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MORPHOLOGICAL CHANGES IN RIPENING FRUIT

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Many events in the ripening of fruit have received intensive study, yet we have little information on some of the structural changes involved. It is of value to summarize the current morphological information in the field of fruit ripening so that it may be considered in relation to the other contributions to this symposium. Not only is attention given here to structure during ripening, but some biochemical and physiological data in which structural changes are strongly implicated are considered. Our attention is limited to studies on the ripening of fleshy horticultural fruits; however, the phenomena considered may apply to other types of fruits or to other plant parts and tissues.

Most fleshy fruits are relatively large organs, an attribute that has received detailed attention by Forward (8) in her review of respiration of bulky organs. Such fruits also are often highly parenchymatous, with cells sometimes 0.5 mm or more in diameter and with walls that may be less than 1 μ thick. The vacuoles in these cells are frequently very large, with the protoplasm and included organelles, such as the mitochondria, nucleus, and plastids, confined to a thin peripheral layer. In *Capsicum annuum*, the pepper, this layer may be as thin as 500 A in some areas (Fig. 1), which includes the plasma membrane and the tonoplast with an interspace between them containing the ground substance of the cytoplasm. Fleshy fruits usually have a weakly developed but extensive vascular system, and their tissues are permeated by a system of intercellular spaces. The skin of the fruit is a protective layer well adapted, in many instances, for the retardation of water loss, even after removal of the fruit from the plant.

In addition to the rather distinctive structural features of the fruit, there are events in its early life that may have a bearing on structural changes during ripening. By the time ripening begins, a fruit has passed through complex developmental stages, some of which are

