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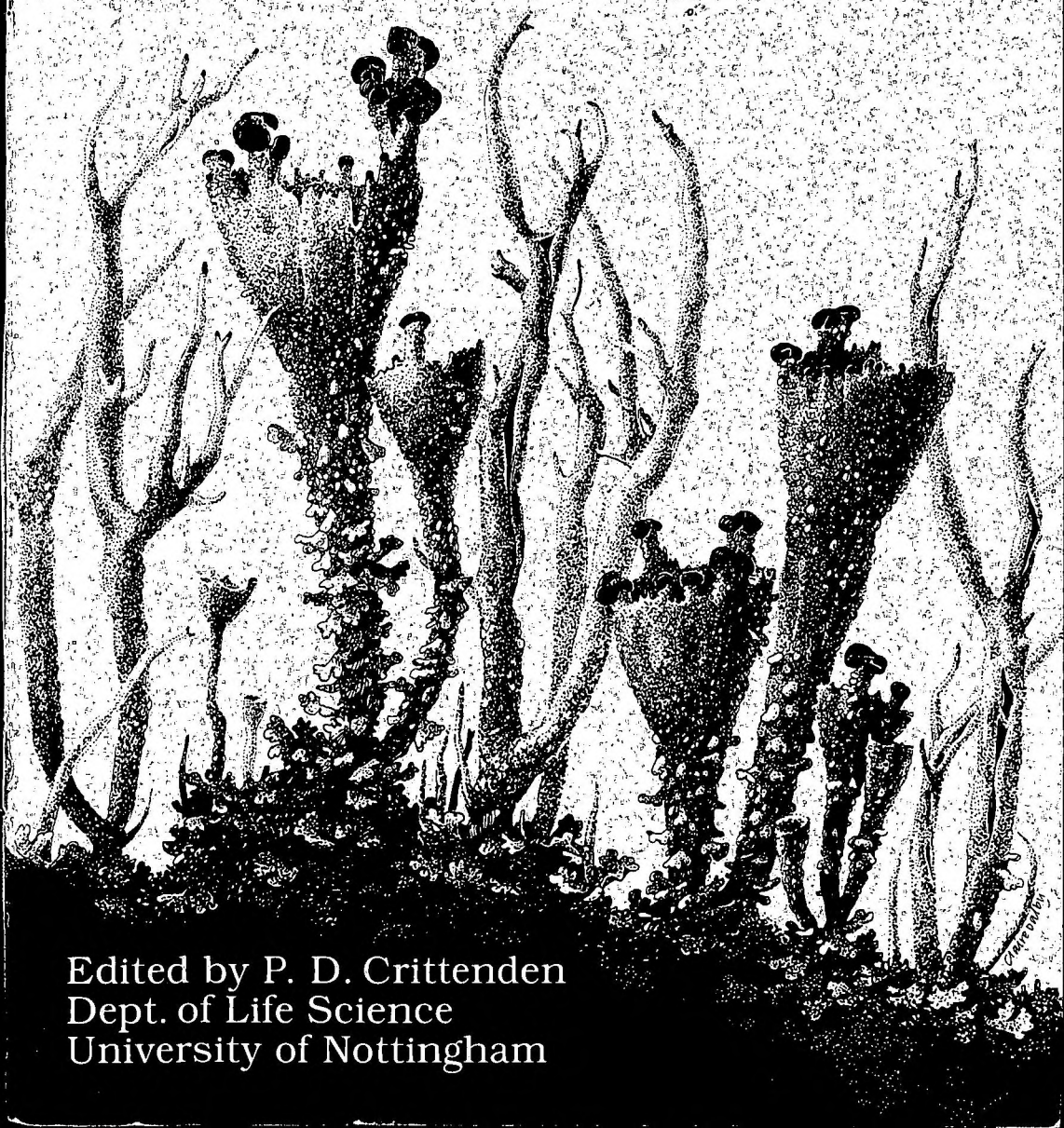

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ULTRACRYOMICROTOMY IN IMMUNOLOGICAL STUDIES ON LICHENS

Ultracryomicrotomy is widely used in immunological studies on animal cells (e.g. Wooding *et al.*, 1991; Hayashi *et al.*, 1994). However, applications of this technique to plant tissue have remained few (e.g. Marttila *et al.*, 1993) mainly because the rigid nature of plant cell walls makes the procedure very difficult to employ. Immunological studies on lichens have been carried out using conventional ultramicrotomy and mounting materials that allow the penetration of antibody. For example, Bergman and Huss-Danell (1983) localized the nitrogen fixation sites in cephalodial cyanobacterium of *Stereocaulon paschale* using labelled antibodies raised against different enzymes of the N_2-fixation pathway. Hällblom *et al.* (1986) used gold-labelled antibodies of GS to localize the enzyme in the cyanobacterium *Nostoc* in two lichens *Peltigera aphthosa* and *P. canina*. Furthermore, Bergman and Rai (1989) used the same method in order to detect nitrogenase, GS, phycoerythrin and ribulose 1,5 -bisphosphate in *Nostoc* from *Nephroma arcticum*.

