CHAPTER 1

# The Enzymology of Cell Organelles

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## I. Introduction

Within the last two years studies of the distribution of enzymes among the different organelles of plant cells and of the activities of individual types of organelle have been extensively reviewed (Bonner, 1965a, b; Hallaway, 1965; Lieberman and Baker, 1965; Koblitz, 1966; Mollenhauer and Morré, 1966; Hall and Whatley, 1967). Since much of the recent information about the isolation and activities of particular organelles is summarized and assessed in the later chapters, this chapter will be restricted almost entirely to discussing some technical aspects of the separation and analysis of organelles, noting especially those points which may cause uncertainty in assigning a site to a particular enzyme and how this uncertainty may be reduced.

## II. The Investigation of the Intracellular Locations of Enzymes

The most direct way to demonstrate unequivocally that an enzyme is present in a particular organelle is to isolate the organelle uncontaminated by other particles or fragments; to obtain a quantitative measure of the activity of enzyme, the organelle should be undamaged as well as uncontaminated. If, however, the organelles are isolated mixed with other subcellular particles then there will usually be some degree of doubt as to which of the enzymes

TABLE I

Distribution of nitrite reductase, nitrate reductase, fumarase and succinoxidase in preparations from leaves.

| *Zea mays** | | | | *Nicotiana tabacum*† | | | |
|---|---|---|---|---|---|---|---|
| | | Ratio of Activity | | | | Ratio of activity | |
| Fraction No. | Chlorophyll μg/ml | $NO_2$ reductase/ Chlorophyll | $NO_3$ reductase/ Chlorophyll | Fraction No. | Chlorophyll μg/ml | Fumarase/ Chlorophyll | Fumarase/ Succinoxidase |
| I | 37 | 0·07 | 0·4 | 1 | 160 | 0·04 | 0·60 |
| II | 13 | 0·07 | 0·8 | 2 | 180 | 0·07 | 0·50 |
| III | 8 | 0·08 | 1·3 | 3 | 130 | 0·13 | 0·27 |
| | | | | 4 | 59 | 0·34 | 0·32 |
| | | | | 5 | 36 | 0·33 | 0·29 |

* Taken from Ritenour *et al.* (1967).
† Calculated from Pierpoint (1962).

present in the fraction is located in each type of particle. This consideration is particularly important if the observed activity of the enzyme is low for low activity could be due to the enzyme being present either in trace amounts in a major component organelle or in larger amounts in a minor component organelle. Reducing the amount of damage which can occur to all types of organelle during homogenizing and fractionation reduces the degree of contamination of one type of particle with fragments of other types, as well as facilitating the study of the quantitative distribution of enzymes among the organelles. Therefore, any refinement of technique which leads to a reduction in damage to organelles, or to an increase in their purity, will be advantageous for localization studies (see de Duve, 1967).

It is also possible to assign an enzyme to a particular organelle if, in different subcellular fractions, the enzyme is present in constant proportion to an enzyme or other component which occurs exclusively in that organelle. For example, the consistency of the ratio of nitrite reductase to chlorophyll in three fractions prepared by a non-aqueous technique from leaves of *Zea mays* (Table I) shows that this enzyme is associated with the chloroplast (Ritenour *et al.*, 1967). Pierpoint (1962) found that the ratio of fumarase to succinoxidase varied little in fractions separated from a "mitochondrial" preparation from tobacco leaves by density gradient centrifugation (Table I). On the assumption that succinoxidase is a mitochondrial enzyme, he was able to conclude that fumarase was located in the mitochondria in the original preparation, even though it was heavily contaminated with chloroplast material. (Over 75% of the protein of the preparation was derived from chloroplasts.) This technique so far can be used only to identify enzymes localized in chloroplasts and mitochondria since these are the only organelles for which suitable reference compounds are known (chlorophyll and $NADP^+$ linked triose-phosphate dehydrogenase for chloroplasts, cytochrome oxidase and succinic oxidase for mitochondria; see also p. 7).

