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DEGRADATION OF THE SPORODERM UNDER NATURAL AND IN VITRO CONDITIONS

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Abstract

This paper survey the most important fields of researches concerning the biopolymer organization of the sporoderm and the up-to-date results.

Key words: spore-pollen wall, biopolymer organization

Remark. — This contribution was presented at the XIV. International Botanical Congress (Berlin West, 1987).

The investigation of the very resistant material of the spore-pollen wall has been the subject of many publications.

The 1st problem: **Studies of the monomers of the sporopollenin.**

Among the comprehensive publications of the early concepts, the work of TOMSOVIC (1960) is worth of mentioning. In this paper on the chemistry, it was established, that sporopollenin is a high-polymerized terpene derivate, similar to the cutin. ROWLEY and PRIJANTO (1977) reviewed the early concepts: a highly cross-linked lipid (FREY-WISSLING, 1953), a high molecular weight of polysaccharide (TRAVERSE, 1968). Several methods were also described. The results of SHAW and YEADON (1968), BROOKS and SHAW (1968, 1971, 1978) and SHAW (1971) fundamentally changed the first concepts. They established, that the precursors of the sporopollenin are β carotene, and oxidizing esters of carotenoids. The monograph, edited by BROOKS et al. (1971) on the concepts of the sporopollenin is very important. Some selected points from this very important monograph: Following POTONIÉ and REHNELT (1971) in the course of coalification the aliphatic part of the sporopollenin becomes more and more aromatised. This compound was named as sporin. On the basis of the results on the exine of recent Epacridaceae, FORD (1971) established the following; p. 131: "The mature pollen wall consists of three chemically and structurally distinct layers; the outermost ectexine is composed of sporopollenin, the endexine has a high lignin content while the intine is cellulosic." The following points are also very important: ROWLEY and SOUTHWORTH (1967) established, that the sporopollenin accumulates on unit membrane dimensions lamellae, and a paracrystalline molecular system may be presumed to be similar to the unit membrane. ROWLEY (1973) wrote: the wall itself is a molecular sieve. In 1975, ROWLEY pointed out, that the sporopollenin cannot any longer be considered as the only major component of the exine, for example lipopolysaccharides are embedded within it. ROWLEY et al. (1980) described the helical substructures of the exine. ROWLEY et al. (1981); Nonsporopolleninous macromolecules embedded within the

sporopollenin matrix of exines as glycocalyx units. SOUTHWORTH (1985, 1986) established that the exine material consists of three different solubilities in 2-amino-ethanol. A pentagonal polygon system was described.

The 2nd problem: **Degradation of the sporoderm during the sedimentation.**

This problem was studied also by several autors. Among the most important publications I cite KIRCHHEIMER (1933, 1935), HAVINGA (1963, 1967, 1984), HEINEN (1960, 1963), ELSIK (1966, 1968, 1971). The fact, that the taphonomical processus may aid in the discovery of the higher organized biopolymer units of the sporopollenin was observed first by KEDVES et al. (1974). Globular biopolymer units were described from the partially degraded exine of *Restioniidites hungaricus* (KDS. 1965) ELSIK 1968, and *Thomsonipollis magnificus* (PF. et TH. 1953) W. KR. 1960 from the Eocene sediments of Mississippi, USA. The same biopolymer units were described from both exines of the tropical grass (*Restioniidites,* Plate I, fig. 1), and from the probably dicotyledonous early angiosperm; Paranormapolles; *Thomsonipollis* (Plate I, fig.2). Recently, KEDVES and WINTER (in print) restudied the first pictures, and the pentagonal polygon substructures are well shown on these pictures too. These substructures were not recognized, and interpreted at the first observations.

On the partially degraded wall of an algal cyst - *Pleurozonaria concinna* — from the carbonate manganese ore layers of Urkut, Hungary, two types of biopolymer units were described. Small, globular particles (Plate I, fig. 3), and helical structures (Plate I, fig. 4) from the organic remnants, which are outer from the algal cysts. These are probably from very degraded pollen grains. Taking into consideration, that the manganese ore layers of Urkut are rich in *Classopollis* pollen grains, the new results of ROWLEY and SRIVASTAVA (1986) on the higher organized exine components of this pollen grain are very important.

