DEVELOPMENT OF THE STRUCTURE OF THE RUSSETED APPLE SKIN

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SUMMARY

The epidermal, cuticular, and periderm layers of the apple "Golden Delicious" were studied electron microscopically in the period during which the skin russets, with special reference to the cutin and suberin containing layers. Naturally occurring russeting and the artificially induced form (by iron or copper compounds or mechanical injury) are identical on the EM level. The stages of development are as follows: Firstly, a strong cutinization is observed in the anticlinal and periclinal inner cell walls of the epidermis forming a cuticular epithelium. Secondly, a suberization of the divided cells under the "second cuticle" is described. Thirdly, the suberized cells become located on the outside and they give rise to russeting.

The suberin wall consists of alternating wax and suberin lamellae. These suberin lamellae appear to end in globules located in the tertiary cellulose wall. The fibrillar structure of the cuticle is discussed in connection with the lamellar structure of the suberin.

1. INTRODUCTION

Russeting is the phenomenon of visible brown spots distributed over the entire apple. From literature summarized earlier (KREMER 1963; LINSKENS & GELISSEN 1966), it is known that russeting is the result of cork formation.

Microscopic examination of russeted spots on apples have revealed that periderm, several layers of cells in thickness, forms the protective layer of the fruit. Only remnants of the original cuticle can be found then. During growth of the apple cork is still being formed by the cork cambium and the flakes sloughing off the surface of the russeted spots are remnants of the cork tissue (TETLEY 1930; SIMONS 1957, 1959, 1960).

The work described in this paper is a continuation of the investigation on the fine structure of the apple cuticle (DE VRIES 1968). It is extended to the russeted cuticle of "Golden Delicious". Russeting observed in the orchard under natural conditions is compared with russeting induced artificially under controlled circumstances. Mechanical wounding was used because some enzymatic changes and free fatty acid movement during cutin synthesis are known to occur in relation to wounding of *Gasteria verrucosa* leaves (HEINEN 1963; BREDEMEIJER & HEINEN 1968). The effect of spraying with copper oxychloride and the influence of iron compounds were observed because of the practical importance and the possibility to induce russeting slowly.

2. MATERIAL AND METHODS

For light microscopy the tissue was fixed in a solution of formalin, acetic acid, and alcohol, dehydrated with tertiary butyl alcohol, embedded in paraffin, and

sectioned with a rotormicrotome (JOHANSEN 1940). By means of Sudan dyes, the cutinized and suberized cell walls in the sections were located.

Techniques of fixation, postfixation with 2% OsO_4 solution, sectioning, and staining for electron microscopy have been described previously (DE VRIES 1968). All the sections were stained with lead citrate.

3. OBSERVATIONS

The russeting occurring on the apple in the orchard has been compared electron microscopically with russeting of known cause, *i.e.*:

1. artificial injury by cutting off a small piece of the skin
2. the presence of a rusted iron-wire above the apples
3. spraying with a 0.3 per cent solution of copper oxychloride after anthesis.

