# ORGANIZATION RESISTANCE AND THE RESPIRATION CLIMACTERIC

By JOAN M. BAIN* and F. V. MERCER*

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*Summary*

Electron micrographs showed extensive disorganization of the protoplasts of Williams pear tissue during ripening. These changes in ultrastructure, characterized by vacuolation of the cytoplasm and its inclusions, were correlated with existing data for respiration during ripening of Williams pears.

Protoplasts of mature but unripe fruit showed slight signs of vacuolation when compared with developing fruit, but disorganization was not obvious until after the initial climacteric period. As the climacteric state progressed, disorganization was extensive; vacuoles increased in size and number. Plastid structure was almost destroyed in the ripe fruit, and the cytoplasm was a mass of small, membrane-bounded vacuoles. Mitochondria did not show appreciable breakdown until the post-climacteric stage.

This extensive loss of organized structure, and the rearrangement and apparent increase of cytoplasmic membranes accompanying ripening, raises the possibility that membrane barriers, by influencing the distribution and orientation of enzymes and substrates, may be important in controlling metabolic rates, and these observations give credence to Blackman's concept of organization resistance.

Data showing impedance changes in ripening pear tissue are appended; these were consistent with the cell membranes becoming more permeable during ripening.

## I. INTRODUCTION

Blackman and Parija (1928) suggested that the respiratory activity of a cell containing excess substrates is controlled by the spatial distribution of enzymes and reactants within the protoplast by semipermeable protoplasmic membranes, and they described this type of control as organization resistance. Although this concept proved useful in describing the respiratory drifts of senescing organs it gradually became neglected, for a variety of reasons, in favour of biochemical theories of the climacteric. One such reason was that plant cytoplasm seemed to be a relatively homogeneous monophasic system which, therefore, could not provide a structural basis for organization resistance.

Recent electron microscope studies, however, have shown that the plant protoplast has an elaborate ultrastructure with many membrane-bounded regions, and this reopens the possibility of organization resistance being a factor affecting metabolic rates. For this reason a submicroscopic examination was made of ripening pear tissue to determine whether or not correlations exist between metabolic processes and cytoplasmic structure. The Williams variety of pear was very suited to this approach. It has a short ripening period during which clear-cut colour changes

* Plant Physiology Unit, Division of Food Preservation, CSIRO, and School of Biological Sciences, University of Sydney.

accompany the marked climacteric rise in respiration, with the maximum respiration of the fully ripe fruit reaching approximately five times that of the green fruit (Bain 1961).

The present studies have shown that an extensive breakdown of structural organization occurs during this period, and consequently alterations in the spatial distribution of enzymes and reactants during the climacteric seem highly probable. These observations give cytological credence to the concept of organization resistance.

## II. Material and Methods

Immature, commercially mature, and post-harvest fruit ripening at 20°C after an initial period in cold storage, were used. A ripening series of hard green, hard yellow-green, firm pale yellow, soft deep yellow, and overripe fruit was obtained. These categories, shown in Figure 1, corresponded to the pre-climacteric, initial, mid-, peak, and post-climacteric condition in previous observations (Bain 1961).

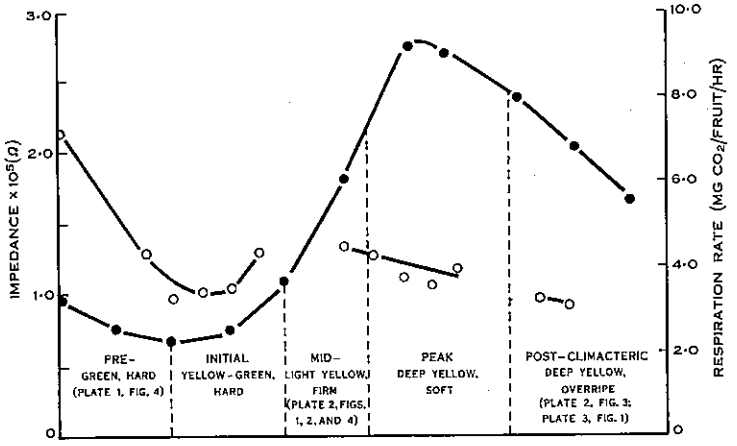


Fig. 1.—Correlation of changes in colour and texture with changes in respiration data at 20°C (●—●) (Bain 1961) and with impedance values measured at 1 kc/s at 23·0–26·7°C (○—○) in ripening Williams pears. Reference is made to electron micrographs which show changes in ultrastructure.

