower activity-concentration curve: this is likely to lead to a serious underestimate of the potential auxin activity of P if it is converted to IAA. The data in Table 1 are not corrected for losses during extraction and chromatography. The average recovery of [1-^{14}C]IAA added to samples of guttation fluid was 26%. Therefore the total amounts of auxin in guttation fluid are likely to be in the order of 4 $\mu g\ l^{-1}$ for *Avena* and 3 $\mu g\ l^{-1}$ for *Zea*.

The coleoptile tip as a site of auxin accumulation

If *Avena* seedlings whose roots have been removed are placed with their bases in a solution of dye, e.g. acid fuchsin, and left to transpire, the dye moves upwards through

the vascular bundles of the coleoptile and accumulates at the coleoptile tip (Plate 1, No. 1). Dyes introduced into the transpiration stream of decapitated coleoptiles accumulate at the new apical region at the tip (Plate 1, No. 2). Apical accumulation of substances introduced into the transpiration stream also occurs at the tips of leaves (Plate 1, No. 3) and at the tips of veins in the petals of flowers; it is particularly easy to observe in white flowers whose stalks are placed in a solution of dye (Plate 1, No. 4). This phenomenon presumably depends on the withdrawal of water from the xylem along the length of the vascular system, resulting in an ever-increasing concentration of substances dissolved in the xylem sap which reaches a maximum at the apex. Apical accumulation can also occur under conditions of guttation; if *Avena* seedlings whose roots have been slightly damaged to facilitate the entry of the dye are watered with a solution of acid fuchsin, the dye appears in the guttation fluid and also accumulates at the coleoptile tip.

Table 2. *Radioactivity in sections of coleoptiles of* Avena *seedlings after 2 hours' transpiration in the dark with their cut roots in a solution of* [1 − ^{14}C]*IAA* (1 × 10^{-6} *M*) (*coleoptile sections from ten seedlings were pooled in each experiment*)

	Intact coleoptiles			Decapitated coleoptiles	
	ct/minute	ct/minute/mm		ct/minute	ct/minute/mm
Apical 2 mm	78	39	Apical 3 mm	99	33
Next 3 mm	66	22	Next 3 mm	60	20
Next 3 mm	44	15	Next 3 mm	51	17
Next 5 mm	56	11	Next 3 mm	42	14
Next 5 mm	77	15			

Table 3. *Radioactivity in apical and subapical sections of* Zea *coleoptiles which were supplied at their bases with a solution of* [1 − ^{14}C]*IAA* (0.5 × 10^{-6} *M*) (*after 4 hours' transpiration ten coleoptiles were divided into sections for counting; ten others were decapitated and agar blocks were placed on their apical cut surfaces before a further* (5-*hour*) *period of transpiration*)

	Ct/minute per ten sections	
	After 4 hours	After 9 hours
Apical 3 mm	212	–
Subapical 3 mm	177	360
Agar blocks	–	4

The introduction of [1-^{14}C]IAA into the transpiration stream of coleoptiles results in an accumulation of the auxin at the coleoptile tip; in decapitated coleoptiles the auxin accumulates at the new apical region (Table 2). If blocks of agar are placed on the apical cut surface while the auxin is accumulating, very little auxin can be detected in the agar even though considerable amounts have accumulated at the new tip; results of a typical experiment are shown in Table 3. Thus, in the experiments of Skoog (1937) where agar blocks were placed on the apex of decapitated coleoptiles, the failure to detect auxin in the agar cannot be regarded as evidence against the acropetal movement of auxin in the coleoptile.

Whitehouse and Zalik (1968) showed that the labelled IAA injected into the endosperm of *Zea* seeds moved acropetally into the coleoptile. I found that [1-^{14}C]IAA injected into the endosperm of *Avena* seeds moves acropetally in the xylem. It can be recovered from the coleoptile tip by extraction and can also be detected in guttation fluid (Fig. 5a).

When coleoptile tips in which the accumulation of acid fuchsin has taken place are

placed in water the dye readily diffuses out of the tips. This process was followed by measuring the optical density of the diffusate at the absorption maximum of acid fuchsin, 542 mμ. Over half the total amount of dye in the coleoptile tips appeared in the aqueous diffusate within 3 hours. Similarly [1-^{14}C]IAA appears in aqueous or agar diffusates from coleoptile tips in which this auxin has accumulated (Fig. 5b).

