THE STRUCTURE OF THE CUTICULAR WAX OF PRUNE PLUMS AND ITS INFLUENCE AS A WATER BARRIER

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Summary

Wax on the surface of prune plums, sampled from 2 weeks before fruit was mature until 2 weeks after, was shown by electron microscopy, using the carbon-replica technique, to occur in a two-layer structure. The inner layer consisted of a matrix of thin platelets, while the outer layer was composed of fragile projections, many of which appeared tubular. The incidence and complexity of the projections in the outer layer increased as the fruit matured.

Deposits of wax remained uniform at about 300 $\mu g/cm^2$ during the time that samples were taken and the thickness was estimated to be 3–5 μ. The influence of this waxy layer in retarding the water loss from prunes during drying by counter-flow and parallel-flow procedures is discussed.

I. INTRODUCTION

The thin layer of wax which covers the surface of many plant organs acts as a barrier to water movement, repelling water externally and conserving it internally. The structure of this waxy layer is important practically in agriculture and food processing since it influences the ease of wetting and penetration of plant surfaces by agricultural chemicals, and the rate of water loss from fruits during natural or artificial drying.

The first attempt to relate the structure of the surface wax of a fruit with drying characteristics was made by Chambers and Possingham (1963) for the sultana berry. Other studies on the structure of plant surface waxes have revealed a wide diversity in physical form (Juniper and Bradley 1958; Hall and Jones 1961; Hall and Donaldson 1963; Skene 1963; Chambers and Possingham 1963; Gunther and Wortmann 1966; Hall 1966).

The present investigation was undertaken to study the nature of the waxy surface of prune plums in relation to their drying characteristics. Prune plums were sampled during development from 2 weeks prior to optimum maturity for drying until 2 weeks after this stage. The physical structure of the surface of the naturally occurring wax layer was revealed by electron microscopy and the amounts of wax present were determined. In addition, changes in the structure of the wax caused by solvent and mechanical treatments were observed on the sample taken at the stage of optimum maturity for drying. Currently the chemical composition of prune wax is being investigated and the influence of a range of temperatures on the structure of the natural wax is being studied.

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II. Materials and Methods

Prunes (*Prunus domestica* cv. D'Agen) were sampled on three occasions at 2-weekly intervals (February 4 and 18, 1966, and March 4, 1966) from a commercial orchard at Kingsvale, N.S.W. The rapid increase in solids content, characteristic of maturing prunes, occurred during the month the samples were harvested, but fruit expansion had ceased by the first sampling. On the basis of solids content, fruit from the respective samplings were considered to be slightly immature (21·5%), at optimum maturity for drying (24·0%), and overmature (31·0%). All fruits were covered with a conspicuous bloom (Plate 1, *A*). Small glossy spots appeared on some of the fruit at the final sampling.

The amount of cuticular wax was estimated and carbon replicas were made of the surface structure at each sampling; in addition, at the second sampling the effects of solvents and polishing on fine structure were observed.

(*a*) *Estimation of Cuticular Wax*

Wax was removed from 5–6 kg of prunes (250–300 fruits) by chloroform in a Soxhlet-type extraction for 16 hr. The average surface area of the ellipsoidal fruits was calculated using measured mean axes.

(*b*) *Electron Microscopy*

(i) *Fine Structure of the Natural Surface*

Unblemished fruits were carefully harvested and carried to the laboratory cradled in cotton wool. Pieces of untouched surface were removed from the fruits with a sharp blade, the incision being made parallel with and as close to the surface as possible; the cut surface was blotted to remove excess moisture. The excised pieces were attached flat to glass slides with Sellotape. The material was than placed in a vacuum-coating unit, directly beneath and about 10 cm from the carbon rod electrodes; a carbon layer (dark grey colour on an adjacent porcelain tile) was evaporated onto the sample at 5×10^{-4}mmHg. Small amounts of material were used at each evaporation to keep the pumping time short and to reduce shrinkage of samples.

The carbon was removed from the surface according to Juniper and Bradley (1958) and the resulting film, representing only a small area of the excised surface, was examined in a Siemens Elmiskop I electron microscope at 80 kV. The image was recorded on N50 thin film half-tone Ilford plates, the negatives being reversed prior to the final printing of the electron-micrographs. Initially some replicas were shadowed with platinum–palladium prior to examination, but much surface detail was lost in this procedure (compare Plate 2, Figs. 1 and 2); the observations reported here, therefore, have been made on unshadowed replicas.