The 3rd problem: **Degradation with *Helix* enzyme method.**

This method was elaborated for the preparation of the protoplasts for microbial genetics experiments. A monograph concerning this problem was published by PEBERDY and FERENCZY (1985).

3.1. Among the recent taxa *Corylus avellana* L., and *Taxus baccata* L. was the subject of our first experiments. *Corylus avellana* L. (KEDVES, 1986b); 16 different procedures were used. The most important results are as follows; p. 59: "*Helix* enzyme with merkapto-ethanol is suitable for the destruction of the ectexine. In this

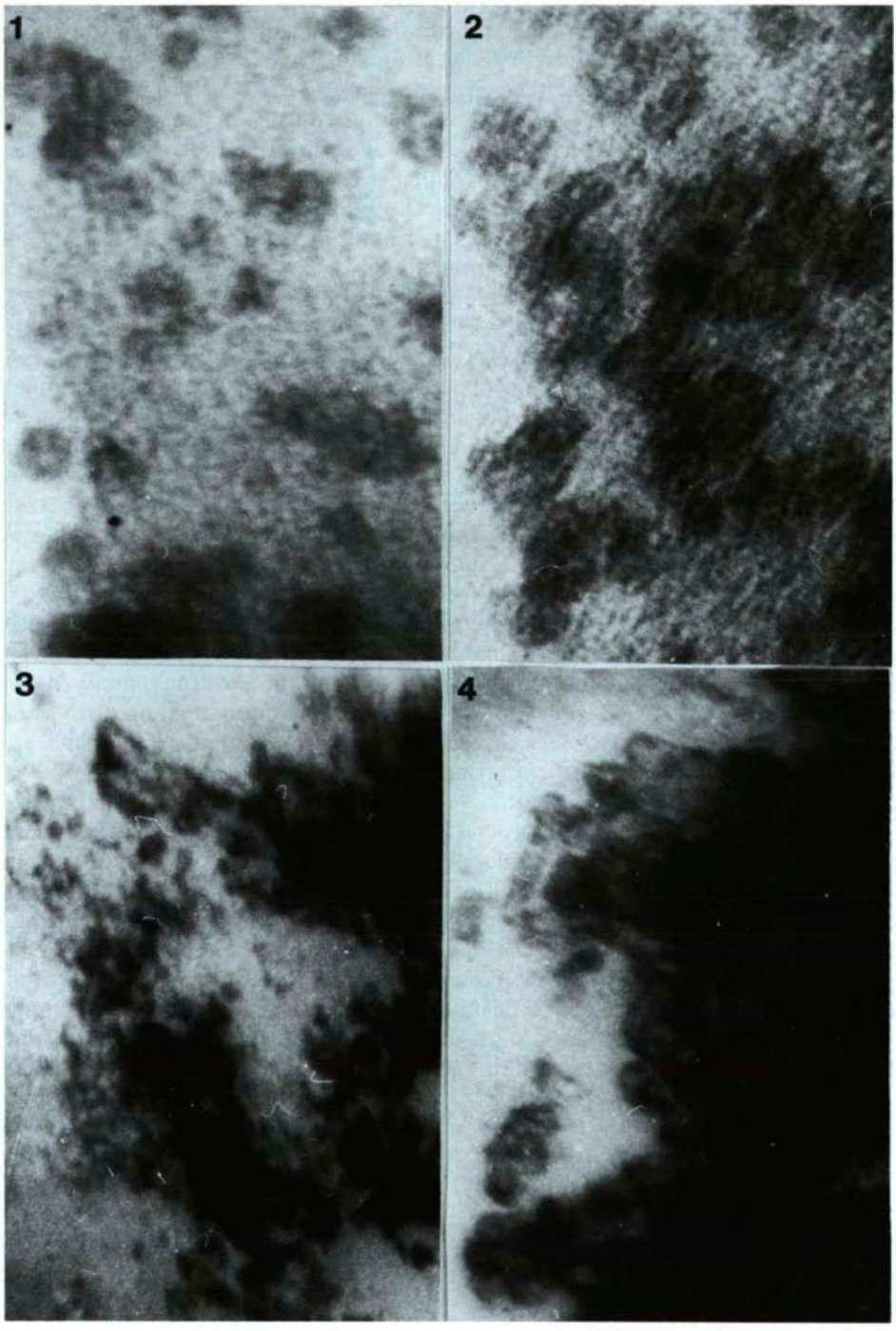
Plate I

1. *Restioniidites hungaricus* (KDS. 1965) ELSIK 1968, Mississippi, Eocene, 76/4, 68-618-17, x500000, following KEDVES et. al. 1974.