Pieces of tissue, approximately 1 mm$^3$, were removed from the fruit surface and fixed for 4 hr at approximately 5°C in a buffered 1% osmium tetroxide solution (Palade 1952). The tissue was dehydrated in an ethanol series, placed in xylol, and embedded in Araldite, using the procedure of Mercer and Birbeck (1961). Sections were cut on a Porter–Blum microtome with a diamond knife and examined in a Siemens Elmiskop I electron microscope. Fixation difficulties were sometimes encountered due to the large vacuole in the cells. The presence of groups of thick-walled stone cells in the tissue was frequently detrimental to embedding and section cutting.

Observations were confined to the hypodermal and outer flesh tissue since the larger flesh cells, with a very thin layer of cytoplasm surrounding a very large vacuole, are unsuited to electron microscopy. The hypodermal and outer cortical cells, though

often highly vacuolated, have a greater proportion of cytoplasm and a greater variety of organelles, being the photosynthetic cells of the fruit. Ultrastructure changes in the fruit surface have been correlated with colour changes in ripening fruit, and thence with respiration data for the whole fruit, on the assumption that all tissues would show similar metabolic trends during the climacteric.

## III. Results

### (*a*) *Protoplasts of Immature Fruit*

The vacuole occupied most of the volume of the protoplasts in fruit developing on the tree. In section, the cytoplasm formed a thin layer, 0·1–0·3 $\mu$ thick, lining the cell wall, with occasional expansion into areas up to 1·0 $\mu$ thick (Plate 1, Fig. 1). The cytoplasmic matrix, bounded by the plasmalemma and tonoplast, was granular and contained very small vacuoles and occasional paired membranes, possibly part of the endoplasmic reticulum. Mitochondria and plastids were found in the thicker areas (Plate 1, Fig. 2). A nucleus was rarely seen in these cells. Patches of electron-dense material in the vacuoles were probably derived from the reaction of osmium with tannins or polyphenols believed to be located in these hypodermal cells.

The mitochondrial profiles were circular with diameter from 0·3–0·8 $\mu$, or elongated with the long axis approximately three times the short axis. The largest mitochondria had a long axis approximately 1·0 $\mu$. The cristae appeared circular to tubular in section and were scattered fairly uniformly through the mitochondria. A homogeneous material was commonly present between the cristae. The mitochondrial membrane usually appeared as a single structure but was sometimes seen to be double. The chloroplasts were elliptical, disk-shaped bodies about 2·0–4·0 $\mu$ long and bounded by an apparently continuous membrane (Plate 1, Fig. 3). Their ultrastructure was poorly developed when compared with that of a chloroplast from a true photosynthetic tissue. The lamellae system was made up of a network of numerous lamellae running roughly parallel to the long axis, with their spacing varying from less than 0·01 to 0·1 $\mu$. Some of the lamellae were grouped together at intervals to form regions resembling poorly developed grana, but it was extremely difficult to decide the relative proportion of grana and intergrana lamellae in these plastids. The parallel arrangement of the lamellae was frequently distorted by the presence of starch grains and small osmiophilic bodies in the stroma.

### (*b*) *Protoplasts of Mature Pre-climacteric Fruit*

Slight changes in submicroscopic structure were detected in protoplasts of hard green, mature fruit following removal from the tree and also after cold storage. The small vacuoles of the cytoplasm appeared to be larger; spaces between the lamellae of some plastids had increased; osmiophilic bodies in the plastids had increased in size and number (Plate 1, Fig. 4).

### (*c*) *Protoplasts of Mature Climacteric Fruit*

During ripening the slight breakdown detected in the mature cells became more extensive, leading to an almost complete disorganization of the protoplast. Loss of

structure in the chloroplasts and cytoplasm became evident at about the same time, obvious breakdown coinciding with the colour change in the skin and therefore with the beginning of the climacteric rise in respiration. The colour change was coincident with an increase in the number and size of the osmiophilic bodies in the chloroplasts; these bodies, which already numbered approximately six to each plastid section in the hard green, preclimacteric fruit, increased to eight or ten in the fully ripe fruit, with their diameter increasing from 0·2 to 0·3 $\mu$. Similar bodies isolated from spinach chloroplasts by Murakami and Takamiya (1962) were shown to be carotenoid in composition.

