6. LM, SEM AND TEM INVESTIGATIONS ON PARTIALLY DEGRADED BOTRYOCOCCUS BRAUNII KÜTZ. COLONIES FROM HUNGARIAN UPPER TERTIARY OIL SHALE II.

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Abstract

The new experiments are the methodological continuation of the previously published ones.The length of time of the degradation with 2-aminoethanol was 4, 5 and 6 days, but the oxidation with $KMnO_4$ and the dissolution with merkaptoethanol remained of 24 hours. In this way the most moderate degradation in this new series was stronger than the strongest degradation of the previous series of experiment. The new LM, TEM and SEM data indicated also this strong degradation. The SEM results of the degradation with 2-aminoethanol and the oxydation with $KMnO_4$ resulted in superficial globular biopolymer units. The diameter of these units is 40-400 Å, much larger than that of discovered by the previous experiments. The TEM method indicated the degradation of the quasi-periodic and quasi-equivalent biopolymer structures. These new results confirmed, that for the biopolymer symmetry operations the experiments No. AKP-99-4,5,7,8 published in the previous publication are the most suitable.

Key words: Alginite, partial degradation, LM, SEM, TEM.

Introduction

In our previous work (KEDVES et al., 2000) an attempt was made to survey the most important papers concerning the structure, chemistry, and EM results of *Botryococcus braunii* KÜTZ. colonies. Particular attention was made to the Hungarian Upper Pannonian oil shale. The partially degraded colonies by different kinds of methods were investigated with the LM, SEM and TEM methods. To continue this series of experiments stronger partial degradations were carried out.

The aim of this paper is to establish more suitable experiments for the symmetry operations of the different biopolymer and /or molecular structures. Taking into consideration the pecularities in the biopolymer symmetry of the *Botryococcus braunii*, cf. e.g.: KEDVES, ROJIK and VÉR, (1991), KEDVES, TRIPATHI, VÉR, PÁRDUTZ and ROJIK, (1998), there are a lot of problem to solve.

Materials and Methods

The temperature for each experiment was 30 °C.
AKP-99-10. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 96 h.
AKP-99-11. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 120 h.
AKP-99-12. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 144 h.
AKP-99-13. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 96 h, after washing + 10 ml $KMnO_4$ 1%, length of time 24 h.
AKP-99-14. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 120 h, after washing + 10 ml $KMnO_4$ 1%, length of time 24 h.
AKP-99-15. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 144 h, after washing + 10 ml $KMnO_4$ 1%, length of time 24 h.
AKP-99-16. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 96 h, after washing + 1 ml merkaptoethanol, length of time 24 h.
AKP-99-17. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 120 h, after washing + 1 ml merkaptoethanol, length of time 24 h.
AKP-99-18. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 144 h, after washing + 1 ml merkaptoethanol, length of time 24 h.

After partial degradation the used LM, SEM and TEM methods are completely identical with the previous ones (KEDVES et al., 2000, p. 67).

Results

1. LM results

Experiments AKP-99-10-12 (Plate 6.1., figs. 1-3)

The partial dissolution with 2-aminoethanol during 4-5-6 days resulted characteristic alterations. Moderate degradation of the wall, and dark amorphous content, probably kerogen appeared in the cups (Plate 6.1., figs. 1-3).

Experiments AKP-99-13-15 (Plate 6.1., figs. 4-6)

After partial degradation and oxidation with $KMnO_4$ the inner part of the colonies turned into very dark. The outest part of the cups of the colonies remained lighter. At this part of the colonies remarkable disintegration was also observed.

Experiments AKP-99-16-18 (Plate 6.1., figs. 16-18)

The effect of the merkaptoethanol after 2-aminoethanol was also perceptible at the first series of experiments (p. 71, in KEDVES et al., 2000). This degradation process is more expressed after the longer treatement with 2-aminoethanol.

2. EM results

Experiment: AKP-99-10

SEM pictures (Plate 6.2., figs. 1,2) illustrate superficial degradation. Debris and small globular biopolymer units on the surface were observed only occasionally. Autospores are well illustrated in the low magnified picture (Plate 6.2., fig. 1). The preservation of the autospores is better than the cups of the colonies. The TEM pictures (Plate 6.4., figs. 1,2) illustrate the degradation of the cups, but there are electron dense granular units within the wall. The ultrastructure of the inner content of the cups is well shown, there are tiny dark globular or linear units in the more or less homogeneous substance.

Experiment: AKP-99-11

The low magnified SEM picture illustrates well the differences in the preservation and in this way the differences in the measure of the degradation of the different colonies (Plate 6.2., fig. 3). In the highly magnified picture (Plate 6.2., fig. 4) the different kinds of debris are shown on the surface. There are globular units within these cellular debris. It is probable that the outest lamella of the cup was destroyed, and the remnants of this layer are perceptible on the surface of an inner lamella. The degradation of the wall is well shown in the TEM pictures (Plate 6.4., figs. 3,4), too. There are globular electron dense particles in the more or less homogeneous wall.

Experiment: AKP-99-12

The SEM results are essentially identical with the previous experiment (Plate 6.2., figs. 5,6). A very well preserved diatom remnant of Pennatae type was observed in this sample (Plate 6.3.). Dr. M. HAJÓS was asked for the nearer determination for this algue, she accepted our request, but she is very ill so we publish this remnant without nearer determination. The TEM investigations (Plate 6.4., 5-7) in contrast to the previous experiment resulted characteristic lamellar structures in the wall of the cups. The lamellae are bordered by electron dense inner surfaces. Sometimes the inner lamella is separated from the others (Plate 6.4., figs. 5,6). There are organic remnants in the cups (Plate 6.4., fig. 5).

