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The Ontogeny and Cytogenesis of Cork Spot in 'York Imperial' Apple Fruit[1]

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Abstract. **Histogenetic studies of the young fruit revealed that incipient cork spot in 'York Imperial' apples (*Malus domestica* Borkh.) may appear as early as 3 weeks after fruit set, and that it initially becomes evident as a sporadic gradual necrosis of several parenchymatous cells of the outer cortex. About 1 month later, healthy enlarging cortical cells contiguous to the necrotic region undergo a redifferentiation via direct nuclear or amitotic divisions that result in a promiscuous intracellular proliferation of daughter cells. These remain within the confines of the original mother cell wall until it ruptures. The amitotic nuclei assume a variety of configurations and divide by cleavage. The method by which cell walls form between the intracellular proliferations is undetermined, and there is no evidence of cell plate formation. Once initiated, cork spot proliferation occurs only within the parenchymatous cells of the fruit cortex and is continuous throughout the growing season. There is no evidence of an internal cork cambium *per se.* Cork spot is not invariably associated with the vascular bundles, and there is no distinct evidence of the senescence of vascular bundles resulting principally from the necrosis. The necrotic condition becomes visible externally as a slightly sunken epidermal discoloration about 3 months after fruit set.**

Apple fruit are subject to a host of diseases and disorders, not the least of which are those non-pathogenic physiological disturbances commonly described as "internal corking." A recent physiological and biochemical review (8) updates the classification, nomenclature, and etiology of some of the more economically important of these corking disorders. One of these is called "cork spot" and is particularly prevalent on the 'York Imperial' apple cultivar, and not uncommon on several others. It was first briefly described in 1914 (17). Its name is derived from the brown spots or plugs of dead tissue that superficially resemble cork within mature fruit. Another physiological disturbance, described in the literature as "bitter pit," superficially resembles cork spot in appearance, structure, and biochemistry. However, among other characteristics, it differs in fruit lesion location, time of occurrence and development, and etiology. Although corking disorders of apples in general have been studied (6, 8, 9, 13, 16, 17, 20, 21), the ontogeny of cork spot in particular has not. The development of cork spot superficially resembles that of bitter pit, and the earlier descriptions were more or less desultory and equivocal. The present investigation was undertaken in the attempt to determine the site and time or origin, and to better clarify the developmental aspects, of cork spot of the 'York Imperial' apple. Primary consideration is given to tissue differentiation relative to the physiological disturbance.

Materials and Methods

Random selections for this study were obtained from 3 vigorous trees of 'York Imperial' apple seedling rootstocks planted in 1966 on the North Farm of the USDA Agricultural Research Center in Beltsville, MD. The soil is a Iuka silt loam type with adequate drainage. The orchard had been limed initially at planting, and received yearly applications of 10N-0.4P-0.8K (10-10-10) fertilizer, and no deliberate additions of B or Mg were made. Mature leaf Ca was at a moderately low level (1.1%), which is purportedly conducive to cork spot development (8). Pesticides had been applied regularly throughout the growing seasons.

Inasmuch as this study is concerned with a necrotic condition developing within otherwise healthy tissue, it was assumed that all sample collections were from fruit that were developing normally. Weekly collections during the 1977 and 1978 growing seasons consisted of 12-18 entire bud and fruit samples that appeared to be uniform in size and configuration. Samples were taken at about 1 month before full bloom (March 16, 1977 and April 6, 1978) and continued through harvest (October 5, 1977 and 1978). Monthly samples were obtained also from cold storage (3°C) apples over a period of 4-6 months. The 1977 growing season at this locality was about 3 weeks earlier than in 1978. Full bloom occurred on April 18, 1977 and May 1, 1978, respectively. Susceptible trees had a higher percentage of fruit set in 1977 than in 1978.

Additional fruit for comparative study was obtained from trees growing on the University of Maryland Farm at Silver Spring, and from the Catoctin Mountain Orchard at Thurmont.

All collections were aspirated, killed, and fixed in FAA and Navashin (CRAF) III solutions. Bud scales were removed to facilitate entry of the processing fluids. Only the central or terminal flower in the bud was chosen for study from the pre-flowering stage through full bloom. Successively larger immature and mature fruit required only the removal of wedges of extracarpellary tissue exhibiting various degrees of cork spot development.

External symptoms of the disorder do not usually appear for 6-8 weeks after full bloom, if at all. The detection of internal symptoms in very young fruit requires the sectioning of innumerable fruits, and is usually fortuitous. To facilitate the earliest detection of symptoms, free-hand sections of young as well as older fruit were both aspirated and cleared, without any additional processing, in an aqueous chloral hydrate solution (250g/100 ml distilled water) for 48 hr, followed by several complete solution changes depending upon the degree of clarification desired. This technique effectively revealed the overall

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endogenous appearance of the disorder within the encompassing healthy fruit tissues.

A standard tertiary butyl alcohol (TBA) schedule was used for dehydration, followed by embedding in 56-58°C Tissuemat and in 56-57°C Paraplast Plus. All processed material was sectioned on a rotary microtome at 8-10 μm. A modified safranin and fast green schedule was used for general staining; a number of slides were triple-stained with safranin, crystal violet, and orange G. Stained slides were made from both transverse and longitudinal sections of young fruit up to 2.5 cm in diameter. Comparisons were made between fresh sections, cleared materials, and stained slide preparations. All cell and most tissue measurements were made with a calibrated 10× ocular micrometer in combination with a 43× objective. A camera lucida was used for detailed nuclear, cell, and tissue illustrations; the diagrammatic illustrations were made from prepared slides with a Bioscope projector. The quantitative histochemical techniques used for identification of the cell contents of fresh material are those found in Jensen (11). All photomicrographs were made on Kodak Panatomic-X 35 mm, Ektachrome-50 35 mm, and Ektapan 4162 10.2 × 12.7 cm (4 × 5 inch) sheet film.

In order to determine where and when the incipient condition of cork spot appears, it was necessary to briefly investigate the development of both the pericarp and extracarpellary tissues prior to and through the fruit "set" stage. Fruit set normally occurs within 24-36 hr after pollination, depending upon environmental and other conditions. Although no previous ontogenetic studies have been made on the fruit wall of this particular apple cultivar, few basic differences were found from those made on other cultivars (2, 4, 5, 14, 23, 25, 26, 29). Although fruit wall histogenesis was observed throughout the growing season, the data presented here concern only that period from 1 month prior to full bloom through 1 month after fruit set (see Table I). For the latter observations, samples of tissue approximately 125 μm^2 were arbitrarily chosen as an area standard in which to consistently measure tissue development (these areas are depicted by rectangles in fruit longisections of Fig. 1-3).

