Protoplasmic Extrusion of Pollen Grains—an Electron Microscope Study (36576)

LEWIS W. MAYRON, RAYMOND J. LOISELLE, TAMARA TATARCHUK,
HIROSHI TONAKI, AND Y. T. OESTER

Research and Education Service, Veterans Administration Hospital, Hines, Illinois 60141

The process of granular exudation, or G formation (Fig. 1), in ragweed pollen grains has been described by us previously (1)[1] and has been shown to be a function, at least partly, of the composition of the saliva. It is likely that protoplasmic extrusion is the same as the process that the botanists refer to as the pushing of an intine papilla through the colpus or germination aperture, followed by the protoplasm of the pollen. If it is true that the two processes are identical, then an explanation is needed for the fact that the percentage occurrence of protoplasmic extrusion in a population of ragweed pollen grains (*ambrosia trifida*) is 1–5% when the pollen is incubated in normal saliva, in isotonic saline, or in distilled water, but the frequency of occurrence increases significantly (to 50% and up) when the pollen is incubated in "inflammatory" saliva, *i.e.*, saliva from an individual who is congested due to a cold or bronchial infection (1). It is also evident that if this process occurs during the hay fever season to an individual who is allergic to ragweed pollen, then the individual is flooded with pollen antigens.

In an attempt to clarify the mechanism(s) of this process, we examined, in the electron microscope, ragweed pollen grains from a population that was undergoing protoplasmic extrusion. It appears that even though the process may be triggered by a substance outside the pollen grain, *i.e.*, in "inflammatory" saliva, the process itself is initiated from inside the grain. This lends credence to the proposition that protoplasmic extrusion is part of a natural process for which the pollen grain is already programmed.

Materials and Methods. The collection and treatment of pollen and salivary fluids and their incubation have been previously described (1).

After the desired incubation time, the incubation chambers were opened and the grains were removed. They were washed with saline, fixed with 3% glutaraldehyde which was buffered to pH 7.4 with Millonig buffer, and embedded in Araldite. The block was sectioned with a Porter-Blum MT-2 Microtome. Sections were then placed on grids and stained with methanolic uranyl acetate and aqueous lead hydroxide. They were examined with the RCA EMU-3H transmission electron microscope. Some sections were stained with methylene blue and azure II to demonstrate the layers in the pollen (Fig. 2A) and the protoplasmic extrusion through the walls of the grain (Fig. 2B).

In order to describe the series of events which are to be shown, there must be names for the parts of the pollen grain wall. This involves some measure of controversy; however, we will follow the nomenclature used by Heslop-Harrison (2, 3).

Figure 3 shows a cross-section of a pollen grain, as seen by transmission electron microscopy. The outer layer is called the exine. The outer, sculptured portion of the exine is called the sexine (se) and the inner, flat portion of this wall is called the nexine (ne). The further division of the nexine into nexine 1 and nexine 2 may be better seen in Fig. 4. In Fig. 3, there are shown three areas of seeming continuity between the sexine and the nexine. In Fig. 4 is shown an enlargement of such an area. Notice how the texture of the sexine extends only to the outer coating of this inner wall, called nexine 1; the remainder of this wall, called nexine 2, contains

