New patterns of vascular development in roots of Pisum recovering from colchicine treatment

P. W. Barlow[1]

Agricultural Research Council Unit of Developmental Botany, University of Cambridge, 181A Huntingdon Road, Cambridge CB3 ODY (England), 10 January 1977

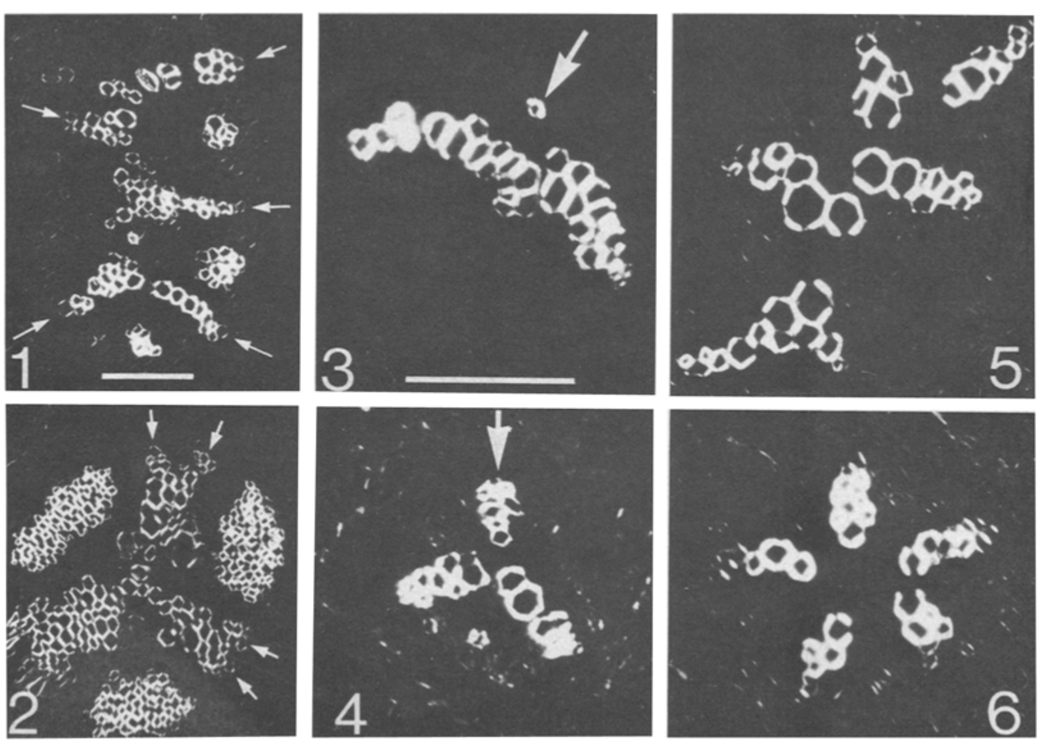
Summary. Abnormal vascular patterns were formed in pea roots recovering from a 3-h treatment with 0.025% of colchicine solution. New, often asymmetrical, patterns arose by the formation of additional protoxylem poles; later the patterns converted to a normal symmetrical tetrarch or triarch condition.

Few studies have been made of the factors that determine vascular patterns within the root apices of plants[2-6]. Such patterns are characteristic for a given species and generally stable, which implies genetic determination and homeostatic maintenance. In the effort towards understanding patterning 2 approaches can be made: one is to explore any normal, ontogenetic, changes that occur in patterning[2,6], the other is to interfere experimentally with vascular pattern formation[3,7,8]; both approaches then require the correlation of anatomical changes with physiological changes within the apex. One problem of the experimental approach is to find an agent that will regularly perturb the mechanism of patterning with the minimum of damage to the root apex. Here described are changes in the vascular pattern of pea roots recovering from treatment with colchicine. This drug, therefore, may be an additional experimental tool with which to probe further the regulation of pattern formation.

