6. BUCKMINSTERFULLERENE-LIKE BIOPOLYMER UNITS FROM THE EXINE OF THALICTRUM FLAVUM L.

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Abstract

On the TEM micrographs of the partially degraded and fragmented exines of *Thalictrum flavum* L., quasi-equivalent biopolymer structures were observed for the fisrst time from *angiosperm* exines. The diameter of the large globular units is 15 – 35 – 85 Å, and the diameter of the superficial electron dense particles is 5 – 10 – 15 Å. The arrangement of these large globular units may be linear, irregular or they may form network systems of different kinds of polygons. Further peculiarities of the pollen grains of the wind-pollinated *Thalictrum* genus were established experimentally. The quasi-equivalent biopolymer structures were discovered in the partially degraded wall of *Botryococcus braunii* KÜTZ. isolated from oil shale. These structures can be modelled with fullerenes.

Key words: Palynology, recent, *Angiospermae,* quasi-equivalent biopolymer structure.

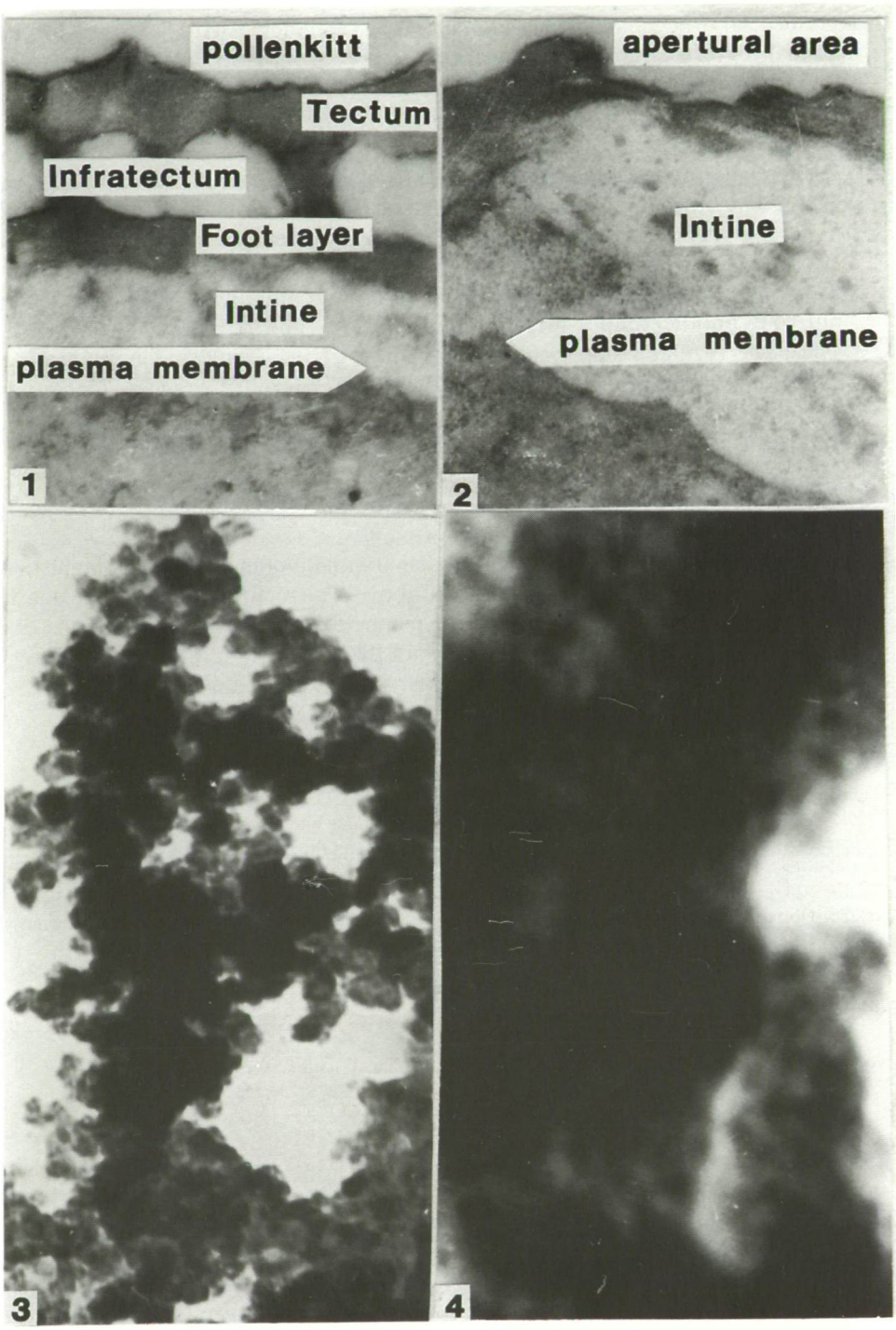
Introduction

During our investigations on the biopolymer structure and organization levels of the plant cell wall, surprising and unusual biopolymer units were observed on the partially degraded and fragmented wall of *Botryococcus braunii* KÜTZ. extracted from the oil shales of Hungary (KEDVES, ROJIK and VÉR, 1991). The peculiarities of the biopolymer organization of the fossil *Botryococcus braunii* wall were the subjects, among others, of further investigations (KEDVES, ROJIK and VÉR, 1992). The previous-

Plate 6.1. ▸

1–4. *Thalictrum flavum* L. Recent.

1. Ultrastructure of the interapertural area of the fresh (non-experimental) pollen grain. Negative no: 8550, 50.000x.
2. Exine ultrastructure of the apertural area of the fresh (non-experimental) pollen grain. Negative no: 8552, 50.000x.
3. Experiment No: 281, globular highly organized biopolymer structures from partially degraded and fragmented exine. Negative no: 9816, 100.000x.
4. Experiment No: 266, detail from the highly organized buckyball-like biopolymer structure. The superficial electron dense molecular systems are well shown. Negative no: 9753, 500.000x.


pollenkitt
Tectum
Infratectum
Foot layer
Intine
plasma membrane
1
apertural area
Intine