The evidence presented so far suggests the simple hypothesis that in the intact seedling, IAA and IAA precursors move acropetally in the xylem from the seed and accumulate at the coleoptile tip, or if this is removed at the 'physiological' tip. The influence of

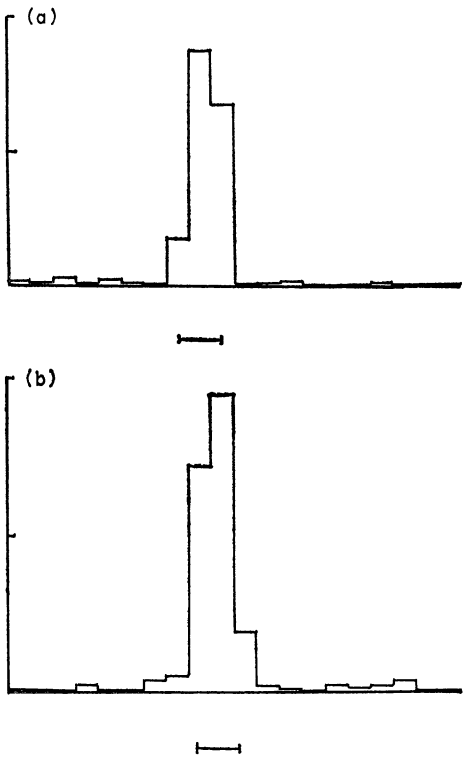

Fig. 5. (a) Radioactivity on a chromatogram of the acidic fraction of guttation fluid collected from coleoptiles of *Avena* seedlings 4 hours after injection of 2 μl of [1-^{14}C]IAA (5×10^{-4} M) into the endosperm of the seeds. (b) Radioactivity on a chromatogram of the acidic fraction of 3.5 hour aqueous diffusate of tips of *Avena* coleoptiles which had been supplied at their bases with [1-^{14}C]IAA (0.5×10^{-6} M) and left to transpire for 3 hours.

root pressure on this process may well explain the role of the roots in the auxin economy of the coleoptile tip, although they may have an additional role as a source of cytokinins (Jordan and Skoog, 1971). The only apparent difficulty in this hypothesis is the relatively low amount of auxin found in guttation fluid: about 4 μg/l as estimated by the straight growth bioassay after corrections for losses in extraction and chromatography. The coleoptile of an *Avena* seedling growing under the conditions used for the collection of guttation fluid produces about 1 μl guttation fluid per hour. Thus about 4×10^{-12} g of auxin (as IAA equivalents) per coleoptile tip per hour appear in guttation fluid. Went and Thimann (1937; their fig. 27) produce data without experimental details indicating that

Avena coleoptile tips can diffuse about 40×10^{-12} g auxin per coleoptile tip per hour. It is impossible to compare these figures directly for several reasons.

(i) Different varieties of *Avena* were used and considerable varietal differences exist. For example, the variety used by Sheldrake and Northcote (1968a) contains about five times as much auxin in its guttation fluid as the variety used in these experiments.

(ii) The auxins other than IAA in guttation fluid may have been under-estimated in their potential auxin activity for the reasons given on p. 439.

(iii) Most important, by no means all the auxin ascending in the xylem sap escapes from the plant in guttation fluid for the majority is retained in the coleoptile. In experiments in which labelled IAA was supplied to the roots of *Avena* seedlings, the apical region of the coleoptile was found to contain between five and twenty times more radioactivity than the guttation fluid. The proportions of non-IAA auxins and IAA esters retained in the coleoptile need not necessarily be the same.

In the absence of further quantitative data it is not possible to decide whether acropetal movement of auxin and auxin precursors in the xylem can account for all or only for part of the accumulation of these substances at coleoptile tips. The possibility that some acropetal movement of auxin and/or auxin precursors also takes place in the phloem cannot be ruled out; but at present there seems no reason to adopt this more complicated explanation.

Auxin in the tips of primary leaves

In the primary leaves of *Avena* the greatest amounts of auxin are obtained by extraction of the basal, meristematic region (van Overbeek, 1938). The leaf tips are non-meristematic and are unlikely to be sites of auxin synthesis.

Substances moving in the xylem accumulate at leaf tips in the same way as they accumulate at the apical limit of the vascular system in other organs (Plate 1, No. 3). Auxin is found in the guttation fluid from primary leaves (Fig. 2d). Therefore the leaf tip might be expected to act as a site of auxin accumulation in the same way as the coleoptile tip. The results in Table 4 indicate that this is so. More extractable auxin was found in the apical sections of young primary leaves than in the subapical sections. The accumulation of auxin at the leaf tip may be of little physiological significance but it is an almost unavoidable consequence of the acropetal movement of auxin in the xylem.

Table 4. *Auxin in the tips of primary leaves of young* Avena *plants grown at 22° C (zones corresponding to the* R_F *of IAA on chromatograms of ether extracts were estimated by the* Avena *mesocotyl bioassay, results are expressed in terms of IAA equivalents)*

Age of plants (days)	Growth conditions	Auxin (ng g^{-1} fresh weight)		
		Terminal 3 mm	Second 3 mm	Third 3 mm
6	Sand, dim daylight	7.8	4.2	2 3
7	Sand, dim daylight	2.1	1.4	1.2
7	Sand, full daylight	3.2	1.7	1.6
9	Sand, full daylight	5.6	4.9	2.9
9	Vermiculite, dark	13.8	6.2	9.4

The evidence for auxin production by isolated coleoptile tips

It has already been pointed out that the comparisons made by van Overbeek (1941) and Wildman and Bonner (1948) of the amounts of auxin which could be obtained by extrac-

tion and by exhaustive diffusion of coleoptile tips depend on the assumptions that no losses of auxin occur during extraction and that bacterial auxin production during the period of diffusion is negligible. These possible errors, if large enough, could mean that the results no longer support the conclusion that auxin production occurs in isolated coleoptile tips.

