5. TRANSMISSION ELECTRON MICROSCOPY OF HUNGARIAN TERTIARY LIGNITES I.

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Abstract

The ultrastructure of two Hungarian Tertiary lignites (*Sequoioxylon gypsaceum* (GÖPPERT) GREGUSS and *Sequoioxylon medullare* GREGUSS 1967) are presented in this paper. The LM structure of the non-experimental and the partially dissolved two lignit samples was published previously. The used two organic solvents (diethylamine, merkaptoethanol) altered the fine structure of the secondary wood. The alterations are not completely identical at the two samples investigated. The ultrastructure of the melanoresin content of the longitudinal parenchyma and the ray cells of *Sequoioxylon medullare* is published herein.

Key words: Xylotomy, transmission electron microscopy, Tertiary, Hungary.

Introduction

As it was emphasized previously (KEDVES, 1997) the light microscopical structure of the Hungarian Tertiary lignites was investigated in several papers. The experimental combined study of several samples by LM and TEM method is a new research program of our Laboratory. This contribution summarizes the first ultrastructure results within this project.

Till this time the TEM method was used for rebedded wood remains isolated from the sediments of the Lake of Soltvadkert (KEDVES and SZEDERKÉNYI, 1988). In this paper the importance of the ultrastructure of the secondary wood fragments in the paleoenvironmental reconstructions was emphasized.

The aim of this paper is to investigate the alterations in the ultrastructure of the lignit samples during the different kinds of sedimentation, and after the partial dissolution with two organic solvents.

General problems

For the fine structure of the wall of the secondary wood there are several schemas. The concept of BOUREAU (1954) was accepted in the great monograph of GREGUSS (1955). Based on the book of JANE (1970) the schema of the microfibrils of the tracheids are as follows:

Middle lamella – there are no microfibrils

Primary wall (P) – the orientation of the microfibrils is rather irregular.
Secondary wall (S)
S1 S helices "right handed"
S2 Z helices "left handed"
S3 S helices

BAMBER and SANGSTER (1977) investigated the sectioning characteristics of normal and gamma-irradiated conifer woods. In this paper the establishments of SEIFERT (1964) were pointed out, namely the lignin is resistant to high levels of gamma irradiation whereas cellulose is rapidly broken down. The SEM pictures of irradiated wood published by ANTOINE, AVELLA and VAN EYSEREN (1971) show better retention of structure than was observed in the sectioned embedded wood. This is the reason why ANTOINE and VAN EYSEREN (1971) proposed a new and easy technique of SEM wood sample preparation using radiation doses higher than 400 Mrad. The polylamellate structure consisting of about 15 successive lamellae is an alternating manner of the parenchyma wall in *Phyllostachys edulis* RIV. was published by PARAMESWARAN and LIESE (1975). CHAFE (1974) emphasized, a considerable variability in parenchyma cell wall of yellow cypress. The TEM method was used for the investigation of the shape and the fine structure of the pits in *Betula alleghaniensis* by KUNG-CHI YOUNG (1978). NEČESANÝ (1979) investigated the fine structure of laser cut surfaces of wood with the SEM method, and the results were compared with the quantity of used energy. FREY-WISSLING (1978) discussed the terminology of the primary cell wall (P), and concluded the following; p. 78: "The term Primary Wall should be reserved for the first lamella of the cell wall." HARADA (1984) summarized the concepts for the fine structure of the wood cell wall. The primary wall (P) in the typically xylem elements consists of two parts, P-outer, P-inner. The microfibrillar orientation of the two parts is different. The S1 or S3 is designated as a "flat helix" the S2 as a "srep helix", although the S1 has a crossed fibrillar structure. SUGIYAMA, HARADA and SAIKI (1984) studied the crystalline ultrastructure of cellulose microfibrils by various electron microscopy techniques. It was established that the cellulose is damaged by electron beam, and the cellulose crystals are destroyed easily by the dose normally required to record lattice images.

Materials and Methods

The previously published two samples were the subject of our TEM study. Non-experimental and partially dissolved secondary wood fragments were ultrathin sectioned. The methods in detail see in the previous paper – KEDVES 1997, p. 57, 61. The lignit samples were postfixed in OsO_4 aqu. dil. and embedded in Araldite (Durcupan, Fluka). The ultrathin sections were made in the EM Laboratory of the Institute of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences. The TEM pictures were taken on a Tesla BS-540 (resolution 6-7 Å).

