X-RAY EFFECT TO THE EXINE ULTRASTRUCTURE OF *ALNUS GLUTINOSA* (L.) GAERTN.

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Abstract: Fresh pollen grains of *Alnus glutinosa* were radiated with the same intensity of CuKα ray for four different lengths of time. The alterations in the exine ultrastructure and particularly in the biopolymer organization were investigated. The most important results are as follows: (1) The originally homogeneous substance of the ectexine layers are altered into finely granular one. (2) In particular on the surface, the degradation of the biopolymer system have been observed with several kinds of organization. (3) The original ultrastructural characteristic features of some exinous layers (e. g. lamellar endexine) disappear. (4) Endexine and the protoplasm may have the same secondary ultrastructure. (5) Peculiar effect to the bordering part of the different exinous layers have been observed with strong electron density. From these results it is concluded that X-ray has effected a heterogeneous character.

INTRODUCTION

There are several papers concerning the secondary alterations in consequence of the X-ray effect. The purpose of these experiments was in the first place to establish the inhibition or the disappearance of the vitality of the protoplasm and/or the fertility of the pollen grains together with further factors (Cf. Gilissen 1978, p. 81: "The RH of the air also determines to what extent ionizing radiations can inhibit pollen germination *in vitro* (Brewbaker and Emery, 1962; Casaret, 1963; Read, 1959))".

During the last years we have started research programs about the secondary alterations of the plant cell wall in consequence of different kinds of effects. For the first step, the high temperature effect was investigated with the (LM) light microscopy (Kedves and Kincsek, 1989; Kedves and Ailer, 1990; Kedves *et al.*, 1991, 1992). The results of these experiments are important from several points of view, such as sporological taxonomy, phylogeny, and palaeontology. The practical aspect of these researches is connected with the investigation of the organic remnants of these sediments, in the first place of those of the more or less metamorphic layers. This plant micropaleontology is in connection with the oil industrial researches.

The LM method was used firstly on the partially degraded dissolved plant cell walls. The quasi-crystalloid basic biopolymer skeleton, and its highly organized structures were investigated. It was established very early, that the chemistry

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and the biopolymer structure of the plant cell wall are of great importance from the following points of view:

1. There are differences between the different taxa, and different organs of the plant cells and tissues.
2. The maturity and the age is also very important.
3. The biopolymer structure of the plant cell wall is not a constant system, a perpetual alteration can be established, the wall resistance has a so-called dynamic stability.
4. The "auto-alteration process" may be influenced, accelerated or decelerated by outer factors.
5. It was established previously that the biopolymer system is very sensitive to high temperature (Sengupta and Rowley, 1974; Kedves, 1990). On the pollen grains of *Thalictrum flavum* L., after a moderately high temperature treatment (100°C for 1 h), highly organized biopolymer structures were observed after partial degradation.
6. Claugher and Rowley (1987) used the fast atom source for the gradual dissection of the exine of the pollen grains of *Betula verrucosa* L. This method is very useful to get three dimensional (SEM) pictures from the highly organized biopolymer structures of the pollen wall.

Recently we started to investigate the irradiation effect of X-ray to different kinds of pollen grains. The aim of our research program is to investigate, on the one hand, the general morphological alterations of the sporomorphs with the LM method, in contrast to the physiological and/or genetical oriented investigations. On the other hand, using the transmission electron microscopy to establish the alterations in the biopolymer system of the sporoderm at different levels.

In connection with the present paper it is necessary to emphasize the following: (1) This is the first attempt, and surely changes can be presumed in the methods, and this kind of investigation will be extended to different taxa. (2) The results concerning the highly organized biopolymer structures of the sporoderm can be used for comparative studies (Abadie *et al.*, 1986-87; Kedves, 1989; Rowley, 1967, 1975; Rowley and Southworth, 1967; Rowley *et al.*, 1980, 1981; Southworth, 1985a, b, 1986a, b).

