SCIENCE

WALTER F. LOEHWING

taking the sale of something really new in the way of botany text-books. Though originally intended primarily as a reference text, its general usefulness will without doubt lead to its adoption as a text in colleges

and universities. The authors have employed a succinct yet animated and lucid style of writing.

UNIVERSITY OF IOWA

SPECIAL ARTICLES

TUMOR PRODUCTION BY HORMONES FROM PHYTOMONAS TUMEFACIENS¹

Phytomonas tumefaciens (Smith and Town.) Bergey et al., incitant of crown-gall, was grown in a medium containing 2 per cent. dextrose and 0.1 per cent. bactotryptophane in distilled water, and in a medium containing, in addition, 1.0 per cent. bacto-peptone. Lacking salts, these media are simpler than those used by Brown and Gardner.² Cultures 2 to 4 days, three and twelve weeks old, also pellicles formed in the latter medium, were extracted with anhydrous peroxide-free ether, yielding crude waxy preparations. The most active extract was obtained from the former medium and organisms cultured twelve weeks. It was applied without wounding to hypocotyls of the red kidney bean (Phaseolus vulgaris) prior to straightening. For comparison, applications of 3 per cent. heteroauxin in anhydrous lanolin also were made in dosages of 0.005 cc and 0.01 cc with a tuberculin syringe. The dosages of bacterial extract were estimated at 0.0025 cc.

Control plants wounded by needle pricking, incision with or without insertion of mica or coverglasses, and complete transection of actively growing hypocotyls were set up to determine effects of moderate and severe wounding. Control inoculations with P. tumefaciens, with ether extracts of uninoculated media and with lanolin also were made.

Bacterial extracts applied unilaterally produced negative bending of 60° or less in from one to two hours. In three to four hours clearing appeared at the site of application, at and below which the hypocotyl first thickened down to soil level and later along all radii, though most abundantly at and below the site of application. Within eighteen hours thickening also extended a short distance upward. Temporary injury was evident in retarded straightening of the arch, elongation of the hypocotyl and development of the epicotyl. Later these general pathic effects were overcome. Whitish tumors appeared beneath and adjacent to the applications. In from three to four days local swellings marked sites of adventitious roots. Similar but more pronounced effects were produced with the heteroauxin-lanolin mixture. The dosages of 0.005 cc produced as marked early results as 0.01 cc dosages, but later effects were weaker. P. tumefaciens produced very small galls, the changes being less rapid than those effected by the extract and heteroauxin and the tissue firmer. Application of ether extracts of non-inoculated media and of lanolin gave negative results. Needle pricks caused slight local callus for-Incisions, especially blocked ones, caused mation. more wound tissue, especially above the incision and, at times, adventitious roots. The basal ends of severed hypocotyls swelled in from two to three days into massive calluses and later developed vigorous roots. The wound responses resembled those produced by extract and heteroauxin applications in intact hypocotyls but were less intense and better integrated than those incited by the latter.

The tumors produced by heteroauxin and bacterial extracts were initiated by cell enlargement, followed by cell division. Cell enlargement often was so excessive that adjoining cells in the cortex separated, forming cavities. These internal wounds led to development of loose callus. In addition or when cavities did not develop, cortical cells, including the endodermis, divided. The latter often was involved in development of extrafascicular vascular tissues^{3, 4} and roots. The vascular tissues, rays and pith also were activated.

Since transection, incision or application of heteroauxin or of bacterial extracts lead to similar effects in the hypocotyl, one may formulate the hypothesis that disturbance of the usual auxone concentration of the affected tissues is one of the causes of cell enlargement characterizing these effects. This disturbance, possibly a hyperauxony, gives hypocotyledonary cells opportunities to realize potentialities of cell enlargement, division and differentiation not stimulated to or inhibited from expression in the course of normal development.

In crown-gall formation this auxone disturbance probably is not a brief but a prolonged condition because the parasite not only produces heteroauxones and disturbs host correlations but also starts new abnormal growing centers, creating additional sites of autoauxone production. Growth substances obtained from P. tumefaciens produce effects similar to those of heteroauxin in the bean hypocotyl, but it can not be stated as yet that they are any of the known auxones.⁵

¹ Supported in part by a grant from the Rockefeller Foundation to the University of Chicago.

