β-Glycerophosphatase and Lateral Root Development

FOR many years plant anatomists[1] have believed that the emergence of lateral roots, which arise endogenously in the pericycle of the stele, occurs principally by mechanical rupture of the surrounding cortex. Torrey[2] expressed the view that emergence is probably assisted by hydrolytic enzymes, although to his knowledge there was no experimental evidence to support the postulate.

When investigating the distribution of β-glycerophosphatase in the roots of *Pisum sativum*, we found a large increase in activity of this enzyme in the region of lateral root development. Enzyme assays were carried out on 1 mm serial sections of 5 day old roots. The sections were ground into ice-cold buffer at *p*H 6·0 and an aliquot of the resultant homogenate was incubated with 10 mM Na β-glycerophosphate at 30° C. The rate of hydrolysis of the substrate was estimated by the rate of appearance of inorganic phosphate in the medium. Supplementary experiments had shown that no inhibitors or activators of the enzyme were present in the homogenates and that the rate of the reaction was proportional to the enzyme concentration.

The distribution of the enzyme activity along the axis of the root is shown in Fig. 1. There is a considerable increase in activity when expressed on a per section or per unit protein basis, in the region 2·5 cm to 4·0 cm from the root apex. This corresponds exactly with the region in which we observed lateral roots to be initiated. The results of Popham[3] show similarly that in 5 day old peas lateral roots were initiated from 2·3 cm behind the apex. This enzyme, in animal cells at least, seems to be associated with cell hydrolysis[4], so we thought the site of activity might be the cortex cells surrounding the lateral initials. To investigate this further, we carried out a histochemical examination of the localization of the enzyme. Cryostat sections were cut through the zone of lateral root development using the technique of Gahan *et al.*[5]. These sections were then incubated in the Gomori acid phosphatase reaction mixture[6] using 10 mM Na β-glycerophosphate buffered at *p*H 5·4. β-glycerophosphatase was localized as a brown–black precipitate of lead sulphide.

Examination of sections passing through the laterals showed activity clearly limited to the stelar cells and those cells of the cortex adjoining lateral roots (Fig. 2). Control sections to which either inhibitors or no substrate had been added showed no such activity. McGregor and Street[7] observed similar activity in the region of lateral root initials in tomato roots but they did not comment on its significance. Examination of sections under high power leaves no doubt that the activity surrounding the lateral occurs in the bordering cortex cells rather than in the outer cells of the lateral root itself (Fig. 3). The absence of activity around very young initials is a good indication that the β-glycerophosphatase activity is induced by wounding of the cortex cells, as a result of mechanical pressure exerted by the lengthening lateral initial. High glycerophosphatase activity has also been observed in the surface layers of roots where radial expansion is occurring, a situation where similar wounding is expected.

Our conclusion is that β-glycerophosphatase and perhaps other hydrolytic enzymes are induced by mechanical stimulation of cells surrounding the emerging lateral roots, and that this activity assists the lateral root in penetrating through the cortex.

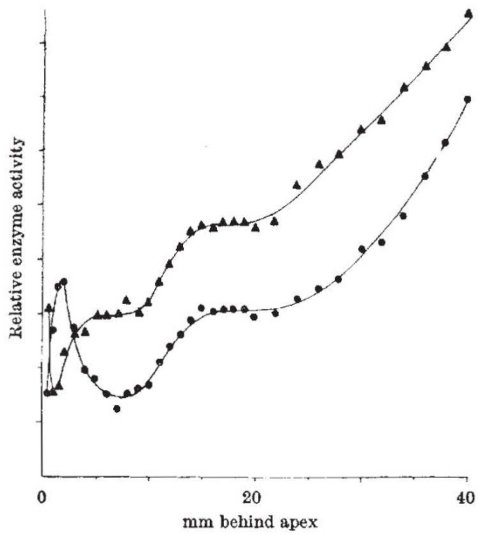

Fig. 1. β-Glycerophosphatase activity along the longitudinal axis of pea roots. Activity is expressed on a per section (●) and as a per unit protein (▲) basis.

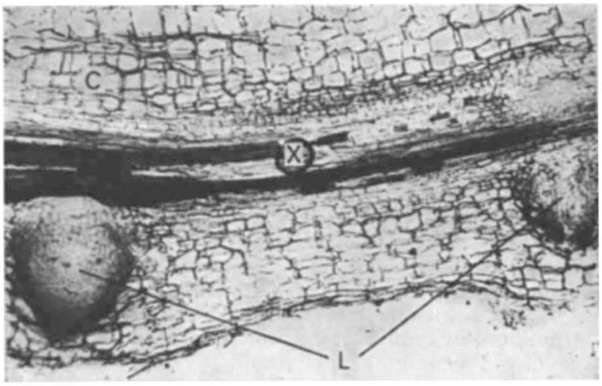

Fig. 2. Longitudinal section of a pea root through the region of lateral root formation. Incubated for 15 min, 30° C, in Gomori acid phosphatase medium. L, Lateral root initial; C, cortex; X, xylem cells. (× 40.)

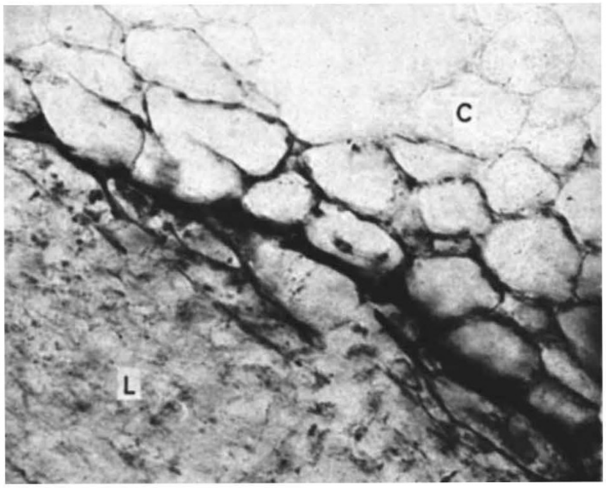

Fig. 3. Transverse section through the junction of a lateral initial and the cortex after incubating as in Fig. 2. L, Lateral root initial; C, cortex. (× 400.)

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