MATERIALS AND METHODS

The pollen material for investigation was collected in the Botanical Garden of the Department of Botany of the J. A. University. Szeged, by Dr. K. Margóczi. The X-ray irradiations were made with the BRON-UM1 in the Radiological Laboratory of the Department of Mineralogy, Petrology and Geochemistry of the J. A. University.

Radiation data: 35 KV, 20 mA, CuKα radiation.

Experiment No.	Length of time in minutes
1062	5
1063	15
1064	35
1065	60

After radiation, the pollen grains were prepared for two kinds of investigations. Slides for LM studies were made, but the largest part was prepared for TEM studies with Post-fixation of OsO_4 aq. dil. and embedding in Araldite (Durcupan, Fluka). The ultrathin sections were made on a Porter Blum ultra-microtome. The TEM pictures were taken on Opton EM-902 (resolution 2-3.5 Å).

This paper present the results of the transmission electron-microscopical investigations only, the LM data will be the subject of another paper.

RESULTS

1. Experiment No. 1062 (Plate 1, Figs. 1-7)

In general, two kinds of alterations can be emphasized after this X-ray dose. (1) There is a remarkable, and interesting alteration in the exine ultrastructure. (2) The developing of the pollen tube in consequence of the X-ray irradiation can be observed on the ultrathin sections. As regards the details, the followings are worth of mentioning.

Intergerminal area of the exine (Plate 1, Figs. 1-5)

It is well shown on the general survey picture, that the originally homogeneous ectexine has been altered. The secondary consistence is finely granular, with light-coloured network system. The size of the electron dense granular elements is 6-12 Å. Regular arrangement of these components has been observed. In the tectum the following arrangement of this kind of molecular system was observed: Linear, which may be parallel or perpendicular to the surface or mass of polygons. The surface is particularly damaged. Surface protective biopolymer system, similar to those observed at the saccate gymnosperm pollen grains (Kedves *et al.*, 1991; Kedves and Párdutz, 1992), has not been observed. Some irregular, linear (marked with an arrow) and globular biopolymer systems (marked with two arrows) have been observed. The diameter of these globular units is about 20-34 Å. The globular or irregular elements of the infratectal layer seems to be much more degraded than the tectum and the foot layer (Plate 1, Figs. 1, and 4). The originally lamellar endexine is extremely damaged (Plate 1, Fig. 3). The altered ultrastructure of the protoplasm is similar to those of the endexine (Plate 1, Fig. 5.)

Germinal (apertural) area of the exine (Plate 1, Figs. 6, 7)

The protrusion of the protoplasm through the exoaperture and the degradation of the aperture covering layer is characteristic. This later mentioned one is granular; the dark, electron dense granules are embedded in a matrix. The tectum surface of the annulus is covered with irregular, and/or granular elements (Plate 1, Fig. 7). The extremely advanced degradation of the infractal layer and of the endexine is well shown in Fig. 7 of Plate 1.

2. Experiment No. 1063 (Plate 2, Figs. 1-5)

At this experiment peculiar secondary alterations have been observed. In this case, the apertural area was particularly investigated. The external and internal bordering parts of the exine degraded in consequence of this kind of X-ray irradiation. A dark electron dense layer appeared.

The general aspect of the secondary ultrastructure of the apertural region is illustrated in Fig. 4, Plate 2. The protrusion of the intine together with the protoplasm is not so characteristic as in the previous experiment. But the remnants of the dark, electron dense granular exoaperture-covering layer were observed. The outer surface of the tectum is generally darker than that of the previous experiment (Plate 2, Fig. 4, 5). But it is interesting, and worth of mentioning, that in several cases the inner delimiting parts of the exine have the same secondary ultrastructure. This can be observed also in the tectum/infratectum, and the infratectum/endexine, occasionally the endexine/intine bordering (Plate 2, Figs. 2-5). On the highly magnified pictures (Plate 2, Fig. 1), the outer surface is not strictly delimited. Neither highly organized globular elements nor filamentous biopolymer structures have been observed. The characteristic elements of the infratectal layer disappeared in consequence of the X-ray effect, and more or less secondary homogeneous layer appeared. The endexine is similar to the above mentioned layers. It may be established that a homogeneisation process happened.

