4. TRANSMISSION ELECTRON MICROSCOPY OF PARTIALLY DISSOLVED EXINES OF DIFFERENT BISACCATE GYMNOSPERM POLLEN GRAINS

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Abstract

In the present paper our TEM observations are published on the bisaccate gymnosperm pollen grains treated previously with different organic solvents. The diethylether is a suitable solvent for studying the biopolymer structure of the inner exine layers: foot layer, endexine and intine. The glycogen molecular structure may be discovered with tetrahydrofuran. N-pentane, and particularly pyrrolidine is the best to dissolve the quasi-crystalloid skeleton of the exine, probably of the plant cell wall. This is a tool to investigate the stabilizing biopolymer system. The detailed study of the stabilizing molecular structures will be the subject of further investigations.

Key words: Palynology, bisaccate gymnosperm, biopolymer structure.

Introduction

The discovery of the quasi-crystalloid biopolymer skeleton in the plant cell wall, first described in the exine of the pollen grains of *Pinus griffithii* McCLELL in 1988, raised several problems. The most important are as follows.
1. Methical investigations in two different, opposite ways;
1.1. The TEM study of the quasi-crystalloid skeleton after dissolving or oxidizing the so-called stabilizing molecular structures. In these experiments we have the opportunity to demonstrate the different levels of organization.
1.2. To dissolve the quasi-crystalloid skeleton, to get more information about the stabilizing biopolymer structures. By the modified MARKHAM rotation method, the "negative PENROSE modell" was planned as another way for the verification of the quasi-crystalloid system of the plant cell wall.
2. Comparisons between recent and fossil data, inside them the different kinds of plant cell wall, not only the "sporopollenin type" biopolymer structures.
3. To pay particular attention to the outer and inner surfaces and to the previously established characteristic features, such as the molecular sieve character (ROWLEY, 1973), and the electrostatic charge of the surface (ROWLEY, 1971).
4. As it was emphasized in our previous paper (KEDVES, 1991) the three dimensional modelling is a necessity and now it seems promising to project

several parallel studies, because these results may have favourable effect on each other.

During one of our new research program the basic concept is to dissolve the quasi-crystalloid biopolymer skeleton. Concerning this subject we have few information. Some data were published in our preliminary report (KEDVES et al., 1991), and in another paper (KEDVES and ROJIK, 1991) on the sclereids of *Armeniaca vulgaris* GAERTN.

Regarding the details the following may be pointed out.
1. Investigating the effect of one solvent on different taxa.
2. The effect of the different solvents on one species and/or sample.
3. Varia.

Material and Methods

The following species were the subject of our investigations:
1. *Pinus mugo* TURRA
   Coll.: Dr. K. MARGÓCZI, Botanical Garden of the J. A. University, 18. 5. 1988.
2. *Pinus silvestris* L.
   Coll.: Dr. K. MARGÓCZI, Botanical Garden of the J. A. University, 18. 5. 1988.
3. *Pinus griffithii* McCLELL
   Coll.: Dr. K. MARGÓCZI, Botanical Garden of the J. A. University, 18. 5. 1988.
4. *Picea glauca* (MOENCH.) VOSS.
   Coll.: Dr. K. MARGÓCZI, Botanical Garden of the J. A. University, 18. 5. 1988.

The experiments were made as follows.

	tetrahydrofuran	n-pentane	pyrrolidine	diethylether
Pinus mugo	634	656	670	682
Pinus silvestris		657		692
Pinus griffithii			669	681
Picea glauca			671	680

20 mg air dried pollen grains+5 ml solvent. The experiment started on 10. 5. 1989 at 16^h, and finished on 24. 5. 1989 at 10^h. Temperature from 10. 5. 1989, 16^h up to 12. 5. 1989 8$^{30'}$ the so-called room temperature after in a refrigerator on +5−+6 °C. The residue material was washed in aethyl alcohol, and fixed in 1% OsO_4 aq. dil., embedded in Araldite. The ultrathin sections were made in the EM Laboratory of the Biological Research Center of the Hungarian Academy of Sciences on a Porter Blum ultramicrotome. The TEM pictures were taken on a TEM instrument of Opton EM−902 (resolution 2−3.5Å).

Submicroscopic morphological nomenclature
of the bisaccate pollen grains

Several kinds of nomenclature are known in this subject. The most important establishments obtained firstly on the basis of TEM data are summarized in the following. Concerning their general morphology (POCKNALL, 1981, and HANSEN and ENGSTROM, 1985) the pollen grain consists of corpus (sensu strictu pollen grain) and of saccus (Plate 4.1., fig. 1). The apertural area is on the distal part, this is a furrow. On the border of the saccus/corpus there is the furrow rim, on the proximal part the cappa (cappus) and the marginal ridge are found. The infratectal layer including the corpus and the sacci is characteristically alveolar (cf. M. VAN CAMPO, 1973). At this place it is necessary to point out that the saccus is not reticulate as it was written in several papers. The former light microscopic description is misleading because the optical section of the alveoli forms seemingly a reticulum. The alveolar structure is essentially a system of lamellar infratectal elements. The orientation of these lamellae may be regular or irregular. Concerning the saccus, following M. VAN CAMPO (1973, p. 98): "On peut dans les Sapins distinguer 3 couches d'alvéoles, les alvéoles a$_1$ petits fermés directement sous le tectum, les alvéoles
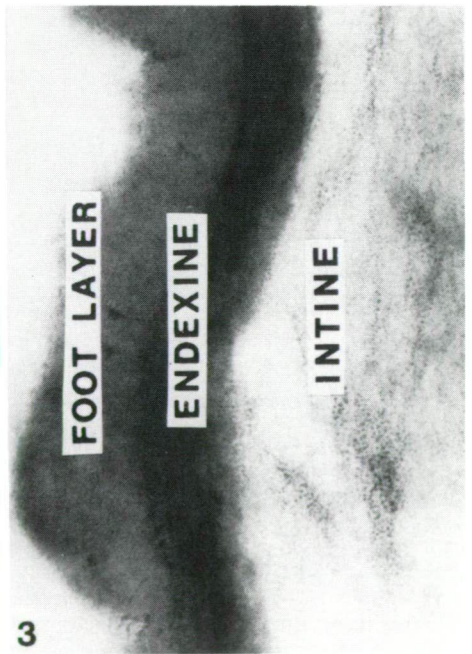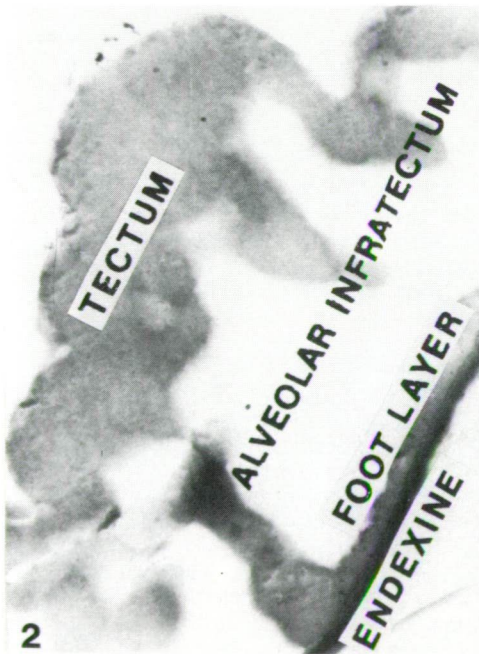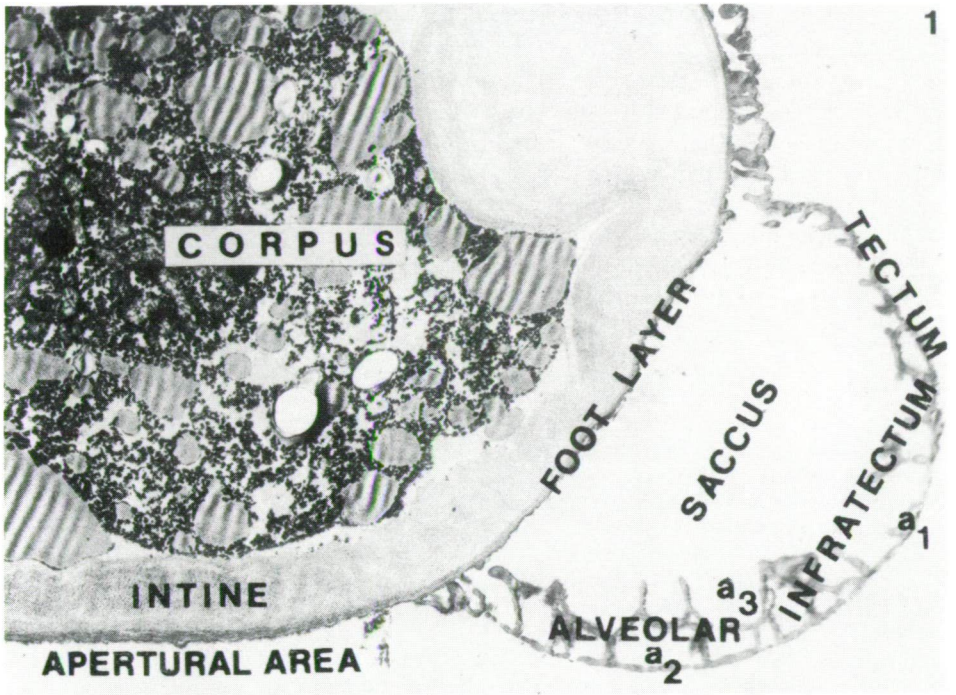
moyens a_2 et les grands alvéoles internes, ouverts a_3." KURMANN (1989) established as follows, p. 2489: "...there are differences in the pollen wall formation between gymnosperms and angiosperms especially in the timing of the wall deposition." P. 2502: "In angiosperms, the ektexine is formed during the tetrad phase and the endexine, if present, in the free spore phase. In gymnosperms, however, both the ektexine and endexine are deposited during the tetrad phase." Following VASIL (1978), p. 118: "...the intine is formed immediately outside the plasmolemma, GOLGI-derived vesicles are again involved in the deposition of the intine, which is a homogeneous layer devoid of any lamellations or fibrillar substructure." MARTENS and WATERKEYN (1961), p. 1390: "...intine — c'est-à-dire la vrai membrane cellulaire — est encore très mal connue." P. 1393: "Nous admettons aussi avec Mme VAN CAMPO (1950), que l'intine de *Picea* est mince et simple, celle de *Pinus* épaisse et complexe,..."