It was rather difficult to follow the sequence of disorganization in the poorly developed chloroplasts of pear during ripening. The plastids appeared swollen and to be highly vacuolated in the protoplasts of firm yellow fruit (mid-climacteric) (Plate 2, Figs. 1 and 2). It was uncertain whether these vacuoles originated in the stroma or in the regions of the poorly developed grana or lamellae, but from the location of the osmiophilic bodies the former view was considered more likely. In soft overripe fruit (post-climacteric) the chloroplasts were completely disorganized into a mass of irregular membranous structures and numerous, apparently unaltered, osmiophilic bodies. These bodies indicated the position of former chloroplasts (Plate 2, Fig. 3).

Vacuoles formed in the cytoplasm possibly from the separation of the parallel membranes of the poorly developed endoplasmic reticulum, from the fusion of the small vacuoles of the cytoplasmic matrix, or *de novo* (Plate 2, Fig. 4). Mitochondria showed no appreciable disorganization even in the ripe fruit; their structure, though somewhat disorganized, was still recognizable in the overripe, post-climacteric cells (Plate 2, Fig. 3; Plate 3, Fig. 1). The fate of the plasmalemma was difficult to follow as disorganization advanced. The plasmalemma appeared to be more stable than the tonoplast, though it was possible for the tonoplast to appear intact even though the climacteric was advancing (Plate 2, Fig. 2).

## IV. Discussion

The electron micrographs show that the climacteric rise in respiration is coincident with an extensive disorganization of the elaborate ultrastructure of the protoplasts. At the structural level, this breakdown appears to be due to extensive vacuolation resulting in rearrangement of the membrane systems. It is essential to know whether these changes in fine structure are real, and if so, whether they are the cause of the climacteric rise, or whether they are brought about by metabolic changes associated with the climacteric.

On the basis of the structure of the mitochondria and plastids in the preclimacteric and climacteric fruit, it is concluded that the structural changes are real, and not the result of an increasing inability to fix the tissue as senescence advanced. Mitochondria and plastids are sensitive indicators of conditions of fixation and their structure is easily altered by poor fixation. Since both these organelles have the same basic structure in pre-climacteric and ripening tissue, and the mitochondrial structure is well preserved even in post-climacteric fruit, it seems unlikely that the extensive vacuolation was an artefact of fixation.

The phenomenon of vacuolation is not well understood. However, there is evidence that the direct cause is an uptake of water, due to a change in osmotic conditions caused by an increase in osmotic pressure of the cytoplasm or by a change in the cohesive properties of the protoplast, or by both of these factors. Such changes could occur at the climacteric, allowing the cytoplasm to swell at the expense of the vacuole, and presumably lead to the vacuolation of the cytoplasm.

One might expect the organelles to shrink if the osmotic pressure of the cytoplasm increased markedly. The mitochondria, however, underwent little change until the late climacteric when they became swollen, while the plastids swelled throughout the climacteric to become completely disorganized by the late climacteric. Such changes would occur if the chloroplast membrane became permeable early in the climacteric and the mitochondrial membrane permeable towards the end of the climacteric, though a swelling of the organelles could result from the gradual loss of structural molecules.

Progressive vacuolation or vesiculation of the cytoplasm and organelles, which characterized the climacteric breakdown, is by no means unique to aging cells, for vacuolation is observed in tissues subjected to a wide range of abnormal conditions, including exposure to hypotonic solutions (Plate 3, Fig. 2), freezing and thawing (Plate 3, Fig. 3), high concentrations of some ions (Plate 3, Fig. 4), chloroform vapour (Plate 3, Fig. 5), fungal invasion (Plate 3, Fig. 6), or from poor fixation and mechanical shearing. In each of these conditions, the osmotic pressure of the cytoplasm increases due to the leakage of vacuolar sap following the loss of permeability of the tonoplasts or, as in Kappen plasmolysis, to a decrease in the cohesive forces of the cytoplasm. With existing electron-microscopy techniques the pattern of vacuolation induced by a variety of treatments appears identical, suggesting that vacuolation may be a generalized response to altered osmotic conditions.