If, however, the ratio of enzyme to reference compound is not constant, this does not necessarily mean that the enzyme is localized outside the particle *in vivo* since it could have been dissociated from the reference compound during fractionation (see Bucke *et al.* (1966) for an example of this type of problem). Taken by itself, the variation in the ratio of fumarase to chlorophyll (Table I) in the "mitochondrial" preparation is not definitive evidence for the absence of fumarase from chloroplasts. However, the variation combined with the other information provided by Pierpoint (1962), i.e. 95% of the fumarase was in the mitochondrial fraction and the small amount present in the "chloroplast" fraction separated from the bulk of the chlorophyll on density gradient centrifugation, does indicate a non-chloroplast site for the enzyme. Nitrate reductase, also, appears to be located outside the chloroplast (see discussion by Ritenour *et al.*, 1967). In investigating

intracellular location of enzymes by this use of reference compounds, the possibility of adsorption of the enzyme on to the particle containing the "marker" may have to be considered (Siebert and Smillie, 1957; Jansen *et al.*, 1960).

### A. PREPARATION OF HOMOGENATES AND SEPARATION OF PARTICLES

The only new technique for separating particles which has been developed in the last few years is stable-flow free boundary sedimentation electrophoresis (Mel, 1964a, b, c) which separates organelles or whole cells by a combination of sedimentation and electric field. In the first application of this technique to organelles, Packer *et al.* (1966) were able to fractionate a chloroplast preparation within 30 min in a gradient of 0·2M to 0·3M sucrose into particles differing in size and activity. Earlier, Gross *et al.* (1964) had subjected chloroplast fragments to electrophoresis in a pH gradient and obtained particles differing in Hill reaction activity and Hannig *et al.* (1964) had succeeded in separating chloroplasts almost completely from mitochondria by electrophoresis. Of other techniques proposed for separating organelles, gel filtration (Tiselius *et al.*, 1963) cannot yet be employed since suitable gels are not available and counter current distribution does not appear to have been extended beyond the initial study by Albertsson and Baltscheffsky (1963). Zonal centrifugation, which permits the processing of relatively large amounts of tissue and which can separate particles on the basis of both sedimentation coefficient and equilibrium density, has not been used widely as yet for isolating plant organelles (Price and Hirvonen, 1966; Still and Price, 1966; Quigley and Price, 1966).

Recent refinements in the techniques for fractionating cells have yielded organelles much less damaged than those prepared by earlier procedures (as assessed using biochemical activity as the criterion for structural integrity) In most instances the degree of contamination is still considerable. Both Jensen and Bassham (1966) and Walker (1967) describe the isolation of highly active chloroplasts and Leech (1964) gives details of how to prepare intact and almost uncontaminated chloroplasts. Active mitochondria showing respiratory control have been isolated from a number of tissues; e.g. from sweet potatoes (Wiskich and Bonner, 1963), potato tubers (Verleur, 1965) and etiolated mung bean hypocotyls (Ikuma and Bonner, 1967). However, any alteration in the tissue, type of plant or growth conditions from those for which a particular procedure was developed may necessitate an alteration in the isolation technique in order to achieve the required type of preparation. Jagendorf (1955) found that chloroplasts from different species, or from leaves of the same species but of different ages, did not behave identically on density gradients so gradients which will separate one type of chloroplast may not separate another. Smillie (1956) showed it was possible to prepare mitochon-

dria from etiolated pea leaves only slightly contaminated with plastids but the same procedure applied to green leaves yielded mitochondria heavily contaminated with chloroplast material. The amount of lipid lost from chloroplast fractions during isolation varied with the tissue, the media and the method by which the leaves were homogenized (Jervis and Hallaway, see page 154).

