# 6. LM AND TEM INVESTIGATIONS ON PARTIALLY DEGRADED POLLEN GRAINS OF CHENOPODIUM ALBUM

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## Abstract

Pollen grains of *Chenopodium album* L. were partially degraded with 2-aminoethanol, $KMnO_4$, and merkaptoethanol. Based on the LM studies, it was established that the molecular system of the sporopollenin of the ectexine of this species is extremely less resistant, and it may be destroyed easily with 2-aminoethanol. Similar experiments on further species of the genus *Chenopodium*, and further taxa of Chenopodiaceae, as well as the pollen of morphologically similar Amaranthaceae, seem to be necessary to better understand the molecular system of the sporopollenin of this kind of periporate pollen grain.

*Key words:* Palynology, angiosperm, *Chenopodium album*, partial degradation, LM, TEM.

## Introduction

The bibliographical data concerning the allergenic character of the pollen grains of the genus *Chenopodium* were summarized by MOLNÁR (1999).

NILSSON, PRAGLOWSKI and NILSSON (1977) investigated with the LM, TEM and SEM method the pollen grains of *Chenopodium album* L. It was established that the pori of the pantoporate pollen grains are covered with operculi. The tectum is perforated, and the surface is covered by spinules.

SEM picture of *Chenopodium ambrosoides* L. was published by HUANG (1998). ERDTMAN (1966) pointed out the morphological similarities between the Chenopodiaceae and the amaranthoid type of the Amaranthaceae. The gomphrenoid type of the Amaranthaceae occurs in the Caryophyllaceae, and is different from the amaranthoid type. The number of the pores of periporate (pantoporate) pollen grains has been used for taxonomic purposes. MCANDREWS and SWANSON (1967) reviewed several methods in this respect and finally they concluded that the C/D ratio is a character of taxonomy, cf. KAPP, DAVIS and KING (2000). In the aeropalynological papers, probably the above mentioned similarities are the reason for the different accuracy in the determinations of this kind of pollen grains. Some selected examples are as follows:

*Chenopodium album:* DE LEONARDIS et al. (1986), LEBBE, VIGNES and HIDEUX (1988), AGNIHOTRI and SINGH (1991), MEZEI et al. (1992), FERREIRO, RODRIGUEZ and AIRA (2001).

*Chenopodium:* WANG XIAN-ZENG (1986), SINGH and DEVI (1991), BEN TIBA et al. (1995), PEHLIVAN (1995), MUNUERA GINER et al. (2000).

Chenopodiaceae-Amaranthaceae: AROBBA (1986), CHAUDHARY and SINGH (1991), MAJUMDAR and CHANDA (1991), JÁRAI-KOMLÓDI (1991), ELVIRA RENDUELES et al.

(2000), RUIZ, CANO and DIAZ DE LA GUARDIA (2000), TORTAJADA and MATEU (2000). Following JÁRAI-KOMLÓDI and MEDZIHRADSZKY (1993), "the late summer-autumn season caused first of all by *Ambrosia* and *Artemisia,* and members of the Chenopodiaceae and Amaranthaceae families." (P: 49).

Amaranthaceae/Chenopodiaceae: CHEN and HUANG (1980), NILSSON (1990), MURRAY, SONAGLIONI and VILLAMIL (2001).

The aim of this paper to establish the solubility of the sporopollenin of the ectexine with our standard method with 2-aminoethanol by light and transmission electron microscopical methods and to investigate the qualitative and quantitative morphological alterations in consequence of the mounting media, stain and the embedding processes for TEM studies.

## Materials and Methods

The material investigated was collected by Miss K. PRISKIN from Szeged, and by Miss J. SASHALMI from Hódmezővásárhely from weedy associations. The partial degradation was made two times, because at the first result the sporopollenin was extremely less resistant, it was degraded very easily. We believed, that with this fresh material, the polymerization of the sporopollenin is not completely finished, in this way, after three months the experiments with 2-aminoethanol were repeated. After the first set of experiments there are two numbers, the second one is the repeated number. Unstained (a) and stained pollen grains with Safranin T (b) were investigated with the LM method. Temperature 30°C

5 mg pollen + 2 ml 2-aminoethanol, length of time 24 hours, T-12-82, T-12-137.

5 mg pollen + 2 ml 2-aminoethanol, length of time 48 hours, T-12-83, T-12-138.

5 mg pollen + 2 ml 2-aminoethanol, length of time 72 hours, T-12-84, T-12-139.