The existence and breakdown of an elaborate ultrastructure and the changes in cytoplasmic membranes raise the possibility of membrane barriers affecting metabolism during the climacteric. The mechanism of respiratory control in fruit is not clearly established (Varner 1961). Control could be excercised by the level of nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, adenosine diphosphate, and inorganic phosphate, and by the availability of substrates. Marked changes in cytoplasmic organization, by altering the permeability of the cells and orientation of enzymes, could lead to changes in the availability of either cofactors or substrates for respiration. Thus it is possible that the observed changes in the cytoplasm caused the climacteric rise in respiration, rather than that they were caused by changes in respiratory metabolism. Sacher (1962) also related the climacteric rise to changes in membrane permeability.

The present observations, though not clearly distinguishing between cause and effect, do indicate that organization resistance could be a significant factor in the control of metabolism in plant cells. Membranes are a conspicuous feature of cell structure, and during the climacteric cytoplasmic membranes are changed and reorientated, and possibly arise *de novo*. As current emphasis is towards biochemical mechanisms of control, other mechanisms tend to be overlooked. The present paper aims at refocussing attention on Blackman's concept of organization resistance.

## V. Acknowledgments

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## VI. References

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## Appendix

The following data support the postulate that changes in permeability of cell membranes occur during ripening. An electrode was used to measure changes in the electrical behaviour of the surface tissue of a Williams pear during ripening after cold storage. Measurements of resistance and capacitance were made twice daily at room temperature (23–27·5°C) in a frequency range of 0·3–30 kc/s. The electrode was removed after each series of readings.

There was little overall change in resistance and capacitance, and therefore in impedance, at high frequency; changes were noted at low frequency. The changes at 1 kc/s are given in Table 1; impedance changes are also given in Figure 1, in relation to fruit colour and to changes in respiration and ultrastructure in ripening pear fruit. During the first 3 days, while the fruit was still hard and green, there was a change of about 50% in all readings, capacitance showing an increase and resistance and impedance a decrease. The values showed little further change as the fruit turned yellow-green (initial climacteric) and then became fully ripe (peak climacteric).

Bean, Rasor, and Porter (1961) have reported an inverse correlation between changes in impedance and respiration in ripening avocado pears and noted a slight rise in impedance during the pre-climacteric period, followed by a steady and continuous fall as the climacteric progressed. Their data, like those for the Williams pear,

suggest that the changes in impedance, which were due to changes in capacitative reactance which arise from the effects of semipermeable membranes and from interfaces between phases, could indicate increasing permeability during ripening.

Although the time sequences of the impedance changes in the Williams pear and the avocado, respectively, were not identical, the electrical data appear to support the assumption that increasing permeability of the cell membranes may be responsible for increased respiration following breakdown of organization resistance during ripening.

TABLE 1

CHANGES IN RESISTANCE, CAPACITANCE, AND IMPEDANCE IN RIPENING WILLIAMS PEAR TISSUE

Data are for measurements at 1 kc/s, and are averages of morning and afternoon readings

| Days of Ripening | Resistance ($\Omega$) | Capacitance ($\mu$F) | Impedance $\times 10^5$ ($\Omega$) | Colour | Temperature (°C) |
|---|---|---|---|---|---|
| 0 | 82,000 | 787 | 2·18 | Green | 26·7 |
| 3 | 45,800 | 1338 | 1·31 | Yellow-green | 26·1 |
| 4 | 36,500 | 1765 | 0·98 | Yellow-green | 26·5 |
| 5 | 40,000 | 1638 | 1·04 | Yellow-green | 26·7 |
| 6 | 42,500 | 1663 | 1·05 | Yellow-green | 25·8 |
| 7 | 51,250 | 1375 | 1·33 | Yellow | 25·2 |
| 10 | 52,350 | 1360 | 1·37 | Yellow | 24·3 |
| 11 | 50,550 | 1375 | 1·28 | Yellow | 24·1 |
| 12 | 46,150 | 1553 | 1·13 | Yellow | 25·7 |
| 13 | 40,000 | 1575 | 1·07 | Bruising obvious | 23·0 |
| 14 | 44,150 | 1463 | 1·19 | Bruising obvious | — |
| 17 | 35,250 | 1750 | 0·99 | Bruising obvious | 25·5 |
| 18 | 33,450 | 1900 | 0·91 | Bruising obvious | 25·5 |