I have estimated the recoveries of [1-^{14}C]IAA added to coleoptile tips extracted by the procedures used by these workers. By the 12-hour micro-Soxhlet method of van Overbeek an average recovery of 58% was obtained; by the procedure of Wildman and Bonner (lyophilization of coleoptile tips, storage in a desiccator over P_2O_5 and then ether extraction) 65% was recovered.

An examination of the results of van Overbeek strongly suggests that bacterial auxin production was involved. The amount of diffusible auxin from *Zea* coleoptile tips declined over the first 4 hours and then rose again; and in one experiment a further rise occurred when a new cut was made in the tissue exposing more damaged cells (his Fig. 1). In coleoptile sections taken from the apex of decapitated seedlings little auxin was obtained in the first 4 hours of diffusion but after this period auxin production began (his Table 4). If the apical cut surface of sections prepared in this way was burnt or treated with silver nitrate the production of auxin was delayed for several hours (his Fig. 5). The most probable interpretation of these results seems to be that the auxin obtained after the first 4 hours of diffusion was produced by bacteria growing mostly on the cut surfaces. On the other hand the auxin obtained within the first 4 hours is likely to have originated from the coleoptile tip; this represents less than half the total auxin obtained from 1-mm tips.

Table 5. *Extractable auxin as a percentage of auxin obtained by exhaustive diffusion of coleoptile tips (results were corrected for probable losses in extraction and for bacterial auxin production)*

Author	Species	Uncorrected results	Corrected results
van Overbeek (1941)	*Zea* (sand grown)	8	25
	Avena (sand grown)	42	72
	Avena (water grown)	14	23
Wildman and Bonner (1948)	*Avena* (water grown)	20	27

The growth of bacteria on cut surfaces is also likely to have occurred in the experiments of Wildman and Bonner. They measured the ability of coleoptile tissues to convert tryptophan to auxin and found a significant increase in the tips of decapitated coleoptiles 3 hours after the cuts were made. Winter (1966) has shown that sterile coleoptile tissues are incapable of bringing about this conversion, indicating that the enzymic activity detected by Wildman and Bonner was due to bacteria.

The results of van Overbeek, and Wildman and Bonner are shown in Table 5 together with corrected results calculated on the basis of the probable recoveries of auxin by extraction and on the assumption that 50% of the auxin obtained by diffusion from *Zea* coleoptile tips and by extraction of the tips at the end of the diffusion period was of bacterial origin. Even after these corrections have been made the results still indicate that auxin is produced during the diffusion period.

Of the compounds with auxin activity detectable in coleoptile tips by the straight-growth bioassay only IAA is active in the *Avena* curvature bioassay (Shen-Miller and

Gordon, 1966). Both van Overbeek, and Wildman and Bonner used the curvature bioassay; the auxin production they observed can therefore be equated with the production of IAA. Evidence that the production of IAA occurs in isolated coleoptile tips is also provided by the data of Shen-Miller and Gordon (1966), who found that increases in IAA were associated with declines in the amounts of compounds P and F. In some experiments increases in the amount of IAA took place while the total auxin activity detectable by the straight growth bioassay remained more or less constant or even declined. Whether a situation such as this can be described as auxin production depends on the criteria used; the straight growth bioassay would indicate that there had been no net auxin production while the curvature bioassay would indicate that there had.

These results are consistent with the accumulation at coleoptile tips of IAA and of the other compounds with auxin activity in the straight growth bioassay detected in guttation fluid, and the subsequent conversion of these compounds and also of IAA esters to IAA.

The autonomous curvature of coleoptiles

Tetley and Priestley (1927) drew attention to the fact that the anatomy of the coleoptile tip is asymmetrical in the dorsi-ventral plane. Just before they terminate the vascular bundles which run up either side of the coleoptile arch over towards each other on the side of the coleoptile facing the seed and away from the side on which the terminal pore is located. The more detailed investigations of the anatomy of this region by O'Brien and Thimann (1965) and Thimann and O'Brien (1965) showed that the xylem terminates about 0.4 mm and the phloem about 0.65 mm below the extreme tip. The distribution of the xylem is consequently more asymmetrical than that of the phloem.

Auxin which moves acropetally in the xylem and accumulates at the apical limit of the vascular system might therefore be expected to be distributed asymmetrically in the coleoptile tip, with more auxin on the side facing the scutellum. If this reasoning is correct the side of the coleoptile facing the scutellum might be expected to grow more than the other side of the coleoptile, resulting in an autonomous curvature. This curvature might not be detected under normal conditions of growth because the geotropic response would tend to correct it; but in the absence of the geotropic response the curvature should develop.

Avena seedlings grown in darkness on a clinostat rotated around the horizontal axis do indeed develop an autonomous curvature of this type (Bremekamp, 1925; Lange, 1925; Pisek, 1926; Dolk, 1936). A similar autonomous curvature has also been observed in *Triticum* seedlings grown on a clinostat and also in gravity-free conditions in a satellite orbiting the earth (Lyon, 1968). These curvatures have so far been unexplained.

