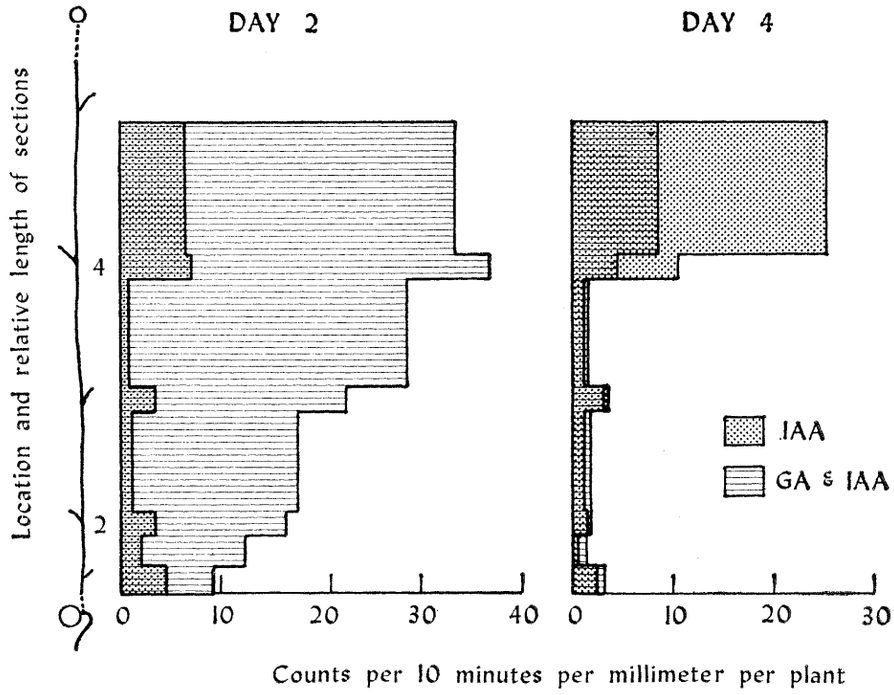

Fig. 1. Average counts obtained from ether extracts of successive nodes and internodes of pea stems, as shown on the left. Five plants were used per treatment. The stem had been decapitated about midway up the 6th internode. For isotope counting the stem just below the paste was discarded (dashed line). The remainder of internode 6 plus node 5 (not graphed) gave counts of 399 and 505 after treatments with IAA-C^{14} for 2 and 4 days, respectively. Corresponding portions from plants treated with gibberellic acid plus IAA-C^{14} gave 322 and 415 counts, respectively.

regions of the stem remote from the site of application (Fig. 2). In the R_F zone typical of IAA was found 33 percent of the radioactivity from chromatographed extracts of the bottom half of pea stems which had IAA-C^{14} plus gibberellic acid substituted for their apical shoot tip. Only 10 percent of the smaller amount of total radioactivity was in the "IAA" zone when IAA-C^{14} alone was substituted for the apical shoot tip.