## EXPLANATION OF PLATES 1–3

All figures, except Plate 3, Figure 4, are electron micrographs of material fixed in 1% buffered osmium tetroxide, embedded in Araldite, and sectioned; material in Plate 3, Figure 4, was fixed in 1% potassium permanganate

### PLATE 1

Figures 1–3: immature pear fruit; Figure 4: mature, pre-climacteric green pear

Fig. 1.—A hypodermal cell showing the relative proportions of cytoplasm (*Cyt*) and vacuole (*Vac*). The aggregated material (*A*) in the vacuole was probably derived from the reaction of osmium with tannins or polyphenols. Part of an epidermal cell (*E*) and the cuticle (*C*) are shown; the cell wall (*CW*) is indicated. ×5000.

Fig. 2.—The detailed structure of cytoplasm as in Plate 1, Figure 1. The granular cytoplasmic matrix (*Cyt*) contains very small vacuoles and a few paired membranes of the endoplasmic reticulum (*ER*). The cytoplasm is bounded by the plasmalemma (*P*) beneath the cell wall (*CW*) and by the tonoplast (*T*) enclosing the vacuole (*Vac*). Mitochondria (*M*) are shown. ×30,000.

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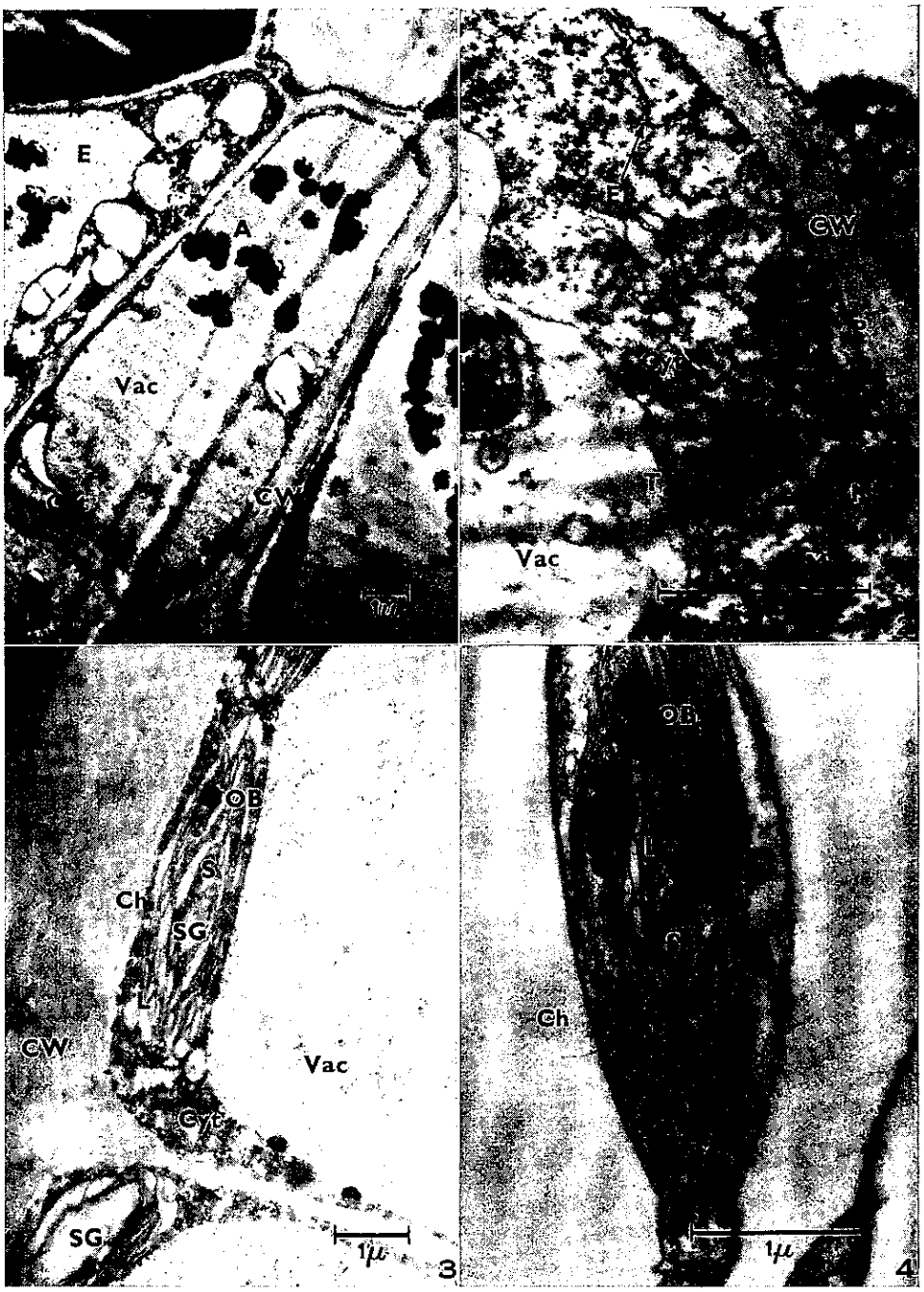