Results

Fruit wall histology – epidermis. The epidermis (Fig. 1-4, Table 1) appears as a uniseriate layer (biseriate in the 'Golden Russet' cultivar; 5) of short, columnar, thin-walled, closely-packed cells whose numbers increase by anticlinal divisions. Based on the observation of mitotic figures, increases in cell size, and cell vacuolation, division gradually ceases by about 3 weeks after fruit set. Some epidermal cells differentiate into unicellular, thick-walled, unbranched, ephemeral trichomes or hairs that vary in length and number. The hair soon breaks and its base gradually becomes occluded and replaced by the pervading cuticle. At this stage the <2 μm cuticle is continuous over the epidermis, including the unbroken hairs and the inner surfaces of the guard cells but not within the stomatal cavity. As the immature fruit enlarges, the cuticle generally thickens, particularly so in the hair-base region and in the inward extensions between the outer radial walls of the pidermal cells. One month after fruit set the cuticle is about 7 μm thick. Relatively few scattered stomata with "horned" guard cells develop on the fruit of this apple cultivar, and then primarily on its calyx half. Development becomes more evident about 2 weeks after fruit set. Lenticels make their initial appearance on the calyx half of the fruit approximately a week later. There is no correlation between lenticel development and the appearance of cork spot in either young or mature fruit. Ontogeny of the stomata and lenticels was not investigated.

Hypodermis. The hypodermis (Fig. 1-4, Table 1), derived from the meristematic cortical parenchyma, consists principally of 2 thin-walled cell layers (4 layers in the 'McIntosh Red' cultivar; 4). During this stage of development the cells appear very similar to those of the adjoining cortical cells in configuration and orientation. Periclinal divisions are common, with occasional anticlinal divisions. Small intercellular spaces uncommonly appear at the cell junctions but may gradually disappear as the cells enlarge. About 3 weeks after fruit set, the hypodermal cells undergo tangential elongation and radial enlargement, their walls begin to thicken, and the cytoplasm becomes more dense in appearance. Chloroplasts are more prevalent in the hypodermis than in the epidermis, and they may be present also in cortical cells adjacent to the hypodermis.

Cortex. The compact cells of the cortex (Fig. 1-4, Table 1) are thin-walled, more or less isodiametric, and less densely cytoplasmic than the dermal tissues. Small, inconspicuous schizogenous intercellular spaces are abundant throughout.

Table 1. Extracarpellary tissue development in 'York Imperial' apple cultivar.[z]

				Epidermis				Hypodermis				Cortex		
Sampling date	Immature fruit[y] (mm)	Cuticle thickness (μm)	Cell no.[x]	Cell size[w] (μm)	Vacuolation	Mitosis	Stomata	Cell size[v] (μm)	Vacuolation	Mitosis	Cell width[u] (μm)	I.S. size[t] (μm)	Vacuolation	Mitosis
March 16	3 × 3	<1	19	7.5 × 12.5	0	+	0	10 × 20	0	+	15	3 × 20	0	+
March 23	4 × 4	1	18	7.5 × 15	0	+	0	12.5 × 17.5	0	+	17.5		0	+
March 30	6 × 6	<2	16	7.5 × 17.5	0	+	0	15 × 15	0	+	20	5 × 30	+	+
April 6	6 × 7	<2	18	7.5 × 17.5	0	+	0	12.5 × 15	0	+	20		+	0
April 13	6 × 8	<2	20	7.5 × 17	0	+	0	15 × 15	0	0	20	6 × 47	+	0
April 20	8 × 11	2-5	21	7.5 × 15	0	+	0	10 × 17.5	0	0	24		+	0
April 27	9 × 13	2-5	16	7.5 × 20	0	0	0	10 × 20	0	0	32.5	30 × 75	+	0
May 4	15 × 17	5-7	12	10 × 20	0	0	+	12.5 × 17.5	0	0	37.5		+	0
May 11	20 × 19	5-7	10	10 × 20	0	0	+	10 × 20	+	0	42.5	40 × 160	+	0
May 18	28 × 24	7	9	10 × 17.5	+	0	+	15 × 27.5	+	0	100		+	0
May 25	33 × 27	7-10	8	12.5 × 20	+	0	+	12.5 × 42.5	+	0	107.5	45 × 171	+	0

[z]Data are averages of 12 different fruits per sampling period (1977). From median longisections of prepared slides.

[y]Includes developing ovary prior to fruit set. Full bloom was 4-8-77. From fresh weekly samples. Width × length.

[x]Per 125 μm area tangent to fruit surface in approximate regions depicted in Fig. 1-3.

[w]Tangential × radial.

[v]Radial × tangential.

[u]Tangential only.

[t]Intercellular spaces. Tangential × radial. Biweekly measurements.

For about 2 weeks prior to full bloom, diffuse cell divisions are common and the cells become more or less tangentially oriented. After this period, divisions are less frequent, the cells gradually undergo vacuolation, and druse crystals as well as a few rhombohedral crystals may form sporadically within some of the cells. Divisions appear to have ended by 2 weeks after fruit set. This is in close agreement with observations on other apple cultivars (25, 29). By that time the cells have become vacuolated, and enlarged to twice their size at fruit set, and the intercellular spaces have become larger and less tangentially oriented. The cells closer to the hypodermis are relatively smaller and more tangentially elongated than the more or less isodiametric cells deeper within the cortex. Small aggregates of variable-sized leucoplasts develop by the third week after fruit set. Starch grains develop about a week later. No sclereids were ever observed in the cortex.

During the early period of fruit wall development all of the non-vascular tissues contain various amounts of lipoidal bodies that gradually disappear by the week of fruit set. Also during this period, an anastomosing reticulum of small, partially differentiated vascular bundles are interspersed throughout the cortex. These secondary bundles are usually ensheathed by small cortical parenchyma cells. The fundamental pattern of vascular development appears to be little different from that of other apple varieties (25, 29). Although cell divisions in various tissues of the young pericarp continue for different lengths of time, they appear to cease entirely by the third week after fruit set. Thereafter, the pericarp increases in size principally by gradual cell enlargement and reorientation, concomitant with a considerable increase in the size and number of intercellular spaces.

About 1 month after fruit set (Fig. 4), the epidermal cells have stopped dividing and are wider tangentially than radially; the cuticle is 9-14 μm thick; and the trichome bases are entirely occluded. The hypodermis is thick-walled and becomes flattened tangentially, along with 3-4 subtending cortical layers. The cortical cells have quadrupled in size since the fruit set stage, are gradually becoming radially oriented, and reach their approximate maximum size ($\pm$ 125 μm) about 2 months later; by that time the intercellular spaces have also assumed a radial orientation.

Necrosis – incipient stage. The initial internal symptoms were first observed no earlier than 3 weeks after fruit set (Fig. 4-5). External symptoms appeared as small, greenish epidermal depressions within 2 months. The cork spot disorder is incident to and develops only in the fleshy cortex, where it usually occurs superficially rather than deep-seated. During its earliest stages it does not encroach upon either the dermal or core tissues. It is both sporadic and promiscuous in its initial appearance and distribution, and for no discernible reason develops among apparently normal tissue. There is no profound condition to indicate that it is incipient.

Although the cork spot appears very distinct and prominent in older fruit, the incipient stage is difficult to recognize in fresh material. The earliest symptoms appear as an inconspicuous gradual disorganization and sporadic disintegration of several localized cortical cells, concomitant with heavier histological staining of their cell contents, and rupturing of their walls (Fig. 4-5). Starch grains are not present in the incipient necrotic cells; only small amounts ever accumulate in the contiguous healthy cortical cells during subsequent proliferation of the necrosis. In developing fruit the cork spot tissue is usually separated from the hypodermis by variable amounts of healthy cortical tissue. In mature and storage fruit it is not uncommon for a cork spot area to have enlarged to the extent that it may approach the hypodermal tissue.