plasma membrane
2
3
4

ly described, large peculiar, and globular units can be modelled with fullerenes having a buckyball-like structure; review of the fullerenes from BECK and BRAUN (1992). Taking into consideration our up-to-date knowledge about chemistry and biopolymer structures, the following scheme can be summarized:

1. Chemical compounds of the plant cell wall.

2.1. Basic regular pentagonal biopolymer units of angstroem dimension (8 – 22 Å). These units from the quasi-crystalloid metastable skeleton of the plant cell wall in pentagon dodecahedrane systems. Such biopolymer systems can be modelled after the theory from PENROSE's tiling patterns (PENROSE, 1979).

2.2. In living systems, the metastable quasi-crystalloid biopolymer skeleton is stabilized by another biopolymer system (Cf. KEDVES and TÓTH, 1992, 1994).

2.3. The basic regular pentagonal dodecahedron of the PENROSE–I units are the elementary components of the units of a further level of organization in nanometer dimension filaments, lamellae, helical, tubular (ROWLEY et al., 1981), globular (HESSE, 1985), irregular polygons (SOUTHWORTH, 1986), etc.

2.4. The colloidal-crystal-like organization (HEMSLEY, COLLINSON and BRAIN, 1992) can be joint to the previous organization level. But this time its lower or elementary biopolymer units are not well known.

3. The biopolymer units, which can be modelled with fullerenes can be distinguished from the biopolymer systems mentioned above. The relations between the quasi-crystalloid and quasi-equivalent biopolymer systems are under elaboration.

Previously it was believed that the buckyball-like biopolymer units are characteristic for the wall of "peculiar living organisms" such as the genus *Botryococcus.*

Materials and Methods

The pollen grains were collected by Dr. L. TÉCSI from the Botanical Garden of the J. A. University, Szeged, on 20th June 1988. Fresh, non-experimental pollen grains were also used in TEM investigations for comparisons. The experiments started on 20th June 1988 and the different kinds of experiments are the following.

No 265: 20 mg air dried pollen grains + 1 ml 2-aminoethanol, temperature 30 °C, length of time 24^h.

No 280: the pollen grains were heated onto 100 °C for 1 hour, after being partially degraded as previously (265).