(ii) *Fine Structure of the Treated Surface*

In an attempt to elucidate further the structure of the waxy layer, fruits from the second sampling were treated to remove different amounts of the bloom: by dipping in pentane (b.p. 35°C) for 10 sec, in chloroform for 10 sec, in chloroform for 10 sec three times, and by polishing the surface with soft paper. Carbon replicas of the treated surfaces were compared with the undisturbed surface structure.

III. Results

At all three sampling dates the prune plums were covered with a relatively heavy layer of wax. Visually this layer appeared to be heavier than in previous years, perhaps because drought conditions prevailed during the growth of this crop.

Although there appeared to be a slight increase in the amount of bloom on the fruit from the first to the second sampling, the amount of wax per unit area (285–314 $\mu g/cm^2$) showed little variation between the three samplings. Since the density of plant waxes is close to 1 g/cm^3, the minimum thickness of the wax layer on prunes at an average loading of 300 $\mu g/cm^2$ would be close to 3 μ. The electron-micrographs, however, suggested that the density was well below 1 g/cm^3. Skene (1963) quoted up to 3 μ as the thickness of the cuticular wax on apples at a loading of 200 $\mu g/cm^2$. If the density was assumed to be as low as 0·6 g/cm^3, the thickness of the waxy layer on prunes would reach 5 μ.

Plate 1 shows the normal appearance of the bloom on prunes picked at optimum maturity for drying and the effect of solvent treatments on this surface. Plates 2 and 3 show the fine structure of the natural surface during the month of observation; increasing complexity is apparent from the immature condition (Plate 2, Fig. 1) to optimum maturity (Plate 2, Fig. 3). The underlying wax occurs as platelets closely packed together (Plate 2, Fig. 1), and they appear to have rolled edges and protuberances, some of which seem tubular. The more complex structure observed at the second and third sampling (Plate 2, Fig. 3, and Plate 3, Fig. 1) is due to the increased size and number of protuberances and tubules; increased visual bloom on the fruit is due to these structural changes, a larger area of wax being present to scatter the light falling on the surface. The tubules appear to have a striated surface and a diameter of about 0·15 μ (Plate 2, Fig. 4). The two-layered structure of the waxy surface of the prune is apparent in Plate 3, Figure 2, where an area of normal bloom is adjacent to an area from which the outer fine projections have been lost, leaving the platelet structure beneath; circular structures in the platelets may be the bases of former tubules.

Electron-micrographs of solvent-treated or polished surfaces supported the proposal that fine projections arose from a basal plate-like matrix of wax. Immersion in cold pentane (10 sec) left most of the wax, but removed some of the bloom (Plate 1, *B*); the fine surface structure was partly removed, but the layer of platelets beneath was little altered (Plate 4, Fig. 1). Immersion in cold chloroform (1 or 3 dips for 10 sec) removed the bloom, leaving shiny surfaces (Plate 1, *C* and *D*); replicas of these surfaces showed a layer of continuous wax with some plate-like formation still evident (Plate 4, Fig. 2). Chambers and Possingham (1963) showed a similar structure on sultana berries treated with chloroform; they also indicated that short solvent dips were not sufficient to strip the entire wax layer from the surface. The effect of polishing is shown in Plate 4, Figures 3 and 4. Light polishing caused some breakdown of the fine surface structure of the waxy layer (Plate 4, Fig. 3), while more severe polishing caused the wax to appear as polygonal structures (Plate 4, Fig. 4). These structures are similar to those observed on polished apple and Oullins Golden Gage Plum by Skene (1963) who suggested that the heat generated during polishing caused recrystallization of the wax.

IV. Discussion

The fine structure of the waxy layer of D'Agen prunes was comparable with that observed by Skene (1963) for other varieties of plums, but there appeared to be a more elaborate structure at the surface than on the varieties previously studied. Similarity of surface structure in members of one family has been claimed previously (Juniper 1961, cited by Skene 1963).

