Tracer Phosphatase Determination in Tissue Sections

THE increasing use of radioactive tracers in biology demands methods for quantitative recording even in tissue sections. Autoradiography furnishes one method, either by simply placing the sections containing the radio-ions towards a photographic plate[1], or by covering the section itself with a film of photographic emulsion[2,3]. In this way α- and even β-rays can be recorded[4].

The Geiger – Müller counter, however, is still the best means for quantitative recording of β-rays. I have used a counter in the way described below to record the amounts of radioactive tracers in tissue sections as well as of tissue extracts according to established principles.

A Geiger – Müller counter, as described by Ambrosen, Madsen, Ottesen and Zerahn[5], is placed within a lead cylinder directly above an aluminium turntable with eight holes for small aluminium dishes which contain the radioactive material. The electrical details of the apparatus, which contains a regulated 2,000-volt power supply, a scale-of-16 and a scale-of-10, a registering device and an arrangement which makes it possible to make counts for each dish or slide automatically as long as desired within the limits of 2 minutes and 30 hours, has been described elsewhere[6].

When tissue sections are to be measured, they are mounted on polished slides and covered by a 'Cellophane' coverslip ; the sections are placed above the holes in the turntable, and by means of a microscope fixed to the apparatus their positions are controlled. Small brass plates, 1 mm. thick, with diaphragms of the desired diameters, ½–2 mm., are then lowered upon the slides and are kept in place by means of small pins fitting into holes in the turntable. Before the plates are pressed into position, it is checked by the microscope that the desired parts of the sections actually are centred beneath the diaphragms.

Now the Geiger – Müller tube with its lead cylinder is raised 2 mm. by means of a half-moon-shaped piece of lead which corresponds to the height of the slide plus brass plate, and the counting may begin.

As the brass plate directly covers the section, except for the 0·05 mm. thick 'Cellophane' coverslip, the amount of tissue in which the radio-ions are measured depends only upon the diameter of the diaphragm and the thickness of the section. Using a common diaphragm of 1 mm.^{2} and sections of 5 μ, the amount of tissue under observation is only 0·005 mgm. Either frozen sections or paraffin or celloidin sections, stained or unstained, may be used. When possible, stained sections are preferred, as the histological examination and the radio-ion counting can then be undertaken in the same section.

As an example of the method, I have made a quantitative estimation of phosphatase in tissue sections by means of a modified Gomori[7] technique. The tissue, fixed in 80 per cent alcohol and imbedded in paraffin wax melting at 42°, is cut in 10 μ sections which are deparaffinized and incubated in a substrate of glycerophosphate containing radiophosphorus and calcium nitrate. The sections, which now contain radioactive calcium phosphate, can then be placed directly in the apparatus, and afterwards—or previously—be stained with cobalt according to Gomori. The Gomori technique thus affords a qualitative, and the method mentioned here a quantitative, means of estimating phosphatase in the same section.

A further report of the results will appear in due course.

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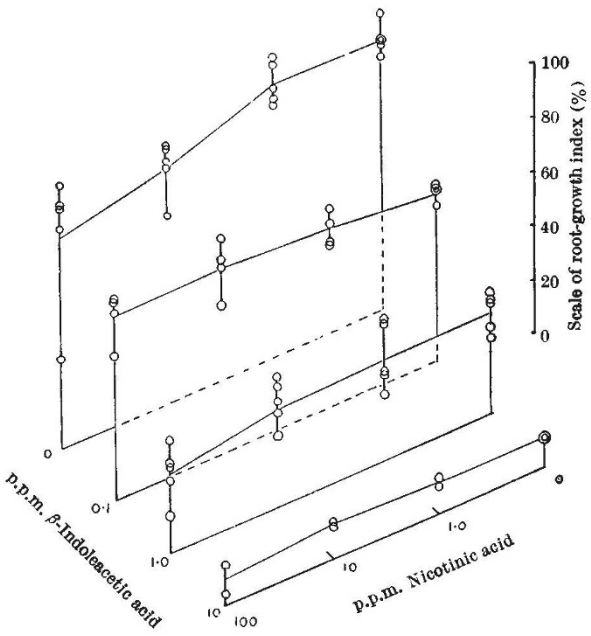
Nicotinic Acid and the Inhibition of Growth by Auxin

IN a recent paper, Galston[1] has shown that β-indoleacetic acid, at concentrations of 1–10 p.p.m., inhibits the growth in the dark of excised stem tips of *Asparagus*, in contrast to its normal stimulating effect in the light. Nicotinic acid, at a concentration of 0·5 p.p.m., was shown greatly to augment this dark inhibition, although without any effect by itself. These results were regarded as indicating that nicotinic acid is involved in the growth-regulating action of β-indoleacetic acid.

Recently, I have been studying the interactions with auxins of a wide range of plant metabolites in the growth inhibition of roots, and a considerable number of observations has been made with nicotinic acid. The technique employed has been fully described in a previous paper[2] and consists essentially of growing samples of cress seedlings in sterile water-culture in a number of different concentrations of the two substances both singly and in all possible combinations of those concentrations. Total root-



Three-dimensional diagram showing the interaction of nicotinic acid and β-indoleacetic acid in the growth-inhibition of cress roots