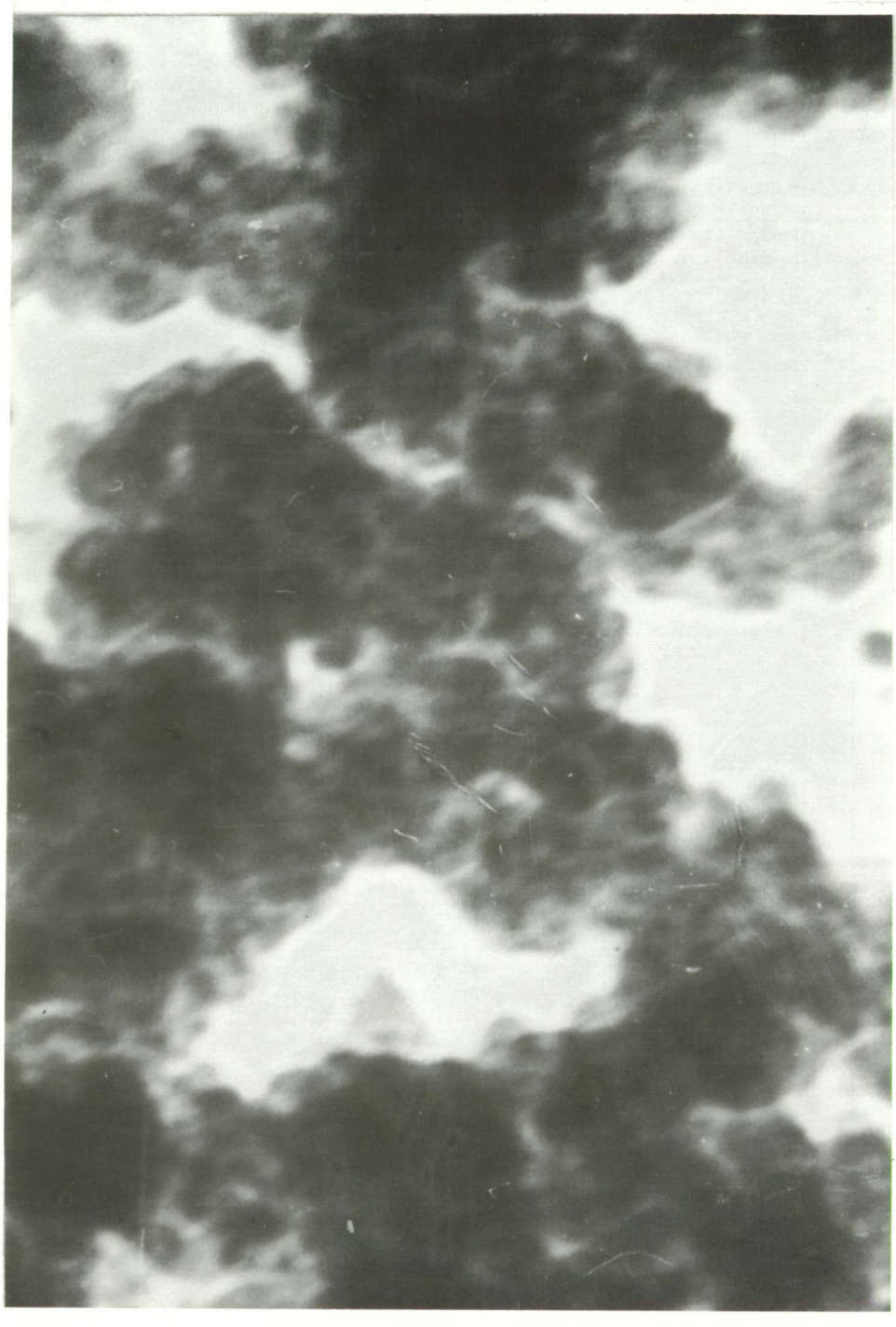
No 266: 20 mg air dried pollen grains + 1 ml 2-aminoethanol, temperature 30 °C, length of time 24^h, washing (H_2O) + 10 ml $KMnO_4$ aq. dil. 1%, temperature 30 °C, length of time 24^h.

No 281: the pollen grains were heated onto 100 °C for 1 hour, after being partially degraded as previously (266).

No 267: 20 mg air dried pollen grains + 1 ml 2-aminoethanol, temperature 30 °C, lenght of time 24^h, washing (H_2O) + 10 ml $KMnO_4$ aq. dil. 1%, temperature 30 °C, lenght of time 48^h.