5 mg pollen + 2 ml 2-aminoethanol, length of time 24 hours washing, + 10 ml $KMnO_4$ 1%, length of time 24 hours, T-12-85.

5 mg pollen + 2 ml 2-aminoethanol, length of time 48 hours, washing, + 10 ml $KMnO_4$ 1%, length of time 24 hours, T-12-86.

5 mg pollen + 2 ml 2-aminoethanol, length of time 72 hours, washing, + 10 ml $KMnO_4$ 1%, length of time 24 hours, T-12-87.

5 mg pollen + 2 ml 2-aminoethanol, length of time 24 hours, washing, + 2 ml merkaptoethanol, length of time 24 hours, T-12-88.

5 mg pollen + 2 ml 2-aminoethanol, length of time 48 hours, washing, + 10 ml $KMnO_4$ 1%, length of time 24 hours, T-12-89.

5 mg pollen + 2 ml 2-aminoethanol, length of time 72 hours, washing, + 10 ml $KMnO_4$ 1%, length of time 24 hours, T-12-90

5 mg pollen + 5 ml glycerin 50%, length of time 30 days.

The pollen grains were mounted in glycerine-jelly hydrated to 39.6%. For TEM studies the investigation material was postfixed with $OsO_4$ aq. dil. 1%, and embedded in Araldite (Durcupan, Fluka). The ultrathin sections were made with glass knives on a Porter Blum ultramicrotome. The pictures were taken on a Tesla BS-540 instument (resolution 6-7 Å). All pictures are unretouched.

## Results

LM results (Plate 6.1., figs. 1-70)

The fresh pollen grains (Plate 6.1., figs. 1-6) are typically periporate, diameter from 18.0 µm - 33.0µm.

After treatment with 2-aminoethanol during 24 hours, (Plate 6.1., figs. 7-13) the degradation of the ectexine are well shown by the LM method. There are some differences in the degree of the degradation between the first (Plate 6.1., figs. 7,8) and the second experiment (Plate 6.1., figs. 9,10). The ectexine of the pollen grains of the second experiment seems to be a little more resistant than that of the first one. The ectexine of the $OsO_4$ postfixed and embedded pollen grains in Araldite was more degraded in the first experiment (Plate 6.1., figs. 11,12) than in the second one (Plate 6.1., fig. 13).

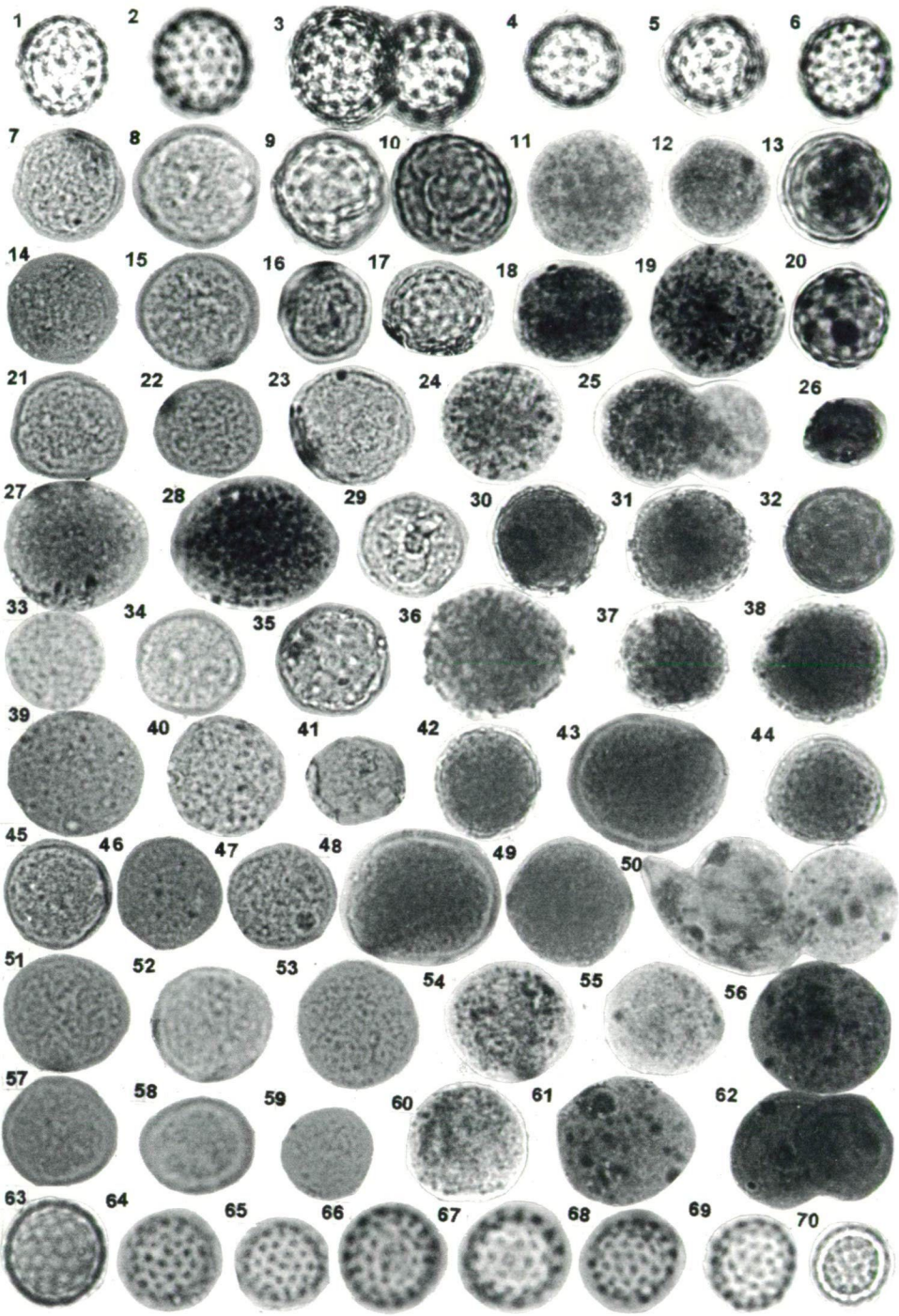
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Plate 6.1.