2. *Thomsonipollis magnificus* (PF. et TH. 1953) W. KR. 1960, Mississippi, Eocene, 74/3, 68-618-8, x500000, following KEDVES et al. 1974.

3. *Pleurozonaria concinna* (COOKSON and MANUM 1960) MÄDLER 1968, Urkut, Jurassic, 85/6, x250000, following KEDVES 1987c.

4. *Pleurozonaria concinna* (COOKSON and MANUM 1960) MÄDLER 1968, Urkut, Jurassic, 85/6, x250000, following KEDVES 1987c.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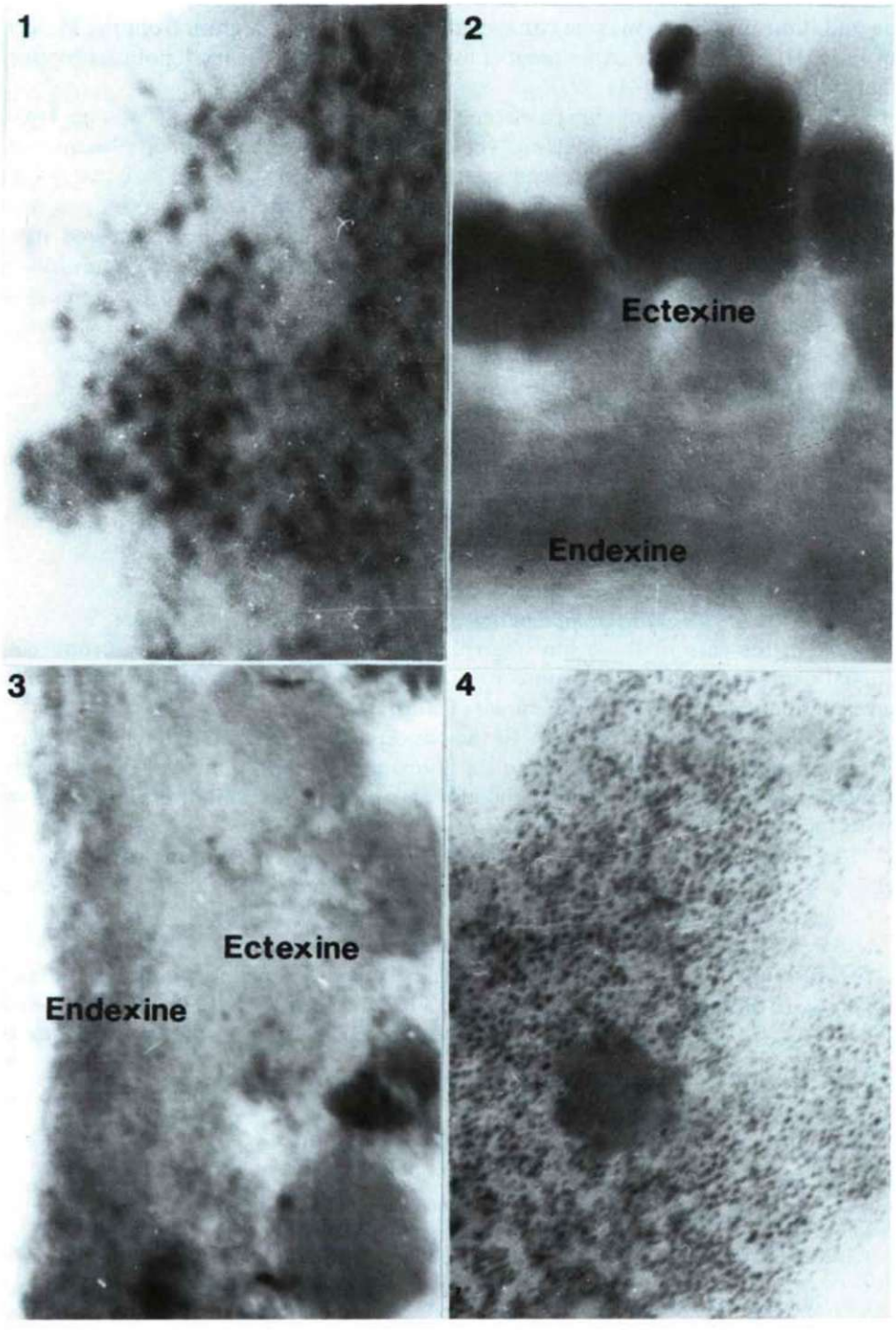
way, combined with the TEM method, the molecular structure of sporopollenin may be demonstrated". Globular units were found (Plate II, fig. 1). "Probably the basic elements are globular, and these elements may be arranged into units of higher order; filaments, helicoide structures, etc." "Because during all experiments there is the risk that the observed structures have been altered during the experiment or the preparation for the TEM investigations. Further experiments of different kinds are necessary on both recent and fossil biological objects before we can understand the details of the molecular structure of the sporopollenin. *Taxus baccata* L. (KEDVES 1987); The experimental degradations methods, which resulted at the exine of *Corylus avellana* L. in well defined globular biopolymer units, in the case of the *Taxus baccata* L. showed a different results. This means, that the chemical composition, and in consequence of this in the molecular structure of the exine of *Corylus* and *Taxus* there are essentially differences. In connection with this it is interesting to cite newly from the paper of UENO (1960) the following; p. 126/127: "The pigments were studied by SUITA (1948), KARRER and LEUMANN (1952) etc., and LUBLINER-MIANOWSKA (1955) investigated pigments in pollen grains of 67 species. According to him the pigments in pollen grains of conifers is not carotenoid, while that of entomophilous pollen of angiosperms is carotenoid." But BROOKS (1971) established the following; p. 351: "The chemical study of various modern and fossil spore walls of gymnosperms, angiosperms, pteridosperms fungi and algae show a majority to be composed of sporopollenin." It is interesting, that the merkapto-ethanol changed the electron affinity of the two principal layers of the exine of *Taxus baccata* L. The exclusive use of the merkapto-ethanol resulted that the electron affinity of the ectexine alternate stronger (Plate II, fig. 2). The merkapto-ethanol, with *Helix* enzyme caused an opposing effect, so that the electron affinity of the endexine became stronger than that of the ectexine (Plate II, fig.3).

