A STUDY OF THE SURFACE WAX DEPOSITS ON APPLE FRUIT

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Summary

Optical and electron micrographs of surface wax deposits on four varieties show varietal differences. In addition to normal structure a lath-like wax can be seen in the fine cuticle cracks that are plentiful on Golden Delicious and sparse on other varieties; another form of wax is always at right angles to abrasion marks on the fruit and is present on all varieties.

The removal of surface wax by solvents exposes a cuticle structure which is related to the deposits and differs in some varieties. The ease of removal of surface wax by wiping differs with variety, Granny Smith wax being much easier to shift than Golden Delicious. When surface deposits are removed by any method the apples lose moisture at a faster rate.

I. Introduction

The exuded waxy coating on apples has been the subject of a number of chemical analyses (Markley and Sando 1931; Kreger 1948; Huelin and Gallop 1951; Batt and Martin 1959; Richmond and Martin 1959; Baker *et al.* 1961; Waldron *et al.* 1961; and Mazliek 1963), but less attention has been paid to its physical characteristics. The few studies which have been made have shown that the waxy surface determines the wetting properties of the fruit and affects the deposition of spray chemicals (Markley and Sando 1931; Van Overbeek 1956).

Others have observed that the removal of insecticide sprays from apples (Marshall, Overley, and Groves 1936) or from citrus fruits (Eaks and Ludi 1960) by brushing combined with chemical treatment increased their transpiration rate and accelerated wilting. Similar results were obtained by Pieniazek (1944) when he wiped the surface of apples until they were shiny and the ensuing increased permeability, which he attributed to the removal of the waxy coating, was found to differ with variety. He concluded that this layer was of great importance in diminishing water losses from apples. Recent work on plant leaves adds support to this view (Hall and Jones 1961; Hall and Donaldson 1963) since with the aid of electron microscopy the removal of surface wax deposits could be observed.

The present investigation is concerned with (1) varietal morphology of the surface wax deposits and epicuticle and (2) the effects of wiping and polishing apples.

II. Materials and Methods

Apples from the Department's experimental orchards at Hastings and Nelson were harvested when mature with as little disturbance to the surface as possible.

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Most of the apples were examined soon after picking, but some were kept in cool storage for 3 months. Bruised apples were rejected as their rate of moisture loss is abnormally high.

The varieties studied were Golden Delicious, Granny Smith, Red Dougherty, and Sturmer, the last variety being available from trees which had been fed with a surplus of phosphorus or nitrogen (Tiller, Roberts, and Bollard 1959).

The following methods were used to examine the properties and structure of the surface wax and cuticle:

(1) Shadowed plastic replicas in the light microscope.

(2) Carbon replicas in the electron microscope.

(3) Contact angle measurements to give a measure of the wettability of the fruit surface.

(4) X-ray diffraction analyses of the wax deposits.

(a) *Optical Microscopy*

Transparent plastic replicas were prepared from the fruit surface in its normal state and also from cuticle which had been immersed in a wax solvent for 2 hr or more. A solution of polyvinyl alcohol when dry formed a negative replica and this was shadowed with antimony in a vacuum chamber. A perusal of replicas from several areas on each apple ensured that only typical structure was photographed. Shadows appear lighter than the background.

(b) *Electron Microscopy*

The following methods were used:

(1) The distribution of wax on the cuticle was revealed by pseudo-replicas which retained some of the surface wax. The surface of a small slice of apple peel was initially shadowed with gold palladium and then coated with a carbon film. A few drops of gum arabic solution were dried on this surface and used to strip the carbon, shadowing material, and wax from the cuticle surface. The gum arabic was then dissolved in warm water.

(2) The surface structure of the wax deposits was obtained in a similar manner but the wax attached to the replica was dissolved in benzene or chloroform.

(3) The cuticle surface underlying the wax was examined by preparing replicas after wax on the apple surface had been dissolved.

The carbon replicas were examined with a Philips EM100B electron microscope and large fields were obtained by recording as many as 25 adjacent areas.

(c) *Contact Angle*

Contact angle measurements were made with the aid of a microprojector having a magnification of 50 diameters. Water droplets of about 2 mm diameter were applied to the outer surface of pieces of apple peel and the image of the droplet in contact with the surface was projected on to a ground-glass screen (Fogg 1947).