3. Experiment No. 1064 (Plate 3, Figs. 1-9)

On the general survey pictures (Plate 3, Figs. 1, 2), the following can be established. The intine is strongly damaged, and in several places completely disappeared. As regards the aperture-covering layers, endexine, intine and outer granular components were observed (Plate 3, Figs. 1-3). In contrast with the previous experiment, the electron density of the bordering parts of the different exine layers is not so characteristic. The homogeneisation of the structured exine layers (infratectum and endexine) is characteristic here too, with the remark only, that in some places of our ultrathin sections the remnants of the infratectal layer can be observed (Plate 2, Fig. 2). But the remnants of the lamellar ultrastructure have not been observed at the endexine. The intine in some parts has not disappeared, and the outermost part of this layer has a strong electron density.

On the basis of the highly magnified TEM pictures, the following may be established. On the surface of the tectum (Plate 3, Figs. 4, 8) there are interesting alterations. Tiny, more or less filamentous units of 14-16 Å in diameter, oriented perpendicular to the surface have been observed. This may be the molecular disintegration of the superficial part of the tectum. The infratectal layer is more or less unstructured except some not so characteristic globular elements of 18-24 Å in diameter. The bordering part part of the foot layer/endexine (Plate 3, Fig. 6) respectively endexine/intine (Plate 3, Figs. 7, 9) is not characteristic. These layers have also nearly the same fine structure, so-called original ultrastructural remnants has observed at the endexine only.

4. Experiment No. 1065 (Plate 4, Figs. 1-9)

The alteration which happened in consequence of this very strong X-ray effect is well illustrated in the general survey picture. The exine changed its character, a fragile electron dense material appeared. It is interesting that the endexine and the protoplasm is not completely degraded. But the alterations in the protoplasm is well shown, this may be characterized by the advanced vacuolisation process.

It is noteworthy, that after this irradiation dose the remnants and the limits of the different ectexinous layers can be newly observed by the fracturation of the exine layers, which happened at the ultrathin sectioning. On the outer surface of the tectum (Plate 4, Fig. 3) in the apertural area globular superficial units of 20-40 Å in diameter have been observed. The inner layer of the exine (Plate 4, Fig. 4) is similar, with the remark, that at this picture some fine molecular elements have also been observed.