3.2 Microfossils.

Botryococcus braunii KÜTZ (KEDVES 1986a) from the oil shale of the Pliocene layers of Pula, Hungary. Globular biopolymer units were described, but on our pictures published earlier on the plate III, the higher organized pentagonal polygon biopolymer structures are well shown (Plate II, fig. 4), similarly to the previously mentioned fossil taxa and of *Corylus avellana* L. It is important to emphasize, that the merkapto-ethanol only reveals the previously mentioned higher organized biopolymer system. In connection of this paper, it was pointed out, that the degradation is complex. The first steps starts during the sedimentation - taphonomical processus — and continued later during the experiments. Naturally, the two kind of

Plate II

1. *Corylus avellana* L. recent, experiment: C-2A, following KEDVES 1986b, x500000.
2. *Taxus baccata* L. recent, experiment: T-4b2, following KEDVES 1986d, x100000.
3. *Taxus baccata* L. recent, experiment: T-11A, following KEDVES 1986d, x100000.
4. *Botryococcus braunii* KÜTZ., Pula, Pliocene, experiment: B.4a.2, following KEDVES 1986a, x100000.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Ectexine
Endexine
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Ectexine
Endexine
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degradations processus may be connected. *Picea* type pollen grain from the Pliocene of Pula, Hungary. These experiments have not shown well defined globular biopolymer units (Plate III, fig. 1).