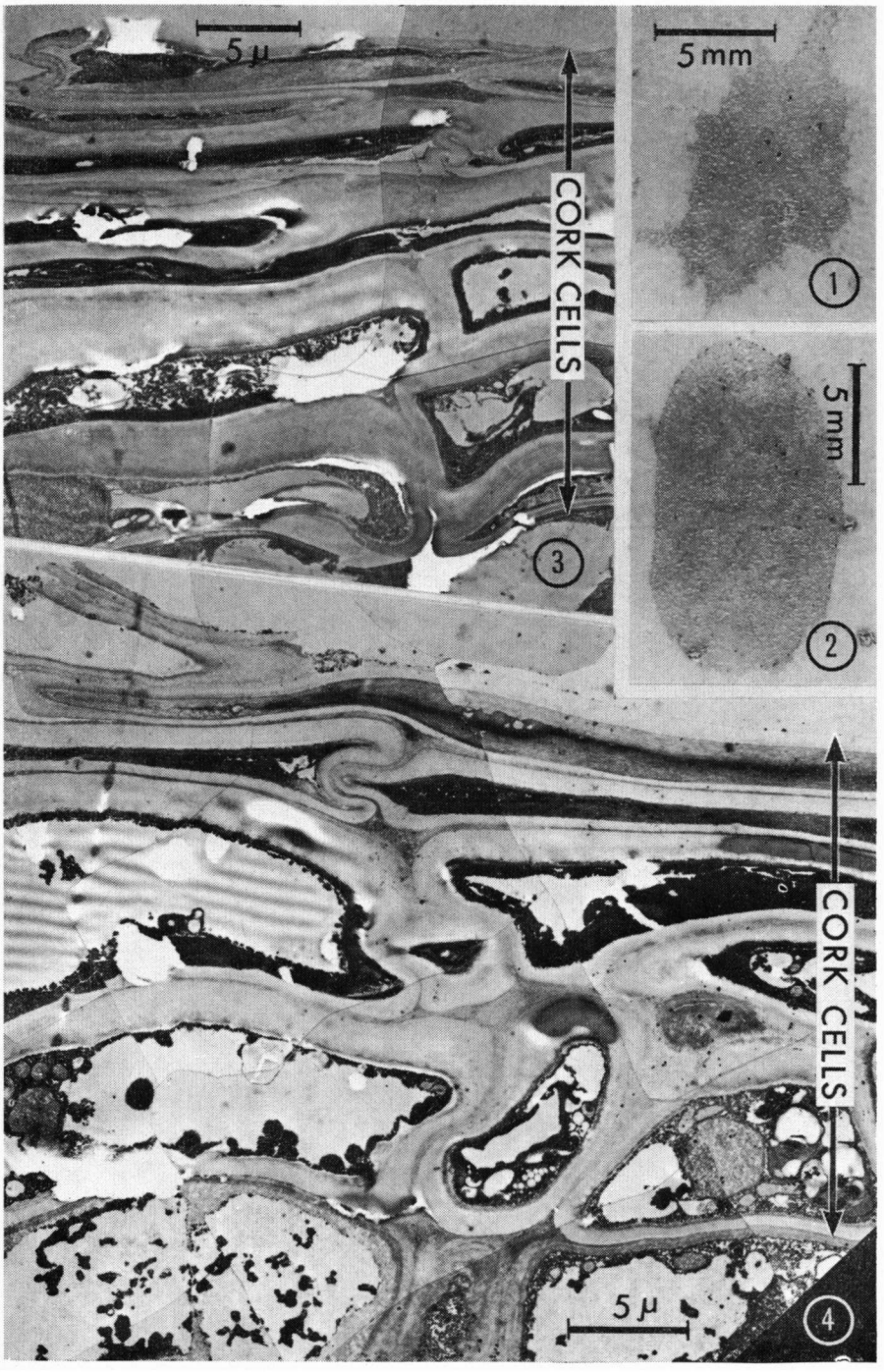
3.1. Russeting after mechanical injury

The mechanical wounding, brought about in the beginning of June, is closed off after several days by a brown layer. This brown layer bursts after three weeks. An even scar tissue can be seen already under it. Five weeks after injury, this protective scar tissue lies on the surface and differs very little in colour from the normal smooth skin ("smooth russeting"). Slowly brown flakes arise, which fall off all the time, and after sixteen weeks the entire wound is coloured light brown ("full russeting"). Macroscopically, a mechanically injured spot can be distinguished from a naturally russeted spot by the smooth border against the healthy tissue *(figs. 1,2)*. It is not possible to distinguish the injured spot from any other naturally russeted spot on the EM level *(figs. 3, 4)*.

The stages mentioned above take a longer time if injury is brought about later on in June. If injury is brought about in the beginning of July, it will take thirteen weeks before the brown layer bursts open. Wounds dating from the beginning of August do not recover, a brown layer appears and stays; below it rot appears. Sections show that, as a result of injury, the exposed cells are now flattened and necrosed. They form a layer of on the average five cells in thickness. No suberized cell walls have been found. A periderm arises beneath the necrosed cells separated from them by some rows of non-flattened cells. That is the reason the outer brown layer loosens easily. At first the cork cells do not have a strong osmiophilic content, they are tightly packed and give rise to the above mentioned smooth russeting. Later on the flakes which cover the wound consist of rejected cork cells. In full russeting the only difference with the first stages appears to be a very electron dense cell content *(fig. 4)*. Sections of naturally russeted spots give exactly the same picture *(fig. 3)*.