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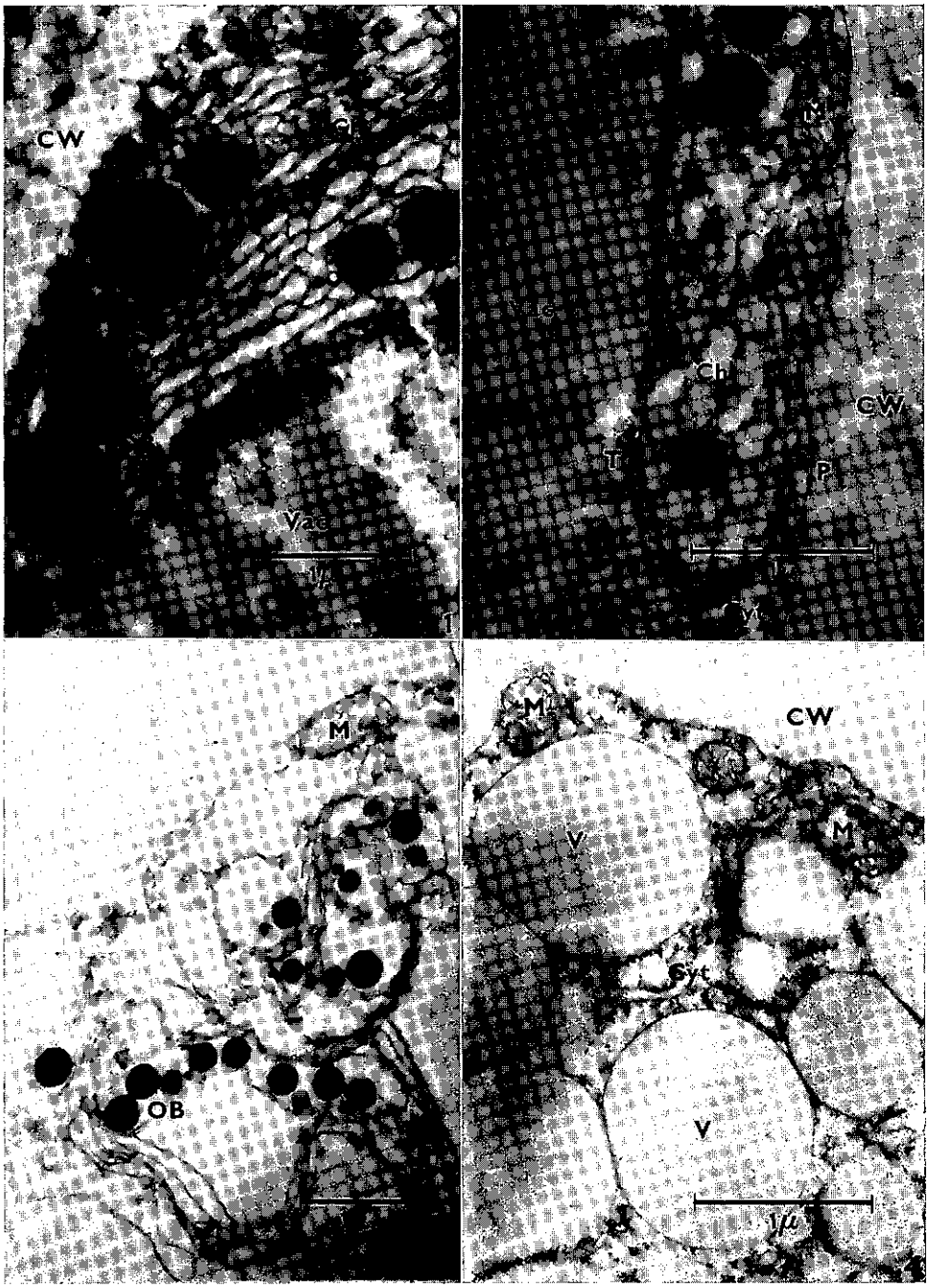

