and fewer spikes per discharge). Both cerebral hemispheres displayed these modifying capabilities, although the cortex ipsilateral to the unit under observation was uniformly less effective than the contralateral cortex.

In this study (*5*) cats were anesthetized with Nembutal and paralyzed with decamethonium bromide (or Flaxedil). Single units in the cuneate and gracile nuclei were isolated by conventional 0.5 to 1.5 μ micropipette techniques. Responses in these units were evoked by needle electrodes in the footpads. Because it is not possible to excite only pyramidal tract elements in the intact nervous system, the bulb was isolated from the midbrain so that only the pyramidal tract was intact. The brain stem was exposed by a ventral approach, and the tissue was removed by suction under direct vision. The transection was begun on each side of the pyramids at the level of the trapezoid body and carried through to the bony tentorium. All tissue, including the middle and the superior cerebellar peduncles, was carefully removed so that there was a gap of a few millimeters between the rostral and caudal stumps of the transected brain stem. Bipolar silver-ball electrodes were then placed on the pericruciate cortex of each hemisphere for excitation of corticofugal elements; only the pyramidal tract, however, could carry activity beyond the transection. At the end of each experiment the brain was perfused with formalin, removed, sectioned, and stained in order to assess the extent of pyramidal tract isolation (Fig. 1*C*).

In spite of this radical surgical intervention, units in the dorsal column nuclei were affected by stimulation of the motor cortex to the same extent as were those in intact preparations. Nearly one-third of the units could be driven and two-thirds could be inhibited from the cortex; occasionally, a unit which could not be affected was isolated. Figure 1 shows the responses of two units, the first inhibited and the second driven by the motor cortex. The histological section shows the extent of pyramidal tract that remained in this animal; examination of other sections revealed nearly complete destruction of the overlying medial lemniscus-trapezoid complex, which can be seen in Fig. 1*C*. The remaining brain stem was totally transected. Evidently both excitation and inhibition can be transmitted to the bulb by way of the pyramidal tract. The excitatory effect appears to be direct. This is deduced from the following of high stimulus frequencies (in excess of 100 per second), the brief latency and the small latency dispersions found in some cortically driven units, and the profound difficulty of demonstrating excitation when the pyramidal tracts alone were transected. The inhibitory pathway, on the other hand, appears to be less direct. Perhaps the pyramidal tract projections to the reticular formation (*6*) are involved; certainly inhibition remains, albeit with altered characteristics, after transection of the pyramidal tract. Nevertheless, it is interesting that both anatomical and functional evidence points to a direct connection between the somatic motor and sensory systems via that phylogenetically recent system, the pyramidal tracts.

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Hormonal Induction of Vascular Tissue in Tobacco Pith in vitro

Abstract. The direct addition of indole-3-acetic acid into sterile-cultured stem pith sections of *Nicotiana tabacum* through inserted glass pipettes has induced cell division and differentiation where such activity would not normally occur. This evidence lends support to the theory that indole-3-acetic acid is the hormone that limits xylogenesis.

There is considerable evidence that indole-3-acetic acid (IAA) plays a major role in the differentiation of vascular tissue in plants. The classical study of Jacobs (*1*) illustrated that vascular tissue can be induced around a wound in vivo when the original vascular strand has been severed and external IAA is introduced through a petiole in the node above the wound. Other workers (*2*) have demonstrated in vitro that vascular tissue can be induced in undifferentiated callus tissue either by grafting a bud into the callus, thus providing it with hormones diffusing from the apical tissue, or by applying indole-3-acetic acid or naphthaleneacetic acid in a lanolin paste into an incision in the callus. Pith tissue which is, essentially, undifferentiated parenchyma presents a region which has a definite orientation in the plant as opposed to the nonpolar orientation of callus. Previous work (*3*) has shown that no vascular tissue was induced in sterile cultures of tobacco pith sections when the culture medium contained only IAA as the growth-promoting substance. In fact, no cell divisions were initiated at any concentration of the hormone. Since it was shown that division could be induced with mixtures of IAA and other growth-promoting substances, the supposition was that, although IAA may cause differentiation, another growth-promoting substance is necessary to promote cell division.

The experiment described in this report is part of a study designed to give quantitative and qualitative evidence of the amount and type of differentiation induced in response to IAA and other differentiation factors in undifferentiated tissues. The observations were made during a study of the response of tobacco pith to IAA supplied through glass pipettes inserted directly into the center of the tissue (Fig. 1). Pith cylinders were removed from *Nicotiana tabacum* by boring through lengths of stem with a sterile cork borer slightly smaller in diameter than the entire pith cylinder. The pith sections were cut into 15 mm pieces and, after insertion of Pyrex glass pipettes into the morphological apical end, were placed in culture tubes containing agar-solidified Riker's tobacco medium (*4*), a nutrient mixture which contains no IAA and does not support growth of the pith. The pipettes were filled with the additives through holes pierced in the polyethylene caps that cover the tubes. The caps were sealed with small squares of cellophane tape. The pipettes were refilled as they emptied. The cultures were maintained in a culture room at 25°C with a 12-hour light-dark cycle to a maximum of 42 days, some sections being terminated each week and fixed for sectioning. In the present experiment the concentration of hormone supplied to the tissue was 0.5 mg/liter. Control cultures were set up with pipettes that contained sterile water or nothing.