In thin slices of fresh immature fruit, the necrotic condition appears as a minute isolated, discolored, amorphous spot embedded in the healthy whitish cortex. It may occur as a single entity in an entire fruit, or several may develop in various areas of the cortex at the same or different times during fruit development. The cork spot disorder usually has no profound effect on the total enlargement of the fruit *per se.* As more cells become affected, small lacunae are formed as a result of the cytolysis of contiguous cells (Fig. 21-23). The lacunae often contain variable amounts of proteinaceous matter derived from the ruptured cells. Larger lacunae often develop in combination with intercellular spaces. Incipient cork spot tissue was observed to develop from less than 200 μm to more than 1500 μm away from any vascular bundle. Considering the widespread network of vascular tissue throughout the cortex, it is not uncommon to find cork spot necrosis also encompassing the vascular bundles. However, only the smaller cortical parenchyma surrounding the vascular tissue may undergo necrosis; the vascular tissue *per se* does not appear to become affected.

Subsequent to cytolysis of the initial cork spot cells, some contiguous healthy, vacuolated cortical cells undergo changes in nuclear size, configuration, and number. As will be elaborated upon below, the normally spheroid nucleus becomes ellipsoidal, spindle-shaped, or fusiform in configuration (Fig. 6-8, 10), and undergoes what appears to be direct nuclear division (amitosis) by cleavage, and finally results in a binucleate cell. These cells may either die shortly or, not uncommonly, undergo a redifferentiation. The incipient stage of necrosis development prevails for approximately 4 weeks following the initial appearance of cork spot symptoms.

Proliferation stage. After about 1 month, while some contiguous cells continue as described above, a differentiation *de novo* becomes clearly evident among other vacuolated cortical cells around the perimeter of the earlier necrotic tissue. This redifferentiation, concomitant with amitotic nuclear divisions, is directly incident to and contributes incrementally to the ultimate size of the necrosis. Although it is more or less sporadic, it continues throughout fruit enlargement and maturation and evidently also to a degree in stored fruit.

Prior to division the amitotic nuclei may be either spheroid, elliptical, or, more commonly, fusiform (Fig. 6-8, 10). The nucelus enlarges, with an attendant increase in both heterochromatin and nucleoplasm. The enlargement is not uncommonly about twice the size of a normal cortical nucleus. The size of the cell itself does not appreciably increase. Usually only one nucleolus (occasionally 2-3) is visible in the resting nucleus of a normal cortical cell. However, in an enlarged pre-amitotic nucleus there are often 2 to several nucleoli (4-7 have been observed) of various sizes, with one almost invariably appearing larger than the others. A fusiform nucleus usually contains only a single enlarged nucleolus (often approximately 2.5-3.75 μm in diameter), which may have resulted from the coalescence of 2 or more smaller nucleoli. The overall appearance of the larger nuclear configurations within cells contiguous to the necrotic tissue is almost always indicative of imminent amitotic divisions (Fig. 6-7, 10).

The nucleus commonly divides amitotically by cleavage (Fig. 8, 11-17). Critical examination of hundreds of these reactivated cells has revealed no mitotic figures, and no semblance of either spindle fibers or phragmoplasts. Upon completion of division, the 2 small nuclei usually become elliptic or rounded and may "migrate" a short distance apart (Fig. 9, 17). Inasmuch as these nuclei are normally considerably smaller than the parent nucleus (or "macronucleus"), they will be referred to as "micronuclei" (Fig. 9, 17-21). Occasionally 3 (rarely 4) micronuclei appear in a single cell, and these as well as those in the binucleate condition, may appear overlapping, attenuated, attached by nuclear strands, or fragmented. The amitotic "macronuclei" may also assume a variety of other configurations, e.g., reniform, scalloped, spheroid, fusiform, convoluted, amoeboid, or triadic (Fig. 6-8, 10-16). Furthermore, regardless of their polymorphisms, they almost invariably