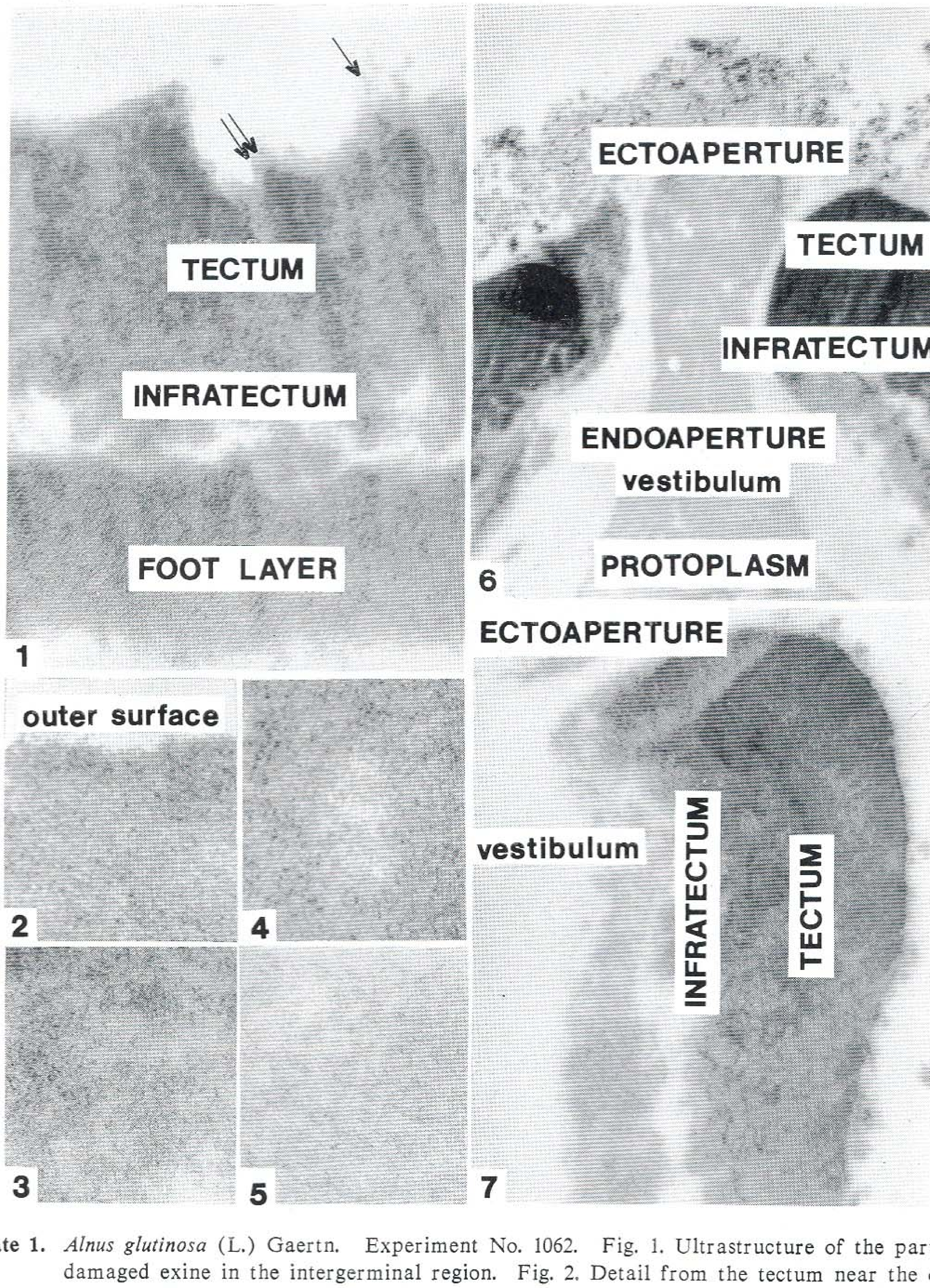

Plate 1. *Alnus glutinosa* (L.) Gaertn. Experiment No. 1062. Fig. 1. Ultrastructure of the partially damaged exine in the intergerminal region. Fig. 2. Detail from the tectum near the outer surface. Fig. 3. Ultrastructure of the damaged endexine. It is worth of mentioning, that the endexine lost its original lamellar ultrastructure. Fig. 4. Detail from the partially degraded infratectum. The original ultrastructural elements are not discernibles at this magnification. Fig. 5. Finely granular damaged protoplasm. Fig. 6. General survey picture from the apertural area altered as consequence of the X-ray irradiation. Fig. 7. Secondary ultrastructure of the apertural area.

Fig. 1, ×100,000; Figs. 2-5, ×250,000; Fig. 6, ×20,000; Fig. 7, ×50,000.