Honda *et al.* (1966) have developed a medium containing sucrose, dextran, albumin and Ficoll, buffered with Tris. Mitochondria and chloroplasts isolated in this medium by their procedure retain the pleomorphic character they have *in vivo* and damage to nuclei is also reduced. Many of the procedures available for isolating relatively intact mitochondria, chloroplasts, nuclei and Golgi apparatus (Morré and Mollenhauer, 1964; Morré *et al.*, 1965) have in common the use of gentle methods for disrupting the cells, e.g. slicing the tissue up with razor blades and then floating out the organelles by stirring the finely sliced tissue in a suitable medium. Close control of pH is often critical, particularly during isolation of mitochondria (Wiskich and Bonner, 1963). Polyvinylpyrrolidone (PVP) in either water soluble form or as the highly cross-linked insoluble "Polyclar" or polyethylene-glycol (PEG) may be added to the isolation media to precipitate tannins (Loomis and Battaile, 1964; Hulme *et al.*, 1964; Badran and Jones, 1965) which otherwise can precipitate and partially inactivate enzymes (Goldstein and Swain, 1965).

The use of PVP or PEG while maintaining the activity of enzymes can give rise to difficulty in determining the intracellular site for enzymes. Lin and Hassid (1966), for example, found that the presence of PVP (at a final concentration of 5–10%) in the isolation media was essential to obtain preparations from *Fucus gardneri* which were active in forming GDP-mannose from mannose-l-phosphate and in oxidizing GDP-mannose to GDP-mannuronic acid. In the presence of PVP the activities were partially sedimented by centrifugation at 40,000 × *g* for 150 min and much of the sedimented activity could be removed by one wash in 0·1M Tris buffer. If the PVP were replaced by Polyclar then the activities remained in the supernatant after centrifugation at 2000 × *g* for 120 min. Sanderson (1964), in a study of the localization of catechol oxidase in tea shoots, observed that adding an insoluble polyamide to the isolation media increased the total activity of the homogenate. In the absence of polyamide all the activity was sedimented in 2 min at 2000 × *g* (Table II) and this activity was not removed by washing. On grinding the tea shoots in the presence of increasing amounts of polyamide, the activity was progressively shifted to the low speed supernatant but, at ratios of amide to tissue above 0·75, the activity in the sediment began to increase again. This sedimented activity was removed by one wash with buffer in the absence of polyamide. An explanation for these observations may lie in the fact that both PVP and PEG can precipitate proteins as a rule at concentrations higher

than the 1–2% normally used to precipitate tannins. The amount of protein precipitated is affected by pH and by the nature and concentration of the ions, proteins and PVP or PEG present in the solution (Zeppezauer and Brishammer, 1965). The precipitated proteins are readily re-dissolved after removing the polymer. In the case of the catechol oxidase of tea shoots, at low concentrations the polymer appeared to be preventing the inactivation

TABLE II

Effect of polycaprolaktam (polyamide) powder on the activity, distribution and recovery of catechol oxidase from tea shoots (calculated from Sanderson, 1964).

| Experiment No. | Level of polyamide in extraction media (g/g fresh wt tissue) | Relative catechol oxidase activity (activity in homogenate without polyamide = 100) | | | |
|---|---|---|---|---|---|
| | | Homogenate | | Fractions Precipitate (2000 × g; 2 min) | Supernatant |
| 1 | 0 | 100 | (61·5)* | 66 | 0 |
| 2 | 0 | 100 | (71·5)* | 78 | 0 |
| 3 | 0 | 100 | (65·1)* | 83 | 0 |
| 1 | 0·25 | 113 | | 81 | 0 |
| 2 | 0·5 | 122 | | 60 | 23 |
| 3 | 0·75 | 141 | | 6 | 80 |
| 1 | 1·0 | 160 | | 8 | 84 |
| 3 | 1·5 | 161 | | 11 | 90 |

* μMoles of catechol oxidized per g fresh wt tissue $min^{-1}$.

and irreversible precipitation of the enzyme caused by tannins but at high levels the polymer itself brought about a reversible precipitation of the activity. Similarly the high levels of PVP necessary to give maximum activity in Lin and Hassid's (1966) preparations may have precipitated the enzymes but the insoluble "Polyclar" seemed to have a predominantly protective effect. From the point of view of determining intracellular localization, it would seem useful if, when PVP or PEG are included in isolation media, the effect on enzyme distribution of varying their concentration and of replacing them with "Polyclar" were determined.