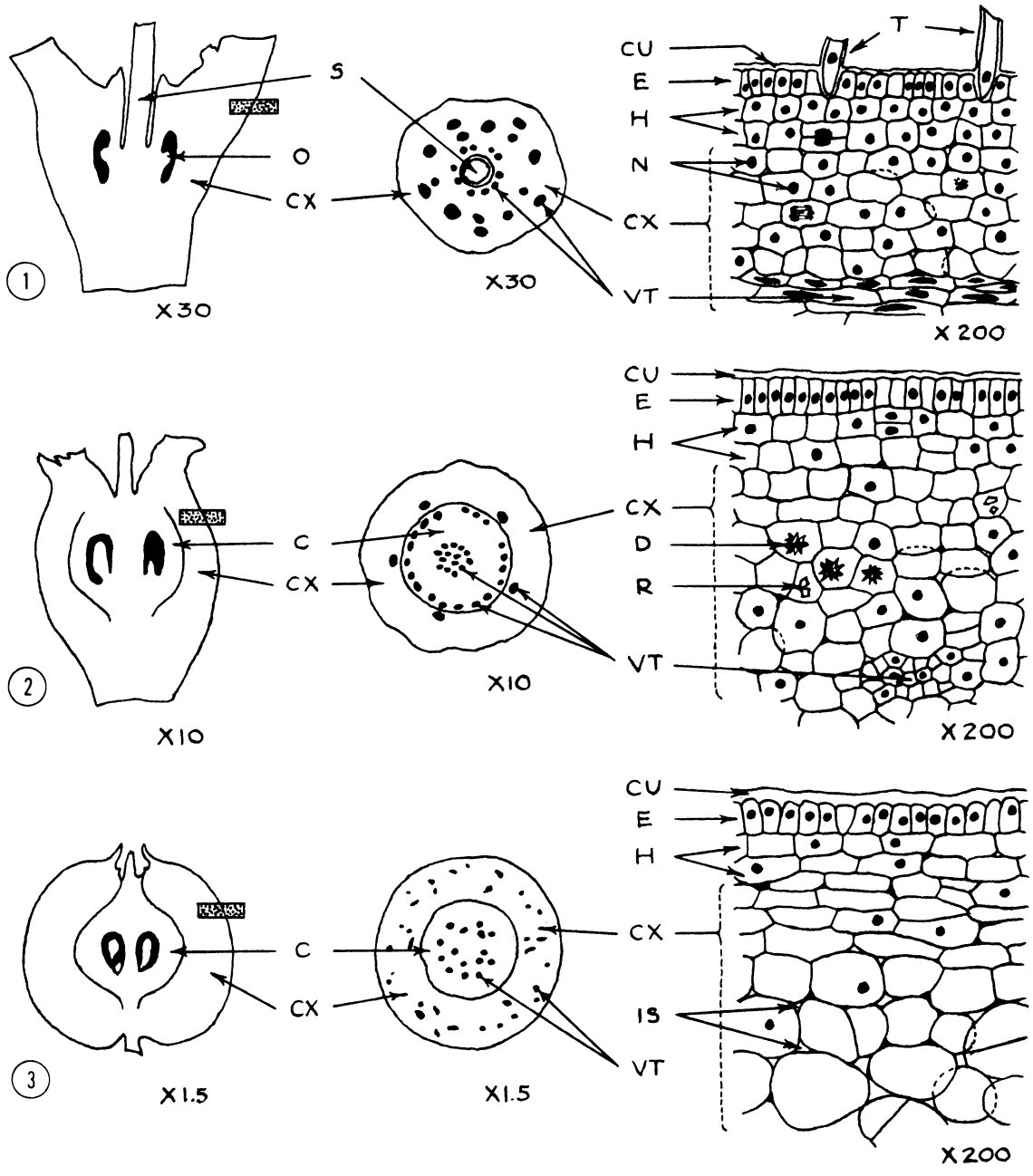


Fig. 1-3. Morphology and anatomy of immature 'York Imperial' apple fruit at 3 stages of development. The left mean longisections and the center transections are diagrammatic; the right tissue longisections are camera lucida renditions. The dotted rectangle in each of the left longisections represents the approximate regions from which the other 2 sections were obtained. Fig. 1. One month prior to full bloom. Fig. 2. A month later, at the fruit set stage. Fig. 3. Twenty-three days after fruit set. The vascular traces depicted in the diagrams are not the same as those shown in the tissue sections. C-core, CU-cuticle, CX-cortex, D-druse crystals, E-epidermis, H-hypodermis, IS-intercellular spaces, N-nuclei, O-ovule, R-rhombohedral crystals, S-style, T-trichome, VT-vascular traces.

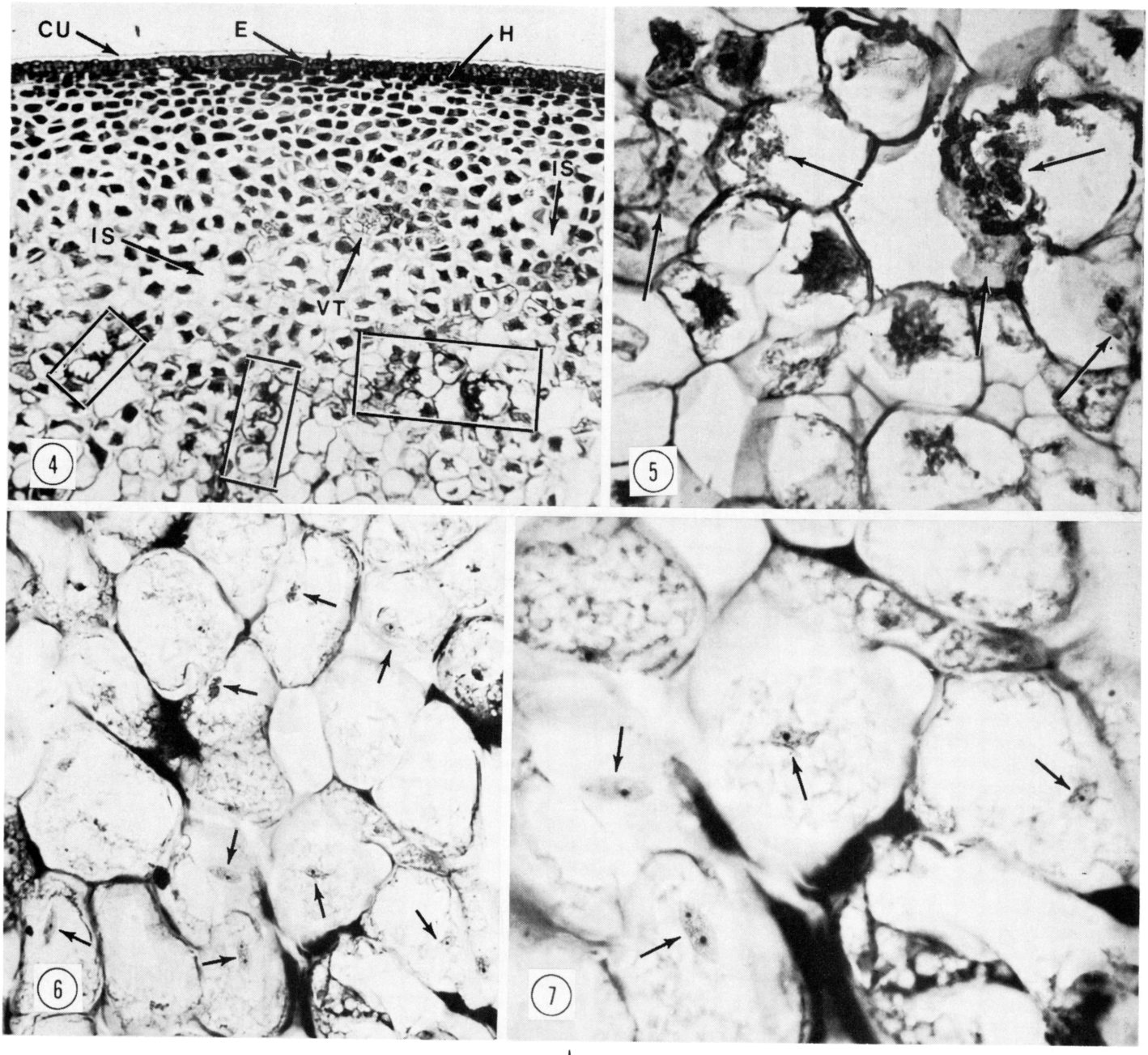
Fig. 4. Longisection of extracarpellary tissue 23 days after fruit set (see Fig. 3 and 5) exhibiting incipient cork spot (within rectangles), ×50. CU-cuticle, E-epidermis, H-hypodermis, IS-intercellular space, VT-vascular traces. The healthy cortical cells are plasmolyzed from tissue processing.

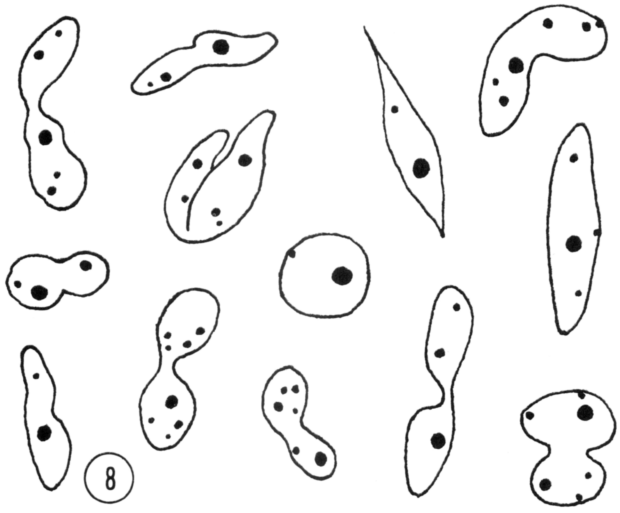
Fig. 5. Incipient cork spot areas (arrows) enlarged from the region demarcated by the largest rectangle in Fig. 4, ×200.