In seedlings growing under normal gravitational conditions the interaction of the autonomous curvature with the geotropic response could account for the nutational movements of coleoptiles in the dorsi-ventral plane, although the more frequent and rapid nutational movements in the lateral plane require a different type of explanation (Anker, 1972).

The autonomous curvature of coleoptiles thus provides circumstantial evidence for the acropetal movement of auxin in the xylem. The accumulation of auxin in the most apical part of the xylem can also explain Went's (1928) finding that the terminal 0.7 mm of the *Avena* coleoptile is particularly rich in auxin; this would not be expected if the acropetal movement of auxin or auxin precursors in the phloem was of major importance since the phloem terminates about 0.65 mm from the apex.

Conclusions

The acropetal movement of auxin and potential precursors of auxin in the xylem from the seed to the coleoptile tip where they accumulate and where IAA esters and other compounds related to IAA may be activated is a mechanism which was proposed in various forms by Pohl (1935, 1936), Avery and Burkholder (1936) and van Overbeek (1937). It is consistent with the classical work on coleoptiles and provides an explanation for the role of the roots, for the regeneration of the physiological tip and for the autonomous curvature of coleoptiles. The views of Pohl (1935, 1936) that auxin itself moves acropetally and of Skoog (1937) and Voss (1939) that 'inactive' forms of auxin move in this way both appear to be correct. The balance between the amounts of auxin and different precursors of auxin seem to be different in different species; for example, IAA esters are apparently of greater importance in the coleoptiles of *Zea* than *Avena*. An unresolved problem is the identity of the labile compounds P and F found by Shen-Miller and Gordon (1966), one of which may be identical with the second auxin of Raadts and Söding (1955) and Ramshorn (1955) and with the substance detected in guttation fluid. However, it seems clear that they should be regarded as closely related to IAA; more closely than its biosynthetic precursor, tryptophan, which is not detectable in guttation fluid (Sheldrake and Northcote, 1968a). The conversion of these 'inactive' auxins to IAA in the coleoptile tip and the hydrolysis of IAA esters is not comparable to the biosynthesis of IAA which takes place in other parts of plants. Considerable confusion has resulted from ignoring this distinction; for example, an elaborate hypothetical pathway of IAA biosynthesis from indole was proposed by Winter (1966) to account for his finding that sterile coleoptile tissue was incapable of converting tryptophan to IAA.

Mer (1969) has drawn attention to a number of inconsistencies in the traditional views of the role of auxin in the control of growth. In particular Dattaray and Mer (1964) have shown that in etiolated *Avena* seedlings grown under different conditions no correlation exists between the auxin content and the growth rate. These results are not so surprising in the light of the mechanism of auxin accumulation at the coleoptile tip put forward in this paper; the amount of auxin moving from the seed and accumulating in the coleoptile will presumably be affected by transpiration, guttation and root pressure and thus be determined by different variables from some of those which influence coleoptile growth.

Dormant seeds of *Zea* contain large quantities of IAA esters from which IAA can be released by alkaline hydrolysis (Ueda and Bandurski, 1969). As the seeds germinate a decline in the amount of bound auxin is associated with an increase in the amount of free IAA (Hemberg, 1955; Ueda and Bandurski, 1969). By contrast, as *Zea* seeds develop there is a decline in the level of free auxin which may be associated with its conversion to auxin derivatives that serve as a source of auxin in the germinating seed (Hemberg, 1958). In developing rye seeds the appearance of alkali-labile auxin complexes is preceded by a large increase in the level of free auxin which declines as the bound auxin is formed (Hatcher and Gregory, 1941; Hatcher, 1943).

Auxin biosynthesis is widely considered to take place in meristematic cells. This view is based not on direct experimental evidence but on the correlation between areas of meristematic activity and auxin production. If the non-meristematic coleoptile tip were a site of auxin biosynthesis it would present a serious difficulty for this hypothesis. But since the auxin economy of coleoptile tips is vicarious and depends on auxin which originates from the seed, the actual biosynthesis of auxin can be traced back to the process

of seed development where it could perhaps be attributed to meristematic cells. An alternative explanation of auxin production is provided by the dying cell hypothesis (Sheldrake and Northcote, 1968b, c; Sheldrake, 1971b), according to which auxin is produced in regions of meristematic activity as a result of the autolysis of differentiating cells (e.g. xylem) or the regression of nutritive tissues. Hatcher (1943) found that in the developing seed most of the auxin was present in the aleurone layer and that none was detectable in the embryo. By a correlation of the appearance of auxin with the anatomical changes which occur during seed development, in particular the regression of nutritive tissues, he concluded: 'As to the immediate source of the auxin, I am inclined to the view that it is derived from the cytoplasm of the disintegrating cells.' This interpretation implies that the auxin of coleoptile tips is ultimately derived from dying cells in the developing seed.