The transition of a naturally russeted spot to a spot where the cuticle is still

Fig. 1. A naturally russeted spot.
Fig. 2. A mechanically injured spot.
Fig. 3. A section through the spot shown in fig. 1.
Fig. 4. A section through the spot shown in fig. 2.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present is seen in *fig. 5*. The partially present cuticle is thinner than on the smooth fruit of the same age, probably because the synthesis of the cutin constituents is delayed or stopped altogether by disconnection from the living cells. It is likely that these cutin constituents are being used for the formation of cork cells found under the epidermis. No lamellar structure has been found where the cuticle is damaged, but instead a fibrillar structure *(fig. 6)* is seen like that of the smooth apple (DE VRIES 1968).

3.2. Russeting caused by iron compounds

It is known that rusted iron wires cause russeted spots on apples of a black-brown color. No direct contact of the apple with the wire is necessary.

In the light microscope an early stage of a russeting apple, which was hanging under an iron-wire and thus continually irrigated by a solution saturated with iron compounds, it appears as shown in *fig. 8*. The cuticle has an irregular appearance. In some places, the cutin wedges reach between and below the epidermal cells forming a cuticular epithelium *(fig. 8*, arrow), which on the smooth fruit of the same age is not to be seen. Many vesicular elements which appear to be empty have been found electron microscopically on the bases of the cutin wedges; also many divided epidermal and hypodermal cells have been found. Suberized cells are seen on the russeted spots. And they are also seen in the transition of the russeted spot to where the cuticle is still present. This way cutinized and suberized cell walls fit closely together. Electron microscopically full russeting is the same as the one shown in *fig. 4*. In addition to natural russeting causes, which can occur in the orchard, russeting was artificially induced by spraying with a defined metal solution, which is known to cause russeting.

3.3. Russeting by spraying with a copper solution

Spraying four times after anthesis, namely at May 18, 22, 26 and June 2, 1967,

ABBREVIATIONS

Cu	Cuticle
SCu	Secondary cuticle
EC	Epidermal cell
C	Cork cell
Cs	Cell suberizes starting at the inner side
PW	Primary wall
SW	Secondary-suberin-wall
TW	Tertiary-cellulose-wall
MVE	Multivesicular elements
W	Wax