We hope that these above mentioned basic establishments are enough for the interpretation and discussion of our experimental studies.

◀ Plate 4. 1.

1—3. *Pinus mugo* TURRA.
   Ultrastructure of the pollen grains after partial degradation with diethylether. Experiment No: 682.
1. General survey picture of the ultrastructure of the pollen grain. Negative no: 808, 2.500x.
2. Fine structure details of the exine of the corpus. Negative no: 816, 25.000x.
3. Ultrastructure of the inner layers of the exine. Negative no: 817, 100.000x.

Results

1. INVESTIGATION OF THE EFFECT OF ONE SOLVENT (DIETHYLETHER) TO DIFFERENT TAXA

Pinus mugo TURRA
Experiment No: 682
(Plate 4.1., figs. 1—3, plate 4.2., figs. 1—5)

In low magnification (so-called general survey picture) (Plate 4.1., fig. 1) the cytological characteristic features of the pollen grain ultrastructure of the proximal and distal surfaces, and those of the saccus are well illustrated. The three kinds of the saccus alveoli, mentioned previously in the paper of M. VAN CAMPO (1973) are also clearly shown. The intine is extremely thick. In the apertural area the ectexine consists of the foot layer only.

Corpus (Plate 4.1., fig. 2, 3)
Fig. 2, illustrates the exine stratification. The foot layer is relatively thin, and the endexine distincts only by its stronger electron density. The finer stratification of the inner layers of the corpus is as follows (Plate 4.1., fig. 3). The endexine has a very strong electron density but there are less characteristic lamellations, too. The endexine/intine border is a lighter part. In the intine fine lamellations have been observed. As regards the biopolymer organization of the exine layers of the corpus this experiment has not yielded sufficient data.

Saccus (Plate 4.2., figs. 1—5)
   Tectum (Plate 4.2., fig. 1)

OUTER SURFACE

1

2

FOOT LAYER

ENDEXINE

INTINE

3

FOOT LAYER

ENDEXINE

4

ENDEXINE

INTINE

5
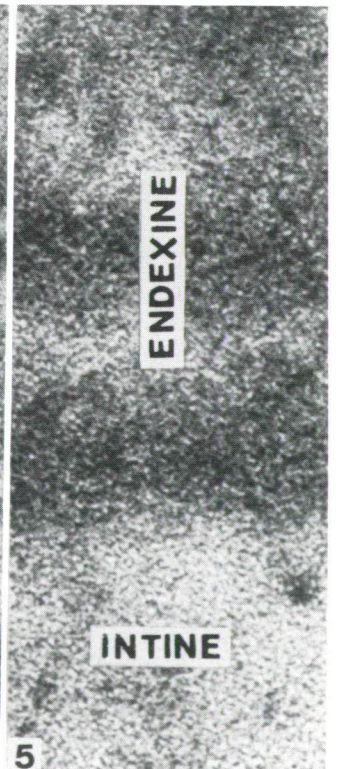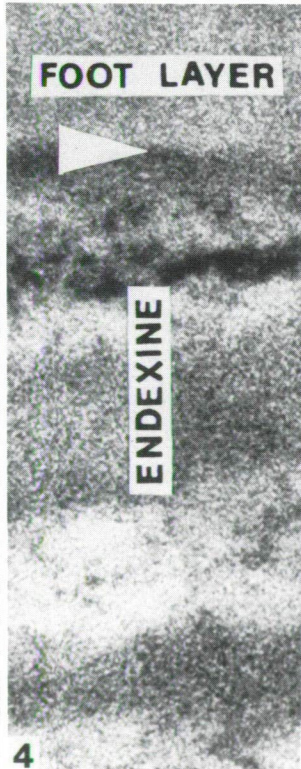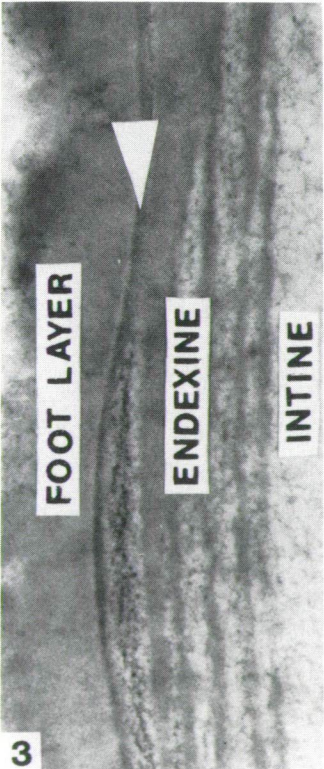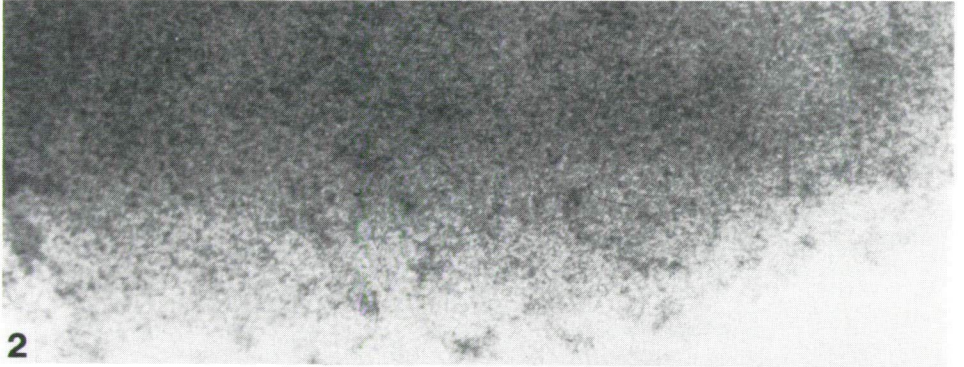
On the surface of the tectum this experiment demonstrated characteristic surface — protective — layer of molecules composed of globular units, connected with tiny arms. The diameter of the granular elements range from 3—7 Å, mostly 3—5 Å. The granular biopolymer units of the inner part of the tectum are a little larger; 3—13 Å (mostly 6—8 Å). The size of the granular elements of the inner surface of the tectum is similar to the previously mentioned; 4—11 Å, mostly 4—8 Å, in this way, it differs from the biopolymer organization of the surface. These filaments can correspond to the glycogen molecular structure (network of chains, cf. DARNELL et al., 1986), but are of different degrees of degradation.

Infratectal layer (Plate 4.2., fig. 2)
The globular biopolymer units are of 4—10 Å in diameter. These elements have often fibrillar or irregular network-like arrangement.

Foot layer (Plate 4.2., fig. 3)
The surface of this layer is also covered with granular biopolymer units of 4—10 Å in diameter (mostly 6—8 Å). The border with the lamellar endexine is characteristic. A narrow bright and a dark layer separate well these two parts, marked with arrows (Plate 4.2., fig. 3, 4).

Endexine (Plate 4.2., figs. 3—5)
This layer is characteristically lamellar, composed of compact and loose layers. The compact lamellae are similar to the foot layer or in general to the endexine, the loose part to the intine. The globular biopolymer units of the compact part of the endexine range from 3 to 10 Å (mostly 6—8 Å), those of the loose part; 4—10 Å (mostly 4—8 Å).

Intine (Plate 4.2., figs. 3—5)
The globular elements of this layer are relatively small, 2—7 Å (mostly 3—4 Å), and are sometimes of linear or network-like arrangement.
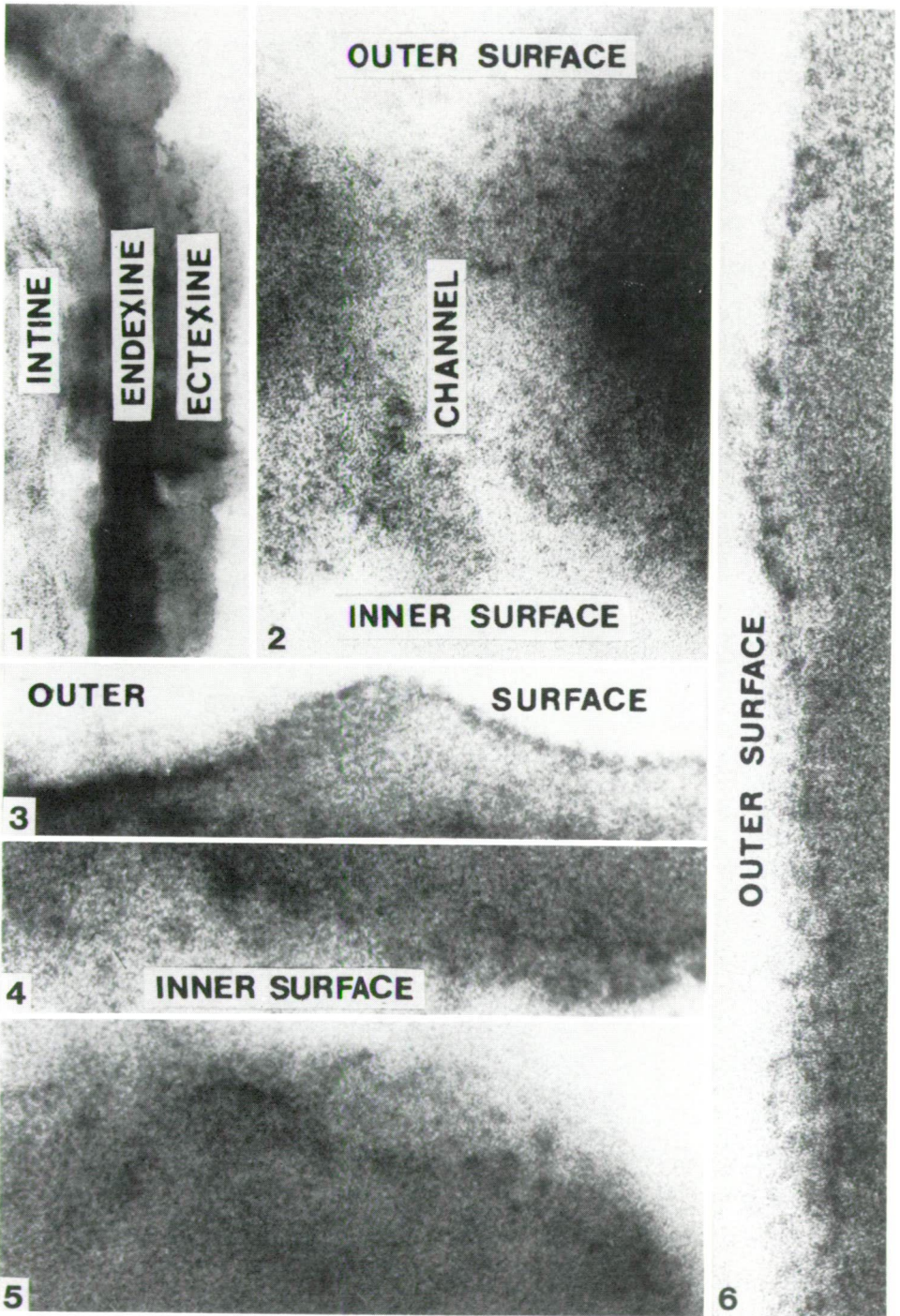
Pinus silvestris L.
Experiment No: 693
(Plate 4.3., figs. 1—6)

◀   Plate 4.2.