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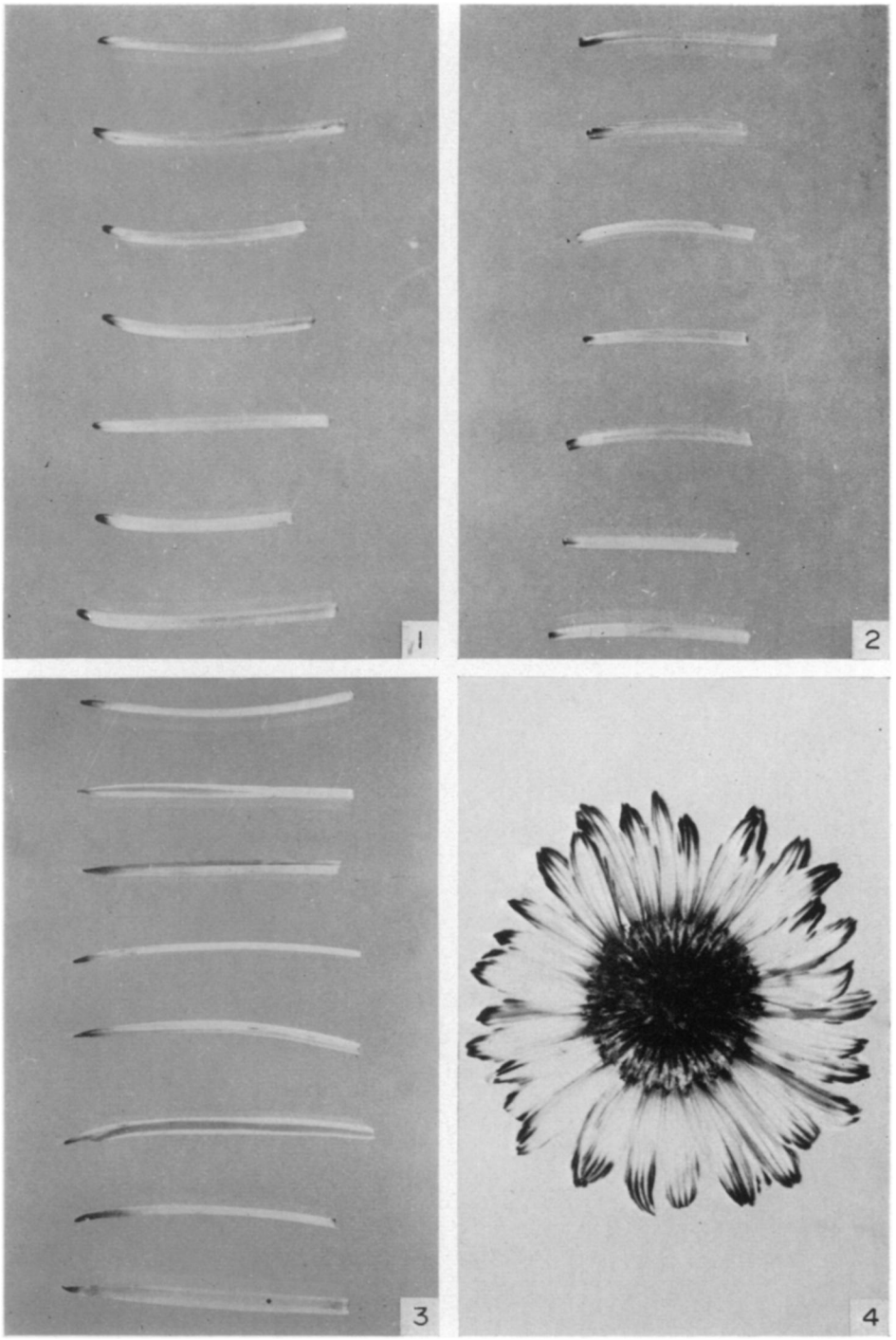

A. R. SHELDRAKE—*COLEOPTILE TIPS AND AUXIN* (*facing page* 446)

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EXPLANATION OF PLATE

Nos. 1–4. The accumulation of acid fuchsin at the apical limit of the vascular system. Dilute (0.05%, w/v) solutions of the dye were introduced into the transpiration stream either through cut roots or the base of the stem; the specimens were left to transpire for 2–4 hours before being photographed. All × 1½. In Nos. 1–3 the apices are shown on the left.

No. 1. Coleoptiles of 5-day-old *Avena* seedlings.

No. 2. Coleoptiles of 5-day-old *Avena* seedlings which were decapitated before dye was introduced into the transpiration stream.

No. 3. Primary leaves of 10-day-old etiolated *Avena* seedlings.

No. 4. A white flower of *Chrysanthemum leucanthemum* L. Note the accumulation of dye at the tips of veins in the petals.

age at different rates but, according to this hypothesis, all cells would be ageing to a greater or lesser extent all the time; all cells would be heading towards senescence and death.

The elimination of membranous material from cells might enable the ageing process to be retarded and there are a few examples of the shedding of membranes by cells which I will discuss further. But, in general, the only way in which cells could avoid their otherwise inevitable mortality would be by growing and dividing, thus diluting the accumulated breakdown products. Although lipid peroxidation may be the most important cause of the formation of such substances, the following general considerations could apply to any deleterious substances which accumulate with age.