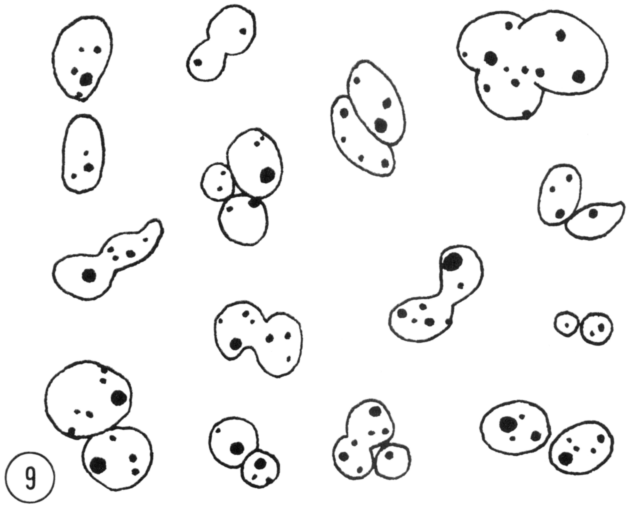
Fig. 6. Healthy cortical cells with variously-shaped enlarged nuclei, "macronuclei" (arrows), prior to amitotic division, ×50.

Fig. 7. An enlargement of Fig. 6 (lower center) depicting pre-amitotic "macronuclei" with their nucleoli, ×200. The lowermost nucleus is about to divide.

Fig. 8-9. Amitotic nuclear configurations in cork spot tissue of 'York Imperial' apple fruit. Camera lucida drawings, all ×738. Fig. 8. Various amitotic configurations and stages of 'macronuclei" and their nucleoli from individual parenchymatous cortical cells contiguous to the necrotic tissue of cork spot (see Fig. 10-17). Fig. 9. Amitotic "micronuclei" and their nucleoli in various configurations as derived from earlier intracellular divisions. Several are undergoing still further cleavages; others appear as if they are in a process of fusing (upper right).







appear flat, and each usually contains one slightly larger nucleolus in addition to a few smaller ones. Uncommonly, one micronucleus may be somewhat larger than the other(s), a possible indication of more of less acentric division.

After the first amitotic division, the binucleate cell either quickly degenerates, or, more commonly, develops a thin wall separating the micronuclei (Fig. 18-23). In the latter case each micronucleus soon may enlarge slightly and again divide. Repeated sequences of cytokinesis result in what appears as a promiscuous intracellular proliferation of parenchymatous cells. The abnormal increase in cell number is invariably confined within the original "mother cell" wall until the latter ruptures (Fig. 21-23). There is no distinct enlargement of the mother cell to accommodate the "daughter" cells within its wall. The exact origin and method of cell wall formation between the micronuclei has not been determined. There is no evidence of either a cell plate or the development of furrows, yet the thin wall is apparently synthesized rapidly from the mother cell cytoplasm. The use of various staining techniques, as well as polarization, phase contrast and darkfield microscopy, did not enable the detection of cell plates or incipient cell wall formation.

Uncommonly, some cells immediately adjoining the cork spot region appear to undergo a series of normal cell divisions that are tangential to the necrotic area. These result in the development of a radial series of 2 to several cambium-like appearing cells that may partially encircle the necrosis (Fig. 24). These cells are limited in their enlargement, with the outermost ones occasionally assuming a hyperplasia-like appearance. The surrounding cortical cells contiguous to these radially-oriented cells often undergo amitosis and intracellular proliferation.

The small nucleated daughter cells vary in number (2 to more than 30), size, and shape; are thin-walled and compacted when numerous; are devoid of intercellular spaces; and contain no starch grains (Fig. 18-23). Starch grains are usually uncommon in the proliferated cells until fruit maturity; and thereafter they may occur in various amounts in cells of stored fruit. They usually occur in small aggregations or clusters (Fig. 16, 25), with each grain appearing lamellated and possessing a distinctly eroded hilum. The daughter cells are further proliferated by a continuation of amitotic cytokinesis. Their micronuclei often assume typical amitotic configurations emulating, to a certain extent, the appearances of the original parent nuclei (Fig. 9). Each proliferated cell invariably contains a nucleus that appears to have enlarged slightly prior to the next division, and each tends to increase its nucleolar number. Occasionally some micronuclei appear to undergo a fusion process (Fig. 9). The 'York Imperial' apple is a diploid (2n=34), the chromosome numbers of the macro- and micronuclei were not determined.

Eventual rupture of the mother cell wall releases the proliferated cells, which may separate, round up and enlarge slightly, and soon disintegrate to form a lacuna; or they may discharge directly into an intercellular space (Fig. 20-23). The intercellular spaces of the healthy cortex are normally distinct from the rhexigenous cavities created by dissolution of the cork spot cell. The former are outlined by the contiguous healthy cell walls and the latter by lysed walls.

Although vacuolated cortical cells anywhere in the vicinity of the disorder may be reactivated, not all cortical cells are involved. The unaffected adjoining tissues surrounding the necrotic region continue to enlarge normally unless they too succumb to the necrosis. Furthermore, the endogenous proliferation occurs only among cortical cells contiguous to or within the necrotic region and not in other cortical, vascular, or dermal tissues. It may also involve some of the small cortical parenchyma cells ensheathing the vascular bundles but appears to have no effect on that tissue itself. There is no *de novo* differentiation of vascular elements.

Tests with Sudan IV on freshly cut sections indicates some suberization of the necrotic tissue. Phellem or cork *per se* does not develop. No "ladder-like arrangement" of healthy cortical cells was found around the "corky portion" as reported by Mix (16). Some of the cork spot cell walls may appear thicker than others, but this is due primarily to the swelling of the middle lamella prior to wall dissolution. When the fresh sections or deparaffined unstained slides are viewed in transmitted light, the developing necrosis appears slightly darker and more dense than the healthy tissue, with a lighter, halo-like area surrounding the denser region. About 3 weeks after the onset of cellular proliferation, pectic extrusions may become observable as minute globular papillations or protuberances into the intercellular spaces or into the lacunae from the walls of cortical cells lying within or contiguous to the cork spot tissue (Fig. 18). Although relatively few at any time, they occur thereafter throughout the development of necrosis. In storage fruit they may also appear in the intercellular spaces of the thick-walled cortical cells adjoining the flattened hypodermis.