1—5. *Pinus mugo* TURRA.
   Ultrastructure of the saccus of the pollen grains after partial degradation with diethylether. Experiment No: 682.
1.   Biopolymer organization of the tectum of the saccus. Negative no: 810, Magnification 1 million.
2.   Biopolymer structure of the infratectum of the saccus. Negative no: 811, 500.000x.
3.   Ultrastructure of the inner layers of the saccus. Negative no: 813, 100.000x.
4.   The border of the ectexine and endexine marked with an arrow. Negative no: 814, 500.000x.
5.   The border of the endexine and the intine of the saccus. Negative no: 815, Magnification 1 million.

1 INTINE ENDEXINE ECTEXINE

2 OUTER SURFACE — CHANNEL — INNER SURFACE

3 OUTER SURFACE

4 INNER SURFACE

5

6 OUTER SURFACE

Corpus (Plate 4.3., fig. 1)

The TEM pictures are very similar to those of the previous experiment (cf. Plate 4.1., fig. 3), this can be consequence of the identic solvent.

Ectexine (Plate 4.3., fig. 1)

The three layers of the ectexine are not so characteristic. Small globular elements with relatively strong electron density are observed on the surface.

Endexine (Plate 4.3., fig. 1)

This layer is well separated from the ectexine by its strong electron density.

Intine (Plate 4.3., fig. 1)

Beneath the endexine a finely lamellar intine is found.

Saccus (Plate 4.3., figs. 2—6)

In this experiment the biopolymer structure of the pollen wall was particularly investigated.

Tectum (Plate 4.3., figs. 2—4, 6)

Fig. 2 on Plate 4.3. represents well the biopolymer structure of the tectum channel. The globular biopolymer units of the surface and the inner part of the tectum were investigated in the pictures of five TEM negatives. The summarized results of their size distribution are presented in the following chart. The first number represents the number of the biopolymer units of a given diameter of the surface, the second ones that of the inner part of the tectum.
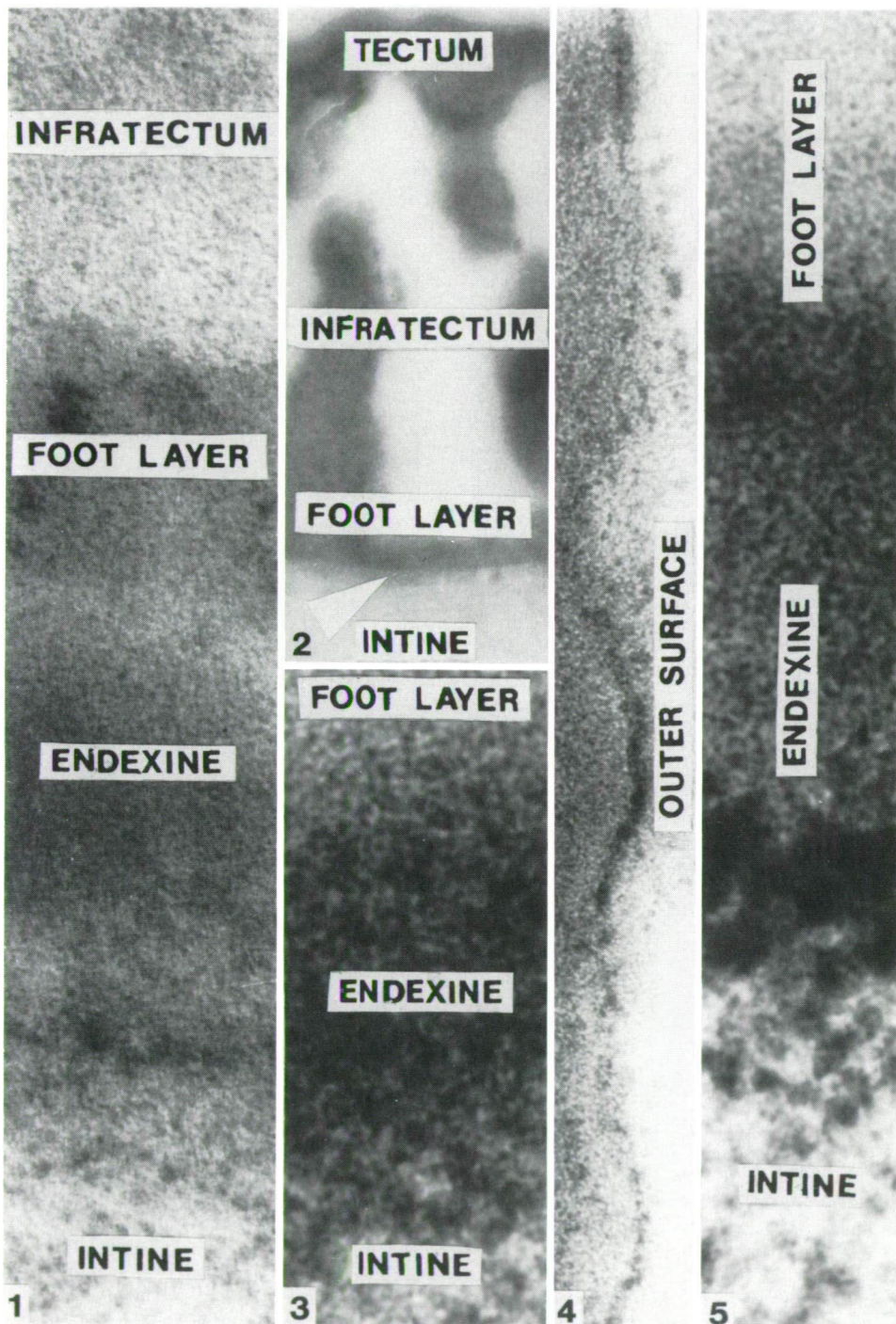
Diameters in Å	Numbers of the negatives				
	818	824	825	826	827
3	3; 0	9; 0	1; 0	2; 0	2; 0
4	6; 1	4; 5	5; 3	5; 1	6; 0
5	5; 1	2; 2	5; 2	4; 2	0; 3
6	4; 7	9; 5	11; 6	6; 6	8; 5
7	1; 0	3; 1	6; 1	1; 0	6; 1
8	4; 5	9; 8	7; 4	4; 7	11; 9
9	0; 1	4; 0	0; 2	2; 0	1; 1
10	1; 5	5; 9	5; 5	1; 10	5; 7
11	0; 1	0; 2	0; 4	0; 5	0; 7

◀ Plate 4.3.

1—6. *Pinus silvestris* L.

TEM pictures of the pollen grains after partial degradation with diethylether. Experiment No: 693.

1. The wall ultrastructure of the corpus. Negative no: 822, 100.000x.
2. The tectum of the saccus. Negative no: 818, 500.000x.
3, 4. The surfaces of the tectum of the saccus. Negative no: 825, 500.000x.
5. Biopolymer organization of the infratectum of the saccus. Negative no: 820, 500.000x.
6. Different kinds of preservation of the biopolymer structure of the outer surface of the tectum of the saccus. Negative no: 827, 500.000x.

INFRATECTUM

FOOT LAYER

ENDEXINE

INTINE

1

TECTUM

INFRATECTUM

FOOT LAYER

2 INTINE

FOOT LAYER

ENDEXINE

INTINE

3

OUTER SURFACE

4

FOOT LAYER

ENDEXINE

INTINE

5

It is shown that the globular units of the surface range from 3−10 Å (mostly 4−8 Å). Those of the inner part of the tectum: 4−11 Å, mostly 4−10 Å.

The protective biopolymer layer of the outer surface is well illustrated on fig. 3 and 6 of the Plate 4.3. Molecular disintegration was observed on the outer, inner and channel surfaces (Plate 4.3, fig. 2) and on the inner surface of the tectum (Plate 4.3., fig. 3).

Infratectum (Plate 4.3., fig. 3)

The disintegration of the biopolymer system was established on the basis of the TEM pictures.

The further layers of the exine were not investigated at this experiment with the TEM method.

Pinus griffithii McCLELL
Experiment No: 681
(Plate 4.4., figs. 1−5)

This species is extremely important in the knowledge of the biopolymer organization of the pollen exine.

Corpus (Plate 4.4., figs. 1−3)

The stratification of the exine is illustrated in the fig. 2. The foot layer is relatively thin. Beneath the foot layer there is a very thin endexine marked with an arrow. It is noteworthy, that after experiment, the intine is not lamellar. Concerning the biopolymer structure of the infratectum, foot layer, endexine and intine (Plate 4.4., fig. 1) the following can be pointed out. The delimitation of the infratectum and the foot layer is characteristic. The molecular structure which is more or less characteristic of the different inner layers, are illustrated in picture 3, Plate 4.4. The diameter distribution of the globular biopolymer structures of the different parts of the foot layer is summarized as follows. (The first number indicates the number of biopolymers of the outer surface, the second and third ones those of the inner part, and inner surface respectively.)

◀   Plate 4.4.