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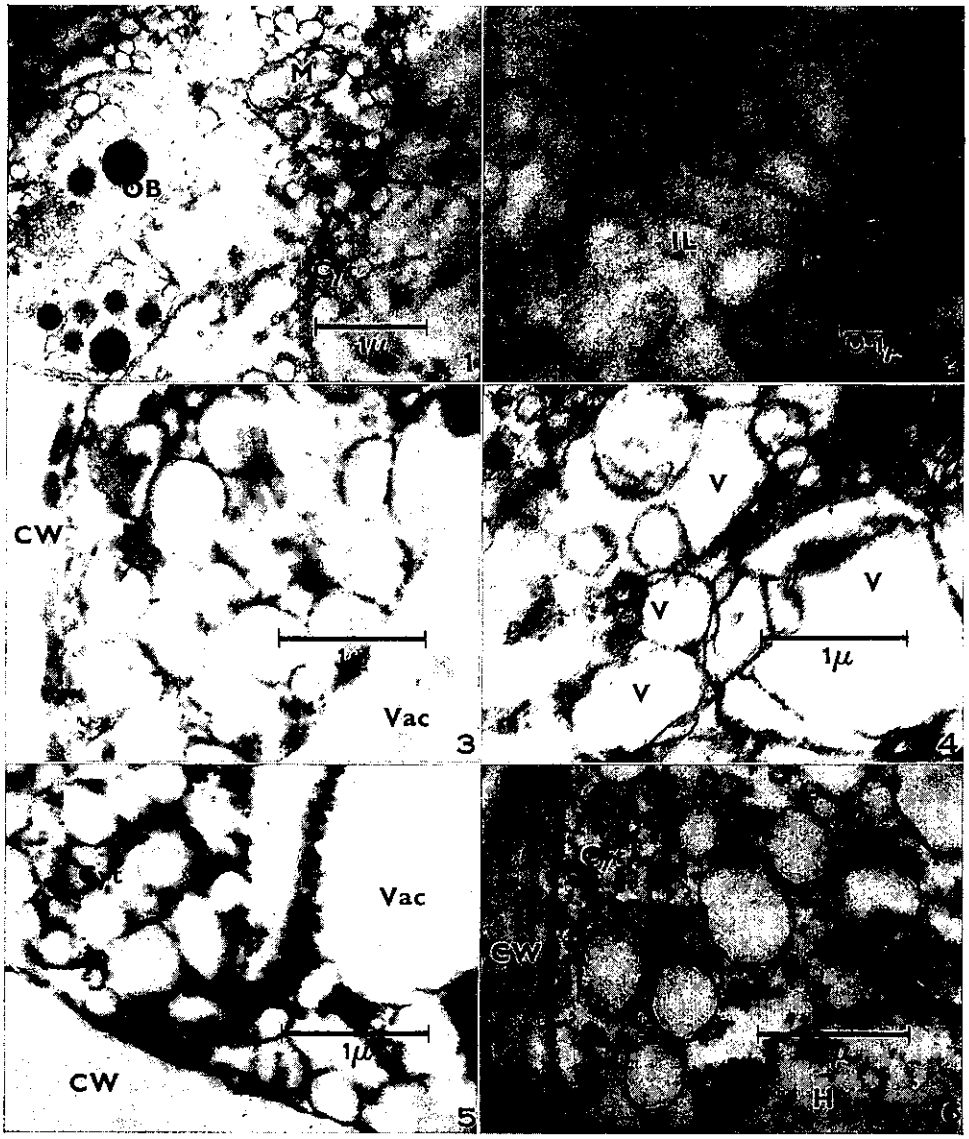