Plate 6.1.

1-70. *Chenopodium album* L., LM pictures, 750x.
1-6. Fresh pollen grains mounted in glycerine-jelly;
7-10. Pollen grains degraded in 2-aminoethanol during 24 hours, mounted in glycerine-jelly. figs. 7,8. first, 9,10 second experiment.
11-13. Pollen grains degraded in 2-aminoethanol during 24 hours, mounted in Araldite, after embedding processes, figs. 11,12, first, 13, second experiment.
14-17. Pollen grains degraded in 2-aminoethanol during 48 hours, mounted in glycerine-jelly, figs. 14,15 first, 16,17 second experiment.
18-20. Pollen grains degraded in 2-aminoethanol during 48 hours, mounted in Araldite after embedding processes, figs. 18,19 first, 20 second experiment.
21-23. Pollen grains degraded in 2-aminoethanol during 72 hours, mounted in glycerine-jelly, figs. 21,22 first, 23 second experiment.
24-26. Pollen grains degraded in 2-aminoethanol during 72 hours, mounted in Araldite after embedding processes, figs. 24,25 first, 26 second experiment.
27-70. Pollen grains prepared with the first experiment.
27-32. Pollen grains degraded in 2-aminoethanol during 24 hours, washed and oxidized with KMnO4 1% during 24 hours. Figs. 27-29 pollen grains mounted in glycerine-jelly, 30-32 mounted in Araldite after embedding processes.
33-38. Pollen grains degraded in 2-aminoethanol during 48 hours, washed and oxidized with KMnO4 1% during 24 hours. Figs. 33-35 pollen grains mounted in glycerine-jelly, 36-38 mounted in Araldite after embedding processes.
39-44. Pollen grains degraded in 2-aminoethanol during 72 hours, washed and oxidized with KMnO4 1% during 24 hours. Figs. 39-41 pollen grains mounted in glycerine-jelly, 42-44 mounted in Araldite after embedding processes.
45-50. Pollen grains degraded in 2-aminoethanol during 24 hours, washed and partially dissolved with merkaptoethanol during 24 hours. Figs. 45-47 pollen grains mounted in glycerine-jelly, 48-50 mounted in Araldite after embedding processes.
51-56. Pollen grains degraded in 2-aminoethanol during 48 hours, washed and partially degraded with merkaptoethanol during 24 hours. Figs. 51-53 pollen grains mounted in glycerine-jelly, 54-56 mounted in Araldite after embedding processes.
57-62. Pollen grains degraded in 2-aminoethanol during 72 hours, washed and partially degraded with merkaptoethanol during 24 hours. Figs. 57-59 pollen grains mounted in glycerine-jelly, 60-62 mounted in Araldite after embedding processes.
63-70. Pollen grains partially dissolved in glycerine 50% during 30 days, mounted in glycerine-jelly. Magnifications: 750x.