III. Results

(a) *Optical Microscopy*

Sturmer apples are covered with wax deposits that are predominantly crescent-shaped at low magnification (Plate 1, Fig. 1). When the wax is dissolved from an apple before a replica is prepared, the contours of the epidermal cells are revealed.

Granny Smith apples viewed at the same magnification have a very different appearance (Plate 1, Fig. 2); long marks are present in their soft wax and are produced even by abrasion with paper wrappers. At least half the surface wax is dissolved when peel is immersed in benzene for 2 hr and very little remains when it is left in chloroform for a longer period. The boundaries of the epidermal cells can be seen in Plate 1, Figure 3, and also a fibrous-like pattern of parallel ridges (in the exposed cuticle) that has a concentric pattern round each lenticel (L). Other markings are often at right angles to these ridges.

The fibrous type of structure was also present on a proportion of the surface of Golden Delicious apples although they had not been in contact with solvents. One of these regions is indicated by an arrow in Plate 1, Figure 4. On this variety of apple, dense wax bands (B) appear to coincide with the edges of many of the epidermal cells and with the minute cracks that are visible when the skin is studied directly with the light microscope (Page 1960). The bands are due to optically dense wax from the apples being retained by the replicas. When this wax is dissolved from the replica and examined at higher magnification, the surface structure of a band is lath-like as in Plate 1, Figure 5.

Replicas of Red Dougherty variety showed no outstanding features and indicated that the wax was evenly distributed.

(b) *Electron Microscopy of Wax Layer*

A low magnification electron micrograph of a pseudo-replica of the surface of a Sturmer apple is shown in Plate 1, Figure 6. The crescent-shaped wax observed in Plate 1, Figure 1, is again present and is indicated by an arrow. When the preshadowed replicas were immersed in wax solvents and observed at higher magnification, the wax was seen to consist of crystals of a flat platy type (Plate 2, Fig. 2).

Different feeding of the Sturmer trees appeared to have no effect on the morphology of the surface wax and chemical extraction gave a value of approximately $0 \cdot 6$ mg/cm^2 when the trees were fed with either nitrogen or phosphorus.

Pseudo-replicas of the surface wax on Granny Smith variety showed few outstanding features, but wax-free replicas of the surface displayed aggregates of well-defined flat plates (P) parallel with the cuticle (Plate 2, Fig. 1). These layers can be seen more clearly in Plate 2, Figure 3, at a higher magnification. This variety of apple is very easily polished and a few rubs with a soft cloth removes some of the wax and distributes the remainder over the surface in the manner shown in Plate 3, Figure 1. The shiny appearance of polished apples is probably due to reflection of light from wax that has been spread over the surface in relatively uniform flat areas.

The wax structure present in Plate 3, Figure 2, was present on all varieties but more abundant on Granny Smith apples. It consists of thin exudates that grow outwards from the epidermis and have their long axes at right angles to abrasion marks on the cuticle. They are small in the early stages, but after a month or so have the appearance of Plate 3, Figure 3. The abrasion probably occurs in windy weather when the apples rub against branches.

On Golden Delicious apples most of the surface is covered with flat crystallized layers similar to Plate 2, Figure 2, but there are few aggregates of the multilayer type shown in Plate 2, Figure 1 (*P*). The outstanding feature of this variety is the irregularly shaped bands of wax mentioned earlier. Electron micrographs of these areas confirmed the lath-like structure (Plate 3, Fig. 4) and indicated that the wax was lying in channels which may exceed 20 μ in width. This type of wax was not profuse on other varieties and seldom seen on Granny Smith apples.

Table 1

CONTACT ANGLE MEASUREMENTS FOR APPLES HARVESTED EARLY IN THE SEASON

Variety	Date Harvested	No. of Apples	No. of Measurements	Mean Value of Contact Angle ±S.E. (deg)
Sturmer (phosphorus fed)*	May 8	19	114	89·9±0·1
Sturmer (nitrogen fed)	May 8	19	110	85·9±0·1
Sturmer (untreated)	May 8	20	120	89·6±0·1
Granny Smith (non-shiny areas)	April 5	5	36	80·0±0·3
Granny Smith (random areas)	April 27	13	78	88·1±0·2
Golden Delicious	April 17	20	58	94·5±0·2
Red Dougherty	July 15	9	96	89·2±0·1

* For details see Tiller, Roberts, and Bollard (1959).