Immunological labelling is a highly specific means for detecting macromolecules in electron micrographs, but unfortunately the method is susceptible to chemical interruption which can lead to non-specific binding of the antibody. Since in cryomicrotomy the tissue is only slightly fixed and mounted by freezing without ethanol dehydration the method is likely to preserve the tissue closer to the natural state than conventional methods. Another advantage of this technique is that sample preparation is much faster. Although it is probable that ultracryomicrotomy will not encounter the same problems in lichens as in plants it has not been attempted with lichen material. In the present study we applied cryomicrotomy for the first time in the immunological detection of GS in the cyanobacterium of a lichen. *Stereocaulon glareosum* was collected on the island of Hailuoto (65°02'N, 24°42'E) near Oulu, Finland. Thalli were stored outdoors in open plastic boxes to maintain the environment as close to natural conditions as possible. Cephalodia containing a *Nostoc* cyanobacterium were excised from fresh thalli with forceps and a knife, fixed in a paraformaldehyde (3%)-glutaraldehyde (0.1%) solution for 2 hours and then infiltrated overnight in 2.1 M sucrose for cryoprotection prior to freezing (Griffiths *et al.* 1984). Prior to sectioning the samples were mounted on specimen holders (copper pins) by rapid freezing in liquid nitrogen where they could also be stored for several days.

Thin sections were cut using LKB Ultratome equipped with a cryo-attachment and a glass knife prepared with a LKB 7800 knife maker

modified according to Stang (1988). The knife and specimen temperatures were –85°C and –92°C respectively. Sections were picked up on nickel grids covered with a Formwar membrane (0.3 %), coated with carbon, and the grids were floated on a phosphate buffer. Staining and immunolabelling were conducted following the procedure of Marttila *et al.* (1993) with the modifications that bovine serum albumin was used instead of fetal calf serum and the gold particle size was 6 instead of 10 nm. The primary antibody was anti-rabbit-IgG raised against legume root nodule specific GS provided by the Soils and Crop Sciences Division, Rothamsted Experimental Station, Harpenden Herts, U.K. The lyophilized antibody was redissolved in 500 ml of water and diluted 10x and 100x with 5% BSA in PBS-glycine. Grids were stained with uranylacetate (0.4%) and covered with a methylcellulose (1.5%) membrane, and samples were studied under a transmission electron microscope (Jeol TemScan 100 Cx, 60 kV). Control samples were prepared in the same way but without antiserum treatment. For comparison of ultrastructural details the same lichen material was also prepared as follows, using conventional methods which do not support immunolabelling. Small sections were cut with a razor blade from the thalli. The tissue was fixed in 2.5 % glutaraldehyde in 0.05 M phosphate buffer (pH 7.0), and postfixed in 1 % buffered OsO_4 solution. Samples were dehydrated in ethanol and embedded in Ladd's LX-112. Ultrathin sections were stained with uranyl acetate and lead citrate and observed under a transmission electron microscope (Jeol 1200 Ex, 80 kV).

Since no heterocysts could be detected in cryomicrotomically or conventionally prepared samples the only images obtained were of vegetative cells. In cryomicrotomy structural details of samples were generally well-preserved, and the resolution was good (Fig. 1). The thickness of the cryosections varied substantially due to the difficulty of controlling the knife advance during sectioning. In a small number of cases samples were so fragile that they tore apart during the electronmicroscopic observation. The immunolabels were abundant all over the tissue, but especially in the vicinity of membrane structures (Fig. 1). They were also found in control samples, which indicated, that binding of the label was relatively non-specific.

Comparisons between differently prepared samples showed that in the cryomicrotome sections the overall picture of the cell was more porous and looked 3-dimensional (Fig. 1), whereas in the conventionally prepared sections the cells looked firmer and 2-dimensional (Fig. 2). In both samples membrane structures looked quite similar, although in conventional sections more were visible. In both samples it was possible to see particles in the cytoplasm. The cell walls and some membranes were more heavily stained