Results

Sample no 1 (Plate 5.1., figs. 1,2, plate 5.2., figs. 1,2, plate 5.3.)
Sequoioxylon gypsaceum (GÖPPERT) GREGUSS 1967

Plate 5.1.

1,2. *Sequoioxylon gypsaceum* (GÖPPERT) GREGUSS 1967, non-experimental sample (T-9-1). Ultrastructure of the secondary wood remains.
1. Negative no: 6199, 50.000x.
2. Negative no: 6192, 50.000x.

Plate 5.2.

1,2. *Sequoioxylon gypsaceum* (GÖPPERT) GREGUSS 1967, TEM pictures of the partially dissolved lignite with diethylamine (T-9-2).
1. Negative no: 6436, 15.000x.
2. Negative no: 6440, 50.000x.

Plate 5.3.

Sequoioxylon gypsaceum (GÖPPERT) GREGUSS 1967. Ultrastructure of the partially dissolved lignit sample (T-9-3) with merkaptoethanol. Negative no: 6446, 10.000x.

Plate 5.4.

1-3. *Sequoioxylon medullare* GREGUSS 1967. TEM pictures from the non-experimental lignit sample (T-9-4).
1. Surface of the secondary wood. Negative no: 6629, 100.000x.
2. Inner part of the secondary woody remnant. Negative no: 6630, 100.000x.
3. Ultrastructure of the fossil resin. Negative no: 6162, 50.000x.

Plate 5.5.

1-4. *Sequoioxylon medullare* GREGUSS 1967, TEM pictures of the surface of the partially dissolved lignit sample (T-9-5) with diethylamine.
1. Negative no: 6633, 15.000x.
2. Negative no: 6634, 50.000x.
3. Negative no: 6635, 50.000x.
4. Negative no: 6635, 100.000x.

Plate 5.6.

1-3. *Sequoioxylon medullare* GREGUSS 1967. Ultrastructure of the lignit sample partially dissolved with merkaptoethanol (T-9-6).
1. Ultrastructure of the fossil resinous material in medullary ray cell. Negative no: 6673, 15.000x.
2. Ultrastructure of the partially dissolved secondary wood remnant. Negative no: 6474, 50.000x.
3. Ultrastructure of the surface of the partially dissolved secondary wood remnant. Negative no: 6474, 100.000x.