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Results after partial degradation with 2-aminoethanol during 48 hours (Plate 6.1., figs. 14-20). The ectexine of the pollen grains of the first experiment mounted in glycerine-jelly (Plate 6.1., figs. 14,15) is hardly damaged. It is well shown that the characteristic periporate character is not perceptible. But on the pollen grains of the second experiment (Plate 6.1., figs. 16,17) there are differences in this respect namely that the polyporate character is more or less preserved. This phenomenon is more characteristic of the pollen grains prepared for TEM studies (Plate 6.1., figs. 18,19), respectively Plate 6.1., fig. 20.

Results after partial degradation with 2-aminoethanol during 72 hours (Plate 6.1., figs. 21-26). The degradation of the sporopollenin is well shown in the pollen grains of the first (Plate 6.1., figs. 21,22) and the second experiment (Plate 6.1., fig. 23) mounted in glycerine-jelly. The pollen grains after partial degradation and embedding processes and mounted in Araldite are similar to the pollen grains mounted in glycerine-jelly, Plate 6.1., figs. 24,25 illustrates the first experiment, Plate 6.1., fig. 26 the pollen grains of the second experiment.

Partial degradation with 2-aminoethanol during 24 hours, washing and oxidizing with $KMnO_4$ 1% during 24 hours (Plate 6.1., figs. 27-32). The degradation of the ectexine is well shown in the pollen grains mounted in glycerine-jelly (Plate 6.1., figs. 27-29) and in Araldite (Plate, 6.1., figs. 30-32). The osmium affinity of the degraded protoplasm is characteristic. Picture 32 on Plate 6.1. illustrate the osmium accumulation around the remnants of the pores.

Partial degradation with 2-aminoethanol during 48 hours, washing and oxidizing with $KMnO_4$ during 24 hours (Plate 6.1., figs. 33-38). The results of this experiment are similar or identical to the previous one.

Partial degradation with 2-aminoethanol during 72 hours, washing and oxidizing with $KMnO_4$ during 24 hours (Plate 6.1., figs. 39-44).

Remnants of the ectexine was observed in these pollen grains. The osmium affinity of the embedded pollen grains was not so intensive than previously (Plate 6.1., figs. 42-44).

Partial degradation with 2-aminoethanol during 24 hours, washing, and continued with merkaptoethanol during 24 hours (Plate 6.1., figs. 45-50)

Characteristic ectexine remnants are in the partially degraded pollen grains mounted in glycerine-jelly (Plate 6.1., figs. 45,47). It is worth mentioning that the remains of the pores were not perceptible. Further strong degradation was observed in the embedded pollen grains (Plate 6.1., figs. 48-50). The measure of the degradation was not completely the same in different specimens. This may be the consequence of several reasons. Near "protoplast" with electron dense granular units in the remnant of the protoplasm is characteristic (Plate 6.1., fig. 50).

Partial degradation with 2-aminoethanol during 48 hours, washing and continued with merkaptoethanol during 24 hours (Plate 6.1., figs. 51-56).

The degraded pollen grains mounted in glycerine-jelly (Plate 6.1., figs. 51-53) are identical to the previous experiment. The ectexine of the embedded pollen grains is nearly destroyed, there are electron dense granular units in the degraded protoplasm (Plate 6.1., figs. 54-56).

Partial degradation with 2-aminoethanol during 72 hours, washing and continued with merkaptoethanol during 24 hours (Plate 6.1., figs. 57-62)

Based on our observations the results of this experiment are identical with the previous one.

Partial dissolution with glycerine (50%) during 30 days (Plate 6.1., figs. 63-70)

The light microscopical morphology of the pollen grains are identical to the fresh untreated specimens.