### B. ASSESSMENT OF CONTAMINATION AND DAMAGE

The usual methods for assessing the degree of contamination or damage to subcellular particles are examination in the light or electron microscope and chemical or enzyme analysis. The presence of some component which is restricted to one organelle or part of the cell can be used as an index of the

degree of contamination of a preparation by that organelle. For example, the determination of pyruvic kinase in chloroplast preparations will give a measure of cytoplasmic contamination (Heber, 1960) and chlorophyll can be used as a measure of the amount of chloroplast material. Similarly, in a preparation of some organelle, the level of a component or activity which is readily lost from it during its separation can indicate the integrity of the organelle; e.g. mitochondria in which phosphorylation and oxidation are tightly coupled are regarded as relatively undamaged.

The number of reference compounds and activities suitable for assessing damage and contamination is rather limited. Reference compounds for mitochondria and chloroplasts are mentioned earlier (p. 3). DNA is concentrated in the nucleus but it is also present in minor amounts in both chloroplasts and mitochondria; however, the presence of more than a small amount of DNA in a preparation is indicative of nuclear contamination. RNA is present in so many organelles (nuclei, mitochondria, chloroplasts and cell wall) as well as in cytoplasmic ribosomes that it can be used only as a measure of gross contamination with ribosomal material. Although cellulose is restricted to the cell wall, it is not simple to assay and does not appear to have been used as a reference compound for cell walls.

Even in those cases where a component is restricted to the one type of organelle the measurement of the level of the reference compound can give only an indication of the range of contamination, not of its precise degree. This follows from the fact that enzymes or other components associated with the marker *in vivo* may become dissociated from it to differing degrees *in vitro*. In chloroplasts, for example, the enzymes catalysing $CO_2$ fixation are much more readily lost than are the enzymes catalysing the Hill reaction (Smillie, 1963) so the amount of chlorophyll in a preparation provides only an approximate guide to the amount of protein of chloroplast origin in the preparation. If the protein/chlorophyll ratio for intact chloroplasts and for washed chloroplast fragments are known for the species concerned (see Leech (1966) for values for *Vicia faba*) then the maximum and minimum values for chloroplast protein can be calculated from the chlorophyll levels. In many cases this refinement may not be necessary but, for those enzymes which occur in more than one sub-cellular location, such quantitative assessment of degree of contamination may be useful for determining the precise distribution of activity within the cell.

### C. THE PRESENCE OF BACTERIA

Sub-cellular fractions from plant tissues frequently contain relatively large numbers of bacteria. Table III lists the total colonies grown from four sub-cellular fractions from three types of leaf after homogenizing in different buffers. The number of colonies is large and very variable; there were 3 to 6

TABLE III

Number of bacterial colonies grown from leaf preparations. The washed leaves were ground in 4 vols of buffer, the homogenate was strained through muslin and centrifuged to give 3 fractions and a final supernatant. Samples of each fraction, the supernatant and the buffer were plated on nutrient broth plates and counted after 72 hr at 34°C.