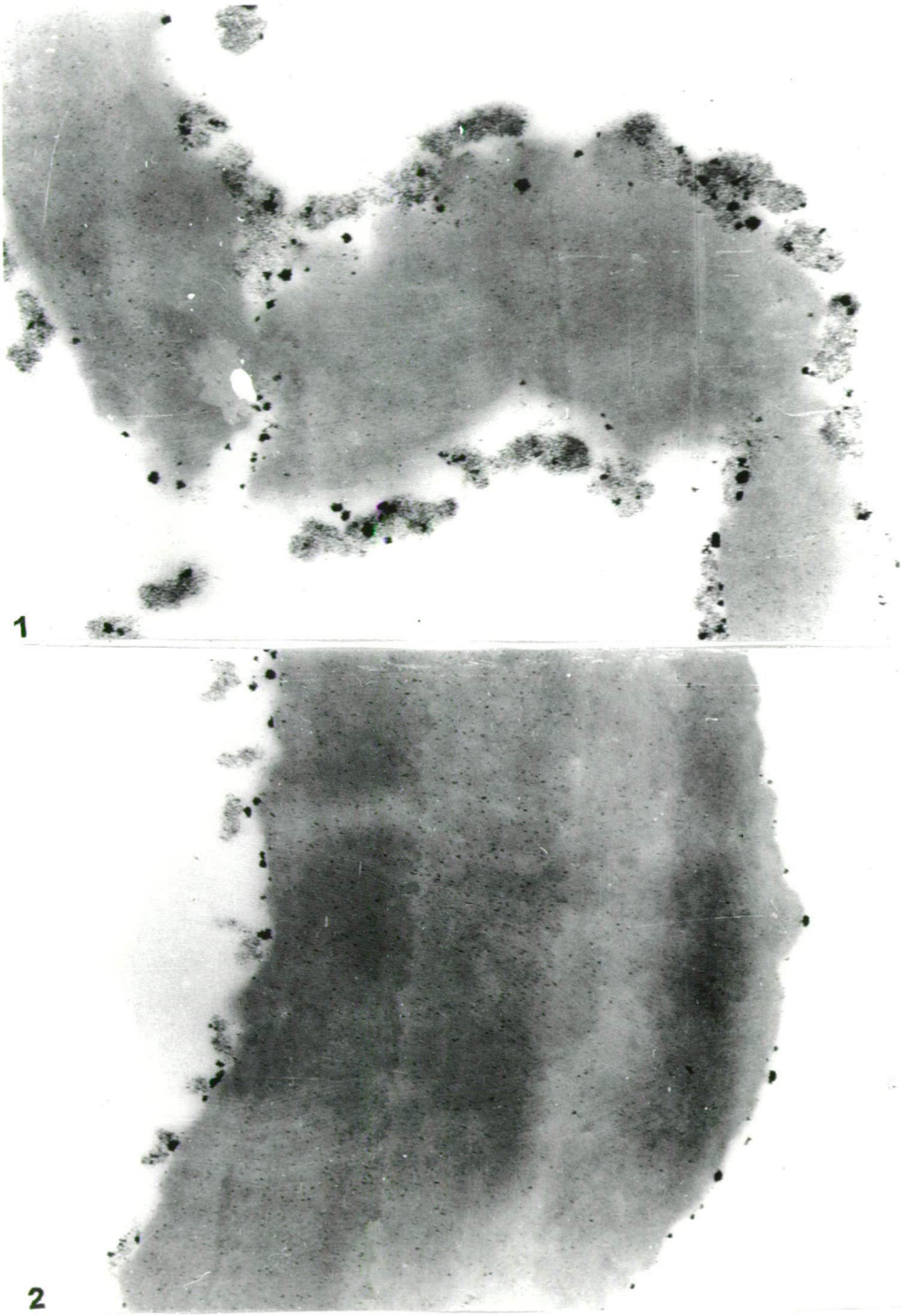

Plate 5.1.