Palynomorphs from the Paleocene sediments of Menat, France. The Upper Thanetian sediments of Menat are very useful for these experiments because the richness of the very well preserved sporomorphs. In this case the only merkapto-ethanol was used, without *Helix* enzyme. Several types of sporomorphs were studied, but untill now the results have not been elaborated or discussed in all details. The preliminary results as it was presented at the APLE Symposium at Salamanca in the last year (1986c) are as follows: The most important groups of pollen grains, which were the subject of investigations:

1. Saccate gymnosperm pollen grains, *Pinus* type
2. Angiosperms, Longaxones
   Monocolpates
   Tricolpates
   Tricolporates

Among the Brevaxones, the following genera:

Plicapollis
Stephanoporopollenites
Platycaryapollenites
Triporopollenites

a) In the case of these sporomorphs, the merkapto-ethanol only produced a partial degradation of the sporomorphs. In several exines the globular higher organized biopolymer units are well shown (Plate III, fig. 2).

b) These results add support to the concept that there are differences between the gymnosperm and angiosperm pollen grains in the point of view of the molecular structure of the exines. In general the globular biopolymer units were not observed at the saccate gymnosperm pollen grains (Plate III, fig. 3).

c) The measure of the degradation of the different types of angiosperm pollen grains is uneven, but at this moment we have not enough data for general conclusions.

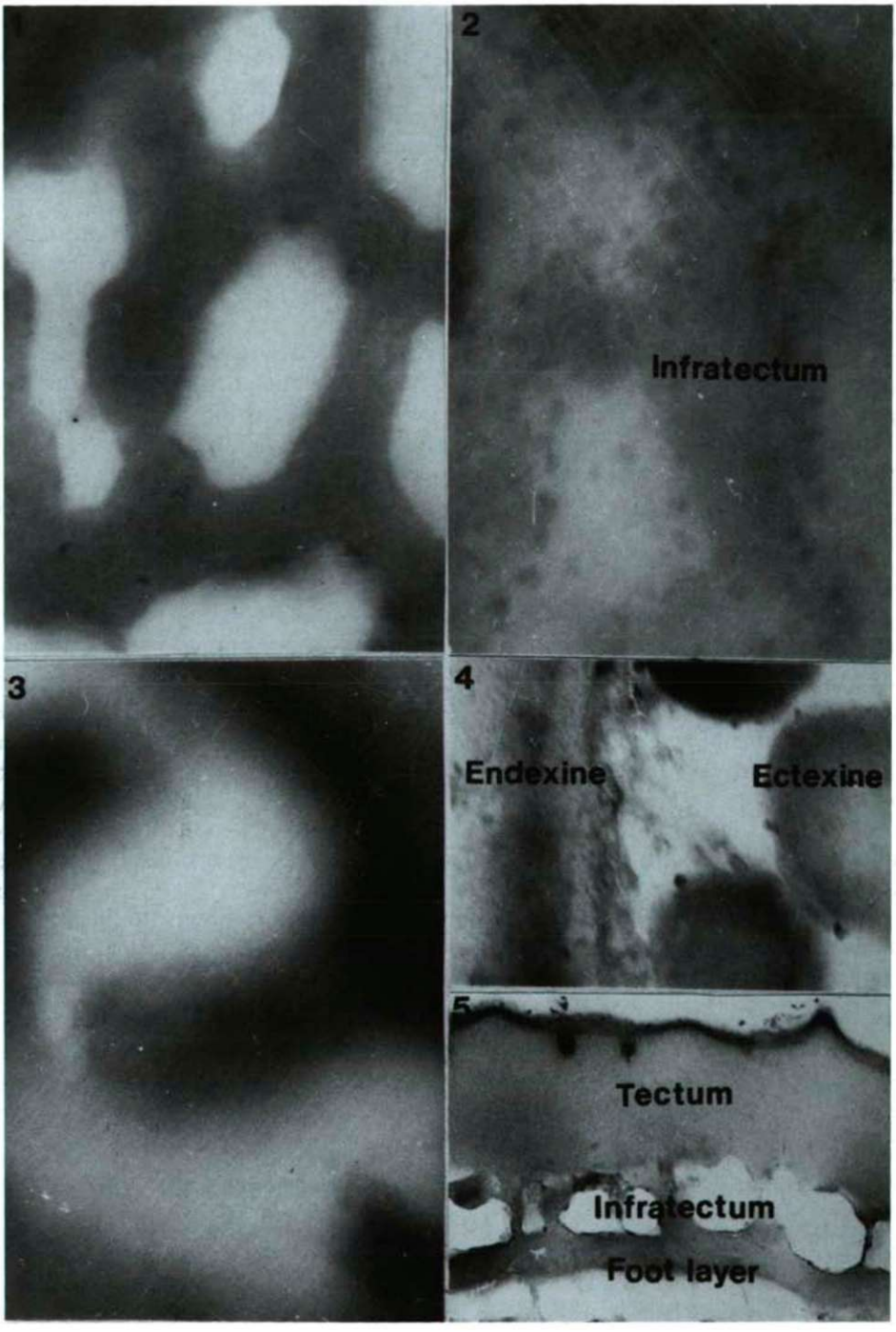
The 4th problem: **Degradation with solvent methods.**