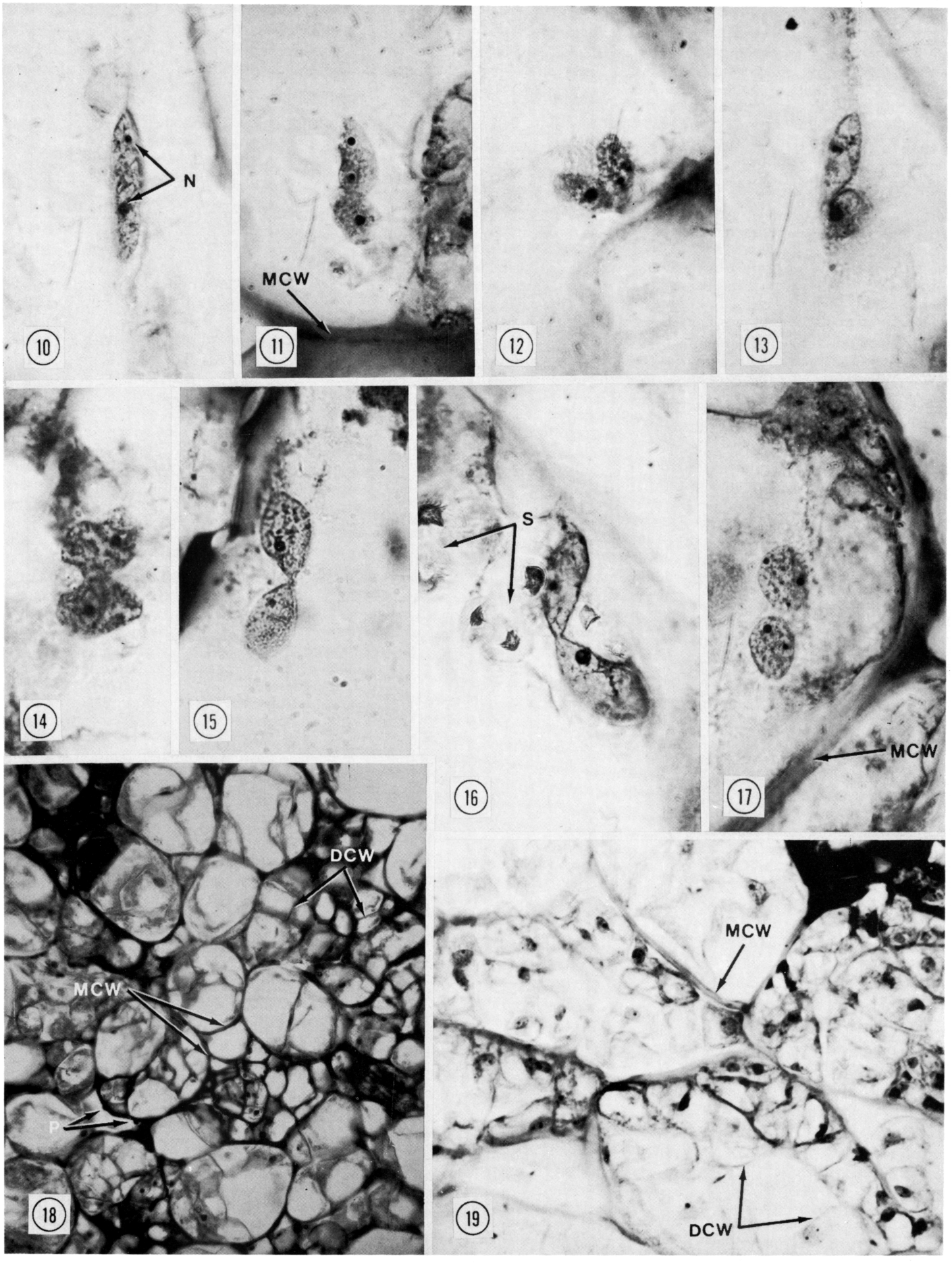
External appearance. Once the cork spot necroses are initiated within the cortex they develop relatively slowly and are confined to comparatively small areas of the fruit. After about 6 months of growth, the overall size and number of necrotic tissues at fruit maturity are still very small. In mature fruit the necroses ranged in size from <1 cm to >1 cm in both depth and diameter. The cork spot first becomes externally visible approximately 3 months after fruit set as a small, rounded, greenish, shallow depression or cavity, usually but not invariably on the calyx half of the fruit surface. This sunken "spot" is not the necrosis itself but only the initial external evidence of its existence. The development of the surface depression is actually a secondary symptom of the necrotic condition. It is the result of a combination of factors leading to overall tissue degeneration within the necrotic region, namely, the cessation of cell enlargement, the dissolution of cell walls and lysis of cells concomitant with the formation of large lacunae, and the inability of the necrotic tissue to maintain a turgidity comparable to that of the encompassing healthy cortical tissue.

As the fruit develops further, the sunken area may enlarge and deepen slowly, concomitant with the enlargement of the necrotic tissue in the cortex below. The dermal tissues within the cavity are not appreciably affected in their configuration; however the depressed surface may become a darker green, or develop various hues due to the presence of anthocyanins. The number

Fig. 10-17. Enlargements of "macronuclei" in various stages of amitosis. The larger black spots within each nuclear half are nucleoli (N). All ×800. The nucleus in Fig. 17 has recently completed division, resulting in the formation of two "micronuclei." MCW-mother cell wall, S-aggregate starch grains. See Fig. 6-9.

Fig. 18. Various intracellular proliferations following amitotic divisions, ×100. Note differences in thickness of primary walls of daughter cells (DCW) in comparison with those of mother cells (MCW). Distinct differences in sizes of "micronuclei" are also apparent. P-pectic protuberances. See Fig. 19-21.

Fig. 19. Enlarged appearance of proliferating intracellular daughter cells with their respective "micronuclei" following amitotic divisions, ×400. Note very thin daughter cell walls (DCW) in comparison to "mother cell" primary walls (MCW).



of visible cork spots appears to increase, but seldom to any appreciable degree, as the fruit reaches maturity. Usually only a few (uncommonly 6 or more) variable-sized, shallow cavities develop; each is usually separate and more or less distinct. In mature fruit the cavities vary in diameter from several millimeters to more than 1 cm. The proximity or confluence of 2 or more cavities may create the appearance of one large cavity (one measured 10 × 17 mm in width and length). However, large or small, the cavity always appears spot-like relative to the fruit as a whole. The depth of the cavity depends upon the size of the necrotic tissue and its distance from the outer cortex and dermal layers. Depressions seldom increase in depth to more than a few millimeters. In the developing fruit the cork spot tissue is normally separated from the dermal layers by a variable amount of healthy cortical tissue. Deep-lying cork spot tissue does not effect a visible depression unless the affected area is quite large, whereas that developing relatively close to the surface almost invariably results in the formation of a sunken area. The number of externally visible symptoms does not necessarily coincide with the total number of cork spots. The total number not uncommonly is twice the number visible externally. In this investigation, cork spot was found most commonly in the outer cortex, occasionally in the middle cortex, and seldom in the innermost cortex (and then primarily in storage apples). The deepest penetration observed in the cortex was 18 mm from the inner hypodermal layer, where it was encroaching upon the core boundary.

No other anomalous tissue is developed either before, during, or after the depression is formed. Neither hyperplasia *per se* nor hypertrophy occurs, there is little or no necrotic cell enlargement, and no callus tissue is produced. Furthermore, there is no evidence of the resorption of the necrotic tissue at any time during its development. Extracarpellary tissues, including the cork spot, do not become lignified. In mature apples the density of the cork spot tissue is approximately twice that of the adjacent healthy cortex tissue (9). In mature fruit and especially storage fruit, it is not uncommon for some of the thick-walled outer cortical cells to undergo a reactivation and subsequent intracellular proliferation to the extent that they encroach directly upon the flattened hypodermal layers.