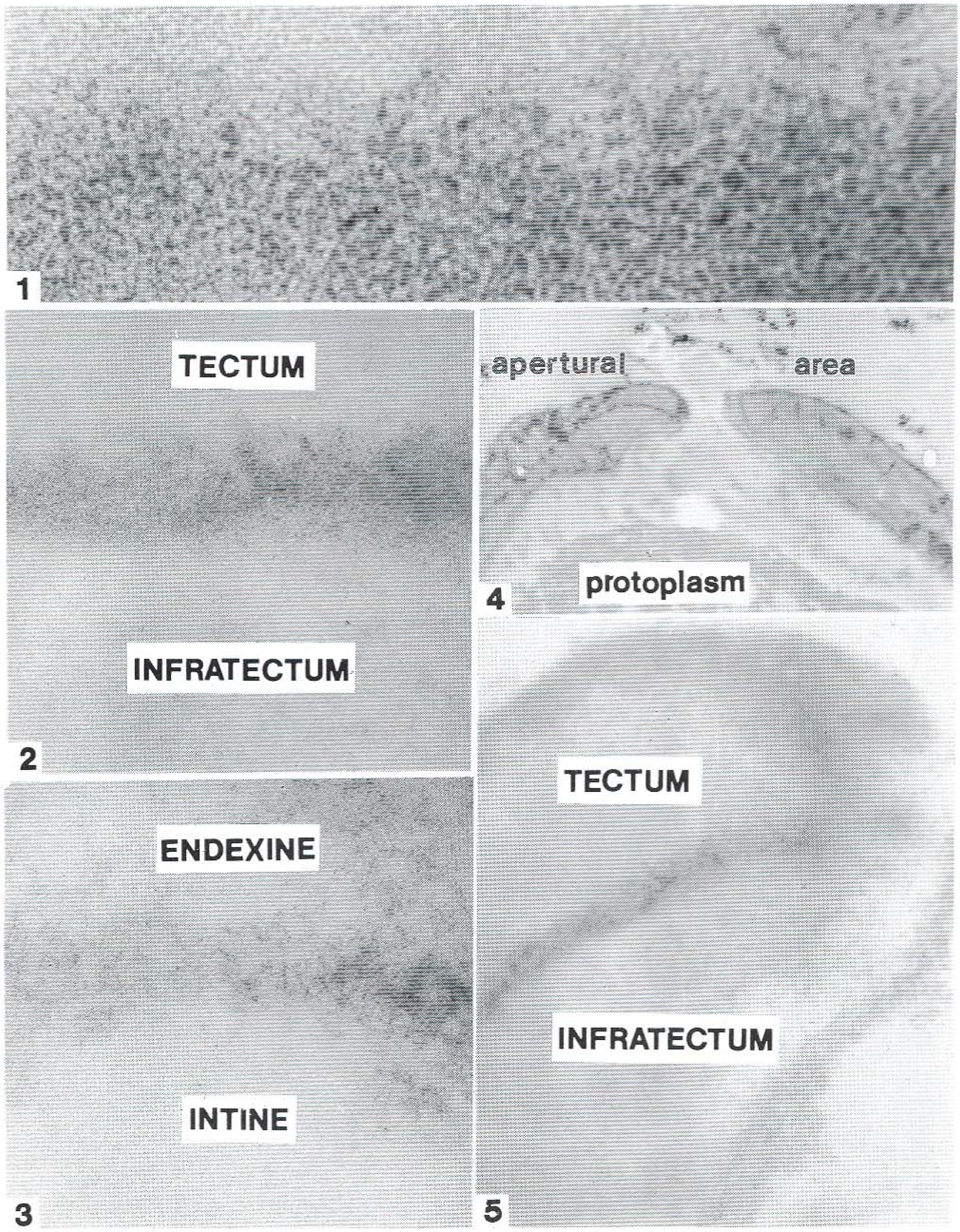

Plate 2. *Alnus glutinosa* (L.) Gaertn. Experiment No. 1063. Fig. 1. The secondary altered surface of the tectum after experiment. Fig. 2. Detail from the highly degraded tectum and infratectum. The bordering line between the exinous layers with strong electron density is characteristic. Fig. 3. Detail from the damaged endexine and intine. The bordering part of the endexine have a strong electron affinity. Fig. 4. General survey picture from the partially degraded pollen grain in the apertural area. Fig. 5. Detail from the damaged apertural exine. The damaged layers, and the electron dense bordering parts are clearly shown.

Fig. 1, ×1,000,000; Figs. 2, 3, ×250,000; Fig. 4, ×10,000; Fig. 5, ×100,000.