More than 120 experiment was carried out. As starting point the classical solvents (BAILEY, 1960, SOUTHWORTH, 1985, DENIZOT, 1978, ROWLEY and PRIJANTO, 1977, ROWLEY, et al., 1981) were used. Our up-to-date results may be summarize as follows:

1. It is interesting, that the benzine, methanol, and ethanol degraded the

Plate III

1. *Picea* type, Pula, Pliocene, experiment: B.1.2., x100000.
2. *Cupuliferoipollenites pusillus* (R. POT. 1934) R. POT. 1960, Menat, Paleocene, 85/58, x250000.
3. *Pityosporites,* type *haploxylon,* Menat, Paleocene, 85/45, x250000.
4. *Taxus baccata* L. recent, x100000.
5. *Corylus avellana* L. recent, x50000.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Ectexine
5
Tectum
Infratectum
Foot layer

lamellar ultrastructure of the endexine of the pollen grains of *Taxus baccata* L. (Plate III, fig. 4). These solvents produced a narrow layer with very strong electron affinity in the tectum of the pollen grains of *Corylus avellana* L. (Plate III, fig. 5).

2. Potassium permanganate aq. dil. degraded the wall of *Botryococcus braunii* KÜTZ. Globular biopolymer units, and pentagonal polygon substructures were observed (Plate IV, fig. 1).

3.1. 2-aminoethanol combined with oxydation of potassium permanganate resulted to pentagonal polygon subunits in the fossil algue *Botryococcus* from the oil shale of Pula Hungary (Plate IV, fig. 3) this is similar to the previous experiments. Rarely lamellar biopolymer organization was observed (Plate IV, fig. 2).

3.2. Recent species

Equisetum arvense L., with J. WINTER. — Globular subunits were observed, arranged in pentagonal polygons in the elateres, the globular forms of the surface, perispore and exospore. The perispore (Plate IV, fig. 4) and the globular forms on the perispore are more resistant than the exospore (Plate IV, fig. 5).

3.3 These experiments strongly degraded the ectexine, in this case the endexine was also more resistant (Plate IV, fig. 6—8).

Conclusions

1. By the different methods of degradation the results and conclusions may be the same — *Botryococcus* from the oil shale, or may be different, for example the *Corylus* and *Taxus* — experiments with *Helix* enzyme method and with degradation of 2-aminoethanol and potassium permanganate.

2. It seems, that the results of all experiments must be taken seriously and are useful.

3. According to the previous results, e.g.: SOUTHWORTH (1985, 1986) by the solubility of the exine by 2-aminoethanol the degrees of organization of the sporoderm may be reconstructed.

4. The pentagonal polygon biopolymer organization seems to be in this moment a general structure. See *Botryococcus, Equisetum, Taxus, Abies, Corylus,* among the fossil angiosperms *Restioniidites, Thomsonipollis.*

Plate IV

1. *Botryococcus braunii* KÜTZ., Pula, Pliocene, 20 mg. air dried material + $KMnO_4$ aq. dil. 4%, length of time: $2^h30'$, x500000.

2,3. *Botryococcus braunii* KÜTZ., Pula, Pliocene, experiment: 56, x500000.