1−5. *Pinus griffithii* McCLELL.
TEM pictures of the pollen grains after partial degradation with diethylether. Experiment No: 681.
1. The biopolymer organization of the inner layers of the corpus. Negative no: 401, 500.000x.
2. General survey picture of the wall of the corpus after the diethylether treatment. The endexine is marked with an arrow. Negative no: 400, 50.000x.
3. Biopolymer organization of the inner layers of the wall of the corpus. Negative no: 429, Magnification 1 million.
4. Biopolymer structure of the surface of the tectum of the saccus. Negative no: 424, 500.000x.
5. Biopolymer organization of the inner layers of the saccus. Negative no: 432, Magnification 1 million.

Diameters in Å	Numbers of the negatives					
	401	402	403	428	429	432
2	5; 6; 1	1; 0; 19	5; 9; 0	1; 0; 4	0; 0; 2	0; 16; 0
3	4; 2; 0	4; 0; 0	2; 0; 0	4; 3; 1	0; 1; 14	0; 1; 14
4	1; 0; 5	9; 0; 13	2; 4; 0	1; 2; 3	0; 2; 10	0; 7; 5
5	10; 4; 2	0; 0; 1	0; 0; 1	4; 2; 7	0; 5; 12	0; 5; 10
6	5; 6; 6	7; 0; 11	5; 15; 4	14; 12; 18	0; 7; 4	0; 6; 12
7	2; 0; 1	0; 0; 0	1; 2; 0	0; 0; 10	0; 2; 5	0; 1; 1
8	4; 7; 10	4; 0; 13	2; 10; 9	6; 6; 12	0; 6; 6	0; 1; 14
9	0; 0; 0	0; 0; 1	0; 0; 0	0; 0; 2	0; 2; 3	0; 0; 1
10	0; 4; 5	0; 0; 2	0; 0; 9	10; 4; 7	0; 5; 2	0; 0; 5
11	0; 0; 3	0; 0; 0	0; 0; 1	0; 0; 1	0; 0; 0	0; 0; 0

The measured values are quite different, but essentially it can be concluded that the globular biopolymer units of the surface are a little larger than those of the outer surface and/or of the inner part of the foot layer. As regards the intine of the corpus, the measured values are as follows.

Diameters in Å	
2	0
3	0
4	10
5	7
6	30
7	12
8	37
9	4
10	16

In this way it may be concluded, that these granular elements are significantly larger than those of the inner parts of the ectexine and the intine.

Saccus (Plate 4.4., fig. 4, 5)
    Tectum (Plate 4.4., fig. 4)
    On the outer surface of the tectum, the protective molecular layer is well shown, it is composed of globular units of 2—9 Å (mostly 4—6 Å) in diameter. It is also well shown in picture 4 of Plate 4.4., that one part of this layer is damaged, and the destruction of the biopolymer system of the tectum has already started.

    Infratectum (not illustrated)
    The globular biopolymer units of this layer are of 2—10 Å (mostly 6 Å) in diameter.

    Endexine (Plate 4.4., fig. 5)
    This layer distincts well from the foot layer by its stronger electron density.

    Intine (Plate 4.4., fig. 5)
    The globular units of the intine in this picture are characteristic and separate quite well from the endexine.

Picea glauca (MOENCH.) VOSS.
Experiment No: 680
(Plate 4.5., figs. 1—4)

In case of this species the corpus and the corpus/saccus border were investiga-
ted in detail. The general survey pictures illustrate well the characteristic ultrastruc-
tural features of the pollen grain. Fig. 1 in the Plate 4.5., illustrates well the uneven
outer surface of the tectum, the characteristic alveolar infratectal layer, and the
relatively thin foot layer. At the bordering part of the corpus/saccus, between the
lamellar endexine and the foot layer there is an interbedded layer with finely
lamellar substance. The thickness of this layer is not uniform, this is particularly well
shown in picture 2, of Plate 4.5. The endexine is lamellar. The endexine/intine
border is not so clear. Regarding the details, the following can be pointed out.

Tectum (not illustrated in larger magnification)
On the surface of the tectum, the protective molecular film is well shown. The
damage of this layer was also observed with the degradation of the biopolymer
system of the tectum. The diameter of the globular biopolymer units is $3-10$ Å,
mostly $4-8$ Å. The inner surface of the tectum including its biopolymer structure is
similar to the outer one.

Infratectum (not illustrated in high magnification)
The observed globular biopolymer units are of $2-10$ Å (mostly of $2-4$ Å) in
diameter, a little smaller than those of the tectum.

Endexine (Plate 4.5., figs. 1—4)
The inner layers, as it was emphasized in our previous paper (KEDVES et al.
1991, Plate 4.1., fig. 4, p. 33) are complicated. The innermost layer of the foot layer
have the strongest electron affinity. This layer is very thin (about $12-15$ Å), layer
"A" in our previous paper. As a first part of the layers of the endexine this layer is
followed by the previously mentioned granular layer; layer "B" in our previous
paper. The biopolymer structure of this layer is heterogeneous. There are globular
units, with strong electron density interbedded in a faint finely granular substance.
The diameter of the granular units is $2-10$ Å, mostly 4 Å. The biopolymer
organization of this endexine layer is similar to that of the intine. The following layer
is more compact than the foot layer, in general it is similar to the ectexine.
Summarizing the above described results, this layer seems to be extremely
interesting and peculiar following the treatment regarding its ultrastructure and
biopolymer organization.

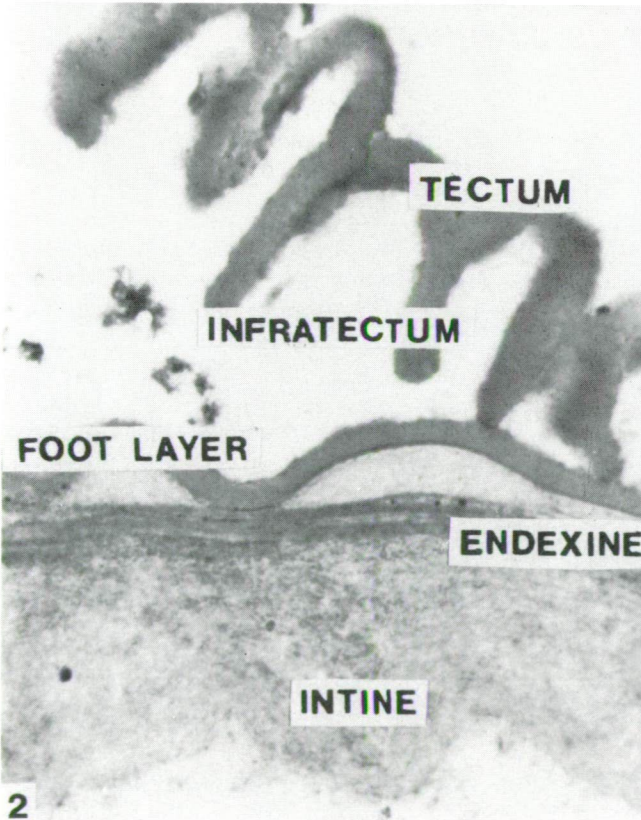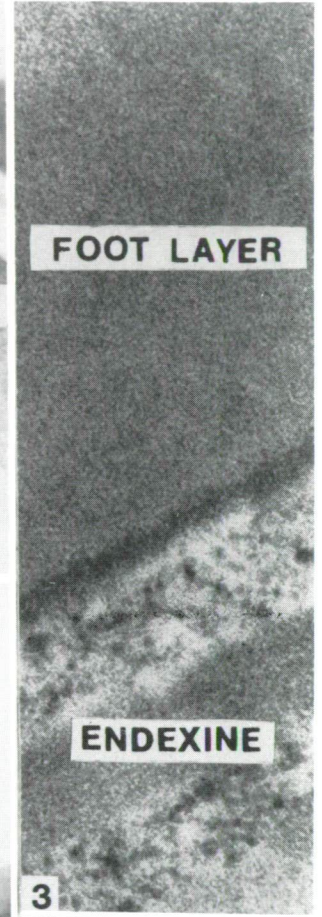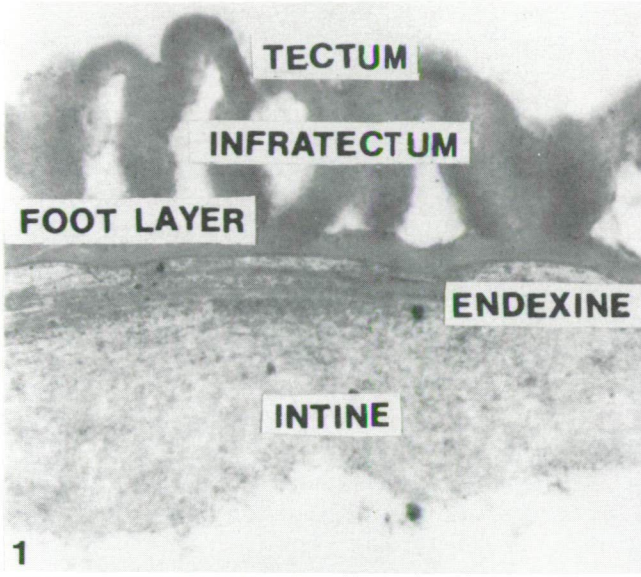## 2. THE EFFECT OF THE DIFFERENT SOLVENTS ON ONE SPECIES AND/OR SAMPLE

Partial degradation with tetrahydrofuran

Pinus mugo TURRA
Experiment No: 634
(Plate 4. 6., figs. 1—4)

49
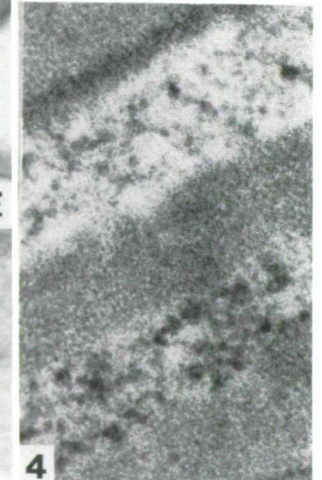
◀ Plate 4. 5.

1—4. *Picea glauca* (MOENCH.) Voss.
    TEM pictures from the pollen grains after partial degradation with diethylether. Experiment No: 680.
1. General survey picture of the wall of the corpus after treatment. Negative no: 525, 25.000x.
2. TEM picture of the wall of the corpus/saccus bordering after degradation. Negative no: 526, 25.000x.
3, 4. Biopolymer organization of the foot layer and the endexine of the corpus. Negative no: 528, 250.000x.