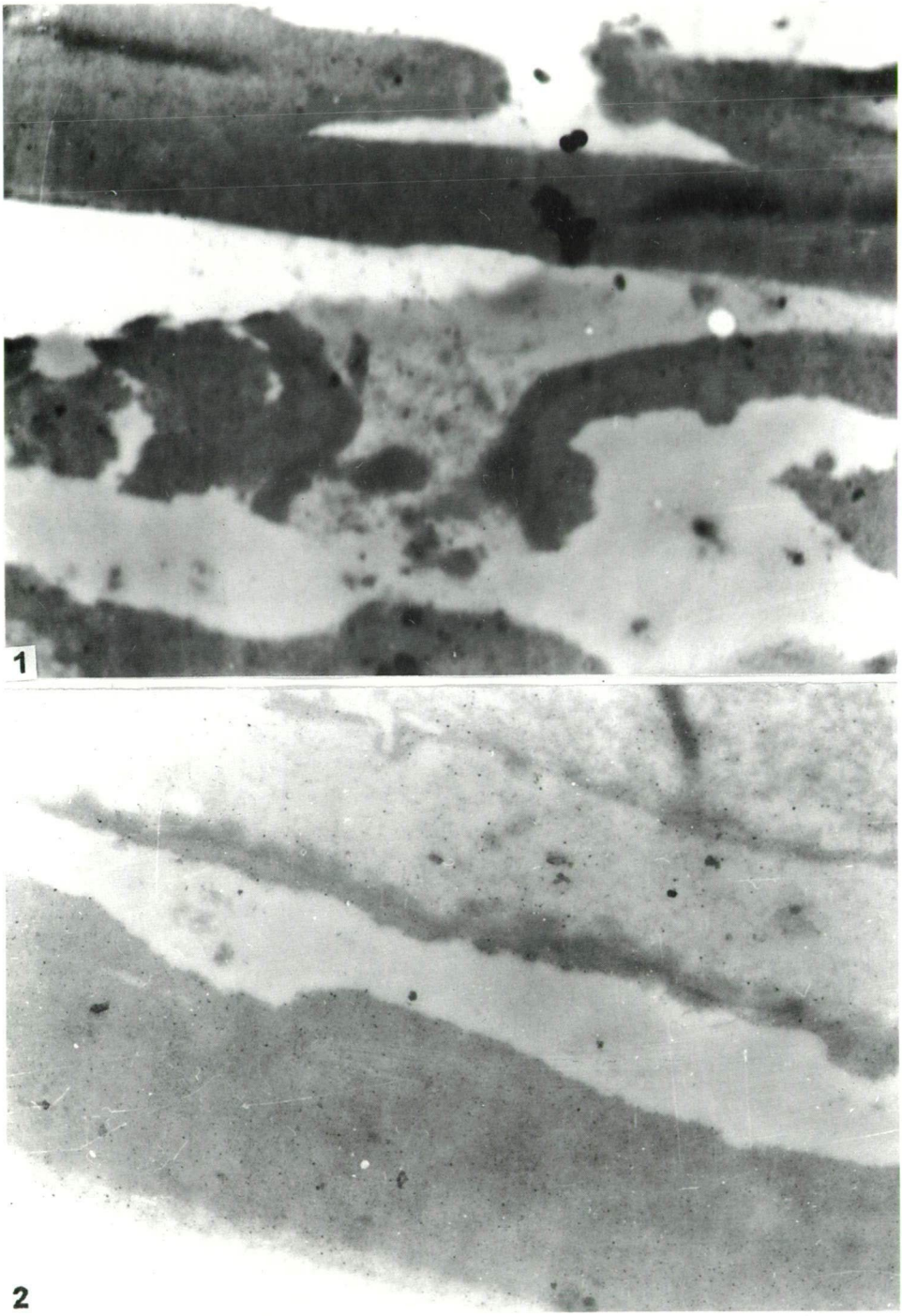

Plate 5.2.