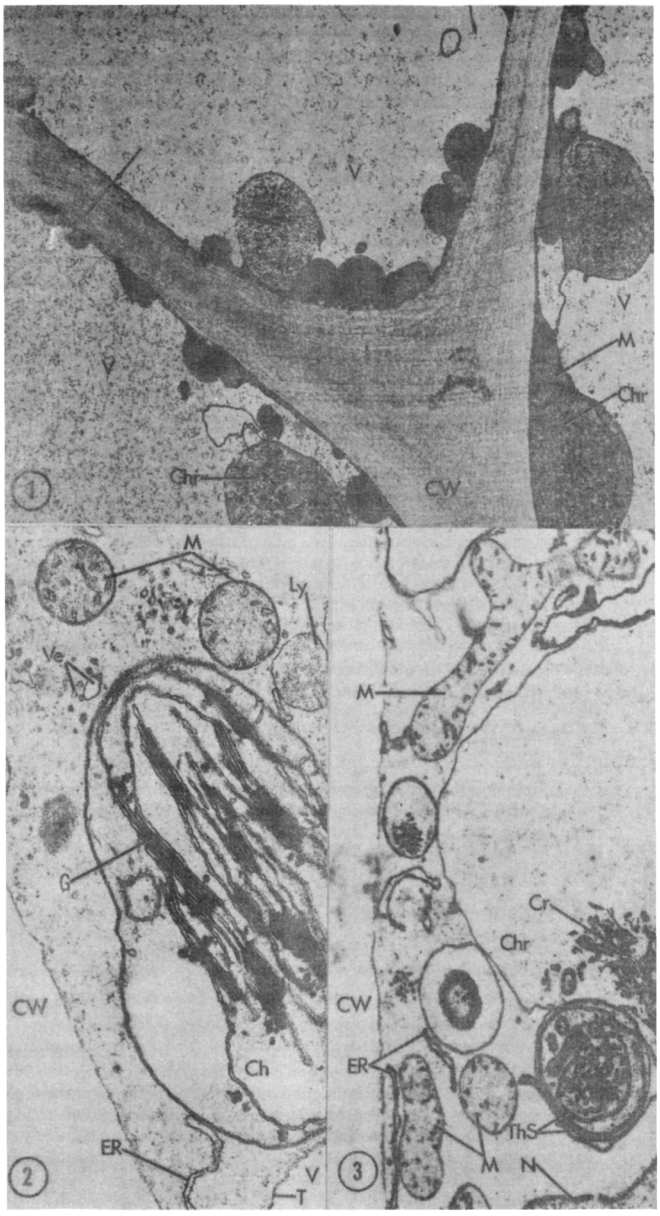

Fig. 1-3. Electron micrographs of the pericarp of *Capsicum annum,* the pepper: *Fig. 1.* Portions of 3 cells from the red-ripe fruit showing some features typical of fleshy fruits. Observe the large principal vacuoles, the relatively thin cell walls, and the thin parietal layer of protoplasm with organelles. The layer of protoplasm between the arrows is about 500 A thick, Glutaraldehyde-Dalton fixed, lead citrate contrasted. (X5800); *Fig. 2.* Organelles of a mature-green fruit. Observe typical mitochondria and portion of a chloroplast (Ch) with grana. The vesiculated bodies (Ve) are probably components of the endoplasmic reticulum or Golgi vesicles. Glutaraldehyde-KMn04 fixed, lead citrate contrasted. (X18900); *Fig. 3.* Organelles of a red-ripe fruit. Note mitochondria with no indications of degredation and portions of chromoplasts (Chr) with fibrillar carotenoid crystalloids (Cr) and thylakoid sheets (ThS). Processing same as *Fig. 2.* (X10360). Additional symbols: cell wall (CW), endoplasmic reticulumn (ER), granum (G), lipid droplet (LD), lysosome (LY), mitochondrion (M), nucleus (N), tonoplast (T), vacuole (V).

involved with reproduction. Although considerable information is available on the ontogeny of fruits, primary attention in such studies has been given to gross morphology, histology, and histochemistry. Information on changes in ultrastructure prior to ripening is very limited (2, 11, 23). As reviewed in one of the papers in this symposium, fruit ripening is also known to be intimately affected by environmental factors prior to ripening. One of these, the nitrogen nutrition of the plant, affects fruit structure (19) and possibly structural changes during ripening. Although emphasis is given here to structure, it is recognized that structural changes reflect the underlying metabolism of the fruit. Observation of such changes may direct attention to special sites and types of metabolic activity.

Cell walls

Changes in structure and texture

Softening of a fruit is usually regarded as a prime indication of ripening. From a structural point of view, ripening in *Prunus persica,* the peach (1, 18), is characterized by a marked decrease in cell wall thickness. Softening, however, may also be due to a variety of complex factors, including changes in turgor or differences in composition (18, 24). In *Pyrus communis,* the pear, some thinning of the cell walls during the initial stages of ripening may be due to the resorption of pectic deposits (16). Deposits of pectic materials occur in the cell walls and as protuberances on the surfaces lining the intercellular spaces of apples (*Malus sylvestris*) and pears (4). In many instances, the deposits may decrease in size or disappear during ripening. Such changes may reflect pectic enzyme activity (12).

Cellular dissociation

Changes in the cell walls may be so extensive that the cells become rounded and tend to separate from one another. This has been studied experimentally by Sacher (21) in explants of *Phaseolus vulgaris*, the bean, and it is a familiar condition in overripe fruits when the tissue becomes mealy. In the placental tissue of some tomato fruits (*Lycopersicon esculentum*), some protoplasts or protoplasmic units may be released from the cells into the locule of the fruit (5).

Skin structure and wax formation

The epidermis of a fruit commonly consists of small cells, often with very thick outer walls rich in pectic and cuticular materials. Thick-walled hypodermal cells commonly underlie the epidermis and, together with the epidermis, form what is frequently referred to as the skin of the fruit. Wax often is present on the epidermal surface and is associated with the epidermal wall materials. In apple the wax deposits, as revealed by light and electron microscopy, have distinctive structural characteristics (10). Cuticular and wax constituents continue to be deposited during storage (17) and are regarded here as a structural change during ripening.

Intercellular space

In fruits such as tomatoes and apples, a prominent intercellular space system occurs. In contrast, the spaces in peaches are small (19). The degree to which the system is developed and its changes during ripening probably have important effects on the postharvest physiology of the fruit. Porosity increases during the initial stages of ripening in pears but decreases in the later stages (16). Liquid-logging in bean is related to the leakage of cellular fluid into the intercellular spaces (21). This gives the tissue a water-soaked appearance and apparently decreases its light reflectivity. The phenomenon is attributed to changes in plasma membrance permeability during ripening (21). Increased hydration of the membranes in *Musa sapientum,* the banana, may cause ion leakage during the climacteric (3). If such changes in the function and state of the membranes occur during ripening, it is likely that they are associated with subtle changes in the ultrastructure of the membranes.

Free space

The portion of the tissue volume into which solutes may freely move by simple diffusion is the free space of the tissue (7). Starting about two days before the respiratory climacteric peak, the free space in banana increases from about 21% to 100% of the tissue volume (21). This is interpreted as a change in permeability mainly to small molecules such as sugars, amino acids, and salts; the membranes affected are presumed to be the plasma membrane and the tonoplast. The change in the free space system during ripening apparently introduces very different relationships in the functioning of the membrane systems, and thus in the compartmentalization of the fruit.