OUTER SURFACE

FOOT LAYER

ENDEXINE

INTINE

INFRATECTUM

FOOT LAYER

OUTER SURFACE
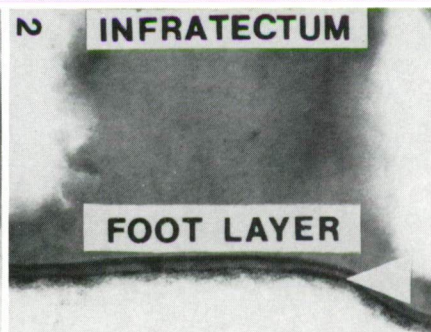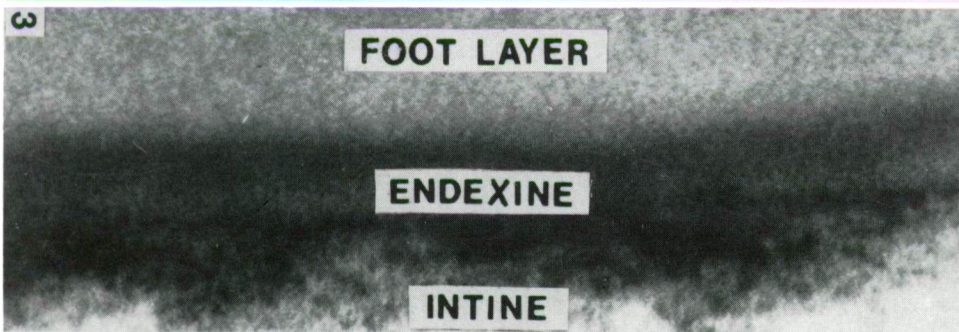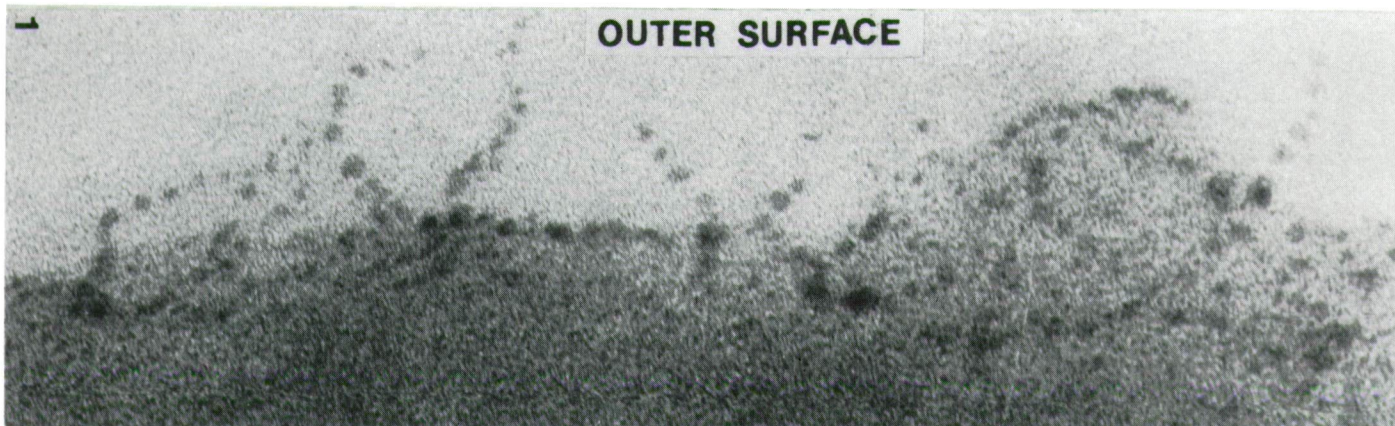
This experiment resulted for the first time in the demonstration of the characteristic glycogen molecular chains published earlier in our preliminary short communication (KEDVES et al., 1991, Plate 4. 1., fig. 2, p. 33). The results in this subject are summarized in detail as follows.

Corpus (Plate 4.6., figs. 1—3)
    Tectum (Plate 4.6., fig. 1)
    The above mentioned glycogen molecular chains are well shown. The diameter of the globular units is 2—10 Å, mostly 4—6 Å. The distance, or better say the "α (1→4) linkage between two glucose units" (cf. DARNELL et al. 1986, p. 99) is about 4—6 Å. We have observed the "α (1→6) linkage between two glucose chains" too, but for the detailed investigations we need further experimental data.

    Infratectal and foot layer (Plate 4. 6., fig. 2, 3)
    From the infratectal layer, a low magnified picture is presented here. In the highly magnified pictures of this layer, glucose chains or globular, highly organized biopolymer units were not observed. The biopolymer organization of the foot layer, is identic with that of the infratectum (Plate 4. 6., fig. 3).

    Endexine and intine (Plate 4. 6., fig. 2, 3)
    This layer well separates from the ectexine by its strong electron density. It is an intermediate zone between these two layers. In the endexine sensu stricto no globular biopolymer structures were observed. At the endexine/intine bordering part, howewer, there are globular units of 2—10 Å in diameter, mostly of 6—8 Å. The "distance" between these units is about 2—3 Å. The globular units are sometimes arranged into fibrillar or lamellar structures. The origin of these elements needs further investigations. The globular units of the outer part of the intine are a little smaller; 2—8 Å, mostly 4—6 Å. Distance between these globular units is 2—6 Å, mostly 2—4 Å.

Saccus (Plate 4. 6., fig. 4)
    Tectum (Plate 4. 6., fig 4)
    On the outer surface of the tectum the glycogen molecular chains are well shown. The diameter of the globular (cf. glucose) units is 2—10 Å, mostly 4—6 Å. The "α (1→4) linkage between two glucose units" is 4—6 Å.

◀   Plate 4.6.

1—4. *Pinus mugo* TURRA.
    TEM pictures of the pollen grains after partial degradation with tetrahydrofuran. Experiment No: 634.
1.   Glycogen molecular chains of the partially degraded tectum of the corpus. Negative no: 399, 500.000x.
2.   TEM picture of the inner layers of the exine after THF treatment. Negative no: 392, 50.000x.
3.   Molecular structure of the inner layers of the pollen wall after the treatment. Negative no: 395, 500.000x.
4.   Biopolymer structure of the partially degraded tectum of the saccus. The glycogen molecular chains are clearly shown. Negative no: 387, 500.000x.

SACCUS

a3

a2 a1

TECTUM

OUTER SURFACE

54
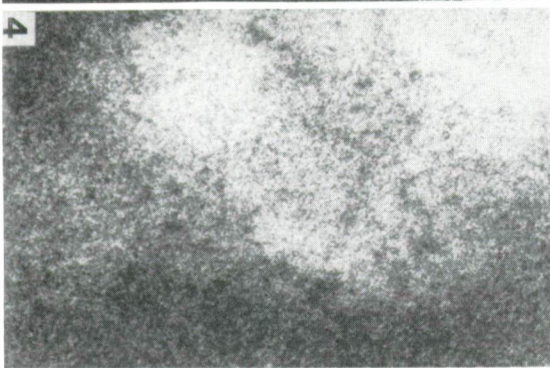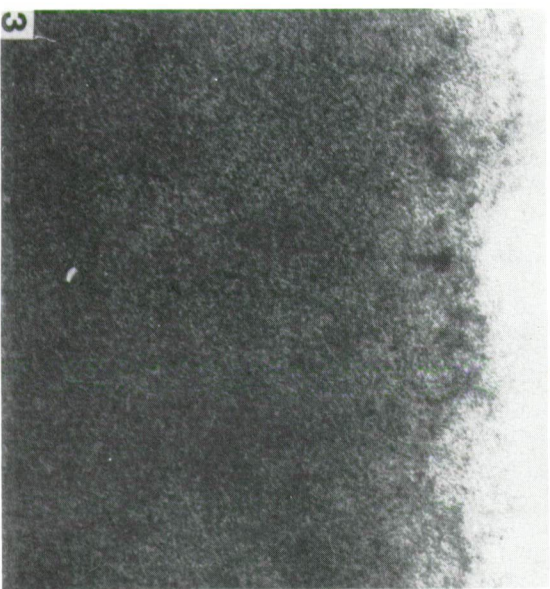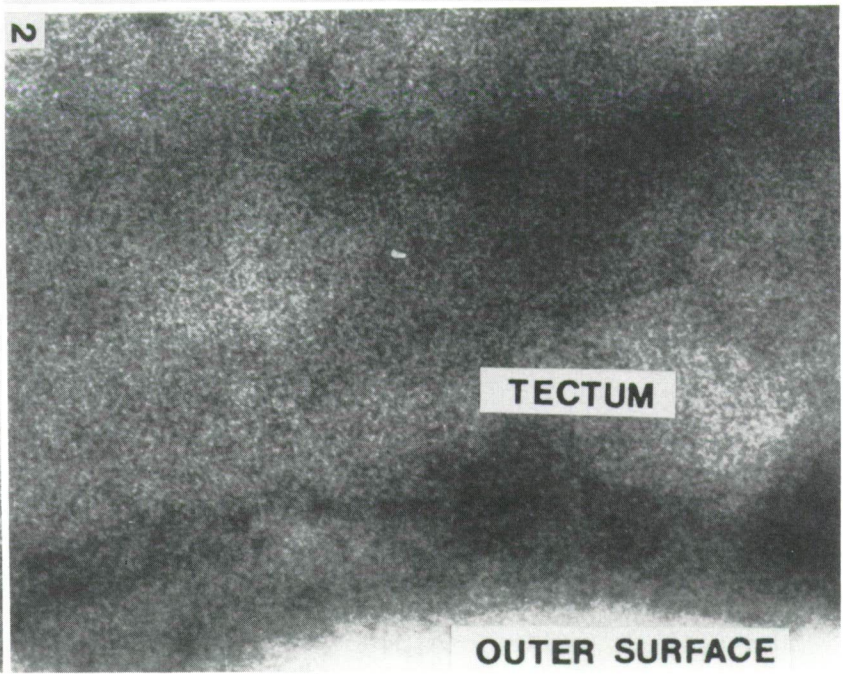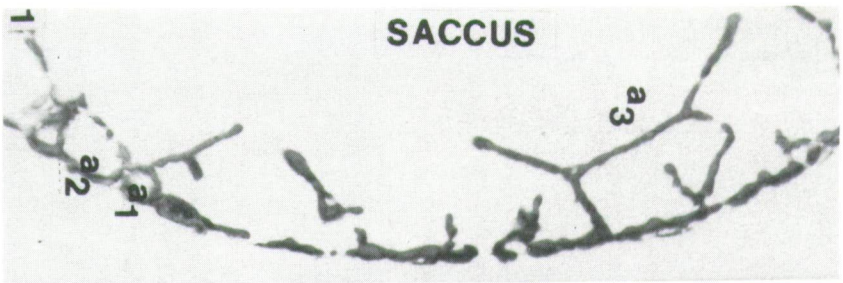
Infratectum

On the basis of our TEM data the biopolymer units were not suitable for measurements.