Experiment: AKP-99-13

The SEM pictures (Plate 6.5., figs. 1,2) illustrate well the remarkable degradation of the wall. The superficial globular units are well shown in the highly magnified picture (Plate 6.4., fig. 2). The distribution of the diameters of the globular units are as follows:

40	80	120	160	200	240	280	320	360	400	Å
22.7	34.0	26.2	10.1	3.9	1.6	0.8	0.5	0.1	0.1	%

Plate 6.1.

1-9. *Botryococcus braunii* KÜTZ. LM pictures. 1. - Experiment number: AKP-99-10, 2. - Experiment number: AKP-99-11, 3. -AKP-99-12, 4.-AKP-99-13, 5.-AKP-99-14, 6.-AKP-99-15, 7.-AKP-99-16, 8.-AKP-99-17, 9.-AKP-99-18. Magnification: 670x.

Plate 6.2.

1-7. *Botryococcus braunii* KÜTZ. SEM pictures. 1,2. - Experiment number: AKP-99-10, 3,4.-AKP-99-11, 5,6,7.-AKP-99-12.

Plate 6.3.

SEM picture of a diatom of Pennatae type.

Plate 6.4.

1-7. Ultrastructure of the partially degraded colonies of *Botryococcus braunii* KÜTZ. 1,2. - Experiment number: AKP-99-10, 1. Negative number: 7878, 5.000x., 2. Negative number: 7879, 15.000x., 3,4. - Experiment number: AKP-99-11, 3. Negative number: 7882, 15.000x., 4. Negative number: 7883, 50.000x., 5,6,7. - Experiment number: AKP-99-12, 5. Negative number: 7889, 5.000x., 6. Negative number: 7886, 15.000x., 7. Negative number: 7887, 50.000x.