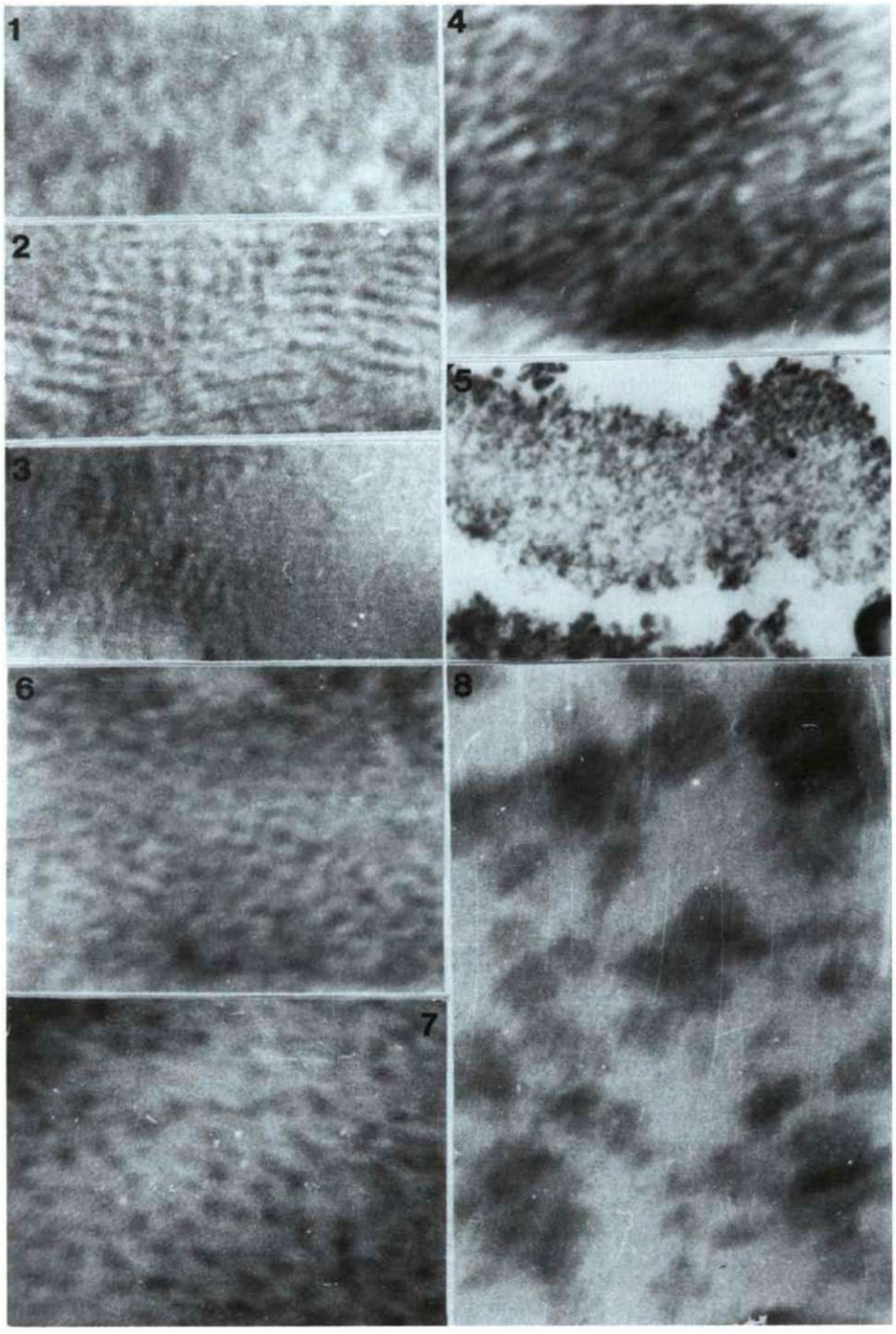
4. *Equisetum arvense* L. perispore, experiment: 73, x500000, following KEDVES and WINTER, in print.

5 *Equisetum arvense* L. exospore, experiment: 73, x50000, following KEDVES and WINTER, in print.

6. *Taxus baccata* L., recent, endexine, experiment. 54, x500000, following KEDVES 1987b.

7. *Abies concolor* HOOPES, recent, tectum, experiment: 81, x500000.

8. *Corylus avellana* L. recent, tectum experiment: 52, x500000.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5. The higher organizations of the sporopollenin may be important with regard to an evolutionary point of view. These structures may be:

lamellar
granular
globular
helical, etc.

6. Further methodical studies are also necessaries not only on the spore and pollen wall, but on the other kind of cell walls.

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