The inner exine layers of the saccus were not investigated in detail.

Partial degradation with n-pentane

Pinus mugo TURRA
Experiment No: 656
(Plate 4. 7., figs. 1—4)

In this experiment the saccus was investigated only. The low magnified picture illustrates well the consequence of the n-pentane treatment the ectexine has a strong electron density.

Tectum (Plate 4. 7., fig. 2)

On the outer surface of the tectum, the protective biopolymer layer is not characteristic. The glycogen chains, which were very characteristic and well demonstrated in the previous experiment of the same species were in all probability destroyed during treatment with n-pentane. Less characteristic globular units of $2-10$ Å were observed (mostly of $4-8$ Å) being probably the remains of the glycogen chains.

Infratectum (Plate 4. 7., fig. 3, 4)

In this ectexine layer the molecular structures (probably glycogen) are better preserved, see fig. 3, in Plate 4—7. The globular units are of $2-10$ Å in diameter, mostly $4-10$ Å.

Partial degradation with pyrrolidine

Pinus mugo TURRA
Experiment No: 670
(Plate 4.8., figs. 1—3, plate 4. 9., figs. 1—6)

In the general survey pictures (Plate 4. 8., figs. 1—3) it is well shown that the electron density of the ectexine is very strong after the treatment. The intine is lamellar, and seemingly with secondary alterations. As regards the details, the

◀   Plate 4. 7.

1—4. *Pinus mugo* TURRA.
   TEM pictures of the pollen grains after partial degradation with n-pentane. Experiment No: 656.
1.   General survey picture of the outer part of the saccus. Negative no: 748, 5.000x.
2.   Biopolymer structure of the partially degraded tectum of the saccus. Negative no: 750, 500.000x.
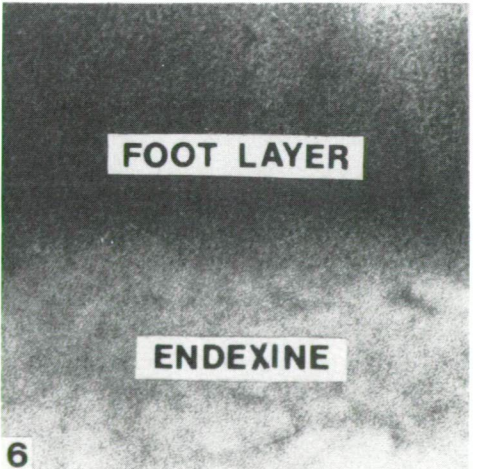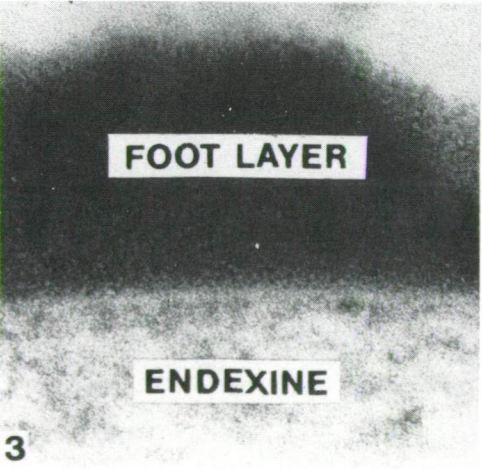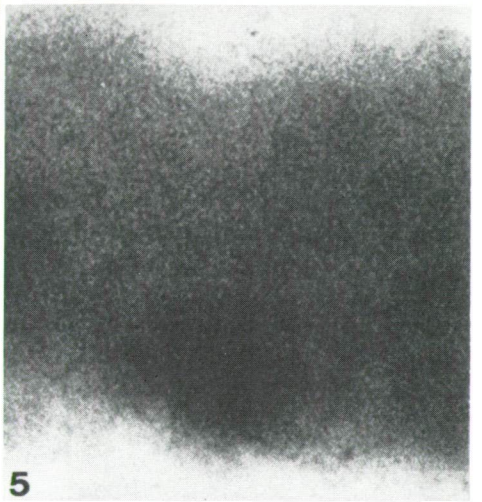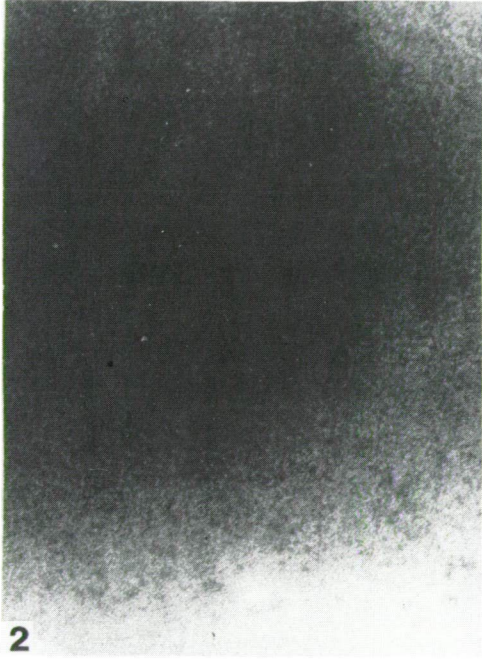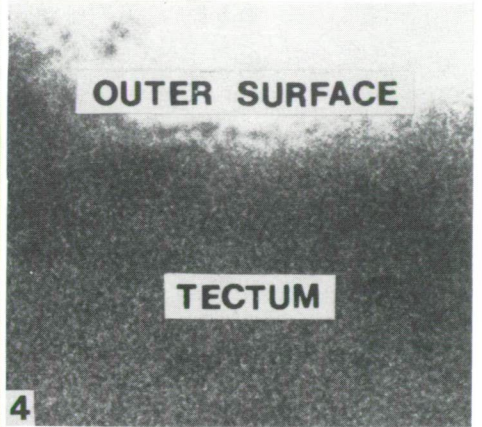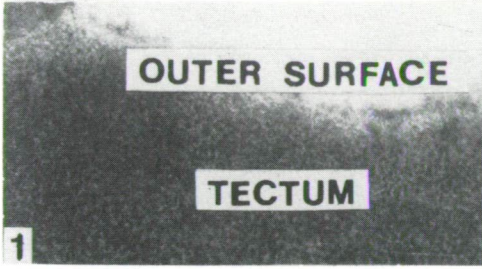3, 4. TEM pictures of the partially degraded infratectum. Negative no: 752 and 751 respectively, 500.000x.

CORPUS

INTINE

FOOT LAYER

SACCUS

1

INTINE

2

CORPUS

SACCUS

3

56
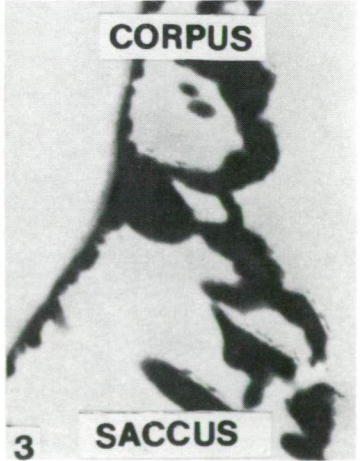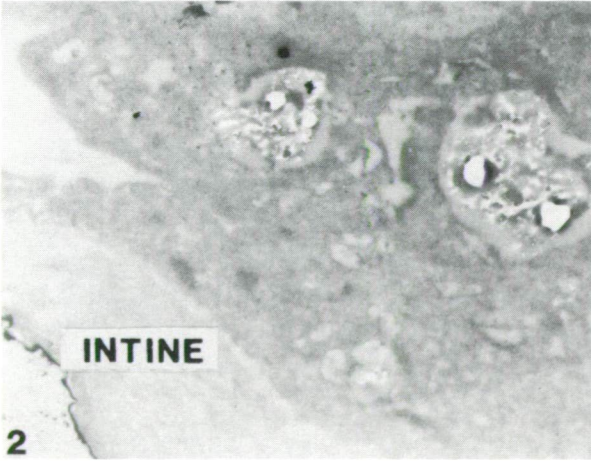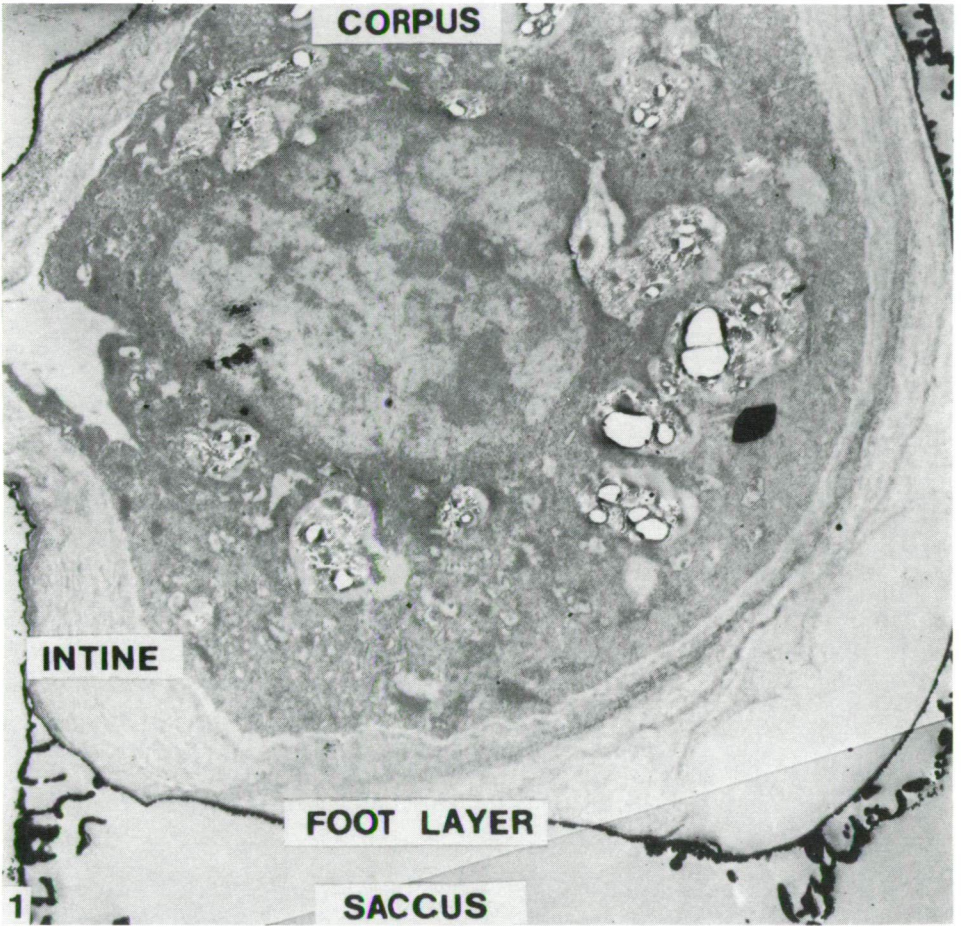
1—3. *Pinus mugo* TURRA.
TEM pictures of the pollen grains after partial degradation with pyrrolidine. Experiment No: 670.
1.   General survey picture of the pollen grain after experiment. Negative no: 755 and 756, 2.500x.
2.   Part of the corpus in the apertural area. Negative no: 757, 5.000x.
3.   Ultrastructure of the corpus/saccus border region. Negative no: 764, 10.000x.