Roots of seedlings of Pisum sativum c.v. Meteor that had reached a length of 3 cm were immersed for 3 h in 0.025% colchicine solution[9]. Then they were washed and grown on in $^1/_2$-strength Hoagland's salt solution. All solutions

1 Acknowledgment. I thank Professor L. W. Roberts, University of Idaho, for the help he gave me during the preparation of this paper.

2 H. Dyanat-Nejad and P. Neville, Revue gén. Bot. *80*, 41 (1973).
3 L. J. Feldman and J. G. Torrey, Can. J. Bot. *53*, 2796 (1975).
4 A. Lux and M. Luxová, Biologia *22*, 166 (1967).
5 B. Samantarai and S. K. Sinha, J. Indian bot. Soc. *36*, 1 (1957).
6 J. G. Torrey and W. D. Wallace, in: The Development and Function of Roots, p. 91. Ed. J. G. Torrey and D. T. Clarkson. Academic Press, London 1975.
7 J. Montezuma-de-Carvalho and M. da G. Pratas do Vale, Bol. Soc. Broter. *45* (2nd ser.), 435 (1971).
8 E. Reinhard, Ber. dt. bot. Ges. *73*, 19 (1960).
9 P. W. Barlow, Protoplasma *68*, 79 (1969).



Figures 1–6. Transverse sections through the regenerating portion of different roots recovering from a colchicine treatment. The sections were photographed using polarized light.

Fig. 1. Immediately distal to the c-tumour; there are 5 protoxylem poles (arrowed). Fig. 2. Splitting of 2 of the protoxylem poles (arrowed) about 0.2 cm distal to the c-tumour. This particular root eventually acquired a pentarch vascular pattern (shown in figs. 5 and 6). Figs. 3 and 4. Successive stages in the development of a triarch pattern from a diarch condition. A new pole is initiated (fig. 3, arrow) 1.7 cm distal to the c-tumour; 1 cm further towards the root tip a symmetrical pattern has been established (fig. 4). [Actually, this particular root became pentarch immediately distal to the c-tumour and then the root split to give 1 root that was triarch and a second that was diarch: it is the latter that is shown here.] Figs. 5 and 6. Change in the symmetry of a pentarch vascular pattern. The asymmetric pattern in figure 5 lies 0.7 cm distal to the c-tumour and the pattern in figure 6 lies 0.5 cm further towards the root tip.

Figs. 1 and 2 are printed at the same magnification; so are figures 3–6. The scale bar represents 100 μm.

were aerated, changed regularly and maintained at room temperature. After the roots had made at least 4 cm of new growth they were fixed in Helly's fluid, embedded in wax and sectioned transversely.

Normally, pea roots have a symmetrical triarch (rarely tetrarch) vascular pattern. Of 13 roots grown after colchicine treatment, 8 showed a deviation from this normal pattern in the regenerating portion of the root. The abnormal patterns resulted from an increase in the number of protoxylem poles from the usual 3 poles to 4, 5 or 6 poles (figure 1). One root showed a reduction of the number of poles from 3 to 2.

Examination of serial sections of the regenerating roots reveals how the abnormal patterns arise and their subsequent histories. The new patterns may originate immediately distal to the c-tumour (i.e. the swelling induced by the colchicine immediately behind the root apex) or they may occur after the root has made up to 2 cm of additional growth. The increases in the number of protoxylem poles occur in 2 ways. One is by the splitting of an existing pole: that is, where there was previously 1 cell, or 1 group of cells, differentiating as a protoxylem pole, 2 arise (figure 2). The sites of inception of the 2 poles then diverge and become independent of one another. The other way is by the inception of a new pole that is unrelated to any pre-existing pole. Here, a single lignified cell is at first seen equidistant from the existing poles (figure 3) and, as root regeneration continues, new metaxylem cells differentiate to complete the additional xylem arm (figure 4).

All the abnormal vascular patterns found in the regenerating roots showed a tendency to revert eventually to the original triarch condition by loss of the supernumerary protoxylem poles. Changes in patterning also involved changes in the symmetry of the placement of the xylem arms. For instance, an asymmetrical pentarch pattern which arose in 1 regenerating root (figure 5) subsequently converted to a symmetrical pattern (figure 6) by changes in both the orientation of metaxylem differentiation and the positioning of the protoxylem poles relative to one another.