Electron micrographs of Red Dougherty variety showed no outstanding features. A pseudo-replica of the wax is shown in Plate 3, Figure 5, and true replicas of the wax surface structure were similar to Plate 2, Figures 1 and 2, showing well-defined platy layers.

(*c*) *Electron Microscopy of Epicuticle*

Replicas of the cuticle were prepared after the wax had been removed from the apple surfaces with solvents. The Sturmer variety is covered with circular shaped ridges of the type shown in Plate 3, Figure 6. This cuticle structure was peculiar to this variety and appeared to be related to the shape of the wax above it.

Granny Smith had no distinctive features, but on Golden Delicious channels were observed to be present under the lath-like wax. The bottoms of the channels were rougher than the cuticle on either side.

On all varieties the numerous pores which exuded the wax were observed in the epicuticle. They were similar to those mentioned in an earlier paper (Hall and Donaldson 1962) and will be described in more detail elsewhere.

(*d*) *Contact Angles*

Contact angle measurements were made on apples harvested early in the season and those picked a few weeks later (Table 1). They show that droplets of water adhere to all four varieties since the highest value of contact angle was 94·5 degrees.

Feeding made little difference to the wettability of Sturmer apples, less in fact than the polishing of Granny Smith apples that occurred during harvesting.

(e) *Role of Surface Wax in Conserving Moisture*

Pieniazek's work made it clear that when apples were wiped with cheese-cloth their transpiration rate increased, and he considered this to be due to the removal of surface wax.

We confirmed that wax was removed when apples were wiped with paper wrappers, by weighing the wrappers before and after this procedure. The results are given in the following tabulation:

	Wax Removed (mg)		
	First Wipe	Second Wipe	Third Wipe
Granny Smith	25	12	4
Golden Delicious	8	4	3
Red Dougherty	21	8	5
Sturmer Pippin	27	10	0

It will be seen that the wax on the Granny Smith and Sturmer Pippin varieties was the easiest to remove by wiping, and Golden Delicious the most difficult.

Different methods of removing some of the surface wax were then applied to Granny Smith and Golden Delicious apples as follows:

(1) Apples were wiped with a cloth saturated by a 1% solution of Teepol in water.

(2) Others were wiped with paper wrappers.

(3) One application of adhesive tape was made to over 90% of the apple surface and then stripped from the surface.

Changes in transpiration for each variety was then measured. Care was taken to ensure that apart from wax removal the epidermis remained undamaged. Six unblemished apples were used in each weight measurement and these were made before and after keeping the fruit in a standardized condition of 25°C and 60% R.H. for 3 days. All fruit were of comparable size and surface area so that transpiration loss on a weight basis was considered justifiable. Results obtained are given in the following tabulation. Control values for Sturmer and Dougherty varieties are also included.

	Transpiration Loss per Day (as % of initial weight)			
	Granny Smith	Golden Delicious	Sturmer	Dougherty
Control values	0·34	0·43	0·41	0·41
Single polish with wrapping paper	0·41	0·46	—	—
1% Teepol on cloth	0·43	—	—	—
Adhesive tape stripping	0·50	0·48	—	—

The results show that the surface wax may be removed by various methods and confirm that its removal causes an increase in transpiration rate. The rate can be further increased by polishing for a longer period or by repeated stripping, but if stripping is repeated too often some epidermis is also removed.

Of the varieties examined Granny Smith has the lowest moisture loss when its surface is undisturbed, but a single polish of this soft wax increased the rate by 20%. Golden Delicious variety had a higher initial rate but because its wax is more difficult to remove, transpiration was not increased to the same extent as Granny Smith.

IV. Discussion

(a) *Wax Morphology*

The use of shadowed plastic replicas was shown by (Page 1960) to be of particular value for the examination of translucent surfaces. It provides a ready means of observing gross wax forms and distribution whereas direct observation of the epidermis with the light microscope is unrewarding in this respect. The replicas showed that (1) the surface of some varieties had a distinctive form of wax, (2) cuticle deformities in Golden Delicious can be observed in detail, and (3) damage to the wax of Granny Smith after handling is easily detected.

Electron micrographs of replicas with wax attached indicated that the morphology of apple wax differs in a manner related to the structure of the epidermis. On Sturmers, for example, the crescent-shaped wax appeared to originate from the circular-shaped areas of the cuticle. The surface structure of apple wax examined by standard replication techniques showed differences in the deposition of the crystalline layers, Granny Smith having aggregates of these layers more clearly defined than other varieties. However, X-ray diffraction analysis did not show any major varietal differences in crystallinity.