Fig. 3.—The detailed structure of a chloroplast as in Plate 1, Figure 1. The chloroplast (*Ch*) is made up of more or less parallel lamellae (*L*) with starch grains (*SG*) and osmiophilic bodies (*OB*) in the stroma (*S*). Cell wall (*CW*), cytoplasm (*Cyt*), and vacuole (*Vac*) are shown. ×15,000.

Fig. 4.—Changes have occurred in the chloroplast (*Ch*) of the mature but pre-climacteric fruit. Starch grains have disappeared from the stroma (*S*) and osmiophilic bodies (*OB*) have increased in size and number. The parallel arrangement of the lamellae (*L*) and the poorly developed grana regions (*G*) are shown. ×25,000.

## Plate 2

Figures 1, 2, and 4: mid-climacteric, yellow pear; Figure 3: post-climacteric, yellow pear

Fig. 1.—Osmiophilic bodies (*OB*) in the chloroplasts have enlarged further and the chloroplast (*Ch*) is swollen and vacuolated. The cell wall (*CW*) and cell vacuole (*Vac*) are indicated. ×25,000.

Fig. 2.—Both cytoplasm (*Cyt*) and chloroplast (*Ch*) are showing considerable vacuolation. A distinct tonoplast (*T*) is seen against the cell vacuole (*Vac*) and a distinct plasmalemma (*P*) is shown withdrawn from the cell wall (*CW*). The mitochondrion (*M*) does not appear disorganized. ×25,000.

Fig. 3.—The cytoplasm and chloroplasts are completely disorganized at this stage but the structure of the mitochondrion (*M*) is still recognizable. The position of former chloroplasts is indicated by osmiophilic bodies (*OB*). ×15,000.

Fig. 4.—The disorganization of the cytoplasm is to be related to Plate 2, Figures 1 and 2. Large vacuoles (*V*) bounded by thin membranes are formed throughout the cytoplasm (*Cyt*). Mitochondria (*M*) close to the cell wall (*CW*) do not appear disorganized. ×20,000.

## Plate 3

A series of figures showing vacuolation, as a general response to altered osmotic conditions, in a variety of tissues

Fig. 1.—Post-climacteric, yellow pear. Disorganization of the protoplast, presumably caused by changes in permeability of the membranes during ripening, is obvious. The cytoplasm (*Cyt*) has become highly vacuolated and the chloroplasts completely disorganized. Osmiophilic bodies (*OB*) mark the former position of the chloroplasts. The structure of the mitochondrion (*M*), however, is still recognizable. ×15,000.

Fig. 2.—Maize leaf. Exposure to hypotonic solution (0·3M glucose) has caused vacuolation in isolated chloroplasts. The grana (*G*) are swollen and the intergrana lamellae (*IL*) disorganized. ×77,000.

Fig. 3.—Granny Smith apple. Vacuolation has occurred in the cytoplasm of a hypodermal cell following rupture of membranes by freezing and thawing. The cell wall (*CW*) and cell vacuole (*Vac*) are shown. ×20,000.

Fig. 4.—Beetroot. High concentration of ions (1·5M $KNO_3$ for 2 hr followed by fixation in 2% $KMNO_4$ in 1·5M $KNO_3$) has caused vacuolation (*V*) of the cytoplasm, as cohesive forces of the cytoplasm decreased in Kappen plasmolysis. ×20,000.

Fig. 5.—Granny Smith apple. Vacuolation has occurred in the cytoplasm (*Cyt*) of a hypodermal cell following breakdown of membrane structure after exposure to chloroform vapour. The cell wall (*CW*) and cell vacuole (*Vac*) are shown. ×20,000.

Fig. 6.—Barley leaf. Vacuolation of the cytoplasm (*Cyt*) has been caused by fungal invasion. A haustorium (*H*) of a powdery mildew is present. The cell wall (*CW*) is shown. ×20,000.