² N. Brown and F. E. Gardner, Phytopathology, 26: 708-733, 1936.

⁸ K. Schilberszky, Ber. d. Bot. Ges., 10: 424, 1892. ⁴ E. J. Kraus, N. Brown and K. C. Hamner, Bot. Gaz., 98: 370-421, 1936.

⁵ After this article was submitted, a new lot of extract made possible application of the hydrochloric acid, ferric

tration.

H. W. WILCOX

Since heteroauxones produced by non-gall-forming organisms incite tumors in plants not parasitized by these organisms and since wounding alone leads to tumor production, it appears that gall production by a particular parasite in a particular host is initially conditioned by factors determining specificities of parasitism. G. K. K. LINK

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SALT ACCUMULATION AND POLAR TRANS-PORT OF PLANT HORMONES¹

It is a well-established fact that a number of ions may be taken up by plant cells against a concentration gradient, or in other words that many cells are able to perform concentration work. The physico-chemical processes responsible for this concentration work are not generally understood, but the experiments have shown that the energy is ultimately furnished by oxygen respiration, in the absence of which no ion accumulation is possible. If the internal concentration of a given ion is plotted against its concentration in the surrounding medium, a more or less logarithmic relationship is obtained. In order to compare the accumulation process with polar hormone transport, it is advantageous to formulate the former as follows: Accumulation consists in the uptake of ions by the cell to such an extent that upon reaching the steady state the internal concentration is increased above that of the surrounding medium by a definite amount. At very low external ion concentrations the equilibrium concentration inside (steady state) may not be reached within the experimental period, which would then account for the initial parabolic rise in the accumulation curve. But from the data of Hoagland and Davis, and Collander for Nitella, and of Steward for potato tuber cells it is evident that for each set of conditions (temperature, rate of respiration, sugar content of cells) there is a constant increment independent of outside concentration. This means that for one given set of conditions the internal ion concentration may be calculated from the external concentration plus a certain amount. Thus the accumulation mechanism is able to raise the internal concentration to a given height above its surroundings. This makes it clear why no constant "accumulation ratio" is found.

The polar transport of auxin in the living plant

behaves in a similar way. It may be defined as the concentration of auxin (an organic acid) from apex towards base of each cell. It does not seem that there are theoretical objections against suggesting a homology between concentration from outside towards inside and concentration from apex to base of a cell. In addition, the facts are in accordance with this view. The accompanying graph shows the amount of indole-3-acetic acid transported in two hours through Avena coleoptile sections (4.2 mm long), as a function of the original concentration applied to one end of the sections. It will be seen that the amount transported from apex to base (normal transport) increases almost linearly with the logarithm of the applied indole acetic acid concentration. Beyond 1 mg/cc the amount transported decreases, probably due to toxicity, proving that the observed values are not caused by "leakage" through vessels or along the surfaces of the sections. The curve for transport from base to apex (inverse transport) is exactly like the normal transport, except that the applied concentrations must be 100 times as high to give numerically the same transport. This means that at each given concentration a certain



amount more of indole acetic acid is transported in

the normal direction than is transported in the inverse

direction or, in other words, that the polar auxin trans-

port mechanism handles a constant amount of indole

acetic acid independent of the existing gradient. This

is in direct parallelism with ion accumulation, in which

also a given amount more of ions are moved from out-

side towards inside of cell than are moved in the

reverse direction, independent of the external concen-

FIG. 1. Amount of indole-3-acetic acid (ordinate) transported in 2 hours through 6 Avena coleoptile sections, from apex towards base (normal) and from base towards apex (inverse), as a function of the applied concentration in mg per l (abscissa).

In addition it might be mentioned that the fact that the auxin concentration must be 100 times higher at the base than at the apex to get equal amounts trans-

chloride, amyl alcohol test. The color reaction characteristic of β -indoleacetic acid (heteroauxin) was obtained, indicating that this substance or a closely related indole compound is the heteroauxone produced by *P. tumefaciens*. We are indebted to our colleague, Professor F. C. Koch, of the department of biochemistry, for advising us and checking these tests.

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