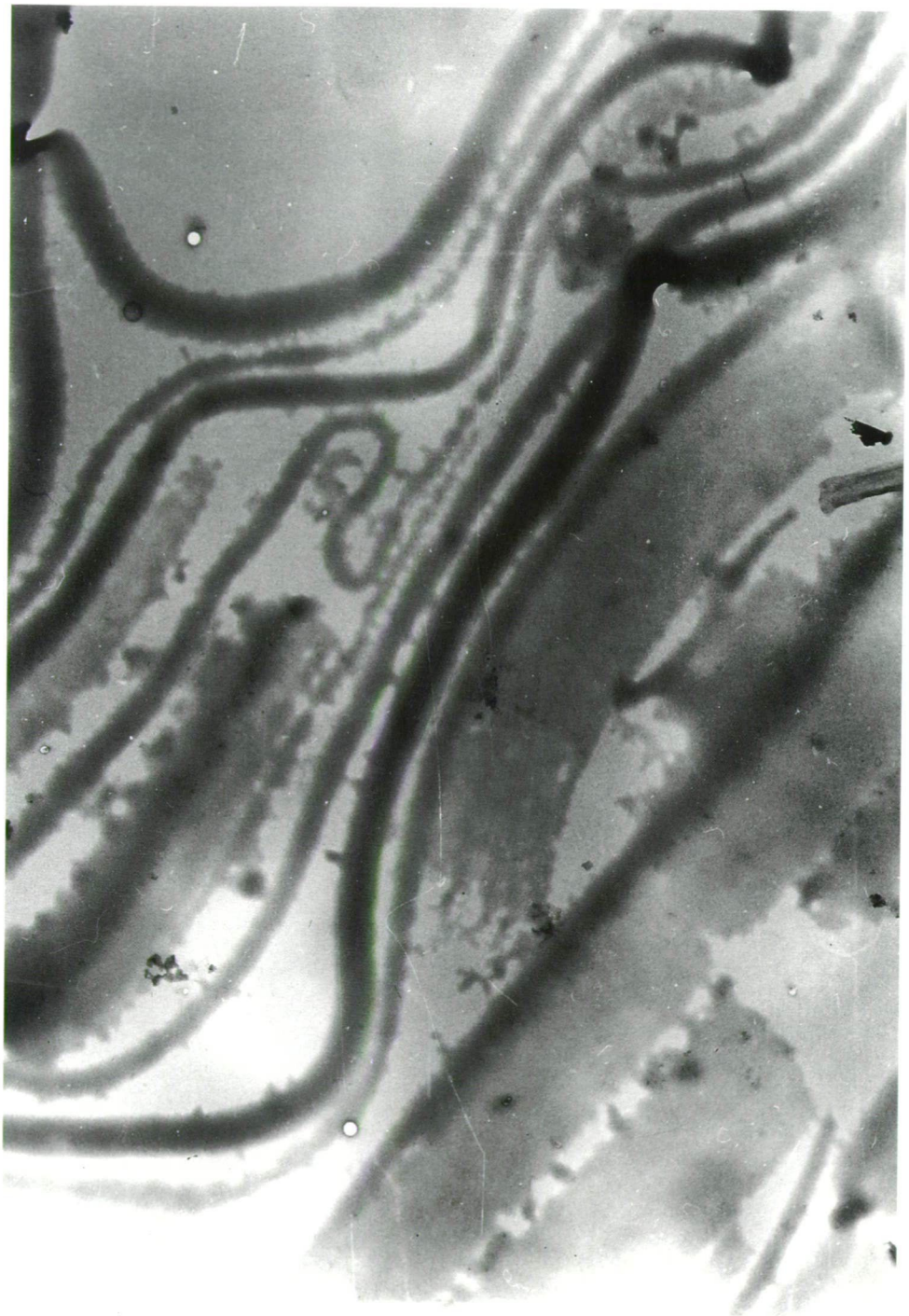

Plate 5.3.