| Species | Weight g | Buffer*,† | Total colonies × $10^{-6}$ Material sedimented at | | | |
|---|---|---|---|---|---|---|
| | | | 200 × *g* 5 min | 1000 × *g* 10 min | 20000 × *g* 30 min | Supernatant |
| *Beta vulgaris* | 8 | Tris-sucrose | 72 | 37 | 6 | 6 |
| *Beta vulgaris* | 11 | Sucrose-phosphate | 67 | 190 | 30 | 45 |
| *Beta vulgaris* | 10 | Sucrose-phosphate (sterile) | 627 | 260 | 459 | 180 |
| *Beta vulgaris* | 4 | Honda | 125 | 133 | 47 | 49 |
| *Phaseolus vulgaris* | 9 | Sucrose-phosphate (sterile) | 1146 | 1050 | 305 | 52 |
| *Phaseolus vulgaris* | 5 | Tris-sucrose (sterile) | 220 | 11 | 29 | 1 |
| *Phaseolus vulgaris* | 4 | Honda | 9 | 2 | 27 | 3 |
| *Vicia faba* | 15 | Sucrose-Phosphate (sterile) | 10 | 210 | 100 | 207 |

* Buffers: Honda *et al.* (1966) pH 7·0;
Tris-sucrose, 0·025 M Tris-HCl and 0·25 M sucrose, pH 7·8;
Sucrose-phosphate, 0·01M phosphate and 0·2M-sucrose pH 7·1.

† Maximum number of colonies derived from non-sterile buffers was $10^5$.

distinct types of colony and the proportion of the types varied from fraction to fraction and from day to day. Freshly prepared but non-sterile buffers did not contribute significantly to the bacterial contamination, the maximum number of colonies derived from the buffer being about $10^5$.

The levels of bacterial infection recorded in literature are the same order, i.e. $10^8$ per mg chlorophyll in chloroplast preparations (App and Jagendorf, 1964), $2 \times 10^6$ per mg protein bodies (Wilson, 1966), $30 \times 10^6$ per disc of sugar beet (Leaver and Edelman, 1965). A rat liver mitochondrial preparation (Roodyn *et al.*, 1961), however, contained fewer—about $10^5$ ml. B. Gyldenholm (private communication) has also found considerable bacteria contamination of chloroplast preparations ranging from 75 to 600 chloroplasts per bacterium.

The significance of bacterial infection in the study of protein synthesis by subcellular fractions is discussed by Cocking (page 198) and by App and Jagendorf (1964) and Wilson (1966). It is relevant to note that Wheeldon (1966), using a rat liver mitochondrial preparation containing $10^6$ bacteria, found that 90% of the amino acid incorporation observed was attributable to the bacteria which had pre-empted the amino acids. The presence of bacteria in large numbers may also interfere with the study of processes other than protein synthesis. The $QO_2$ (dry wt) of bacteria is 10 to 100 times greater than that of plant tissues so infection by bacteria could contribute significantly to the oxygen uptake of tissue slices or of subcellular fractions. Heavy bacterial infection ($10^{10}$ bacteria) would add about 500 $\mu$g RNA (including ribosomal and transfer RNA) and 100 $\mu$g DNA to a subcellular fraction (McQuillen, 1965). Finally, as noted earlier, low enzyme activities exhibited by a fraction containing a mixture of organelles cannot easily be assigned to one or other of the components and the possibility that bacteria may contribute to some of the activities may have to be considered.

Ways to reduce or eliminate bacterial infection of preparations have been investigated repeatedly. In Wilson's (1966) experiments, the use of sterile media reduced the number of bacteria 100-fold but in the experiments summarized in Table III the infection did not come from the media but from the plants themselves. App and Jagendorf (1964) also considered that the plant material was a major source of infection, particularly if the tissues were bought, and they contrasted the bacterial count in their preparations with the much lower levels of Stephenson *et al.* (1956). Leaver and Edelman (1965) surveyed a range of antibiotics for use in controlling bacterial infection of beet discs; chloramphenicol (at 50 $\mu$g/ml) in the media in which the discs were aerated almost entirely eliminated bacteria without altering the metabolic processes they examined. None of the other antibiotics, which included penicillins, tetracycline, neomycin, terramycin and polymixin, were satisfactory.