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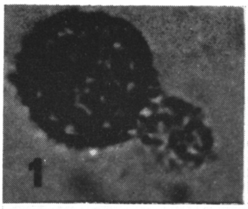
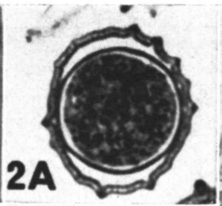
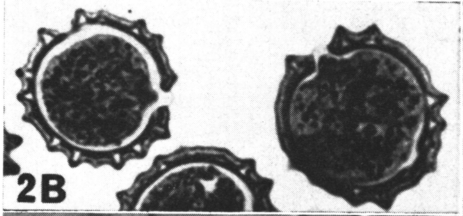
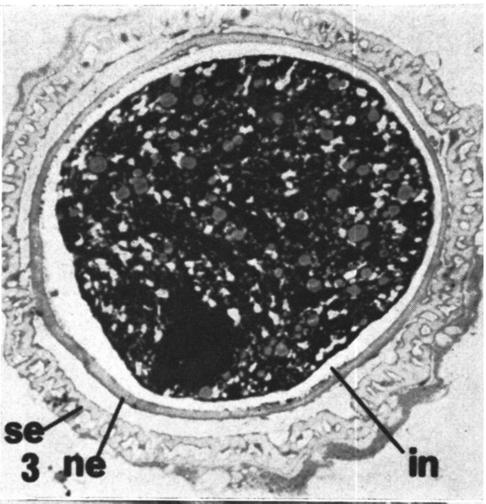
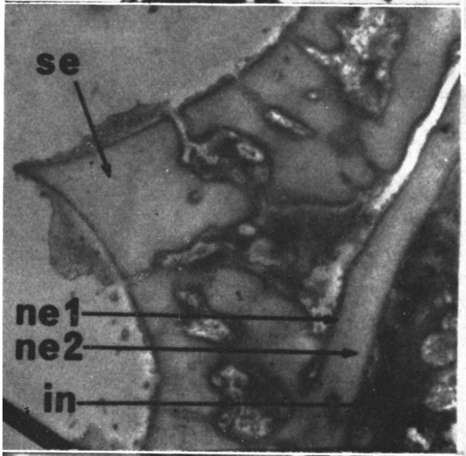
[1] Because of the possible confusion between the medical term "exudation" and our use of the term in the present context, this is changed to "extrusion"; and "granular" is changed to "protoplasmic" because it is more explicit.

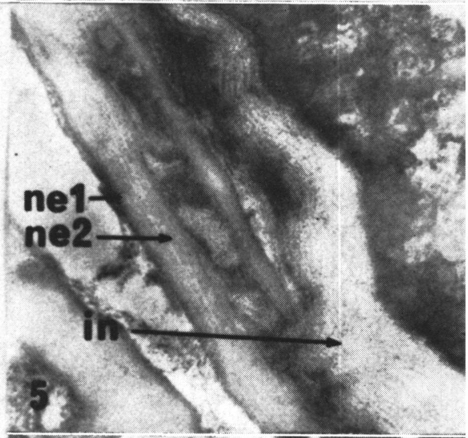


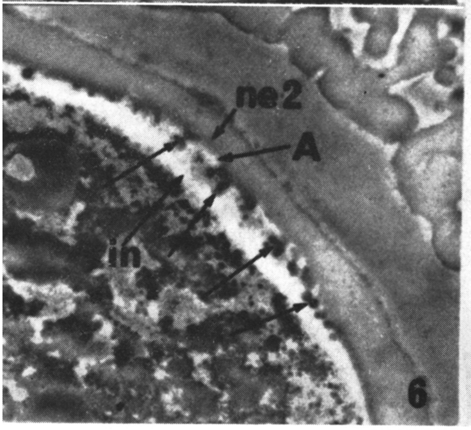












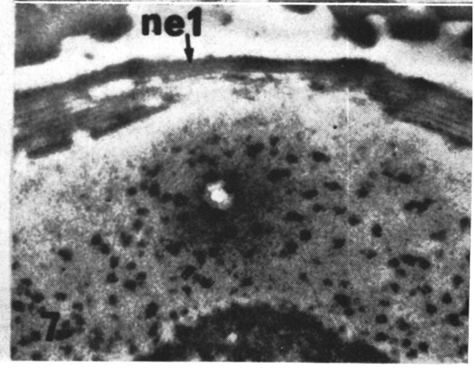


FIG. 1. Protoplasmic extrusion taking place from a grain of giant ragweed pollen. 900×.

FIG. 2. Giant ragweed pollen (*ambrosia trifida*) grains were fixed in 3% glutaraldehyde, embedded in Araldite and sectioned. The sections were stained with methylene blue-azure to demonstrate the wall layers (A) and the initiation of protoplasmic extrusions (B). 900×.