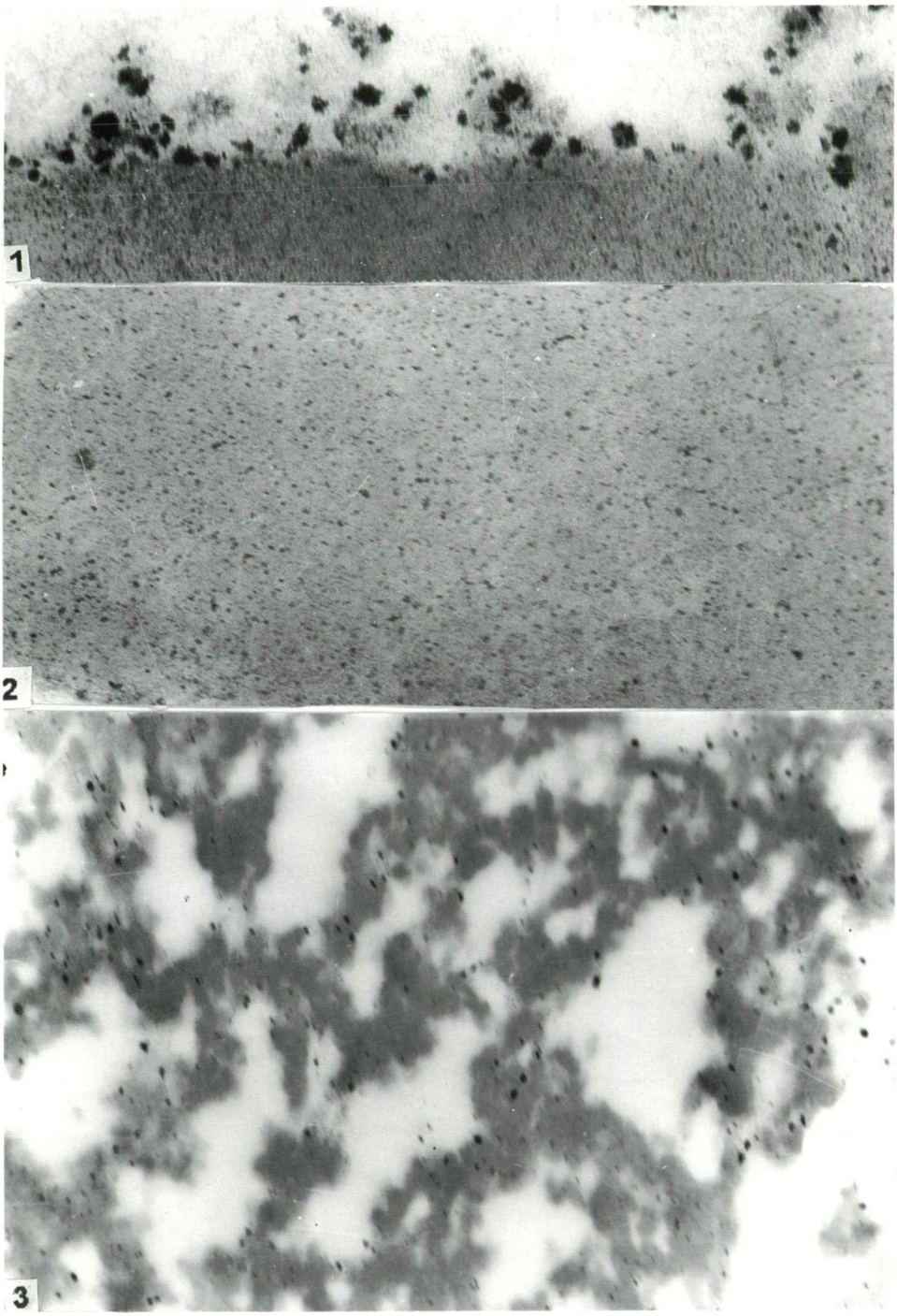

Plate 5.4.