Chloramphenicol (at 50 $\mu$g/ml) inhibited amino acid incorporation by contaminated protein bodies but did not reduce the bacterial count (Wilson, 1966). In Wheeldon's (1966) experiments with rat liver mitochondria, chloramphenicol at this concentration inhibited amino acid incorporation by the mitochondria themselves. Although antibiotics are useful in preventing extensive growth of bacteria in prolonged experiments, taking into account the number of types of bacteria which may be present and their probable differences in sensitivity to antibiotics as well as the multiple effects of many antibiotics on biological systems, the use of sterile tissue as recommended by Cocking (p. 198) and Boulter (1965) seems the ideal.

Bacteria stick firmly on to subcellular particles and cannot be removed completely by washing or by differential or density gradient centrifugation (App and Jagendorf, 1964; Boulter, 1965; B. Gyldenholm, personal communication; L. Jervis and M. Hallaway, unpublished work).

If non-sterile tissues are used, the degree of bacterial contamination is readily measured and, if necessary, suitable tests can be carried out to exclude the possibility that an observed activity or component is bacterial in origin (see Van Huyster and Cherry, 1966; Wheeldon, 1966; Phethean *et al.*, 1968). If subcellular fractions are prepared by non-aqueous techniques most of any bacteria present will be rendered non-viable and to measure the degree of contamination it would be necessary to estimate some bacterial component, possibly $\alpha$, $\varepsilon$-diaminopimelic acid (Holm-Hanson *et al.*, 1965).

## D. ASSAY OF THE ENZYMES

### 1. *Latent Enzymes*

The determination of the quantitative distribution of enzyme activity among subcellular organelles can be complicated by the existence of latent activity and by the related phenomenon of the changes in enzyme activity which may occur when an enzyme is bound to (or released from) subcellular particles. The question of latent enzymes in connection with the occurrence of lysosomes in plants is discussed fully by Gahan in a later chapter (p. 228). Siekevitz (1962) has summarized the effects of siting enzymes in membrane systems but there have been so far only a few studies on the changes in enzyme activity consequent on binding or release from particles, although the degree of change is often striking (Table IV).

Arnold (1965) has shown that about 25% of the $\beta$-fructofuranosidase of grape berries is recovered in the cell wall fraction; if the walls were treated with 0·2M borate at pH 8·5, $\beta$-fructofuranosidase was dissolved from the wall and the total activity (i.e. dissolved plus a trace remaining in the wall) was 90% higher than the original activity in the wall fraction (Arnold, 1966a). The increase in activity was not due to a fall in Km of the enzyme on changing from a bound to a free state, since both bound and solubilized activities have

approximately the same Km and Vmax (Arnold, 1966b) and both were assayed at saturating levels of sucrose. Like β-fructofuranosidase, some of ascorbic oxidase activity of cell walls is masked and has been released by treating the cell walls with purified cellulase (J. Taggart and M. Hallaway, unpublished results) and with a consequent increase in total activity.

Cell walls and other subcellular particles can bind soluble enzymes and this may be accompanied by a change in activity. When soluble ascorbic oxidase is bound to cell walls, we have found a small but reproducible drop

TABLE IV

| Enzyme | Treatment | Activity change |
|---|---|---|
| β-Fructofuranosidase (Arnold, 1966) | release of cell-wall enzyme by 0·2M borate | +90% |
| ADPG-starch transglucosylase (Akazawa and Murata, 1965) | adsorption of soluble enzyme on to starch grains | −15% |
| Ascorbic oxidase | release of cell-wall enzyme by cellulase | +60% |
| Ascorbic oxidase | adsorption of soluble enzyme on to cell walls | −15% |
| Adenosine triphosphatase (Brown *et al.*, 1966) | binding of soluble enzyme on to cellulose matrix | −50% |
| Hexokinase (Siekevitz, 1962) | adsorption of soluble enzyme on to mitochondria | +100% |

in activity which is not attributable to denaturation or change in Km and a similar fall in activity was noted by Akazawa and Murata (1965) when the soluble ADPG-starch transglucosylase was adsorbed on to starch grains. Potato adenosine triphosphatase showed a drop in activity and a change in pH optimum on binding to a cellulose matrix (Brown *et al.*, 1966). Adsorbing enzymes on to particles does not always produce a reduction in activity since the activity of hexokinase was increased by up to 100% by adsorption on to mitochondria (Siekevitz, 1962, Ruchti and McLaren, 1965). The explanation for the change in activity in these experiments is not known.