FIG. 3. Cross-section of giant ragweed pollen (*ambrosia trifida*) that had been fixed in glutaraldehyde, embedded in Araldite, and sectioned. The sections were stained with uranyl acetate and lead hydroxide and then examined by transmission electron microscopy. Note the sculptured sexine (se), the nexine (ne), and the intine (in). 3700×.

laminations. These laminations may be better seen in Fig. 5, which primarily shows breakdown of this laminar area, the nexine 2. Here it is shown that this laminar area is a mixture of interbedding of nexine and intine (in), which is the next inner wall and composed only of cellulose. This intine appears as a thick, white band between the nexine and the protoplasmic material of the grain in Fig. 3. It is not nearly so pronounced in Fig. 4 but it shows up well in Figs. 5 and 6. In Fig. 5, especially, one can see the fibrillar material in the intine laminations (2). In Figs. 3, 4, and 5, there appear to be materials on top of and in the channels of the sexine. These materials may be debris or, as has been stated elsewhere (3, 4), it may be substance from the tapetum of the anther (tryphine). If some of this is tapetal material, then it could contain enzymes, as has been shown for other pollens, and the plant incompatibility substance, which prevents interspecies pollination (4). This material would be one of the first pollen substances to be in contact with the mucous membranes of the eyes, nose and mouth and their adjacent fluids.

Results and Discussion. In Fig. 6, particles are shown lying just outside of the intine and inside nexine 2 (arrows). There appears to be a digestion of nexine 2 in the area of these particles. We do not know the nature of these electron-dense particles but they seem to be concentrated in areas of nexine destruction and it is likely that they are enzyme carriers (5). Immediately below these particles lies a membrane that appears to be adjacent to the intine. The particles appear to lie between this membrane and nexine 2. We shall refer to this membrane as membrane A. Figures 5 and 7 show more of this breakdown and give a greater view of the separation of layers as the wall is breaking down. In Fig. 7, it appears as though breakdown has proceeded as far as the nexine 1 layer.

We have no photomicrographic evidence of the breakdown of nexine 1 but Figs. 8 and 9 show that nexine 1 has been removed from the path of the extrusion. However, Fig. 8 does show a cap on the protrusion that consists of electron opaque particles (arrow). These particles could be the same as those shown in Fig. 6 that are concentrated at the area of intine breakdown. They may also be components of the nexine, which is being destroyed. This photomicrograph also shows a mass of debris [or tapetal material (T)] that the protrusion is just beginning to disturb. In Fig. 9, this cap is gone and just a small amount of the tapetal debris remains.

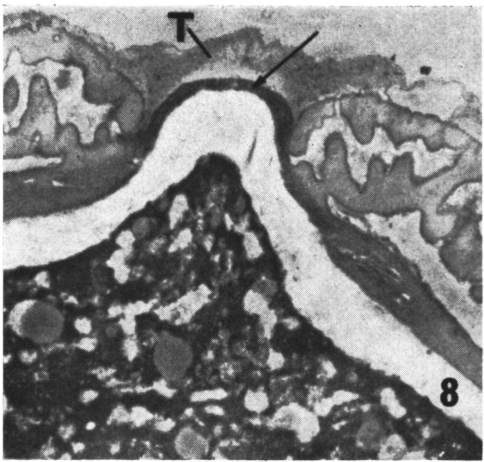
The problem of the passage of the protoplasm through the sexine merits some discussion. From our earlier work (1) and from the work of others (5, 6), we know that there are enzymes which can attack the substance of the sexine and nexine, the sporopollenin, and thus there may be sexine breakdown at the point of emergence of the protoplasm. Another possibility is that the protrusion disrupts the sexine by pushing against it. However, if this were so, the direction of the sexine walls should be that of the outward flow of the protrusion, which they are not (Figs. 8 and 9). A third and most likely possibility is that the protoplasm simply pushes through a preexisting hole or pore in the sexine (a germination aperture).

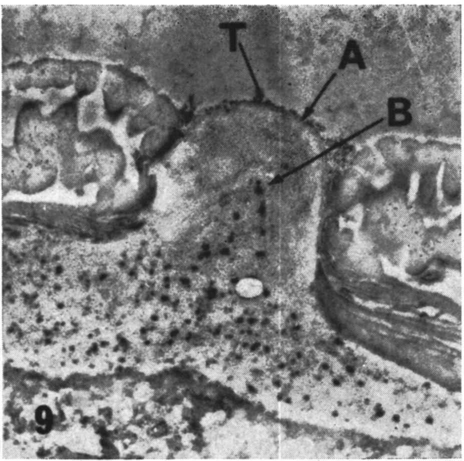
FIG. 4. Electron micrograph of a section of a pollen grain with particular emphasis on the exine, showing the outer, sculptured sexine (se), the outer, nexine 1 layer (ne 1), the inner, laminated nexine 2 layer (ne 2), and the intine (in). Note the debris (tapetal material) on and in the channels of the sexine. 18,000×.