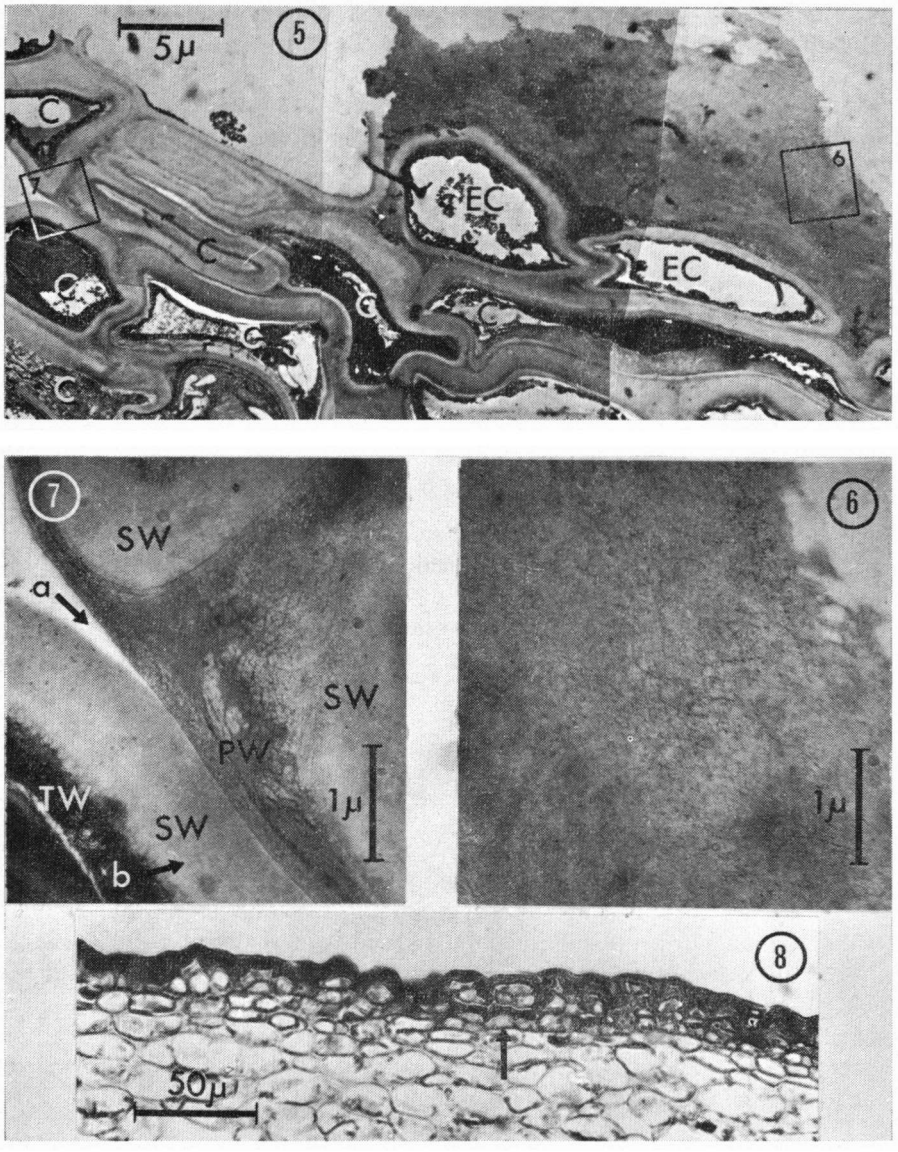


Fig. 5. Transition of a naturally russeted spot to a spot where the cuticle is present.
Fig. 6. Magnification of the damaged cuticle shown in fig. 5. Note the fibrillar structure.
Fig. 7. Magnification of the cork cells of fig. 5. Note that the suberin wall loosens from the primary wall (arrow a); and the alternatingly thin layers of the suberin wall (arrow b).
Fig. 8. A cuticular epithelium in an early stage of russeting caused by iron compounds as seen through the light microscope (arrow). Stained with Sudan black.

with a 0.3% copper oxychloride solution gives rise to russeting in the second week of June which stays on till harvest time. Full russeting is alike again both macroscopically and electron microscopically *(figs. 2, 4)*. However if sprayed at the end of September or the beginning of October, no periderm occurs; instead, red coloured lenticles ("Lentizellenröte" BOHNEN 1964) appear.

3.3.1. Change in the epidermal outer cell wall layer

Electron microscopically we see a very clear fibrillar structure in the cuticle next to the very thick wax layer *(fig. 9)* while the fruit is still smooth macroscopically. This structure is much more distinct than in the cuticle of smooth fruit (DE VRIES 1968, fig. 4b). Supposedly the clear structure of the fibrils is due to the incorporation of Cu-ions, which results in an better contrast. The thickness of the cuticle varies, but it is usually less than the cuticle of smooth fruit.

3.3.2. Formation of a cuticular epithelium

Locally an epidermal cell is cutinized on all sides, while the apple is not yet russeted macroscopically. The epidermal inner cell wall, which is cutinized very little as yet, contains many vesicular elements *(fig. 10)*. The cutinous material and the vesicular elements have been observed in the invaginations of the plasmalemma of the epidermal and hypodermal cells *(fig. 11*, arrows*)*. The very thick wax layer found in an earlier stage has decreased considerably.

3.3.3. Localisation of the suberized cell walls

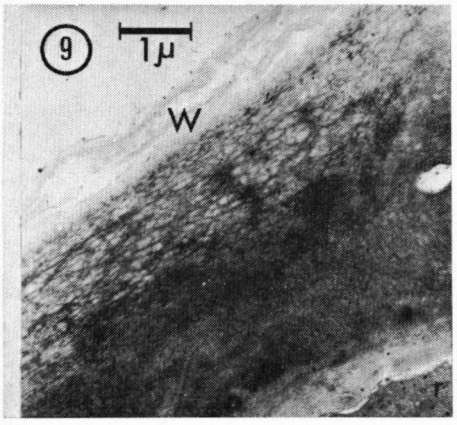
Eight weeks after spraying, the apples show many transitory forms from cuticle formation to full russeting *(fig. 12)*. A cutinized wall ("secondary cuticle") has developed below the epidermal cell which wall is as thick as the cuticle. Within this second cuticle and in the cutin wedges, we find many multivesicular elements. Going from the smooth to the russeted skin, we observed an increasing number of cork cells, the result of which is closely packed cutinized and suberized cell walls. Primarily, cells beneath the second cuticle are forming cutinous material and multivesicular elements, and later on, they suberize starting at the inner side (*fig. 12*, arrows). Finally, the suberized outer cell wall is thicker than the inner. Full russeting shows only when the cuticle is no longer present. Five

Fig. 9. A very clear fibrillar structure in the cuticle of an apple sprayed with copper oxychloride.