The wettability of plant or fruit surfaces is governed to a large extent by the type of wax deposits and their distribution; a surface with numerous flat deposits possesses moderate wetting properties that retains water droplets. The measured value for the apple fruit surfaces, therefore, was of the order anticipated as the type of surface in electron micrographs such as Plate 2, Figure 1, is associated with contact angles between 80 and 100°. This form of coating has a semi-transparent look as distinct from the glaucous appearance of some plant species with different forms of wax.

The lath-like wax on Golden Delicious apples was shown to occur in channels in the cuticle and is of especial interest as it has not been observed in plant leaves. Plate 3, Figure 4, only tells part of the story of these cuticular cracks. Both Smock and Neubert (1950) and Pieniazek (1944) have observed that higher transpiration than normal occurs when such cracks are present and one would therefore expect that the lath-like wax forms a permeable layer. This could only be verified by sections that retained the surface wax (a technique that is still being developed). It will be seen from the second tabulation in Section III(*e*) that the transpiration rate of Golden Delicious is higher than other varieties due mainly to the cuticular cracks; however, Table 1 also shows it to be the least wettable. This suggests that the lath-like wax above the cracks together with the main deposits prevent water droplets from adhering to the cuticle as firmly as on other varieties.

The wax layer shown in Plate 3, Figures 2 and 3, is always at right angles to abrasion marks and it is possible that cuticular damage or stress may be responsible for its form. Similar deposits have occasionally been seen on plant leaves.

(*b*) *Effect of Wax Removal*

The surface on the Granny Smith variety was the easiest to damage or distort by handling, and surface changes due to this were observed with micrographs and the increased contact angles of water droplets (see Table 1). Areas with the surface wax intact are more wettable than shiny areas, where wax has been removed, and this is the only plant surface we have examined which has shown this effect.

The wettability of plant leaf surfaces covered with wax rodlets or platelets always increases because the wax is readily broken and removed by abrasion. Granny Smith apples have wax deposits consisting of crystalline layers that lie flat on the cuticle and when rubbed slide over one another very easily. Although abrasion causes wax to be removed from the apple surface [see first tabulation in Section III(*e*)] the remaining deposits are evidently redistributed to cause a higher wettability. How this is done and at the same time accounts for a higher transpiration rate is not clear. Skene (1963) has shown that the effect of polishing an apple surface differs not only with variety but with individual apples.

Recent work in this laboratory by Horrocks (1964) has shown that the use of solvents to remove not only surface deposits but also wax embedded in apple cuticle increased cuticular permeability to water vapour by 70 times in Granny Smith and 30 times in Golden Delicious; this lower value was due to the higher initial permeability of Golden Delicious. It is clear that the cuticular wax is a very efficient barrier to moisture loss and it is possible that the varietal differences of fruit with undamaged surface wax can be accounted for by other factors. For example, Pieniazek has shown that moisture loss from the lenticels of Golden Delicious was greater than that of the other varieties he examined and that russeting increased this loss.

Increases in transpiration readily occur, however, when surface wax is removed from apples that lose their wax easily. A single twist of wrapping paper held in contact with the surface of a Granny Smith apple is all that is needed to increase its transpiration to a similar value given by undamaged Golden Delicious apples.

Loss of wax may occur not only during harvesting and packing but by abrasion while the apples are growing. The increased transpiration that results is only partially offset by further exudation of wax during storage (Markley and Sando 1931; Pieniazek 1944; Huelin and Gallop 1951; and Richmond and Martin 1959) so there is little doubt that their storage life is reduced.

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EXPLANATION OF PLATES 1–3

PLATE 1

Fig. 1.—Optical replica of a Sturmer apple surface. ×200.

Fig. 2.—Optical replica of the surface of a Granny Smith apple. ×100.