### 2. *Effects of Ions*

An effect of ions on the localization of enzymes has occasionally been noted. Lipetz and Garro (1965), for example, observed that the cell walls of crown

gall contained a peroxidase which was released by ions (in order of effectiveness $Ca^{2+} > Ba^{2+} > Mg^{2+}$) and they suggested that the concentration of ions could control lignin formation by controlling peroxidase location. The adsorption of phosphorylase on to starch grains *in vitro* is promoted by a number of ions including $Na^+$ $Cl^-$, $NH_4^+$ and $Na^+$ $EDTA^-$ and is inhibited by sucrose. It is possible that the location of phosphorylase *in vivo* is regulated by the relative concentrations of ions and sucrose (de Fekete, 1966). The localization of phosphorylase is uncertain; most of the phosphorylase of leaf homogenates remains in the supernatant after centrifuging down the plastids but, if non-aqueous techniques are used, about half the phosphorylase is recovered with the chloroplasts (Stocking, 1959). The phosphorylase activity of starch grains was not investigated in these experiments.

## III. Localization of Enzymes by Cytochemical Techniques

Although there have been a few studies of the intracellular localization of enzymes in plants using cytochemical techniques, the results obtained from such studies can remove much of the uncertainty about the subcellular location of enzymes studied in cell free preparations (see Chapter 13 for a review of investigations on lysosomal enzymes using cytochemical techniques). Recent work on the localization of the pectin synthesizing enzymes illustrates this point well. Many of the enzymes involved in pectin synthesis are present in a fraction sedimented from homogenates of mung bean seedlings in 10–20 min by forces between 10,000 × *g* and 35,000 × *g*. These enzymes include those catalysing (*a*) the conversion of UDP-glucuronic acid to UDP-galacturonic acid (Feingold *et al.*, 1961); (*b*) the incorporation of UDP-galacturonic acid into pectin (Villemez *et al.*, 1965) and (*c*) the methylation of pectin (Kauss *et al.*, 1967). The same fractions also catalyse the incorporation of GDP-glucose into cellulose and GDP-mannose into glucomannan (Barber *et al.*, 1964; Elbein and Hassid, 1966). This fraction is likely to contain fragments of membrane systems and nuclei, mitochondria and plastids as well as some cell wall material (Bailey *et al.*, 1967). In 1961 Mollenhauer *et al.* suggested that the Golgi apparatus was implicated in the synthesis of some cell wall components and in 1966 Northcote and Pickett-Heaps demonstrated by radioautography that tritiated glucose, fed to root tips of wheat, is rapidly incorporated into a high molecular weight material in the Golgi apparatus. Much of the activity is in galactosyl residues and this is incorporated into the cell wall and slime layer. This cytochemical study both compliments and extends the work on isolated cell free preparations and suggests that in the mung bean preparation it may be the fragments of Golgi apparatus that carry the pectin synthesizing enzymes. In a preliminary study of isolated dictyosomes from the Golgi bodies of mung bean and onion,

Morré (1966) noted that they accumulated lipids, apparently in the membranes, and glucose, apparently in the cisternal lumen.

## IV. Conclusions

The precise intracellular distribution of many enzymes is unknown and difficulties in determining distribution may arise partly from the techniques used to isolate and assay the organelles and partly from features of the binding of the enzyme to particular sites *in vivo*. Ideally, the study of the enzymology of organelles requires the use of cytochemical techniques as well as the analysis of subcellular particles.

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