Plate 6.2. ▸

Thalictrum flavum L. Recent.
Experiment No: 282, buckyball-like highly organized biopolymer structures from partially degraded and fragmented exine. The superficial electron dense molecular units and the partial degradation process are well illustrated.



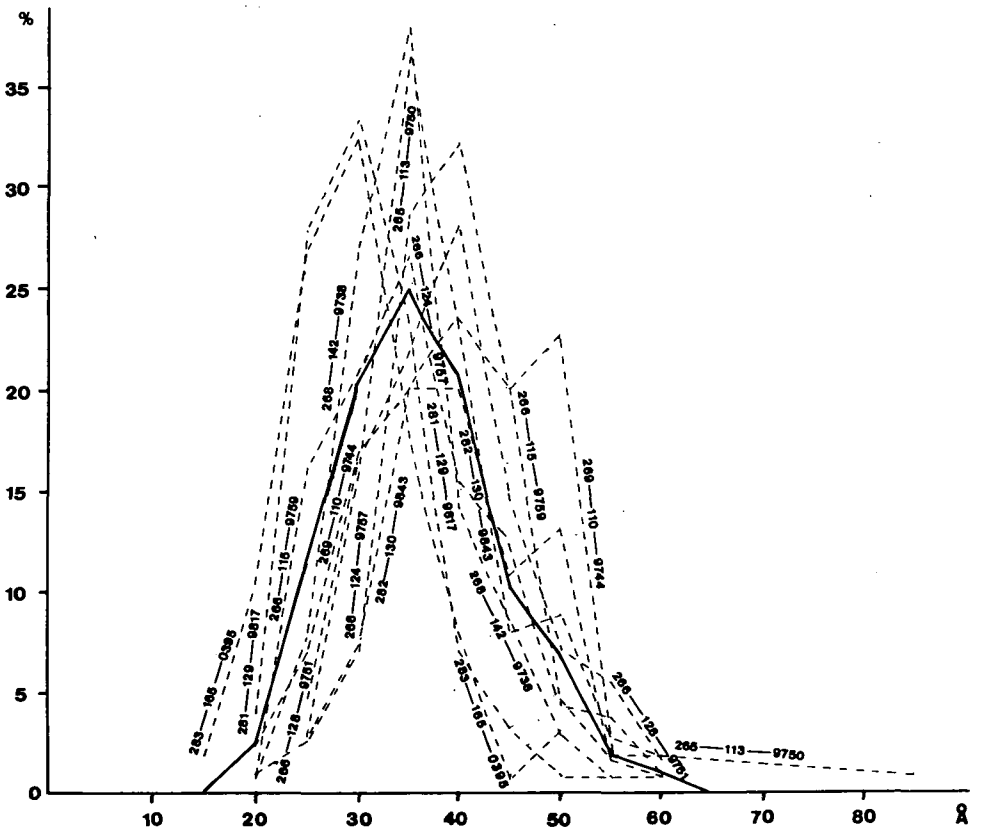
No 282: the pollen grains were heated onto 100 °C for 1 hour, after being partiallly degraded as previously (267).

No 268: 20 mg air dried pollen grains + 1 ml 2-aminoethanol, temperature 30 °C, lenght of time 24^h, washing (H_2O) + 10 ml $KMnO_4$ aq. dil. 1%, temperature 30 °C, lenght of time 24^h, washing (H_2O) + 2 ml acetic acid anhydride, temperature 30 °C, lenght of time 24^h.

No 283: the pollen grains were heated onto 100 °C for 1 hour, after being partially degraded as previously (268).

No 269: 20 mg air dried pollen grains + 1 ml 2-aminoethanol, temperature 30 °C, lenght of time 24^h, washing (H_2O) + 10 ml $KMnO_4$ aq. dil. 1%, temperature 30 °C, lenght of time 24^h, washing (H_2O) + 5 ml methanol, temperature 30 °C, lenght of time 24^h.

No 284: the pollen grains were heated onto 100 °C for 1 hour, after being partially degraded as previously (289).



Text-fig. 6.1.

Variation statistical graphs of the diameter of the highly organized buckyball-like biopolymer structures. The thick line represents the general average of all measurements, the broken lines represent that of the results by experiments or sometimes by negatives. The numbers of the graphs represent the following: number of experiment, number of the measured biopolymer units, number of the negative.