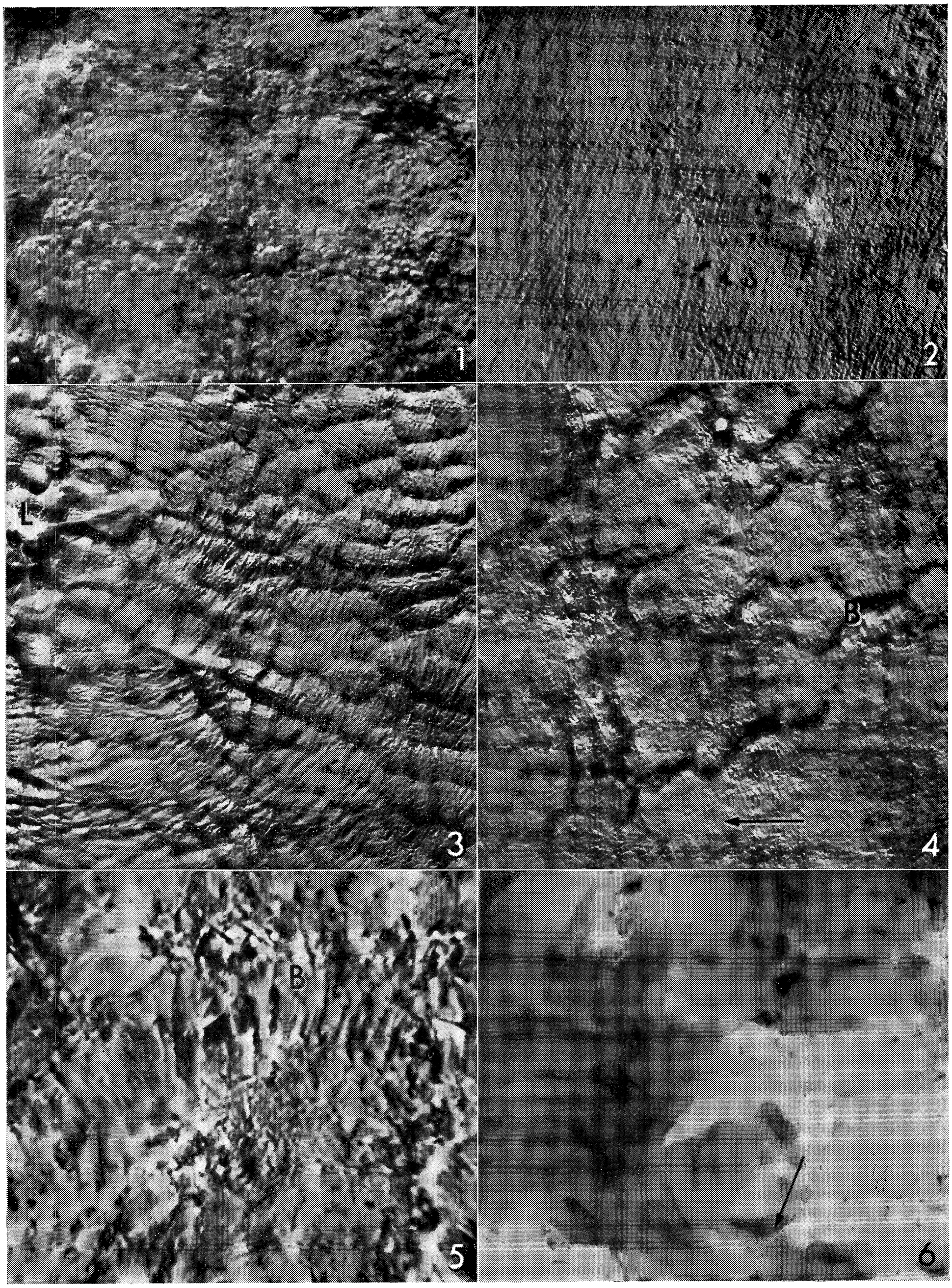
Fig. 3.—Optical replica of the cuticle of a Granny Smith apple after removal of the surface wax by solvents. ×100.

Fig. 4.—Optical replica of a Golden Delicious apple showing attached irregular bands of wax (*B*) and fibrous structure indicated by an arrow. ×100.

Fig. 5.—One of the bands at higher magnification after dissolving the wax from the optical replica. ×650.

Fig. 6.—Electron micrograph of pseudo-replica of a Sturmer apple. Crescent-shaped wax indicated by an arrow. ×3000.