Wax is secreted on plant surfaces in a variety of forms (Juniper 1959), but little is known of the way in which it is transported and deposited. It is possible that the fine outer surface layer was formed by the weathering of platelets: many of the prune tubules looked as though they were formed by curling of the edges of thin platelets during exposure to sun and wind. On the other hand there might be a continuous passage of substances through the cuticular layer (Juniper 1959); the projections on the prune surface might then be newly generated wax forced up through the platelets. Some of the structures shown in Plate 2, Figure 4, show indications of phasic growth, suggesting exudation or extrusion. The structure of the "smoother" patch shown in Plate 3, Figure 2, indicates the possible origin of tubules from holes in the wax platelets. It has been shown that regeneration of the waxy layer occurs soon after plant surfaces have been disturbed, but that the form of the new deposit may be different; the wax forming on the surface of polished apples appeared as fine projections, not in the form of the original platelets (Skene 1963). The smooth patches, developed on the prune surface at the third sampling (Plate 3, Fig. 2), did not show signs of rejuvenation, but these fruit were approaching senescence, when the wax flow is known to decline.

Prunes are dried as whole fruit and water loss is retarded by several factors: the distance through which water must diffuse to the surface to evaporate; the high solids content (25–30%), which is mainly carbohydrate and which on concentration during drying retards water movement; and the cuticular wax, which is strongly hydrophobic. The normal method of drying prunes in Australia is by the counter-flow system in which fruit is introduced into a drying tunnel at the cooler end (air temperature 50–55°C) and progresses stepwise towards the outlet, being moved counter to the air flow. The temperature of the drying air at the hotter end is limited to 75°C, the maximum which near-dry prunes can withstand without heat damage. Drying times range from 22–30 hr. Unpublished data of Johnson and McBean (1966) show that the lowest melting point of any component of prune wax is 56°C, while the wax, as a whole, does not melt until about 65°C. From temperatures measured in drying tunnels by Gentry, Miller, and Claypool (1965) and McBean *et al.* (1966), it was estimated that the temperature at the surface of prunes would not reach 65°C in a counter-flow tunnel until 10–12 hr after the start of drying. During this time, the cuticular wax would keep much of its original structure, thereby retaining its ability to act as a water barrier and retard drying rate. It has become a common commercial practice to spray prunes with near-boiling 0·2% sodium hydroxide solution before they are dried. As this treatment removes about half the total wax (McBean and Johnson, unpublished data 1966) the residue must be thoroughly disorganized. As a result, a reduction in drying time of 10% or 2–3 hr is achieved.

In California recently, Gentry, Miller, and Claypool (1965) showed that prunes could be dehydrated in much shorter times by introducing them into the hotter end of a drying tunnel and moving them parallel with the air flow towards the cooler end. Due to initial high evaporative cooling at the surface of the fruits, drying temperatures up to 90°C could be used without causing heat damage to the tissue. Using this system in Australia, McBean *et al.* (1966) dried prunes in 12–16 hr.

The faster drying rate in the parallel-flow system is due to increased rates of evaporation and diffusion at the higher temperatures as well as changes in the structure of the wax layer. The temperature of the surface of the prunes rises to more than 65°C soon after the fruits enter the tunnel and as this is above the melting point of the wax it is expected that the resulting change in the natural structure of the wax layer destroys its water-barrier properties. Data from McBean *et al.* (1966) support this by showing that untreated prunes dried at the same rate, under parallel-flow conditions, as those which had been exposed to light petroleum to remove the wax layer.

Although breakdown of the natural structure of the hydrophobic prune wax layer due to melting is postulated in both methods of dehydration discussed above, destruction of the natural barrier to water loss is not the only way in which quicker drying of fruit can be achieved. Chambers and Possingham (1963) showed that the dipping of sultanas in alkaline emulsions increased the rate of sun-drying without altering the natural structure of their cuticular wax. They postulated that the emulsions, by flooding the minute spaces in the wax layer, make it hydrophilic instead of hydrophobic and they showed that the original wax structure remained unimpaired after the emulsion was removed by washing.

V. Acknowledgments

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EXPLANATION OF PLATES 1–4

All plates show the surface structure of the prune (cv. D'Agen). Plates 2–4 are electron-micrographs taken from carbon replicas of the fruit surface; all are unshadowed, except for Plate 2, Figure 2

PLATE 1

Showing (*A*) the natural surface at optimum maturity of the fruit for drying (sample 2) compared with that of similar fruit after treatment with pentane for 10 sec (*B*), chloroform for 10 sec (*C*), and chloroform for 10 sec three times (*D*). Solvent treatment has removed different amounts of bloom.

PLATE 2

Fig. 1.—Immature for drying (sample 1). The wax layer is in the form of platelets with rolled edges and protuberances, some of the latter appearing tubular.