Fig. 10. An epidermal cell cutinized on all sides caused by spraying with copper oxychloride. The inner cell wall contains many multivesicular elements.

Fig. 11. Magnification of an inner cell wall of the same stage as mentioned under fig. 10. Note the osmiophilic material and the elements in the invagination of the plasmalemma (arrows).

Fig. 12. A drawing of a section of a transitory form from cuticle formation to full russeting caused by spraying with copper oxychloride. The cells suberize starting at the inner side (arrow). Location of figs. 13, 14 and 15 are indicated.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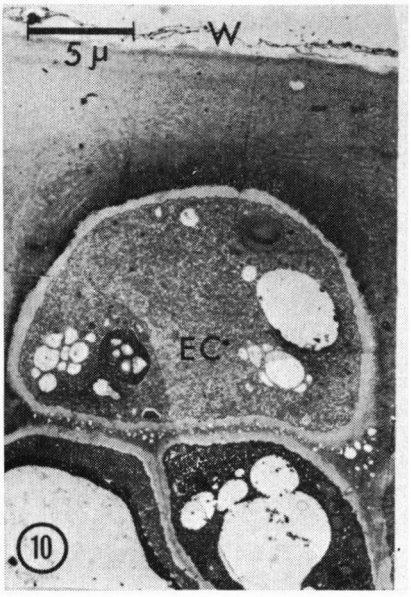
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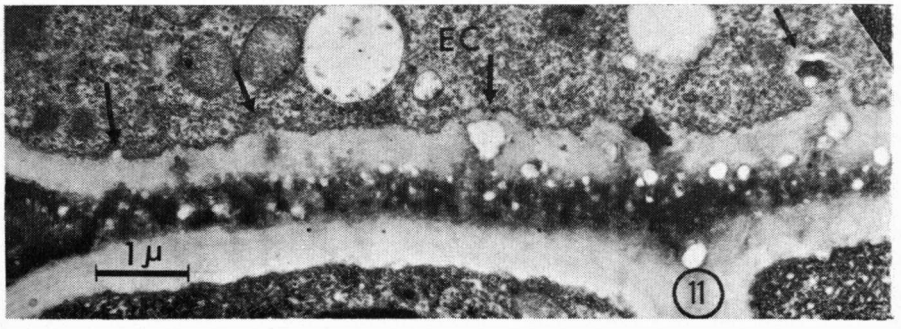
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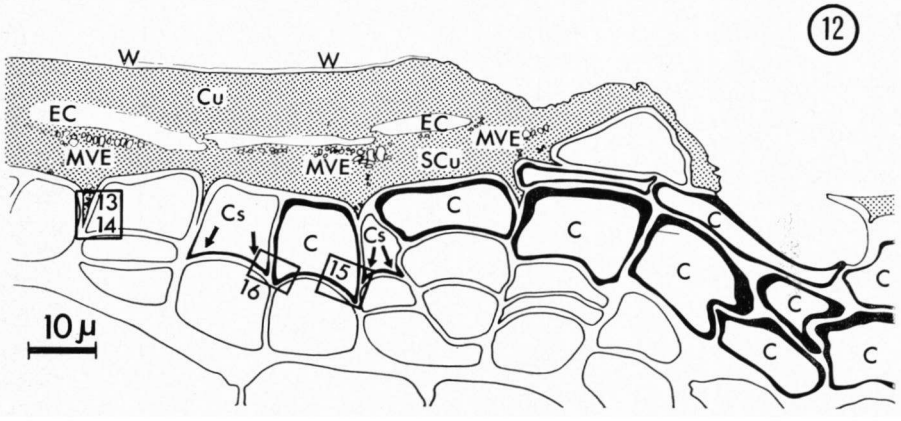
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layers of flattened cork cells of which the upper four have electron dense contents are present.