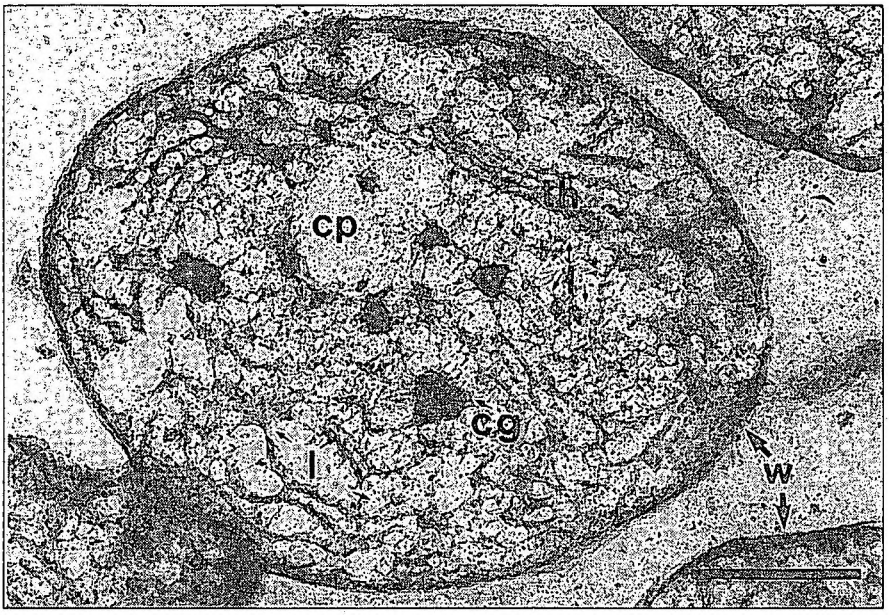

Fig. 1. Transmission electron micrograph of a cephalodial vegetative *Nostoc* cell of *Stereocaulon glareosum* prepared by means of ultracryomicrotomy and labelled with protein-A-gold conjugate (GS-antiserum treatment with a 10 x dilution). Abbreviations: cb = carboxysome, cg = cyanophycin granule, cp = centroplasm, l = label(s), s = polysaccharide sheat, th = thylakoids,w = cell wall. Scale bar = 1 µm.

in cryomicrotomically prepared samples than in those prepared using conventional methods whereas no differences between the two approaches could be found in the staining intensity of the cytoplasm and the organelles.

The results of this experiment confirmed that cryomicrotomy has great potential in immunological studies on lichens. It should be noted that, as the application of this technique to lichens becomes established, it should be possible to further improve the quality of the cryomicrotome samples. There are some details requiring further study, especially those concerning strength of the sections, resolution, and preventing the non-specific labelling. It has been suggested that non-specific binding could result from the affinity of protein-A in the labelled conjugate to glycoproteins (Mau & Clarke 1983). To overcome this kind of non-specificity an IgG-conjugate has been used instead of protein-A (Bergman *et al.* 1985). It is likely that the use of antiserum raised against the GS of *Nostoc* rather than that of legume nodules could have improved the binding specificity. The structure of GS varies from species to species to such an extent that enzymes may be

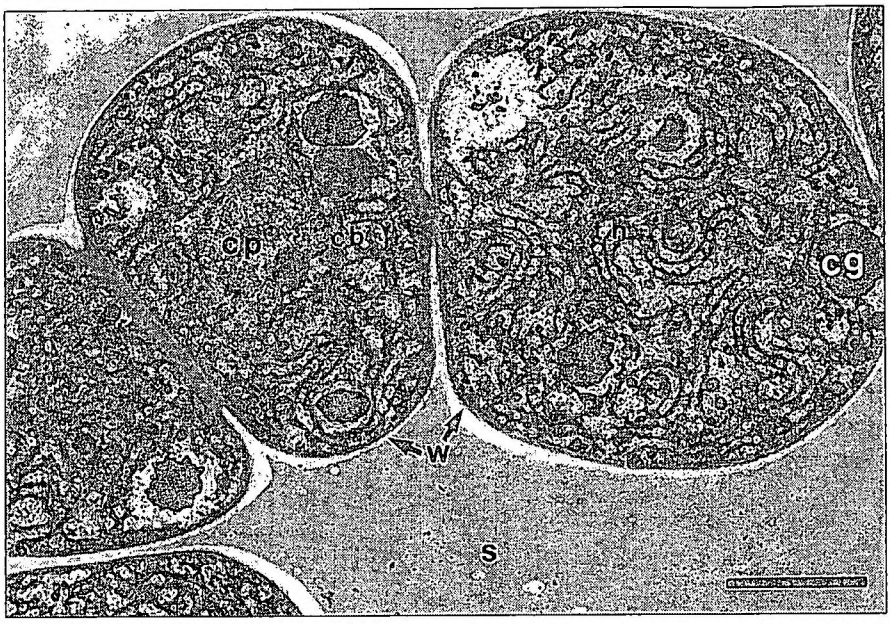

Fig. 2. Transmission electron micrograph of conventionally prepare (i.e. method does not support immunolabelling) cephalodial vegetative *Nostoc* cells. Abbreviations: see Fig. 1. Scale bar = 1 μm.

immunologically different and the antibody against root nodule specific GS2 (Cullimore & Miflin 1984) may not bind so closely to GS from the lichen cyanobacterium.

Acknowledgements

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JANUARY 1996 MEETING AT ASHTEAD COMMON

A field meeting to Ashtead Common, Surrey, will be held on Sunday, January 7 1996 (the day after the AGM). Ashtead Common is a promising area of open woodland near Leatherhead and should prove interesting especially since the levels of pollution in the area have been dropping. It has now been taken over by the Corporation of London and both they and Vikki Forbes, their local woodlands officer, are very interested in obtaining a lichen survey for the Common. We also hope to visit Ashtead church which has a large churchyard with gravestones dating back to the 17th century. We will, no doubt, also visit a local pub for lunch.

We will meet at about 10.15 am by the gate to the Common. This is opposite the level crossing next to the station (grid reference 181591). For those coming by train, the 9.19 from Victoria to Dorking arrives at Ashtead at 10.16.

Frank Dobson