Growth-promoting Effects of Ethylenediamine Tetra-acetic Acid

The recent communication by Heath and Clark[1] prompts us to present some of our observations of presumed plant hormone effects of the chelating agent ethylenediamine tetra-acetic acid. Reference to these observations was made in a review on the application of chelation to agriculture[2]. Our interest in this problem developed from observations, made in the summer of 1953, on massive growth response to ethylenediamine tetra-acetic acid by sunflower plants in water culture; metal complexing effects in the nutrient medium were involved, but there was also evidence of a direct effect of ethylenediamine tetra-acetic acid itself on growth.

To investigate possible growth-regulator effects of ethylenediamine tetra-acetic acid, a series of experiments on straight growth of hypocotyl sections of etiolated white lupin seedlings was undertaken late in 1953. Sections 2 cm. long were cut from central portions of hypocotyls 6–7 cm. in length (one section per seedling) from seedlings germinated in the dark at 24°–26° C. under a continuous tap-water spray. For each treatment twenty-five sections were floated on the appropriate solution in a large Petri dish which was then stored in the dark at 25° C. All solutions were made up with de-ionized water without added nutrients. Measurements were made after 24 hr. In preliminary experiments, all treatments included indole acetic acid at $1 \times 10^{-5} M$. It was found that either indole acetic acid at this concentration or disodium ethylenediamine tetra-acetate at $5 \times 10^{-5} M$, or both in combination, caused increases in elongation and water uptake of about the same magnitude, 30–50 per cent. In contrast with the studies using the wheat coleoptile[1], in which growth of controls on water alone gave elongations of about 80 per cent in 19 hr. at 25° C., the lupin hypocotyl sections elongated only about 10 per cent in 24 hr. at the same temperature. The relative magnitude of effects attributable to these compounds thus appears greater with the lupin material than with wheat, presumably because of a lower content of native hormone in this tissue.

When hypocotyl sections were floated on solutions of disodium ethylenediamine tetra-acetate substantial increases in both length and water uptake were observed at concentrations of 3×10^{-4}, 1×10^{-4} and $5 \times 10^{-5} M$, with maximum increases of more than 50 per cent occurring at either 5×10^{-5} or $1 \times 10^{-4} M$. Addition of *p*H 5 phosphate buffer to the solutions did not influence these results. With disodium ethylenediamine tetra-acetate at $5 \times 10^{-5} M$, respiration was stimulated to the extent of a 50 per cent increase in oxygen uptake.

The experiments with disodium ethylenediamine tetra-acetate are presumed to demonstrate effects of exposure of the plant part to uncomplexed chelating agent, so that responses observed are attributed to its action within the tissues. To study the response to complexed chelating agent in the medium, experiments were carried out with the iron chelate, ferric disodium ethylenediamine tetra-acetate, which at most physiological hydrogen-ion concentrations is the most stable known chelate of ethylenediamine tetra-acetic acid. At $10^{-4} M$ ferric disodium ethylenediamine tetra-acetate, there was a 10 per cent decrease in length and 15 per cent loss of water from the tissues, and more gradual but continued decreases at higher concentrations. Respiration was stimulated about 10 per cent by $5 \times 10^{-5} M$ of the ferric disodium compound, as contrasted with 50 per cent stimulation by the disodium compound at this same molar concentration. It seems evident from these results that growth-stimulating effects of ethylenediamine tetra-acetic acid are associated only with the addition of uncomplexed chelating agent to the growing medium.

The association of growth-promoting effects with uncomplexed ethylenediamine tetra-acetic acid in the medium, but not with the iron chelate, can be interpreted as supporting the concept of growth promotion by removal of calcium from the cell-wall structure. This would presume that uncomplexed ethylenediamine tetra-acetic acid, in passing through the cell wall, forms a chelate with calcium removed from the wall structure, which is thereby enabled to expand.

A further observation of hormone effects exerted by ethylenediamine tetra-acetic acid has been seen in epinastic response of soybean leaves on plants in solution cultures supplied with disodium ethylenediamine tetra-acetate at concentrations of $6 \cdot 7 \times 10^{-5} M$ or higher. The response characteristically took the form of an opening of the angle between petiole and stem and twisting of the leaflets through angles ranging from 90° to 270°.

This investigation will be presented for publication in more detail in a subsequent issue of the *Contributions from Boyce Thompson Institute*. It was supported by grants from the Refined Products Corp., Lyndhurst, N.J., and by Versenes Inc., Dow Chemical Co., Framingham, Mass.

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[1] Heath, O. V. S., and Clark, J. E., *Nature*, **177**, 1118 (1956).
[2] Haertl, E. J., *Down to Earth*, **2** (1), 6 (Dow Chemical Co., Midland, Michigan, 1955).

Seed-coat Structure of Ethiopian 'Rape'

Samples have been obtained of seed described as Ethiopian 'rape', imported into Europe for oil extraction. Plants grown from the seed corresponded with specimens in the Kew Herbarium named *Brassica integrifolia* (West) O. E. Schulz var. *carinata* (A.Br.). This plant is cultivated in Ethiopia and adjacent north-east African territories. Most taxonomists regard this species as a mustard, and some include it in the *B. juncea* complex which contains a number of mustards of commercial importance.

Fig. 1 is a drawing of a cross-section of the testa. All layers characteristic of the cruciferous seed-coat[1] are present. The uneven height of the palisade cells is responsible for a distinct reticulation which is shown by the whole seed and, also, by surface preparations of the palisade layer. The subepidermal layer, although not so distinct as that of *B. nigra*, is often quite obvious. So far as the mustards of commercial