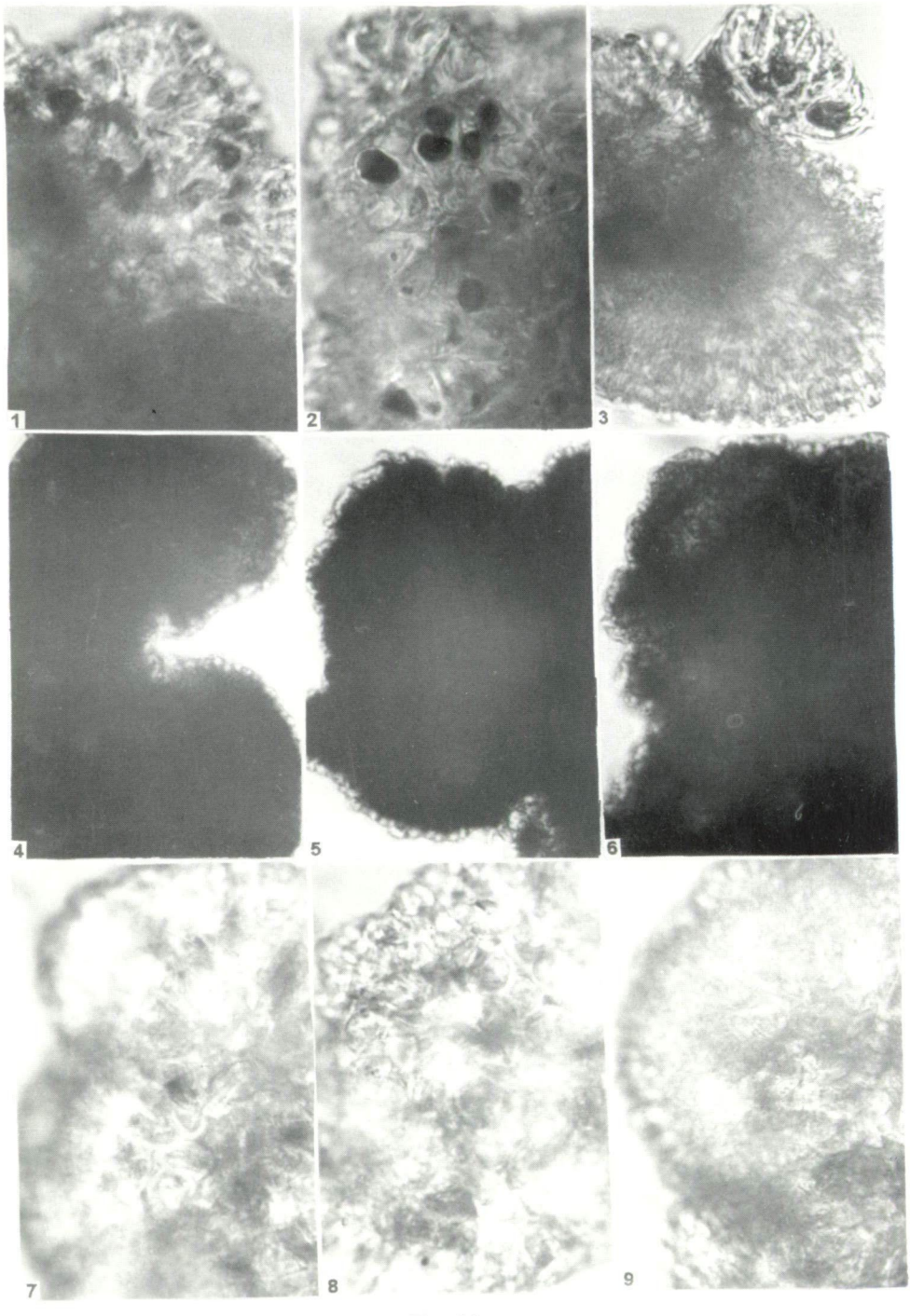
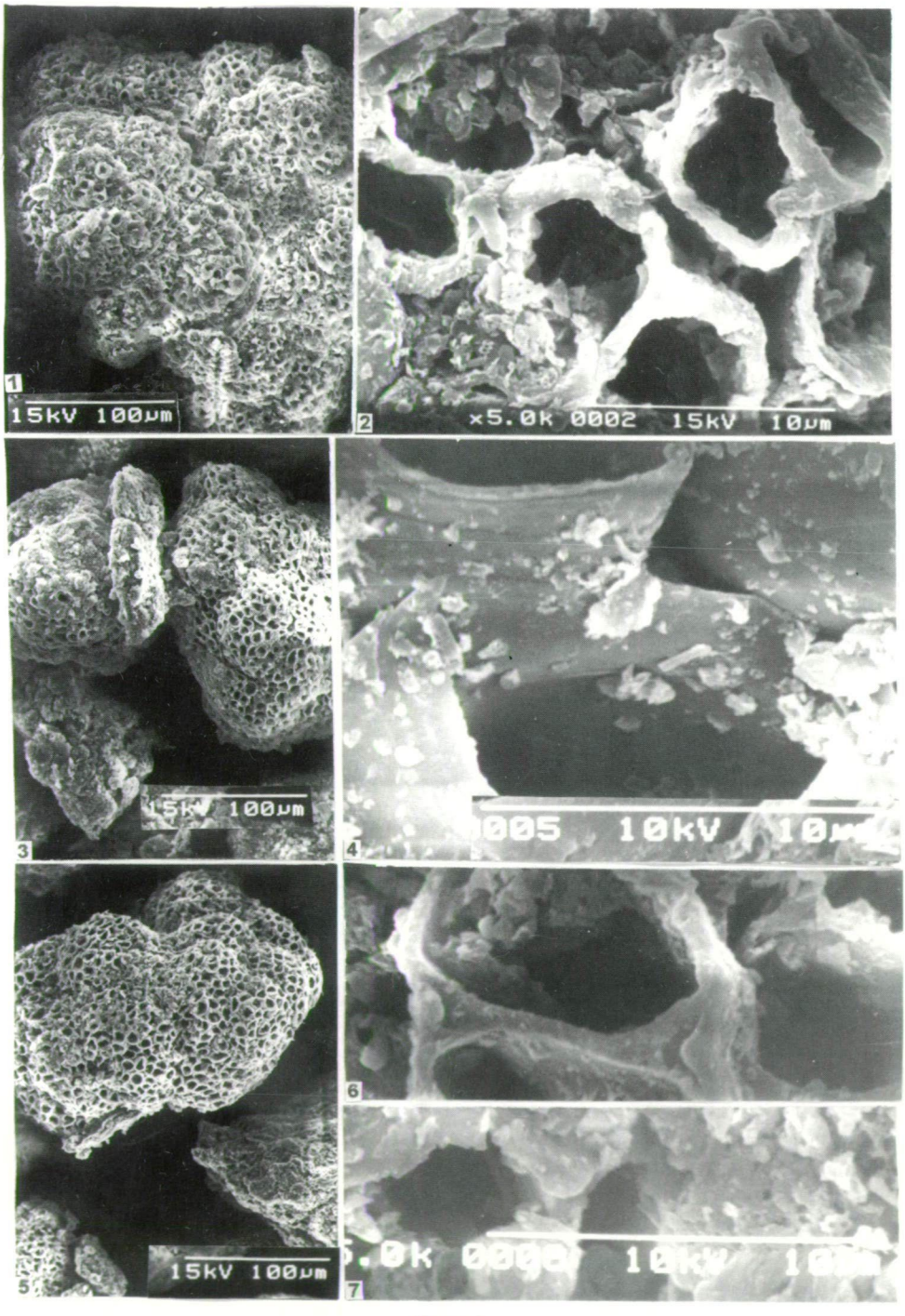


Plate 6.1.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2
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3
15kV 100µm
4
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15kV 100µm
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Plate 6.2.

