GROWTH INHIBITION IN PEA SEEDLINGS

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THE inhibition of the growth of lateral buds by the application of auxins in high concentrations to the apical surface of cut stems is well known, and it is often associated with marked swelling of the cut stems. Borgström¹ explains both the inhibition and the swelling as being due to increased supplies of auxin set free in a transverse flow from the phloem, induced by the decapitation of the main stem. He considers that the swelling is a secondary effect of the increased auxin supply and not otherwise related to the inhibition of the buds. In the experiments which are described below observations have been made on seedlings of *Pisum sativum* after treatment with *H 11* extract of urine, three fractions of *H 11* and some pure substances. The effect of the various applications on the growth of the cut epicotyls both in height and width and on the development and growth of lateral buds was examined. The plants were grown in pots of soil in the light until three or four internodes high, and then placed in the dark for twenty-four hours before experimenting and for forty-eight hours after treatment, being kept throughout the experiment under glass covers in a moist atmosphere. The inhibiting effect was calculated as the percentage difference between the averages for the total length of the side shoots in treated and control plants.

H 11 extract is an alcoholic extract of urine prepared by the Hosa Research Laboratories and used for treatment of tumour growth. The concentration of paste used for these experiments was equivalent to 3.5 c.c. *H 11* extract in 6 gm. of pure lanoline. Tests were made with various dilutions of this. Treatment with concentrated paste when epicotyls were decapitated just below the terminal bud caused increased growth of the cut stems and marked swelling at the top of the stems. In plants decapitated at the node below which growth has ceased, treatment caused no increase in the height of cut stems nor did any swelling occur, but lateral buds were inhibited rather more than in the plants which had only the apical part removed.

Three fractions of *H 11* extract were prepared by the Hosa Laboratories: (1) 80 *K* was prepared from the filtrate after the precipitation of neutral *H 11* with copper sulphate; (2) 80 *L* was prepared from the soluble fraction after extracting the precipitate with dilute hydrochloric acid; (3) 80 *M* was prepared by dissolving the tarry substance which remained in 4 *N* sodium hydroxide. Lanoline paste was made with each fraction using 5 c.c. in 3.5 c.c. paraffin mixed with 2 gm. of lanoline. When applied to cut epicotyls 80 *K* and 80 *M* resulted in inhibition to side shoots, but 80 *L* had no inhibitory effect. 80 *L* and 80 *M* both caused greater swelling of the cut stem than 80 *K*. It appears as if 80 *K* contained an inhibitory substance which did not cause stems to swell as the other fractions did. Thompson *et al.*² found that 80 *M* was stimulatory to tumour growth.

As it was known that *H 11* extract contained β -indole acetic acid and its sodium salt³ a comparison was made between the effects of different concentrations of pure β -indole acetic acid and *H 11*, and it was found that a paste containing approximately 6,250 units β -indole acetic acid caused equivalent

inhibition to the concentrated *H 11* paste but less swelling of the stems. Series of experiments, in which paste made with a solution of β -indole acetic acid neutralized with sodium hydroxide was used, showed more swelling in the stems treated with the sodium salt than with the acid. The presence of the sodium salt in *H 11* extract would therefore account for the marked swelling noted in the cut stems. Avery⁴ has recorded that the potassium salt of β -indole acetic acid is twice as active as the acid in the *Avena* test.

Analysis of *H 11* extract⁵ having indicated the presence of a member of the quinone series, it was considered useful to test the effect of similar substances on cut stems. The following solutions were therefore used: (1) a saturated solution of anthraquinone in distilled water; (2) anthraquinone 2.6 di-sulphonic acid refined sodium salt, 0.1 per cent in distilled water; (3) 1.5 di-hydroxy anthraquinone, 0.1 per cent in distilled water. Results of these experiments showed a slight increase in the height of cut stems and inhibition of the growth of side shoots after treatment with anthraquinone and anthraquinone 2.6 di-sulphonic acid refined sodium salt. 1.5 di-hydroxy anthraquinone caused less increase in the height of the cut stems and increased growth of side shoots. No swelling of the stems occurred.

Pisum sativum epicotyls

Applications to cut stems	Percentage inhibition of lateral shoots	Percentage increase in width of cut stems
<i>H 11</i> extract	70.2	150.0
Fractions of <i>H 11</i>		
80 <i>K</i>	42.86	50.0
80 <i>L</i>	—	100.0
80 <i>M</i>	41.67	100.0
β -indole acetic acid, 6,250 units per gram lanoline	69.6	76.8
Anthraquinone saturated solution	73.4	—
Anthraquinone 2.6 di-sulphonic acid refined sodium salt 0.1 per cent	54.4	—
1.5 di-hydroxy anthraquinone 0.1 per cent	—	—

A comparison of the amount of inhibition and swelling caused by these different applications is given in the accompanying table. It is evident from these results that substances which cause inhibition may not cause swelling in the stem and that auxin application may inhibit without causing swelling if the actively growing internodes of the stem are removed.

Snow⁶ states that the inhibitive influence travelling up the lateral shoot is a hormone other than auxin and finds evidence in his experiments that another substance is active in inhibiting lateral buds in pea seedlings. No previous record has been found of inhibition by substances other than auxin when applied apically to the cut stem, and it is hoped to carry out more experiments to test the inhibition of other substances along these lines.

I am indebted to Mr. J. H. Thompson, director of research of Hosa Research Laboratories, for providing the *H 11* extracts and other substances.

¹ Borgström, Georg, "The Transverse Reactions of Plants", 126-131 (Lund: C. W. K. Gleerup; Copenhagen: Ejnar Munksgaard; London: Williams and Norgate, Ltd., 1939).

² Thompson, J. H., Holt, P. F., and Jones, R. Forbes, *Nature*, **151**, 23 (1943).

³ Thompson, J. H., *Brit. Med. J.* (July 31, 1943).

⁴ Avery, George S., Jr., Buckholder, P. R., and Creighton, Harriet B., *Amer. J. Bot.*, **24**, 226 (1937).

⁵ Thompson, J. H., "Cancer Research", *Medical World* (Oct. 13, 1944).

⁶ Snow, R., *New Phyt.*, **39** (2), 177 (1940).