3.3.4. Changes in the cell in the period during which the skin russets

Many changes occur during this period. The most obvious ones are listed chronologically below. Fifteen days after treatment, the epidermal cells have a large central vacuole with osmiophilic material on the edges. Sometimes these cells contain electron-dense cytoplasm. Twenty five days afterwards, we observed epidermal cells with many small vacuoles and an increasing amount of endoplasmic reticulum. Eight weeks after spraying, those cells which are surrounded by cutin material have an almost obliterated lumen where only some degenerated plastids are discernable. The cells located below the second cuticle divide rapidly, and have many small vacuoles. When they are synthesizing cuticular material they contain much endoplasmic reticulum. These cells have an irregular, strongly osmiophilic plasmalemma with many invaginations which enclose the same material as in the wall nearby (pinocytosis), *i.e.*, osmiophilic material and multivesicular elements of the second cuticle (*figs. 13, 14*, arrows). But when they start forming suberin they contain a smooth endoplasmic reticulum and a smooth plasmalemma without invaginations *(figs. 15, 16)*. After this suberin formation the cork cells synthesize a tertiary cellulose-containing layer; the electron density of the lumen increases and hardly any organelles are discernable.

4. NATURALLY OCCURRING RUSSETING

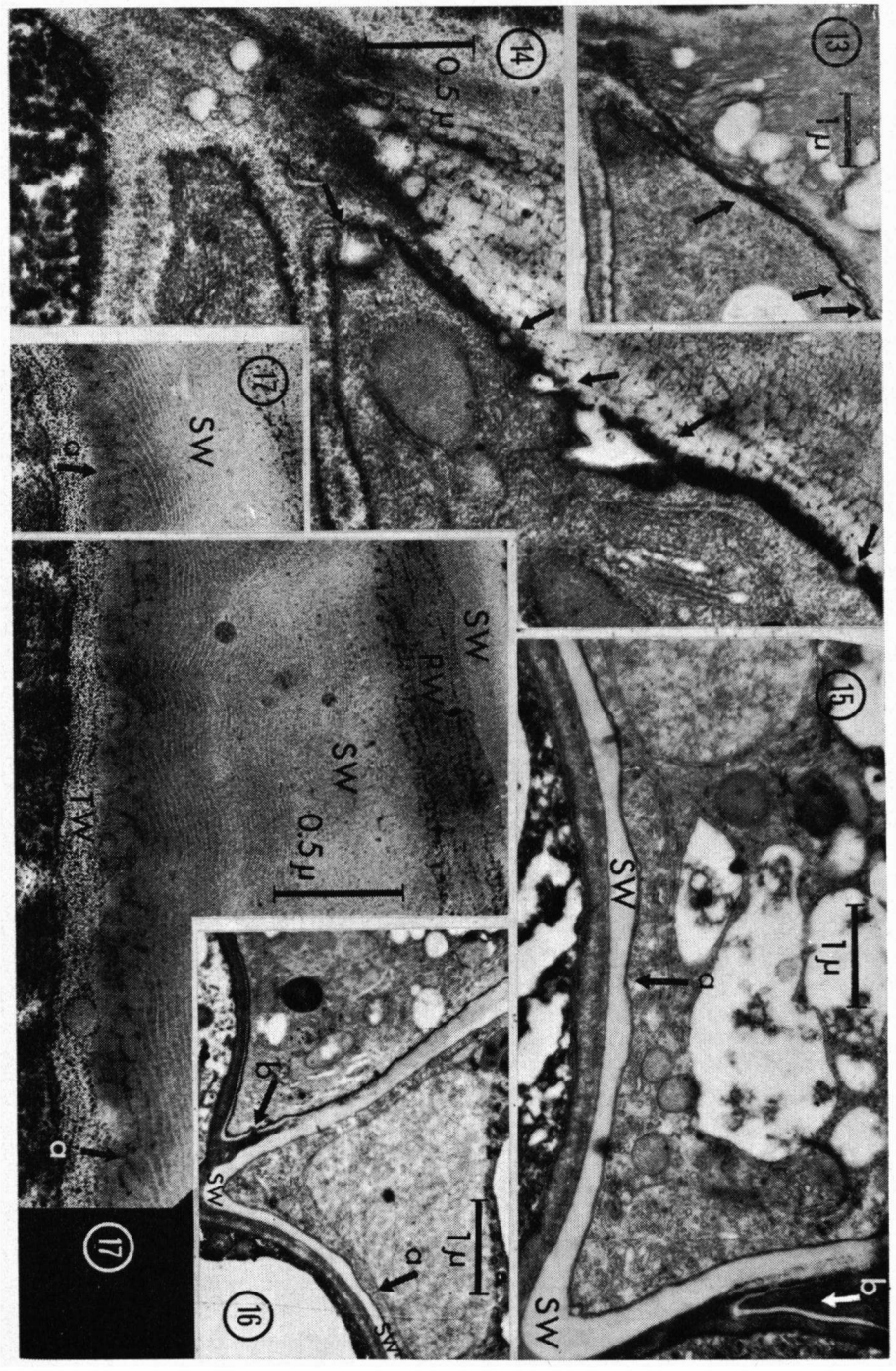
We observed that natural russeting is identical with full russeting mentioned under 3.1. electron microscopically *(figs. 3, 5)*.