Fig. 2.—Immature for drying (sample 1). Shows loss of structural detail by shadowing carbon replica with platinum palladium. Compare with Figure 1.

Fig. 3.—Optimum maturity for drying (sample 2). Increased complexity of surface structure is evident, due to increased size and number of protuberances and tubules.

Fig. 4.—Optimum maturity for drying (sample 2). Greater detail of structure in Figure 3. Surface of tubules appear striated.

PLATE 3

Fig. 1.—Slightly overmature for drying (sample 3). Similar structural pattern to sample 2 (Plate 2, Fig. 3).

Fig. 2.—Slightly overmature for drying (sample 3). Two structural patterns indicate the two-layered structure of the waxy surface. The normal pattern is shown on the left-hand side of the figure (compare Fig. 1); the adjacent area shows the underlying platelet structure with the outer fine projections removed, the circular structures in the platelets possibly being the bases of former tubules.

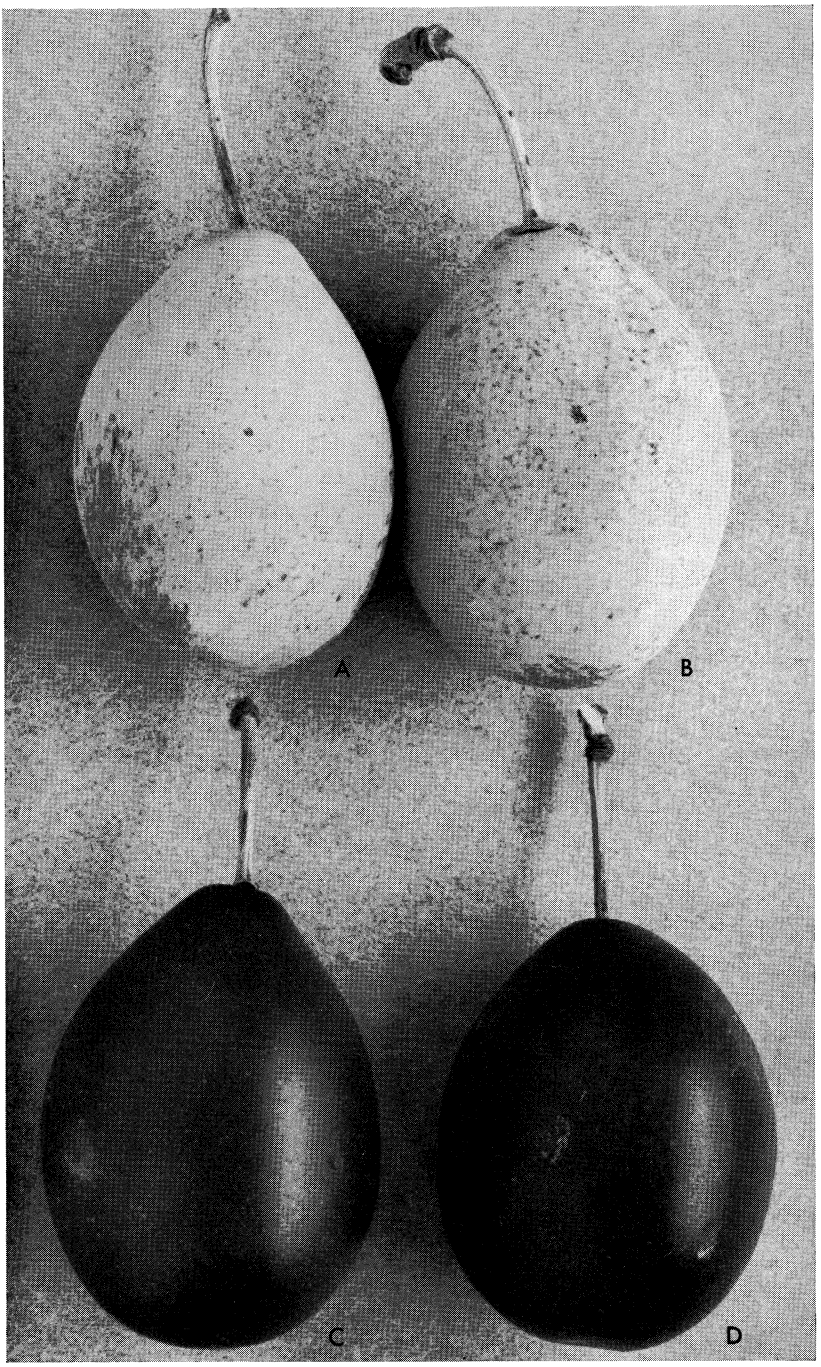
PLATE 4

Fig. 1.—Immersion in cold pentane (10 sec) has partly removed the fine outer surface structure; the layer of plates beneath is comparable to the condition in Plate 3, Figure 2.