Growth and division of cells

An artificially simple case is provided by cells dividing symmetrically with a fixed generation time if these accumulate deleterious breakdown products linearly with time, an amount, x, being formed per cell generation time (Fig. 1). Successive generations contain more of the accumulated breakdown products but the increments become smaller and smaller. If the rate of accumulation is not linear, but proportional to the amount already accumulated, the content per cell will increase exponentially; and if there is a progressive lengthening of the cell generation time, there will be a greater accumulation within individual cells in succeeding generations. With either or both of these assumptions, it can be seen that the whole population will undergo senescence and sooner or later die out.

But another type of cell division is possible, an asymmetrical division in which one of the daughter cells receives all or most of the accumulated breakdown products (becoming more 'mortal') while the other is rejuvenated, receiving little or none. The more 'mortal' of the daughter cells might die or differentiate directly, or it might divide again unequally, producing a rejuvenated cell and a cell even more 'mortal' than itself, or it might undergo one or more sequential symmetrical divisions (as discussed above) to produce a population of cells which sooner or later die (unless they can undergo further asymmetrical divisions to produce rejuvenated cells).

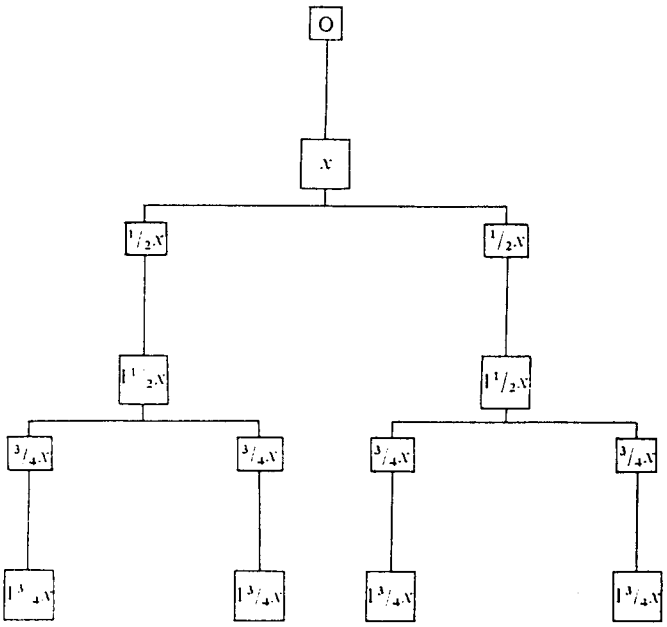

Fig. 1 Cells dividing symmetrically with a fixed generation time showing the accumulation of deleterious breakdown products linearly with time, an amount, x, being formed per cell generation time.

I shall now consider a few aspects of the growth and development of higher plants and higher animals in the light of these ideas. Dicotyledonous trees illustrate the pattern of indefinite growth that is characteristic of plants. (There are of course plants, such as herbaceous annuals, which die after they have flowered. But annuals are capable of growing for much longer than their normal life-span if they are prevented from flowering, indicating that they die because they flower and not because of an innate inability to go on growing[15].) The life span of trees is limited by a variety of mechanical factors, but cuttings taken from old trees can give rise to healthy young trees, and this process can be repeated indefinitely. The growing points of the tree, the apical meristems, remain perpetually young.

Cell divisions within the apical meristems of the shoots give rise to daughter cells with different fates: some remain meristematic, others give rise to the differentiated structures of the stems and the leaves. Some of these cells die as they differentiate into vascular tissues and fibres, others, for example the leaf mesophyll and pith parenchyma, remain alive for some time, but, unless they are stimulated to divide again in a regenerative response to wounding or damage, they eventually die. The leaves senesce and fall from the tree; the pith breaks down. The root meristems give rise to the primary tissues of the root which, apart from those which divide to produce further root meristems, sooner or later die. In secondarily thickening stems the divisions of the cambial cells give rise to cells which die as they differentiate into xylem or undergo further asymmetrical divisions to produce phloem companion cells and sieve tubes. These cells eventually die and are sloughed off in the bark. Cell divisions in the cork cambium give rise to cork cells which die as they differentiate; divisions of the root cap initials give rise to root cap cells which die and are sloughed off. Thus, in the various meristems of the plant the continued growth and continued rejuvenation of the meristems is associated with the production of cells which die during or after differentiation.