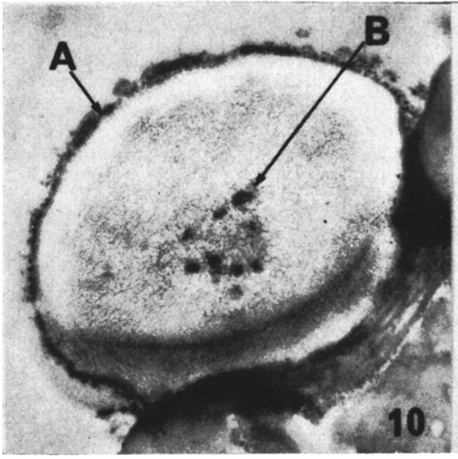
Fig. 5. Electron micrograph of a section of a pollen grain with particular emphasis on the two nexine layers, nexine 1 (ne 1) and nexine 2 (ne 2), and the intine (in). Note the lamellae in the nexine 2 layer and in the intine. 34,000×.

FIG. 6. Electron micrograph of a section of a pollen grain showing electron-dense particles (arrows) between the intine (in) and the nexine 2 (ne 2) layers. Nexine 2 has begun to break down. Note the membrane (A) separating these particles from the intine. 15,500×.

FIG. 7. Electron micrograph of a section of a pollen grain. Note the increased breakdown of the nexine 2 layer compared to Fig. 6 and the laminations that are now so prominent in this layer. Nexine 1 still seems to be intact. 22,600×.







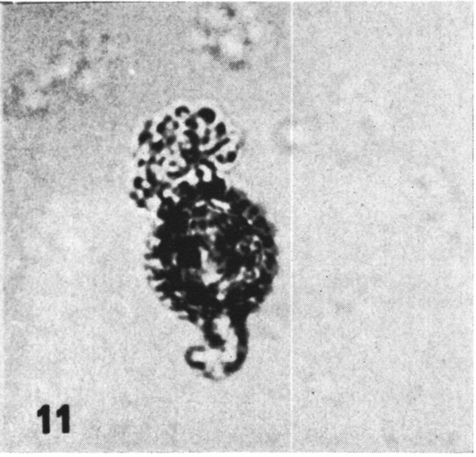


FIG. 8. Electron micrograph of a section of a pollen grain showing the beginning of a protoplasmic extrusion. Note the electron-dense particles (arrow) that cap the protrusion and the debris [tapetal material (T)] at the surface of the grain. 10,700×.

FIG. 9. Electron micrograph of a section of a pollen grain showing the beginning of an extrusion. Note the small amount of (T) present compared to Fig. 8 and the membrane (A) and boundary (B). 13,300×.

FIG. 10. Electron micrograph of a section of a pollen grain showing the extrusion from an oblique view. Note the membrane (A) and boundary (B) and the tapetal debris in comparison to Fig. 9. 24,700×.

FIG. 11. Photograph of a protoplasmic extrusion that is curling as it emerges from the pollen shell. 9200×.

In Fig. 9, the cap of the protrusion appears to be bound by a membrane which we feel is identical to membrane A. A second zone of distinction is seen which separates the area below A from a second area containing particulates; this delineating layer or boundary is labeled B. A third zone of larger particles can be identified below this but no distinct separation entity, such as a membrane, can be discerned.

An extrusion is seen from a different angle in Fig. 10, almost as though it is being seen from the outside. Notice the outer membrane with tapetal debris on it and the small, differentiated area in the center that contains the particulates. This outer membrane appears to be identical to membrane A and the inner zone to the delineating layer B.