Fig. 2.—Immersion in chloroform for 10 sec three times has removed most of the wax from the surface but there is still a continuous layer showing some plate-like formation.

Figs. 3 and 4.—Effect of polishing. Light polishing (Fig. 3) has caused some breakdown of the fine structure; more severe polishing has caused the wax to appear as polygonal structures (Fig. 4).

CUTICULAR WAX OF PRUNE PLUMS



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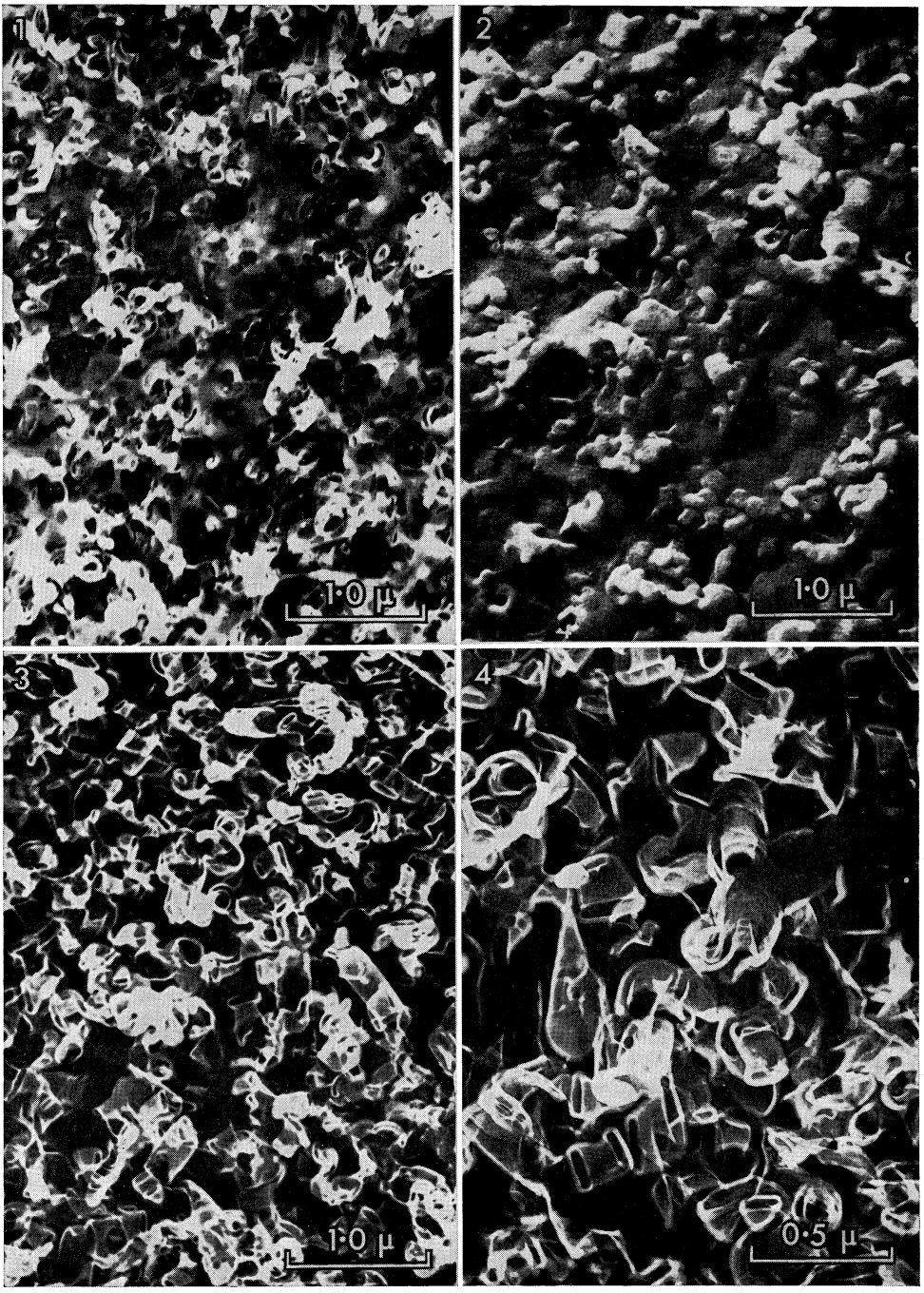