While corkspot does not apparently develop *de novo* in stored fruit, it does continue to enlarge slowly even after harvest. Fruit stored for 3-4 months at 3°C may exhibit somewhat larger internal spots than freshly harvested fruit. The cork spot tissue is not invariably directly associated with the vascular bundles; however, the prevalence of the vascular bundles increases the likelihood that they will be in contact with the necrotic tissue. No distinct obstruction or plugging of either xylem or phloem elements due to the cork spot condition was observed; and there was no evidence of "senescing" of vascular bundles (21). From none to several small pustulate lenticels may appear within the depressed area (8 were found in one cavity of a storage fruit); however, these usually develop prior to the formation of the depression. Lenticels have no direct relation to cork spot, and the cork spot does not extrude either into or through a lenticel. The cork cells of the lenticels and adjoining cells on each side are suberized. At time of fruit harvest the cuticle is 12-18 μm thick.

Discussion

No correlations were found between the cytohistogenesis of normal fruit development and that of cork spot. In the course of this study, 2 uncommon cytological anomalies were discovered that have not heretofore been described for this disorder. The 2 are related and both contribute directly to the proliferation of cork spot. The first of these is what appears to be direct nuclear division or amitosis within cells of the cortex, and it gives rise to the second, namely, intracellular proliferation or "endocytogenesis." Both phenomena are described and discussed.

Not uncommonly, multinucleate cells in addition to tapetal and endosperm cells and certain specialized coenocytes occur normally in many plants (3, 18, 27). However, the multinucleate condition in most instances is the result of mitotic divisions, and it rarely arises from amitosis. Although there are numerous reports and reviews on purportedly direct nuclear divisions occurring as a result of either pathological (galls) or naturally occurring physiological disturbances in plant tissues (1, 10, 12, 15, 18, 19, 22, 24, 27, 30, et al.), apparently relatively few cases (1, 15, 18) are well authenticated; many appear to have been observed principally in tapetal and embryo sac tissues. Wanscher's (30) illustrations (p. 63) of the amitotic nuclei in the endosperm of 'Wøldike's Pigeon' apple cultivar closely resemble several of the amitotic nuclear configurations observed in the present investigation. Reactivated cell division was observed also by Tetley (25) in apparently non-necrotic vacuolated cortical cells of 'Wagener' apples, although her single illustration indicates the occurrence of normal mitosis. The amitotic phenomenon in cork spot can be interpreted as either an early regeneration phase of stimulated cell activity or an abnormal process of degeneration.

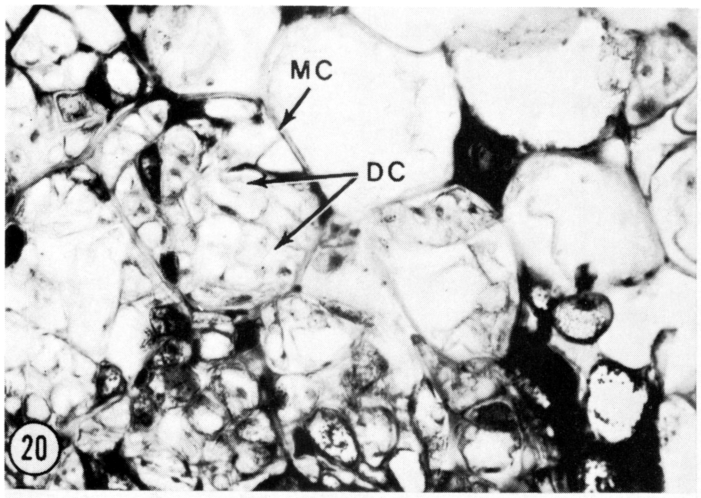
The singularly intracellular proliferation phenomenon *per se* is unusual. An appropriate term for this phenomenon, "endocytogénèse" or endocytogenesis, was coined by Collin (7) while he was investigating human pituitary tissue. An intriguing aspect of endocytogenesis in cork spot tissue is that the proliferated cells (whether 2 or many) all invariably remain within the original "mother" cell until it ruptures. Although several such instances have been reported for animal cells (7, 10, 28, et al.), this appears to be the first report for plant tissue. Although cork spot cell proliferation has been reported previously (21), the so-called "proliferations" observed were actually the "end" results due to the rupturing of the mother cell walls along with the concomitant release of a prodigious number of daughter cells, and not the initial intracellular proliferations themselves. There is neither hypertrophication by unusual cell enlargement or a condition of hyperplasia by unusual cell multiplication. Furthermore, there is no abnormal meristematic activity in the sense that a condition of hyperplasia *per se* occurs, except perhaps in a delayed mode upon the rupturing of the mother cell wall. This may explain why the necrotic area is never very large and only spot-like in overall configuration.

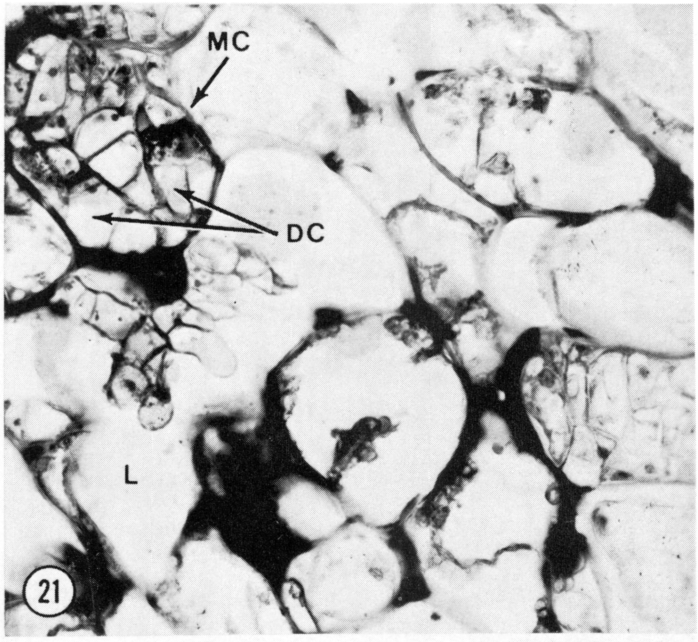
The knowledge that incipient cork spot can develop shortly after fruit set may change concepts of appropriate control measures. However, still unknown are the factors that directly contribute to the occurrence of this physiological disturbance and the concomitant cytological aberrations.

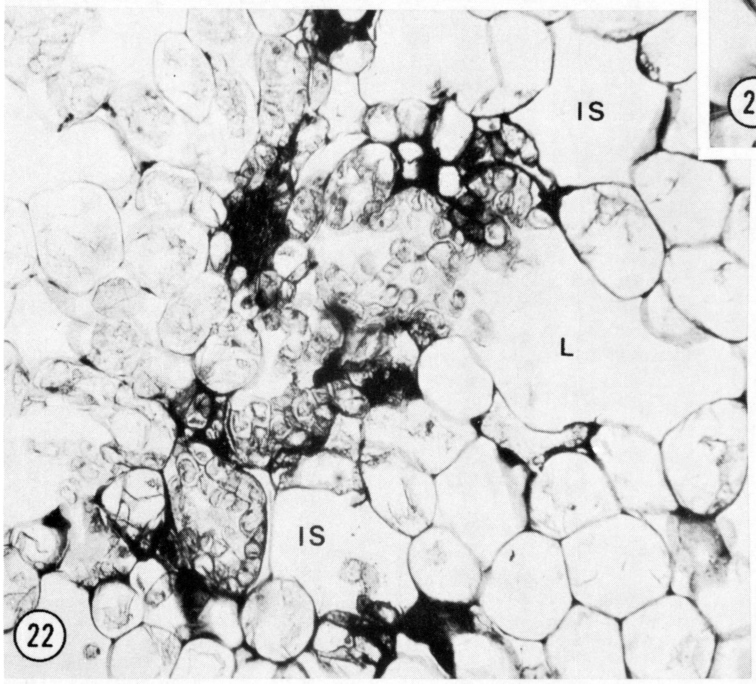
Fig. 20-23. Cork spot tissue in various stages of proliferation and dissolution as the necrotic condition progresses. Note the mother cells (MC) in Fig. 20, 21, and 23 containing the endogenous proliferated daughter cells (DC). In Fig. 20-23 several mother cells have ruptured and discharged the daughter cells into one or more lacunae (L) or into intercellular spaces (IS). Fig. 20-21, ×100; Fig. 22-23, ×50.