The results indicate that no cell divisions occurred until the tissue had been subjected to a prolonged exposure to IAA, for no divisions were observed

before 32 days in culture. At that time longitudinal sections of the fixed tissue revealed small areas of dividing cells directly under the pipette incisions (Fig. 1). Contrary to what was expected—that is, that long files of xylem would be differentiated as in Jacobs' study—xylem tracheids were found in a haphazard arrangement among the dividing cells with occasional spherical areas of a more organized arrangement. This compares favorably with the findings of vascular tissue arrangement in callus tissue. Apparently, when pith is removed from the physiological orientation of the plant it loses its polarity and reacts basically in the same way as the nonpolar callus tissue. Since divisions were restricted to the area close to the site of the pipette insertion, it may be that there is no basipetal transport of auxin in the pith. On the other hand, this may mean merely that a relatively long period of time in culture is necessary for basipetal vascular differentiation. The latter possibility seems more likely since the sections cultured for 42 days had a much wider and longer extension of the dividing and differentiating area than the sections cultured for 32 days. The possibility that cell division was initiated by a wound hormone response was obviated by the fact that no divisions occurred in the controls. These experiments are at variance with results of previous work in which it was shown that cytokinesis and differentiation did not occur in pith sections placed on an IAA-containing medium (*3*), but did occur when the medium contained kinetin as well as IAA (*5*). It is possible that the effect of IAA on the tissue is determined by the method of application. Thus, a direct application (as through the pipettes) of IAA rather than diffusion from the medium may be necessary for cell division.

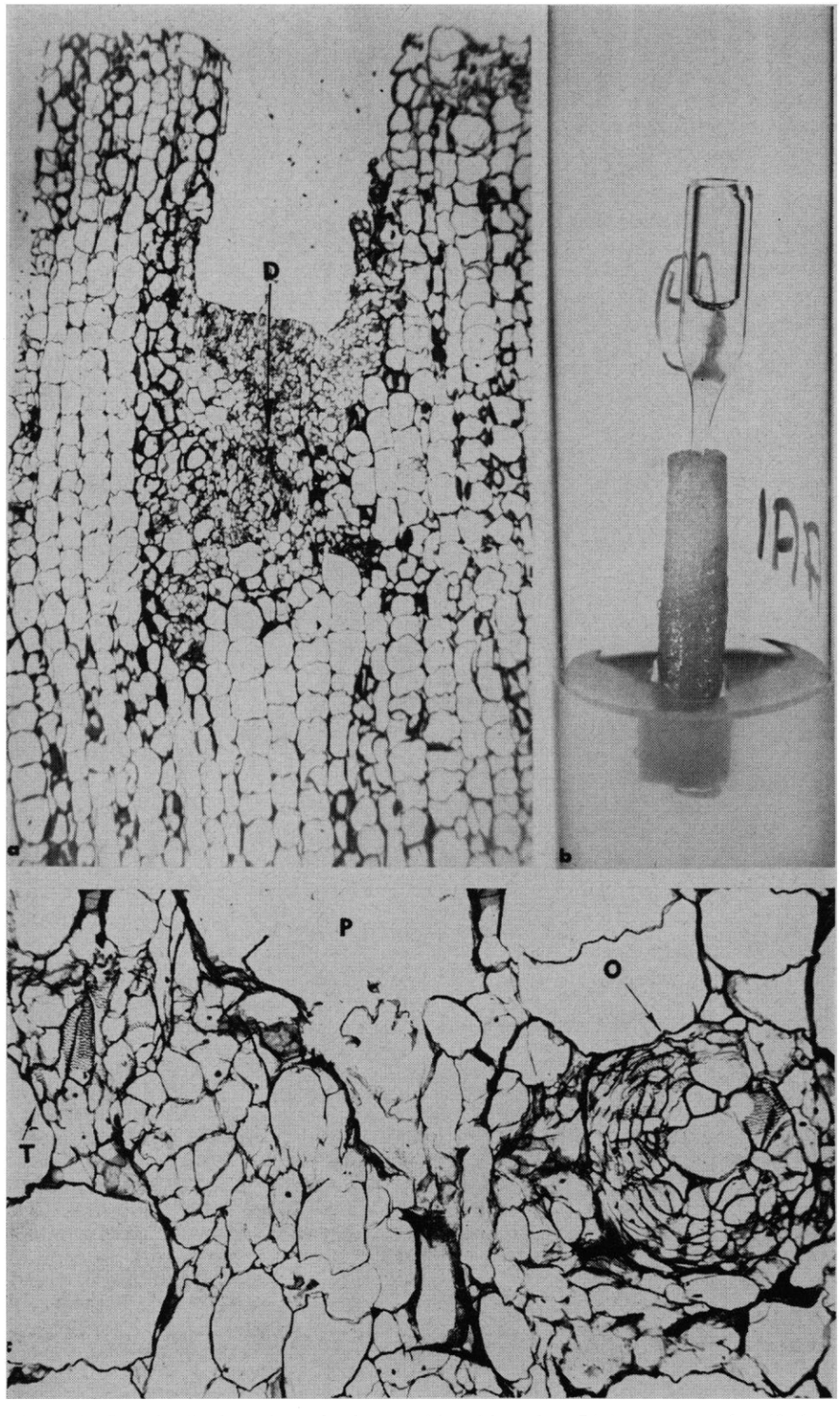

Fig. 1. (*a*) Longitudinal section of tobacco pith subjected to IAA treatment for 42 days (× 14). (*b*) Pith cylinder with pipette in place (× 1.1). (*c*) Longitudinal section of tobacco pith subjected to IAA treatment for 32 days (× 54). *P*, pipette incision; *D*, area of cell division; *T*, tracheids; *O*, organized area.

On the basis of the foregoing results it was concluded that vascular tissue can be induced in undifferentiated pith sections by the addition of IAA alone as the division-initiating and differentiation factor, provided an extended period is given in culture. Other experiments are being conducted to test this hypothesis as well as the effects of other growth-promoting substances and differentiation factors (*6*).

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