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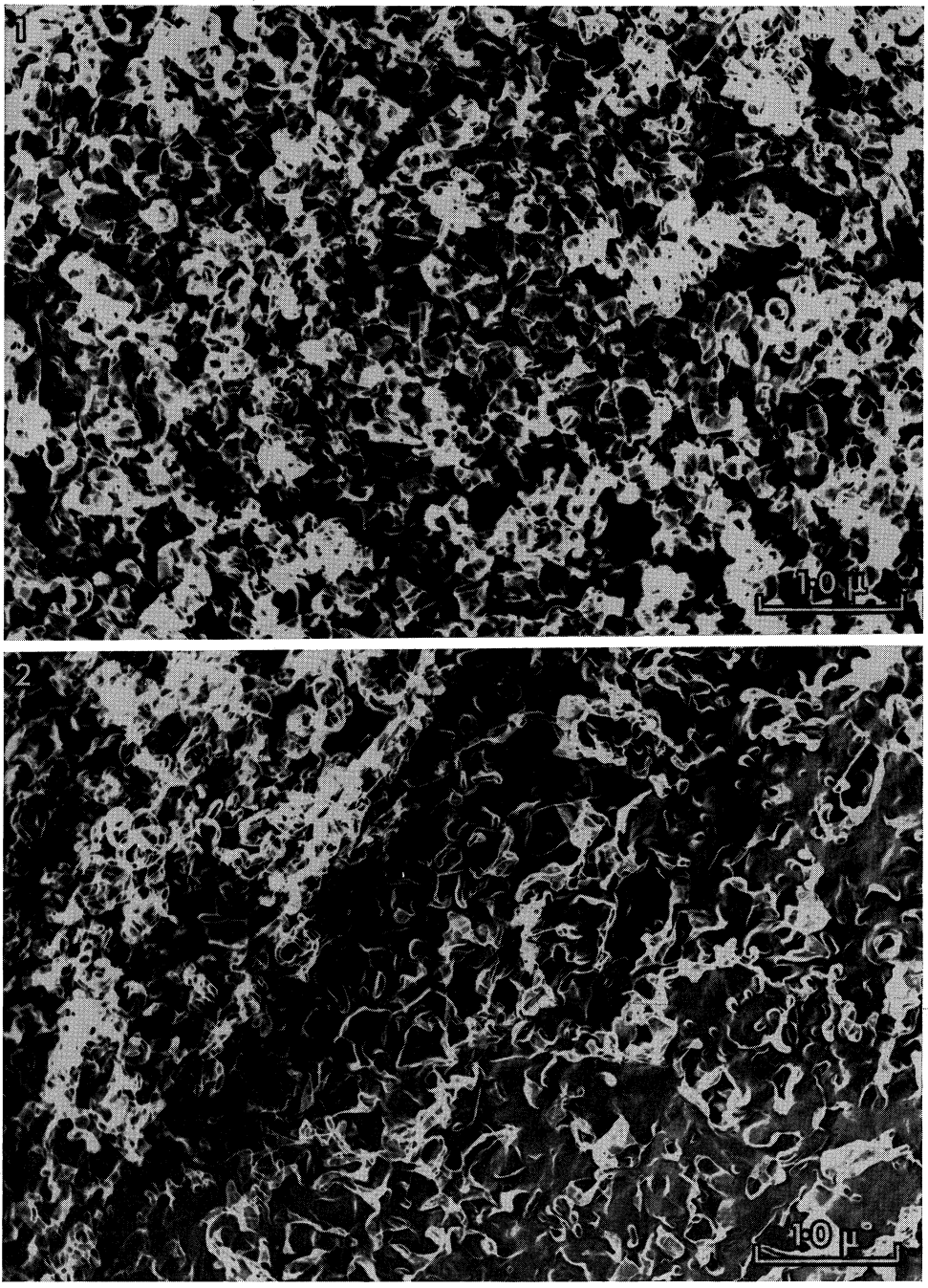

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