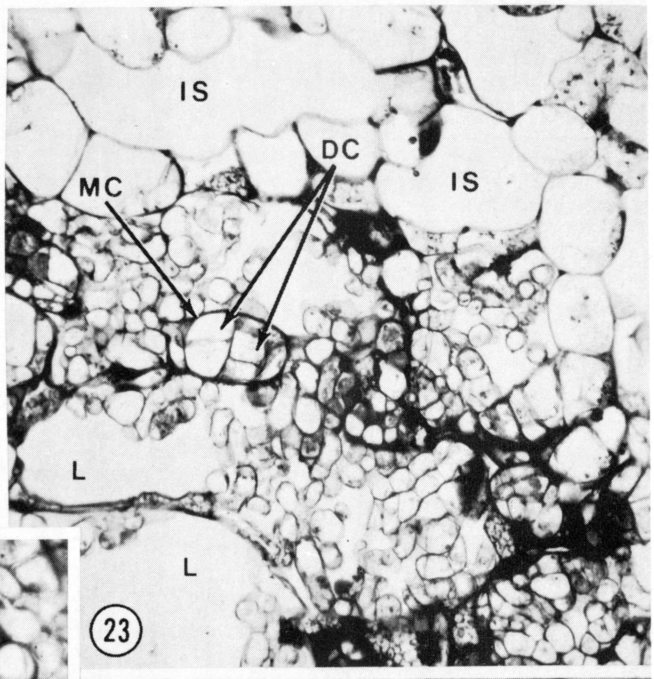
Fig. 24. An uncommon cambium-like (arrows) cellular arrangement of cortical cells encompassing the cork spot necrotic region, ×100.

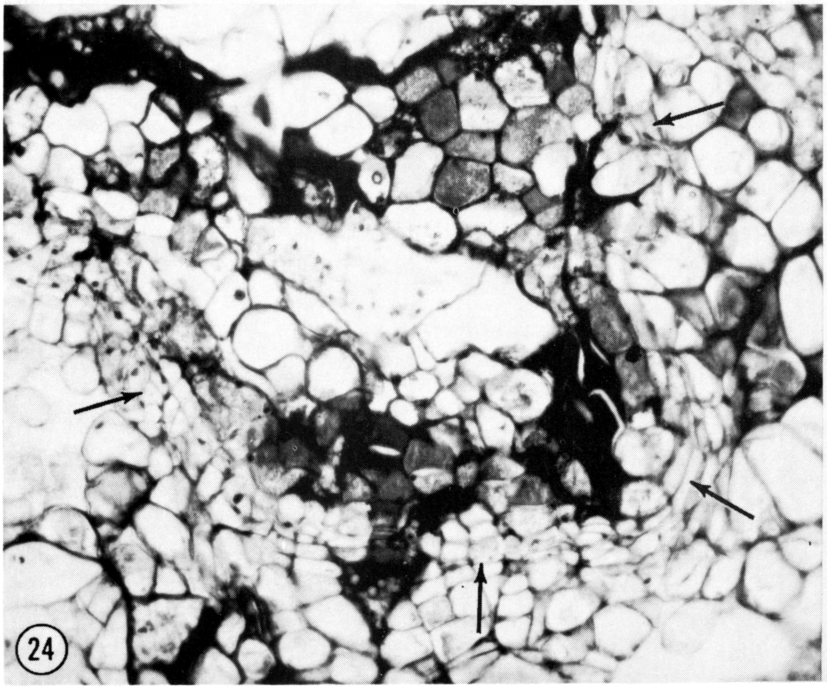
Fig. 25. The usual occurrence of fewer starch grains (arrows) in cork spot tissue (left) than in the contiguous healthy cortical cells (right) as seen in polarized light, ×50. Intercellular spaces (IS), lacuna (L).

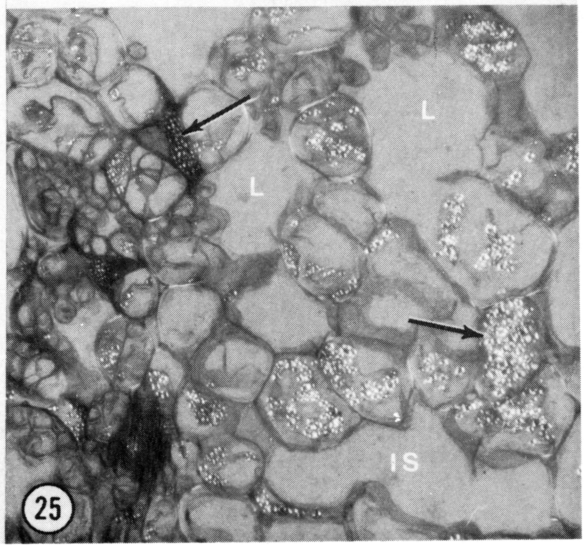












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Light-stimulated Ethylene Production by Germinating Cucumber Seeds[1]

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Additional index words. vegetable breeding, sexual phenotype, *Cucumis sativus*

Abstract. **The degree to which white light stimulated ethylene production in germinating seeds of cucumber (*Cucumis sativus* L.) was influenced by the length of time the seed had been germinated in the dark before being exposed to light. Maximum stimulation occurred when 24 hour old dark-grown seedlings were exposed to 40 μE m^{-2} sec^{-1} light for 24 hours. Ethylene production increased with the duration and intensity of light exposure at all seedling ages. Neither the light or dark rates of ethylene, or carbon dioxide production, nor their ratios, were highly correlated with the sexual phenotype of the 9 cultivars examined.**

Ethylene has been reported to influence the sexual phenotype of cucumber plants (2, 14). Reducing the physiologically active concentration of ethylene with inhibitors of its synthesis or action, or with hypobaric ventilation caused gynoecious plants of the closely related species *Cucumis melo* L. to exhibit more maleness; i.e. to produce perfect flowers as well as pistillate flowers (2). In contrast, elevating the endogenous concentration of ethylene by the application of (2-chloroethyl)phosphonic acid promoted more femaleness in monoecious lines of cucumber plants *(C. sativus)* (11, 18). The natural rate of ethylene production has been shown to be higher in gynoecious than in monoecious cucumber plants (2, 12, 14). Androecious cucumber plants produced less ethylene than monoecious plants (16). These phenotypic differences in the rate of ethylene production were present in 1- to 2-day-old seedlings and generally persisted in the adult plant.

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