Vertebrates

Vertebrates, unlike trees, do not go on growing indefinitely, nor can they be propagated vegetatively. At first, fertilised eggs undergo cleavages which rapidly increase the number of cells, but this rate of increase of cell number declines progressively as the animal develops, and as cells and tissues differentiate[16]. Throughout the development of the embryo many tissues and groups of cells regress and die[17,18]. Some of these cell deaths are associated with tissue differentiation[19], some occur during morphogenetic processes[20], and others may represent the regression of phylogenetically vestigial structures[17], but the significance of other cell deaths is obscure[17]. As the animal develops, the cells of some tissues, such as nerve and muscle, differentiate and to a large extent lose the ability to undergo further division. Some of these cells die as the animal grows older and are not replaced[21,22] but in the adult animal a number of other tissues continue to grow, for example the epidermis, the intestinal lining, the liver and blood cells continue to be formed. In all these examples the production of new cells is offset by cell death. Cell divisions in the basal layers of the mammalian epidermis give rise to daughter cells which remain in the basal layers and divide again, and other daughter cells which differentiate and keratinise, dying as they do so. Cell divisions in the crypts of the intestinal villi replenish the population of crypt cells capable of further division and produce other daughter cells which move up the villi where they die and are sloughed off[23]. Asymmetrical divisions of the early precursors of all cells of the blood occur throughout life and give rise to further precursor cells as well as to the maturing and mature cells of the blood, all of which have a limited life span. During

the formation of red blood cells[24] and granulocytes[25] in the bone marrow, and lymphocytes in the thymus[26], considerable numbers of cells die *in situ* soon after they are formed. The reasons for this 'ineffective' erythropoiesis, granulopoiesis and lymphopoiesis are unknown.

The mortality of at least some of the cells which die in developing animal embryos and in mature animals may represent the price that is paid for the rejuvenation of other cells which continue to grow and divide. But unfortunately too little is known about cell lineages in animals, especially in embryos, for it to be possible to decide how general is the phenomenon of asymmetrical cell divisions giving rise to daughter cells of unequal mortality. The recognition of this pattern is complicated by the fact that by no means all cell death takes place as a result of cellular senescence. Some cells die as they differentiate and others may die because they find themselves in the wrong places at the wrong times[19]. Cell deaths may be controlled chemically, for example by steroid hormones: the injection of glucocorticoids can cause large numbers of lymphocytes to die[27], the regression of Mullerian and Wolffian ducts is controlled by androgens and oestrogens[19,28] and the regression of the lining of the female genital tract is under the control of oestrogens[28]. But, under the hypothesis that asymmetrical cell divisions lead to a rejuvenation of 'meristematic' daughter cells at the price of the increased mortality of their sister cells, it does not matter whether the latter die as a result of senescence, or whether they die as they differentiate or for any other reason.

Sexual reproduction

In the sexual reproduction of both higher plants and higher animals almost all the cytoplasm from which the embryo and the new organism develops is provided by the egg. In both cases, the egg cells are formed as a result of asymmetrical divisions of the egg mother cell. In the great majority of higher plants, the meiotic divisions of the egg mother cell produce four cells, three of which die. The fourth undergoes further divisions to produce the cells of the embryo sac, most of which die before or shortly after fertilisation. In some species, one or more of the three sister cells of the cell which gives rise to the egg may undergo further division to produce short-lived embryo sac cells[29]. In animals the first and second meiotic divisions of the egg mother cell give rise to the first and second polar bodies, which regress and die[30,31].

It is particularly striking that in both plants and animals, only one of the progeny of the egg mother cell gives rise to an egg while the sister cells die (or if they divide give rise to short-lived progeny). By contrast, there is no comparable cell loss in male gametogenesis associated with the meiotic divisions of the pollen mother cells and spermatogonia.

The many examples in both higher plants and higher animals (and many more can be found in the lower plants and lower animals) of the production of rejuvenated meristematic, stem or egg cells by asymmetrical divisions do not of course prove that these divisions involve an asymmetrical distribution of deleterious breakdown products; but the available facts appear to be consistent with this hypothesis.

Loss of membranous material by animal cells

If the accumulation of deleterious breakdown products of membrane lipids is one of the causes of cellular senescence, the loss of membranous material might be of considerable importance in enabling cells to rid themselves of such substances. The shedding of membranous material by living cells does not seem to be of common occurrence but can take place in mammalian cells as follows.

First, in apocrine secretions part of the cell membrane is lost. The best example, and the only one for which conclusive ultrastructural evidence exists, is in the secretion of lipid droplets by the cells of lactating mammary glands. The secreted lipid droplets are surrounded by a unit membrane derived in part from the surface membrane and in part from Golgi vesicle membranes[32].

Second, membrane-bounded vesicles of cytoplasm can break away from mammalian macrophages both *in vitro* and *in vivo*[33]. This process, known as clasmotosis, is of unknown significance. Lymphocytes which are activated in immunological reactions or as a result of phytohaemagglutinin stimulation form 'tails' (uropods) which can bleb off vesiculated buds *in vivo* and *in vitro*[34]. Again, the significance of this process is unknown. Clasmotosis is also frequently observed in cultures of fibroblasts.

Third, many types of animal viruses are budded off from host cells in membrane-bounded vesicles. The protein in the membrane of the vesicles is largely viral, at least in the case of RNA tumour viruses, but the lipids are derived from the host cell membrane[35]. Viral particles bounded by membrane are also budded off from the cells of a number of spontaneously cancerous tissues[36] and from many of the cell strains and permanent cell lines which are commonly cultured in laboratories[37].