Gradually, this protoplasmic material is extruded from the enclosure of the pollen grain (Fig. 11) until it is completely free of the walls (1). Its integrity appears to be retained so it is probable that the membranes have remained intact. In cases where the membranes break, the globular material is released, leaving the membranes and pollen shell (1).

Our use of the term membrane above is as a bimolecular leaf, which is similar to the model as proposed by Singer and Nicolson (7). These workers concluded that a mosaic structure of alternating globular proteins and phospholipid bilayer was the only membrane model that was consistent both with all the available experimental data and with thermodynamic restrictions. Although we are not attempting to prove the structure, we would evaluate what we call membrane A as "fitting the description," with reference to this mosaic model. Support for this stand comes from examining Figs. 5 and 6 with a magnifying glass, especially in the area of the electron-dense particles in Fig. 6. We have indicated above that these particles appear to lie between the membrane and nexine 2. It is also possible that these particles are situated in the membrane, such as between the layers, or they may be placed so that a base is located in or on the membrane while the active portion is protruding above the membrane and against the nexine 2 layer.

Our boundary B seems to delineate the intine from a second area which contains large, electron-dense granules. As of now, we do not know the nature of boundary B.

We have shown that protoplasmic extrusion takes place to a very small extent (1–5%) in water, saline and normal saliva (1). In "inflammatory" saliva, the degree of frequency of this occurrence is much higher, demonstrating a stimulation from the medium in which the pollen grain is incubated (1). During the hay fever season, the challenges of the pollen grains to the allergic individual results in the production of a coryza. The "inflammatory" saliva which is a part of this condition is now capable of triggering an extrusion of internal materials from the pollen, thus flooding the allergic individual with pollen antigens.

The differences between "inflammatory" saliva and normal saliva that could cause this extrusion of internal materials from the pollen grain is a matter of conjecture at this time. It is likely that the effective differences center about the enzyme activities of saliva and the respective origins of these enzymes. Chauncey (8) has classified, according to origin, the enzymes found in saliva. These origins consist of the major and minor salivary glands, the cells of the oral epithelium, microorganisms, and leukocytes.

The salivary glands will increase their output of secretions and enzyme content with nervous system stimulation (9), but this may not necessarily be the case under inflammatory conditions. Such stimulation thus affects the amount of the enzyme being secreted and does not produce an all-or-none situation, but rather one of degree.

Unless there is a condition that results in a large amount of desquamation, the contribution of enzymes from desquamated epithelial cells is unlikely to change. Usually, inflammation associated with allergic events will not change the normal flora of the oral cavity to any great extent.

Under conditions of inflammation, leukocytes invade the area and release their lysosomal enzymes (10). Boyden (11) has demonstrated the relevance of antigen–antibody reactions to polymorphonuclear infiltration. In this situation, the increases in enzyme activities are so great as to constitute an all-or-none comparison. In addition, the neutrophil contains an enzyme which can fragment the fifth component of complement, resulting in chemotactic fragments (12). These neutrophils also contain kinin-producing enzymes (13), which tend to exacerbate the inflammation. Kinins are also found in submaxillary (14) and submandibular (15) fluids, which would contribute to the effect under proper nervous system stimulation.

Thus, it is likely that enzymes from polymorphonuclear leukocytes are suspect in the initiation of protoplasmic extrusion from the pollen grain, either by triggering the internal breakdown of the shell components or by softening the outside layers of the shell by external attack (6). Because of the report by Brooks and Shaw (16) on the nature of pollen exine as a polymerization of carotenoids and carotenoid esters, it is likely that an attacking enzyme would be an esterase, which are present in the lysosomal granules of the polymorphonuclear leukocytes.

The process of protoplasmic extrusion is hereby shown to be dependent upon an internal system, which can be triggered by an external factor. It is likely that this process is a natural one for the pollen grain, for which it has already been programmed. Thus, it is possible that it is a part of the germination process and that the pollen grain has been incorrectly signaled by the "inflammatory" saliva to "go ahead" with the process. Normally, this "go ahead" signal would be selectively given by the species-specific environment of the ovule which is to be fertilized.

Summary. Protoplasmic extrusion has been described previously and has been shown to be at least partly influenced by the salivary contents. An electron microscope study is hereby presented. These results demonstrate an active participation of pollen components in this process.

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