Ultrathin sections were made on a Porter Blum ultramicrotome with glass knives (post-fixation with OsO_4 aq. dil. embedding in Araldite Durcupan, Fluka). The fragmentation was made with a magnetic stirrer in watered medium, during 30 minutes. The fragmented exines were dropped on a grid covered with collodium pellicle and then they were dried. The electron microscopical investigations were made on a Tesla BS-500 transmission electron microscope, resolution 6 Å.

Results

LM morphology: "16μ. – Grains cribellate with about 8 pores. Exine with reticulate texture." (ERDTMAN, 1954, p. 120); "6–12 pantoporate" (CLARKE, PUNT and HOEN, 1991, p. 146).

TEM structure of the fresh pollen grains.

Interapertural exine (Plate 6.1., fig. 1). – Tectate, tectum perforated with channels, and ornamented with coni of different size and shape. The surface of the tectum is covered with pollenkitt (cf. HESSE, 1978, p. 20 .."auf dem Tectum liegen geringfügige Mengen teiweise granulären Pollenkitts...") Infratectal layer columellar. The foot layer is a bit thinner than the tectum. Beneath the foot layer, the interbedded zone was observed (cf. FREAN, 1973, KEDVES and ANTUNOVICS, 1979). The intine is thick, the plasma membrane is thin.

Apertural exine (Plate 6.1., fig. 2). – The ectexine is extremely reduced, and pro parte fragmented (cf. ROLAND, 1966). The intine in this area is thicker than extragerminally; oncus-like.

TRANSMISSION ELECTRON MICROSCOPY OF THE PARTIALLY DEGRADED AND FRAGMENTED EXINES

Experiment No: 265 (Text-fig. 6.1.)

Globular biopolymer units were observed in several kinds of arrangements, linear, network-like, irregular polygons. Diameter: 25 – 35 – 85 Å. The typical buckyball-like biopolymer characteristic features were not observed.