Quantitative results

Remark. - a = unstained, b = stained with Safranine T, Ar = pollen grains mounted in Araldite.

| Nos of experiments | 13.0 | 15.0 | 18.0 | 20.0 | 23.0 | 25.0 | 28.0 | 30.0 | 33.0 | 35.0 | 38.0 | 40.0µm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T-12-81 | | | 0.5 | 6.0 | 13.0 | 26.0 | 37.5 | 15.5 | 1.5. | | | % |
| T-12-82a | | | 0.5 | 12.0 | 15.0 | 37.0 | 20.5 | 9.0 | 3.5 | 1.0 | 0.5 | 5.0 |
| T-12-82b | | | 3.5 | 12.0 | 12.0 | 42.5 | 16.5 | 10.0 | 3.5 | | | |
| T-12-137a | | 3.0 | 13.5 | 15.0 | 33.0 | 22.0 | 8.5 | 3.5 | 1.5 | | | |
| T-12-82Ar | 2.5 | 2.0 | 5.0 | 15.0 | 17.0 | 26.0 | 16.5 | 16.0 | 8.0 | 0.5 | 1.5 | |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| T-12-83a | | | | 6.0 | 12.5 | 17.5 | 34.5 | 19.0 | 5.5 | 5.0 | |
| T-12-83b | | | 11.0 | 18.0 | 10.0 | 36.0 | 21.0 | 4.0 | | | |
| T-12-138a | | | 4.5 | 16.0 | 19.5 | 28.0 | 18.0 | 11.0 | 2.5 | 0.5 | |
| T-12-83Ar | | | 4.0 | 13.0 | 14.0 | 33.0 | 13.5 | 11.5 | 7.5 | 3.5 | |
| T-12-84a | | 2.0 | 9.0 | 18.5 | 22.0 | 31.0 | 11.5 | 5.0 | 0.5 | 0.5 | |
| T-12-84b | | 2.0 | 4.5 | 16.5 | 11.0 | 36.0 | 18.0 | 12.0 | | | |
| T-12-139a | | 0.5 | 3.5 | 14.5 | 17.0 | 25.5 | 18.0 | 14.0 | 4.0 | 3.0 | |
| T-12-84Ar | | 1.6 | 3.0 | 16.0 | 15.0 | 33.0 | 10.0 | 14.0 | 5.7 | 1.7 | |
| T-12-85a | | 1.5 | 3.0 | 6.0 | 5.5 | 22.0 | 15.5 | 25.5 | 13.5 | 6.5 | 1.0 |
| T-12-85Ar | | 0.8 | 0.8 | 2.2 | 4.5 | 15.0 | 25.4 | 26.0 | 14.1 | 9.0 | 2.2 |
| T-12-86a | | 1.0 | 3.0 | 13.0 | 13.0 | 25.0 | 18.0 | 18.0 | 6.0 | 3.0 | |
| T-12-86Ar | | 1.2 | 4.6 | 2.3 | 6.9 | 20.7 | 26.4 | 18.4 | 9.2 | 8.0 | 2.3 |
| T-12-87a | | | 0.5 | 6.5 | 6.5 | 26.5 | 20.0 | 23.0 | 9.5 | 6.0 | 1.5 |
| T-12-87Ar | | | 1.0 | 5.0 | 12.5 | 21.5 | 20.0 | 19.0 | 11.5 | 5.5 | 4.0 |
| T-12-88a | | | 0.5 | 10.5 | 10.0 | 27.5 | 22.5 | 16.0 | 9.5 | 3.0 | 0.5 |
| T-12-88Ar | | | 4.6 | 20.0 | 10.8 | 26.1 | 13.8 | 17.0 | 4.6 | 3.1 | |
| T-12-89a | | | 0.5 | 9.0 | 11.0 | 23.0 | 19.0 | 20.0 | 12.0 | 5.0 | 0.5 |
| T-12-89Ar | | | 5.0 | 13.0 | 9.0 | 27.5 | 24.0 | 10.0 | 8.5 | 2.5 | 0.5 |
| T-12-90a | | | 0.5 | 12.0 | 11.5 | 33.5 | 20.0 | 16.5 | 6.0 | | |
| T-12-90Ar | 1.0 | 1.5 | 4.0 | 13.0 | 16.5 | 22.5 | 15.5 | 17.0 | 6.0 | 3.0 | |
| T-12-91 | | | | 9.0 | 44.5 | 31.0 | 14.5 | 1.0 | | | |

Based on our results, the smallest pollen grain was 13.0 µm, the largest was 40.0 µm. As regards the quantities of the different sizes it may be established, that this is nearly the same in all experiments, except the partial dissolution with diluted glycerine. The greatest part of the pollen grains are of 25.0 or between 20.0 - 30.0 µm. It is interesting that the pollen grains dissolved in diluted glycerine are from 18.0 to 28.0 µm, so the size range is relatively short, and 44.5% is of 20.0 µm.