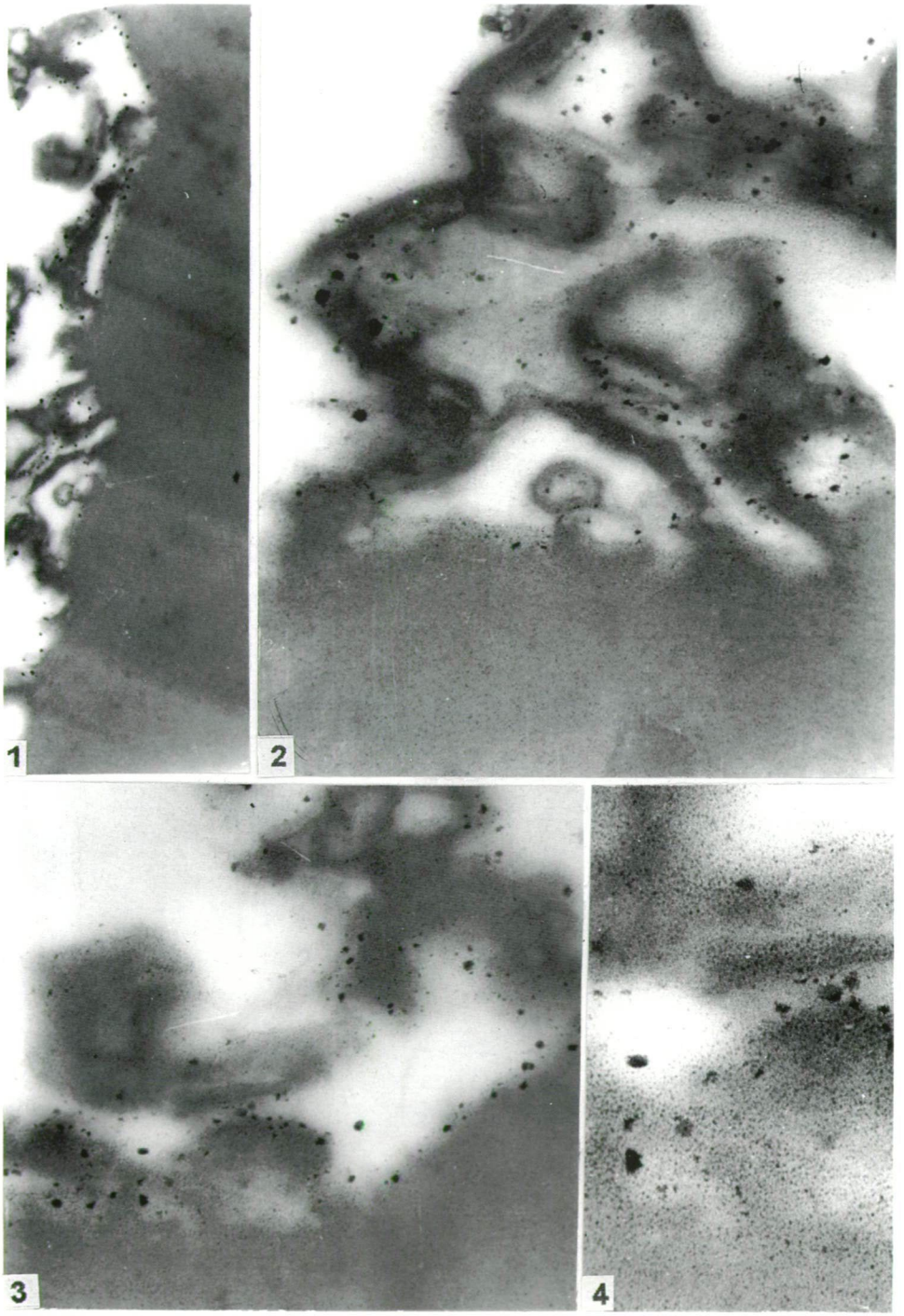

Plate 5.5.