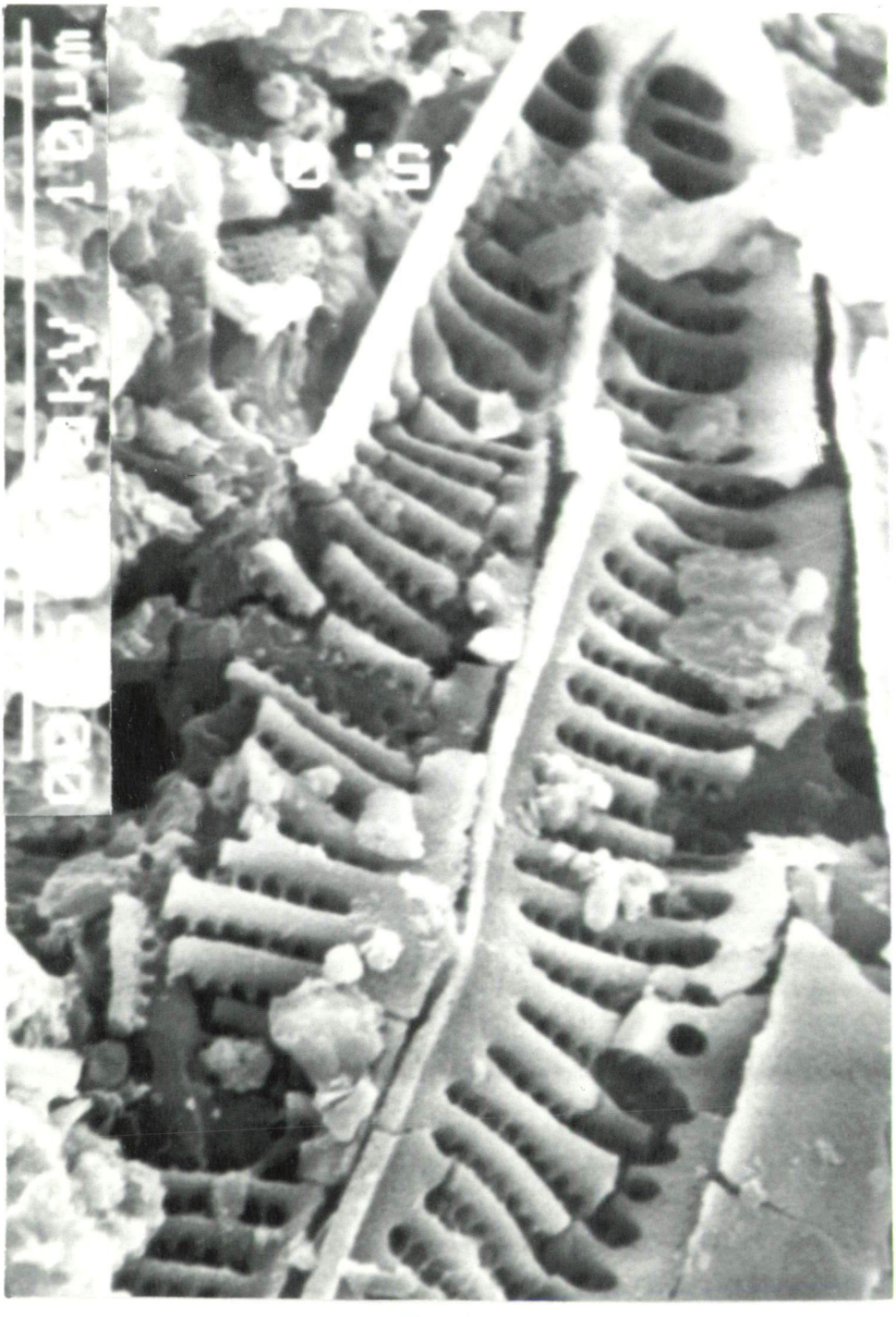


Plate 6.3.