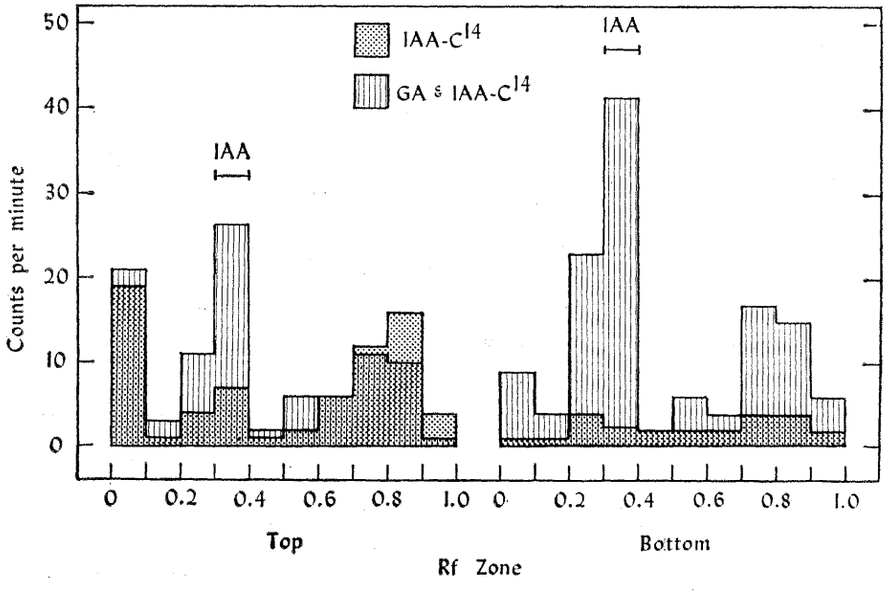

Fig. 2. Average counts from chromatograms of ether extracts of plants treated for 2 days. The solvent was isopropanol-ammonia-water (8 : 1 : 1). The dried zones were counted directly in scintillation fluid (*8*). The calibration IAA-C^{14} was run at the same time in a separate chromatogram tube. "Bottom" designates extracts from the bottom portion of the stem (nodes 3 and below). "Top" designates extracts of node 4 plus internodes 4 and 5 ($N = 15$).

The time-course of this gibberellic acid effect is closely correlated with the time-course of side-shoot inhibition (*5*).

There is no reason to think that gibberellic acid is inhibiting side shoots indirectly by stimulating elongation of the main shoot. There were no significant differences in total lengths of the treated main shoots on either day 2 or day 4 (Table 1). Nor was there any significant difference in the average stump length of internode 6 at the start of the experiment. [The plants were decapitated about half-way up the 6th internode. Stumps used for IAA treatment averaged 14.4 mm ± 0.6 ($N = 15$); those treated with gibberellic acid plus IAA treatment averaged 15.3 ± 0.6 ($N = 14$).]

This work provides evidence that both gibberellins and IAA are normally involved in maintaining apical dominance in pea plants, and that gibberellic acid acts by increasing the concentrations of IAA functional at a distance from the site of production.

The physiological-biochemical mechanism by which gibberellic acid exerts this effect remains to be investigated. One of the most attractive possibilities is that gibberellic acid increases the transport of IAA. In addition to the earlier reports of gibberellic acid decreasing the growth of side shoots (*7*), a number of otherwise puzzling observations can be explained if gibberellic acid increases auxin transport in other situations. Among these are: (i) the frequent need for adding auxin before the growth effects of gibberellic acid become apparent (*7*); (ii) the stimulation by gibberellic acid of xylem differentiation in the roots of intact bean seedlings (*9*), whereas auxin is the normal limiting factor for the differentiation of xylem cells in *Coleus* stems and stimulates the process in pea roots, fern leaves, and lilac callus (*3, 10*); (iii) the increase in the amount of endogenous "diffusible" auxin collected after gibberellic acid application (*11*). Although Kuraishi and Muir (*11*) suggested that gibberellic acid may directly increase the *formation* of auxin, it is well to remember that the collection of diffusible auxin requires transport. Even the mysterious fact that gibberellic acid added to an intact plant causes growth, whereas IAA does not (*7*), seems explicable as an effect of gibberellic acid on auxin transport, since quantitative evidence exists that

C'2 activity suggested at first that different groups in the C'2 molecule might be affected. This hypothesis became less probable when it was found that treatment with iodoacetamide prevented subsequent inactivation by *p*-CMB and failed to enhance the activity of *p*-CMB-treated C'2. The most probable explanation is that both compounds react with the same group in the C'2 molecule, the chemical nature of which is as yet undetermined. Whereas this group does not seem to be essential for C'2 activity, it appears to be located in a critical region of the molecule which governs part of the C'2 function.

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References and Notes

1. Terminology conforms to that recommended at the Complement Workshop, Bethesda, Maryland, 1963, as summarized by H. J. Rapp and T. Borsos, *Science* **141**, 738 (1963).
2. M. M. Mayer, L. Levine, H. J. Rapp, A. A. Marucci, *J. Immunol.* **73**, 443 (1954); T. Borsos, H. J. Rapp, M. M. Mayer, *ibid.* **87**, 326 (1961).
3. M. M. Mayer, Ciba Symposium on Complement, London (1964), Proceedings in preparation.
4. M. A. Leon, *Science* **147**, 1034 (1965); personal communication.
5. M. J. Polley and H. J. Müller-Eberhard, in preparation.
6. M. M. Mayer, in E. A. Kabat and M. M. Mayer, *Experimental Immunochemistry* (Thomas, Springfield, Ill., ed. 2, 1961).
7. Kindly supplied by Dr. I. H. Lepow, Cleveland, Ohio. I. H. Lepow, G. B. Naff, E. W. Todd, J. Pensky, C. F. Hinz, Jr., *J. Exp. Med.* **117**, 983 (1963).
8. H. J. Müller-Eberhard and C. E. Biro, *J. Exp. Med.* **118**, 447 (1963).
9. Publication No. 113 from the Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California. M. J. P. is supported by a grant from the National Foundation. This work was supported in part by PHS grant No. Al-05671.