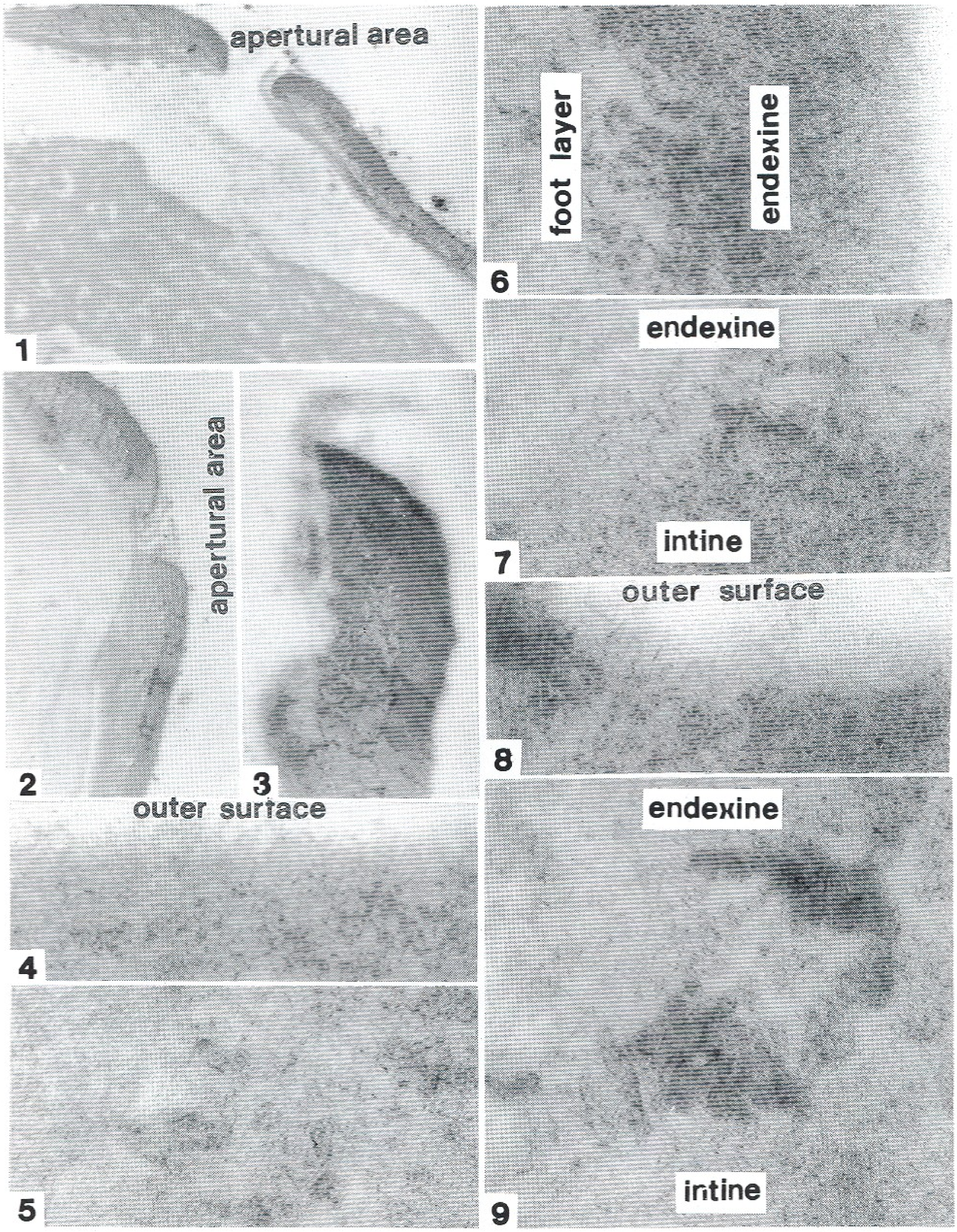

Plate 3. *Alnus glutinosa* (L.) Gaertn. Experiment No. 1064. Figs. 1, 2. General survey pictures from the ultrastructure in the apertural area after experiment. Fig. 3. TEM picture from the detail of the degraded apertural exine. Fig. 4. Biopolymer Organization after experiment of the tectum in the apertural area. Fig. 5. TEM picture from the secondarily altered infratectum in the apertural region. Fig. 6. The extremely damaged foot layer and endexine in the apertural region. Fig. 7. Detail from the damaged endexine and intine in the apertural area. Fig. 8. The partially damaged outer superficial part of the tectum in the interapertural area. Fig. 9. Detail from the damaged endexine and intine in the interapertural area.