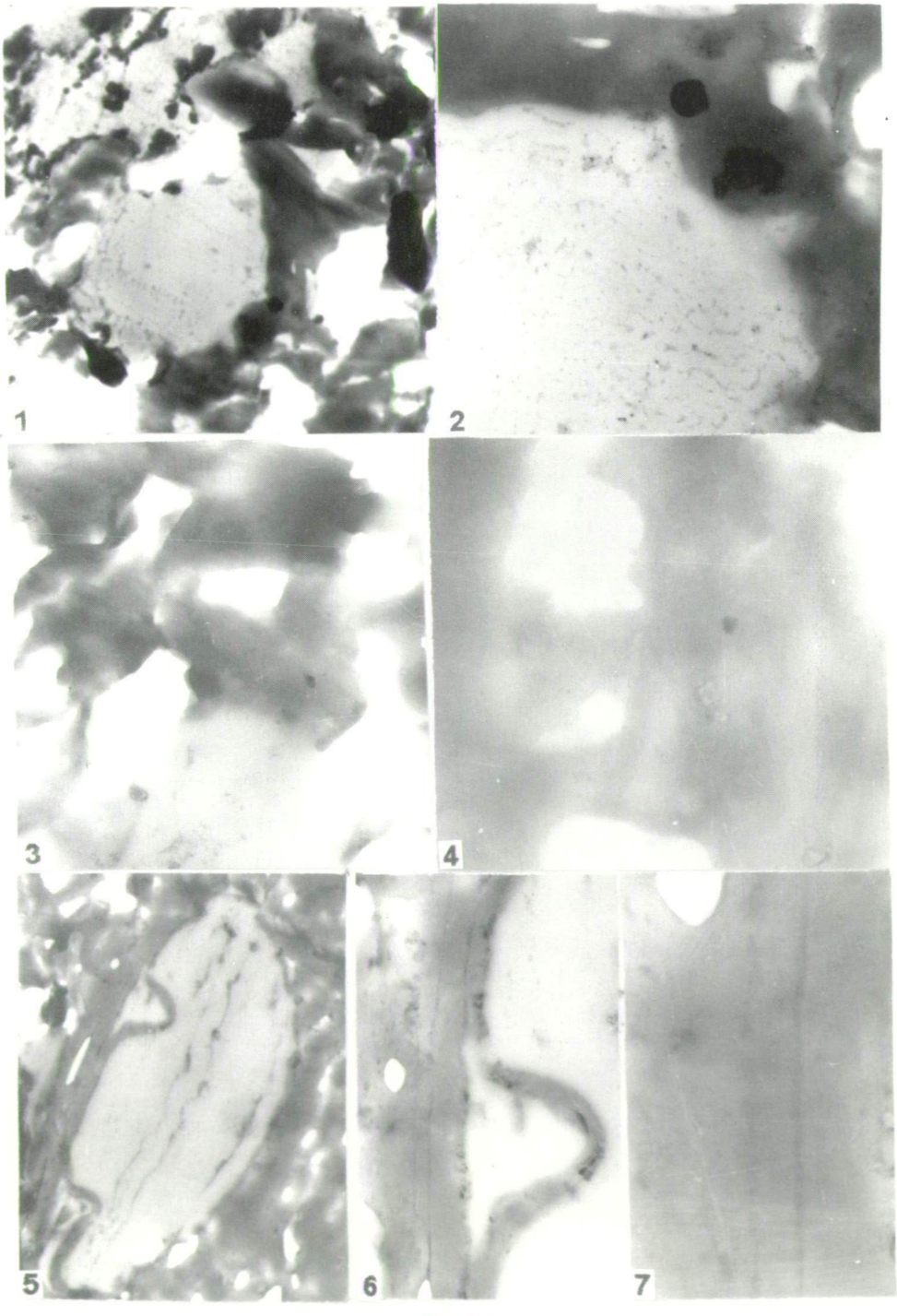


Plate 6.4.

The TEM pictures (Plate 6.6., figs. 1,2) illustrate the advanced degradation of the ultrastructure of the wall, but damaged lamellae are perceptible in particular in the low magnified picture (Plate 6.6., fig. 1).

Experiment: AKP-99-14

Based on the SEM pictures (Plate 6.5., figs. 3-5) this experiment revealed the globular biopolymer structures better than at the previous one. The distribution of the globular superficial units are as follows:

40	80	120	160	200	240	280	320	Å
17.5	36.1	26.3	12.2	4.7	2.3	0.4	0.5	%

The ultrastructure is severely damaged (Plate 6.6., figs. 3,4), sometimes less characteristic electron dense particles are in the substance of the wall.

Experiment: AKP-99-15

The SEM (Plate 6.5., figs. 6-8) and the TEM results (Plate 6.6., figs. 5,6) are essentially identical with those of the previous experiment. The distribution of the diameter of the globular biopolymer units are as follows:

40	80	120	160	200	240	280	320	360	Å
19.6	33.6	27.8	2.0	5.0	1.2	0.4	-	0.4	%