SURFACE WAX DEPOSITS ON APPLE FRUIT



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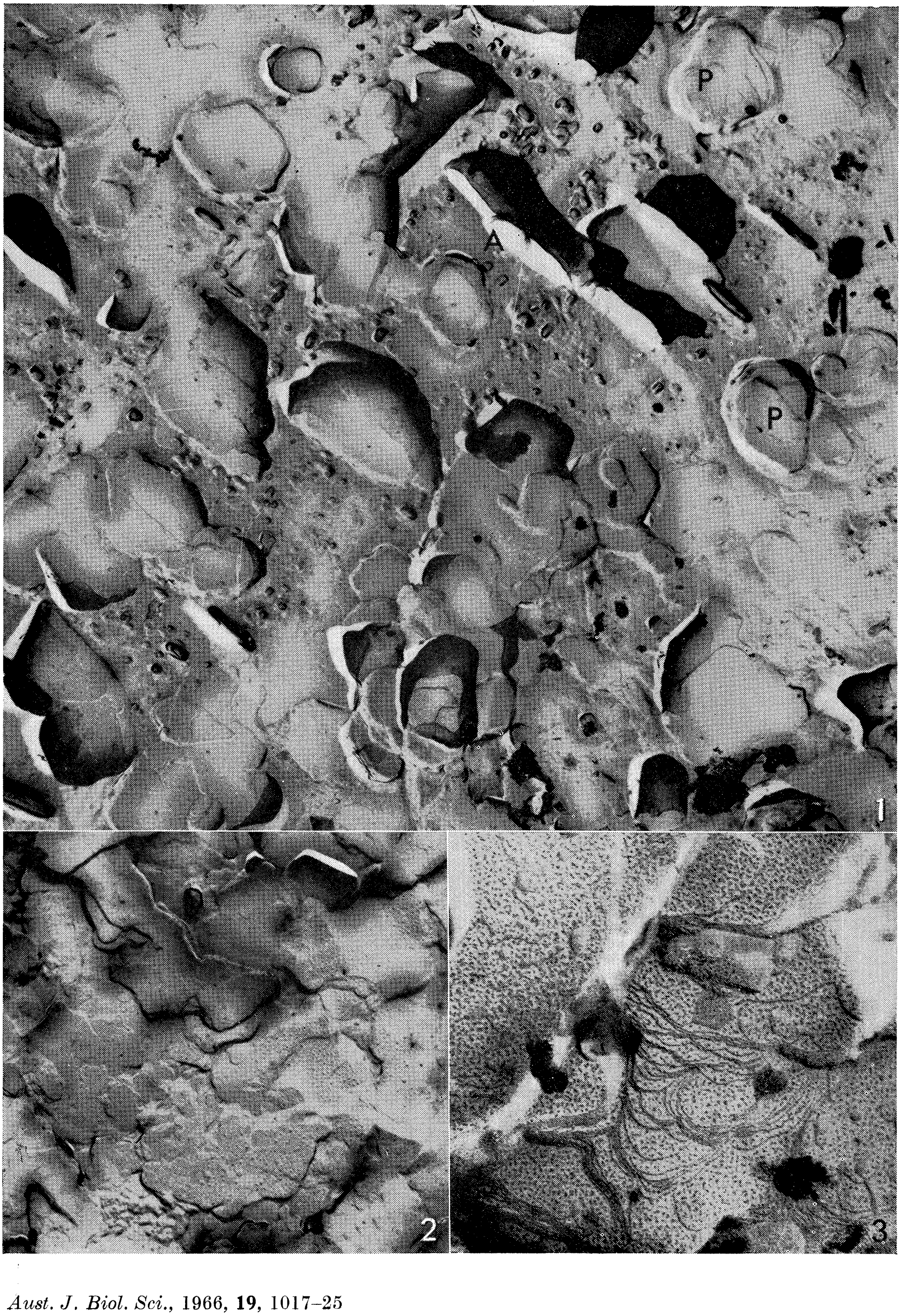