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Auxin Transport, Gibberellin, and Apical Dominance

Abstract. *Substitution of indoleacetic acid plus gibberellic acid for* Pisum *shoot apices restored apical dominance more effectively than indoleacetic acid alone. Studies of translocation, in which carbon-14–labeled indoleacetic acid was used (as determined by paper chromatography and scintillation counting) revealed that gibberellic acid caused more indoleacetic acid to be present and effective far from the site of application.*

In many plant species the presence of the apical shoot tip prevents side shoots from elongating. This phenomenon is called apical dominance. It was reported 30 years ago that the growth hormone, auxin, which can be obtained from growing buds, could substitute for the apical shoot tip in this effect (*1*). Thimann and Skoog hypothesized that auxin from the apical shoot tip moved down the stem in sufficient quantities to inhibit the side shoots directly. A recent critical analysis of the evidence called attention to the fact that exact studies of replacement were lacking (*2*). When exact substitution of synthetic auxin for the endogenous auxin was demonstrated in *Coleus* by adding 1 percent indoleacetic acid (IAA) in lanolin, there was concomitant exact replacement of the apical shoot tip with respect to the differentiation of xylem cells but not with respect to apical dominance (*2, 3*). This suggests either that auxin is not involved directly in apical dominance or that some other substance(s) from the shoot tip is also necessary. A review of the many unresolved problems of apical dominance was written in 1961 by Champagnat (*4*).

To investigate this further, we used light-grown young legumes, both because these plants had been used by the early workers and because they show particularly strong apical dominance (that is, side shoots grow out very little in the intact plant). Twelve-day old plants of *Pisum sativum* L. (var. "Alaska") were used. Confirming earlier studies on legumes, we found that when auxin was substituted for the apical shoot tip of peas it caused a significant decrease in the growth of the side shoots; but no auxin concentration by itself would restore full dominance for more than a few days (*5*) (Table 1). If a second substance is acting in the shoot tip along with auxin, then the gibberellins are a likely choice. They have been extracted from various parts of pea plants (*6*), and, although gibberellic acid causes faster growth of side shoots when added to decapitated plants, it has also been reported to enhance apical dominance when added to various intact plants (*7*).

We found that gibberellic acid, when added with the auxin IAA as a substitute for the apical shoot of peas, significantly increased the duration of inhibition of side shoots (*5*) (Table 1). The optimum concentration in lanolin paste was 1 percent gibberellic acid and 1 percent IAA. Gibberellic acid added alone at a concentration of 1 percent stimulated growth of the side shoots rather than inhibiting them.

Indoleacetic acid-C^{14}, 1 percent in lanolin, was substituted for the apical shoot, with and without the optimal concentration of gibberellic acid. At 2-day intervals, the peas were harvested, all side shoots were measured, and various nodes and internodes were measured, diced, then extracted three times with diethyl ether. The combined extracts were evaporated to dryness, scintillation fluid containing 25 percent ethanol was added, and the samples were counted twice for at least 10 minutes each in a liquid scintillation counter (*8*). Typical results from one of the three experiments are shown in Fig. 1. The presence of gibberellic acid in the apical paste caused much more C^{14} to be extractable farther down the stem after 2 days. Only from the section nearest the site of application (node 5 plus lower portion of internode 6) was more C^{14} extractable from the plants treated with IAA-C^{14} alone. By day 4 this effect of gibberellic acid was no longer apparent: below node 4 there was no significant difference between treatments in the amount of C^{14} extractable after the treatment with IAA-C^{14}. This confirms the finding of Case (*5*).