Experiment: AKP-99-16

Characteristic superficial degradation was observed in the SEM pictures (Plate 6.7., figs. 1,2) but the globular biopolymer units are not so well revealed, and are not suitable for statistical investigations. The TEM pictures (Plate 6.8., figs. 1,2) illustrate a very damaged wall structure. This may be the consequence of an individual characteristic feature.

Plate 6.5.

1-8. *Botryococcus braunii* KÜTZ. SEM pictures. 1,2. - Experiment number: AKP-99-13, 3,4,5. - Experiment number: AKP-99-14, 6,7,8. - Experiment number: AKP-99-15.

Plate 6.6.

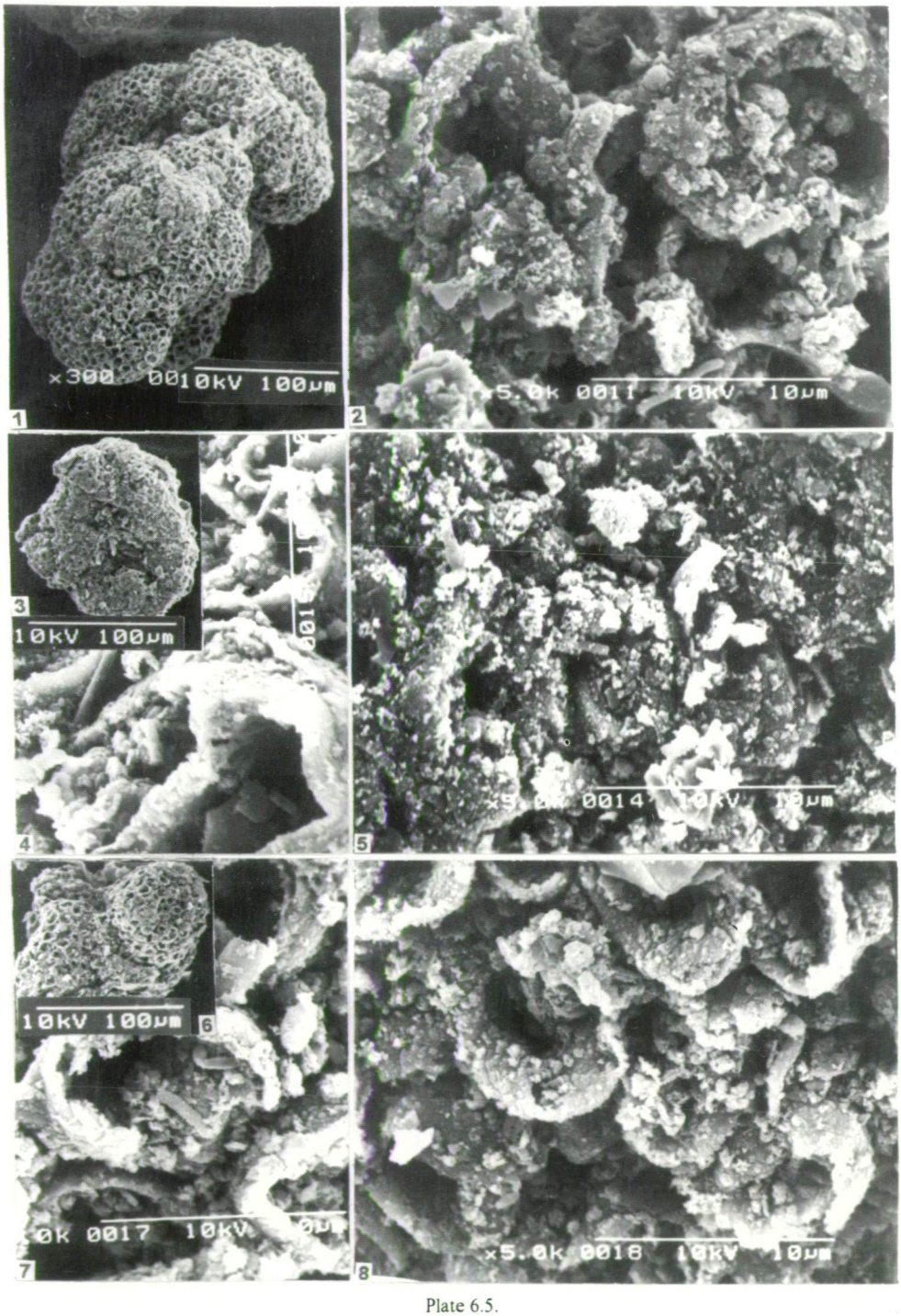
1-6. Ultrastructure of the partially degraded colonies of *Botryococcus braunii* KÜTZ. 1,2. -Experiment number: AKP-99-13, 1. Negative number: 7890, 50.000x., 2. Negative number: 7889, 50.000x., 3,4. - Experiment number: AKP-99-14, 3. Negative number: 7891, 5.000x., 4. Negative number: 7892, 15.000x., 5,6. - Experiment number: AKP-99-15, 5. Negative number: 7896, 15.000x., 6. Negative number: 7897, 50.000x.