OUTER SURFACE

TECTUM

1

2

FOOT LAYER

ENDEXINE

3

OUTER SURFACE

TECTUM

4

5

FOOT LAYER

ENDEXINE

6

following can be emphasized. In general, the biopolymer structure, firstly the surface protecting biopolymer system is damaged.

Corpus (Plate 4.9., figs. 1—3)
    Tectum (Plate 4. 9., fig. 1)
    The more or less damaged unilayered protective biopolymer system is also composed of globular units of 2—10 Å in diameter, mostly of 4—6 Å.

    Infratectum (Plate 4.9., fig. 2)
    On the surfaces of this layer only very damaged biopolymer structures were observed. The preservation of these units is insufficient for exact measurements.

    Foot layer and endexine (Plate 4.9., fig. 3)
    Taking into consideration the results of the previous experiment, it is interesting that the endexine was strongly dissolved, and its electron density is extremely low compared to the foot layer. Damaged globular units of 3—8 Å in diameter were observed which form filaments or lamellae, or irregular network system.

Saccus (Plate 4.9., figs. 4—6)
    Tectum (Plate 4.9., fig. 4)
    Similar to those of the corpus, the surface protective layer is composed of globular units of 3—10 Å in diameter, mostly of 4—10 Å.

    Infratectum (Plate 4.9., fig. 5)
    On the surface of this layer no highly organized biopolymer structures were observed.

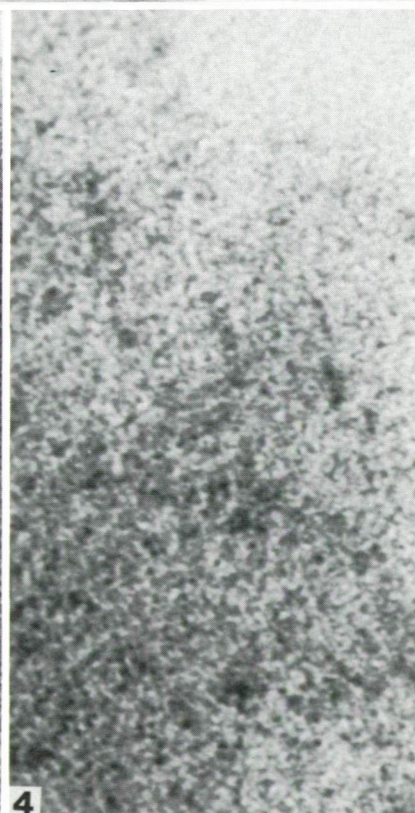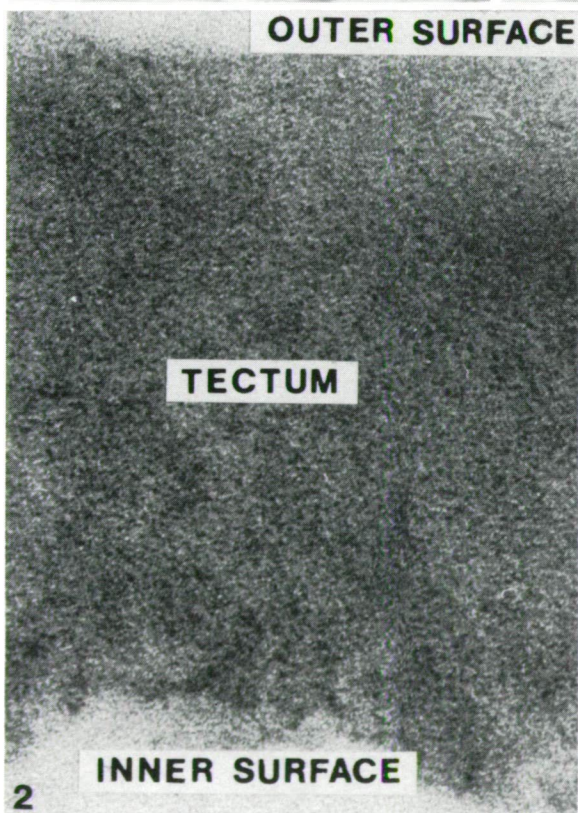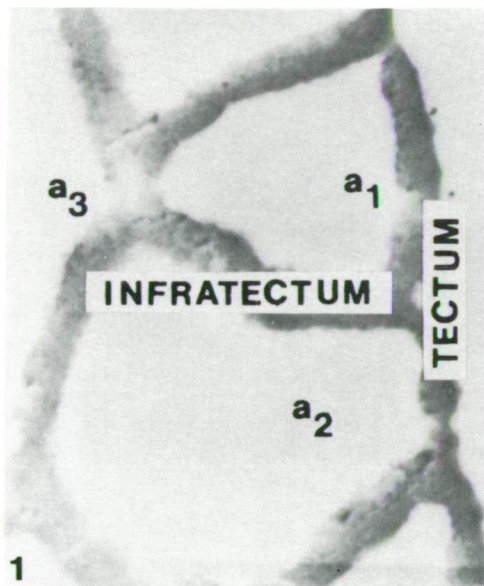   Foot layer and endexine (Plate 4.9., fig. 6)
    Beneath the relatively electron dense foot layer, the endexine is a little more damaged than those of the corpus.

◀   Plate 4.9.

1—6. *Pinus mugo* Turra.
    Biopolymer structure of the exine of the pollen grains after partial degradation with pyrrolidine. Experiment No: 670.
1. Detail of the tectum of the corpus. Negative no: 765, 250.000x.
2. Partially degraded infratectum of the corpus. Negative no: 766, 250.000x.
3. Biopolymer structure of the inner layers of the exine after treatment. Negative no: 767, 250.000x.
4. Tectum surface of the saccus after treatment. Negative no: 759, 250.000x.
5. TEM picture of the partially degraded infratectum of the saccus. Negative no: 761, 250.000x.
6. Detail of the biopolymer structure of the foot layer and the endexine of the saccus. Negative no: 762, 250.000x.

3. VARIA

Partial degradation with n-pentane

Pinus silvestris L.
Experiment No: 513
(Plate 4.10., figs. 1—4)

In this experiment only the saccus was investigated with the TEM method. The strong degradation of the ectexine is well shown in the low magnified picture (Plate 4. 10., fig. 1).

Tectum (Plate 4.10., fig. 2)
The protective biopolymer units of the surface are damaged. In the outer surface globular units of 2—6 Å (mostly 2—4 Å) diameter were observed. The measured units of the inner surface are of 4—10 Å in diameter, mostly 6—8 Å. This difference in size of these units may be the consequence of the extensive degradation.