Fig. 1, 2, ×10,000; Fig. 3, ×25,000; Figs. 4-9, ×25,000.

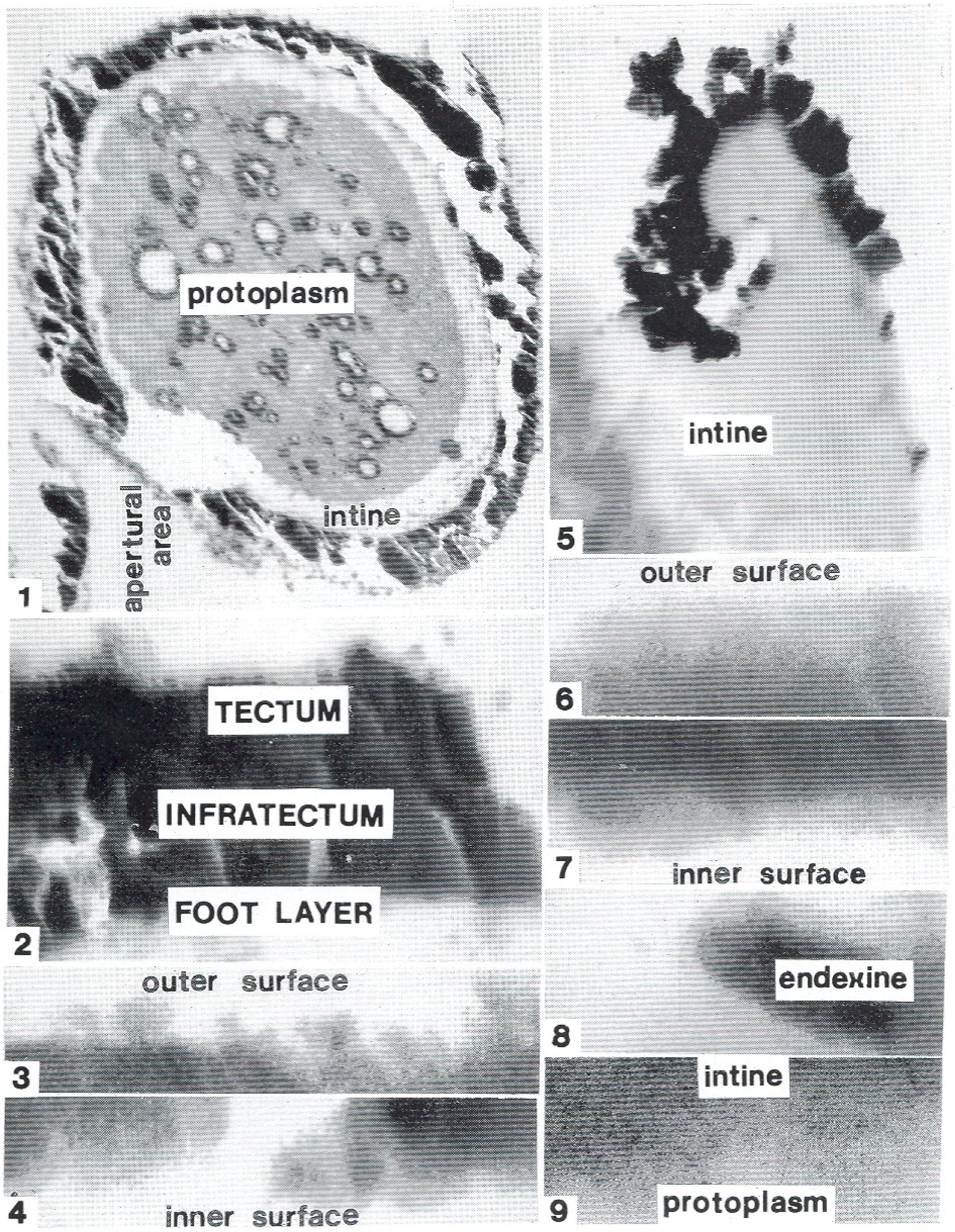

Plate 4. *Alnus glutinosa* (L.) Gaertn. Experiment No. 1065. Fig. 1. General survey picture from the pollen grain after experiment. The greatly damaged and fractured exine is well illustrated. Fig. 2. Detail from the ultrastructure of the exine in the interapertural region. Fig. 3. Detail from the damaged bioplymer structure of the outer surface of the tectum in the intergerminal area. Fig. 4. Inner surface of the secondarily altered exine in the apertural area. Fig. 5. The exinous remnants in the apertural area. The degradation of the intine is relatively moderated. Fig. 6. Detail from the ultrastructure of the damaged tectum in the apertural area. Fig. 7. TEM picture from the inner surface of the damaged exine in the apertural area. Fig. 8. Detail from the characteristic endexine in the apertural area. Fig. 9. Detail from the ultrastructure of the damaged intine and protoplasm in the apertural area.

Fig. 1, ×5,000; Fig. 2, 50,000; Figs. 3, 4, 6-8, ×250,000; Fig. 5, ×20,000.

The apertural area is hardly damaged (Plate 4, Figs. 5-9). Ectexine remnants of strong electron density are connected to the light intine. The tectum and the foot layer surfaces are similar to those of the interapertural exine. The characteristic endexine in the vestibulum is essentially homogeneous with tiny biopolymer (molecular) units.

DISCUSSION AND CONCLUSIONS

During our first experiments about the alterations of the exine ultrastructure including the biopolymer system to the pollen grains of *Alnus glutinosa*, in consequence of different doses of CuKα irradiation we have established the followings:

1. Five minutes of irradiation resulted important alterations. The originally homogeneous exine changed into granular one, and the lamellar endexine have lost this ultrastructural characteristic feature. It is also noteworthy that the network system was established in the endexine of *Taxus baccata* L. originally