Tissue cultures

Many plant callus cultures can be grown indefinitely *in vitro*. During the early stages of the growth of some calluses, an exponential increase in cell number takes place at a rate which suggests that many of the cells may undergo a limited number of sequential symmetrical divisions before the growth rate declines[38,39] but in most plant tissue cultures the rate of increase of cell number is more or less linear for most of the growth period[39,40]. Linear growth characteristics would be compatible with a meristematic pattern of cell division such that some daughter cells continue to grow and divide while their sister cells age and sooner or later die. Unfortunately nothing is known in detail about cell lineages within these cultures, nor are there any quantitative data on cell death. Nevertheless, dead and dying cells are by no means uncommon.

'Permanent' mammalian cell lines capable of indefinite propagation *in vitro* can be derived from cancerous tissues and also from cells which have undergone a spontaneous 'transformation' during culture. Diploid fibroblast cultures can be propagated, however, only for a finite number of subculturings, more (up to about 60) if the cells are derived from embryonic tissues, fewer if they are derived from mature organisms[41]. The number of generations through which the cells can be passed before the population senesces and dies out is reduced if the period of time between the subculturings is increased[42]. Fibroblasts of the mouse L strain have been observed to divide symmetrically over six to seven cell generations with a more or less constant generation time[43]; if the cells in the diploid fibroblast cultures also divide symmetrically, deleterious breakdown products might accumulate in the cells of succeeding generations, as discussed above, and account for the senescence of these cultures. It is impossible, however, to make any detailed interpretation of the senescence of these cultures in the absence of quantitative information about the proportions of dividing and nondividing cells, the incidence of cell death, and the extent and significance of clasmotosis within these cultures—or indeed with cultures of 'transformed' and 'permanent' cell lines.

Cancer

Malignancy must not only involve the freeing of cells from the normal controls on their proliferation, but also the avoidance of senescence by at least a part of the cell population. Many animal tumours contain a stem cell or

'meristematic' population which gives rise to daughter cells which may or may not differentiate, but which sooner or later die[44]. There are numerous examples of cell death within cancerous tissues[45–48]. Some of the cell deaths can be explained in terms of an inadequate vascularisation of the tumour tissue, but in most tumours this is by no means the only cause and does not apply at all to leukaemias; many of the cells may die as a result of ageing[44].

Little attention has been paid to the incidence of cell death within cultures of cancerous cells and it is therefore at present impossible to know to what extent the patterns of cell division, ageing and death within these cultures resemble those within *in vivo* cancers. It is sometimes assumed, if only implicitly, that overall exponential growth characteristics of cell cultures mean that there is a homogeneous population of symmetrically dividing cells. This assumption is not justified: a heterogeneous population containing proliferating, nonproliferating and dying cells can also grow exponentially if the proportion of cells that die is constant with time[49,50].

It is conceivable that the loss of membranous material either spontaneously, as in certain types of mammary gland tumours[51], or as a result of the budding off of viruses (such as RNA tumour viruses) could play a significant role in the retardation of cellular senescence in certain types of cancer.

Effects of cell death

Very little is known about the biochemistry of dying cells. Such cells probably release all sorts of proteins, glycoproteins, peptides, amino acids, amino acid breakdown products, nucleic acids and nucleic acid breakdown products, lipids and lipid breakdown products as well as salts and other substances which were sequestered inside the cells.

It has recently been found that in higher plants the hormone auxin (indole-3-acetic acid) is formed as a consequence of cell death as tryptophan, released by proteolysis, is broken down. Dying cells in differentiating vascular tissue, regressing nutritive tissues and so on, are probably the major source of this hormone within the plant[52]. Other plant hormones may also be produced by damaged and dying cells: ethylene from the breakdown of methionine and cytokinins by the hydrolysis of transfer RNA[52]. In higher plants the normal production of hormones as a consequence of cell death and the production of 'wound hormones' by damaged cells can be seen as two aspects of the same phenomenom[52].

Wound and regenerative responses in vertebrates cannot be explained simply in terms of wound hormones, but there is evidence that dying cells release substances that stimulate phagocytosis[53], and affect growth and development in both normal[54,55] and cancerous tissues[56]. And at least some of the cell deaths which occur during normal embryonic development may well result in the production or release of substances involved in the control of differentiation and development.

Dying cells may not only have a chemical effect on neighbouring cells but also a physical effect as cell to cell contacts are broken. Cell deaths within a tissue may also affect the functioning of the tissue as a whole: for example, the death of nerve cells within the brain[22] seems likely to affect pathways or patterns of nervous conduction, perhaps leading to the formation of new pathways or patterns. Such cell deaths could act as a source of random change within the nervous system that might not always be deleterious[57].

So little attention has been paid to the ageing and death of cells during growth and development, both normal and abnormal, that detailed information about these processes is scarce. Where facts are few, speculation can flourish. Most of the speculations advanced in this article could be opposed by alternative speculations, but they illustrate the view that growth and development cannot be understood in isolation from ageing and death. This is by no means an original concept, but at the cellular level it provides a perspective in which many familiar facts take on a new significance and suggests a new approach to familiar problems.

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