Plate 6.7.

1-6. *Botryococcus braunii* KÜTZ. SEM pictures. 1,2. - Experiment number: AKP-99-16, 3,4. - Experiment number: AKP-99-17, 5,6. - Experiment number: AKP-99-18.

Plate 6.8.

Ultrastructure of the partially degraded colonies of *Botryococcus braunii* KÜTZ. 1,2. -Experiment number: AKP-99-16, 1. Negative number: 7924, 15.000x., 3,4. - Experiment number: AKP-99-17, 3. Negative number: 7927, 15.000x., 4. Negative number: 7928, 50.000x., 5,6. - Experiment number: AKP-99-18, 5. Negative number: 7930, 15.000x., 6. Negative number: 7932, 50.000x.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2
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3
10kV 100µm
4
5
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8
×5.0k 0018 10kV 10µm

Plate 6.5.

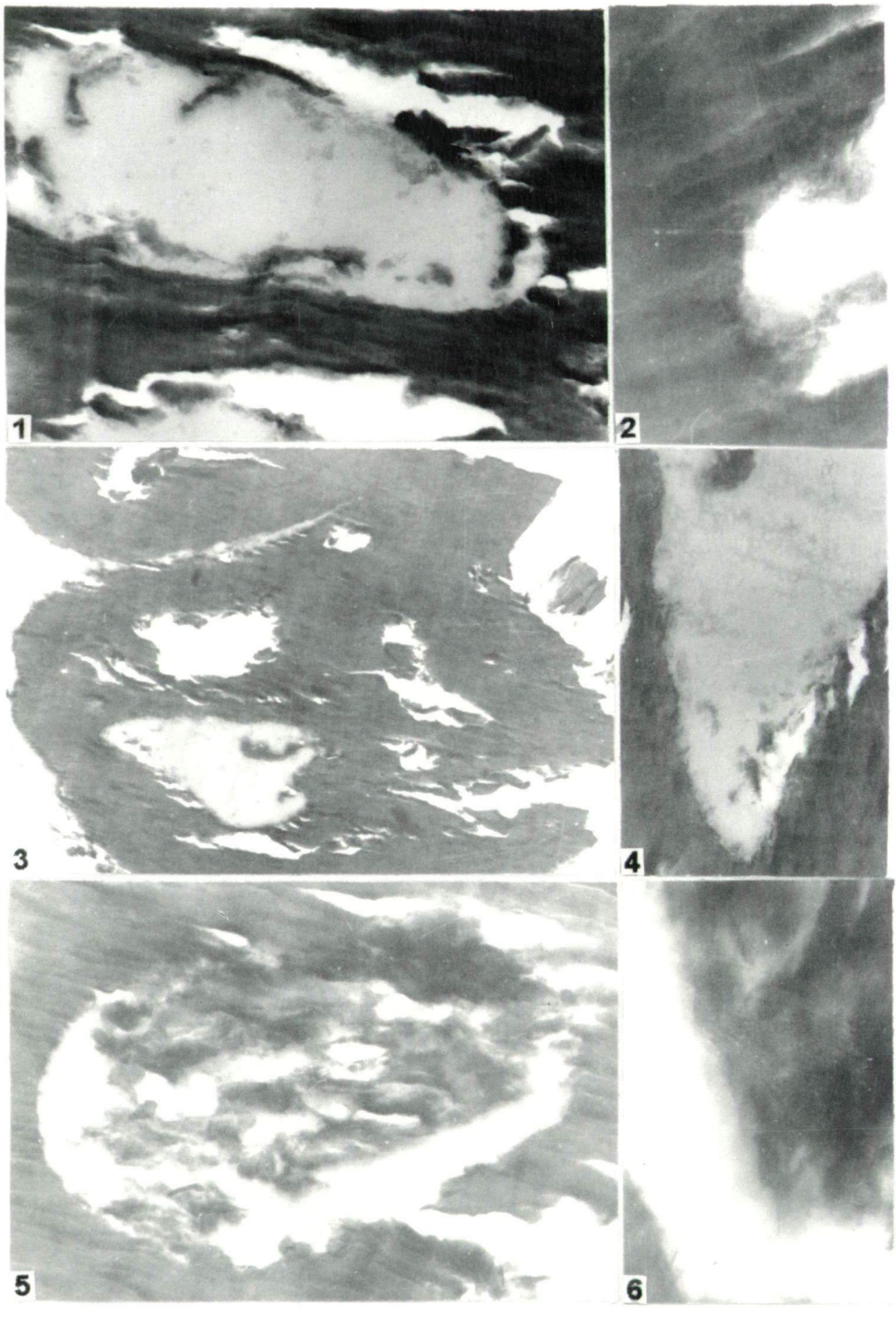

Plate 6.6.

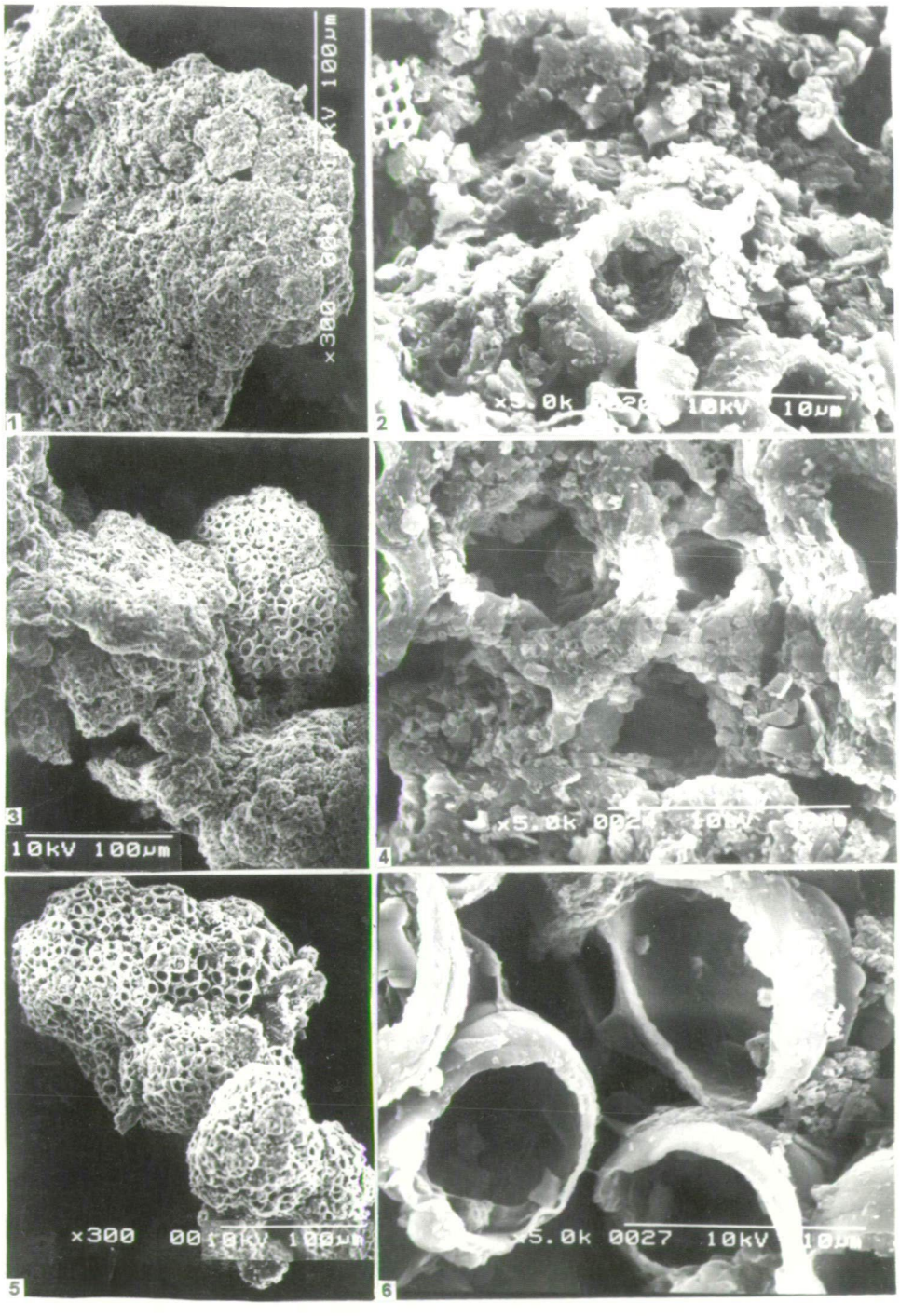

Plate 6.7.