In spite of these reported changes in membrance function, respiratory control is maintained by the mitochondria in the ripe fruit of *Persea gratissima*, the avocado (25).

Organelles

Mitochondria

The intensive respiratory activity exhibited by fruits during ripening indicates that mitochondrial structure is probably maintained. Some early electron microscope observations lead to the belief that mitochondria in apple disappear in the post climacteric stage (13). This feature, however, was not clearly evident in the electron micrographs. In pear, the mitochondria do not undergo appreciable breakdown until the post climacteric overripe stage (2). This is in contrast to other structural features in pear that are almost completely disorganized at an earlier stage of ripening. Our observations on the pericarp of *Capsicum annuum*, the pepper, indicate that mitochondria retain their general structure from the mature-green through the full-red-ripe stages (Fig. 2, 3). The cells also retain their general organization during ripening. We have also found a similar condition with respect to the mitochondria and other organelles in the tomato. In homogenates of ripening fruit tissue of pear and of cherry (*Prunus avium*) Romani *et al.* (20) found a decrease in particulate yields. They reported changes in the fatty acid composition of the lipid fraction of mitochondria of ripening pears and apples. Their results were interpreted as an indication of possible membrane changes in the mitochondria. The effects of ethylene on the rate of swelling of isolated mitochondria has been interpreted as an effect on membrane permeability (15). Changes in mitochondrial structure are known to occur during aging of other tissues (6). Although mitochondria remain intact during ripening, it seems likely, especially in view of possible changes in membrane permeability, that they may undergo subtle changes in ultrastructure during this period.

Plastids

Color changes in fruits during ripening, especially those due to a loss in chlorophyll and an increase in carotenoids, are associated with the transformation of chloroplasts into chromoplasts. The chloroplast membranes (thylakoids) of the grana may persist if the plastids remain green, but more commonly they are variously modified, vesiculated, separated, or, as in some peppers (23), undergo lysis. Other membranes (intergranal thylakoids) may undergo similar changes, and in pepper they may become extensively branched plexes or even concentrically arranged membranous sheets (23). The carotenoids may form crystalloids in association with the membranes, as in the case of lycopene in tomatoes (11). Carotenoid crystalloids may also form in association with the lipid globules, as in pepper (23). In some instances in pepper the carotenoids appear to be mainly confined to enlarged globules or to increased number of globules. An increase in the size and number of the globules is also reported in pears during ripening (2). Such changing features denote a marked alteration in the metabolism of lipids during chromoplast development.

Another very common change in plastids during ripening is the loss or depletion of much of the starch. This occurs, for example, in the plastids of tomato (11), pepper (23), and apple (24). It is also common during chromoplast differentiation for plastids to change markedly in size and form, often in relation to the formation of carotenoid crystalloids which impinge on the plastid envelope, as in the tomato (11).

Plastid are also reported to undergo degredation during ripening (2, 9), even to the extent of breakage of the plastid envelope with the release of the contents of the plastid into the cytoplasm (9). It is difficult to judge, however, to what extent such changes represent events during ripening or are indicative of post ripening senescent changes. Also, some of the presumed degradative changes seem to reflect damage to the organelles due to handling very soft fruit tissue by the usual techniques for election microscopy. It is evident that the many structural changes centered in the plastids during ripening are indicative of intensive metabolic activities in these organelles. Other components of the cell, notably the nucleus (22), are probably indirectly involved with such changes.

Other organelles

A progressive vesiculation of the cytoplasm and other organelles occurs during the climacteric breakdown of pears (2). In our observation of pepper and tomato, the cytoplasmic and plastid ribosomes were not as deeply contrasted in the ripe fruits as in younger material. As shown by Ku *et al.* (14), well-defined cytoplasmic ribosomes are present in mature-green tomato fruits. The extent and manner that the ribosomes change during ripening has not been studied in detail.

It is evident that a wide range of structural changes occur during ripening. One of these, the change in texture or softening, though very complex and involving changes in cell-wall thickness and in deposits of pectin, may prove to be a universal feature in the ripening of fleshy fruits. Other structural features, particularly those in some plastids, undergo a multiplicity of rapid changes during chromoplast formation. When these and other changes at all levels from gross morphology through ultrastructure are more fully integrated with other types of experimental evidence, a more complete notion of fruit ripening will be attained.

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