by the way of solvent and oxidizing method (Kedves, 1987). This network system in Å dimension, which is essentially different to those in nanometer dimension described in several papers of Southworth (1985a, b, 1986a, b) can be dissolved by different way. During this time the solvent and oxidizing methods were the best to discover the quasicrystalloid biopolymer system. For the future investigations the use more moderate irradiation effect and combine with solvent and/or oxidizing methods seems to be more promising. For TEM investigations the ultrathin sections and the fragments are also very important. As it was emphasized previously several times the fragmented partially degraded exines, investigated with the TEM method are useful to investigate the basic biopolymer system and its highly organized degrees on different levels.

2. The irradiation during one hour is a very destructive dose to the exine layers. Probably this kind of extremely altered exine after the dissolution of some of their components may add several new data to the knowledge of the biopolymer system of the pollen wall.

3. Interesting alterations were observed after the irradiation of during 15 minutes. The dark, electron dense bordering layers are characteristic, but similar phenomenon was observed on fossil pollen grains of the Eocene layers of the Mississippi Formation (cf. Kedves and Stanley, 1976), particularly on the TEM pictures of the pollen grains of *Platycaryapollenites* and *Caryapollenites*. It may be presumed, that this kind of characteristic ultrastructural features can provide useful data to the ionizing (or radioactive) effects during the sedimentation processes.

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X射線照射對赤楊花粉壁微細構造的影響

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摘　　要

以相同的銅 Kα 射線強度但四種不同時間處理赤楊的新鮮花粉。其花粉壁的微細構造，特別是生物聚合物的構造產生改變。重要改點如下：(1)改變原均質性之外壁外層成爲細粒狀。(2)在花粉表面，生物聚合物系統崩潰成多種結構。(3)原本層狀的外壁內層微細構造消失。(4)外壁內層和原生質具有相同的次級構造。(5)不同的外壁層產生强電子强度。依據我們初步的結果，使用 X-射線照射具有異質性結果。