5. DISCUSSION

The progressive development from smooth to russeted skin can be pictured as follows.

A strong cutinization takes place between, and sometimes below, the epidermal cells resulting in a cuticular epithelium. The deposit of cutin above the epidermal cells is retarded compared to the smooth fruit. Cutinization all

Fig. 13. A cell beneath the second cuticle (cf. fig. 12) forming osmiophilic material and multivesicular elements. Note the strongly osmiophilic plasmalemma with the invaginations (arrows).
Fig. 14. See fig. 13.
Fig. 15. A cell forming a suberin wall (arrow a). The very first formation indicated by arrow b.
Fig. 16. See fig. 15.
Fig. 17. Magnification of a full-grown suberin wall with alternatingly osmiophilic and non-osmiophilic layers. Note that the suberin lamellae sometimes appear to end in globules (arrow a).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around also occurs normally, but much more towards the full-grown stage (DE VRIES 1968). The cells beneath the cuticular epithelium divide and form the periderm. Rejection of the cuticle and the appearence of the cork cells result in russeting. Finally brown flakes arise because cork cells loosen; the growth of phellogen is necessary for the growth of the apple.

Most striking is the strong cutinization preceeding formation of periderm, also observed by SIMONS (1957) on frost-injured "Golden Delicious".

Russet caused by the presence of iron-wires develops less rapidly than the one caused by copper oxychloride. A similar result was obtained by SIMONS (1960) concerning frost-injured apples and genetically-controlled russet sports of "Golden Delicious". However all the stages of russeting are about the same anatomically. The conclusion reached by TETLEY (1930), that russeting occurs often in the variety that has a great cutin deposit, is also valid for our object. In "Golden Delicious" great cutin deposits lead to russeting.

The cuticular constituents released pinocytotically by the cell (see 3.3.4.) become located within the cuticles, which are separated from the cells by a layer of cellulose (DE VRIES 1968). The suberin constituents synthesized by a smooth plasmalemma become located next to this membrane. After this secondary suberin wall has been finished, the cell synthesizes a tertiary cellulose-wall after which the cell dies. The suberin wall loosens very easily from the primary wall (*fig. 7*, arrow a).

Cutinized cell walls show the above mentioned fibrillar structure. The secondary cell wall of all the cork cells described above consists of alternatingly thin electron dense and non-electron dense layers *(figs. 7*, arrow b, *17)* as described by SITTE (1962, 1965). The suberin wall is built the same way in all our observations. Non-osmiophilic wax lamellae alternate with osmiophilic suberin lamellae, the suberin lamellae being 70–90 Å thick. This is in accordance with the results of SITTE (1962), who found suberin lamellae varying in thickness from 25–200 Å in bottle cork, and with the results of FALK & NABIL EL HADIDI (1961), who observed the variation of 25–100 Å in cork cells of Acacia seyal and of the potato. The suberin lamellae themselves appear to consist of small granules *(fig. 17)* as supposed by SITTE (1955) in his theory: "vernetzten Aggregaten globulärer Makromoleküle". The suberin lamellae sometimes appear to end in globules located in the tertiary cellulose layer (*fig. 17*, arrow a), but usually we see an almost hexagonal structure. Both the globules and the hexagons have a diameter of 600–900 Å. The hexagonal structure found in the cuticles of smooth apples varies in diameter from 400–2600 Å (DE VRIES 1968).

An interesting question is whether or not the structure of the fatty acid material of both the suberized and cutinized cell walls arises because of accompanying material. Although the fatty acids occurring in the cuticle of "Cox Orange Pippin" apples (EGLINTON & HUNNEMAN 1968) have already been analyzed (about thirty), our own research shows that both in smooth and russeted outer cell walls of "Golden Delicious" apples the same groups of fatty acids occur as shown by thin layer chromatography.

In further experiments we shall try to find out whether the lamellar structure of

the suberin is caused by the presence of wax molecules and whether the above mentioned fibrillar structure arises because of polar molecules.

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