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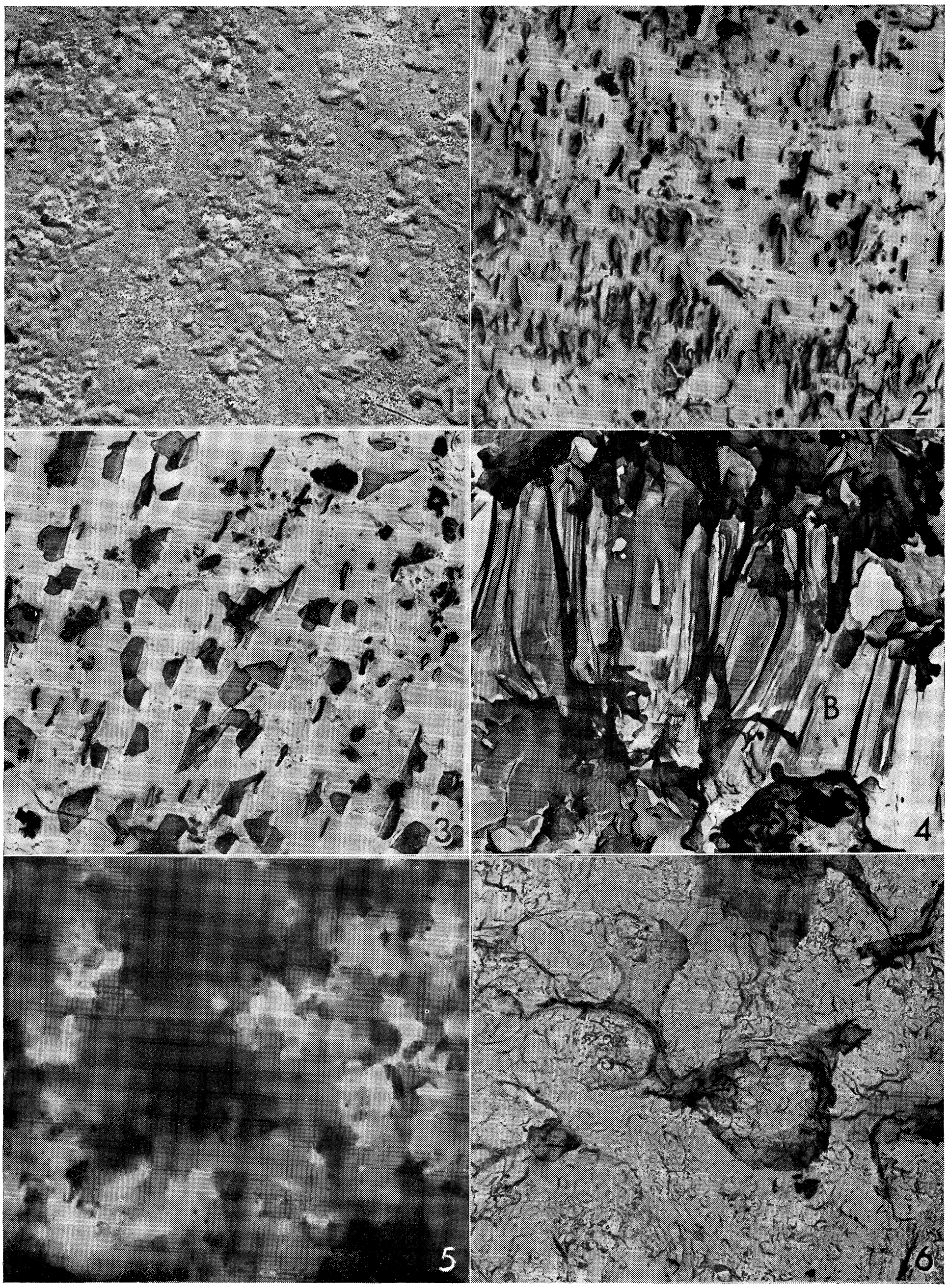

Plate 2

Fig. 1.—Electron micrograph of Granny Smith apple showing aggregates of platelets (*P*) and wax (*A*) peculiar to abraded surfaces. ×6500.

Fig. 2.—Electron micrograph of Sturmer wax surface after immersion of replica in wax solvents. ×6500.

Fig. 3.—Similar surface to Figure 2 at higher magnification, showing crystalline layers of wax platelets. ×33,000.

Plate 3

Fig. 1.—Surface of Granny Smith apple after polishing. ×8000.

Fig. 2.—Young wax in abraded region. ×2600.

Fig. 3.—Mature wax in abraded region. ×2500.

Fig. 4.—Electron micrograph of the lath-like wax occurring in the bands on Golden Delicious apples. ×2500.

Fig. 5.—Electron micrograph of a pseudo-replica of the surface of a Red Dougherty apple. ×3000.

Fig. 6.—Electron micrograph of the cuticle of a Sturmer apple showing structure underlying crescent-shaped wax. ×3000.