Although the period of exposure to colchicine is brief, the drug probably persists in cells of the apex for some time afterwards[10]. This persistence may account for some of the long-term changes in mitotic activity found in root meristems following a 3 h treatment[11,12], and, in part, for the changes in vascular pattern. Alterations to the meristem include a reduction of its size[13] and the possible stimulation into division of cells located in the quiescent centre (QC)[12,14]. The cells from the QC eventually repopulate the meristem.[14,15]. The size of the QC has been postulated to control the complexity of the vascular pattern[3,6]; therefore, the change in the number of xylem arms in the regenerating root may reflect changes in the number, or activity, of cells in the reforming QC after its stimulation by colchicine. It is also conceivable that cells in the procambial cylinder that would never normally become lignified are stimulated to do so by the colchicine treatment[16]. The patterning of the vascular system is likely to involve some type of interaction between presumptive vascular cells in the region of their inception. Whether the repatterning that is observed relates to changes in the ploidy of the presumptive xylem initials or their neighbours, or to disturbances to gradients of morphogenetic determinants within the apex, remains to be elucidated.

10 J. S. R. Nelson, Can. J. Genet. Cytol. *14*, 279 (1972).
11 R. D. MacLeod, Öst. bot. Z. *120*, 15 (1972).
12 R. D. MacLeod, Chromosoma *35*, 217 (1971).
13 R. D. MacLeod, Planta *71*, 257 (1966).
14 D. Davidson, Chromosoma *12*, 484 (1961).
15 T. Shimamura, Cytologia *9*, 486 (1939).
16 E. R. Witkus and C. A. Berger, Bull. Torrey bot. Club *77*, 301 (1950).

A new drug against Paragonimus infection

J. P. Dubey, P. C. Stromberg and M. J. Toussant

Department of Veterinary Pathobiology, College of Veterinary Medicine, The Ohio State University, Columbus (Ohio 43210, USA), 14 March 1977

Summary. An oral dose of 50–100 mg/kg b.wt of a new anthelmintic, albendozole, for 2–3 weeks killed adult flukes and stopped shedding of ova of Paragonimus kellicotti in experimentally infected cats. No clinical signs related to treatment were recognized. This low toxicity of albendozole may be useful in treating human paragonimiasis.

Paragonimiasis is a serious parasitic disease of human beings in Asia, Africa and South America[1]. 3 or more hosts are involved in the life cycle: the definitive host (man) becomes infected by ingesting intermediate hosts (crabs, crayfish) or paratenic hosts[2] (wild pigs) that have eaten crabs. Several carnivorous hosts (cats, dogs) also act as the definitive host for species of Paragonimus that infect man. Young flukes migrate to the lungs of the definitive host via intestinal wall, peritoneum and diaphragm. The most common symptoms are a cough, profuse expectoration, hemoptysis, and chest pains[3,4]. Infection may not be confined to the lungs as Paragonimus frequently invades eyes and brain causing vision impairment, seizures and paralysis[3,4]. Bithionol is considered the drug of choice at the present time[3,4]. However, this drug causes unpleasant side effects such as diarrhea, nausea, vomiting and urticarial eruptions[3,4]. We report promising paragonimocidal properties of a recently discovered anthelmintic, albendazole[5], in cats experimentally infected with Paragonimus kellicotti.

Each of 6 specific-pathogen free cats were inoculated orally with 25 metacercariae dissected from the hearts of naturally infected crayfish. The cats developed radiographically demonstrable cysts in their lungs 28 days after

1 I. Miyazaki, Jap. J. Med. *10*, 8 (1969).
2 I. Miyazaki and S. Habe, J. Parasit. *62*, 646 (1976).
3 M. Yokogawa, Adv. Parasit. *3*, 99 (1965).
4 M. Yokogawa, Adv. Parasit. *7*, 374 (1969).
5 V. J. Theodorides, R. J. Gyurik, W. D. Kingsbury and R. C. Parish, Experientia *32*, 702 (1976).