TEM results (Plate 6.2., figs.1-8)

Pollen grains from all experiments were embedded and investigated with the transmission electron microscope. As it was established during the light microscopical studies, the ectexine was unusually damaged. Because of the hardly damaged ectexine we present from our documents some selected examples as follows:

Pollen grains treated with 2-aminoethanol during 24 hours (Plate 6.2., figs. 1,2)

The general survey picture illustrates well the more or less homogenized protoplasm, and the electron dense and extremely damaged ectexine (Plate 6.2., fig. 1). The foot layer is not completely destroyed, fragments of the infratectal layer are also present. In the highly magnified picture (Plate 6.2., fig. 2) the outer part of the foot layer is granular, which may be the biopolymer units of the ectexine. The inner part is more or less homogeneous.

Pollen grains treated with 2-aminoethanol during 48 hours (Plate 6.2., fig. 3)

Damaged protoplasm and the fragment of the foot layer are illustrated. Between the remnant of the plasma membrane and the foot layer there is a light zone.

Pollen grains treated with 2-aminoethanol during 72 hours (Plate 6.2., fig. 4-6)

Ultrastructure of ectexine lost "inner body" is illustrated in fig. 4, Plate 6.2. The protoplasm is after treatment homogeneous the organelles were degraded. The ultrastructure of the ectexine remnants (Plate 6.2., figs. 5,6) is granular, and there are more or