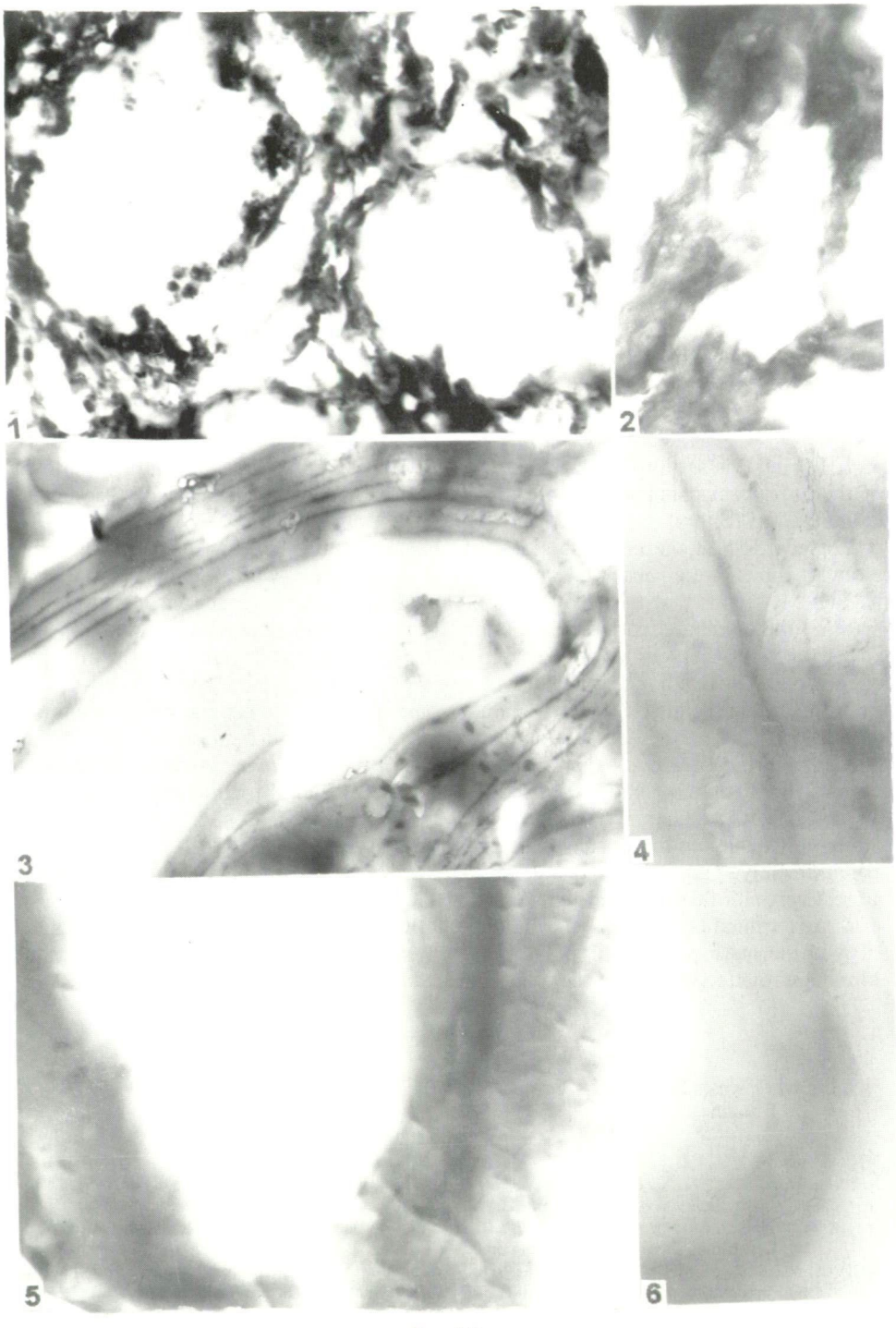

Plate 6.8.

Experiment: AKP-99-17

The SEM results are essentially identical with the previous one (Plate 6.7., figs. 3,4). The different kind of preservation is well illustrated in the picture 3, in Plate 6.7.

It is interesting that the TEM method resulted characteristic lamellar ultrastructure of the wall (Plate 6.8., figs. 3,4). Electron dense globular particles are well shown in the substance of the cups.

Experiment: AKP-99-18

In contrast to the previous experiments the surface is more or less smooth, globular units are very rare, particularly on the damaged parts of the colony (Plate 6.7., figs. 5,6). It may presumed that the superficial partially degraded layer was destroyed. The TEM pictures (Plate 6.8., figs. 5,6) support this supposition. The lamellar ultrastructure is not so characteristic, the surfaces are severely damaged.

Discussion and Conclusions

1. During this series of experiments we have observed several times the importance of the individual characteristic of the colonies, e.g.: ontogenetical stage, ecological, taphonomical factors.

2. According to the previous series of experiments by the SEM method superficial globular units were observed after partial degradation with 2-aminoethanol, and $KMnO_4$. It seems, that to discover the biopolymer system of recent and fossil organic material the combined partial degradation, namely 2-aminoethanol and $KMnO_4$ is suitable. But it is necessary to emphasize, that there are several exceptions also in consequence of the different molecular structure of the investigated biopolymer structures.

3. Regarding the diameter of the globular superficial units in general we can establish, that this stronger partial degradation discovered larger units than previously. In our classification in our previous paper, p. 82, was the largest globular structures of diameter more than 130 Å. The diameter of the partially degraded and fragmented colonies was about 224-240 Å. In this way the present experiments (AKP-99-13,14,15) discovered the largest units, but the greatest part of the units is between 40-120 Å. These units are approximately comparable to the data of AKP-99-6, with the remark that the diameter of a great percent of the globular units is 20 and 30 Å.

4. As terminal conclusion we can point out that these strongest partial degradation destroyed the quasi-periodic biopolymer system which can be studied with the modified Markham rotation method of the ultrathin sections.

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