To see how much of the extracted radioactivity was still with IAA after 2 days, paper chromatograms were run. The results confirmed the hypothesis that gibberellic acid increases the amount of IAA-C^{14} extractable from

Table 1. Average lengths of main shoots (mean ± standard error; $N=5$ in each instance) and of all side shoots in "Alaska" pea plants decapitated midway up internode 6 (counting from the base) and treated with 1 percent IAA or with 1 percent gibberellic acid (GA) plus 1 percent IAA in lanolin paste. When compared by Student's *t*-test, the values for the main shoots on day 2 yielded 1.61 ($t_{0.1}=1.86$) and on day 4, 0.235 ($t_{0.5}=0.706$).

Treatment	Length of main shoots (mm)	Mean summated length of side shoots (mm)
	Day 2	
Decapitated only		16.3
IAA	110.4 ± 2.5	1.4
GA + IAA	106.0 ± 1.0	0.3
	Day 4	
Decapitated only		45.6
IAA	116.2 ± 3.3	21.0
GA + IAA	115.2 ± 2.7	9.2

the amount of auxin which can move in the plant is strictly limited by the transport capacity of the tissues (see *3, 12*).

On the other hand, almost all these observations as well as others in the literature (*13*) can be explained as easily on the basis of an "auxin-sparing" action of gibberellic acid (*7, 14*). In fact, Scott and Briggs (*15*) have presented evidence, also based on ether extractions of light-grown "Alaska" peas, that IAA in lanolin added to the 5th internode moves basipetally at about 10 mm/hr for at least 80 mm. Hence, the IAA added in our experiments should have reached the bottoms of the 90 to 100-mm long stems by 10 hours after application. The apparent lack of IAA-C^{14} in the bottom portion of the stem 2 days after the application of IAA alone (Figs. 1 and 2) therefore supports the argument for an effect of gibberellic acid on "auxin-saving" rather than directly on auxin-transport.

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References and Notes

1. F. Laibach, *Ber. Deut. Bot. Ges.* **51**, 336 (193→ K. V. Thimann and F. Skoog, *Proc. Roy. Soc. London Ser. (B)* **114**, 317 (1934).
2. W. P. Jacobs, *Develop. Biol.* **1**, 527 (1959); W. P. Jacobs, J. Danielson, V. Hurst, P. Adams, *ibid.*, p. 534.
3. W. P. Jacobs, *Amer. Natur.* **90**, 163 (1956).
4. P. Champagnat, in *Encyclopedia of Plant Physiology*, W. Ruhland, Ed. (Springer, Berlin, 1961), vol. 14, p. 872.
5. D. B. Case, thesis, Princeton University (1964); D. B. Case, T. K. Scott, W. P. Jacobs, in preparation.
6. M. Radley, *Ann. Bot.* **22**, 297 (1958).
7. P. W. Brian, *Biol. Rev.* **34**, 37 (1959).
8. S. Naqvi, thesis, Princeton University (1963).
9. C. Odhnoff, *Physiol. Plantarum* **16**, 474 (1963).
→ W. P. Jacobs and I. B. Morrow, *Amer. J. Bot.* **44**, 823 (1957); T. A. Steeves and W. R. Briggs, *J. Exp. Bot.* **11**, 45 (196→ J. G. Torrey, *Amer. J. Bot.* **40**, 525 (1953); R. H. Wetmore and S. Sorokin, *J. Arnold Arboretum* **36**, 305 (1955).
11. S. Kuraishi and R. M. Muir, *Science* **137**, 760 (1962).
12. W. P. Jacobs, *Amer. J. Bot.* **37**, 248, 551 (1950); *Amer. Natur.* **88**, 327 (1954); in *Plant Growth Regulation*, R. Klein, Ed. (Iowa State Univ. Press, Ames, 1961), p. 397; T. K. Scott and W. P. Jacobs, *Science* **139**, 589 (1963).
13. J. P. Nitsch, *Proc. Amer. Soc. Hort. Sci.* **70**, 512 (1957); I. D. J. Phillips, A. J. Vlitos, H. Cutler, *Contrib. Boyce Thompson Inst.* **20**, 111 (1959).
14. B. O. Phinney and C. A. West, *Ann. Rev. Plant Physiol.* **11**, 411 (1960); in *Plant Growth Regulation*, R. Klein, Ed. (Iowa State Univ. Press, Ames, 1961).
→ T. K. Scott and W. R. Briggs, *Amer. J. Bot.* **49**, 1056 (1962).
16. Supported by NSF undergraduate science education program to D.B.C., NSF research grant 24166 to W.P.J., and by funds from the Eugene Higgins Trust allocated to Princeton University.