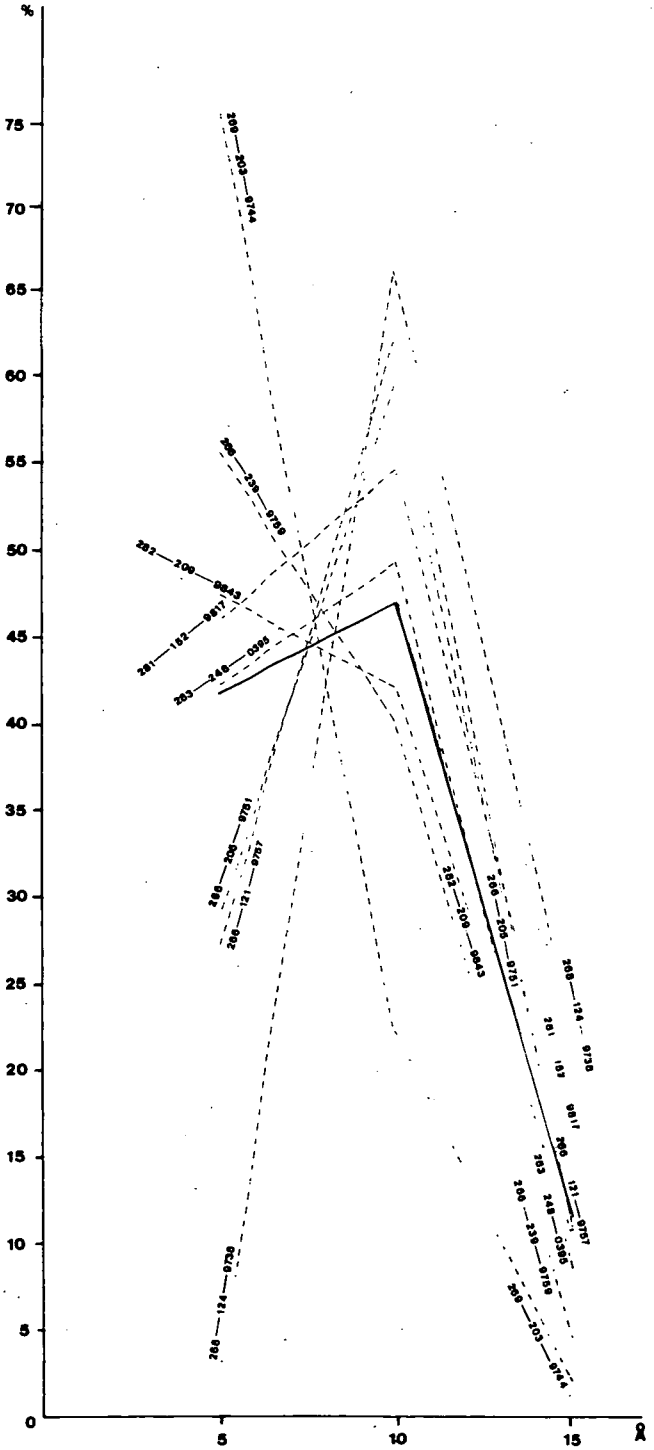
Experiment No: 280

Our TEM data are not sufficient in this respect, this may be the consequence of methodical problems.

Experiment No: 266 (Plate 6.1., fig. 4, text-fig. 6.1., 6.2.)

Globular units of several kinds of arrangements were observed. In highly magnified pictures, not so characteristic buckyball type biopolymer units were observed, large globular units with smaller electron dense superficial molecular systems. In this experiment, the measurements were made on three negatives, the data are not the same;

Negative number	Diameter of the globular units in Å	Diameter of the superficial molecular systems in Å
9751	25 – 40 – 60	5 – 10 – 15
9757	20 – 35 – 60	5 – 10 – 15
9759	20 – 45 – 60	5 – 10 – 15


%
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
5
10
15
A

Experiment No: 281 (Plate 6.1., fig. 3, text-fig. 6.1., 6.2.)
This experiment resulted essentiallly the previously discussed one; diameter of the globular biopolymer units: 20 – 30 – 60 Å, diameter of the superficial electron dense molecular systems: 5 – 10 – 15 Å.
Experiment No: 267
The qualitative results are similar to those of the experiment No 266, but the biopolymer units were not suitable for quantitative measurements.
Experiment No: 282 (Plate 6.2., text-fig. 6.1., 6.2.)
The buckyball-like biopolymer structures are well shown in our pictures but in several particles of the fragmented exine advanced degradation process was observed. Diameter of the large globuar units: 20 – 35, 40 – 60 Å, diameter of the superficial electron dense molecular systems: 5 – 10 – 15 Å.
Experiment No: 268 (Text-fig. 6.1., 6.2.)
Similar to the previously mentioned ones. Diameter of the large globular units: 20 – 35 – 60 Å, diameter of the superficial electron dense molecular systems: 5 – 10 – 15 Å.
Experiment No: 283 (Text-fig. 6.1., 6.2.)
The globular biopolymer units are relatively smaller than in the previous experiments; 15 – 30 – 50 Å, the size of the superficial electron dense molecular systems is constant; 5 – 10 – 15 Å.
Experiment No: 269 (Text-fig. 6.1., 6.2.)
Diameter of the large globular biopolymer units: 25 – 40 – 60 Å. Diameter of the superficial electron dense molecular systems: 5 – 10 – 15 Å.
Experiment No: 284
Advanced disintegration of the biopolymer structure was observed. The quasi-equivalent structures were not in a measurable preservation.

Discussion and Conclusions

1. The buckyball-like biopolymer structure was first observed on *angiosperm* exines. In this way this may occur in other taxa too.
2. The new results underlined newly that the molecular system of the plant cell wall is extremely complicated. Several kinds of structures can be established.
3. The sporopollenin-type plant cell wall is resistant, but as we have pointed it out several times previously this extremely complex molecular system is a dinamically and perpetually altering structure.
4. The chemical compound of the plant cell wall, and its highly organized structures on several organization levels are not unique, either, and it seems that after last time's obtained results, several details need more and more investigations.

◂ Text-fig. 6.2.

Variation statistical graphs of the diameter of the superficial electron dense molecular systems. Thick line represents the general average of all measurements, the broken lines represent of the results by experiment or sometimes by negatives. Numbers of the graphs represent the following: number of experiment, number of the measured electron dense superficial molecular systems, number of the negative.

Acknowledgements

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