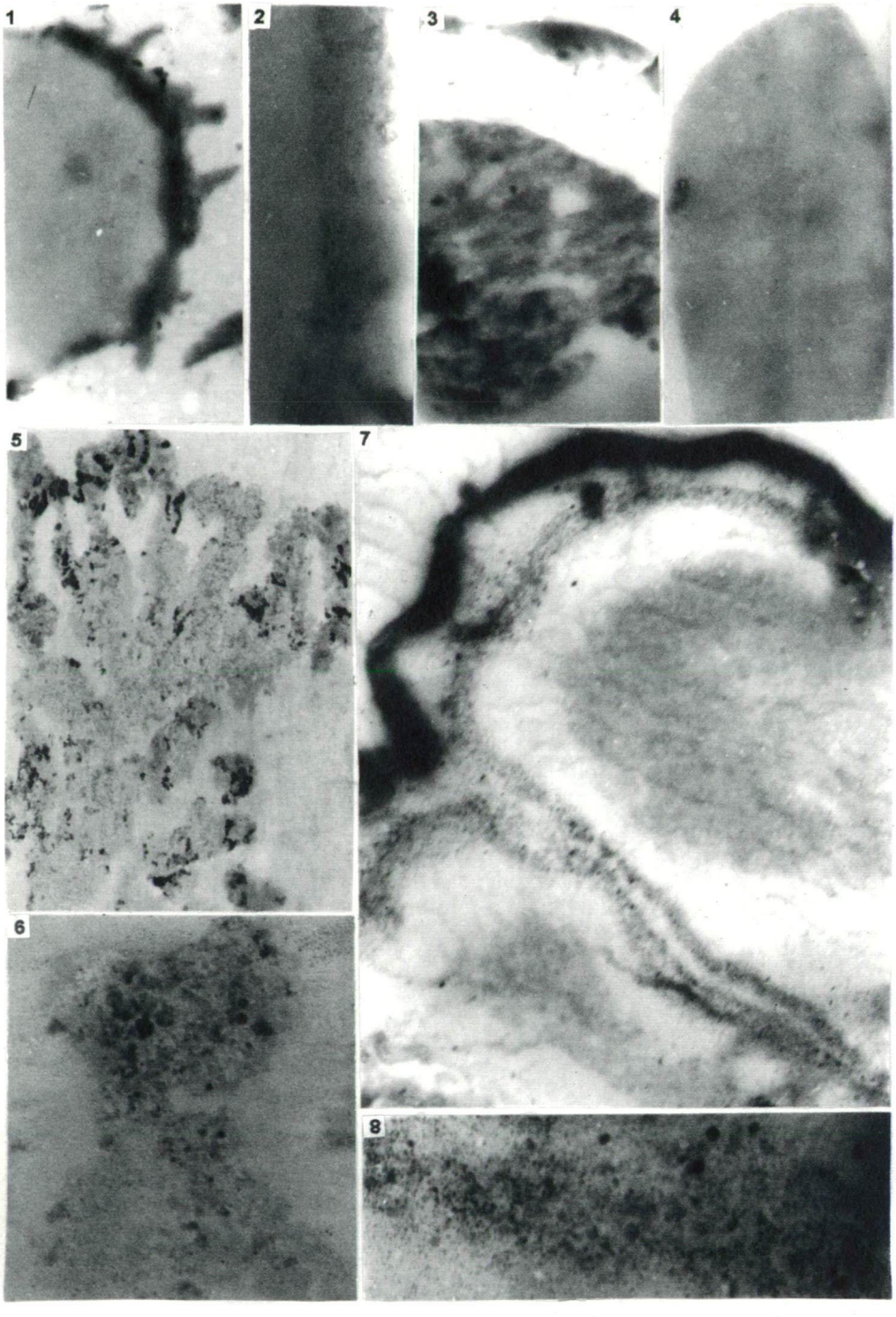


Plate 6.2.

Plate 6.2.

*Chenopodium album L.* TEM pictures.

1. General survey picture from the partially degraded pollen grain (T-12-82), Negative No.: 8445, 15.000x.
2. Detail from the partially degraded inner layer of the ectexine (foot layer). The globular elements of the biopolymer structure are well shown. (T-12-82), Negative No.: 8446, 75.000x.
3. Degraded wall and protoplasm with experiment T-12-83, Negative No.: 8451, 15.000x.
4. Degraded "inner body" with experiment T-12-84, Negative No.: 8448.
5. Detail from the damaged ectexine, experiment T-12-84, Negative No.: 8490, 15.000x.
6. Highly magnified picture from the degraded ectexine. The globular biopolymer units are well shown. T-12-84, Negative No.: 8492, 75.000x.
7. General survey picture from the partially degraded pollen grain. T-12-85, Negative No.: 8452, 15.000x.
8. Detail from the biopolymer units of the partially degraded ectexine. T-12-85. Negative No.: 8451, 75.000x.

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less globular electron dense units. The large units may be elements of the biopolymer system of the quasi-periodic arrangement.

Pollen grains treated with 2-aminoethanol during 24 hours, washing and oxidized with $KMnO_4$ during 24 hours (Plate 6.2., figs. 7,8)

Damaged protoplasm a light zone and a granular layer, followed by an electron dense outer layer is illustrated in picture 7, Plate 6.2. The granular layer may be the endexine or the inner part of the foot layer. In the highly magnified picture (Plate 6.2., fig. 8) the ultrastructure of this inner layer is granular with electron dense globular units, similar to the previous ultrastructure (Plate 6.2., fig. 6).

## Discussion and Conclusions

1. The organization of the biopolymer system of the spore-pollen wall, based on the newest results, is more complicated than was believed earlier. It is well established that this question may not be resolved with one model. The composition change in the different taxa, the different part of the ectexine within one species and several further factors.

SOUTHWORTH (1974) established that the old pollen of dicots dissolves readily in 2-aminoethanol. During our different kind of experiments we established that the walls of some sporomorphs are easily soluble in organic solvents. As a good example, the ectexine of *Quercus* and the exospore of the spores of *Equisetum*, may be mentioned, cf. KEDVES and GÁSPÁR (1994, 1996). KEDVES et al. (1998) established that the pollen grains of *Platanus hybrida* BROT. and *Tilia platyphyllos* SCOP. dissolved easily in diethylamine, but were resistant to merkaptoethanol and further alcohols (methanol, ethanol, n-propanol, n-pentanol and i-amyl alcohol). These new experiments verified again the extremely heterogeneous and dynamic character of the molecular system of the sporopollenin.

2. It may be emphasized again, the extremely multifaceted aspect of the study of the molecular and highly organized biopolymer system of the spore-pollen wall. Ecological factors are extremely important. The molecular transformation of the sporopollenin may be taken into consideration in the evaluations of the experimental results.

3. In the polyporate pollen grains, the resistance of the pollen grains of the *Juglans* genus may be emphasized (KEDVES and KINCSEK, 1989, KEDVES, KÁROSSY and BORBOLA, 1997). In this way, the polyporate morphological character may not be the first factor of the less resistant ectexine of the genus *Chenopodium.*

4. In the future, control investigations will be necessary. In this respect, the partial dissolution of the pollen grains further species of the genus *Chenopodium,* and different taxa of the Chenopodiaceae and Amaranthaceae will be important.

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