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Reversion in Hamster Cells Transformed by Rous Sarcoma Virus

Abstract. *Hamster cells of the BHK-21 line are transformable by Rous sarcoma virus (Schmidt-Ruppin strain). The transformed cells form colonies in agar suspension culture, grow on glass in disarray, and initiate tumors in hamsters and chickens, but extracts do not induce tumors in chickens. Chickens bearing tumors develop neutralizing antibody against the virus. Transformed cell clones give rise to "revertants" which form colonies on glass with cells oriented parallel to each other like the original uninfected cells. These revertants do not grow in agar or initiate chicken tumors, and they regain the original low transplantability of untransformed cells in hamsters.*

When hamster cells of the BHK21 C13 line (*1*) are infected with polyoma virus and suspended in soft agar medium, only cells which have undergone transformation have a high colony-forming capacity. The use of this property in a selective assay for polyoma-transformed hamster cells has been described (*2, 3*). It was of interest to determine whether the same technique was applicable to the study of transformation by other oncogenic viruses. This report describes the use of such technique for isolation of C13 cells transformed by Rous sarcoma virus, Schmidt-Ruppin strain (RSV-SR) (*4*), with particular emphasis on the loss of transformed character by some of these cells.

A suspension of C13 cells was infected with RSV-SR virus at an input multiplicity of 0.05 focus-forming units per cell (*5*). Ten agar suspension cultures were each seeded with 10^4 infected cells. After 14 days at 37°C five colonies about 0.1 to 0.2 mm in diameter and 11 colonies less than 0.1 mm in diameter had developed. Four control plates each containing 10^4 uninfected cells had only a few minute colonies. The five largest colonies from the infected cell cultures were picked and cultured on glass in 10 percent calf serum medium (*1*). The cells in all five cultures grew in disarray like C13 cells transformed by polyoma virus (*1*). When the culture derived from one of these colonies (designated C13/SR) had grown to about 5×10^5 cells, clones were prepared by isolating single cells in microdrops under paraffin (*6*). Three of the 16 isolated cells formed colonies, which were designated C13/SR1, C13/SR2, and C13/SR3. When grown on glass these clones formed randomly oriented cell layers. Cells of the C13/SR2 and C13/SR3 clones grew rapidly and made the medium very acid; C13/SR1 cells grew more slowly. The clones and original C13/SR cells were plated at low cell density to produce discrete colonies in petri dishes containing x-irradiated C13 cells as "feeders" (*7*) (Table 1). A small proportion of the C13/SR, C13/SR2, and C13/SR3 cells formed colonies with well-defined parallel orientation ("revertants"). It is very unlikely that these revertant cells were derived from untransformed C13 cells which had contaminated the transformed colony in the agar culture and which had been carried through the cloning procedure. The micromanipulations of cloning involve two separate visual checks on the discreteness of the isolated cell. The possibility that the revertants were derived from the x-irradiated feeder cells was excluded when platings of C13/SR3 cells with and without feeder cells gave essentially the same proportion of transformed and reverted colonies. Clone C13/SR1 cells gave rise to only transformed colonies, that is, with random orientation. Intermediate colonies with some degree of parallel orientation in the center of the colony were present in all platings except those of C13/SR1 cells.

This gradation in morphology made classification of the colonies difficult, but in the results presented here only colonies with very well-marked parallel orientation, like the original C13 cells, have been classified as "reverted." A completely random transformed colony and a revertant colony in a plating of C13/SR3 cells are shown in Fig. 1. When platings were made in fetal instead of postnatal calf serum the proportion of transformed colonies was increased, but well-marked reverted colonies were still present in platings of C13/SR2 and C13/SR3 (Table 2). This observation is of particular interest since Rubin (*8*) has shown that

Table 1. Colony formation by C13/SR cells and its derivative clones in early passage in 10 percent serum medium.

Cells	Plating efficiency (%)	Colonies with parallel orientation (revertants) (%)
C13/SR	35	10
C13/SR1	21	0
C13/SR2	55	1.3
C13/SR3	55	9.4