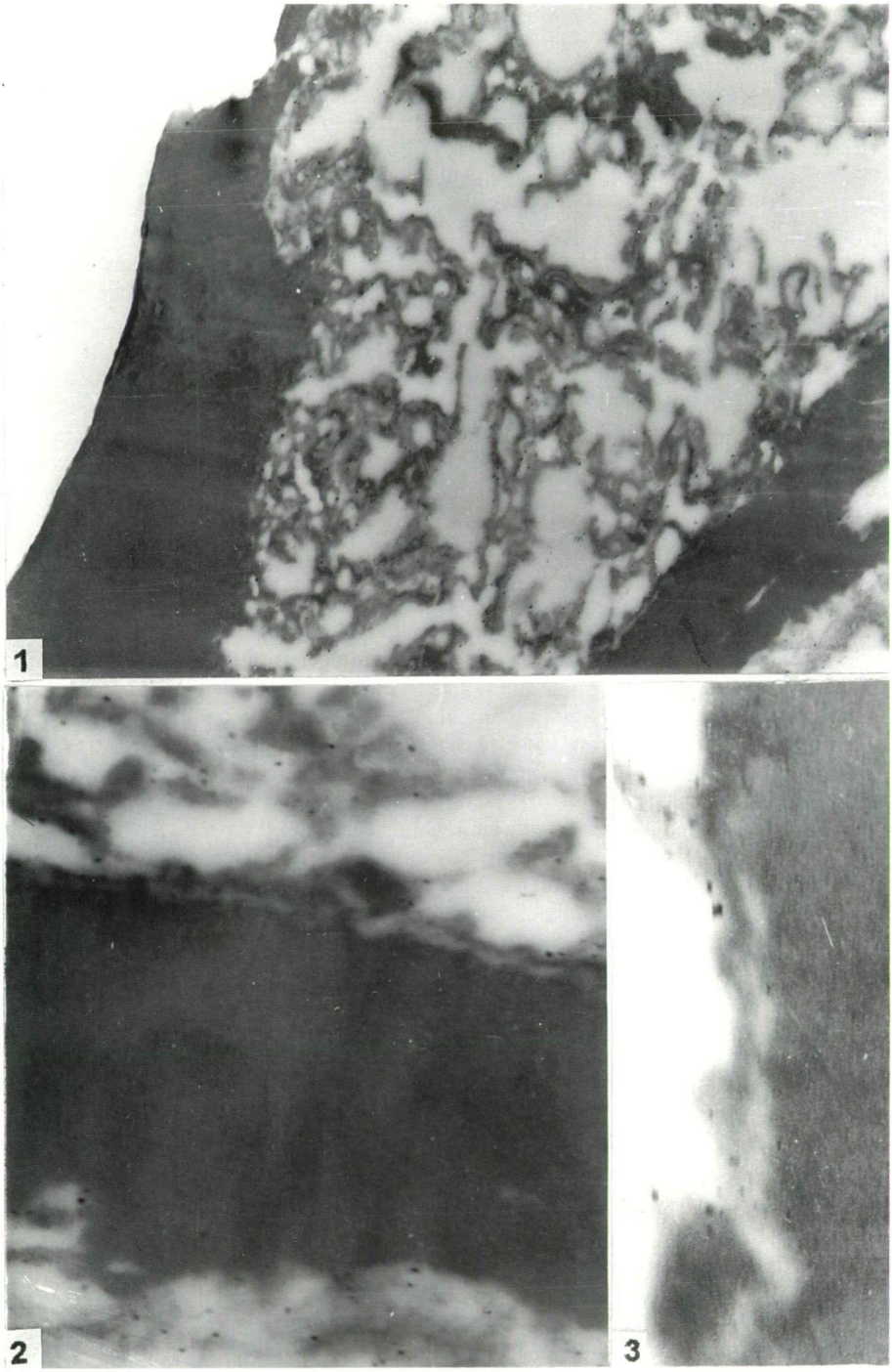

Plate 5.6.

The ultrastructure of the secondary wall of the non-experimental tracheids (Plate 5.1., figs. 1,2) is more or less homogeneous, but tiny granular elements are also present. Interesting alterations are on the surface. Extremely electron dense globular particles of different size are on the uneven surface. Finely granular particles are also on the surface or detached from the secondary wall.

Experiment T-9-2, dissolution with diethylamine during 30 days at 30 °C (Plate 5.2., figs. 1,2). After this experiment the electron dense particles, and the greatest part of the finely granular particles were dissoluted. No important alterations were observed at the secondary wall of the tracheids. On several parts of the tracheids, continuous in all probability secondary thin layer appeared on the surface. This layer is very clear in contrast to the secondary wall. Well shown on the lower part of picture 2 in Plate 5.2. Section of pits was also observed (Plate 5.2., fig. 1).

Experiment T-9-3, dissolution with merkaptoethanol during 30 days at 30 °C (Plate 5.3). The superficial electron dense particles dissolved. The outer part of the secondary wall is electron dense. In some parts of the ultrathin sections lamellar ultrastructure may be observed on the secondary wall. The detached lamellae of the secondary wall are electron dense.

Sample no 2 (Plate 5.4., figs. 1-3, plate 5.5., figs. 1-4, plate 5.6., figs. 1-3)
Sequoioxylon medullare GREGUSS 1967

The ultrastructure of the non-experimental tracheids (Plate 5.4., figs. 1-3). On the surface of the secondary wall of the tracheids there are electron dense particles of different size (Plate 5.4., fig. 1). Sometimes these granules are embedded in less electron dense particles which separate from the wall. The outer and the inner part of the secondary wall is full of tiny electron dense particles (Plate 5.4., figs. 1,2). The ultrastructure of the fossil resin (Plate 5.4., fig. 3) contains also tiny electron dense particles. The pattern of the resin has irregular network, composed of anastomosing drops.

Experiment T-9-5, dissolution with diethylamine during 30 days at 30 °C (Plate 5.5., figs. 1-4). The secondary wood seems to be more or less homogeneous without electron dense particles. The surface is interesting, different kind of lamellae and/or irregular particles separate from the compact secondary wall (Plate 5.5., 1-4). On the surface (Plate 5.5., fig. 3) in several places, there are tiny electron dense particles. These electron dense granular systems are present within the detached lamellae of the surface.

Experiment T-9-6, dissolution with merkaptoethanol during 30 days at 30 °C (Plate 5.6., figs. 1-3). After this kind of dissolution the wall of the secondary wood is more or less homogenous. (Plate 5.6., figs. 2,3). The outer lamellae on several part of the xylem remnant detached, well shown in pictures 2 and 3 in Plates 5.6. The ultrastructure of the resin remnant was observed in the ray cells (Plate 5.6., fig. 1). The fine structure of the resin is sometimes lamellar, but in general an irregular network.

Discussion and Conclusions

Based on our first TEM data of this research program of the Laboratory we can point out the following:

1. The peculiar submicroscopic pattern of the fossil resin was the same at the non-experimental and at the partially dissolved material.

2. The fossilization process results in remarkable alterations in the ultrastructure of the lignit samples. To this problem we need the results of further investigations within this program.

3. To investigate the microfibrillar structure of the fossil wall the partial dissolution with merkaptoethanol was the most successful.

Acknowledgements

This work was supported by the Grant OTKA T/9 023208.

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