The Use of Radioautography for Investigating Wall Secretion in Plant Cells

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Introduction

Classical histological procedures for radioautography involve coating mounted sections of radioactively-labelled tissue with photographic emulsion. Recent refinements to the technique have allowed it to be used successfully on specimens prepared for electron-microscopy. The results of such experiments can be of great significance to cytologists, since by observing the sites of incorporation of labelled metabolites within the cell, the possibility exists of relating ultrastructure to function and to the sites of some biochemical processes. In some cases, sequences of events can be inferred from the positioning within the cytoplasm of labelled material; consequently a time factor can occasionally be introduced into the otherwise static picture of cellular morphology generally produced by the electron microscopist. Interest in the technique has therefore been increasing, and the following paper presents some results, experimental considerations and conclusions that may be of interest to workers not familiar with the method.

Plant tissues often offer biologists excellent experimental material for attempting to relate cytoplasmic structure and function. In higher plants, the cytoplasm is normally contained within a rigid, or elastic/plastic cell wall which both dictates the shape of the cell, and also forms an integral unit in a clearly defined pattern of development (e.g. as in a root tip). The wall is secreted by the cell; both in chemical nature and morphological form, it can reflect profound and predictable changes resulting from the cells’ growth and differentiation, the predictability resulting from the cells’ position in the pattern of organisation. For example, Thornber and Northcote (1961 a, b, 1962) have described the changes in the chemical composition of the walls of cambial cells as they differentiate into phloem and xylem. Expansion of the wall during growth of the cell and later
secondary wall deposition, can be explained to some extent in terms of the chemical nature of the constituents of the wall (e.g. Northcote 1963a, b, Gould, Rees, Richardson, and Steele 1965). The electron microscopist can present an entirely different picture of wall formation. Study of the xylem cell for example shows how the form of its characteristic wall is built up.

It has long been postulated that secretion of some wall material could occur by reverse micro-pinocytosis, whereby vesicles containing wall precursor material are preformed in the cytoplasm, and then passed to the cell surface. Mollenhauer, Whaley, and Leech (1961) clearly demonstrated the hypertrophied nature of the Golgi apparatus, and its relationship to secretion of wall material in root cap cells. Other workers have described a similar process occurring in diverse plant cells (e.g. Sievers 1963, Whaley and Mollenhauer 1965, Gantt and Arnot 1965, Larson 1965, Wooding and Northcote 1964, 1965, Bisalputra, Ashton, and Weier 1966). However, the time sequence of events could not be established unambiguously, although the likelihood of a reverse process occurring (i.e. vesicles coming from the wall, and travelling to the Golgi apparatus) was obviously remote.

The introduction of radioautographic techniques has enabled clarification of some aspects of wall deposition. The following paper summarises the results of a series of comparatively simple experiments on several different plant tissues using tritiated glucose as precursors. In fortunate circumstances such work has even been extended to include a preliminary chemical characterisation of a secreted product (Northcote and Pickett-Heaps 1966).

Methods and Materials

Since these have been described in detail elsewhere (Northcote and Pickett-Heaps 1966), only a brief summary will be given.

Wheat seeds (Triticum vulgare) were germinated for appropriate periods on damp filter paper. D-glucose-6-H\textsuperscript{3} (1.5 C/mM) or D-glucose-1-H\textsuperscript{3} (550–500 mc/mM) were made up in glass distilled water to concentrations of between 0.25 and 5 mC/ml. Excised root tips and excised coleoptiles (ca. 0.5 cm long) were incubated in the radioactive solutions contained in small vials. Intact root-tips were placed in small drops of the radioactive solutions (ca. 0.05 ml) placed on glass slides; in pulse experiments, they were then rinsed and placed in a "cold" glucose solution (0.5–1 mg/ml).

Following the incubation procedures, the tissues were excised (if necessary) and fixed in either 4% unbuffered KMnO\textsubscript{4} containing NaCl, CaCl\textsubscript{2} (Pickett-Heaps and Northcote 1966) or in glutaraldehyde/osmium (containing ca. 0.001 M CaCl\textsubscript{2}; method of Ledbetter and Porter 1963). Following standard methods of dehydration, embedding in araldite, sectioning and staining with lead and/or uranium acetate, the sections were coated a second time with carbon (Koehler, Mühlethaler, and Frey-Wyssling 1963), to prevent leaching of the stain from the sections by