Infratectum (Plate 4.10., fig. 3, 4)
On the surface of this layer no molecular structure was observed. The superficial degradation of the biopolymer system can be clearly seen. Only the remnants of the protective biopolymer layer were observed.
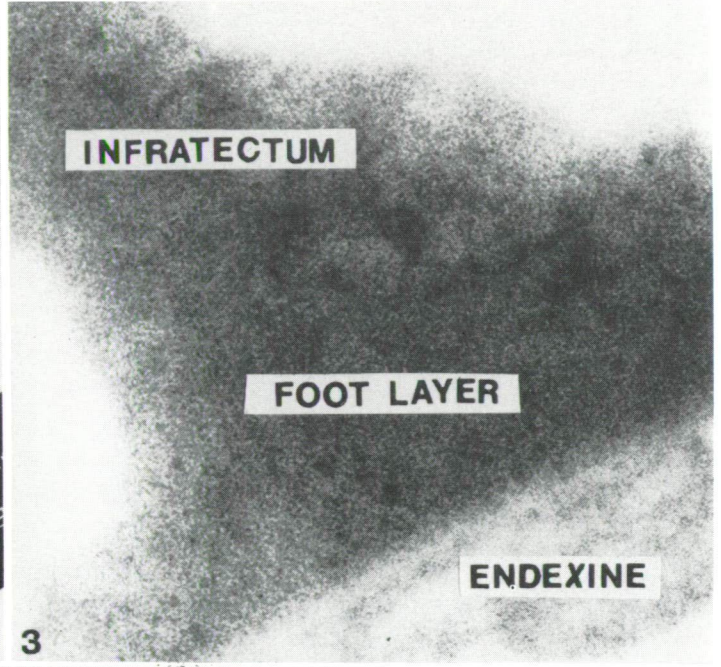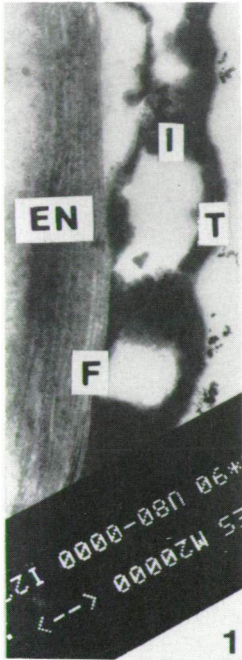
Partial degradation with pyrrolidine
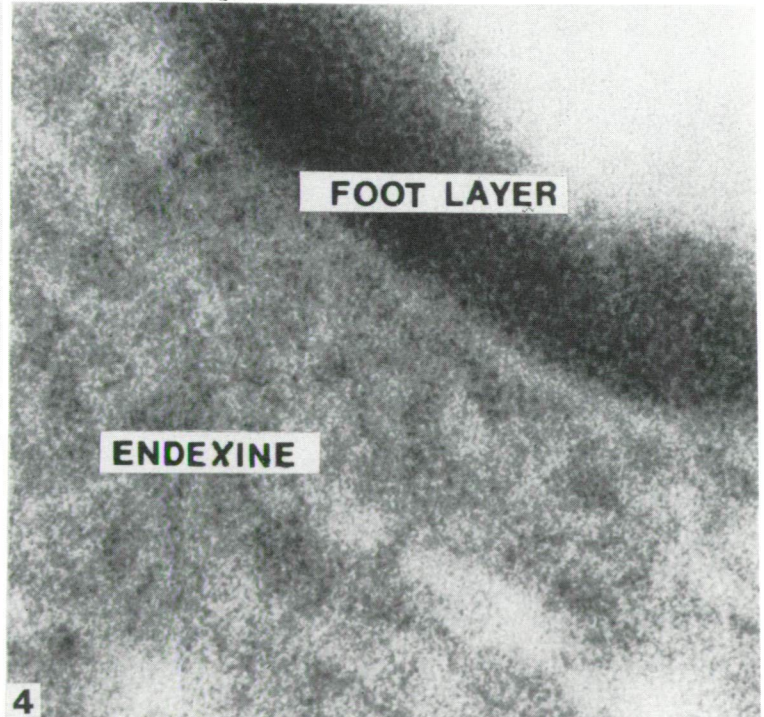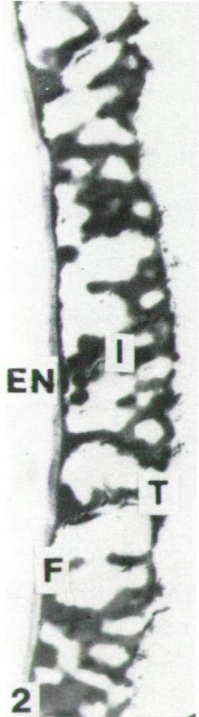
Pinus griffithii McCLELL
Experiment No: 668
(Plate 4. 11., figs. 1—4)

The strong electron density of the ectexine layers is well shown in the low magnified pictures (Plate 4.11., fig. 1, 2). It is worth of mentioning that the dissolution of the lamellar endexine is not uniform. In fig. 1 of Plate 4.10., the lamellar endexine is well shown. The disappearance of this layer is illustrated in Plate 4.11., fig. 2. At this experiment the corpus was investigated only by the TEM method.

INFRATECTUM

FOOT LAYER

ENDEXINE

FOOT LAYER

ENDEXINE

Plate 4.11.

Tectum (not illustrated)
On the surface, extremely damaged globular biopolymer units were observed. Diameter of these units is between 2—10 Å, mostly of 4—6 Å.

Infratectum, foot layer and endexine (Plate 4.11., fig. 3, 4)
The small stabilizing units are well shown. The highly organized globular structures however are not so characteristic. Their diameter is between 2—10 Å, mostly of 4—8 Å. These values are measured from the endexine. The difference in the electron density of the foot layer and the endexine is very characteristic.

Partial degradation with pyrrolidine

Picea glauca (Moench.) Voss.
Experiment No: 671
(Plate 4.12., figs. 1—4)

Corpus (Plate 4.12., fig. 1, 2)
In the low magnified picture, the strong electron density of the ectexine and the degradation of the endexine and the intine are well illustrated.

Tectum (not illustrated in high magnification)
On the surface, damaged globular units were observed, as the remnants of the protective molecular layer. The diameter of these globular elements is 2—10 Å, mostly 4—6 Å.

Infratectum (Plate 4.12., fig. 2)
The biopolymer structure of this layer is identical with that of the tectum.
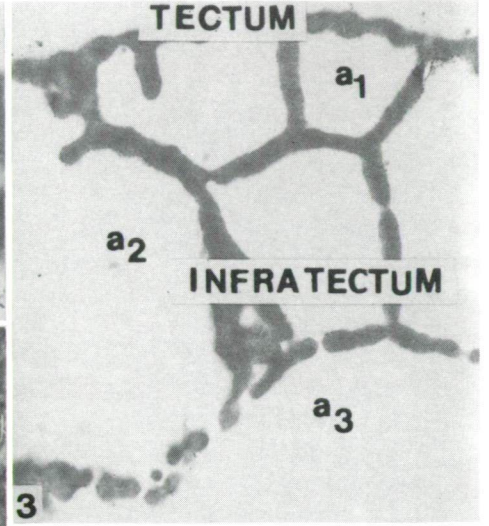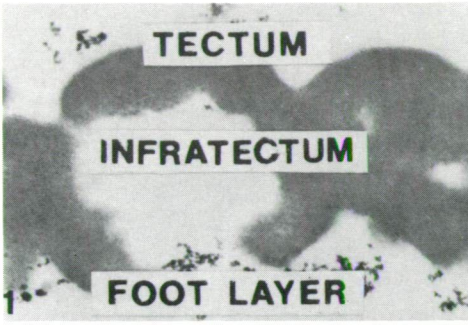
Foot layer (not illustrated)
In the foot layer, and probably in the endexine/or intine border larger globular elements were observed, they are not so well preserved. The electron density is very strong, the diameter is 8—20 Å, mostly 14—20 Å.

Saccus (Plate 4.12., fig. 3, 4)
Tectum (Plate 4.12., fig. 4)
The different kinds of alveoli are illustrated in fig. 3, Plate 4.12. The globular

63
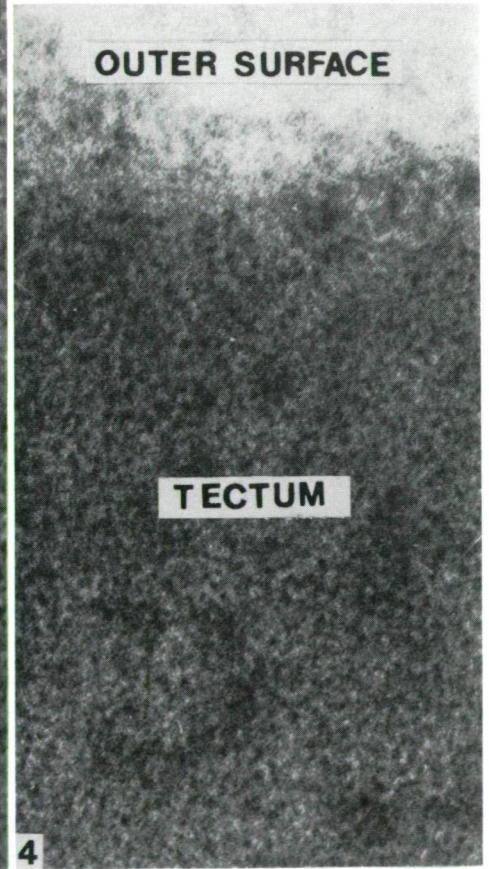
molecular remnants are of 2—8 Å in diameter, mostly of 3—6 Å. These structures are extremely damaged.

Infratectum (not illustrated)
The damaged biopolymer units are of 2—8 Å in diameter, mostly of 4—6 Å. The further inner layers were not investigated in this respect.

◀ Plate 4.12.

1—4. *Picea glauca* (Moench.) Voss.
   Partially degraded pollen grains with pyrrolidine. Experiment No: 671.
1. Detail of the exine stratification of the corpus after treatment. Negative no: 516, 25.000x.
2. Biopolymer organization of the partially degraded infratectum of the corpus. Negative no: 519, 500.000x.
3. Detail from the ultrastructure of the tectum after degradation. Negative no: 522, 10.000x.
4. Biopolymer structure of the tectum after pyrrolidine treatment. Negative no: 523, 500.000x.

Discussion and Conclusions

1. The effect of the diethylether on all of the saccate pollen grains investigated can be summarized as follows.
1.1. This solvent is particularly suitable for the investigation of the biopolymer and/or molecular structure of the inner wall layers of the saccate gymnosperm pollen grains. Foot layer, endexine, the different layers of the intine can be investigated. Its effect on the intine, as it is well illustrated in Plate 4.1., fig. 1. (*Pinus mugo* Turra) is similar to the effect on the pollen grains of *Corylus avellana* L. of the experiment C-2A (20 mg air dried pollen grains+2 ml *Helix* enzyme 2%,+1 ml merkapto-ethanol, temperature 30 °C, length of time 5ʰ, cf. Kedves, 1986).
1.2. The globular biopolymer units — probably the glucose chains on the surfaces are in general damaged, this is the first sign of the degradation of the tectum.
2. We have only one data about the effect of the tetrahydrofuran on the exine of the bisaccate gymnosperm pollen grains. This seems the best solvent to demonstrate the glycogene chains of the surface. This method with this solvent may be a basis for another research program of different taxa of sporomorphs and different kinds of plant cell walls.
3. The n-pentane dissolved the protective biopolymer system of the surfaces. The strong electron density of the ectexine layers indicates important changes inside the exine. The dissolution of the quasi-crystalloid skeleton may be presumed.
4. Pyrrolidine seems to be the best to dissolve the quasi-crystalloid biopolymer skeleton. On the basis of our up-to-date knowledge by the dissapearance of the surface protecting layer or in general the highly organized units we can presume that in the greatest part the stabilizing biopolymer system is present. High magnified negatives, as 250.000x and 400.000x seems to be suitable to give sufficient data for the investigation of the molecular organization of the stabilizing system of the quasi-crystalloid biopolymer skeleton. The detailed investigation and evaluation of

the pictures of high magnification (2.5 and 5 million) will be the subject of further investigations. We hope that on the basis of these data we will have the opportunity to start the combined modelling of the biopolymer structure of the sporoderm.

Acknowledgements

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